Method Validation of Per- and Polyfluoroalkyl Substances (PFAS) Extraction from Shellfish Tissue

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Abstract

Per- and polyfluoroalkyl substances (PFAS) are synthetic chemical compounds that are characterized by their nonpolar fluorine saturated carbon tail and polar head that vary corresponding with legacy or emerging PFAS compounds as carboxylate, phosphate, or sulfonate groups. PFAS are surface active compounds and cannot be prevented from adhering to protein matrices of organic life. This has led to the unavoidable bioaccumulation and biomagnification of some of these compounds within biota, particularly those exposed to the marine food web. Ongoing research includes refining extraction and analytical methods for monitoring PFAS and its impact on biological matrices, as well as methodologies to appropriately remove PFAS contamination from areas that may lead to direct human exposure. The overall research objectives are to quantify concentrations of PFAS contamination in mussel samples across the Puget Sound region and to assess levels of background exposure. In addition, the project aims to provide data to assess exposure levels from consumption of shellfish. Mussel tissue samples were provided by the Department of Fish and Wildlife (n>30) from artificially planted mussels from cages in various urban and non-urban bays across the Puget Sound and its waterfronts. Kept at -80 C and these will be used for the QuEChERS extraction, LCMSMS detection and quantification. Improving the extraction is vital to detection, recently we have processed and analyzed a random sample of store-bought mussels from a local market and spiked with a known concentration of PFAS. Experiments using these samples was used to validate recovery and define the limits of our detection for further quantification of Puget Sound mussel samples.

Introduction

On going research throughout environmental organic chemistry includes refining extraction and analytical methods for monitoring PFAS and its impact on biota, and methodologies to appropriately remove PFAS contamination from sources of food consumption. Due to PFAS ability to penetrate or bind to membrane layers; extraction and detection of both legacy and emerging PFAS from biomass have been challenging. A widely accepted method of extraction is Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS). This method was originally utilized for pesticide detection and has since been modified and refined for PFAS detection and monitoring. The QuECHERS PFAS method is illustrated below (Figure 1) coupled with liquid chromatography mass spectrometry (LCMSMS).

Objectives

- Demonstrate efficiency of QuECHERS extraction method with high PFAS recovery and detection via liquid chromatography-tandem mass spectrometry (LCMSMS).
- Using LCMSMS, quantify several PFAS from mussel tissue obtained from various urban and non-urban bays across the Puget Sound region
- Utilize data to validate and adjust methodology for mussel specific anatomy
- Utilize data to create a contamination model of the Puget Sound and reanalyze the designated hotspots biota for toxicity and exposure to the general public.

Table 1: Summary of PFAS analyzed in this study and structural information.

Classification	Compounds	Acronyms	Molecular Weights	Molecular Weights (transition measured)	General Structure	Analysis
Perfluorinated Sulfonic Acids (PFSAs)	Perfluorobutane Sulfonic Acid	PFBS (n=3)	300	299>80	$CF_3(CF_2)_n-S(=O)(=O)-OH$	LCMSMS
	Perfluorohexane Sulfonic Acid	PFHXS (n=5)	400	399>80		
	Perfluorooctane Sulfonic Acid	PFOS (n=7)	500	499>80		
	Perfluorodecane Sulfonic Acid	PFDS (n=9)	600	599>80		
Perfluorinated Carboxylic Acids (PFCAs)	Perfluorobutanoic Acid	PFBA (n=2)	214	213>169	$CF_3(CF_2)_n-C(=O)-OH$	LCMSMS
	Perfluoropropanoic Acid	PFPeA (n=3)	264	263>219		
	Perfluorohexanoic Acid	PFHxA (n=4)	314	313>269		
	Perfluoroheptanoic Acid	PFHpA (n=5)	364	363>319		
	Perfluorooctanoic Acid	PFOA (n=6)	414	413>369		
	Perfluorononanoic Acid	PFNA (n=7)	464	463>419		
	Perfluorodecanoic Acid	PFDA (n=8)	514	513>469		
	Perfluoroundecanoic Acid	PFUnDA (n=9)	564	563>269		
	Perfluorododecanoic Acid	PFDoA (n=10)	614	613>569		
	Perfluorotridecanoic Acid	PFTrDA (n=11)	664	663>619		
	Perfluorotetradecanoic Acid	PFTeDA (n=12)	714	713>669		
	Perfluorohexadecanoic Acid	PFHxDA (n=14)	814	813>769		
	Perfluorooctadecanoic Acid	PFODA (n=16)	914	913>869		

