Application note | 001502

Mass spectrometry

Detection and quantitation of PFAS in animal tissue using Orbitrap Exploris 120 high-resolution mass spectrometer

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Keywords

Per- and polyfluoroalkyl substances (PFAS), PFOS, PFOA, GenX, PFCs, QuEChERS Extraction, Orbitrap Exploris 120 mass spectrometer, Vanquish Flex Binary UHPLC system, high resolution accurate mass (HRAM), TraceFinder software

Goal

To develop a robust method that can efficiently extract, identify and quantify target per- and polyfluoroalkyl substances (PFAS) at pg/g (parts-per-trillion) levels in animal tissues using a LC-Orbitrap high resolution mass spectrometer. Thirty-four target PFAS compounds were chosen based upon available reference standards that are cited in various regulated USEPA methods. Pork muscle meat was used as a test matrix to demonstrate applicability.

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[1] (with more PFAS being actively discovered) and only a very limited number of certified reference standards commercially available for routine targeted analysis. High-resolution accurate-mass (HRAM) analysis by

LC-Orbitrap has an advantage over triple quadrupole MS because, in addition to quantification and identification of target PFAS, it also enables retrospective analysis of sample data files for other untargeted PFAS. The higher mass selectivity of HRAM MS, due to the low parts per million (ppm) mass accuracy and high mass resolution, can help to overcome matrix interferences observed in the analysis of animal tissue extracts. This work describes the development of a LC-HRAM method for the analysis of PFAS in pork meat. The method shows excellent sensitivity and specificity and is fit for purpose with the potential to be an excellent platform for expanded PFAS target compounds as well as into more complex matrices.

The sample extraction method was based upon the USFDA Foods Program Compendium of Analytical Laboratory Methods: Chemical Analytical Manual (CAM) Method Number: C-010.01[2]. SANTE/12682/2019[3] guideline criteria for pesticides were adopted as a means to evaluate method performance regarding identification, reproducibility and accuracy of the analysis.

Experimental

Reagents and consumables

- Acetonitrile, UHPLC-MS grade (P/N A956-1)
- Ammonium Acetate, Optima LC-MS grade (P/N A114-50)
- Methanol, UHPLC-MS grade (P/N A458-1)
- Formic acid, LC-MS grade (P/N 28905)
- Water, UHPLC-MS grade (P/N W8-1)
- Thermo Fisher Scientific PFAS HPLC Vial Kit (P/N C4015-100)
- Fisherbrand™ Easy Reader™ 50 mL Conical Polypropylene Centrifuge Tubes (P/N 05-539-6)
- Fisherbrand™ Easy Reader™ 15 mL Conical Polypropylene Centrifuge Tubes (P/N 05-539-5)
- Thermo Scientific™ HyperSep™ dSPE Centrifuge Tube (6G $MgSO_4$, 1.5 g $C_2H_3NaO_2$) (P/N 60105-210)
- Thermo Scientific™ HyperSep™ dSPE Clean-up Tube (900 mg $MgSO_4$, 300 mg PSA, 150 mg Graphitized Carbon Black(GCB) (P/N 60105-205)
- Kit, Upgrade, PFAS Analysis, Thermo Scientific™ Vanquish™ Flex UHPLC System, (P/N 80100-62142)
- Thermo Scientific™ Accucore™ C18 HPLC Column (Analytical Column) (P/N 17126-102130)

Standards

Thirty-four target PFAS analytes and 23 labeled compounds at 50 µg/mL in methanol were obtained from Cambridge Isotope Laboratories. See Table 4 for the identity of all PFAS investigated for targeted quantitative analysis.

LC-MS/MS setup

Vanquish Flex UHPLC system, consisting of:

- Vanquish Flex Binary Pump F (P/N VF-P10-A-01)
- Split Sampler FT (P/N VF-A10-A-02)
- Column Compartment H (P/N VH-C10-A-03)
- System Base (P/N VF-S01-A-02)
- Sample Loop, 100 µL (P/N 6850.1913)
- Thermo Scientific™ Viper™ Capillary, 0.18 × 350 mm, MP35N (P/N 6042.2337)
- Set, Inline Filter, 35 µL, VF-P1 (P/N 6044.3870)
- Thermo Scientific™ nanoViper™ Capillary 75 µm × 750 mm (P/N 6041.5780)
- Thermo Scientific™ Orbitrap Exploris™ 120 high-resolution mass spectrometer (P/N BRE725531) equipped with the Thermo Scientific™ OptaMax™ NG source housing

