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PROGRAM AREA: Chemical Contaminants

METHOD TITLE: Determination of 30 Per and Polyfluoroalkyl Substances (PFAS) in Food and Feed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

VALIDATION STATUS: Single Laboratory Validation (L2) per the Guidelines for the Validation of Chemical Methods for the FDA Foods Program 3rd Edition

AUTHOR(S): Susan Genualdi and Lowri deJager

METHOD SUMMARY/SCOPE:

The method describes a procedure for measuring 30 PFAS in food and feed using LC-MS/MS. The method has been single laboratory validated in the following food matrices:

Matrices	Validation	Date	Analyst
lettuce, chocolate milk, salmon, bread, eggs, clams, blueberries, silage, corn snaplage	Single lab validation[1]	2023	Susan Genualdi Wendy Young Elsie Peprah Cynthia Srigley Brian Ng

Analyte(s): Perfluorobutanoic acid, Perfluoropentanoic acid, Perfluorohexanoic acid, Perfluoroheptanoic acid, Perfluorooctanoic acid, Perfluorononanoic acid, Perfluorodecanoic acid, Perfluoroundecanoic acid, Perfluorododecanoic acid, Perfluorotridecanoic acid, Perfluorotetradecanoic acid, Perfluorobutanesulfonic acid, Perfluoropentanesulfonic acid, Perfluorohexanesulfonic acid, Perfluoroheptanesulfonic acid, Perfluorooctanesulfonic acid, Perfluorononanesulfonic acid, Perfluorodecanesulfonic acid, Perfluoroundecanesulfonic acid, Perfluorododecanesulfonic acid, Perfluorotridecanesulfonic acid, Perfluorooctanesulfonamide, 4,8-Dioxa-3*H*-perfluorononanoic acid, Hexafluoropropylene oxide dimer acid, 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid, 11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid, 1*H*,1*H*, 2*H*, 2*H*-Perfluorohexane sulfonic acid, 1*H*,1*H*, 2*H*, 2*H*-Perfluorooctane sulfonic acid, 1*H*,1*H*, 2*H*, 2*H*-Perfluorodecane sulfonic acid, 1*H*,1*H*, 2*H*, 2*H*-Perfluorododecane sulfonic acid.

Matrices: Lettuce, chocolate milk, salmon, bread, eggs, clams, blueberries, silage, corn snaplage. These matrices were chosen based on those that have been previously validated, those with known interferences (chocolate milk, eggs) and those with known analytical challenges due to matrix effects and interferences (silage, corn snaplage).

REVISION HISTORY: Version 010.03 replaces version 010.02 (2021).

This method has been modified from C-010.02 to include 14 additional analytes (30 total) and the extension of the method to feed samples (corn snaplage and silage).

OTHER NOTES:

- A new custom mix of 30 native analytes from Absolute Standards is used for analysis.
- Two new custom mixes of isotopically labeled standards (16 from Wellington and 4 from Cambridge Isotopes) are used for isotope dilution mass spectrometry.
- Solid phase extraction (SPE) is now performed on all samples.
- Strata™-XL-AW 100 µm column (200 mg/3 mL) was replaced with a Strata™-XL-AW 100 µm column (200 mg/6 mL) to reduce time spent transferring sample extract. The sample dilution was adjusted from 15 mL to 12 mL (approximate volumes) for ease of use with the 6 mL cartridge.
- An additional clean-up step has been added for samples containing both PFOS and cholic acids (specifically TCDCA).
- HRMS validation was performed for the confirmation of PFBA and PFPeA.
- The following analyte/matrix combinations did not meet the criteria for validation and are considered qualitative findings:
    - PFTrDS in eggs.
    - PFDS in bread.
    - PFTrDA in silage.
- Matrix interferences were identified with 4:2 FTS in both silage and corn snaplage samples.

Title: Determination of 30 Per and Polyfluoroalkyl Substances (PFAS) in Food and Feed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

Version 3.0 (2024)
Authors: Susan Genualdi and Lowri deJager

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2024.1 Method Title

Determination of 30 Per and Polyfluoroalkyl Substances (PFAS) in Food and Feed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

2024.2 Scope of Application

This method describes a procedure for measuring 30 PFAS in food and feed using LC-MS/MS. The method has been single laboratory validated in the following food matrices:

Matrices	Validation	Date	Analyst
lettuce, chocolate milk, salmon, bread, eggs, clams, blueberries, silage, corn snaplage	Single lab validation	2023	Susan Genualdi Wendy Young Elsie Peprah Cynthia Srigley Brian Ng

This method applies to analysts experienced in the use of LC-MS/MS, including but not limited to operation of the instrumentation and software, data analysis, and reporting results. Analysts shall be able to identify chromatographic and mass spectrometric interferences during sample analysis and take necessary actions following validated procedures for their correction to achieve reliable identification and quantification. The method shall be used only by personnel thoroughly trained in the handling and analysis of samples for the determination of trace contaminants in food and beverage products. PFAS chemicals are prevalent in all laboratory environments and special care must be taken to prevent false positives due to accidental and/or routine laboratory contamination.

Additional considerations:

- Only LC-MS grade solvents should be used unless otherwise noted in the procedure below. All solvents and complete method blanks should be analyzed on the LC-MS/MS instrument prior to sample analysis. All measures should be taken to reduce method blanks from background contamination in solvents, lab equipment, etc. If there is unavoidable background contamination, subtraction of method blanks may be performed by subtracting out the concentration of the method blank from the concentration of the sample. Complete method blanks should be performed and analyzed daily, preferably in the same instrument sequence as the samples. Sources of potential contamination during sample preparation include solvents, syringe filters, centrifuge tubes, SPE sorbents, septa, and others.
- A delay column should be used between the mobile phase mixer and sample injector to temporarily trap any system related interferences, which results in their elution at a later retention time than the analyte. This eliminates contamination from instrument tubing, mobile phase solvents, and solvent bottles.
- Due to the extremely low concentrations of detection required for this analysis, choice of MS/MS instrumentation is critical. Our analysis has been performed using a Sciex QTRAP® 6500+ instrument platform. We also validated a method for the quantification of PFBA and PFPeA on a Thermo Orbitrap™ MS system and determined the lower limits of quantification (LLOQ).

- Some analyte/matrix combinations did not meet the criteria for validation (minimum of three spike levels)
    - Results where only one spike level did not meet acceptance criteria, the analyte/matrix combination may still be quantified for the concentration range that passed.
        - PFUdS for the 15 ng/kg spike in lettuce
        - PFUdS for the 15 ng/kg spike in eggs
        - PFTrDS for the 0.15 ng/kg spike in salmon and clams
        - PFTrDA and PFDoS for the 15 ng/kg spike and PFUdS for the 0.15 ng/kg spike in bread
    - Results where two or more spike levels did meet acceptance criteria, the analyte/matrix combination would be considered qualitative.
        - PFTrDS in eggs (all spike levels)
        - PFDS for the 0.15 ng/kg or 1 ng/kg spike in bread
        - PFTrDA for the 1 ng/kg or 15 ng/kg spike in silage
    - There was an interference in the 4:2 FTS for the 0.15 ng/kg spike in silage preventing accurate quantification using triple quadrupole mass spectrometry. Any 4:2 FTS analyte in silage that cannot be resolved from matrix interferences must be quantified using high resolution mass spectrometry.

2024.3 Principle

The test sample is homogenized and fortified with isotopically labeled surrogates prior to the addition of water. The PFAS are extracted from the food samples using acetonitrile and formic acid. Following extraction, a modified QuEChERS (Quick, easy, cheap, effective, rugged, safe) extraction technique is performed and further clean-up using solid phase extraction is required. The resulting extract is filtered and fortified with internal standard solution and analyzed using LC-MS/MS. The PFAS compounds are identified by multiple reaction mode (MRM) transitions and retention time matching with the calibration standards. Ion ratios are used to confirm the identity. If two MRM transitions are not available (e.g., PFBA and PFPeA), then HRMS is necessary for confirmation. The concentration of each PFAS is determined using the response ratio of the PFAS quantitation transition to that of the relevant labeled surrogate standard (SS). The concentration is calculated by preparing a calibration curve using response ratios versus concentration ratios for native analytes to that of their labeled-SS. During analysis, quality control samples and method blanks must be analyzed. Analyte concentration in method blanks must be subtracted from the sample concentration prior to final quantification. After determination of the concentration from the curve, the concentration must be adjusted for dilution and starting sample mass. The new standard solution mix has concentrations in their ionic (not salt) form so salt corrections are no longer needed.

2024.4 Reagents

The use of trade names in this method constitutes neither endorsement nor recommendation by the U.S. Food and Drug Administration (FDA). Equivalent performance may be achievable using apparatus and materials other than those cited here.

(1) Formic acid, reagent grade >95% (Sigma Aldrich St. Louis, MO)
(2) LC/MS grade Optima water (Fisher Scientific, Hampton, NH)
(3) LC/MS grade Optima acetonitrile (Fisher Scientific, Hampton, NH)
(4) LC/MS grade Optima methanol (Fisher Scientific, Hampton, NH)
(5) Acetic acid, ammonium salt, 98% for analysis (Acros Organic, Geel, Belgium)
(6) 1-methyl piperidine 99% (Alfa Aeser, Tewksbury, MA)
(7) Original QuEChERS extraction salt ECMSSCFS-MP with 6000 mg MgSO4 and 1500 mg NaCl (UCT, Bristol, PA)
(8) QuEChERS dSPE ECMPSCB-MP with 900 mg MgSO4, 300 mg PSA, 150 mg graphitized carbon black (UCT, Bristol, PA) or ECMPSCB15-CT prefilled units
(9) Ammonium hydroxide, certified ACS Plus 14.8N (Fisher Scientific, Hampton, NH)

2024.5 Standards

(1) Isotopically labeled PFAS analytical standards custom mix FDA12-MPFAS (Wellington laboratories, Guelph, ON, Canada) and FTSs custom mix ES-5661 from Cambridge Isotopes (Tewksbury, MA).
(2) Native PFAS analytical standards 30 analyte custom mix (Absolute Standards, Hamden CT) Part Number: 65947.
(3) PFHxS, PFOA, and PFOS are reported as the sum of linear and branched isomers. A one peak integration is used to integrate any branched and linear isomers present in samples and standards. All other analytes are reported as the concentration of the linear isomer (if applicable).
(4) Optional – Standards to identify cholic acid interferences: TUDCA – tauroursodeoxycholic acid (580549-1GM), TCDCA – sodium taurochenodeoxycholate (T6260-250MG), TDCA – taurodeoxycholic acid, sodium salt (580221-5GM) from Fisher Scientific (Hampton, NH).

