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An Automated Magnetic Dispersive Solid-phase Extraction Method for Detection of Cocaine and its Metabolites in Human Urine

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ABSTRACT

In this work, an automated magnetic dispersive solid-phase extraction (AMDSPE) sampling method followed by high performance liquid chromatography-mass spectrometry (HPLC-MS) was developed for quantitative enrichment of cocaine and its metabolites (COCs) from human urine, using modified magnetic nanoparticles as absorbents. The methodology was validated according to internationally accepted criteria in order to establish the validity of the combination of the AMDSPE and use of HPLC-MS for quantitative analysis. The proposed device significantly improved the sampling preparation efficiency with 32 samples in one batch within 40 mins. Optimization of the preparation procedure for the magnetic nanoparticles was explored and the performances of magnetic nanoparticles were characterized by scanning electron microscopy, vibrating sample magnetometer and infrared spectra measurements. Several analytical experimental parameters were studied, including amount of particles and elution solvent. The automated procedure exhibited acceptable linearity ($r>0.9972$) over the concentration range of 10 to 200ng/mL. The limits of detection for the cocaine and its metabolites were 0.23-1.5 $ng{\cdot}mL^{-1}$ with recoveries ranging from 77.1 to 94.7%. Compared to traditional sampling method, this method is time-saving and environmentally friendly.

Keywords: Magnetic dispersive solid-phase extraction, Magnetic nanoparticles, Automation, Drug detection, High performance liquid chromatography.

1. Introduction

Computer Cocaine (COC) is the third most common substance of abuse after cannabis and alcohol. The use of cocaine as an illicit substance is implicated as a causative factor for multisystem derangements ranging from an acute crisis to chronic complications [1]. Growing consumption trend of abused COC and drug crimes are also a great concern, therefore, it is necessary to devote to find a simple, rapid and efficient methods for detection of cocaine and its metabolites (COCs) in human body in criminal technical field [2-7].

Urine drug testing is a noninvasive sampling whereas drugs and metabolites are usually present in high concentrations and relatively long detection windows [8]. However, direct analysis of urine samples is not feasible because urine complex medium often causes low sensitivity and selectivity of the determination [6,8]. Several manual pretreatment methods have been used for the extraction and clean-up of drugs or pesticide residue in complex matrices, such as liquid -liquid extraction [9], dispersive liquid–liquid microextraction [10-11], solid-phase extraction (SPE) [12-14], solid-phase microextraction and dispersive solid-phase extraction [15]. However, these techniques have many disadvantages such as tedious operation, long extraction time and large consumption of eluents. Especially when the amount is large, the pretreatment step would be more tedious and time-consuming. Nowadays, multi-analyte screening

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methods can benefit from the development of the automated sample clean-up based on SPE to overcome the drawbacks of liquid-liquid extraction, while it still need to excitation and rinse steps. So, automation is very important in practical application of sample pretreatment, which can perform the extraction and solvent desorption steps of multiple samples in parallel and presents a time-efficient method.

In this work, a new automated method which can reduce the manual pretreatment steps and the risk of human error was developed. This kind of automated magnetic dispersive solid-phase extraction (AMDSPE) sampling method followed by high performance liquid chromatography-mass spectrometry (HPLC-MS) was researched for quantitative enrichment of COCs from human urine, using modified magnetic nanoparticles as absorbents. The nanoparticles were prepared by silanizing magnetic Fe_3O_4 nanoparticles and modifying them with divinyl benzene and vinyl pyrrolidone, which possesses the ability for specific adsorption of COCs. And this kind of magnetic particle facilitated the pretreatment steps by electromagnetically controlled extraction to achieve full automation.