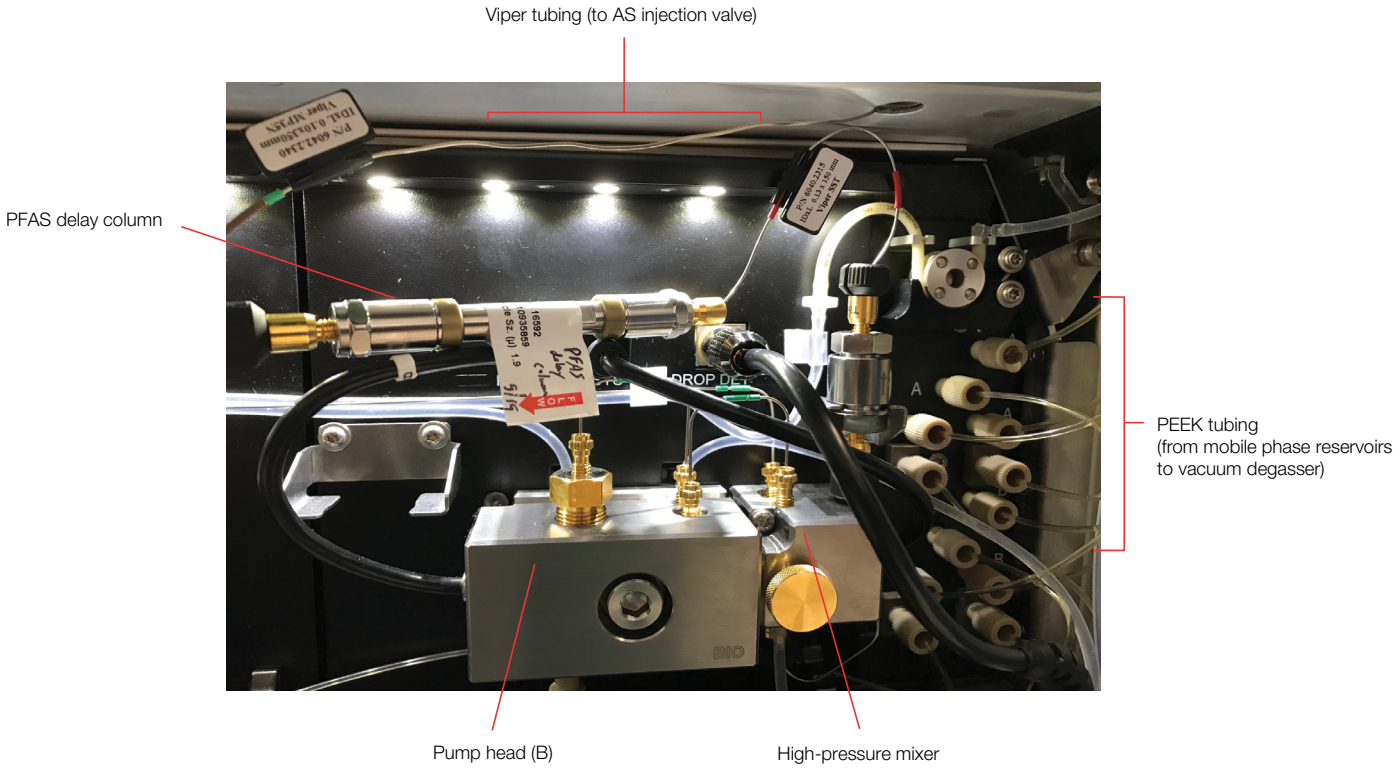

Figure 1. The Vanquish Flex UHPLC system shown here fitted with a PFAS Analysis Kit (P/N 80100-62142) that replaces wetted Teflon™ surfaces with comparable PEEK components and installing a PFAS trapping column.

LC and autosampler injection conditions

Table 1A. LC conditions

Parameter	Setting
LC analytical column	Thermo Scientific™ Accucore™ C18 column, 100 × 2.1 mm, 2.6 µm
LC trap column	Thermo Scientific™ Hypersil GOLD™ C18 column, 50 × 4.6 mm, 1.9 µm
Mobile phase A	5 mM ammonium acetate in water
Mobile phase B	Methanol
Flow rate	0.4 mL/min
Gradient	See Table 2
Column oven	40 ˚C forced air mode
Sample loop volume	100 µL
Sample injection volume	15 µL
Solvent sandwich volume	2 × 30 µL
Solvent sandwich composition	Mobile phase A
Needle wash solution	50:50:50 MeCN:MeOH:0.1% Formic acid in water vol/vol/vol

Table 1B. Autosampler custom injection program for sandwich injection technique

Command	Parameter setting
Prepare liquid handling	Volume = 100 µL
Needle wash	Duration = 20 sec, depth = 30,000 µm
Draw	Position = SB1, 30 µL (Mobile phase A) Specified sample tray position (15 µL)
Needle wash	Duration = 20 sec, depth = 30,000 µm
Draw	Position = SB1, 30 µL (Mobile phase A)
In needle mix	Volume = 15 µL, draw speed = 20 uL/s, dispense speed = 20 uL/s; cycles = 40
Needle wash	Duration = 20 sec, depth = 30,000 µm
Wait	10 sec
Prepare/inject	Sample injected

