Quantitative Extraction and Analysis of PFAS from Plastic Container Walls with Cut Coupons by LC/MSMS

Table of Contents

1.0 Scope and Applications
    1.1 Target Analyte List and Limits of Quantitation
2.0 Summary of Method
3.0 Interferences
4.0 Health and Safety
5.0 Materials
    5.1 Equipment and Supplies
    5.2 Reagents
    5.3 Standards
6.0 Sample Collection, Holding Time, and Storage
7.0 Quality Control
    7.1 Blanks
    7.2 Spikes
    7.3 Calibration Standards
    7.4 Sample Set
    7.5 Continuing Calibration Verification
    7.6 Analytical Sequence
8.0 Sample Preparation and Analysis
    8.1 Part A: coupon cutting, control blanks and matrix spikes, and extraction
    8.2 Part B: preparation of coupon extracts for instrument analysis
9.0 LC/MSMS Instrumentation and Consumables
10.0 Data Analysis & Calculations
    10.1 Peak Identification
    10.2 Integration
    10.3 Peak Quantitation
    10.4 Over-spike Recovery Calculation
    10.5 Results Reporting
11.0 Data Review
12.0 Method Performance and Validation Data
13.0 References

1.0 Scope and Application

This document describes the laboratory procedures for sample preparation, analysis and quantification of per- and polyfluoroalkyl substances (PFAS) in plastic (e.g., high-density polyethylene (HDPE)) containers, by liquid chromatography with tandem mass spectrometry (LC/MSMS). The method may be used, with modifications, if necessary, for other similar types of solid samples (e.g., fabric and packaging paper) for the analysis of PFAS. The full names and the abbreviated names of all targeted PFAS analytes are listed in Table 1, along with their Chemical Abstract Service Registry Number (CASRN).

Note: This method has been single lab validated at the Analytical Chemistry Branch (ACB) of the Biological and Economic Analysis Division (BEAD), Office of Pesticide Programs (OPP). It is recommended that the method be validated at each laboratory before use.

1.1 Target Analyte List and Limits of Quantitation (LOQ)

The lowest achievable Limit of Detection (LOD) and Limit of Quantitation (LOQ) for target analytes using this method are provided in Table 1. The LOD and LOQ for each species were determined by fortifying control matrix (non-fluorinated HDPE coupons) according to the internal Standard Operating Procedure (SOP) No. ACB-030[1] guidelines.

Normally, the LOQ is validated at three times the LOD; however, due to the presence of some PFAS in the background, the LOQs for this method were validated at ten times of the lowest LOD.

2.0 Summary of Method

Plastic containers are cut to small sizes and extracted with methanol. Three options for sample preparation procedures are available for instrument analysis, depending on the expected concentration of PFAS and matrix interferences in the tested container:

Option 1. If PFAS concentrations are expected to be high, aliquots of the extracts can be analyzed directly after extraction. This option can also be used initially to screen for presence of PFAS.

Option 2. If PFAS concentrations are expected to be low (low parts-per-billion (ppb) to parts-per-trillion (ppt) levels), aliquots of the extracts can be concentrated and then analyzed.

Option 3. If PFAS concentrations are expected to be low (low ppb to ppt levels) and there is matrix interference in sample analysis, solid phase extraction (SPE) cleanup can be used after concentration of the extracts.

Analyses of the extracts are conducted with a LC/MSMS in the multiple reaction monitoring (MRM) mode. Sample concentrations are determined by internal standard quantification using

isotopically labeled PFAS analytes added to the samples after preparation and prior to instrument analysis. Results are reported on the basis of weight by weight – (one ppb is equivalent to one nanogram (ng) of a PFAS analyte per gram (g) of sample container, or 1000 ppt).

Detailed description of the method is available in section 8.0 "**Sample Preparation and Analysis**".

3.0 Interferences

Solvents, reagents, glassware, SPE cartridges and manifold, and other sample processing materials may have PFAS contamination or intentionally added PFAS, yielding artifacts and elevated baselines in instrument chromatograms. Testing and selection of clean reagents, solvents, and laboratory supplies is necessary to minimize these interferences. Clean all equipment prior to and after each use to avoid PFAS cross-contamination.

Note: Severe interference for compound 6:2 FTS (6:2 fluorotelomer sulfonate) was encountered during the method development with several brands of Florisil SPE cartridges. No substitute was yet found.

PFAS can occur in lines, tubing, pump heads, frits, seals and valves in an analytical instrument. In addition to utilizing a delay column, the LC plumbing should be changed to PFAS-Free components.

All materials used in sample storage, sample processing and analysis must be demonstrated to be free from interferences by running appropriate blanks during instrument analysis. Matrix interferences inherent of the samples, can result in analyte signal suppression or enhancement during analysis. These interferences may be removed or reduced with appropriate clean-up techniques (e.g., SPE) or dilution.

