United States Department of Agriculture

Food Safety and Inspection Service

CLG-PFAS 2.04

Screening, Determination, and Confirmation of PFAS by UHPLC-MS-MS

This method describes the laboratory procedure screening, confirmation, and determination of per- and polyfluorinated alkyl substances (PFAS) residues in bovine (cattle), porcine (swine), poultry, and *Siluriformes* muscle and bovine plasma at levels in Table 13 and Table 14.

Notice of Change

The method has changed:

- Abbreviation used for Perfluorotridecanoic Acid was changed from PFTrDA to PFTriA
- Instruction for labeling solvent blank vial.

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Safety Precautions

The personnel performing the analysis are to read the Safety Data Sheets for the standards and reagents used in this method. Follow all applicable federal, state, and local regulations regarding the disposal of chemicals listed in this method.

Introduction

Per- and polyfluorinated alkyl substances (PFAS) are a broad group of man-made chemicals or manufactured chemicals. PFAS have amphiphilic chemical properties, which allow for water and oil repellency[1] and therefore were, and still are, widely used in industrial and consumer applications. Some examples of applications of PFAS include the food packaging industry, household cleaning products, fire retardants, and wastewater treatment plants.[2]

PFAS are resistant to natural degradation and therefore are environmental persistent and bioaccumulate in nature. Studies have shown that exposure to PFAS at high levels can have detrimental health effects. As a result, many countries have begun to phase out the use of PFAS.

The National Residue Program (NRP) is an interagency program designed to identify, rank, and analyze chemical residues in meat, poultry, and egg products. FSIS publishes an Annual Sampling Plan to provide information on the process of sampling meat, poultry, and egg products for animal drugs of public health concern. The NRP is monitored and modified annually to set future priorities if data shows trends in detected residues.

Method Overview

The following method describes the laboratory procedure for screening, confirmation, and determination of selected per- and poly-fluorinated compounds in bovine (cattle), porcine (swine), poultry, and *Siluriformes* muscle and bovine plasma at levels in Table 13 and Table 14.

In brief, PFAS residues are extracted through a protein precipitation extraction through use of methanol and stored in a freezer to aid precipitation. The extracted residues are examined using Ultra-High Performance Liquid Chromatography Coupled with Tandem Mass Spectrometry (UHPLC-MS-MS).

Key Definitions

Protein-precipitation: An extraction technique resulting in solid material being left at the bottom of an extraction vessel with the extract or liquid layer containing the analyte. The liquid layer can be separated out for further analysis.

UHPLC-MS-MS: An analytical technique where there is a physical separation of target compounds followed by their mass-based detection.

MLA: Lowest level at which an FSIS method has been successfully validated for a residue in each matrix.

[1] *Zhanyun Wang, Jamie C DeWitt, Christopher P. Higgins, Ian T Cousins. A Never-Ending Story of Per- and Polyfluoroalkyl Substances (PFASs) Environ*. Sci. Technol. 2017 Feb; 51(5): 2508-2518

[2] *Hage DS, Carr JD, Analytical Chemistry and Quantitative Analysis*. Pearson, 2010

