

Determination of PFAS in Fish Oil, Offal and Coffee by LC-MS/MS

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1. Introduction

Foods contaminated with PFAS pose a risk to consumers, especially in products used as supplements, stimulants, or eaten for specific nutritive purposes. PFAS have been linked to serious health effects, accurate testing methods are needed. In this work, we describe a single laboratory validation study with a rapid extraction of low concentrations of 30 PFAS in fish and animal foods. For a simplified extraction, QuEChERS reagents were not used prior to SPE cleanup for these matrixes. Samples were analyzed using the Shimadzu Nexera Liquid Chromatograph coupled to the LCMS-8060NX triple quadrupole mass spectrometer in less than nine minutes (Figure 1 and 2).

In this study, we spiked samples at three concentrations in triplicate. Standards were matrix-matched and extracted, spikes were quantified using isotope dilution. Recovery and precision were compared to the requirements of AOAC SMPR 2023.003. All recovery, precision, and LOQ's met the acceptance criteria of the SMPR; including the stricter requirements for EU-regulated compounds in edible offal.

2. Methods

Offal samples were prepared by cutting locally purchased beef kidney into slices, freezing and grinding with dry ice. Organic brewed coffee was purchased locally. Coffee samples were prepared by shaking the bottle and removing aliquots directly from the original container. Fish oil soft gels were purchased locally and prepared by cutting the capsule and dispensing the oil into a 50 ml tube. Test portions were spiked in triplicate for the calibration with 30 native PFAS and 16 isotopically labeled internal standards.

All samples were prepared in the same manner; 10-gram portions were weighed, spiked with target analytes and internal standards, and 10 mL of acetonitrile was added. The samples were vortexed for 1 minute and then centrifuged for 5 minutes at 4000 rpm. An aliquot of the acetonitrile layer was transferred to a tube and diluted 5 times with PFAS-free reagent water. The sample was then passed through a weak anion exchange (WAX) Solid Phase Extraction (SPE) cartridge and the PFAS were eluted with basic methanol-water mixture.



