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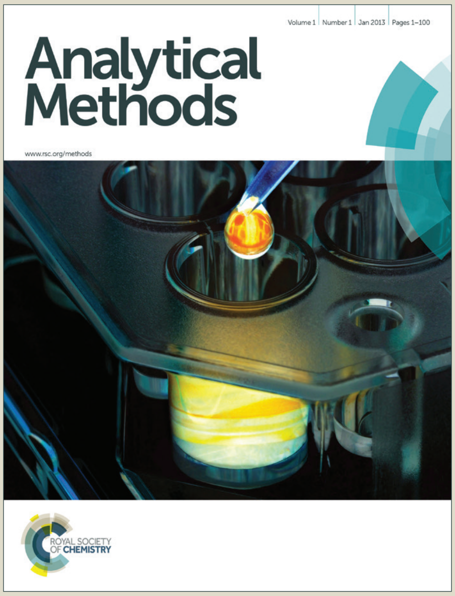

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Preparation of fluorine functionalized magnetic nanoparticles for fast extraction and analysis of perfluorinated compounds from traditional Chinese medicine samples

Zhihong Yan[a,b], Genhua Zhu[b],Ying Cai[a], Jinbin Yuan[*b], Shouzhuo Yao[*a]

This paper demonstrates the preparation and application of 4-(trifluoromethyl)-benzoyl chloride functionalized magnetic nanoparticles (Fe_3O_4@SiO_2@TFBC MNPs) as adsorbent for magnetic solid-phase extraction (MSPE) of perfluorinated compounds (PFCs) in 3 typical traditional Chinese medicine samples (TCMs). With the synthesized MNPs as the adsorbents, a novel magnetic solid-phase extraction (MSPE) method was developed, by which a permanent magnet was added into container to collect magnetic adsorbents. The new method can saved time of dumping sample solution and dumping eluent, which greatly accelerate the extraction processes. The main influencing parameters on MSPE, including adsorbent amount, desorption solvent, extraction time, sample volume and pH value of solution samples were investigated in detail. Ultra-high performance liquid chromatography coupled to tandem triple quadrupole mass spectrometry (UHPLC-MS/MS) was used for the analysis of the extracted solution. The proposed MSPE showed good extraction performances in terms of efficiency,

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[a] State Key Laboratory of Chemo/Biosensing & Chemometrics, College of Chemistry & Chemical Engineering, Hunan University, Changsha 410082, China. E-mail: szyao@hnu.edu.cn (S. Yao); Tel.: +86-731-88821968

[b] Key Laboratory of Modern Preparation of TCM, Ministry of Education, Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, China. E-mail: kings2008@163.com (J. Yuan), Tel.: +86-791-87118658

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adsorbent amount and extraction time. The linear ranges of six PFCs were 0.2-20 ng L^{-1} with the limits of detection (S/N = 3) ranging from 0.010 to 0.025 ng L^{-1}, and relative standard deviation in the range of 2.1-5.0%. The recoveries were in the range of 83.6-107.4%, and contamination at low levels was detected for some PFCs in the TCMs. These results indicated that the whole analytical method based on $Fe_3O_4@SiO_2@TFBC$ adsorbents is a simple, fast, effective and sensitive.

Keywords Magnetic nanoparticles; Perfluorinated compounds; traditional Chinese medicine samples; Ultra-high performance liquid chromatography coupled to tandem triple quadrupole mass spectrometry

1 Introduction

Perfluorinated compounds (PFCs) are a class of anthropogenic fluorinated organic substances. Owning to unique and useful chemical properties including surface activity, thermal and acid resistance, and repellency of water and oil, they have been used in the treatments of textiles, paper, food containers, leather, carpets, upholstery, firefighting foams, and semiconductor.[1] During the treatment, these compounds may enter the environment. Their long persistence in the natural and built environment, bioaccumulation potential, and prevalence in wildlife and human populations have raised serious environmental and human health concerns.[2] Numerous monitoring studies revealed that PFCs have been detected in nearly all environmental media and biota,[3] such as Air,[4,5] water,[6-9] soil,[10] sediment,[11] sewage sludge,[12,13] biological,[14,15] food[16,17] and human samples.[18,19] Toxicological studies on animals have indicated that human exposure to PFCs resulting a range of adverse outcomes including hepatic,

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immunotoxic, reproductive, neurobehavioral, developmental, hormonal, and other effects.[20,21] Since PFCs have been found in nearly all environmental media and biota, traditional Chinese medicine (TCM) as natural products growing in soil and waters may also be contaminated. Up to now, the monitoring study of PFCs in TCM was rarely available.

The most frequently used instrument for the measurement of PFCs is the high performance liquid chromatography coupled with a tandem mass spectrometry operated in a negative electrospray mode (HPLC/(–)ESI-MS/MS) or high resolution time-of-flight (TOF)-MS. In order to improve the detection sensitivity in environment samples, an enrichment procedure is often indispensable prior to chromatography analysis. Hitherto, various pretreatment methods including liquid-liquid extraction (LLE),[22] liquid-liquid microextraction (LLME),[23] and solid-phase extraction (SPE)[24,25] have been applied to trace analysis, in which SPE has become more popular due to its high recovery, short extraction time, high enrichment factor, low consumption of organic solvents and ease of operation.

The adsorbent material is the core of SPE which determines the selectivity and sensitivity of the method. There has been an increasing interest in magnetic nanoparticle adsorbents for the preconcentration of target analytes from environmental or biological samples. Owing to its high extraction efficiency and rapid extraction kinetics, it has been successfully applied to extract different analytes.[26-36] Magnetic nanoparticles (MNPs) with functional groups as adsorbents have been the subject of intense research. The unique superparamagnetic property enables the

