Summary of FDA Method C-010.01:

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

UCT Part Numbers:

ECMSSCFS-MP
Mylar pouch containing 6g $MgSO_4$ and 1.5g NaCl

ECMPSCB15CT
15mL dSPE tube with 900mg $MgSO_4$, 300mg PSA and 150mg GCB

or

ECMPSCB-MP
Mylar Pouch with 900mg $MgSO_4$, 300mg PSA and 150mg GCB

organofluorine compounds that have been widely used in industrial applications and consumer products such as non-stick cookware, food packaging, fire-fighting foams, carpeting, apparels and metal plating. PFASs are persistent in the environment and are extremely resistant to degradation due to heat, acids or bases. They are also bioaccumulative in humans and wildlife and are known to cause reproductive and developmental toxicity in laboratory animals and wildlife.

This application note summarizes a validated procedure developed by the US Food and Drug Administration (FDA) for the measurement of 16 PFASs in food using a QuEChERS sample preparation approach and LC-MS/MS analysis. Representative food matrices tested include milk, bread, lettuce, and fish.

Due to the extremely low concentrations of detection required for this analysis, the choice of MS instrumentation is critical to hit necessary cutoff concentrations. In some cases, further clean-up using solid-phase extraction may be required (e.g. using Enviro-Clean® WAX SPE cartridges, p/n ECWAX126-P). The agency reports that the method's release is "*an important step in furthering collaboration between the FDA and states in assessing the safety of human and animal food from specific areas potentially affected by environmental contamination*". Complete method details, including instrumental parameters and validation criteria/results, can be found on the FDA website (https://www.fda.gov/media/131510/download).

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List of Analytes Covered in Method:

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
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		
Acronym	**Name**	**MW**
d5N-EtFOSAA	N-ethyl-d5-perfluoro-1-octanesulfonamidoacetic acid *(internal standard)*	590
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

Precautions:

- 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.
- The analyte 11Cl-PF3OUdS exhibits known issues with recovery in certain matrices, which may reduce the confidence in this result in certain food types.

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QuEChERS Procedure:

Sample Extraction:

The edible portion of the food sample is collected and homogenized using an IKA tube mill with a disposable 100 mL polypropylene grinding chamber (an alternative homogenizer may also be used for this step). Samples are ground at 5000 rpm for approximately 2 minutes. The minimum sample size for analysis is 5 grams.

1. Add amount of sample and LC/MS grade water based on Table 1 and commodity type to a 50 mL polypropylene (PP) centrifuge tube.
2. Add 10 µL of 1 µg/mL isotopically labeled surrogate standard solution to the sample.
3. Add 10 mL acetonitrile and 150 µL formic acid to the 50 mL PP conical centrifuge tube.
4. Shake vigorously for 1 minute.
5. Add QuEChERS salt packet (**ECMSSCFS-MP**) and shake for 5 minutes.
   - Glas-Col® digital pulse vortexer at 1500 rpm with pulse set to 70
6. Centrifuge the samples at 10000 rcf for 5 minutes.

Sample Clean-up:

1. Transfer supernatant to a 15 mL dSPE tube (**ECMPSCB15CT**).
2. Vortex/shake for 2 minutes.
3. Centrifuge the samples at 10000 rcf for 5 minutes.
4. Filter 5 mL of the extract with a 0.2 µm nylon syringe filter and transfer to a 15 mL conical centrifuge tube.
5. Add internal standard:

   <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 1ng/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.
6. Briefly vortex/shake
7. Transfer sample to a polypropylene autosampler vial for analysis by LC-MS/MS.

Table 1. Sample Preparation Conditions Based on Food Commodity Type

Commodity	Sample Amount	Water Added (mL)	ACN Added (mL)	Concentrate to Dryness
Fruits & Vegetables	5 g	5	10	No
Bread	5 g	15	10	No
Milk	5 mL	5	10	Yes – take 5mL of extract to 0.5 mL
Cheese	1 g	5	10	No
Other Dairy	5 g	5	10	No
Meat	5 g	5	10	No

Additional SPE Cleanup Procedure (*Optional*):

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 were run through an additional SPE clean-up step following the initial QuEChERS protocol.

SPE Procedure:

NOTE: The original FDA method employed the use of Strata™-XL-AW 100 µm Polymeric Weak-Anion 200 mg / 3 mL (Phenomenex, Torrance, CA) SPE cartridges. However, any equivalent polymeric weak-anion exchange column can be used as an alternative. UCT's equivalent for this chemistry and configuration is noted below.

1. Take 1 mL of filtered QuEChERS extract and dilute to approximately 15 mL with LC/MS grade water in a clean 15 mL mL polypropylene (PP) centrifuge tube.
2. Condition a Enviro-Clean® WAX SPE cartridge (ECWAX126-P, 200 mg/ 6mL) with 9 mL of 0.3% ammonium hydroxide in acetonitrile.
3. Load sample onto SPE cartridge and let pass through slowly (apply a low vacuum if necessary).
4. Wash cartridge with 5 mL of LC/MS grade water.
5. Dry cartridge for approximately 1 minute.
6. Elute sample with 4 mL of 0.3% ammonium hydroxide in acetonitrile.
7. Evaporate sample to near dryness.
8. Reconstitute to 1 mL with methanol and transfer to a polypropylene autosampler vial for analysis by LC-MS/MS.

Results:

A level 2 single lab 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.

References:

FDA Foods Program Compendium of Analytical Laboratory Methods: Chemical Analytical Manual (CAM); method number C-010.01; Determination of 16 Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) in Food using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS); Susan Genualdi and Lowri deJager, CFSAN/ORS/DAC/MDB; https://www.fda.gov/media/131510/download.

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