Determination of PFOS and PFOA in Food Matrix of Animal Origin Using UHPLC Hyphenated Triple Quadrupole Tandem Mass Spectrometry*

Application Note

Food

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Abstract

An ultrasensitive method has been developed for simultaneous determination of perfluorooctane sulfonates (PFOS) and perfluorooctanoic acid (PFOA) in food using an Agilent UHPLC 1290 hyphenated Triple Quadrupole 6460 tandem mass spectrometer. The samples were first extracted using methanol, followed by cleanup with weak cation-exchange cartridge. The resultant solutions were subjected to reverse-phase UHPLC separation and triple quadrupole MRM detection. The dynamic linear range for PFOA and PFOS was 0.01–10 ng/mL and 0.1–40 ng/mL, respectively, with linear correlation regression coefficient of 0.999 or above. The limit of detection (LOD) (signal-to-noise (S/N) = 3) was 0.002 and 0.02 µg/kg for PFOA and PFOS, respectively, and the limit of quantitation (LOQ) (S/N=10) was 0.01 and 0.1 µg/kg, respectively. The average spiking recoveries at three levels in the matrix of fish, shrimp, and eggs ranged from 79.2% to 113.1%, with the relative standard deviation (RSD) within 3.2–10.7% (n=6). Inter-laboratories validation further proves the accuracy and reliability of the method. The developed method has the advantages of high sensitivity, high recovery, and good selectivity. Therefore, it can be applied for routine inspection of PFOA and PFOS in food of animal origin.

*The primary work has been presented in the China Annual National Conference of Organic Mass Spectrometry in 2012.

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Introduction

Perfluoro alkylated substances (PFASs) are a large group of highly fluorinated aliphatic compounds with high thermal and chemical stability, as well as high surface activity [1]. They have been widely applied in industrial manufacturing over decades such as refrigerant, anesthetic, insecticide, herbicide, lubricant, and coatings. PFASs are resistant to hydrolysis, photolysis, and biodegradation, and thus belong to the persistent organic pollutants. PFOA and PFOS are the two primary compounds increasingly found in the food chain due to environmental pollution from industrial practices. They have been detected in matrix like water [2,3], sediment, and silt [4], birds, fish, other aqueous life, as well as egg white [5,6]. Bioaccumulation leads to increasing risk of human exposure to PFOA and PFOS, which has been found in human milk [3].

Studies on experimental animals have demonstrated that PFASs can induce a range of adverse health effects including hepatotoxicity, developmental toxicity, neurobehavioral toxicity, immunotoxicity, reproductive toxicity, lung toxicity, hormonal effects, and so on [1]. A recent epidemiological study indicated that elevated exposure to PFASs was associated with reduced humoral immune response to routine immunization in children [7]. EFSA has recommended a tolerable daily intake (TDI) of 150 ng/kg b.w. per day for PFOS and of 1,500 ng/kg b.w. per day for PFOA in 2008 [8]. Hence, it is critical to develop a highly sensitive, selective, and reliable method for routine monitoring for the level of PFASs in food products.

To determine the level of PFASs in food, a number of methods have been developed with the main focus on the environmental settings. We attempted to develop a rapid reference method, with high sensitivity and accuracy, for monitoring the levels of PFOS and PFOA in the food originating from animal, throughout China.

Experimental

Reagents and materials

PFOA (MW: 414.10) and PFOS (MW: 538.22) were purchased from AccuStandard Inc. (USA). Figure 1 shows the molecular structures. $^{13}C_8$-PFOA and 1, 2, 3, 4-$^{13}C_4$- PFOS standard compounds were obtained from Wellington Laboratories Inc (Guelph, ON, Canada) and Cambridge Isotope Laboratories Inc (USA), respectively. Methanol, ammonia acetate, acetic acid, and ammonia hydroxide were of HPLC grade and from Fisher Scientific (Fair Lawn, NJ, USA). Mill-Q water was used throughout the experiment. All other reagents were of analytical grade.

The stock standard solutions of PFOA, PFOS, $^{13}C_8$-PFOA, and 1, 2, 3, 4-$^{13}C_4$- PFOS were prepared from the purchased standards using methanol to form the final concentrations of 100.00, 100.00, 10.00, and 50.00 ng/mL, respectively.

