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ORIGINAL

Analysis of Methamphetamine in Human Urine Using Ionic Liquid Dispersive Liquid-Phase Microextraction Combined with HPLC

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Abstract A specific and sensitive method using ionic liquid dispersive liquid-phase microextraction coupled with high-performance liquid chromatography was developed and applied in the analysis of methamphetamine in human urine. In this experiment, 1-butyl-3-methylimidazolium bis (trifluoromethyl) imide ([BMIM]TF_2N) was selected as the extraction solvent and parameters affecting the extraction efficiency such as the volume of ionic liquid, percentage of dispersant, dissolving temperature, extraction time, sample pH, centrifuging time and salting-out effect have been investigated in detail. Under optimized conditions, good linear relationship of methamphetamine was obtained in the range 0.04–20.00 $\mu g\ mL^{-1}$, the correlation coefficient (r) was 0.9987, the limit of detection and limit of quantitation were 0.01 and 0.04 $\mu g\ mL^{-1}$, respectively, and the enrichment factor was 44. The results showed that the method was simple, time-saving, special, clean, sensitive and it could be applied in the analgesics analysis of forensic trace evidences and addicted cases.

Keywords Dispersive liquid-phase microextraction · Methamphetamine · Ionic liquid · High-performance liquid chromatography · Urine

Introduction

Drug abuse is increasing among people around the world, especially among the young people, and most drug abusers are under the age of 30. Drug abuse not only affects human nature and causes numerous crimes, but also causes serious social problems throughout the world [1]. Cannabis is the most widely abused drug in all parts of the world, but the abuse of methamphetamine (MA) is commonly rising in our country at present, because it can be obtained easily and the price is much lower than other illicit drugs [2]. MA is a powerful stimulant of the central nervous system and the most frequently abused drug, and there are more reports on the addict cases of MA [3]. Therefore, it is important to establish a simple, direct and sensitive preliminary screening method for the determination of it. Several analytical approaches have been applied in the determination of MA in pharmaceutical samples and biomaterials, including gas chromatography [4], gas chromatography–mass spectrometry [5], high-performance liquid chromatography (HPLC) [6] and liquid chromatography–mass spectrometry [7]. Generally, a sample pretreatment step is commonly applied before an effective method is developed; up to now, many methods have been established for the enrichment of MA in urine, including liquid–liquid extraction [8], solid-phase extraction [9], etc. All these techniques often needed a large volume of the sample of interest, toxic organic solvents and also were labor intensive, expensive, and time-consuming. As new extraction solvents, ionic liquids (ILs) have many interesting properties including wide liquid ranges, low volatility, good stability, reusability [10], and they have been widely used in organic chemistry [11, 12] and analytical chemistry [13–15].

In the present study, a specific and sensitive method for the analysis of analgesics using ionic liquid dispersive liquid-phase microextraction combined with HPLC was developed and applied in the identification of MA in human urine. The factors influencing the extraction efficiency have been investigated in details. Under the optimized conditions, good linear relationship of MA was obtained, and the results showed the potential in the analgesics analysis of forensic trace evidences and addicted cases.

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Experimental

Chemicals and Reagent

Chromatography grade methanol, acetonitrile and acetone were purchased from Shield Co., Ltd. (Tianjin, China). Triethylamine, diethyl ether, acetic acid, sodium carbonate, sodium bicarbonate and sodium chloride were all of analytical grade and purchased from Guoyao Group Chemical Reagent Shenyang Co., Ltd. (Shenyang, China). C_{18} solid-phase extraction column (200 mg, 10 μm) was purchased from Supelco Co. (PA, USA). MA standard was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) and the purity was above 98 %. All kinds of ionic liquids including 1-octyl-3-methylimidazolium tetrafluoroborate ([OMIM]BF_4), 1-butyl-3-methylimidazolium chlorate ([BMIM]Cl), 1-butyl-3-methylimidazolium bis [(trifluoromethyl) sulfonyl] imide ([BMIM]TF_2N), 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM]PF_6), 1-hexyl-3-methylimidazolium tetrafluoroborate ([HMIM]BF_4), 1-hexyl-3-methylimidazolium hexafluorophosphate ([HMIM]PF_6) and 1-octyl-3-methylimidazolium hexafluorophosphate ([OMIM]PF_6) were purchased from Lanzhou Institute of Chemical Physics of the Chinese Academy of Sciences (Lanzhou, China). Human drug-free urine was obtained from volunteers and all of the experiments were performed in compliance with the relevant laws and institutional guidelines of National Police University of China, and the institutional committee approved the experiments.