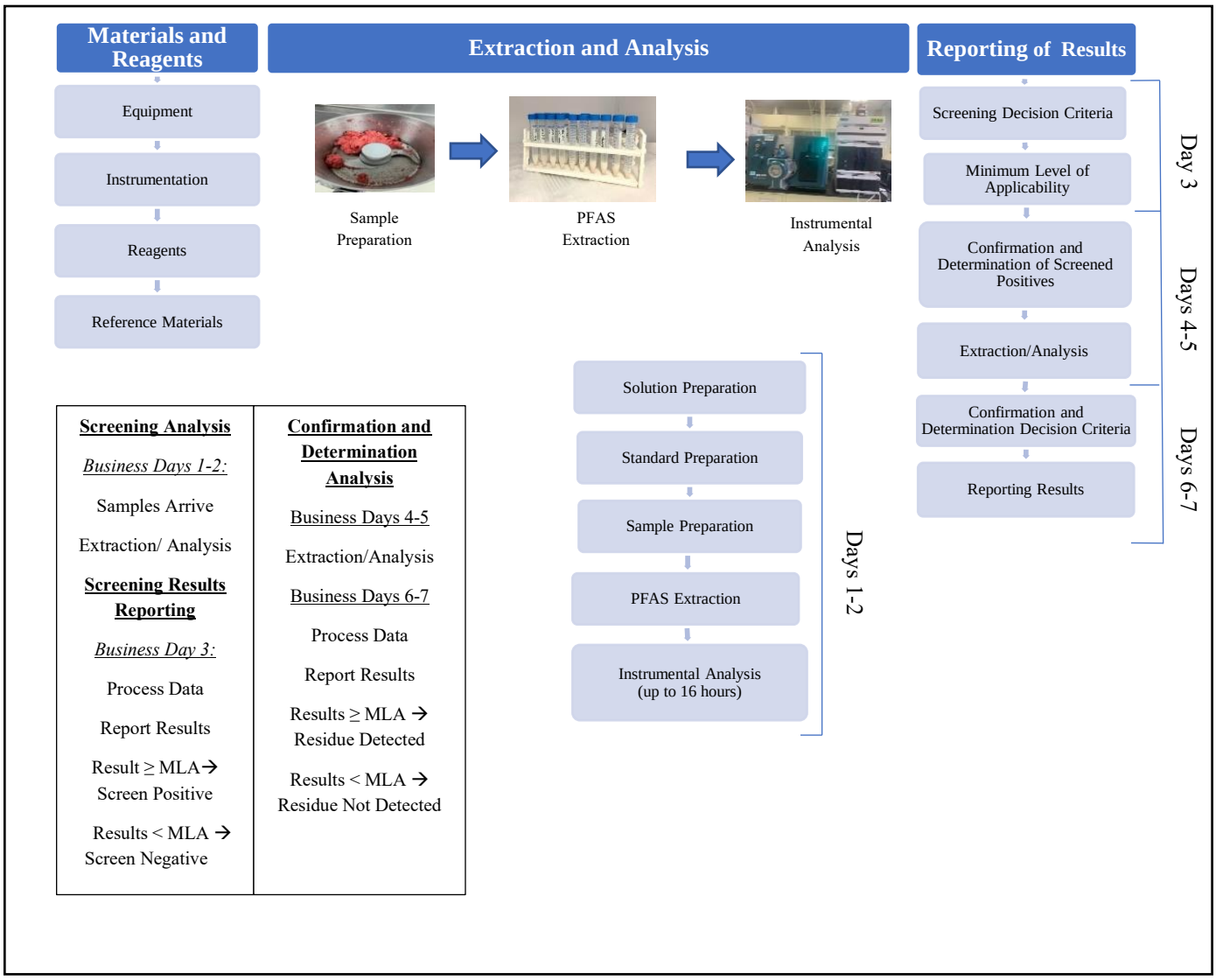

Figure 1: Overview and timeframe of PFAS analysis. Materials and reagents are obtained and utilized to prepare solutions and standards. The samples arrive at laboratory, are prepared into a homogenized mixture, weighed, extracted, and analyzed by UHPLC-MS/MS on business days 1-2. Screening results are reported on business day 3. Confirmation analysis is done on business days 4-5. Confirmation and determination results are reported on business days 6-7.

Decision Criteria

Screening and confirmation are based on the comparison of sample LC retention time and ion response against those obtained for a positive control (recovery). Quantitation (determination) is based on comparing ion response from samples against the standard curve and method Minimum Level of Applicability (MLA). Due to no stable confirmation ions, PFPeA is applicable for screening and determination only.

Disclosure Statement

FSIS does not specifically endorse any test products listed in this method. FSIS acknowledges that equivalent equipment, reagents, or solutions may be suitable for laboratory use. The FSIS laboratory system uses method performance requirements when evaluating the equivalence of an alternative equipment, reagent, or solution for a given analyte and sample matrix pair. A significant equivalence issue would require FSIS laboratory leadership approval.

Table 1: Name and Structure of PFAS Analytes Analyzed by CLG-PFAS2

Analyte	Name	Structure	CAS No.
PFPeA	PERFLUOROPENTANOIC ACID	$CF_3(CF_2)_3COOH$	2706-90-3
PFHxA	PERFLUOROHEXANOIC ACID	$CF_3(CF_2)_4COOH$	307-24-4
PFHpA	PERFLUOROHEPTANOIC ACID	$CF_3(CF_2)_5COOH$	375-85-9
PFOA	PERFLUOROOCTANOIC ACID	$CF_3(CF_2)_6COOH$	335-67-1
PFNA	PERFLUORONONANOIC ACID	$CF_3(CF_2)_7COOH$	375-95-1
PFDA	PERFLUORODECANOIC ACID	$CF_3(CF_2)_8COOH$	335-76-2
PFUnA	PERFLUOROUNDECANOIC ACID	$CF_3(CF_2)_9COOH$	2058-94-8
PFDoA	PERFLUORODODECANOIC ACID	$CF_3(CF_2)_{10}COOH$	307-55-1
PFTriA	PERFLUOROTRIDECANOIC ACID	$CF_3(CF_2)_{11}COOH$	72629-94-8
PFTeA	PERFLUOROTETRADECANOIC ACID	$CF_3(CF_2)_{12}COOH$	376-06-7
PFHxDA	PERFLUOROHEXADECANOIC ACID	$CF_3(CF_2)_{14}COOH$	67905-19-5
PFODA	PERFLUOROOCTODECANOIC ACID	$CF_3(CF_2)_{16}COOH$	16517-11-6
PFBS	PERFLUOROBUTANESULFONIC ACID	$CF_3(CF_2)_3SO_3H$	375-73-5
PFHxS	PERFLUOROHEXANESULFONIC ACID	$CF_3(CF_2)_5SO_3H$	355-46-4
PFOS	PERFLUOROOCTANESULFONIC ACID	$CF_3(CF_2)_7SO_3H$	1763-23-1
PFDS	PERFLUORODECANESULFONIC ACID	$CF_3(CF_2)_9SO_3H$	335-77-3

Materials and Reagents

Equipment

Table 2: Equipment for CLG-PFAS2

Equipment	Supplier and Part Number	Purpose
Analytical Balance	General lab supplier	Record weight of standard reagent. Minimum accuracy ±0.0001g.
Top Loading Balance	General lab supplier	Record weight of quality controls and samples. Minimum accuracy ±0.01g.
Centrifuge tubes, Polypropylene (PP), 15 mL and 50 mL	General lab supplier	Contain sample material and extraction vessel
Vortex Mixer	General lab supplier	Facilitates extraction of residue from the sample
Centrifuge	General lab supplier	Separates the solid sample material from the extraction solution. Capable of ~ 4600 RPM and -5 °C
Crimp-top polypropylene autosampler vials	Agilent 5182-0567	Storage of final sample extracts
Clear snap caps with polypropylene septa	Agilent 5182-0542	Capping of final sample extracts
Glassware, Class A	General lab supplier	Measuring standards and reagents
Food Processor	Robot Coupe USA Inc.	Homogenize sample
Refrigerator	General lab supplier	Storage of standards and reagents
Freezer - ≤ 20 °C	General lab supplier	Storage of samples Aid in protein precipitation

Instrumentation

Table 3: Instrumentation

Instrument	Supplier and Model Number	Purpose
1290 Infinity UHPLC with Sciex QTrap 6500+ mass spectrometer	Agilent/Sciex	Extract analysis
Phenomenex Luna C8(2), 3 µm, 2 x 50 mm	Phenomenex, 008-4248-B0	Extract analysis
ZORBAX RR Eclipse Plus C18, 95Å, 4.6 x 50 mm, 3.5 µm	Agilent, 959943-902	Extract analysis
UltraShield- Pre-column Filter	MAC-MOD Analytical MMUS1510	Extract analysis

Reagents

Table 4: Reagents

Reagent	Supplier and Part Number
Methanol, LC-MS Grade	Burdick & Jackson, 230-4
Water, LC-MS Grade	Burdick & Jackson, 365-4
Ammonium Acetate Optima, LC-MS	Fisher Chemical, A11450
Bovine Plasma in Potassium EDTA	VWR, 10802-208
Activated Charcoal Norit SX Ultra	Millipore Sigma, 53663

Reference Materials

Table 5: Reference Materials

Standard	Supplier	Catalog Number
Selected PFAS Compounds in Methanol, 2.0 µg/mL, approximately 98% pure, stored in sealed ampoule	Wellington Laboratories	PFAC-MXB
Mass-Labeled PFAS Compounds in Methanol, 2.0 µg/mL, stored in a sealed ampoule	Wellington Laboratories	MPFAC-MXA

Purity and counterions are to be taken into account when calculating standard concentrations. In-house prepared standards shall be assigned an expiration date that is no later than the stability stated in the method.

