Determination of 28 Perfluorinated Compounds in Aquatic Products by High Performance Liquid Chromatography-Tandem Mass Spectrometry

Fang LI[1], **Sitong QUAN**[2], **Yaodong QIU**[2]

1. Dandong Ande Biotechnology Co., Ltd., Dandong 118300, China; 2. College of Marine Science and Environment, Dalian Ocean University, Dalian 116023, China

Abstract [**Objectives**] To extract and purify perfluoroalkyl and polyfluoroalkyl substances (PFAS) from aquatic products and determine the optimal pretreatment conditions. [**Methods**] The QuEChERS method was employed for the extraction and purification of PFAS in aquatic products. The detection of PFAS was carried out using liquid chromatography-tandem mass spectrometry (LC-MS/MS) in ESI negative ion mode with multiple reaction monitoring (MRM), and quantification was performed using the external standard method. By optimizing instrument parameters such as ion source temperature, spray voltage, and collision energy, high selectivity and sensitivity of the detection were ensured. [**Results**] The established calibration curves for 28 PFAS compounds compounds, covering a range of 0.02 to 20 ng/mL, demonstrated excellent linearity. Spike recovery tests yielded limits of detection (LOD) between 0.02 and 0.5 μg/kg for the 28 PFAS compounds compounds, with recovery rates ranging from 72.2% to 113.0%. [**Conclusions**] The method established in this study demonstrates excellent linearity, and is accurate, reliable, efficient, simple, and rapid. It possesses considerable practical applicability and can be used for the quality and risk assessment of 28 PFAS compounds compounds in aquatic products, indicating high practical utility.

Key words High Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS), Perfluoroalkyl and polyfluoroalkyl substances (PFAS), Aquatic products, QuEChERS, Limit of detection (LOD)

0 Introduction

The perfluoroalkyl and polyfluoroalkyl substances (PFAS) have been produced and used for over 70 years[1]. Throughout various stages including production, application, and subsequent waste treatment and disposal, PFAS are inevitably released into the environment, where they undergo varying degrees of transport and transformation[2-4]. In 2023, the European Union published a proposal to ban the production and use of PFAS, covering more than 10 000 PFAS compounds. Although long-chain PFAS such as perfluorooctanesulfonic acid or perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been restricted in production and use, numerous short-chain and other novel PFAS alternatives continue to be developed and applied, leading to a continuous increase in the types of PFAS detectable in environmental media[5]. As the world's largest producer and consumer of fluorinated compounds, China accounts for approximately 64.9% of the global production capacity of fluoropolymers[6-8].

At present, the primary detection methods for perfluoroalkyl substances include gas chromatography (GC)[9], gas chromatography-tandem mass spectrometry (GC-MS/MS)[10-12], and liquid chromatography-tandem mass spectrometry (LC-MS/MS)[13-15]. Gas chromatography (GC) overcomes the low volatility of PFAS through pre-column derivatization and is commonly used for analyzing short-chain PFAS (C4-C8). This method is cost-effective and suitable for detecting volatile precursors (such as FTOHs)[9]. However, it involves cumbersome derivatization steps (derivatization efficiency <80%) and cannot detect long-chain or polar PFAS. Gas chromatography-tandem mass spectrometry (GC-MS/MS) can detect nine volatile precursors in food contact materials, with detection limits of 0.05 – 0.5 μg/kg, outperforming traditional GC combined with liquid-liquid extraction techniques. Nevertheless, the high-temperature injection port leads to thermal decomposition of long-chain PFAS (*e.g.*, PFOS decomposition rate >30%). Liquid chromatography-tandem mass spectrometry (LC-MS/MS), as the current mainstream technique, dominates due to its high sensitivity and broad applicability. Using a C_{18} column, baseline separation of 28 PFAS compounds can be achieved (resolution > 1.5), and dynamic multiple reaction monitoring (dMRM) improves the signal-to-noise ratio by 2 – 3 times.