2. Material and Methods

2.1 Chemicals and materials

COCs and its metabolites with 1.0 mg·mL^{-1} of Benzoylecgonine (BE), 1.0 mg·mL^{-1} of Norcocaine (NC), 1.0 mg·mL^{-1} of Ecgonine (ECG), 1.0 mg·mL^{-1} of m-Hydroxybenzoylecgonine (m-HOBE), 1.0 mg·mL^{-1} of Benzoylnorecgonine (BN), 1.0 mg·mL^{-1} of Norcocaethylene (NCE), 1.0 mg·mL^{-1} of Cocaine (COC), 1.0 mg·mL^{-1} of Cocaethylene (CE) and the internal standards 1.0 mg·mL^{-1} of BE-d^3, 100 μg·mL^{-1} of NC-d^3 hydrochloride were obtained from the National Institute for food and drug control (Beijing, China) and Cerilliant Corporation (Darmstadt, Germany). The methyl alcohol, acetone, acetonitrile (ACM), ethyl acetate, ethylene glycol, sodium acetate, ferric chloride, dimethylformamide, tetraethyl orthosilicate (TEOS), divinyl benzene (DVB), vinyl pyrrolidone (VP), isopropanol, 2,2-azobisisobutyronitrile, 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.

The magnetic Fe_3O_4 particles were prepared according to our previous reported synthetic process [16]. Firstly, the silyl reagents MPS and TEOS were coated on the surface of Fe_3O_4 by hydrolysis in order to form SiO_2 layer. 1g as synthesized Fe_3O_4 was dispersed in 200 mL ethanol under ultrasonication, then 50mL water was added to the above dispersion with ultrasonication for 5 min. After stirred for 30min, 2mL ammonia was added to the solution. The temperature was set to 45°C, kept it for 20 minutes, then 4.5mL TEOS and 1.5mL MPS was added and stirred for another 12 h to obtain the SiO_2 layer. Secondly, 2 g as prepared particles were dispersed in 500 mL acetonitrile 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 8h. Later the isolated material was washed 3 times with water and 3 times with ethanol. Finally, the product was dried at room temperature.

Scanning electron micrographs (SEM) were obtained with a S3400N scanning electron microscope (Hitachi, Japan). Infrared spectra were recorded by a Nicolet 6700 FT-IR spectrophotometer (Nicolet, USA). The magnetic properties were analyzed through a vibrating sample magnetometer (VSM, PPMS-9), which was purchased from Quantum Design, Ltd., USA.

2.2 Chromatography conditions and mass spectrometric conditions

Chromatographic separation was performed on an Agilent HPLC 1200 system with a C18 column (Agilent Eclipse XDB C18, 4.5 mm × 150 mm, 5μm) equipped with a guard column (Agilent Eclipse XDB C18, 4.5 mm × 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 96% water and 4% acetonitrile) and solvent B (2 mM ammonium formate and 0.05% formic acid in acetonitrile). 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 run–time was 18 min at a flow rate of 0.20 mL/min. The temperature of the auto–sampler prior to analysis was maintained at 8°C. The injection volume was fixed at 5 μL in the partial loop with needle overfill mode. Mass spectrometry was performed on AB SCIEX API 4000 linear ion QTRAP quadrupole mass spectrometer (USA) equipped with an electrospray ionization (ESI) interface in the positive ion mode. The tandem mass spectrometer was operated under the multiple reaction monitoring mode, Q1 and Q3. Diluted stock solutions of each analyte and the internal standards were prepared in order to obtain the appropriate multiple reaction monitoring mode parameters. The optimal parameters were as follows: ion spray 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 (CV) 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.