Table 2

Time (min)	Flow rate (mL/min)	%A	%B
0.0	0.400	95	5
0.5	0.400	95	5
1.0	0.400	50	50
9.0	0.400	0	100
11.0	0.400	0	100
11.01	0.400	95	5
12.0	0.400	0	100
13.0	0.400	95	5
14.0	0.400	0	100
15.0	0.400	95	5
16.0	0.400	0	100
17.0	0.400	95	5
19.0	0.400	95	5

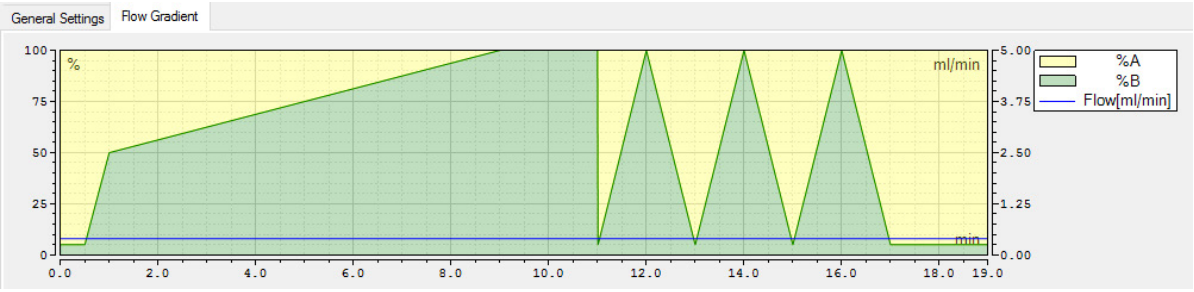

Figure 2. Gradient profile used for the analysis. A 'saw tooth' gradient at the end of run was found to reduce carryover of low-level PFAS from standards or matrix spikes.[3]

MS conditions and PFAS compounds

Table 3. MS conditions

Parameter	Setting
Run time	19 min
Ion source	H-ESI
Source positioning	Between M and L; 1.0
Spray voltage	Negative mode, 1,000 V
Sheath gas	35
Auxiliary gas	5
Sweep gas	1
Ion transfer tube temperature	220 ˚C
Vaporizer temperature	450 ˚C
Experiment type	Full MS with data-independent acquisition (DIA)
Chromatography peak width	6 s
HCD collision energies	Stepped 10,50
Full scan mass resolution	60,000 FWHM @ *m/z* 200
RF lens setting	50
Full scan mass range	*m/z* 100–1000
MS^2 mass resolution	15,000 FWHM @ *m/z* 200
DIA *m/z* windows	5 × 210 *m/z* windows

