Journal of Chromatography A, 1341 (2014) 50–56

Contents lists available at ScienceDirect

Journal of Chromatography A

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

# 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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ARTICLE INFO

*Article history:*
Received 18 December 2013
Received in revised form 8 March 2014
Accepted 11 March 2014
Available online 16 March 2014

*Keywords:*
Perfluorooctanoic acid
Perfluorooctane sulfonate
Reversed-phase liquid–liquid extraction
Cooking oil
Adipose tissue
Liquid chromatography tandem mass spectrometry

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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http://dx.doi.org/10.1016/j.chroma.2014.03.032
0021-9673/

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