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convenient separation of these adsorbents from the mixture with an external magnet after adsorption, which avoids the difficulties of solid-liquid separation or the high back-pressure when passing through the solid-phase extraction (SPE) column. For extraction of PFCs, Zhang *et al.* synthesized chitosan-coated octadecyl-functionalized MNPs (Fe_3O_4-C_{18}-chitosan) and used them as an adsorbent to extract trace PFCs from environmental water samples.[37] Liu *et al.* synthesized magnetic nanoparticle-decorated graphene (magnetic-MG) and applied for SPE of perfluoroalkyl and polyfluoroalkyl substances.[38] Yang *et al.* synthesized decyl-perfluorinated functionalized magnetic mesoporous microspheres (F_{17}-Fe_3O_4@$mSiO_2$).[39] Yan *et al* prepared and characterized 3-fluorobenzoyl chloride functionalized magnetic nanoparticles (Fe_3O_4@SiO_2@FBC) for extraction and determination of PFCs from water samples.[40] Very recently, fluorocarbon-bonded MNPS were reported for the analysis of PFCs in human serum.[41] To our knowledge, almost all studies employing magnetic nanoparticle adsorbents use the following processes.[26-36] Firstly, magnetic adsorbents were dispersed in the sample solution and analytes were adsorbed onto them, then a piece of permanent magnet was attached to the outside bottom of the vial to separate the adsorbents from the solution. After standing for several minutes, the solution was decanted through magnetic separation. Secondly, the eluent was added in and analytes were desorbed from MNPs, then the eluent was separated from adsorbents by the permanent magnet. Finally, the eluate was filtered and injected to the instrument for analysis. The procedure contains a standing and two dumping processes, which was time-consuming, and what's more,

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dumping process may bring error.

In this work, we synthesized a fluorous functionalized magnetic adsorbent, 4-(trifluoromethyl)-benzoylchlorid functionalized magnetic silica nanoparticles (Fe_3O_4@SiO_2@TFBC). The MNPs were well characterized with transmission electron microscopy (TEM), vibrating sample magnetometer (VSM), X-ray diffraction (XRD) and Fourier transform infrared spectrometry (FTIR). Secondly, we introduced a novel MSPE method, in which the magnet was added into container to collect magnetic adsorbents. The proposed MSPE method saved time of dumping sample solution and dumping eluent, which greatly accelerates the MSPE procedure. Finally, ultrahigh-performance liquid chromatography (UHPLC-MS/MS) was introduced for the fast and sensitive detection of the analytes. To validate the efficiency, reliability and robustness, six PFCs namely perfluoroheptanoic acid (PFHpA), perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoA), perfluorotetradecanoic acid (PFTeDA), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) were selected as model compounds. To investigate the contamination possibility of TCM, three typical TCM samples were selected as screening objectives including *Pheretima vulgaris* Chen*, Hirudo nipponica* whitman and *Syngnathus acus* Linnaeus growing in soil, fresh water and seawater, respectively.

2 Experimental

2.1 Chemicals and materials

Ferric chloride hexahydrate ($FeCl_3$•$6H_2O$, 99%), ferrous sulfate heptahydrate ($FeSO_4$•$7H_2O$, 99%), ammonia (26%), hydrazine hydrate (99%), isopropanol (99%),

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triethylamine (99%), acetic acid (99%), ammonium acetate (99%) and toluene (99%) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). HPLC grade methanol and acetonitrile (99%) were from Merck (Darmstadt, Germany). Triethylamine (TEA, 99.5%), tetraethoxysilane (TEOS, 99%), (3-aminopropyl) triethoxysilane (APTES) and 4-(trifluoromethyl)-benzoyl chloride (TFBC) were obtained from Adamas-beta Co. Ltd. (Shanghai, China). Chemicals including n-hexane, dichloromethane, ethyl acetate, acetone, toluene and ethanol were analytical grades and purchased from Merk (Darmstadt, Germany).

Six PFCs including PFHpA, PFDA, PFDoA, PFTeDA, PFOA and PFOS were obtained from Alfa Aesar (Ward Hill, Massachusetts, USA). Stock solutions containing 1.0 mg mL^{-1} of each analyte were prepared in methanol and stored at 4 ºC. Mixed stock solutions containing six analytes were prepared with methanol at a concentration of 0.1 mg mL $^{-1}$ for each, and stored at 4 ºC. The working solutions were diluted with methanol at known concentrations and stored at 4 ºC.

2.2 Sample collection

Traditional Chinese medicine (TCM) samples were obtained from Huangqingren Warehouse Pharmacy (Nanchang city, Jiangxi province, China), including *Pheretima vulgaris* Chen*, Hirudo nipponica* whitman and *Syngnathus acus* Linnaeus. All TCMs were pulverized to fine powder in a pulverizer and sieved to 100-200 µm particles. A sample of TCM was used for method optimization and 100 g of the ground sample was spiked with 1.0 mL of mixed working solution containing all PFCs (50 ng mL^{-1}). The mixture was stirred mechanically and allowed to dry at room temperature

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thoroughly for 24 h, and then used to survey extraction variables under different conditions.

2.3 Instrumental analysis

The size and morphological characterization of the particles were observed by transmission electron microscopy (TEM, JEM-2100F, JEOL Co., Tokyo, Japan). Fourier transform infrared spectra (FTIR) were recorded on Vertex 70 (Bruker Optics, Ettlingen, Germany). PFCs were extracted with the assistantce of an ultrasonicator (KQ-600KDE, Kunshan, China) at a frequency of 45 Hz at room temperature. The magnetic property was investigated using a vibrating sample magnetometer (VSM, Model 7410, Lake Shore Cryotronics, Ohio, USA). The X-ray diffraction (XRD) pattern was collected by a D/max2550 VB+18KW (Rigak International Corporation, Tokyo, Japan).

Liquid chromatography-tandem quadrupole mass spectrometry(LC–MS/MS) analyses were performed on a UHPLC system equipped with a DGU-20A5R degasser, a CTO-30A column oven, a LC-30AD pump, a SIL-30AC autosampler (Shimadzu Corporation, Tokyo, Japan) and an AB SCIEX TRIPLE QUADTM 5500 mass spectrometer (Applied Biosystems, Foster City, CA, USA). The UPLC–MS/MS system was controlled, and data were analyzed on a computer equipped with AB SciexTM Analyst 1.6 (Applied Biosystems, Foster City, CA, USA).

2.4 Synthesis of Fe_3O_4@SiO_2@TFBC

First, APTES coated magnetic nanoparticles (MNPs) were prepared according to our previous method.[40] Briefly, bare Fe_3O_4 nanoparticles were prepared by

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coprecipitation method. Then, $Fe_3O_4@SiO_2$ nanoparticles were prepared by dispersing Fe_3O_4 in the mixture of ammoniua (10 mL) and TEOS (8 mL), stirred for 12 h at 45 ºC. Next, $Fe_3O_4@SiO_2$ nanoparticles (3 g) were suspended in a mixture of toluene (60 mL), triethylamine (1 mL) and APTES (6 mL) under argon atmosphere, mechanically stirred and refluxed at 110 ºC for 24 h. The reaction was then stopped and the APTES coated magnetic particles were cooled to room temperature, washed with toluene, acetone and ethanol respectively, dried under vacuum at 60 ºC.

