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Simultaneous quantification of amphetamines, caffeine and ketamine in urine by hollow fiber liquid phase microextraction combined with gas chromatography-flame ionization detector

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ABSTRACT

A method of hollow fiber (HF) liquid phase microextraction (LPME) combined with gas chromatography (GC)-flame ionization detection (FID) was developed for the simultaneous quantification of trace amphetamine (AP), methamphetamine (MA), methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), caffeine and ketamine (KT) in drug abuser urine samples. The factors affecting on the extraction of six target analytes by HF-LPME were investigated and optimized, and the subsequent analytical performance evaluation and real sample analysis were performed by the extraction of six target analytes in sample solution containing 30% NaCl (pH 12.5) for 20 min with extraction temperature of 30 °C and stirring rate of 1000 rpm. Under such optimal conditions, the limits of detection (LODs, S/N = 3) for the six target analytes were ranged from 8 μg/L (AP, KT) to 82 μg/L (MDA), with the enrichment factors (EFs) of 5–227 folds, and the relative standard deviations (RSDs, $n = 7$) were in the range of 6.9–14.1%. The correlation coefficients of the calibration for the six target analytes over the dynamic linear range were higher than 0.9958. The application feasibility of HF-LPME-GC-FID in illegal drug monitoring was demonstrated by analyzing drug abuser urine samples, and the recoveries of target drugs for the spiked sample ranging from 75.2% to 119.3% indicated an excellent anti-interference capability of the developed method. The proposed method is simple, effective, sensitive and low-cost, and provides a much more accurate and sensitive detection platform over the conventional analytical techniques (such as immunological assay) for drug abuse analysis.

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1. Introduction

Up to now, the illegal market offers a growing number of narcotic substances such as amphetamine (AP), methylamphetamine (MA), methylene-dioxy derivatives, ketamine (KT) and so on to so many people for the increasing of sensory stimulation or physical performance, or as anorexic drug for the treatment of obesity. As is well known, so-called drug abuse would lead to a variety of disorders of human body and sometimes even death; on the other hand, different kinds of criminal activities are always involved in, resulting in an unstable social order. Accordingly, drug abuse has become a major social issue world widely and various measures have been taking to combat with it, as well as a series of regulations for drug control.

According to the U.S. federal standard for urine drugs set by Substance Abuse and Mental Health Services Administration (SAMHSA), the cutoff concentration of abuse is 1000 ng/mL of total amphetamines by initial test, and 500 ng/mL of AP, or 500 ng/mL of MA with at least 200 ng/mL AP by confirmatory test [1]. With the help of modern analytical instrument, the real sample analysis with such concentration level of illegal drugs can be fulfilled easily. Whereas the new analytical method with much higher sensitivity and anti-interference capability is still expected because the concentration level of the abused drug in human body will be declining gradually within its metabolism process and the complex matrix of biological sample (such as body fluid) will introduce serious interference to the quantification of target illegal drugs. Besides, simultaneous quantification of multi-abused drugs with different characteristics is needed urgently because of the serious situation of mixing-drugs abuse presently. Different analytical techniques including immunological assay (IA) [2], liquid chromatography (LC) [3–7] and gas chromatography (GC) [8–10] have been employed for illegal drug testing. Among them, IA is the most commonly used one, but it exhibits poorer sensitivity than chromatographic techniques [2]. LC has been successfully applied in the quantification of MA and KT and their metabolites in biological samples [3–7], while

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its application potential is limited to some extent due to its high solvent consumption and time-consuming isolation procedures. By comparison, GC is widely used in forensic drug testing because of its easy operation, high separating efficiency, high selectivity and high sensitivity.

Traditionally the drug testing program involved the process of urine testing to monitor drug use [11] because urine samples can reflect the drug consumption or exposure during the preceding 1–4 days [12] and are simple, non-invasive to collect and available in relatively large quantities [13]. In addition, urine samples contain relatively high concentrations of drugs and metabolites compared with other biological specimens. Due to the complicated matrix of urine and other biological specimens, an appropriate sample pretreatment prior to instrumental analysis of abused drugs is often required for sample cleanup, which is always used for the preconcentration of target drugs simultaneously.

Liquid–liquid extraction (LLE) [8,14] and solid phase extraction (SPE) [7,9,10,15–18] are commonly used sample pretreatment techniques in the analysis of illegal drugs. However, LLE is time-consuming, labor-intensive and consuming large amount of solvent, which often leads to the formation of emulsions. SPE is less time-consuming than LLE but requires column conditioning and elution with organic solvents. Moreover, both techniques require an appreciable amount of extraction or desorption solvent, leading to possible environmental pollution and threat to human health. In recent years, much attention has been focused on the miniaturized sample pretreatment techniques such as solid phase microextraction (SPME), stir bar sorptive extraction (SBSE) and liquid phase microextraction (LPME), the common features of all these techniques are their advantages of easy operation and solvent free or minimal solvent consumption. Because of the semivolatile characteristics of all amphetamines, the methods based on SPME for amphetamines analysis could be processed in either direct-immerse [19–23] or headspace [24–29] mode, and various SPME fiber coatings were investigated and successfully applied in the extraction and preconcentration of amphetamines [4,30,31]. Although SPME is simple, portable and has been demonstrated to be an effective sample pretreatment technique for amphetamines analysis, it suffers from the comparatively expensive and fragile fiber with limited lifetime, and the sample carry-over effect. SBSE exhibits much higher sensitivity and better detection limit than SPME, but the problems of carry-over effect and the limited lifetime of stir bar are still associated with it. Furthermore, complex sample matrix will definitely affect the partitioning of target analytes within SPME and SBSE.