Table 4. PFAS native and labeled analytes

Compound name	Formula	CAS number	Retention time (min)	Adduct	*m/z* (Expected)	*m/z* (Apex)	*m/z* (Delta) (ppm)
11Cl-PF3OUdS	$C_{10}F_{20}ClSO_4K$	83329-89-9	8.00	M-K	630.8892	630.8901	1.4039
3,6-OPFHpA	$C_5HF_9O_4$	151772-58-6	4.78	M-H	294.9658	294.9661	.7609
4:2 FTS	$C_6H_4F_9SO_3Na$	27919-93-8	4.84	M-Na	326.9744	326.9745	.2670
6:2 FTS	$C_8H_4F_{13}SO_3Na$	27619-94-9	6.18	M-Na	426.9679	426.968	.3469
8:2 FTS	$C_{10}H_4F_{17}SO_3Na$	27619-96-1	7.33	M-Na	526.9615	526.9619	.6320
9Cl-PF3ONS	$C_8F_{16}ClSO_4K$	73606-19-6	7.11	M-K	530.8956	530.8959	.5538
br-NEtFOSAA	$C_{12}H_8F_{17}NO_4S$	2991-50-6	7.78	M-H	583.983	583.9839	1.6572
br-NMeFOSAA	$C_{11}H_6F_{17}NO_4S$	909405-48-7	7.56	M-H	569.9673	569.9682	1.5276
br-PFHxSK	$C_6HF_{13}SO_3$	355-46-4	5.62	M-H	398.9366	398.9367	.2671
br-PFOSK	$CF_3(CF_2)_7SO_3K$	1763-23-1	6.83	M-K	498.9303	498.9302	-.1266
d3-N-MeFOSAA	$C_{11}D_3H_3F_{17}NO_4S$	1400690-70-1	7.55	M-H	572.9862	572.9874	2.1410
d5-N-EtFOSAA	$C_{12}D_5H_3F_{17}NO_4S$	2991-50-6	7.77	M-H	589.0144	589.0147	.5933
HFPO-DA	$C_6HF_{11}O_3$	13252-13-6	5.09	M-H	328.9677	328.9681	1.1525
L-PFBS	$C_4F_9SO_3K$	29420-49-3	4.38	M-K	298.943	298.943	-.0915
L-PFDS	$C_{10}F_{21}SO_3Na$	2806-15-7	7.79	M-Na	598.9238	598.9241	.4045
L-PFHpS	$C_7F_{15}SO_3Na$	21934-50-9	6.26	M-Na	448.9334	448.9337	.6812
L-PFHxS	$C_6F_{13}SO_3Na$	82382-12-5	5.62	M-Na	398.9366	398.9367	.2671
L-PFOSK	$CF_3(CF_2)_7SO_3K$	2795-39-3	6.83	M-K	498.9302	498.9302	.0738
L-PFPeS	$C_5F_{11}SO_3Na$	630402-22-1	4.98	M-Na	348.9398	348.9396	-.5568
M2-4:2FTS	$^{13}C_2C_4H_4F_9SO_3Na$	NA	4.84	M-Na	328.981	328.9812	.5187
M2-6:2FTS	$^{13}C_2C_6H_4F_{13}SO_3Na$	NA	6.17	M-Na	428.9746	428.9746	-.0726
M2-8:2FTS	$^{13}C_2C_8H_4F_{17}SO_3Na$	NA	7.33	M-Na	528.9682	528.9706	4.5600
M2PFOA	$^{13}C_2C_6HF_{15}O_2$	335-67-1	6.22	M-H	414.9732	414.9732	-.0601
M3HFPO-DA	$^{13}C_3C_3HF_{11}O_3$	NA	5.09	M-H	331.9778	331.978	.7149
M3PFBA	$^{13}C_3CHF_7O_2$	NA	3.66	M-H	215.9893	215.9894	.4844
M3PFBS	$^{13}CC_3F_9SO_3Na$	NA	4.38	M-Na	301.9532	301.953	-.7537
M3PFHxS	$^{13}C_3C_3F_{13}SO_3Na$	3871-99-6	5.62	M-Na	401.9467	401.9471	.9504
M4PFHpA	$^{13}C_4C_3HF_{13}O_2$	6130-43-4	5.57	M-H	366.983	366.9831	.0620
M5PFHxA	$^{13}C_5CHF_{11}O_2$	NA	4.90	M-H	317.9896	317.9899	.8748
M5PFPeA	$^{13}C_5HF_9O_2$	2706-90-3	4.27	M-H	267.9927	267.9929	.8206
M6PFDA	$^{13}C_6C_4HF_{19}O_2$	335-76-2	7.34	M-H	518.9802	518.9803	.2228
M7PFUdA	$^{13}C_7C_4HF_{21}O_2$	NA	7.80	M-H	569.9803	569.981	1.2072
M8PFOA	$^{13}C_8HF_{15}O_2$	335-67-1	6.22	M-H	420.9933	420.9935	.5695
M8PFOS	$^{13}C_8F_{17}SO_3Na$	2795-39-3	6.83	M-Na	506.9571	506.9572	.3045
M9PFNA	$^{13}C_9HF_{17}O_2$	375-95-1	6.81	M-H	471.9941	471.9939	-.4312
MPFBA	$^{13}C_2C_2HF_7O_2$	375-95-1	3.66	M-H	216.9926	216.9928	.7008
MPFDA	$^{13}C_2C_8HF_{19}O_2$	375-22-4	7.34	M-H	514.9668	514.9668	.0910
MPFDoA	$^{13}C_2C_{10}HF_{23}O_2$	307-55-1	8.20	M-H	614.9604	614.9611	1.2368
MPFHxA	$^{13}C_2C_4HF_{11}O_2$	307-24-4	4.90	M-H	314.9795	314.9798	.8806
MPFOS	$^{13}C_4C_4F_{17}SO_3Na$	NA	6.83	M-Na	502.9438	502.9438	-.0266
NaDONA	$C_7HF_{12}O_4Na$	958445-44-8	5.64	M-Na	376.9689	376.9689	.0055
PF4OPeA	$C_4HF_7O_3$	377-73-1	3.90	M-H	228.9741	228.9742	.1817
PF5OHxA	$C_5HF_9O_3$	863090-89-5	4.45	M-H	278.9709	278.9709	-.2304
PFBA	$C_4HF_7O_2$	375-22-4	3.66	M-H	212.9792	212.9793	.5838
PFDA	$C_{10}HF_{19}O_2$	335-76-2	7.34	M-H	512.96	512.9601	.2028
PFDoA	$C_{12}HF_{23}O_2$	307-55-1	8.20	M-H	612.9537	612.9543	1.1350
PFEESA	$C_4F_9SO_4K$	117205-07-9	4.61	M-K	314.9379	314.938	.2486
PFHpA	$C_7HF_{13}O_2$	375-85-9	5.57	M-H	362.9696	362.9695	-.4631
PFHxA	$C_6HF_{11}O_2$	307-24-4	4.90	M-H	312.9728	312.9731	.9714
PFHxDA	$C_{16}HF_{31}O_2$	67905-19-5	9.36	M-H	812.9409	812.9417	.9477
PFNA	$C_9HF_{17}O_2$	375-95-1	6.81	M-H	462.9632	462.9636	.7831
PFOA	$C_8HF_{15}O_2$	335-67-1	6.22	M-H	412.9664	412.9665	.0755
PFODA	$C_{18}HF_{35}O_2$	16517-11-6	9.77	M-H	912.9345	912.935	.5450
PFPeA	$C_5HF_9O_2$	2706-90-3	4.27	M-H	262.976	262.976	.1282
PFTeDA	$C_{14}HF_{27}O_2$	376-06-7	8.86	M-H	712.9473	712.9482	1.3776
PFTrDA	$C_{13}HF_{25}O_2$	72629-94-8	8.55	M-H	662.9505	662.9515	1.5341
PFUdA	$C_{11}HF_{21}O_2$	2058-94-8	7.80	M-H	562.9568	562.9574	.9902

