

## Total Workflow for the Sensitive Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Fish, Meat, Edible Offal, and Eggs

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### Abstract

Environmental release and contamination of per- and polyfluorinated alkyl substances (PFAS) has resulted in contamination of a variety of food sources. 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. High sensitivity LC-MS/MS analysis was performed on an ACQUITY UPLC I-Class PLUS coupled to Xevo TQ-XS. 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 the European Food Safety Authority (EFSA) and the US Food and Drug Administration (FDA).

### Benefits

- A single extraction method that can be utilized for a large suite of PFAS from a variety of food matrices

- Sensitive analysis on the Xevo TQ-XS to detect PFAS at sub-ng/g levels to match the challenging concentrations published in reports by EFSA and FDA
- Confidence in results with the utilization of the PFAS Kit for LC modification minimize possible system and solvent contaminants to assure accurate results

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## Introduction

Rising concerns about the long-term impacts of human exposure to per- and polyfluoroalkyl substances (PFAS) have 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. To protect the public and understand dietary exposure, analytical methods for the analysis of a large variety of food products are required.

In response, many agencies around the globe have been studying the impact of PFAS in food. The most recent report published in 2020 by the European Food Safety Authority (EFSA) have identified fish, meat, fruit/fruit products, and eggs/egg products to contribute most to human PFAS exposure through diet during the study period of 2007 to 2018.<sup>1</sup> From this study, EFSA set a recommended tolerable weekly intake (TWI) of 4.4 ng per kg of body weight for a total of 4 PFAS: PFOA, PFNA, PFHxS, and PFOS.

Among food derived from animal origin, the EFSA study found the mean concentration of PFAS identified varied by sample type. PFOA, PFNA, PFHxS, and PFOS were found in livestock and wild game meats in the range of 0.02–1.59 ng/g, in farm raised edible offal in the range of 0.087–1.18 ng/g, in salmon and trout in the range of 0.003–0.83 ng/g and in eggs and egg products in the range of 0.06–0.35 ng/g. The median detected concentration in all sample types considered was 0.32 ng/g. In addition to the EFSA study, the FDA monitor contaminants, including PFAS, in highly consumed foods in their Total Diet Study (TDS).<sup>2</sup> In the most recent study from 2019, the TDS reported a finding of 0.087 ng/g PFOS in tilapia. These studies and reports provide evidence for the sensitivity required in a method suitable to analyze these types of samples.

A strategy for the extraction of PFAS from edible produce was discussed in a previous application note,<sup>3</sup> therefore the focus of this study will be meat, edible offal, fish, and eggs. These food commodities were separated due to differences in sample preparation needs. Produce is a simpler matrix to extract and utilized a QuEChERS

extraction and dispersive SPE protocol. Animal based samples add complexity to sample preparation needs due to the presence of proteins and fats that can bind PFAS. There are a variety of approaches available for the extraction of PFAS from meat, fish, and similar matrices. One of the most effective methods is to perform an alkaline extraction (typically sodium hydroxide in methanol or acetonitrile) of the sample followed by solid phase extraction (SPE) clean-up.<sup>4,5</sup> This is the approach this study has taken to evaluate the extraction of a suite of 30 PFAS from six different food matrices: salmon, tilapia, ground beef, beef liver, beef kidney, and egg. Weak Anion Exchange (WAX) chemistry was utilized for the SPE clean-up. Analysis was performed on an ACQUITY UPLC I-Class PLUS modified with PFAS Kit and coupled to a Xevo TQ-XS to fit the highly sensitive analysis requirements.

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## Experimental

### Sample Preparation

Samples of frozen salmon, frozen tilapia, 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. These samples were stored in a freezer (-20 °C) and thawed in a refrigerator (4 °C) overnight prior to extraction. Eggs were prepared fresh. After removing from the shell, the egg white and yolk were mixed before subsampling. All standards were obtained from Wellington Laboratories. The method contained a total of 30 PFAS including the following compounds: Carboxylates: C4–C14; Sulfonates: C4–C10; Ethers: GenX, ADONA, 9Cl-PF3ONS, 11Cl-PF3OUdS; Precursors: FBSA, FHxSA, FOSA, NMeFOSAA, NEtFOSAA, 4:2 FTS, 6:2 FTS, 8:2 FTS.

Prior to extraction, a 2 g sample was weighed into a 50 mL centrifuge tube and spiked with 1 ng/g extraction standard (MPFAC-24ES and M3-HFPODA). 10 mL methanol containing 0.02 M sodium hydroxide was added to each sample. Samples were shaken for 1 hour using a SPEX Sample Prep Geno/Grinder at 500 RPM. After shaking, samples were centrifuged for 10 minutes at 4000 RPM at room temperature. Following extraction, 0.5 mL of supernatant was diluted in 14.5 mL water in preparation for solid phase extraction (SPE) using Oasis WAX for PFAS, 6 cc, 150 mg Cartridges (p/n: [186009345 <https://www.waters.com/nextgen/global/shop/sample-preparation--filtration/186009345-oasis-wax-for-pfas-analysis-6-cc-vac-cartridge-150mg-sorbent-per.html>](https://www.waters.com/nextgen/global/shop/sample-preparation--filtration/186009345-oasis-wax-for-pfas-analysis-6-cc-vac-cartridge-150mg-sorbent-per.html) ). The full SPE procedure is detailed in steps 2–5 of Figure 1.

