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Title: Screening, Determination and Confirmation of PFAS by UPLC-MS-MS		
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A. INTRODUCTION

1. Background / Summary of Procedure

Per- and polyfluorinated alkyl substances (PFAS) are extracted from muscle or plasma with methanol, kept in the freezer to aid in protein precipitation, and centrifuged to reduce particulates. 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. Analysis is performed by UPLC/MS/MS on a triple quadruple mass spectrometer in negative ESI mode.

2. Applicability

This method is suitable for the 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 6 and Table 7. in Appendix J.4. Note: PFPeA is applicable for screening and determination only, and at levels between 0.50 to 125 ppb (ng PFAS/g matrix).

Note: This method may be performed using standards/solutions that contain fewer analytes than the method is applicable for, if the excluded analytes will not be included in the reported results.

B. EQUIPMENT

Note: Equivalent equipment may be substituted.

1. Apparatus

a. Analytical Balance – Readable to 0.01 mg, Model MS303TS, Mettler Toledo.

b. Centrifuge Tubes – 15 mL (polypropylene) Falcon tubes, Cat. No. 352096.

c. Centrifuge Tubes - 50 mL (polypropylene) Falcon tubes, Cat. No. 352070.

d. Variable volume pipettors capable of accurately delivering 2 – 5,000 µL.

e. Vortex Mixer – Vortexer-2, Model G-560, VWR.

f. Centrifuge – Hettich Rotanta 460 R, capable of ~3500 RPM and -5 °C.

g. Crimp-top polypropylene autosampler vials – Agilent 5182-0567.

h. Clear snap caps with polypropylene septa – Agilent 5182-0542.

Note: Avoid using glass autosampler vials or vial caps with PTFE septa. Vials and caps made of polypropylene (PP) or high density polyethylene (HDPE) are recommended.

2. Instrumentation

   a. LC/MS/MS – Agilent 1290 Infinity II UPLC with AB Sciex Qtrap 6500+ mass spectrometer. UPLC should be re-plumbed as suggested in Agilent Publication Number 5991-7863EN.

   b. HPLC Column – Phenomenex Luna C8(2), 3 µm, 2 x 50 mm.

   c. Delay Column – Zorbax Eclipse Plus C18, 3.5 µm, 4.6 x 50 mm.

   d. Pre-column filters – UltraShield MMUS-1510.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents / solutions may be substituted.

1. Reagents

   a. Methanol LC-MS Grade, Cat. No 230-4, Burdick & Jackson.

   b. Water LC-MS Grade, Cat. No 365-4, Burdick & Jackson.

   c. Ammonium Acetate Optima LC-MS, Cat. No A11450, Fisher Chemical.

   d. Bovine Plasma in Potassium EDTA, Cat. No 10802-208, VWR.

   e. Activated Charcoal Norit SX Ultra, Cat. No 53663, Millipore Sigma.

2. Solutions

   a. Mobile Phase A (2 mM ammonium acetate aqueous):

      Weigh 0.1542 g ammonium acetate and place into a 1 L volumetric flask. Bring to 1 L volume with LC-MS grade water.

      Note: Since it is recommended to bypass the degasser of the LC system, it is also recommended to sonicate Mobile Phase A for 5 minutes after preparation to minimize the risk of outgassing in the solvent lines or the LC pump.

   b. Mobile Phase B:

      100% LC-MS grade methanol.

D. STANDARD(S)

Note: Equivalent standards / solutions may be substituted. 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.

1. Standard Information

   a. Stock mixed Standards

      Reference standard solution containing selected PFAS compounds in methanol with a concentration of 2.0 µg/mL, approximately 98% pure, stored in sealed ampoule. Cat. No. PFAC-MXB, Wellington Laboratories (Guelph, Ontario, Canada).

   b. Internal Standards

      Reference standard solution containing mass-labeled PFAS compounds in methanol with a concentration of 2.0 µg/mL, stored in a sealed ampoule. Cat. No. MPFAC-MXA, Wellington Laboratories (Guelph, Ontario, Canada).

