# *Shaken Not Sonicated*: Optimization of Extraction and Clean-up Methods for Measuring Perfluorinated Alkyl Substances in Mussel Samples.

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## Introduction

- Perfluorinated alkyl substances (PFAS) are a class of man-made organic chemicals found in everyday objects such as umbrellas and rain jackets to cookware.
- PFAS are used to make materials resistant to water, oils and grease.
- Due to the chemical and physical properties of these compounds, carbon and fluorine bonds are very strong, it is difficult to break apart even with heat and a catalyst.
- PFAS have been given the common name of forever chemicals for this reason. The problem now lies that there are varying levels of PFAS found in waterways, various environmental matrices (soil, water and tissues) and human blood.
- PFAS have been linked to adverse health outcomes such as infertility, organ failure, changes in liver enzymes and increased risk of various cancers.
- With heavy usage there has been movement of PFAS from products to the soil, air and water for some carbon chain links.
- The unique shape of the Puget Sound provides many sites along the waterways with varying conditions (i.e population density, water traffic, tides and diverse marine life) potentially leading to various levels of PFAS contamination. (Figure 1A)

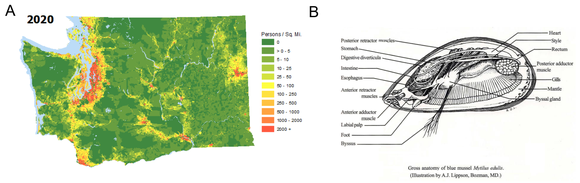


Figure 1: A. Population density map of Washington state in 2020 from the Office of Financial Management. B. Anatomy of a blue mussel. Image received from bbshellfish.org

## Study Objectives

- Compare two slightly different extraction procedures to determine the most efficient method to measure PFAS from *Mytilus edulis,* blue mussels tissue samples.
- We used blue mussels as an indicator species for water quality. Indicator species are organisms that are sensitive to changes in their environment and are used as a proxy in research to assess the environment's health. (Figure 1B)
- Mussels were collected with the assistance from the WA department of Ecology (ECY) Mussel Watch program from various urban and remote bays along the Puget Sound region.
- In order to validate and optimize extraction and clean-up methods for mussel samples spike and recovery experiments were performed with the goal to recovery >70% for majority of the analytes of interest (Table 1).

Table 1: Listed classification, name, acronym, molecular weight and general structure for the analytes of interest.

| Classification | Compounds | Acronyms | Molecular Weights (transition measured) | General Structure |
|---|---|---|---|---|
| Perfluorinated Sulfonic Acids (PFSAs) | Perfluorobutane Sulfonic Acid | PFBS (n=3) | 299>99 | $CF_3(CF_2)_n$—S(=O)(=O)—OH |
| | Perfluorohexane Sulfonic Acid | PFHXS (n=5) | 399>99 | |
| | Perfluorooctane Sulfonic Acid | PFOS (n=7) | 499>99 | |
| Perfluorinated Carboxylic Acids (PFCAs) | Perfluorohexanoic Acid | PFHxA (n=4) | 313>269 | $CF_3(CF_2)_n$—C(=O)—OH |
| | Perfluoroheptanoic Acid | PFHpA (n=5) | 363>319 | |
| | Perfluorooctanoic Acid | PFOA (n=6) | 413>369 | |
| | Perfluorononanoic Acid | PFNA (n=7) | 463>419 | |
| | Perfluorodecanoic Acid | PFDA (n=8) | 513>469 | |
| | Perfluoroundecanoic Acid | PFUnDA (n=9) | 563>519 | |
| | Perfluorododecanoic Acid | PFDoA (n=10) | 613>569 | |
| | Perfluorotridecanoic Acid | PFTrDA (n=11) | 663>619 | |
| | Perfluorotetradecanoic Acid | PFTeDA (n=12) | 713>669 | |

## Methods

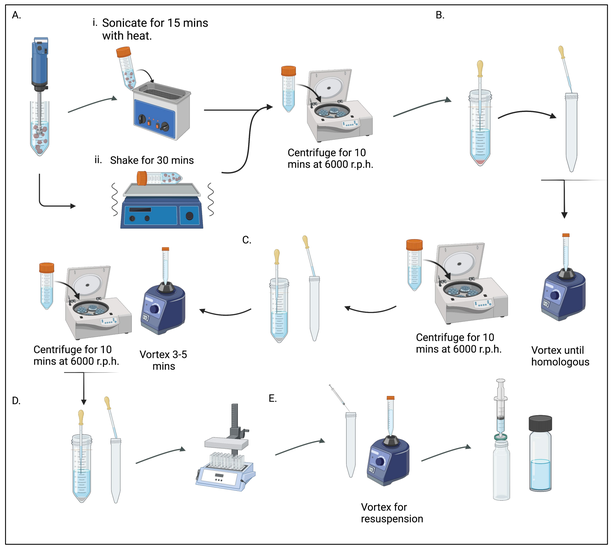


Figure 2: A. Spiked samples contained 50 µl of stock PFAS and 4 mL of Acetonitrile (ACN) in a falcon tube with ~1g of mussel tissue; blank samples had 4 mL of ACN with ~1g of mussel tissue. Samples were homogenized then placed on their method of extraction. i. Falcon tubes were sonicated with heat for 15 mins and then centrifuged. ii. Falcon tubes were shaken for 30 mins and then centrifuged. B. 2 g $MgSO_4$ and 0.5 g NaCl were added to supernatant and vortexed until homogenous. C. Removed liquid from salts (8mL total) were transferred into falcon tubes filled with 0.15g charcoal and 0.3g C18 then vortexed and centrifuged. Once spun samples were transferred into a new tube and were nitrogen evaporated to dryness. E. 500 µl of CAN was syringed into each nitro-evaporated falcon tube, then vortexed; samples from falcon tube were syringed and filtered tip into a 1mL vial, seal by crumpling the vial and analyzed using liquid chromatograph-tandem mass spectrometry (LCMSMS). Created with Biorender.com