LPME is an emerging technique developed from LLE, in which a small amount of solvent is employed to extract analytes from sample matrices. As a simple, quick, inexpensive and virtually solvent-free sample pretreatment technique, LPME has attracted increasing attention and has been widely used for the analysis of organic compounds and inorganic trace elements in environmental, biological, and food samples. The two main methodologies that evolve from the LPME approach are single drop microextraction (SDME) [32,33] and hollow fiber liquid phase microextraction (HF-LPME) [34,35]. SDME in two-phase [32] or three-phase [33] operation mode was developed for the extraction of amphetamines from urine samples, whereas a filtration prior to extraction is necessary. Moreover, since there is no support for the organic solvent except for the tip of the microsyringe, SDME is not very robust, and the droplet may be lost from the needle tip of the microsyringe during the extraction. HF-LPME employed hollow fiber to contain and protect the extraction solvent during extraction and it was much more robust than SDME, no need for sample filtration prior to extraction. In different operation modes, three-phase system [34] and headspace (HS) [35], HF-LPME have been applied for the determination of amphetamines in urine samples. For three-phase concept, strongly acidic or basic target analytes are preferred generally and subsequent analytical techniques are limited to HPLC or capillary electrophoresis (CE). HS-HF-LPME for the determination of AP and methylenedioxyamphetamine (MDA) [35] involved a derivatization reaction which may not be effective for other illegal drugs and complicate the pretreatment process.

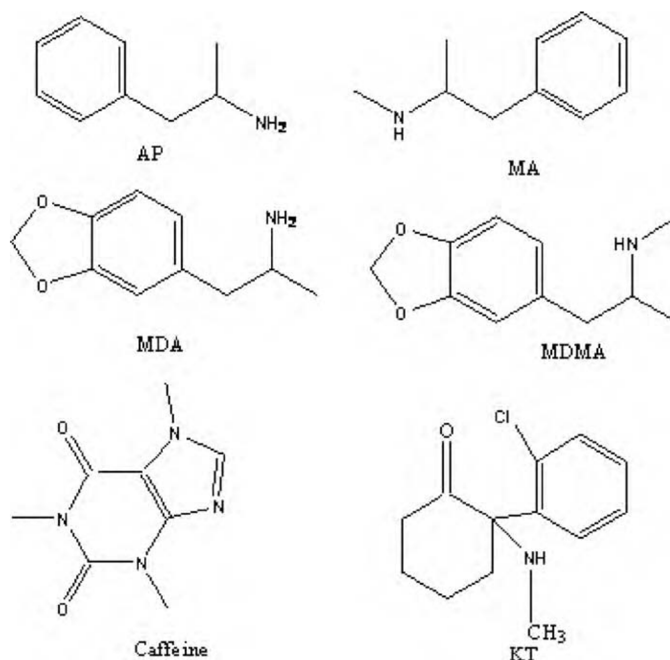

Fig. 1. Chemical structure of target drug analytes.

The aim of this paper is to develop a simple, rapid and sensitive method based on direct immerse HF-LPME combining with GC-FID detection for simultaneous quantification of various abused drugs in urine samples. Six target drugs, AP, MP, MDA, MDMA, caffeine and KT, which are popular abused drugs in regional recreational places presently, are chosen as the target analytes. To obtain an optimal condition for simultaneous extraction of target drugs, a series of influencing factors including pH of sample solution, type of extraction solvent, extraction time, extraction temperature, stirring rate and ion strength have been investigated. Under the optimized condition, the analytical performance of the proposed HF-LPME-GC-FID method was evaluated and the application feasibility was also investigated by applying the method to illegal drugs analysis in real urine samples.

2. Experimental

2.1. Reagents and materials

AP sulfate, MA and methylenedioxymethamphetamine (MDMA) as hydrochloride salts were obtained from National Drug Lab (Beijing, China). MDA and KT as hydrochloride salts were purchased from Second Institute of the Ministry of Public Security (Beijing, China), and their purity was 97.0%. Caffeine was from Verification of Chinese Bio-pharmaceutical Products (Beijing, China). The chemical structures of all six target drugs are shown in Fig. 1, with their partition coefficients and pK_a values listed in Table 1. Standard stock solution (10 mg/mL) of each analyte was prepared in methanol and stored in refrigerator (4 °C). A mixture of standard solution containing all the target analytes was prepared from their respective stock solution and subsequently diluted with methanol. Working solutions used in further studies were prepared by diluting the standard solution with doubly distilled

Table 1
log *P* and pK_a values of target drug compounds.

