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pH-resistant titania hybrid organic–inorganic coating for stir bar sorptive extraction of drugs of abuse in urine samples followed by high performance liquid chromatography–ultraviolet visible detection

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ABSTRACT

An organic–inorganic hybrid titania-hydroxy-terminated silicone oil (titania-OH-TSO) stir bar coating was prepared by sol–gel method. The extraction performance of titania-OH-TSO coated stir bar was evaluated and compared with poly(dimethysiloxane) (PDMS), poly(dimethysiloxane)–divinylbenzene (PDMS–DVB), poly(dimethysiloxane)–β-cyclodextrin (PDMS–β-CD) and C_{18} coated stir bar with five polar drugs of abuse including amphetamine (PA), methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA) and ketamine (Ke) as the model analytes. The experimental results revealed that the titania-OH-TSO coated stir bar exhibited highly pH-resistant ability, good preparation reproducibility, superior selectivity and high extraction efficiency for the target compounds. Based on this fact, a new method of titania-OH-TSO coated stir bar sorptive extraction (SBSE) combined with high performance liquid chromatography (HPLC)–ultraviolet visible (UV) detection was developed for the analysis of five drugs of abuse in urine samples. The factors affecting the extraction efficiency of SBSE such as sample pH, desorption solvent, sample volume, extraction time, desorption time, stirring rate and ionic strength were investigated and the optimal extraction conditions were established. Under the optimized conditions, the limits of detection (LODs) for titania-OH-TSO coated SBSE–HPLC–UV determination of five polar drugs of abuse were in the range of 2.3–9.1 μg/L with relative standard deviations (RSDs) ranging from 7.3 to 8.9% (c = 300 μg/L, n = 6), and all of the target compounds exhibited good linearity over a concentration range of 30–3000 μg/L. The developed method was applied to the determination of amphetamines and Ke in urine samples of drug abusers with satisfactory results.

1. Introduction

The abuse of drugs is a major social issue all over the world and imperils the public security seriously. Amphetamine (PA), methamphetamine (MA) and their methylenedioxy derivatives are among the list of the most commonly drugs of abuse in recent years, and they are always used in combination with ketamine (Ke) in many cases. In order to obtain accurate and effective information on the testing of drugs of abuse, the development of sensitive, selective and rapid methods for the simultaneous quantification of multiple drugs of abuse in biological samples is required urgently. This would be of benefit not only for clinical research, but also for forensic analysis.

The majority of methods for the determination of amphetamines in biological samples are based on gas chromatography (GC) or gas chromatography–mass spectrometry (GC–MS) [1,2]. However, there generally should be a derivatization step in order to improve the volatility of these compounds before GC analysis. Nowadays, high performance liquid chromatography (HPLC) and liquid chromatography coupled to mass spectrometry (LC–MS) have been successfully applied to the analysis of a wide variety of small molecules in biological matrices, such as amphetamine-like compounds [3–5]. Compared with GC, HPLC can avoid derivatization and is especially suitable for the analysis of non-volatile or semi-volatile compounds. As well known, the drug testing program has traditionally involved the urine testing as urine samples are simple, non-invasive to collect and available in relatively large quantities. However, urine matrices are very complex, and therefore, a suitable sample pretreatment method aimed at separating the matrix and enriching the target analytes is necessary to obtain the reliable analytical results. The conventional sample pretreatment techniques for drugs of abuse analysis in urine samples are liquid–liquid extraction (LLE) and solid phase extraction (SPE). However, they are rather laborious, time-consuming and using large amounts of toxic solvents. Therefore, solventless sample preparation techniques such as liquid-phase

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microextraction (LPME) [6–8], supercritical fluid extraction (SFE) [9] and solid phase microextraction (SPME) [10–14] had already been proposed for the analysis of drugs of abuse in the last decade.

Stir bar sorptive extraction (SBSE) which was developed from SPME is a kind of novel and solvent-free sample pretreatment technique with high concentration factors, good reproducibility and high sensitivity. In the past few years, SBSE has been developed rapidly and successfully applied to the trace analysis of various analytes in environmental, food and biological samples [15,16]. However, to the best of our knowledge, only poly(dimethysiloxane) (PDMS) coating is commercially available for SBSE now, and it has some inherent shortcomings such as low recovery for relative high polarity compounds and the limited tolerance of pH range, which have limited the application of SBSE technique to a certain extent, especially for the analysis of polar compounds and basic compounds. To overcome the above-mentioned limitation and to extend the application field of SBSE, it is important to develop novel extraction phases that have better affinities to polar compounds. In recent years, different kinds of new SBSE coatings have been proposed for the extraction of semi-polar and polar compounds, these include polyurethane (PU) foams [17], alkyldiol-silica (ADS) [18] poly(phthalazine ether sulfone ketone) (PPESK) [19] and PDMS/polypyrrole (PPY) [20].

