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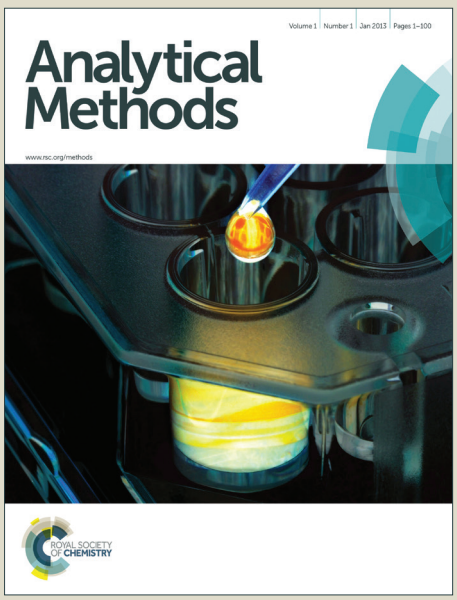

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ARTICLE

Magnetic solid-phase extraction based on trimethylstearylammonium bromide coated Fe_3O_4/SiO_2 composite for determination of adriamycin hydrochloride in human plasma and urine by HPLC-FLD

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A novel trimethylstearylammonium bromide (TSAB) coated Fe_3O_4/SiO_2 magnetic nanocomposite was fabricated and used as the adsorbent for the magnetic solid-phase extraction of adriamycin hydrochloride (ADR) in human plasma and urine with high performance liquid chromatography-fluorescence detection (HPLC-FLD). The factors influencing the extraction efficiency were examined including the pH value, extraction time and elution solvent *etc*. A good linearity was presented by HPLC-FLD in the range of 0.5-10.0 μg mL^{-1} for ADR, with the correlation coefficient of 0.998 (R^2). The relative standard deviation was 4.27%. The limit of detection and limit of quantitation were 5.05 and 16.82 ng mL^{-1}, respectively. The feasibility of developed method was further validated by extraction of ADR in plasma and urine samples. The recoveries were in the range of 76.5-94.0% and 77.9-96.0% for plasma and urine samples, respectively.

1. Introduction

Adriamycin hydrochloride (ADR) is a widely used anthracicline which has been certified to be effective against many types of human malignancies [1], such as leukemia and breast cancers [2]. Unfortunately, the process of using ADR treatment is accompanied by the appearance of serious cardiac toxicity [3, 4], inhibition of bone marrow hematopoietic function and drug resistances [5-7] which may result from cellular processes involving the parent compound or drug metabolites [8]. Accordingly it is necessary and important to detect the contents of ADR in biological samples. Some analytical methods have been reported for ADR detection, such as HPLC with fluorescence, ultra-violet or diode array detection [9-13], ultra high performance liquid chromatography (UPLC) [14], fluorescence/bioprode [15], mass spectrometry (MS) [16], HPLC-MS [17-19], and microchip-based capillary electrophoresis with laser-induced fluorescence detection [20, 21]. Among them, either the complicated and expensive instruments or cockamamie and tedious sample pretreatment are needed. For example, a home-built CCD camera setup based on a nucleic acid-dye bio-probe was applied for detection of Adriamycin by Liu and Danielsson [15]. Also, HPLC and UPLC, as the frequently used method, are always developed along with the performance of sample pretreatment before determination [9-14].

Sample pretreatment, which often requires pre-concentration of the analytes from large volumes of solutions and/or suspensions, is a crucial step in the analysis of complicated sample. As commonly applied pre-concentration methods, liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are used to the extraction of target compounds from composite samples. However, LLE is time-consuming and needs large amounts of organic solvents that are potentially toxic. SPE demands much less number of organic solvents than LLE, but it is still tedious and relatively expensive. In order to overcome these problems, extensive efforts have been made to the development of some new sample pretreatment techniques, which possess high enrichment performance, low solvent-cost and easy automation. Rezaee et al. [22] proposed a new solid-phase extraction combined with dispersive liquid-liquid micro-extraction method for the determination of carbamazepine in biological fluids. Rezazadeh [23] introduced electromembrane surrounded solid phase micro-extraction as the sample pretreatment method for detection of amitriptyline and doxepin in human plasma and urine samples. Other methods such as dispersive liquid-liquid micro-extraction method based on solidification of floating organic droplets technique [24] and molecular imprinted SPE combined with dispersive liquid-liquid micro-extraction method [25] have been developed for the analysis of trace level analytes with HPLC.

