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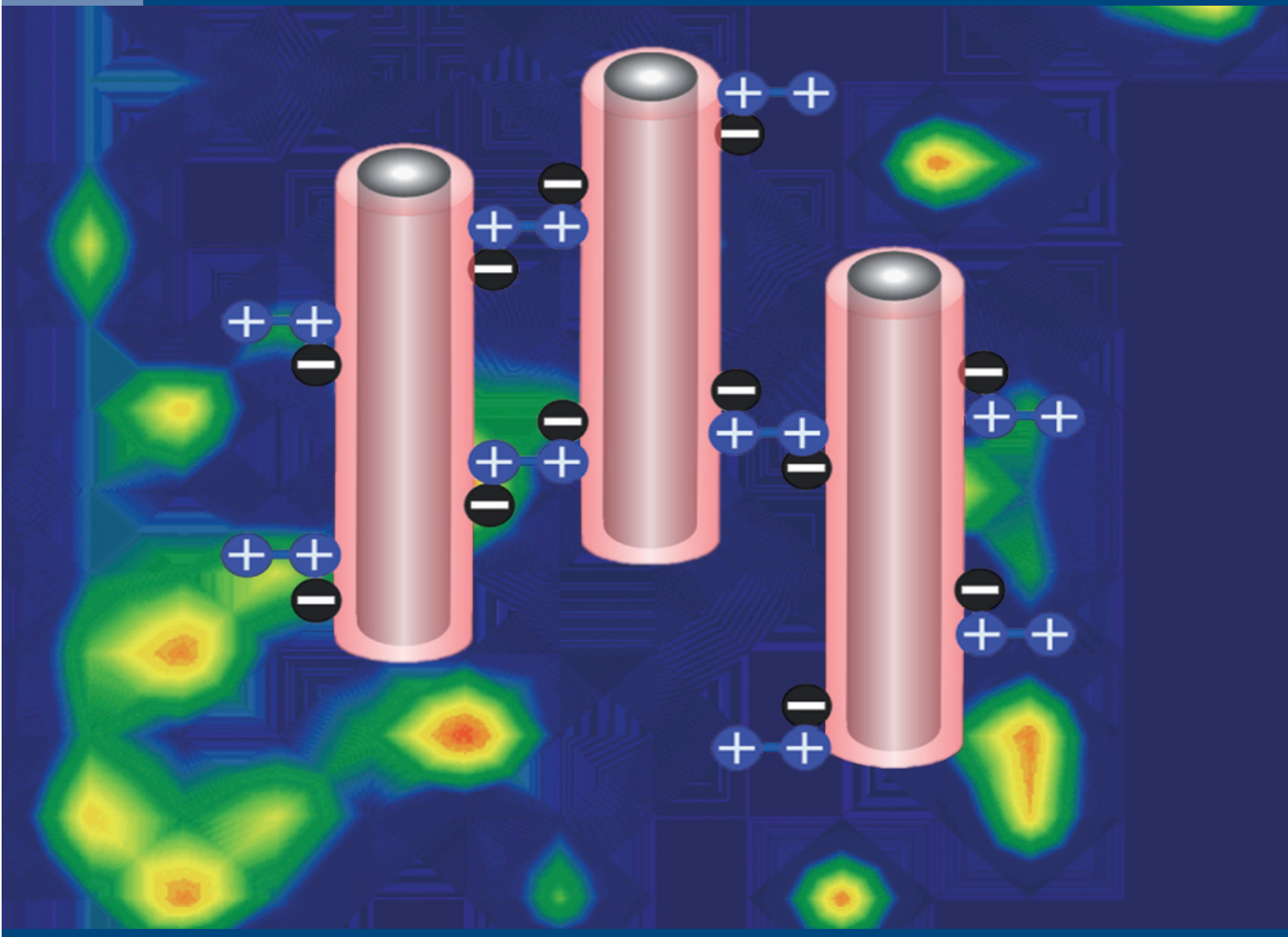

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RESEARCH ARTICLE

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Magnetic dispersive solid-phase extraction based on modified magnetic nanoparticles for the detection of cocaine and cocaine metabolites in human urine by high-performance liquid chromatography–mass spectrometry

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An easy-to-handle magnetic dispersive solid-phase extraction procedure was developed for preconcentration and extraction of cocaine and cocaine metabolites in human urine. Divinyl benzene and vinyl pyrrolidone functionalized silanized Fe_3O_4 nanoparticles were synthesized and used as adsorbents in this procedure. Scanning electron microscopy, vibrating sample magnetometry, and infrared spectroscopy were employed to characterize the modified adsorbents. A high-performance liquid chromatography with mass spectrometry method for determination of cocaine and its metabolites in human urine sample has been developed with pretreatment of the samples by magnetic dispersive solid-phase extraction. The obtained results demonstrated the higher extraction capacity of the prepared nanoparticles with recoveries between 75.1 to 105.7% and correlation coefficients higher than 0.9971. The limits of detection for the cocaine and cocaine metabolites were 0.09–1.10 ng/mL. The proposed magnetic dispersive solid-phase extraction method provided a rapid, environmentally friendly and magnetic stuff recyclable approach and it was confirmed that the prepared adsorbents material was a kind of highly effective extraction materials for the trace cocaine and cocaine metabolites analyses in human urine.

KEYWORDS
cocaine, high-performance liquid chromatography-mass spectrometry, magnetic dispersive solid-phase extraction, metabolites, urine

1 | INTRODUCTION

Cocaine (COC) is among the most widely used illicit drugs, following cannabis and amphetamine-type stimulants and opioids, with the global annual prevalence of COC used ranging from 0.3 to 0.4% of the adult population aged 15–64 years. It is estimated that the illegal market for COC is 100 to 500 billion USD each year. With further processing crack COC can be produced from COC [1–4]. Its use increases the risk of stroke, myocardial infarction, lung problems in those who smoke it, blood infections, and sudden cardiac death. COC

Abbreviations: ACM, acetonitrile; BE, benzoylecgonine; BE-d^3, the internal standards of benzoylecgonine; BN, benzoylnorecgonine; CE, cocaethylene; COC, cocaine; COCs, cocaine and cocaine metabolites; DVB, divinyl benzene; ECG, ecgonine; MDSPE, magnetic dispersive solid-phase extraction; m-HOBE, *m*-Hydroxybenzoylecgonine; MPS, methacrylic acid-3-(trimethoxysilyl) propyl ester; NC, norcocaine; NC-d^3, the internal standards of norcocaine; NCE, norcocaethylene; PLS, divinyl benzene and vinyl pyrrolidone; SMPS, SiO_2 and methacrylic acid-3-(trimethoxysilyl) propyl ester; TEOS, tetraethyl orthosilicate; VP, vinyl pyrrolidone

Conflict of interest: The authors declare no potential conflict of interest.

sold on the street is commonly mixed with local anesthetics, cornstarch, quinine, or sugar which can result in additional toxicity [1,3,5]. Following repeated doses a person may have decreased ability to feel pleasure and be very physically tired [6].

Growing consumption trend of abused COC and drug crimes are a great concern, therefore, it is necessary to devote to find simple, rapid and efficient methods for detection of cocaine and its metabolites (COCs) in human body in criminal technical field [6–11]. Urine drug testing is a noninvasive sampling whereas drugs and metabolites are usually present in high concentrations and relatively long detection windows [12]. However, direct analysis of urine samples is not feasible because urine complex medium often causes low sensitivity and selectivity of the determination [10,12]. On the other hand, presence of low doses of analytes in urine makes an extraction and pretreatment step important before determination.

