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METHOD TITLE: Determination of 16 Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) in Food using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

VALIDATION STATUS: Single-laboratory validation per the Guidelines for the Validation of Chemical Methods for the FDA FVM Program 2nd Ed.

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METHOD SUMMARY/SCOPE:

Analyte(s): Perfluorobutanoic acid, Perfluoropentanoic acid, Perfluorohexanoic acid, Perfluoroheptanoic acid, Perfluorooctanoic Acid, Perfluorononanoic acid, Perfluorodecanoic acid, Perfluorobutanesulfonic acid, Perfluoropentanesulfonic acid, Perfluorohexanesulfonic acid, Perfluoroheptanesulfonic acid, Perfluorooctanesulfonic acid, Sodium dodecafluoro-3H-4, 8-dioxanonanoate, 2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy) propanoic acid (GenX), Potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate, 11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid

Matrices: Bread, Lettuce, Milk, and Fish

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 extraction technique is performed. The resulting extract is filtered and fortified with internal standard solution and analyzed using LC-MS/MS. Some matrices require the extract to be concentrated using nitrogen prior to addition of the internal standard solution. The PFAS compounds are identified by multiple reaction mode (MRM) transitions and retention time matching with the calibration standards. Ion ratios, when available, are used to confirm the identity. In some cases, further clean-up using solid phase extraction may be required. 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 response in method blanks must be subtracted from the sample response prior to final quantitation. After determination of the concentration from the curve, the concentration must be adjusted for dilution and starting sample mass.

REVISION HISTORY:

OTHER NOTES:

Determination of 16 Perluoroalkyl and Polyfluoroalkyl Substances (PFAS) in Food using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

Version 2019 (2019)

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GLOSSARY

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2019.1 METHOD TITLE: Determination of 16 Per and Polyfluoroalkyl Substances (PFAS) in Food using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

2019.2 SCOPE OF APPLICATION

The method describes a procedure for measuring 16 PFAS in food using LC-MS/MS. The method has been single laboratory validated in the following food matrices:

Matrices	Validation	Date	Analyst
Lettuce, Milk, Bread, Fish,	Single lab validation	2019	Susan Genualdi and Wendy Young

- This method should be used by 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 should also 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 quantitation.
- The method should 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.
- 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. If PFAS compounds are determined, complete method blank results should be subtracted from samples. 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, dSPE 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 extreme low concentrations of detection required for this analysis, choice of MS/MS instrumentation is critical. Our analysis has been performed using Sciex 6500 and 6500 plus instrumentation platforms. We have not fully evaluated any Orbitrap MS systems, but preliminary investigations have not demonstrated adequate lower levels of quantitation (LLOQ) for these systems.
- The analyte 11Cl-PF3OUdS exhibits known issues with recovery in certain matrices, which may reduce the confidence in this result in certain food types.

2019.3 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 extraction technique is performed. The resulting extract is filtered and fortified with internal standard solution and analyzed using LC-MS/MS. Some matrices require the extract to be concentrated using nitrogen prior to addition of the internal standard solution. The PFAS compounds are identified by multiple reaction mode (MRM) transitions and retention time matching with the calibration standards. Ion ratios, when available, are used to confirm the identity. In some cases, further clean-up using solid phase extraction may be required. 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 response in method blanks must be subtracted from the sample response prior to final quantitation. After determination of the concentration from the curve, the concentration must be adjusted for dilution and starting sample mass.

2019.4 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.