2. Preparation of Standard Solution(s)

   a. Standard Solutions

      i. Standard 8 (500 ng/mL)

         Pipet 1000 µL of the PFAS stock standard (2.0 µg/mL) (PFAC-MXB) F.1.a. into a polypropylene vial. Add 3000 µL methanol into the polypropylene vial.

      ii. Standard 7 (250 ng/mL)

         Pipet 1000 µL of the Standard 8 (500 ng/mL) solution into a polypropylene vial. Add 1000 µL methanol into the polypropylene vial.

      iii. Standard 6 (50 ng/mL)

         Pipet 200 µL of the Standard 8 (500 ng/mL) solution into a polypropylene vial. Add 1800 µL methanol into the polypropylene vial.

      iv. Standard 5 (25 ng/mL)

         Pipet 200 µL of the Standard 7 (250 ng/mL) solution into a polypropylene vial. Add 1800 µL methanol into the polypropylene vial.

      v. Standard 4 (5 ng/mL)

         Pipet 200 µL of the Standard 6 (50 ng/mL) solution into a polypropylene vial. Add 1800 µL methanol into the polypropylene vial.

vi. Standard 3 (2.5 ng/mL)

Pipet 200 µL of the Standard 5 (25 ng/mL) solution into a polypropylene vial. Add 1800 µL methanol into the polypropylene vial.

vii. Standard 2 (1.0 ng/mL)

Pipet 400 µL of the Standard 4 (5 ng/mL) solution into a polypropylene vial. Add 1600 µL methanol into the polypropylene vial.

viii. Standard 1 (0.5 ng/mL)

Pipet 200 µL of the Standard 4 (5 ng/mL) solution into a polypropylene vial. Add 1800 µL methanol into the polypropylene vial.

b. Internal Standard (IS) Standard (150 ng/mL)

Pipet 300 µL of the Wellington MPFAC-MXA solution (2.0 µg/mL) into a polypropylene vial and add 3700 µL methanol.

E. SAMPLE RECEIPT AND PREPARATION

1. Muscle

Allow sample to thaw and trim sample of extraneous connective tissue from muscle. If only a sub-sample is to be prepared, assure the sub-sample is representative of the entire sample. Muscle samples may be homogenized by either blending or dry ice grinding.

a. Using a food processor, thoroughly blend muscle tissue to homogenize. Immediately transfer mixture to a suitable container and store in a freezer at ≤ -20 °C. Avoid any unnecessary freeze/thaw cycles.

b. Alternatively, muscle sample preparation may be done by dry ice grinding as follows:

i. Chop 0.5 - 1 lb of muscle tissue into small pieces and homogenize with an equal amount of dry ice in a large food processor. The resulting sample homogenate will be a frozen powder.

ii. Transfer a portion of the homogenized sample into a loosely capped sample cup until the dry ice has sublimed. Excess sample from step E.1.a may be discarded.

iii. For any retained sample, tighten the caps and store sample cups at ≤ -20 °C. Avoid any unnecessary freeze/thaw cycles.

2. Preparation of plasma blank tissue

   a. Allow plasma to thaw and mix to ensure homogeneity.

   b. Prepare 50 mL polypropylene tube by adding 2 g activated charcoal.

   c. Add plasma to 50 mL polypropylene tube and fill to the top.

   d. Mix plasma and charcoal manually or by using a vortexer.

   e. Incubate plasma with charcoal overnight in a 4°C refrigerator, vortexing occasionally to mix.

   f. Centrifuge plasma at 4600 rpm (4708 RCF) for 30 minutes at 5°C, forming a charcoal pellet.

   g. Decant supernatant plasma into clean 50 mL polypropylene tube (combining portions, if necessary) and centrifuge at 4600 rpm (4708 RCF) for 30 minutes at 5°C, again. Repeat, if necessary.

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

   h. Divide into aliquots, as necessary, and store frozen at ≤ -20°C.

F. ANALYTICAL PROCEDURE

1. Preparation of Controls and Samples

   a. 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.

   b. Remove the tubes containing tissue from the freezer and allow to thaw at room temperature for 30 minutes.

   c. To prepare samples, spike each sample tube with 250 µL of Methanol and 50 µL of IS. Cap the tubes and set them aside. (Refer to Table 1).

Table 1: Spiking of Samples and QC Samples

Sample	IS to add (µL)	MeOH to add (µL)	Mixed Std. to add (µL)
Solvent Blank	0	500	0
Tissue Blank	50	250	0
Recovery (0.5 ng/g)	50	0	250 (of Standard 2)
Recovery (1.25 ng/g) (for muscle only)	50	0	250 (of Standard 3)
Recovery (12.5 ng/g) (for plasma only)	50	0	250 (of Standard 5)
Samples	50	250	0

d. To prepare controls, spike the extracted curve and QC tissue tubes with 250 µL of the appropriate standard solution (Standard 1 - Standard 7, refer to Table 2) and 50 µL of IS. Cap the tubes and set aside.