Sample Extraction Procedure

200 μL of acetonitrile was selected as dispersant and 70 μL ([BMIM]TF_2N) as extractant in our work, they were added in 3 mL human urine, the pH value was adjusted to 8.0 with Na_2CO_3–$NaHCO_3$ buffer and NaCl was added in urine with the concentration of 20 % (w/v), and then the mixture was incubated in 50 °C water bath for 6 min after vortex-mixed for 1 min, then was extracted in the ice bath for 11 min, and then the mixture was centrifuged at 8,000 rpm for 4 min, the upper aqueous phase was removed with a syringe, the sediment phase was dissolved in 200 μL methanol and 10 μL was injected into the HPLC system for analysis.

Chromatographic Conditions

HPLC separation was performed with HITACHI-655 liquid chromatography system equipped with HITACHI ultraviolet detector (Hitachi Ltd., Tokyo, Japan). Thermo Gold ODS column (150 × 4.6 mm, 5 μm) was used and the mobile phase consisting of methanol, 0.1 % (v/v) triethylamine (adjusted to pH 4.50 with acetic acid) aqueous solution (45:55) was chosen and the flow rate was 1.0 mL min^{-1}. Detection wavelength was set at 210 nm and the injection volume was 10 μL.

Results and Discussion

In this study, experimental parameters affecting the extraction efficiency of MA including ionic liquid, dispersant, salting-out effect, sample pH valve, extraction time and temperature, and centrifuging time were all investigated in details.

Selection of Ionic Liquid

ILs are composed of an organic cation and either an organic or an inorganic anion. They remain liquid over a wide range of temperatures, including room temperature, and they are a new group of solvents of great interest, which have been recently studied as potential "green solvents" [16]. There are many kinds of ionic liquids for us to choose in analytical chemistry. Seven kinds of ionic liquids including [OMIM]BF_4, [BMIM]Cl, [BMIM]TF_2N, [BMIM]PF_6, [HMIM]BF_4, [HMIM]PF_6 and [OMIM]PF_6 were examined in our study, and the results are shown in Fig. 1. As shown in Fig. 1, the results showed that the extraction efficiency of [BMIM]TF_2N was higher than others, so it was utilized as the extractant in the liquid-phase microextraction of MA in human urine.

Effect of Amount of Ionic Liquid

The amount of ionic liquid plays an important role in the extraction procedure. Different volumes of ionic liquid were added in 3 mL urine aiming to evaluate the effect