2.3 AMDSPE procedure for high-throughput analysis

Table 1. Optimum mass spectrometry (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	45	105.1	0.3
NC	290.3	7.74	23	168.3	0.3
ECG	186.3	2.60	25	168.3	0.3
m-HOBE	306.1	7.58	29	168.1	0.3
BN	276.1	7.70	23	154.3	0.3
CE	318.0	8.15	30	196.1	0.3
NCE	304.1	7.98	35	136.2	0.3
COC	304.3	7.99	28	182.3	0.3
BE-d^3	293.4	7.72	42	105.3	0.3
NC-d^3	293.4	8.11	35	136.4	0.3

The sample preparation equipment with 96 holes location and touch display screen is shown in Fig.1a. Software was designed to fill the dialog boxes with the extraction process parameters directly. The sample plate with magnetic particles (green color), urine (yellow) and eluent (red) was shown with different colors in Fig1b. The AMDSPE procedure is shown in Fig.1c. The whole automatic pretreated procedures included the magnetic dispersion, solid phase extraction and elution steps. Firstly, the magnetic nanoparticles (50mg/ml) with 400 μL water solution was added into the tubes, and the particles were stirred up and down in water for about 1 min. Then the magnetic nanoparticles were moved and dispersed into the sample solution (urine or spiked solution) for extraction for 20 min with stirring rod up and down mixing. After the extraction step was completed, the particles were translated into desorption tube for eluting the target with stirring rod up and down mixing for 15 min by 1 ml desorption solvent (methanol: acetonitrile (3:7, v/v)). The tube containing the desorption solvent was then transferred into the HPLC-MS by syringe filter for analysis. The magnetic particles can be collected and reused after cleaning with acetone and 70% ethanol solution.

2.4 Method validation

The stock solutions of NCE, CE and NC were diluted with acetonitrile to 0.1 mg·mL^{-1}, and those of COC, BE, ECG, BN and m-HOBE were diluted with methanol to 0.1 mg·mL-1. The stock solutions of BE-d^3 and NC-d^3 were prepared in acetonitrile at 0.1mg·mL-1. 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^{-1} 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^{-1}. 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.

A method validation covering all aspects (selectivity, linearity, accuracy, precision, matrix interferences, recovery, carry over and stability) required to establish the feasibility of a validated AMDSPE approach for analysis of the Cocaine and its metabolites was performed according regulatory guidelines. The working standard mixture solution at a concentration of 10 μg·mL^{-1} 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 of 50 ng·mL^{-1} 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 acetonitrile with concentrations 20.0, 25.0, 50.0, 100.0, 150.0 and 200.0 ng·mL^{-1} were prepared for the calibration curves. The limit of detection (LOD) and the limit of quantification (LOQ) were determined based on a signal-to-noise ratio of 3 (S/N=3) and 10 (S/N=10), respectively.

3. Results and discussion

3.1 Characterization of magnetic particles

The magnetic Fe_3O_4 particles were prepared according to our previous reported synthetic process [16]. The core-shell microstructure of the magnetic particles was shown in Fig.2. Fig. 2a was core particle of Fe_3O_4 with the diameter of 500 nm, and Fig.2b was the particle coated with SiO_2 medium layer with slippy surface, and Fig 2c showed the final functional particles with outer coating layer of divinyl benzene and vinyl pyrrolidone.

FT-IR spectra of the magnetic particles were shown in Fig.3. The peaks at 580 cm^{-1} and 1092 cm^{-1} are attributed to the stretching vibrations of Fe-O (Fig3a) and Si-O (Fig3b) respectively. In comparison to the curve of Fig3c, the characteristic peaks of C-H were found at approximately 1280 cm^{-1}. The strong absorption at 1518 cm^{-1} indicated the presence of C-O group in particle surface. All peaks showed that the synthesis was achieved and the ideal groups were obtained for sorbents.

Fig. 4 shows the hysteresis curve of magnetic particles 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 69.3 emu·g^{-1} for Fe_3O_4 and 59.2 emu·g^{-1} for Fe_3O_4 with medium layer. This difference might be attributed to the non-magnetic SiO_2 shell surrounding the magnetite particles. After the outer layer was grafted on the particles, saturation magnetization value was 36.1 emu·g^{-1}. This indicated the new outer layer was formed.