Finally, $Fe_3O_4@SiO_2@TFBC$ magnetic nanoparticles were prepared as follows (Fig.1). Toluene (40 mL) and triethylamine (1 mL) were added to APTES coated magnetic particles (3 g) under argon atmosphere, after stirred for 30 min in ice-bath, TFBC (2.5 mL) was added. The mixture was refluxed for 4 h at room temperature and then for 12 h at 110 ºC. After cooled to room temperature, the prepared $Fe_3O_4@SiO_2@TFBC$ magnetic particles were washed with water/ethanol (1/1, v/v), and dried under vacuum at 60 ºC for 12 h.

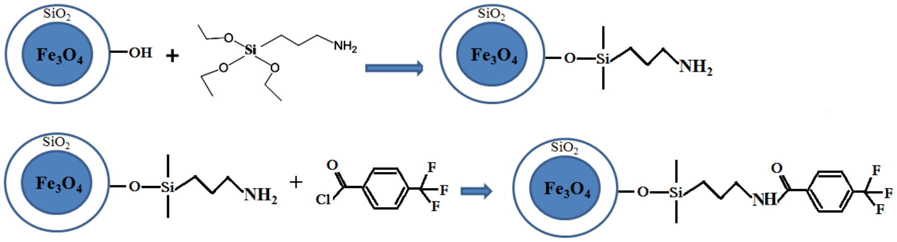

Fig.1. Scheme for the preparation of the $Fe_3O_4@SiO_2@TFBC$

2.5 Sample treatment and MSPE procedure

The powdered TCMs (1.0 g) spiked with 0.5 ng g^{-1} PFCs were dispersed in acetonitrile (10 mL), and the mixture was sonicated for 3 min. Afterwards, the solution was centrifuged for 5 min at 10,000 rpm. The final solution was filtered

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through a 0.22 μm nylon membrane syringe filter, and evaporated to dryness under a gentle stream of nitrogen at room temperature. The dried residues were reconstituted with 100 mL of pure water and subjected to MSPE procedure.

The novel magnetic solid-phase extraction procedure was conducted as follows (Fig.2): Firstly, 100 mL of aqueous sample spiked with PFCs (Before extraction, the samples were adjusted to pH 5 with 20 mM acetic acid solution) was added in a 250 mL vial, then 10 mg of Fe_3O_4@SiO_2@TFBC adsorbent and a magnet were placed in. The mixture was stirred for 3 min to disperse the adsorbent uniformly and facilitate the adsorption of analytes. When the stirring process was over, the dispersed MNPs carrying with PFCs were rapidly retrieved by the magnetic field of the magnet. Finally, the magnet was taken out and placed it into a sample tube with a plastic thumb forceps, and 1.0 mL of acetonitrile was added to desorb the PFCs. After the elution, the supernatant was transfered to an autosamper vial, and 5 μL was used for UHPLC-MS/MS analysis.

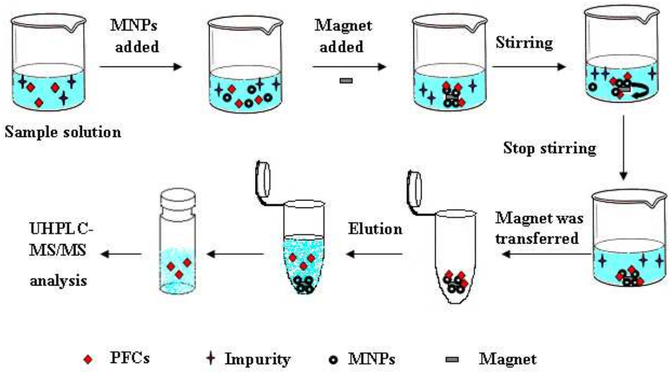

Fig.2 The novel magnetic solid-phase extraction procedure

2.6 UHPLC-MS/MS condition

The chromatographic analysis was performed using a Phenomenex C18 column

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(4 µm particle diameter, 2.0 mm i.d. × 50 mm length). Column temperature was set at 30 ºC. The mobile phase used for the chromatographic separation consisted of aqueous ammonium acetate 10 mM (A) and acetonitrile (B).

Table 1 MS/MS parameters.

Compoud	Precursor (m/z)	Product ion(m/z)	Declustering Potential(v)	Collision Energy(v)
PFDoA	613.0	568.9	−51.0	−16.0
	613.0	319.0	−51.0	−27.0
PFHpA	362.9	319.0	−55.0	−13.0
	362.9	119.0	−55.0	−26.0
PFDA	512.8	468.9	−81.0	−16.0
	512.8	268.8	−81.0	−23.0
PFTeDA	712.9	669.0	−60.0	−22.0
	712.9	168.8	−60.0	−36.0
PFOA	413.0	369.0	−85.0	−14.0
	413.0	168.9	−85.0	−24.0
PFOS	499.0	98.9	−140.0	−98.0
	499.0	79.8	−140.0	−97.0

The percentage of acetonitrile varied during the chromatographic run to give the following values at the specified times: 0-0.01 min, 40% B; 0.01-0.6min, linear gradient to 50% B; 0.6-0.8 min, linear gradient to 63% B; 0.8-1.7 min, linear gradient to 90% B; 1.7-2.0 min, 90% B; 2.0-2.1 min, linear gradient to 40% B; 2.1-3.8 min, 40% B. The flow rate of the mobile phase was 0.45 mL min^{-1}, and injection volume was 2 µL. The mass spectrometer was operated in negative electrospray ionization multiple reaction monitoring (MRM) mode. Source parameters were as follows: Curtain gas (CUR), 30.0 L min^{-1}; Collision Gas (CAD), Medium; Ionspray Voltage (IS), -4500 V; Temperature (TEM), 450 ºC; Ion Source Gas 1 (GS1), 40 L min^{-1}; Ion source gas 2 (GS2), 40 L min^{-1}. The dwell time of each MRM transition was 200 ms.