Calibration standards were prepared from the stock standard solution (100 ng/mL) diluting with methanol to final concentration of PFOA at 0.010, 0.020, 0.10, 1.00, 2.00, and 10.0 ng/mL, and those of PFOS at 0.10, 0.20, 1.00, 10.0, 20.0, and 100.0 ng/mL. $^{13}C_8$-PFOA and 1, 2, 3, 4-$^{13}C_4$- PFOS were added to the above calibration solutions to make the final concentration of each at the levels of 1.00 ng/mL and 5.00 ng/mL, respectively.

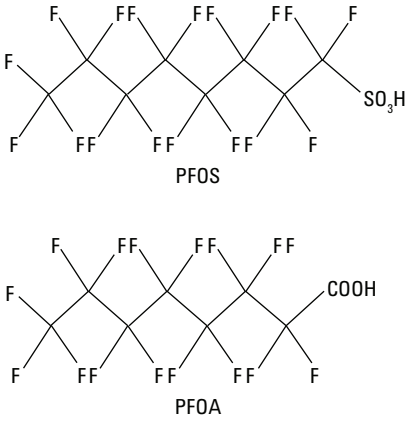

Figure 1. The molecular structure of PFOS and PFOA.

Fish, shrimp, and egg samples

A number of food samples from three types of matrixes, fish, shrimp, and egg, were used in the experiment. The matrixes, with skin removed, were initially blended in a polyethylene container and frozen at –18 °C for further use. The concentration of PFOS and PFOA were determined. The matrixes with the lowest level of PFOS and PFOA were selected as the blank matrixes for the recovery test.

Sample preparation

Initially, 5.00 g (± 0.01 g) of samples were mixed with 1.0 ng $^{13}C_4$-PFOA and 5.0 ng 1, 2, 3, 4-$^{13}C_4$-PFOS as internal standards for calibration. The samples were then extracted with 10 mL of methanol, and the methanol layer was collected. The residue was extracted with 10 mL of methanol two more times, and all the methanol layers were mixed together. The combined methanol extract was subjected to nitrogen evaporation until the final volume reached 1 mL. The extract was then adjusted with 5 mL of 2% methanol followed with sonication for 15 minutes before cleanup.

The extracted samples were subjected to weak anion exchange column cleanup (WAX). Initially, the column was activated with 2 mL methanol and 1 mL water respectively, followed by equilibration with 2 mL of 2% formic acid. The sonicated sample was then transferred into the column and subjected to washing with 2 mL of 2% formic acid and elution with 2 mL of methanol and 4 mL of 4% ammonia hydroxide sequentially. The eluate was collected and evaporated to dry with nitrogen. The residue was redissolved in 1 mL of methanol for analysis.

LC and MS Conditions

LC configuration and conditions

- Agilent 1290 Infinity Binary Pump (G4220A)
- Agilent 1260 Infinity High Performance Autosampler (G4226A)
- Agilent 1200 Series Autosampler Thermostat (G1330B)
- Agilent 1200 Series Thermostatted Column Compartment SL (G1316B)

Column	Agilent ZORBAX Eclipse Plus C18, 100 mm × 2.1 mm, 1.8 µm
Column temperature	30 °C
Injection volume	5 µL
Needle wash	Flushport (100% methanol), 5 seconds
Mobile phase	A = 5 mM ammonia acetate in water B = methanol
Gradient flow rate	0.2 mL/min

Table 1 shows the gradient elution profile.

Table 1. The Gradient Elution Profile

Time	Sol. A (%)	Sol. B (%)
0	70	30
1.0	70	30
1.01	50	50
9.0	20	80
9.01	70	30
10.0	70	30

Total run time: 10 minutes (including 1 minute equilibration time).

MS Configuration and conditions

Agilent 6460 Triple Quadrupole LC/MS with JetStream ionization source

Ionization mode	Negative ionization
Scanning mode	Multiple reactions monitoring (MRM)
Capillary voltage	–3,500 V
Nozzle voltage	–500 V
Nebulizer pressure	45 psi
Dry gas temperature	300 °C
Dry gas flow rate	6 L/min
Sheath gas temperature	260 °C
Sheath gas flow rate	11 L/min

The quantitative and qualitative ions, as well as the relevant collision energies, were optimized and shown in Table 2.

