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Determination of perfluorooctanoic acid and perfluorooctane sulfonate in cooking oil and pig adipose tissue using reversed-phase liquid–liquid extraction followed by high performance liquid chromatography tandem mass spectrometry

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ABSTRACT

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) are two perfluorinated compounds (PFCs) ubiquitously present in the environment, which could pose potential adverse effects on human health. Contamination and presence of PFOA and PFOS should be eliminated or rigidly restricted in food stuffs such as cooking oils and lard (from pig adipose tissue). This work describes a rapid, simple, reliable and sensitive method for quantitative analysis of PFOA and PFOS in cooking oils and pig adipose tissue with liquid chromatography tandem mass spectrometry (LC–MS/MS). The pretreatment mainly included a one-step reversed-phase liquid–liquid extraction using the mixture of basified water/methanol as the aqueous system, and dichloromethane (DCM) as the non-polar system. PFOA and PFOS can be successfully separated from the two lipid-rich matrices, i.e., cooking oil and adipose tissue, and extracted into the aqueous system, and then directly analyzed with LC–MS/MS. This method was validated in terms of accuracy (both intra- and inter-batch), precision, recovery, linearity, sensitivity and applicability. The intra-batch accuracies for PFOA and PFOS in cooking oil samples were within 93.9–101.9% with relative standard deviation (RSD) no more than 10.9%, and the inter-batch accuracies were 91.2–96.2% with RSD not exceeding 10.0%. The intra-batch accuracies of the analytes in pig adipose tissue samples were 102.9–113.0% with RSD of 8.8–13.1%. And the quantification ranges of PFOA and PFOS were 0.01–25 ng/mL. This method has been applied to the analysis of PFOA and PFOS in real samples collected from local markets in Guangzhou, China.

1. Introduction

Perfluorinated compounds (PFCs), also named as perfluorinated alkylated substances (PFAS), are a group of synthetic chemicals that have been widely used in various domestic and industrial products [1], including stain repellents, waxes, textile, adhesives, polishes, paints, electronics and food packaging [2,3]. It was reported that about 7300 tons of PFCs were released into the environment since more than 60 years ago when they were firstly manufactured [2]. As a result, PFCs have been reported to be ubiquitous in the environment. Besides, they were also found in human blood and liver [4]. PFCs are not readily metabolized or degraded due to the strong strength of the carbon-fluorine bonds in their structures [5]. In addition, they have been reported to be bioaccumulative and biomagnifiable [6], particularly those with eight or more fluorinated carbons, such as perfluorooctane sulfonate (PFOS) [7].

Perfluorooctanoic acid (PFOA) and PFOS have attracted more concerns relative to other PFCs due to their adverse effects to human health as well as the large consumption and production. The toxicity and adverse effects of PFOA and PFOS in terms of hepatotoxicity, developmental toxicity, immune-toxicity, hormonal effects and carcinogenic potency have been well studied [8–13]. PFOA and PFOS are frequently detected in a variety of food products, beverages, biological tissues and water, e.g., sea food [14,15], meat [16,17], fish [16], packaged food [16,18], blood and liver of farm

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animals [19], vegetables [17], milk [17,20], fruits [21], pulses [21], cereals [21], eggs [21], tea [22] and drinking water [23]. Intake and risk assessment of PFOA and PFOS via food and drinking water have also been extensively studied [16,21,24–29]. However, to our knowledge, only a few studies have reported the data about PFCs in adipose tissue of animals [30,31], and no study has been devoted to the PFC contamination in cooking oil yet.

Cooking vegetable oils are daily consumed food, and adipose tissue of some livestock (e.g. pig) has been used for refining edible cooking oil or consumed as other meat food for a very long time in some regions [32]. Accordingly, cooking oil and edible adipose tissue of livestock should be free of hazardous substances, e.g., mycotoxins and persistent organic pollutants (POPs) [33,34], or with restricted concentration of them. Previous publications have reported several toxic POPs in cooking oils [35,36] and pig adipose tissue [37]. PFOA and PFOS may also be present in cooking oils and edible adipose tissue of livestock. Therefore, a reliable and feasible analytical method for the determination of PFOA and PFOS in cooking oils and edible adipose tissue of livestock is needed in order to reveal the contamination of PFOA and PFOS in these food products.