- Formic acid, reagent grade >95%-- (Sigma Aldrich St. Louis, MO)
- LC/MS grade Optima water (Fisher Scientific, Hampton, NH)
- LC/MS grade Optima acetonitrile (Fisher Scientific, Hampton, NH)
- LC/MS grade Optima methanol (Fisher Scientific, Hampton, NH)
- Acetic acid, ammonium salt, 98% for analysis (Acros Organic, Geel, Belgium)
- Original QuEChERS extraction salt ECMSSCFS-MP with 6000 mg $MgSO_4$ and 1500 mg NaCl (UCT, Bristol, PA)
- QuEChERS dSPE ECMPSCB-MP with 900 mg $MgSO_4$, 300 mg PSA, 150 mg graphitized carbon black (UCT, Bristol, PA) or ECMPSCB15-CT prefilled units
- Ammonium hydroxide, certified ACS Plus 14.8N (Fisher Scientific, Hampton, NH)

2019.5 STANDARDS

- Isotopically labeled PFAS analytical standards (Wellington laboratories, Guelph, ON, Canada)
- Native PFAS analytical standards (Wellington laboratories, Guelph, ON, Canada)

2019.6 PREPARATION OF CALIBRATION STOCK SOLUTIONS AND SOLUTIONS FOR ANALYSIS

2019.6.1 Prepare native PFAS stock solution at 1 µg/mL and 0.01 µg/mL

- Add 0.2 mL of each 50 µg/mL PFAS analytical standard (16 native compounds in Table 1) to 6.8 mL methanol. In the resulting solution, each compound has a concentration of 1 µg/mL in methanol. Individual PFAS 50 µg/mL methanol standards were purchased from Wellington, but other sources are acceptable.

- Add 0.1 mL of 1 µg/mL stock solution to 9.9 mL of methanol to produce a 0.01 µg/mL stock solution.

2019.6.2 Prepare isotopically labeled PFAS surrogate stock solution (SS) at 1 µg/mL

- Add 0.2 mL of each 50 µg/mL analytical standard (7 isotopically labeled PFAS in Table 1) to 8.6 mL methanol. Individually labeled PFAS 50 µg/mL methanol standards were purchased from Wellington but other sources are acceptable. This stock solution was used for both sample analysis and calibration curve preparation.

2019.6.3 Prepare isotopically labeled internal standard solution (IS) at 1 µg/mL

- Add 0.2 mL of N-ethyl-d5-perfluoro-1-octanesulfonamidoacetic acid (d5-N-EtFOSAA) 50 µg/mL analytical standard to 9.8 mL methanol. The individual d5-N-EtFOSAA standard was purchased from Wellington but other sources are acceptable.

2019.6.4 Prepare mobile phase A (5 mM ammonium acetate in water)

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

2019.6.5 Prepare mobile phase B (100% methanol)

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

2019.6.6 Continuing Calibration Verification (CCV) standard

- The stock solution of the 1 ng/mL calibration standard was used as the CCV standard (Table 2).

2019.6.7 0.3 % ammonium hydroxide solution in acetonitrile

- Add 6 mL of a 14.8 N ammonium hydroxide solution to 1000 mL volumetric flask and fill to volume with acetonitrile

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

Acronym	Name	CAS	Formula	MW
	Native PFAS			
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
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
NaDONA	Sodium dodecafluoro-3H-4, 8-dioxanonanoate	958445-44-8	$C_7H_5F_{12}NO_4$	395
HFPO-DA	2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy) propanoic acid (GenX)	62037-80-3	$C_6HF_{11}O_3$	330
9Cl-PF3ONS	Potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	73606-19-6	$C_8ClF_{16}KO_4S$	570
11Cl-PF3OUdS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	763051-92-9	$C_{10}HClF_{20}O_4S$	632
	Internal Standard/Surrogates			
M3 PFBA	Perfluoro-n-[2,3,4-13C3] butanoic acid			217
MPFHxA	Perfluoro-n-[1,2-13C2] hexanoic acid			316
13C PFOA	Perfluoro-n-[13C8] octanoic acid			422
M3 PFBS	Sodium perfluoro-1-[2,3,4-13C3] butane sulfonate			303
MPFHxS	Sodium perfluoro-1-hexane[18O2] sulfonate			404
13C PFOS	Sodium perfluoro-[13C8] octane sulfonate			508
M3 HFPO	2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-13C3-propanoic acid			333
d5N-EtFOSAA	N-ethyl-d5-perfluoro-1-octanesulfonamidoacetic acid			590

2019.6.8 Calibration Standards

Calibration standards are prepared at concentrations of 0.01, 0.05, 0.10, 0.50, 1.0, 5.0, 10, 25, and 50 ng/mL according to the table below.