Methods

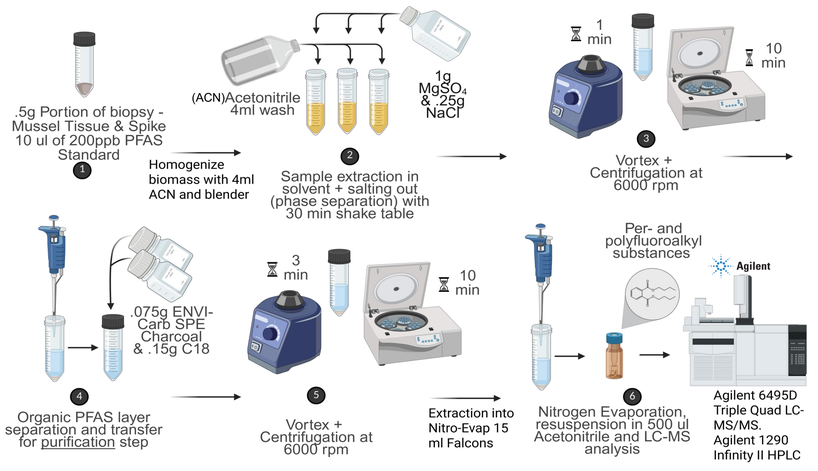

Figure 1: Schematic of QuEChERS extraction method used to analyze PFAS from mussel tissue samples.

Results and Discussion

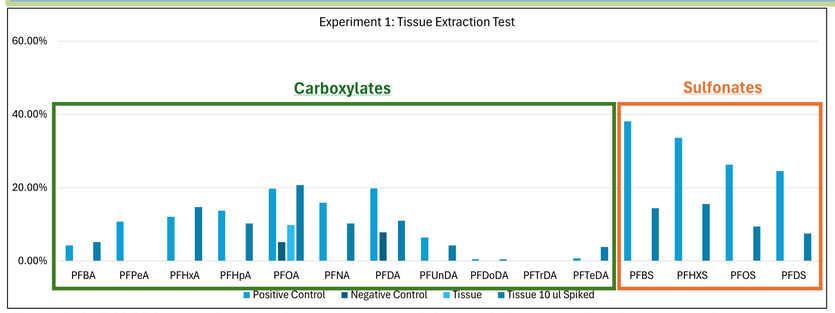

Figure 2: QuEChERS extraction experiment on commercial mussel tissue samples. Poor recoveries were observed with all compounds having <50% of spiked PFAS recovered.

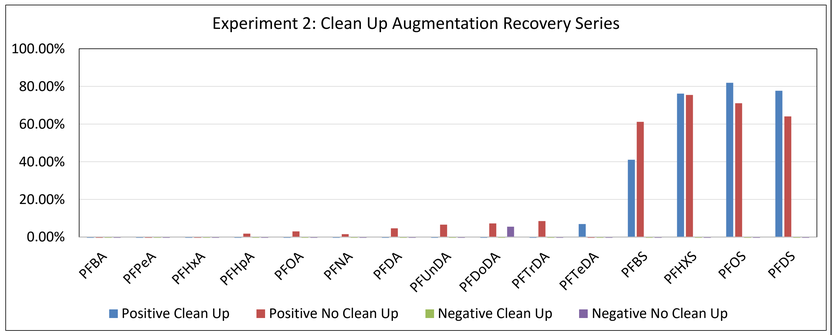

Figure 3: QuEChERS extraction follow up experiment to procedurally improve recovery rates without tissue samples.