Due to their unique chemical properties, PFAS have been produced on a large scale worldwide and are extensively used across numerous industrial and daily life applications, such as water- and oil-resistant fabrics, food packaging, and firefighting foams. However, with the continuously increasing usage of PFAS, they have caused severe pollution to the global environment[16-19], and China has also been significantly affected. Natural water bodies in many regions of China have been contaminated by PFAS, which subsequently enter the food chain, leading to a certain degree of contamination in aquatic products[20-22]. Given that PFAS are persistent, bioaccumulative, and potentially toxic, posing potential risks to human health and the ecological environment, the accurate detection of PFAS levels in aquatic products is particularly crucial.

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Fang LI, master's degree, associate senior engineer, research fields: physical and chemical analysis, detection of pesticides and veterinary drugs, additives, hazardous substances, and toxins in food, water, and soil, as well as the application of detection instruments.

1 Materials and methods

1.1 Materials and reagents Acetonitrile (chromatographic grade, Merck, USA); Ammonium acetate (chromatographic grade, Merck, USA); Sodium chloride (analytical grade, Tianjin Yongda Chemical Reagent Co., Ltd.); Anhydrous magnesium sulfate (analytical grade, Tianjin Yongda Chemical Reagent Co., Ltd.); PAS powder (40 – 60 μm, Tianjin Bonna – Agela Technologies Co., Ltd.); Polyethersulfone (PES, 0.22 μm): Jinteng. Glass or polytetrafluoroethylene (PTFE) utensils should be avoided during the experiment process.

1.2 Instruments and equipment Triple quadrupole mass spectrometer (4500): AB SCIEX, USA; High-performance liquid chromatograph (ExionLC): Shimadzu, Japan; Ultrapure water system (DI Pro): Sartorius, Germany; Vortex mixer (VORTEX): Qilinbeier Instrument Manufacturing Co., Ltd., Haimen City; One-over-ten-thousand electronic balance (BCE224-1CCN): Sartorius, Germany; High-speed refrigerated centrifuge (H1750R): Xiangyi Centrifuge Instrument Co., Ltd., Changsha; Nitrogen blow-down instrument (HSC-24B): Tianjin Heng'ao Technology Co., Ltd.

1.3 Working conditions of instruments

1.3.1 Chromatographic conditions. Phenomenex LC Column C_{18} column (50 mm × 3 mm, 5 μm); Phenomenex LC Column C_{18} column (100 mm × 2 mm, 5 μm); injection volume: 10 μL; column temperature: 30 ℃; flow rate: 0.4 mL/min; The mobile phase A was acetonitrile and B was 2 mmol/L ammonium acetate. The gradient elution procedure is detailed in Table 1.

Table1 Parameters for procedure gradient elution

Time//min	Mobile phase A//%	Mobile phase B//%
0	20	80
1	20	80
5.5	50	50
12.5	95	5
14	99	1
17	99	1
17.1	20	80
22	20	80

1.3.2 Mass spectrum conditions. Electrospray ionization (ESI) source, negative ion mode; Scanning mode: Multiple reaction monitoring (MRM). Curtain gas (CUR), 35 psi; Ion source gas 1 (GS1), 55 psi; Ion source gas 2 (GS2), 55 psi; Ion Spray voltage (IS), 4 500 V; Ion source temperature (TEM), 550 ℃. The characteristic ions are listed in Table 2, with the " * " indicating the quantitative ion.