Table 2: Calibration Levels and Equivalent Concentrations in Tissue (Applicable for determination only)

Calibration Curve Components	Vol. to add of each Standard Solution in D.2.a. (µL)	Conc. of Std (ng/mL)	Conc. in Final Extract (ng/mL)	Equivalent Conc. In Sample (ng/g)
S1	250 of Standard 1	0.5	0.05	0.25
S2	250 of Standard 2	1.0	0.10	0.50
S3	250 of Standard 3	2.5	0.25	1.25
S4	250 of Standard 4	5	0.5	2.5
S5	250 of Standard 5	25	2.5	12.5
S6	250 of Standard 6	50	5	25
S7	250 of Standard 7	250	25	125

e. Let the tubes equilibrate at room temperature for 30 minutes.

2. Extraction Procedure

a. Add 2.20 mL of methanol to all tubes. Cap and vortex for 30 seconds. Set aside extracts at room temperature for 30 minutes.

b. Place samples in ≤ -20 °C freezer for 60 minutes to aid in protein precipitation.

c. Remove samples from the freezer and centrifuge them at ~- 5 °C and at least ~3500 rpm for 22 minutes. (The centrifuge should be set and coming to temperature when the samples are placed in the freezer.)

d. Label auto sampler vials, one for each sample and QC. Label one additional vial as “MeOH” for a solvent blank. Remove the samples from the centrifuge and pipet 500 µL of each supernatant to the corresponding autosampler vial. Add 500 µL of methanol to a vial labeled “MeOH”. Cap all vials and place in the sample rack. Freeze vials until analysis - extracts are stable overnight.

e. Dispose of the samples and tubes in the hazardous waste container.

3. Instrumental Settings

Note: The instrument parameters may be optimized to ensure system suitability.

Note: Frequent instrument cleaning is recommended.

Note: Tune the instrument as needed.

a. To minimize possible PFAS background levels, sonicate mobile phases and

bypass instrument degasser and install and use PFAS delay column (B.2.c). Additionally, re-plumb the LC system according to Agilent publication 5991-7863EN.

Column temperature:	40 °C
Autosampler temperature:	10 °C
Injection volume:	10 µL
Needle wash:	20 sec

Table 3: Gradient conditions

Time (min)	Flow Rate (mL/min)	(A) Aqueous (%)	(B) Organic (%)
0.01	0.4	95	5
0.50	0.4	95	5
1.00	0.4	50	50
9.00	0.4	0	100
11.00	0.4	0	100
11.01	0.4	95	5
14.00	0.4	95	5

b. MS/MS Parameters

Scan Type:	MRM(MRM)
Source Temperature:	300 ºC
Capillary Voltage:	-4500 V
Collision Gas:	Medium
Curtain Gas:	30
Gas 1:	50
Gas 2:	50
Scheduled MRM:	Enabled
MRM detection window:	45 sec
Target Scan Time:	0.7 sec
Resolution Q1:	Unit
Resolution Q3:	Unit
Duration:	14.00 min
Cycles:	1200

Table 4: 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
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**	**PFTrDA-1**	**-30**	**-10**	**-19**	**-13**	N/A
663	319	7.56	PFTrDA-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

Q1	Q3	RT	Name	DP	EP	CE	CXP	IS
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

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

4. Sample Set for Screening and Confirmation
    a. Solvent Blank
    b. For muscle sets:
        i. Reference standard 0.50 ng/g (See Table 6 for applicable compounds and species)
        ii. Reference standard 1.25 ng/g (See Table 6 for applicable compounds and species)
    c. For plasma sets:
        i. Reference standard 0.50 ng/g (See Table 6 for applicable compounds and species)
        ii. Reference standard 12.5 ng/g (See Table 6 for applicable compounds and species)
    d. Positive Controls (recoveries)
    e. Negative Control
    f. Intra-laboratory check sample (if needed)
    g. Samples, up to a maximum of 32
    h. Reinjection of standard or positive control
5. Sample Set for Determination
    a. Solvent Blank
    b. Standard Curve
    c. Positive Controls (recoveries)
    d. Negative Control
    e. Intra-laboratory check sample (if needed)
    f. Samples, up to a maximum of 32

g. Reinjection of standard or positive control

G. DECISION CRITERIA / CALCULATIONS

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

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.

a. Screening

i. The screening ion for a given analyte must be present. The required ion for each compound is listed in Table 4.

ii. Retention time of the sample must be ± 5% of the recovery/positive control.

iii. 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.

iv. 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 6).