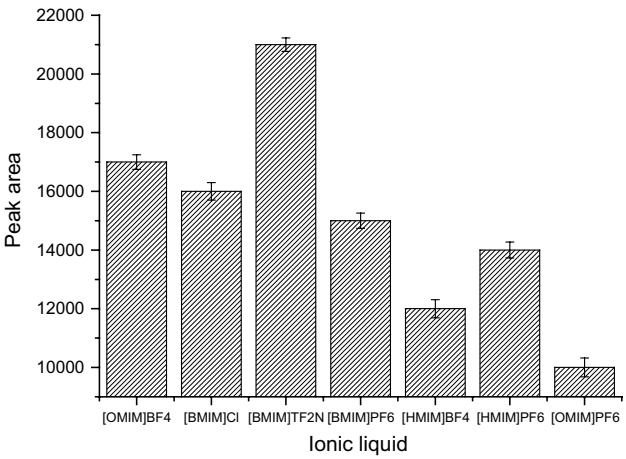

Fig. 1 Comparison of different ion liquid

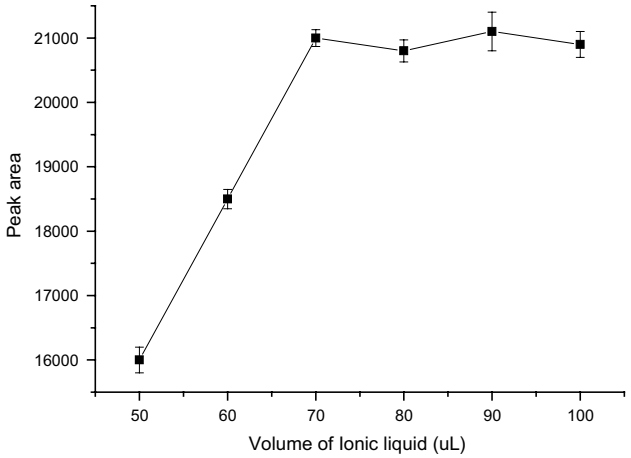

Fig. 2 Effect of amount of ionic liquid

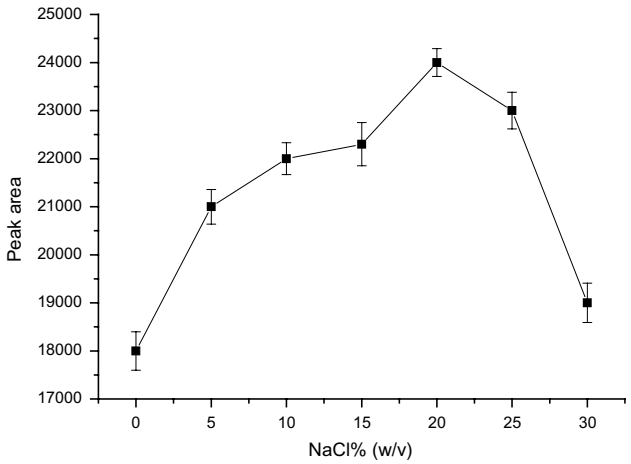

Fig. 3 Effect of ionic strength

of amount of ionic liquid in the range 50–100 μL. The detailed results can be found in Fig. 2. When amount of [BMIM]TF_2N is lower than 70 μL, [BMIM]TF_2N could not foam in the solution well. In addition, if the amount is too high, the ability of extraction is limited. Therefore, the concentration of [BMIM]TF_2N was optimized as 70 μL adding in 3 mL sample.

Optimization of Dispersant

To obtain excellent extraction efficient, the type of dispersant should be selected and the percentage of dispersant in sample also needed to be optimized in the analysis of MA in human urine. According to the corresponding report in Reference [16], the dispersant was employed as acetonitrile by comparing the extraction efficiencies of methanol, acetonitrile and acetone, which were dispersants and commonly utilized in the ionic liquid dispersive liquid-phase microextraction separation. The percentage of dispersant in sample affected the extraction efficiency of target compound. Aiming to select the correct percentage of acetonitrile in urine, different volumes of acetonitrile were individually added in 3 mL urine, respectively. And then the concentration was selected as 200 μL in the extraction procedure based on the comparing results of different concentrations of acetonitrile to the extraction efficiencies of MA in human urine, it mean that the percentage of acetonitrile in urine was 2.85 to the usage of ionic liquid in the extraction procedure.

Effect of Ionic Strength

The salting-out effect is often utilized to enhance the enrichment or preconcentration performance in separation science. Addition of salt could decrease the solubility of analytes in the aqueous sample and enhance their partitioning into the adsorbent or organic phase [17]. A series of experiments with different concentrations of NaCl in human urine were designed aiming to investigating the salting-out effect, and the results are shown in Fig. 3. The results demonstrated that a significant increase of the peak area occurred as the ionic strength of the solution increased from 0 to 20 % (w/v), but the peak area decreased when the ionic strength of the solution was further increased in the range 20–30 %. So, 20 % (w/v) NaCl was added in the following experiments.