Table 2 Mass spectrometry parameters

No.	Compound name	Abbrev.	Parent ion/mass-to-charge ratio	Product ion/mass-to-charge ratio	Taper hole voltage//V	Collision energy//eV
1	1H,1H, 2H, 2H- Perfluorohexanesulfonic acid	4:2FTS	326.9	306.8*, 81.1	–50	–29.0, –52.0
2	Perfluorohexanoic acid	PFHxA	312.9	268.9*, 119.0	–35	–11.0, –26.0
3	7H – Perfluoroheptanoic acid	7HPFHpA	344.9	280.7*, 131.0	–30	–15.0, –39.0
4	Nonafluorobutane-1-sulfonic acid	PFBS	298.7	79.8*, 98.8	–70	–60.0, –56.0
5	Perfluoro(2-methyl-3-oxahexanoic) acid	PFPrOPrA	329.0	308.9*, 83.0	–60	–28.0, –51.0
6	Perfluoroheptanoic acid	PFHpA	362.9	318.9*, 168.9	–30	–13.0, –21.0
7	1H, 1H, 2H, 2H- Perfluorooctanesulfonic acid	6:2FTS	426.8	407.0*, 80.9	–20	–34.0, –74.0
8	Pentadecafluorooctanoic acid	PFOA	412.9	368.9*, 168.9	–30	–15.0, –25.0
9	2-perfluorooctylethanoieque	2-PFOA	476.9	392.7*, 413.1	–30	–20.0, –12.0
10	Perfluorohexane-1-sulphonic acid	PFHxS	398.9	80.0*, 98.9	–80	–79.0, –66.0
11	Perfluorononanoic acid	PFNA	462.9	418.9*, 218.9	–27	–14.0, –23.0
12	Glycine, N-[(heptadecafluorooctyl)sulfonyl]-	N-PFOS	555.9	497.8*, 418.8	–80	–43.0, –36.0
13	1H, 1H, 2H, 2H- henicosafluorodecanesulphonic acid	8:2FTS	526.9	506.8*, 80.9	–50	–37.0, –84.0
14	Perfluoroheptanesulfonic acid	PFHpS	448.6	79.9*, 98.9	–100	–92.0, –70.0
15	Heptadecafluoro-N-methyloctanesulphonamide	N-MeFOSAA	569.8	419.0*, 483.1	–60	–28.0, –23.0
16	Perfluorodecanoic acid	PFDA	512.9	468.9*, 218.9	–33	–16.0, –24.0
17	N-Ethylperfluorooctane sulfonamidoacetic acid-d5	N-EtFOSAA	583.8	418.9*, 525.8	–60	–30.0, –28.0
18	Perfluorooctanesulfonic acid	PFOS	498.6	80.0*, 99.0	–60	–96.0, –81.0
19	Perfluoroundecanoic acid	PFUdA	562.9	518.9*, 268.9	–29	–15.0, –26.0
20	1H, 1H, 2H, 2H- Perfluorododecane sulphonicacid	10:2FTS	626.8	606.8*, 80.9	–80	–44.0, –108.0
21	Perfluorododecanoic acid	PFDoA	612.8	568.8*, 168.9	–30	–16.0, –31.0
22	Perfluorooctane sulfonamide	FOSA	497.9	77.8*, 478.0	–60.0	–75.0, –38.0
23	Perfluorotridecanoic acid	PFTrDA	662.8	618.8*, 168.9	–60.0	–17.0, –34.0
24	Perfluorotetradecanoic acid	PFTeDA	712.8	668.8*, 168.9	–30.0	–17.0, –37.0
25	Perfluorooctadecanoic acid	PFSA	912.8	869.1*, 218.8	–20.0	–27.0, –40.0
26	2H, 2H, 3H, 3H- Perfluoroundecanoic acid	PFUnA	490.9	367.1*, 387.1	–40.0	–34.0, –20.0
27	Bis[1H, 1H, 2H, 2H-perfluorodecane] phosphate	DFDP	989.1	96.8*, 542.8	–15.0	–105.0, –37.0
28	Perfluorohexane sulfonamide	FHxSA	398.0	77.9*, 169.1	–60	–36.0, –36.0

1.4 Sample processing We weighed 5.00 g of the sample (accurate to 0.01 g) and transferred it into a 50 mL plastic centrifuge tube. Precisely added 5 mL of acetonitrile, then extracted by ultrasonication for 30 min. After extraction, added 1.0 g of sodium chloride, 2.0 g of anhydrous magnesium sulfate, and 0.1 g of PSA powder. Mixed thoroughly, then centrifuged at 5 500 r/min for 10 min. Used a plastic pipette to transfer the supernatant acetonitrile layer into a plastic centrifuge tube. Evaporated to dryness under a nitrogen stream at 50 ℃. Added 1 mL of acetonitrile to dissolve the residue, vortex to mix well, and filtered through a 0.22 μm polyethersulfone (PES) membrane. The resulting solution is now ready for instrumental analysis.