(b) The sample response ratio equals or exceeds the applicable recovery/positive control level (See Table 6).

v. 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 6).

Note: 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.

b. Confirmation

i. 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 6) 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.

Note: 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:

The recovery/positive control of the analyte must exceed 10% of the applicable standard level (See Table 6).

The sample response equals or exceeds the applicable recovery/positive control level (See Table 6).

(e) The blank (negative control) must be less than 10% of the applicable standard for the analyte (See Table 6).

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

c. Determination

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

ii. 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.

iii. Each calibration curve is created using the response ratio (quan ion of Analyte/Internal Standard) as outlined in Table 2 in F.1.d, plotted against the concentration of the respective analyte. The concentration of each analyte present in each sample is calculated using the applicable curve.

iv. 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 section I.1.

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

2. Screening, Determination, and Confirmation Criteria for the following analytes: PFHxDA, PFODA, PFTeA, PFTrDA (no Internal Standard used)

a. Screening

i. The screening ion for a given analyte must be present. The required ion for each compound is listed in Table 4.

ii. Retention time of the sample must be ± 5% of the recovery/positive control.

iii. 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.

iv. 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 6).

(b) The area equals or exceeds the applicable recovery/positive control level (See Table 6).

v. The blank (negative control) area response for the screening ion must be less than 10% of the applicable standard calibration standard (See Table 6).

Note: 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.

b. Confirmation

i. 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 6) within 5%. Retention time for the samples must match the retention time of the applicable recovery/positive control (See Table 6) 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.

Note: 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:

The recovery/positive control of the analyte must exceed 10% of the applicable calibration standard level (See Table 6).

The sample response equals or exceeds applicable recovery/positive control level (See Table 6).

(e) The blank (negative control) must be less than 10% of the applicable calibration standard level (See Table 6).

c. Determination

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

ii. 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.

iii. Each calibration curve is created using the peak area as outlined in Table 2 in F.1.d. plotted against the concentration of the respective analyte. The concentration of each analyte present in each sample is calculated using the applicable curve.

iv. 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 section I.1.

(c) Peak areas should fall within the range of the curve. Dilute and re-analyze if necessary.

H. SAFETY INFORMATION AND PRECAUTIONS

1. Personal Protective Equipment — Protective clothing, eyewear, and gloves.

2. Hazards

   Consult all Safety Data Sheets (SDS) associated with the method.

3. Disposal Procedures

   Follow federal, state and local regulations.

I. QUALITY ASSURANCE PLAN

1. Performance Standard
   - a. Screening Criteria
     - i. 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.
     - ii. 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 criteria in Section G for samples containing corresponding presumptive positive analytes.
   - b. Confirmation Criteria
     - i. 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.
     - ii. The blank (negative control) must be negative using the criteria in Section G for the analytes of interest.
   - c. Determination Criteria
     - i. For set acceptance, the analyte(s) of interest in the recovery/positive control must meet the acceptable recovery range for those analytes in Table 6.
     - ii. The blank (negative control) must be negative using the criteria in Section G for the analyte(s) of interest.
     - iii. The correlation coefficient for the standard curve for the analyte(s) of interest must be ≥ 0.995.

2. Intralaboratory Check Samples

   a. Acceptability criteria.

      Refer to I. 1.

      If unacceptable values are obtained, then:

      i. Investigate following established procedures.

      ii. Take corrective action as warranted.

J. APPENDIX

1. References

   a. 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*

   b. 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

   c. 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.

2. Chromatograms/spectra

   {RESERVED}

3. Structure

Table 5 Name and Structure

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
PFTrDA	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

4. Minimum Level of Applicability

a. Minimum Level of Applicability (MLA) for Screening and Confirmation

Table 6: Screening and Confirmation Levels

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
PFTrDA	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	0.50	0.50	0.50	0.50

b. Applicability for Determination (Quantitation)

Table 7: Determinative (Quantitative) 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%
PFTrDA	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%	0.50 – 125	75 – 127%	0.50 – 125	67 – 128%

K. APPROVALS AND AUTHORITIES

1. Approvals on file.

2. Issuing Authority: Director, Laboratory Quality Assurance Staff