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A Robust Method for Routine Analysis of Perfluorooctane Sulfonate (PFOS) and Perfluorohexane Sulfonate (PFHxS) in Various Edible Crop Matrices

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Abstract A reliable, sensitive, and efficient method was developed for routine analysis of perfluorooctane sulfonate (PFOS) and perfluorohexane sulfonate (PFHxS) in various edible crop matrices including cereal (grain), root vegetable (carrot), leafy vegetable (lettuce), and melon vegetable (pumpkin). The target analytes were extracted by ion-pair approach followed by solid-phase extraction clean-up and HPLC-MS/MS. The type of extraction solvent, clean-up cartridge, and the usage of Supelclean graphitized carbon were evaluated to reach an optimized pretreatment procedure. The matrix-matched standard calibrations relative to the isotope-labeled internal standard were used in the developed method to obtain more reliable quantitative results. The average recoveries at four spiked levels (0.5, 10, 25, 50 ng/g) in the diverse matrices ranged from 70.9 to 114.6% with relative standard deviations (RSD) lower than 11.5%. The matrix-dependent method detection limits using the common equipment (HPLC-MS/MS) were between 0.020 and 0.140 ng/g (dw), equivalent to the 3–130 pg/g (fw), corresponding to the sensitivity of superior equipment (e.g., UPLC-MS/MS and HPLC-QTOF-HRMS). Furthermore, the developed method was conferred with the practicality through determination of the analytes in actual crops sampled from several farms in China's Pearl River Delta.

Keywords Perfluoroalkyl sulfonic acids (PFSAs) · Edible crops · Ion-pair extraction · Solid-phase extraction clean-up · HPLC-MS/MS · Matrix effect

Introduction

Perfluorinated compounds (PFCs) are a kind of emerging organic pollutants arousing a growing concern because of their persistence, bioaccumulation, and high toxicity (Zabaleta et al. 2014). Perfluoroalkyl sulfonic acids (PFSAs) are a type of classical PFCs, consisting of a fully fluorinated hydrophobic carbon chain linked to a sulfonic acid group (Richardson 2011, Zabaleta et al. 2014). The special properties including amphiphilic character and inertness made PFSAs manufactured massively and applied widely in industrial applications and consumer goods (e.g., surfactants, fighting foam, food containers, clothing, adhesives, cosmetics) during the last six decades (Ullah et al. 2012). Large amounts of PFSAs introduced into environment have been widely detected in various environment media, wildlife, and humans (Ullah et al. 2012). PFSAs can produce various toxicities to animals and humans, such as birth defects, growth retardation, hormonal effects, immunotoxicity, and carcinogenicity (Young et al. 2013, Yang et al. 2015). Accordingly, concerns on PFSAs raised rapidly in the entire international community, especially on the perfluorooctane sulfonate (PFOS), which is one of the most frequently applied and toxic PFSAs with the longest persistence as the ultimate degraded products of perfluorooctanoic acid (PFOA) and other homologous PFCs (Poothong et al. 2012). Norway regulated the content of PFOS

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in textiles, impregnation agent, and fighting foam lower than 0.005% (Herzke et al. 2012). The European Union (EU) has prohibited the general use of PFOS and its derive since June 2008 (Zabaleta et al. 2014). In May 2009, PFOS was listed as "restricted use" persistent organic pollutants in Stockholm Convention. Sequentially, PFCs were proclaimed as emerging contaminant in the food chain by European Food Safety Authority (EFSA), and the tolerable daily intake (TDI) of PFOS was established as 0.15 μg/kg/day (Llorca et al. 2009). Moreover, EFSA recommended that the Member States should monitor the presence of PFOS and its derive in environment (Lacina et al. 2011). China is also a member state to Stockholm Convention, and thus a National Implementation Plan invested by 145.3 millions dollars to phase out PFOS in key industries (e.g., electroplating, pesticide, and fire-fighting industry) was initiated in China in 2015. Meanwhile, the criteria of PFOS including criteria maximum concentration (3.78 mg/L) and criteria continuous concentration (0.25 mg/L) for protection of aquatic organism in China was derived by Chinese scientist recently (Yang et al. 2014).

Owing to their water-soluble property as the ionic surfactants, PFSAs are readily absorbed and bio-accumulated in crops from contaminated soils with industrial discharge, land application of sludge, and the use of water and pesticides containing PFSAs (Stahl et al. 2009, Blaine et al. 2013, Lu et al. 2015). PFOS uptake in grain, potato, and rye grass was found directly proportional to soil pollution, and much more PFOS in vegetative compartments than in storage organs was observed (Stahl et al. 2009). Bioaccumulation factors (BCFs) of PFOS in crops varied greatly, with up to 3.8 of BCF reported (Yoo et al. 2011; Lecher et al. 2011). Besides the part uptake from the contaminated soils, PFSAs in crop origins foods could be derived from food processing or the surface-treated food contact materials (Picó et al. 2011). As a result, PFSAs could exert a great threat to human health via food chain of edible crops.

To date, very limited data are available on concentration, fate, and risk of PFSAs in soils and edible crops compared to those in water and aquatic organisms (Ericson et al. 2009; Haug et al. 2010; Houde et al. 2011; Feilizeter et al. 2014). This was likely ascribed to the fact that the analysis of PFSAs in soils and plants is challenging due to the influence of complicated matrices on analytical method (Richardson et al. 2011; Xiang et al. 2015; Yang et al. 2015). Moreover, previous analytical methods related to PFSAs in crops were based on limited crops other than various crops such as cereal, root vegetable, leafy vegetable, and melon vegetable (Ballesteros-Gómez et al. 2010; Ulah et al. 2012; Vestergren et al. 2012; Zabaleta et al. 2014). Meanwhile, matrix effect (ME) and its potential influencing factors that affected the accuracy and precision of an analytical method were hardly discussed (Ulah et al. 2012; Vestergren et al. 2012; Zabaleta et al. 2014). Thus, a reliable analytical method is urgently needed to be developed for routine analysis of PFSAs in various kinds of edible crops.