Table 2. MRM Parameters for Detection of PFOS and PFOA

Compounds	Precursor ion	Product ion	Frag. voltage (V)	CE (V)	Dwell time (msec)
PFOA	412.9	368.9*	-80	-4	100
		219.0	-80	-12	100
		169.0	-80	-16	100
PFOS	498.9	130.0*	-200	-50	100
		99.0	-200	-55	100
		80.0	-200	-90	100
$^{13}C_4$-PFOA	417.1	372.0*	-85	-3	100
		172.1	-85	-12	100
1, 2, 3, 4-$^{13}C_4$- PFOS	503.1	80.1*	-170	-65	100
		99.1	-170	-50	100

Note: * is for Quantification ion. Resolution for Q_1 and Q_2 was set at unit resolution.

Spiking Recovery

Blank samples were spiked with PFOA and PFOS at three levels with PFOA concentrations at 0.01, 0.05, and 0.10 µg/kg, while PFOS concentrations were ten fold of those for PFOA (0.1, 0.5, and 1.0 µg/kg) in each spiked sample. The spiked samples were then vortexed at room temperature for 5 minutes. 1.0 ng $^{13}C_4$-PFOA and 5.0 ng 1, 2, 3, 4-$^{13}C_4$-PFOS were added to the spiked samples. The resultant samples were vortexed for another 5 minutes and were then subjected to sample extraction and cleanup with the protocol in the sample preparation section followed by LC Triple Quadrupole MRM measurement.

Results and Discussion

LC/MS analysis condition

The concentration of PFOS and PFOA in the solvents can be high enough to impact accurate and sensitive analysis. To reduce interference derived from the aqueous solvent, the LC system was slightly modified. A trapping C18 column (30 × 3.5 mm, 5 µm) was connected between the aqueous pump channel and the mixing point of the gradient pump [9]. The connection tubing was stainless steel tubing. With such modifications, the interference from the LC system was significantly reduced. PFOA and PFOS standard solutions were initially subjected to a Q_1 MS scan under a negative ionization mode to find the correct precursor ions of the two compounds. The selected precursor ions were then subjected to product ion scanning. The fragments for the standard compounds are shown in Figure 2 and Figure 3.