Several pretreatment methods can be used for the extraction and clean-up of COCs in human urine with complex matrices, such as LLE [13], dispersive liquid–liquid microextraction [14,15], SPE [16–18], SPME, and dispersive SPE [19]. However, these techniques have many disadvantages such as tedious operation, long extraction time, and large consumption of eluents. To overcome these drawbacks, magnetic dispersive solid-phase extraction (MDSPE) has been proposed as a novel, rapid, and simple technique for the extraction of target analytes from matrix. In MDSPE, the magnetic nanoadsorbents are dispersed into the sample solution and phase separation can be conveniently carried out by applying an external magnetic field outside the sample solution. MDSPE reveals many advantages in sample preparation such as short extraction time, easy operation, which takes the advantages of both nanoadsorbents structure and magnetic property of material at the same time [20,21].

The main aim of this study was to investigate the applicability of the MDSPE using magnetic nanoparticles immobilized divinyl benzene (DVB) and vinyl pyrrolidone (VP) on SiO_2 surface for the extraction and determination of COCs in human urine by HPLC–MS. The different structures of COCs and the metabolic architecture diagram are provided in Fig. 1. Their structures have a hydrophobic chain and a heterocycle, which can cause rapid partitioning of target analytes on the surface of DVB and VP. The surfaces of the pure Fe_3O_4 nanoparticles are often coated with different compounds such as macromolecule polymer, silica, and titanium [22–26]. The nanostructured SiO_2 has been considered as one of the most suitable material for widespread environmental application due to its biocompatibility, chemical stability, and cost effectiveness [22,27]. Moreover, the easily modifiable surface of SiO_2 film coated on Fe_3O_4 allows surface modification with various functional groups. The schematic drawings of magnetic adsorbents are shown in Fig. 2A and the detailed MDSPE process is shown in Fig. 2B. Meanwhile, separation is quickly carried out by the application of an external magnetic field, overcoming the need for centrifugation or manual collection of the extractant. To the best of our knowledge, this is the first time that MDSPE with modified Fe_3O_4 nanoparticles followed by HPLC–MS has been applied for the separation and determination of COCs in human urine samples. The

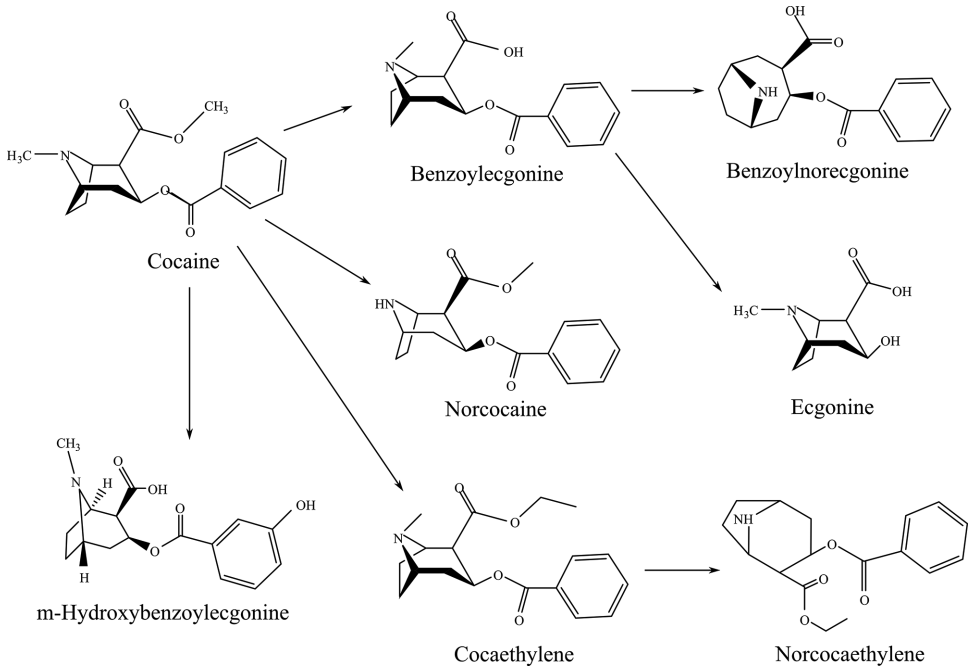

FIGURE 1 The different structures of COCs and the metabolic architecture diagram

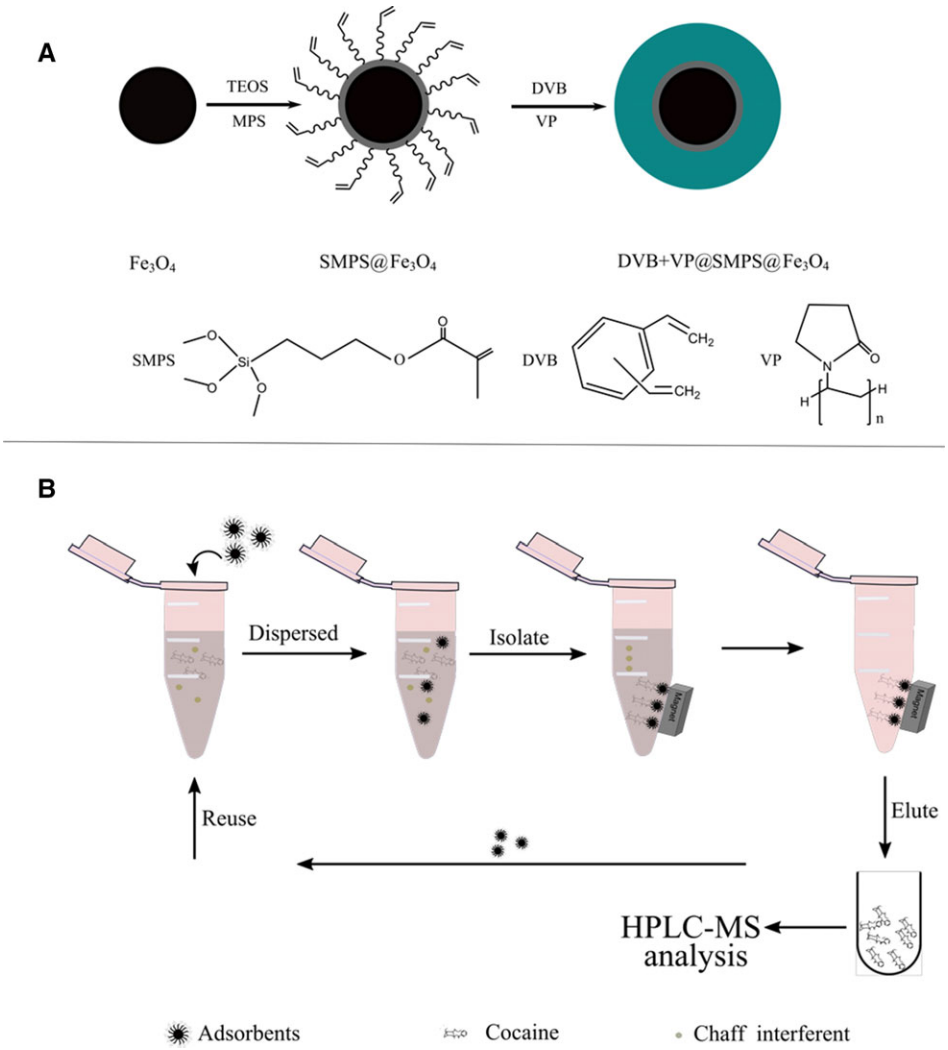

FIGURE 2 Synthesis strategy of PLS@SMPS@Fe_3O_4 (A); process of magnetic dispersive SPE (B)

main experimental parameters affecting the extraction procedure were investigated in detail and the analytical characteristics of the method were evaluated.