Table 2. Calibration standard preparation

Final concentration	Native stock solution concentration	Volume of stock solution	Volume of 1 µg/mL surrogate stock solution*	Methanol	Final volume	Volume of 1 µg/mL IS stock solution*
ng/mL	µg/mL	mL	mL	mL	mL	mL
0.01	0.01	0.01	0.1	9.89	10	0.1
0.05	0.01	0.05	0.1	9.85	10	0.1
0.1	0.01	0.1	0.1	9.8	10	0.1
0.5	1	0.005	0.1	9.895	10	0.1
1	1	0.01	0.1	9.89	10	0.1
5	1	0.05	0.1	9.85	10	0.1
10	1	0.1	0.1	9.8	10	0.1
25	1	0.25	0.1	9.65	10	0.1
50	1	0.5	0.1	9.4	10	0.1

*This curve was initially created to match surrogate and internal standard concentrations (10 ng/mL) in the final extracts of samples that require N_2 concentration. If this method is going to be primarily used for samples that are not concentrated, a modification may be preferred to match the surrogate and internal standard concentrations (1 ng/mL) in these extracts.

2019.7 PREPARATION OF SAMPLES OR TEST PORTIONS

The edible portion of the food sample was collected and homogenized using an IKA tube mill with a disposable 100 mL polypropylene grinding chamber. Samples were ground at 5000 rpm for approximately 2 minutes. The minimum sample size for analysis is 5 grams. Sample composites from different brands of the same product is acceptable.

2019.8 APPARATUS/INSTRUMENTATION

Equipment:

- IKA tube mill 100 control (IKA Works Inc, Wilmington, NC)
- Digital pulse mixer/vortexer (Glas-Col, Terre Haute, IN) capable of 1500 rpm with pulse
- Sorvall legend XTR centrifuge (Thermo Fisher Scientific, Waltham, MA)
- Nitrogen evaporation system (Organomation, Berlin, MA)
- Nexera X2 (Shimadzu, Kyoto, Japan) with binary pump, degasser, autosampler, and thermostatted column compartment
- A Sciex 6500 plus QTRAP hybrid triple quadrupole/linear ion trap mass spectrometer with an electrospray ESI ion source (Sciex, Toronto, ON Canada)
- Analyst® Software version 1.6.3

Supplies:

- 100 mL polypropylene grinding chamber (IKA Works, Inc, Wilmington, NC)
- Falcon 50 mL polypropylene (PP) conical centrifuge tubes (Thermo Fisher Scientific, Waltham, MA)
- Falcon 15 mL polypropylene (PP) conical centrifuge tubes (Thermo Fisher Scientific, Waltham, MA)
- 300 µL PP autosampler vials (SUN Sri, Rockwood, TN)
- PP autosampler vial caps (SUN Sri, Rockwood, TN)
- 0.2 µm Acrodisc nylon syringe filters (Pall Corporation, Port Washington, NY)
- 5 mL PP/PE luer lock syringes (Sigma Aldrich, St. Louis, MO)
- Nano filter vials 0.2 µm nylon (Thomson Instrument Company, Oceanside, CA)
- Analytical column – 150 mm x 2.1 mm 5 µm Atlantis T3 C18 (Waters Corp, Milford, MA)
- Guard column – 2.1 mm x 5 mm, 5 um Vanguard™ Atlantis T3 (Waters Corp, Milford, MA)
- Delay column – 2.1 mm x 50 mm, 5 um Atlantis T3 (Waters Corp, Milford, MA)
- SPE cartridge – Strata™-XL-AW 100 µm Polymeric Weak Anion 200 mg / 3 mL, Tubes (Phenomenex, Torrance, CA)

2019.9 METHOD

QuEChERS (Quick, easy, cheap, effective, rugged, safe) is used for the extraction of PFAS from foods. Due to the high variability of the sample matrix, sample preparation steps are based on food type.