Both ion-pair extraction method (IPE) and solid-liquid extraction method (SLE) were often used to determine PFSAs in crops (Ullah et al. 2012; Felizeter et al. 2014), and thus they were compared to obtain the optimized extraction approach for the analytes in various edible crops. In terms of clean-up, solid-phase extraction was an effective and powerful technique to separation of PFSAs in the complex matrix samples such as sludge, vegetable, usually giving satisfied recoveries (Ullah et al. 2012; Martínez-Moral et al. 2013). In the aspects of samples, use of freeze-dried samples was more favorable than fresh ones due to reducing volume of both extractant and sample, and lowering interference of water content in fresh samples in the pretreatment procedures, etc. (Li et al. 2014; Xiang et al. 2015). As for the detection equipment, high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was the most commonly used to the analysis of PFSAs (Ballesteros-Gómez et al. 2010; Zabaleta et al. 2014). More recently, the superior analytical equipments such as ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and HPLC combined a quadrupole time-of-flight QToF Premier HRMS instrument (HPLC-QTOF-HRMS) were successfully used to detect PFSAs at pg/g levels (Ullah et al. 2012; Vestergren et al. 2012). But they were too expensive to be widely used in ordinary laboratory for routine analysis.

In the present study, two typical PFSAs including PFOS and perfluorohexane sulfonate (PFHxS) that is another one of the most used and detected PFSAs were selected as the analytes (Ullah et al. 2012; Vestergren et al. 2012). Sequentially, a robust method with high sensitivity and accuracy for routine analysis of the analytes in various edible crop matrices including cereal, root vegetable, leafy vegetable, and melon vegetable was successfully developed using the common analytical equipment (HPLC-MS/MS). Besides, the ME and its potential affecting factors in the developed method were discussed comprehensively.

Materials and Method

Materials

Standards perfluoro-n-octane sulfonate (PFOS) and perfluoro-n-hexane sulfonate (PFHxS), and isotopically labeled standards (ISs), i.e., perfluoro-n-[1,2,3,4-$^{13}C_4$]-octanoic sulfonate ($^{13}C_4$-PFOS), were bought from Wellington Laboratory (Ontario, Canada). The purities of the standards were ≥98%. HPLC-grade methanol, tetrabutyl ammonium hydrogen sulfate (TBA), methyl tertbutyl ether (MTBE), acetonitrile (ACN), ammonium hydroxide (NH_4OH), and tetrahydrofuran (THF) were purchased from Sigma-Aldrich (Steinheim,

Table 1 The optimal HPLC-MS/MS parameters for PFHxS and PFOS, and their internal isotopically labeled standards

Analytes	Retention (min)	Precusor ion	Product ion	IS used	DP	CE
PFHxS	7.73	399	80[a] /99	$^{13}C_4$-PFOS	−80	−75
PFOS	8.61	499	80[a] /99	$^{13}C_4$-PFOS	−70	−85
$^{13}C_4$-PFOS	8.68	515	80[a] /99	–	−45	−15

DP decluster potential, *CE* collision energy voltage

[a] Quantitative ion

Germany). Analytical grade sodium hydrogen (NaOH) and sodium hydrogen carbonate ($NaHCO_3$) were purchased from Guangzhou Chemical Reagent Co., Ltd. (Guangzhou, China). Florisil cartridges (6 mL, 1000 mg), Oasis WAX cartridges (6 mL, 150 mg), and Oasis HLB (6 mL, 150 mg) were purchased from Waters Corporation (Milford, MA). The solid-phase extraction (SPE) instrument with 24-port vacuum manifolds was obtained from Sigma-Aldrich (St. Louis, MO). Supelclean graphitized carbon (ENVI-Carb) was bought from Supelco (Bellefonte, PA, USA). Acrodisc LC13 GHP Pall filter (0.2 μm) was bought from Pall Corp (Port Washington, NY, USA). Ultrapure water was prepared by a Unique-R20 equipment (Research Scientific Instruments Corporation, Xiamen, China) and used in the entire experiment.

Mixture stock solution (1000 μg/L) of PFHxS and PFOS was dissolved in methanol and then kept in a refrigerator at 4 °C for use in 1 month. Working standard solutions of the two standards at seven concentrations (i.e., 0.5, 1, 2.5, 5, 10, 25, 50 μg/L) for calibrations were gained by diluting the stock solution using methanol. Various edible crops including cereal (grain), root vegetable (carrot), leafy vegetable (lettuce), and melon vegetable (pumpkin) were chosen to assess the ME of PFHxS and PFOS in crops, considering the effect of crop matrices on the analyte responses to detector (Li et al. 2014; Xiang et al. 2015). Accordingly, matrix-matched standard solutions at seven concentrations including 0.5, 1, 2.5, 5, 10, 25, and 50 ng/mL were prepared by diluting the standard solution with the extract of each edible crop. All the edible crops used to develop the analytical method were free of the target analytes, which were bought from organic farms in Guangzhou in October 2015.

Recommended Sample Preparation

Spiked Sample Preparation

About 2 kg of each crop sample was homogenized using a crusher (Jiu Yang Co., Ltd., China) after washed by tap water and ultrapure water in turn. These homogenized samples were lyophilized using a vacuum freeze drier (Jiangsu Hengfeng equipment manufacture Co., Ltd., China), ground to powders (0.45 mm) in a mill, and then stored in brown glass bottles in a refrigerator at 4 °C. The spiked samples at four spiked levels (0.5, 10, 25, 50 ng/g) were obtained by delivering 1 mL of mixed standard working solutions at 0.25, 5, 12.5, and 25 ng/mL in methanol to the lyophilized powder samples (0.5 g), respectively. After homogenized for 2 h, the spiked samples were kept on in a fume hood at room temperature for releasing the solvent (methanol).

