Talanta 106 (2013) 321–327

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Restricted access magnetic core-mesoporous shell microspheres with C8-modified interior pore-walls for the determination of diazepam in rat plasma by LC-MS

Xiaodan Liu [a,1], Yingjia Yu [a,1], Yan Li [a,*], Suli Ning [b], Tingting Liu [a], Fajie Li [a], Gengli Duan [a,*]

[a] Department of Pharmaceutical Analysis, School of Pharmacy, Fudan University, No. 826 Zhangheng Road, Shanghai 201203, China
[b] Department of Dermatology of Shanghai Eighth People's Hospital, No. 8, Caobao Road, Shanghai 200235, China

ARTICLE INFO

Article history:
Received 29 August 2012
Received in revised form
7 November 2012
Accepted 8 November 2012
Available online 16 November 2012

Keywords:
C8-modified interior pore-walls
Magnetic mesoporous microspheres
Diazepam
Rat plasma
Restricted access material

ABSTRACT

In this study, a novel enrichment technique based on magnetic core-mesoporous shell microspheres with C8-modified interior pore-walls (C8-Fe_3O_4@$mSiO_2$) was successfully developed for the determination of diazepam in rat plasma by LC-MS. Due to the unique properties of the synthesized C8-Fe_3O_4@$mSiO_2$ microspheres (C8-modified magnetic mesoporous microsphere), small drug molecules like diazepam can enter the mesopore channels and be efficiently absorbed through hydrophobic interaction by interior C8-groups (Octyl functional groups). Large molecules like proteins are excluded from the mesopore channels as a result of size exclusion effect, leading to direct extraction of drug molecules from protein-rich biosmaples such as plasma without any other pretreatment procedure. Moreover, diazepam adsorbed C8-Fe_3O_4@$mSiO_2$ microspheres could be simply and rapidly isolated through placing a magnet on the bottom of container, and then diazepam could be easily eluted from C8-Fe_3O_4@$mSiO_2$ microspheres for further LC-MS analysis. Extraction conditions such as amounts of C8-Fe_3O_4@$mSiO_2$ microspheres added, adsorption time, elution solvent and elution time were investigated. Method validations including linear range, the limit of detection, precision, and recovery were also studied. The results indicated that the proposed method based on C8-Fe_3O_4@$mSiO_2$ microspheres was simple and accurate for the analysis of diazepam in the rat plasma. And it will provide new ideas for analyzing plasma concentration and pharmacokinetics of similar drugs.

1. Introduction

The explosive request of rapid and reliable methods for screening analysis of drugs and poisons in complex biological samples (urine, serum) is of great importance in clinical and forensic toxicology laboratories. Sample preparation plays an important role in the analysis of complex samples before instrumental determination and often involves elaborate procedures [1–3]. The most common and wide spread sample preparation and preconcentration method is liquid–liquid extraction (LLE) [4]; however, it is time-consuming and environment unfriendly.

Solid-phase extraction (SPE) as an alternative offers a wide field with numerous applications [5–7]. One technical problem severely hampered the successful development of SPE based sample preparation method is that biological fluids such as plasma and serum contain perhaps tens of thousands of proteins and peptides. Most of these macromolecules will compete with small molecule analytes for binding sites on the particles, resulting in a reduction in binding capacity. At the elution step, the adsorbed proteins may elute together with small molecule analytes, causing interference with subsequent separation and detection [8].

To overcome this problem, one option is to use restricted access materials (RAM) as sorbents. The RAM sorbents typically consist of porous particles with dual functional groups located on inner and outer surfaces. The hydrophilic and biocompatible outer surface combined with a controlled pore size serves as a physical barrier to exclude marcomolecules whereas the hydrophobic or ion exchange inner surface provides binding sites for small molecules which can freely into and out of the porous particle. Due to these unique properties, RAM has great potential to isolate and enrich trace analytes in the presence of abundant serum proteins [9].

Compared with normal porous particles, mesoporous materials possess large surface area and uniform pore size distribution, and thus have high extraction efficiency and good anti-interference ability, making them ideal RAM for SPE [10–12]. Recently, mesoporous materials have been incorporated into magnetic

* Corresponding authors. Tel.: +86 21 51980057; fax: +86 21 51980053.
E-mail addresses: yanli@fudan.edu.cn (Y. Li), glduan@shmu.edu.cn (G. Duan).
[1] These two authors contributed equally to this work.

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.11.015

separation supports, and have been widely applied in various areas, such as enrichment of peptide [13,14] or DNA [15].

In our previous work, magnetic core-mesoporous shell microspheres with alkyl groups-modified interior pore walls (Cn–Fe_3O_4@$mSiO_2$) were synthesized through a one-pot sol–gel approach without the need for pre-coating of nonporous silica middle layer [16] and they were successfully applied for extraction and analysis of organic compounds in water samples [11]. The aim of this work was to demonstrate the feasibility of using this novel material as RAM for extraction and analysis of drugs in protein-rich biological samples. Diazepam, a benzodiazepine (BDZ) derivative drug, was selected as the model drug to investigate the extraction ability of the as-prepared materials. In addition to its anxiolytic action, diazepam has sedative and hypnotic effects. Abuse of diazepam can have serious consequences including drowsiness, reducing psychomotor activity and attention, as well as anterograde amnesia [17], even causing death when taken in overdose [18,19], so the determination and monitoring of benzodiazepines in biological fluids, such as plasma and urine is the object of great interest. Various analytical methods have been reported for the determination of diazepam in biological sample (plasma, urine), involving LC-MS [20], GC–MS [21], LC-MS/MS [22,23], and GC–MS/MS [24]. In this study, diazepam in rat plasma was identified by extraction using C8-Fe_3O_4@$mSiO_2$ microspheres followed with LC-MS analysis. The C8-Fe_3O_4@$mSiO_2$ microspheres possess large surface area, uniform mesopores, high magnetization and super paramagnetism, and numerous C8 groups anchored in the interior pore walls, making them ideal SPE absorbents for simple, fast and efficient extraction and enrichment of hydrophobic drugs in protein-rich biosamples. Compared with other reported works, there is no need for remove of proteins prior to extraction, thus offering a valuable alternative to simplify and speed up the sample preparation step.

