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Fluorocarbon-bonded magnetic mesoporous microspheres for the analysis of perfluorinated compounds in human serum by high-performance liquid chromatography coupled to tandem mass spectrometry

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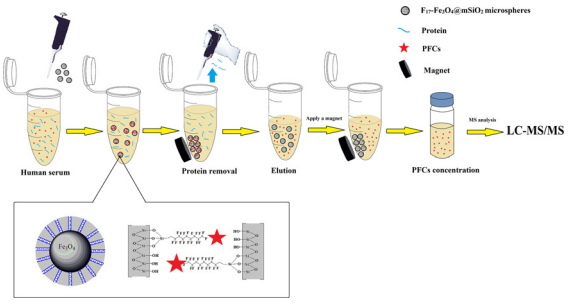
HIGHLIGHTS

- New SPE method was developed for analysis of PFCs in human serum.
- Fluorocarbon-bonded magnetic mesoporous microspheres were used as SPE absorbents.
- PFCs in serum were directly extracted without any other pretreatment procedure.
- The PFCs-adsorbed microspheres were simply and rapidly isolated by using a magnet.

GRAPHICAL ABSTRACT



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ABSTRACT

We report herein an extraction method for the analysis of perfluorinated compounds in human serum based on magnetic core–mesoporous shell microspheres with decyl-perfluorinated interior pore-walls (Fe_3O_4@$mSiO_2$-F_{17}). Thanks to the unique properties of the Fe_3O_4@$mSiO_2$-F_{17} microspheres, macromolecules like proteins could be easily excluded from the mesoporous channels due to size exclusion effect, and perfluorinated compounds (PFCs) in protein-rich biosamples such as serum could thus be directly extracted with the fluorocarbon modified on the channel wall without any other pretreatment procedure. The PFCs adsorbed Fe_3O_4@$mSiO_2$-F_{17} microspheres could then be simply and rapidly isolated by using a magnet, followed by being identified and quantified by LC–MS/MS (high-performance liquid chromatography coupled to tandem mass spectrometry). Five perfluorinatedcarboxylic acids (C6, C8–C11) and perfluorooctane sulfonate (PFOS) were selected as model analytes. In order to achieve the best extraction efficiency, some important factors including the amount of Fe_3O_4@$mSiO_2$-F_{17} microspheres added, adsorption time, type of elution solvent, eluting solvent volume and elution time were investigated. The ranges of the LOD were 0.02–0.05 ng mL^{-1} for the six PFCs. The recovery of the optimized method varies from 83.13% to 92.42% for human serum samples.

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1. Introduction

Recent scientific research suggested that several organic perfluorinated compounds (PFCs), as a group of emerging contaminants, which are derived from surfactants, paints, food packaging, and fire-retarding foams in commercial applications, are ubiquitous in human worldwide [1,2]. The strong carbon–fluorine (C–F) bonds of PFCs make them resistant to hydrolysis, photolysis, metabolism, and biodegradation. Recently, more and more evidences showed that PFCs are highly bioaccumulative because of their existence in environmental sample [3,4], sediments [5], wildlife [6–10] and humans [11,12]. PFCs have also received great concern due to their accumulation toxicity [13] in human tissues [14–16] for human health concern. The toxicological studies of perfluoroalkylsulfonic acids (PFOS) and perfluoroalkylcarboxylic acids (PFOA), the two of the most common PFCs, suggested that their effects on lipid metabolism and transport resulted in a reduction of cholesterol and triglycerides in animal serum and an accumulation of lipids in liver, so the experimental study of PFCs exposure is vital to the risk assessment for public health.

Blood, serum and breast milk have been the most utilized matrices for PFCs biomonitoring. The determination of PFCs in blood samples (i.e., serum and plasma) is mostly made by liquid chromatography (LC)–tandem mass spectrometry (MS/MS) with negative electrospray ionization (ESI(−)) interfaces [17,20–22]. Currently, the most commonly applied sample preparation methods for the analysis of PFCs are based on solid phase extraction (SPE), while others are ion pair extraction, organic solvents precipitation etc. [17–24]. Acidification [17,18,22,23], alkaline digestion or addition of organic solvent to samples [21] are usually carried out prior to SPE in order to prevent the clogging of pre/columns caused by the precipitation of blood proteins. A modification of the on-line SPE coupled to HPLC–MS/MS approach was described [19]. Comparison of extraction and quantitation methods for PFCs in human plasma, serum and whole blood has been reported [25]. Although these approaches are of high efficiency both from qualitative and quantitative point of view, some limitations may be underlined in terms of sensitivity (with regard to the reduced sample volumes available in the frame of human studies) and/or specificity (with regard to the high stability of PFCs leading to limited fragmentations in MS/MS). In addition, due to co-eluting interferents presenting the same diagnostic signals as certain PFCs, some problems in quantification were reported [26,27]. Chan et al. [27] reported a possible overestimation of PFOS and perfluorohexane (PFHxS) concentrations in serum samples using LC–MS/MS because of the matrix interferences. In order to analyze these compounds with better specificity in biota, the application of a new sample preparation strategy based on functionalized magnetic materials have become an alternative [28,29]. Many previous works based on designed synthesis of functionalized magnetic materials and their application as the sample preparation techniques have garnered much research interest for biological analysis over the past decades [30–32]. Magnetic silica-based materials possess the excellent characteristics of strong magnetization, high surface areas, uniform mesopores distribution, adjustable pore sizes, and extra adsorption capacity [33–35]. By employing those materials as solid-phase extraction sorbents, the special advantages of high adsorption capacity of mesoporous silica and separation convenience of magnetic nanopaticles could make extraction easy. Due to the specific force of functional groups on the sorbents, selective extraction of targeted analytes and high anti-interference ability can be readily achieved. The exterior shell of mesoporous silica contributes to preventing the interference of macromolecules in matrices from entering into the interior pore walls. More recently, our group developed a very simple approach for the extraction and separation of low concentration medicinal molecules from rat serum and cow milk [36,37]. This method is flexible and can be widely used in various matrices, such as egg, blood plasma, liver and other biological tissues. The development of F-functionalized magnetic mesoporous for extraction and enrichment of PFCs in biota is both fascinating and of great significance. However, to the best of our knowledge, few related work was reported. Recently, Bailey et al. [38,39] used fluorous solvents such as methyl perfluorobutyl ether, trifluoroethanol and perfluorohexane to partition PFCs from food matrices, which was a successful application of fluorous affinity in the extraction of PFCs with high selectivity. However, the extracted liquor needs to be stored at −80 °C overnight to reform the triphasic system. Therefore, the lack of effective and selective extraction method of PFCs in complex biosample calls for the development of new techniques based on fluorous affinity.