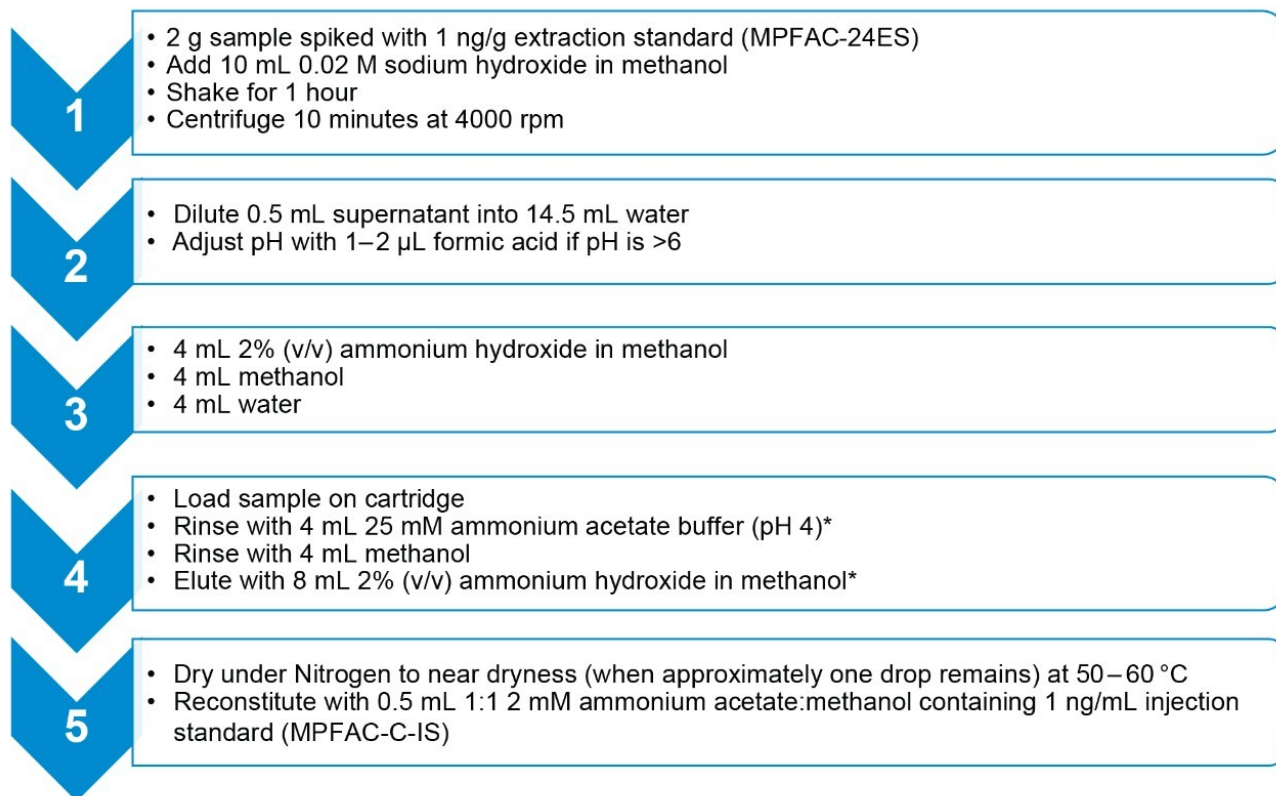


Figure 1. Procedure for SPE clean-up of extracts. Steps denoted with (\*) indicate the solvent used in this step should be used to rinse the sample tube prior to addition to the SPE cartridge.

A solvent calibration curve in the range of 0.01–5 ng/mL (equivalent to 0.05–25 ng/g) was used for sample analysis. For limit of detection (LOD) and limit of quantitation (LOQ) assessment, matrix post spiked (or matrix matched) calibration curves in the range of 0.001–5 ng/mL were used. Isotope labelled extraction (MPFAC-24ES and M3-HFPODA) and injection standards (MPFAC-24ES) were used during extraction and analysis to perform isotope dilution calculations. The extraction standard was spiked in the samples prior to sample preparation and used to correct the native compounds for recovery and matrix effects. The injection standard was added to the sample after clean-up when the sample was reconstituted and used to correct the extraction standards for reconstitution variations, matrix effects, and injection variation. With the presence of the extraction and injection standards, matrix matching was not necessary for routine sample analysis, but is an option in place of performing the isotope dilution method.

## LC Conditions

LC system:	ACQUITY UPLC I-Class PLUS with PFAS Analysis Kit
Vials:	Polypropylene autosampler vial with polyethylene cap (p/n: 186005230)
Column(s):	ACQUITY UPLC BEH C <sub>18</sub> 2.1x 100 mm, 1.7 um (p/n: 186002352) with ACQUITY Column In Line Filter (p/n: 205000343)
Column temp.:	35 °C
Sample temp.:	4 °C
Injection volume:	10 µL
Flow rate:	0.3 mL/min
Mobile phase A:	Water + 2 mM Ammonium acetate
Mobile phase B:	Methanol + 2 mM Ammonium acetate

## Gradient Table

Time (min)	%A	%B	Curve
0	95	5	initial
1	75	25	6
6	50	50	6
13	15	85	6
14	5	95	6
17	5	95	6
18	95	5	6
22	95	5	6

## MS Conditions

MS system:	Xevo TQ-XS
Ionization mode:	ESI-
Source temp.:	100 °C
Capillary voltage:	0.50 kV
Desolvation temp.:	350 °C
Desolvation flow:	900 L/hr
Cone flow:	150 L/hr
MRM method:	See Appendix for Full MRM Method details

## Data Management

Chromatography software:	MassLynx v4.2
MS software:	MassLynx v4.2
Informatics:	MassLynx v4.2 with TargetLynx v4.2

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## Results and Discussion

The 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. Example chromatograms of the extracted quantitation ion trace of each PFAS spiked into salmon prior to extraction at the lowest level of 0.1 ng/g can be seen in Figure 2. Limit of detection and quantitation (LOD and LOQ) were also determined for all samples except for egg and are reported in the Appendix Table 2. LOD and LOQ values were calculated from matrix blank extracts of each sample post spiked with PFAS standards. Calculations were performed in TargetLynx using the criteria of S:N of 3 for LOD and 10 for LOQ.