2 | MATERIALS AND METHODS

2.1 | Reagents and equipment

One milligram per milliliter of benzoylecgonine (BE), 1.0 mg/mL of norcocaine (NC), 1.0 mg/mL of ecgonine (ECG), 1.0 mg/mL of *m*-hydroxybenzoylecgonine (m-HOBE), 1.0 mg/mL of benzoylnorecgonine (BN), 1.0 mg/mL of norcocaethylene (NCE), 1.0 mg/mL of COC, 1.0 mg/mL of cocaethylene (CE), and the internal standards 1.0 mg/mL of benzoylecgonine (BE-d^3), 100 μg/mL of NC-d^3 hydrochloride were obtained from the National Institute for food and drug control (Beijing, China) and Cerilliant (Darmstadt, Germany). The acetone, acetonitrile (ACM), ethyl acetate, ethylene glycol, sodium acetate, ferric chloride, dimethylformamide, tetraethyl orthosilicate (TEOS), DVB, VP, isopropanol, 2,2-azobisisobutyronitrile, and methacrylic acid-3-(trimethoxysilyl) propyl ester (MPS) were purchased from Beijing Chemicals Corporation (Beijing, China). The syringe filters were purchased from Xingya (Shanghai). All other chemicals were used as received without further purification.

SEM images were obtained with a S3400N scanning electron microscope (Hitachi, Japan). IR spectra were recorded by a Nicolet 6700 FTIR spectrophotometer (Nicolet, USA). The magnetic properties were analyzed through a vibrating sample magnetometer (VSM, PPMS-9), which was purchased from Quantum Design, USA. The TEM image was obtained with a H600 transmission electron microscope (Hitachi, Japan).

2.2 | Preparation of standard solutions

The stock solutions of NCE, CE, and NC were diluted with ACM to 0.1 mg/mL, and those of COC, BE, ECG, BN, and

m-HOBE were diluted with methanol to 0.1 mg/mL. The stock solutions of BE-d^3 and NC-d^3 were prepared in ACM at 0.1 mg/mL. The mixed working solutions for urine samples containing NCE, CE, NC, COC, BE, ECG, BN, and m-HOBE at 0.1 and 1 μg/mL were prepared in methanol. The internal standard working solutions for urine samples containing BE-d^3 and NC-d^3 were prepared in methanol at 0.1 μg/mL. All solutions were stored at 4°C. To produce the desired concentrations for validation of each experiment and internal standardization, further dilutions in water were prepared on the same day.

2.3 | Chromatography conditions

Chromatographic separation was performed on an Agilent HPLC 1200 system with a C18 column (Agilent Eclipse XDB C18, 4.5 × 150 mm, 5μm) equipped with a guard column (Agilent Eclipse XDB C18, 4.5 × 12.5 mm, 5 μm), a G1311A quaternary pump, a G1329A autosampler, and a G1316A column oven.

The mobile phase was composed of solvent A (2 mM ammonium formate and 0.05% formic acid in water) and solvent B (2 mM ammonium formate and 0.05% formic acid in ACM). The column was maintained at 45°C and eluted with a gradient of 10% B (0–1 min), 10–30% B (1–2 min), 30–50% B (2–6 min), 50–70% B (6–13 min), and 70–95% B (13–13.5 min), and the column was then flushed with 95% B (13.5–16.5 min), 95–10% B (16.5–18 min). The total runtime was 18 min at a flow rate of 0.20 mL/min. The temperature of the autosampler before analysis was maintained at 8°C. The injection volume was fixed at 5 μL in the partial loop with needle overfill mode.

2.4 | Mass spectrometric conditions

MS was performed on AB SCIEX API 4000 linear ion QTRAP quadrupole mass spectrometer (USA) equipped with an ESI interface in the positive ion mode. The tandem mass spectrometer was operated under the multiple reaction monitoring modes, Q1 and Q3. Diluted stock solutions of each analyte and the internal standards were prepared to obtain the appropriate multiple reaction monitoring mode parameters. The optimal parameters were as follows: ionspray voltage was 5500 V, entrance potential was 10 V, collision cell exit potential was 10 V, curtain gas flow was 30 psi, Nebulizer gas and heating gas pressures (GS1 and GS2) were 50 and 60 psi respectively, collisional activated dissociation gas setting was medium, and source temperature was set at 600°C. Cone voltage was optimized to get the maximum intensity of the protonated molecular species $[M+H]^+$. The specific parameters for each analyte are shown in Table 1.

2.5 | Synthesis of the magnetic adsorbents

The magnetic Fe_3O_4 particles were prepared according to our previous reported synthetic process [22,23]. The preparation procedure of SMPS@Fe_3O_4 (where SMPS is SiO_2 and methacrylic acid-3-(trimethoxysilyl) propyl ester) included the following steps. Firstly, the silyl reagents MPS and TEOS, were coated on the surface of Fe_3O_4 by hydrolysis to form SMPS@Fe_3O_4. One gram as-synthesized Fe_3O_4 was dispersed in 200 mL ethanol under ultrasonication, then 50 mL water was added to the above dispersion with ultrasonication for 5 min. After stirred for 30 min, 2 mL ammonia was added to the solution. The temperature was set to 45°C, kept it for 20 min, then 4.5 mL TEOS and 1.5 mL MPS was added and stirred for another 12 h to obtain the SMPS@Fe_3O_4. Secondly, 2 g SMPS@Fe_3O_4 were dispersed in 500 mL ACM in the 1000 mL stand-up round bottom flask, then DVB (3.6 mL), VP (7.2 mL), and 2,2-azobisisobutyronitrile (0.4 g) were added to the solution. The mixture was allowed to react with vigorous stirring for 1 h with N_2. Then, the temperature was set to 75°C and this mixture was allowed to react for another 8 h. Later the isolated material was washed three times with water and three times with ethanol. Finally, the product was dried under room temperature.

2.6 | Sample preparation and preprocessing process

For the present study, 1 mL of urine sample was diluted with 20 μL of 5% sodium hydroxide solution and 10 μL of the respective internal standard working solutions. The sample was subjected to vortex treatment for 30 s in a centrifuge tube. The MDSPE procedure is the critical step for selective adsorption and enrichment. For the adsorption step, an optimum weight of the adsorbents (10 mg) were added into above centrifuge tube. The mixture was stirred under ultrasonication for 1 min and stirred vigorously at 300 rpm for another 20 min. Subsequently, a strong magnet was placed at the bottom of the centrifuge tube so that adsorbents with the COCs were isolated from the solution. The solution became limpid after about 10 s and the supernatant was removed carefully with the magnet. The above adsorbents loaded with the COCs were eluted from the adsorbents with 1 mL methanol/ACM (4:1, v/v) for 10 min. Finally, 5 μL of this solution was injected into the HPLC–MS system for analysis.