2. Experimental

2.1. Instrumentation

Chromatographic analyses were carried out on an Agilent LC-MS series 1200 system (Agilent, USA) equipped with a G1312A Bin Pump, a G1379B vacuum degasser, a G1367B ALS autosampler, G1316A column oven, and a G1315D UV–vis diode-array detector (DAD) and a quadrupole mass spectrometer equipped with electro spray ionization (ESI) source. The separation steps were performed at 30 °C on a TSK gel C18 column (150 mm × 4.6 mm, id 5 μm; TOSOH, Japan) with a security guard cartridge C_{18} (10 mm × 4.6 mm id) Dikma (Tianjin, China). Elution was carried out on the initial mobile phases consisting of methanol (solvent A) and 0.025% TFA in H_2O (solvent B) in the first 12 min, initially ratio is 65:35 for A/B, at the flow rate of 1.0 mL min^{-1}. Gradient HPLC was used with a changing solvent ratio from 65:35 over 12 min to 95:5 for A/B, respectively, then, maintained for 3 min, finally returned to initial conditions. The injection volume was 20 μL and the detector wave length was set at 228 nm. Quantification of diazepam and I.S. was achieved by MS detection in the positive ion mode, with ESI as an interface, a drying gas flow of 12 L min^{-1}, a drying gas temperature of 300 °C, a nebulizer pressure of 35 psi, a capillary voltage of 3000 V, and a fragment of 70 V. Protonated molecular ions were observed at m/z 285 for diazepam and at m/z 316 for the I.S.

2.2. Reagents and materials

$FeCl_3 \cdot 6H_2O$, TEOS (Tetraethyl orthosilicate), ethylene glycol, ethanol, concentrated ammonia solution (28 wt%) and CTAB (Cetyltrimethyl ammonium bromide) are of analytical grade and purchased from Sinopharm (Shanghai, China). C8TES (n-octyltriethoxysilane, Purity > 95%) was purchased from Alfa Aesar

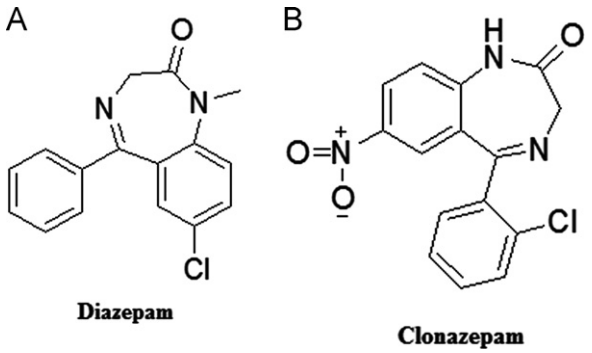
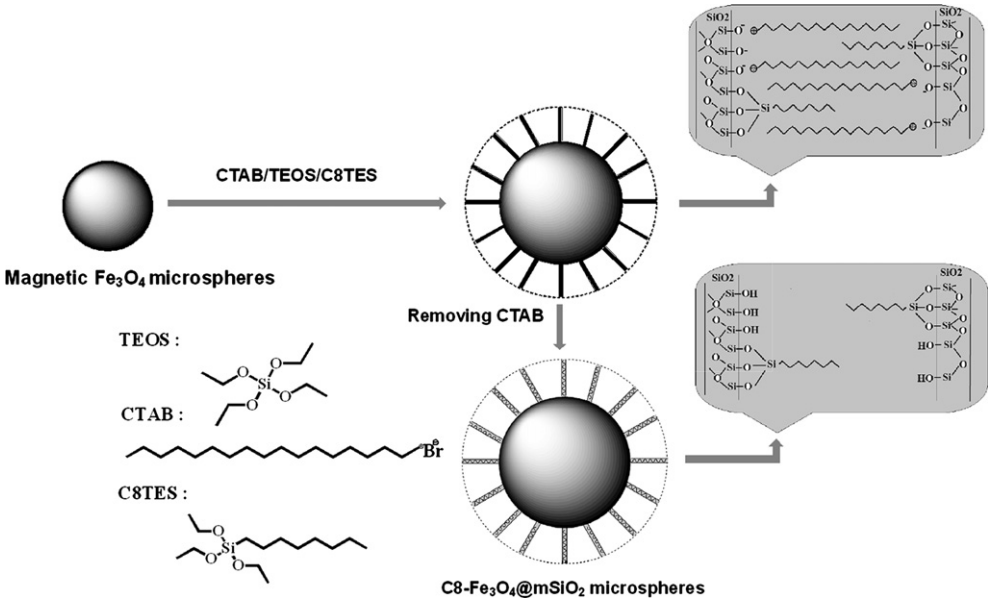


Fig. 1. Chemical structures of diazepam and clonazepam.