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MRM transitions and corresponding declustering Potentials and collision energies for PFCs are listed in Table 1.

3 Results and discussion

3.1 Characterization of the synthesized materials

Characterizations of the synthesized magnetic nanoparticles were performed, including TEM, VSM, XRD and FT-IR.

The morphology of the synthesized material is examined by TEM, and the images of Fe_3O_4 and Fe_3O_4@SiO_2@TFBC MNPs are shown in Fig. 3. TEM investigation showed that the naked Fe_3O_4 nanoparticles exhibit spherical morphologies with an average diameter of 20 nm (Fig. 3a). After being functionalized with TFBC, the Fe_3O_4@SiO_2@TFBC magnetic nanoparticles (Fig. 3b) are nearly 450 nm in diameter and monodisperse, and exhibit a smooth surface (Fig. 3b).

The magnetic properties of the prepared microspheres were investigated with a VSM. Fig. 3c shows the magnetization curves of Fe_3O_4, Fe_3O_4@SiO_2 and Fe_3O_4@SiO_2@TFBC at 300 K, and the magnetic saturation values were 68.03, 29.42 and 25.46 emu g^{-1}, respectively. This result indicated that the Fe_3O_4@SiO_2@TFBC possessed excellent magnetic responsiveness, and the magnetic nanoparticles can be rapidly attracted to the surface of an external magnet.

The crystal phases of Fe_3O_4, Fe_3O_4@SiO_2 and Fe_3O_4@SiO_2@TFBC MNPs were investigated by XRD, and the obtained XRD patterns are shown in Fig. 3d, these three kinds of magnetic nanoparticles all match well with each other. It indicates that the TFBC layer has been synthesized successfully without damaging the Fe_3O_4

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nanoparticles during the coating process.

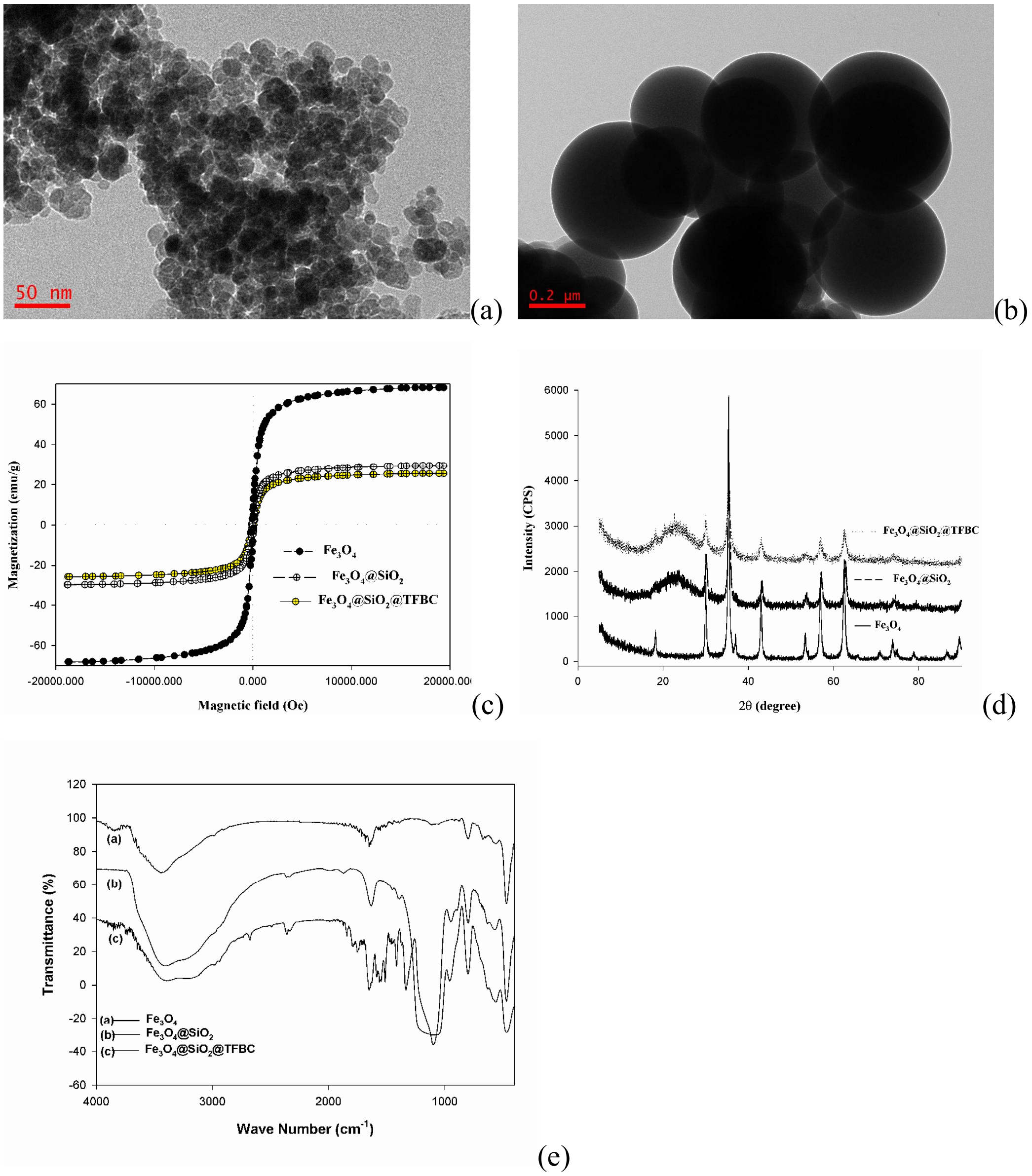

Fig.3. Characterization of the MNPs: TEM image of Fe_3O_4 MNPs (a); TEM image of Fe_3O_4@SiO_2@TFBC MNPs (b); VSM magnetization curves of Fe_3O_4, Fe_3O_4@SiO_2 and Fe_3O_4@SiO_2@TFBC MNPs (c); X-ray diffraction patterns of Fe_3O_4, Fe_3O_4@SiO_2 and Fe_3O_4@SiO_2@TFBC MNPs (d) and FTIR of Fe_3O_4, Fe_3O_4@SiO_2 and Fe_3O_4@SiO_2@TFBC MNPs (e).