Table 5-1. PFAS native, surrogate, and internal standard compounds

Native PFAS

Acronym	Name	CAS	Formula	MW
PFBA	Perfluorobutanoic acid	375-22-4	$C_4F_7O_2$	214
PFPeA	Perfluoropentanoic acid	2706-90-3	$C_5HF_9O_2$	264
PFHxA	Perfluorohexanoic acid	307-24-4	$C_6HF_{11}O_2$	314
PFHpA	Perfluoroheptanoic acid	375-85-9	$C_7HF_{13}O_2$	364
PFOA	Perfluorooctanoic Acid	335-67-1	$C_8HF_{15}O_2$	414
PFNA	Perfluorononanoic acid	375-95-1	$C_9HF_{17}O_2$	464
PFDA	Perfluorodecanoic acid	335-76-2	$C_{10}HF_{19}O_2$	514
PFUnA	Perfluoroundecanoic acid	2058-94-8	$C_{11}HF_{21}O_2$	564
PFDoA	Perfluorododecanoic acid	307-55-1	$C_{12}HF_{23}O_2$	614

PFTrDA	Perfluorotridecanoic acid	72629-94-8	$C_{13}HF_{25}O_2$	664
PFTeDA	Perfluorotetradecanoic acid	376-06-7	$C_{14}HF_{27}O_2$	714
PFBS	Perfluorobutanesulfonic acid	375-73-5	$C_4HF_9O_3S$	300
PFPeS	Perfluoropentanesulfonic acid	2706-91-4	$C_5HF_{11}O_3S$	350
PFHxS	Perfluorohexanesulfonic acid	355-46-4	$C_6HF_{13}O_3S$	400
PFHpS	Perfluoroheptanesulfonic acid	375-92-8	$C_7HF_{15}O_3S$	450
PFOS	Perfluorooctanesulfonic acid	1763-23-1	$C_8HF_{17}O_3S$	500
PFNS	Perfluorononanesulfonic acid	68259-12-1	$C_9HF_{19}O_3S$	550
PFDS	Perfluorodecanesulfonic acid	335-77-3	$C_{10}HF_{21}O_3S$	600
PFUnDS	Perfluoroundecanesulfonic acid	749786-16-1	$C_{11}HF_{23}O_3S$	650
PFDoS	Perfluorododecanesulfonic acid	79780-39-5	$C_{12}HF_{25}O_3S$	700
PFTrDS	Perfluorotridecanesulfonic acid	791563-89-8	$C_{13}HF_{27}O_3S$	750
FOSA	Perfluorooctanesulfonamide	754-91-6	$C_8H_2F_{17}NO_2S$	499
DONA	4,8-Dioxa-3*H*-perfluorononanoic acid	919005-14-4	$C_7H_2F_{12}O_4$	378
HFPO-DA	Hexafluoropropylene oxide dimer acid	13252-13-6	$C_6HF_{11}O_3$	330
9Cl-PF3ONS	9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	756426-58-1	$C_8HClF_{16}O_4S$	532
11Cl-PF3OUdS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	763051-92-9	$C_{10}HClF_{20}O_4S$	632
4:2FTS	1*H*,1*H*, 2*H*, 2*H*-Perfluorohexane sulfonic acid	757124-72-4	$C_6H_5F_9O_3S$	328
6:2FTS	1*H*,1*H*, 2*H*, 2*H*-Perfluorooctane sulfonic acid	27619-97-2	$C_8H_5F_{13}O_3S$	428
8:2FTS	1*H*,1*H*, 2*H*, 2*H*-Perfluorodecane sulfonic acid	39108-34-4	$C_{10}H_5F_{17}O_3S$	528
10:2 FTS	1*H*,1*H*, 2*H*, 2*H*-Perfluorododecane sulfonic acid	120226-60-0	$C_{12}H_5F_{21}O_3S$	628

Surrogate/Internal Standards

Acronym	Name	CAS	Formula	MW
M3 PFBA	Perfluoro-n-[2,3,4-$^{13}C_3$]butanoic acid			217
M3 PFPeA	Perfluoro-n-[3,4,5-$^{13}C_3$]pentanoic acid			267
M5PFHxA	Perfluoro-n-(1,2,3,4,6-$^{13}C_5$)hexanoic acid			316
M8PFOA	Perfluoro-n-[$^{13}C_8$]octanoic acid			422
MPFUdA	Perfluoro-n-(1,2-$^{13}C_2$)undecanoic acid			566
MPFDoA	Perfluoro-n-(1,2-$^{13}C_2$)dodecanoic acid			616
M2PFTeDA	Perfluoro-n-(1,2-$^{13}C_2$)tetradecanoic acid			716
M3PFBS	Sodium perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonate			303
M3PFHxS	Sodium perfluoro-1-(1,2,3-$^{13}C_3$)hexanesulfonate			404
M8PFOS	Sodium perfluoro-[$^{13}C_8$]octanesulfonate			508
M3HFPO-DA	2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-$^{13}C_3$-propanoic acid			333
M8FOSA	Perfluoro-1-($^{13}C_8$)octanesulfonamide			507
Labeled 4:2 FTS	1H,1H,2H,2H-Perfluorohexane Sulfonic Acid (4:2 FTS)(13C2,99%;D4,98%)			356
Labeled 6:2 FTS	1H,1H,2H,2H-Perfluorooctane Sulfonic Acid (4:2 FTS)(13C2,99%;D4,98%)			456
Labeled 8:2 FTS	1H,1H,2H,2H-Perfluorodecane Sulfonic Acid (4:2 FTS)(13C2,99%;D4,98%)			556
Labeled 10:2 FTS	1H,1H,2H,2H-Perfluorododecane Sulfonic Acid (4:2 FTS)(13C2,99%;D4,98%)			656
d5-N-EtFOSAA	N-ethyl-d5-perfluoro-1-octanesulfonamidoacetic acid			590

2024.6 Preparation of Standards, Samples, and Test Portions

2024.6.1. Prepare native PFAS stock solution at 1000 ng/mL, 100 ng/mL, and 10 ng/mL

(1) Add 2 mL of 2000 ng/mL PFAS analytical standard custom mix from Absolute Standards (30 native compounds in Table 5-1) to 2 mL methanol for a final volume of 4 mL. In the resulting solution, each compound has a concentration of 1000 ng/mL. This solution will be used for calibration curve preparation and single laboratory validation (SLV) spikes. This standard was purchased from Absolute Standards, but other sources are acceptable.

(2) Add 1 mL of 1000 ng/mL stock solution to 9 mL of methanol to produce a 100 ng/mL stock solution. This solution will be used for calibration curve preparation and SLV spikes.

(3) Add 1 mL of 100 ng/mL stock solution to 9 mL of methanol to produce a 10 ng/mL stock solution. This solution will be used for SLV and method detection limit (MDL) spikes.

2024.6.2 Prepare isotopically labeled PFAS surrogate stock solution (SS) at 200 ng/mL

Combine 2 mL of Cambridge Isotope mass-labeled PFAS mixture (4 FTSs) at 1000 ng/mL and 1 mL of Wellington mass-labeled PFAS mixture (FDA12-MPFAS) at 2000 ng/mL and add 7 mL of methanol. This solution will have a concentration of 200 ng/mL. This stock solution was used for both sample analysis and calibration curve preparation. These standards were purchased from Wellington and Cambridge, but other sources are acceptable.

2024.6.3 Prepare isotopically labeled internal standard solution (IS) at 200 ng/mL

Add 0.04 mL of N-ethyl-d5-perfluoro-1-octanesulfonamidoacetic acid (d5-N-EtFOSAA) 50 µg/mL analytical standard to 9.96 mL methanol. The individual *d5*-N-EtFOSAA standard was purchased from Wellington, but other sources are acceptable.

2024.6.4 Prepare mobile phase A (5 mM ammonium acetate in water) and 5mM 1-methyl piperidine

Weigh out 0.38 ± 0.01 g of ammonium acetate. Add to mobile phase bottle with 1000 mL of LC/MS Optima water. Then add 0.5 mL of 1-methyl piperidine. Invert several times to mix.

2024.6.5 Prepare mobile phase B (100% methanol)

Add approximately 1000 mL of LC/MS Optima methanol to a mobile phase bottle.

2024.6.6 Continuing Calibration Verification (CCV) standard

A duplicate solution was prepared of the 1 ng/mL calibration standard and used as the CCV standard (Table 6-1).

2024.6.7 Solution for (SPE) clean-up

Add 6 mL of a 14.8 N ammonium hydroxide solution to 1000 mL volumetric flask and fill to volume with methanol to make up a 0.3 % w/v solution.

2024.6.8 Calibration Standards

Calibration standards are prepared at concentrations of 0.01, 0.05, 0.10, 0.50, 1.0, 5.0, 10, and 25 ng/mL according to Table 6-1 below.

Table 6-1. Calibration standard preparation

Final concentration (ng/mL)	Native stock solution concentration (ng/mL)	Volume of stock solution to add (mL)	Volume of 200 ng/mL surrogate stock solution (mL)	Methanol (mL)	Final volume (mL)	Volume of 200 ng/mL IS stock solution (mL)
0.01	10	0.01	0.05	9.94	10	0.05
0.05	10	0.05	0.05	9.9	10	0.05
0.1	10	0.1	0.05	9.85	10	0.05
0.5	100	0.05	0.05	9.9	10	0.05
1	100	0.1	0.05	9.85	10	0.05
5	1000	0.05	0.05	9.9	10	0.05
10	1000	0.1	0.05	9.85	10	0.05
25	1000	0.25	0.05	9.7	10	0.05

2024.6.9 Preparation of Samples or Test Portions

(1) Most of the samples used for method validation (lettuce, salmon, bread, clams, blueberries) were homogenized using an IKA tube mill with a disposable 100 mL polypropylene grinding chamber. Samples were ground at 5000 rpm for approximately 2 minutes.
(2) Some of the samples used for the validation were previously homogenized by FDA's Kansas City Human and Animal Food Laboratory (chocolate milk, egg).
(3) The feed samples were homogenized with dry ice in a Robot Coupe Blixer.