The long-chain fluorinated carbon backbone presents highly hydrophobic nature for PFOA and PFOS. And these two compounds exhibit relatively low acid-dissociation values (pK_a) due to the strong electronegativity of fluorine atoms, which may lead to hydrophilic property. The hydrophobic nature of the fluorinated carbon backbone and the hydrophilic property of the anionic functional groups contribute to the surfactant properties of these compounds, creating amphiphilic molecules which exhibit repellency and stain resistance for both water and oil [38,39]. The physicochemical properties of PFOA and PFOS suggest the relatively high water solubility (higher than 0.5 g/L for PFOA and PFOS) and low sorption to organic matter of these compounds [40].

So far, the main-stream pretreatments for the analysis of PFCs generally involve solid phase extraction (SPE) purification, which mainly use weak anion exchange (WAX) cartridges [41]. Meanwhile, very few studies have concerned the analytical methods for detecting PFCs in adipose tissue. Greaves et al., used the mixture of 10-mM potassium hydroxide in acetonitrile/water (80:20, v/v) for homogenizing adipose tissue of polar bears and cleared up the extracts with WAX cartridges [30,31].

In this work, we proposed a rapid and simple method for analyzing PFOA and PFOS in cooking vegetable oils and pig adipose tissue using a reversed-phase extraction coupled with LC–MS/MS detection. The developed and validated method was further applied to the analysis of PFOA and PFOS in cooking vegetable oils and pig adipose tissue collected from local markets in Guangzhou, China.

2. Experimental

2.1. Chemicals and materials

Standards PFOA (100 μg/mL in MeOH) and PFOS (100 μg/mL in MeOH) were bought from AccuStandard, Inc. (New Haven, CT, USA). The isotopically labeled internal standards (ISs) $^{13}C_4$-PFOA (50 μg/mL in MeOH) and $^{13}C_4$-PFOS (50 μg/mL in MeOH) were purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada). MeOH, DCM and acetonitrile (ACN) were of HPLC grade and purchased from Merck Crop. (Darmstadt, Germany). HPLC-grade ethyl acetate (EtAc) and ammonium acetate (NH_4Ac) were bought from J&K Scientific Ltd. (Beijing, China). Hydroxide ammonia (NH_3H_2O) was of HPLC grade and obtained from Tedia Inc. (Fairfield, OH, USA). The water used in this study was of ultrapure grade (with electrical resistivity of 18.2 MΩ cm) and produced with a Millipore water purification system (Millipore Corporation, Bellerica, MA, USA).

2.2. Working solutions

A cocktail solution containing both PFOA and PFOS with the respective concentration of 5 μg/mL was prepared by diluting the standards with MeOH. Calibration working solutions and quality control (QC) working solutions were prepared by serial dilution with MeOH/H_2O (1:1, v/v) from the cocktail solution. The IS working solution containing $^{13}C_4$-PFOA and $^{13}C_4$-PFOS with the respective concentration of 500 ng/mL was prepared by diluting the ISs with MeOH/H_2O (1:1, v/v).

2.3. Samples

Two types of cooking vegetable oil, i.e., peanut oil and mixed vegetable oil were purchased from local supermarkets in Guangzhou. Six pig adipose tissue samples were purchased from local food markets. All the samples were taken from the subcutaneous fat of the slaughtered pigs. These samples were skinned, cut up and weighted immediately after they were carried into laboratory. The cooking oil samples selected in this study only covered single vegetable oil (peanut oil) and mixed vegetable oil, of which the main content was fatty acid glycerides (99.9%). For peanut oil, the main composition fatty acid glycerides include polyunsaturated fatty acid glycerides, monounsaturated fatty acid glycerides and saturated fatty acid glycerides, with the ratio of 38.3%, 40.0% and 18.5%, respectively. The mixed vegetable oil contained soybean oil, corn oil, sunflower seed oil, colza oil, peanut oil, linseed oil, and safflower oil, and the individual proportion of each kind of oil was not available. According to the specification of this mixed vegetable oil, the composition ratio of polyunsaturated fatty acid glycerides, monounsaturated fatty acid glycerides and saturated fatty acid glycerides was 1:1:1. And the main composition of the pig adipose tissue is saturated fatty acid glycerides.