2.7 | Method validation

The working standard mixture solution at a concentration of 10 μg/mL was prepared by appropriate dilution of the stock standard solutions with methanol. These solutions were stored at 4°C in the dark. Spiked recoveries for method precision and accuracy and matrix effects were performed at concentrations

TABLE 1 Optimum MS conditions used for determination of COCs

Compound	$[M+H]^+$ (*m/z*)	Retention time (min)	Collision energy(eV)	Quantitation (*m/z*)	Scan time (s)
BE	290.3	7.77	27	168.3	0.3
			43	105.1	
NC	290.3	8.00	23	168.3	0.3
			33	136.2	
ECG	186.3	2.63	25	168.3	0.3
			39	82.0	
m-HOBE	306.1	7.61	29	168.1	0.3
			41	121.0	
BN	276.1	7.72	23	154.3	0.3
			31	136.0	
CE	318.0	8.18	30	196.3	0.3
			45	82.2	
NCE	304.1	8.15	22	182.3	0.3
			34	136.2	
COC	304.3	8.02	28	182.3	0.3
			45	82.0	
BE-d^3	293.4	7.74	28	171.3	0.3
			42	105.3	
NC-d^3	293.4	7.99	22	171.4	0.3
			34	136.4	

of 20 and 100 ng/mL for COCs in urine samples. The spiked samples were homogenized in a tube and stored at 4°C for about 24 h. The method was evaluated by linearity, LOD and LOQ, precision, and accuracy. Calibration standards in ACM with concentrations 5.0, 10.0, 25.0, 50.0, 100.0, 150.0, and 200.0 ng/mL were prepared for the calibration curves. The LOD and LOQ were determined based on S/N = 3 and 10, respectively.

2.8 | Precision and accuracy

Three validation batches were assayed to assess the accuracy and precision of the method. Each batch included a set of calibration standards and four replicates of spiked samples at two concentration levels (20.0 and 100.0 ng/mL), and was processed on three separate days. Intra-assay precision was evaluated by replicate ($n = 5$) analysis of the spiked samples in one run. Interassay precision was evaluated by replicate analysis of the spiked samples in experiments performed on three different days. The accuracy of the assay was expressed by comparing the calculated concentrations of spiked samples to their respective nominal values × 100% and the precision was evaluated by RSD.

3 | RESULTS AND DISCUSSION

3.1 | Characterization of magnetic adsorbents

3.1.1 | SEM and TEM analysis

The detailed morphological and structural features of the as-prepared Fe_3O_4, SMPS@Fe_3O_4, and PLS@SMPS@Fe_3O_4 (where PLS is divinyl benzene and vinyl pyrrolidone) were characterized using SEM. Figure 3A indicates that Fe_3O_4 particles are well dispersed with the average size of 500 nm by hydrothermal method. After coating the SiO_2 shell, the obtained microspheres show slippery surface in Fig. 3B, which is consistent with [22]. After coating the polymer shell, the microspheres grow up and the shells change relatively rough (Fig. 3C) because DVB and VP have been polymerized onto the surface of the microspheres. The detailed morphological features of the as-prepared Fe_3O_4, SMPS@Fe_3O_4, and PLS@SMPS@Fe_3O_4 were also characterized using TEM (Supporting Information Fig. S1). After coating the SiO_2 shell (~20 nm), the obtained microspheres show a slippery surface. After coating the polymer shell (DVB and VP), the thickness of the microspheres increased by ~5 nm.

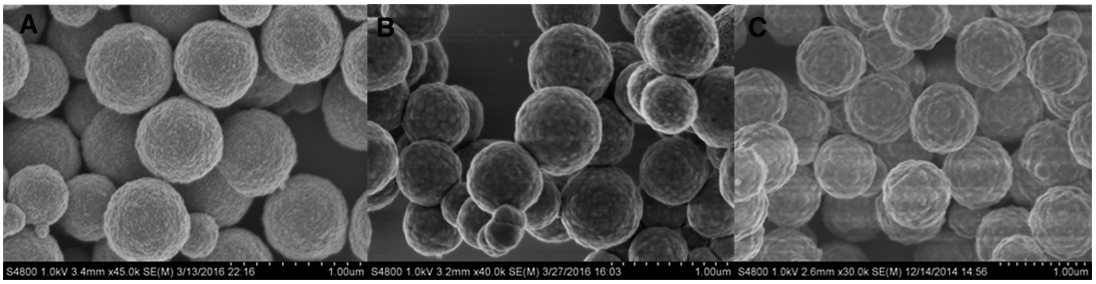

FIGURE 3 The SEM of (A) Fe_3O_4, (B) SMPS@Fe_3O_4, and (C) PLS@SMPS@Fe_3O_4

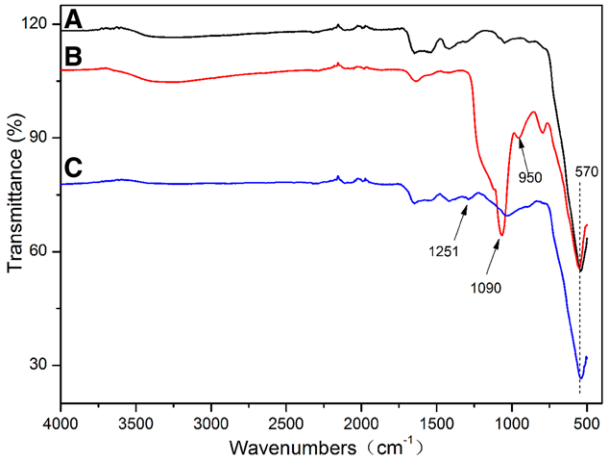

FIGURE 4 The FTIR spectroscopy results of (A) Fe_3O_4, (B) SMPS@Fe_3O_4, and (C) PLS@SMPS@Fe_3O_4

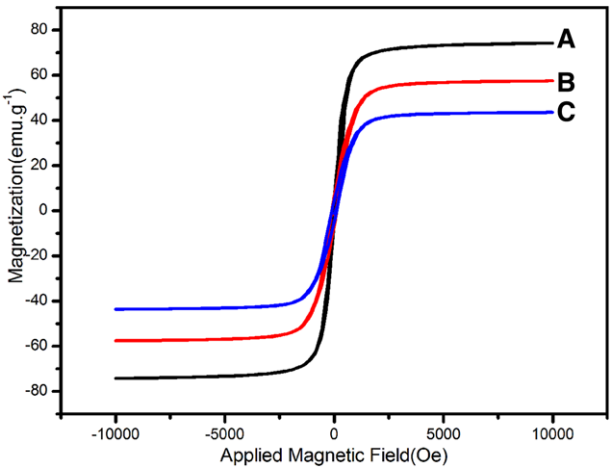

FIGURE 5 The magnetic property of (A) Fe_3O_4, (B) SMPS@Fe_3O_4, and (C) PLS@SMPS@Fe_3O_4 investigated by vibrating sample magnetometry

3.1.2 | FTIR spectroscopy and vibrating sample magnetometry

FTIR spectroscopy was used to characterize the chemical interaction between Fe_3O_4 and functional groups. As can be seen from Fig. 4, the adsorption band of Fe–O is at 570 cm^{-1} (Fig. 4A) [22,23,28], which is the characteristic peak of Fe_3O_4 nanoparticles. The two bands at 950 and 1090 cm^{-1} (Fig. 4B) are the stretching vibration of Si–O bonds of the SiO_2 shell [22]. This proves that the SiO_2 shell is linked to the surface of the magnetic Fe_3O_4. In Fig. 4C, the peak of 1090 cm^{-1} has been almost invisible. And peaks in the region of 1100–1300 cm^{-1} are attributed to C–H and C–C stretching vibrations from DVB and VP [29]. These results indicate that the DVB and VP are successful chemisorbed on the surface of SMPS@Fe_3O_4 nanoparticles.