Scheme 1. The synthetic procedure of C8-Fe_3O_4@$mSiO_2$ microspheres .

(Tianjin, China). Acetonitrile, n-hexane, methanol, acetone, isopropanol, all HPLC grade, were obtained from TEDIA (Ohio, USA), Sinopharm (Shanghai, China), Merck (Darmstadt, Germany), Fisher Science (New Jersey, USA). The standard of diazepam (Fig. 1A) and clonazepam (internal standard, I.S., Fig. 1B) were acquired from China drug's biology assay Office. Water used for HPLC was purified with a Milli-Q plus system (Millipore, Bedford, MA, USA). All of other reagents were analytical grade and acquired from Sinopharm (Shanghai, China).

A stock solution (1 mg mL^{-1}) of diazepam was prepared in methanol. The calibration solutions of diazepam (0.1, 0.5, 1, 5, 10, 50 μg mL^{-1}) were prepared by dilution of the standard stock solution with methanol. Internal standard working solution was prepared at the concentration of 10 μg mL^{-1}. They were both stored at 4 °C. Drug-free plasma was collected from healthy rats and stored at −20 °C. Mobile phases were filter through a 0.45 μm cellulose acetate (water) and ultrasonically degassed for 15 min before use.

2.3. Preparation of C8-Fe_3O_4@$mSiO_2$ microspheres

Magnetic Fe_3O_4 microspheres with a mean diameter of 250 nm and the C8-Fe_3O_4@$mSiO_2$ microspheres were synthesized through a surfactant involved sol–gel process (Scheme 1) according to the reported method [16,25,26]. The prepared Fe_3O_4 and CTAB with a ratio of 50 mg/500 mg were dispersed in 50 mL of H_2O and ultrasonically treated for 30 min. The resultant solution was further diluted with 450 mL of NaOH aqueous solution (1.1 mM) in and further sonicated for 5 min to form a stable dispersion, and the resultant dispersion was then heated at 60 °C for 30 min. Later, 2.55 mL of TEOS/C8TES/ethanol (v/v/v: 10/1/4) solution was added by injection under mechanical stirring, and subsequently the dispersion was further heated at 60 °C for 12 h. The product was collected by magnetic separation and washed with ethanol repeatedly, and redispersed in acetone (50 mL) for refluxing at 50 °C to remove the CTAB. The refluxing process was repeated 5 times to achieve a complete removal of CTAB, and the resulting C8-Fe_3O_4@$mSiO_2$ microspheres were dried at 50 °C for 24 h in vacuum for the future use.

2.4. Extraction using C8-Fe_3O_4@$mSiO_2$ microspheres

The route for the extraction of diazepam was shown in Fig. 2. Extraction conditions like added amounts of C8-Fe_3O_4@$mSiO_2$ microspheres, extraction time, eluting solvents and elution time, were investigated. Drug-free plasma (180 μL) was centrifuged at 4000 rpm for 10 min and then added with 20 μL of diazepam (0.1, 0.5, 1, 5, 10, 50 μg mL^{-1}) and 20 μL of clonazepam (10 μg mL^{-1}). Then, certain amounts of suspension of C8-Fe_3O_4@$mSiO_2$ microspheres (10 mg mL^{-1}) were appended to the mixed liquid and continuously vibrated for certain minutes. Thereafter, the diazepam adsorbed C8-Fe_3O_4@$mSiO_2$ microspheres were isolated by placing a magnet in the bottom of EP tube. The captured diazepam/C8-Fe_3O_4@$mSiO_2$ microspheres were collected and rinsed with 200 μL of water twice, and water was decanted with the help of magnet. Subsequently, the conditions of eluting solvents including acetonitrile, n-hexane, methanol, acetone, isopropanol and elution time were also investigated. The captured diazepam was then eluted from the C8-Fe_3O_4@$mSiO_2$ microspheres by eluting solvents (40 μL) for certain minutes. Then 20 μL of sample solution eluted from microspheres was injected into LC-MS system for analysis.

2.5. Linear range, detection limit, precision, and recovery

Spiked plasma samples were prepared by adding 20 μL of standard working solutions of diazepam to 0.18 mL of drug-free plasma to a final concentration from 0.01 to 5 μg mL^{-1}. Then, 20 μL of I.S. solutions were appended into the above plasma. The calibration curve was obtained by plotting the peak area ratio between diazepam and I.S. using optimal C8-Fe_3O_4@$mSiO_2$ microspheres extracted conditions. Limit of detection (LOD) was determined by triplicate analyses of the lowest concentration in spiked

