Development and Performance Evaluation of a Method for the Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Foods of Animal Origin

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INTRODUCTION

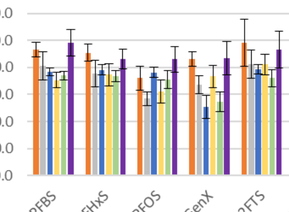
Cases of per- and polyfluoroalkyl substances (PFAS) contamination of foods have become more prominent in the media, causing a steep rise in concerns about the long-term impacts of human exposure. This has propelled the scope of PFAS analysis from just environmental matrices into the field of food analysis as well. Over the last decade, cases of PFAS contamination being found in foods such as, but not limited to, eggs, milk, chocolate cake, and fast-food have become more prominent in the media. In order to protect the public and understand dietary exposure, analytical methods for the analysis of a large variety of food products are required.

Complex food commodities such as fish, meat, edible offal, and eggs require a comprehensive sample extraction and clean up. To accommodate these types of samples, an alkaline digestion and extraction was implemented followed by Weak Anion Exchange (WAX) SPE to produce a suitable sample for analysis. The method was evaluated in six different commodity types including salmon, tilapia, ground beef, beef liver, beef kidney, and chicken eggs. This approach proved to be accurate, sensitive and robust for a range of 30 PFAS compounds of varying chemistry classes to match the challenging concentrations published in reports by EFSA and the FDA. To further characterize the reproducibility and robustness of the method, an interlaboratory study was performed to evaluate method performance for PFHxS, PFOS, PFOA, and PFNA quantitation in fish.

SAMPLE PREPARATION

Samples of frozen salmon, frozen tilapia, FAPAS fish QC material, ground beef, beef liver, beef kidney, and whole chicken eggs were purchased from local grocery stores. Fish and meat were homogenized using a Ninja® kitchen blender. After removing from the shell, the egg white and yolk were mixed before subsampling. Samples were prepared using the method described in Figure 1.

1. 2 g homogenized heat
   - Spike 1 ng/g surrogates (PFAC-24ES and M3HFPO-DA)
2. Add 10 mL 0.02 M NaOH in MeOH
   - Shake for 1 hr
   - Centrifuge for 10 min at 4000 rpm
3. Dilute 0.5 mL extract in 14.5 mL water
   - Adjust pH with 2 µL formic acid if pH is >6
4. Perform SPE



Prep Samples
- Adjust pH < pKa of WAX – activates ion exchange when sample loaded, holds PFAS on sorbent

Condition Cartridge
- 4 mL 2% ammonium hydroxide in methanol
- 4 mL methanol
- 4 mL water

Load Sample

Wash Cartridge
- Rinse with 4 mL 25 mM ammonium acetate buffer (pH 4)
- Rinse with 4 mL methanol

Elute
- Elute is 8 mL 2% ammonium hydroxide in methanol
- Eluent with pH > pKa of WAX – "turns off" ion exchange to release PFAS

Dry and Reconstitute
- Dry and reconstitute to 0.5 mL with 1:1 MeOH:water containing internal standard (PFAC-C-IS)

Figure 1. Extraction protocol with for foods of animal origin using alkaline methanol and solid phase extraction procedure using Oasis™ WAX SPE Cartridges for PFAS analysis (6 cc, 150 mg, p/n 186009345).

LC/MS-MS METHOD

LC System	ACQUITY™ UPLC™ I-Class PLUS System fitted with the PFAS Analysis Kit
MS System	Xevo™ TQ-XS Mass Spectrometer, ESI-
Column	ACQUITY UPLC BEH™ C_{18}, 2.1 x 100 mm, 1.7 µm with ACQUITY Column In Line Filter
Separation	Gradient, 22 min, 0.3 mL/min
Mobile Phase A	Water, 2 mM ammonium acetate
Mobile Phase B	Methanol, 2 mM ammonium acetate
Software	MassLynx™ Software and the QuanOptimize tool

Xevo™ TQ-XS

Table 1. Instrument configuration, ionization mode, and chromatographic conditions.

METHOD DEVELOPMENT RESULTS

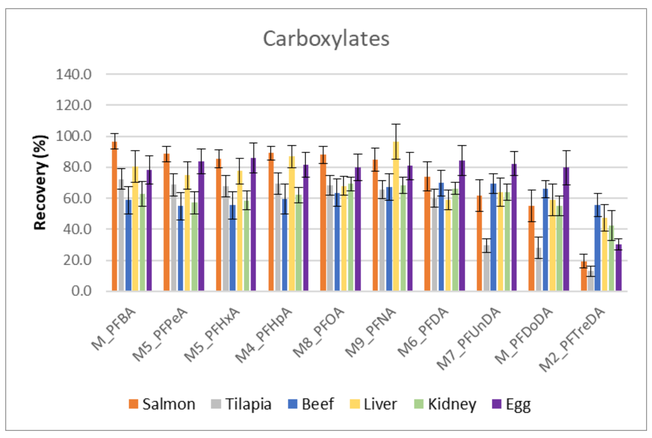


Figure 2. Percent recovery in each matrix evaluated. **143% standard deviation shown off scale. Orange highlight demonstrates the FDA guidelines for recovery at 1 ng/g (40 – 120%).