Fig. 1 Nexera™ and LCMS™-8060NX

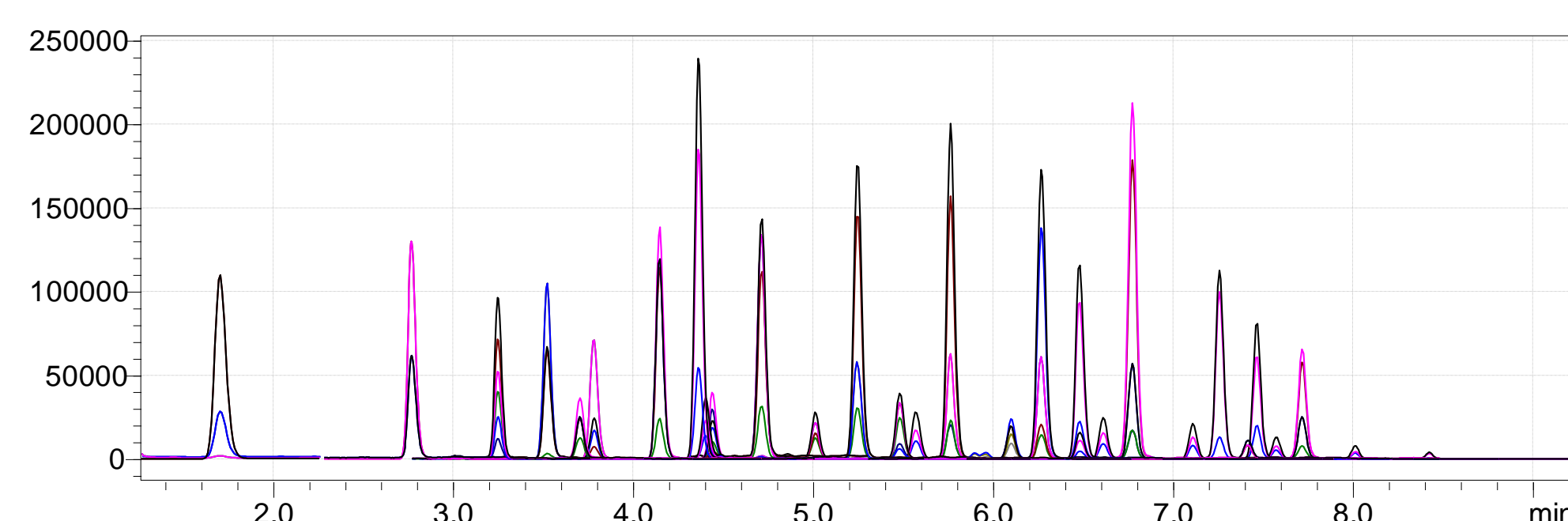


Fig. 2 Chromatogram of 0.4 ng/g PFAS in an offal matrix with separation of all peaks in nine minutes

3. Results

Calibration standards were processed the same as samples. A linear model provided the best fit and best recoveries of analytes. Residuals of each point in the curve were $\pm 25\%$ of the expected value (Figure 3).