Compound	log *P*	pK_a
AP	1.76	9.9
MA	2.07	9.87
MDA	1.64	9.67
MDMA	2.12	10.38
Caffeine	−0.07	10.4
KT	3.12	7.8

P-octanol/water partition coefficient; log *P* and pK_a values were obtained from web site: http://chemfinder.cambridgesoft.com and web databases from Syracuse Research Corporation.

water to the required concentrations. All solutions were stored at 4 °C in a refrigerator prior to use.

O-xylene, toluene, carbon tetrachloride and cyclohexane were obtained from Shanghai Experiment Reagent Co., Ltd. (Shanghai, China). Ethyl acetate, chloroform, dimethyl phthalate, isopropanol and methanol were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All reagents used were at least of analytical reagent grade. Doubly distilled water was used throughout this work.

A Q 3/2 Accurel polypropylene hollow fiber membrane (600 μm id, 200 μm wall thickness, and 0.2 μm pore size) was purchased from Membrana GmbH (Wuppertal, Germany). A hollow fiber was cut into 1.0 cm pieces. The approximate internal volume of each section was about 3 μL. The hollow fiber sections were ultrasonicated for 5 min in acetone to remove the contaminants in the fiber. After ultrasonication, the fibers were removed from the acetone and dried in air. The prepared hollow fiber was used for subsequent extraction.

2.2. HF-LPME procedure

A 3.0 mL of sample was placed in a 7.0 mL vial (1.1 cm id × 7.0 cm H) containing a stir bar. The vial was placed on an 85-2A constant temperature magnetic stirrer (Ronghua, Jiangsu, China). A 10 μL microsyringe (Gaoge, Shanghai, China) was rinsed at least five times with the extraction solvent. 5 μL of o-xylene was drawn into a 10 μL microsyringe. The needle tip was inserted into the hollow fiber and the assembly was then immersed in o-xylene for about 20 s to impregnate the pores of the hollow fibers. After impregnation, the o-xylene in the syringe was injected to the lumen of hollow fiber, and the plunger was pulled and pushed repeatedly until there was no bubble in the hollow fiber. Then, the syringe needle was removed from the organic solvent and inserted into the sample solution immediately and the end of hollow fiber was located about 1.0 cm above the surface of the stir bar. After extraction for 20 min, the extraction solvent was withdrawn and 1.0 μL was injected into the GC-FID for analysis.

2.3. GC-FID analysis

Chromatographic analysis was made on an Agilent 6890 gas chromatography equipped with flame ionization detection (FID) system (Agilent Technologies, Palo Alto, CA, USA). An HP-5 capillary column (30 m × 0.53 mm id and 1.5 μm film thickness) purchased from J&W Scientific (Folsom, CA, USA) was employed. The injection was made in the splitless mode at 250 °C. The temperature of the detector was 300 °C and it was fed with 35 mL/min of hydrogen, 400 mL/min of synthetic air and 10 mL/min of nitrogen as auxiliary gas. Nitrogen was used as carrier gas with constant flow of 4.0 mL/min. The column oven temperature program was as follows: first held at 110 °C, then programed at 10 °C/min to 150 °C, then 20 °C/min to 280 °C and held for 1.0 min. The total analytical time is 11.5 min.

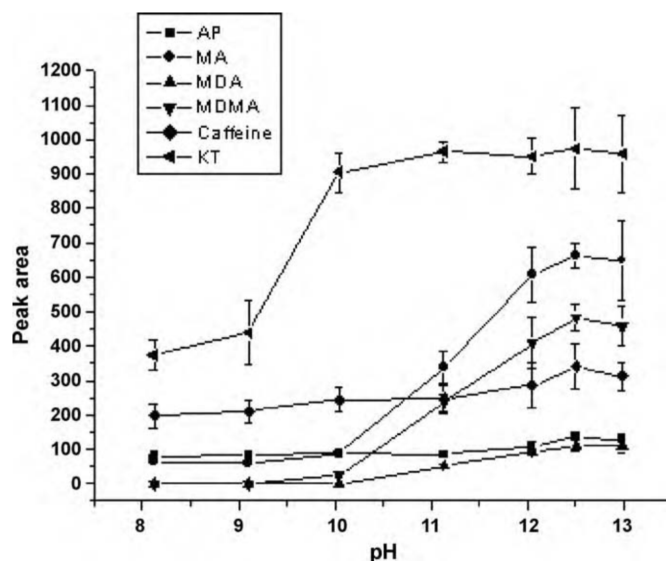

Fig. 2. Effect of pH on HF-LPME. Conditions: sample volume: 3.0 mL; length of HF: 1.0 cm; extraction solvent: o-xylene; extraction time: 20 min; extraction temperature: room temperature; stirring speed: 1000 rpm; sample solution without salt addition; concentration of AP, MA, MDA, MDMA, caffeine and KT are 2.02, 1.93, 2.53, 1.82, 2.50 and 1.83 mg/L, respectively.