Extraction

The analytes were extracted by ion-pair extraction method. Briefly, half a gram of each lyophilized sample powder was weighed and added into a 50 mL of polypropylene centrifugal tube, and then 50 μL of internal standard with 100 ng/mL of $^{13}C_4$-PFOS was spiked to the each sample powder. After equilibrium for half an hour, 0.2 mL of NaOH (0.5 M) was added to the each sample to release analytes from the sample matrices, and then the samples were mixed in a MS3 digital vortex (IKA group, German) for 2 min and left for 8 h. Afterwards,

Table 2 Matrix-matched calibrations and solvent calibration of PHxS and PFOS

Analyte	Matrix	Linear range (ng/mL)	R^2	Slope ratio (matrix/methanol)	IDL (ng/mL)	MDL (ng/g, dw)	MDL (ng/g, fw)
PFHxS	Methanol	0.5–50	0.999	–	0.08	–	–
	Lettuce	0.5–50	0.999	1.19	–	0.067	0.003
	Pumpkin	0.5–50	0.998	0.74	–	0.067	0.015
	Carrot	0.5–50	0.999	2.42	–	0.020	0.004
	Grain	0.5–50	0.999	1.55	–	0.033	0.031
PFOS	Methanol	0.5–50	0.999	–	0.08	–	–
	Lettuce	0.5–50	0.997	1.03	–	0.120	0.012
	Pumpkin	0.5–50	0.999	1.45	–	0.150	0.035
	Carrot	0.5–50	0.999	1.10	–	0.100	0.020
	Grain	0.5–50	0.998	1.11	–	0.140	0.130

dw dry weight, *fw* fresh weight

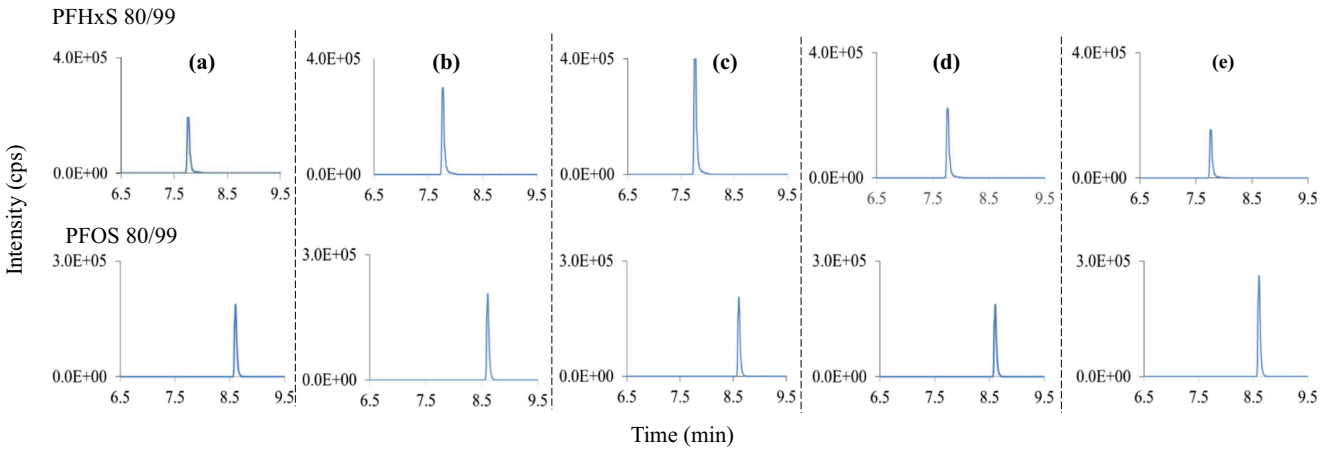

Fig. 1 Typical HPLC-MS/MS chromatograms of PFHxS and PFOS in methanol standard solution at 5 ng/mL (**a**), grain (**b**), carrot (**c**), lettuce (**d**), and pumpkin (**e**) spiked at 5 ng/g

2 mL of 0.25 M TBA used as ion-pairing agent and 4 mL of Na_2CO_3/$NaHCO_3$ buffer (pH = 10 adjusted by 1 M NaOH) was added to each the sample and then vortex mixed for 2 min. Five milliliters of MTBE used as extractant was added, vortex mixed again for 1 min, sonicated for 10 min, and then centrifuged at 8000 rmp for 10 min. The supernatant of each sample (MTBE layer) was transferred to a 15 mL of polypropylene tube, and the extraction was repeated twice again with 5 mL MTBE for each time. The combined extracts of the each sample were concentrated to near dryness using a gentle stream of dry nitrogen gas and then redissolved in 1 mL of methanol.