2019.9.1 Sample Preparation

Table 3. Sample preparation conditions based on food commodity type

Commodity	Amount of sample used:	mL of water added:	mL of CH_3CN added:	Concentrate with nitrogen?
Fruits/vegetables	5.0 g	5	10	no
Breads	5.0 g	15	10	no
Milk	5.0 mL	5	10	yes -take 5 mL of extract to 0.5 mL
Cheese	1.0 g	5	10	no
Other Dairy	5.0 g	5	10	no
Meat	5.0 g	5	10	no

- Add amount of sample based on Table 1 and commodity type to a 50 mL polypropylene (PP) centrifuge tube
- Add 10 µL of 1 µg/mL isotopically labeled surrogate standard solution to the sample.
- Add amount of LC/MS grade Optima water based on Table 3 to the 50 mL PP conical centrifuge tube
- Add 10 mL acetonitrile to the 50 mL PP conical centrifuge tube
- Add 150 µL formic acid to the 50 mL PP conical centrifuge tube
- Shake vigorously for 1 minute
- Add QuEChERS salt packet (Original extraction salt ECMSSCFS-MP from UCT with 6000 mg $MgSO_4$ and 1500 mg NaCl)
- Place on Glas-Col shaker at 1500 rpm with pulse set to 70 for 5 minutes
- Centrifuge for 5 minutes at 10000 rcf
- 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)
- Vortex/shake for 2 minutes
- Centrifuge 5 minutes at 10000 rcf
- Filter 5 mL of the extract with a 0.2 µm nylon syringe filter and transfer to a 15 mL conical centrifuge tube
- <u>For samples that do not require nitrogen concentration</u>:
    - Add 5 µL of 1 µg/mL d5-N-EtFOSAA internal standard solution to the 5 mL extract to give a final concentration of 1 ng/mL. Surrogates will also have a final concentration of 1 ng/mL in the final extract.
- <u>For samples that require nitrogen concentration</u>:
    - Concentrate to near dryness with nitrogen and reconstitute to 0.5 mL with methanol.
    - Add 5 µL of the 1 µg/mL d5-N-EtFOSAA internal standard solution to give a final concentration of 10 ng/mL in solution. Surrogates will also have a final concentration of 10 ng/mL in the final extract.
- Briefly vortex/shake.
- Transfer 100 µL to a Thomson nano filter vial with 0.2 µm nylon® filter and a PP screw cap (Sun Sri) to run using LC-MS/MS

2019.9.2 Clean-up of extract using weak anion exchange solid-phase extraction (SPE) column

Due to the complexity of food samples and the possibility of matrix interferences, any samples with a positive detection above the method detection limit for any compound was run through an additional SPE step.

- Take 1 mL of filtered QuEChERS extract and dilute to ~ 15 mL with LC Optima water in a clean 15 mL PP conical centrifuge tube
- Condition a Strata™-XL-AW 100 µm column with 9 mL of 0.3% ammonium hydroxide in acetonitrile
- Add sample to column and let pass through
- Add 5 mL of LC Optima water to wash column
- Let column dry 1 minute
- Add 4 mL of 0.3% ammonium hydroxide in acetonitrile to elute analytes into a clean 15 mL PP conical centrifuge tube
- Blow to near dryness

- Reconstitute to 1 mL with methanol and transfer to a clean PP nano filter vial

2019.9.3 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 an AB Sciex 6500 plus QTRAP.

Set up the LC-MS/MS method with the following parameters and monitor for the transitions using the information in the table below.