2.4. Preparation of samples

Five urine specimens were collected from five drug taking suspects held by anti-drug squad of regional Public Security Bureau (Wuhan, China). All specimens were kept frozen or at 4 °C until analysis. Before extraction, the pH of the samples was adjusted to 12.5 by 1 mol L^{-1} NaOH solution. Then samples were directly processed according to HF-LPME procedure as specified in Section 2.2. For the recovery study, the samples were prepared by spiking certain amount of the target analytes into the urine sample, and 3.0 mL of spiked urine sample was pipetted into a 7.0 mL glass vial and directly subjected to the same HF-LPME procedure.

3. Results and discussion

3.1. Optimization of HF-LPME conditions

In order to obtain a simultaneous extraction of AP, MA, MDA, MDMA, caffeine and KT with maximal extraction efficiencies, different parameters affecting on HF-LPME including sample pH, organic solvent type, extraction time, extraction temperature, ionic strength and stirring speed have been investigated and optimized.

3.1.1. pH of sample solution

The pH of the sample solution affects the dissociation equilibrium and existence form of target acidic or basic analytes. To study the effect of sample pH on the extraction efficiency of six drug analytes, the solutions containing target analytes were adjusted to pH 8.0–13.0 by using 0.1 mol L^{-1} HCl or 1.0 mol L^{-1} NaOH solution, and then extracted for about 20 min at a stirring speed of 1000 rpm. Fig. 2 was the effect of sample pH on the extraction efficiencies of target analytes. As could be seen, when the pH of the sample solution was increased from 10.0 to 12.5, the responses was increased dramatically for MA and MDMA, while the further increase of pH brought to an indistinctive arise of their responses. The signal intensity of KT was increased dramatically along with the increase of sample solution pH in the range of 9.0–10.0, and maintained almost constant with pH further increasing. The responses of AP, MDA and caffeine were increased slowly with the pH of the sample solution increasing from 11.0 to 12.5. This phenomenon can be explained

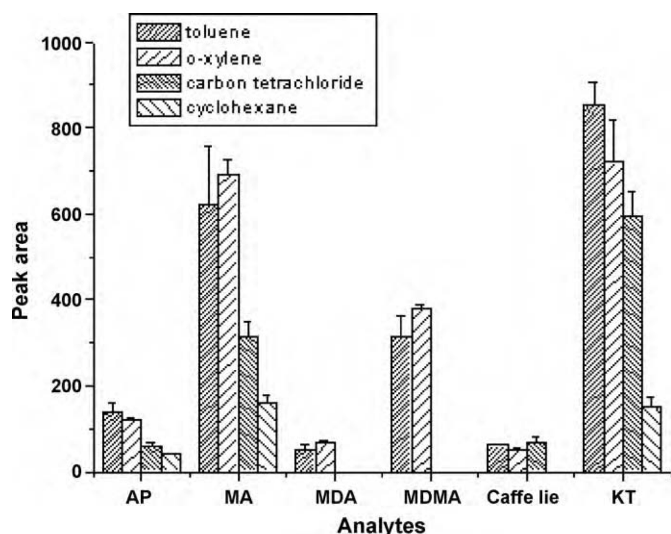

Fig. 3. Effect of organic solvent type on HF-LPME. Conditions: sample volume: 3.0 mL; length of HF: 1.0 cm; pH: 12.5; extraction time: 10 min; extraction temperature: room temperature; stirring speed: 1000 rpm; sample solution without salt addition; concentration of AP, MA, MDA, MDMA, caffeine and KT are 2.02, 1.93, 2.53, 1.82, 2.50 and 1.83 mg/L, respectively.

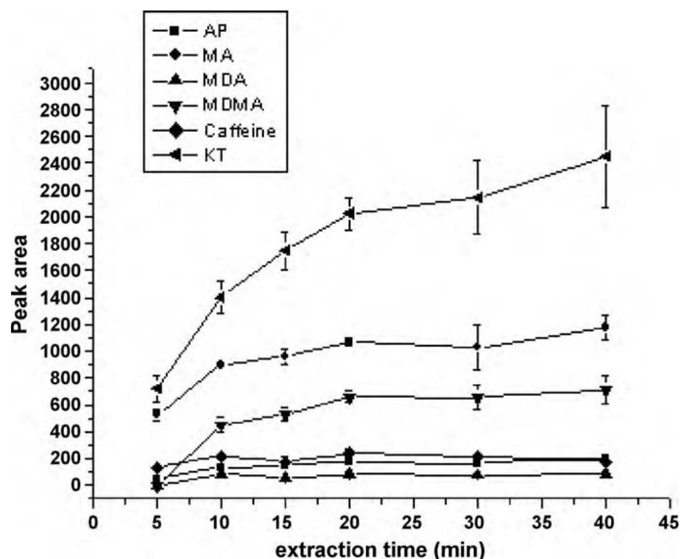

Fig. 4. Effect of extraction time on HF-LPME. Conditions: sample volume: 3.0 mL; length of HF: 1.0 cm; pH: 12.5, extraction solvent: o-xylene; extraction temperature: room temperature; stirring speed: 1000 rpm; sample solution without salt addition; concentration of AP, MA, MDA, MDMA, caffeine and KT are 2.02, 1.93, 2.53, 1.82, 2.50 and 1.83 mg/L, respectively.

by the pK_a values of target analytes. The pK_a value of AP, MA, MDA, MDMA and caffeine are all around 10.0 and they are mainly existed as the undissociated molecular forms approximately at pH higher than 12 (pK_a + 2), which contributed to the extraction, and for KT, its pK_a value is 7.8, for similar reason, pH higher than 10 is preferred for its extraction. To keep AP, MA, MDA, MDMA, caffeine and KT in their undissociated forms in the aqueous solution and simultaneously increase their extraction efficiencies as possible, pH 12.5 was selected for further experiments.