The purpose of this study was to develop a facile method based on F_{17}-functionalized magnetic core–mesoporous shell microspheres as the novel adsorbent for analysis of PFCs in human serum. Five perfluorinatedcarboxylic acids (C6, C8–C11) and one perfluorooctane sulfonate (PFOS), which are the most commonly studied PFCs, were selected as the model analytes to investigate the extraction ability of the as-made materials.

2. Experimental

2.1. Chemicals

Six PFCs were analyzed in this study. PFOS, perfluorohexanoic acid (PFHxA), PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDeA) and perfluorododecanoic acid (PFDoA) were purchased from Alfa Aesar (Ward Hill, MA, USA) with chemical purities of >98%. Perfluorotetradecanoic acid (PFTeA, I.S.) were also purchased from Alfa Aesar (Ward Hill, MA, USA). Methanol, acetonitrile, dimethyl formamide and tetrahydrofuran, which are all HPLC grade, were obtained from TEDIA (Ohio, USA), Merck (Darmstadt, Germany) and Sinopharm (Shanghai, China). Standard stock solutions ($1\,\mu g\,L^{-1}$) containing the PFCs were prepared in acetonitrile and stored at 4 °C. Working solutions were prepared daily by appropriate dilution of the stock solutions with acetonitrile.

$FeCl_3{\cdot}6H_2O$, tetraethylorthosilicate (TEOS), ethylene glycol-ethanol, cetyltrimethylammonium bromide (CTAB), ammonium formate and ammonium acetate were purchased from Shanghai Chemical Corp (Shanghai, China). 1H,1H,2H,2H-perfluorodecyltriethoxysilane (PDTES, purity 97%) was purchased from Alfa Aesar (Tianjin, China). Deionized water used for LC–MS/MS was purified with a Milli-Q plus system (Millipore, Bedford, MA, USA).

2.2. Sample collection

Human serum samples were collected from volunteer donors of Shanghai Changzheng Hospital. Individual informed consent was obtained. All serum samples used for testing the method were obtained from the general population of Shanghai. One pooled serum samples were mixed thoroughly and used as a non-spiked sample and as preparation for the calibration standards.

2.3. Preparation of standard solutions

Individual stock solutions of PFCs (including I.S.) were prepared by dissolving 1 μg of each compound in 1 mL of acetonitrile in stained glass stopper bottles. Six working standard solutions containing all analytes were prepared by serial dilutions in acetonitrile and 10 mM ammonium formate aqueous solution

(1/1 v/v). Calibration curves for all analytes and spiking tests were prepared daily. All stock solutions were divided into small aliquots and stored in a refrigerator at −70 °C after preparation.

2.4. Synthesis of Fe_3O_4@mSiO_2-F_{17} microspheres

The process of the preparation of Fe_3O_4@mSiO_2-F_{17} microspheres is illustrated in Scheme 1. First, the magnetic Fe_3O_4 microspheres were synthesized through solvothermal reaction according to the previously reported method [40], and dried in vacuum at 50 °C for 24 h. Secondly, the Fe_3O_4@mSiO_2-F_{17} microspheres were synthesized through a surfactant involved sol–gel process according to the previous reports [41,42]. Briefly, the prepared Fe_3O_4 microspheres and CTAB with a ratio of 75 mg/750 mg were dispersed in 75 mL of deionized water and sonicated for 30 min. The resultant dispersion was mixed with 675 mL of alkaline aqueous solution and further sonicated for 5 min to form a stable dispersion. The mixed dispersion was then heated at 60 °C for 30 min. Afterwards, 3.75 mL of TEOS/ethanol (1/4 v/v) solution was added drop by drop under vigorous stirring, followed by heating at 60 °C for 30 min with stirring. Subsequently, 225 μL of TEOS/1H,1H,2H,2H-PDTES (2/1 v/v) mixed solution was injected into the dispersion, and the dispersion was further heated at 60 °C for 12 h. The product was collected by magnetic separation and refluxed in ethanol at 60 °C to remove the CTAB templates. Finally, the resulting Fe_3O_4@mSiO_2-F_{17} microspheres were dried at 50 °C for 24 h in vacuum for future use.