Titania exhibits a rich surface chemistry. It represents anion-exchange properties at acidic condition and cation exchange properties at alkaline condition [21]. In recent years, titania [21], alumina [22] and zirconia [23] based hybrid organic–inorganic sol–gel coatings have been reported for SPME. These hybrid coatings have excellent pH stability and suitable for the analysis of polar compounds. In this work, an organic-inorganic hybrid titania-hydroxy-terminated silicone oil (titania-OH-TSO) SBSE coating was prepared by sol–gel method and compared with other SBSE coatings including poly(dimethysiloxane) (PDMS), poly(dimethysiloxane)–divinylbenzene (PDMS–DVB), poly(dimethysiloxane)–β-cyclodextrin (PDMS–β-CD) and C_{18} for the extraction of five polar compounds such as PA, MA, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA) and Ke. The titania-OH-TSO SBSE coating showed excellent chemical stability, good preparation reproducibility, highly pH-resistant ability, superior selectivity and high extraction efficiency for five target polar compounds. Based on this fact, a new method of titania-OH-TSO coating SBSE–HPLC–UV was developed for the determination of five drugs of abuse in urine samples. The analytical performance of the proposed method was evaluated in terms of linear range, precision and limits of detection (LODs). Finally, the developed method was applied to the determination of amphetamines and Ke in drug taken suspect's urine samples.

2. Experimental

2.1. Instrumentation

Agilent 1100 series HPLC–UV system (Agilent Technologies, USA) consisting of vacuum degasser, a quaternary pump and a variable wavelength detector was used for identification and quantification of the target analytes. The separation was performed on a reverse phase C_{18} HPLC column (Lichrospher ODS, 5 μm, 200 mm × 4.6 mm, Hanbon, Jiangsu, China).

The optimized mobile phase was consisted of 14% (v/v) acetonitrile and 86% (v/v) buffer solution containing 0.02 mol/L KH_2PO_4 and 0.015 mol/L triethylamine (pH = 3.0). The flow rate of the mobile phase was 0.8 mL/min and the detection was performed at 205 nm with UV detector.

A Quanta 200 scanning electron microscope (FEI, Holland) was used to characterize the surface of the titania-OH-TSO coated stir bar, with the accelerator voltage at 30 kV.

A magnetic stirrer 85-2A (Ronghua Electrical Apparatus Works, Jiangsu, China) was employed for stirring the sample solution during extraction.

2.2. Chemicals and reagents

Hydroxy-terminated silicone oil (OH-TSO) was purchased from Chenguang Research Institute of Chemical Industry (Chengdu Silicone Research Centre, Chengdu, China). Titanium(IV) butoxide (chemical grade) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). β-Cyclodextrin was purchased from Aldrich (Milwaukee, WI, USA). C_{18} (200–300 mesh) was obtained from Qingdao Ocean Chemical Industry (Qingdao, China). Methyltrimethoxysilane (MTMS), divinylbenzene (DVB), γ-glycidoxypropyltrimethoxysilane (KH-560), and trifluoroacetic acid (TFA) were purchased from China Medicine (group) Shanghai Chemical Reagent Corporation (Shanghai, China), and poly(methylhydrosiloxane) (PMHS) was obtained from the Chemical Plant of Wuhan University (Wuhan, China). High purity deionized water (18.2 MΩ cm) purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used for the preparation of mobile phase. Acetonitrile (HPLC grade) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The buffers with various pH values were prepared by mixing 0.1 mol/L KH_2PO_4 with NaOH. The capillary glass bars (1 mm I.D. and 0.1 mm wall thickness) were obtained from Apparatus Factory of West China University of Medical Sciences (Sichuan, China).

Amphetamine (PA), methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA) and ketamine (Ke) were provided by Wuhan Public Security Bureau (Wuhan, China), and their molecular structures are shown in Table 1. A stock standard solution of 1000 mg/L for each analyte was prepared by dissolving certain amount of respective standard in methanol. The mixed standard solution containing 50 mg/L of each analyte was prepared by diluting the stock solution with methanol. These stock solutions were then further diluted with high purity water to obtain the appropriate working solutions.

2.3. Sample preparation

The urine samples of drug abusers were provided by Wuhan Public Security Bureau (Wuhan, China), and blank urine samples were collected from drug-free, healthy volunteers. All samples were maintained at −18 °C in a refrigerator until further treatment.

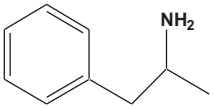
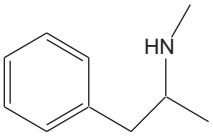
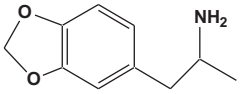
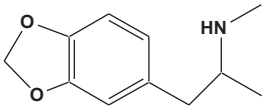
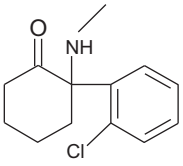
2.4. Preparation of the stir bars

In order to reduce the friction loss of coatings resulting from high stirring rate, dumbbell shape stir bars as described in Ref. [24] were used in this work. The bared bars were cleaned by water and CH_2Cl_2 in turn, followed by soaking in 1 mol/L NaOH for 3 h and 0.1 mol/L HCl for 15 min, respectively. After being washed by water, the bars were dried at room temperature.