Optimization of Sample pH Value

The sample pH value is also a key factor in the sample pretreatment techniques, which determines the present form of the analytes and the extraction efficiency. Therefore, the effect of sample pH value also needs to be investigated. In this study, it was carried out in the pH range 7–11 via adding different volumes of Na_2CO_3–$NaHCO_3$ buffer in urine samples and the results are shown in Fig. 4. The peak areas increased with the increase of pH value in the pH range 7–8, and decreased in the pH range 8–11. This phenomenon was closely related to the chemical properties of MA. Based on the results, pH value of sample was adjusted as 8 with Na_2CO_3–$NaHCO_3$ buffer in all subsequent experiments.

Effect of Extraction Temperature

In this established process, temperature is a crucial factor influencing the extraction efficiency, because it is the driving force for the dispersion of ionic liquid into the aqueous solution. To achieve the best temperature for extraction, the

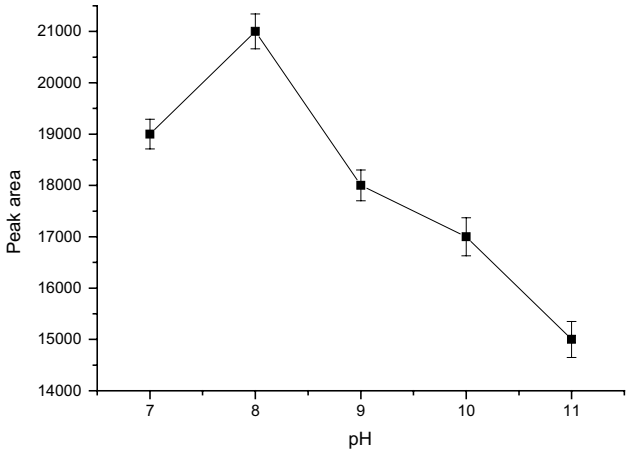

Fig. 4 Effect of sample pH

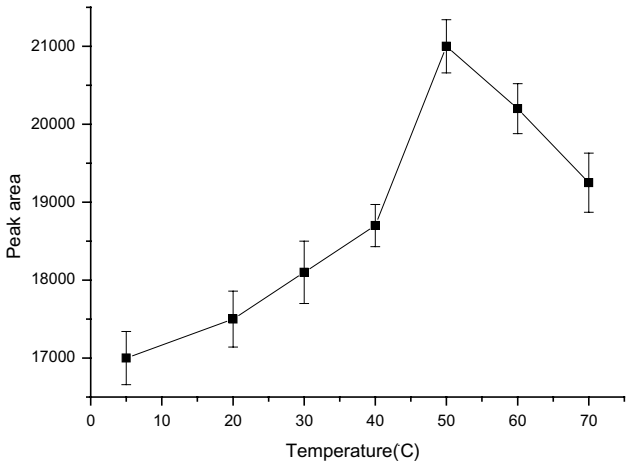

Fig. 5 Effect of temperature

effect of temperature was investigated in the range 5–65 °C and the results are listed in Fig. 5. As shown in Fig. 5, 50 °C was the reasonable temperature because the largest peak area occurred at the temperature. The reason was that at low temperature, the performance of dispersing of ionic liquid was poor and the diffused rate of MA was very low, so the mass transfer coefficient was low. But at higher temperature, the diffused rate and mass transfer rate were increased, the amounts of MA increased with the rise of temperature. However, the rise of temperature also resulted in increasing MA migrating out from the ionic liquid phase. From Fig. 5, we can see that the transferring rate into the ionic liquid was larger than the migrating out rate when the temperature was lower than 50 °C and a reverse result was obtained when the temperature was higher than 50 °C, so the dissolving temperature was set at 50 °C. Furthermore, the time for heating was also tested in the range 2–10 min. The results exhibited that the extraction efficiency of MA increased with the time up to 6 min, and then remained constant with further time, so the heating time was chosen as 6 min in the experiments.