Software

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software to ensure full automation from instrument setup to raw data collection, processing, and reporting. A PFAS library was created for the target compounds using myLibrary™ Enterprise, a novel cloud hosted application.

Sample preparation

The stock concentration of all PFAS native and labeled standards were 50 µg/mL in methanol. Various mixed intermediate stock concentrations were prepared and used to create calibration and matrix spiking standards. In addition, a 1 µg/mL substock of each individual standard was prepared in methanol for subsequent acquisition of mass spectral data.

A five-hundred-gram portion of ground pork was purchased at a local organic market. Five-gram homogenized portions were taken for the analysis with detailed sample preparation steps described in Table 5.

Table 5. Sample preparation steps

Step	Action
1	Weigh 5 g ground pork sample into a 50 mL polypropylene (PP) centrifuge tube
2	Add isotopically labeled PFAS compounds (500 ppt)
3	Add 5 mL 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 min, then add a QuEChERS salt packet (Thermo Fisher Scientific product #60105-210 with 6000 mg $MgSO_4$ and 1500 mg $C_2H_3NaO_2$)
7	Place on benchtop shaker at 1500 rpm with pulse set to 70 for 5 min
8	Centrifuge for 5 min at 10,000 rcf
9	Add 6 mL supernatant to 15 mL PP conical centrifuge tube with dSPE sorbent (Thermo Scientific #60105-205 900 mg $MgSO_4$, 300 mg PSA, 150 mg graphitized carbon black)
10	Vortex/shake for 2 min; 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

Native calibration standard preparation

Standards were prepared in neat solvent closely matching the final QuEChERS extraction solvent composition. Calibration levels prepared were 5, 10, 25, 50, 100, 500, 1000, and 5000 pg/mL.

Labeled standard preparation

A labeled analyte intermediate stock standard was prepared in 100% methanol with a final concentration of 150 ng/mL. All matrix extracted samples (MES) and neat calibrants were spiked at a final concentration of 500 pg/mL.

Results and discussion

Creation of a target PFAS library with the myLibrary Enterprise application

All standards, each at 1 µg/mL were individually injected into the LCMS system configured using a short piece of PEEK tubing between the autosampler injection valve and the HESI source along with a fritted union to provide backpressure. This allowed for rapid acquisition of compounds to capture mass spectral information. myLibrary Enterprise is a SaaS (Software as a Service) application designed for collaborative creation and management of proprietary spectral libraries. First, the compounds and metadata are imported into the cloud application for each PFAS native and labeled compound (name, molecular formula, CAS#, compound class, etc). Next, the raw data files are uploaded, and batch processed by extracting and assigning the MS^2 spectra into spectral trees. The batch processing includes spectral curation and fragment structure annotation, ensuring high spectral fidelity is obtained using the same algorithms that are used in Thermo Scientific™ mzCloud™ mass spectral library. The final library can be exported in mzVault format for use in TraceFinder software.

Custom injection program for LC peak shape optimization

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 with the extract composition. Use of a custom injection program on the Vanquish Split Autosampler[5] improves LC peak shapes when injecting larger volumes of extract, since stronger solvents like acetonitrile are in the final QuEChERs extract. This “sandwich injection” works by bracketing the injected sample volume between aqueous plugs of mobile phase A that allows the sample solvent strength to be reduced prior to entering the column. Table 1b describes the custom injection program used. The technique was optimized to yield acceptable peak shapes for all the compounds in the method. Figure 3 displays extracted ion chromatograms of each of the precursor ions for a solvent calibration standard at 100 pg/mL (500 pg/mL for labeled compounds).