The titania-OH-TSO sol solution was prepared as follows [21]: 100 mg OH-TSO, 150 μL titanium butoxide, 10 μL PMHS, 150 μL CH_2Cl_2 and 150 μL isopropanol were mixed with agitation. The mixture was ultrasonicated for 10 min and then aged for 1 h.

The titania-OH-TSO coated stir bar was prepared as follows: a mechanical pipette (4–40 μL) was employed to precisely control the volume of sol and also to avoid the sol being coated on the two spherical bubbled ends of the "dumbbell-shaped" stir bar. This procedure was repeated three times to ensure the surface of bars

Table 1
Structures, log $K_{o/w}$ and pK_a values of target analytes.

Analyte	Structure	Log *P*	pK_a
Amphetamine (PA)		1.806	9.9
Methamphetamine (MA)		1.944	9.87
3,4-Methylenedioxyamphetamine (MDA)		1.667	9.67
3,4-Methylenedioxy-methamphetamine (MDMA)		1.806	10.38
Ketamine (Ke)		2.180	7.8

has been coated thoroughly. Then the coated bars were placed into a muffle and the temperature was programmed from room temperature to 250 °C at 1 °C/min and maintained at 250 °C for 2 h. After cooling down, the titania-OH-TSO coated stir bars were taken out and ultrasonicated in methanol for 20 min, 0.1 mol/L HCl for another 20 min prior to their use to get rid of the organic impurities and activate the coating.

The PDMS sol solution was prepared as follows [25]: 100 mg OH-PDMS, 100 μL MTMS, 50 μL KH-560, 10 μL PMHS, 100 μL 95% TFA and 100 μL CH_2Cl_2 were mixed with agitation. The mixture was ultrasonicated for 10 min. The PDMS–DVB [26] sol solution contained the same components as the PDMS sol solution and 100 μL DVB. And the PDMS–β-CD [11] sol solution contained the same components as the PDMS sol solution and 30 mg β-CD. PDMS, PDMS–DVB and PDMS–β-CD coated stir bars were prepared by the same method as the titania-OH-TSO coated stir bar. Then the coated bars were placed into a constant temperature drier at 60 °C for 1 day. The C_{18} stir bars were prepared by using epoxy as the adhesive glue [18]. Then the coated bars were placed into a constant temperature drier at 60 °C for 1 day. Prior to use, these stir bars were ultrasonicated in methanol for 20 min in order to get rid of the organic impurities in the coating.

2.5. Stir bar sorptive extraction

In a typical assay, a 3 mL aliquot of aqueous solution containing the target analytes was introduced into a glass vial. The stir bar was immersed into the sample solution, and the extraction was performed at the stirring rate of 700 rpm for 20 min. After extraction, the stir bar was removed from the aqueous sample, washed with water and then gently dried with a filter paper. Then it was placed into a small test tube containing 60 μL desorption solvent (80/20 (v/v) methanol/phosphate buffer, pH = 1.5) to desorb the target analytes by ultrasonication for 20 min. Finally, the stir bar was taken out to dry its surface carefully and placed into 1 mL methanol and 1 mL water in turn for ultrasonic cleaning before the next use. Twenty microliters of the eluted solution was injected into the sample loop for HPLC–UV analysis.

3. Results and discussion

3.1. Relative extraction efficiencies of various SBSE coatings

According to the literatures, PDMS [1,10,27], PDMS–DVB [13] and PDMS–β-CD [11] coatings as well as poly(methacrylic acid-ethylene glycol dimethacrylate) (PMAA-EGDMA) monolith [12] are the suitable extraction phases for the exaction of amphetamines by SPME. Therefore, five kinds of stir bar with different coatings including titania-OH-TSO, PDMS, PDMS–β-CD, PDMS–DVB and C_{18} were prepared as described in Section 2.4 and initially evaluated for the extraction of target analytes. The experimental results in Fig. 1 revealed that titania-OH-TSO stir bar coating provided much better extraction performance than the other four coatings for the target analytes. Besides the hydrophobic interaction, the special extraction capability of titania-OH-TSO coating to the target compounds could be mainly attributed to the electrostatic interaction and the Lewis acid–base interaction [21] between the analytes and the titania-OH-TSO coating. On one hand, at pH 10, amphetamines are partly protonated, and the surface of titania-OH-TSO coating is negatively charged, the electrostatic interaction between amphetamines and the coating results in an improved extraction performance. On the other hand, the Lewis basic group (amines) of the target compounds can be served as an electron-pair donor and the Lewis acid sites on the surface of titania-OH-TSO coating (Ti^{4+}) can served as an electron-pair acceptor, the Lewis acid–base interaction between the electron-pair donor and the electron-pair acceptor also leads to an improved extraction performance. For PDMS, PDMS–DVB and C_{18} coatings, except hydrophobic interaction, there is no electrostatic interaction or Lewis acid–base interaction between the target compounds and the coating due to the apolar characteristics of coatings, as a result, a poor extraction performance was observed. Sol–gel hybrid titania coating also

