IADN Per- and polyfluoroalkyl substances Standard Operating Procedures

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Table of Contents

Introduction

This manual describes the techniques and standard operating procedures used for the Integrated Atmospheric Deposition Network (IADN) laboratory for Per- and polyfluoroalkyl substances (PFAS). It includes media and supply preparation for in-field deployment of precipitation samples, sample handling, lab processing techniques and data analysis and quantification..

Field Supplies

Precipitation samples. The follow supplies are needed at each sampling site, every month:

- 0 – 2 pre-cleaned high-density polyethylene (HDPE) buckets (with the "950-mL" grade)
- 0 – 2 pre-cleaned 1-L polypropylene (PP) bottles
- 0 – 2 pre-cleaned 10-L PP carboys
- 0 – 2 pre-cleaned 1-L PP graduated cylinders
- 0 – 2 pre-cleaned 100 mL graduated cylinders
- Parafilm
- Aluminum foil
- Field data sheet/Site visit sheet
- A few pre-cleaned Zip ties
- 0 – 2 coolers

Cleaning.
The HDPE buckets are wiped clean with a Kim wipe that is saturated with reagent grade water, three times. They are then rinsed with methanol three times.

The PP graduated cylinders are to be rinsed three times with water then three times with methanol.

The PP 10-L carboys should be rinsed with 100 mL of water, three times, followed by 100 mL of methanol, three times.

Precipitation Sample Deployment and Collection

PFAS sampling via N-con ADS-120 precipitation sampler

Precipitation Sample Set up (1st of month)

1. Trigger the infrared sensor, by shaking hand, until lid is fully open and in final rest position. Set the power switch to "off" position until ready to install bucket, (maximum capacity: 13.5 L).
2. Remove the lock for the bucket.
3. Remove the bucket from the sampler. If a sample is to be collected see "Sample Collection" below.
4. Replace the collected bucket with a pre-cleaned bucket sent with supplies.
5. Switch the unit back "on" and lid will return to cover position.
6. Secure the bucket by mounting the lock.

Weekly activities during each site visit

1. Check if the sampler works properly: Trigger the sensor and see if the cover lifts off the sampling bucket. Note operation on the weekly Site Visit Sheet.

Precipitation Sample Collection:

Carried out on 1st of each month, and again mid-month (During a Tuesday site visit). Pre-cleaned containers will arrive with supplies close to the 1st of each month.

Trigger the sensor to lift the cover off the bucket. Once lid is in the fully open and resting position set power switch to "off" position so that unit is ready for bucket removal and replacement.

1. Remove the lock for the bucket
2. Remove the bucket from the sampler if a sample has been collected
3. Sample preparation:
   - If the precipitation collected is **< 200 mL**, note the volume on your field forms and discard the entire sample. No sample needs to be returned to IU. **Proceed to Step 9.**
   - If the precipitation collected is **< 1000 mL**, pour entire sample into a **1-L** polypropylene (PP) bottle. (*Pre-cleaned PP bottles and funnels will be included in the supplies)*
   - If the precipitation collected is **> 1000 mL**, pour entire sample into the pre-cleaned **10-L** PP carboy and shake thoroughly to homogenize the sample. Measure out 1000 mL of the

thoroughly mixed sample using a pre-cleaned 1-L PP graduated cylinder, transferred this 1000-mL sample into a **1-L** PP bottle. **Record the total collected volume on both the field data sheet and site visit sheet.**

Note: If the precipitation collected is snow or frozen, cover the bucket with foil and leave it in the refrigerator for melting. Perform the preceding steps during the next site visit.

4. Screw the cap on tightly to preserve precipitation inside;
5. Seal the top of the sample bottle by wrapping a strip of parafilm around the interface of the lid and bottle. (Strip should be approximately **4 × 20** inch (i.e., 2 × 10 parafilm squares; each square has a size of 2 × 2 inch); This will prevent possible loosening of cap while in transit.
6. Label the bottle in the form as:
   "Site code"- "Matrix code, (i.e. Air: S; Precipitation: B)" – "Ending date for sampling"
   For example: EB-200915 means: Precipitation collected at Eagle Harbor; The sampling ends on September 15, 2020 for PFA's analyses.
7. Wrap the bottle with aluminum foil
8. Place sealed and wrapped bottle **vertically** into the site refrigerator
9. Place new pre-cleaned bucket onto the sampler, power unit "on"
10. Secure the bucket by mounting the lock.