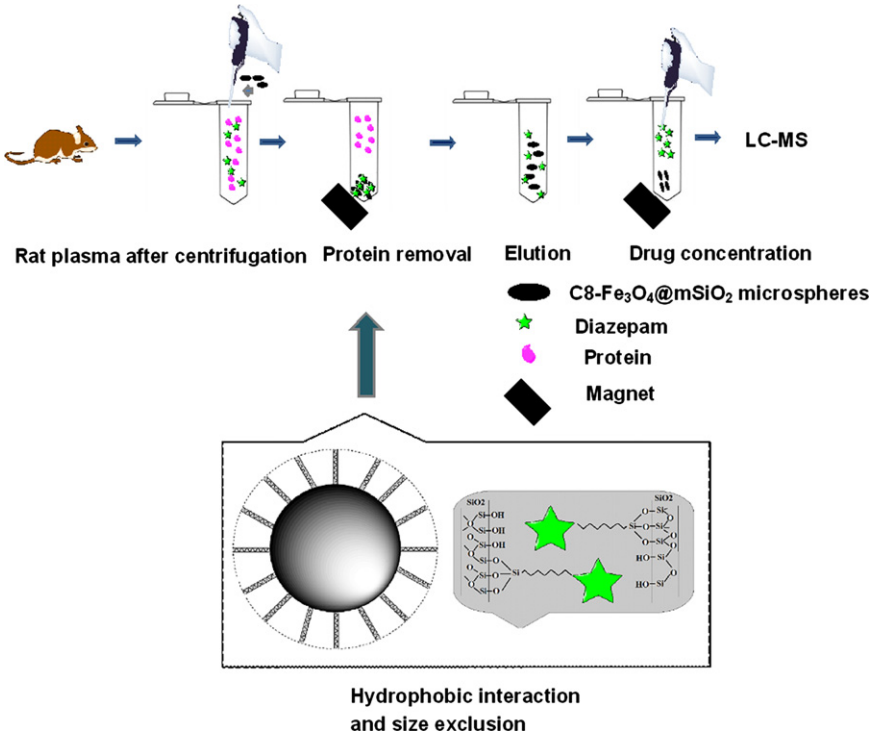


Fig. 2. Extraction and enrichment of diazepam from rat plasma using C8-Fe_3O_4@$mSiO_2$ microspheres.

rat plasma (0.003 μg mL^{-1}). LOD was calculated on the basis of extrapolation to a signal-to-noise ratio of 3. The intra- and inter-run precision and accuracy of the assay were determined by five replicate analyses of the diazepam concentration in spiked rat plasma (0.01, 0.1, 5 μg mL^{-1}) plus I.S. solution intra-day and on continuous 5 days. The obtained peak areas were used for the calculation of the relative standard deviations (RSDs). Recoveries were carried out by adding 20 μL diazepam calibration solutions (0.1, 1, 50 μg mL^{-1}) to the 0.18 mL rat plasma containing known content of diazepam. Triplicate measurements were performed.

2.6. Determination of diazepam in rat samples by the optimal method using LC-MS

Normal male SD rats were purchased from center of experimental animals, Fudan University (Shanghai, China). During the experiment, five rats were injected 0.8 mg kg^{-1} diazepam via the tail vein. Blood samples (0.35 mL) were collected from the jugular vein catheter at 0 (before drug administration), 5, 10, 15, 30, 60, 90, 120 min (n=5 at each time point). Blood was collected in EDTA containing syringes and stored at −20 °C until further analysis. The remaining procedure was the same as described in Section 2.4 under the optimized conditions.

3. Results and discussion

3.1. Characterization of C8-Fe_3O_4@mSiO_2 microspheres

By using the facile one-pot sol–gel approach, the C8-Fe_3O_4@mSiO_2 microspheres were synthesized through the simultaneous condensation of TEOS and n-octyltriethoxysilane (C8TES) in the presence of Fe_3O_4 microspheres as the seeds and CTAB as the template. Precise control of the pore size was achieved due to the addition of CTAB and silane agents. According to our previous work, the mesopore channels in the shell are perpendicular to the surface of the as-prepared microspheres, and the pore size is about 3.7 nm with narrow pore size distribution [16]. Theoretically, these mesopores on the surface of the microspheres can prevent irreversible adsorption of the proteins from the sample matrix due to size exclusion effect.

Modification of the inner walls of the pore channel with C8 groups was accomplished in the one-pot synthesis approach to extract small drug molecules from the samples through hydrophobic interaction. It is worth mentioning that compared to post-modification, the novel simultaneous-modification approach ensured that only interior surface of mesoporous microspheres was modified with the hydrophobic C8 groups, leading to an excellent dispersibility and biocompatibility of the as-prepared microspheres in aqueous solution [16], which is beneficial for their application in analysis of biosamples. Also, according to our previous work [16], the as-prepared microspheres exhibited a superparamagnetic behavior, that is, no remanence was detected at room temperature, which ensured a fast magnetic separation process with a magnet. The magnetic C8-Fe_3O_4@mSiO_2 microspheres in their homogenous dispersion (Fig. 3A) showed fast movement to the applied magnetic field (Fig. 3B) and redispersed quickly with a slight shake once the magnetic field was removed. It demonstrated that the magnetic C8-Fe_3O_4@mSiO_2 microspheres possessed excellent magnetic responsivity and redispersibility, which is an advantage to its further applications.

In conclusion, the obtained C8-Fe_3O_4@mSiO_2 microspheres possess large surface area, uniform mesopores, high magnetization and superparamagnetism, and numerous C8 groups anchored in the interior pore walls, making them ideal SPE absorbents for simple, fast and efficient extraction and enrichment of hydrophobic drugs in protein-rich biosamples.

Fig. 3. Photos of: (A) aqueous dispersion of the magnetic C8-Fe_3O_4@mSiO_2 microspheres (B) after separation with a magnet for a few seconds.