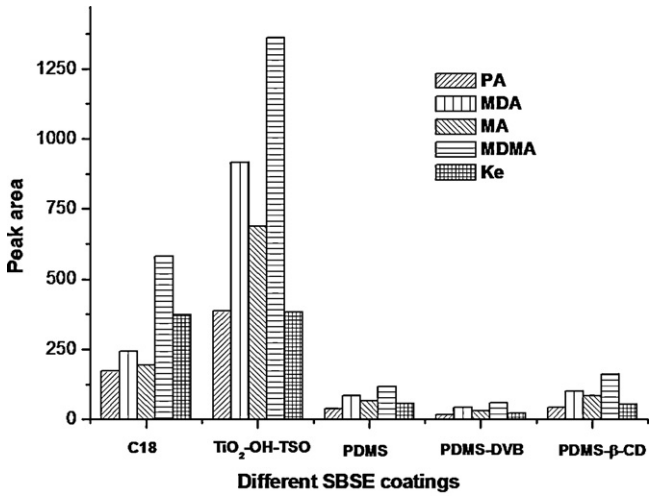


Fig. 1. The effect of SBSE coatings on the extraction efficiency of five drugs of abuse. Conditions: extraction time: 25 min; desorption time: 15 min; pH: 10; NaCl: 0% (m/v); stirring speed: 700 rpm; concentration of each target analyte: 500 μg/L.

exhibited an excellent pH stability and thermal stability [21]. Therefore, titania-OH-TSO coated stir bar was chosen for the following experiments.

3.2. Characterization of the titania-OH-TSO coated stir bar

The morphology of the titania-OH-TSO coated stir bar was assessed by scanning electron microscope (SEM), and its scanning electron micrographs under different magnifications were shown in Fig. 2. As could be seen, small pores at micrometer size in the stir bar coating was observed with 5000× magnification (Fig. 2a). These pores greatly enlarge the surface area of titania-OH-TSO coating and thus improve the extraction performance. Fig. 2b was obtained at a magnification of 20,000×, and it could be seen that the titania-OH-TSO coating represented a highly dense structure.

Table 2 was the bar-to-bar in one batch and batch-to-batch reproducibility for the preparation of titania-OH-TSO coatings. Three titania-OH-TSO coated stir bars prepared in one batch and four titania-OH-TSO coated stir bars prepared in four batches were tested for the extraction of the target analytes from aqueous solution. The experimental data in Table 2 demonstrated an excellent reproducibility for the preparation of titania-OH-TSO coated stir bars not only within batch (RSDs, 4.0–7.5%) but also among different batches (RSDs, 5.6–10.6%). The life time of the prepared titania-OH-TSO coating stir bar was evaluated, it was found that the prepared titania-OH-TSO coating stir bar could be reused for at least 40 times at pH 11 without obvious decrease in extraction efficiency, indicating that the prepared titania-OH-TSO coated stir bar has highly pH-resistant ability.

Table 2
The preparation reproducibility of the sol–gel titania-OH-TSO coatings.

Analyte	RSD (%)	
	Bar-to-bar (c = 300 μg/L, n = 3)	Batch-to-batch (c = 300 μg/L, n = 4)
PA	5.1	6.2
MDA	7.3	5.9
MA	4.0	10.6
MDMA	6.1	5.6
Ke	7.5	9.6

3.3. Optimization of extraction parameters

3.3.1. The effect of pH value

Alkaline conditions are preferred for the extraction of PA, MA and their derivatives since PA, MDA, MA and MDMA are all basic analytes with pK_a values ranging from 9.6 to 10.4. Considering the limited pH resistant ability of the conventional SPME fiber and the characteristics of target analytes, most of the SPME methods adopted head space extraction mode [1,11,14], low sample pH [13,27] or derivatization reagents [28–30] to improve the extraction efficiency. Since hybrid titania-OH-TSO coating exhibited excellent pH stability, the effect of sample pH on the extraction efficiency of the target analytes was investigated within the pH range of 8–12 by direct stir bar sorptive extraction mode. As shown in Fig. 3, the extraction efficiency for PA, MDA, MA and MDMA was increased drastically with increasing pH from 8 to 11, and then decreased with further increasing pH to 12. The possible reason for this triangle-like trend in Fig. 3 was electrostatic, Lewis acid–base and hydrophobic interaction. From pH 8 to 11, the protonated amphetamines gradually transform into neutral forms according to their pK_a values (in the range of 9.6–10.4). Besides the electrostatic interaction between protonated target analytes and titania, the hydrophobic interaction between neutral analytes and OH-TSO and the Lewis acid–base interaction between amines of the target compounds and Ti^{4+} in the coating also play great role in extraction. However, the target analytes shift significantly to their neutral