2024.7 Apparatus/Instrumentation

The use of trade names in this method constitutes neither endorsement nor recommendation by the U. S. Food and Drug Administration (FDA). Citation of sample preparation equipment is based on the original method validation. Equipment of equivalent specifications also accepted, however, all chemistry (e.g., analytical column) must be maintained with the original method.

(1) Digital pulse mixer/vortexer (Glas-Col, Terre Haute, IN) capable of 1500 rpm with pulse 70
(2) Sorvall legend XTR centrifuge (Thermo Fisher Scientific, Waltham, MA)
(3) Nitrogen evaporation system (Turbovap LV, Biotage, Uppsala, Sweden)
(4) Nexera X2 (Shimadzu, Kyoto, Japan) with binary pump, degasser, autosampler, and thermostatted column compartment
(5) Agilent positive pressure manifold 48 processor (PPM-48)
(6) Sciex QTRAP® 6500+ hybrid triple quadrupole/linear ion trap mass spectrometer with an electrospray ESI ion source (Sciex, Toronto, ON Canada)
(7) Analyst® Software version 1.7.1
(8) SCIEX OS Version 2.0.0.45330
(9) Q-Exactive Orbitrap™ mass spectrometer (Thermo Fisher Scientific, Waltham, MA).
(10) VWR 50 mL polypropylene (PP) ultra-high performance centrifuge tubes (VWR, Radnor, PA) or equivalent
(11) Falcon 15 mL polypropylene (PP) conical centrifuge tubes (Thermo Fisher Scientific, Waltham, MA) or equivalent
(12) Falcon 14 mL round-bottom polypropylene (PP) test tubes with cap (Falcon 352059) or equivalent
(13) 300 µL PP screw top vials (MicroSolv, Wilmington, NC) – vials for calibration standards

(14) Screw caps, silicone rubber/PP Septa (Microsolv, Wilmington, NC) – caps for calibration standards
(15) 2 mL PP screw top vials (MicroSolv, Wilmington, NC) – vials for standard storage
(16) Screw caps, solid, silicone rubber/PP liners (Microsolv, Wilmington, NC) – caps for standard storage
(17) 0.2 µm Acrodisc nylon syringe filters (Pall Corporation, Port Washington, NY)
(18) 5 mL PP/PE luer lock syringes (Sigma Aldrich, St. Louis, MO)
(19) Nano filter vials 0.2 µm nylon without cap (Thomson Instrument Company, Oceanside, CA)
(20) Nano filter vial caps for PFAS use (Part # 14638, Thermo Scientific)
(21) Analytical column – 150 mm x 2.1 mm, 3.5 µm XBridge C18 (Waters Corp, Milford, MA)
(22) Guard column – 2.1 mm x 5 mm, 1.7 µm Vanguard™ Acquity BEH C18 (Waters Corp, Milford, MA)
(23) Delay column – 2.1 mm x 50 mm, 5 µm XBridge C18 (Waters Corp, Milford, MA)
(24) SPE cartridge – Strata™-XL-AW 100 µm Polymeric Weak Anion 200 mg/6 mL, Tubes (Phenomenex, Torrance, CA)
(25) ENVIcarb cartridge - SupelClean ENVI-Carb, 250 mg/6 mL (Millipore Sigma, St. Louis, MO)

2024.8 Method

QuEChERS is used for the extraction of PFAS from foods. Due to the high variability of the sample matrix, sample preparation steps may vary by food type. Two separate protocols are given for food and for feed matrices.

2024.8.1 Sample Preparation Food

(1) Add 5 grams of food sample to a 50 mL PP centrifuge tube.
(2) Add 50 µL of 200 ng/mL isotopically labeled surrogate standard solution to the sample to give a final concentration of 1 ng/mL in the final extract.
(3) Add 5 mL of LC/MS grade Optima water if the sample is fruit or vegetable based to the 50 mL PP conical centrifuge tube. Dry samples (< 25% water content) will need additional water. Descriptions of low water content commodity groups can be found in Appendix 4 of FDA Foods Program Guidelines for Chemical Methods. For most dry foods, the addition of 15 mL of water is sufficient. In some cases (e.g. protein powder) up to 25 mL of additional water is needed to adequately swell the matrix. In the cases of very dry food samples (e.g. protein powder), the protocol for feed samples may be preferred.
(4) Add 10 mL acetonitrile to the 50 mL PP conical centrifuge tube.
(5) Add 150 µL formic acid to the 50 mL PP conical centrifuge tube.
(6) Shake vigorously for 1 minute.
(7) Add QuEChERS salt packet (Original extraction salt ECMSSCFS-MP from UCT with 6000 mg $MgSO_4$ and 1500 mg NaCl).
(8) If clumping occurs, the sample should be hand shaken or vortexed until homogenous.
(9) Place on Glas-Col shaker at 1500 rpm with pulse set to 70 for 5 minutes.
(10) Centrifuge for 5 minutes at 10000 rcf.
(11) Add supernatant to 15 mL PP conical centrifuge tube with dSPE sorbent (ECMPSCB-MP from UCT with 900 mg $MgSO_4$, 300 mg PSA, 150 mg graphitized carbon black).
(12) Vortex/shake for 2 minutes.
(13) Centrifuge 5 minutes at 10000 rcf.
(14) Filter the supernatant with a 0.2 µm nylon syringe filter and transfer to a 15 mL conical centrifuge tube.

(15) For SPE clean-up, take 1 mL of the filtered supernatant and transfer to a 15 mL centrifuge tube. Then add 11 mL of water.

2024.8.2 Sample Preparation Feed/Dry Foods

(1) Add 1 gram of feed or dry food sample to a 50 mL PP centrifuge tube.
(2) Add 10 µL of 200 ng/mL isotopically labeled surrogate standard solution to the sample to give a final concentration of 1 ng/mL in the final extract.
(3) Add 15 mL of LC/MS grade Optima water to the centrifuge tube.
(4) Add 10 mL acetonitrile to the 50 mL PP conical centrifuge tube.
(5) Add 150 µL formic acid to the 50 mL PP conical centrifuge tube.
(6) Shake vigorously for 1 minute.
(7) Add QuEChERS salt packet (Original extraction salt ECMSSCFS-MP from UCT with 6000 mg $MgSO_4$ and 1500 mg NaCl).
(8) If clumping occurs, the sample should be hand shaken or vortexed until homogenous.
(9) Place on Glas-Col shaker at 1500 rpm with pulse set to 70 for 5 minutes.
(10) Centrifuge for 5 minutes at 10000 rcf.
(11) Add supernatant to 15 mL PP conical centrifuge tube with dSPE sorbent (ECMPSCB-MP from UCT with 900 mg $MgSO_4$, 300 mg PSA, 150 mg graphitized carbon black).
(12) Vortex/shake for 2 minutes.
(13) Centrifuge 5 minutes at 10000 rcf.
(14) Filter 5 mL of supernatant with a 0.2 µm nylon syringe filter and transfer to a 15 mL conical centrifuge tube.
(15) Blow to approximately 1 mL with nitrogen in a 60 °C water bath.
(16) Take the centrifuge tube with the approximately 1 mL extract and add 11 mL of water in preparation for SPE analysis.

2024.8.3 Clean-up of Extract Using Weak Anion Exchange (SPE) Column (Same for Food and Feed)

The SPE step is used for all samples.

(1) Take final extract diluted with water (approximately 12 mL total volume) from QuEChERS step to be used for SPE clean-up.
(2) Condition a Strata™-XL-AW 100 µm column (200 mg/6 mL) with 6 mL of 0.3% w/w ammonium hydroxide in methanol.
(3) Add 5 mL of LC Optima water to equilibrate column.
(4) Add sample (approximately 12 mL total volume) to column and let pass through.
(5) Add 5 mL of LC Optima water to wash column.
(6) Let column dry 1 minute.
(7) Add 4 mL of 0.3% ammonium hydroxide in methanol to the empty sample tube to rinse the sides and then add to SPE cartridge to elute analytes into a clean 14 mL round bottom PP tube.
(8) Blow to just below 1 mL with nitrogen in a 60 °C water bath.
(9) Bring volume to 1 mL with methanol and add 5 µL of 200 ng/mL *d5* N-EtFOSAA to the tube.
(10) Transfer approximately 100 µL to a Thomson nano filter vial with 0.2 µm nylon® filter and a nano filter screw cap to run using LC-MS/MS.

2024.8.4 LC-MS/MS Analysis

All samples were analyzed using a liquid chromatograph (Nexera X2, (Shimadzu, Kyoto, Japan)). The MS/MS data was acquired using scheduled MRM with a Sciex QTRAP® 6500+.