3.2. Optimization of C8-Fe_3O_4@mSiO_2 microspheres extraction conditions

To investigate the enrichment efficiency of the C8-Fe_3O_4@mSiO_2 microspheres, extraction conditions were optimized in the following work. To acquire the maximum extraction efficiency of the target analyte, the amounts of C8-Fe_3O_4@mSiO_2 microspheres required were optimized first ranged from 10 μL to 50 μL (10 mg mL^{-1}). According to the results shown in Fig. 4A, 20 μL of C8-Fe_3O_4@mSiO_2 microspheres was enough to obtain satisfactory results because of the large surface and high adsorption efficiency of the mesopores on the shell, so the optimal amount of C8-Fe_3O_4@mSiO_2 microspheres was 0.2 mg.

For the sake of enhancing extraction efficiency, the selection of extraction time is important after the sorbents are dispersed into the solution. In the study, different extraction time, *i.e*, 1 min, 3 min, 5 min, 8 min and 10 min were investigated. As shown in Fig. 4B, the extraction efficiency of diazepam was enhanced until the time increased to 3 min, whereas after 3 min, as the time prolonged, no remarkable increase of extraction efficiency was observed. It may be due to that the large surface area of the C8-Fe_3O_4@mSiO_2 microspheres and numerous C8 groups anchored in the pore walls resulted in a strong hydrophobic interaction between diazepam and C8-Fe_3O_4@mSiO_2 microspheres, making the distribution equilibrium easy to be achieved in a short time. According to the results, 3 min was selected as the extraction time in the following experiments.

The selection of an appropriate elution solvent is quite important in a SPE procedure. In this study, five solvents including acetonitrile, ethyl acetate, methanol, acetone and isopropanol were investigated for improving the extraction efficiency of diazepam. As depicted in Fig. 4C, the extraction efficiency reached the maximum when using methanol as the eluting solvent. It could be explained as that methanol has the similar polarity

with diazepam, thus it can elute diazepam more easily than the other four solvents. Therefore methanol was selected as the eluting solvent in the following work.

The volume of elution solvent and elution time are also important factors to obtain reliable and reproducible analytical results. To investigate the volume of elution solvent, 40, 80 and 120 μL methanol were selected, and the results indicated that the maximum extraction efficiency was obtained when the elution volume reached to 40 μL. Also, five different elution time (1 min, 3 min, 5 min, 8 min and 12 min) were investigated and it seemed that 5 min was enough for elution of diazepam from the C8-Fe_3O_4@$mSiO_2$ microspheres as be seen in Fig. 4D.

3.3. Validation of the method

The method validations such as linearity, repeatability, and limit of detection were studied. The calibration curve exhibited good linearity with the corresponding value (R^2) which were more than 0.9993 in the range from 0.01 to 5 μg mL^{-1} ($y=2.5039x-0.011$) ($n=3$), where y is the peak area ratio of diazepam and clonazepam (the internal standard), x is the concentration of diazepam in rat plasma.

Precision of the method varied from 2.4% to 7.9%. The LOD values was 0.003 μg mL^{-1} calculated on the basis of $S/N=3$, which had enough sensitivity for the analysis of diazepam in