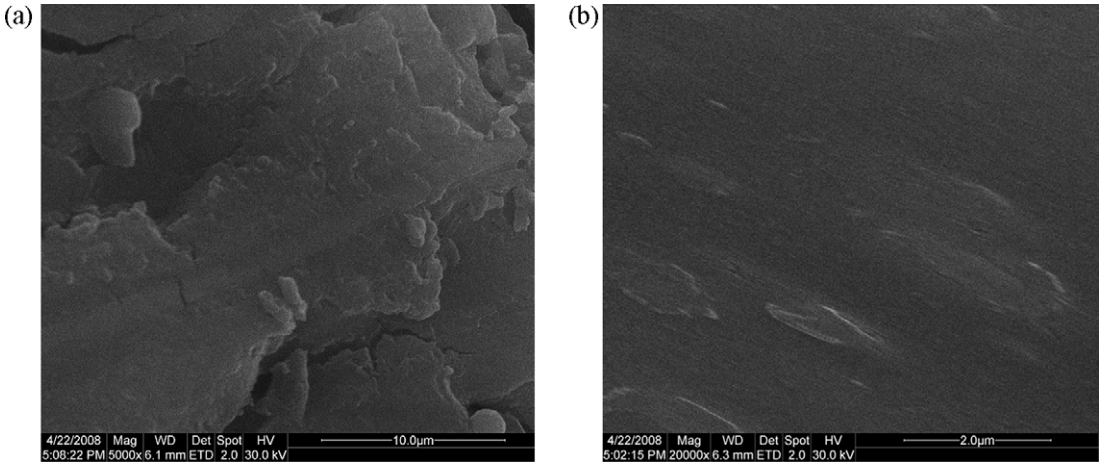


Fig. 2. Scanning electron microscopic images of the titania-OH-TSO coating. (a) Magnification 5000× (Accelerator voltage 30 kV) and (b) magnification 20,000× (Accelerator voltage 30 kV).

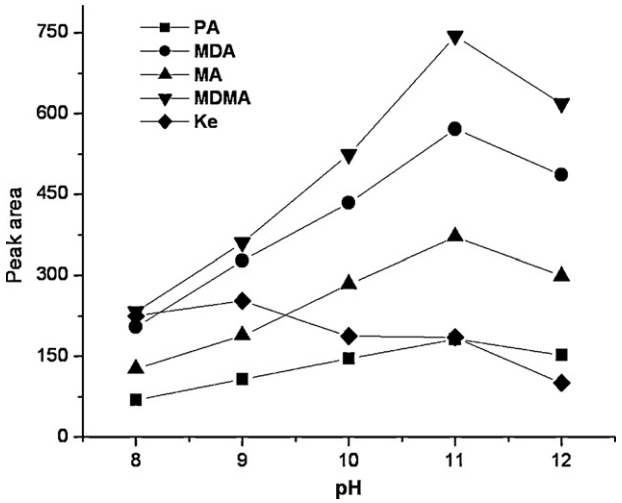


Fig. 3. Effect of pH on the extraction efficiency of the target drugs of abuse Conditions: extraction time: 30 min; desorption time: 20 min; NaCl: 0% (m/v); stirring speed: 700 rpm; concentration of each target analyte: 300 μg/L.

forms at higher pH, resulting in the decrease of electrostatic interaction and finally the lower extraction efficiency.

The pK_a value of Ke is 7.8, so the best extraction efficiency was expected at pH 8–9, which is in accordance with the experimental results obtained in Fig. 3. More and more Ke exist as their neutral forms at higher pH, resulting in a weak electrostatic interaction between the coating and Ke and thus poor extraction performance. For simultaneous extraction of Ke, PA, MA and their derivatives, a compromised pH value of 11 was chosen for the following experiments.

3.3.2. Effect of desorption solvent

Desorption of target polar analytes was performed with methanol–phosphate buffer by ultrasonication. The effect of methanol percentage on the desorption efficiency of the target analytes was studied by varying methanol percentage from 60% to 90% (v/v) and the results were shown in Fig. 4. As could be seen, the desorption efficiency was increased with the increase of methanol percentage from 60% to 80% and the best desorption efficiency was obtained with the 80% methanol addition. Based on like dissolves like, the desorption efficiency of the target analytes was enhanced

