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Quantification of PFAS in Oyster Tissue Using a Rapid QuEChERS Extraction Followed by UPLC-MS/MS Analysis

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ABSTRACT

Per- and poly-fluoroalkyl substances (PFAS) are a large group of man-made chemicals that repel oil, stains, grease, and water and are fire resistant. PFAS are known to be persistent in the environment and bioaccumulate in human and animal tissue, soil, and water. To mitigate human exposure to these chemicals, PFAS analysis by ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) was used to monitor their presence in the environment. This study focused on analyzing oysters harvested from coastal New England for 14 PFAS. The validated method addressed in this study utilized a quick, easy, cheap, effective, rugged, and safe (QuEChERS) approach using various sorbents and showed that the combination of primary secondary amine/graphitized carbon black (PSA/GCB) provided the most effective sample clean-up prior to analysis. The recoveries for the targeted analysis ranged from 61% to 116.3% with relative standard deviations from 2.4% to 13.3% at the 125.0 ng mL^{-1} analyte level. The method detection limits were from 0.33 ng g^{-1} to 6.75 ng g^{-1}. In a targeted analysis of five unknown samples, two samples had a detectable level of the legacy compound, perfluorooctanesulfonic acid (PFOS).

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Introduction

Per- and poly-fluoroalkyl substances (PFAS) are a group of environmentally persistent chemicals that are characterized by their strong fluorinated carbon bonds which make the parent and terminal transformation products incredibly stable and resistant to biotic and abiotic degradation (Buck et al. 2011). PFAS are unique in that they have hydrophobic, oleophobic, and amphiphilic properties and therefore are widely used as surfactants and in consumer and industrial products. There are over 9,000 known PFAS compounds and PFAS have been included in products such as waterproof gear, aqueous film-forming foams, cosmetics, anti-fog products, food packaging, plastics, textiles, ski wax, and pesticides (Tittlemier et al. 2007; Kotthoff et al. 2015; Lang et al. 2016; Barzen-Hanson et al. 2017; Robel et al. 2017; U.S. Environmental Protection Agency

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2021; Whitehead et al. 2021; Herkert et al. 2022; Rodgers et al. 2022). Although PFAS are highly beneficial from a manufacturing standpoint, they demonstrate environmental persistence and mobility, bioaccumulate in food webs, and are associated with numerous adverse health outcomes in wildlife and humans (Schulz, Silva, and Klaper 2020).

Due to their ubiquity and resistance to degradation, PFAS are often detected in environmental media including dust, air, and water, which may then travel to other regions *via* atmospheric or oceanic transport and aquatic discharges. Humans are primarily exposed to PFAS through their diet and exposure has been associated with birth defects, reduced antibody response to vaccines, elevated cholesterol, and increased risk of some cancers (Looker et al. 2014; Ou et al. 2021; Shearer et al. 2021). Dietary exposure may occur directly through bioaccumulation and trophic transfer in aquatic and terrestrial food chains or indirectly through consuming food items that were in contact with PFAS-containing food packaging or cookware. Fish, meat, eggs, and seafood are considered the main contributors to PFAS ingestion and have been detected in samples worldwide (Haug et al. 2010; Christensen et al. 2017; De Silva et al. 2021; Lemos et al. 2022).

Shellfish are of particular interest due to their sessile nature, filter feeding mechanisms, and high consumption by humans. Additionally, shellfish commonly accumulate high levels of contaminants and are commonly used as bioindicators of environmental quality. For example, high levels of arsenic, zinc, and microplastics were detected in oysters from Brazil (Vieira et al. 2021) and Pacific oysters and mussels had average tissue concentrations that exceeded California advisory levels for PCBs (Talley et al. 2022). The complex nature of human consumption coupled with the compound diversity, pervasiveness, and potential toxicity of PFAS warrants timely, sensitive, and dependable analytical methods are required to monitor their levels in the environment and dietary items.