Table 4. MS/MS Conditions for the Monitored Transitions on a 6500 plus QTRAP

Internal Standard							
ID	**Retention Time (min)**	**Q1 mass (m/z)**	**Q3 mass (m/z)**	**DP (volts)**	**EP (volts)**	**CE (volts)**	**CXP (volts)**
NN EtFOSAA[a]	21.2	589	419	-50	-10	-30	-20
NN EtFOSAA	21.2	589	219	-50	-10	-38	-20
Surrogates							
M3 PFBA[a]	3.8	216	172	-17	-8	-12	-14
MPFHxA[a]	10.5	315	270	-13	-10	-14	-12
13C PFOA[a]	15.9	421	172	-19	-5	-25	-7
13C PFOA	15.9	421	376	-36	-8	-13	-20
M3 PFBS[a]	7.7	302	99	-85	-6	-36	-8
M3 PFBS	7.7	302	80	-88	-6	-73	-9
MPFHxS[a]	13.6	403	103	-60	-10	-81	-15
MPFHxS	13.6	403	169	-60	-10	-42	-15
13C PFOS[a]	18.1	507	80	-100	-5	-125	-15
13C PFOS	18.1	507	99	-100	-5	-100	-15
M3 HFPO[a]	11.6	332	287	-10	-8	-9	-13
M3 HFPO	11.6	332	169	-10	-8	-17	-11

Table 4. MS/MS Conditions for the Monitored Transitions on a 6500 plus QTRAP (cont.)

Natives							
ID	**Retention Time (min)**	**Q1 mass (m/z)**	**Q3 mass (m/z)**	**DP (volts)**	**EP (volts)**	**CE (volts)**	**CXP (volts)**
PFBA[a]	3.8	213	169	-10	-6	-13	-19
PFPeA[a]	7.1	263	219	-20	-8	-11	-20
PFHxA[a]	10.7	313	269	-25	-8	-14	-26
PFHpA[a]	13.7	363	319	-17	-10	-14	-26
PFOA[a]	16.2	413	219	-20	-5	-20	-20
PFOA	16.2	413	369	-20	-5	-13	-20
PFNA[a]	18.1	463	419	-38	-11	-15	-37
PFNA	18.1	463	269	-40	-5	-24	-13
PFDA[a]	19.8	513	469	-15	-10	-16	-29
PFDA	19.8	513	269	-20	-10	-26	-17
PFBS[a]	7.7	299	99	-35	-4	-36	-15
PFPeS[a]	11.1	349	99	-80	-9	-80	-12
PFPeS	11.1	349	119	-53	-10	-40	-18
PFHxS[a]	13.9	399	99	-108	-6	-84	-8
PFHxS	13.9	399	169	-66	-5	-42	-20
PFHpS[a]	16.2	449	99	-58	-8	-84	-24
PFHpS	16.2	449	169	-68	-8	-41	-27
PFOS[a]	18.1	499	80	-150	4	-120	-10
PFOS	18.1	499	99	-150	4	-100	-10
NaDONA[a]	13.9	377	251	-25	-8	-15	-20
NaDONA	13.9	377	85	-20	-7	-39	-10
HFPO-DA[a]	11.8	329	285	-8	-12	-10	-10
HFPO-DA	11.8	329	169	-7	-7	-20	-10
9Cl-PF3ONS[a]	19.1	531	351	-87	-9	-36	-28
11Cl-PF3OUdS[a]	21.9	631	451	-34	-9	-40	-12
11Cl-PF3OUdS	21.9	631	199	-20	-10	-36	-11

[a] Primary MRM transition used for quantitation

Table 5. Gradient Profile for the LC Conditions

Time (min)	Concentration of B
0.01	40%
1	40%
25	90%
25.1	40%
26.1	40%

The following conditions are for the 6500 plus Q-trap:

- Curtain gas: 40 au
- Collisionally activated dissociation (CAD) gas: medium
- Ion spray voltage: -4500 V
- Source temperature: 350 °C
- Gas 1 pressure: 50 au
- Gas 2 pressure: 50 au
- Injection volume: 5 µL
- Column temperature: 40 °C
- Flow rate: 0.30 mL/min

Run the samples using the following template:

- Blank (MeOH) injection
- Standard curve
- Blank (MeOH) injection
- 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 can typically be within 70-120 % 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 remeasured.