Extraction and Analysis

Solution Preparation

Table 6: Solutions

Mobile Phase A (2 mM ammonium acetate aqueous)	1) Weigh 0.1542 g ammonium acetate. 2) Quantitatively dissolve and transfer the ammonium acetate to a 1 L volumetric flask with water. 3) Dilute to volume with water. 4) Mix well and transfer to glass storage container for use.

Standard Preparation

Table 7: Standard Solutions

Standard 8 (500 ng/mL)	1) Pipet 1000 µL of the PFAS stock standard (2.0 µg/mL) (PFAC-MXB) into a polypropylene vial. 2) Add 3000 µL methanol into the polypropylene vial. 3) Mix well.
Standard 7 (250 ng/mL)	1) Pipet 1000 µL of the Standard 8 (500 ng/mL) solution into a polypropylene vial. 2) Add 1000 µL methanol into the polypropylene vial. 3) Mix well.
Standard 6 (50 ng/mL)	1) Pipet 200 µL of the Standard 8 (500 ng/mL) solution into a polypropylene vial. 2) Add 1800 µL methanol into the polypropylene vial. 3) Mix well.
Standard 5 (25 ng/mL)	1) Pipet 200 µL of the Standard 7 (250 ng/mL) solution into a polypropylene vial. 2) Add 1800 µL methanol into the polypropylene vial. 3) Mix well.
Standard 4 (5 ng/mL)	1) Pipet 200 µL of the Standard 6 (50 ng/mL) solution into a polypropylene vial. 2) Add 1800 µL methanol into the polypropylene vial. 3) Mix well.
Standard 3 (2.5 ng/mL)	1) Pipet 200 µL of the Standard 5 (25 ng/mL) solution into a polypropylene vial. 2) Add 1800 µL methanol into the polypropylene vial. 3) Mix well.

Standard 2 (1.0 ng/mL)	1) Pipet 400 µL of the Standard 4 (5 ng/mL) solution into a polypropylene vial. 2) Add 1600 µL methanol into the polypropylene vial. 3) Mix well.
Standard 1 (0.5 ng/mL)	1) Pipet 200 µL of the Standard 4 (5 ng/mL) solution into a polypropylene vial. 2) Add 1800 µL methanol into the polypropylene vial. 3) Mix well.

Table 8: Preparation of Internal Standard

Internal Standard (IS) Standard (150 ng/mL)	1) Pipet 300 µL of MPFAC-MXA solution (2.0 µg/mL) into a polypropylene vial. 2) Add 3700 µL methanol into the vial. 3) Mix well.

Sample Preparation

Muscle Preparation

Samples must be kept cold before and during shipping to the laboratory. Once received at the laboratory, samples must be frozen (≤ -10°C) prior to grinding if they cannot be prepared on the day of receipt. Once frozen, temper (partially thaw) while keeping it as cold as possible. Trim away fat and connective tissue. Grind tissue in blender or vertical cutter-mixer until homogeneous. Store samples frozen (≤ -10 °C) prior to analysis.

Figure 2: Prepared lean muscle sample with connective tissue removed. Photo courtesy of Hue Quach, USDA FSIS.

Figure 3: Homogenized sample. Photo courtesy of Hue Quach, USDA FSIS

Plasma

1) Allow plasma to thaw and mix to ensure homogeneity.
2) Prepare 50 mL polypropylene tube by adding 2 g activated charcoal.
3) Add plasma to 50 mL polypropylene tube and fill to the top.
4) Mix plasma and charcoal manually or by using a vortex.
5) Incubate plasma with charcoal overnight in a 4 °C refrigerator, vortexing occasionally to mix.

6) Centrifuge plasma at 4600 rpm (4708 RCF) for 30 minutes (min) at 5 °C, forming a charcoal pellet.
7) Decant supernatant plasma into clean 50 mL polypropylene tube (combining portions, if necessary) and centrifuge at 4600 rpm (4708 RCF) for 30 min at 5 °C, again. Repeat, if necessary.
8) Divide into aliquots, as necessary, and store frozen at ≤ -20 °C.

Technical Fact:

Some residual charcoal may be present but the plasma should be mostly clean after centrifugation.

PFAS Extraction

Samples

Weigh 0.50 g ± 0.01 g of thawed muscle tissue or pipet 500 µL of plasma into 15 mL polypropylene centrifuge tubes. Place all the tubes containing tissue into a ≤ -20 °C freezer and store until needed. As shown in Figure 4.

Figure 4: Weighed controls and samples. Photo courtesy of Qing Delgado, USDA-FSIS

QUALITY CONTROL

Either bovine or corresponding blank muscle tissues are to be used for used for quality control samples for screening purposes of muscle in all species. Use corresponding blank control bovine plasma for plasma analysis.

For screening

- Weigh five 0.50 ± 0.01 g portions of blank tissue or pipet 500 µL of plasma into 15 mL polypropylene centrifuge tubes.
    - Blank (negative control) - one
    - Recoveries (positive control) - one each for the 0.5 ng/g and 1.25 ng/g (muscle only) or 12.5 ng/g (plasma only)
    - Matrix match (reference standard) - one each for the 0.5 ng/g and 1.25 ng/g (muscle only) or 12.5 ng/g (plasma only)
- Weigh one additional portion for a check sample, if necessary.

For confirmation/determination

- Weigh ten 0.50 ± 0.01 g portions of blank tissue or pipet 500 µL of plasma into 15 mL polypropylene centrifuge tubes.
    - Blank (negative control) - one
    - Recoveries (positive control) - one each for the 0.5 ng/g and 1.25 ng/g (muscle only) or 12.5 ng/g (plasma only)
    - Standard curve - one each for each standard
- Weigh one additional portion for a check sample, if necessary.

KEY DEFINITIONS

Negative control (Blank): A quality control sample that is negative for all analytes of interest.