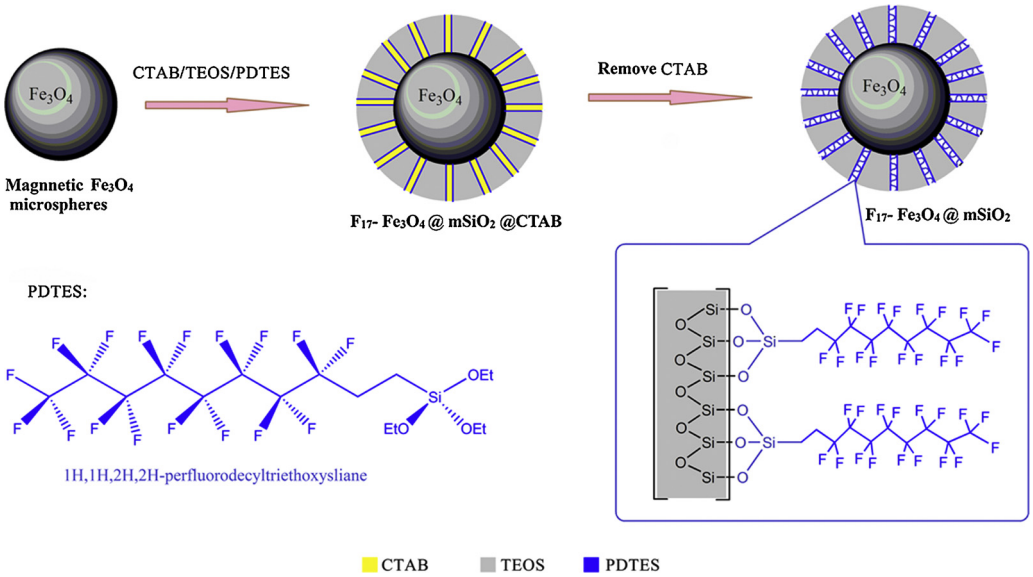
2.5. Enrichment procedure by Fe_3O_4@mSiO_2-F_{17} microspheres

The entire extraction route of PFCs from serum samples with the Fe_3O_4@mSiO_2-F_{17} microspheres is shown in Scheme S1. 100 μL of serum sample was spiked into 40 μL of solutions, which contains six standard PFCs and 10 μL of I.S. solution with a certain concentration. It was diluted with acetonitrile and 10 mM ammonium formate aqueous solution (1/1 v/v) before it was added to 100 μL of serum, encompassing the entire linear range of the method (0.25–1000 ng mL^{-1} or 0.20–1000 ng mL^{-1} especially for PFDoA) to construct daily calibration curves. Then, certain amounts of suspension of Fe_3O_4@mSiO_2-F_{17} microspheres (0.5 mg μL^{-1}) pretreated by acetonitrile were appended to the mixed liquid and continuously vibrated for certain minutes. Thereafter, the PFCs-captured Fe_3O_4@mSiO_2-F_{17} microspheres were isolated with a magnet bar (40 × 20 × 10 mm) in the bottom of EP tube. The adsorbed PFCs/Fe_3O_4@mSiO_2-F_{17} microspheres were gathered and rinsed twice with 200 μL of deionized water, and supernatant liquid was pipetted off. Subsequently, the type and volume of eluting solvents (e.g., acetonitrile, tetrahydrofuran, methanol and dimethyl formamide), and the elution time were also investigated. The captured PFCs were then eluted from the Fe_3O_4@mSiO_2-F_{17} microspheres by eluting solvents for certain minutes. The elution solvent was evaporated under a stream of dry nitrogen in Pressure Blowing Concentrator (Aosheng MD200-2, China) at 55 °C. 20 μL of acetonitrile was added to the evaporative extract, and then it was transferred into a poly propylene auto-samples vial. Subsequently, 10 μL of the above solution was injected into the LC–MS/MS system. Using a method similar to the enrichment and analysis procedure described above, optimization of the extraction of PFCs with the Fe_3O_4@mSiO_2-F_{17} microspheres was conducted under a variety of experimental conditions. The optimum conditions were established in terms of the recoveries of the target compounds.

2.6. Liquid chromatography tandem mass spectrometry analysis

Liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) was used for separation and quantification of PFCs. Chromatographic analyses were carried out on the Agilent 1200 series HPLC system (Agilent, USA) equipped with a G1312A Bin Pump, a G1322A vacuum degasser, a G1329A ALS autosampler and G1316A column oven. The chromatographic separation of the compounds was on a Ultimate XB-C18 column (150 × 2.1 mm, id 5 μm; Welch Materials, China) equipped with a security guard cartridge XB-C18 (10 × 2.1 mm id 5 μm; Welch Materials, China). Elution was carried out on the mobile phases consisting of (A) 2 mM aqueous ammonium acetate in HPLC water (containing 0.05% acetic acid) and (B) methanol. The elution gradient conditions for the LC mobile were based on A/B (20/80 v/v). The flow rate was kept at 0.20 mL min^{-1} throughout the run, and the sample volume was 10 μL. The total chromatographic run had duration of 8 min.

The LC system was coupled to an Applied Biosystem 4000Q TRAP mass spectrometer (Applied Biosystems-Sciex, Foster City,



Scheme 1. The synthetic procedure of Fe_3O_4@mSiO_2-F_{17} microspheres.

CA), equipped with a Turbo ion Spray source which was carried out in the negtive electrospray ionization mode (ESI(−)). Acquisition was performed in multiple reaction monitoring (MRM) mode to obtain sufficient quantification points for confirmation of each analytes. For the sake of optimizing all the MS parameters, standard mixture solutions (1 μg mL^{-1}) of target analyte and I.S. were infused into the mass spectrometer.