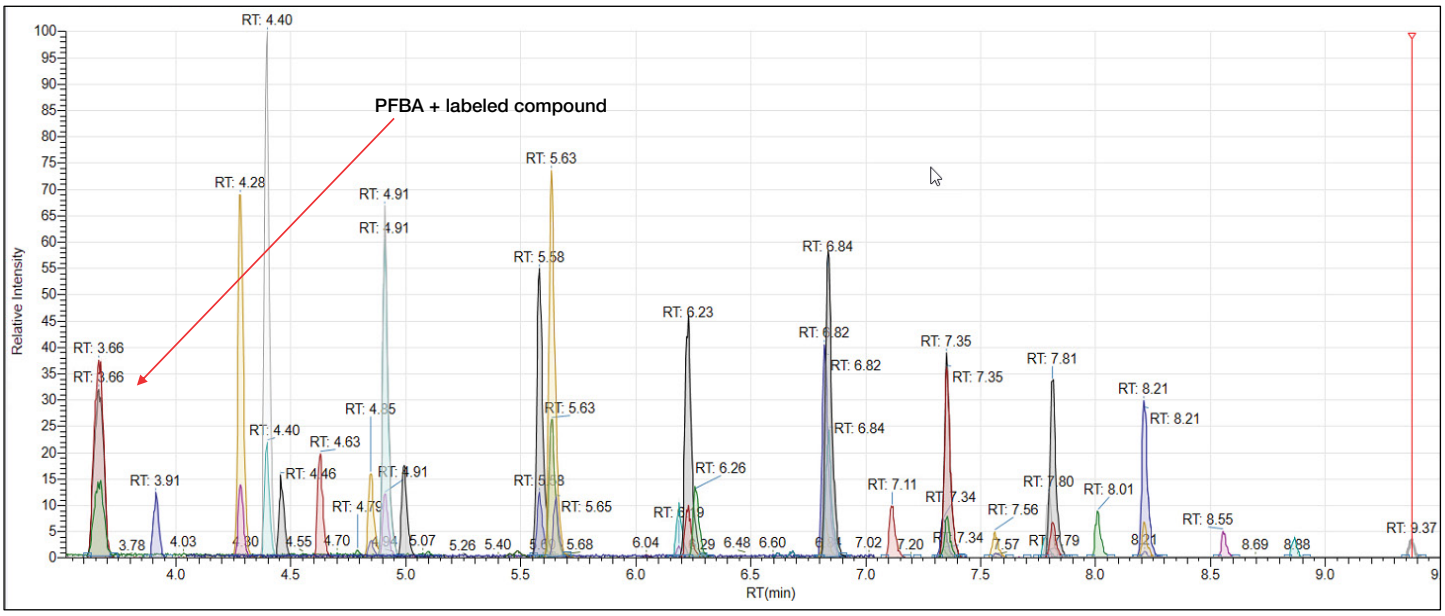

Figure 3. Extracted full scan precursor ions for PFAS compounds at 500 pg/mL in a solvent standard. Early eluting peaks show good peak shape despite high solvent concentration (70:30 MeCN:mobile phase A) in the extract.

Calibration

Calibration statistics are shown in Table 6. Standards were prepared in neat solvent to closely match the final QuEChERS extraction solvent composition (70:30 MeCN:H_2O + 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. Most analytes had excellent linearity from 5 to 5000 pg/mL with r^2 values >0.995 and RSDs over the specified calibration range of <7%. Retention times for all analytes were very reproducible indicating that the optimized custom injection program did not add any variability to the analysis.

Recovery experiments and LOQ

Four 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 pg/g, and the replicates (N=4 at each concentration) had native PFAS levels at 25, 50, 100, and 500 pg/g. [Note, the final concentrations of native PFAS in the extracts were 8.3, 16.7, 33.3, and 167 pg/mL 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 Figures 4 and 5.

High biased recovery was observed for PFOA at the lower concentrations due to contamination coming from the dSPE tube reagents, as approximately 15 pg/mL was detected in that blank, and 17 pg/mL was detected in the pork meat blank. PFBA was also detected in the same blanks (30–50 pg/mL 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 clean-up reagent. The limits of quantitation (LOQ) distribution for the native PFAS are shown in Figure 6. LOQ is defined here as MES % Average recovery 60–130% and RSD < or = 25%.