Figure 5 shows the hysteresis curve of Fe_3O_4, SMPS@Fe_3O_4, and PLS@SMPS@Fe_3O_4 at room temperature. As can be seen, the three curves have a similar shape and symmetry about the origin. The saturation magnetization value was found to be 56.3 emu/g for SMPS@Fe_3O_4 and 74.2 emu/g for Fe_3O_4. This difference might be attributed to the nonmagnetic SiO_2 shell surrounding the magnetite particles. After DVB and VP were grafted on SMPS@Fe_3O_4, saturation magnetization value for PLS@SMPS@Fe_3O_4 was 40.1 emu/g. This indicated the formation of PLS shell on the surface of SiO_2 shell.

3.2 | Optimization of magnetic dispersive SPE conditions

3.2.1 | Amount of the magnetic adsorbents

It is of importance to employ a suitable amount of the PLS@SMPS@Fe_3O_4 without affecting the recoveries of COCs. To investigate the optimum amount of adsorbents needed for the preconcentration and extraction of COCs, batch experiments were performed by 1 mL of urine sample being spiked at 100.0 ng/mL of COCs with the amount of the adsorbents from 1 to 50 mg. The results showed that the extraction efficiency increased with increasing amount of adsorbent up to 10 mg and then leveled off (Supporting Information Fig. S2). The recoveries of the eight COCs were in the range of 76.5–95.8%. Thus, 10 mg of adsorbent was used for further experiment.

3.2.2 | Adsorption time

The time needed for the interaction between the adsorbate and the adsorbent is crucial. Therefore, the effect of

adsorption time on the recoveries was studied. Batch experiments were performed by mixing 10 mg of PLS@SMPS@Fe_3O_4 in 1 mL of urine sample spiked at 100 ng/mL with adsorption time in the range of 2 to 30 min (Supporting Information Fig. S3). The experimental results indicated that the recoveries of eight COCs gradually increased and reached an equilibrium of 78.2–101.3%with the increasing of the adsorption time from 2 to 10 min. Furthermore, the similar results were also obtained in the case of low concentration level of 20.0 ng/mL. Therefore, 10 min was chosen as the optimal adsorption time for further studies.

3.3 | Optimization of elution procedure

The elution solvent and volume of the elution solvent are necessary parameters in elution procedure to obtain reliable results. To select the best eluent for desorbing analytes from the adsorbent, acetone, methanol, ACM, ethyl acetate, water and their mixture solution were examined. As depicted in Supporting Information Fig. S4, the best elution of analytes was mixture solution of methanol/ACM (4:1, v/v), which ensures an efficient and robust elution of COCs while maintaining satisfactory recoveries of the target. And it was selected as the elution solvent in the subsequent experiments. Furthermore, considering the minimum usage of organic solvents for reducing environment pollution, the volume of the elution solvent should be as small as possible in the desorption step. The effect of desorbing solvent volume on the recovery of COCs was investigated in the range of 0.1–3 mL (Supporting Information Fig. S5). The maximum recoveries in the range of 78.4–99.8% were obtained with 1 mL. Therefore, 1 mL of elution solvent (methanol/ACM, 4:1, v/v) was selected for the next experiments.

In summary, the optimal PLS@SMPS@Fe_3O_4 MDSPE procedure conditions are as follows: 1 mL of urine sample was diluted with 20 μL of 5% sodium hydroxide solution and 10 μL of the respective internal standard working solutions. The alkaline solution can reduce the solubility of COCs in water and it will cause COCs to be more easily adsorbed onto the solid support. The sample was subjected to vortex treatment for 30 s in a centrifuge tube. For the adsorption step in MDSPE procedure, 10 mg of PLS@SMPS@Fe_3O_4 was added into the final sample and the mixture was stirred under ultrasonication for 1 min and stirred vigorously at 300 rpm for 20 min to facilitate adsorption of the COCs on the surface of the adsorbent at room temperature.

Then the magnetic adsorbent was magnetically separated using an external magnet and the supernatant solution was decanted. Afterward, the adsorbed analytes were desorbed from the adsorbent by addition of 1 mL of a mixture solution (methanol/ACM, 4:1, v/v) for 10 min vortex treatment. After desorption, the magnetic adsorbent was separated using the magnet and the target analytes in the desorbed solution were determined by HPLC–MS. Compared with traditional method, the MDSPE sample preparation procedure shows the advantages of simple operation, less time, and solvent consuming.

3.4 | Analytical performances

For the analysis of urine samples, the linearity of calibration curves made by peak area (y) versus concentration (x, ng/mL) was studied using calibration standards in freshly prepared urine samples at seven concentrations of 5.0, 10.0, 25.0, 50.0, 100.0, 150.0, and 200.0 ng/mL. The response function was found to be linear. For eight kinds of COCs, the correlation coefficients were higher than 0.9971 in the tested range listed in Table 2. The LOD and LOQ, which were calculated on the analysis of eight COCs in blank extracts spiked at low level in blank samples that yielded an S/N ratio of 3 and 10, were in the range of 0.09–1.1 and 0.31–3.5 ng/mL, respectively. The stability, accuracy, and precision were assessed based on the analysis of COCs spiked at 20.0 and 100.0 ng/mL in blank

TABLE 2 Linear ranges, correlation coefficient (r), LOD, LOQ, intraday/interday variation, recovery, and RSD for COCs studied

Analytes	Internal standard	Linear range (ng/mL)	r	LOD (ng/mL)	LOQ (ng/mL)	Intraday/ interday variation (%)	Recovery (%) 20 ng/mL[a] (%RSD)	Recovery (%) 100 ng/mL[b] (%RSD)
BE	BE-d^3	5–200	0.9971	0.09	0.31	3.5/2.2	79.3 (4.5)	83.6 (2.4)
NC	NC-d^3	5–200	0.9991	0.20	0.63	2.1/2.4	84.4 (3.4)	79.6 (6.6)
ECG	BE-d^3	5–200	0.9982	1.1	3.2	3.2/2.5	105.7(4.4)	96.3 (5.8)
m-HOBE	BE-d^3	5–200	0.9995	0.13	0.42	2.1/4.4	84.3 (4.8)	82.3 (4.7)
BN	BE-d^3	5–200	0.9981	0.26	0.81	2.5/5.4	87.1 (4.7)	77.3 (4.9)
CE	NC-d^3	5–200	0.9982	0.36	1.15	2.7/3.2	83.3 (3.6)	85.3 (2.4)
NCE	NC-d^3	5–200	0.9989	0.21	0.65	3.9/3.2	91.3 (1.5)	75.1 (2.6)
COC	NC-d^3	5–200	0.9972	0.29	0.92	4.8/4.3	80.5 (3.2)	82.3 (3.9)

[a]Spiked at 20 ng/mL.
[b]Spiked at 100 ng/mL.

urine sample. Table 2 shows that the majority of mean recoveries are in the range of 75.1–105.7% at the two spiking levels, wherein the associated intraday RSDs vary from 2.1 to 4.8% and the interday (RSDs) vary from 2.2 to 6.4%.