3.1.2. Extraction solvent

The selection of the extraction solvent should be based on the principle of "like dissolve like", which directly affects the extraction efficiency. Considering the similarity of target analytes, extraction stability and compatibility of organic solvent with hollow fiber and GC analysis, eight organic solvents including o-xylene, toluene, ethyl acetate, chloroform, dimethyl phthalate, chloroform/isopropanol (9/1, v/v), carbon tetrachloride and cyclohexane have been investigated for simultaneous extraction of the six target analytes. Compared with other extraction solvents, the LLE and SPE commonly used solvent of ethyl acetate, chloroform and chloroform/isopropanol revealed higher solubility in water and much serious loss after extraction for 10 min at room temperature with a stirring rate of 1000 rpm. Additionally, the solvent peak of dimethyl phthalate interfered with the analytical peak of caffeine during GC separation. Accordingly, the influence of extraction solvent on HF-LPME was investigated among o-xylene, toluene, carbon tetrachloride and cyclohexane, and the results were shown in Fig. 3. As could be seen, both o-xylene and toluene provided good extraction for all six target analytes. In consideration of the lower toxicity and water solubility of o-xylene than toluene under the same extraction condition, o-xylene was selected as the extraction solvent in subsequent experiments.

3.1.3. Effect of extraction temperature

Extraction temperature could obviously affect the extraction efficiency in two ways. On one hand, increasing the extraction temperature could accelerate the mass transfer rates of analytes and increase extraction efficiency within the same extraction time; on the other hand, a rising temperature could lead to a rising solubility of analytes in aqueous solution and a declining partition coefficient in extraction phase. To study the effect of extraction temperature on HF-LPME, the extraction responses of target six drug analytes were investigated over a temperature range of 23–40 °C. The experimental results showed that no obvious variation of the signal intensities was observed during the tested temperature interval. It was also found that the volume of extraction phase withdrawn was declined from 1.8 μL to 1.1 μL when the extraction temperature was increased from 23 °C to 40 °C, due to an improved solubility of the extraction solvent in aqueous phase. And air bubbles adhering to the hollow fiber were more likely to occur under higher temperature, affecting the operation reproducibility seriously. To ensure the extraction efficiency and operation reproducibility, room temperature was employed for further studies.

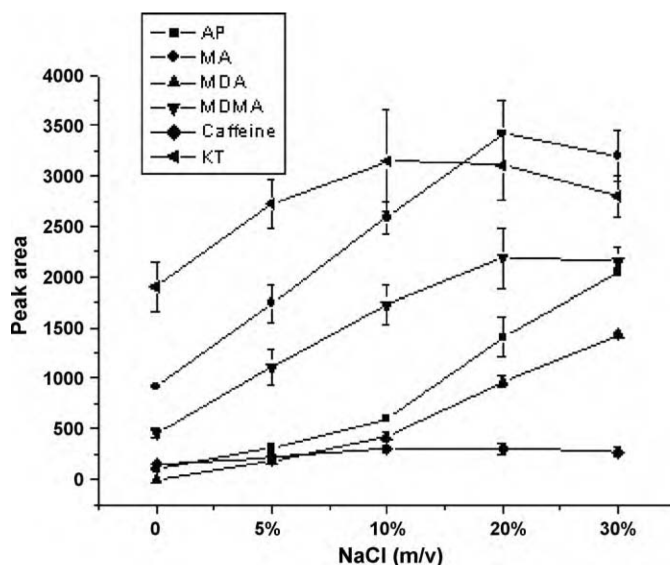

Fig. 5. Effect of NaCl concentration on HF-LPME. Conditions: sample volume: 3.0 mL; length of HF: 1.0 cm; pH: 12.5, extraction solvent: o-xylene; extraction time: 20 min; extraction temperature: room temperature; stirring rate: 1000 rpm; concentration of AP, MA, MDA, MDMA, caffeine and KT are 2.02, 1.93, 2.53, 1.82, 2.50 and 1.83 mg/L, respectively.

Table 2
Analytical performance of HF-LPME-GC-FID for the determination of amphetamines, caffeine and ketamine.