The optimal ESI source settings were used as follows:

Turbo heater temperature (TEM) at 350 °C; curtain gas pressure (CUR) at 10 psi; ion spray transfer voltage (IS) at 3.0 kV; ion source gas 1 (GS1) at 40 V and gas 2 (GS2) at 40 V; interface heater on; collision gas (CAD) medium; fragment ions, and optimal values for declustering potential (DP), collision energy (CE), entrance potential (EP) and collision cell exit potential (CXP), for each target transition, are showed in Table S1.

2.7. Data analysis

Data acquisition and analysis for all samples including blank and standard were performed with Analyst software versions 1.6 (Applied Biosystems). The data analysis program automatically selected and integrated the signals of each analyte's transition in the chromatogram. The integrations was manually corrected, if necessary.

2.8. Analytical validation

The analytical validations of the proposed procedure were carried out with blank serum samples spiked with six PFCs standard solution of certain concentrations. The seven-point calibration curve was obtained by plotting the peak area ratio (y) between each PFCs and I.S. versus corresponding concentration of the analyte (x) using optimal Fe_3O_4@mSiO_2-F_{17} microspheres extracted conditions. Concentrations of six PFCs in serum sample were calculated from each resulting peak area ratios and each regression equation of the calibration curve. Six replicate injections of same standard samples of each PFCs were analyzed by the LC–MS/MS method to determine repeatability (system precision). The limit of detection (LOD) (S/N = 3) of the method was calculated by the analysis of the standard solution with the lowest concentration that the analytical process can reliably differentiate from background levels. And the limit of quantification (LOQ) was calculated on the basis of S/N = 10, which is the lowest concentration of analyte that can be quantified. Accuracy and precision were estimated by analytical recovery from spiked samples.

In addition, the recoveries of the target compounds were studied by extracting the spiked serum samples under the optimized conditions for five times. Recovery experiments were performed by comparing results for extracted standard samples of serum and internal standards added before the extraction procedures with the non-extracted standards prepared at the same concentrations in blank extracts representing 100% recovery. Intra-day precision was assessed by comparing the results of six replicates prepared in the same day at three different concentrations. The procedure was also repeated to determine the inter-day precision and a pooled serum sample was spiked at three different concentrations. For each concentration, we analyzed a set of six samples on two different days. The relative standard deviations (% RSD) were calculated.

3. Results and discussion

3.1. Characterization of Fe_3O_4@mSiO_2-F_{17} microspheres

The procedure for synthesis of Fe_3O_4@mSiO_2-F_{17} microspheres is illustrated in Scheme 1. On the basis of the facile one-pot synthesis approach, the Fe_3O_4@mSiO_2-F_{17} microspheres were synthesized through the synchronous condensation of TEOS and 1H,1H,2H,2H-perfluorodecyltriethoxysilane in the presence of Fe_3O_4 microspheres as the seeds and CTAB as the template [35,38].

According to the SEM image (Fig. S1), the obtained microspheres have a narrow size distribution with the mean diameter of 250 nm. The TEM image (Fig. 1) indicates a dark magnetic core (darker section) with the diameter of approximately 200 nm and a gray porous silica shells (lighter section) with the thickness of approximately 30 nm [38]. Fig. 1 also implies that the dense pore channels in the shell were perpendicular to the microsphere's surface, which was favorable to the capacity of extraction as SPE absorbents.

The porosity of the Fe_3O_4@mSiO_2-F_{17} was studied by N_2 adsorption–desorption measurement at 77 K. The pore size distribution derived from the adsorption branch using the Barrett–Joyner–Halenda (BJH) method indicates that the obtained materials have a pore size of about 2.1 nm with a narrow pore size distribution (Fig. S2, inset). The BET surface area and total pore volume were calculated to be 275.2 m^2 g^{-1} and 0.18 cm^3 g^{-1}, respectively, indicating a high porosity.

The FT-IR experiments of Fe_3O_4@mSiO_2-F_{17} were taken to confirm the presence of F_{17} groups in the Fe_3O_4@mSiO_2-F_{17} microsphere. As depicted in Fig. S3, the peaks at 1070 and 1629 cm^{-1} were attributed to the characteristic absorption of Si–O–Si and O–Si–C vibrations, respectively. Two obvious new absorption peaks at 1150 and 1208 cm^{-1} were designated to C–F vibration of CF_2 and CF_3, indicating that the F_{17} groups were successfully modified in the synthesized microsphere.

Also, the as-prepared Fe_3O_4@mSiO_2-F_{17} microspheres in their homogenous dispersion indicated fast movement when a magnetic field is applied, and redispersed quickly with a slight shake once the magnetic field was removed (Fig. S4). The magnetization should be sufficient to provide rapid solid–liquid separation. It demonstrated that the magnetic Fe_3O_4@mSiO_2-F_{17} microspheres possessed excellent magnetic response and redispersibility which means an advantage to its further application.