While it is nearly impossible to completely avoid all potential PFAS contamination, great care should be taken to limit contaminated materials contacting the sample directly.

4.0 Health and Safety

Handling, storage, and disposal of laboratory wastes, chemicals, and reagents must follow the appropriate safety procedures and precautions described in the ACB SOPs. Proper PPE must be worn when handling scissors, box cutters or other sharp objects for container size reduction. To avoid accidental cuts, wear cut resistant gloves.

5.0 Materials

Reference to specific brands and manufacturer names does not imply endorsement of products. Similar or equivalent materials and equipment may be used in place of those mentioned in this method.

5.1 Equipment and Supplies

- Analytical balance and top loading balance capable of weighing to the nearest 0.0001 g and 0.01 g, respectively
- Ultrasonic bath with temperature control
- Sample evaporator water bath with nitrogen gas
- Vortex mixer or equivalent
- Calibrated variable volume pipettes with disposable plastic tips, used for quantitative solution transfer and small volume solvent transfers
- Class A glass pipettes, used for solvent transfer only
- Metal box cutter, metal scissors and other appropriate cutting tools
- Optional: Ruler and cutting template, e.g., 1.5 inch x 2 inch. Cutting coupons to specific size using the cutting template is needed only for expressing the measured PFAS concentration per unit area of the container walls. If there is no need to express the concentration per unit area, the coupon sizes can be any size provided it fits in the extraction bottle.
- Ziploc bags or non-fluorinated bottles, for storage of cut coupons
- 15-mL and 50-mL conical polypropylene centrifuge tubes with caps for preparing and storing extracts and analytical standards
- Polypropylene or polyethylene LC vials and caps, with vial inserts if needed
- Non-fluorinated bottles with lids for coupon extraction
- Non-fluorinated HDPE containers or equivalent, used as control matrix
- SPE manifold. Ensure SPE manifold does not have PTFE valves, PTFE dripping tubes, or other fluorinated parts that may contact sample
- Florisil SPE cartridges – Agilent Technologies, BondElut Mega BE-FL, 1 g, 6 mL, or equivalent. Ensure cartridges have non-PFTE frits and do not contain any fluorinated components

5.2 Reagents

- Water – LC/MS grade or equivalent
- Acetonitrile (ACN) – HPLC grade or equivalent, store at room temperature
- Methanol (MeOH) – LC/MS grade or equivalent, 99.9% purity, store at room temperature
- Acetone – HPLC grade or equivalent, store at room temperature
- Hexane – HPLC grade or equivalent, store at room temperature
- Ethyl Acetate – HPLC grade or equivalent, store at room temperature
- Acetic acid – glacial or equivalent, store under manufacturer specified conditions
- Ammonium acetate – HPLC grade or equivalent; store under manufacturer specified conditions

- Sodium hydroxide (NaOH) – pellets, ACS grade or equivalent, store under manufacturer specified conditions
- 0.005 M NaOH in water. Add approximately 0.2 grams NaOH to 1 L water
- Methanol/0.005 M NaOH in water (99/1, v/v). Add 10 mL 0.005 M NaOH in water to 990 mL MeOH
- Hexane/ethyl acetate (9/1, v/v). Add 100 mL ethyl acetate to 900 mL hexane
- 1% acetic acid in methanol/acetone (9/1, v/v). Add 1 mL glacial acetic acid to 900 mL MeOH and 100 mL acetone

5.3 Standards

Mixtures of PFAS standards were purchased from Wellington Laboratories (e.g., PFAC-MXC, PFAC-MXF, MPFAC-C-ES, MPFAC-C-IS). Individual PFAS analytes that were not in the premixed standard solutions were purchased separately.

If the standards purity is 98% or greater, the weight/concentration can be used to calculate solution concentrations without purity correction. For analytes obtained in a salt form, correction to acid form may be applied.

Prepare intermediate and working standard solutions from stock PFAS standards and mixtures purchased from commercial sources (e.g., Wellington Laboratories, AccuStandard). These standards should have known purity and composition with certification to the purity and concentration. When not in use, store stock standards and standard solutions at 2-10°C, unless otherwise recommended by vendor.

Native Spike (NS) Solution, 10 ng/mL – Prepare a 10 ng/mL spiking solution of target native PFAS analytes (Table 1) in methanol/0.005 M NaOH in water (99/1, v/v) from standard stock solutions. This solution is used to prepare over-spiked samples and can be used to prepare calibration standards and matrix spikes.

Mass-labeled Spike (MLS) Solution, 10 ng/mL – Prepare a 10 ng/mL spiking solution of isotopically mass-labeled PFAS analytes (Table 2) in methanol/0.005 M NaOH in water (99/1, v/v) from stock solution. This solution is used to prepare over-spiked samples and can be used to spike samples before extraction, prepare calibration standards and matrix spikes.