Effect of Extraction Time

Extraction time is another critical parameter in most extraction process. It is defined as the time from the moment that the solution containing completely dissolved ionic liquid was put into ice water bath to the moment to start to centrifuge. Experimental results indicate that the extraction efficiency of MA increased with the extraction time up to 13 min, and then remained constant with further prolongation of extraction time. Therefore, 11 min was chosen as the optimal extraction time.

Effect of Centrifugation Time

Centrifugation is a critical step to obtain two distinguishable phases, so it will affect the size of the settled phase and the concentration of analytes in extraction phase. The centrifugation time was checked in the range 1–8 min at 8,000 rpm after extraction in our study. The results indicated that the peak area of MA increased as the centrifugation time increased from 2 to 4 min, and decreased after 4 min. The sediment volume increased along with the time, but longer centrifuging time resulted in heat generation, which led to the re-dissolution of some ionic liquid into the water phase and a loss of extraction performance. Finally, 4 min was used for the centrifugation time in the experiment.

Comparison with Liquid–Liquid Extraction and Solid-Phase Extraction

To evaluate the extraction efficiency, the comparison of ionic liquid with liquid–liquid extraction and solid-phase extraction were performed. Liquid–liquid extraction and solid-phase extraction experiments were performed using 5.0 mL of diethyl ether and C_{18} solid-phase sample-preparation column to extract 3.0 mL urine samples spiked with 1.0 μg mL^{-1} of MA according to the reports extraction procedures in literatures [9, 18], respectively. The results shown in Table 1 demonstrated that the detection concentration and limit of detection of these three approach were very similar, while the extraction recovery obtained with ionic liquid was a little higher than that obtained with solid-phase extraction and a little bit lower than the result obtained with liquid–liquid extraction, but the experimental costs and the solvent usages of the approach mentioned here were lower than traditional extraction methods.

Table 1 Comparison of ionic liquid dispersive liquid-phase microextraction with traditional liquid–liquid extraction and solid-phase extraction

Method	Detection result ($\mu g\ mL^{-1}$)	Extraction radio (%)	Limit of detection ($\mu g\ mL^{-1}$)
Ionic liquid dispersive liquid-phase microextraction	0.87 ± 0.03	87 ± 3	0.01
Liquid–liquid extraction with ethyl ether	0.91 ± 0.02	91 ± 2	0.008
Solid-phase extraction with C_{18} column	0.85 ± 0.04	85 ± 3	0.01

Method Validation

Under the optimal conditions, the proposed method was applied to the analysis of MA in human urine, and the HPLC chromatogram is shown in Fig. 6. Calibration curve was constructed by analyzing the spiked urine samples with MA at seven concentrations prepared in the range 0.010–30.00 μg mL^{-1}. The samples were treated with ionic liquid and analyzed by HPLC. The obtained calibration curve exhibited good linearity in the range 0.040–20.00 μg mL^{-1} and the coefficient was 0.9987 as shown in Table 2. To estimate the limit of detection (LOD) and the limit of quantitation (LOQ), spiked urine samples at different concentrations were analyzed. The LOD and LOQ of MA developed in the present work are also listed in Table 2, which were calculated on the basis of the chromatographic peak for which the signal-to-noise ratio was 3 ($S/N = 3$) for qualitative analysis and 10 ($S/N = 10$) for quantitative analysis, respectively. As shown in Table 2, LOD and LOQ for MA were 0.010 and 0.040 μg mL^{-1} in spiked human urine, respectively.