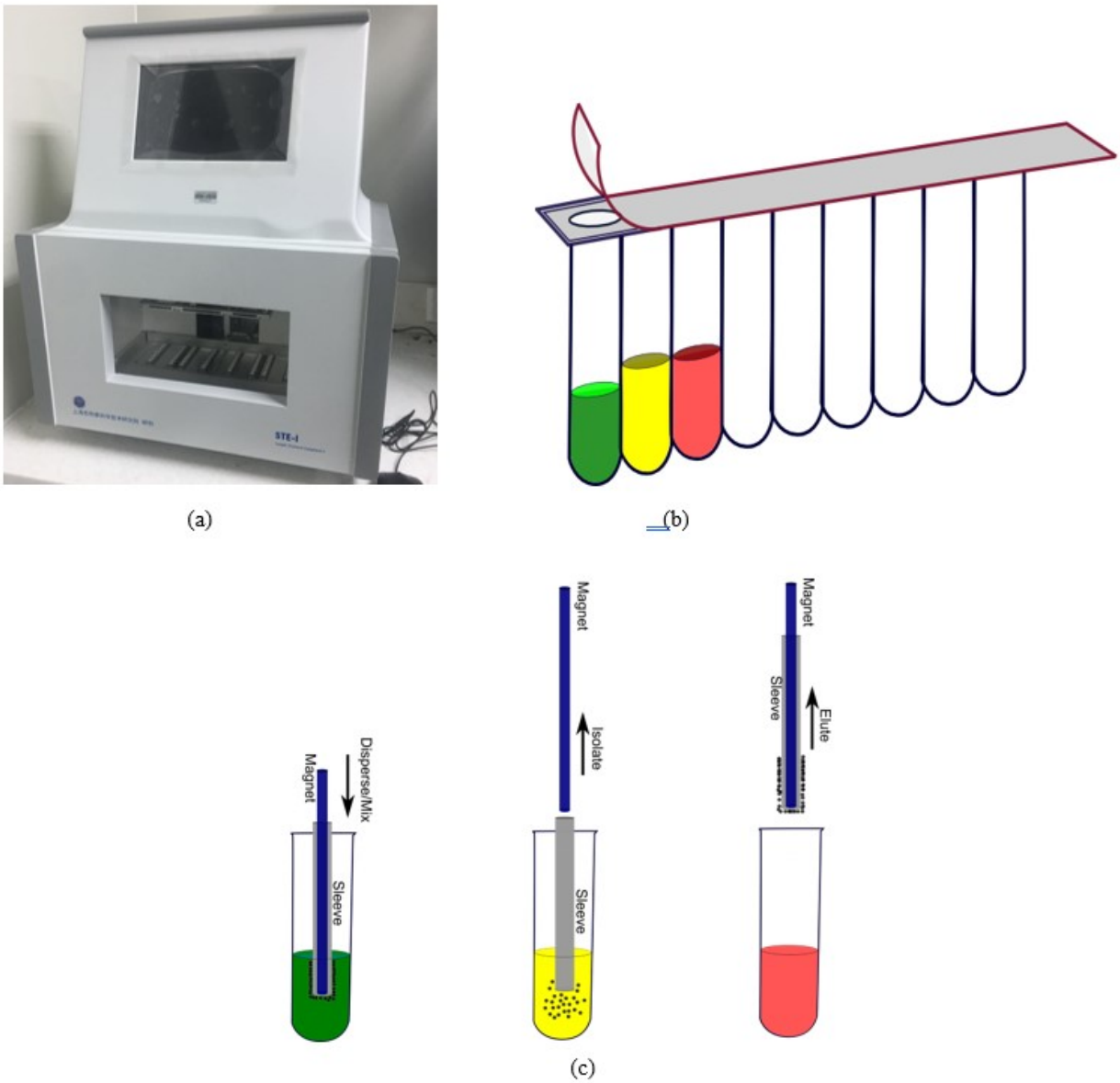

Figure 1. Automated sampling equipment(a), sample plate(b) and process of magnetic dispersive solid-phase extraction(c).

