Optimization of Fish Homogenization Procedures for EPA Method 1633 to Measure Per- and Polyfluoroalkyl Substance (PFAS) in Fish

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Introduction

Per- and polyfluoroalkyl substances (PFAS) are man-made, aliphatic compounds that persist in the environment due to their stability and resistance to degradation. These persistent environmental pollutants are associated with adverse health effects such as liver damage, reduced immune response, and increases in certain types of cancer. Bioaccumulation and biomagnification in fish, particularly those inhabiting local contaminated water sources, exacerbate the PFAS contamination risk to humans.

In addressing this concern, the EPA has recently released its final version of EPA Method 1633, which enables the analysis of 40 different short and long-chained PFAS compounds using liquid chromatography/mass spectrometry across various matrices, including tissue samples. The New Jersey Department of Health (NJDOH) aims to adopt this method to monitor PFAS levels in fish sourced from the Delaware River.

The tissue sample preparation process starts with tissue homogenization, followed by solvent extraction and solid-phase extraction (SPE) purification. Homogenization is a crucial initial step in sample processing, which in turn can lead to improved extraction efficiency, reduced matrix effects, and enhanced accuracy and precision of the method. The objective of this poster is to assess various grinders/technologies and optimize homogenization conditions to achieve this goal.

Methodology
Part I:Homogenization

Optimization of tissue sample homogenization was performed by evaluating sample homogeneity, ease of operation and cleanup, safety consideration, contamination assessment and matrix effect measurement. Chicken breast was selected as blank matrix according to EPA Method 1633 for fish sample analysis.

Experiment 1: Grinding Devices Comparison

Benchmark Scientific D1000 Homogenizer

Robot Coupe R2B Ultra

Robot Coupe Blixer 2

Waring Commercial Lab Blender

250g semi-thawed chicken breast was divided equally then ground using the Benchmark Scientific D1000 Homogenizer, Robot Coupe R2B Ultra, Robot Coupe Blixer 2, and Waring Commercial Lab blender. Devices were compared for overall performance.

Experiment 2a: Homogenization Condition Optimization

250g chicken breast samples were ground with different grinding speed and cycles. 2g aliquots were removed after each stage to observe the sample homogeneity under each condition.

Experiment 2b: Temperature Control

250g chicken breast samples were ground with and without dry ice to observe the sample homogeneity, ease of cleanup and potential contamination.

Experiment 3: Matrix Effect Evaluation

Matrix effect was used to evaluate and optimize the homogenization conditions.

It was calculated using the following equation:

$$\text{Matrix Effect (\%)} = \mathbf{A}/\mathbf{B}*100$$

Where:

A: Peak area of the analyte spiked to the homogenized and SPE extracted tissue samples.

B: Peak area of the analyte spiked to elution solvent.

Methodology
Part II: Sample Preparation- Tissue Extraction

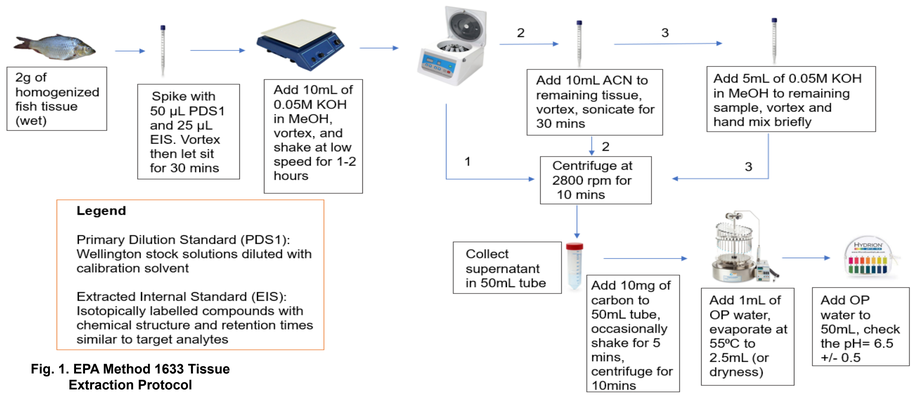

Fig. 1. EPA Method 1633 Tissue Extraction Protocol

SPE Protocol

1. Pre-condition cartridges half-packed with salinized wool with 15mL of 1% MeOH NH4OH.
2. Condition cartridge with 5 mL of 0.3M formic acid.
3. Load 50 mL sample with vacuum.
4. Wash with 5mL OP water (twice) and 1:1 0.1M formic acid/MeOH buffer.
5. Dry cartridge for 15 seconds.
6. Elute analytes with 5mL of 1% NH4OH in MeOH.
7. Add 25 µL of non-extracted internal standard (NIS).
8. Add 25µL of concentrated acetic acid to each sample extract.
9. Filter sample with 25-mm, 0.2-µm nylon membrane for LC-MS/MS injection.