Table 6. Calibration statistics

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

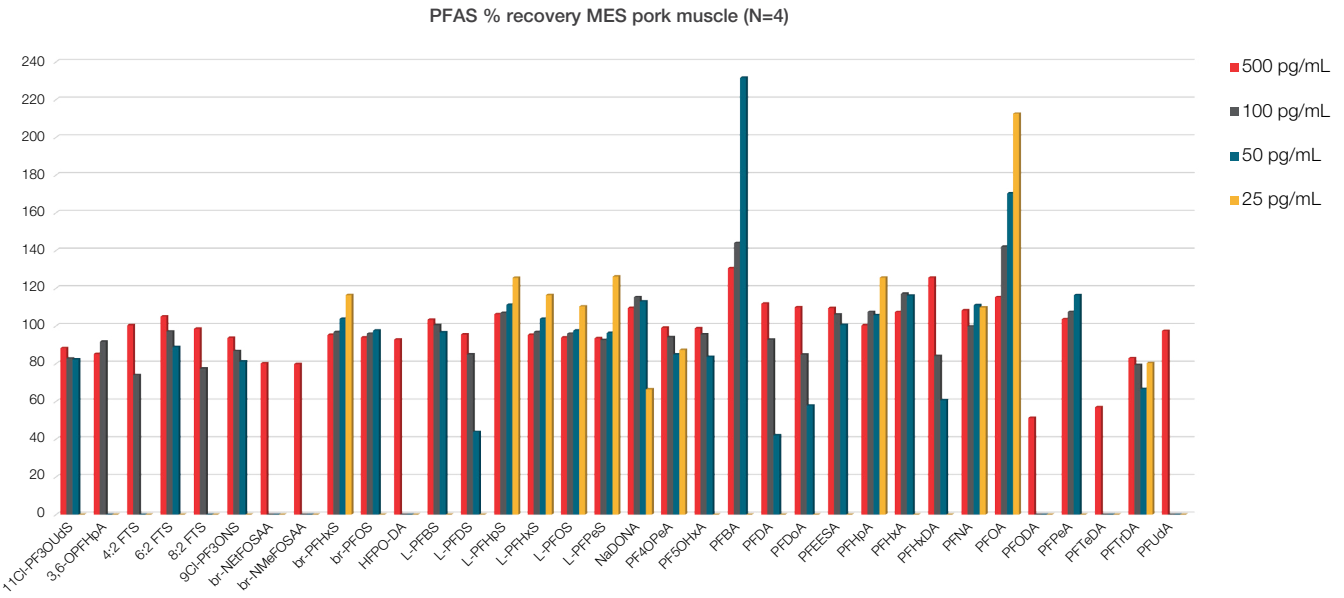

Figure 4. Average percent recovery of PFAS compounds based on four separate matrix extracted spikes of pork muscle meat at each concentration.

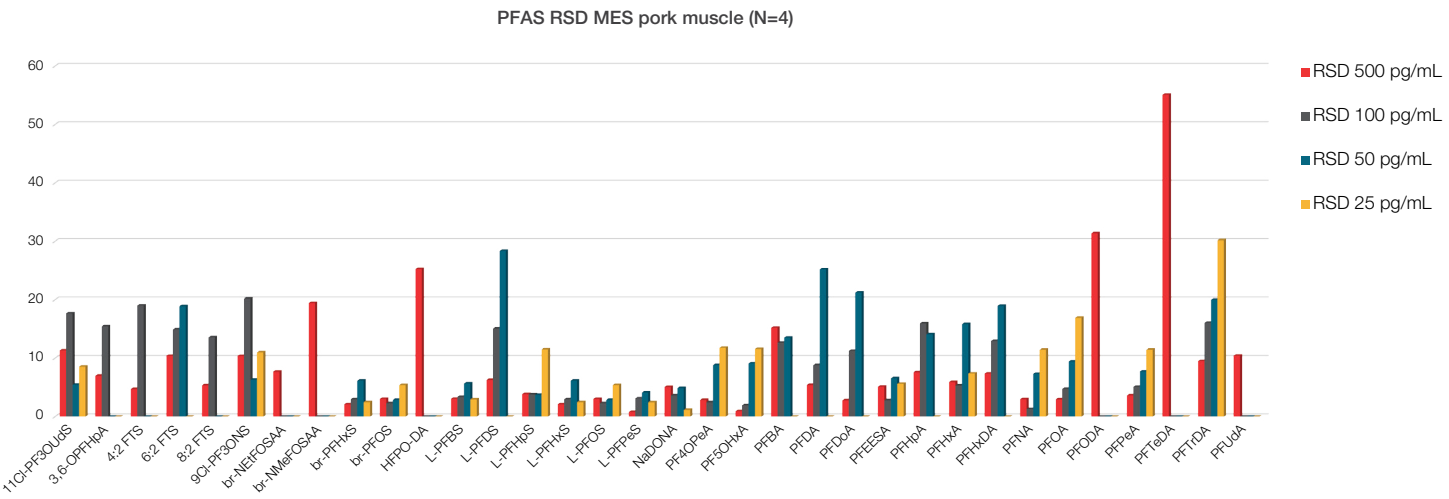

Figure 5. Average RSD of PFAS compounds based on four separate matrix extracted spikes of pork muscle meat at each concentration.

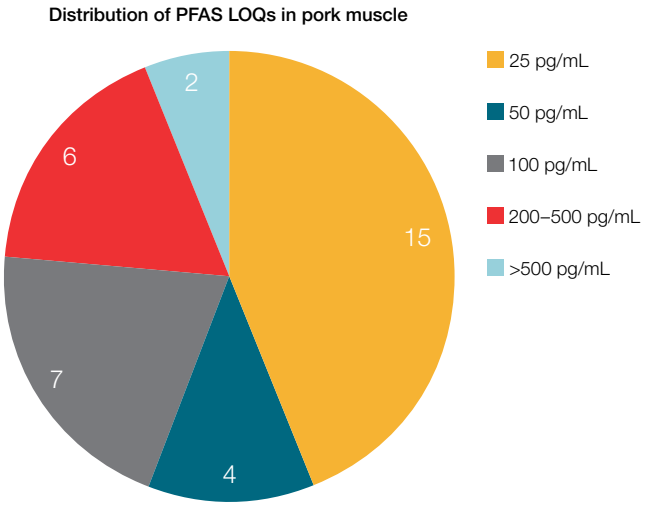

Figure 6. The limit of quantification (LOQ) distribution for the native PFAS are shown in the pie chart above. LOQ is defined here as MES % Average Recovery 60–130% and RSD < or = 25%.