3.5 | Verification of the proposed method

To further verify the feasibility of this method, three batches of urine sample (12 samples for each batch) were analyzed by the developed method. Each batch of samples was processed together with a matrix blank (COCs-free sample), which was confirmed by using HPLC–MS method. The blank matrix was used to eliminate the false positive in the extraction process and instrument. The eight COCs were identified by comparison of their retention time and fragment ions with the related standard compound. The chromatogram for eight COCs spiked at concentrations 100.0 ng/mL were given in Fig. 6, with all the targets of satisfactory recoveries. No COCs were detected in the 12 real urine samples in first batch. Only one sample in third batch was detected containing BE and COC, and the typical positive sample HPLC–MS chromatogram is shown in Supporting Information Figs. S6 and S7.

Furthermore, a comparison study among different methods in literatures for the determination of COCs applied in various samples was also picked out, and the results were shown in Table 3. Comparing the proposed procedure with other procedures, the PLS@SMPS@Fe_3O_4 does not need to be packed into the SPE cartridge but dispersed in sample extraction instead. The proposed method gives a faster way for the extraction of COCs in urine samples and provides a relatively lower LOD. This good performance was due to the larger specific surface area and selective absorption ability of the absorbent.

3.6 | Recycling and reproducibility of PLS@SMPS@Fe3O4

The PLS@SMPS@Fe_3O_4 could be regenerated by treating the adsorbents sequentially by ultrasonic washing with ethanol and water. In our study, the effectiveness of regeneration of PLS@SMPS@Fe_3O_4 on the urine sample was evaluated by spiking eight kinds of COCs at a concentration of 100.0 mg/mL and two internal standard working solutions. The results are shown in Fig. 7. The PLS@SMPS@Fe_3O_4 could be reused at least five times without much sacrifice of the recoveries (>76.9%). Furthermore, the batch stability of the PLS@SMPS@Fe_3O_4 was also evaluated. Supporting Information Figure S8 displayed the recoveries of eight kinds of COCs by the three batches of Fe_3O_4@SMPS@PLS, which indicated that the absolute deviation in the recovery of each COCs from the three batches was <6.5%. All the results demonstrated that the preparation of the PLS@SMPS@Fe_3O_4 had satisfactory reproducibility and repeatability.

3.7 | Adsorbents specificity validation

The functional groups on PLS@SMPS@Fe_3O_4 that play a vital role in the adsorption of COCs are DVB and VP. The DVB is a hydrophobic functional group and can adsorb hydrophobic compound containing a benzene ring. The VP is a relatively hydrophilic functional group and can adsorb most of the heterocycle. Since most of the COCs molecules are composed of a benzene ring and a heterocyclic ring, the combination of DVB and VP could adsorb the COCs. In our study, different proportions for DVB and VP (5:1, 2:1, 1:1, 1:2, 1:5) were studied to find the optimal ratio of DVB and VP. The experimental results indicated that the DVB:VP (1:2) was the most preferred ratio to obtain satisfactory recoveries of the eight COCs.

In addition, the adsorbents specificity for COCs was further validated. Batch experiments were performed by mixing 10 mg of optimized adsorbents in a 1 mL of urine sample spiked at 100 ng/mL with eight target COCs and three interferences (clozapine, triazolam, and zolpidem). Supporting Information Fig. S9 showed that the recoveries were in the range of 76.1–95.7% for eight COCs and in the range of 17.2–37.8% for clozapine, triazolam, and zolpidem. That means the adsorbents have high selectivity towards COCs.

3.8 | Adsorption isotherms and adsorption capacity

The adsorption isotherm can give the most significant information, which points out how the adsorbate molecules (COCs) are distributed between the liquid phase and the solid phase when the adsorption process reaches equilibrium. Adsorption capacity at different aqueous equilibrium concentrations can be illustrated by the adsorption isotherm. In our study, the adsorption isotherm of BE on PLS@SMPS@Fe_3O_4 was investigated to disclose the adsorption mechanism. The experimental data of BE were fitted by employing Langmuir and Freundlich model. The mathematical representations of the Langmuir and Freundlich models are given below:

$$\frac{1}{q_e} = \frac{1}{q_m} + \frac{1}{K_L q_m C_e} \quad (1)$$

$$\log q_e = \log K_F + n \log C_e \quad (2)$$

where q_e is the amount of analyte adsorbed at equilibrium and C_e is the equilibrium concentration of BE in urine. q_m is the Langmuir constants related to the theoretical

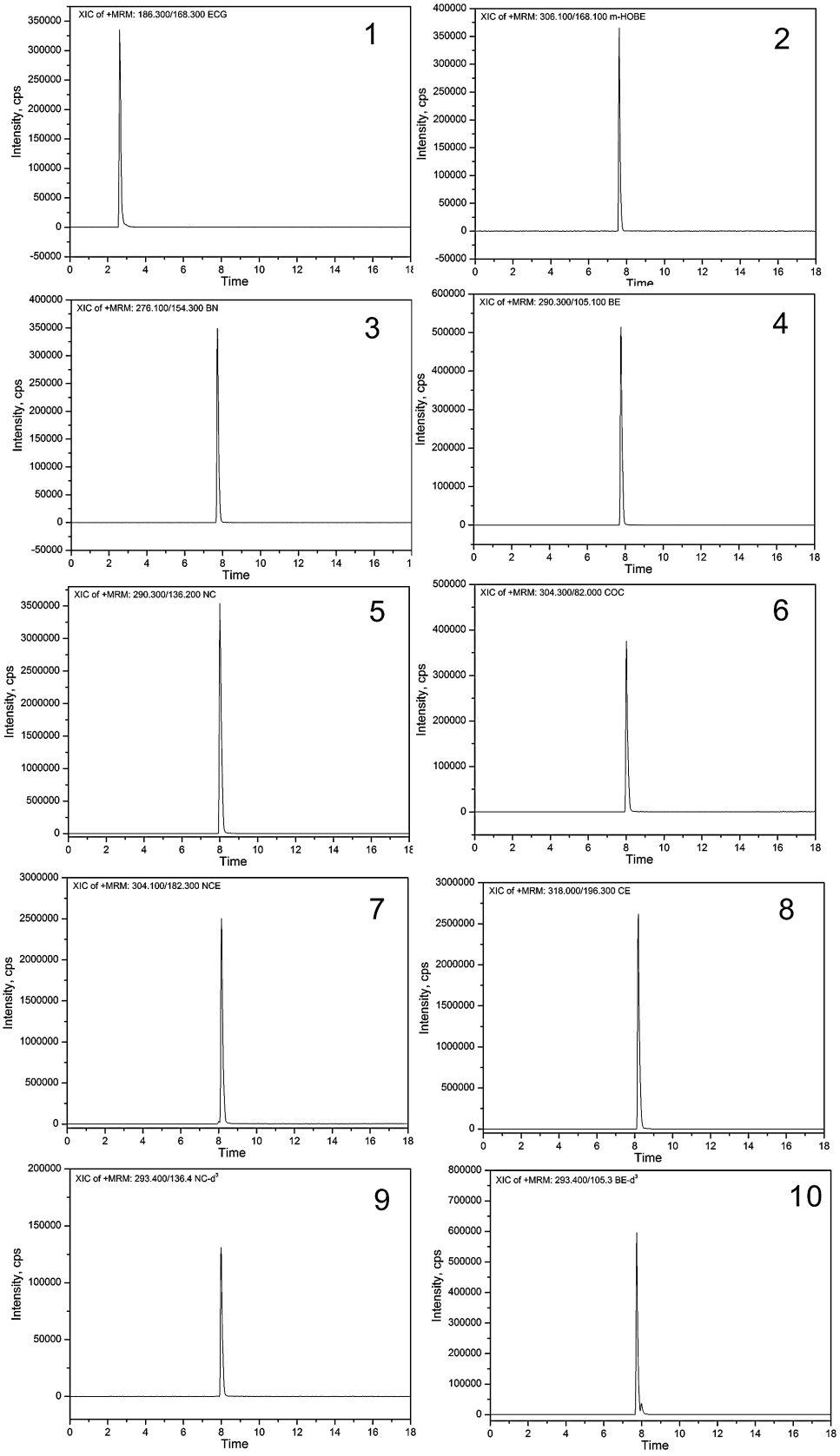

FIGURE 6 The HPLC–MS chromatogram for eight COCs spiked at concentrations 100.0 ng/mL