To be shipped back to IU lab (on a monthly basis):

1. Completed forms with documented sample information
2. Coolers with sealed sample bottles containing precipitation
3. 10 L carboy, graduated cylinder(s), buckets used during month for re-cleaning.

(Note: Operator's may ship MIC samples in the same box containing PFA's samples)

Lab Processing

This method describes the analysis of per- and polyfluoroalkyl substances (PFAS) in aqueous samples, including both precipitation and lake water matrices in the [redacted] Laboratory. A flowchart of the procedure is shown in Figure 1. In brief, water samples are prepared, extracted, and cleaned to remove any interference and then analyzed by LC-MS/MS. Internal standard quantification is used to determine PFAS concentrations using isotopically labeled standards. The procedure is described in detail below.

Reagents and Standards

1. Prepare chemical solutions before sample preparation. The required volumes of solutions will vary depending on the number of samples being processed.
   - 3% formic acid (aqueous)
   - 3% ammonium hydroxide (aqueous)
   - 0.3 M formic acid (aqueous)
   - 0.1 M formic acid (aqueous)
   - 0.1 M formic acid in LC/MS methanol (1:1).
   - 1% ammonium hydroxide in LC/MS methanol (v/v)
2. Remove PFAS surrogate and matrix spike standards from storage and allow them to reach ambient temperature, shielded from light.
3. Complete the PFAS laboratory processing form and record the catalog and lot numbers of reagents and supplies.

Sample pH Adjustment

1. Remove samples from storage and allow them to reach ambient temperature.
2. Measure the pH of the sample and record the pH.
3. If necessary, adjust the pH by adding 3% formic acid or 3% ammonium hydroxide (aqueous) to a pH of 6.5 ± 0.5. Mix and allow it to equilibrate before re-measuring.
4. Record the final pH.

Extraction Setup

1. Rinse the extraction materials (e.g., tubes, reservoirs with 27 mm frit, bottles, and graduated cylinders) three times with LC/MS grade methanol and allow to dry in the fume hood.
2. Use 10 mL Milli-Q water for blank (BLK) and matrix spike (MS) quality control samples.
   - Prepare a BLK and MS for each SPE manifold in use. For Batches > 12 samples, utilize two SPE manifolds, placing one BLK and MS on the first SPE manifold and another BLK and MS on the second.
3. Label the columns and reservoirs.
4. Install the WAX/carbon SPE columns (6 cc, 150 mg, Phenomenex-Strata PFAS (WAX/GCB; Part No. CS0-9207), pairing adapter, and 60 mL reservoirs with frit for each sample onto an SPE manifold, alternating positions to allow room for the larger 60 mL reservoirs.
5. Rinse and condition the SPE column:
   - Add 15 mL 1% ammonia hydroxide in LC/MS methanol (MeOH), then elute.
   - Add 5 mL 0.3 M formic acid aqueous solution, then elute.

i. Do not allow the WAX SPE to go dry.
ii. Discard the wash solvents.

6. The volume of the sample should be between 500 mL – 1 L. Add the sample to a cleaned graduated cylinder and record the initial volume.
   - Note: if using 500 mL graduated cylinders and the sample is more than 500 mL, you will have to wait to record the initial volume until the first 500 mL is finished eluting.