PFAS have been determined in drinking water, non-potable water, groundwater, soil, sediment, various food items (i.e., fruits and vegetables), and aquatic and terrestrial biota. Several methods have been used to detect and quantify PFAS mixtures; however, liquid chromatography – tandem mass spectrometry is most used for targeted analyses due to its high sensitivity and selectivity (Huset and Barry 2018). Previous studies have also used liquid chromatography coupled to high resolution mass spectrometry for untargeted approaches (Piva et al. 2022). Sample matrices can be complex, including shellfish, and require efficient sample clean-up to reduce matrix effects. Numerous extraction methods have been used for shellfish such as accelerated solvent extraction (Galvao et al. 2012), pressurized liquid extraction (Rodrigues et al. 2016), microwave decomposition (Yoda, Ichinohe, and Yokosawa 2021), and ultrasonic-assisted extraction (Aquilina-Beck et al. 2020). These methods are typically followed by solid phase extraction (SPE), which may involve various sorbent materials. However, each method has its own advantages and disadvantages including being time intensive, yielding low analyte recoveries due to multiple steps, and increased risk for contamination due to extensive sample manipulation (Langford 2022; Li et al. 2022).

The quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction is an alternative to other methods due to its speed, consistent results, and low cost. This method was originally developed by Anastassiades et al. (2003) for the determination of pesticides in fruits and vegetables and has been successfully modified to analyze a wide range

of contaminants in shellfish (Provatas et al. 2014; Cruzeiro et al. 2016; Álvarez-Muñoz et al. 2019; Diallo et al. 2022). Moreover, QuEChERS has also been used to determine PFAS in dairy products, baked goods, fruits, vegetables, meat, seafood, honey, barbecue sauce, and bottled water (Genualdi et al. 2017; Chiesa et al. 2018; Huset and Barry 2018; Scordo et al. 2020; Genualdi et al. 2021). QuEChERS has proven to be a reliable and sensitive extraction and clean-up method for PFAS; however, we are unaware of any studies that have used it with ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) to measure these concentrations in oysters.

The purpose of this study was to develop and validate a QuEChERS-based method for detection and quantification of 14 PFAS in Eastern oyster (*Crassostrea virginica*) tissue from the Northeastern United States. Eastern oysters were chosen due to their high economic and environmental importance. The proposed method provides a rapid and reliable technique to detect PFAS in oyster tissue and can help assess the risk to human health from shellfish consumption.

Experimental

Materials and reagents

Methanol ($\geq$ 99.9%) was purchased from Fisher Scientific (Fair Lawn, NJ, USA). The internal standard, EPA-537IS, and the surrogate, EPA-537SS-R1, were purchased from Wellington Laboratories (Guelph, Ontario, Canada). Ammonium acetate ($\geq$ 97%) was purchased from Fisher Scientific (Fair Lawn, NJ, USA). LC vials with polypropylene caps were purchased from Waters (Milford, MA, USA). Standards of perfluorobutanesulfonic acid (PFBS), perfluorohexanesulfonic acid (PFHxS), perfluorotetradecanoic acid (PFTA), and perfluorotridecanoic acid (PFTrDA) were purchased from Sigma Aldrich (St. Louis, MO, USA). N-ethyl perfluorooctanesulfonamidoacetic acid (NEtFOSAA), N-methyl perfluorooctanesulfonamidoacetic acid (NMeFOSAA), perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoA), perfluoroheptanoic acid (PFHpA), perfluorohexanoic acid (PFHxA), perfluorononanoic acid (PFNA), perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluoroundecanoic acid (PFUnA) were purchased in the form of EPA 537 Method Standard from AccuStandard, Inc. (New Haven, CT, USA). QuEChERS ($MgSO_4$/NaCl) and QuEChERS ($MgSO_4$/PSA/GCB) were purchased from UCT (Bristol, PA, USA).