Internal Standard (IS) Solution, 5 ng/mL – Prepare a 5 ng/mL spiking solution of internal standard PFAS analytes (Table 3) in methanol/0.005 M NaOH in water (99/1, v/v) from standard stock solution. This solution is used as an injection standard and can be used to prepare calibration standards.

Calibration Standards – Prepare a series of calibration standards with target native analyte concentrations between 0.025 – 20 ng/mL, isotopically mass-labeled analyte concentrations of 0.5 ng/mL and IS analyte concentrations of 0.5 ng/mL. Different concentrations of calibration standards may be used depending on instrument performance and quantification goals. Prepare calibration standards in methanol/0.005 M NaOH in water (99/1, v/v) and a minimum of five different levels.

6.0 Sample Collection, Holding Time, and Storage

Collected plastic sample containers are stored at ambient temperature in cardboard boxes or plastic bags until analysis. Procedures for sample receiving, storage, and disposal described in internal SOPs No. ACB-028[2] and ACB-030 should be followed.

Sample extracts may be retained in capped centrifuge tubes at lab room temperature for up to 28 days or in refrigerated conditions (2-10°C) after extraction.

7.0 Quality Control (QC)

Various quality control measures are needed to demonstrate the performance of the procedure, suitability of materials, and potential sample interferences.

7.1 Blanks

Blanks are used to demonstrate materials and instrument used are free of target analytes at various steps in sample storage, processing, and analysis. All blanks (solvent, control matrix or procedural) should be free of target analytes or the detected concentrations should be below the method LOD. If higher levels are detected, causes of contamination should be investigated and corrective actions taken before continuing sample analysis.

Solvent Blank – A solvent blank (or instrument blank) is analyzed at the beginning of each analytical sequence, to demonstrate clean instrumental background, and after samples containing high levels of target compounds (e.g., calibration, continuing calibration verification (CCV)) to monitor carryover from the previous injection. The solvent blank is methanol or other solvent similar to the sample solvent.

Control Blank (Matrix Blank) – Control blanks, also known as matrix blanks, are control materials that are similar to sample matrices and are processed through the entire procedure in the same batch as the sample materials. Control blanks are used to demonstrate the sample matrices do not interfere with target analytes and the procedures do not introduce target analytes. Non-fluorinated plastic containers (and cut coupons) can be used as control materials.

Procedural Blank – Procedural blanks are solvents that are processed through the entire procedure in the same batch as the sample materials. Procedural blanks are used to demonstrate the sample processing procedure and reagents do not result in significant amounts of target analytes.

7.2 Spikes

Matrix Spikes – Matrix spikes are control material spiked with native analytes and mass-labeled analytes that are processed through the entire procedure before analysis. Matrix spikes are used to evaluate method performance and to assess potential matrix effects in sample analyses.

Over-Spikes – Over-spikes are native analyte and mass-labeled analyte spikes of split blank and split sample extracts after processing but before instrument analysis. This type of spike is used to assess matrix effects of individual samples and used as a quality control on a per sample level.

7.3 Calibration Standards

The calibration curve, used for sample concentrations quantitation, should consist of at least four different concentration levels for linear curve fits or at least five different concentration levels for quadratic fits. The concentration of the lowest standard of the calibration curve must be at or below the method validated LOD. Curve fits must have a $R^2 \geq 0.98$. If curve criteria cannot be met, the potential causes need to be investigated and corrected.

7.4 Sample Set

Calculated analyte concentrations must be from a discernable peak (>3x of noise level) and be within ± 10% of method validated range and calibration curve range. If the analyte concentrations are above the calibration range, reanalysis is required after sample dilution. Detected analytes must also meet retention time and ion ratio criteria to qualify as a positive hit. Retention time must be within ± 0.1 minute of that of the calibration standards. The ion ratio of two product mass transitions must be within ± 30% of that of the calibration standards.

Over-spikes should have recoveries between 60 – 120%. Recoveries observed outside criteria should be examined and noted. Reanalysis should be considered for over-spike recoveries that are significantly outside criteria (>300%) or negative.

Matrix spikes should have recoveries between 60 – 120%. Recoveries observed outside criteria should be investigated and noted.

7.5 Continuing Calibration Verification

The calculated concentrations of Continuing Calibration Verifications (CCVs) standards should not deviate more than ± 20% from that of the expected concentrations. If the criteria are not met, the causes need to be investigated and samples bracketed by the failing CCV be re-injected. Additional corrective actions may need to be taken or the reported data of relevant analytes be flagged with nonconformance.