Targets	Regression equation	Linear range (mg/L)	R	Enrichment factor	LOD (μg/L)	RSD/% (n = 7)
AP	$y = 1159.9x - 14.106$	0.03–35	0.9983	42	8	7.4
MA	$y = 1562.3x + 158.26$	0.03–40	0.9991	85	9	6.9
MDA	$y = 697.16x - 73.75$	0.30–40	0.9993	80	82	14.1
MDMA	$y = 1294.2x + 4.0608$	0.08–20	0.9991	227	21	11.5
Caffeine	$y = 22.397x + 36.61$	0.03–45	0.9958	5	9	10.0
KT	$y = 1227.9x + 182.5$	0.03–35	0.9988	110	8	8.9

RSD: The concentration of AP, MA, MDA, MDMA, caffeine and KT are 0.35, 0.39, 0.38, 0.22, 0.44 and 0.36 mg/L, respectively.

3.1.4. Stirring rate and extraction time

Sample stirring would enhance the extraction efficiency and shorten the extraction time, because the partitioning equilibrium of analytes between donor phase and acceptor phase would be established more rapidly under this case. In this work, the effect of stirring rate on the extraction of target drugs was investigated by agitating 3.0 mL sample solution at different stirring rates (600, 800, 1000, and 1200 rpm) using magnetic stirrer. The experimental results showed that the peak area of KT was increased obviously with the increase of stirring rate from 600 to 1200 rpm, while the peak area of the other five target analytes almost revealed negligible fluctuation over the tested range. Subsequently, a stirring rate of 1000 rpm was chosen for further studies.

Generally, increasing extraction time could increase the extraction efficiency until extraction equilibrium is reached. Whereas it would take too long to achieve equilibrium for those analytes with low partition coefficient, and extended extraction time would result in a serious loss of the extraction solvent. Fig. 4 showed the effect of extraction time ranging from 5 to 40 min on the extraction of six target drugs by HF-LPME. As could be seen, the peak area for each drug analytes was increased by increasing the exposure time from 5 to 10 min. When the extraction time was continually increased up to 40 min, the peak area for each analyte except KT almost maintained constant. The peak area of KT was increased continually with further increase of the extraction time. In practice, with the prolongation of the extraction time the volume of extraction phase withdrawn was kept decreasing from 2.1 μL (5 min) to 1.3 μL (40 min). To ensure acquisition of enough volume of extraction phase and better reproducibility for the subsequent determination, and also to compromise the analytical speed and the extraction efficiency, an extraction time of 20 min was selected for the subsequent experiments.

3.1.5. Effect of ionic strength

The effect of salt addition on the extraction efficiency of HF-LPME was evaluated by increasing NaCl concentration from 0% to 30% (m/v) in sample solution and the experimental results were shown in Fig. 5. As could be seen, the signal intensities of AP and MDA were increased gradually with increasing the concentration of NaCl from 0% to 30%, but the signal intensities of MA, MDMA, caffeine and KT were initially increased and then decreased slowly within the increase of the concentration of NaCl. These results could be explained from two aspects. Firstly, the dissolution of NaCl in water might change the physical properties of the Nernst diffusion film and slow down the diffusion rate of the target analytes into the extraction solvent. Secondly, the addition of salt could lead to an increase in the ionic strength of the solution and then decrease the solubility of the target analytes in the aqueous phase, resulting in an increase of their partition coefficient in the organic phase. These two factors lead to exactly opposite effects. Based on the above experimental data, 30% (m/v) of NaCl was employed in subsequent experiments.

3.2. Evaluation of the HF-LPME-GC-FID method for abused drug analysis

Taking AP, MA, MDA, MDMA, caffeine and KT as the target abused drugs, sample solution containing 30% NaCl (m/v) with pH of 12.5 was extracted for 20 min at room temperature with a stirring rate of 1000 rpm, the analytical performance of the proposed method was evaluated, and the obtained data were listed in Table 2. Enrichment factor (EF) was defined as the ratio between the slope of calibration curve after and before extraction. The limits of detections (LODs) were obtained by determining the minimum amount of each analyte required to give a signal of S/N = 3 by GC-FID after HF-LPME procedure. As could be seen, the EFs obtained for the tar-

Table 3
Comparison of detection limits found in literatures for the determination of illegal drugs in real samples.

Method	Analytes	Sample	LODs (μg/L)	References
HF-LPME-GC-FID	AP, MA, MDA, MDMA, caffeine, KT	Urine	8–82	This work
In-tube SPME-HPLC-UV	AP, MA, MDA, MDMA	Urine	1.4–4.0	[4]
SPE-GC–MS	13 Amphetamine related drugs[a]	Whole blood	5–50	[9]
SPE-GC–MS	MA, AP	Urine	0.08–0.10	[10]
SPE-GC–MS	AP, MA, MDA, MDMA	Urine	2–4	[18]
SPME-LC–MS/MS	AP, MA	Serum	0.04–0.3	[21]
SPME-HPLC-FLD	AP, MA, MDMA	Urine	50–100	[22]
SPME-HPLC-FLD	AP, MDA, norephedrine	Urine	100–250	[23]
HS-SPME-GC-FID	AP, MA	Urine	3–9	[24]
HS-SPME-GC–MS	AP, MA, MDA, MDMA, MDEA[b]	Urine	0.016–0.193	[28]
HS-SPME-GC-FID	Ephedrine, MA	Urine	0.33–0.60	[30]
Three-phase SDME-HPLC-UV	AP, MA	Urine	0.5	[33]
Three-phase HF-LPME-CE-UV	MA, citalopram	Urine, plasma, blood	2.0	[34]
HS-HF-LPME-GC–MS	AP, MDA	Urine	0.25–1.00[c]	[35]