The magnetic solid-phase extraction (MSPE) technique was first introduced for the pre-concentration of safranin O and crystal violet based on silanized magnetite particles with copper phthalocyanine dye by Šafaříková [26]. MSPE offers an excellent alternative, which could be faster and easier by placing an external magnet without any additional centrifugation or filtration, compared with the conventional LLE and SPE methods. Fe_3O_4 magnetic nanoparticles (Fe_3O_4 MNPs) have received increasing attention due to their unique properties including good biocompatibility, non-toxic side effects and easy modification and so on [27, 28]. Especially, Fe_3O_4 MNPs was used in separation and pretreatment by virtue of special superparamagnetism [29] and high surface area of the Fe_3O_4

MNPs [30]. However, the ease of aggregation and poor adsorption of pure Fe_3O_4 MNPs limited their further application. Many attempts have been made to explore effective chemical modification to Fe_3O_4 MNPs. As far as we know, some biomacromolecules [31, 32], surfactants [33-35] and carbon materials like graphene or MWCNT [36-39], were utilized to modify Fe_3O_4 MNPs to improve their functions. Liu and coworkers [31] investigated the adsorption of BSA functionalized MNPs for active constituents from Puerariae lobata flower coupled with HPLC-MS/MS. Zhu et al. [33] proposes a mixed hemimicells of cetyltrimethyl ammonium bromide (CTAB) on Fe_3O_4/SiO_2 NPs for extraction and pre-concentration of herbal bioactive constituents from biological samples. Zhao et al. [36] developed a facile route for the immobilization of graphene on magnetic nanoparticles and applied to determine triazine herbicides from environmental water samples. Among these modifiers, the surfactants were highly effective in terms of favorable adsorption, amphipathy and good dispersibility. Song et al. [40] reported chlorodimethyloctylsilane was used to modify amine-Fe_3O_4 MNPs for the determination of hexanal and heptanal in the urine. Rajabi et al. [41] prepared magnetic nanoparticles with CTAB to determine antidepressants from biological fluids. Cheng et al. [42] assemblied 1-hexadecyl-3-methylimidazolium bromide coated Fe_3O_4 MNPs for the pre-concentration of chlorophenols in environmental water samples. However, few surfactants with hydrocarbon chain longer than C_{16} were introduced to modify Fe_3O_4 MNPs.

In this study, we tried to adopt trimethylstearylammonium bromide (TSAB) with long carbon chain as a modifier, which is expected to express favorable adsorption capacity. First of all, the Fe_3O_4 MNPs were successfully synthesized by the in situ chemical coprecipitation of Fe^{2+} and Fe^{3+} in an alkaline solution. Then, the bare hydroxyls on Fe_3O_4 MNPs surface were modified by hydrolysis and poly-condensation of Ethyl silicate (TEOS) and formed a core-shell protective layer of negatively charged. Based on the electrostatic attractive interactions between cationic surfactants and oppositely charged groups on the Fe_3O_4 MNPs, the Fe_3O_4/SiO_2 were modified by TSAB, whose head-group adsorbs to an oppositely charged on silanoxide surface while the C18-chain tail-group protrude into the solution. As a result, we fabricated the TSAB-coated Fe_3O_4/SiO_2 MNPs by combining the magnetic properties of MNPs and the high affinity capacity of TSAB, as-prepared composite was characterized by transmission electron microscopy (TEM), Fourier transform infrared spectrometer (FTIR) and vibrating sample magnetometer (VSM). Further it was applied to effective separation and pre-concentration of ADR from the plasma and urine of healthy human.

2. Experimental

2.1. Reagents and chemicals

The standard of ADR was purchased from Melonepharma Biology Technology Co., Ltd (Dalian, China). Acetonitrile and methanol (HPLC-grade) were obtained from Yongda Chemical Reagent Development Center (Tianjin, China). All of the other reagents were analytical reagent grade. Ferric chloride and iron chloride tetrahydrate were supplied by Fuchen Chemical Reagents Company (Tianjin, China). TEOS and TSAB were got from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Ethanol, formic acid, sodium phosphate monobasic dehydrates, disodium hydrogen phosphate dodecahydrate, and all other reagents were supplied by Fengchuan Chemical Reagent Science and Technology Co., Ltd. (Tianjin, China). Ultrapure water (18.25 MΩ resistivity, 25 ℃) used in all experiments was prepared by molecular ultrapure systems (Shanghai, China).