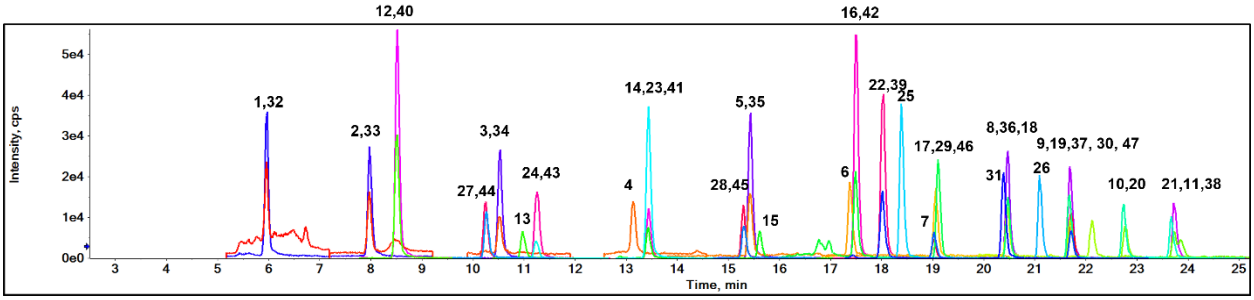

Figure 8-1. Example chromatogram of a spiked single-laboratory validation sample (salmon) with native and labeled PFAS concentrations at 5 µg/kg.
1. PFBA, 2. PFPeA, 3. PFHxA, 4. PFHpA, 5. PFOA, 6. PFNA, 7. PFDA, 8. PFUnA, 9. PFDoA, 10. PFTrDA, 11. PFTeDA, 12. PFBS, 13. PFPeS, 14. PFHxS, 15. PFHpS, 16. PFOS, 17. PFNS, 18. PFDS, 19. PFUnDS, 20. PFDoS, 21. PFTrDS, 22. FOSA, 23. DONA, 24. HFPO-DA, 25. 9Cl-PF3ONS, 26. 11Cl-PF3OUdS, 27. 4:2 FTS, 28. 6:2 FTS, 29. 8:2 FTS, 30. 10:2 FTS 31. d5 N-EtFOSAA, 32. M3 PFBA, 33. M3 PFPeA 34. M5PFHxA, 35. M8PFOA, 36. MPFUdA, 37. MPFDoA, 38. M2PFTeDA, 39. M8 FOSA 40. M3 PFBS, 41. M3PFHxS, 42. 13C PFOS, 43. M3 HFPO-DA 44. Labeled 4:2 FTS, 45. Labeled 6:2 FTS, 46. Labeled 8:2 FTS, 47. Labeled 10:2 FTS

The following conditions were used during LC analysis:

(1) Analytical column – 150 mm x 2.1 mm, 3.5 µm XBridge C18 (Waters Corp, Milford, MA)
(2) Guard column – 2.1 mm x 5 mm, 1.7 µm Vanguard™ Acquity BEH C18 (Waters Corp, Milford, MA)
(3) Delay column – 2.1 mm x 50 mm, 5 µm XBridge C18 (Waters Corp, Milford, MA)
(4) Mobile phase A: 5mM ammonium acetate and 5mM 1-MP in water
(5) Mobile phase B: 100% methanol
(6) Injection volume: 3 µL
(7) Column temperature: 40 °C
(8) Flow rate: 0.30 mL/min

The LC gradient and the MS/MS monitored transitions can be found in Tables 8.1 and 8.2.

Table 8.1. Gradient Profile for the LC Conditions

Time (min)	Concentration of Mobile Phase B
0.01	10%
3.0	10%
3.1	40%
26.0	90%
26.1	10%
28.0	10%

Table 8-2. MS/MS Conditions for the Monitored Transitions on a Sciex QTRAP® 6500+.

Internal Standards

ID	Retention Time (min)	Q1 mass (*m/z*)	Q3 mass (*m/z*)	DP (volts)	EP (volts)	CE (volts)	CXP (volts)
NN EtFOSAA[a]	20.52	589	419	-50	-10	-30	-20
NN EtFOSAA	20.52	589	219	-50	-10	-38	-20

[a]Primary MRM transition used for quantification.

Surrogate Standards

ID	Retention Time (min)	Q1 mass (*m/z*)	Q3 mass (*m/z*)	DP (volts)	EP (volts)	CE (volts)	CXP (volts)
M3PFBA[a]	5.9	216	172	-17	-8	-12	-14
M3PFPeA[a]	7.9	266	222	-17	-6	-11	-28
M5PFHxA[a]	10.52	318	273	-13	-10	-14	-12
M8PFOA[a]	15.5	421	376	-36	-8	-13	-20
M8PFOA	15.5	421	172	-19	-5	-25	-7
MPFUdA[a]	20.61	565	520	-32	-4	-17	-45
MPFUdA	20.61	565	269	-35	-5	-27	-22
MPFDoA[a]	21.86	615	570	-48	-3	-19	-45
MPFDoA	21.86	615	169	-51	-5	-38	-17
M2PFTeDA[a]	23.85	715	670	-38	-3	-23	-52
M2PFTeDA	23.85	715	169	-36	-3	-38	-18
M3PFBS[a]	8.49	302	80	-88	-6	-73	-9
M3PFBS	8.49	302	99	-85	-6	-36	-8
M3PFHxS[a]	13.48	402	80	-60	-10	-81	-15
M8PFOS[a]	17.6	507	80	-100	-5	-125	-15
M8PFOS	17.6	507	99	-100	-5	-100	-15
M3HFPO[a]	11.25	287	169	-19	-12	-10	-25
M3HFPO	11.25	287	185	-41	-5	-35	-35
M8FOSA[a]	18.29	506	78	-101	-9	-100	-8
M8FOSA	18.29	506	64	-120	-9	-165	-28
13C2,D4 4:2 FTS[a]	10.39	333	312	-60	-10	-30	-29
13C2,D4 4:2 FTS	10.39	333	82	-60	-10	-63	-12
13C2,D4 6:2 FTS[a]	15.38	433	412	-80	-10	-33	-13
13C2,D4 6:2 FTS	15.38	433	82	-80	-10	-67	-13
13C2,D4 8:2 FTS[a]	19.09	533	512	-100	-10	-49	-23
13C2,D4 8:2 FTS	19.09	533	82	-100	-10	-81	-34
13C2,D4 10:2 FTS[a]	21.82	633	612	-100	-10	-45	-19
13C2,D4 10:2 FTS	21.82	633	82	-100	-10	-85	-10

[a]Primary MRM transition used for quantification.

Native Standards

ID	Retention Time (min)	Q1 mass (*m/z*)	Q3 mass (*m/z*)	DP (volts)	EP (volts)	CE (volts)	CXP (volts)
PFBA[a]	5.9	213	169	-10	-6	-13	-19
PFPeA[a]	7.9	263	219	-20	-8	-11	-20
PFHxA[a]	10.52	313	269	-10	-12	-13	-45
PFHxA	10.52	313	119	-23	-12	-27	-14
PFHpA[a]	13.17	363	319	-17	-10	-14	-26
PFHpA	13.17	363	169	-32	-11	-25	-24
PFOA[a]	15.5	413	369	-43	-7	-16	-25
PFOA	15.5	413	219	-24	-5	-23	-25
PFNA[a]	17.48	463	419	-38	-11	-15	-37
PFNA	17.48	463	269	-40	-5	-24	-13
PFDA[a]	19.18	513	469	-15	-10	-16	-29
PFDA	19.18	513	269	-20	-10	-26	-17
PFUdA[a]	20.61	563	519	-38	-9	-18	-45
PFUdA	20.61	563	269	-39	-7	-27	-25
PFDoA[a]	21.86	613	569	-33	-7	-19	-52
PFDoA	21.86	613	169	-33	-8	-39	-17
PFTrDA[a]	22.94	663	619	-39	-7	-21	-18
PFTrDA	22.94	663	169	-39	-7	-39	-18
PFTeDA[a]	23.85	713	669	-42	-12	-25	-51
PFTeDA	23.85	713	169	-49	-6	-40	-19
PFBS[a]	8.49	299	80	-44	-10	-70	-11
PFBS	8.49	299	99	-35	-4	-36	-15
PFPeS[a]	11	349	99	-80	-9	-80	-12
PFPeS	11	349	119	-53	-10	-40	-18
PFHxS[a]	13.48	399	99	-108	-6	-84	-8
PFHxS	13.48	399	169	-66	-5	-42	-20
PFHpS[a]	15.69	449	99	-58	-8	-84	-24
PFHpS	15.69	449	169	-68	-8	-41	-27
PFOS[a]	17.6	499	80	-150	-4	-120	-10
PFOS	17.6	499	99	-150	-4	-100	-10
PFNS[a]	19.25	549	80	-72	-10	-142	-8
PFNS	19.25	549	99	-110	-10	-124	-12
PFDS[a]	20.69	599	80	-136	-8	-171	-14
PFDS	20.69	599	99	-139	-7	-141	-14
PFUdS[a]	21.82	649	80	-180	-4	-160	-12
PFUdS	21.82	649	99	-170	-4	-140	-11
PFDoS[a]	22.95	699	80	-79	-11	-171	-9

PFDoS	22.95	699	99	-130	-10	-158	-10
PFTrDS[a]	23.76	749	80	-76	-11	-177	-37
PFTrDS	23.76	749	99	-79	-13	-174	-40
FOSA[a]	18.3	498	78	-138	-11	-93	-7
FOSA	18.3	498	478	-130	-13	-41	-36
DONA[a]	11.25	377	251	-25	-8	-15	-20
DONA	11.25	377	85	-20	-7	-39	-10
HFPO-DA[a]	13.47	285	169	-78	-6	-11	-27
HFPO-DA	13.47	285	185	-20	-5	-21	-27
9Cl-PF3ONS[a]	18.49	531	351	-100	-14	-38	-13
9Cl-PF3ONS	18.49	531	83	-100	-4	-92	-9
11Cl-PF3OUdS[a]	21.24	631	451	-34	-9	-40	-12
11Cl-PF3OUdS	21.24	631	199	-20	-10	-36	-11
4:2 FTS[a]	10.27	327	307	-50	-6	-28	-24
4:2 FTS	10.27	327	81	-40	-11	-62	-10
6:2 FTS[a]	15.38	427	407	-31	-11	-34	-38
6:2 FTS	15.38	427	81	-41	-12	-88	-12
8:2 FTS[a]	19.15	527	507	-69	-11	-37	-29
8:2 FTS	19.15	527	81	-70	-11	-109	-36
10:2 FTS[a]	21.88	627	607	-54	-6	-46	-48
10:2 FTS	21.88	627	81	-68	-6	-129	-15

[a]Primary MRM transition used for quantification.

Cholic acid transitions monitored for potential interferences (covers TDCA, TUDCA, and TCDCA)

ID	Retention Time (min)	Q1 mass (*m/z*)	Q3 mass (*m/z*)	DP (volts)	EP (volts)	CE (volts)	CXP (volts)
cholic acid 1	17.6	498	124	-161	-14	-62	-16
cholic acid 2	17.6	498	80	-107	-11	-152	-9

[a]Primary MRM transition used for quantification.

The following conditions are for the Sciex QTRAP® 6500+:

(1) Curtain gas: 40 au
(2) Collisionally activated dissociation (CAD) gas: medium
(3) Ion spray voltage: -4500 V
(4) Source temperature: 350 °C
(5) Gas 1 pressure: 50 au
(6) Gas 2 pressure: 50 au

The samples are run using the following template:

(1) Blank (MeOH) injection
(2) Standard curve
(3) Blank (MeOH) injection
(4) Samples

For every 6 samples analyzed, a CCV standard (typically 1 ng/mL) is run to check for accuracy. The accuracy of the calculated concentration of the CCV should be statistically evaluated, which should be within 70-130 % of the original value. If the accuracy falls outside this range, the calibration curve is rerun, and any test samples run since the last successful CCV are reinjected.