FT-IR was employed to examine the surface groups of the as synthesized Fe_3O_4, Fe_3O_4@SiO_2, and Fe_3O_4@SiO_2@TFBC magnetic nanoparticles, as shown in Fig. 3

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(e). The adsorption peaks around 3410 cm^{-1} and 1654 cm^{-1}can be assigned to the -OH group on the surface of magnetite. The absorption peak around 580 cm^{-1} is assigned to Fe-O-Fe vibration, and 1100 cm^{-1} is attributed to the Si-O-Si stretching vibration. After the modification with TFBC, it displays a prominent peak at 1334 cm^{-1} which is characteristic of C-F stretching vibration. These signals indicate the successful modification of Fe_3O_4 magnetic nanoparticles surface with TFBC.

3.2 Optimization of extraction conditions

Several parameters that may affect the MSPE extraction efficiency of the adsorbent were optimized, including the adsorbent amount, type of desorption solvent, extraction time， the solution volume，pH value and ionic strength of the sample. The influence of all these parameters was evaluated in terms of recovery rate. The optimization experiments were conducted using spiked standard PFCs aqueous solution containing 2.5 ng L^{-1} of each analyte. Each experiment was performed in triplicate.

To obtain the maximum recovery rate of target analyte, the adsorbent amount was optimized by varying amount of Fe_3O_4@SiO_2@TFBC magnetic nanoparticles from 5 to 80 mg in 100 mL solution sample. Fig. 4a shows that the recoveries of all the tested PFCs reached the maximum when the amount of adsorbent was 10 mg, and then decreased with the following increase of the adsorbent amount. On the basis of this finding, 10 mg of adsorbent was sufficient to extract PFCs, and the more the adsorbent was involved, the more the analyte was retained. Therefore, 10 mg adsorbent amount was selected as the final amount of magnetic adsorbent used in the

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following experiments.

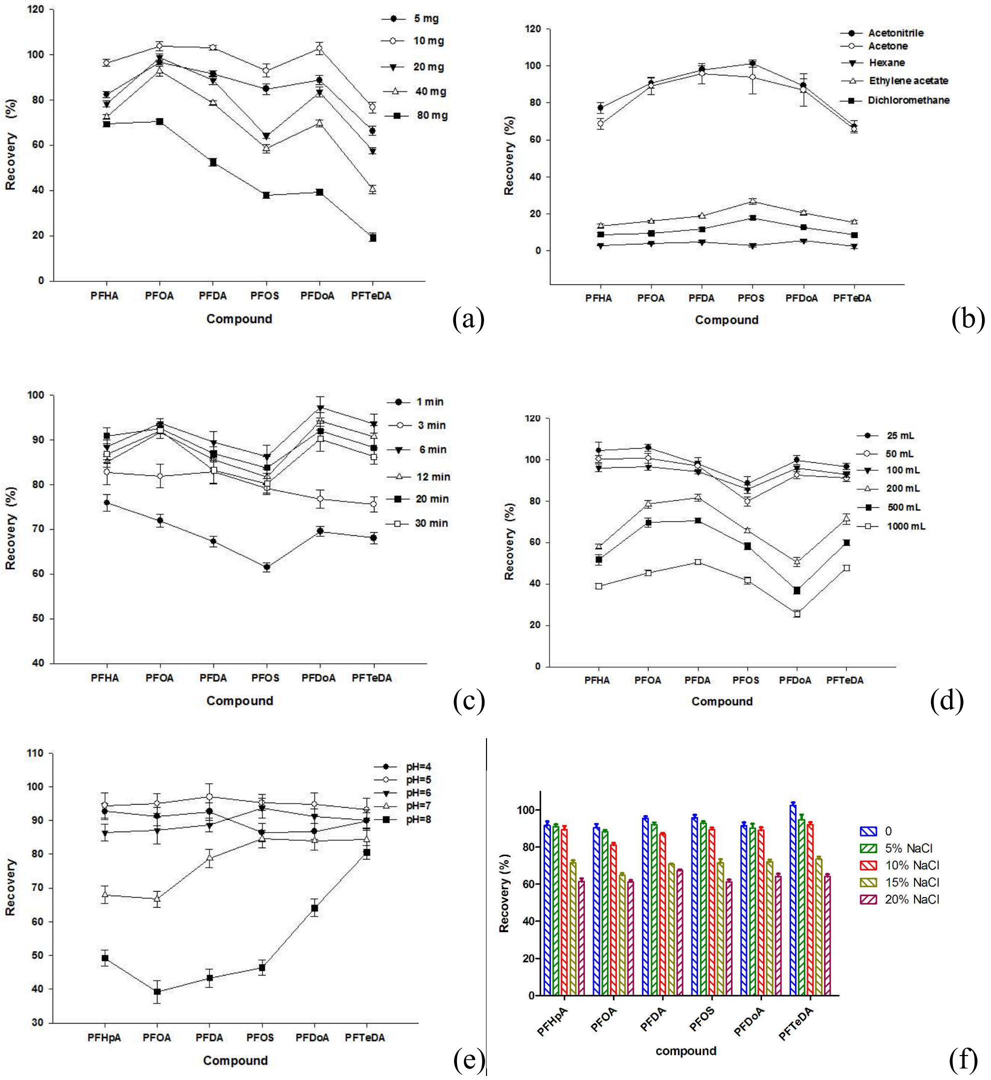

Fig.4 Effect of (a) amount of adsorbent; (b) type of desorption solvent; (c) extraction time; (d) sample volume; (e) pH value and (f) ionic strength on the extraction recoveries and relative standard deviations (*n* = 3) of the PFCs.

Type of desorption solvents was studied using n-hexane, dichloromethane, ethyl acetate, acetone and acetonitrile. In order to achieve better recoveries, Fe_3O_4@SiO_2@TFBC adsorbents were sonicated for 30 s in desorption solvents. As shown in Fig. 4b, acetonitrile and acetone yield higher recoveries than other desoption

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solvents, and acetonitrile exhibited the highest recovery.