TABLE 3 Method comparisons for analysis of COCs in real sample

Sample	Extraction method	Adsorbent	Detecting instrument	Pretreatment time	Method LOD, (ng/g) recoveries	Reference
Blood	SPE	Oasis MCX	UHPLC-QTOF–MS/MS	90 min	0.2–16 41–114.3%	[1]
Blood	Automated SPE	Hysphere MM anion	LC–MS/MS	~30 min	3–16	[30]
Hair	SPE	MCX, Oasis	LC–ESI-MS/MS	80 min	5–30	[31]
Urine	SPE	Unkown	GC–MS	60 min	~1 >80%	[32]
Plasma	SPE	C18, HLB	LC–MS/MS	65 min	71–92/4%	[33]
Urine	Magnetic SPE	Magnetic adsorbents	HPLC-MS	40 min	0.09–1.10 75.1–105.7%	This work

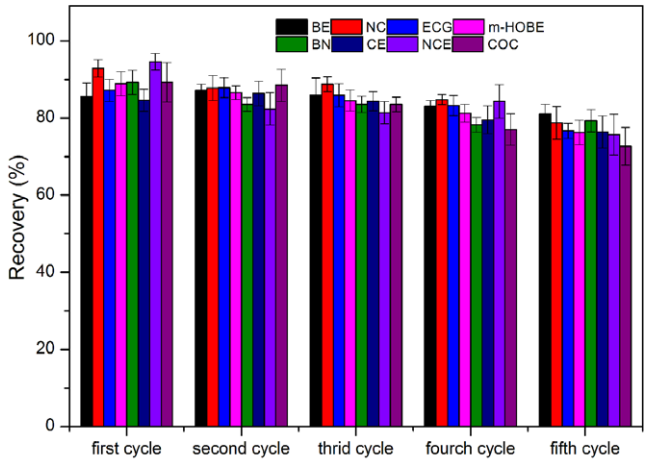

FIGURE 7 Recovery effect of eight COCs under the MDSPE procedure by reusing PLS@SMPS@Fe_3O_4

maximum adsorption capacity of the adsorbents. K_L and K_F are adsorption constants of Langmuir and Freundlich models, respectively. The Langmuir isotherm is an ideal model, which describes a monolayer adsorption process based on the adsorption sites at equilibrium. The Freundlich isotherm is an empirical isotherm model, which depicts a monomolecular coverage layer of the adsorbents by the solutes. The values of r for the Langmuir and Freundlich isotherm model are 0.996 and 0.963, respectively. The theoretical maximum adsorption capacity is 20.4 mg/g, while the experimental maximum adsorption capacity is 19.1 mg/g. This shows that the Langmuir isotherm model fitted well with the experimental data (Supporting Information Fig. S10) and the adsorption behavior of BE on particle surface is probably a monolayer adsorption at equilibrium.

4 | CONCLUDING REMARKS

The development, optimization, and validation of an analytical methodology for the determination of eight COCs in urine samples were the main objectives of this study. For this purpose, the MDSPE procedure with modified magnetic nanoparticles followed by HPLC–MS was successfully applied for the target analytes analysis. The MDSPE extraction procedure was performed by a kind of novel adsorbents (PLS@SMPS@Fe_3O_4). The proposed method possesses many advantages including high adsorption capacity, low solvent consumption, low cost, easy operation, and relatively low matrix effect for the COCs in complex urine. Simultaneously, acceptable recoveries for the studied eight COCs ranged from 75.1 to 105.7% and the accuracy and precision of the proposed PLS@SMPS@Fe_3O_4 MDSPE coupled with HPLC–MS method were satisfactory. Furthermore, the present work provides a promising application for the analysis of other persistent drug in complex biological sample.