[a] AP, MA, dimethylamphetamine (DMA), phenylpropanolamine (PPA), ephedrine, methylephedrine (ME), MDA, MDMA, N-methyl-1-(3,4-methylene dioxyphenyl)-2-butanamine (MBDB), *p*-methoxyamphetamine (PMA), *p*-methoxymethamphetamine (PMMA), 4-methylthioamphetamine (4MTA) and β-phenethylamine (PEA).
[b] MDEA: methylen-dioxyethylamphetamine.
[c] LOQ: limit of quantification.

Table 4
Analytical results of real urine samples by HF-LPME-GC-FID.

Targets	Urine-1 Added (mg/L)	Found (mg/L)	Recovery (%)	RSD[a] (%)	Urine-2 Added (mg/L)	Found (mg/L)	Recovery (%)	RSD[a] (%)	Urine-3 Added (mg/L)	Found (mg/L)	Recovery (%)	RSD[a] (%)	Urine-4 Added (mg/L)	Found (mg/L)	Recovery (%)	RSD[a] (%)	Urine-5 Added (mg/L)	Found (mg/L)	Recovery (%)	RSD[a] (%)
AP	0	n.d.[b].	–	–	0	3.08	–	9.6	0	0.50	–	8.0	0	0.24	–	13.9	0	0.23	–	10.0
	1.76	1.45	82.3	3.8	1.76	4.34	89.6	10.7	1.76	2.26	100.0	5.5	1.76	1.80	90.1	4.4	1.76	1.61	81.1	7.9
MA	0	1.56	–	5.9	0	11.93	–	12.8	0	5.82	–	9.3	0	2.06	–	9.8	0	3.38	–	12.5
	1.94	3.90	111.5	4.1	1.94	11.55	83.3	12.3	1.94	7.91	101.9	11.1	1.94	4.34	108.6	0.9	1.94	6.35	119.3	9.2
MDA	0	n.d.	–	–	0	n.d.	–	–	0	n.d.	–	–	0	n.d.	–	–	0	n.d.	–	–
	1.89	1.40	75.2	0.6	1.89	1.62	85.5	12.6	1.89	1.78	94.0	4.4	1.89	1.54	81.6	2.8	1.89	1.43	75.5	6.5
MDMA	0	0.34	–	7.1	0	n.d.	–	–	0	n.d.	–	–	0	n.d.	–	–	0	n.d.	–	–
	1.11	1.26	87.1	1.0	1.11	1.03	93.0	13.1	1.11	1.12	100.9	2.4	1.11	0.98	88.0	0.1	1.11	1.19	107.5	5.7
Caffeine	0	n.d.	–	–	0	2.98	–	5.8	0	3.56	–	6.8	0	1.49	–	0.8	0	0.42	–	13.0
	2.20	2.48	112.8	3.8	2.20	4.70	90.7	1.5	2.20	5.39	93.6	3.9	2.20	3.63	98.4	3.4	2.20	2.36	90.1	9.6
KT	0	0.80	–	0.8	0	n.d.	–	–	0	n.d.	–	–	0	n.d.	–	–	0	0.23	–	9.6
	1.82	2.89	110.4	1.1	1.82	1.79	98.5	12.1	1.82	1.98	109.1	3.9	1.82	1.50	82.6	4.3	1.82	2.08	101.3	5.9

[a] $n = 3$.
[b] n.d.: not detected.

get analytes were between 5 (caffeine) and 227 (MDMA), and the lowest EF for caffeine is probably due to the extremely low log *P* (octanol/water partition coefficient) value as listed in Table 1. The obtained LODs for the target analytes were ranging from 8 μg/L (AP and KT) to 82 μg/L (MDA), and the limit of quantifications (LOQs, defined as the lower limit of the linear range) were ranging from 0.03 mg/L (AP, MA, caffeine and KT) to 0.3 mg/L (MDA), meeting the analytical requirements of drug abuse in real sample analysis as specified in Section 1. Good linearity was obtained for each target analytes over the reported linear range with the correlation coefficients varying in 0.9958–0.9993. The reproducibility, expressed as relative standard deviations (RSDs) for six replicate analyses at the concentration level of 0.35 mg/L for AP, 0.39 mg/L for MA, 0.38 mg/L for MDA, 0.22 mg/L for MDMA, 0.44 mg/L for caffeine and 0.36 mg/L for KT, ranged from 6.9% to 14.1% for HF-LPME-GC-FID.