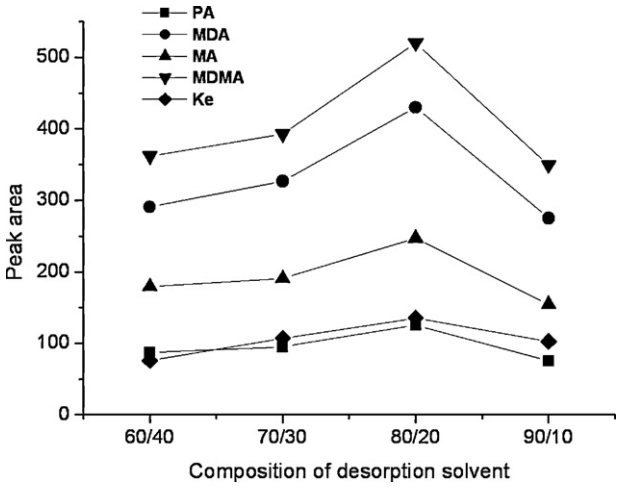


Fig. 4. Effect of desorption solvent (methanol/phosphate buffer, v/v) Conditions: extraction time: 30 min; desorption time: 20 min; pH: 11; NaCl: 0% (m/v); stirring speed: 700 rpm; concentration of each target analyte: 200 μg/L.

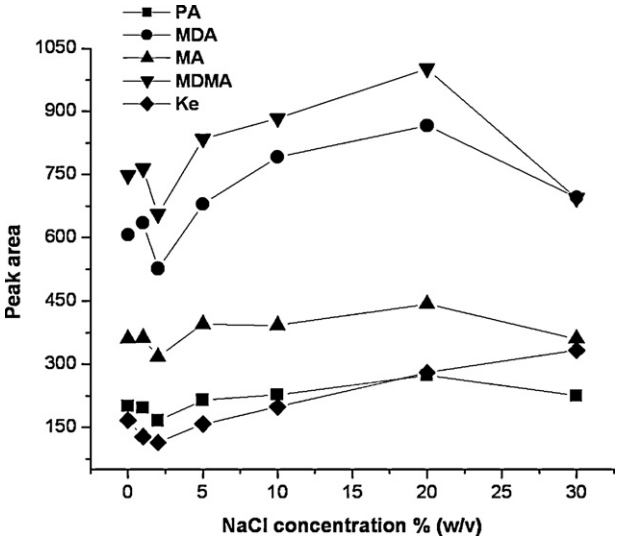


Fig. 5. Effect of NaCl concentration on the extraction efficiency of target drugs of abuse. Conditions: extraction time: 30 min; desorption time: 20 min; pH: 11; stirring speed: 700 rpm; concentration of each target analyte: 300 μg/L.

with the increase of methanol percentage. At the same time, the acidic phosphate buffer (pH = 1.5) provides an acidic condition in which both the hybrid titania coating and the target analytes are prone to be positively charged, the electrostatic repulsion between the target analytes and the coating resulted in an increased desorption efficiency. When the percentage of methanol was higher than 80% (acidic phosphate buffer lower than 20%), the desorption efficiency was decreased. Finally, 80% methanol and 20% buffer solution was chosen as the optimal desorption solvent.

3.3.3. Effect of extraction and desorption time

The influence of extraction time was evaluated with extraction time varying from 10 to 50 min, the experimental results indicated that the extraction equilibrium for the target analytes was reached after 40 min extraction. To trade off the sensitivity and the analytical speed, an extraction time of 30 min was selected for subsequent analyses.

The effect of desorption time was investigated within 5–40 min, and the experimental results demonstrated that 20 min could completely desorb the target analytes. Therefore, desorption time of 20 min was adopted for the subsequent experiments.

3.3.4. The effect of ionic strength

Generally, the addition of salt may increase the extraction efficiency of the target analytes from aqueous solutions due to the salting-out phenomenon in which the addition of salt decreases the solubility of target analytes, resulting in the extraction equilibrium towards adsorption of the analytes onto the coating. The effect of ionic strength on the extraction efficiency of target analytes was studied by adding different amounts of NaCl (from 0% to 30%, m/v) into the aqueous solution, and the experimental results were shown in Fig. 5. It could be seen that the highest extraction efficiency for the target analytes was obtained at salt concentration of 20%. Therefore, 20% (m/v) NaCl concentration was selected for subsequent experiments. The signal decrease for the target analytes with NaCl concentration varying from 0% to 2% can be attributed to the electrostatic interaction of the coating, which is sensitive to the inorganic salt concentration. When the NaCl concentration was in the range of 2–20%, the hydrophobic interaction between the target analytes and coating was enhanced with the increase of NaCl concentration, thus, the extraction efficiency was increased. This phenomenon further confirmed the mix-mode extraction mecha-

Table 3
Analytical performance of the proposed SBSE–HPLC–UV method for the analysis of drugs of abuse.

Analyte	Linear range (μg/L)	Correlation coefficient (R)	RSD (%, n = 6)	LOD (S/N = 3, μg/L)
PA	20–3000	0.9977	8.1	6.6
MDA	20–3000	0.9983	8.3	2.3
MA	20–3000	0.9979	7.3	4.9
MDMA	20–3000	0.9982	9.0	2.5
Ke	30–3000	0.9984	8.9	9.1

Table 4
Comparison of LODs for amphetamines in biological samples.