Generally, sufficient contact time is required to obtain desorption equilibrium for target analyte in adsorbent. In our MSPE, the magnet was palced inside the vial, which make the magnetic adsorbents dispersed in the sample solution homogeneously when stirring. For studying the effect of extraction time, it was evaluated within the range from 1 to 30 min. The recoveries of all the PFCs increased significantly when extraction time increased from 1 to 6 min (Fig. 4c). Further increase in the extraction time resulted in no significant variation in the recoveries. Therefore, extraction time of 6 min was applied in the following study.

In order to test the effect of volume on extraction efficiency of PFCs, the sample volume from 25 to 1000 mL was tested. As shown in Fig. 4d, the recoveries of all the PFCs do not change significantly with the sample volume in the range of 25-100 mL. When the sample volume increased to the range of 200-1000 mL, the recoveries of all PFCs decreased obviously. Considering above result, a sample volume of 100 mL was utilized in the following experiments.

The pH value of sample solution plays an important role in the analysis of organic compounds, and is a major factor affecting their extraction performance. In view of stability of the MNPs, the effect of pH value was evaluated in the range of 4-8. As shown in Fig. 4e, it is obviously that the effect of the pH value of the solution sample solution on the recoveries is significant. Due to the small pKa of the investigated PFCs, they are mainly present in anionic form in the water sample, and higher extraction efficiency is achieved in a more acidic solution. [6,42-44] The extraction

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efficiency is affected by pH mainly through the ways to affect the dissociation of the target compounds. To obtain the best efficiency, pH 5 is selected for all experiments.

In general, addition of salts to the solution can affect the extraction efficiency of sorbent coating by two ways: one is to affect the interface property between adsorbent coating and sample solution, and then affect the partition coefficient between analytes and sorbent coating; and another is to decrease the solubility of organic compound in water via the salting-out effect. With regard to PFCs, a salting-out effect was reported in environmental waters, in which the partitioning of PFCs between water and particle increased with the increasing in water salinity.[45] The effect of ionic strength on the extraction efficiency was investigated with addition of NaCl at concentrations ranging from 0 to 20% (w/v). As shown in Fig. 4f, the extraction efficiency for all PFCs decreases with increasing the salt concentration. Therefore, no salt was added to the sample solution in the subsequent experiments.

Based on the above experimental results, the optimal conditions for the determination of PFCs were found to be: 10 mg of Fe_3O_4@SiO_2@TFBC magnetic adsorbents, acetonitrile as desorption solvent, 100 mL of sample solution, 6 min of the extraction time and pH 5 of the sample solution.

3.3 Investigation of the extraction mechanism

To prove that fluorine on the surface played an important role on the extraction of PFCs, the extraction capacities of naked Fe_3O_4, Fe_3O_4@SiO_2 and Fe_3O_4@SiO_2@TFBC were compared under the same conditions. The results are shown in Fig. 5. It can be seen that bare Fe_3O_4 has little enrichment ability towards

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PFCs, while $Fe_3O_4@SiO_2$ has better extraction capacity but recoveries of PFCs were all below 30%. $Fe_3O_4@SiO_2@TFBC$ shows the best extraction performance towards 7 PFCs, Due to fluorine atoms existing on the surface of magnetic particle, $Fe_3O_4@SiO_2@TFBC$ provided fluorous - fluorous interaction with the target analyte, so it exhibited good extraction performance and high recoveries.

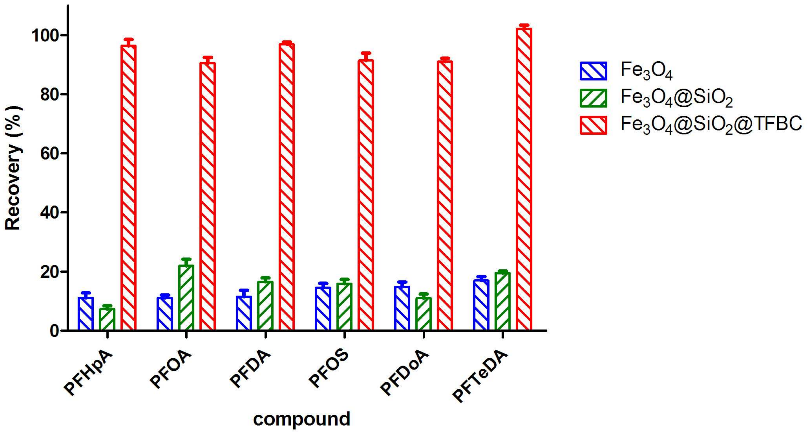

Fig.5 Comparison of different sorbents on the extraction efficiencies of PFCs

3.4 Method validation

The method was validated for a series of experiments with regard to the linearity, limit of detection (LOD), limit of quantitation (LOQ) and precision. The results are listed in Table 2.

With optimized MSPE procedure, the calibration curves for standard solutions were found to be linear in the range of 0. 1-20 ng L^{-1}, with coefficient of determination (R^2) ranging from 0.9980 to 0.9992. The limit of detection (LOD) and the limit of quantification (LOQ) are calculated as the concentrations of the analytes at a signal-to-noise ratio (S/N) of 3 and 10, respectively. Our results show that the

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LOD and LOQ values of the PFCs range from 0.010 to 0.025 ng L^{-1} and from 0.033 to 0.083 ng L^{-1}, respectively. The relative standard deviations (RSDs) for the PFCs were below 5.0%, illustrating the good repeatability achieved by the suggested procedure. These results imply that the proposed method can be applied to the analysis of real samples containing PFCs at trace level.

3.5 Application of Fe_3O_4@SiO_2@TFBC for the analysis of PFCs in TCMs

The method was applied to analyze three types of TCMs including *Pheretima vulgaris* Chen*, Hirudo nipponica* whitman and *Syngnathus acus* Linnaeus under optimized conditions. Before extraction, the samples were adjusted to pH 5 with 20 mM acetic acid solution. Under optimized conditions, chromatograms of *Pheretima vulgaris* Chen sample spiked with PFCs after extraction are shown in Fig. 5. The results of spiked PFCs are listed in Table 3, giving recoveries of 83.6-107.4%, and RSDs within 7.3%. And the analytical results of unspiked TCMs samples are also summarized in Table 3. PFOA was detected in *Hirudo nipponica* whitman samples but not quantified, and PFOS was found in *Syngnathus acus* Linnaeus samples. These results imply that the established method can be applied to the analysis of PFCs at trace level in real samples.