Matrix match control: Sample is spiked with analytes at the end after extraction. The analyte concentration is comparable to MLA. Negative and positive controls are compared to "Matrix match control."

Recovery (positive control): Sample is prepared with addition of analytes that have a concentration level comparable to MLA. Samples are compared to recovery.

Extraction

1) Prior to analysis, all samples are to thaw at room temperature for 30 min.
2) Spike each tube according to Table 9 (screening) or Table 10 (confirmation / determination)

Table 9: Spiking of Samples and QCs for Screening Analysis

Sample	IS to add (µL)	MeOH to add (µL)	Mixed Std. to add (µL)
Solvent Blank	0	500	0
Negative Control / Samples	50	250	0
Recovery (0.5 ng/g)	50	0	250 (of Standard 2; 1.0 ng/mL)
Recovery (1.25 ng/g) (for muscle only)	50	0	250 (of Standard 3; 2.5 ng/mL)
Recovery (12.5 ng/g) (for plasma only)	50	0	250 (of Standard 5; 25 ng/mL)
Matrix Match (0.5 ng/g)	50	0	250 (of Standard 2; 1.0 ng/mL)
Matrix Match (1.25 ng/g) (for muscle only)	50	0	250 (of Standard 3; 2.5 ng/mL)
Matrix Match (12.5 ng/g) (for plasma only)	50	0	250 (of Standard 5; 25 ng/mL)

Table 10: Spiking of Samples and QCs for Confirmation/Determination Analysis

Sample	IS to add (µL)	MeOH to add (µL)	Mixed Std. to add (µL)
Solvent Blank	0	500	0
Negative Control / Samples	50	250	0
Recovery (0.5 ng/g)	50	0	250 (of Standard 2; 1.0 ng/mL)
Recovery (1.25 ng/g) (for muscle only)	50	0	250 (of Standard 3; 2.5 ng/mL)
Recovery (12.5 ng/g) (for plasma only)	50	0	250 (of Standard 5; 25 ng/mL)
Standard 1 (0.25 ng/g)	50	0	250 (of Standard 1; 0.5 ng/mL)
Standard 2 (0.50 ng/g)	50	0	250 (of Standard 2; 1.0 ng/mL)
Standard 3 (1.25 ng/g)	50	0	250 (of Standard 3; 2.5 ng/mL)
Standard 4 (2.50 ng/g)	50	0	250 (of Standard 4; 5.0 ng/mL)
Standard 5 (12.5 ng/g)	50	0	250 (of Standard 5; 25 ng/mL)
Standard 6 (25.0 ng/g)	50	0	250 (of Standard 6; 50 ng/mL)
Standard 7 (125 ng/g)	50	0	250 (of Standard 7; 250 ng/mL)

3) Let the tubes equilibrate at room temperature for 30 min. As shown in Figure 5

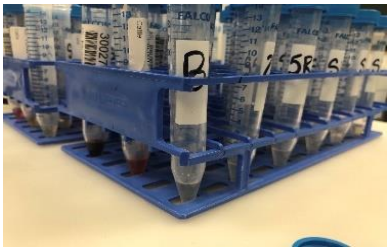

Figure 5: Plasma samples equilibrate at room temperature. Photo courtesy of Noelle Lebow, USDA-FSIS

Figure 6: Samples undergoing vortex for extraction. Photo courtesy of Qing Delgado, USDA-FSIS

4) Add 2.20 mL of methanol to all tubes. As shown in Figure 6, cap and vortex for 30 seconds (sec). Set aside extracts at room temperature for 30 min.

5) As shown in Figure 7, place samples in ≤ -20 °C freezer for 60 min to aid in protein precipitation.

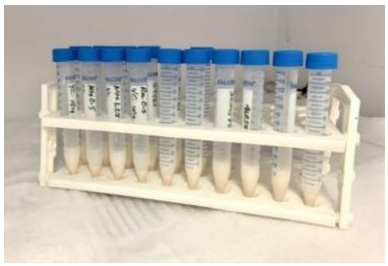

Figure 7: Samples in freezer for protein precipitation. Photo courtesy of Qing Delgado, USDA-FSIS

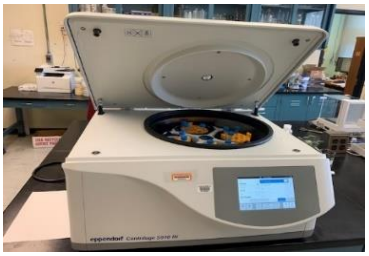

Figure 8: Samples in centrifuge. Photo courtesy of Qing Delgado, USDA-FSIS

6) As shown in Figure 8, remove samples from the freezer and centrifuge them at ~ - 5 °C and at least ~3500 rpm for 22 min. Plasma samples after centrifugation are shown in Figure 9.

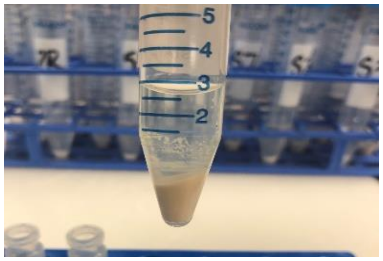

Figure 9: Plasma samples, after the centrifugation. Photo courtesy of Noelle Lebow, USDA-FSIS.

Figure 10: Samples in autosampler vials that are ready for analysis. Photo courtesy of Qing Delgado, USDA-FSIS.

Technical Note:

Due to the low temperature requirement, some centrifuges may require an extended period of time to reach a stable temperature.

7) Remove the samples from the centrifuge and pipet 500 µL of each supernatant to a labeled autosampler vial.

8) Add 500 µL of methanol to a labeled vial for a solvent blank. As shown in Figure 10, cap all vials and place in the sample rack. Freeze vials until analysis. (Extracts are stable overnight.)

9) Dispose of the samples and tubes in the hazardous waste container.

Instrumental Analysis

An example of a sample tray for an UHPLC-MS-MS system and an example of an UHPLC-MS-MS instrument are shown in Figure 11 and Figure 12, respectively.

Instrumental Note:

To minimize possible PFAS background levels; sonicate mobile phases, bypass instrument degasser, and install a PFAS delay column (ZORBAX RR Eclipse Plus C18, 95Å, 4.6 x 50 mm, 3.5 µm). Additionally, re-plumb the UHPLC system according to Agilent publication 5991-7863EN.