2.2. Instruments

Agilent 1200 HPLC systems (Agilent Technologies, Germany) were used for determining analytes. The equipment consisted of a G1311A quaternary pump, a G1322A on-line degasser, a G1321A FLD detector，a 7725i manual injector (Rheodyne, USA) and an AT-330 column heater (Autoscience Instrument Co., Ltd, Tianjin). The analytes were separated on Eclipse XDB-C18 column (4.6mm×250mm, 5μm), which was purchased from Agilent Technologies (Germany). Agilent ChemStation program was applied to control HPLC systems and process chromatographic data. The mobile phase was a mixture of 0.5% formic acid in water (A) and acetonitrile (B) in the ratio of 70:30 (v/v) at a flow rate of 0.8 mL min^{-1}. The excitation wavelength and emission wavelength were 498 nm and 554 nm, respectively. The column temperature was set at 30 °C and the injection loop volume was 20 μL.

The morphology and size of MNPs were observed by TEM using a JEM-1011 TEM instrument (JEOL, Japan). FTIR spectra of the MNPs were recorded with a Bruker VERTEX 70 (Bruker Optics, Germany). Fourier transform infrared spectrometer in the wavelength range of 4000-500 cm^{-1} by pressing a small amount of MNPs into a KBr pellet. The magnetic properties were measured by Lake Shore 7304 vibrating sample magnetometer (VSM) (Lakeshore, USA).

The pH of solution was detected by a FE20 pH meter (Mettler-Toledo Intrument Co., Ltd, Shanghai, China). QL-901 vertex that was purchased from Kylin-bell Lab Instruments Co., Ltd (Jiangsu, China) was used to shake the mixture.

2.3. Synthesis of TSAB-coated Fe_3O_4/SiO_2 MNPs

The procedure for synthesis of TSAB-coated Fe_3O_4/SiO_2 MNPs involves three steps. Firstly, Fe_3O_4 MNPs were synthesized via chemical coprecipitation of $FeCl_3$ and $FeCl_2$ in alkaline conditions. According to the method reported by Indira [43] and his/her co-workers with partial changes, 2.379 g of $FeCl_3 \cdot 6H_2O$ was dissolved in 20 mL of ultrapure water, followed by addition of 0.4 mL concentrated hydrochloric acid and 0.994 g $FeCl_2 \cdot 4H_2O$. The mixture was placed in a water bath pot at 70 ℃ and stirred vigorously at approximately 1000 rpm simultaneously. In the meantime, the solution was purged by N_2 gas to remove the oxygen. Next, 100 mL of 3.75 mol L^{-1} ammonium hydroxide in ultrapure water was added gradually to maintain the pH at 9.0, then the reaction proceeded during 2 h under a nitrogen atmosphere. After reacting, the black precipitates (Fe_3O_4 MNPs) were obtained.

Secondly, the obtained Fe_3O_4 MNPs were modified with TEOS and TSAB. Under the previously described condition, 2 mL of TEOS was added into the reaction solution to react for 1 h, then 0.863 g of TSAB was added to react for 30 min. After the reaction, the resulting solution was allowed to stand at room temperature for 1 h.

Finally, the obtained black precipitates were separated from the reaction medium using a magnet, and washed with ultrapure water several times until the pH of the washing became close to neutral, followed by ethanol to wash three times. Then, the produced TSAB-coated Fe_3O_4/SiO_2 MNPs were dried at 60 ℃ for 12 h by vacuum drying.

2.4. Preparation of standard solutions and real samples

The stock solutions of ADR in the concentration of 0.2 mg mL^{-1} and 10 mg mL^{-1} were prepared by dissolving proper amount ADR in ultrapure water. The calibration solution of ADR was prepared by

dilution of the standard stock solution with ultrapure water. All the standard solutions were stored at 4 ℃ and re-prepared every 2 weeks.

Blank plasma and urine samples were collected from healthy volunteers in Shanxi University Hospital and stored at -20 ℃. The complete ethical approval has been obtained, and all the healthy volunteers gave written informed consent. The study was approved by the Institutional Review Board of Shanxi University Hospital. In the analysis, the plasma sample was added with saturated sodium phosphate monobasic dehydrates solution in the ratio of 1:2 (v/v) and centrifuged at 12000 rpm for 5 min to remove proteins. The urine sample was used without further pretreatment. To test the recoveries of method, opportune microliter of stock solution of ADR were added to the blank plasma and urine samples to the final concentrations of 0.5, 5.0 and 10.0 μg mL^{-1}, respectively.