QuEChERS is an extraction method created for compounds from a wide breadth of environmental and biological tissues. Better recoveries were observed for the sulphonate group of compounds (Figure1) although no recoveries exceeded 50%. To validate extraction method, tissues were removed from Experiment #2 with consistent poor recoveries observed for the carboxylates and better recoveries for the sulphonate group of PFAS. A simple dilute and analyze experiment was performed to determine whether instrument was selective or responded better for the sulphonate group of compounds after the LCMSMS undergone a maintenance procedure. No extraction was used in these samples. Data below (Figure 4) show consistent recoveries for all PFAS of interest indicating the instrument is responding well and that the selectivity of the recoveries is likely as result of the extraction procedure.

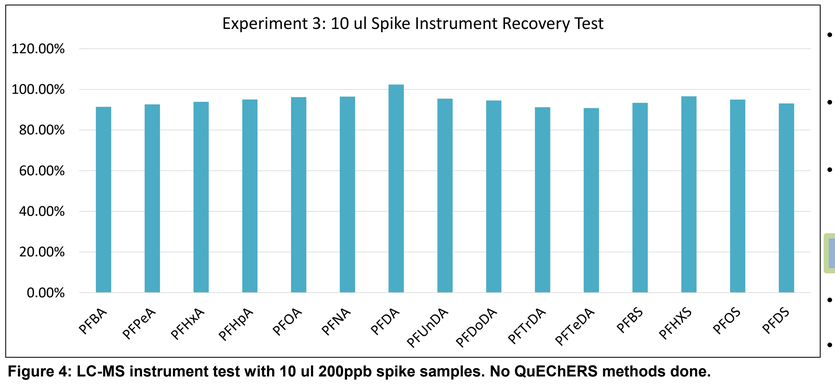

Figure 4: LC-MS instrument test with 10 ul 200ppb spike samples. No QuEChERS methods done.

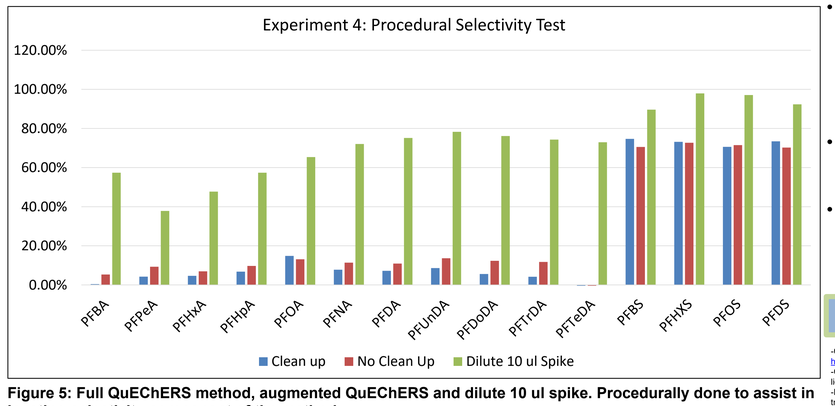

Figure 5: Full QuEChERS method, augmented QuEChERS and dilute 10 ul spike. Procedurally done to assist in locating selectivity component of the method.

After confirming that the LCMSMS was equal in detection and quantifiable recovery show an average recovery >90% as shown in Figure 4, the next experiment was to determine which aspect of the QuEChERS method was causing the observed selectivity and higher recovery of sulfonates versus carboxylates. Two hypothesis were derived:

- Carbon clean up C18 and CARB-Envi SPE was adhering the nonpolar fluorinated tails and not allowing the PFAS compounds to remain in the organic ACN layer.
- Salting out phase was interacting with the polar carboxylate heads more then the sulfonates and causing the carboxylates to remain with mussel tissue mass.