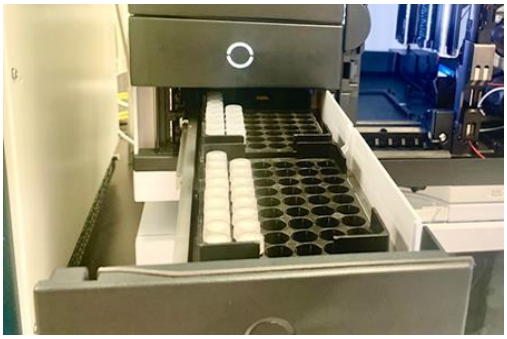

Figure 11: Prepared samples in UHPLC-MS-MS instrument. Photo courtesy of Qing Delgado, USDA-FSIS

Chromatographic Parameters

1) Mobile phases for PFAS analysis
    a) Mobile Phase A – 2 mM ammonium acetate aqueous
    b) Mobile Phase B – Methanol
2) Flow rate: 0.4 mL/min
3) Run time: 14.00 min
4) Gradient Program

Table 11: UHPLC Gradient Program

Time (min)	% Mobile Phase A	% Mobile Phase B
0.01	95	5
0.50	95	5
1.00	50	50
9.00	0	100
11.00	0	100
11.01	95	5
14.00	95	5

5) Autosampler program
    a) Run time: 14.0 min
    b) Injection Volume: 10 µL
    c) Weak wash solvent: 50:50 Methanol:Water
    d) Needle Wash: 20 sec
    e) Sample temperature: 10 °C

Instrumental Note:

Autosampler parameters can be modified or optimized if needed to ensure that all chromatographic peaks are present.

6) Column manager
    a) Column valve position: To match column location
    b) Column manager temperature: 40 °C

Mass Spectrometry Parameters

1) MS Method Parameters
    a) Scan Type: Scheduled MRM
    b) Polarity: Negative
    c) MRM Detection Window: 45 sec
    d) Target Scan Time: 0.7000 sec
    e) Resolution Q1: Unit
    f) Resolution Q3: Unit
    g) Duration: 14.00 min
    h) Cycles: 1200
    i) Collision Energy: See Table 12 below
2) Electrospray Source Parameters
    a) Curtain Gas: 30
    b) Collision Gas: Medium
    c) Temperature: 300 °C
    d) Source Gas 1: 50
    e) Source Gas 2: 50
    f) Ion Spray Voltage ESI: -4500 V
    g) MRM Transitions

Instrumental Note:

Mass spectrometer parameters are optimized and adjusted as needed during annual preventative maintenance and calibration.

Figure 12: 1290 Infinity UHPLC with Sciex QTrap 6500+ mass spectrometer. Photo courtesy of Qing Delgado, USDA FSIS.

Table 12: Summary of (MRM) transitions and parameters

Q1	Q3	RT	Name	DP	EP	CE	CXP	IS
263	**219**	**3.62**	**PFPeA-1**	**-8**	**-10**	**-12**	**-12**	MPFHxA
313	**269**	**4.25**	**PFHxA-1**	**-10**	**-10**	**-14**	**-7**	MPFHxA
313	119	4.25	PFHxA-2	-10	-10	-28	-7	MPFHxA
363	**319**	**4.9**	**PFHpA-1**	**-15**	**-10**	**-14**	**-15**	MPFOA
363	169	4.9	PFHpA-2	-15	-10	-24	-10	MPFOA
413	**369**	**5.51**	**PFOA-1**	**-20**	**-10**	**-15**	**-20**	MPFOA
413	169	5.51	PFOA-2	-15	-10	-24	-10	MPFOA

Q1	Q3	RT	Name	DP	EP	CE	CXP	IS
463	**419**	**6.08**	**PFNA-1**	**-20**	**-10**	**-15**	**-25**	MPFNA
463	169	6.08	PFNA-2	-20	-10	-26	-9	MPFNA
513	**469**	**6.54**	**PFDA-1**	**-20**	**-10**	**-16**	**-10**	MPFDA
513	269	6.54	PFDA-2	-20	-10	-25	-15	MPFDA
563	**519**	**6.96**	**PFUnA-1**	**-25**	**-10**	**-16**	**-30**	MPFUnA
563	269	6.96	PFUnA-2	-25	-10	-25	-15	MPFUnA
613	**569**	**7.29**	**PFDoA-1**	**-25**	**-10**	**-17**	**-30**	MPFDoA
613	319	7.29	PFDoA-2	-25	-10	-28	-19	MPFDoA
663	**619**	**7.56**	**PFTriA-1**	**-30**	**-10**	**-19**	**-13**	N/A
663	319	7.56	PFTriA-2	-30	-10	-29	-16	N/A
713	**669**	**7.89**	**PFTeA-1**	**-30**	**-10**	**-19**	**-15**	N/A
713	319	7.89	PFTeA-2	-30	-10	-29	-20	N/A
813	**769**	**8.51**	**PFHxDA-1**	**-30**	**-10**	**-21**	**-19**	N/A
813	319	8.51	PFHxDA-2	-35	-10	-35	-20	N/A
913	**869**	**8.99**	**PFODA-1**	**-35**	**-10**	**-23**	**-19**	N/A
913	269	8.99	PFODA-2	-35	-10	-41	-17	N/A
299	**80**	**3.72**	**PFBS-1**	**-60**	**-10**	**-64**	**-10**	MPFHxS
299	99	3.72	PFBS-2	-60	-10	-36	-10	MPFHxS
399	**80**	**4.94**	**PFHxS-1**	**-75**	**-10**	**-84**	**-10**	MPFHxS
399	99	4.94	PFHxS-2	-75	-10	-69	-10	MPFHxS
499	**80**	**6.07**	**PFOS-1**	**-100**	**-10**	**-105**	**-10**	MPFOS
499	99	6.07	PFOS-2	-100	-10	-93	-12	MPFOS
599	**80**	**6.93**	**PFDS-1**	**-130**	**-10**	**-123**	**-10**	MPFOS
599	99	6.93	PFDS-2	-130	-10	-110	-10	MPFOS
315	270	4.25	MPFHxA	-15	-10	-13	-15	N/A
417	372	5.51	MPFOA	-20	-10	-14	-21	N/A
468	423	6.06	MPFNA	-20	-10	-16	-25	N/A
515	470	6.54	MPFDA	-25	-10	-16	-29	N/A
565	520	6.96	MPFUnA	-25	-10	-18	-28	N/A
615	570	7.3	MPFDoA	-30	-10	-19	-33	N/A
403	84	4.94	MPFHxS	-85	-10	-85	-10	N/A
503	80	6.07	MPFOS	-85	-10	-105	-10	N/A

Quan ion (most abundant product ion, used for quantitation) is in **Bold**.