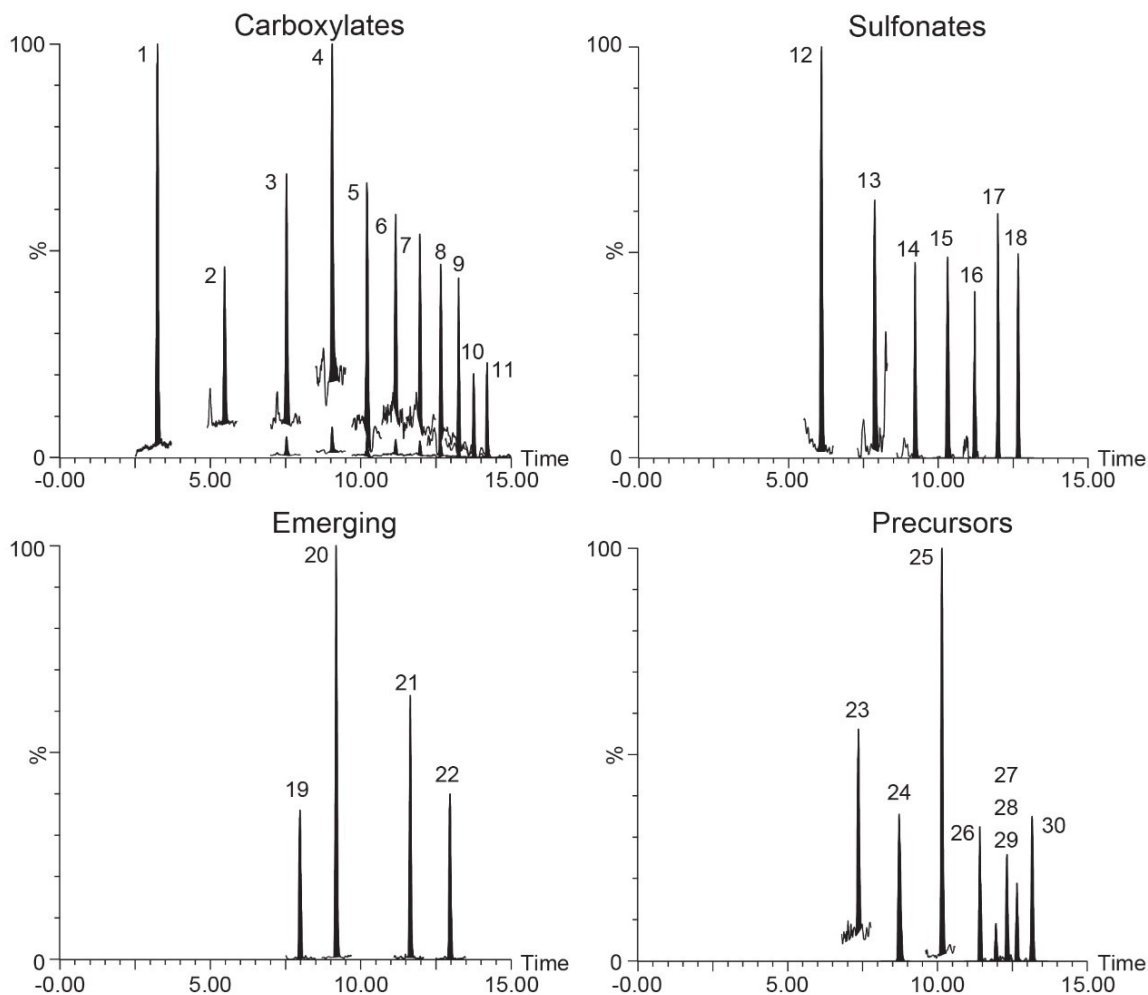


Figure 2. Extracted ion chromatograms of the quantitation ion for each PFAS in salmon spiked at 0.1 ng/g (Peak 23 shown at 1.0 ng/g). Peak assignments are listed in the Appendix. Note: Peaks 3–11 shown zoomed in to display on same view as Peaks 1 and 2.

A well-known matrix interferent compound in meat and meat-like samples is taurodeoxycholic acid (TDCA). This is a group of isomers that have a nominal molecular mass of 499.7 amu and share the same 499 > 80 MRM transition commonly used as the PFOS quantitative transition. TDCA was detected in beef liver and chicken eggs during this study, but using 499 > 91 MRM as the quantitative transition was specific to PFOS. Figure 3A demonstrates the use of the 499 > 91 MRM transition for PFOS in liver and eggs as well as the comparison to ground beef, which did not contain this interferent (fish and kidney did not either). In addition to the alternate transition, Figure 3B demonstrates the presence of TDCA did not cause any matrix suppression or enhancement



in liver (due to the chromatographic resolution and specificity of the 499 > 91 transition) as demonstrated by the graphed peak areas of the injection standard M-PFOS during a continuous sample analysis including salmon, tilapia, ground beef, liver, and kidney. Ground beef did appear to have another source of matrix suppression, as demonstrated in Figure 3B, but this was not from TDCA and not an isobaric interferent that could be detected in the M-PFOS MRM transition. Even with the presence of matrix effects demonstrated in beef, utilization of the injection and extraction internal standards allowed for accurate and reproducible quantitation, as demonstrated and discussed later in Table 1.