Instrumentation

A Precisa XT 220 A analytical balance (Princeton, NJ, USA) with a resolution of 0.001 g was used to determine sample weights. A Branson 5510 R-DTH ultrasonic cleaner (Danbury, CT, USA) was used to sonicate samples, a Fisher Scientific 02215452 multitube vortex (Waltham, MA, USA) was used to mix samples, and a Thermo Scientific CL10 centrifuge (model 11210901) and Thermo Scientific Legend Micro 21 R centrifuge (model 75002446) (Waltham, MA, USA) were used for centrifugation. Polypropylene centrifuge tubes (2.0 mL) were used for extraction and sample clean-up and purchased from Eppendorf (Hauppauge, NY, USA). Samples were prepared in a PFAS-free cleanroom and all glassware was rinsed three times with deionized water, followed by an acetone rinse (Ricca Chemical Company; Arlington, TX, USA), and heated at 500 °C

overnight to prevent contamination. Additionally, polyethylene gloves (Fisher Scientific; Waltham, MA) were worn during sample preparation to prevent potential cross contamination from nitrile gloves. Analysis was performed on a Waters Acquity UPLC-MS/MS (Waters Corp., Milford, MA, USA) coupled with a triple-quadrupole (TQD) tandem mass spectrometer and retrofitted with a PFAS installation kit (Waters Corp., Milford, MA, USA) to replace PFAS-containing components within the instrument.

Preparation of standard solutions

Starting at the 2000 µg mL^{-1} concentration of EPA 537, the mixed PFAA stock solution was diluted to a volume of 2.0 mL with 96:4% (vol/vol) methanol:water to achieve a concentration of 1000 ng mL^{-1}, herein referred to as *PFAA_Mix_1000*. Serial dilutions for this solution are described in Table S1. The surrogate stock solution was prepared by diluting 200 µL of EPA-537SS-R1, which was purchased as a mixture of MPFHxA, MPFDA, M3HFPO-DA, and d5-N-EtFOSAA, at concentrations of 1000 ng mL^{-1}, 1000 ng mL^{-1}, 1000 ng mL^{-1}, and 4000 ng mL^{-1}, respectively. This solution was used for the calibration of the surrogates as shown in Tables S2 and S3 (Supplemental). The remaining 1.0 mL of *EPA-537SS-R1* was used as the surrogate spiking solution. The internal standard solution (*EPA-537IS*) was added to each sample without any further dilution.

Sample preparation and clean-up

Eastern oysters (*Crassostrea virginica*) were obtained from a commercial source in the Northeastern United States and stored at −20 °C until analysis. Oyster tissue samples ($n = 5$) were thawed overnight at 4 °C and 0.5 ± 0.0001 g (ww; wet weight) of homogenized tissue was weighed and added to a 2.0 mL polypropylene centrifuge tube. Sample preparation efficacy was determined by spiking each sample with 20 µL of the surrogate stock solution (*EPA-537SS-R1*) to give a final concentration of 20 ng mL^{-1} for MPFHxA, MPFDA, and M3HFPO-DA, and 80 ng mL^{-1} for d5-N-EtFOSAA. Previously validated, PFAS-free oyster tissue was used for the matrix spike (MS), matrix spike duplicate (MS-DUP), and laboratory control samples (LCS). The MS and MS-DUP were used to determine precision and bias. MS, MS-DUP, and LCSs were fortified with 100 µL of the 1000 ng mL^{-1} standard solution (*PFAA_Mix_1000*) to give a final concentration of 100 ng mL^{-1}.

Samples were subsequently vortexed for 1 min at 2,500 rounds per minute (rpm). An aliquot of 980 µL of HPLC-grade methanol was added to all samples, whereas 880 µL was added to LCS and MSs. Samples were vortexed for 1 min at 2,500 rpm, followed by 1 min of sonication. After sonication, 0.1-0.3 g of QuEChERS ($MgSO_4$/NaCl) was added to each sample, shaken vigorously, and vortexed for 10 min at 2,500 rpm. Samples were cooled to −20 °C for one hour and centrifuged for 10 min at 14,000 rpm. 500 µL of supernatant was transferred to a 2.0 mL polypropylene centrifuge tube and 0.1-0.3 g of QuEChERS clean-up powder ($MgSO_4$/PSA/GCB) was added. Samples were vortexed for 10 min at 2,500 rpm, centrifuged for 10 min at 14,000 rpm, and 200 µL of the supernatant was transferred to a 300 µL LC vial that were spiked with 10 µL of the internal standard (*EPA-537IS*), which gave a final concentration of 40 ng mL^{-1} for M2PFOA, 120 ng mL^{-1} for MPFOS, and 160 ng mL^{-1} for d3-N-MeFOSAA. Samples were vortexed and analyzed by UPLC-MS/MS.