Method	Analytes	Instrument	Matrix	LOD (μg/L)	References
SBSE	PA, MA, MDA, MDMA, Ke	HPLC–UV	Urine	2.3–9.1	This work
SPME	PA, MA, MDMA	HPLC–FLD	Urine	50–100	[29]
SPME	Enantiomeric analysis of PA and MDA	HPLC–FLD	Urine	100–250	[30]
Monolithic in-tube SPME	PA, MA, MDA, MDMA	HPLC–UV	Urine	1.4–4.0	[12]
SPME	PA, MA	LC–ESI-MS/MS	Serum	0.3–0.04	[13]
SPME	PA, MA	GC–FID	Urine	3–9	[14]
LPME	PA, MDA	GC–MS	Urine	0.25–1	[8]
LPME	PA, MA, MDA, MDMA, MDEA, MBDB	FI–MS/MS	Urine Blood	2–10 0.4–14	[32]
SPE	13 amphetamines	GC–MS	Whole Blood	5–50	[31]

nism of the prepared hybrid coating for the target analytes. As the concentration of salt was increased from 20% to 30%, the extraction efficiency was decreased due to the increase of viscosity of the aqueous solution.

3.3.5. *Effect of stirring speed and sample volume*

Stirring speed is another parameter that influences extraction, because it causes turbulence in the aqueous solution and enhances the extraction efficiency. The effect of stirring speed on the extraction of five drugs of abuse was studied and the results demonstrated that 700 rpm provided the best extraction performance. Hence, the stirring speed of 700 rpm was employed in the following experiments.

The influence of sample volume was examined by changing the sample volume in the range of 2–5 mL. When the sample volume was 2 mL, a poor reproducibility was observed owing to the violent agitation. Therefore, the effect of sample volume on the extraction efficiency was investigated with sample volume of 3 mL and 5 mL and the experimental results indicated that the sample volume had no obvious effect on the extraction of five target analytes and a smaller sample volume of 3 mL was adopted for the following experiments due to a limited biological sample amount available.

3.4. *Linear range, limits of detection, recovery and precision*

Based on the above experimental results, the optimized extraction conditions were as follows: 3 mL aqueous sample solution (pH = 11) containing 20% (m/v) NaCl was extracted for 30 min by a titania-OH-TSO coated stir bar at 700 rpm, and the stir bar was desorbed for 20 min with 60 μL 80/20 (v/v) methanol/phosphate buffer mixtures. Under the above optimal conditions, the analytical performance of the proposed method was evaluated and the results were summarized in Table 3. A good linearity was obtained for the target analytes within concentration range of 20–3000 μg/L or 30–3000 μg/L with correlation coefficients all above 0.9977. Limits of detection (LODs), calculated on the basis of a ratio of signal to noise of 3 (S/N = 3), were in the range of 2.3–9.1 μg/L for five target analytes with the relative standard deviations (RSDs) in the range of 7.3–8.9% (c = 300 μg/L, n = 6).

Table 4 is the comparison of LODs for the determination of amphetamines obtained by this method and by other approaches with different sample pretreatment techniques. As could be seen, although the ultraviolet absorption response of target analytes are not sensitive enough, LODs obtained by the proposed SBSE–HPLC–UV method are better than that obtained by SPME–HPLC–FLD (fluorescence detector) [29,30] and SPE–GC–MS [31], comparable with that obtained by monolithic in-tube SPME–HPLC–UV [12], SPME–GC–FID [14] and LPME–FI–MS/MS [32], but a little bit poorer than that obtained by SPME–LC–ESI-MS/MS [13] and LPME–GC–MS [8]. However, the proposed SBSE method could also be combined with MS, probably resulting in similar LODs to Ref. [32] or even better LODS than Ref. [32].

3.5. *Analysis of amphetamines and ketamine in urine samples*

Under the optimized conditions, the developed method of titania-OH-TSO coating SBSE–HPLC–UV was applied for the determination of target drugs of abuse in the suspect drug-taken urine samples. Before extraction, the urine was adjusted to pH 11 using 1 mol/L NaOH solution and then NaCl was added with final NaCl amount of 20% (m/v). According to Ref. [12], the extraction recovery was calculated by comparing the extraction efficiency obtained by extracting spiked blank urine sample containing 300 μg/L of