Extraction

1. Pour ~10 mL water sample into the reservoir.
2. Spike 50 µL PFAS surrogate standards (0.04 µg/mL) into the 10 mL sample. Use 10 mL Milli-Q water for BLK, and MS and spike 50 µL PFAS matrix spike standards (0.1 µg/mL) into MS.
3. Elute 10 mL but don't let the cartridge go dry.
4. Load the samples to the reservoirs.
   a. Adjust the vacuum and pass the sample through the cartridge at 5 mL/min.
   b. Elution time may vary depending on the sample and some SPE cartridges may stop eluting. Be sure to elute at least 250 mL of water and record the details.
      i. If the cartridge clogs and stops eluting (i.e., <250 mL of sample volume), then use a second cartridge and note details on the lab form.
   c. If you elute less than the total initial volume of the sample, please record the extracted volume on the sample prep sheet.
5. Wash the graduated cylinder with 5 mL Milli-Q water and pour it into the reservoir.
   a. Repeat once.
   b. Omit this step if less than 250 mL of the sample volume passes through the column.
6. Add rinsed or previously tested 15 mL PP tubes to the manifold for collection.
7. Add 5 mL of 0.1 M (1:1) formic acid in methanol to the reservoir and elute using a vacuum.
8. Add 5 mL of 1% ammonium hydroxide in MeOH solution and elute using vacuum into the same tube.

Neutralization and concentration of solvent through the cartridge and into the collection tubes.

1. Add 25 µL of concentrated acetic acid to each sample, BLK, and MS eluted in the collection tubes and vortex.
2. Concentrate the sample to 0.3 mL using N_2 blowdown.
3. Label the LC vials, spike with 50 µL internal standard (0.1 µg/mL), and cap vials.
4. Transfer the sample into a centrifugal filter (0.3 µm) and filter using a microcentrifuge at 3000 rpm for 5 minutes.
5. Transfer the filtrate into the IS-spiked vials.
6. First, rinse the original collection tube (15 mL PP) with 0.4 mL MeOH, vortex, and transfer to the centrifugal filter.
   a. Filter at 3000 rpm for 5 minutes.
   b. Transfer filtrate into IS-spiked sample vials.
7. Second, rinse the bottom of the centrifugal filter with 0.2 mL of MeOH.
   a. Transfer to the IS-spiked sample vials.
8. Dilute samples, BLK, and MS vials to 1 mL, if needed.

9. Perform instrumental analysis on LC/MS-MS.

Data Acquisition and Analysis

Analyzed Compounds, Abbreviations, and Ions.
The full names, abbreviations, precursor, and product ions of individual PFAS analytes analyzed on the LC/MS/MS in ESI (-) mode are given in Table 12.

Table 12. PFAS compounds analyzed on LC-MS/MS under ESI (-) mode.

Abbr.	Compound Name	CAS #	Formula	Retention time (min)	Mol. Wt.	Precursor ion [M-H/D]-	Fragmentor (volts)	Product ions (m/z)	Collision energy (volts)	Structure
PFBA	Perfluorobutanoic acid	375-22-4	$C_4HF_7O_2$	2.243	214.04	213.0	64	169 \	5 \	$F_3C(CF_2)_2COOH$
PFPeA	Perfluoropentanoic acid	2706-90-3	$C_5HF_9O_2$	3.518	264.05	263.0	64	218.9 140.8	5 5	$F_3C(CF_2)_3COOH$
PFHxA	Perfluoro-n-hexanoic acid	307-24-4	$C_6HF_{11}O_2$	5.008	314.05	313.0	73	268.9 119	5 21	$F_3C(CF_2)_4COOH$
PFHpA	Perfluoro-n-heptanoic acid	375-85-9	$C_7HF_{13}O_2$	6.646	364.06	363.0	78	319 169	5 17	$F_3C(CF_2)_5COOH$
PFOA	Perfluoro-n-octanoic acid	335-67-1	$C_8HF_{15}O_2$	8.186	414.07	413.1	83	369 169	5 17	$F_3C(CF_2)_6COOH$
PFNA	Perfluoro-n-nonanoic acid	375-95-1	$C_9HF_{17}O_2$	9.542	464.08	463.1	83	419 218.9	5 17	$F_3C(CF_2)_7COOH$
PFDA	Perfluoro-n-decanoic acid	335-76-2	$C_{10}HF_{19}O_2$	10.712	514.08	513.0	93	468.9 269	5 17	$F_3C(CF_2)_8COOH$
PFUnDA	Perfluoro-n-undecanoic acid	2058-94-8	$C_{11}HF_{21}O_2$	11.725	564.09	563.0	102	518.9 268.9	5 17	$F_3C(CF_2)_9COOH$
PFDoDA		307-55-1	$C_{12}HF_{23}O_2$	12.601	614.10	613.0	102	569	9	$F_3C(CF_2)_{10}COOH$