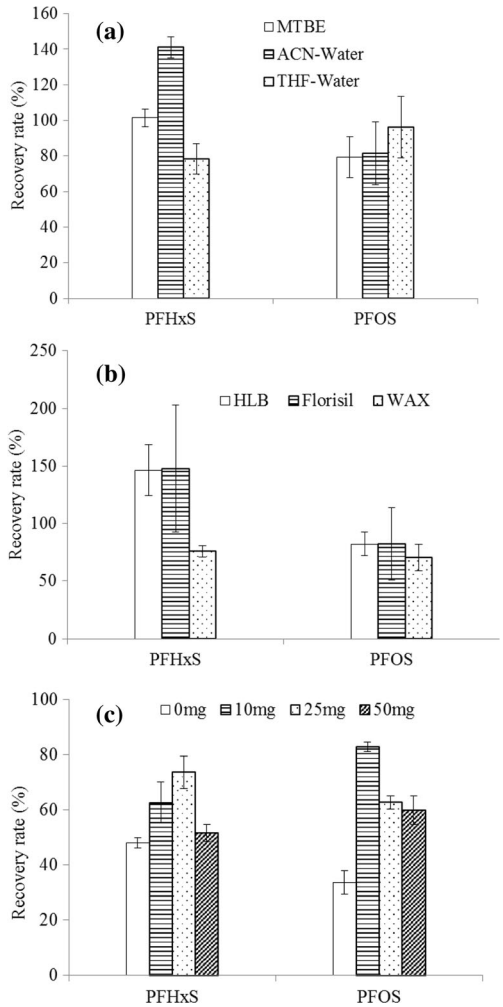

Fig. 2 Effects of extractant (**a**), clean-up cartridge (**b**), and ENVI-Carb usage (**c**) on the recoveries of PFHxS and PFOS in lettuce sample spiked at 10 ng/g

Solid-Phase Extraction Clean-Up

Ten milligrams of ENVI-Carb was loaded into each WAX cartridge (6 mL, 150 mg) used for solid-phase extraction clean-up. The WAX cartridge was preconditioned with 5 mL of methanol and 5 mL of ultrapure water before clean-up, respectively. The obtained extract (1 mL) of each sample in the polypropylene tube was diluted with 3 mL of ultrapure water and then introduced to the WAX cartridge. The polypropylene tube was rinsed two times with 6 mL (3 mL× 2) of ultrapure water, and then the rinsed solution was introduced to the WAX cartridge. The eluent got from the above conditions was discarded. The target analytes were eluted with 4 mL of methanol and 4 mL of 0.1% of ammonia methanol (ammonia/methanol, *v*/*v*), respectively. The combined eluent was evaporated to near dryness by a gentle stream of dry nitrogen gas, and then redissolved in 1 mL of methanol, vortex mixed for 1 min, and filtered using an acrodisc LC13 GHP Pall filter (0.22 μm) for HPLC-MS/MS analysis.

Chromatographic and Mass Spectrometric Condition

A HPLC-MS/MS composed of a HP1200 liquid chromatograph (Agilent, USA) and an API 4000 Q-Trap spectrometer (Applied Biosystems, Foster city, CA, USA) was used to determine the target analytes. Sample was injected with 5 μL of volume and separated on a C_{18} column (4.6 × 100 mm, i.d.,

Table 3 Recoveries and RSD ($n = 5$) of target PFHxS and PFOS in various matrices

Analyte	Spiked levels ng/g	Lettuce		Pumpkin		Carrot		Grain	
		Recovery	RSD	Recovery	RSD	Recovery	RSD	Recovery	RSD
PFHxS	0.5	81.7	2.9	90.2	1.4	75.6	9.6	70.9	2.0
	10	101.4	4.9	101.5	4.5	114.6	4.3	101.8	4.6
	25	74.7	5.0	112.7	10.5	93.9	4.4	114.3	2.7
	50	78.4	1.7	110.7	6.6	90.9	9.6	81.8	7.6
PFOS	0.5	71.2	6.1	86.1	6.3	80.2	9.7	71.2	4.4
	10	79.4	11.5	99.3	5.8	95.0	8.6	100.8	2.0
	25	99.1	7.3	76.5	2.6	98.4	3.8	111.5	9.8
	50	96.3	7.6	77.8	1.2	101.8	5.8	113.4	2.4

2.7 μm, Phenomenex, USA). The C_{18} column was eluted with a linear gradient of methanol in an ammonium acetate solution (10 mM) at 500 μL/min of flow rate. The linear gradient of methanol start at 3% (keep for 0.5 min), increase to 95% at 6 min (keep for 3.1 min), and then back to 3% at 9.5 min (keep for 3 min), in a total run time of 12.5 min. All the target analytes were eluted within 9 min. They were identified and quantified by the electrospray ionization source in scheduled negative multiple-reaction-monitoring (MRM) mode. The typical parameters of the electrospray ionization source were exhibited as follows: entrance potential (EP), −10 V, air curtain gas pressure (CUR), 200 psi (nitrogen), ion source spray voltage (IS), −4500 V, collision gas (CAD), high, collision cell exit potential (CXP), −15 V, atomization gas pressure (GAS1), 45 psi (nitrogen), auxiliary gas pressure (GAS2), 50 psi (nitrogen), atomization temperature (TEM), 550 °C. The used ISs and the optimized HPLC-MS/MS parameters for the analytes were showed in Table 1.