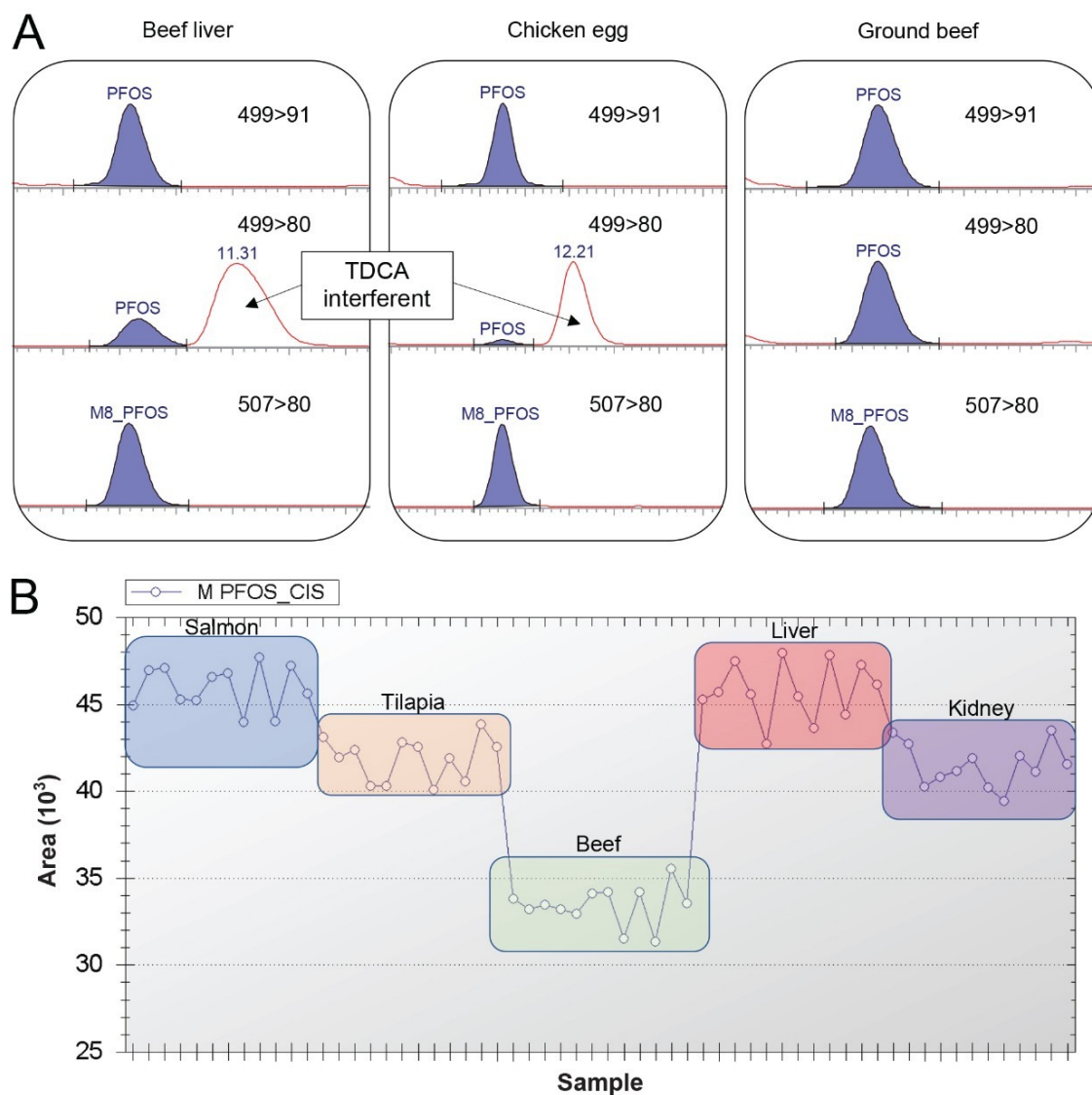


Figure 3. (A) chromatograms of PFOS MRM transitions 499 > 91 and 499 > 80 and M8-PFOS in beef liver, chicken egg, and ground beef showing taurodeoxycholic acid (TDCA) as an interferent in 499 > 80 transition in liver and egg. (B) M-PFOS injection standard peak area (y-axis) over 12 sample injections of each sample matrix (x-axis): salmon, tilapia, beef, liver, and kidney.

The isotope labelled extraction standards were used to evaluate method recovery due to lack of a truly blank matrix and issues with extraction solvent contamination. Recovery values are shown in Figure 4, with standard deviation for n=15 extracts. There currently are no official guidelines or standards for PFAS in food to compare

the recovery results to. The FDA has a set of general guidelines regarding validation of chemical methods in various matrices, including food, that can be referenced for recovery guidelines.<sup>6</sup> This document allows for a recovery range of 40–120% at a concentration of 1 ng/g. 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 4 for <sup>13</sup>C<sub>8</sub>-FOSA (perfluorooctane sulfonamide). Alternate SPE using Oasis HLB can be utilized if recovery of the sulfonamides is required but is not suitable for the full range of PFAS compounds covered in this study. 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 problematic compounds, the remaining PFAS recoveries were within the FDA guidelines.