2 Results and analysis

2.1 Selection of pretreatment method In this study, two sample pretreatment methods were compared: direct extraction with acetonitrile, and reconstitution of the sample with 10 mL of water followed by acetonitrile extraction. Since aquatic products inherently have high water content, additional water for reconstitution is unnecessary. After reconstitution, the emulsion layer between the acetonitrile and aqueous phases appeared blurred, with poor phase separation. In addition, the volume of the acetonitrile extract was reduced and insufficiently separated from the aqueous phase, ultimately resulting in lower recovery rates.

2.2 Effects of the purification step The purification process involving the addition of sodium chloride, anhydrous magnesium sulfate, and PSA powder effectively removed impurities from the sample. Sodium chloride facilitated the phase separation between acetonitrile and the aqueous layer, while anhydrous magnesium sulfate served as a drying agent to eliminate residual moisture. PSA powder adsorbed interfering substances such as fatty acids and pigments from the sample. Comparative analysis of pre- and post-purification results demonstrated higher recovery rates in purified samples. Furthermore, the extract obtained after nitrogen blowdown exhibited cleaner composition and more stable baseline, significantly enhancing the accuracy and sensitivity of the detection method.

2.3 Optimization of chromatographic conditions In this experiment, two chromatographic columns were employed for method development. When using only the Phenomenex LC Column C_{18} (100 mm × 2 mm, 5 μm), chromatographic peak splitting (double peaks) occurred after a period of usage, with one of the peaks appearing as a broad peak, which interfered with both qualitative and quantitative analysis. By connecting two columns in series, using the Phenomenex LC Column C_{18} (50 mm × 3 mm, 5 μm) for pre-concentration followed by the Phenomenex LC Column C_{18} (100 mm × 2 mm, 5 μm) for separation, the phenomenon of double peaks was effectively resolved.

Due to the presence of carboxylic acid and sulfonic acid groups in PFAS compounds, which are analyzed in negative ion mode, ammonium acetate is generally employed as a mobile phase component to improve peak shape and enhance ionization efficiency. Commonly used mobile phase systems include methanol-ammonium acetate, acetonitrile-ammonium acetate, and isopropanol-methanol-ammonium acetate. Compared to the methanol-ammonium acetate and isopropanol-methanol-ammonium acetate systems, the acetonitrile-ammonium acetate mobile phase system yields superior chromatographic peak shape, reduced tailing, and enhanced response, thereby providing better separation performance. Consequently, the acetonitrile-ammonium acetate mobile phase system was selected for this analysis.

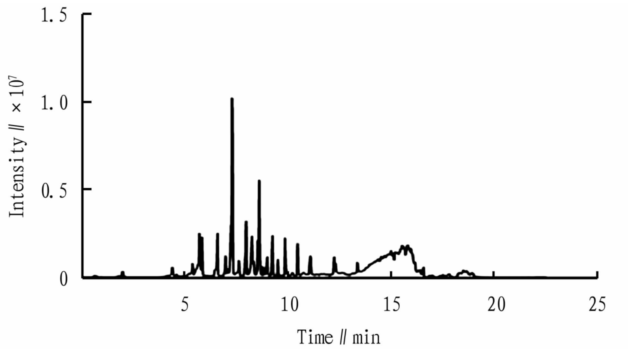

Fig. 1 Total ion chromatogram (TIC) of 28 PFAS compounds

2.4 Spiked recovery The QuEChERS method was selected as the sample pretreatment approach in this study due to its rapidity, simplicity, and high efficiency. Spiked recovery tests were performed on live clams (*Ruditapes variegata*) by fortifying them with 28 PFAS compounds at different concentrations. The results showed average recoveries of 72.2% – 113.0% with six replicates, and the precision (*RSD*) was below 9.56%. The linear ranges and correlation coefficients obtained for each compound are presented in Table 3. As shown in the table, the limits of detection (LOD) for the 28 PFAS compounds ranged from 0.02 to 0.5 μg/kg, and good linearity ($R^2 \geq 0.99$) was achieved within the concentration range of 0.02 – 20 ng/mL.