Fig. 2. Phenomenex Strata PFAS (GCB/WAX), 50mg/200mg/6mL cartridge

Methodology
Part III: LC-MS/MS

A modified LC-MS/MS method was developed to measure 40 PFAS and their internal standards in a 20 minute-run as demonstrated in Fig 4. Three Bile Salt interference were separated from target analysis.

LC Conditions

Column: Waters Acquity UPLC C18 column, 1.7µm, 100 x 2.1mm.
Column Temp : 40 ºC.
Flow rate: 0.4mL/min.

Mobile Phase A: 5mM ammonium acetate in water.
Mobile Phase B: Acetonitrile.

Fig. 3. Agilent uHPLC-MS/MS 6495C

Gradient Table

Time [min]	A [%]	B [%]
0	90	10
2	70	30
8.5	55	45
11.5	25	75
13.3	0	100
13.5	90	10
15	90	10

LC-MS/MS

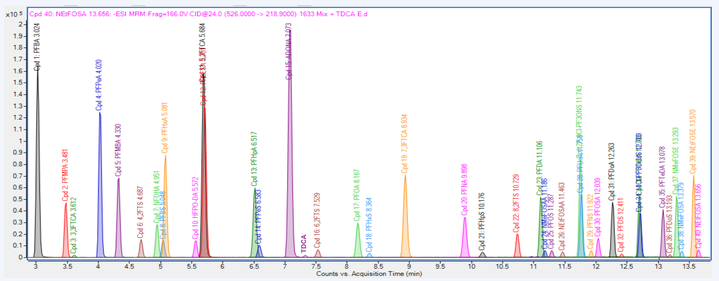

Fig. 4 Example of chromatograph depicting analyte and bile salt (TDCA) peaks

Results

Experiment 1. Grinding Device Comparison

Blender Type	Sample Homogeneity	Anti-clogging	Ease of Operation & cleanup	Safety Considerations	Overall Efficiency
Robot Coupe R2B Ultra	Excellent	Excellent	Excellent	Excellent	Excellent
Robot Coupe Blixer 2	Excellent	Excellent	Good	Excellent	Good
Benchmark Scientific D1000 Homogenizer	Poor	Poor	Poor	Poor	Poor
Waring Commercial Lab Blender	Poor	Good	Excellent	Good	Poor

- Robot Coupe R2B Ultra was selected for tissue homogenization based on overall performance.

Experiment 2. Homogenization Condition Optimization

- 20 secs and 2 cycle (mix in-between) was sufficient to homogenize the chicken tissue samples.
- Adding dry ice facilitated the homogenization process for fully thawed tissues and was easier for cleanup, however resulted in less homogeneity.
- Semi-solid tissue without dry ice is ideal for homogenization.

Experiment 3. Matrix Effect Evaluation

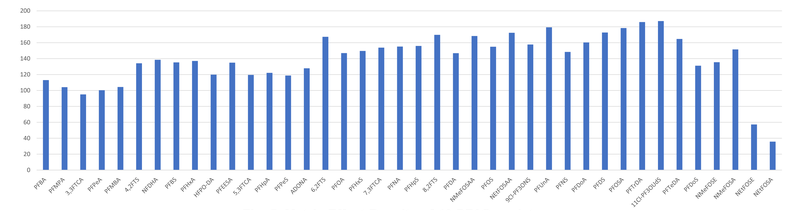

Fig. 5. Matrix Effect Results of 40 PFAS analytes

- 15 out of 40 analytes showed severe matrix effect (< 50% or >150%).
- Optimization for sample processing is needed to improve the matrix effect.

Conclusion and Future Plan

A method was set up in our lab based on EPA Method 1633 and the homogenization conditions were optimized by selecting proper homogenization device and conditions.

Future plans:

- ❑ To evaluate fish homogenization (Salmon and Tilapia).
- ❑ To compare recovery between EPA Method 1633 and other PFAS fish methods.
- ❑ To optimize the overall method to achieve required validation criteria.
- ❑ To validate the PFAS fish method and analyze fish samples.

References

1. United States Environmental Protection Agency. (2024) Final Draft Method 1633 Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS.

Acknowledgements

- This study was supported by APHL and New Jersey Department of Health (NJDOH)
- I am very grateful to my colleagues in the Chemical Terrorism Lab and Organics Lab and members of the NJ State Biomonitoring team for their endless support.