7.6 Analytical Sequence

The LC/MSMS analytical sequence should include the following:

- Solvent blank(s) at start of sequence
- Calibration standards at the beginning of sequence followed by solvent blank(s)
- Sample set which includes control blank, procedural blank, matrix spikes, samples, over-spiked control/procedural blanks, and over-spiked samples
- The CCV standards interspersed in the sequence, one CCV for a maximum of ten samples injections and at the end of the sequence

- Solvent blank(s) following samples or standards containing high target analyte concentrations and as needed

8.0 Sample Preparation and Analysis

8.1 Part A: coupon cutting, control blanks and matrix spikes, and extraction

Container Coupon Cutting

1. Weigh entire container (without lid) and record mass.
2. To cut coupons, first decontaminate tools (boxcutter, scissors, etc.) by rinsing the tool surfaces with high purity methanol and drying with Kimwipe. Decontaminate working surfaces likewise. Perform decontamination procedure three times.
3. Using a template (e.g., 1.5 x 2 inches), if needed, cut coupons to size.
4. Store extra cut coupons in labeled Ziploc bag or non-fluorinated bottles.

Control Blanks and Matrix Spikes

5. Prepare procedural blank, control blank and matrix spike using non-fluorinated container coupons.
6. Place non-fluorinated coupons into non-fluorinated extraction bottle with lid. Record mass and area of coupons to be used for extraction.
7. Spike coupons in extraction bottle with NS Solution and MLS Solution. Do not spike control blank(s).
8. Allow spike, along with control and procedural blanks, to dry overnight (or between four hours to up to two days) in extraction bottle with lid off.
9. Follow coupon extraction procedure below starting at step 13.

Coupon Extraction

10. Place sample coupons into non-fluorinated extraction bottle with lid. Record mass and area of coupons used for extraction.
11. Note: the number of coupons used for extraction can be varied. A target mass of 6 – 9 grams is suggested if using the volume of extraction solvent suggested below. If a larger mass of coupons is used for extraction, the extraction solvent volume should be increased accordingly. The area of the coupons can be used to express concentrations in per unit area of surface, useful for comparing containers varying in size, shape, and thickness.
12. Spike coupons in extraction bottle with 0.05 mL of the 10 ng/mL MLS Solution.
13. Fill extraction bottle with 10-20 mL of methanol, making sure entirety of coupons are submerged and note volume of extraction solvent used.
14. Sonicate in a water bath at 50°C for 30 minutes, leaving lid only slightly untightened to allow for pressure to release.
15. Decant the coupon extract into labeled centrifuge tubes.
16. Fill extraction bottle with another 10-20 mL of methanol, making sure entirety of the coupons are submerged. Repeat the sonication a second time.
17. Decant second coupon extracts into the labeled centrifuge tubes to combine the extracts of the same samples.

8.2 Part B: preparation of coupon extracts for instrument analysis

Coupon extracts can be prepared for instrument analysis (Section 9.0) with one or more of the procedures outlined below, depending on the expected PFAS concentration levels in the extracts and matrix interference level.

Option 1 (for high PFAS levels in coupon extract): Shoot Straight of Extracts

1. Aliquot 450 µL of coupon extract from section 8.1, step 17 into polypropylene or polyethylene LC vial with cap.
2. Add 50 µL of IS Solution (5 ng/mL) and mix. Final IS concentration of this sample is 0.50 ng/mL.
3. Prepare over-spike of sample to evaluate matrix effects. Over-spiked amount of analyte should not be excessively high compared to what is found in sample. An over-spike of 0.5 - 1 ng/mL is suggested if analyte concentration is currently unknown or spans a large range.
4. Suggested over-spike: to prepare a 1 ng/mL over-spike, aliquot 200 µL of sample (from 8.2, step 2), add 25 µL of NS Solution (10 ng/mL), add 25 µL of MLS Solution (10 ng/mL), mix to homogenize. Final IS concentration of this over-spiked sample is 0.40 ng/mL.
5. Dilutions: if concentration of PFAS in coupon extracts are above method validated upper limit, dilutions of the extracts should be performed.

Note: If no PFAS is detected by Option 1, proceed to Option 2 or 3.

Option 2 (for low PFAS levels in coupon extract with minimum matrix interference): Concentration and Reconstitute of Extracts

1. Concentrate entire volume of extract from section 8.1, step 17. Prepare QC samples alongside. Concentrate to dryness at 50°C under a stream of N_2 on the sample evaporator. Do not over-dry.
2. Add 50 µL of IS Solution (5 ng/mL).
3. Reconstitute with 450 µL of methanol/0.005 M NaOH in water (99/1, v/v) and vortex. Transfer to polypropylene or polyethylene LC vial with cap for analysis. Final IS concentration of this sample is 0.50 ng/mL.
4. Prepare over-spike of sample to evaluate matrix effects. Over-spiked amount of analyte should not be excessively high compared to what is found in sample. An over-spike of 0.5 - 1 ng/mL is suggested if analyte concentration is currently unknown or spans a large range.
5. Suggested over-spike: to prepare a 1 ng/mL over-spike, aliquot 200 µL of sample, add 25 µL of NS Solution (10 ng/mL), add 25 µL of MLS Solution (10 ng/mL), mix to homogenize. Final IS concentration of this over-spiked sample is 0.40 ng/mL.

Note: If no PFAS is detected by Option 2, or if there is matrix interference preventing the proper and reliable identification of PFAS, proceed to Option 3.

