Nota de aplicación

Evaluation of the Performance of a Total Workflow Approach for the Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Fish, Using an Interlaboratory Study

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Waters™ previously developed a method for the determination of per- and polyfluoroalkyl substances (PFAS) in complex food commodities such as fish, meat, edible offal, and eggs which requires a comprehensive sample extraction and clean-up. This application brief shows the successful evaluation of the performance of this method by interlaboratory study. It is based upon an alkaline extraction, sample clean-up using Oasis™ WAX for PFAS Analysis SPE cartridge and determination using ACQUITY™ UPLC™ System fitted with the PFAS Analysis Kit, Isolator Column and Xevo™ TQ-XS Tandem Quadrupole Mass Spectrometer. A reference material of fish containing PFHxS, PFOS, PFOA, and PFNA was sent to seven Waters laboratories, along with standard solutions containing native PFAS compounds and isotopically labelled analogues. For the four PFAS analytes, trueness of the method was determined to be within the range of 102 to 121%. Repeatability within laboratories was <20% and between laboratory reproducibility ≤30%.

Benefits

- The interlaboratory study has shown that a single extraction method, with selective clean-up, can be utilized to give accurate results for PFAS from an independently prepared QC material

- Sensitive analysis to determine PFAS at sub µg/Kg levels suitable for monitoring compliance with regulatory limits and to provide data for risk assessment purposes

Introduction

Due to rising concerns about the long-term impacts of per- and polyfluoroalkyl substances (PFAS) on human health, many agencies across the globe have been studying the occurrence of PFAS in food. A report published by the European Food Safety Authority (EFSA) identified fish, amongst others, as one of the foods that contribute the most to human PFAS exposure through diet during the study period of 2007 to 2018.[1] Recently, the European Commission in Commission Recommendation (EU) 2022/1431 introduced recommendations for PFAS monitoring and indicative levels in a range of foodstuffs with method LOQs of 0.1 µg/Kg in fish meat and meat of terrestrial animals.[2] Commission Implementing Regulation (EU) 2022/1428 details required method performance and additional information on this has been released by the European Union Reference Laboratory for halogenated Persistent Organic Pollutants in Feed and Food (EURL-POPs) listing PFHxS, PFOS, PFOA, and PFNA in the method requirements as main compounds.[3,4] This guidance document method lists an LOQ for fish meat of 0.1 µg/Kg. We have previously reported method performance that meets this required LOQ for the four listed PFAS main components and 26 additional PFAS related chemicals in fish and other animal products.[5]

Here we have assessed the performance of that method using the results from an interlaboratory study following the criteria defined by the EURL-POPs. The method used, in brief, was an alkaline methanol extraction, followed by sample clean-up using Oasis WAX for PFAS Analysis SPE cartridges and determination using an ACQUITY BEH C_{18} Column on an ACQUITY UPLC I-Class PLUS System with a Xevo TQ-XS Tandem Quadrupole Mass Spectrometer. The laboratories analyzed for a total of 30 PFAS analytes (see appendix I), with data for the PFAS with assigned values in the FAPAS Fish QC material (PFHxS, PFOS, PFOA, and PFNA) reported here.

Results and Discussion

Seven laboratories (two located in the US, three located in the UK, one in Germany, and one in Singapore) were supplied with:

- An analytical protocol, including a list of the analytes of interest and internal standards, the instrument configuration, the method, and parameters to be used, guidance documents and the analytical run sequence. This was taken from a previously published application.[5]
- A FAPAS Fish QC material (T0696QC) and a FAPAS Fish Blank material (T0696b).
- Both native and isotopically labelled PFAS stock solutions supplied by Wellington Laboratories.

Each of the laboratories successfully implemented the chromatographic method to provide sufficient retention of the most polar PFAS and exhibited Gaussian chromatographic peak shape and stable retention times throughout. The participating laboratories demonstrated method sensitivity by achieving the method LODs as reported in the previous published application note.[5] The method was able to readily detect the PFAS in the FAPAS Fish QC material with sufficient signal to noise as can be seen from the chromatograms (Figure 1).