2.5. Magnetic solid-phase extraction procedure

The performance of MSPE is illustrated in Fig. 1. In brief, 10 μL of 0.2 mg mL^{-1} ADR standard stock solution was diluted to 2 mL by PBS buffer (pH 5.0) in a sealed glass test tube. Then, 10 mg TSAB-coated Fe_3O_4/SiO_2 MNPs was added into the solution and shaken for 3 min. To make sure that the ADR was extracted effectively, the MNPs were separated from the sample solution by placing a strong magnet at the bottom of the tube. The supernatant was totally removed. After that, 2 mL of ethanol was added into the tube as elution solvent and vortexed for 1 min. During the process, the ADR adsorbed on the surface of TSAB-coated Fe_3O_4/SiO_2 MNPs were desorbed. After being isolated with the assistance of the magnet force again, the supernatant was transferred to another tube and evaporated with N_2 to dryness in water bath (55 ℃). Finally, the residual was redissolved in 1 mL of ultrapure water and analyzed by HPLC-FLD.

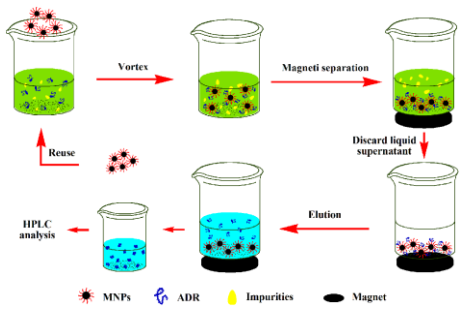

Fig. 1 Schematic procedures for magnetic SPE of ADR from samples

3. Results and discussion

3.1. Characterization of MNPs

The Fourier transform infrared spectroscopy (FTIR) has been applied to qualitatively estimate adsorption of TSAB onto Fe_3O_4 MNPs surface. Fig. 2 illustrates the FTIR spectra of pristine (a) Fe_3O_4 MNPs and (b) TSAB-coated Fe_3O_4/SiO_2 MNPs. The spectra data verified that TEOS and TSAB were successfully modified onto the surface of Fe_3O_4 MNPs. The characteristic bands at 2924, 2855 and 1070 cm^{-1} can be distinguished in the TSAB-coated Fe_3O_4/SiO_2 MNPs IR spectrum, while not observed in the IR spectrum of the pristine Fe3O4 MNPs. According to the standard spectrum, the adsorption bands at 2924 and 2855 cm^{-1} can be ascribed to C-H stretching-vibration. The typical band at 1070 cm^{-1} can be attributed to the Si-O band. It should be noted that the characteristic band of Fe-O is at 586 cm^{-1}, and the adsorption band at 3435 cm^{-1} belongs to the stretching-vibration of O-H from the bare hydroxyl on Fe_3O_4 MNPs surface.

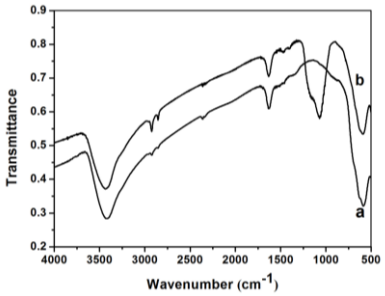

Fig. 2 FTIR spectrogram of (a) Fe_3O_4 MNPs and (b) TSAB-coated Fe_3O_4/SiO_2 MNPs

To investigate the morphology and size of MNPs, the typical TEM images of Fe_3O_4 MNPs, Fe_3O_4/SiO_2 MNPs and TSAB-coated Fe_3O_4/SiO_2 MNPs are given in Fig. 3. As observed in TEM images, most of the particles are quasi-spherical as reported before. In addition, Fig. 3A shows that the mean diameters of Fe_3O_4 MNPs are mainly distributed in the range of 6-10 nm. In Fig. 3B, Fe_3O_4/SiO_2 was covered by TSAB, and the diameters of most TSAB-coated Fe_3O_4/SiO_2 MNPs are distributed in the range of 20-30 nm.

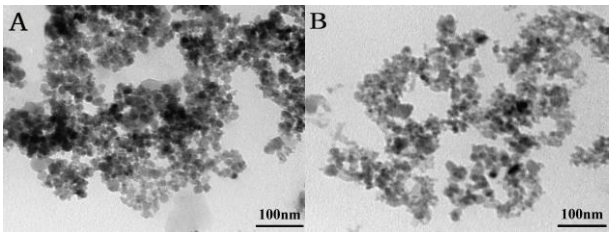

Fig. 3 TEM images of (A) Fe_3O_4 MNPs and (B) TSAB-coated Fe_3O_4/SiO_2 MNPs

Magnetism of Fe_3O_4 MNPs and TSAB-coated Fe_3O_4/SiO_2 MNPs were carried out by vibrating sample magnetometer (VSM). The magnetization values were measured to be 50.06 and 30.16 emu g^{-1} for Fe_3O_4 MNPs and TSAB-coated Fe_3O_4/SiO_2 MNPs, respectively. Fig. 4 shows the magnetization curves at room temperature. Both Fe_3O_4 MNPs and TSAB-coated Fe_3O_4/SiO_2 MNPs exhibit typical superparamagentic behavior due to no remanence and coercivity.