Validation Study

Validation was conducted following the recent studies (Li et al. 2014; Xiang et al. 2015). Different edible crop matrices including cereal (grain), root vegetable (carrot), leafy vegetable (lettuce), and melon vegetable (pumpkin) were separately applied for validation of the developed method with linearity, instrument detection limits (IDLs), method detection limits (MDLs), specificity, accuracy, and precision. The analyte concentrations were determined by internal standard isotope approach with relative to $^{13}C_4$-PFOS (Table 1). The linearity was assessed for each analyte by measuring the standards containing 10 ng/mL of $^{13}C_4$-PFOS in methanol and the four edible crop matrices, respectively, in triplicate at seven concentration levels, i.e., 0.5, 1, 2.5, 5, 10, 25, and 50 ng/mL (Table 2). Matrix effect (ME) was estimated by determining the slope ratios between each matrix-matched calibration curve ($n = 3$) and methanol curve ($n = 3$) (Li et al. 2014; Xiang et al. 2015). The IDLs were determined as the concentration producing a signal-to-noise (S/N) ratio of three in methanol (Yang et al. 2015). Because of no the analytes detected in all the four edible crop matrix blanks, the matrix-dependent MDLs were determined to be the instrument minimum detectable concentration creating a signal-to-noise (S/N) ratio of three for the analytes in the each edible crop matrix blank (Zhang et al. 2010; Yang et al. 2015). The absence of interfering peaks related to the characteristic *m/z* at the retention time of the analytes in the procedure blank and the edible crop matrix blanks were used to evaluated to the specificity of the developed method (Li et al. 2014; Xiang et al. 2015). Recovery tests using the four crop matrices spiked with the analytes at four concentrations, namely 0.5, 10, 25, and 50 ng/g, were conducted to evaluate the accuracy and precision of the developed method. The accuracy was measured by determining the percentage ratios of actual levels to theoretical levels in the spiked recovery tests (Vestergren et al. 2012). The precision was set as the percentage relative standard deviation (RSD) for five replicates (Vestergren et al. 2012). The evaluation for accuracy and precision was based on the requirements of DGSANCO/12459/2011 guidelines with recovery from 70 to 120% and RSD lower than 20%. All experimental utensils were rinsed by ultrapure water and HPLC-grade methanol in turn before analysis to avoid sample contamination (Yang et al. 2015). A sample matrix spiked and a procedural blank were included into the analytical procedures for every batch of five samples to control the analysis quality (Yang et al. 2015).

Data Analysis

The data of analyte concentrations obtained from HPLC-MS/MS analysis were calculated by AB Sciex Analyst 1.6 software (Applied Bioscience). Determination of mean RSD (%), Pearson correlation, and regression equation were conducted by SPPSS 21.0 (International Business Machines Co, USA). Tables and figures listed in the present study were finished by Microsoft Excel 2013 (Microsoft Co., Redmond, WA, USA).

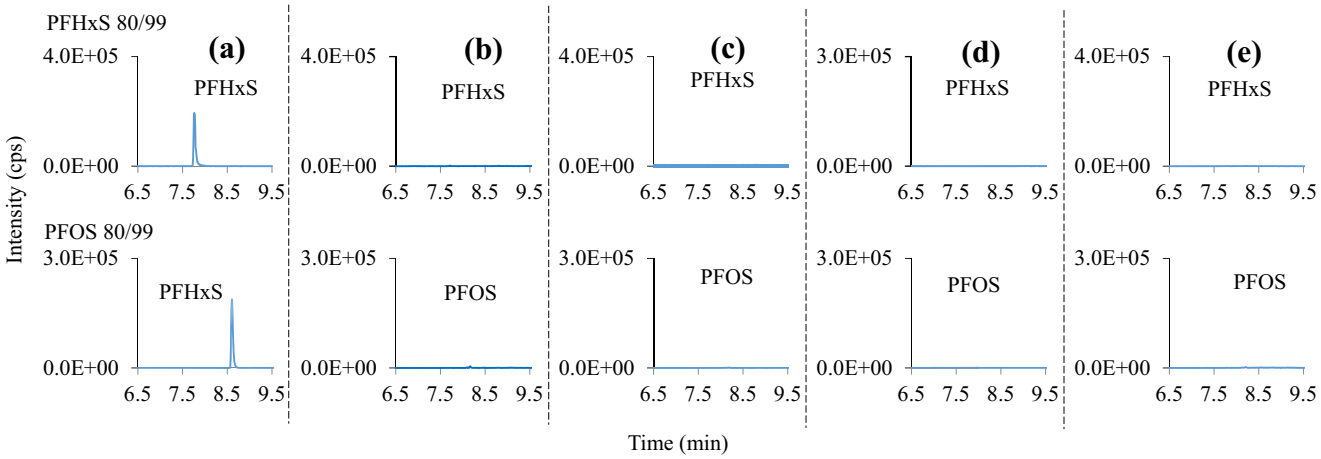

Fig. 3 Typical HPLC-MS/MS chromatograms of PFHxS and PFOS at 5 ng/mL in methanol standard solution (**a**), grain blank (**b**), carrot blank (**c**), lettuce blank (**d**), and pumpkin blank (**e**)

Results and Discussion

HPLC-MS/MS Optimization

The reverse-phase HPLC equipment with a mobile phase (methanol) and a stationary phase (C_{18}) was often applied for separating PFSA variants (Young et al. 2013; Yang et al. 2015). Three commonly used C_{18} columns, namely Agilent C_{18} column (2.1 × 150 mm, i.d., 5 μm), Agilent C_{18} column (2.1 × 150 mm, i.d., 3.5 μm), and Phenomenex C_{18} column (4.6 × 100 mm, i.d., 2.7 μm), were tested to obtain optimized separation of PFOS and PFHxS. Considering the best sensitivity, resolution, and reproducibility of the analytes obtained using Phenomenex C_{18} column, it was selected as the stationary phase in the present study. Regarding mobile phase, a methanol with a linear gradient in a certain concentration of ammonium acetate was able to separate PFSA variants with more satisfactory results compared with the pure methanol. This was explained by the fact that ammonium acetate increased both the protonation of sulfonyle in the analytes and the interactions of the alkane groups between the C_{18} column and the analytes (Taniyasu et al. 2005). Different levels of ammonium acetate solution, namely 5, 10, and 20 mM were tested to gain the optimized sensitivity. It can be found that 10 mM of ammonium acetate solution showed optimal sensitivity. Sequentially, several linear gradients of methanol in 10 mM of ammonium acetate solution were investigated to obtain the best separation. Satisfactory separation of PFHxS and PFOS were achieved (Fig. 1) when the linear gradient of methanol was set as follows: start at 3% (held 0.5 min), increase to 95% at 6 min (held 3.1 min), and then back to 3% at 9.5 min (held for 3 min). The retention times were 7.13 and 8.18 min for PFHxS and PFOS, respectively, when the optimal HPLC condition was used.