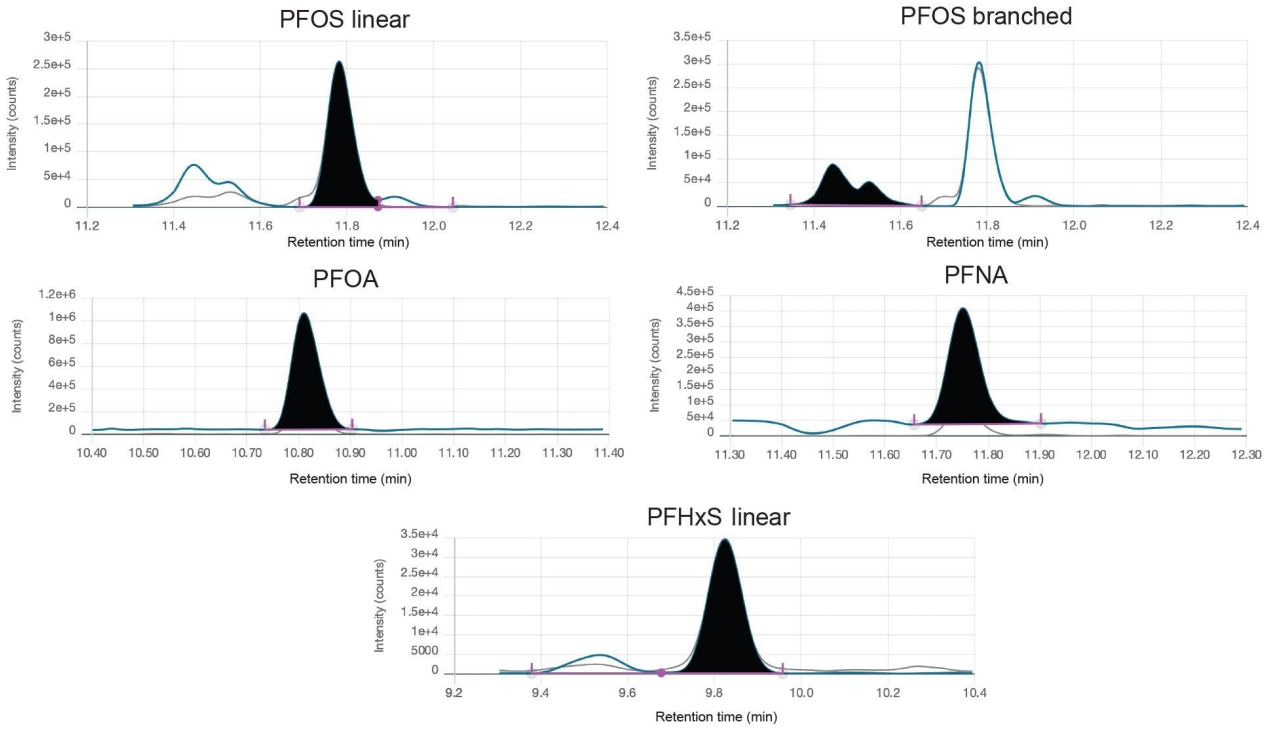

Figure 1. Chromatograms of PFHxS (0.58 µg/Kg), PFOS (linear and branched 4.55 µg/Kg), PFOA (1.47 µg/Kg), and PFNA (0.53 µg/Kg) quantified in FAPAS FISH QC material, assigned concentrations in brackets.

The calibration approach used was as outlined in our previous application note where solvent calibration

standards, over a suitable concentration range, containing both native and labelled PFAS analytes were used to bracket the sample extracts. The calibration graphs were quadratic in some cases and linear in other cases for the four PFAS analytes measured with r_2 values above 0.99 and residuals were within ±20% for most of the laboratories. Figure 2 shows the calibration graphs from laboratory 7 for the PFHxS, PFOS, PFOA, and PFNA analytes present in the FAPAS Fish QC material.