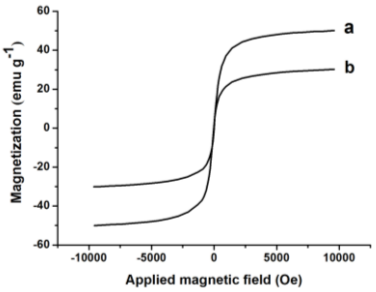

Fig. 4 VSM magnetization curves of (a) Fe_3O_4 MNPs and (b) TSAB-coated Fe_3O_4/SiO_2 MNPs

3.2. Optimization of extraction conditions

In order to obtain the optimal extraction efficiency of target analytes, several conditions that affecting the extraction efficiency of analytes were optimized including the pH, extraction time, elution solvent, elution volume, desorption time, maximum adsorption quantities and the reuse property of TSAB-coated Fe_3O_4/SiO_2 MNPs.

3.2.1. Effect of solution pH

pH is one of the prime influencing factors on the adsorption of the analytes by affecting both existing forms of the target compounds and the charge species on the sorbent surface. In our work, the effect of solution pH was investigated by varying the pH values between 3.0 and 9.0 with 0.05 mol L^{-1} PBS buffer. As shown in Fig. 5, TSAB-coated Fe_3O_4/SiO_2 MNPs exhibited highest extraction efficiencies for ADR when the solution pH was acidic. From pH 3.0 to 5.0, the adsorption amount gradually increased and reached the maximum value at pH 5.0. With the increase of pH value, the percentage of ADR adsorbed was obvious declined, which may be explained as that ADR was easily degraded in neutral or strong alkaline conditions. Furthermore, according to the experimental results, the TSAB-coated Fe_3O_4/SiO_2 MNPs were also stable under this pH condition. For this reason, the pH 5.0 was selected for the following experiments.

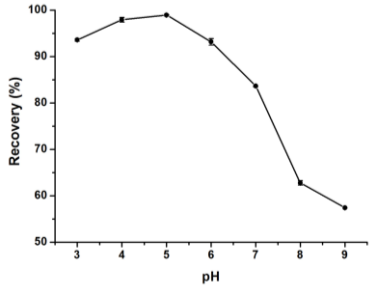

Fig. 5 Effect of solution pH (n=3 for each point). Conditions: extraction time, 3 min; elution solvent, ethanol; elution volume, 2 mL; desorption time, 1 min. Other conditions were the same as the section of magnetic solid-phase extraction procedure (Section 2.5)

3.2.2. Effect of extraction time

The extraction time plays an important role in the adsorption equilibrium of the analytes for the MSPE performance. In this experiment, the effect of extraction time was tested within 0.17-120 min. According to the result in Fig. 6, when the sample solution was shaken for 3 min, the extraction amount of ADR reached maximum, indicating that the adsorption equilibrium can be achieved after about 3 min. Consequently, the extraction time was set at 3 min.

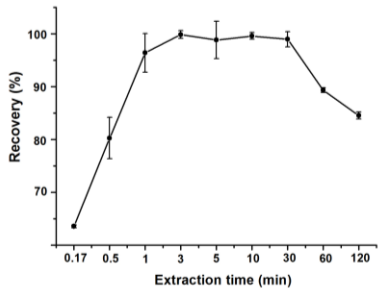

Fig. 6 Effect of extraction time (n=3 for each point). Conditions: pH value, 5; elution solvent, ethanol; elution volume, 2 mL; desorption time, 1 min. Other conditions were the same as the section of magnetic solid-phase extraction procedure (Section 2.5)

3.2.3. Selection of elution solvents

In the MSPE process, retrieve of target analytes retained on the surface of MNPs was an essential procedure. The organic solvents could disrupt the aggregation of surfactant on the surface of Fe_3O_4 MNPs. Thus in this study, different organic solvents, including methanol, ethanol, acetonitrile, acetone and chloroform, were examined as elution solvents. Fig. 7 shows the recoveries of some solvents to ADR, revealing that the desorption ability of ethanol was superior to that of other organic solvents. So ethanol was selected as the elution solvent in the following studies.