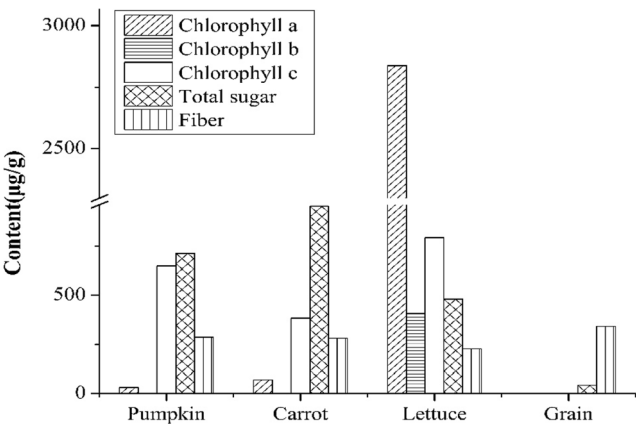

Fig. 4 The constituents of the edible crop matrices

The single standard solutions of PFHxS and PFOS were separately infused into the electrospray ionization source to create an ion transition in the scheduled negative MRM mode. The deprotonated molecules ($[M-H]^-$) with *m/z* of 399 and 499 were formed by PFHxS and PFOS in the full-scan mass spectrum, respectively (Young et al. 2013; Yang). All the deprotonated molecules were used as the precursor ions and then suffered from fragmentations to form fragment ions $[SO_3]^-$ and $[SO_3\ F]^-$ with *m/z* of 80 and 99, respectively (Table 1). The fragment ion $[SO_3]^-$ derived from the cleavage of the sulfonyle and α-linked alkane residue of the analytes was chosen as the quantitative ion for both PFHxS and PFOS, because it was major product ion and displayed higher sensitivity than the fragment ion $[SO_3\ F]^-$ (de Voogt et al. 2006). The precusor ions ($[M-H]^-$), fragment ions ($[SO_3]^-$ and $[SO_3\ F]^-$), and optimal MS/MS parameters for PFHxS and PFOS were exhibited in Table 1.

Sample Preparation Optimization

Based on recovery tests using leaf vegetable matrix (lettuce) spiked with analyte at 10 ng/g, various factors including extraction approach, clean-up cartridge, and ENVI-Carb usage were investigated to gain an optimized pretreatment procedure. Whereupon, the optimized procedure was evaluated in

Table 4 The correlation coefficients (R^2) between matrix effect (ME = $Slope_{matrix}$/$Slope_{methanol}$) and crop matrix contents (n = 12)

Matrix effect	Chlorophyll a	Chlorophyll b	Carotenoids	Total dissolved sugar	Fiber
ME_{PFHxS}	−0.064	−0.006	−0.405	−0.875**	0.504*
ME_{PFOS}	−0.062	−0.075	0.479	0.107	−0.124
ME_{PFOS} = −0.875 × total dissolved sugar[a], R^2 = 0.75, P < 0.05.					

ME matrix effect

*P < 0.05; **P < 0.01

[a] A lineal regression equation between total dissolved sugar and the ME of PFHxS

other three edible crop matrices (Yang et al. 2015; Xiang et al. 2015). Firstly, two frequently used extraction approach including ion-pair extraction method (IPE) using TBA as ion-pairing agent and MTBE as extractant and solid-liquid extraction method (SLE) using ACN/water (90:10, *v*/*v*) or THF/water (75:25, *v*/*v*) as extractant were compared (Ballesteros-Gómez et al. 2010; Ullah et al. 2012; Felizeter et al. 2014). Apart from ion-pairing procedure using TBA, the extraction conditions in IPE were identical to those in SLE. As displayed in Fig. 2a, unsatisfactory recovery of PFHxS (141%) was observed when SLE with ACN/water (90:10, *v*/*v*) was used. However, satisfactory recoveries of both PFHxS and PFOS (78.3–101.4%) were achieved when either IPE or SLE with THF/water (75:25, *v*/*v*) was applied, and higher recoveries of PFHxS were achieved when using the former. This was explained by the fact that the positively charged TBA reacted with the negatively charged analyte in solution to form ion pairing readily extracted by the low polar solvent (MTBE) (Vestergren et al. 2012). So, the IPE with TBA ion pairing and MTBE extraction were selected in the present study.

Clean-up using solid-phase extraction can efficiently decrease the influences of the sample matrix constituents on the analysis of PFSAs (Ullah et al. 2012; Martínez-Moral et al. 2013). Three commonly used clean-up cartridges for PFSAs determination including Florisil cartridge, WAX cartridge, and HLB cartridges were compared to obtain a satisfied clean-up cartridge (Vestergren et al. 2012; Zabaleta et al. 2014). Figure 2b indicates the excessive recoveries of PFHxS (146.4–147.8%) with high RSDs (22.0–55.3%) and good recoveries (82.2–82.3%) of PFOS with RSDs (11.5–31.6%) when HLB cartridge and Florisil cartridge were separately used. On the other hand, satisfactory recoveries (70.4–75.9%) with acceptable RSDs (5.0–11.5%) for both PFOS and PFHxS were found when using WAX cartridge, owing to the mixed retention mechanism for the acidic analytes including reverse phase and anion exchange (Zabaleta et al. 2014). Thus, WAX cartridge was chosen in the solid-phase extraction in the present study.