## Results and Discussion

- In order to calculate the levels of the analytes, standards were made using a stock solution of PFAS (200 ng/mL) using a MeOH solvent. Final concentration of PFAS standards were 50, 25, 10, 1, 0.1 and 0 parts per billion (ppb). (Figure 3)
- Results indicate that shaker tables are better to use for the extraction in mussels in comparison to the use of sonication.
- Standards and samples chromatograph were analyzed and evaluated through the use of Agelint's: Qualitative Analysis B.06.00 program.
- The standards were then plotted to make a calibration curve with the aim to have an $R^2$= 1.
- The linear equation of the calibration curve was then rearranged and used to determine concentration of the analyte of interest.

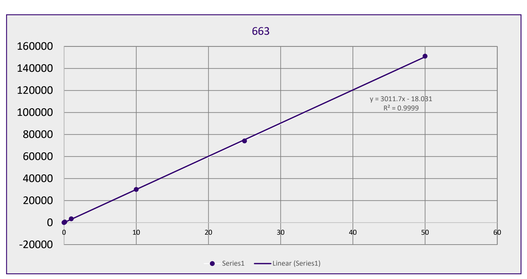


Figure 3: Calibration Curve for analyte mass 663, Perfluorotridecanoic Acid.

## Results and Discussion Continued

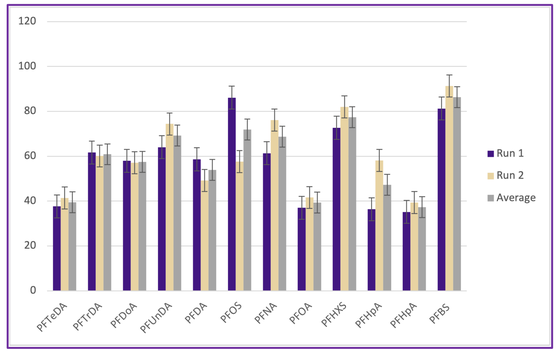


Figure 4: Shaker Table percent recoveries of different PFAS from 2 experimental trials and their average. Yielding a 49.01% recovery range from 37.27 to 86.28 %.

- The shaker table yielded an average percent recovery range from 37.27 to 86.28% (Figure 4) depending on chain length of analyte. The standard deviation ranged from 0.065 to 20.15.
- Sonication yielded an average percent recovery range from 27.16 to 76.24% (Figure 5) also depending on chain length. The standard deviation ranged from 28.63 to 49.03.
- No clear trends based on chain length were observed while using the shaker table.
- Analyte mass 413, perfluorooctanoic acid, was removed from Figure 4 for having two major outliers that greatly skewed the data. Having an average recovery of -408.64% and a standard deviation of 895.04.
- The standard deviation for replicated samples for shaker tables were smaller signifying better precision.
- Having a lower standard deviation and a better percent recovery the use shaker table provides more precise and replicable data.
- Compared to a run completed by another student averages were similar to their recoveries but variable pattern observed for individual chain lengths. (Figure 6)
- Provided more time having one more shaker table run would allow a complete comparison of 6 runs instead of 5.
- Visit QR code to view all data gathered: standards, calibration curves, calculations, standard deviations and sample recoveries.

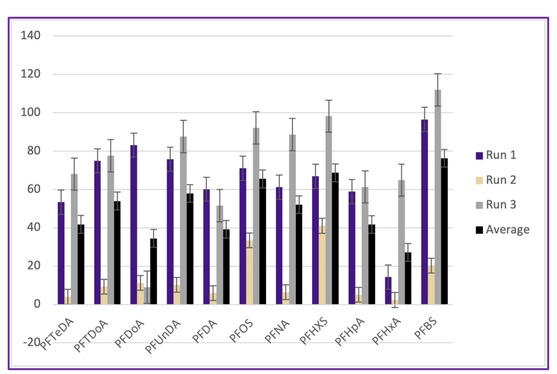


Figure 5: Sonication percent recoveries of different PFAS from 3 experimental trials and their average. Yielding a 49.08% recovery range from 27.16 to 76.24%.

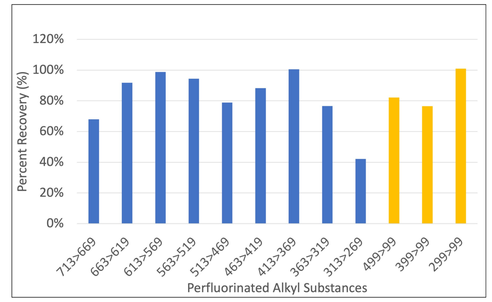


Figure 6: Shaker table recoveries from another study under the same conditions yielding a recovery range from ~40-100%. Perfluorinated sulfonic acids in yellow and in blue are perfluorinated carboxylic acids.

## Conclusion

- Sonication is likely too intense and can release proteins and other macromolecules, such as DNA from tissue that could bind the analytes of interest, leading to lower and less precise extraction recoveries.
- These findings imply that using a shaker table would be the better method when quantifying and tracking levels of PFAS in mussel tissue .
- Future work will look at the accumulation of PFAS in different types of shellfish and if similar method will be applicable.

## Acknowledgments

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