2019.10 CALCULATIONS

Example calculation for concentration measured on LC-MS/MS to concentration in 5 grams of food sample blown down to 0.5 mL:

- The lowest calibration curve point is 0.01 ng/mL in 0.5 mL of solution.

$$\frac{0.01\ ng}{mL} * 0.5\ mL = 0.005\ ng$$

- The amount (ng) in 0.5 mL is also the same amount (ng) in 5 mL of extract since this solution was concentrated from 5 mL to 0.5 mL. Because the total extract was 10 mL, the amount (ng) in the 10 mL extract is equal to twice that of the 5 mL extract.

$$0.005\ ng * 2 = 0.01\ ng$$

- With a 5 gram food sample, this is equivalent to 2 ng/kg in foods

$$\frac{0.01\ ng}{5\ g} = 0.002\frac{ng}{g} or\ 2\frac{ng}{kg}$$

Example calculation for concentration measured on LC-MS/MS to concentration in 5 grams of food with a final extract of 10 mL:

- The lowest calibration curve point is 0.01 ng/mL in 0.5 mL of solution.

$$\frac{0.01\ ng}{mL} * 0.5\ mL = 0.005\ ng$$

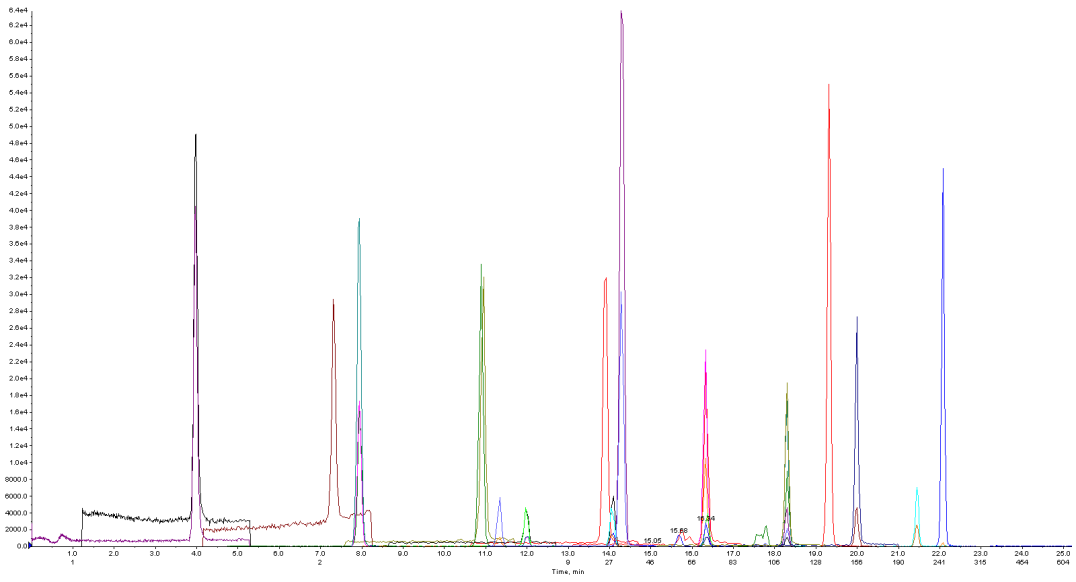
- Since there are 0.005 ng in 0.5 mL of extract, there would be 0.1 ng in the total 10 mL extract

$$0.005\ ng * \frac{10\ mL}{0.5\ mL} = 0.1\ ng$$

- With a 5 gram food sample, this is equivalent to 20 ng/kg in foods

$$\frac{0.1\ ng}{5\ g} = 0.02\frac{ng}{g} or\ 20\frac{ng}{kg}$$

An example chromatogram is included below of a calibration standard with native and labeled PFAS concentrations at 10 ng/mL.