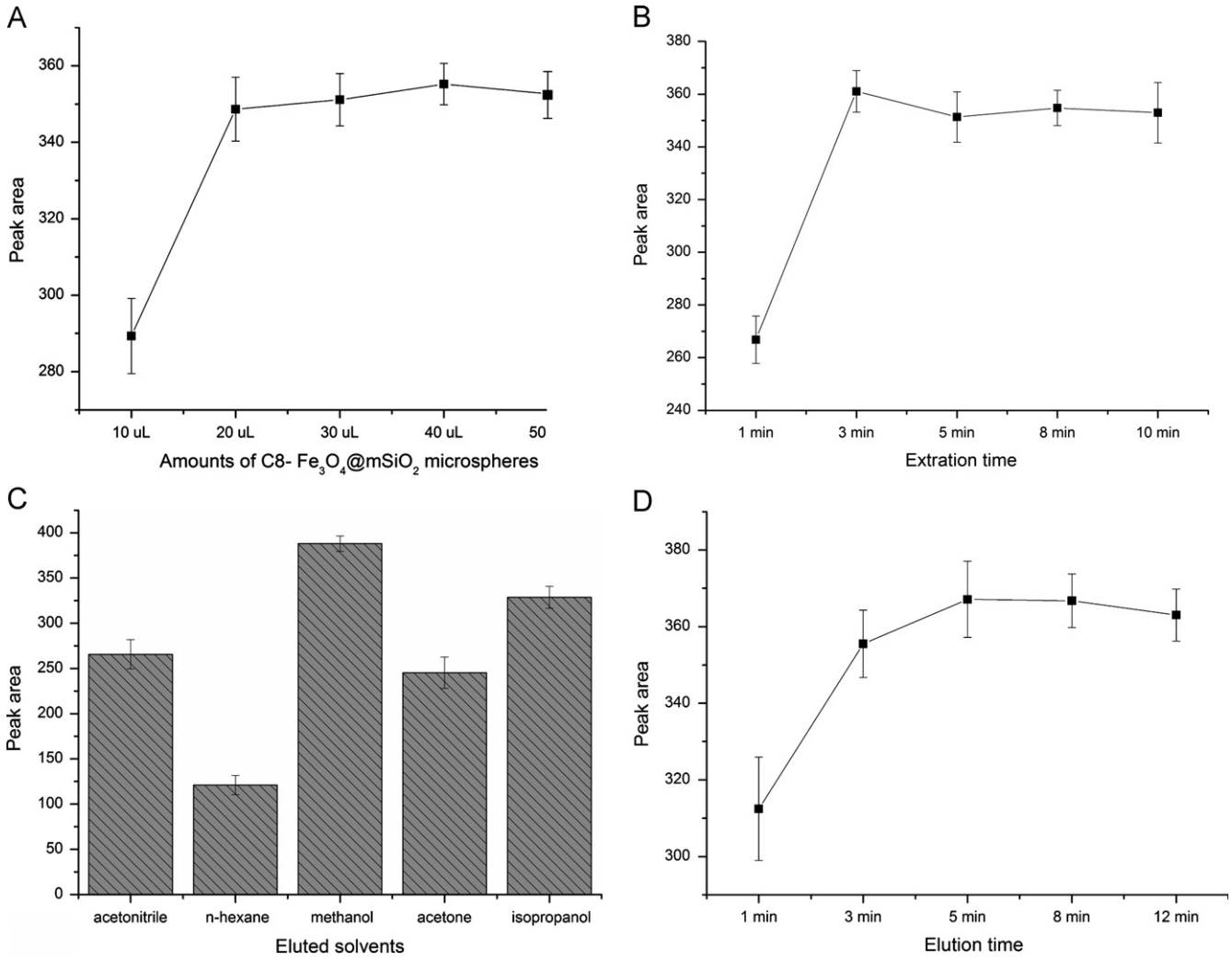


Fig. 4. Parameters influence extraction efficiency. (A) Amounts of C8-Fe_3O_4@$mSiO_2$ microspheres; (B) extraction time (1, 3, 5, 8 and 10 min); (C) eluting solvents (acetonitrile, n-hexane, methanol, acetone and isopropanol) and (D) elution time (1, 3, 5, 8 and 12 min) ($n=3$).

Table 1
The precision, recovery, RSD acquired in the analysis of diazepam in rat plasma.

Concentration un-spiked (ng/ml)	Concentration spiked (ng/ml)	Concentration measured (ng/ml)	Recovery (%)	RSD (%, $n=5$)	
				Intra-assay precision	Inter-assay precision
–	**10 (Low)**	**9.28**	**92.8**	**7.8**	**7.9**
–	**100 (Medium)**	**95.2**	**95.2**	**6.6**	**7.2**
–	**5000 (High)**	**4715**	**94.3**	**2.4**	**5.1**

'-' indicates that no diazepam was determined in the blank sample.

the rat plasma sample. The precision was determined by the percentage coefficient variation of inter- and intra-day variations at three different concentrations. The precision was ranged from 2.4% to 7.8% for inter-day measurement, and for intra-day variation was in the range of 5.1%–7.9%. Recoveries were obtained from 92.8% to 95.2% by comparing the calculated content with the real values of the added diazepam (Table 1). The results showed that the proposed method was reliable for the analysis of diazepam in the rat plasma samples. Compared to other published works (Table 2), the LOD of our work equals to those methods with LC-MS or GC–MS but higher than those with LC-MS/MS or GC–MS/MS which may be due to the high sensitivity

Table 2
The comparison of different methods in the analysis of diazepam in biological samples.

Method	Biological source	Sample preparation procedure	Solvent used	Sample amount (μL)	LOD (ng mL^{-1})	Linearity (ng mL^{-1})	Ref.
LC-MS	Rat plasma	SPE with C8-Fe_3O_4@$mSiO_2$ microspheres	Elution:Methanol (40 μL)	180	3	10–5000	Our work
LC-MS	Rat serum	alkaline extraction	Extraction:Diethylether	-	4.37	10–2000	[20]
GC–MS	Human plasma	SPME with polydimethylsiloxane fiber	-	250	10	10–1000	[21]
LC-MS/MS	Human plasma	Protein precipitation	Acetonitrile (150 μL)	100	1	1–500	[22]
LC-MS/MS	Human urine	Protein precipitation	10 mM ammonium acetate (1000 μL)	1000	0.1	0.5–500	[23]
GC–MS/MS	Human urine	LLE with TOXI-TUBES® A extraction tubes	Extraction:Methylene Chloride, heptane, 1,2-dichloroethane, isopropyl alcohol (3300 μL); Wash: Methanol (200 μL)	1000	0.15	1–500	[24]