The calibration samples were prepared by diluting the calibration working solutions into MeOH/H_2O (1:1, v/v) and the concentrations were 25.0, 5.00, 2.50, 0.500, 0.250, 0.0500, 0.0200 and 0.0100 ng/mL for each level, respectively. The QC samples (QCs) were prepared by spiking the QC working solutions in the pooled matrix of cooking vegetable oils and the mixture of pig adipose tissue. The fortified concentrations were 4.00 ng/mL and 2.00 ng/mL for cooking oil QCs and pig adipose tissue QCs, respectively.

2.4. Sample extraction

2.4.1. Cooking oil

Aliquot 6 μL of IS working solution (500 ng/mL) was added into each polypropylene tube (2-mL). For blank samples, only 6 μL of MeOH/H_2O (1:1, v/v) was added. Then, 300 μL of cooking oil sample was placed into the tube and vortex-mixed well with a Polytron® PT 1200E homogenizer (Kinematica, Lucern, Switzerland), followed by the addition of 600 μL of basified MeOH/H_2O (1:1, v/v, containing 0.5% NH_3H_2O) and 600 μL of DCM, successively. The mixture was then vortex-mixed for 5 min and centrifuged with a rotation rate of 12,000 rpm at 4 °C for 5 min using a Thermo Multifuge X3R centrifuge (Thermo Fisher Scientific, Waltham, MA, USA). After centrifugation, 500 μL of the clear supernatant was transferred to an amber glass injection vial. Ten μL of the extract was injected onto the LC–MS/MS system.

2.4.2. Pig adipose tissue

The sliced and weighted adipose tissue sample was placed into a 50-mL polypropylene centrifugal tube, followed by the addition of 2-fold volume (compared with the used adipose tissue) of EtAc. The sample was then sufficiently homogenized until all the adipose tissue was dissolved in the solvent EtAc. Then 300 μL of EtAc-dissolved sample was transferred into a 2-mL polypropylene

Table 1
Liquid chromatography gradient program.

Time (min)	Flow rate (μL/min)	Mobile phase A (%)	Mobile phase B (%)
0.00	350	72.0	28.0
3.00	350	10.0	90.0
4.00	350	10.0	90.0
4.10	350	0.0	100.0
5.10	350	0.0	100.0
5.20	350	72.0	28.0
8.00	350	72.0	28.0

tube containing IS working solution, and vortex-mixed well. The following steps were the same as those for the cooking oil samples.

2.5. LC–MS/MS analysis

The LC–MS/MS system was comprised of an Agilent 1290 HPLC (Agilent Technologies, Palo Alto, CA, USA) and an API-4000 Q-Trap mass spectrometry (Applied Systems, Forster City, CA, USA) equipped with an electrospray ionization (ESI) interface. Separation of PFOS and PFOA was performed with an Atlantis® dC18 (2.1 mm × 100 mm, 3 μm) column (Waters, Milford, MA, USA). The mobile phase consisted of ultrapure water containing 2 mM NH_4AC (A) and ACN (B). The gradient elution program and flow rate are provided in Table 1. The thermostatic column oven was kept at 25 °C and the auto-sampler was maintained at 10 °C. The optimized working conditions of mass spectrometry are provided as the following: ESI interface was operated in negative mode; curtain gas, gas 1 and gas 2 were set at 30, 50 and 50 units, respectively, and collision gas was set at medium; the resolution of Q1 and Q3 was set at unit (0.7 amu); source temperature was 500 °C and the ionization voltage was −4000 V. The data acquisition was performed with multiple reaction (MRM) mode. The optional MRM parameters including declustering potential, entrance potential, collision energy, and collision cell exit potential and dwell time are documented in Table 2. Nitrogen of high-purity grade was used for mass spectrometry. The instrument control, data acquisition and data processing were carried out with the software Analyst 1.6.0 (Applied Systems).

2.6. Method validation

2.6.1. Calibration, accuracy and precision

Quantification of PFOA and PFOS were carried out with internal standard method. Two sets of calibration samples, prepared with $MeOH/H_2O$ (1:1, v/v), were analyzed at the beginning and the end of each analytical batch, respectively. The two-set calibration samples were plotted to construct an eight-point calibration curve for each analyte with the weight factor of $1/x^2$ to calculate the concentration of the analytes.