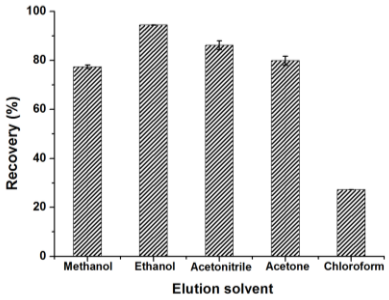

Fig. 7 Effect of elution solvent (n=3 for each point). Conditions: pH value, 5; extraction time, 3 min; elution volume, 2 mL; desorption time, 1 min. Other conditions were the same as the section of magnetic solid-phase extraction procedure (Section 2.5)

3.2.4. Effect of elution volume

Under the same other experimental conditions, the effect of elution volume was investigated in the volume range of 0.3-3 mL and the other experimental conditions were kept the same. Fig. 8 depicts the desorption percentage of adsorbed ADR increases with the increase of the elution volume. As a result, the best elution efficiency was acquired when 2 mL of elution solvent was used to elute.

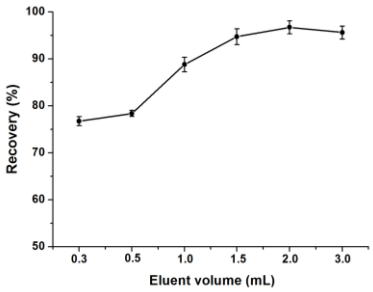

Fig. 8 Effect of eluent volume (n=3 for each point). Conditions: pH value, 5; extraction time, 3 min; elution solvent, ethanol; desorption time, 1 min. Other conditions were the same as the section of magnetic solid-phase extraction procedure (Section 2.5).

3.2.5. Effect of desorption time

As mentioned in the section of Magnetic solid-phase extraction procedure, after adding elution solvent into the sample solution, the sealed glass test tube was vortexed for a definite time to insure the sufficient desorption. For this reason, the influence of desorption time was also evaluated from 0.17 to 10 min under vortexing. As given in Fig. 9, the result demonstrated that the elution equilibrium reached quickly within 0.5-1 min, and 1 min was enough for eluting the loaded ADR from the surface of MNPs, and it was selected as the optimum desorption time.

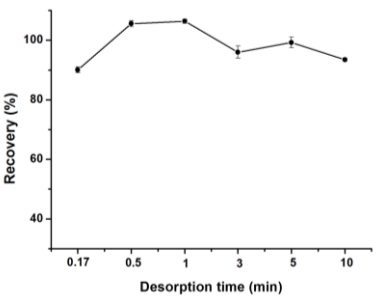

Fig. 9 Effect of desorption time (n=3 for each point). Conditions: pH value, 5; extraction time, 3 min; elution solvent, ethanol; elution volume, 2 mL. Other conditions were the same as the section of magnetic solid-phase extraction procedure (Section 2.5)

3.2.6. Adsorption property

For the MSPE method, the adsorption quantity of magnetic sorbent is a crucial parameter that evaluates the adsorbent of adsorption ability. Maybe fewer amount of complex magnetic adsorbent could achieve satisfactory results due to their greater surface areas. In order to gain the maximum adsorption quantities, 5 mg TSAB-coated Fe_3O_4/SiO_2 MNPs were added respectively into 2 mL of PBS (pH 5.0) buffer spiked with proper stock solution of ADR, which the final amount of ADR in every PBS buffer were 0.005, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg, respectively. The other experimental conditions were to remain unchanged. A graph of peak areas of ADR versus addition was shown in Fig. 10. As illustrated in the curves, the peak areas of ADR increased at first and levelled off gradually. The data generation into the equation as provided in the section of method validation was calculated. In consequence as a result, the maximum adsorption quantity of TSAB-coated Fe_3O_4/SiO_2 MNPs was 0.104 mg per 5 mg.