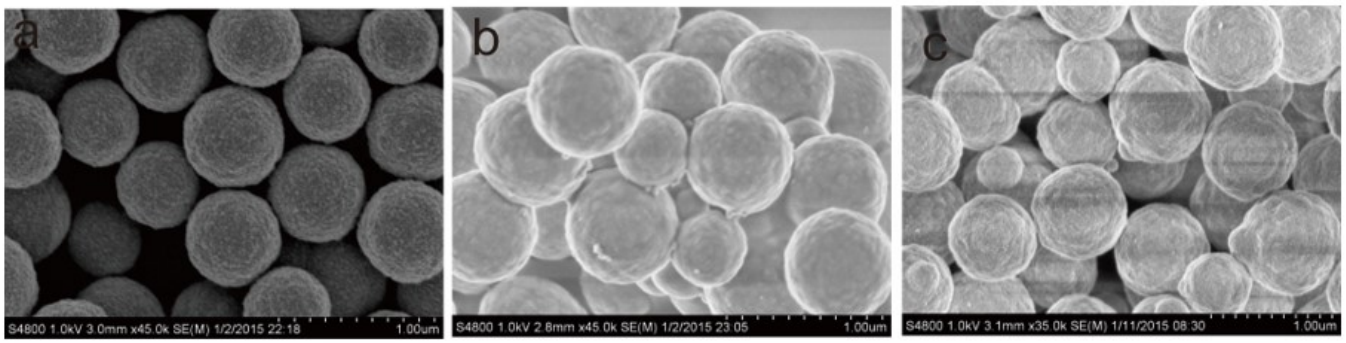

Figure 2. The SEM of (a) Fe3O4, (b) Fe3O4 with medium layerand (c) Fe3O4 with outer layer.

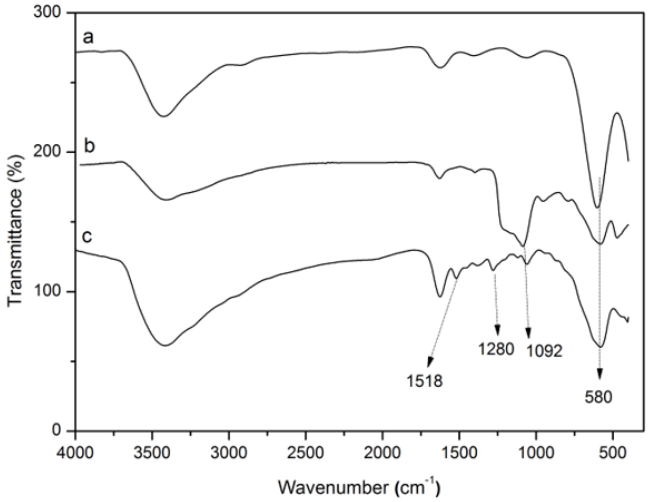

Figure 3. The Fourier transform infrared spectroscopy results of (a) Fe_3O_4, (b) Fe_3O_4 with medium layerand (c) Fe_3O_4 with outer layer.

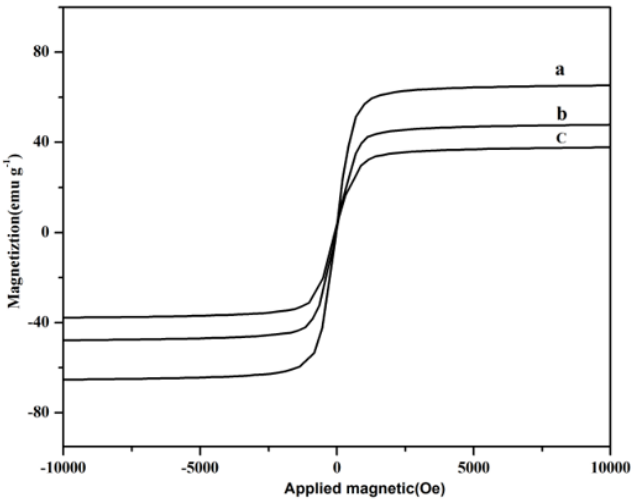

Figure 4. The magnetic property of (a) Fe_3O_4, (b) Fe_3O_4 with medium layerand (c) Fe_3O_4 with outer layer investigated by vibrating sample magnetometer.