Table 3 Linear range, correlation eoefficient, recovery, LOD, and precision for 28 PFAS compounds in live clams (*Ruditapes variegata*)

Compound name	Addition concentration // μg/kg	Average recovery // %	LOD // μg/kg	Linear equation	Correlation coefficient R^2	Precision // %
4:2FTS	0.05	102.6	0.05	$y=1.700\ 68e5x+3\ 950.735\ 80$	0.997 73	5.74
PFHxA	0.05	101.1	0.05	$y=6.446\ 87e5x+20\ 220.557\ 6$	0.997 15	6.25
7HPFHpA	0.05	81.7	0.05	$y=9.385\ 03e5x+21\ 403.904\ 3$	0.996 55	3.48
PFBS	0.05	88.5	0.05	$y=3.001\ 60e5x+3\ 316.141\ 96$	0.997 27	7.89
PFPrOPrA	0.50	116.2	0.50	$y=12\ 152.804\ 02x+681.872\ 9$	0.997 59	8.25
PFHpA	0.10	87.2	0.10	$y=1.053\ 57e6x+28\ 288.407\ 9$	0.997 55	7.61

(To be continued)

(Continued)

Compound name	Addition concentration // μg/kg	Average recovery // %	LOD // μg/kg	Linear equation	Correlation coefficient R^2	Precision // %
6:2FTS	0.05	88.7	0.05	$y = 3.280\ 22e5x + 5\ 396.796\ 11$	0.998 35	2.69
PFOA	0.01	109.4	0.01	$y = 1.913\ 87e6x + 4.084\ 96e4$	0.994 47	5.26
2-PFOA	0.10	91.3	0.10	$y = 1.066\ 30e5x + 1\ 611.086\ 30$	0.994 55	6.16
PFHxS	0.05	97.1	0.05	$y = 4.056\ 01e5x + 6\ 749.611\ 60$	0.998 99	9.56
PFNA	0.02	98.6	0.02	$y = 1.360\ 80e6x + 18\ 020.073\ 7$	0.993 92	8.31
N-PFOS	0.05	89.5	0.05	$y = 9.789\ 48e4x + 2\ 205.415\ 50$	0.993 64	3.33
8:2FTS	0.05	99.6	0.05	$y = 2.422\ 39e5x + 6\ 033.698\ 91$	0.982 97	4.51
PFHpS	0.10	100.5	0.10	$y = 3.306\ 74e5x + 5\ 855.139\ 36$	0.999 04	2.56
N-MeFOSAA	0.02	113.0	0.02	$y = 2.459\ 08e5x + 2\ 595.720\ 60$	0.999 28	5.69
PFDA	0.05	90.6	0.05	$y = 1.675\ 10e6x + 2\ 9695.363\ 8$	0.996 54	6.49
N-EtFOSAA	0.05	94.3	0.05	$y = 2.393\ 35e5x + 1\ 855.701\ 59$	0.997 38	7.51
PFOS	0.10	86.7	0.10	$y = 3.143\ 84e5x + 7\ 515.226\ 34$	0.999 19	5.12
PFUdA	0.05	108.2	0.05	$y = 1.486\ 99e6x + 21\ 666.358\ 0$	0.993 92	4.96
10:2FTS	0.05	86.5	0.05	$y = 3.037\ 23e5x + 4\ 458.239\ 58$	0.998 32	5.69
PFDoA	0.05	72.2	0.05	$y = 1.376\ 56e6x + 22\ 608.391\ 4$	0.995 68	7.69
FOSA	0.05	82.6	0.05	$y = 6.175\ 93e5x + 6\ 217.396\ 17$	0.996 04	8.64
PFTrDA	0.05	98.5	0.05	$y = 1.480\ 15e6x + 27\ 923.542\ 4$	0.994 74	9.11
PFTeDA	0.05	100.0	0.05	$y = 1.510\ 02e6x + 3.418\ 24e4$	0.993 63	6.61
PFSA	0.02	89.7	0.02	$y = 1.251\ 96e5x + 893.364\ 94$	0.999 38	7.26
PFUnA	0.05	78.6	0.05	$y = 3.283\ 83e5x + 2\ 562.253\ 81$	0.999 78	8.26
DFDP	0.05	94.6	0.05	$y = 1.306\ 14e5x + 1\ 851.010\ 78$	0.999 01	5.64
FHxSA	0.02	92.4	0.02	$y = 2.314\ 44e5x + 12\ 997.768\ 0$	0.999 57	6.40