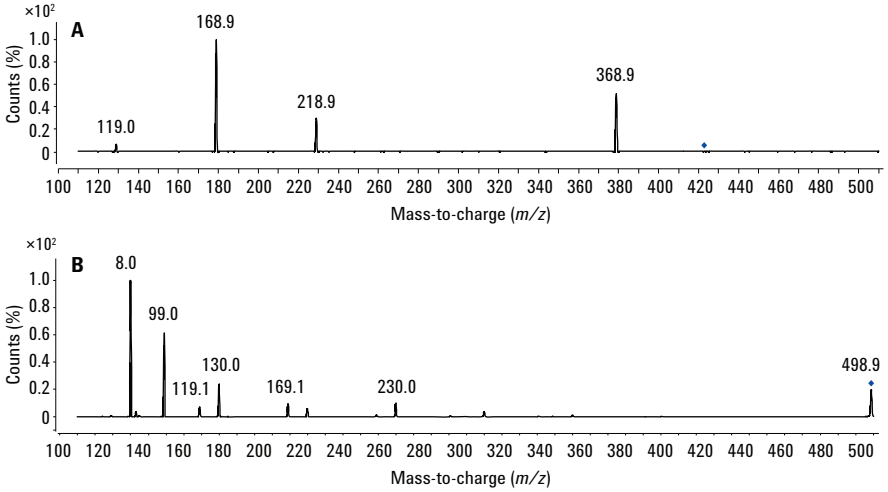

*Figure 2. Product spectra for PFOA (**A**) and PFOS (**B**).*

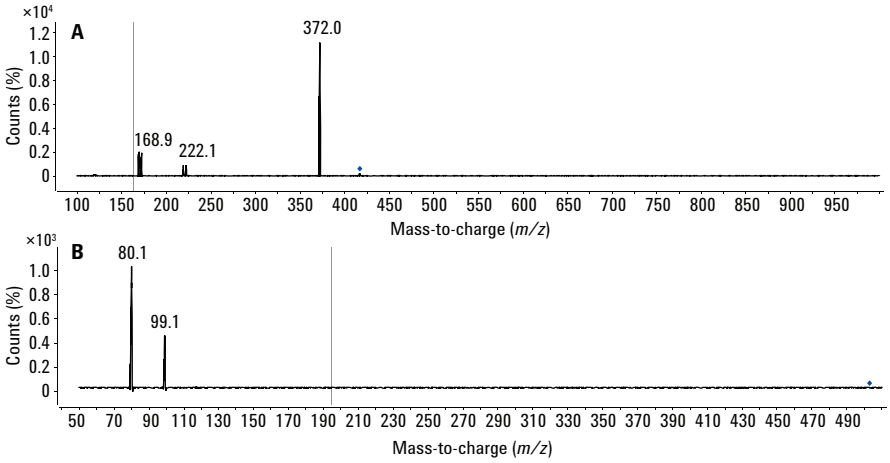

*Figure 3. Product spectra for $^{13}C_8$-PFOA (**A**) and 1, 2, 3, 4-$^{13}C_4$-PFOS (**B**).*

By ramping the fragmentor voltage for best precursor transmission, and collision energy for optimal fragmentation, transmissions of 498.9/130 and 412.99/368.9, which gave highest response were selected to quantitate PFOS and PFOA, respectively, while the other ion transmissions were selected for confirmation as shown in Table 2. Table 2 also shows the established transmissions for isotopic standards. With the MRM selective monitoring, typical chromatograms for PFOS and PFOA were obtained as shown in Figure 4. The double peaks for PFOS standard indicate that PFOS standard was present in both linear and branched forms. Hence, the quantitation of PFOS was based on the sum-up peak area.

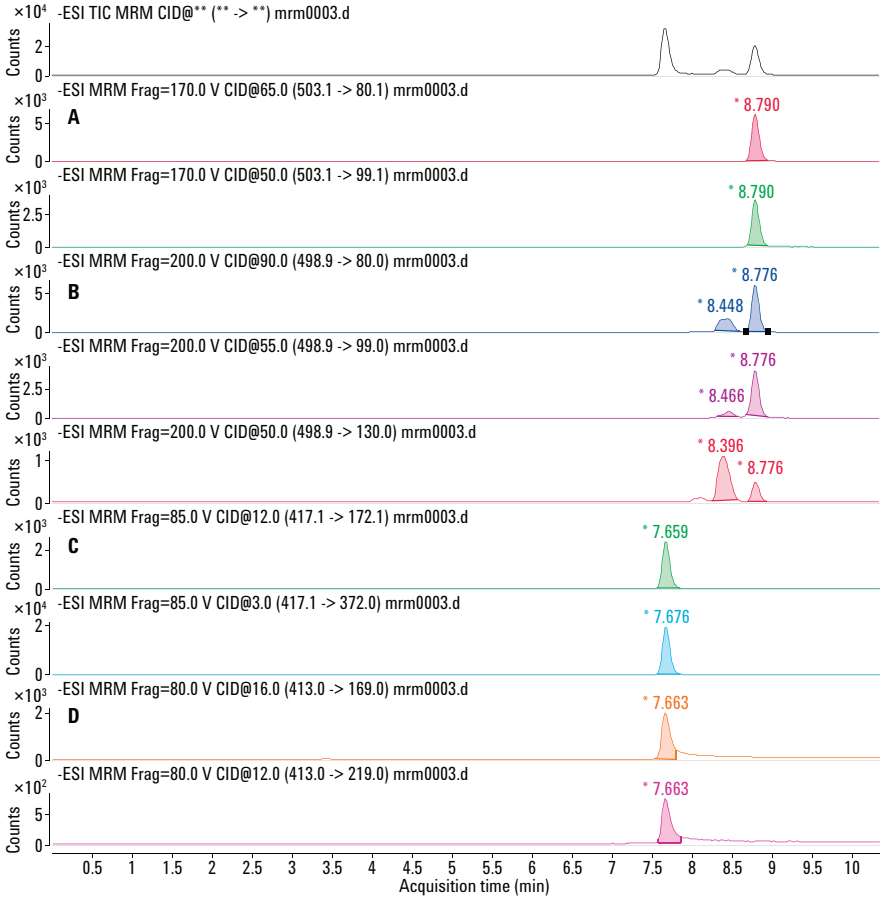

*Figure 4. MRM chromatograms for 1, 2, 3, 4-$^{13}C_4$-PFOS (**A**), PFOS (**B**), $^{13}C_8$-PFOA(**C**), and PFOA (**D**).*