Option 3 (for low PFAS levels in coupon extract with matrix interference): SPE Clean-up of Extracts

1. Concentrate entire volume of extract from section 8.1, step 17. Prepare QC samples alongside. Concentrate to dryness at 50°C under a stream of N_2 on the sample evaporator, do not over-dry.
2. Reconstitute with 5 mL of hexane/ethyl acetate (9/1, v/v), vortex to mix, allow to sit then vortex to mix two more times.
3. Prepare SPE cartridges on SPE manifold.
4. Condition the SPE cartridge by passing through 15 mL of 1% acetic acid in methanol/acetone (9/1, v/v), followed by 5 mL of hexane/ethyl acetate (9/1, v/v).
5. Load the samples onto the SPE cartridges. Apply a vacuum to the manifold to ensure the flow through the SPE is dropwise (1-2 mL/min).
6. After all the samples pass through the SPE (do not let the SPE go to dry), rinse the sample tubes with three aliquots of 4 mL of hexane/ethyl acetate (9/1, v/v). Each time transfer the rinse solution to the SPE and let the hexane/ethyl acetate pass through the SPE. On the last aliquot, continue to pull the vacuum to ensure all solvent has passed through (no more dripping).
7. Place labeled clean 15 mL polypropylene collection tubes under the SPE. Add 10 mL of 1% of acetic acid in methanol/acetone (9/1, v/v) to each SPE and collect the eluate.
8. Remove the collected eluates from the manifold and concentrate to just dry/near dryness under a stream of N_2 in a water bath (40-50°C).
9. Add 50 µL of IS Solution (5 ng/mL) to each sample tube.
10. Add 450 µL of methanol/0.005 M NaOH in water (99/1, v/v) to each sample tube and vortex. Final IS concentration of this sample is 0.50 ng/mL.
11. Transfer the samples to polypropylene or polyethylene LC vials with caps for instrumental analysis.
12. Prepare solvent blank(s) in polypropylene LC vial(s) with polyethylene caps.
13. Over-spikes can be prepared if desired: Suggested over-spike: to prepare a 1 ng/mL over-spike, aliquot 200 µL of sample, add 25 µL of NS Solution (10 ng/mL), add 25 µL of MLS Solution (10 ng/mL), mix to homogenize. Final IS concentration of this over-spiked sample is 0.40 ng/mL.

9.0 LC/MSMS Instrumentation and Consumables

The analytical instrument setup is adapted from EPA Method 8327[3], with some modifications.

- LC/MSMS instrumentation – Agilent Technologies 1290 Infinity LC system (fitted with Agilent Technologies PFC-Free HPLC conversion kit) coupled with Sciex QTRAP 6500+ mass spectrometer, operating in negative ion electrospray ionization (ESI) mode; or equivalent instrument.
- Delay column – Agilent, InfinityLab PFC delay column, 4.6 x 30 mm, or equivalent.
- Analytical Column – Waters, XBridge C18, 2.1 x 150 mm, 3.5 µm, or equivalent.
- Mobile Phase A – 5mM ammonium acetate in water.
- Mobile Phase B – acetonitrile.
- LC needle rinse solution – methanol/water (75/25).

LC parameters
Column temperature: 35°C
Injection volume: 10 µL
Flow rate: 300 µL/min

Gradient Table

Total Time (min)	Flow Rate (µL/min)	Mobile A (%)	Mobile B (%)
0	300	90	10
5	300	80	20
20	300	10	90
25	300	5	95
25.1	300	90	10
27	300	90	10

Mass spectrometer parameters
MRM scan mode
ESI, negative polarity
Source temperature: 450°C
Curtain gas: 30 psi
Collision gas: 9 psi
Ion spray voltage: -4500 V
Ion source gas 1: 45 psi
Ion source gas 2: 40 psi
Entrance potential: -10 V
For all target native analytes (Table 1), two mass transitions are monitored, except for PFBA and PFPeA where only one mass transition is available.

10.0 Data Analysis & Calculations

10.1 Peak Identification

Peak identification in samples is performed using retention time and ion ratio matching to that of standards (section 7.3). Peaks should be distinctly discernable in shape and size. Broad peaks that do not resemble the peak shape of standards should not be considered as valid peaks unless there are clear causes for peak broadening in the sample for that analyte. Peaks with signal to noise ratio below 3:1 are not discernable peaks.

10.2 Integration

Automated peak integrations by software should be performed whenever possible. Manual integrations are sometimes necessary to correct inaccurate automated integrations. Manual

integrations of the chromatographic peaks must closely match peak integrations of standards. All manual integrations must be noted.

10.3 Peak Quantitation

Sample concentrations of the target native analytes are determined with respect to an IS analyte using the response ratios or response factors of the most recent set of calibration standards. In cases where the IS analyte signal in the sample being used for quantification is significantly suppressed or enhanced compared to the responses in calibration standards, reanalysis should be attempted using techniques to eliminate these interferences.