Above all, the obtained F-functionalized Fe_3O_4 microspheres posses numerous silanol groups on the exterior surface and inner modification of F_{17} groups anchored in the interior pore-walls, large surface area, superparamagnetism and uniform mesopores,

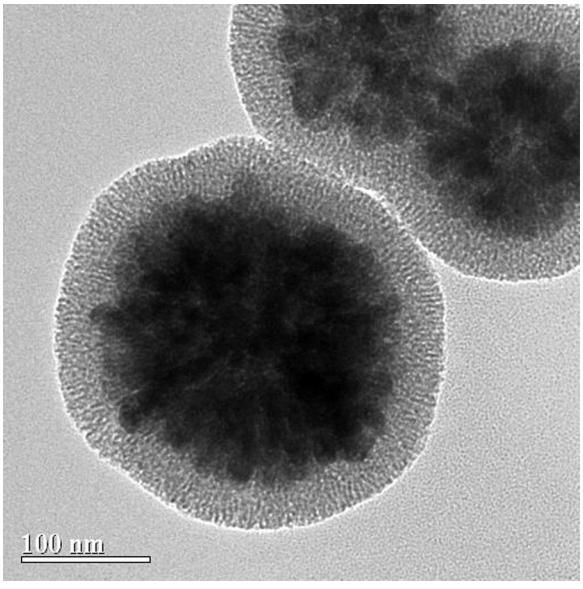


Fig. 1. TEM image of the synthesized Fe_3O_4@mSiO_2-F_{17} microspheres.

making them satisfactory absorbent for highly selective and effective enrichment of PFCs in protein-rich biological samples.

3.2. Optimization of extraction conditions

The effects of some parameter on extraction of PFCs such as the amount of Fe_3O_4@mSiO_2-F_{17} microspheres, extraction time, the type of eluting solvent, the volume of elution solvent and elution time, which can affect the performance of extraction, were studied. Analytical recovery of each PFCs added to samples at given concentration was assessed. Recovery, which is defined as calculating the peak area ratio relative to that of the I.S. and comparing this result with that obtained for standard solution containing the same nominal analyte quantities and the I.S. [43]. The optimization of extraction condition was evaluated by extracting the serum samples fortified with 2.5 ng mL^{-1} for each analyte and I.S. (2.0 ng mL^{-1}). The effect of the amount of Fe_3O_4@mSiO_2-F_{17} microspheres (5–50 mg) was investigated. Extraction time, the volume of elution solvent, eluting solvent and desorption time were fixed as 8 min, 300 μL of acetonitrile and 5 min. As shown in Fig. S5, the extraction efficiencies of all six PFCs increased with the amount of microspheres, reached a plateau when the amounts of adsorbents were 20 mg. Based on the above results, 20 mg of Fe_3O_4@mSiO_2-F_{17} microspheres were added in the following experiments.

A certain standing time is required for balance after the adsorbents are dispersed into the solution. In the research, different extraction time (2–30 min) were studied. The amounts of Fe_3O_4@mSiO_2-F_{17} microspheres, the volume of elution solvent, eluting solvent and desorption time were fixed as 20 mg, 300 μL of acetonitrile and 5 min. As shown in Fig. S6, the recovery increased with extraction time and maximum recovery was obtained at around 8 min of the extraction time. As the time prolonged, the extraction efficiency maintained at a stable level. It may be interpreted that the fluorous affinity between the PFCs and Fe_3O_4@mSiO_2-F_{17} microspheres was so strong that the adsorption equilibrium could be achieved in a short time. Finally, for the purpose of developing a rapid analytical procedure, 8 min was chosen in the following experiments.

An appropriate type of elution solvent is fundamental for developing an optimal extraction procedure by Fe_3O_4@mSiO_2-F_{17} microspheres. The amounts of Fe_3O_4@mSiO_2-F_{17} microspheres, extraction time, the volume of eluting solvent and desorption time were fixed as 20 mg, 8 min, 300 μL and 5 min, respectively. According to the previous research, the ability of methanol and acetonitrile in destroying the fluorous affinity were already affirmed [44]. The results in this study are shown in Fig. S7, and from which the desorption ability of acetonitrile was superior to other kind of solvents. It could be explained as that the dissolving capacity of acetonitrile which can break the fluorous affinity existing between the fluorous molecules is higher. Therefore, acetonitrile was finally selected as the elution solvent.

The volume of elution solvent is also an important parameter to obtain reliable and reproducible analytical results in pre-concentration of serum samples. In this work, for the sake of investigating the effect of the extraction recovery, 100, 200, 300 and 500 μL of acetonitrile were added for elution. The amounts of Fe_3O_4@mSiO_2-F_{17} microspheres, extraction time, the eluting solvent and desorption time were fixed as 20 mg, 8 min, acetonitrile and 5 min, respectively. Fig. S8 shows that the maximal extraction efficiency of these PFCs was obtained when the elution solvent volume reached to 300 μL. Also, different desorption time (3, 5, 8 and 10 min) was investigated. The amounts of Fe_3O_4@mSiO_2-F_{17} microspheres, extraction time, the volume of elution solvent and eluting solvent were fixed as 20 mg, 8 min, 300 μL of acetonitrile. As shown in Fig. S9, 5 min was enough for elution.