3.2 Optimization of automated magnetic dispersive solid-phase extraction conditions

It is of importance to employ a suitable amount of the magnetic particles without affecting the recoveries of COCs.To investigate the optimum amount of adsorbents needed for the preconcentraion and extraction of COCs, batch experiments were performed by 1 mL of urine sample being spiked at 50.0 ng·mL-1 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 20 mg and then levelled off . The recoveries of the 8 COCs were in the range of 73.5- 94.7%. Thus, 20 mg of adsorbent was used for further experiment.

The type and the 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, acetonitrile, ethyl acetate, water and their mixture solution were examined. The best elution of analytes was mixture solution (methanol: acetonitrile (3:7, v/v)), which ensure efficient and robust elute COCs while maintaining satisfactory recoveries of the target; 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 to 3 mL. The maximum recoveries in the range of 78.4-99.8% were obtained with 1 mL. Therefore, 1 mL of elution solvent (methanol: acetonitrile (3:7, v/v)) was selected for the next experiments.

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 20 mg of magnetic particles in 1 mL of urine sample spiked at 50 ng·mL^{-1} with adsorption time in the range of 2 to 30 min. The experimental results indicated that the recoveries of eight COCs gradually increased and reached to an equilibrium of 78.2–101.3% with the increasing of the adsorption time from 2 to 20 min. Furthermore, the similar results were also obtained in the case of low concentration level of 20.0 ng·mL^{-1}. Therefore, 20 min was chosen as the optimal adsorption time for further studies.

3.3 Method validation

For the analysis of urine samples, the linearity of calibration curves made by peak area (y) vs. concentration (x, ng·mL^{-1}) was studied using calibration standards in freshly prepared urine samples at six concentrations of 10.0, 25.0, 50.0, 100.0, 150.0 and 200.0 ng·mL^{-1}. The response function was found to be linear. For eight kinds of COCs, the correlation coefficients were higher than 0.9972 in the tested range listed in Table 2. The LOD and LOQ, which were calculated on the analysis of 8 COCs in blank extracts spiked at low level in blank samples that yielded a signal-to-noise (S/N) ration of 3 and 10, were in the range of 0.23-1.5 ng·mL^{-1} and 0.69-4.7 ng·mL^{-1}, respectively. The stability, accuracy and precision were assessed based on the analysis of COCs spiked at 50 ng·mL^{-1} in blank urine sample. Table 2 shows that the majority of mean recoveries are in the range of 77.1–94.7% at the spiking levels, wherein the associated intra-day relative standard deviations (RSDs) vary from 3.2 to 6.8% and the inter-day (RSDs) vary from 2.1 to 6.2%.

To further verify the feasibility of this method, three batches of urine sample (32 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 8 COCs were identified by comparison of their retention time and

Table 2. Linear ranges, correlation coefficient(r), LOD, LOQ, intra-day/inter-day variation, recovery and RSD for COCs studied .