Abbr.	Compound Name	CAS #	Formula	Retention time (min)	Mol. Wt.	Precursor ion [M-H/D]-	Fragmentor (volts)	Product ions (m/z)	Collision energy (volts)	Structure
	Perfluoro-n-dodecanoic acid							269	21	
PFTrDA	Perfluoro-n-tridecanoic acid	72629-94-8	$C_{13}HF_{25}O_2$	13.347	664.11	663.1	107	619 169	9 29	$F_3C(CF_2)_{11}COOH$
PFTeDA	Perfluoro-n-tetradecanoic acid	376-06-7	$C_{14}HF_{27}O_2$	13.998	714.11	713.1	112	668.9 169	13 29	$F_3C(CF_2)_{12}COOH$
PFHxDA	Perfluoro-n-hexadecanoic acid	67905-19-5	$C_{16}HF_{31}O_2$	15.041	814.13	813.1	121	768.9 168.9	13 37	$F_3C(CF_2)_{14}COOH$
PFPrS	Perfluoro-1-propanesulfonic acid	423-41-6	$C_3HF_7SO_3$	2.748	250.09	249.1	140	80 98.9	37 33	$F_3C(CF_2)_2SO_3H$
PFBS	Perfluoro-1-butanesulfonic acid	375-73-5	$C_4HF_9SO_3$	3.876	300.10	299.0	149	80 98.9	37 37	$F_3C(CF_2)_3SO_3H$
PFPeS	Perfluoro-1-pentanesulfonic acid	2706-91-4	$C_5HF_{11}SO_3$	5.336	350.11	349.0	175	80 98.9	45 37	$F_3C(CF_2)_4SO_3H$
PFHxS	Perfluoro-1-hexanesulfonic acid	355-46-4	$C_6HF_{13}SO_3$	6.885	400.11	399.0	179	80 98.9	45 41	$F_3C(CF_2)_5SO_3H$
PFHpS	Perfluoro-1-heptanesulfonic acid	375-92-8	$C_7HF_{15}SO_3$	8.357	450.12	449.0	183	80 98.9	49 45	$F_3C(CF_2)_6SO_3H$
PFOS	Perfluoro-1-octanesulfonic acid	1763-23-1	$C_8HF_{17}SO_3$	9.647	500.13	499.0	208	80 98.9	101 49	$F_3C(CF_2)_7SO_3H$
PFNS	Perfluoro-1-nonanesulfonic acid	68259-12-1	$C_9HF_{19}SO_3$	10.776	549.93	549.0	218	80 98.9	105 49	$F_3C(CF_2)_8SO_3H$
PFDS	Perfluoro-1-decanesulfonic acid	335-77-3	$C_{10}HF_{21}SO_3$	11.764	600.14	598.9	232	80 98.9	137 53	$F_3C(CF_2)_9SO_3H$
FBSA	Perfluoro-1-butanesulfonamide	30334-69-1	$C_4H_2F_9NO_2S$	5.165	299.11	298	98	78 48.1	25 93	C(C(C(F)(F)S(=O)(=O)N)(F)F)(C(F)(F)F)(F)F
4:2 FTA	1H,1H,2H,2H-perfluorohexan		$C_6H_5F_9O_3S$	4.870	328.15	327.1	136	306.9	21	$F_3C(CF_2)_3(CH_2)_2SO_3H$