Calibration curve and detection sensitivity

Calibration solutions were prepared from the stock standard solution, with PFOA concentration ranging from 0.010 to 10 ng/mL, and that for PFOS ranging from 0.1 to 40 ng/mL. Under the experimental condition, the obtained peak areas over those of the corresponding isotope standard for the quantitative ions were plotted against the concentrations of the compounds. As shown in Table 3, excellent linear calibration curve has been obtained with regression coefficients of 0.999 or higher.

The LOD and LOQ were determined by decreasing the standard concentrations in the blank sample gradually. The LOD was determined at the concentration when S/N was 3 while the LOQ was determined at the concentration when S/N was 10. As shown in Table 3, the LOD and LOQ for PFOS were 0.02 and 0.1 µg/kg, respectively, while those for PFOA were 10x lower correspondingly, suggesting that the method is highly sensitive for the detection of PFOS and PFOA in the food matrixes studied.

Method Accuracy and Precision

The spiking experiments were conducted in three matrixes including fish, shrimp, and egg. Under the optimal LC/MS condition, the analytes were well separated from the matrix, indicating less background interference. The average recovery in the three matrixes for the two compounds under three spiking levels ranged from 86.2%–111.6% with a relative standard deviation of 3.2–10.7%, demonstrating that the developed method is reliable and meets the requirement for residue analysis.

Table 3 Calibration Curve and Detection Sensitivity

Analytes	Linear range (µg/L)	Linear equation	R^2	LOD (µg/kg)	LOQ (µg/kg)
PFOA	0.01–10	$Y = 1.798e3x + 4501.1$	0.9990	0.002	0.01
PFOS	0.1–40	$Y = 6.819e2x + 383.92$	0.9993	0.02	0.1

Table 4. Spiking Recovery and Precision for PFOA and PFOS in Fish, Shrimp and Egg Matrixes (n=6)

	PFOA			PFOS		
Analytes	Spiked (µg/kg)	Recovery (%)	RSD (%)	Spiked (µg/kg)	Recovery (%)	RSD (%)
Fish	0.01	87.2~113.1	6.0	0.1	82.2~90.6	8.2
	0.05	92.8~101.0	3.2	0.5	79.9~97.2	5.8
	0.1	85.8~102.5	4.5	1.0	81.0~99.6	6.0
Shrimp	0.01	95.7~107.2	6.3	0.1	79.2~88.8	10.7
	0.05	96.3~111.2	6.6	0.5	80.2~89.2	8.12
	0.1	85.2~101.6	6.5	1.0	85.0~88.4	6.0
Egg	0.01	85.7~98.6	4.8	0.1	89.7~93.4	7.6
	0.05	95.2~108.8	8.8	0.5	88.3~93.6	6.9
	0.1	84.2~111.6	7.1	1.0	90.8~112.0	4.2

Inter Laboratory Validation

The method was further validated by five independent laboratories. Spiking recovery and precision were evaluated at three spiking levels in three separate matrixes. Table 5 shows that the overall average recoveries ranged from 87.1%–105.8%, with the overall RSD ranging from 10.8%–31.4%. The results demonstrated that the developed method met the requirement as a reference standard method.

Real sample analysis

The developed method was further applied to real sample analysis. Fish, shrimp, and egg samples from the local grocery stores were randomly selected, with each matrix of 50 samples. Among the total 150 samples, 10 samples were found to contain PFOS, with the highest positive percentage in fish and relatively low in eggs. The level of PFOS in the positive samples ranged from 0.04 to 3.0 µg/kg.