This method was evaluated using five replicates of each commodity spiked at 3 concentration levels; 0.1 ng/g, 1.0 ng/g, and 5 ng/g. The isotope labelled extraction standards were used to evaluate method recovery due to lack of a truly blank matrix. Recovery values are shown in Figure 2, with standard deviation for n=15 extracts. The neutral sulfonamides are not recovered using the WAX SPE protocol as they are lost to waste during the methanol wash step required to remove matrix, resulting in the low recoveries in Figure 2 for $^{13}C_8$-FOSA. Besides the sulfonamides, the long chain carboxylates were difficult to recover from egg, salmon, and tilapia, resulting in recoveries below the FDA guidelines of 40%. Additionally, NEtFOSAA had recovery of 30% in tilapia. Besides these particularly problematic compounds, the remaining PFAS recoveries were within the FDA recovery guidelines of 40-120%.

PFAS	Liver ng/g	Egg ng/g
PFOS linear	0.52	-
PFOS branched	0.24	-
PFOS (total)	0.76	-
PFPeA	-	0.18
PFHxA	-	0.25
PFHpA	-	0.29
PFOA	-	0.13

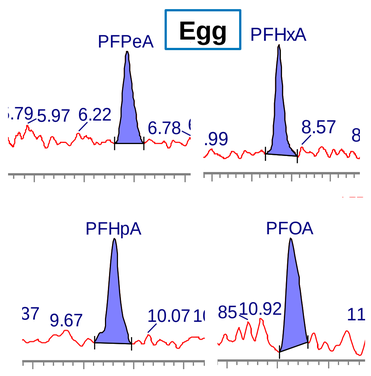


Figure 3. PFAS detected in samples of beef liver and egg purchased at a MA grocery store.

PERFORMANCE EVALUATION

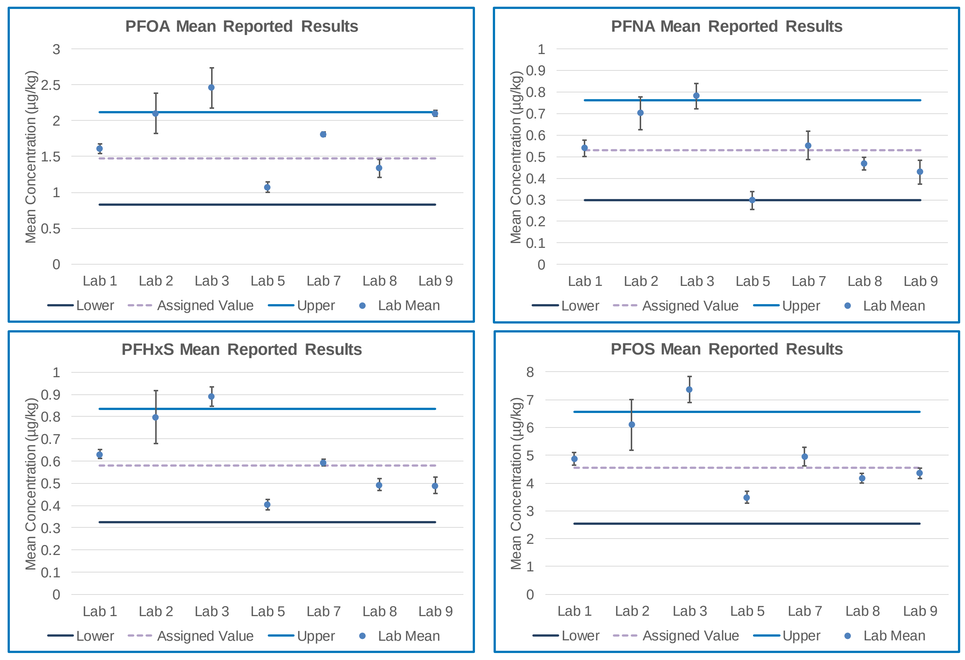


Figure 4. Results from the analysis of FAPAS Fish QC Material T0696QC by the participating laboratories.

Seven laboratories in different places around the world tested FAPAS Fish QC material and demonstrated good accuracy for the quantification of the four PFAS analytes.

Mean reported results are shown in Figure 4. Laboratory 3 results were all outside of the upper range of values of the four PFAS analytes which suggests a systemic error. The results from laboratory 3 were included in the overall method performance calculations.

CONCLUSIONS

- **Alkaline methanol extraction followed by clean up with Oasis WAX SPE for PFAS analysis was successfully implemented to extract and concentrate most PFAS analytes for analysis.**
- **Low recoveries were observed for the sulfonamides – Oasis HLB could be a useful alternative but would require a separate method.**
- **Sensitive analysis on the Xevo TQ-XS mass spectrometer successfully detected and quantified PFAS at sub-ng/g levels required to meet the maximum dietary levels of PFAS recommended by the EFSA.**
- **Recoveries were within FDA criteria except for the C_{13} and C_{14} carboxylates (acceptable recovery range of 40–120% for concentrations at 1 ng/g and a maximum %RSD of 22%).**
- **PFOA, PFNA, PFHxS, and PFOS in fish tissue were investigated using an interlaboratory study, resulting in trueness between 102 and 121%, repeatability within each lab as <20%, and reproducibility between labs as ≤30%.**
- **This method is suitable for compliance testing when PFAS levels in food become more heavily regulated.**

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