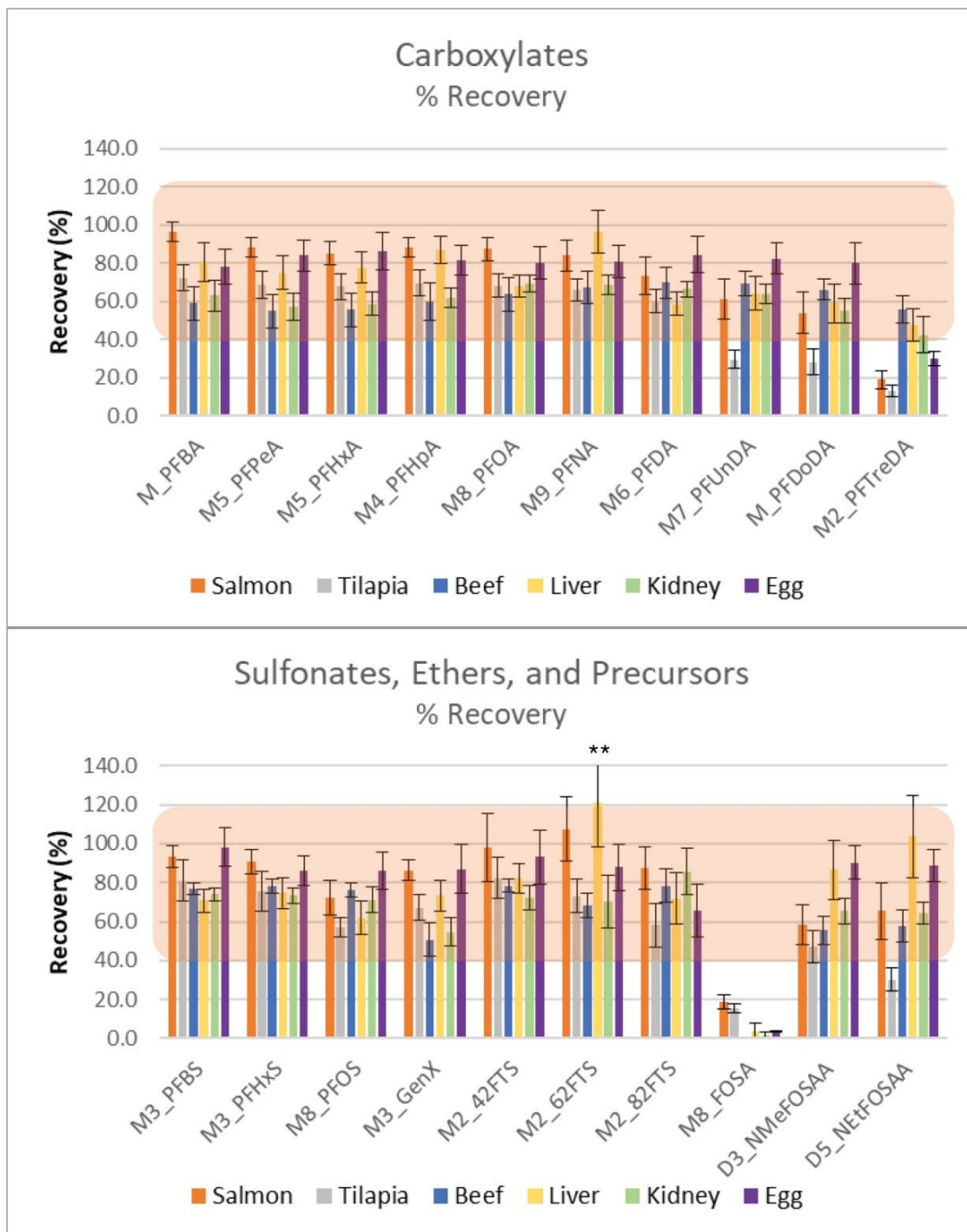


Figure 4. Percent recovery in each matrix evaluated, n=15 for all matrices except egg where n=5. \*\*143% standard deviation shown off scale. Orange highlight demonstrates the FDA guidelines for

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recovery at 1 ng/g (40–120%).

Accuracy of the method was evaluated by percent bias of the comparison of calculated concentration to the expected concentration for each PFAS at the three different spike levels. Table 1 lists the mean calculated concentrations (using the isotope dilution method) for each type of sample as well as the percent bias, or accuracy. For the 1.0 and 5.0 ng/g spike levels, as well as the 0.1 ng/g spike level for kidney, liver, tilapia, and beef, mean concentrations were within 17% of the expected value. Liver and salmon were within 30% of the expected value for the 0.1 ng/g level. The %RSD values for five replicates of each concentration is also shown in Table 1. RSDs were below 20% for all samples except for the 0.1 ng/g spike level of liver and salmon having RSDs of 21.9 and 28.4, respectively. The bias and RSD values demonstrate both precision and reproducibility of the method.

		Egg	Kidney	Liver	Tilapia	Salmon	Beef
0.1 ng/g	mean (ng/g)	–	0.11	0.10	0.12	0.076	0.11
	% bias of mean	–	14	2.0	19	24	8.0
	% RSD	–	12	22	16	28	13
1.0 ng/g	mean (ng/g)	1.0	1.1	1.1	1.2	1.0	1.1
	% bias of mean	3.0	11	14	16	3.0	14
	% RSD	7.1	5.7	6.7	7.9	9.0	4.3
5.0 ng/g	mean (ng/g)	–	5.5	5.7	5.8	5.6	5.5
	% bias of mean	–	9.0	13	16	12	9.8
	% RSD	–	6.9	17	15	6.7	3.7

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*Table 1. Mean calculated concentration, %bias of mean, and %RSD (n=5) for each spike level in egg, kidney, liver, tilapia, salmon, and beef.*

In addition, NIST standard reference material 1947, Lake Michigan Fish Tissue, was extracted and analyzed alongside the spiked samples to gauge the accuracy of the method. This reference material reports NIST determined concentrations for four PFAS (PFNA, PFDA, PFUnDA, PFTriDA), but does not indicate uncertainty values due to lack of data. During analysis, n=8 replicates of NIST 1947 were extracted and analyzed and the comparison data is reported in Figure 5. While the uncertainty values aren't available for the NIST SRM, the experimental results are not significantly different to the NIST values, further demonstrating method accuracy.

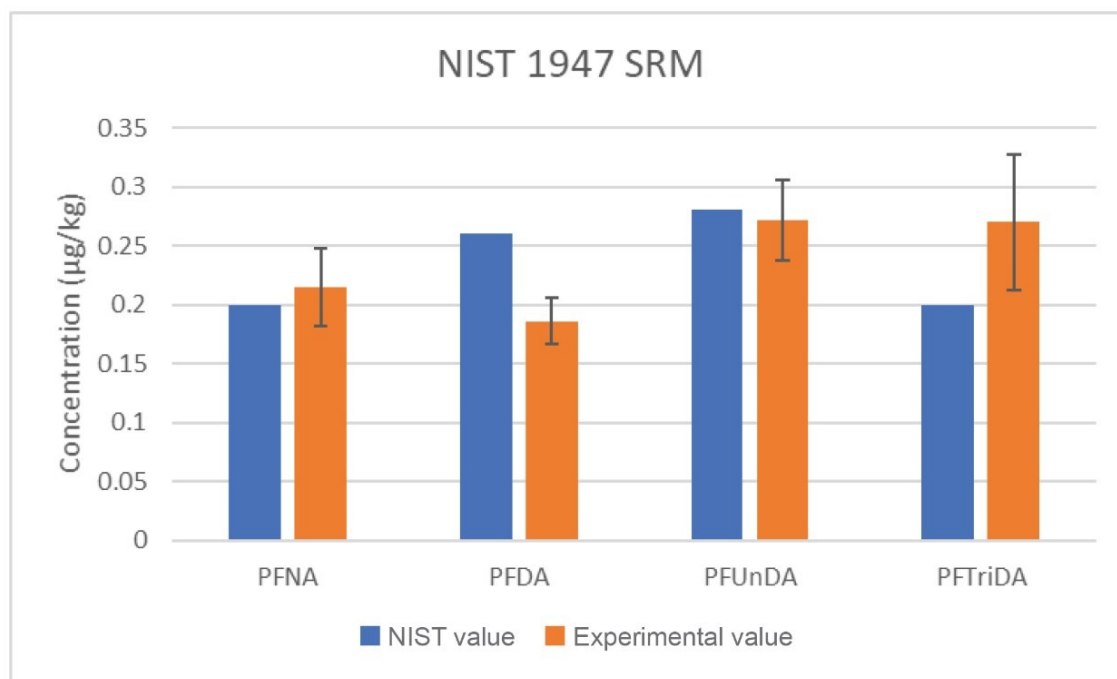


Figure 5. Comparison of NIST reported values to experimental values of four PFAS in NIST 1947 SRM, Lake Michigan Fish Tissue.  $n=8$  for experimental values.