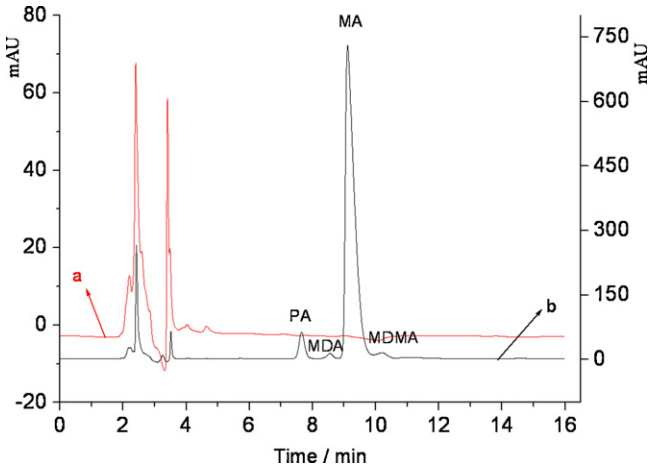


Fig. 6. Chromatograms for urine samples obtained by SBSE–HPLC–UV. (a) Blank urine (corresponding to left ordinate) and (b) abusers' urine (corresponding to right ordinate).

Table 5
Analytical results of drug testing in abusers' urine samples by the proposed method.

	PA (μg/L)	MDA (μg/L)	MA (μg/L)	MDMA (μg/L)	Ke (μg/L)
Abuser 1	3359.6	n.q.	24522.6	88.5	n.d.
Abuser 2	3282.2	n.q.	15740.5	n.q.	n.d.

n.d., not detected; n.q., not quantified.

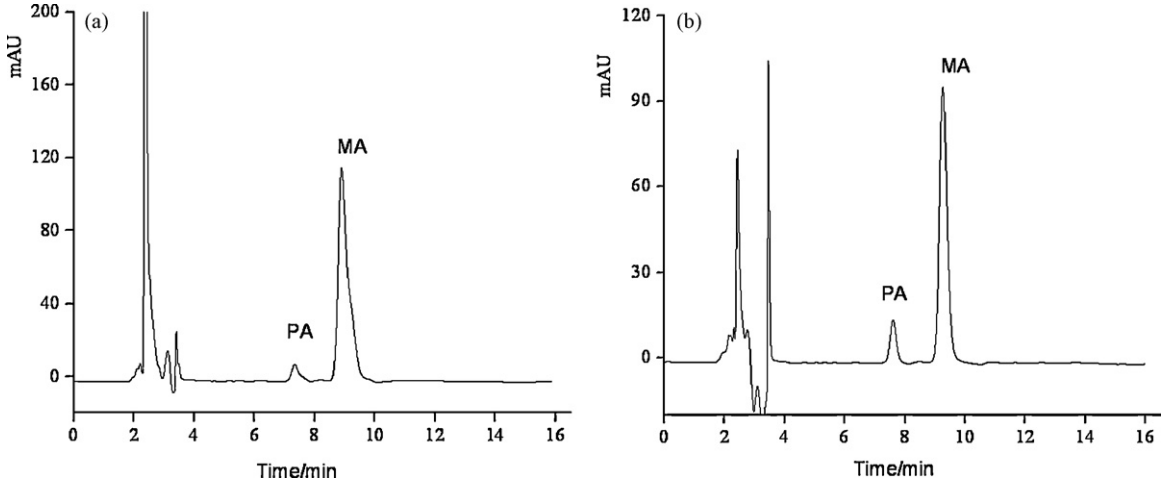


Fig. 7. Chromatograms for abusers' urine samples obtained by SBSE–HPLC–UV (diluted by 10 times). (a) Abuser 1 and (b) abuser 2.

the target analytes to that of the aqueous solution sample containing the same concentration of the target analytes and the results obtained were 108.8, 101.7, 114.2, 117.3 and 90.8% for PA, MDA, MPA, MDMA and Ke, respectively, indicating an excellent clean-up capability of titania-OH-TSO coating stir bar sorptive extraction. Fig. 6 represented chromatograms of blank urine and drug abuser urine samples after SBSE pretreatment, and no interfering peaks at the retention time of the target analytes were found, which further confirmed that the urine matrix hardly affected the extraction. The detected drugs of abuse in abuser urine samples were mainly MA and PA with small amount of MDA and MDMA. For drug abuser urine samples quantification analysis, the samples were diluted by 10 times prior to analysis because the concentration of MA was beyond the upper linear limit of this method. Table 5 is the analytical results for two drug abusers urine samples and Fig. 7 is titania-OH-TSO coating SBSE–HPLC–UV chromatograms for two drug abusers urine samples after 10 times dilution.

4. Conclusion

A hybrid organic–inorganic titania-OH-TSO coated stir bar was prepared by sol–gel method and was used in combination with HPLC–UV for the determination of drugs of abuse in urine samples. LODs at μg/L level were obtained with RSDs (c = 300 μg/L, n = 6) ranging from 7.3 to 8.9%. Titania-OH-TSO coated stir bar showed a highly pH-resistant ability, superior selectivity and high extraction efficiency for the polar compounds. Compared with other four different SBSE coatings, titania-OH-TSO coating exhibited the best extraction properties for amphetamines and ketamine. The developed method is potentially applicable for the analysis of other polar/weak polar drugs of abuse in similar samples.

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