Based on the obtained detection data, nearest neighbor analysis was performed using SPSS Statistics 17.0 software, with the LOD concentration as the target variable and recovery rate, precision, and correlation coefficient as feature variables. The results showed 22 valid samples, achieving an effective rate of 71.0%. This indicates that the detection results for the 28 PFAS compounds were relatively stable, with no significant outliers observed. The specific analytical plot is shown in Fig. 2.

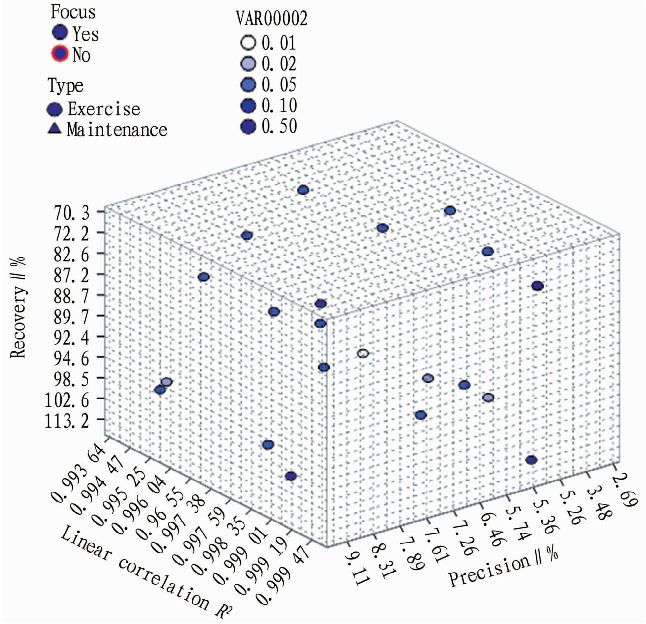

Fig. 2 Detection results of 28 PFAS compounds

2.5 Sample analysis Analysis of nearly 100 samples revealed a PFAS detection rate exceeding 80%. The detected perfluorinated compounds were primarily identified as Perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), Perfluorohexane-1-sulphonic acid (PFHxS), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluorooctanesulfonic acid (PFOS), Perfluoroundecanoic acid (PFUdA), Perfluorotridecanoic acid (PFTrDA), and Perfluorotetradecanoic acid (PFTeDA). According to a relevant study conducted in the major economic sea areas of Liaoning Province, PFOA was detected in 100% of various fish samples, while PFOS reached a 100% detection rate in fish viscera[23]. These findings demonstrate that aquatic products in coastal regions are universally contaminated with PFAS, warranting significant attention.

3 Conclusions

In this study, 28 PFAS compounds in aquatic product samples were extracted with acetonitrile and purified of pigment impurities using the QuEChERS method[24]. The analysis was performed using HPLC-MS/MS in multiple reaction monitoring (MRM) mode. The established method is accurate, reliable, efficient, simple, and rapid, demonstrating high practical value for the quality and risk assessment of PFAS in aquatic products.

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