Analyst 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 6, 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 6 with their curve fit. Adjustments are made in Analyst for the internal standard concentration. The calibration curve has surrogate and internal standard concentrations of 10 ng/mL as well as samples concentrated with nitrogen. Samples that are not concentrated with nitrogen have surrogate and internal standard concentrations of 1 ng/mL.

Table 6. Analytes with calibration curve fit and surrogates used as the internal standard

Surrogates			
M3 PFBA[a]	NN EtFOSAA[a]	mean response factor	none
MPFHxA[a]	NN EtFOSAA[a]	mean response factor	none
13C PFOA[a]	NN EtFOSAA[a]	mean response factor	none
13C PFOA	NN EtFOSAA[a]	mean response factor	none
M3 PFBS[a]	NN EtFOSAA[a]	mean response factor	none
M3 PFBS	NN EtFOSAA[a]	mean response factor	none
MPFHxS[a]	NN EtFOSAA[a]	mean response factor	none
MPFHxS	NN EtFOSAA[a]	mean response factor	none
13C PFOS[a]	NN EtFOSAA[a]	mean response factor	none
13C PFOS	NN EtFOSAA[a]	mean response factor	none
M3 HFPO[a]	NN EtFOSAA[a]	mean response factor	none
M3 HFPO	NN EtFOSAA[a]	mean response factor	none

Table 6. Analytes with calibration curve fit and surrogates used as the internal standard (cont.)

Natives			
PFBA[a]	M3 PFBA[a]	Linear	1/x
PFPeA[a]	MPFHxA[a]	Linear	1/x
PFHxA[a]	MPFHxA[a]	Linear	1/x
PFHpA[a]	MPFHxA[a]	Linear	1/x
PFOA[a]	13C PFOA[a]	Linear	1/x
PFOA	13C PFOA[a]	Linear	1/x
PFNA[a]	13C PFOA[a]	Linear	1/x
PFNA	13C PFOA[a]	Linear	1/x
PFDA[a]	13C PFOA[a]	Linear	1/x
PFDA	13C PFOA[a]	Linear	1/x
PFBS[a]	M3 PFBS[a]	Linear	1/x
PFPeS[a]	MPFHxS[a]	Linear	1/x
PFPeS	MPFHxS[a]	Linear	1/x
PFHxS[a]	MPFHxS[a]	Linear	1/x
PFHxS	MPFHxS[a]	Linear	1/x
PFHpS[a]	MPFHxS[a]	Linear	1/x
PFHpS	MPFHxS[a]	Linear	1/x
PFOS[a]	13C PFOS[a]	Linear	1/x
PFOS	13C PFOS[a]	Linear	1/x
NaDONA[a]	13C PFOA[a]	Linear	1/x
NaDONA	13C PFOA[a]	Linear	1/x
HFPO-DA[a]	M3 HFPO[a]	Linear	1/x
HFPO-DA	M3 HFPO[a]	Linear	1/x
9Cl-PF3ONS[a]	MPFHxS[a]	Linear	1/x
11Cl-PF3OUdS[a]	MPFHxS[a]	Linear	1/x
11Cl-PF3OUdS	MPFHxS[a]	Linear	1/x

[a] Primary MRM transition used for quantitation

2019.11 VALIDATION INFORMATION/STATUS

Single lab validation. A level 2 validation was conducted under the Guidelines for the Validation of Chemical Methods for the FDA FVM Program 2nd Ed. A total of 4 different types of foods and beverages were evaluated. These include produce, milk, fish, and bread. The method was validated at 6 concentrations (0.05, 0.15, 0.5, 1.5, 2, 5 ng/mL) in 4 food matrices. Acceptable recovery ranges for these compounds based on the FDA guidelines for the validation of chemical methods is 40-120% for concentrations spiked at 1 ng/mL. All compounds were within the acceptable range except for 11Cl-

PF3OUdS in bread samples which were on the lower side at 26-42% recovery. Raw data may be examined by contacting the study director.