2024.8.5 Cholic acid monitoring and clean-up

During the analysis of PFAS in foods, bile acids (TDCA, TCDCA, and TUDCA) can be present in certain food types (e.g., eggs, egg derived products, seafood, milk, meat). The current dSPE clean-up step with 150 mg GCB is sometimes not enough to remove all cholic acids in a sample. Cholic acids also produce the 499→80 MRM transition and can result in false positive detections of PFOS. However, they do not have the 499→99 confirmatory PFOS MRM transition. To identify cholic acid interferences in samples, two MRM transitions are also monitored that are unique to cholic acids and not PFOS (Table 8-2). If there is a suspected false positive, these ions are used to confirm the detection of cholic acids. In the cases where both PFOS (499→99) and cholic acids (498→80 and 499→124) are confirmed, and if the cholic acids interfere chromatographically, an additional clean-up step with Envi-Carb is performed to be able to accurately quantify PFOS using the 499→80 MRM transition. In this method, only TCDCA interferes chromatographically, while TUDCA and TDCA are completely resolved from PFOS branched and linear isomers. TDCA, TCDCA, and TUDCA analytical standards are analyzed with samples with suspected interferences for confirmation.

(1) An ENVIcarb cartridge 250 mg/6 mL (SupelClean ENVI-Carb, Millipore Sigma, St. Louis, MO) is conditioned with 4 mL of methanol.
(2) Three mL of the QuEChERS extract (acetonitrile) is eluted through the cartridge.
(3) The eluent is blown to near dryness and reconstituted with 3 mL of methanol.
(4) Internal standard is added (15 µL of 0.2 ng/µL d_5-N-EtFOSAA, totaling 3 ng) and vortexed.
(5) A portion is pipetted into a filter vial for LC-MS/MS analysis for PFOS only.

2024.8.6 Verification of PFBA and PFPeA using liquid chromatography/high resolution mass spectrometry (LC-HRMS)

Due to matrix interferences and the potential for false positives for PFBA and PFPeA given that they only have one MS/MS transition, any positive detection of PFBA or PFPeA must be confirmed by LC-HRMS. The LC-HRMS instrument includes a Nexera LC (Shimadzu, Columbia, MD) coupled to a Q-Exactive Orbitrap™ mass spectrometer (Thermo Fisher Scientific, Waltham, MA). The LC separation is performed using the same conditions as described for the LC-MS/MS analysis (2024.8.4).

The mass spectrometer is operated using a negative ion polarity, full scan (100-1200 *m/z*) method, with 70k resolving power, an AGC target of 1E6, and a maximum injection time of 250 ms. The heated electrospray ionization (HESI) source parameters should be tuned to minimize in-source fragmentation of PFBA and PFPeA with a sheath gas flow rate of 35 au, auxiliary gas flow rate of 10 au, a spray voltage of -2.5 kV, capillary temperature of 350 °C, S-lens RF level of 25, and an auxiliary gas heater temperature of 310 °C. Extracted ion chromatograms are generated for the exact mass of PFBA (*m/z* 212.9792) and PFPeA (*m/z* 262.9760) with a ±5 ppm mass accuracy tolerance and the peak intensity values are used for quantification. PFBA and PFPeA concentrations are calculated following the same isotope dilution procedure described for the LC-MS/MS data. PFBA and PFPeA can be verified above the LLOQ of 0.2 ng/mL. The calibration curve ranged from 0.2 ng/mL to 25 ng/mL.

2024.9 Calculations

Example calculation for concentration measured on LC-MS/MS (using the calibration curve) to concentration in 5 grams of food and 1 gram of feed with a final extract of 10 mL:

(1) **For food:** If the calculated concentration off the instrument is 1 ng/mL, this represents a 10 mL extraction volume and 5 grams of food, the concentration in food is 2 ng/g.

$$\frac{1\ ng}{mL} * \frac{10\ mL}{5\ grams} = 2\ ng/g$$

(2) **For feed:** If the calculated concentration off the instrument is 1 ng/mL, this represents a 10 mL extraction volume (where 5 mL of extract is concentrated to 1 mL) and 1 gram of food, the concentration in food is 2 ng/g.

$$\frac{1\ ng}{mL} * \frac{10\ mL}{1\ grams} * \frac{1\ mL}{5\ mL} = 2\ ng/g$$

Sciex OS software is used to prepare a linear standard curve where x is the concentration ratio (analyte/SS) and y is the instrument response ratio (analyte/SS) with 1/x weighting. Surrogates and their internal standard pairs are listed in Table 9-1 which are used to calculate absolute recoveries of the surrogate standards over the entire extraction method. Surrogates and their native analyte pairs are also listed in Table 9-1 with their curve fit. The calibration curve has surrogate and internal standard concentrations of 1 ng/mL. In Sciex OS, equations can be used to generate the surrogate recovery calculation instead of generating individual curves for each surrogate. Peak integration of all analytes is reviewed and compounds with branched and linear isomers may require manual integration.

Table 9-1. Analytes with calibration curve fit and surrogates used as the internal standard

Surrogate Standards

Surrogate	Internal Standard	Calibration Curve Type	Weighting
M3PFBA	N-EtFOSAA	mean response factor	none
M3PFPeA	N-EtFOSAA	mean response factor	none
M5PFHxA	N-EtFOSAA	mean response factor	none
M8PFOA	N-EtFOSAA	mean response factor	none
MPFUdA	N-EtFOSAA	mean response factor	none
MPFDoA	N-EtFOSAA	mean response factor	none
M2PFTeDA	N-EtFOSAA	mean response factor	none
M3PFBS	N-EtFOSAA	mean response factor	none
M3PFHxS	N-EtFOSAA	mean response factor	none
M8PFOS	N-EtFOSAA	mean response factor	none
M3HFPO	N-EtFOSAA	mean response factor	none
M8FOSA	N-EtFOSAA	mean response factor	none
13C2,D4 4:2 FTS	N-EtFOSAA	mean response factor	none
13C2,D4 6:2 FTS	N-EtFOSAA	mean response factor	none
13C2,D4 8:2 FTS	N-EtFOSAA	mean response factor	none
13C2,D4 10:2 FTS	N-EtFOSAA	mean response factor	none

Native Standards

Native	Surrogate Standard	Calibration Curve Type	Weighting
PFBA	M3PFBA	Linear	1/x
PFPeA	M3PFPeA	Linear	1/x
PFHxA	M5PFHxA	Linear	1/x
PFHpA	M5PFHxA	Linear	1/x
PFOA	M8PFOA	Linear	1/x
PFNA	M8PFOA	Linear	1/x
PFDA	M8PFOA	Linear	1/x
PFUdA	MPFUdA	Linear	1/x
PFDoA	MPFDoA	Linear	1/x
PFTrDA	MPFDoA	Linear	1/x
PFTeDA	M2PFTeDA	Linear	1/x
PFBS	M3PFBS	Linear	1/x
PFPeS	M3PFHxS	Linear	1/x
PFHxS	M3PFHxS	Linear	1/x
PFHpS	M3PFHxS	Linear	1/x
PFOS	M8PFOS	Linear	1/x
PFNS	M8PFOS	Linear	1/x
PFDS	M8PFOS	Linear	1/x
PFUdS	MPFUdA	Linear	1/x
PFDoS	MPFDoA	Linear	1/x
PFTrDS	M2PFTeDA	Linear	1/x
FOSA	M8FOSA	Linear	1/x
DONA	M8PFOA	Linear	1/x
HFPO-DA	M3HFPO	Linear	1/x
9Cl-PF3ONS	M3PFHxS	Linear	1/x
*11Cl-PF3OUdS	M3PFHxS *MPFDoA for bread	Linear	1/x
4:2 FTS	13C2,D4 4:2 FTS	Linear	1/x
6:2 FTS	13C2,D4 6:2 FTS	Linear	1/x
8:2 FTS	13C2,D4 8:2 FTS	Linear	1/x
10:2 FTS	13C2,D4 10:2 FTS	Linear	1/x

2024.9.1 Corrections for salts and technical mixture

The custom mix from Absolute Standards was obtained with assigned values in anion concentrations. Therefore, no salt corrections are necessary.

2024.10. Quality Control and Confirmation Criteria

2024.10.1 Quality Control Criteria

(1) The r^2 values for all calibration curves must be ≥ 0.990.
(2) Matrix Spike and Matrix Spike Duplicate Recoveries: 40-120%, Relative Percent Difference (RPD%) must be < 22%.
(3) ICV and CCV Recoveries: 70-130% of native analytes.
(4) When performing spike/recovery experiments for validation, if no acceptable matrix blank is available, then matrix blanks can be subtracted for recovery calculations.
(5) All measures should be taken to reduce method blanks from background contamination in solvents, lab equipment, etc. If there is unavoidable background contamination, subtraction of method blanks may be performed by subtracting out the concentration of the method blank from the concentration of the sample.

2024.10.2 Confirmation Criteria

(1) The ion ratio(s) of the diagnostic ions shall correspond to those in the calibration points of the same sequence with +/- 30% relative tolerance.
(2) The relative retention time matching should be ≤ 5% relative to the retention time in the standard as outlined in the CVM Guidance for Industry 118.[2]
(3) The S/N ratio of the confirmation transition for the lowest calibration point (0.01 ng/mL) must be ≥ 3.
(4) In the case of PFBA and PFPeA where no confirmatory transition is available, all positive detects must be quantified using LC-HRMS.

2024.10.3 Duplicate Analysis Requirements

Samples should be analyzed in duplicate when confirmation is required for regulatory analysis. A percent relative difference should be calculated for the two extracts and the percent difference should be less than 20%.

2024.11 Instrument Performance Criteria Evaluation

Instruments must achieve the following performance criteria to successfully run the method. Three replicates of the protocol below should be completed prior to implementing the method in your laboratory.

(1) Prepare a spike of the lowest calibration point (0.01 ng/mL).
(2) Analyze the samples using the parameters included in this compendial method on your instrumentation.
(3) Determine the signal to noise ratio of the confirmatory transition using instrument software. This technique may vary among vendors. This must be greater than or equal to 3.