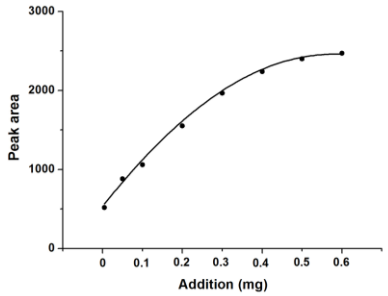

Fig. 10 The test of maximum adsorption quantities (n=3 for each point)

Possible mechanism of the TSAB-coated Fe_3O_4/SiO_2 MNPs-based magnetic solid-phase extraction is considered from the hemimicelles of TSAB on silica-coated Fe_3O_4 MNPs (Fig.11). When concentration of TSAB is less than critical micelle concentration (CMC), TSAB will form hemimicelles on the surface of silica-coated Fe_3O_4 magnetic nanoparticles. TSAB-coated magnetic nanoparticle was selected as a sorbent because the sorbent with the long alkyl chain of C18 has a strong hydrophobicity and affinity interaction with ADR. In addition, the core-shell magnetic nanoparticles have high surface areas. Silica is used as one of the most ideal shell materials due to its reliable chemical stability, biocompatibility and versatility in surface modification. It can be easily isolated by using an external magnetic field placed outside of the extraction container, which offers feasible magnetic solid-phase extraction separation.

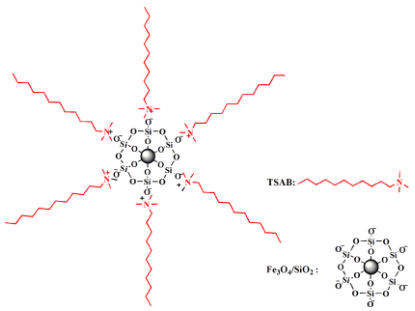

Fig. 11 Schematic of hemimicelles on the surface of silica-coated Fe_3O_4 magnetic nanoparticles

3.2.7. Reuse property

One of the reasons that a complex magnetic nanoparticles is considered to be good adsorbent is that they can be reused. The reuse property of TSAB-coated Fe_3O_4/SiO_2 MNPs for extraction of ADR was tested. In this experiment, the same MNPs were dried and used again for extraction and desorption for six consecutive cycles. As shown in Fig. 12, the recovery of ADR is greater than 65% even after six used, suggesting that the TSAB-coated Fe_3O_4/SiO_2 MNPs has a good recycle property.

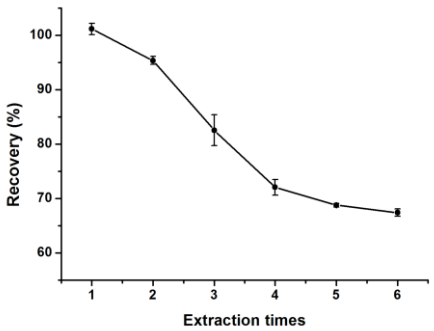

Fig. 12 Reuse property of TSAB-coated Fe_3O_4/SiO_2 MNPs (n=3 for each point)

3.3. Method validation

The analytical method of ADR was investigated in terms of its linearity, limit of detection (LOD), limit of quantification (LOQ) and inter-assay and intra-assay precisions. A series of standard solution containing ADR at six concentration levels of 0.50, 1.0, 2.5, 5.0, 7.5 and 10.0 μg mL^{-1} were prepared for the establishment of the calibration curve. The calibration curve was obtained by plotting peak area (Y) against the corresponding concentration (X) of ADR, and each point on the calibration plot was the mean value of six peak area measurements. Calibration curve exhibited good linearity with a correlation coefficient (R^2) above 0.998 in the range from 0.50 to 10.0 μg mL^{-1}. The LOD and LOQ were calculated, as a concentration that produced a signal-to-noise ratio of 3 (S/N=3) and 10 (S/N=10), respectively. The precision of the proposed method, reported as relative standard deviation (RSD), was assessed based on the intra-assay and inter-assay precisions. Results of validation parameters were summarized in Table 1. In addition, another standard calibration curve in the range from 50 to 2000 μg mL^{-1} was achieved to test the maximum adsorption capacity. It exhibited similarly good linearity with the correlation coefficient (R^2) over 0.999.

Table 1 Linear equation, LOD, LOQ and RSD of ADR

Linear range (μg mL^{-1})	Linear equation	R^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	RSD (%) (n=6)	
					Inter-day	Intra-day
0.50-10.0	Y=13.33X-5.10	0.9984	5.05	16.82	≤4.27	≤2.86
50-2000	$Y=2.51\times10^4X-1.37\times10^2$	0.9991				

3.4. Analysis of human plasma and urine samples

In order to evaluate the practical applicability of proposed TSAB-coated Fe_3O_4/SiO_2 MNPs in the real samples, the proposed method was applied to extract of ADR from human plasma and urine samples, respectively. The performance was carried out by extracting at three different concentrations of 0.50, 5.0 and 10.0 μg mL^{-1} plasma and urine samples spiked with ADR, meanwhile, same concentrations of ultrapure water solutions of ADR were used as controls, and the results were summarized in Table 2. The recoveries of plasma sample spiked with ADR were from 76.5% to 94.0%. The

urine sample recoveries were in the range of 77.9%-96.0%, and the reproducibility indicated good precision.