The fourth experiment (Figure 5) shows the observed selectivity persisted between the two groups of compounds. This made the second hypothesis more probable and led to the design of Experiment 5 (results shown in Figure 6).

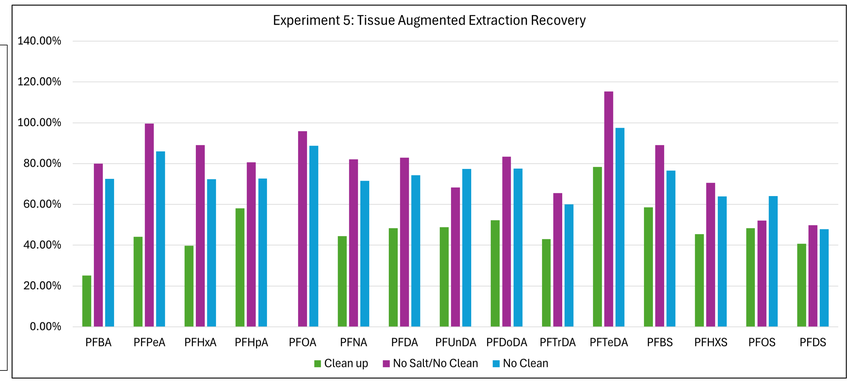

Figure 6: Mussell tissue samples (0.5g), 10 ul 200 ppb PFAS spike. Determine which component of QuEChERS method is selecting for sulfonates.

- An overall greater recovery was observed, likely attributed to improved application of extraction procedure.
- 25-60% recoveries were observed for full extraction method and improved recoveries of 50-95% observed in the set of samples where the clean up step was omitted.
- Recoveries for both sulphonates and carboxylates increased, however, a 41.89% increase in carboxylate recover was found when salting and clean up was removed.
- However, a marked improvement of PFAS recovery from the mussel tissue was observed when no salting out nor clean up steps were utilized. (add range)
- Salting out makes the tissue become more polar and pushes the relatively hydrophobic PFAS to go into the organic top layer with ACN to be extracted.
- Use of C18 and ENVI-Clean in the cleanup phase, non-specifically binds to hydrophobic contaminants, but doesn't bind to charged compounds like PFAS. Allowing for a purification of PFAS in solution to be recovered.
- It's hypothesized that some ionic interaction may be occurring between the salted solvents **OR** the tissue is not being ionized enough to create the environment in the solution to push the PFAS of the carboxylates and sulfonates into the organic layer.
- Salting out appears to have a greater effect on the carboxylates then the sulfonates. This suggest that some ionic interaction could be happening between the polar heads of the PFAS compounds and the $MgSO_4$/NaCl salting out phase.

Conclusion and Future Works

- Our study using the modified QuECHERs method (no salting out and no clean-up) resulted in 36% greater PFAS recovery than full QuEChERS method.
- Our 50-115% recovery rates were comparable to other published studies (Fu et al. 2022 and Campbell et al. 2024) that both reported recoveries ranging from 52-105.2%.
- Future work, include investigation of the more labor-intensive EPA 1633 PFAS extraction method, in addition we intend to investigate anatomical differences between mussel tissue and oyster tissues. Oysters are the general sample tissue in shellfish experiments around the globe, and this tissue difference may be the underlying cause of the variance in recoveries within the studies. Potential differences in the shellfish include lipid content, protein content or anatomical filtration method and different regions within the tissues for uptake.
- Potential modification will include blending all tissue in one batch and weighing out the frozen tissue, this could ensure more homogenous mixture in each sample. Samples will be held in -80 C before pelleting and extraction of organic ACN or methanol layer.
- The more efficient extraction method will be used in analyzing mussel samples from the Department of Ecology and potentially assess the most contaminated bays around the Puget Sound region. This data will be useful in modelling potential exposure and toxicity to the surrounding environment from these persistent contaminants.

Literature Cited

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