Instrumental Note:

Retention time windows and collision energies were set and utilized at time of method validation.

- Retention time windows may be adjusted to account for aging of UHPLC columns or for improved separation to ensure that all chromatographic peaks are present.
- Collision energies may be adjusted and optimized for improved mass spectrometry detection.
- Target masses for precursor and product ions can be optimized to a *m/z* value that falls within the unit mass resolution of the exact mass, but not to exceed the next integer value (e.g., if the exact mass is 787.5, an allowable target mass range includes 787.0-787.9).

Sample Set

The injection sequence below can be modified, as needed, but must include required controls.

For Screening

1) Solvent Blank
2) Negative Control
3) For muscle sets:
   - Matrix match 0.50 ng/g
   - Matrix match 1.25 ng/g

   For plasma sets:
   - Matrix match 0.50 ng/g
   - Matrix match 12.5 ng/g
4) Positive control (Recoveries)
5) Intra-laboratory check sample (if applicable)
6) Samples, up to a maximum of 32
7) Reinjection of standard or positive control

INTRA-LABORATORY CHECK SAMPLE

Defined on the CLG website here.

For Confirmation/Determination

1) Solvent Blank
2) Negative Control
3) Positive control (Recoveries)
4) Standard Curve
5) Intra-laboratory check sample (if applicable)
6) Samples, up to a maximum of 32
7) Reinjection of standard or positive control

Instrumental Note:

Internal standards are used to monitor injection sequence performance within a set. For issues observed, such as inconsistent internal standard area counts, samples may be reinjected or reanalyzed as needed.

Reporting of Results

Decision Criteria

Screening

Screening Criteria for the following analytes: PFBS, PFDA, PFDoA, PFDS, PFHpA, PFHxA, PFHxS, PFNA, PFOA, PFOS, PFPeA, PFUnA (with Internal Standard)

1) The screening ion for a given analyte must be present. The required ion for each compound is listed in Table 12.
2) Retention time of the sample must be ± 5% of the recovery/positive control.
3) The screening ion must have a signal-to-noise ratio ≥ 3. This may be verified by visual inspection. Visual inspection for detection may also include assessment of peak shape or drift in relation to standard peaks or evaluating the presence or absence of monitored fragment ions.
4) For a sample to be screened positive for an analyte, the following criteria must be met:
    a. The recovery/positive control of the analyte must exceed 10% of the applicable calibration standard level (See Table 13).
    b. The sample response ratio equals or exceeds the applicable recovery/positive control level (See Table 13).
5) The blank (negative control) response ratio (product ion/IS ion) for the screening ion must be less than 10% of the response ratio for the applicable calibration standard (See Table 13).

If a sample shows a positive response for a compound that did not meet screening criteria in the associated QC samples, then further testing of that sample is warranted.

Screening Criteria for the following analytes: PFHxDA, PFODA, PFTeA, PFTriA (no Internal Standard used)

1) The screening ion for a given analyte must be present. The required ion for each compound is listed in Table 12.
2) Retention time of the sample must be ± 5% of the recovery/positive control.
3) The screening ion must have a signal-to-noise ratio ≥ 3. This may be verified by visual inspection. Visual inspection for detection may also include assessment of peak shape or drift in relation to standard peaks or evaluating the presence or absence of monitored fragment ions.
4) For a sample to screen positive for an analyte the following criteria must be met:
    a. The recovery/positive control of the analyte must exceed 10% of the applicable calibration standard level (See Table 13).
    b. The area equals or exceeds the applicable recovery/positive control level (See Table 13).
5) The blank (negative control) area response for the screening ion must be less than 10% of the applicable standard calibration standard (See Table 13).

If a sample shows a positive response for a compound that did not meet screening criteria in the associated QC samples, then further testing of that sample is warranted.

QUALITY ASSURANCE PLAN

Quality Control Procedures

Screening Criteria

a. For set acceptance, ≥ 90% of the monitored analytes in the recovery/ positive control must meet screening criteria. For sample reporting purposes, the analytes of interest in the recovery/ positive control must meet screening criteria.

b. For set acceptance, ≥ 90% of the monitored analytes in the blank (negative control) must not meet screening criteria. The blank (negative control) must be negative using the screening criteria for samples containing corresponding presumptive positive analytes.

Intra-Laboratory Check Samples (If applicable)

Acceptability criteria.

a. ≥ 90% of the monitored analytes in a fortified Intra-Laboratory Check must meet screening criteria.

b. ≥ 90% of the monitored analytes in an unfortified Intra-Laboratory Check must be negative using the screening criteria.

c. FSIS Field Service Laboratories are to refer to internal FSIS Quality Control Procedures, when unacceptable values are obtained:

i. Refer to LW-Q1002, Chemistry Non-Conformance Tables, for how to proceed and whether to take corrections or corrective actions.

Confirmation

Confirmation Criteria for the following analytes: PFBS, PFDA, PFDoA, PFDS, PFHpA, PFHxA, PFHxS, PFNA, PFOA, PFOS, PFPeA, PFUnA (with Internal Standard)

1) Monitored ions for each analyte will be assessed as follows:

a. Recovery/positive control retention times must match the retention time of the applicable standard (See Table 13) within 5%. Retention time for the samples must match the retention time of the recovery/positive control within 5%.

b. All product ions specified for ratio matching are present with a signal-to-noise ratio ≥ 3. This may be verified by visual inspection. Visual inspection for detection may also include assessment of peak shape or drift in relation to standard peaks or evaluating the presence or absence of monitored fragment ions.

c. The ion abundance ratio should match the calculated average ratio of the calibration curve standards within a ± 10% absolute difference. For example, if the average product ion abundance ratio of the standards is 0.74 the acceptance range for the samples is 0.64 – 0.84.

i. Ratios are calculated by dividing the area count of each confirming ion by the area count of the quan ion. Ion abundance ratios should be less than 1. If the ratio is not less

than 1 for a sample set, the inverse of this ratio may be used.

d. A sample is confirmed positive for an analyte if the following criteria are met:

i. The recovery/positive control of the analyte must exceed 10% of the applicable standard level (See Table 13).

ii. The sample response equals or exceeds the applicable recovery/positive control level (See Table 13).

e. The blank (negative control) must be less than 10% of the applicable standard for the analyte (See Table 13).