Table 2 Results of ADR determination in human plasma and urine samples

Samples	Added (μg)	Found (μg)	Recovery (%)	RSD (%) (n=6)
Control	0.50	0.52	104.0	3.02
	5.0	4.99	99.8	4.40
	10.0	9.63	96.3	3.38
Plasma	0.50	0.47	94.0	4.84
	5.0	4.68	93.6	1.22
	10.0	7.65	76.5	1.56
Urine	0.50	0.48	96.0	5.89
	5.0	4.48	89.6	1.25
	10.0	7.79	77.9	0.58

Fig. 13 illustrates the typical chromatogram of (A) plasma sample and (B) urine sample spiked with ADR at concentration of 5 μg mL^{-1}. The pictures show that the retention time for ADR is at about 3.9 min. The interfering peaks were found at about 4.5 and 3.6 min in chromatogram of plasma and urine sample, respectively. By comparing extraction and non-extraction, obviously, interfering peaks decreased significantly after the proposed pretreatment, and has no interference on detection of ADR. These satisfactory results exhibit the practicability of TSAB-coated Fe_3O_4/SiO_2 MNPs sorbent in the real samples.

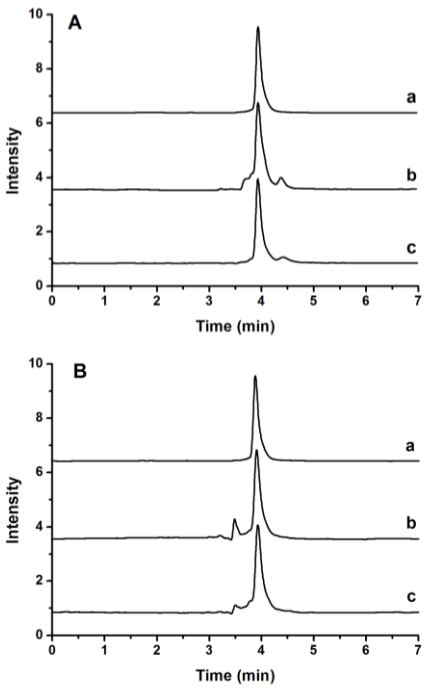

Fig. 13 Typical chromatogram of (A) plasma sample and (B) urine sample spiked with ADR at concentration of 5 μg mL^{-1}. (a: extraction of ADR from ultrapure water; b: spiked plasma or urine sample without extraction; c: extraction of ADR from plasma or urine sample)

As shown in Table 3, compared with the previously reported methods, HPLC-FLD was obvious superior to HPLC-UV method. The retention time is shorter than other methods. The sensitivity is relatively low. Recovery results can meet the need for the detection of body fluid samples. Our study provides a simple, rapid and effective method for ADR analysis by using TSAB-coated Fe3O4/SiO2 MNPs-based magnetic solid-phase extraction.

Table 3 Comparison of proposed method with analytical methods reported for the determination of ADR

Methods	Extraction	Retention time (min)	LOD/LOQ (ng/mL)	Recovery (%)	
				Plasma	Urine
HPLC-UV [11]	LLE	6.5	630/1700	101.3-104.2	/
HPLC-FLD [9]	SPE	5.0	6.0/11.04	/	97.4-109.1
HPLC-MS/MS [18]	SPE	4.7	3.6/7.2	69.0-71.0	/
This work	MSPE	3.9	5.05/16.82	76.5-94.0	77.9-96.0

4. Conclusions

In summary, a magnetic TSAB-coated Fe_3O_4/SiO_2 NPs coupled with HPLC-FLD was successfully applied for the efficient extraction and determination of ADR from human plasma and urine samples. The obtained TSAB-coated Fe_3O_4/SiO_2 MNPs adsorbent combined the advantages of large surface area of the magnetic nanoparticles and the strong affinity property of TSAB surfactant. It exhibit excellent extraction ability to ADR and could be easily and quickly separated with an external magnet. On this basis, a method of MSPE combined with HPLC-FLD has been developed for the analysis of ADR. The good recoveries, precisions and reuse property were obtained, which suggests that the TSAB-coated Fe_3O_4/SiO_2 NPs-based MSPE method has anticipated application prospect for the enrichment of drugs from complicated samples.

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