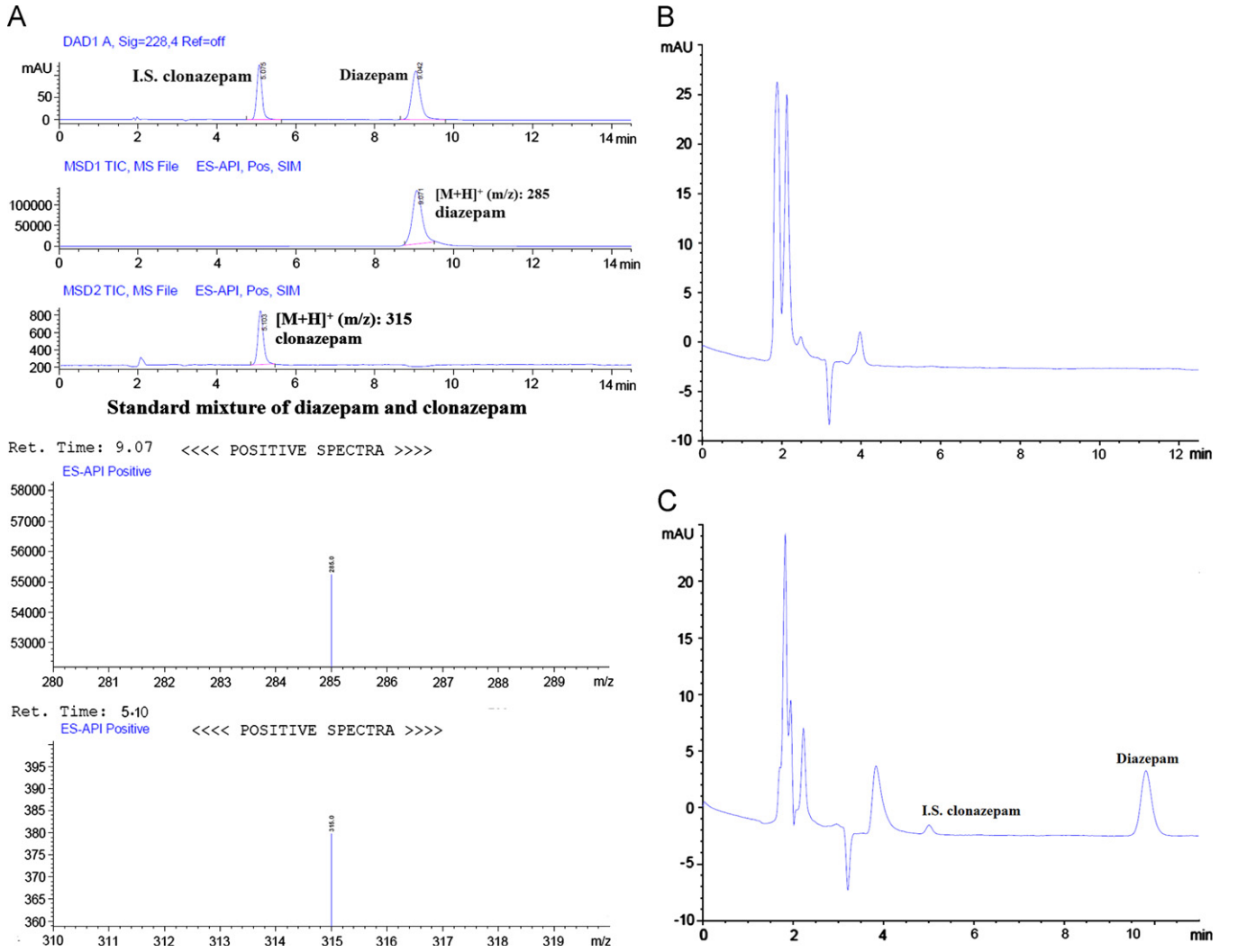


Fig. 5. LC-MS Chromatograms: (A) standard of the diazepam and clonazepam mixture, (B) blank rat plasma, and (C) rat plasma after intravenous administration of a single 0.8 mg kg^{-1} dose of diazepam.

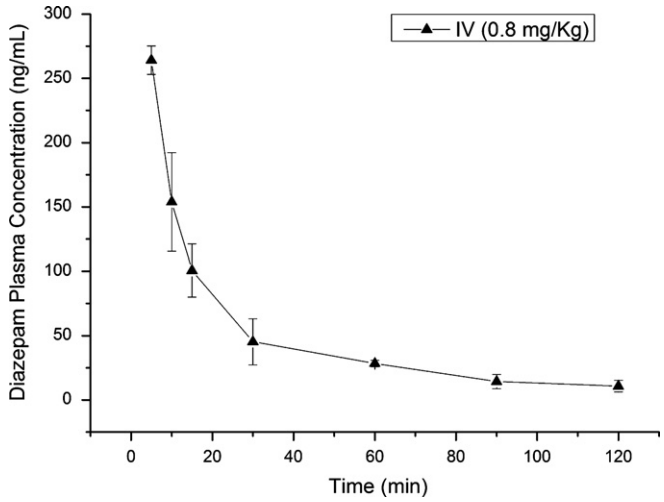


Fig. 6. Mean plasma concentration–time profiles of diazepam after tail intravenous injection administration of 0.8 mg Kg^{-1} diazepam to five healthy SD rats. The symbols and bars represent the mean and SD values, respectively ($n=5$ for each point).

of tandem MS. However, less toxic extraction solvent was consumed in our work, and the sample preparation procedure was simplified with no need for protein precipitation prior to extraction.

3.4. Application to the determination of diazepam in rat plasma samples

The optimal method established above was applied to the determination of diazepam in rat plasma samples from five male SD rats after given 0.8 mg Kg^{-1} diazepam via the jugular vein catheter. Fig. 5A shows that retention times of diazepam and clonazepam (I.S.) was 5.07 min and 9.04 min, respectively. The chromatogram of blank rat plasma after the proposed pretreatment was represented in Fig. 5B. The representative chromatogram of rat plasma after the injection of 0.8 mg Kg^{-1} of diazepam is depicted in Fig. 5C. No interferential peaks and background noise was observed around the retention time of diazepam and clonazepam (I.S.). Using internal standard method, diazepam concentrations in rat plasma samples collected at different times after administration are calculated and presented in Fig. 6. The concentration of diazepam reached its maximum peak value 264 ng mL^{-1} with an elimination half-life of 15.2 min, and the results are in accordance with the reported references [27–29].