2024.12 Validation Information/Status

2024.12.1 Accuracy and Precision Data

Use of this method for the determination of PFAS in food has been previously validated with acceptable results. Per the Guidelines for the Validation of Chemical Methods for the FDA Foods Program, acceptable recovery percentages should fall between 40 and 120% with $\%RSD_r$ less than 22%.[1]

2024.12.2 Single Laboratory Validation

A Level 2, Single Laboratory Validation was conducted under the Guidelines for the Validation of Chemical Methods for the FDA Foods Program, 3rd Edition.[1] A total of 9 different types of foods and beverages were evaluated. These included lettuce, milk, salmon, bread, eggs, clams, blueberries, silage, and corn snaplage. The method was validated at 4 concentrations (0.15, 1, 5 and 15 µg/kg) in triplicate in 7 food matrices and 2 feed matrices. Acceptable recovery ranges based on the CODEX criteria in Appendix 2 of the FDA Guidelines for the Validation of Chemical Methods for the FDA Foods Program are 40-120% for native analytes at a method level of 1 ng/mL. The following analyte/matrix combinations did not meet the criteria for validation (minimum of three spike levels): PFTrDS in eggs, PFDS in bread, and PFTrDA in silage. Recovery and precision data can be found in Appendix Table 1A for Sciex QTRAP® 6500+ data and Appendix 1B for HRMS data. Average surrogate recovery data can be found in Appendix 1C.

2024.12.3 Method Detection Limits

Method detection limits were calculated by performing a minimum of 7 low-level spikes at 0.05 ng/g for all food and feed matrices. The standard deviation of the replicates was multiplied by 3.14 (t-value for seven replicates where 1-α =0.99). The MDL is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the measured concentration is distinguishable from method blank results. This procedure is published in the Code of Federal Regulations.[3] MDLs are recalculated yearly and are dependent on instrumental conditions at the time of the study. The limit of quantification (LOQ) can be calculated by multiplying the standard deviation of the replicates by 10. Both the MDLs and LOQs can be found in Appendix 1D.

2024.13 References

(1) FDA Guidelines for the Validation of Chemical Methods for the FDA Foods Program; http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM298730.pdf.

(2) Guidance for Industry: Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues; Availability. 68 FR 25617. https://www.federalregister.gov/documents/2003/05/13/03-11771/guidance-for-industry-mass-spectrometry-for-confirmation-of-the-identity-of-animal-drug-residues

(3) Definition and Procedure for the Determination of the Method Detection Limit, Revision 2. 40 CFR Appendix B to Part 136. Washington (DC). https://www.epa.gov/cwa-methods appB

Appendix I: Single Laboratory Validation Data

Appendix Table 1A: Single laboratory validation recoveries and precision data (% RSD) using the Sciex QTRAP® 6500+. Shaded values indicate the analyte did not meet the criteria for validation in that matrix since the recovery was not within the acceptable range. Precision values greater than 22% were also shaded. A co-eluting interference (INT) was identified in bread, corn, and silage matrices with 4:2 FTS. Recoveries of PFOS in eggs indicate the recovery reported after the cholic acid clean-up step.

lettuce average (% RSD)	0.15 µg/kg	1 µg/kg	5 µg/kg	15 µg/kg
PFBA	90 (4)	95 (6)	96 (3)	107 (2)
PFPeA	94 (2)	101 (4)	97 (3)	101 (1)
PFHxA	104 (8)	86 (5)	86 (4)	93 (1)
PFHpA	89 (4)	90 (9)	87 (6)	93 (1)
PFOA	114 (3)	90 (6)	93 (1)	97 (3)
PFNA	95 (1)	88 (5)	93 (3)	104 (3)
PFDA	99 (2)	86 (6)	83 (3)	103 (0)
PFUdA	96 (8)	87 (2)	90 (2)	99 (2)
PFDoA	107 (2)	100 (3)	96 (3)	113 (3)
PFTrDA	49 (4)	89 (1)	84 (5)	78 (5)
PFTeDA	100 (1)	93 (3)	100 (6)	100 (2)
PFBS	104 (3)	96 (3)	95 (1)	100 (1)
PFPeS	89 (5)	93 (2)	93 (3)	95 (1)
PFHxS	98 (5)	92 (3)	93 (2)	98 (4)
PFHpS	111 (3)	92 (1)	92 (3)	98 (4)
PFOS	72 (6)	86 (4)	96 (4)	90 (1)
PFNS	97 (9)	92 (4)	94 (4)	99 (4)
PFDS	93 (4)	81 (4)	86 (2)	95 (2)
PFUdS	109 (6)	113 (5)	103 (3)	130 (2)
PFDoS	99 (3)	115 (2)	101 (4)	89 (3)
PFTrDS	104 (10)	118 (5)	104 (6)	99 (6)
HFPO-DA	99 (4)	103 (7)	97 (1)	106 (4)
DONA	98 (1)	92 (6)	96 (2)	99 (2)
9Cl-PF3ONS	92 (4)	87 (4)	85 (2)	99 (7)
11Cl-PF3OUdS	94 (1)	79 (5)	80 (3)	96 (8)
4:2FTS	104 (8)	80 (6)	96 (10)	104 (1)
6:2FTS	106 (4)	93 (9)	90 (3)	97 (1)
8:2FTS	103 (8)	91 (8)	90 (2)	102 (4)
10:2FTS	92 (7)	95 (6)	98 (4)	88 (1)
FOSA	94 (5)	99 (7)	98 (3)	96 (2)

blueberries average (% RSD)	0.15 µg/kg	1 µg/kg	5 µg/kg	15 µg/kg
PFBA	94 (5)	88 (1)	98 (13)	98 (1)
PFPeA	85 (15)	92 (3)	99 (12)	97 (4)
PFHxA	68 (2)	79 (4)	85 (13)	84 (5)
PFHpA	81 (9)	79 (2)	89 (13)	86 (7)
PFOA	97 (7)	88 (1)	94 (12)	93 (7)
PFNA	83 (2)	88 (3)	93 (12)	94 (8)
PFDA	74 (4)	73 (2)	76 (12)	86 (11)
PFUdA	100 (9)	89 (7)	94 (13)	93 (11)
PFDoA	97 (4)	89 (4)	96 (15)	114 (2)
PFTrDA	109 (6)	94 (4)	101 (15)	81 (6)
PFTeDA	74 (7)	104 (0)	98 (11)	100 (4)
PFBS	97 (7)	93 (2)	96 (14)	99 (3)
PFPeS	86 (10)	87 (3)	93 (12)	93 (4)
PFHxS	93 (6)	90 (1)	94 (14)	96 (4)
PFHpS	85 (11)	86 (1)	92 (12)	96 (4)
PFOS	79 (5)	105 (4)	97 (12)	91 (5)
PFNS	90 (2)	82 (2)	88 (12)	88 (8)
PFDS	81 (10)	76 (1)	81 (12)	90 (2)
PFUdS	104 (6)	99 (2)	111 (6)	95 (16)
PFDoS	106 (2)	105 (1)	117 (9)	90 (8)
PFTrDS	106 (7)	109 (6)	111 (5)	95 (9)
HFPO-DA	104 (8)	93 (5)	99 (11)	103 (2)
DONA	99 (8)	92 (1)	96 (13)	95 (4)
9Cl-PF3ONS	68 (2)	72 (1)	76 (14)	95 (10)
11Cl-PF3OUdS	74 (3)	65 (1)	70 (14)	84 (18)
4:2FTS	81 (4)	90 (4)	97 (15)	94 (5)
6:2FTS	82 (8)	90 (2)	96 (12)	96 (4)
8:2FTS	85 (7)	96 (2)	96 (10)	96 (8)
10:2FTS	70 (19)	89 (5)	96 (11)	90 (6)
FOSA	105 (8)	94 (1)	99 (13)	100 (9)

chocolate milk average (% RSD)	0.15 µg/kg	1 µg/kg	5 µg/kg	15 µg/kg
PFBA	91 (8)	61 (5)	80 (1)	107 (2)
PFPeA	92 (4)	94 (3)	89 (2)	102 (0)
PFHxA	103 (10)	95 (2)	82 (3)	91 (1)
PFHpA	95 (4)	96 (4)	85 (3)	93 (1)
PFOA	113 (4)	93 (2)	90 (1)	99 (1)
PFNA	100 (3)	96 (1)	89 (4)	94 (4)
PFDA	92 (4)	83 (3)	79 (7)	93 (9)
PFUdA	96 (8)	97 (5)	91 (2)	91 (21)
PFDoA	105 (7)	80 (5)	90 (6)	115 (3)
PFTrDA	91 (14)	75 (5)	79 (5)	96 (5)
PFTeDA	111 (3)	74 (5)	93 (4)	112 (3)
PFBS	101 (3)	94 (3)	92 (2)	101 (0)
PFPeS	102 (11)	93 (2)	90 (2)	95 (1)
PFHxS	102 (5)	87 (2)	89 (2)	100 (2)
PFHpS	100 (8)	86 (1)	90 (2)	100 (2)
PFOS	68 (10)	81 (7)	88 (2)	91 (5)
PFNS	92 (5)	82 (1)	84 (0)	91 (2)
PFDS	84 (6)	81 (3)	83 (2)	87 (8)
PFUdS	81 (6)	100 (8)	100 (3)	99 (9)
PFDoS	59 (18)	69 (9)	75 (7)	87 (4)
PFTrDS	99 (9)	63 (6)	68 (2)	82 (4)
HFPO-DA	97 (4)	100 (5)	94 (1)	110 (2)
DONA	102 (3)	95 (3)	91 (2)	98 (1)
9Cl-PF3ONS	91 (1)	80 (1)	81 (3)	99 (2)
11Cl-PF3OUdS	83 (7)	82 (0)	79 (3)	83 (7)
4:2FTS	101 (5)	107 (4)	99 (3)	101 (0)
6:2FTS	109 (2)	113 (3)	97 (5)	104 (2)
8:2FTS	98 (14)	112 (2)	91 (2)	111 (5)
10:2FTS	95 (4)	86 (5)	92 (4)	87 (11)
FOSA	98 (6)	88 (4)	91 (1)	97 (5)