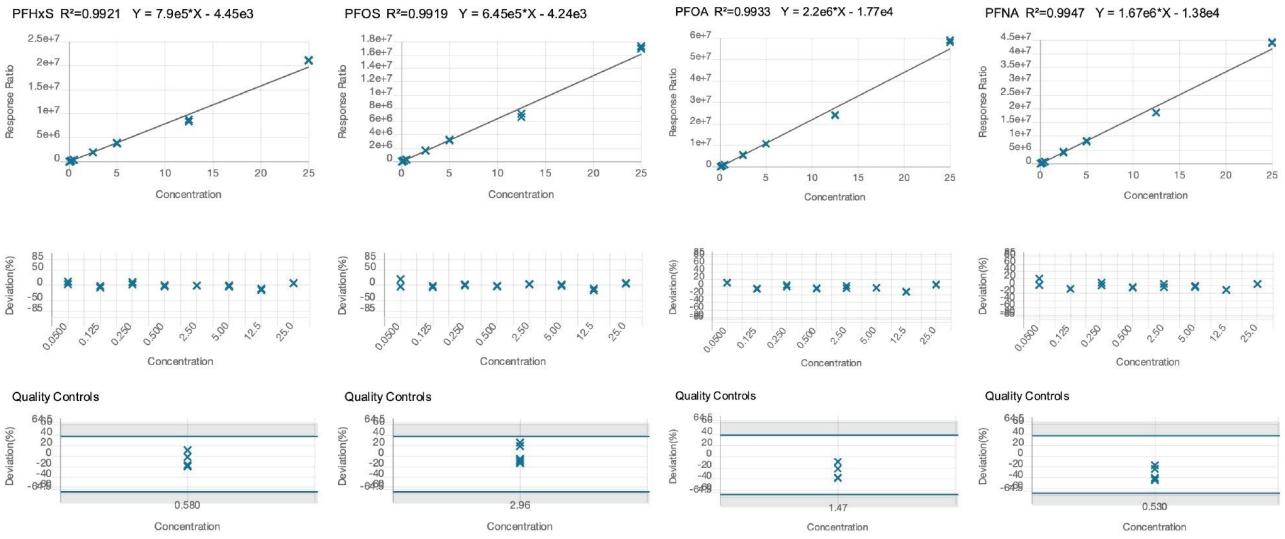

Figure 2. Calibration graphs for PFHxS, PFOS (Linear), PFOA, and PFNA (µg/Kg).

The laboratories demonstrated good accuracy for the quantification of the four PFAS analytes in the FAPAS Fish QC material (Table 1) except for laboratory 3 where all results were outside the upper range of values of the four PFAS analytes, which suggested a systemic error. The results from laboratory 3 were included in the overall method performance calculations (Table 1 and Figure 3) to give a true reflection of expected method performance. Trueness for the four PFAS analytes with assigned values was shown to be between 102 and 121% repeatability, within each laboratory (RSD_r) between 12 and 16% and values for reproducibility between laboratories (RSD_{RL}) were between 25 and 30%. In each case, ion ratios and retention times from the analysis of the FAPAS Fish QC material passed the criteria set out in the EURL-POPs guidance document for PFAS analysis. No residues of PFHxS, PFOS, PFOA, and PFNA were detected in the FAPAS Fish blank sample (T0696b).

T0696QC	PFHxS	PFOS	PFOA	PFNA
Internal standard	$^{13}C_3$-PFHxS	$^{13}C_8$-PFOS	$^{13}C_8$-PFOA	$^{13}C_9$-PFNA
Assigned values (µg/kg)	0.58	4.55	1.47	0.53
Mean of the measured values (µg/kg)	0.61	5.04	1.78	0.54
Range for [z] ≤2 (µg/kg)	0.325–0835	2.55–6.56	0.83–2.12	0.297–0.763
Range of measured values (µg/kg)	0.39–0.94	3.09–7.68	1.03–2.59	0.25–0.85
Trueness (%)	106	111	121	102
Within lab repeatability (%RSD_r)	12	13	12	16
Between lab reproducibility (%RSD_{RL})	28	25	26	30

Table 1. Results of analysis of FPAS QC material T0696QC by the seven participating laboratories.