4. Conclusion

In this paper, magnetic core-mesoporous shell microspheres with C8-modified interior pore-walls were successfully prepared through a one-pot synthesis approach and applied for pre-column extraction and enrichment of diazepam in rat plasma. The unique properties of the C8-Fe_3O_4@mSiO_2 microspheres enables the capturing of small drug molecules by hydrophobic interactions with the C8 groups modified on the interior surface of the mesoporous channels while screening macromolecules due to size exclusion of the orderly mesopores with limited pore sizes. Moreover, the excellent magnetic responsibility of the microspheres made the sample preparation procedure easy and fast. By simply adding different silane agents during the one-pot synthesis approach, other functional groups like phenyl or ion exchange groups could also be bonded on the internal surface of the magnetic microspheres, enabling the extraction of drugs over a wide range of properties. Selectivity for the extraction of target compounds can be further enhanced by incorporating molecular recognition centers in the pores, such as molecular imprinted polymers. Given its simplicity, versatility and compatability with analytical systems (LC or LC-MS), the magnetic core-mesoporous shell microspheres based sample preparation procedure developed in this study may have great potential in routine determination of pharmaceutical compounds in biological fluids.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Project no. 21105016), the Research Fund for the Doctoral Program of Higher Education of China (Project no. 20100071120053), and the Shanghai Municipal Natural Science Foundation (no. 11ZR1403200).

References

[1] D. Vuckovic., Anal. Bioanal. Chem. 403 (2012) 1523.
[2] L. Zhao, H.Q. Qin, R.A. Wu, H.F. Zou, J. Chromatogr. A 1228 (2012) 193.
[3] L. Ramos, J. Chromatogr. A 1221 (2012) 84.
[4] B.K. Bastos, U.T. de Souza, F.E. Figueiredo, Anal. Lett. 44 (2011) 2922.
[5] M. Li Kong, P. Rivory Laurent, J.Clarke Stephen, Curr. Pharm. Anal. 2 (2006) 95.
[6] F.G. Tamayo, E. Turiel, A. Martín-Esteban, J. Chromatogr. A 1152 (2007) 32.
[7] I. Liška, J. Chromatogr. A 885 (2000) 3.
[8] Y. Wang, Y.X. Wang, L. Chen, Q.H. Wan, J. Magn. Magn. Mater. 324 (2012) 410.
[9] S. Souverain, S. Rudaz, J.L. Veuthey, J. Chromatogr. A 801 (2004) 141.
[10] H.P. Yiu, P.A. Wright, J. Mater. Chem. 15 (2005) 3690.
[11] Z.B. Li, D.N. Huang, C.F. Fu, B.W. Wei, W.J. Yu, C.H. Deng, X.M. Zhang, J. Chromatogr. A 1218 (2011) 6232.
[12] X.L. Zhang, H.Y. Niu, W.H. Li, Y.L. Shi, Y.Q. Cai, Chem. Commun. 47 (2011) 4454.
[13] X.S. Li, J.H. Wu, Y. Zhao, W.P. Zhang, Q. Gao, L. Guo, B.F. Yuan, Y.Q. Feng, J. Chromatogr. A. 1218 (2011) 3845.
[14] Q.Q. Gai, F. Qu, T. Zhang, Y.K. Zhang, Talanta 85 (2011) 304.
[15] Z.M. Saiyed, M. Parasramka, S.D. Telang, C.N. Ramchand, Anal. Biochem. 363 (2007) 288.
[16] S.S. Liu, Y. Li, C.H. Deng, Y. Mao, X.M. Zhang, P.Y. Yang, Proteomics 11 (2011) 4503.
[17] Z. Munõz-Torres, J.L. Armonya, D. Trejo-Martínez, R. Conde, M. Corsi-Cabrer, Neurosci. Res. 70 (2011) 260.
[18] J.L. Richardson, S. Stephens, D. Jones, Clin. Toxicol. 48 (2010) 281.
[19] J. Rupasinghe, M. Jasinarachchi, J. Clin. Neurosci. 18 (2011) 710.
[20] S. Dordevic, V. Kilibarda, Vojnosanit. Pregl. 64 (2007) 659.
[21] M.H.D. Oliveira, M.E.C. Queiroz, D. Carvalho, S.M. Silva, F.M. Lancas, Chromatographia 62 (2005) 215.
[22] C. Abbara, I. Bardot, A. Cailleux, G. Lallement, A.L. Bouil, A. Turcant, P. Clair, B. Diquet, J. Chromatogr. B 874 (2008) 42.
[23] H. Umezawa, X.P. Lee, Y. Arima, C. Hasegawa, A. Marumo, T. Kumazawa, K. Sato, Rapid Commun. Mass Spectrom 22 (2008) 2333.
[24] S. Kinani, S. Bouchonnet, N. Milan, I. Ricordel, J. Chromatogr. A 1141 (2007) 131.
[25] H. Deng, X.L. Li, Q. Peng, X. Wang, J.P. Chen, Y.D. Li, Angew. Chem. Int. Ed. 44 (2005) 2782.
[26] K.L. Ding, B.J. Hu, Y. Xie, G.M. An, R.T. Tao, H.Y. Zhang, Z.M. Liu, J. Mater. Chem. 19 (2009) 3725.
[27] P. Kaur, K. Kim, Int. J. Pharm. 364 (2008) 27.
[28] K. Lindhardt, R.D. Ólafsson, S. Gizurarson, E. Bechgaard, Int. J. Pharm. 231 (2002) 67.
[29] F.M. Musteata, I.D. Lannoy, B. Gien, J. Pawliszyn, J. Pharm. Biomed. Anal. 47 (2008) 907.