Sample analysis

All samples were analyzed for 14 PFAS (Table S4) using a Waters Acquity UPLC-MS/MS. Analytes were separated on a Waters Acquity UPLC BEH C18 column (1.7 µm, 2.1 × 50 mm) heated to 25 °C. The injection volume was 5.0 µL. Prior to use, the column was flushed with 100% methanol for at least 20 min. The mobile phase consisted of 95:5 H_2O/MeOH with 2.0 mM ammonium acetate (solvent A) and MeOH with 2.0 mM ammonium acetate (solvent B). Run time was nine minutes with a constant flow rate of 0.4 mL/min. The dualistic gradient was initiated at 40% B, held for 0.3 min, increased linearly to 90% B until 8 min, after which the column was reconditioned to initial state until 9 min. The detection was performed in negative electrospray ionization mode (ESI-). The multiple reaction mode (MRM) for the target PFAS is listed in Table 1 and the MRM chromatograms are depicted in Figure 1. Parameters for the mass

Table 1. Multiple-reaction mode (MRM) transitions (m/z) for the identification and quantification of 14 PFAS in Eastern oyster tissue (*Crassostrea virginica*). The analytes are defined in Table S4 in the supplemental material.

Analyte	MRM Transition (m/z)	Correlation coefficient (R^2)
$13C_2$-HFPO-DA (sur)	287.0 > 169.0	
PFBS	298.8 > 79.9	0.9984
	298.8 > 99.1	
PFHxA	313.0 > 119.0	0.9993
	313.0 > 269.0	
$13C_2$-PFHxA (sur)	315.0 > 270.0	0.9996
PFHpA	363.0 > 169.0	0.9990
	363.0 > 319.0	
PFHxS	399.0 > 80.0	0.9992
	399.0 > 99.1	
$13C_2$-PFOA (IS)	415.0 > 370.0	
	415.0 > 415.0	
PFOA	413.0 > 169.0	0.9991
	499.0 > 99.0	
PFOS	499.0 > 80.0	0.9991
	499.0 > 99.0	
PFNA	463.0 > 219.0	0.9990
	463.0 > 419.0	
$13C_4$-PFOS (IS)	502.8 > 79.9	
	502.8 > 99.8	
PFDA	513.0 > 219.0	0.9996
	513.0 > 469.0	
$13C_2$-PFDA (sur)	514.8 > 469.8	0.9967
d_5-NEtFOSAA (sur)	589.0 > 419.0	0.9990
	589.0 > 506.9	
d_3-NMeFOSAA (IS)	573.0 > 419.0	
	573.0 > 482.7	
NMeFOSAA	570.0 > 219.1	0.9995
	570.0 > 419.0	
NEtFOSAA	584.0 > 419.0	0.9994
	584.0 > 525.9	
PFUnA	563.0 > 319.0	0.9992
	563.0 > 519.0	
PFTA	712.8 > 169.0	0.9991
	712.8 > 668.8	
PFDoA	613.0 > 169.0	0.9991
	613.0 > 569.0	
PFTrDA	662.8 > 169.0	0.9991
	662.8 > 618.8	

Definitions: sur, surrogate; IS, internal standard.

