Detection and Quantitation of Per- and Polyfluoroalkyl Substances (PFAS) in Pork Meat using an LC-Orbitrap High-Resolution Mass Spectrometer

Ed George, Maciej Bromirski, and Charles Yang, Thermo Fisher Scientific, San Jose, CA 95134

ABSTRACT

Purpose: To develop a robust method that can efficiently extract, identify and quantify target PFAS at pg/g (parts-per-trillion) levels in pork meat using an LC-Orbitrap high resolution mass spectrometer.

Methods: A modified QuEChERS (Quick, easy, cheap, effective, rugged, and safe) extraction [1] followed by dispersive solid phase cleanup was applied to ground pork samples. Analysis was carried out on a Thermo Scientific™ Orbitrap Exploris™ 120 high-resolution accurate-mass mass spectrometer coupled to a UHPLC system operated in Full Scan-Data-Independent Acquisition (DIA) mode. Compound identification was performed with fragment ion and spectral matching. The PFAS spectral library was created with unique software that provided built-in spectral curation. The method was tested on 34 target PFAS compounds and based upon sample preparation procedures outlined in USFDA Method C-010.01 [2].

Results: The developed method was able to provide excellent quantitation and linearity (r2>/=0.995, RSD and CV <10%) over a calibration range from 5 to 5000 ppt for most compounds with limits of quantitation (LOQs) in pork meat ranging from 25 to 500 ppt. Fragment matches with at least one matching fragment ion and/or library search scores > 80 in pork meat spikes demonstrated that the developed method is fit for purpose and can likely be extended to other matrices that are listed in C-010.01.

INTRODUCTION

PFAS were first developed in the 1940s and have been used by numerous industrial and commercial sectors for products that required thermal and chemical stability, water resistance, and stain resistance. Awareness of PFAS contamination in the environment first emerged in the late 1990s following developments in tandem LC-MS/MS instrumentation which enabled low-level target detection. Most regulations have been focused on environmental contamination of PFAS that have leached into water and soil samples from a variety of sources, such as landfills or Aqueous Film Forming Foam (AFFF) used to extinguish flammable liquid fires.

The need to analyze PFAS in other matrices is growing rapidly since these 'forever chemicals' are very stable and readily bioaccumulate in plant and animal tissues. Moreover, there are over 9000 known PFAS (with more PFAS being actively discovered) and only a very limited number of certified reference standards commercially available for routine targeted analysis. HRAM analysis by LC-Orbitrap has an inherent advantage over triple quadrupole MS because it can provide both quantification and identification of target PFAS, along with the option of retrospective analysis on samples that may contain other untargeted PFAS. It can also overcome challenges of matrix interferences that have been observed in animal tissue extracts by tandem MS [3] due to the low ppm mass accuracy and high mass resolution capability of orbitrap instrumentation. This work describes an LC-HRAM method with excellent sensitivity and specificity demonstrated in pork meat, which is fit for purpose and has the potential to be an excellent platform for expanded PFAS analysis into more complex matrices.

MATERIALS AND METHODS

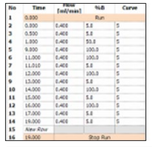
Sample Preparation

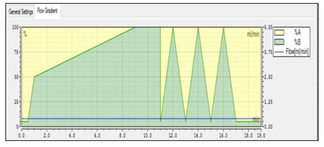
Step	Action
1	Weigh 5g ground pork sample into a 50 mL polypropylene (PP) centrifuge tube
2	Add isotopically labeled PFAS compounds (500 ppt)
3	Add 5mL UHPLC-MS Ultra Pure Water (P/N W8-1) to the 50 mL PP conical centrifuge tube
4	Add 10 mL acetonitrile (Ultra Pure grade P/N A956-1) to the centrifuge tube
5	Add 150 µL Formic Acid, 99% Ultra-Pure LCMS Grade
6	Vortex for 2 minutes, then add a QuEChERS salt packet (Thermo Fisher Product #60105-210 with 6000 mg MgSO4 and 1500 mg C2H3NaO2)
7	Place on benchtop shaker at 1500 rpm with pulse set to 70 for 5 minutes
8	Centrifuge for 5 minutes at 10000 rcf
9	Add 6 mL supernatant to 15 mL PP conical centrifuge tube with dSPE sorbent (Thermo Scientific #60105-205 900 mg MgSO4, 300 mg PSA, 150 mg graphitized carbon black)
10	Vortex/shake for 2 minutes; Centrifuge 5 min at 10000 rfc
11	Transfer 300 µL to a PFAS free polypropylene vial with cap and septa (Thermo Scientific #C4015-100)
12	Add 50 µL Ultra Pure Water, vortex, and place in A/S ready for injection

MATERIALS AND METHODS- cont.