PFPeA has insufficient fragment ions, so this analyte cannot be confirmed following this procedure.

Confirmation Criteria for the following analytes: PFHxDA, PFODA, PFTeA, PFTriA (no Internal Standard used)

1) Monitored ions for each analyte will be assessed as follows:

a. Recovery/positive control retention times must match the retention time of the applicable calibration standard (See Table 13) within 5%. Retention time for the samples must match the retention time of the applicable recovery/positive control (See Table 13) within 5%.

b. All product ions specified for ratio matching are present with a signal-to-noise ratio ≥ 3. This may be verified by visual inspection. Visual inspection for detection may also include assessment of peak shape or drift in relation to standard peaks or evaluating the presence or absence of monitored fragment ions.

c. The ion abundance ratio should match the calculated average ratio of the calibration curve standards within a ± 10% absolute difference. For example, if the average product ion abundance ratio of the standards is 0.74 the acceptance range for the samples is 0.64 – 0.84.

i. Ratios are calculated by dividing the area count of each confirming ion by the area count of the quan ion. Ion abundance ratios should be less than 1. If the ratio is not less than 1 for a sample set, the inverse of this ratio may be used.

d. A sample is confirmed positive for an analyte if the following criteria are met:

i. The recovery/positive control of the analyte must exceed 10% of the applicable calibration standard level (See Table 13).

ii. The sample response equals or exceeds applicable recovery/positive control level (See Table 13).

e. The blank (negative control) must be less than 10% of the applicable calibration standard level (See Table 13).

QUALITY ASSURANCE PLAN

Quality Control Procedures

Confirmation Criteria

a. For set acceptance, ≥ 90% of the monitored analytes in the recovery/positive control must meet confirmation criteria. For sample reporting purposes, the analytes of interest in the recovery/ positive control must meet confirmation criteria.

b. The blank (negative control) must be negative using the confirmation criteria for the analytes of interest.

Intra-Laboratory Check Samples (If applicable)

Acceptability criteria.

a. ≥ 90% of the monitored analytes in a fortified Intra-Laboratory Check must meet confirmation criteria.

b. ≥ 90% of the monitored analytes in an unfortified Intra-Laboratory Check must be negative using the confirmation criteria.

c. FSIS Field Service Laboratories are to refer to internal FSIS Quality Control Procedures, when unacceptable values are obtained:

i. Refer to LW-Q1002, Chemistry Non-Conformance Tables, for how to proceed and whether to take corrections or corrective actions.

Determination

Determination Criteria for the following analytes: PFBS, PFDA, PFDoA, PFDS, PFHpA, PFHxA, PFHxS, PFNA, PFOA, PFOS, PFPeA, PFUnA (with Internal Standard)

1) Ensure that all quan ions used for constructing the standard curve are present at a signal to noise ratio > 10 by visual inspection.

2) Using linear regression analysis with 1/x weighting, calculate the slope, intercept, and correlation coefficient of a standard curve for each compound. This is constructed by plotting peak area ratios using the quan ion (most abundant ion of the Analyte/Internal Standard) versus concentration (ng/mL) for the calibration curve standards.

3) Each calibration curve is created using the response ratio (quan ion of Analyte/Internal Standard) as outlined in Table 7, plotted against the concentration of the respective analyte. The concentration of each analyte present in each sample is calculated using the applicable curve.

4) Calculate results when the following conditions are met:

   a. The correlation coefficient for the standard curve is greater than or equal to 0.995.

   b. The recovery/positive control falls within the limits specified in Quality Assurance Plan.

   c. Response ratio (quan ion for analyte/IS ion) is to fall within the range of the curve. Dilute and re-analyze if above the range of the curve.

Determination Criteria for the following analytes: PFHxDA, PFODA, PFTeA, PFTriA (no Internal Standard used)

a. Ensure that all quan ions used for constructing the standard curve are present at a signal to noise ratio > 10 by visual inspection.

b. Using quadratic regression analysis on the instrument software, with 1/x weighting, calculate the coefficients of the quadratic equation and correlation coefficient of a standard curve for each compound. This is constructed by plotting peak area versus concentration (ng/mL) for the calibration curve standards.

c. Each calibration curve is created using the peak area as outlined in Table 10 plotted against the concentration of the respective analyte. The concentration of each analyte present in each sample is calculated using the applicable curve.

d. Calculate results when the following conditions are met:

   i. The correlation coefficient for the standard curve is greater than or equal to 0.995.

   ii. The recovery/positive control falls within the limits specified in the Quality Control Procedure.

   iii. Peak areas are to fall within the range of the curve. Dilute and re-analyze if above of the range of the curve.

QUALITY ASSURANCE PLAN

Quality Control Procedures

Determination Criteria

a. For set acceptance, the analyte(s) of interest in the recovery/positive control must meet the acceptable recovery range for those analytes in Table 14.

b. The blank (negative control) must be negative using the determination criteria for the analyte(s) of interest.

Intra-Laboratory Check Samples (If applicable)

Acceptability criteria

a. ≥ 90% of the monitored analytes in a fortified Intra-Laboratory Check must meet determination criteria.

b. ≥ 90% of the monitored analytes in an unfortified Intra-Laboratory Check must be negative using the determination criteria.

c. FSIS Field Service Laboratories are to refer to internal FSIS Quality Control Procedures, when unacceptable values are obtained:

   i. Refer to LW-Q1002, Chemistry Non-Conformance Tables, for how to proceed and whether to take corrections or corrective actions.