10.4 Over-spike Recovery Calculation

Recovery calculation for 1 ng/mL over-spike:

$$\%\, recovery = \frac{(C_{OS} \times 0.25) - (C_S \times 0.2)}{0.25} \times 100$$

C_{OS} = concentration of over-spiked sample; C_S = concentration of sample.

10.5 Results Reporting

Sample results for analytes above the LOQ that meet the identification criteria (section 7.4 and 10.1) are reported as numerical values. Sample results for analyte that do not meet the identification criteria can be reported only if flagged with nonconformance indicators. Additionally, if significant IS analyte signal suppression or enhancement is observed and unresolvable (section 10.3), results should also be flagged with nonconformance indicators. Concentrations of detected PFAS analytes are reported on the basis of weight by weight.

11.0 Data Review

Data are reviewed by a peer and a quality assurance officer following guidelines set forth in the internal SOP No. ACB-004[4]. The data review process and any findings should be documented.

12.0 Method Performance and Validation Data

Table 1. List of targeted native PFAS analytes. Lowest validated LOD and LOQ are presented in ppb (ng (of analyte) / g (of container)).

Analyte	Abbreviation	CASRN	LOD	LOQ
Perfluorobutanoic Acid	PFBA	375-22-4	0.005	0.02
Perfluorobutanesulfonic Acid	PFBS	375-73-5	0.002	0.02
Perfluoropentanoic Acid	PFPeA	2706-90-3	0.002	0.02
Perfluoropentanesulfonic Acid	PFPeS	2706-91-4	0.002	0.02
Perfluorohexanoic Acid	PFHxA	307-24-4	0.002	0.02
Perfluorohexanesulfonic Acid	PFHxS	355-46-4	0.002	0.02
Perfluoroheptanoic Acid	PFHpA	375-85-9	0.002	0.02
Perfluoroheptanesulfonic Acid	PFHpS	375-92-8	0.002	0.02
Perfluorooctanoic Acid	PFOA	335-67-1	0.002	0.02
Perfluorooctanesulfonic Acid	PFOS	1763-23-1	0.002	0.02
Perflurononanoic Acid	PFNA	375-95-1	0.002	0.02
Perfluorononanesulfonic Acid	PFNS	68259-12-1	0.002	0.02
Perfluorodecanoic Acid	PFDA	335-76-2	0.002	0.02
Perfluorodecanesulfonic Acid	PFDS	335-77-3	0.002	0.02
Perfluoroundecanoic Acid	PFUdA	2058-94-8	0.002	0.02
Perfluorododecanoic Acid	PFDoA	307-55-1	0.002	0.02
Perfluorododecanesulfonic Acid	PFDoS	79780-39-5	0.002	0.02
Perfluorotridecanoic Acid	PFTrDA	72629-94-8	0.002	0.02
Perfluorotetradecanoic Acid	PFTeDA	376-06-7	0.002	0.02
Perflurohexadecanoic Acid	PFHxDA	67905-19-5	0.002	0.02
Perfluorooctadecanoic Acid	PFODA	16517-11-6	0.005	0.02
4:2 Fluorotelomer sulfonic acid	4:2 FTS	757124-72-4	0.002	0.02
6:2 Fluorotelomer sulfonic acid	6:2 FTS*	27619-97-2	0.2	0.4
1H,1H,2H,2H-Perfluorodecanesulphonic acid	8:2 FTS	39108-34-4	0.02	0.4
Perfluorooctane sulfonamidoacetic Acid	FOSAA	2806-24-8	0.002	0.02
N-Methyl Perfluorooctane sulfonoamidoacetic Acid	N-MeFOSAA	2355-31-9	0.005	0.4
N-Ethyl Perfluorooctane sulfonoamidoacetic Acid	N-EtFOSAA	2991-50-6	0.02	0.4
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	0.002	0.02
4,8-Dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4	0.002	0.02
9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1	0.002	0.02
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9	0.002	0.02

* Available Florisil SPE cartridges used in this method validation had high levels of 6:2 FTS interference, SPE clean-up with tested Florisil SPEs could not be validated for this analyte.

Table 2. List of isotopically mass-labeled PFAS analytes.