Finally, there were detectable amounts of PFAS in the chicken egg and beef liver samples used in this study that were confidently identified and quantified (Figure 6). Beef liver contained 0.76 ng/g PFOS (0.52 ng/g linear, 0.24 ng/g branched), whereas chicken eggs contained PFPeA, PFHxA, PFHpA, and PFOA in amounts of 0.18, 0.25, 0.29 and 0.13 ng/g, respectively.

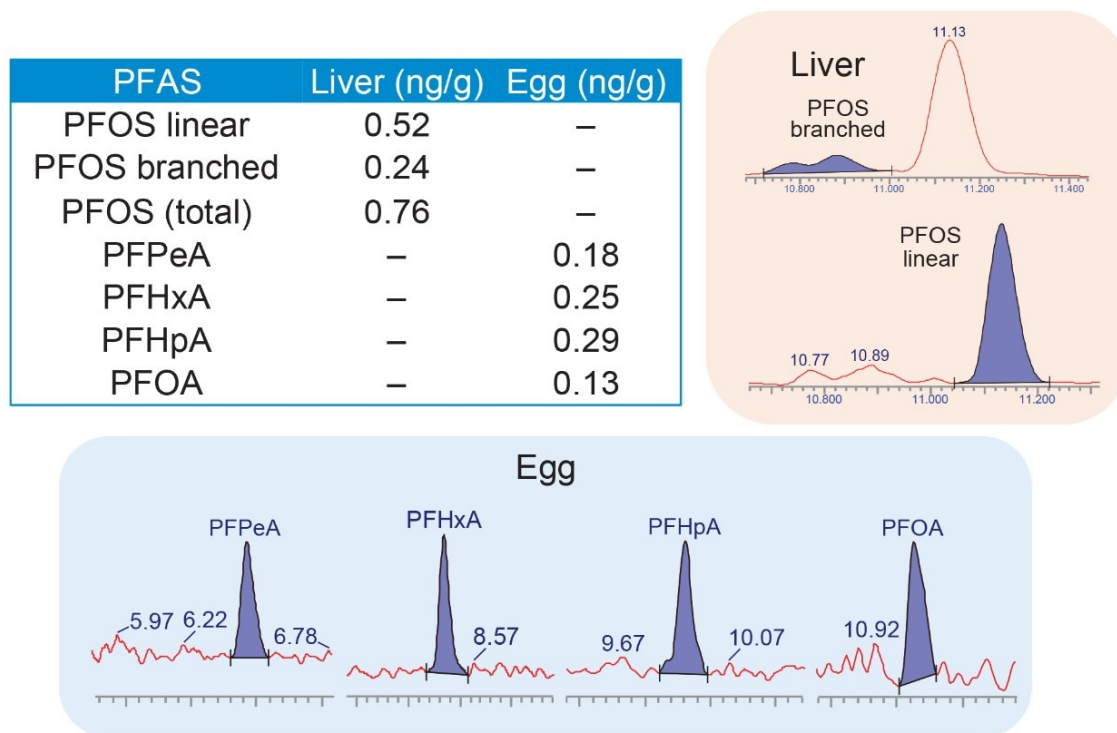


Figure 6. PFAS detected in samples of beef liver and egg purchased in local grocery stores.

## Conclusion

Rising concerns about the long-term impacts of human exposure to per- and polyfluoroalkyl substances (PFAS) have propelled the scope of PFAS analysis from just environmental matrices into the field of food analysis. To protect the public and understand dietary exposure, analytical methods for the analysis of a large variety of food products are required. EFSA has identified food from animal origin to contribute the most to human exposure to PFAS. In response, an alkaline digestion extraction followed by WAX SPE clean-up was successfully implemented to extract PFAS from challenging food samples of meat, fish, edible offal, and eggs. Sensitive and accurate analysis of extracts was performed using the Xevo TQ-XS allowing for detection and quantitation limits in the sub-ng/g range required to meet the maximum dietary levels of PFAS recommended by EFSA. Utilization of the PFAS Kit for LC modification also provides confidence in results by minimizing system and solvent

contaminants. Recoveries were within FDA criteria for all compounds except the C13 and C14 carboxylates. Utilization of the isotope dilution method does take the recovery into account and allows for accurate correction of recovery during calculation of PFAS concentration in samples. Five PFAS were detected in two different food samples purchased from local grocery stores. This comprehensive method allows for high confidence in results of PFAS in complex food matrices to allow for better monitoring and understanding of the environmental impact of PFAS on our food sources.

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## References

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<https://www.fda.gov/food/chemical-contaminants-food/analytical-results-testing-food-pfas-environmental-contamination>> .
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6. Food and Drug Administration Foods Program. Guidelines for the Validation of Chemical Methods in Food, Feed, Cosmetics, and Veterinary Products, 3<sup>rd</sup> edition. October 2019.