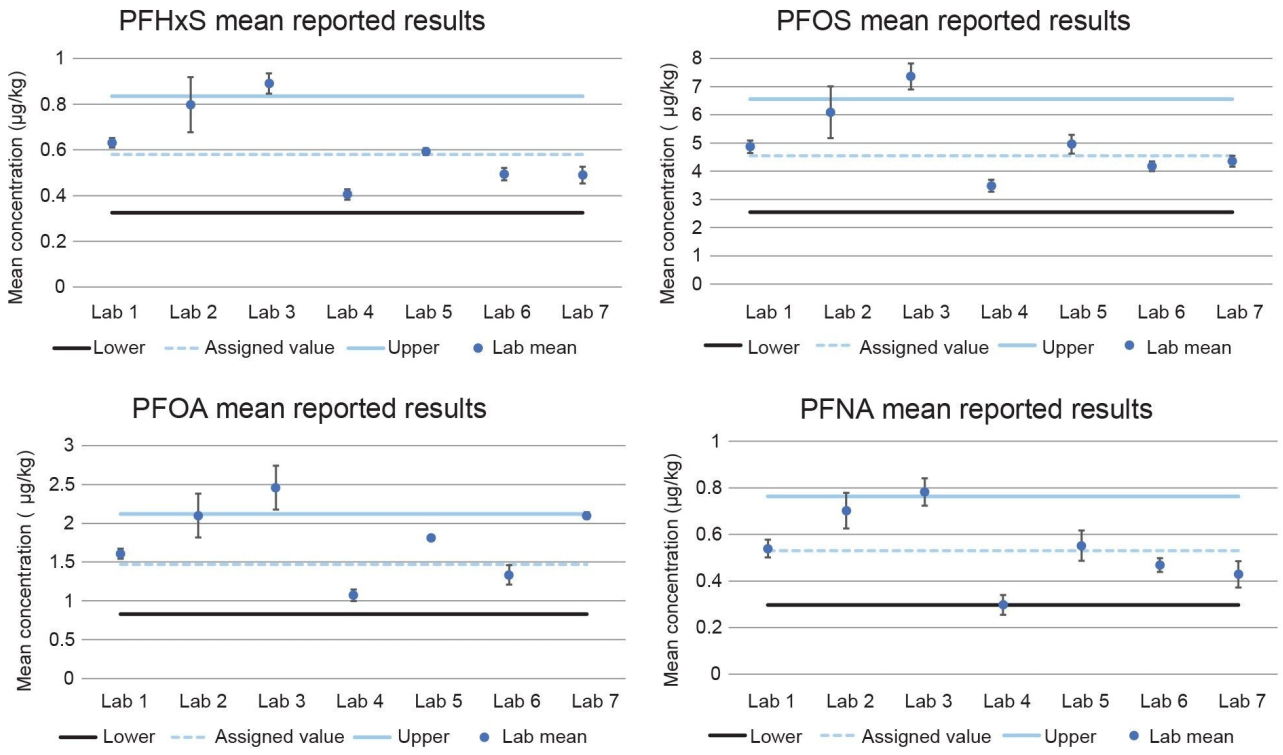

Figure 3. Results of analysis of FPAS Fish QC material T0696QC by the seven participating laboratories.

Conclusion

The performance of the method for the determination of PFOA, PFNA, PFHxS, and PFOS in fish tissue was investigated using an interlaboratory study. Each laboratory successfully implemented the method, including installation of the ACQUITY UPLC PFAS Kit, using the PFAS kit installation guide, and demonstrated satisfactory method performance and sensitivity. Participants demonstrated good accuracy for the determination of the four PFAS analytes in the FAPAS QC material. Trueness for PFHxS, PFOS, PFOA, and PFNA was shown to be between 102 and 121%, values for repeatability within each laboratory were all <20% and reproducibility between laboratories ≤30%. This study confirms that the previously published application by Waters is suitable for assessing PFAS contamination in fish and is suitable for compliance testing when PFAS levels in food become more heavily regulated.[5]

References

1. Schrenk D, Bignami M, ***et al***. EFSA Panel on Contaminants in the Food Chain (EFSA CONTAM Panel), Risk to Human Health Related to the Presence of Perfluoroalkyl Substances in Food. EFS2. 2020;18(9).

2. Commission Recommendation (EU) 2022/1431 of 24 August on the monitoring of perfluoroalkyl substances in food, Official *Journal of the European Union*, L 221, 65, 105–109.

3. Commission Implementing Regulation (EU) 2022/1428 of 24 August 2022 laying down methods of sampling and analysis for the control of perfluoroalkyl substances in certain foodstuffs, Official *Journal of the European Union*, L 221, 65, 66–73.

4. EURL for halogenated POPs in feed and food (2022): Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Fee, version 1.2 of 11 May 2022. Available online under https://eurl-pops.eu/core-working-groups#_pfas <https://eurl-pops.eu/core-working-groups#_pfas> .

5. Orantini K, Hird S, Adams S, Jandova R, Total Workflow for the Sensitive Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Fish, Meat, Edible Offal, and Eggs. Waters Application Note, 720007482, 2022.