Minimum Level of Applicability

Table 13: Minimum Level of Applicability (MLA) for Screening and Confirmation level per species

Analyte	Bovine Muscle Screening (ng/g)	Bovine Muscle Confirmation (ng/g)	Bovine Plasma Screening (ng/g)	Bovine Plasma Confirmation (ng/g)	Poultry Muscle Screening (ng/g)	Poultry Muscle Confirmation (ng/g)	Porcine Muscle Screening (ng/g)	Porcine Muscle Confirmation (ng/g)	Siluriformes Muscle Screening (ng/g)	Siluriformes Muscle Confirmation (ng/g)
PFPeA	0.50	No levels	0.50	No Levels	0.50	No Levels	0.50	No Levels	0.50	No Levels
PFHxA	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
PFHpA	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
PFOA	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
PFNA	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
PFDA	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
PFUnA	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.25	1.25
PFDoA	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
PFTriA	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.25	1.25
PFTeA	0.50	0.50	0.50	0.50	0.50	0.50	1.25	1.25	0.50	0.50
PFHxDA	1.25	1.25	12.5	12.5	1.25	1.25	N/A	N/A	1.25	1.25
PFODA	0.50	0.50	0.50	0.50	0.50	0.50	1.25	1.25	0.50	0.50
PFBS	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
PFHxS	0.50	0.50	12.5	12.5	1.25	1.25	1.25	1.25	1.25	1.25
PFOS	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
PFDS	0.50	0.50	0.50	0.50	0.50	0.50	1.25	1.25	0.50	0.50

Table 14: Determination (Quantitation) Ranges and Acceptable Recoveries

Analyte	Analytical Range Bovine Muscle (ng/g)	Bovine Muscle Acceptable Recovery	Analytical Range Bovine Plasma (ng/g)	Bovine Plasma Acceptable Recovery	Analytical Range Poultry Muscle (ng/g)	Poultry Muscle Acceptable Recovery	Analytical Range Porcine Muscle (ng/g)	Porcine Muscle Acceptable Recovery	Analytical Range Siluriformes Muscle (ng/g)	Siluriformes Muscle Acceptable Recovery
PFPeA	0.50 -125	87 – 116%	0.50 - 125	84 – 116%	0.50 – 125	76 – 124%	0.50 – 125	80 – 128%	0.50 – 125	77 – 125%
PFHxA	0.50 -125	86 – 117%	0.50 - 125	85 – 115%	0.50 – 125	64 – 133%	0.50 – 125	79 – 128%	0.50 – 125	75 – 130%
PFHpA	0.50 – 125	83 – 116%	0.50 - 125	83 – 119%	0.50 – 125	74 – 126%	0.50 – 125	80 – 127%	0.50 – 125	75 – 129%
PFOA	0.50 - 125	88 – 114%	0.50 - 125	84 – 118%	0.50 – 125	79 – 123%	0.50 – 125	81 – 126%	0.50 – 125	75 – 129%
PFNA	0.50 - 125	88 – 114%	0.50 - 125	82 – 119%	0.50 – 125	78 – 124%	0.50 – 125	76 – 131%	0.50 – 125	75 – 129%
PFDA	0.50 - 125	82 – 118%	0.50 - 125	83 – 120%	0.50 – 125	78 – 122%	0.50 – 125	75 – 134%	0.50 – 125	74 – 128%
PFUnA	0.50 - 125	83 – 119%	0.50 - 125	81 – 121%	0.50 – 125	74 – 124%	0.50 – 125	75 – 132%	1.25 – 125	75 – 129%
PFDoA	0.50 - 125	79 – 125%	0.50 - 125	81 – 122%	0.50 – 125	76 – 124%	0.50 – 125	78 – 132%	0.50 – 125	76 – 126%
PFTriA	0.50 - 125	55 – 126%	0.50 - 125	73 – 130%	0.50 – 125	78 – 120%	0.50 – 125	67 – 133%	1.25 – 125	69 – 138%
PFTeA	0.50 - 125	65 – 134%	0.50 - 125	68 – 132%	0.50 – 125	68 – 133%	1.25 – 125	66 – 128%	0.50 – 125	70 – 144%
PFHxDA	1.25 - 125	62 – 173%	12.5 - 125	53 – 138%	1.25 – 125	74 – 119%	N/A	N/A	1.25 – 125	70 – 141%
PFODA	0.50 - 125	76 – 120%	0.50 - 125	63 – 131%	0.50 – 125	80 – 117%	1.25 – 125	61 – 120%	0.50 – 125	74 – 142%
PFBS	0.50 - 125	91 – 117%	0.50 - 125	83 – 118%	0.50 – 125	79 – 122%	0.50 – 125	79 – 126%	0.50 – 125	76 – 128%
PFHxS	0.50 - 125	87 – 116%	12.5 - 125	88 – 112%	1.25 – 125	80 – 122%	1.25 – 125	79 – 125%	1.25 – 125	73 – 130%
PFOS	0.50 - 125	86 – 120%	0.50 - 125	85 – 115%	0.50 – 125	82 – 118%	0.50 – 125	78 – 129%	0.50 – 125	77 – 122%
PFDS	0.50 - 125	75 – 116%	0.50 - 125	72 – 131%	0.50 – 125	81 – 121%	1.25 – 125	75 – 127%	0.50 – 125	67 – 128%

References

1) Geiger, M.; Caterino, M.; Stagliano, M. Analysis of Tissue for Perfluorinated Compounds (PFCs) by Reversed Phase High Performance Liquid Chromatography Multiple Reaction Monitoring Tandem Mass Spectrometry. *Bureau of Laboratories, MDHHS, AC.51*

2) Recommended Plumbing Configurations for Reduction in Per/Polyfluoroalkyl Substance Background with Agilent 1260/1290 Infinity (II) LC Systems Application Note, Agilent Technologies Inc., Publication Number 5991-7863EN, 2017

3) Zabaleta, I.; Bizkarguenaga, E.; Prieto, A.; Ortiz-Zarragoitia, M; Fernandez, L.A.; Zuloaga, O. Simultaneous determination of perfluorinated compounds and their potential precursors in mussel tissue and fish muscle tissue and liver samples by liquid chromatography-elect.

Contact Information and Inquiries

Inquiries about methods can be submitted through the USDA website via the "Ask USDA" portal at https://ask.usda.gov or please contact:

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This method has been validated, reviewed, approved, and deemed suitable and fit for purpose for use in the USDA FSIS Field Service Laboratories.

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