The QCs were performed in triplicates in each batch to calculate the intra-batch accuracy and precision. The inter-batch accuracy and precision for cooking oil samples were evaluated with the experimental data obtained from two different analytical batches in two different occasions. The accuracy was calculated based on the following equation:

$$\text{Accuracy} = \frac{C_{QC} - C_{QC0}}{C_S} \times 100\% \quad (1)$$

where C_{QC} was the observed concentration in the QC sample; C_{QC0} was the detected concentration of QC0; C_S was the nominal spiked concentration. QC0 was the control blank sample which was prepared with the same matrix of the corresponding QCs and only IS working solution was spiked.

Precision was evaluated with relative standard deviation (RSD). For the acceptable calibration samples, the deviation of the corresponding accuracy should be within ±20%.

2.6.2. Limits of quantification and detection

The concentrations of the analytes in lower limit of quantification (LLOQ) samples were set as low as possible provided the instrumental sensitivity was high enough, and the quantification ranges were set as large as possible to cover the probably predicted concentrations after referencing the concentration levels of the two analytes reported in previous publication [30,31]. The ratio of the signal to noise (S/N) for LLOQ samples should be higher than 10. The instrumental limits of quantification (LOQs) of PFOA and PFOS were 0.01 ng/mL. The instrumental limit of detection (LOD) was defined as the concentration in the sample which can give rise to a signal with S/N value just no less than 3. If the concentration in the sample was calculated within the range of LOD to LOQ with software Analyst 1.6.0, then it was not reported, and the analytes were considered to be detectable but not quantifiable in the sample.

2.6.3. Recovery and carryover effect

The absolute recovery experiment was conducted with the isotope-labeled ISs, which exhibit the same physicochemical properties of the native analytes. The recovery experiment was carried out with the normal QCs and the calibration samples. The signal response of the internal standards $^{13}C_4$-PFOA and $^{13}C_4$-PFOS was used for calculating the recovery results. The recovery was calculated as the ratio of the IS signal response (peak area) in the QCs to that in the calibration samples. In fact, this recovery results combined the absolute recovery and matrix effect of the ISs. Because the ISs used in this study were isotopically labeled standards of the analytes, the recovery results of the ISs can be regarded as the recoveries of the native analytes.

The carryover effect was calculated with the ratio of the signal response at the retention time of the analyte in blank sample, injected just next to the highest-concentration calibration sample, to that in the LLOQ sample (0.01 ng/mL). The carryover effect should be no more than 20%.

3. Results and discussion

3.1. Sample preparation

Two types of cooking oil were used to conduct the method development in this study. For preparing QCs, these cooking oils were mixed and the mixture was used as the sample matrix. For the pig adipose tissue, the solvent EtAc with two-fold volume of the adipose tissue was added in the homogenizing tube along with the adipose tissue sample prior to homogenization. The Addition of EtAc during homogenization was necessary because EtAc could dissolve the adipose tissue to get a well homogenized mixture, which considerably simplified the pretreatment procedure. After homogenization, six samples were mixed well for preparing the sample matrix for QCs. The use of mixed samples for preparing the QCs would be useful to reduce the deviation of the analysis results.

In order to analyze PFOA and PFOS in cooking oils and adipose tissue, it was essential to separate the two analytes from the lipid-abundant samples. Based on the calculation with software MarvinSketch 5.12.4 and the reported data [42], the octanol–water partition coefficients (Log *Kow*) are from 2.50 to 5.11 for PFOA and 4.13 to 5.43 for PFOS, respectively, indicating that PFOA and PFOS may tend to be easily dissolved in non-polar solvents. Thus, it seemed difficult to separate PFOA and PFOS from the lipids which were abundant in the samples if merely non-polar solvents were

Table 2
Optimized mass spectrometric parameters for perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and their internal standards.

Analyte	Retention time (min)	MRM transition (*m/z*)	Declustering potential (DP, V)	Collision energy (CE, eV)	Dwell time (ms)	Entrance potential (EP, V)	Collision cell exit potential (CXP, V)
PFOA	3.34	**412.9 → 368.9**[a]	−36	−14	60	−10	−15
		412.9 → 168.6	−36	−24	30	−10	−15
$^{13}C_4$-PFOA (IS)	3.34	**416.9 → 372.0**	−38	−18	40	−6	−8
PFOS	3.78	**499.0 → 79.7**	−80	−104	60	−10	−15
		499.0 → 98.8	−80	−80	30	−10	−15
$^{13}C_4$-PFOS (IS)	3.78	**502.9 → 130.8**	−115	−66	40	−10	−10