APPENDIX I

Compound	PFAS group	Internal standard	Type of internal standard
PFBA	carboxylate	^{13}C-PFBA	
PFPeA	carboxylate	$^{13}C_5$-PFPeA	
PFHxA	carboxylate	$^{13}C_5$-PFHxA	
PFHpA	carboxylate	$^{13}C_4$-PFHpA	
PFOA	carboxylate	$^{13}C_8$-PFOA	
PFNA	carboxylate	$^{13}C_9$-PFNA	
PFDA	carboxylate	$^{13}C_6$-PFDA	
PFUnDA	carboxylate	$^{13}C_7$-PFUnDA	
PFDoDA	carboxylate	^{13}C-PFDoDA	
PFTriDA	carboxylate	^{13}C-PFDoDA	
PFTreDA	carboxylate	$^{13}C_2$-PFTreDA	
PFBS	sulfonate	$^{13}C_3$-PFBS	
PFPeS	sulfonate	$^{13}C_3$-PFHxS	
PFHxS	sulfonate	$^{13}C_3$-PFHxS	
PFHpS	sulfonate	$^{13}C_8$-PFOS	
PFOS	sulfonate	$^{13}C_8$-PFOS	
PFNS	sulfonate	$^{13}C_8$-PFOS	
PFDS	sulfonate	$^{13}C_8$-PFOS	
GenX (HFPO-DA)	ether	$^{13}C_3$-GenX	
ADONA	ether	$^{13}C_3$-GenX	
9Cl-PF3ONS	ether	$^{13}C_8$-PFOS	
11Cl-PF3OUdS	ether	$^{13}C_8$-PFOS	
4:2 FTS	precursor	$^{13}C_2$-4:2 FTS	
6:2 FTS	precursor	$^{13}C_2$-6:2 FTS	
8:2 FTS	precursor	$^{13}C_2$-8:2 FTS	
FBSA	precursor	$^{13}C_8$-FOSA	
FHxSA	precursor	$^{13}C_8$-FOSA	
FOSA	precursor	$^{13}C_8$-FOSA	
N-MeFOSAA	precursor	D_3-N-MeFOSAA	
N-EtFOSAA	precursor	D_5-N-EtFOSAA	
$^{13}C_3$-PFBA	–	$^{13}C_2$-PFOA	Extraction (MPFAC-24ES)
$^{13}C_5$-PFPeA	–	$^{13}C_2$-PFOA	Extraction (MPFAC-24ES)
$^{13}C_5$-PFHxA	–	$^{13}C_2$-PFOA	Extraction (MPFAC-24ES)
$^{13}C_4$-PFHpA	–	$^{13}C_2$-PFOA	Extraction (MPFAC-24ES)
$^{13}C_8$-PFOA	–	$^{13}C_2$-PFOA	Extraction (MPFAC-24ES)
$^{13}C_9$-PFNA	–	$^{13}C_2$-PFOA	Extraction (MPFAC-24ES)
$^{13}C_6$-PFDA	–	^{13}C-PFDA	Extraction (MPFAC-24ES)
$^{13}C_7$-PFUnDA	–	^{13}C-PFDA	Extraction (MPFAC-24ES)
^{13}C-PFDoDA	–	^{13}C-PFDA	Extraction (MPFAC-24ES)
$^{13}C2$-PFTreDA	–	^{13}C-PFDA	Extraction (MPFAC-24ES)
$^{13}C3$-PFBS	–	^{13}C-PFOS	Extraction (MPFAC-24ES)
$^{13}C3$-PFHxS	–	^{13}C-PFOS	Extraction (MPFAC-24ES)
$^{13}C8$-PFOS	–	^{13}C-PFOS	Extraction (MPFAC-24ES)
$^{13}C8$-FOSA	–	$^{13}C_2$-PFOA	Extraction (MPFAC-24ES)
D5-N-EtFOSAA	–	$^{13}C_2$-PFOA	Extraction (MPFAC-24ES)
D3-N-MeFOSAA	–	$^{13}C_2$-PFOA	Extraction (MPFAC-24ES)
$^{13}C2$-4:2 FTS	–	^{13}C-PFOS	Extraction (MPFAC-24ES)
$^{13}C2$-6:2 FTS	–	^{13}C-PFOS	Extraction (MPFAC-24ES)
$^{13}C2$-8:2 FTS	–	^{13}C-PFOS	Extraction (MPFAC-24ES)
$^{13}C3$-GenX	–	$^{13}C_2$-PFOA	Extraction (MPFAC-24ES)
$^{13}C2$-PFOA	–	–	Injection (MPFAC-C-IS)
^{13}C-PFOS	–	–	Injection (MPFAC-C-IS)
^{13}C-PFDA	–	–	Injection (MPFAC-C-IS)

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ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

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