Analyte	Abbreviation	CASRN
Perfluoro-n-[1,2-13C2]tetradecanoic acid	M2PFTeDA	NA
Sodium perfluoro-1-[2,3,4-13C3]butanesulfonate	M3PFBS	2708218-84-0
Sodium perfluoro-1-[1,2,3-13C3]hexanesulfonate	M3PFHxS	2708218-86-2
Perfluoro-n-[1,2,3,4-13C4]heptanoic acid	M4PFHpA	2328024-55-9
Perfluoro-n-[1,2,3,4,6-13C5]hexanoic acid	M5PFHxA	2328024-54-8
Perfluoro-n-[1,2,3,4,5-13C5]pentanoic acid	M5PFPeA	2283397-79-3
Perfluoro-n-[1,2,3,4,5,6-13C6]decanoic acid	M6PFDA	2328024-56-0
Perfluoro-n-[1,2,3,4,5,6,7-13C7]undecanoic acid	M7PFUdA	NA
Perfluoro-n-[13C8]octanoic acid	M8PFOA	1350614-84-4
Sodium perfluoro-[13C8]octanesulfonate	M8PFOS	2522762-16-7
Perfluoro-n-[13C9]nonanoic acid	M9PFNA	2283397-80-6
Perfluoro-n-[1,2,3,4-13C4]butanoic acid	MPFBA	1017281-29-6
Perfluoro-n-[1,2-13C2]dodecanoic acid	MPFDoA	960315-52-0
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-13C2]-hexane sulfonate(4:2)	M 4:2 FTS	2708218-88-4
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-13C2]-octane sulfonate(6:2)	M 6:2 FTS	2708218-89-5
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-13C2]-decane sulfonate(8:2)	M 8:2 FTS	2708218-90-8
N-methyl-d3-perfluoro-1-octanesulfonamidoacetic acid	d3 N MeFOSAA	NA
N-ethyl-d5-perfluoro-1-octanesulfonamidoacetic acid	d5 N EtFOSAA	1265205-97-7
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-13C3-propanoic acid (13C-GenX)	M3HFPO-DA	NA

Table 3. List of PFAS internal standards.

Analyte	Abbreviation	CASRN
Perfluoro-n-[2,3,4-13C3]butanoic acid	M3PFBA	2483735-33-5
Perfluoro-n-[1,2-13C2]decanoic acid	MPFDA	960315-50-8
Perfluoro-n-[1,2-13C2]octanoic acid	M2PFOA	864071-08-9
Sodium perfluoro-1-[1,2,3,4-13C4]octanesulfonate	MPFOS	960315-53-1

Table 4. Average recoveries at tested ppb levels using Shoot Straight of Extracts (Option 1). LOD and LOQ for all analytes were validated at 2.13 ppb, except PFBA (high background level). PFBA LOD and LOQ were validated at 13 ppb and 16 ppb, respectively.

Analyte	LOD & LOQ at 2.13 ppb	16 ppb	10x LOQ at 160 ppb
PFBA	448%	129%	93%
PFBS	96%	101%	94%
PFPeA	88%	95%	90%
PFPeS	86%	95%	89%
PFHxA	90%	96%	92%
PFHxS	88%	95%	87%
PFHpA	77%	90%	89%
PFHpS	88%	98%	92%
PFOA	67%	81%	90%
PFOS	86%	96%	95%
PFNA	85%	97%	100%
PFNS	92%	105%	101%
PFDA	70%	84%	91%
PFDS	85%	92%	94%
PFUdA	91%	92%	101%
PFDoA	86%	85%	92%
PFDoS	90%	90%	92%
PFTrDA	87%	93%	93%
PFTeDA	82%	91%	93%
PFHxDA	75%	81%	82%
PFODA	71%	84%	86%
4:2 FTS	74%	93%	83%
6:2 FTS	81%	92%	87%
8:2 FTS	76%	87%	92%
FOSAA	81%	83%	88%
N-MeFOSAA	105%	84%	87%
N-EtFOSAA	84%	87%	95%
HFPO-DA	103%	114%	105%
NaDONA	95%	101%	92%
9Cl-PF3ONS	92%	96%	96%
11Cl-PF3OUdS	87%	94%	92%

Table 5. Average recoveries at tested validation levels using Concentration and Reconstitute of Extracts. The % recovery at LOD shown in this table is for data evaluation purpose only and should be treated as estimated because quantification at LOD is not reliable.