[a] The MRM transitions with bold font were used for quantification.

used. However, the relatively low pK_a values enable PFOA and PFOS likely to be ionized in neutral or basic solutions, which may significantly enhance the solubility of these compounds in aqueous solution. Theoretically, if the partition factor of the ionized analytes in aqueous solution to non-polar solution is high enough, the analytes can be readily extracted by the aqueous solution from the non-polar solution or mixture system. The separation can be achieved by the optimization and selection of the aqueous solution system and the non-polar solution system. In this study, basified MeOH/H_2O (1:1, v/v, containing 0.5% NH_3H_2O) was used as the aqueous solution system to facilitate the ionization of PFOA and PFOS for enhancing their solubility in the aqueous compartment. Selection of the basified MeOH/H_2O (1:1, v/v) to conduct the reversed-phase extraction was based on the following considerations: (1) The aqueous compartment should have higher solubility for the analytes compared with that of the non-polar system containing high-ratio lipids and non-polar solvent; and (2) the aqueous solution system should be directly suitable for the following analysis using LC–MS/MS and therefore simplify the procedure and improve the work efficiency. DCM was selected as the solvent for the non-polar mixture system due to its hydrophobic properties. In addition, it can be conveniently separated from the aqueous solution. The extraction mechanism is concisely illustrated in Figure S-1.

The results showed that the absolute recoveries of $^{13}C_4$-PFOA and $^{13}C_4$-PFOS were satisfactory with the used extraction system, suggesting that PFOA and PFOS could also be successfully extracted from the cooking oil and adipose tissue as their isotopically labeled counterparts.

3.2. LC–MS/MS optimization

PFOA is one of the carboxylic compounds, which are liable to be dissociated in the collision-induced-dissociation process in mass spectrometer, giving rise to a relatively high-intensity characteristic decarboxylation product ion. In this work, the product ion used for the quantification of PFOA was also a decarboxylation ion. In addition, numerous carboxylic compounds are widely present in the environment, even in the solvents and devices of the laboratory. And some of these carboxylic compounds may possess the same molecular weight of PFOA, which would lead to the elevated base line and unknown interference peaks in the MRM transition. Therefore, the background interference caused by these carboxylic compounds would pose a big challenge for the analysis of PFOA using LC–MS/MS. Moreover, PFCs are widely used chemicals, which could result in heavy contamination issues [20]. It has been reported that PFCs could be present in HPLC system, such as chromatographic system tubes [41] and degasser membrane [43], which could result in interference that is difficult to be eliminated. To reduce this interference, several reagent blank samples (MeOH/H_2O, 1:1, v/v) were injected onto the LC–MS/MS system prior to the sample analysis. The signal response of PFOA and PFOS generated in the reagent blank samples was used to evaluate the background contamination. If the peak area of the analytes in the reagent blank samples was no more than 20% of that in the LLOQ samples (0.01 ng/mL), it could be concluded that the background contamination insignificantly influenced the accuracy of sample analysis. The signal response of the analytes caused by the background contamination in HPLC system decreased as the injections of the reagent blank samples increased. After about four injections of the reagent blank samples, the peak area of the analytes in the last reagent blank sample was less than 20% of that in the LLOQ sample. Additionally, PFOA and PFOS could possibly be adsorbed onto the surface of the pre-column peek pipeline and the packing particles of the chromatographic column. This problem was settled by the relatively high initial proportion of the organic mobile phase (28% ACN) and the relatively long-time elution with high-ratio ACN (90–100%) in this study (Table 1). After the injection of the highest-concentration calibration sample (25 ng/mL), two reagent blank samples were injected for reducing the deviation caused by the carryover effect.

An Atlantis® dC18 (2.1 mm × 100 mm, 3 μm) column was used for chromatographic separation to reduce potential interference caused by fat acids which are abundant in cooking oils and pig adipose tissue, and thus ensuring the chromatographic resolution. With the optimized LC working conditions, the chromatograms with insignificant interference and satisfactory peak shape were achieved for PFOA in all samples and PFOS in cooking oil samples (Fig. 1). A chromatographic peak, not baseline-separated from the peak of PFOS, was observed in pig adipose tissue samples, QCs and even calibration samples. This peak was identified to be generated by a side chain isomer of PFOS. This side chain isomer was very difficult to be separated from the target analyte, i.e., the normal PFOS. Fortunately, the presence of this side chain isomer could not negatively affect the experimental results in terms of accuracy, precision and linearity. It may be of some interest to conduct a further study to identify and quantify this isomer, thus elucidating its chemical structure and determining its concentration.