Compound	PFAS group	Parent	Fragment	Quan*	CV	CE	Internal standard	Type of internal standard	Fig 2 peak assignment
PFBa	carboxylate	213.0	169	x	10	10	<sup>13</sup> C <sub>7</sub> -PFBa		1
PFPaA	carboxylate	262.9	219	x	10	5	<sup>13</sup> C <sub>7</sub> -PFPaA		2
PFHxA	carboxylate	312.9	269	x	5	10	<sup>13</sup> C <sub>7</sub> -PFHxA		3
			119		5	20			
PFHpA	carboxylate	362.9	319	e, s	15	10	<sup>13</sup> C <sub>7</sub> -PFHpA		4
			169	b, k, l, t	15	15			
PFOA	carboxylate	412.9	369	e, s	10	10	<sup>13</sup> C <sub>7</sub> -PFOA		5
			169	b, k, l, t	10	15			
PFNA	carboxylate	462.9	419		10	10	<sup>13</sup> C <sub>7</sub> -PFNA		6
			219	x	10	15			
PFDA	carboxylate	512.9	469.9		15	9	<sup>13</sup> C <sub>7</sub> -PFDA		7
			219	x	15	15			
PFUnDA	carboxylate	562.9	519.9	e, l, s, t	25	10	<sup>13</sup> C <sub>7</sub> -PFUnDA		8
			269	b, k	25	20			
PFDoDA	carboxylate	612.9	569.9		30	10	<sup>13</sup> C <sub>7</sub> -PFDoDA		9
			169	x	30	25			
PFTrIDA	carboxylate	662.9	619.9	e, s	5	10	<sup>13</sup> C <sub>7</sub> -PFTrIDA		10
			169	b, k, l, t	5	30			
PFTreDA	carboxylate	712.9	669.9	e, s	10	25	<sup>13</sup> C <sub>7</sub> -PFTreDA		11
			169	b, k, l, t	10	15			
PFBS	sulfonate	298.9	80.1	x	15	30	<sup>13</sup> C <sub>7</sub> -PFBS		12
			99.1		15	30			
PFPeS	sulfonate	348.9	79.9	x	10	30	<sup>13</sup> C <sub>7</sub> -PFPeS		13
			99.9		10	30			
PFHxS	sulfonate	398.9	80.1	x	10	35	<sup>13</sup> C <sub>7</sub> -PFHxS		14
			99.1		10	30			
PFHpS	sulfonate	448.9	80.1	x	15	35	<sup>13</sup> C <sub>7</sub> -PFHpS		15
			99.1		15	35			
PFOS	sulfonate	498.9	80.1	b, k, s, t	15	40	<sup>13</sup> C <sub>7</sub> -PFOS		16
			99.1	e, l	15	40			
PFNS	sulfonate	548.9	80.1	x	20	40	<sup>13</sup> C <sub>7</sub> -PFNS		17
			99.1		20	40			
PFDS	sulfonate	598.9	80.1	x	46	46	<sup>13</sup> C <sub>7</sub> -PFDS		18
			99.1		46	46			
GenX (HFPO-DA)	ether	285.0	169	x	5	7	<sup>13</sup> C <sub>7</sub> -GenX		19
			119		5	35			
ADONA	ether	376.9	251	x	10	10	<sup>13</sup> C <sub>7</sub> -GenX		20
			85		10	25			
9Cl-PF3ONS	ether	530.9	350.9	x	15	25	<sup>13</sup> C <sub>7</sub> -PFOS		21
			82.9		15	25			
1Cl-PF3OUdS	ether	630.9	450.9	x	30	30	<sup>13</sup> C <sub>7</sub> -PFOS		22
			82.9		30	30			
4:2 FTS	precursor	326.9	306.9	x	15	15	<sup>13</sup> C <sub>7</sub> -4:2 FTS		23
			80.9		15	35			
6:2 FTS	precursor	426.9	407	x	10	20	<sup>13</sup> C <sub>7</sub> -6:2 FTS		25
			80.1		12	32			
8:2 FTS	precursor	526.9	506.8	x	15	25	<sup>13</sup> C <sub>7</sub> -8:2 FTS		27
			80.9		15	37			
FBSA	precursor	297.9	78	x	25	25	<sup>13</sup> C <sub>7</sub> -FOSA		24
			118.9		25	15			
FhXSA	precursor	398.0	78.1	x	30	25	<sup>13</sup> C <sub>7</sub> -FOSA		26
			169		30	25			
FOSA	precursor	497.9	78	x	40	30	<sup>13</sup> C <sub>7</sub> -FOSA		30
			418.9	x	35	25			
N-MeFOSAA	precursor	569.9	219.1		35	20	D <sub>7</sub> -N-MeFOSAA		28
			418.9	x	15	20			
N-EtFOSAA	precursor	584.0	525.9		15	20	D <sub>7</sub> -N-EtFOSAA		29
			418.9	x	15	20			
<sup>13</sup> C <sub>7</sub> -PFBa	-	216.9	172	x	10	10	<sup>13</sup> C <sub>7</sub> -PFOA	Extraction (MPFAC-24ES)	-
<sup>13</sup> C <sub>7</sub> -PFPaA	-	267.9	223	x	10	5	<sup>13</sup> C <sub>7</sub> -PFOA	Extraction (MPFAC-24ES)	-
<sup>13</sup> C <sub>7</sub> -PFHxA	-	317.9	272.9	x	10	5	<sup>13</sup> C <sub>7</sub> -PFOA	Extraction (MPFAC-24ES)	-
			119		10	20			
<sup>13</sup> C <sub>7</sub> -PFHpA	-	366.9	321.9	x	15	10	<sup>13</sup> C <sub>7</sub> -PFOA	Extraction (MPFAC-24ES)	-
			169		15	15			
<sup>13</sup> C <sub>7</sub> -PFOA	-	420.9	375.9	x	5	15	<sup>13</sup> C <sub>7</sub> -PFOA	Extraction (MPFAC-24ES)	-
			172		5	10			
<sup>13</sup> C <sub>7</sub> -PFNA	-	471.9	426.9	x	10	10	<sup>13</sup> C <sub>7</sub> -PFOA	Extraction (MPFAC-24ES)	-
			223		10	15			
<sup>13</sup> C <sub>7</sub> -PFDA	-	519	473.9	x	5	10	<sup>13</sup> C <sub>7</sub> -PFDA	Extraction (MPFAC-24ES)	-
			219		5	15			
<sup>13</sup> C <sub>7</sub> -PFUnDA	-	569.9	524.9	x	5	10	<sup>13</sup> C <sub>7</sub> -PFDA	Extraction (MPFAC-24ES)	-
			274		5	15			
<sup>13</sup> C <sub>7</sub> -PFDoDA	-	614.9	569.9	x	10	10	<sup>13</sup> C <sub>7</sub> -PFDA	Extraction (MPFAC-24ES)	-
			169		10	25			
<sup>13</sup> C <sub>7</sub> -PFTreDA	-	714.9	669.9	x	25	35	<sup>13</sup> C <sub>7</sub> -PFDA	Extraction (MPFAC-24ES)	-
			169		25	35			
<sup>13</sup> C <sub>7</sub> -PFBS	-	301.9	80.1		10	30	<sup>13</sup> C <sub>7</sub> -PFOS	Extraction (MPFAC-24ES)	-
			99.1		10	25			
<sup>13</sup> C <sub>7</sub> -PFHxS	-	401.9	80.1		10	40	<sup>13</sup> C <sub>7</sub> -PFOS	Extraction (MPFAC-24ES)	-
			99.1		10	35			
<sup>13</sup> C <sub>7</sub> -PFOS	-	506.9	80.1		15	40	<sup>13</sup> C <sub>7</sub> -PFOS	Extraction (MPFAC-24ES)	-
			99.1		15	40			
<sup>13</sup> C <sub>7</sub> -FOSA	-	505.9	78.1		35	25	<sup>13</sup> C <sub>7</sub> -PFOA	Extraction (MPFAC-24ES)	-
			418.9		30	20			
D <sub>7</sub> -N-EtFOSAA	-	589	506.9		30	15	<sup>13</sup> C <sub>7</sub> -PFOA	Extraction (MPFAC-24ES)	-
			418.9		35	20			
D <sub>7</sub> -N-MeFOSAA	-	572.9	482.7		35	15	<sup>13</sup> C <sub>7</sub> -PFOA	Extraction (MPFAC-24ES)	-
			418.9		35	15			
<sup>13</sup> C <sub>7</sub> -4:2 FTS	-	328.9	306.9		40	15	<sup>13</sup> C <sub>7</sub> -PFOS	Extraction (MPFAC-24ES)	-
			81		40	25			
<sup>13</sup> C <sub>7</sub> -6:2 FTS	-	428.9	409		10	20	<sup>13</sup> C <sub>7</sub> -PFOS	Extraction (MPFAC-24ES)	-
			80.9		10	27			
<sup>13</sup> C <sub>7</sub> -8:2 FTS	-	528.9	506.9		10	35	<sup>13</sup> C <sub>7</sub> -PFOS	Extraction (MPFAC-24ES)	-
			81		10	35			
<sup>13</sup> C <sub>7</sub> -GenX	-	287	169		5	12	<sup>13</sup> C <sub>7</sub> -PFOA	Extraction (MPFAC-24ES)	-
			119		5	12			
<sup>13</sup> C <sub>7</sub> -PFOA	-	415	370		10	10	-	Injection (MPFAC-C-IS)	-
			169		10	15			
<sup>13</sup> C <sub>7</sub> -PFOS	-	503	80.1		5	40	-	Injection (MPFAC-C-IS)	-
			99.1		5	40			
<sup>13</sup> C <sub>7</sub> -PFDA	-	515	470		20	10	-	Injection (MPFAC-C-IS)	-
			219		20	15			