Abbr.	Compound Name	CAS #	Formula	Retention time (min)	Mol. Wt.	Precursor ion [M-H/D]-	Fragmentor (volts)	Product ions (m/z)	Collision energy (volts)	Structure
	e sulfonic acid (4:2)	757124-72-4						81	33	
6:2 FTA	1H,1H,2H,2H-perfluorooctane sulfonic acid (6:2)	27619-97-2	$C_8H_5F_{13}O_3S$	8.091	428.17	427.0	164	406.9 81	25 41	$F_3C(CF_2)_5(CH_2)_2SO_3H$
8:2 FTA	1H,1H,2H,2H-perfluorodecane sulfonic acid (8:2)	39108-34-4	$C_{10}H_5F_{17}O_3S$	10.676	528.18	527.0	179	506.9 81	29 41	$F_3C(CF_2)_7(CH_2)_2SO_3H$
M3PFBA (Surrogate standard) SS)	Perfluoro-n-[2,3,4-$^{13}C_3$]butanoic acid		$CHF_7O_2 + {}^{13}C_3$	2.242	217.04	216.0	64	172	5	
MPFHxA (SS)	Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid		$C_4HF_{11}O_2 + {}^{13}C_2$	4.999	316.05	315.1	78	270	5	
MPFOA (SS)	Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid		$C_4HF_{15}O_2 + {}^{13}C_4$	8.185	418.07	417.1	83	372	5	
MPFUnDA (SS)	Perfluoro-n-[1,2-$^{13}C_2$]undecanoic acid		$C_9HF_{21}O_2 + {}^{13}C_2$	11.725	566.09	565.1	97	520	9	
M2PFTeDA (SS)	Perfluoro-n-[1,2-$^{13}C_2$]tetradecanoic acid		$C_{12}HF_{27}O_2 + {}^{13}C_2$	13.997	716.11	715.1	116	669.9	13	
M3PFBS (SS)	Perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonic acid		$CHF_9SO_3 + {}^{13}C_3$	3.874	303.10	302.0	149	80	45	
MPFHxS (SS)	Perfluoro-1-hexane[$^{18}O_2$]sulfonic acid		$C_6HF_{13}SO + {}^{18}O_2$	6.882	404.11	403.0	169	84	49	
MPFOS (SS)	Perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonic acid		$C_4HF_{17}SO_3 + {}^{13}C_4$	9.646	504.13	503.0	198	80	93	
M2-8:2 FTSA (SS)	1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]-decane sulfonic acid (8:2)		$C_8H_5F_{17}O_3S + {}^{13}C_2$	10.675	530.18	529.0	195	509	33	
MPFBA (Internal standard, IS)	Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid		$HF_7O_2 + {}^{13}C_4$	2.240	218.04	217.0	64	172	5	
M8PFOA (IS)	Perfluoro-n-[$^{13}C_8$]octanoic acid		$HF_{15}O_2 + {}^{13}C_8$	8.184	422.07	421.1	83	376	5	

Abbr.	Compound Name	CAS #	Formula	Retention time (min)	Mol. Wt.	Precursor ion [M-H/D]-	Fragmentor (volts)	Product ions (m/z)	Collision energy (volts)	Structure
M7PFUnDA (IS)	Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid		$C_4HF_{21}O_2 + {}^{13}C_7$	11.724	571.09	570.0	97	525	9	
M3PFHxS (IS)	Perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonic acid		$C_3HF_{13}SO_3 + {}^{13}C_3$	6.883	403.11	402.0	184	80	45	
M8PFOS (IS)	Perfluoro-[$^{13}C_8$]octanesulfonic acid		$HF_{17}SO_3 + {}^{13}C_8$	9.637	508.13	507.0	203	79.9	97	

LC/MS Preparation and Run.
LC/–S/MS - Agilent 1290 Infinity II UPLC – 6470 QQQ-MS for PFAS_LC

1. Gather the 1 mL PP vials with IS spiked extract from lab processing procedure, allow to reach ambient temperature.
2. Ensure the Acquity BEH C18 column (50 × 2.1 mm, 1.7 µm thickness, Waters) column is installed.
3. Equilibrate column with the mobile phases. **A** - 2 mM ammonium acetate (NH_4Ac) in water and **B** - 2 mM NH_4Ac in MeOH.
4. Gather calibration standard, CRS, and blank methanol vials. Methanol blanks should be run between sample sites on the worklist. CRS vials are used to detect changes in instrument status and should be run every batch of precipitation samples.
5. Prepare and Run the worklist.

The latest instrumental acquisition methods can be obtained in Methods folder on the computer connected to LC/MS/MS.

LC/MS/MS: “IADN iPFAS - 28Dec2023.m”

Analysis Methods.
The latest quantitation method “IADN iPFAS 28Dec2023.m can be obtained the methods folder on the Shared Project drive of the Workgroup.