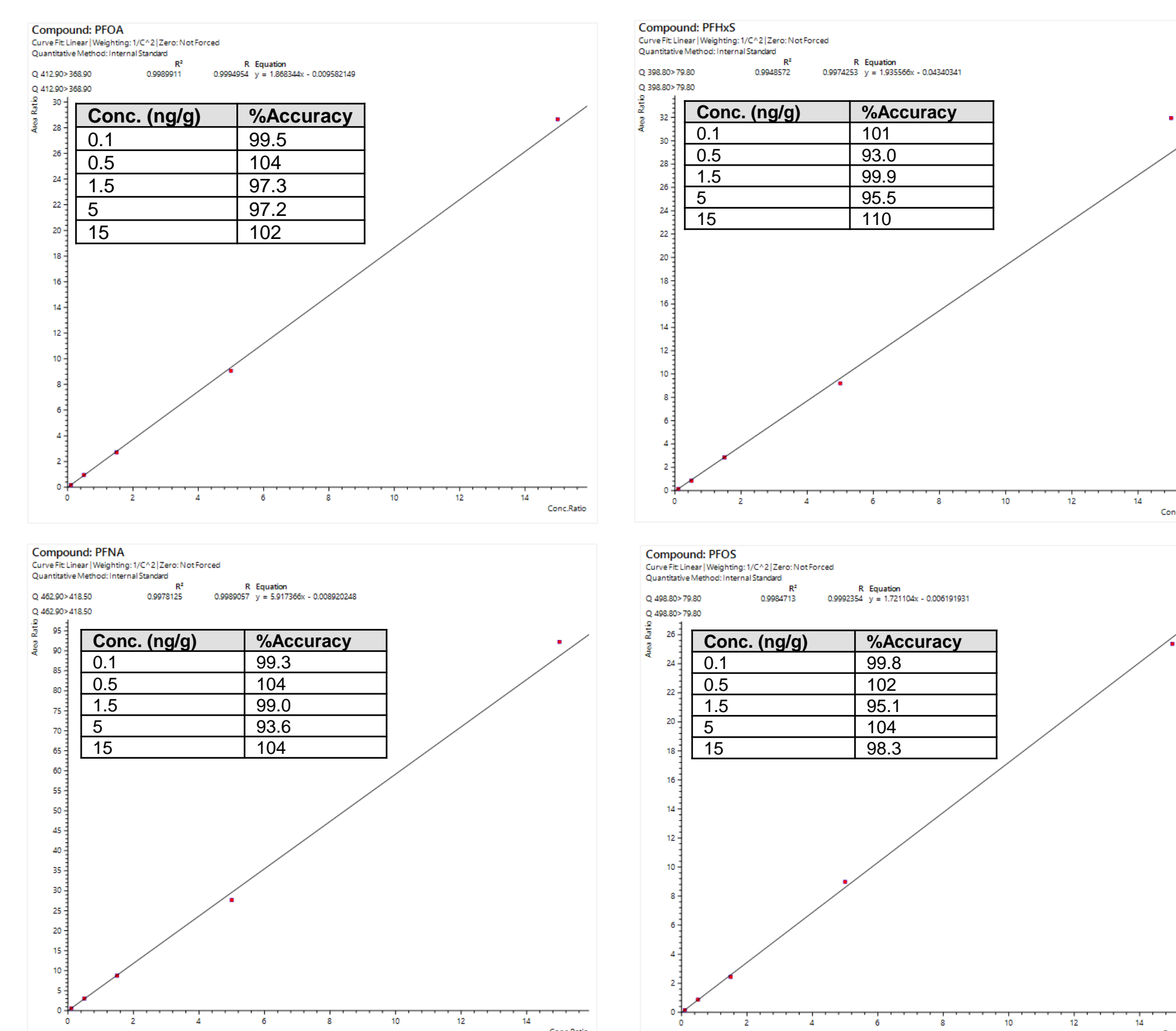


Fig. 3 Calibration curves for offal for each PFOA, PFHxS, PFNA, and PFOS, which are regulated in the EU and have stricter requirements in AOAC's SMPR.

Branched and linear isomers of PFHxS and PFOS were integrated together. Blank matrixes and three different concentrations ranging from below SMPR required LOQ to 20 - 25 times the estimated LOQ were analyzed in triplicate. Recovery and repeatability for PFOA, PFNA, PFOS, and PFHxS at each spike concentration is given for edible offal in Table 1. These compounds are regulated in the EU for offal and have stricter LOQ requirements. Recovery and repeatability data for the remaining 26 PFAS, can be found in application news corresponding to this poster.

The LOQs for all matrixes and compounds were compared each of the requirements of the SMPR including retention time, recovery, repeatability, S/N > 3 for the qualifier ion and an ion ratio of $\pm 30\%$. PFBA, PFPeA, and PFOSA LOQ were set at the minimum concentration, meeting recovery and repeatability requirements and S/N > 10, peaks with internal standards for offal are shown in Figure 4. The lowest concentration to meet all the requirements of the SMPR was set as the LOQ.