3.3. Matrix effects

To determine matrix effects in extraction, equal concentrations of standard solutions were prepared in unspiked serum. The solutions were analyzed and the absolute signal areas and in pure acetonitrile/water (1/1 v/v; 10 mM ammonium formate aqueous solution). The solutions were analyzed consecutively and the absolute signal areas in the two chromatograms were compared. The matrix effect, the possible enhancement or suppression of ionisation induced by the endogenous substances, was assessed by comparing the peak areas of standards spiked after post-extraction matrix with standards in the pure solution. Recovery (RE), matrix effect (ME) and process efficiency (PE) are calculated according to the following equations of reported method by B. K. Matuszewski [45], and the results are listed in Table 1. The peak areas obtained in neat solution standards are defined as *A*, the corresponding peak areas for standards spiked after extraction into serum extracts are defined as *B*, and peak areas for standards spiked before extraction are defined as *C*, the ME, RE, and PE values can be calculated as follows:

$$\mathrm{ME}(\%) = \frac{B}{A} \times 100\% \quad (1)$$

$$\mathrm{RE}(\%) = \frac{C}{B} \times 100\% \quad (2)$$

$$\mathrm{PE}(\%) = \frac{C}{A} \times 100 = \frac{\mathrm{ME} \times \mathrm{RE}}{100\%} \quad (3)$$

Table 1
Recovery, matrix effect and process efficiency ($n = 3$).

Analyte	ng mL^{-1}	RE[a] (%)	ME[b] (%)	PE[c] (%)
PFOS	0.5	89.02	82.17	73.15
	10	83.13	86.26	71.71
	200	91.43	87.26	79.78
	Mean	87.86	85.23	74.88
PFHxA	0.5	83.77	89.21	74.73
	10	88.26	82.13	72.49
	200	83.52	86.95	72.62
	Mean	85.18	86.10	73.28
PFOA	0.5	85.46	91.29	78.02
	10	84.16	89.37	75.21
	200	92.42	86.34	79.80
	Mean	87.35	89.00	77.68
PFNA	0.5	85.46	90.65	77.47
	10	83.25	84.39	70.25
	200	89.42	87.23	78.00
	Mean	86.04	87.42	75.24
PFDoA	0.5	89.36	88.79	79.34
	10	83.67	87.53	73.24
	200	90.17	81.38	73.38
	Mean	87.73	85.90	75.32
PFDeA	0.5	89.66	82.53	74.00
	10	88.24	81.18	71.63
	200	85.47	88.25	75.43
	Mean	87.79	83.99	73.69
PFTeA (I.S.)	10	89.68	84.32	75.62

A: the peak areas obtained in the pure solution standards; B: the corresponding peak areas for standards spiked after extraction into serum extracts; and C: the peak areas for standards spiked before extraction.

[a] Recovery was calculated by the following formula: RE (%) = C/B × 100.

[b] Matrix effect was calculated by the following formula: ME (%) = B/A × 100.

[c] Process efficiency was calculated by the following formula: PE (%) = C/A × 100.

Table 2
Linearity, LOD, LOQ and accuracy, and absolute recovery of the analytical method.

Analyte	LOD ($ng\,mL^{-1}$)	LOQ ($ng\,mL^{-1}$)	Linear range ($ng\,mL^{-1}$)	The calibration curves	Linearity (R^2)	Accuracy ($ng\,mL^{-1}$)	(%)
PFOS	0.05	0.16	0.25–1000	$y=(1.11\pm0.06)\times10^5x-(6.25\pm0.04)\times10^4$	0.9938	10.0	98
PFHxA	0.04	0.15	0.25–1000	$y=(1.72\pm0.02)\times10^3x-(5.40\pm0.09)\times10^4$	0.9952	10.0	102
PFOA	0.04	0.12	0.25–1000	$y=(1.06\pm0.05)\times10^5x-(8.28\pm0.02)\times10^4$	0.9971	10.0	96
PFNA	0.05	0.17	0.25–1000	$y=(1.30\pm0.02)\times10^5x-(1.34\pm0.24)\times10^5$	0.9967	10.0	94
PFDeA	0.02	0.09	0.25–1000	$y=(1.08\pm0.11)\times10^5x+(1.31\pm0.31)\times10^4$	0.9982	10.0	103
PFDoA	0.03	0.11	0.20–1000	$y=(3.60\pm0.12)\times10^3x-(2.28\pm0.22)\times10^3$	0.9975	10.0	105

Accuracy, LOD and recovery: $n=5$.

The ME calculated may be referred to as an matrix effect since the signal response of the standard present in the serum extract is compared to the response of a standard made directly in a neat mobile phase [45]. The matrix effect assessment that is highly relevant for the development of selective LC–MS/MS methods. The mean matrix effect at concentrations of PFOS, PFHxA, PFOA, PFNA, PFDoA, PFDeA (0.5, 10, 200 $ng\,mL^{-1}$) and I.S. were 85.23%, 86.10%, 89.00%, 87.42%, 85.90%, 83.99% and 84.32%, respectively, which is well within acceptable limits (<20%). The results suggested that the presence of a minimal matrix effect indicated the selective extraction using synthesized Fe_3O_4@$mSiO_2$-F_{17} microspheres was more satisfactory. The proposed method was able to reduce the matrix effect and achieve the LLOQ signal intensity.

3.4. Validation of the method

3.4.1. Accuracy and sensitivity

Standard reference materials for determination of PFCs in human serum were not available; therefore, the accuracy of the method were assessed using serum samples fortified at some concentration levels, such as 10.0 $ng\,mL^{-1}$ for each analyte containing 5 $ng\,mL^{-1}$ of the I.S. Method calibration accuracy requirements of 100 ± 25% were met for all analytes. Similarly, the method accuracy (94–105%) was acceptable for all target analytes. The results are shown in Table 2, where limits of detection (LOD), quantification (LOQ), and range and slope of method linearity are also reported. The LOQ values were calculated on the basis of S/N = 3, and the values of all the analytes were from 0.09 to 0.17 $ng\,mL^{-1}$. On the basis of S/N = 10, the LOD value of the analytes were from 0.02 to 0.05 $ng\,mL^{-1}$.