	LOD		LOQ		20x LOQ	
Analyte	Level (ppb)	Ave. Recovery	Level (ppb)	Ave. Recovery	Level (ppb)	Ave. Recovery
PFBA	0.005	234%	0.02	151%	0.4	114%
PFBS	0.002	109%	0.02	100%	0.4	136%
PFPeA	0.002	130%	0.02	91%	0.4	115%
PFPeS	0.002	100%	0.02	95%	0.4	135%
PFHxA	0.002	116%	0.02	99%	0.4	125%
PFHxS	0.002	98%	0.02	100%	0.4	142%
PFHpA	0.002	147%	0.02	106%	0.4	127%
PFHpS	0.002	96%	0.02	92%	0.4	136%
PFOA	0.002	341%	0.02	168%	0.4	99%
PFOS	0.002	129%	0.02	99%	0.4	110%
PFNA	0.002	139%	0.02	98%	0.4	115%
PFNS	0.002	100%	0.02	95%	0.4	99%
PFDA	0.002	112%	0.02	83%	0.4	98%
PFDS	0.002	259%	0.02	95%	0.4	101%
PFUdA	0.002	131%	0.02	94%	0.4	112%
PFDoA	0.002	92%	0.02	96%	0.4	113%
PFDoS	0.002	273%	0.02	94%	0.4	105%
PFTrDA	0.002	195%	0.02	110%	0.4	119%
PFTeDA	0.002	262%	0.02	110%	0.4	132%
PFHxDA	0.002	211%	0.02	103%	0.4	115%
PFODA	0.005	183%	0.02	96%	0.4	111%
4:2 FTS	0.002	139%	0.02	104%	0.4	130%
6:2 FTS	0.2 [β]	270%	0.4	202%	0.4	202%
8:2 FTS	0.02	152%	0.4	101%	0.4	101%
FOSAA	0.002	188%	0.02	97%	0.4	116%
N-MeFOSAA	0.005	367%	0.4	114%	0.4	114%
N-EtFOSAA	0.02	216%	0.4	117%	0.4	117%
HFPO-DA	0.002	81%	0.02	82%	0.4	122%
NaDONA	0.002	91%	0.02	82%	0.4	128%
9Cl-PF3ONS	0.002	217%	0.02	96%	0.4	99%
11Cl-PF3OUdS	0.002	242%	0.02	102%	0.4	100%

[β] 2 replicates.

Table 6. Average recoveries at tested validation levels using SPE Clean-up of Extracts. The % recovery at LOD shown in this table is for data evaluation purpose only and should be treated as estimated because quantification at LOD is not reliable.

	LOD		LOQ		20x LOQ	
Analyte	Level (ppb)	Ave. Recovery	Level (ppb)	Ave. Recovery	Level (ppb)	Ave. Recovery
PFBA	0.005	206%	0.02 [β]	119%	0.4	144%
PFBS	0.002	108%	0.02 [β]	71%	0.4	107%
PFPeA	0.002	119%	0.02 [β]	58%	0.4	93%
PFPeS	0.002	94%	0.02 [β]	64%	0.4	101%
PFHxA	0.002	117%	0.02 [β]	84%	0.4	108%
PFHxS	0.002	87%	0.02 [β]	67%	0.4	96%
PFHpA	0.002	133%	0.02 [β]	77%	0.4	89%
PFHpS	0.002	87%	0.02 [β]	56%	0.4	96%
PFOA	0.007	335%	0.4	122%	0.4	122%
PFOS	0.002	142%	0.02 [β]	96%	0.4	106%
PFNA	0.002	142%	0.02 [β]	91%	0.4	107%
PFNS	0.002	102%	0.02 [β]	83%	0.4	104%
PFDA	0.002	138%	0.02 [β]	88%	0.4	110%
PFDS	0.002	263%	0.02 [β]	89%	0.4	101%
PFUdA	0.002	159%	0.02 [β]	86%	0.4	108%
PFDoA	0.002	134%	0.02 [β]	104%	0.4	103%
PFDoS	0.002	277%	0.02 [β]	88%	0.4	100%
PFTrDA	0.002	218%	0.02 [β]	98%	0.4	99%
PFTeDA	0.005	342%	0.02 [β]	111%	0.4	124%
PFHxDA	0.002	275%	0.02 [β]	89%	0.4	101%
PFODA	0.002	236%	0.02 [β]	78%	0.4	99%
4:2 FTS	0.002	163%	0.02 [β]	77%	0.4	109%
6:2 FTS *						
8:2 FTS	0.05	321%	0.4	193%	0.4	193%
FOSAA	0.002	204%	0.4	54%	0.4	54%
N-MeFOSAA	0.07	250%	0.4	153%	0.4	153%
N-EtFOSAA	0.05	229%	0.4	144%	0.4	144%
HFPO-DA	0.002	79%	0.02 [β]	86%	0.4	100%
NaDONA	0.002	77%	0.02 [β]	56%	0.4	87%
9Cl-PF3ONS	0.002	220%	0.02 [β]	92%	0.4	98%
11Cl-PF3OUdS	0.002	266%	0.02 [β]	91%	0.4	90%

* Available Florisil SPE cartridges used in this method validation had high levels of 6:2 FTS interference, SPE clean-up with tested Florisil SPEs could not be validated for this analyte.
[β] 4 replicates.

13.0 References

1. *"Standard Operating Procedures for Pesticide Residue Chemistry Sample Analysis"* – SOP No. ACB-030, Revision 3.1, March 31, 2022 - Internal QA document.
2. *"Standard Operating Procedures for Expedited Chemical Residue Analysis in Support of States, Federal and/or Other Organizations"*. - SOP No. ACB-028, Revision 1.2, December 26, 2020 – Internal QA document.
3. *"Per- and Polyfluorinated Alkyl Substances (PFAS) by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)"* – EPA Method 8327, Version 0, July 2021.
4. *"Standard Operating Procedures for Auditing Laboratory Projects"* – SOP No. ACB-004, Version 3.1, December 22, 2012 – Internal QA document.