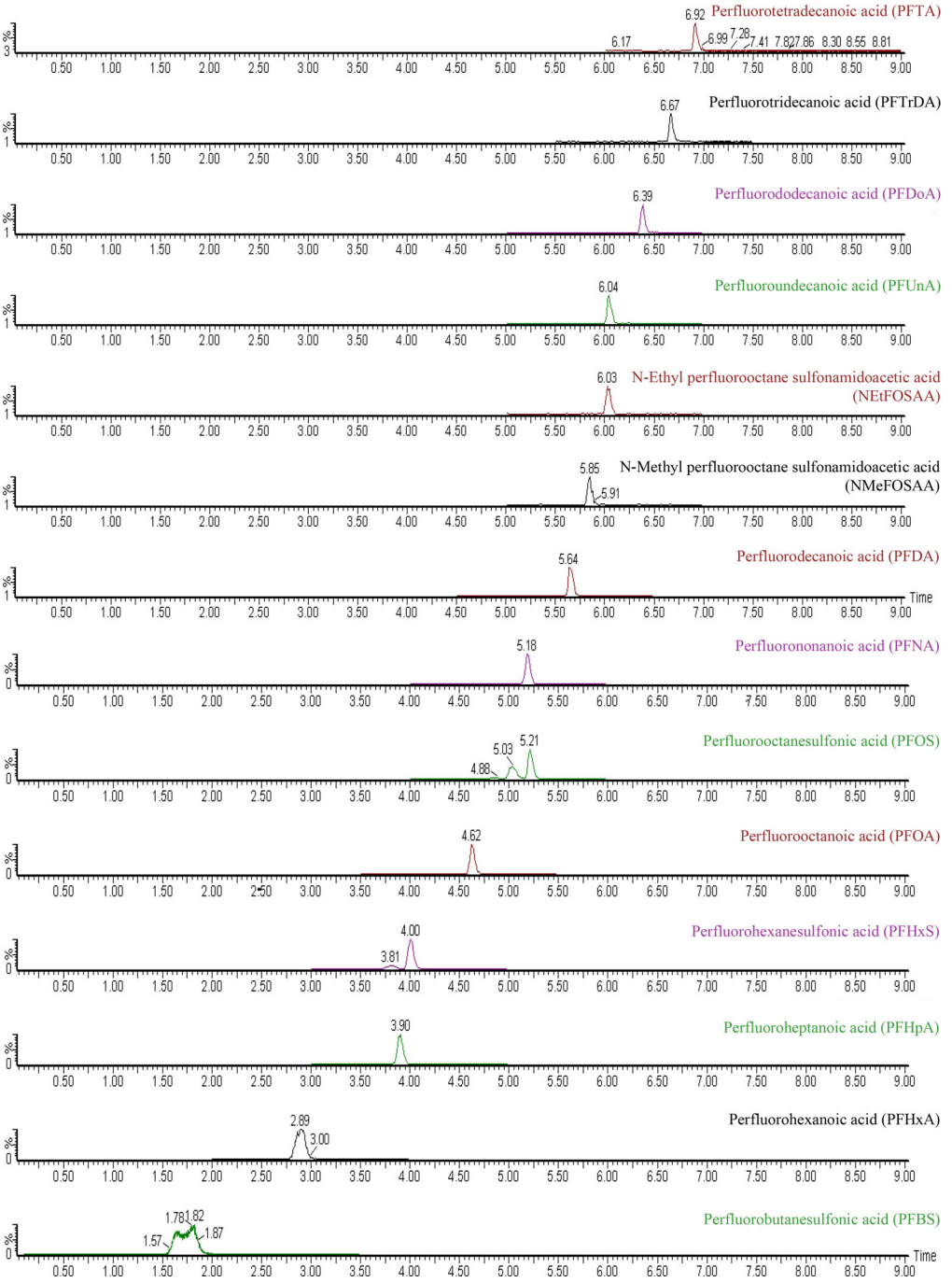

Figure 1. Chromatogram showing multiple reaction monitoring (MRM) of 14 perfluoroalkyl acid (PFAA) analytes at concentrations of 100 ng mL^{-1}.

spectrometer were as follows: capillary voltage 3.6 kV; cone voltage 40 V; desolvation temperature 350 °C; source temperature 150 °C, desolvation gas flow 700 L/Hr; collision gas

flow 0.2 mL/min. Statistical analysis and data processing were performed using Waters MassLynx software (Version 4.1).