3.4.2. Selectivity

The selectivity of the surface of the Fe_3O_4@$mSiO_2$-F_{17} microspheres was investigated. For this purpose, 100 μL of serum was spiked with six targeted perfluorinated compounds (PFOS, PFHxA, PFOA, PFNA, PFDeA and PFDoA, each 2.5 $ng\,mL^{-1}$), two nonperfluorinated straight chain fatty acids (octanoic acid, decanoic acid, each 1 $\mu g\,mL^{-1}$) and PFTeA (2.5 $ng\,mL^{-1}$) as I.S. The spiked serum sample was then pretreated using Fe_3O_4@$mSiO_2$-F_{17} microspheres. The captured analytes were then eluted from the Fe_3O_4@$mSiO_2$-F_{17} microspheres by eluting solvents and were subsequently analyzed by LC–MS/MS. According to the results (Fig. S10), despite the mass ratio of nonperfluorinated straight chain fatty acid to perfluorinated compound was 400:1, only targeted perfluorinated compounds could be detected after extraction, indicating a strong fluorous affinity of the Fe_3O_4@$mSiO_2$-F_{17} microspheres.

3.4.3. Linerary and precision

The LC–MS/MS chromatograms (MRM mode) of the seven PFCs standard mixture spiked at concentrations 5.0 $ng\,mL^{-1}$ are given in Fig. S11. The validation model was built based on the literature researches [46–48]. The method validations such as linearity, accuracy, recoveries, precision and limit of detection were studied. A good linearity was obtained for all target compounds, with a coefficient of determination (R^2) more than 0.993 indicating good fits. Absolute recoveries were estimated on the basis of five replicates of human serum samples fortified at three concentration levels under the optimized condition. PFCs recoveries ranged between 83% and 92%. The values expressed as relative standard deviation (RSD), were between 2.6% and 14.2% for all the PFCs. The intra-day variability was examined six times within 1 day at three different concentrations (0.5, 10, 200 $ng\,mL^{-1}$) and the result showed that the RSD of intra-day variability was in the range of 2.6–12.2% (Table 3). The inter-day precision was calculated from nine trials over 3 days for each concentration and the results show in the range of 5.2–14.2% for the compounds as displayed in Table 3. The results showed that the proposed method based on the novel magnetic micro solid-phase extraction technique was reliable and efficient.

3.5. Comparison with other methods

To illustrate the advantages of Fe_3O_4@$mSiO_2$-F_{17} microspheres as a novel extraction material, the comparative study of our developed method with other reported sample preparation procedures [17–24]

Table 3
Precision of the analytical method for PFCs measured with Fe_3O_4@$mSiO_2$-F_{17} microspheres based on LC–MS/MS.

Analyte	Intra-day precision ($n=6$)			Inter-day precision ($n=6$)		
	RSD%			RSD%		
	0.5 ($ng\,mL^{-1}$) (Low)	10 ($ng\,mL^{-1}$) (Medium)	200 ($ng\,mL^{-1}$) (High)	0.5 ($ng\,mL^{-1}$) (Low)	10 ($ng\,mL^{-1}$) (Medium)	200 ($ng\,mL^{-1}$) (High)
PFOS	8.4	6.2	2.6	9.2	5.2	5.5
PFHxA	9.7	8.3	4.1	7.6	9.2	7.9
PFOA	13.1	11.5	9.4	12.2	8.4	6.2
PFNA	12.5	10.2	7.2	13.0	11.6	9.3
PFDeA	13.1	12.2	9.5	14.2	8.4	8.8
PFDoA	9.9	7.3	5.4	13.1	13.7	5.8

Table 4
Comparison of different methods in the analysis of PFCs in human biological samples and comparison with other published data.

Method	Samples type	Sample preparation procedure	LOD (ng mL^{-1})	Linearity (ng mL^{-1})	Ref.
HPLC–MS/MS	Human serum	SPE with Fe_3O_4@mSiO_2-F_{17} microspheres	0.03–0.05	0.25–1000 (0.20–1000 for PFDoA)	Proposed method
HPLC–MS/MS	Human serum and milk	SPE cartridges with Oasis-HLB column (Waters)	0.1–0.4	/	[17]
HPLC–MS/MS	Human breast milk	SPE cartridges with the Oasis WAX	0.3–9	2–100	[18]
HPLC–MS/MS	The serum and milk of breastfeeding women	Oasis-HLB SPE column	0.05–0.1	/	[19]
HPLC–MS/MS	Human serum and breast milk	The Oasis WAX SPE columns	0.13–0.16	/	[20]
HPLC–MS/MS	Human serum and breast milk	Organic solvents added for protein precipitation, centrifuged for the supernatant	0.03–0.3 (serum) 0.01–0.07 (milk)	0.4–1000 (serum) 0.15–100 (milk)	[21]
UPLC–MS/MS	Human liver and milk samples	SPE cartridges with Oasis® WAX (Waters)	0.02–0.77	/	[22]
UPLC–MS/MS	Human milk	SPE cartridges with Oasis® WAX (Waters)	0.01–0.03	0.005–5	[23]
LC–QqLIT–MS	Breast milk and commercial baby food	SPE cartridges with Oasis HLB and Strata	0.001–0.005 (human breast milk)	0.021–0.907 (0.021–1.289 ng/kg)	[24]

Table 5
Concentrations (ng mL^{-1}) of selected PFCs measured in human serum samples collected from volunteer donors in Shanghai, in May 2013 from 10 adults.