Liquid Chromatography and Mass Spectrometry Conditions

The assays in this study were carried out using a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system and a Thermo Scientific™ Orbitrap Exploris™ 120 high resolution accurate mass spectrometer. The Vanquish system was fitted with a PFAS Analysis Kit (P/N 80100-62142) that replaces wetted Teflon™ surfaces with comparable PEEK components and installing a PFAS trapping column. Thermo Scientific™ TraceFinder™ software was used for instrument control, analysis, data review, and reporting. The LC/MS conditions are shown below.





Inj. Volume	15 µL (With solvent sandwich injection)
Col Temp. and Flowrate	40 C; 400 uL/min
Analytical Column	Thermo Scientific™ Accucore™ C18, 100 x 2.1 mm, 2.6 µm
Trap Column	Thermo Scientific™ Hypersil Gold™ C18, 50 x 4.6 mm, 1.9 µm
Run Time	19 minutes
Mobile Phase A	5mM Ammonium Acetate in H2O
Mobile Phase B	Methanol

Modern LC systems are designed to reduce dead volume through the sampling valve and syringe, resulting in a sharp solvent plug arriving at the head of the column. This is a problem if the starting conditions of a highly aqueous mobile phase do not match up with the extract composition. A sandwich injection was employed for this analysis, which works by bracketing the injected sample volume (15 µL) between mobile phase A plugs (30 µL for each solvent plug) that allows solvent/sample mixing prior to the column head. This greatly improves peak shapes for the early eluting PFAS.

Mass Spectrometer API and Scan Settings

Spray voltage	1.0 kV
Sheath gas	35 arb
Aux gas	5 arb
Sweep gas	1 arb
Capillary temp.	220 ° C
Vaporizer temp.	450 ° C
Ion polarity	Negative

Full scan range	100-1000 m/z
Full scan resolution	60,000
MS2 resolution	15,000
HCD collision energy:	Stepped 10,50
RF Lens	50
DIA m/z windows	5 @ 200 m/z

Standards and Spectral Library Creation

34 target PFAS analytes with 23 labeled compounds were obtained from Cambridge Isotope Laboratories at 50 ug/mL in methanol. All standards were injected individually into the LCMS system at 1 ppm using a piece of PEEK tubing between the autosampler injection valve and the API source. This allowed rapid acquisition of compounds to create the mass spectral library. Raw data files were directly imported into a novel cloud hosted application called myLibrary™ Enterprise (Figure 1) which allows users to easily extract spectra and create MS(n) trees to build fit-for-purpose spectral libraries. Curation is done directly in the application, and the final library can be exported for use in TraceFinder Software with mzVault.

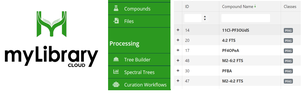


Figure 1: The myLibrary Enterprise Interface allows users to easily create curated spectral libraries for use in HRAM applications.

RESULTS

Calibration

Calibration statistics are shown in the table below. Standards were prepared in neat solvent to closely match the initial QuEChERS extraction solvent composition (70:30 MeCN:H2O + 1% Formic Acid). The branched and linear isomers of PFOS and PFHxS were summed together in this study. Some labeled compounds were not available for certain targets during the development of the method. In those cases, either an external standard calculation method was used, or another closely eluting labeled compound was used.