In consideration of the physicochemical properties of PFOA and PFOS including acidity, polarity and electronegativity, negative ESI interface was preferentially chosen as the ion source for ionization. The deprotonated molecules of PFOA and PFOS were selected as the precursor ions. The MRM transitions (Table 2) used for the quantification and qualification of the analytes were consistent with those in literature [44,45]. The product ion of PFOA with the *m/z* value of 368.9 was generated via the decarboxylation process of the precursor ion of PFOA. For PFOS, its product ion with *m/z* value of 79.7 was SO_3^-. The fragmental pathways for PFOA and PFOS in mass spectrometry are illustrated in Figure S-2. For the isotopically labeled IS $^{13}C_4$-PFOS, the product ion used for quantification was *m/z* 130.8, which contained a ^{13}C atom in the moiety. This used ion would be more specific compared with the common product ions (non-specific) i.e., *m/z* 79.7 and *m/z* 98.8, of the labeled and the native PFOS, which could be helpful to avoid the potential occurrence of crosstalk in the detection using MRM mode.

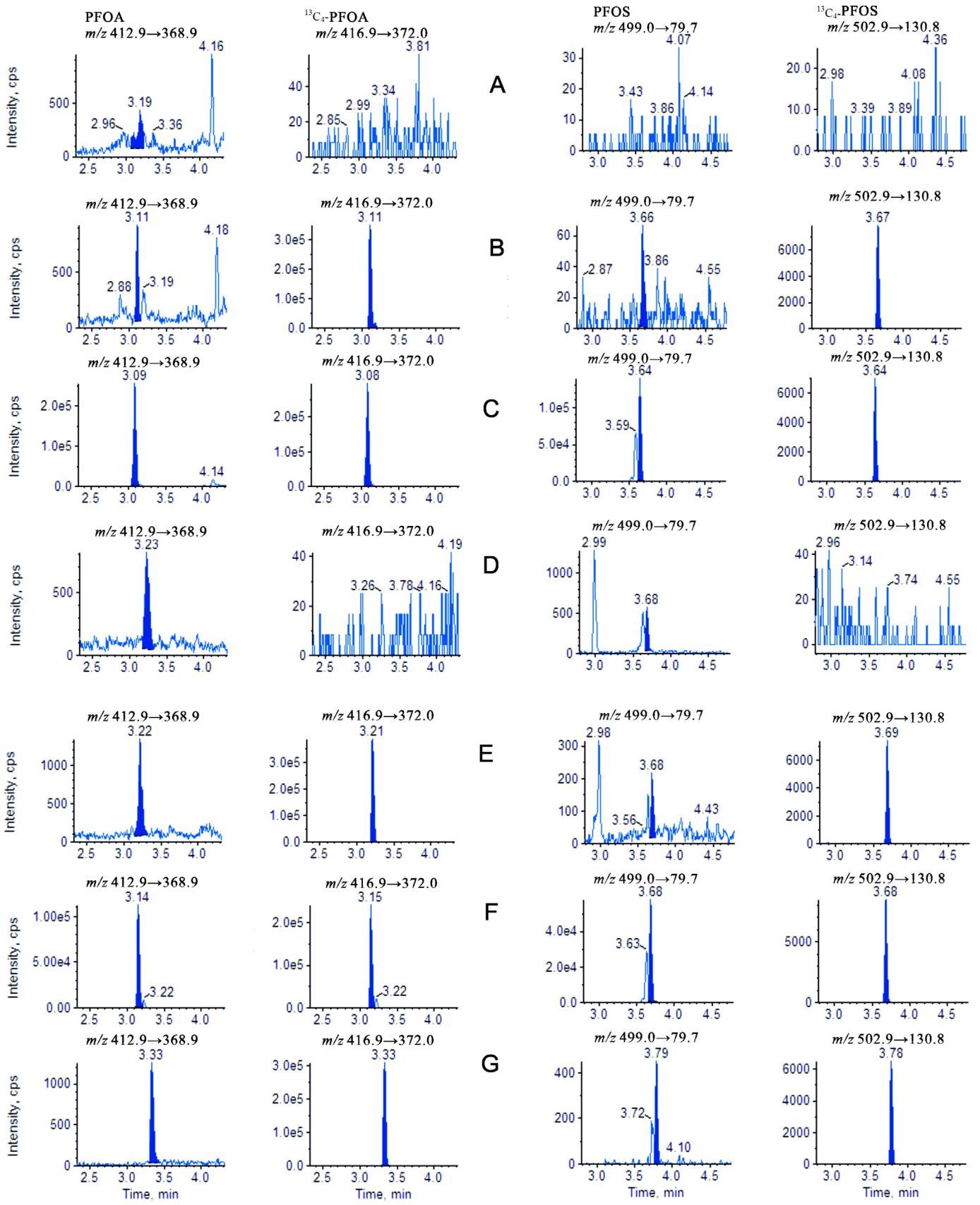

Fig. 1. Representative MRM chromatograms of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS) and the isotope-labeled counterparts. (A) Cooking oil blank sample, (B) cooking oil control blank sample (QC0), (C) cooking oil quality control sample (QC, 4.00 ng/mL), (D) adipose tissue blank sample, (E) adipose tissue control blank sample (QC0), (F) adipose tissue quality control sample (QC, 2.00 ng/mL), (G) lower limit of quantification sample (LLOQ, 0.01 ng/mL). The color-filled peaks represent the analytes and internal standards. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 3
Precision and accuracy of the assay for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) in cooking oil and pig adipose tissue with quality control samples.

Sample matrix	Analyte	Spiked concentration (ng/mL)	Intra-batch ($n=3$)			Inter-batch ($n=6$)	
			Mean calculated concentration (ng/mL)	Mean accuracy (%)	RSD (%)	Mean accuracy (%)	RSD (%)
Cooking oil	PFOA	4.00	4.08	101.9	6.2	96.2	10.0
	PFOS	4.00	3.75	93.9	10.9	91.2	9.6
Pig adipose tissue	PFOA	2.00	2.27	113.0	8.8	NA	NA
	PFOS	2.00	2.06	102.9	13.1	NA	NA

NA, not available.