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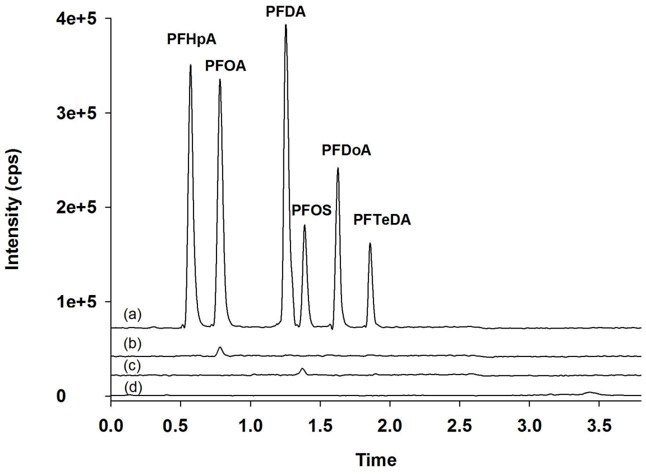

Fig.6 Magnetic solid-phase extraction UHPLC-MS/MS chromatograms of *Pheretima vulgaris* Chen sample spiked with 2.5 ng L^{-1} of each analyte (a), *Hirudo nipponica* whitman sample (b), *Syngnathus acus* Linnaeus (c) and *Pheretima vulgaris* Chen sample (d).

Table 2 Analytical performances of the proposed method.

PFCs	Linear range (ng L^{-1})	Calib. Curve [a] (n=3)	R^2	Method LOD (ng L^{-1})	Method LOQ (ng L^{-1})	RSD(%)[b] (*n*=3)
PFHpA	0.2-20	$Y=9.05\times10^{4}X+5.09\times10^{3}$	0.9991	0.025	0.083	2.1
PFDA	0.2-20	$Y=1.74\times10^{5}X-1.40\times10^{3}$	0.9987	0.020	0.067	3.2
PFDoA	0.2-20	$Y=1.13\times10^{5}X-5.53\times10^{3}$	0.9992	0.012	0.040	3.2
PFTeDA	0.2-20	$Y=1.02\times10^{5}X-9.79\times10^{3}$	0.9988	0.010	0.033	2.4
PFOA	0.2-20	$Y=8.76\times10^{4}X+2.56\times10^{4}$	0.9980	0.020	0.067	5.0
PFOS	0.2-20	$Y=2.92\times10^{4}X-1.23\times10^{3}$	0.9991	0.015	0.050	3.7

[a] X is compound concentration (ng L^{-1}) and Y is peak area.

[b] Determined at a concentration of 2.5 ng L^{-1} for each analyte.

3.6 Comparison of proposed method with previously reported methods with magnetic functionalized adsorbents

Table 4 summarizes the analytical characteristics of previously reported

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magnetic functionalized adsorbents including Fe_3O_4-C_{18}-chitosan,[37] magnetic-MG,[38] F_{17}-Fe_3O_4@mSiO_2,[39] Fe_3O_4@SiO_2@FBC[40] and Fe_3O_4@mSiO_2-F_{17} [41] as compared to the proposed adsorbent. As can be seen, the proposed adsorbent is highly efficient, and only 10 mg was sufficient to extract PFCs, which was less than Fe_3O_4-C_{18}-chitosan, F_{17}-Fe_3O_4@mSiO_2, Fe_3O_4@SiO_2@FBC and Fe_3O_4@mSiO_2-F_{17}. And the extraction time of Fe_3O_4@SiO_2@TFBC adsorbent was only 6 min, which makes the extraction procedure faster than all the other adsorbents. What's more, LOD of the proposed method was the lowest. In addition, our UHPLC analysis was achieved within 3.8 min, and also faster than other chromatographic analysis.[38-41] In brief, the whole method, termed as MSPE combined with UHPLC/MS/MS analysis, was simple, convenient and efficient. Fe_3O_4@SiO_2@TFBC adsorbent showed a very good sensitivity and low detection limits, which can be attributed to the rapid dynamics due to the fluorous - fluorous interaction of fluorine atoms on surface of MNPs adsorbents and the analytes.

4 Conclusion

In the current study, a novel magnetic solid-phase extraction method was presented, by which a permanent magnet was added into container to collect magnetic adsorbents. The new method saved time of dumping solution sample and dumping eluent, which greatly accelerates the magnetic solid-phase extraction. For the extraction of PFCs, a novel kind of magnetic adsorbent, termed as 4-(trifluoromethyl)-benzoyl chloride functionalized magnetic nanoparticles (Fe_3O_4@SiO_2@TFBC) was successfully synthesized. Compared to other magnetic

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functionalized adsorbents, the proposed magnetic nanoparticles displayed better extraction performance, due to the fluorous-fluorous interaction. And only 10 mg of Fe_3O_4@SiO_2@TFBC magnetic nanoparticles was needed. The MSPE coupled with UHPLC-MS/MS is a simple, quick, sensitive and effective method for the determination of PFCs. In conclusion, the whole analytical method including preconcentration and chromatographic analysis was sufficiently sensitive and suitable for determination of very low concentrations of PFCs in real samples.

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Table 3 Detection of PFCs by SPE fromTCMs