Identification and library search

Data acquired were analyzed with an extraction mass tolerance of <5 ppm for both precursor and product ions. Full MS with DIA (data independent acquisition) was used. In DIA mode, the user enables a selective and sensitive method for a global view of MS^2 data over a user-defined mass range. The quadrupole isolates a relatively narrow mass range which is advanced to the collision cell where all ion fragmentation is performed. The entire ion cloud is injected into the Orbitrap mass analyzer, which monitors the full MS^2 mass range. In the following scan, the quadrupole shifts the isolation mass range upwards to the next incremental range.

All isolated ions are again fragmented and analyzed by the Orbitrap mass analyzer. Continuous upward shifting of the isolation mass range continues until the entire selected mass range is covered. The fragment ion spectra are therefore obtained from a wider user-defined mass range, instead of isolated unit mass precursors. Identification of target PFAS compounds was performed using fragment matching and library search results. A detected and identified analyte is defined as the native PFAS precursor ion detected at <5 ppm mass accuracy with S/N ≥ 3, AND at least one MS^2 fragment detected at <5 ppm. A spectral library search result also adds confidence in the identification process. Figures 7a and 7b show the fragment ions match for L-PFPeS at 25 pg/mL and a library search result at 500 pg/mL in the pork meat MES.

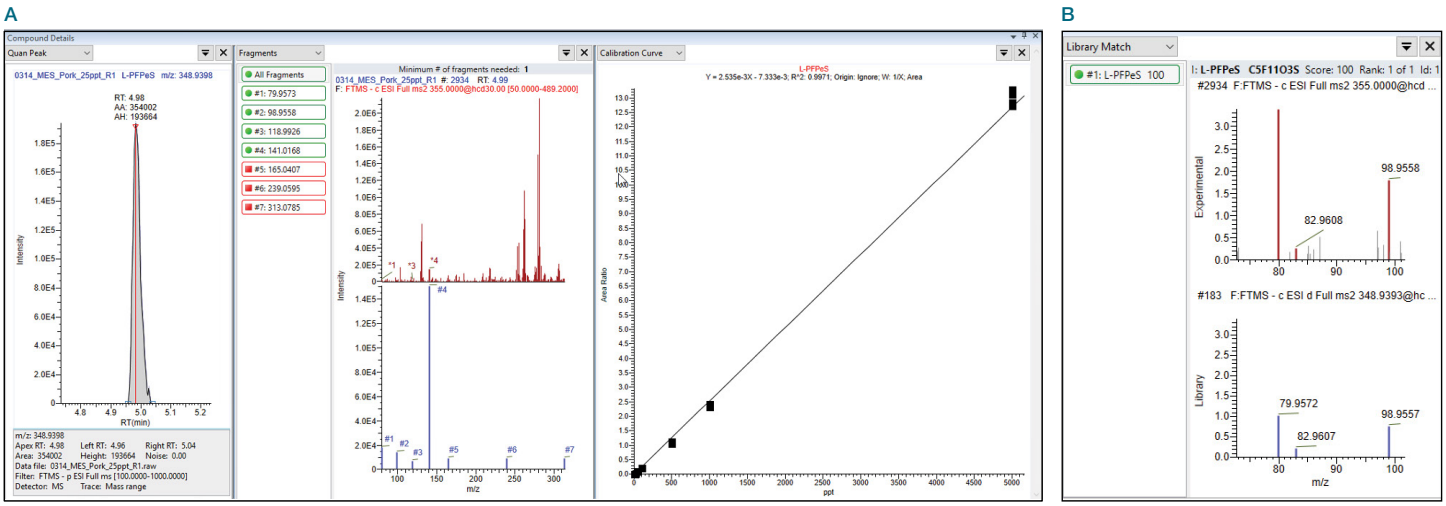

Figure 7. (A) Extracted precursor ion of L-PFPeS in pork muscle matrix at 25 pg/mL, with fragment match and calibration curve. (B) The library search result of a pork extract spiked at 500 pg/mL (zoomed into *m/z* 70–100 range for clarity).

Conclusion

- The Vanquish Flex UHPLC system using solvent sandwich injection technique coupled to the Orbitrap Exploris 120 mass spectrometer provided excellent quantitative sensitivity with qualitative confirmation in Full MS with DIA mode, with most PFAS LOQs in pork meat matrix less than 50 pg/g (16.7 pg/mL in the final extract), without the need for further extract concentration.
- The myLibrary Enterprise application allows users to easily create and share 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.

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