Results and discussion

Chromatographic separation of the 14 PFAS was achieved using a Waters Acquity UPLC BEH C_{18} (1.7 µm, 2.1 × 50 mm) column; therefore no other column options were explored. The validated method displayed good linearity over a concentration range from 1.0 to 500.0 ng mL^{-1} for all analytes and the correlation coefficient R^2 of the regression equations was 0.9984 or higher (Table 1). Method validation was confirmed by performing method detection limit (MDL), precision, and accuracy studies according to the United States Environmental Protection Agency (EPA) guidelines (U.S. Environmental Protection Agency 2015). Seven oyster replicates at the 15.0 ng mL^{-1} concentration level were used in the MDL study, whereas four replicates spiked with 125.0 ng mL^{-1} of the analytes were used in the precision and accuracy study. The MDL was determined to be 3.143 (the Student's t-value for a single tailed 99th percentile for seven replicates) times the standard deviation (SD) of the replicate analysis.

Accuracy (recovery %) was calculated as the mean calculated concentration of the analyte relative to the nominal concentration of the spike, whereas precision was calculated as the relative standard deviation (RSD). Measurement uncertainty (MU) was calculated by dividing RSD (%) from the precision and accuracy study by 100 and multiplying by 2. All sample concentrations are reported as measured and were uncorrected for observed analyte recovery.

The resulting MDLs were from 0.33 ng g^{-1} to 6.75 ng g^{-1} and accuracy ranged from 61% to 116.3%, which was within the acceptable recovery range of 60% to 125% (U.S. Environmental Protection Agency 2011). MDLs may vary for different PFAS due to matrix effects; however, this was not assessed in the present study. Recovery results were greater than 100% for PFNA (100.2%), PFHxS (112.7%), PFDoA (113.5%), and PFOS (116.3%). RSD ranged from 1.9% to 13.3% at the 125.0 ng mL^{-1} spike level. NEtFOSAA and PFTA had the highest RSD values of 12.3% and 13.3%, respectively, whereas all other analyte RSD values were $< 7.1\%$. The presented method is an alternative to existing QuEChERs based extraction methods (Abafe et al. 2021; Álvarez-Ruiz et al. 2021; Genualdi et al. 2021; Lemos et al. 2022) and demonstrated low MDLs at the 15.0 ng mL^{-1} concentration level and good recovery as evidenced by well-established chromatographic peaks. MDL, precision, and accuracy results are shown in Tables 2 and 3.

A non-QuEChERS approach and various dispersive SPE (dSPE) sorbents, including Thermo Scientific, Oasis HLB, Supel Que, and UCT, were compared for secondary QuEChERS clean-up. The results indicated that the combination of primary secondary amine/graphitized carbon black (PSA/GCB) was the most efficient, minimizing matrix interferences while retaining good analyte recoveries. As a result, it was reported that PFNA, PFHxS, PFDoA, and PFOS had the highest recovery. However, PSA/GCB resulted in lower recoveries for PFTA (61%), NMeFOSAA (73.3%), and PFTrDA (75.2%). Lower recovery for these compounds may be due to their ability to readily adsorb to the clean-up sorbent (Boone et al. 2014). GCB is known for its strong

Table 2. Method detection limits (MDLs) and recovery ± relative standard deviation (RSD) for 14 per- and polyfluoroalkyl substances (PFAS) in Eastern oyster (*Crassostrea virginica*) tissue ($n = 7$). The analytes are defined in Table S4 in the supplemental material.

PFAS*	Spiked concentration (ng mL^{-1})	Recovery ± RSD	MDL (ng g^{-1})
PFBS	15	89.6 ± 8.8	0.3296
PFHxA	15	72.1 ± 11.8	0.4691
PFHpA	15	69.6 ± 19.9	1.2980
PFHxS	15	91.7 ± 10.7	0.4903
PFOA	15	57.0 ± 21.1	1.1980
PFOS	15	112.8 ± 14.5	1.1150
PFNA	15	77.6 ± 16.5	0.9978
NMeFOSAA	15	79.4 ± 23.2	2.0080
PFDA	15	79.0 ± 17.5	1.1320
NEtFOSAA	15	72.5 ± 26.0	2.3150
PFUnA	15	69.0 ± 24.3	1.9140
PFTA	15	39.0 ± 44.3	3.5960
PFDoA	15	110.1 ± 36.1	6.7470
PFTrDA	15	52.3 ± 11.9	0.3510

*Mass used for all samples is 0.5 g.