Compound	Retention Time	Calculation Type	ISTD Used	Calibration Range (ppt)	r^2	CAL Ave RSD
11Cl-PF3OUdS	8.00	Internal	d5-N-EtFOSAA	5-5000	0.9982	3.9
3,6-OPFHpA	4.79	Internal	M4PFHpA	20-5000	0.9976	4.7
4:2 FTS	4.85	Internal	M2-4:2FTS	5-5000	0.9992	5.3
6:2 FTS	6.19	Internal	M2-6:2FTS	10-1000	0.9958	6.4
8:2 FTS	7.33	Internal	M2-8:2FTS	10-5000	0.9982	5.6
9Cl-PF3ONS	7.11	Internal	d5-N-EtFOSAA	5-5000	0.9985	4.6
br-NEtFOSAA	7.78	Internal	d5-N-EtFOSAA	20-5000	0.9956	3.7
br-NMeFOSAA	7.57	Internal	d3-N-MeFOSAA	20-5000	0.9977	4.6
br-PFHxS	5.63	Internal	M3PFHxS	5-5000	0.9985	2.0
br-PFOS	6.83	Internal	MPFOS	5-5000	0.9962	2.0
HFPO-DA	5.09	Internal	M3HFPO-DA	100-5000	0.998	6.4
L-PFBS	4.39	Internal	M3PFBS	5-5000	0.9993	2.6
L-PFDS	7.80	Internal	MPFOS	5-5000	0.9986	2.9
L-PFHpS	6.26	Internal	M3PFHxS	5-5000	0.9982	3.0
L-PFHxS	5.63	Internal	M3PFHxS	5-5000	0.9985	2.0
L-PFOS	6.83	Internal	MPFOS	5-5000	0.9962	2.0
L-PFPeS	4.99	Internal	M3PFHxS	5-5000	0.9971	2.5
NaDONA	5.65	Internal	M4PFHpA	5-5000	0.9991	3.0
PF4OPeA	3.91	Internal	M5PFPeA	5-5000	0.9992	3.6
PF5OHxA	4.45	Internal	MPFHxA	5-5000	0.9994	2.5
PFBA	3.67	Internal	M3PFBA	10-5000	0.9516	2.2
PFDA	7.35	Internal	MPFDA	5-5000	0.999	4.8
PFDoA	8.21	Internal	MPFDoA	5-5000	0.9986	5.6
PFEESA	4.62	External	NA	5-5000	0.9992	2.1
PFHpA	5.58	Internal	M4PFHpA	5-5000	0.9991	2.8
PFHxA	4.91	Internal	MPFHxA	5-5000	0.9995	2.5
PFHxDA	9.37	External	NA	5-5000	0.9966	5.2
PFNA	6.82	Internal	M9PFNA	5-5000	0.9994	3.3
PFOA	6.22	Internal	M2PFOA	5-5000	0.9996	4.4
PFODA	9.78	Internal	M2PFOA	10-5000	0.9963	3.8
PFPeA	4.28	Internal	M5PFPeA	5-5000	0.9993	3.2
PFTeDA	8.85	Internal	M7PFUdA	10-5000	0.9986	4.9
PFTrDA	8.55	Internal	M7PFUdA	5-5000	0.9978	5.6
PFUdA	7.81	Internal	M7PFUdA	5-5000	0.9993	5.0

The solvent sandwich injection technique with aqueous mobile phase A was used in conjunction with 'in needle mixing' to help mix the highly organic extract prior to injection. The technique was optimized to yield acceptable peak shapes for all the compounds in the method. Figure 2 represents the extracted full scan precursor ions for a solvent calibration standard at 100 ppt (500 ppt for labeled compounds).

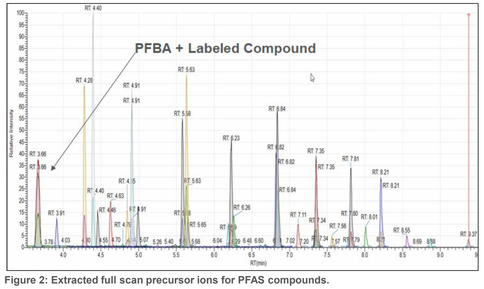
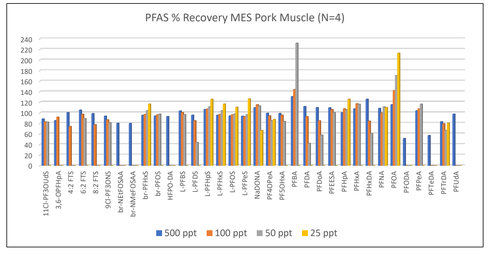


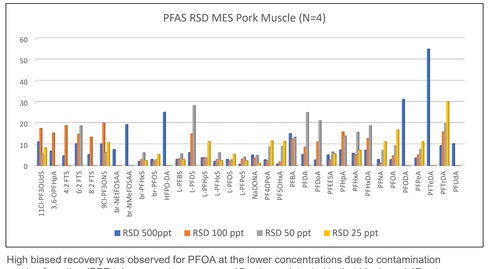
Figure 2: Extracted full scan precursor ions for PFAS compounds.