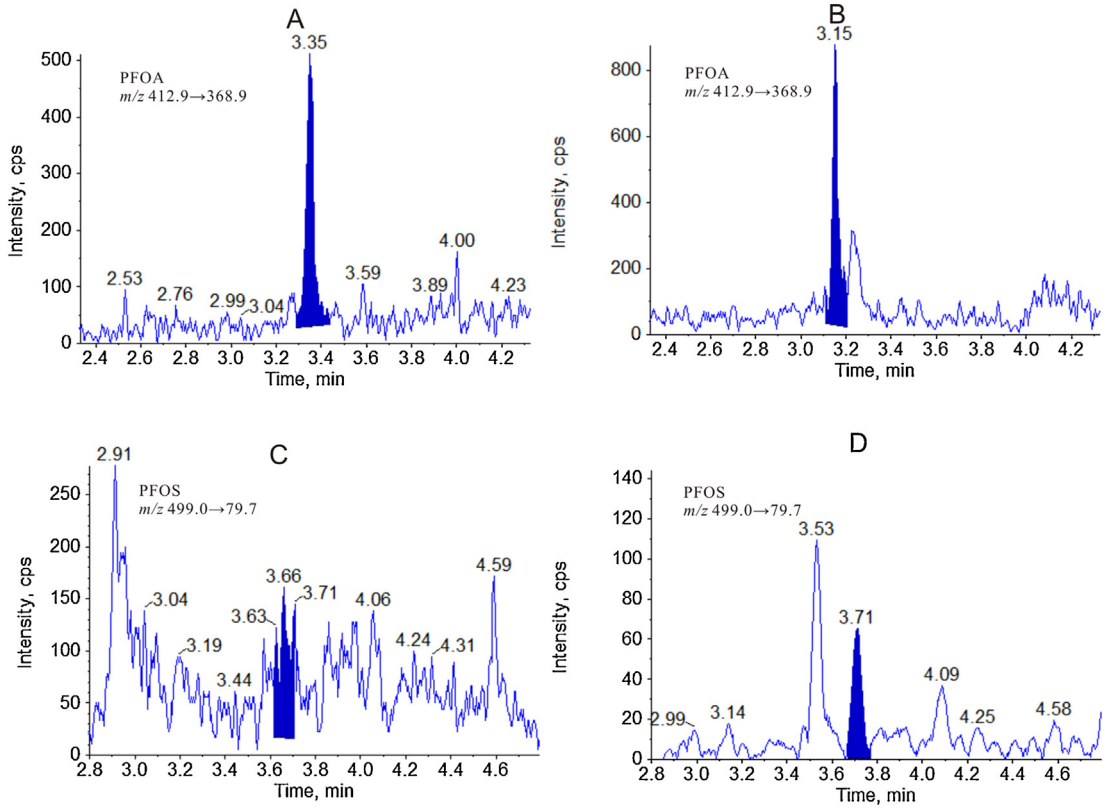

Fig. 2. Representative MRM chromatograms of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) in real samples. (A) PFOA in cooking oil sample, (B) PFOA in pig adipose tissue sample, (C) PFOS in cooking oil sample, and (D) PFOS in pig adipose tissue sample. The peaks of the analytes were color-filled. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3.3. Method validation

The linear ranges of PFOA and PFOS were 0.01–25 ng/mL. The instrumental LOQs of the analytes were 0.1 pg on column. The method LOQs of the analytes were 0.02 ng/mL for cooking oil and 0.06 ng/mL for pig adipose tissue, respectively. The intra-batch accuracies for PFOA and PFOS in cooking oil samples were 93.9–101.9% (RSD ≤ 10.9%) and the results of inter-batch accuracy for cooking oil samples were from 91.2% to 96.2% with RSD no more than 10.0%. The intra-batch accuracies obtained with pig adipose tissue samples were 102.9–113.0% with RSD within 8.8–13.1%. The results of the accuracy and precision are detailed in Table 3.

The recoveries obtained for $^{13}C_4$-PFOA and $^{13}C_4$-PFOS were 106.6–109.8% (RSD 8.8–23.3%) and 91.0–129.4% (RSD ≤ 8.8%) (Table 4), respectively, indicating good extraction efficiency as well as low matrix effect of this method.

3.4. Application to real samples

Two cooking oil samples and six adipose tissue samples were analyzed with the developed method. PFOA was detected (peaks

Table 4
Absolute recovery of isotopically labeled perfluorooctanoic acid and perfluorooctane sulfonate in cooking oil and pig adipose tissue.

Sample matrix	Compound	Spiked concentration (ng/mL)	Signal response in neat solution (mean peak area, counts, $n=6$)	Signal response in QC sample (mean peak area, counts, $n=6$)	Mean absolute recovery ($n=6$, %)	RSD ($n=6$, %)