Table 5. Levels of PFOS and PFOA, Measurement Accuracy and Precision in Matrixes of Fish, Shrimp, and Egg Determined by Five Independent Laboratories

			Determination results from five individual laboratories (µg/kg)												
Samples	**Analytes**	**Spiked level (µg/kg)**	**A**	**A**	**B**	**B**	**C**	**C**	**D**	**D**	**E**	**E**	**Ave conc. (µg/kg)**	**Ave. recovery (%)**	**RSD (%)**
Fish	PFOA	0.01	0.0086	0.0091	0.0076	0.0088	0.0089	0.0096	0.012	0.0112	0.012	0.018	0.01	105.8	28.43
	PFOS	0.1	0.108	0.099	0.111	0.107	0.068	0.073	0.096	0.095	0.099	0.112	0.10	101.3	15.67
	PFOA	0.05	0.048	0.047	0.045	0.047	0.0511	0.0508	0.0513	0.0508	0.0302	0.0395	0.05	92.1	14.45
	PFOS	0.5	0.451	0.485	0.496	0.509	0.514	0.518	0.396	0.372	0.458	0.483	0.47	93.6	10.65
	PFOA	0.1	0.068	0.079	0.089	0.087	0.075	0.086	0.087	0.096	0.101	0.103	0.09	87.1	12.73
	PFOS	1	1.02	1.12	1.04	1.05	1.13	1.14	0.88	0.86	0.79	0.76	0.98	97.9	14.71
Shrimp	PFOA	0.01	0.0096	0.0099	0.0079	0.0068	0.0102	0.0097	0.0093	0.0086	0.0094	0.0096	0.01	96.8	11.48
	PFOS	0.1	0.088	0.089	0.103	0.108	0.083	0.078	0.087	0.085	0.105	0.112	0.09	92.3	12.76
	PFOA	0.05	0.045	0.048	0.043	0.047	0.0501	0.0509	0.0508	0.0511	0.0399	0.0368	0.05	92.5	10.83
	PFOS	0.5	0.463	0.483	0.476	0.502	0.504	0.049	0.456	0.468	0.498	0.483	0.44	87.6	31.43
	PFOA	0.1	0.097	0.089	0.069	0.077	0.102	0.113	0.097	0.086	0.091	0.103	0.09	92.4	14.02
	PFOS	1	0.86	0.96	0.96	0.96	1.13	1.04	0.87	0.76	0.99	0.86	0.94	93.9	11.22
Egg	PFOA	0.01	0.0076	0.0081	0.0086	0.0089	0.0086	0.0098	0.0102	0.0112	0.0086	0.0095	0.01	87.8	11.75
	PFOS	0.1	0.088	0.079	0.103	0.106	0.078	0.083	0.096	0.103	0.105	0.112	0.10	83.3	12.98
	PFOA	0.05	0.0478	0.0467	0.0358	0.0372	0.0501	0.0508	0.0515	0.0513	0.0487	0.0495	0.05	93.9	12.18
	PFOS	0.5	0.481	0.496	0.466	0.369	0.514	0.512	0.397	0.482	0.512	0.516	0.47	94.9	10.85
	PFOA	0.1	0.097	0.089	0.076	0.079	0.105	0.096	0.112	0.096	0.111	0.118	0.10	97.9	14.24
	PFOS	1	1.12	1.03	1.17	0.963	0.896	0.789	0.868	0.856	0.792	0.765	0.92	92.5	15.34

Conclusions

An analytical method using LC/MS/MS MRM technology with weak cation-exchange cleanup for the determination of PFOA and PFOS in fish, shrimp, and egg by isotope internal standard calibration was established. The dynamic range of the method can reach three orders of magnitude, with linear correlation coefficients of 0.999 or above. The LOD for PFOA and PFOS are 0.002 µg/kg and 0.02 µg/kg, respectively, while the LOQ for both are 0.01 µg/kg and 0.1 µg/kg, respectively. Spiking test at the levels of LOQ, 5-fold of LOQ and 10-fold of LOQ showed that the method is highly accurate, with spiking recovery ranging from 79.2–113.1% and RSD within 3.2–10.7% (n=6). Inter-laboratory validation further proved the reliability of the method with overall spiking recovery of 87.1%–105.8%, and RSD within 10.8%–31.4%. With the developed method for determination of 150 samples, the positive rate was 15%. The method has the advantage of high sensitivity, high recovery, and excellent precision and, therefore, can be applied for routine inspection of food matrix of animal origin.

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