Analytes	*Pheretima vulgaris* samples				*Hirudo nipponica* whitman samples				*Syngnathus acus* Linnaeus samples			
	Found (pg g^{-1})	Added (pg g^{-1})	Recovery (%)	RSDs (%,n=3)	Found (pg g^{-1})	Added (pg g^{-1})	Recovery (%)	RSD(%,n=3)	Found (pg g^{-1})	Added (pg g^{-1})	Recovery (%)	RSDs (%,n=3)
PFHpA	N.D[a]	0.2	86.9	3.9	N.D.	0.2	85.8	5.1	N.D.	0.2	93.4	3.6
		0.5	87.7	4.7		0.5	93.2	4.7		0.5	97.1	4.9
		1	97.9	3.8		1	95.9	3.2		1	95.7	5.3
PFOA	N.D.	0.2	87.7	5.3	0.21±0.05	0.2	88.9	4.9	N.D.	0.2	95.8	3.8
		0.5	94.3	7.3		0.5	97.4	3.4		0.5	107.4	6.8
		1	89.2	4.4		1	89.9	4.3		1	94.2	3.7
PFDA	N.D.	0.2	85.2	3.8	N.D.	0.2	91.1	5.6	N.D.	0.2	90.4	5.4
		0.5	96.3	4.6		0.5	104.9	5.2		0.5	94.6	5.2
		1	93.3	5.7		1	92.9	4.9		1	105.1	3.6
PFOS	N.D.	0.2	87.1	4.2	N.D.	0.2	96.4	5.2	0.43±0.05	0.2	86.3	5.3
		0.5	89.6	3.6		0.5	87.1	6.1		0.5	97.9	4.0
		1	93.6	5.7		1	94.6	3.9		1	85.4	5.3
PFDoA	N.D.	0.2	86.3	3.6	N.D.	0.2	91.9	4.9	N.D.	0.2	95.6	4.6
		0.5	88.5	4.4		0.5	84.8	5.2		0.5	95.1	5.0
		1	90.2	5.1		1	106.9	4.8		1	83.6	5.1
PFTeDA	N.D.	0.2	87.7	5.3	N.D.	0.2	88.8	4.5	N.D.	0.2	104.1	5.8
		0.5	91.7	3.5		0.5	85.2	3.9		0.5	93.4	4.3
		1	105	4.7		1	92.7	4.6		1	90.7	4.7

[a] Not detected

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Table 4 Comparison of the analytical performance of different functionalized magnetic adsorbents

Magnetic adsorbents	PFCs	Adsorbent amount (mg)	Extraction time (min)	Desorption solvent (mL)	Elution time (min)	Recovery (%)	Limits of detection (ng L^{-1})	Linear range (ng L^{-1})	RSDs (%)	Reference
Fe_3O_4-C_{18}-chitosan	7	100	20	12	100	56-112	0.033-0.19	0.5-50	3.0-9.4	37
magnetic-MG	5	1[a]	20	1.5	16	56.3-91.4	0.15-0.50	1-500	3.6-8.4	38
F_{17}-Fe_3O_4@$mSiO_2$	4	100	10	0.8	4	93.4–105.7	8 -125	500-50000	2.6–7.6	39
Fe_3O_4@SiO_2@FBC	6	40	12	1.2	6	89.34–111.32	0.01-0.06	0.25-25	0.8-4.1	40
Fe_3O_4@$mSiO_2$- F_{17}	6	20	8	0.3	8	83.13-92.42	20-50	250-10^6	2.6-14.2	41
Fe_3O_4@SiO_2@TFBC	6	10	6	1.2	3.8	82.0-105	0.010-0.025	0.2-20	2.1-5.0	This work

[a] 1 mL of MG aqueous dispersion (1 mg mL^{-1}) and 1 mL of cetyltrimethylammonium bromide (CTAB) aqueous solution (1 mg mL^{-1}).

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Table captions

Table 1 MS/MS parameters

Table 2 Analytical performances of the proposed method.

Table 3 Detection of PFCs by MSPE fromTCMs

Table 4 Comparison of the analytical performance of different functionalized magnetic adsorbents

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Figure captions

Fig.1. Scheme for the preparation of the Fe_3O_4@SiO_2@TFBC

Fig.2 The novel Magnetic solid-phase extraction procedure

Fig.3. Characterization of the MNPs: TEM image of Fe_3O_4 MNPs (a); TEM image of Fe_3O_4@SiO_2@TFBC MNPs (b); VSM magnetization curves of Fe_3O_4, Fe_3O_4@SiO_2 and Fe_3O_4@SiO_2@TFBC MNPs (c); X-ray diffraction patterns of Fe_3O_4, Fe_3O_4@SiO_2 and Fe_3O_4@SiO_2@TFBC MNPs (d) and FTIR of Fe_3O_4, Fe_3O_4@SiO_2 and Fe_3O_4@SiO_2@TFBC MNPs (e).

Fig.4 Effect of (a) amount of adsorbent; (b) type of desorption solvent; (c) extraction time; (d) sample volume; (e) pH value and (f) ionic strength on the extraction recoveries and relative standard deviations (*n* = 3) of the PFCs.

Fig.5 Comparison of different sorbents on the extraction efficiencies of PFCs

Fig.6 Magnetic solid-phase extraction UHPLC-MS/MS chromatograms of Pheretima vulgaris Chen sample spiked with 2.5 ng L^{-1} of each analyte (a), Hirudo nipponica whitman sample (b), Syngnathus acus Linnaeus (c) and Pheretima vulgaris Chen sample (d).

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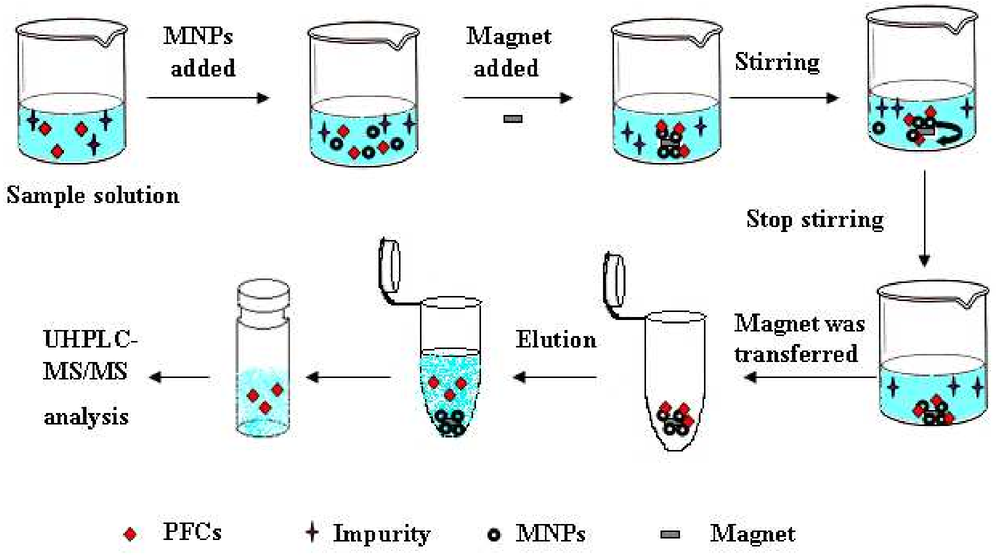
Graphical Abstract

Magnetic solid-phase extraction for the enrichment of PFCs from sample solution



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