The precision referred by relative standard deviation (RSD) was determined by analyzing spiked urine samples with different concentrations of MA, which were set with low, medium and high level of the calibration range as 0.10, 1.00 and 10.00 μg mL^{-1} for MA, respectively. The intra-day precision was calculated by analyzing the samples within 1 day ($n = 3$), while the inter-day precision was determined by analyzing MA at the same concentrations in three consecutive days ($n = 9$), and the results are listed in Table 2. Both RSDs were <4.7 % as shown in Table 1, indicating that the method has good precision and repeatability in the quantitative analysis of MA in urine samples.

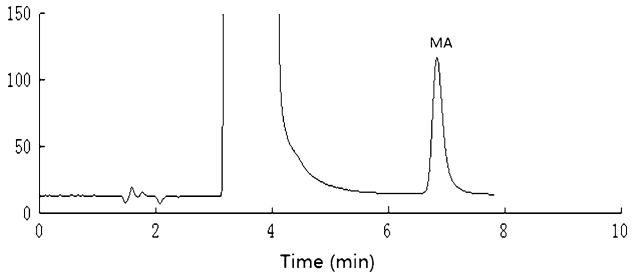

Fig. 6 Chromatogram of MA

While method accuracy was evaluated with the spiked urine samples at known levels of MA, the recovery experiments were also performed by analyzing the samples with HPLC analysis. The analysis data were obtained by comparing the average determined concentrations with the known spiked levels and are listed in Table 2. The average recovery was 87.4 % obtained from the analyzing of samples at low, medium and high concentrations of MA in human urine samples; the result indicated that the method provided good accuracy for the analysis of MA in biological materials and revealed that the approach could be considered as a good candidate for a method to determine the abuse drugs.

To evaluate the extraction effect of ionic liquid, enrichment factor (EF) and enrichment recovery (ER) were examined in our study, and the calculation formulas were as follows [17]:

$$EF = C_{sed}/C_0,$$

$$ER = C_{sed}V_{sed}/C_0V_0 \times 100\% = EF \times V_{sed}/V_0 \times 100\%$$

where C_0 is the concentration of MA before extraction, V_0 is the volume of urine, C_{sed} is the concentration of MA in extraction solvent, V_{sed} is the volume of extraction solvent. The EF and ER of MA were calculated according to the formulas and the results are shown in Table 2.

Urine Samples Analysis of Drug Abusers

Aiming to verify the feasibility of the analysis method developed in this study, volunteers who had been proved as

Table 2 Linearity range, LOD, LOQ, precision, recovery, enrichment factor and enrichment recovery of MA in human urine

Compound	Linearity range ($\mu g\ mL^{-1}$)	Coefficient (r)	LOD ($\mu g\ mL^{-1}$)	LOQ ($\mu g\ mL^{-1}$)	Precision (RSD, %)		Recovery (%)	EF	ER (%)
					Intra-day ($n = 3$)	Inter-day ($n = 9$)			
MA	0.04–20	0.9987	0.01	0.04	4.1	4.7	87.4	44	88.7

drug abusers of MA in the Institute of Forensic Science of National Police University of China were asked to provide urine samples, and urines of donors who had been proved as drug-free users were obtained as comparative blank samples. The results revealed that MA could be detected in all urines from drug abusers, moreover, the amounts of MA in most urine samples were above 0.1 μg mL^{-1}, which beyond the LOQ of the approach, while the analgesic could not be detected in urines of drug-free users, which means that the selectivity of the approach was good.

Conclusion

This paper described a simple and sensitive method using ionic liquid dispersive liquid-phase microextraction in combination with HPLC for the analysis of MA in human urine. This developed extraction system was a cheap, convenient sample pretreatment technique, and reduced the exposure danger to the toxic solvents used in the conventional extraction procedure. Under the best extraction conditions, low limit of detection, good linearity, repeatability and high enrichment factor were achieved. The analysis of drug abusers' urine samples indicated that the method was excellent. Furthermore, the application of developed method showed the potential in the analgesic analysis of forensic trace evidences and addicted cases, because the testing for drug abusing was indeed to confirm the abuse and the testing procedure was usually performed by laboratory investigation of urine.

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