Solid-phase extraction followed by ENVI-Carb clean-up could further improve the recoveries of PFSA in environmental samples such as sludge, soil, and vegetable, because interfering matrix constituents especially those with aromatic structure could be efficiently retained by ENVI-Carb via π-electron interaction (Powley et al. 2005; Vestergren et al. 2012). The influences of ENVI-Carb usages (i.e., 0, 10, 25, and 50 mg) on the analytes were investigated in the present study. As exhibited in Fig. 2c, significantly improved recoveries of the analytes (51.6–82.9%) were found using ENVI-Carb with 10–50 mg of usage compared to the control (33.7–48.1%). However, the recoveries of the analytes generally decreased with increasing ENVI-Carb usage. It might be attributed to the adsorption of analytes to the superfluous ENVI-Carb, i.e., 25 and 50 mg. So, 10 mg of ENVI-Carb was chosen for clean-up. Considering that more loss of analyte in pretreatment procedures could occur when solid-phase extraction clean-up and ENVI-Carb clean-up were set in two single procedures as previous report (Powley et al. 2005; Felizeter et al. 2014), the ENVI-Carb was directly loaded in the WAX cartridge with simultaneous clean-up process. Accordingly, much more satisfied recoveries (70.7–82.9) and RSD (0.1–11.4%) were observed in the present study compared with the previous report (recoveries 54–129%, RSD 6–17%) (Felizeter et al. 2014).

Once the pretreatment procedure for both PFHxS and PFOS was optimized in lettuce spiked at 10 ng/g, it was tentatively used to detect the analytes in other three edible crop matrices spiked at 10 ng/g. Very satisfactory recoveries ranging from 95.0–101.8% with acceptable RSDs (<6%) for both PFHxS and PFOS in the other three matrices, i.e., carrot, pumpkin, and grain (Table 3), were observed, indicating high feasibility of the optimized pretreatment procedure.

Method Validation

Linearity, IDLs, MDLs, and Specificity

The linearity and MDLs were evaluated using the peak area ratios of analyte peak area to IS peak area gained from the MS/MS mode. The linearity was obtained by measuring the calibration curves of both the solvent (methane) and various matrices (lettuce, carrot, pumpkin, and grain) at seven concentrations including 0.5, 1, 2.5, 5, 10, 25, and 50 ng/mL for the analytes. Satisfied linearities with correlation coefficients (R^2 > 0.997) for both PFHxS and PFOS were found in all edible crop matrices within the concentration range of 0.5–50 ng/mL. The IDLs for both FHxS and PFOS were 0.08 ng/mL on account of the peak-to-peak S/N = 3 (Table 2). The matrix dependent MDLs (dry weight, dw) for the analytes

Table 5 Concentrations of PFOS and PFHxS in real edible crops

Analyte	Chinese cabbage[a]		Lettuce[b]		Mustard leaf[c]		Pakchoi[d]		Celery[e]	
	Detection rate (%)	Range (dw/fw) (ng/g)	Detection rate (%)	Range (dw[b]/fw[c]) (ng/g)	Detection rate (%)	Range (dw[b]/fw[c]) (ng/g)	Detection rate (%)	Range (dw[b]/fw[c]) (ng/g)	Detection rate (%)	Range (dw[b]/fw[c]) (ng/g)
PFHxS	50	0.10 ± 0.07/0.02 ± 0.01	14	0.40 ± 0.08/0.10 ± 0.01	0	< MDL	50	0.18 ± 0.09/0.02 ± 0.02	0	< MDL
PFOS	50	0.21 ± 0.08/0.04 ± 0.01	57	0.23 ± 0.15/0.06 ± 0.04	0	< MDL	0	< MDL	0	< MDL
∑PFHxS + PFOS	50	0.31 ± 0.13/0.06 ± 0.03	43	0.63 ± 0.14/0.16 ± 0.04	0	< MDL	50	0.18 ± 0.09/0.02 ± 0.01	0	< MDL

dw dry weight, *fw* fresh weight, *MDL* method detection limit

[a] $n = 2$

[b] $n = 7$

[c] $n = 9$

[d] $n = 3$

[e] $n = 3$

ranged from 0.020 to 0.150 ng/g, up to 100 times lower than previous results (0.3–8.4 ng/g), indicating the high sensitivity of the developed method for determining the analytes in various edible crops (Zabaleta et al. 2014; Ciccotelli et al. 2016). Taking water content into consideration, the matrix dependent MDLs (fresh weight, fw) were calculated by the following equation:

$$\mathrm{MDLs(fw)} = \mathrm{MDLs(dw)} \times (100\text{-W}) \Big/ 100 \quad (1)$$

where *W* indicates water content (%) which were 99.5 ± 4.3, 88.5 ± 5.2, 90.1 ± 3.3, and 7.3 ± 2.4% for lettuce, pumpkin, carrot, and grain, respectively. As described in Table 2, the matrix dependent MDLs (fw) for both PFHxS and PFOS using the common equipment (HPLC-MS/MS) ranged from 3 to 35 pg/g and 31 to 130 pg/g for various vegetables and grain, respectively, achieving the sensitivity (pg/g level) of superior analytical equipment (e.g., UPLC-MS/MS and HPLC-QTOF-HRMS) (Ullah et al. 2012; Vestergren et al. 2012). Regarding the specificity, it was satisfied due to no interfering peaks related to the characteristic *m/z* of both the PFHxS and PFOS observed in procedure blank and each edible crop matrix blank (Figs. 1 and 3).