Table 7. Single Lab validation recovery ranges. "Not validated" indicates that for this compound and matrix, the compound fell below the acceptable recovery range.

	Milk	Lettuce	Salmon	Bread	Bread with WAX clean-up
PFOA	0.05 - 5 ng/mL	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g
PFOS	0.05 - 5 ng/mL	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g
PFBA	0.15 - 5 ng/mL	0.15 - 5 ng/g	0.15 - 5 ng/g	0.5 - 5 ng/g	0.15 - 5 ng/g
PFHpS	0.05 - 5 ng/mL	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g
PFPeA	0.15 - 5 ng/mL	0.15 - 5 ng/g	0.15 - 5 ng/g	0.15 - 5 ng/g	0.5 - 5 ng/g
PFHxA	0.05 - 5 ng/mL	0.15 - 5 ng/g	0.05 - 5 ng/g	0.15 - 5 ng/g	0.05 - 5 ng/g
PFHxS	0.05 - 5 ng/mL	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g
PFHpA	0.05 - 5 ng/mL	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g
PFBS	0.05 - 5 ng/mL	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g
PFPeS	0.05 - 5 ng/mL	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g
NaDONA	0.05 - 5 ng/mL	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g
HFPO-DA	0.15 - 5 ng/mL	0.5 - 5 ng/g	0.5 - 5 ng/g	0.5 - 5 ng/g	0.5 - 5 ng/g
PFDA	0.15 - 5 ng/mL	0.15 - 5 ng/g	0.15 - 5 ng/g	0.15 - 5 ng/g	0.15 - 5 ng/g
PFNA	0.05 - 5 ng/mL	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g
11Cl-PF3OUdS	0.05 - 5 ng/mL	0.05 - 5 ng/g	0.05 - 5 ng/g	Not validated	Not validated
9Cl-PF3ONs	0.05 - 5 ng/mL	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g	0.05 - 5 ng/g

Method detection limits were calculated by performing 7 low-level spikes at 0.25 ng/mL for produce, fish, and bread and at 0.075 ng/mL for milk. 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 reported value is greater than 0. This procedure is published in the Code of Federal Regulations, see references.

Table 8. Method detection limits in ng/kg. *Note that for PFBA, no MDL could be calculated for cheese due to an interference.

ppt	PFOA	PFOS	PFBA	PFHpS	PFPeA	PFHxA	PFHxS	PFHpA	PFBS	PFPeS	NaDONA	HFPO-DA	PFDA	PFNA	11Cl-PF3OUdS	9Cl-PF3ONs
Bread	41	23	20	49	76	93	58	62	52	83	53	74	46	87	90	62
Lettuce	20	33	70	40	43	45	88	36	56	40	70	60	48	56	107	91
Salmon	90	82	66	32	44	26	59	73	21	69	95	83	43	28	90	65
Milk	42	24	29	13	15	7	17	27	14	17	22	24	28	39	28	23
Cheese	419	344	Int*	242	681	376	421	197	416	481	488	888	901	261	386	372

2019.12 REFERENCES

FDA Guidelines for the Validation of Chemical Methods for the FDA Foods Program;
https://www.fda.gov/food/laboratory-methods-food/foods-program-methods-validation-processes-and-guidelines.
(https://www.fda.gov/media/81810/download)

Definition and procedure for the determination of the method detection limit-revision 1.11. Code of Federal Regulations. 40 CFR Appendix B to Part 136. Washington (DC). https://www.govinfo.gov/app/details/CFR-2011-title40-vol23/CFR-2011-title40-vol23-part136-appB