RESULTS- cont.

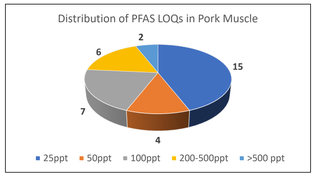
Recovery Experiments

Biological replicates of ground pork meat samples were spiked with PFAS labeled compounds along with native analytes and taken through the entire extraction and cleanup process (Matrix Extracted Spikes-MES). The labeled analytes were spiked at 500 ppt, and the replicates (N=4 at each concentration) had native PFAS levels at 25, 50, 100, and 500 pg/g). [Note that the final concentrations of native PFAS in the extracts are 8.3, 16.7, 33.3, and 167 ppt respectively based on the solvent volumes used for extraction]. In addition, a pork meat method blank and several process blanks containing the QuEChERS salts and dispersive SPE reagents were prepared to determine if PFAS background contamination or incurred residues were present. Recovery and RSD results are shown in the bar graphs below.





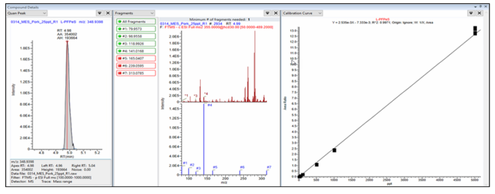
High biased recovery was observed for PFOA at the lower concentrations due to contamination coming from the dSPE tube reagents, as approx. 15 ppt was detected in that blank, and 17 ppt was detected in the pork meat blank. PFBA was also detected in the same blanks (30-50 ppt in both). PFDOA and PFTeDA had poor recovery and high RSD overall. It is suspected that these compounds may have been absorbed by the graphitized carbon black material present in the dSPE reagent. The limit of quantitation (LOQ) distribution for the native PFAS are shown in the pie chart below. LOQ is defined here as MES % Average Recovery 60-130% and RSD < or = 25%.

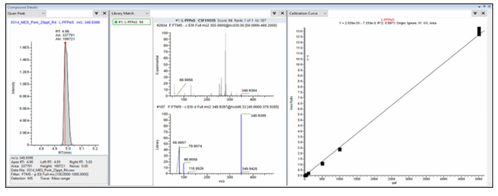


RESULTS- cont.

Identification and Library Search

A detected and confirmed analyte is defined as the native PFAS precursor ion detected at <5 ppm mass accuracy with S/N ≥ 3, AND at least one MS2 fragment detected at < 5 ppm. A spectral library search result also adds confidence in the confirmation process. The top plot shows the fragment ions match for L-PFPeS at 25 ppt in the pork meat MES. The lower plot is a library search result vs. the user created spectral library (created in myLibrary Enterprise application) for the same spike level.





CONCLUSIONS

- The Vanquish Flex UHPLC using solvent sandwich injection technique coupled to the Orbitrap Exploris 120 Mass Spectrometer provided excellent quantitative sensitivity with qualitative confirmation in FS-DIA mode, with most PFAS LOQs in pork meat matrix less than 50 pg/g (16.7 ppt in final extract), without the need for further extract concentration.
- myLibrary Enterprise allows users to easily create highly curated spectral libraries for added confidence in confirmation.
- The method was shown to be fit-for-purpose and may be explored for future expansion into other food matrices.

REFERENCES

1. Anastassiades M, Lehotay SJ, Stajnbaher D, Schenck FJ (2003) Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. J AOAC Int 86:412–431.
2. Method C-010.01-Determination of 16 Per and Polyfluoroalkyl Substances (PFAS) in Processed Food using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) https://www.fda.gov/media/131510/download
3. LCGC- The PFAS Summit: A Virtual Symposium, 2022. [Requires site registration].

TRADEMARKS

PO66152-EN0422S