Matrix Effect and Its Potential Influencing Factors

Despite an optimized pretreatment procedure gained, matrix effect (ME) inevitably emerges in analysis of trace organic pollutants in complex sample by HPLC-MS/MS, which depends on type and amount of the sample matrix, physicochemical property of analyte, equipment configuration, and so on (Vestergren et al. 2012; Li et al. 2014). Considering that it is very likely to cause a significant impact on reliable quantification of the organic pollutants (Vestergren et al. 2012), ME in each edible crop matrix for PFHxS and PFOS was evaluated using the slope ratios of the standard calibration curves, i.e., $\mathrm{ME} = \mathrm{slope_{matrix}} / \mathrm{slope_{methanol}}$, where $\mathrm{slope_{matrix}}$ and $\mathrm{slope_{methanol}}$ indicate the slope of standard calibration curves for matrix and methanol, respectively. Based on previous reports, an accepted ranking criterion was applied, i.e., strong enhancement effect (ME >1.5), medium enhancement effect (1.2 < ME <1.5), mild enhancement effect (1.1 < ME <1.2), negligible effect (0.9 < ME <1.1), mild suppression effect (0.8 < ME <0.9), medium suppression effect (0.5 < ME <0.8), and strong suppression effect (ME <0.5) (Li et al. 2014). Table 2 indicates that ME for the analytes varied greatly with different compounds and crop matrices. ME for PFHxS in various crop matrices was generally greater than PFOS. Regarding PFHxS, mild enhancement effect on lettuce, strong enhancement effect on carrot and grain, as well as medium suppression effect in pumpkin, were observed. While as for PFOS, mild enhancement effect (carrot and grain) and medium enhancement effect (pumpkin) were found in the various edible crop matrices expect negligible effect in lettuce.

According to previous studies, the constituents of the edible crop matrices (Fig. 4) are one of the most crucial factors influencing the ME for the analytes (Li et al. 2014; Xiang et al. 2015). As shown in Table 4, ME for PFHxS displayed significantly negative and positive correlations with total dissolved sugar and fiber, respectively. However, no correlations were found between the ME for PFOS and the constituents of the edible crop matrices due to lower ME levels. The lineal regression equations between the ME for PFHxS and the matrix constituents were deduced to better understand their relationships. It can be observed that total dissolved sugar was the crucial factor affecting the ME for PFHxS (Table 4).

In addition, the carbon chain length also significantly affected the ME for the analytes. Specifically, opposite ME (in pumpkin) or much higher ME (in carrot and grain) for PFHxS were observed in the same edible crop matrices compared with PFOS that has two more [$-CF_3$] than PFHxS. Considering that the ME varied with the analytes and the crop matrices, more reliable quantitative results in the present study were obtained by matrix-matched standard calibrations relative to isotope-labeled IS compounds.

Accuracy and Precision

In order to evaluate the accuracy and precision of the developed method, recovery experiments using the various edible crop matrices spiked with analytes at four analyte-spiked concentrations, i.e., 0.5, 10, 25, and 50 ng/g, were carried out. Dada in Table 3 indicate that fairly satisfied recoveries and RSDs for the analytes in all cases were obtained. Specifically, 74.7–101.4% with RSD < 5.0 in lettuce, 90.2–112.7% with RSD < 11.0 in pumpkin, 75.6–114.6% with RSD < 10.0 in carrot, and 70.9–114.3% with RSD < 8.0 in grain for PFHxS, and as well as 71.2–99.1% with RSD < 12.0 in lettuce, 76.5–99.3% with RSD < 7.0 in pumpkin, 80.2–101.8% in with RSD < 10.0 carrot, and 71.2–113.4% with RSD < 10.0 in grain for PFOS were obtained. All the obtained recoveries and RSDs fulfilled the requirements of DG SANCO/12459/2011 guidelines with recovery from 70 to 120% and RSD lower than 20%. Moreover, the obtained recoveries for the analytes were generally better than previous report (60–86%) (Vestergren et al. 2012; Zabaleta et al. 2014). Accordingly, the developed method exhibited high accuracy and precision, making it possible to be used to routinely determine both PFHxS and PFOS in various edible crop matrices.

Method Application

Various actual crops including Chinese cabbage (n = 2), lettuce (n = 7), mustard leaf (n = 9), pakchoi (n = 3), and celery

($n = 3$) were sampled from several farms near a large fluoride factory in a prosperous city (Foshan) in Pearl River Delta, south China. These actual crops were used to assess the practicality of the developed method. In analysis procedures, four quality controls at 0.5, 10, 25, and 50 ng/g were performed for each actual crop with the recoveries ranging from 81 to 105% and RSDs lower than 12%. As shown in Table 5, PFHxS or PFOS was found in detectable concentrations in Chinese cabbage (50%), lettuce (14–57%), and pakchoi (50%) with the total concentrations between 0.18 and 0.63 ng/g (dw). However, the levels of both PFHxS and PFOS in mustard leaf and celery were lower than the MDLs. It was noted that the concentrations of PFOS in the crops were higher than those of PFHxS. The prevalence of PFOS compared with PFHxS was due to its more use and frequent occurrence in agricultural soil and irrigation water than the latter (Blaine et al. 2013; Lu et al. 2015).

Conclusion

In the present study, a robust method was achieved for analysis of PFHxS and PFOS in various edible crop matrices including cereal, root vegetable, leafy vegetable, and melon vegetable using ion-pair extraction combined with solid-phase extraction clean-up and HPLC-MS/MS, with significantly improved accuracy and sensitivity at pg/g level (fw), corresponding to the sensitivity of superior equipment (e.g., UPLC-MS/MS and HPLC-QTOF-HRMS). The matrix-matched standard calibrations relative to the isotope-labeled ISs were needed to obtain the more reliable quantitative results. The successful application of the method to determine the analytes in actual crops collected from several farms demonstrated the feasibility of routine analysis of the analytes in various edible crops.

Compliance with Ethical Standards The manuscript has not been published previously (partly or in full). The manuscript has not been submitted to more than one journal for simultaneous consideration. Consent to submit has been received explicitly from all co-authors, as well as from the institute/organization where the work has been carried out, before the work is submitted. Authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

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Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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