eggs average (% RSD)	0.15 µg/kg	1 µg/kg	5 µg/kg	15 µg/kg
PFBA	86 (4)	95 (4)	92 (4)	92 (9)
PFPeA	81 (5)	98 (1)	92 (2)	99 (2)
PFHxA	88 (7)	98 (1)	91 (4)	99 (1)
PFHpA	93 (15)	94 (1)	94 (2)	104 (1)
PFOA	92 (15)	94 (2)	92 (1)	100 (4)
PFNA	87 (10)	96 (4)	91 (2)	95 (8)
PFDA	87 (18)	91 (2)	86 (7)	96 (4)
PFUdA	85 (7)	104 (5)	95 (4)	92 (1)
PFDoA	94 (7)	96 (9)	98 (1)	119 (1)
PFTrDA	71 (13)	67 (7)	62 (7)	79 (5)
PFTeDA	97 (6)	111 (1)	112 (6)	104 (3)
PFBS	91 (5)	94 (1)	91 (3)	101 (1)
PFPeS	75 (16)	89 (4)	87 (3)	95 (6)
PFHxS	86 (4)	94 (3)	91 (3)	99 (3)
PFHpS	92 (5)	95 (4)	89 (3)	99 (3)
PFOS	93 (5)	97 (2)	94 (2)	90 (3)
PFNS	82 (8)	89 (1)	85 (3)	96 (5)
PFDS	76 (8)	82 (2)	76 (5)	82 (1)
PFUdS	81 (8)	100 (9)	80 (4)	137 (12)
PFDoS	72 (16)	67 (15)	59 (3)	108 (11)
PFTrDS	146 (21)	130 (13)	123 (3)	131 (10)
HFPO-DA	91 (12)	94 (1)	94 (3)	104 (2)
DONA	90 (9)	102 (0)	98 (1)	98 (7)
9Cl-PF3ONS	80 (7)	81 (5)	77 (4)	100 (7)
11Cl-PF3OUdS	70 (2)	77 (2)	71 (3)	78 (7)
4:2FTS	75 (14)	93 (2)	90 (3)	97 (1)
6:2FTS	77 (13)	97 (1)	97 (2)	99 (3)
8:2FTS	82 (6)	89 (1)	87 (1)	93 (3)
10:2FTS	71 (9)	91 (5)	86 (3)	96 (3)
FOSA	88 (4)	97 (4)	91 (2)	90 (5)

salmon average (% RSD)	0.15 µg/kg	1 µg/kg	5 µg/kg	15 µg/kg
PFBA	62 (7)	80 (5)	87 (9)	101 (4)
PFPeA	76 (7)	88 (2)	90 (6)	100 (1)
PFHxA	75 (8)	79 (1)	81 (7)	93 (1)
PFHpA	79 (6)	85 (4)	86 (5)	95 (3)
PFOA	85 (4)	88 (2)	87 (8)	101 (3)
PFNA	79 (6)	91 (2)	92 (9)	100 (3)
PFDA	68 (7)	84 (7)	87 (7)	105 (6)
PFUdA	82 (6)	89 (1)	88 (8)	89 (15)
PFDoA	92 (12)	90 (6)	92 (7)	115 (2)
PFTrDA	57 (20)	73 (13)	75 (11)	73 (6)
PFTeDA	90 (8)	102 (6)	94 (4)	100 (4)
PFBS	95 (3)	91 (2)	91 (11)	101 (1)
PFPeS	92 (1)	93 (3)	87 (6)	93 (2)
PFHxS	88 (8)	93 (3)	90 (8)	98 (2)
PFHpS	81 (13)	91 (3)	88 (7)	98 (2)
PFOS	113 (5)	85 (1)	86 (8)	94 (7)
PFNS	91 (4)	90 (4)	90 (8)	96 (8)
PFDS	72 (15)	79 (6)	82 (8)	91 (11)
PFUdS	65 (11)	101 (4)	100 (13)	89 (9)
PFDoS	60 (18)	80 (8)	82 (7)	67 (7)
PFTrDS	139 (9)	107 (5)	101 (7)	98 (0)
HFPO-DA	76 (10)	88 (4)	88 (11)	106 (1)
DONA	80 (6)	88 (3)	89 (9)	97 (4)
9Cl-PF3ONS	87 (2)	87 (2)	85 (7)	102 (1)
11Cl-PF3OUdS	62 (8)	77 (3)	80 (10)	81 (8)
4:2FTS	68 (14)	93 (2)	91 (5)	97 (1)
6:2FTS	76 (17)	90 (6)	88 (7)	97 (1)
8:2FTS	75 (6)	91 (2)	91 (5)	101 (7)
10:2FTS	87 (13)	91 (5)	88 (6)	95 (5)
FOSA	86 (9)	89 (1)	88 (8)	96 (2)

clams average (% RSD)	0.15 µg/kg	1 µg/kg	5 µg/kg	15 µg/kg
PFBA	66 (6)	85 (2)	96 (1)	93 (7)
PFPeA	74 (3)	95 (1)	94 (1)	98 (1)
PFHxA	78 (17)	84 (3)	80 (1)	84 (1)
PFHpA	98 (18)	92 (2)	87 (1)	87 (1)
PFOA	90 (3)	97 (2)	97 (1)	96 (1)
PFNA	86 (2)	99 (1)	99 (1)	91 (3)
PFDA	76 (5)	89 (1)	87 (3)	91 (10)
PFUdA	89 (4)	86 (3)	86 (3)	83 (1)
PFDoA	84 (9)	89 (5)	87 (3)	109 (9)
PFTrDA	57 (6)	73 (6)	68 (2)	76 (14)
PFTeDA	105 (12)	99 (2)	93 (4)	99 (6)
PFBS	102 (0)	94 (1)	94 (1)	99 (1)
PFPeS	101 (2)	93 (5)	87 (2)	91 (2)
PFHxS	106 (4)	94 (3)	92 (2)	95 (1)
PFHpS	101 (2)	93 (6)	91 (1)	95 (1)
PFOS	104 (4)	97 (2)	96 (3)	89 (4)
PFNS	97 (4)	90 (3)	90 (1)	98 (6)
PFDS	95 (5)	88 (4)	88 (3)	90 (4)
PFUdS	103 (7)	103 (12)	100 (2)	93 (4)
PFDoS	76 (3)	76 (9)	67 (2)	73 (8)
PFTrDS	162 (2)	105 (2)	92 (9)	87 (6)
HFPO-DA	107 (5)	101 (3)	101 (5)	103 (1)
DONA	101 (1)	99 (2)	97 (1)	96 (2)
9Cl-PF3ONS	94 (5)	85 (5)	86 (1)	96 (6)
11Cl-PF3OUdS	89 (7)	82 (3)	82 (1)	92 (8)
4:2FTS	68 (4)	96 (2)	95 (1)	95 (1)
6:2FTS	65 (5)	94 (2)	96 (1)	96 (2)
8:2FTS	80 (10)	99 (3)	97 (2)	95 (6)
10:2FTS	77 (15)	96 (3)	99 (2)	92 (4)
FOSA	56 (11)	98 (1)	96 (2)	99 (2)

bread average (% RSD)	0.15 µg/kg	1 µg/kg	5 µg/kg	15 µg/kg
PFBA	99 (9)	87 (4)	95 (10)	108 (0)
PFPeA	109 (1)	90 (4)	101 (8)	98 (0)
PFHxA	88 (3)	76 (4)	87 (9)	86 (1)
PFHpA	86 (4)	77 (3)	86 (9)	84 (2)
PFOA	104 (4)	87 (2)	95 (11)	94 (3)
PFNA	88 (5)	76 (4)	85 (13)	78 (2)
PFDA	62 (13)	49 (6)	57 (18)	63 (5)
PFUdA	97 (11)	82 (6)	92 (6)	77 (20)
PFDoA	110 (7)	76 (11)	83 (9)	87 (16)
PFTrDA	65 (11)	48 (16)	48 (23)	35 (6)
PFTeDA	110 (12)	68 (14)	82 (11)	65 (8)
PFBS	106 (5)	97 (5)	106 (10)	100 (0)
PFPeS	101 (4)	87 (6)	96 (9)	98 (2)
PFHxS	108 (3)	87 (9)	98 (10)	98 (1)
PFHpS	98 (6)	86 (7)	96 (10)	98 (1)
PFOS	96 (2)	95 (4)	105 (8)	93 (5)
PFNS	65 (4)	58 (4)	66 (12)	66 (7)
PFDS	31 (5)	34 (4)	40 (13)	42 (6)
PFUdS	37 (22)	58 (8)	54 (6)	42 (7)
PFDoS	44 (8)	45 (18)	44 (23)	36 (9)
PFTrDS	97 (15)	68 (5)	68 (19)	56 (4)
HFPO-DA	100 (4)	99 (6)	104 (12)	102 (3)
DONA	113 (5)	101 (1)	109 (9)	105 (3)
9Cl-PF3ONS	95 (6)	64 (6)	72 (15)	94 (6)
11Cl-PF3OUdS	78 (13)	80 (6)	85 (5)	66 (8)
4:2FTS	99 (8)	92 (5)	96 (9)	95 (1)
6:2FTS	104 (5)	89 (4)	98 (10)	93 (1)
8:2FTS	98 (17)	86 (8)	98 (7)	97 (5)
10:2FTS	83 (3)	73 (13)	88 (13)	77 (2)
FOSA	89 (8)	85 (3)	95 (10)	90 (7)

silage average (% RSD)	0.15 µg/kg	1 µg/kg	5 µg/kg	15 µg/kg
PFBA	95 (6)	91 (9)	97 (3)	95 (0)
PFPeA	75 (8)	76 (6)	86 (1)	92 (1)
PFHxA	111 (6)	65 (8)	75 (3)	82 (3)
PFHpA	94 (11)	48 (2)	76 (1)	88 (4)
PFOA	96 (9)	74 (3)	87 (2)	91 (3)
PFNA	90 (17)	68 (4)	90 (0)	91 (3)
PFDA	85 (17)	44 (12)	76 (5)	86 (2)
PFUdA	87 (12)	56 (3)	85 (1)	91 (1)
PFDoA	91 (19)	61 (4)	84 (6)	90 (3)
PFTrDA	52 (21)	26 (38)	47 (7)	173 (4)
PFTeDA	98 (17)	86 (5)	98 (3)	96 (4)
PFBS	90 (12)	68 (2)	91 (1)	92 (1)
PFPeS	88 (21)	60 (1)	88 (1)	91 (3)
PFHxS	83 (14)	66 (0)	93 (2)	94 (3)
PFHpS	99 (12)	62 (6)	90 (2)	98 (2)
PFOS	102 (10)	89 (2)	97 (1)	89 (1)
PFNS	86 (12)	60 (3)	86 (2)	91 (1)
PFDS	87 (13)	54 (6)	80 (5)	88 (2)
PFUdS	78 (14)	70 (8)	78 (8)	60 (5)
PFDoS	57 (17)	42 (27)	53 (5)	53 (5)
PFTrDS	88 (11)	112 (7)	102 (3)	102 (3)
HFPO-DA	95 (12)	57 (7)	95 (2)	97 (1)
DONA	102 (17)	59 (1)	88 (1)	93 (2)
9Cl-PF3ONS	88 (8)	56 (5)	79 (0)	91 (2)
11Cl-PF3OUdS	85 (14)	48 (13)	73 (7)	80 (2)
4:2FTS	**INT** (12)	62 (5)	102 (4)	107 (3)
6:2FTS	91 (13)	49 (6)	81 (0)	92 (1)
8:2FTS	96 (14)	66 (5)	88 (0)	89 (5)
10:2FTS	95 (12)	80 (4)	97 (2)	100 (3)
FOSA	91 (18)	65 (3)	91 (0)	94 (2)