Appendix Table 1. MS Method conditions for PFAS included in method. (\*) x indicates MRM used as quan ion for

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*all matrices. The following letters indicate the MRM was used as the quan ion for the type of designated matrix (s) salmon, (t) tilapia, (b) ground beef (l) liver (k) kidney, and (e) egg.*

	LOD (ng/g)					LOQ (ng/g)				
	Kidney	Liver	Beef	Tilipia	Salmon	Kidney	Liver	Beef	Tilipia	Salmon
PFBA	N/A	N/A	N/A	N/A	N/A	0.072	0.049	0.059	N/A	0.067
PFPeA	N/A	0.010	N/A	N/A	N/A	0.049	0.053	0.021	N/A	0.034
PFHxA	0.083	0.016	N/A	0.033	0.028	0.312	0.086	N/A	0.130	0.099
PFHpA	N/A	N/A	N/A	N/A	N/A	0.016	0.016	0.010	0.038	0.088
PFOA	0.014	0.017	0.012	0.010	0.027	0.054	0.059	0.036	0.033	0.049
PFNA	0.019	0.031	0.012	0.006	0.005	0.021	0.040	0.016	0.007	0.012
PFDA	0.040	0.017	0.021	0.009	0.010	0.044	0.021	0.037	0.015	0.022
PFUnDA	0.029	0.013	0.014	0.016	0.026	0.044	0.089	0.026	0.078	0.080
PFDoDA	0.031	0.010	0.022	0.005	0.009	0.070	0.013	0.027	0.007	0.014
PFTriDA	0.015	0.020	0.015	0.015	0.014	0.025	0.034	0.028	0.030	0.030
PFTreDA	0.005	0.020	0.020	0.006	0.005	0.006	0.024	0.025	0.013	0.008
PFBS	0.021	0.016	0.018	0.012	0.012	0.035	0.032	0.032	0.025	0.025
PFPeS	0.024	0.031	0.017	0.011	0.029	0.034	0.053	0.022	0.013	0.047
PFHxS	0.008	0.016	0.016	0.007	0.011	0.015	0.018	0.018	0.017	0.024
PFHpS	0.015	0.025	0.020	0.018	0.008	0.021	0.031	0.024	0.022	0.016
PFOS	0.016	0.023	0.005	0.008	0.010	0.019	0.033	0.006	0.010	0.029
PFNS	0.065	0.050	0.045	0.020	0.021	0.065	0.050	0.045	0.020	0.021
PFDS	0.035	0.035	0.070	0.010	0.030	0.035	0.035	0.070	0.010	0.030
GenX	0.020	0.020	0.015	0.015	0.005	0.026	0.025	0.021	0.022	0.010
ADONA	0.022	0.011	0.015	0.010	0.005	0.029	0.015	0.017	0.010	0.009
9CIPF3ONS	0.038	0.022	0.025	0.013	0.008	0.045	0.031	0.035	0.022	0.018
11CIPF3OUds	0.025	0.019	0.019	0.013	0.017	0.030	0.023	0.024	0.018	0.021
4_2 FTS	0.259	0.163	0.186	0.134	0.131	0.576	0.469	0.542	0.458	0.448
6_2 FTS	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
8_2 FTS	0.070	0.034	0.051	0.037	0.036	0.106	0.062	0.094	0.088	0.082
FBSA	0.022	0.035	0.015	0.006	0.007	0.023	0.035	0.015	0.007	0.007
FHxSA	0.030	0.020	0.025	0.010	0.021	0.030	0.020	0.025	0.010	0.022
FOSA	0.025	0.020	0.015	0.005	0.004	0.025	0.021	0.019	0.005	0.004
NMeFOSAA	0.040	0.020	0.017	0.018	0.025	0.052	0.036	0.030	0.052	0.021
NEtFOSAA	0.023	0.029	0.016	0.010	0.033	0.043	0.044	0.045	0.022	0.084

Appendix Table 2. LOD and and LOQ for each matrix reported in ng/g.

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