Table 3 made a comparison of the analytical performance obtained by the proposed method and others for the determination of illegal drugs in biological samples. As could be seen, the LODs obtained by this method is lower than that reported in references [22,23], comparable with those obtained in the references [4,9,18,24,34], but slightly higher than that given in references [10,21,28,30,33,35]. Ref. [35] employing HF-LPME and GC for the determination of only AP and MDA provided lower LODs than that obtained by the proposed method, probably due to the applied dynamic extraction mode and derivatization reaction, both of which will improve the extraction efficiency. Whereas the derivatization would lead to high background and poor precision for the quantification. Compared with SPE, HF-LPME is more simple and convenient with much less solvent/sample consumption; compared with SPME, HF-LPME is less costly and has no carry-over effect. Besides, the hollow fiber used in HF-LPME will stabilize and protect the organic drop during extraction, prevent impurities in the matrix from entering into the extraction phase and interfering with the extraction process and shorten the extraction time due to higher stirring speed allowed. In a word, the proposed HF-LPME-GC-FID method is effective, sensitive, convenient and low-cost, and provides an alternative for the analysis of abused drugs.

3.3. Urine sample analysis

The proposed HF-LPME-GC-FID method was applied for the simultaneous quantification of six target drugs in real urine samples, and the analytical results are listed in Table 4. As could be seen, by using an external standard calibration, all six target drugs except MDA were measured to be at different concentration level, indicating a drug abuse fact for the held suspects on preceding days according to the standard set by SAMHSA. To validate the proposed method, a recovery test was performed and the results are also given in Table 4. The recovery was defined as the ratios of the determined concentration difference between unspiked and spiked sample to the spiked concentration of the analytes. The recoveries for the spiked real urine samples varied from 75.2% to 119.3% with RSDs ranging from 0.1% to 13.9% ($n = 3$), illustrating a good anti-interference capability and the application feasibility of the proposed method in drug abuse analysis. Fig. 6 depicted typical chromatograms obtained by HF-LPME-GC-FID for the real urine sample (urine-2) and the spiked real urine sample (urine-2).

The main abused drug popular in regional recreational places presently is so-called "Maguo", of which the essential constituent is MA. Due to the variation of origin and producer, as well as other ingredient (such as caffeine and KT) added by drug vendor, various drugs would be detected in urine sample even if only one kind of abused drug was taken by the suspect. As could be seen in Table 4, three or more abused drugs (MA, MDMA and KT were found in urine-1; AP, MA and caffeine were detected in urine-2, urine-3 and urine-4; AP, MA, caffeine and KT were found in urine-5) were found

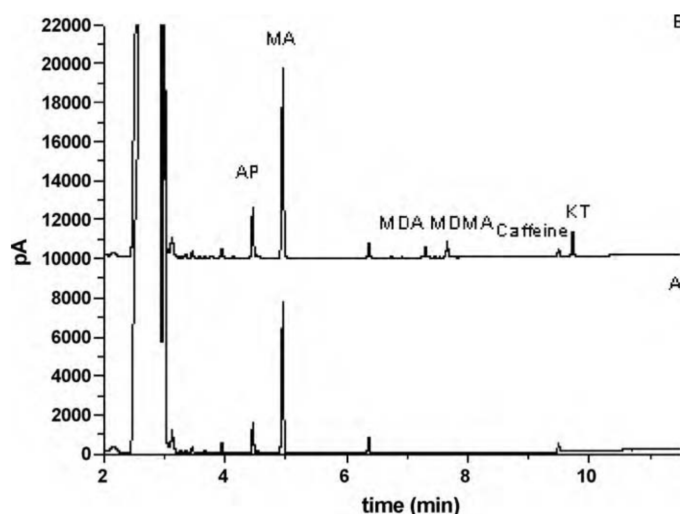

Fig. 6. Chromatogram of illegal drugs obtained by HF-LPME under optimized conditions. (A) Urine-2; (B) spiked urine-2. (The spiked concentration of AP, MA, MDA, MDMA, caffeine and KT are 1.76, 1.94, 1.89, 1.11, 2.20 and 1.82 mg/L, respectively.)

in all five urine samples, MA was detected in all five urine samples and its concentration is the highest in each urine specimen, other abuse drugs found in urine samples, such as AP, MDMA, caffeine and KT are all at lower level. Based on these analytical results and the local illegal market information, the five suspected drug abusers were assumed to have ever taken "Maguo" before being arrested. Additionally, these urine samples were also analyzed by IA, the main technique which is used by local public security organization to determine whether the suspect drug abusers have taken the illegal drugs, and the positive results obtained by IA agreed with that obtained by the proposed method. However, IA only provides qualitative information with a detection capability of higher than 300 μg/L for MA and AP.

4. Conclusion

The simultaneous determination of six abused drugs (AP, MP, MDA, MDMA, caffeine and KT) in real urine samples by HF-LPME combined with GC-FID detection is realized. Compared with the commonly used IA technique in drug abuse analysis, the proposed method provided much higher sensitivity and more accurate quantitative information. Besides, it reveals so many practical advantages such as microliter solvent consumption, small amount of sample required, simple device, convenient operation, low-cost and an outstanding anti-interference capacity. The proposed method provides an effective, sensitive and simple alternative detection platform for practical drug abuse monitoring.

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