Table 3. Precision (relative standard deviation, RSD), accuracy (recovery %), and measurement uncertainty (MU) for 14 per- and polyfluoroalkyl substances (PFAS) in Eastern oyster (*Crassostrea virginica*) tissue ($n = 4$). The analytes are defined in Table S4 in the supplemental material.

PFAS*	Concentration used (ng mL^{-1})	Recovery, %	RSD (%)	MU
PFBS	125	94.4	3.3	0.07
PFHxA	125	90.2	3.4	0.07
PFHpA	125	99.4	5.7	0.11
PFHxS	125	112.7	3.1	0.06
PFOA	125	96.2	1.9	0.04
PFOS	125	116.3	4.6	0.09
PFNA	125	100.2	3.4	0.07
NMeFOSAA	125	73.3	4.1	0.08
PFDA	125	97.4	4.8	0.10
NEtFOSAA	125	82.0	12.3	0.25
PFUnA	125	91.4	7.1	0.14
PFTA	125	61.0	13.3	0.27
PFDoA	125	113.5	2.4	0.05
PFTrDA	125	75.2	5.0	0.10

*The mass used for all samples is 0.5 g.

Table 4. Concentrations (ng g^{-1}) of 14 per- and polyfluoroalkyl substances (PFAS) in Eastern oyster (*Crassostrea virginica*) tissue ($n = 5$) using rapid QuEChERS by UPLC-MS/MS. The analytes are defined in Table S4 in the supplemental material.

	Oyster Sample				
Analyte	Oyster #1	Oyster #2	Oyster #3	Oyster #4	Oyster #5
PFBS	ND	ND	ND	ND	ND
PFHxA	ND	ND	ND	ND	ND
PFHpA	ND	ND	ND	ND	ND
PFHxS	ND	ND	ND	ND	ND
PFOA	ND	ND	ND	ND	ND
PFOS	3.6	ND	3.5	ND	ND
PFNA	ND	ND	ND	ND	ND
PFDA	ND	ND	ND	ND	ND
NMeFOSAA	ND	ND	ND	ND	ND
NEtFOSAA	ND	ND	ND	ND	ND
PFUnA	ND	ND	ND	ND	ND
PFTA	ND	ND	ND	ND	ND
PFDoA	ND	ND	ND	ND	ND
PFTrDA	ND	ND	ND	ND	ND

ND, Not detected (< MDL).

adsorption properties which can lead to reduced recovery of these PFAS and this effect may increase with increasing PFAS C-F chain length (Organtini, Hird, and Adams 2021).

The legacy compound, PFOS, was detected in two out of five oyster samples (3.5 and 3.6 ng g^{-1}); however, all other PFAS results were below the detection limits (Table 4). Indeed, previous studies have also detected PFOS in wild, commercial, and farmed oyster samples throughout the world, including the Greater Bay Area in China (0.030 – 0.149 ng g^{-1} ww; Wang et al. 2022), South Africa (0.024 ng g^{-1} ww; Abafe et al. 2021), and Galveston Bay, Houston, Texas (mean = 1.69 ± 0.19 ng g^{-1} ww; Nolen et al. 2022).

Conclusions

The combination of QuEChERS extraction and clean-up proved to be effective, efficient, and rapid for the determination of 14 PFAS in a complex biological matrix. This method provided good recoveries from the fortified oyster samples while minimizing matrix effects. In comparison to traditional extraction methods, which use gel-permeation chromatography (GPC) followed by SPE clean-up and take several days to complete, this validated method reduced sample preparation time to just three hours for a batch of 20 samples with relatively few preparation steps.

The reduced preparation time and high throughput resulted in increased laboratory productivity and a significant reduction in sample preparation costs. This method also required significantly less solvent volume which resulted in a more environmentally friendly process. The simplicity, rapidness, and reproducibility of our developed and validated method make it a viable alternative to more traditional approaches such as GPC and other clean-up strategies.

This method was applied to a pilot study of five commercial oyster samples, in which one of the 14 target PFAS were detected in two out of five samples. Our results indicate little to no contamination was present in these commercial shellfish while providing a less time consuming and inexpensive way to routinely test the safety. Expanded study of the efficacy of oysters as an indicator of long-term PFAS exposure may be required.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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