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REFERENCES

1. Xiong, L. J., Wang, R., Liang, C., Cao, F. Q., Rao, Y. L., Wang, X., Zeng, L. B., Ni, C. F., Ye, H. Y., Zhang, Y. R., Determination of ecgonine and seven other cocaine metabolites in human urine and whole blood by ultra-high-pressure liquid chromatography–quadrupole time-of-flight mass spectrometry. *Anal. Bioanal. Chem.* 2013, *405*, 9805–9816.
2. Concheiro, M., Gray, T. R., Shakleya, D. M., Huestis, M. A., High-throughput simultaneous analysis of buprenorphine, methadone, cocaine, opiates, nicotine, and metabolites in oral fluid by liquid chromatography tandem mass spectrometry. *Anal. Bioanal. Chem.* 2010, *398*, 915–924.
3. Castro, A. D., Concheiro, M., Shakleya, D. M., Huestis, M. A., Development and validation of a liquid chromatography mass spectrometry assay for the simultaneous quantification of methadone, cocaine, opiates and metabolites in human umbilical cord. *J. Chromatogr. B* 2009, *877*, 3065–3071.
4. Boermans, P. A. M. M., Go, H. S., Wessels, A. M. A., Uges, D. R. A., Quantification by HPLC-MS/MS of atropine in human serum and clinical presentation of six mild-to-moderate intoxicated atropine-adulterated-cocaine users. *Ther. Drug Monit.* 2006, *28*, 295–298.
5. Fernandez, P., Morales, L., Vazquez, C., Bermejo, A. M., Tabernero. M. J., HPLC–DAD determination of opioids, cocaine and their metabolites in plasma. *Forensic Sci. Int.* 2006, *161*, 31–35.
6. Clauwaert, K. M., Van-Bocxlaer, J. F., Lambert, W. E., De-Leenheer, A. P., Segmental analysis for cocaine and metabolites by HPLC in hair of suspected drug overdose cases. *Forensic Sci. Int.* 2000, *110*, 157–166.
7. Fernandez, P., Vazquez, C., Morales, L., Bermejo, A. M., Analysis of opiates, cocaine and metabolites in urine by high-performance liquid chromatography with diode array detection (HPLC-DAD). *J. Appl. Toxicol.* 2005, *25*, 200–204.
8. Jeanville, P. M., Estape, E. S., Torres-Negron, I., Marti, A., Rapid confirmation/quantitation of ecgonine methyl ester, benzoylecgonine, and cocaine in urine using on-line extraction coupled with fast HPLC and tandem mass spectrometry. *J. Anal. Toxicol.* 2001, *25*, 69–75.
9. Shakleya, D. M., Huestis, M. A., Simultaneous quantification of nicotine, opioids, cocaine, and metabolites in human fetal postmortem brain by liquid chromatography tandem mass spectrometry. *Anal. Bioanal. Chem.* 2009, *393*, 1957–1965.
10. Gareri, J., Klein, J., Koren, G., Drugs of abuse testing in meconium. *Clin. Chim. Acta* 2006, *366*, 101–111.
11. Paulsen, R. B., Wilkins, D. G., Slawson, M. H., Shaw, K., Rollins, D. E., Effect of four laboratory decontamination procedures on the quantitative determination of cocaine and metabolites in hair by HPLC-MS. *J. Anal. Toxicol.* 2001, *25*, 490–496.
12. Zuo, Y. G., Zhang, K., Wu, J. P., Rego, C., Fritz, J., An accurate and nondestructive GC method for determination of cocaine on US paper currency. *J. Sep. Sci.* 2008, *31*, 2444–2450.
13. Zhao, X. N., Liu, X. J., Zhao, Z. X., Huang, C. J., Zhang, M. H., Wang, H. L., Wang, X. D., Homogeneous liquid–liquid extraction combined with high performance liquid chromatography–fluorescence detection for determination of polycyclic aromatic hydrocarbons in vegetables. *J. Sep. Sci.* 2009, *32*, 2051–2057.
14. Farajzadeh, M. A., Seyedi, S. E., Shalamzari, M. S., Bamorowat, M., Dispersive liquid–liquid microextraction using extraction solvent lighter than water. *J. Sep. Sci.* 2009, *32*, 3191–3200.
15. Zhao, R. S., Diao, C. P., Chen, Q. F., Wang, X., Sensitive determination of amide herbicides in environmental water samples by a combination of solid-phase extraction and dispersive liquid–liquid microextraction prior to GC–MS. *J. Sep. Sci.* 2009, *32*, 1069–1074.
16. Jiang, F. L., Rao, Y. L., Wang, R., Johansen, S. S., Ni, C. F., Liang, C., Zheng, S. Q., Ye, H. Y., Zhang, Y. R., Sensitive, automatic method for the determination of diazepam and its five metabolites in human oral fluid by online solid-phase extraction and liquid chromatography with tandem mass spectrometry. *J. Sep. Sci.* 2016, *39*, 1873–1883.
17. Liu, X. Y., Zheng, S. Q., Jiang, Z., Liang, C., Wang, R., Zhou, Z., Zhang, Y. R., Yu, Y. Q., Rapid separation and identification of Strychnos alkaloids metabolites in rats by ultra high performance liquid chromatography with linear ion trap Orbitrap mass spectrometry. *J. Sep. Sci.* 2014, *37*, 764–774.
18. Teng, X. M., Liang, C., Wang, R., Sun, T., Rao, Y. L., Ni, C. F., Zeng, L. B., Xiong, L. J., Li, Y., Zhang, Y. R., Screening of drugs of abuse and toxic compounds in human whole blood using online solid-phase extraction and high-performance liquid chromatography with time-of-flight mass spectrometry. *J. Sep. Sci.* 2015, *38*, 50–59.
19. Snow, N. H., Solid-phase micro-extraction of drugs from biological matrices. *J. Chromatogr. A* 2000, *885*, 445–455.
20. Peng, X. T., Jiang, L., Gong, Y., Hu, X. Z., Peng, L. J., Feng, Y. Q., Preparation of mesoporous ZrO_2-coated magnetic microsphere and its application in the multi-residue analysis of pesticides and PCBs in fish by GC–MS/MS. *Talanta*. 2015, *132*, 118–125.
21. Qi, P. P., Wang, Z. W., Yang, G. L., Shang, C. Q., Xu, H., Wang, X. Y., Zhang, H., Wang, Q., Wang, X. Q., Removal of acidic interferences in multi-pesticides residue analysis of fruits using modified magnetic nanoparticles prior to determination via ultra-HPLC-MS/MS. *Microchim. Acta* 2015, *182*, 2521–2528.
22. Zhang, J. B., Gan, N., Pan, M. Y., Lin, S. C., Cao, Y. T., Wu, D. Z., Long, N. B., Separation and enrichment of six indicator polychlorinated biphenyls from real waters using a novel magnetic multi-walled carbon nanotube composite absorbent. *J. Sep. Sci.* 2015, *38*, 871–882.
23. Zhang, J. B., Gan, N., Chen, S., Pan, M. Y., Wu, D. Z., Cao, Y. T., β-cyclodextrin functionalized meso-/macroporous magnetic titanium dioxide adsorbent as extraction material combined with gas chromatography-mass spectrometry for the detection of chlorobenzenes in soil samples. *J. Chromatogr. A* 2015, *1401*, 24–32.
24. Ding, Y., Hu, Y., Jiang, X. Q., Zhang, L. Y., Yang, C. Z., Polymer–monomer pairs as a reaction system for the synthesis of magnetic Fe_3O_4–polymer hybrid hollow nanospheres. *Angew. Chem.* 2004, *116*, 6529–6532.

25. Liu, D., Zhang, L., Li, M. K., Yang, W. T., Deng, J. P., Magnetic Fe_3O_4-PS-polyacetylene composite microspheres showing chirality derived from helical substituted polyacetylene. *Macromol. Rapid Commun.* 2012, *33*, 672–677.
26. Zhong, W., Liu, P., Shi, H. G., Xue, D. S., Ferroferric oxide/polystyrene (Fe_3O_4/PS) superparamagnetic nanocomposite via facile in situ bulk radical polymerization. *Express Polym. Lett.* 2010, *4*, 183–187.
27. Deng, Y. H., Qi, D. W., Deng, C. H., Zhang, X. M., Zhao, D. Y., Superparamagnetic high-magnetization microspheres with an Fe_3O_4@SiO_2 core and perpendicularly aligned mesoporous SiO_2 shell for removal of microcystins. *J. Am. Chem. Soc.* 2008, *130*, 28–29.
28. Lin, S. C., Gan, N., Qiao, L., Zhang, J. B., Cao, Y. T., Chen, Y. J., Magnetic metal-organic frameworks coated stir bar sorptive extraction coupled with GC–MS for determination of polychlorinated biphenyls in fish samples. *Talanta* 2015, *144*, 1139–1145.
29. Wang, Z., Hong, R. Y., Preparation of monodisperse cross-linked PS-DVB-GMA-amino-Fe_3O_4 magnetic microspheres with Cu (II) ions removal property. *J. Polym. Res.* 2016, *23*, 1–10.
30. Jagerdeo, E., Montgomery, M. A., LeBeau, M. A., Sibum, M., An automated SPE/LC/MS/MS method for the analysis of cocaine and metabolites in whole blood. *J. Chromatogr. B* 2008, *874*, 15–20.
31. Imbert, L., Dulaurent, S., Mercerolle, M., Morichon, J., Lachatre, G., Gaulier, J. M., Development and validation of a single LC-MS/MS assay following SPE for simultaneous hair analysis of amphetamines, opiates, cocaine and metabolites. *Forensic Sci. Int.* 2014, *234*, 132–138.
32. Wang, W. L., Darwin, W. D., Cone, E. J., Simultaneous assay of cocaine, heroin and metabolites in hair, plasma, saliva and urine by gas chromatography-mass spectrometry. *J. Chromatogr. B* 1994, *660*, 279–290.
33. Popa, D. S., Vlase, L., Leucuta, S. E., Loghin, F., Determination of cocaine and benzoylecgonine in human plasma by LC-MS/MS. *Farmacia* 2009, *57*, 301–308.

SUPPORTING INFORMATION

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