Sex	Age	PFOS	PFHxA	PFOA	PFNA	PFDeA	PFDoA
F	65	20.42 ± 0.03	<LOQ (0.15)	3.90 ± 0.07	2.43 ± 0.05	0.91 ± 0.06	1.52 ± 0.02
M	58	30.12 ± 0.07	1.41 ± 0.06	5.21 ± 0.06	3.73 ± 0.04	<LOQ (0.09)	2.01 ± 0.04
F	41	22.20 ± 0.05	0.83 ± 0.07	1.10 ± 0.08	5.60 ± 0.05	1.32 ± 0.07	1.41 ± 0.09
F	49	13.20 ± 0.04	<LOQ (0.15)	1.42 ± 0.07	4.10 ± 0.12	0.72 ± 0.03	<LOQ (0.11)
F	59	32.71 ± 0.06	<LOQ (0.15)	10.81 ± 0.02	2.12 ± 0.06	1.51 ± 0.05	<LOQ (0.11)
M	60	22.03 ± 0.08	1.61 ± 0.16	<LOQ (0.12)	2.01 ± 0.04	<LOQ (0.09)	<LOQ (0.11)
M	66	64.10 ± 0.05	0.72 ± 0.08	1.03 ± 0.03	3.52 ± 0.06	<LOQ (0.09)	<LOQ (0.11)
F	57	3.32 ± 0.06	2.02 ± 0.05	12.50 ± 0.04	3.23 ± 0.13	<LOQ (0.09)	0.17 ± 0.10
M	22	55.20 ± 0.07	5.11 ± 0.07	27.32 ± 0.01	0.83 ± 0.04	1.82 ± 0.06	1.32 ± 0.05
M	52	9.30 ± 0.02	<LOQ (0.15)	12.20 ± 0.10	9.62 ± 0.09	1.21 ± 0.07	<LOQ (0.11)

<LOQ indicates that the concentration was below the limit of quantification.

is preformed and the results are shown in Table 4. As presented in Table 4, the LODs of the proposed method were 0.03–0.05 ng mL^{-1}. These values, which reflect the good sensitivity, are substantially lower than the detection limits achieved with the commercial Waters Oasis HLB [17,19] and WAX [18,20] SPE extraction columns reported in other papers. This method realized wide linear limit, high sensitivity, precision which are also superior to or comparable with the commercially available columns. It can be seen that the developed method is rapid and convenience, sensitive and low consumption in the sample and organic solvents in the pretreated process. The developed method for exact quantitation of PFCs, which feature straightforward and reduced sample handling (thus reducing risk of contamination and loss of analytes) is necessary for large-scale human biomonitoring.

3.6. Determination of PFCs in human serum

Inspired by those satisfying enrichment results above, we further investigated the feasibility of the Fe_3O_4@mSiO_2-F_{17} microspheres for the extraction of PFCs in real human serum and the applicability of the validated method. For this purpose, ten human serum samples were analyzed. The detected concentrations of six targeted PFCs are listed in Table 5. PFOS (3.32–64.10 ng mL^{-1}), PFOA (0.12–27.32 ng mL^{-1}) were detected in all 10 samples, however, PFHxA (0.15–5.11 ng mL^{-1}), PFNA (0.83–9.62 ng mL^{-1}), PFDeA (0.09–1.82 ng mL^{-1}) and PFDoA (0.11–2.01 ng mL^{-1}) were found less frequently and at lower concentrations. The concentration levels and order of PFOS, PFOA, PFNA and PFDeA were in accordance with human serum levels previously reported [17,19–21]. Our results suggested that exposure to long-alkyl-chain perfluorocarboxylates may be widespread.

In the proposed method, the synthesized Fe_3O_4@mSiO_2-F_{17} microspheres were successfully applied as adsorbents to determine six PFCs in human serum. Under the optimized conditions, the total time consisting of extraction with Fe_3O_4@mSiO_2-F_{17} microspheres as absorbents and chromatographic analysis by LC–MS/MS is 21 min. Moreover, it proved the suitability of the technique to assess the relevance of environmental human exposure to PFCs, and it will also be helpful for future biomonitoring study of a more broad range of PFCs exposure in biological sample.

4. Conclusions

Due to the fluorous affinity of the synthesized Fe_3O_4@mSiO_2-F_{17} microspheres (the interior-walls decyl-perfluorinated functionalized magnetic mesoporous), small molecules like PFCs can enter the mesopore channels and be efficiently absorbed through high-selective preconcentration by interior F-groups. Macromolecules like proteins are excluded from the mesopore channels as a result of size exclusion effect, leading to direct extraction of PFCs from protein-rich biosample. Moreover, PFCs adsorbed Fe_3O_4@mSiO_2-F_{17} microspheres could be simply and rapidly isolated through placing a strong magnet on the bottom of container, and then could be easily eluted from Fe_3O_4@mSiO_2-F_{17} microspheres for further LC–MS/MS analysis in the negative electrospray ionization mode (ESI(−)). To summarize, the main advantages of the proposed technique are highly selective extraction and enrichment with magnetic F-SPE, and

rapid analysis by LC–MS/MS. The proposed method based on Fe_3O_4@mSiO_2-F_{17} microspheres is proved to be efficient and authentic for analysis of PFCs in human serum samples. It is a potential tool for the assessments of PFCs exposure in other biological samples such as plasma, milk, food and so on.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.2014.07.032.

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