Cooking oil	$^{13}C_4$-PFOA	5.00	545,000	581,000	106.6	23.3
	$^{13}C_4$-PFOS	5.00	10,600	13,700	129.4	8.1
Pig adipose tissue	$^{13}C_4$-PFOA	5.00	545,000	599,000	109.8	8.8
	$^{13}C_4$-PFOS	5.00	10,600	9640	91.0	5.5

observed with $S/N > 3$) but could not be quantified in all the samples (Fig. 2A and B). PFOS was only observed in some adipose tissue samples with the concentration lower than LOQ (Fig. 2D).

4. Conclusion

A simple, feasible and sensitive method has been developed for the rapid quantitative determination of PFOA and PFOS in cooking oil and pig adipose tissue using a simple reversed-phase liquid–liquid extraction followed by LC–MS/MS detection. Since PFOA and PFOS are two typical PFC species, this developed method can be extendedly applied to the analysis of other PFCs with carboxylic or sulfonic group in cooking oil and adipose tissue, or other lipid-rich matrices. The method has been evaluated in terms of accuracy (both intra- and inter-batch), precision, recovery, linearity and sensitivity, showing it is suitable and reliable for analyzing PFOA and PFOS in cooking oil and adipose tissue samples. The application of this method has been conducted with two groups of real samples collected from local markets in Guangzhou, China, indicating good applicability and suitability of this method to the real samples. PFOA was detected (but not quantified) in all the cooking oil and pig adipose tissue samples, and PFOS was detected (but not quantified) in some pig adipose tissue samples.

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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.chroma.2014.03.032.

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