INT: ***co-eluting matrix interference***

corn snaplage average (% RSD)	0.15 µg/kg	1 µg/kg	5 µg/kg	15 µg/kg
PFBA	81 (7)	98 (1)	97 (2)	94 (1)
PFPeA	92 (4)	76 (3)	88 (0)	92 (1)
PFHxA	95 (2)	78 (2)	80 (2)	87 (1)
PFHpA	100 (3)	71 (1)	80 (1)	90 (1)
PFOA	111 (4)	72 (4)	91 (2)	90 (1)
PFNA	112 (2)	66 (2)	92 (1)	90 (2)
PFDA	105 (5)	61 (2)	86 (4)	89 (2)
PFUdA	97 (9)	60 (3)	84 (4)	88 (1)
PFDoA	98 (7)	66 (6)	81 (2)	88 (2)
PFTrDA	81 (7)	32 (16)	64 (2)	55 (4)
PFTeDA	100 (6)	98 (2)	98 (4)	94 (1)
PFBS	114 (5)	71 (0)	90 (1)	91 (2)
PFPeS	103 (11)	68 (0)	91 (1)	92 (2)
PFHxS	96 (6)	67 (4)	93 (1)	92 (3)
PFHpS	112 (12)	67 (1)	91 (2)	93 (1)
PFOS	103 (7)	72 (2)	91 (1)	92 (1)
PFNS	100 (4)	66 (3)	91 (1)	90 (3)
PFDS	97 (2)	61 (4)	83 (1)	84 (3)
PFUdS	112 (5)	51 (1)	69 (1)	76 (5)
PFDoS	100 (7)	43 (2)	62 (5)	58 (10)
PFTrDS	92 (10)	93 (4)	105 (2)	105 (9)
HFPO-DA	99 (4)	70 (4)	92 (3)	93 (1)
DONA	110 (1)	67 (3)	91 (1)	92 (1)
9Cl-PF3ONS	105 (4)	58 (1)	79 (1)	90 (4)
11Cl-PF3OUdS	96 (3)	56 (2)	75 (1)	76 (3)
4:2FTS	111 (9)	59 (7)	92 (4)	100 (1)
6:2FTS	111 (3)	85 (3)	85 (1)	83 (3)
8:2FTS	108 (4)	74 (1)	91 (1)	89 (3)
10:2FTS	111 (7)	91 (1)	99 (3)	94 (4)
FOSA	113 (4)	70 (2)	91 (1)	89 (2)

Appendix 1B. Single laboratory validation average recoveries and precision data (% RSD) for PFBA and PFPeA using LC-HRMS.

PFBA	**1 µg/kg**	**5 µg/kg**	**15 µg/kg**
lettuce	115 (19)	94 (3)	93 (6)
blueberries	78 (9)	91 (12)	88 (5)
chocolate milk	104 (9)	93 (4)	88 (5)
eggs	99 (2)	91 (4)	96 (3)
salmon	83 (2)	88 (12)	93 (4)
clams	93 (1)	87 (1)	91 (2)
bread	84 (11)	93 (10)	94 (2)
silage	81 (7)	88 (4)	86 (4)
corn snaplage	131 (9)	97 (3)	87 (1)

PFPeA	**1 µg/kg**	**5 µg/kg**	**15 µg/kg**
lettuce	99 (15)	91 (4)	96 (4)
blueberries	89 (6)	97 (11)	94 (4)
chocolate milk	114 (4)	95 (4)	94 (2)
eggs	89 (3)	89 (3)	95 (1)
salmon	84 (4)	88 (11)	97 (3)
clams	91 (9)	92 (3)	90 (5)
bread	90 (3)	99 (10)	95 (4)
silage	90 (9)	95 (1)	90 (2)
corn snaplage	132 (11)	104 (3)	94 (0)

Appendix 1C. Average surrogate recoveries of spiked sample matrices

Average surrogate recoveries	lettuce	blueberries	chocolate milk	egg	salmon	clam	bread	silage	corn snaplage
M3 PFBA	62	64	59	43	54	66	95	53	46
M3 PFPeA	65	69	63	46	60	71	71	64	52
M5PFHxA	67	72	66	48	64	74	71	65	54
13C PFOA	70	76	69	52	72	79	70	68	55
MFUdA	69	61	63	46	72	74	29	61	53
MPFDoA	69	60	56	41	66	71	22	60	54
MPFTeDA	58	52	29	16	36	38	12	22	25
M3 PFBS	80	83	79	76	89	89	77	83	65
M3 PFHxS	83	87	78	80	95	94	76	79	59
13C PFOS	82	78	75	75	96	88	63	76	58
M3 HFPO	68	74	70	56	71	76	72	66	52
M8 FOSA	86	57	68	71	79	64	43	62	42
13C2D4 4:2 FTS	60	58	53	30	43	58	86	69	69
13C2D4 6:2 FTS	55	63	55	33	55	64	101	81	86
13C2D4 8:2 FTS	62	59	53	31	58	64	55	70	75
13C2D4 10:2 FTS	120	55	50	27	112	65	28	94	75

Appendix 1D. Method detection limits (MDLs) ng/kg and limits of quantification (LOQs). Quantitative data are only reported if the value is also above the calibration curve lower limit of quantification (20 ng/kg)

MDLs (ng/kg)	lettuce	blueberries	chocolate milk	eggs	salmon	clams	bread	silage	corn snaplage
PFHxA	9	19	32	19	30	32	17	54	126
PFHpA	12	10	13	29	15	8	15	11	62
PFOA	14	8	27	47	15	51	7	29	19
PFNA	15	13	14	12	27	25	14	9	21
PFDA	15	19	16	27	23	25	9	29	13
PFUdA	14	17	22	20	17	26	24	13	14
PFDoA	13	13	19	21	19	17	24	18	20
PFTrDA	18	17	34	32	43	77	29	11	14
PFTeDA	10	10	31	29	14	17	40	10	10
PFBS	6	4	5	7	4	6	8	7	9
PFPeS	18	18	18	23	21	17	16	18	18
PFHxS	6	11	7	13	15	9	19	59	15
PFHpS	12	22	18	22	11	19	21	11	27
PFOS	14	15	9	19	16	14	16	14	18
PFNS	10	8	4	10	10	5	10	8	12
PFDS	8	8	12	26	9	9	15	18	8
PFUdS	4	8	10	28	16	9	13	25	18
PFDoS	12	9	23	33	13	10	7	22	26

PFTrDS	10	10	23	35	8	10	6	14	35
HFPO-DA	10	14	21	19	19	10	13	8	16
DONA	8	5	3	37	2	5	13	5	10
9Cl-PF3ONS	10	11	7	7	9	7	9	12	11
11Cl-PF3OUdS	6	9	10	13	22	11	17	19	12
4:2FTS	34	12	12	13	23	8	***35***	***481***	***28***
6:2FTS	18	15	21	31	15	25	11	35	37
8:2FTS	13	17	11	38	9	14	10	13	19
10:2FTS	11	17	21	45	11	12	19	6	13
FOSA	7	14	14	13	31	8	23	8	12

LOQs (ng/kg)	**lettuce**	**blueberries**	**chocolate milk**	**eggs**	**salmon**	**clams**	**bread**	**silage**	**corn snaplage**
PFHxA	31	64	109	67	104	109	60	181	421
PFHpA	43	33	45	99	51	26	53	36	206
PFOA	47	27	92	161	53	177	24	96	65
PFNA	51	46	49	40	94	86	48	31	68
PFDA	51	66	57	92	79	88	32	96	44
PFUdA	48	59	76	71	60	89	85	42	47
PFDoA	44	43	66	72	64	58	84	61	68
PFTrDA	61	59	116	111	148	267	102	38	45
PFTeDA	36	35	108	100	47	57	140	34	34
PFBS	22	14	17	23	13	20	28	23	30
PFPeS	63	61	63	80	73	60	56	61	60
PFHxS	22	37	24	44	52	32	64	197	50
PFHpS	40	75	62	75	39	66	72	38	89
PFOS	48	52	31	66	56	49	56	47	60
PFNS	34	27	14	35	33	17	34	28	40
PFDS	28	27	41	90	30	32	52	61	26
PFUdS	13	27	35	95	57	30	45	85	61
PFDoS	43	32	80	114	44	36	25	72	88
PFTrDS	34	36	80	122	28	33	21	47	117
HFPO-DA	36	49	72	67	66	34	44	25	52
DONA	28	17	10	128	7	17	46	18	33
9Cl-PF3ONS	35	37	23	24	31	24	30	40	37
11Cl-PF3OUdS	20	32	33	44	77	37	58	64	39
4:2FTS	117	41	42	43	78	29	***121***	***1603***	***92***
6:2FTS	62	52	74	106	51	85	38	117	122
8:2FTS	45	60	39	131	31	49	35	43	65
10:2FTS	37	59	74	154	38	41	66	19	43
FOSA	25	50	47	45	106	28	81	28	41

co-eluting matrix interference