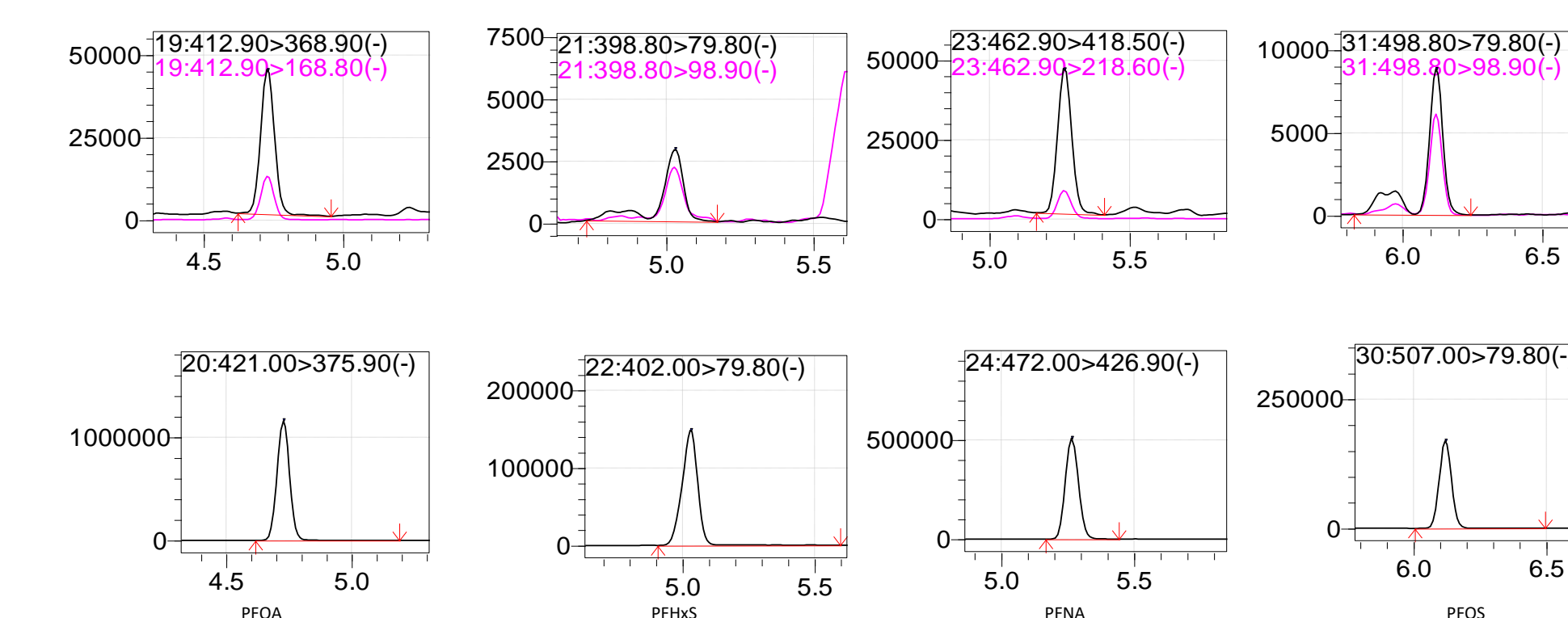


Fig. 4 LOQ peaks with internal standards in offal for PFOA, PFHxS, PFNA, and PFOS

The LOQ for each analyte was estimated by spiking at concentrations at, or below, the required LOQs listed in SMPR 2023.003. Further details, including recovery and repeatability data, as well as LOQ for coffee and fish oil can be found in application news corresponding to this poster.

For this work, we evaluated 1984 different instrument settings, and 6 different column and gradient combinations, to achieve excellent peak shape and resolution between peaks, as well as to maximize the signal-to-noise ratio of PFOA, PFHxS, PFNA, and PFOS. Mass spectrometry was performed on a Shimadzu LCMS-8060NX with heated electrospray ionization operated in negative mode. Chromatography was adjusted to provide sufficient separation of PFOA from potential cholic acid interferences, and to provide baseline resolution of isomers.

4. Conclusion

The Shimadzu LCMS-8060NX Triple Quadrupole Mass Spectrometer coupled with a Shimadzu Nexera Liquid Chromatograph measured 30 PFAS compounds in fish oil, offal and coffee, meeting criteria set by AOAC SMPR 2023.003, including stricter requirements for 4 PFAS that are regulated in the EU for offal. All samples were prepared using the same preparation method and instrument method. Chromatography conditions and the mass spectrometer were optimized to achieve excellent separation of all analytes, baseline resolution between isomers, and a separation between PFOS and potentially interfering cholic acids in only nine minutes.

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Table. 1 Recovery and repeatability for select PFAS: PFOA, PFNA, PFOS, and PFHxS at each concentration level in offal matrix.

Analyte	Spike conc. (ng/g)	Average conc. (ng/g)	Standard Deviation	%RSD	Average Recovery%
PFOA	Blank	0.012			
	0.2	0.221	3.89	3.52	110.7
	0.4	0.453	2.65	2.34	113.3
	1	1.174	6.21	5.29	117.4
	4	4.331	2.69	2.48	108.3
	10	10.503	3.84	3.65	105
PFNA	Blank	0.007			
	0.2	0.205	3.75	3.67	102.1
	0.4	0.435	2.77	2.55	108.9
	1	1.139	7.18	6.3	113.9
	4	4.204	4.53	4.31	105.1
	10	10.421	2.91	2.79	104.2
PFOS	Blank	0.027			
	0.2	0.212	1.9	1.8	105.9
	0.4	0.429	1.97	1.84	107.1
	1	1.092	4.66	4.27	109.2
	4	4.071	5.24	5.15	101.8
	10	10.156	7.04	6.94	101.6
PFHxS	Blank	0.026			
	0.2	0.203	6.37	6.29	101.3
	0.4	0.383	1.65	1.72	95.7
	1	0.962	5.37	5.58	96.2
	4	3.953	4.07	4.12	98.8
	10	9.357	3.12	3.34	93.6