Analytes	Internal Standard	Linear range ng·mL^{-1}	r	LOD (ng·mL^{-1})	LOQ (ng·mL^{-1})	Intra-day/ Inter-day variation (%)	Recovery (%) 50ng·mL^{-1} (%RSD)
BE	BE-d^3	10-200	0.9981	0.23	0.79	4.1/6.2	81.3 (3.2)
NC	NC-d^3	10-200	0.9992	0.31	0.98	4.6/3.4	87.1 (4.5)
ECG	BE-d^3	10-200	0.9972	1.5	4.7	3.2/5.5	94.7(5.2)
m-HOBE	BE-d^3	10-200	0.9982	0.23	0.69	6.3/2.1	82.3 (3.8)
BN	BE-d^3	10-200	0.9977	0.36	1.11	3.5/5.2	77.1 (4.2)
CE	NC-d^3	10-200	0.9972	0.46	1.58	3.3/2.7	81.3 (5.6)
NCE	NC-d^3	10-200	0.9983	0.41	1.45	4.8/4.2	79.3 (3.5)
COC	NC-d^3	10-200	0.9982	0.59	2.02	6.8/4.8	87.5 (4.9)

fragment ions with the related standard compound. No COCs were detected in the 32 real urine samples in first batch. Only one sample in third batch was detected containing ECG, and the typical positive sample HPLC-MS chromatogram is shown in Fig. 5.

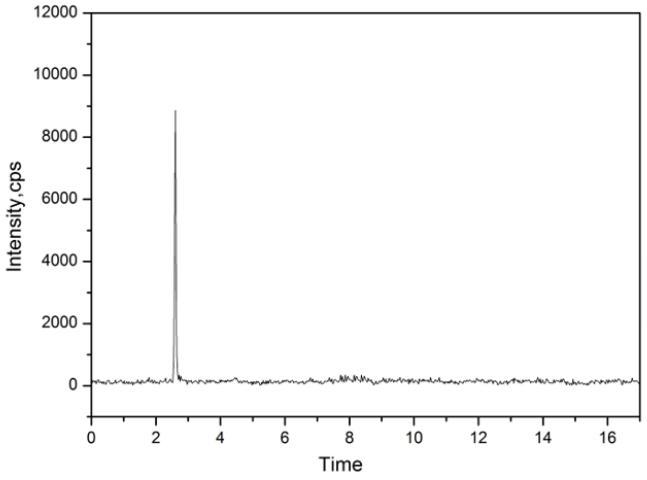

Figure 5. The positive sample HPLC-MS chromatogram with ECG detected.

4. Conclusion

In this study, the special magnetic material was prepared for selectively adsorb the COCs by electromagnetic immobilization and enrichment, rendering the sample preparation easier and controllable. The automated sample preparation equipment provided reproducilbe and reliable results, and allowed for high throughput simultaneous analysis of up to 32 samples, which corresponds to approximately 1.2 min per sample, while manual manipulation is 60 min for about six samples. The proposed method was validated by samples spiked with analytes mixture of satisfactory recovery, repeatability and efficiency, which help to fulfill a simple, fast and automated way for determination COCs in human urine. In quantitive analysis and high-throughput cases, this newly designed method will provide an effective tool for direct extraction of target analytes in complex mixtures with low matrix interference and limit of detection. Meanwhile, the present work provides a promising application for the analysis of other persistent drugs or toxicant 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] 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.

3] 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.

4] 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.

5] 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.

6] Gareri, J., Klein, J., Koren, G., Drugs of abuse testing in meconium. Clinica Chimica Acta. 2006,366, 101-111.

7] 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.

8] Esteveromero J., Albiolchiva J., Perisvicente J., A review on development of analytical methods to determine monitorable drugs in serum and urine by micellar liquid chromatography using direct injection. Anal Chim. Acta. 2016, 926, 1-16.

9] 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.

10] 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.

11] 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.

12] 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.

13] 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.

14] 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.

15] Snow, N. H., Solid-phase micro-extraction of drugs from biological matrices. J. Chromatogr. A 2000, 885, 445-455.

16] YangF.Y., Zou Y., Ni C.F. ,Wang R., Wu M., Liang C., Zhang J.B., Yuan X.L., Liu W.B., Magnetic dispersive solid-phase extraction based modified magnetic nanoparticles for the detection of cocaine and cocaine metabolites in human urine by HPLC-MS, J. Sep. Sci..DOI: 10.1002/jssc.201700457.