U.S Environmental Protection Agency
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National Exposure Research Laboratory
Exposure Methods and Measurement Division
Environmental Chemistry Branch

STANDARD OPERATING PROCEDURE

SOP Title: **Exhaustive Extraction of Charged Per- and Polyfluorinated Alkyl Substances (PFASs) from Contaminated Soil & Sediment Samples with Ion-Pairing Cleanup**

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Revision History

Version No.	Name	Date of Revision	Description of Change(s)
1	John Washington	09/14/17	SOP developed in August of 2016, now being placed into the NERL SOP system

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SOP Title: *Exhaustive Extraction of Charged Per- and Polyfluorinated Alkyl Substances (PFASs) from Contaminated Soil & Sediment Samples with Ion-Pairing Cleanup*

1. *Scope and Applicability*

This method for extraction of charged per- and polyfluoroalkyl substances (PFASs) from soils and sediments includes improvements upon five previous peer-reviewed published methods. The method is expected to be effective for soils and sediments, surface soils and sub-surface soils, pristine soils/sediments and contaminated soils/sediments. The method has been used with acceptable spike and recoveries on a test soil with chain lengths as long as C12 (perfluorododecanoic acid; Figure 1) and on soils from every continent and several ocean islands with C8 (perfluorooctanoic acid).

Our first soils method paper reported a simple 60/40 acetonitrile/water (ACN/H_2O) extraction (Washington et al. 2007). Noting a noisy matrix in this extraction, we modified this method to include an extract cleanup (Washington et al. 2008). In 2010, we reported on our attempt to use this method on agricultural surface and subsurface soils that had been amended with biosolids; in these papers we reported that shaker-table mixing of subsurface soils can lead to the clay packing and poor recoveries, so we modified the mixing to use rotisserie spits which yielded satisfactory recoveries (Washington et al. 2010, Yoo et al. 2010). In 2011, we used this same method on pristine and PFAS-contaminated river sediments with success (Lasier et al. 2011). At this point, our method for charged PFASs in soils/sediments was, 60/40 ACN/H_2O, rotisserie mixing, extract cleanup and inject on an LC/MS/MS – and this worked on surface and subsurface soils, pristine and contaminated soils, and river sediments.

Then in 2013/2014 we tested this method on soils having fluorotelomer-based polymers (FTPs) present (Washington et al. 2014, Washington et al. 2015). FTPs are the major product of the fluorotelomer industry and they commonly have been applied to articles of clothing, among other uses, to impart anti-staining properties; hence, soils treated with biosolids or soils possibly contaminated near landfills or refuse-disposal areas can be expected to contain FTPs commonly. Also, FTPs degrade to form PFASs, so when PFAS contamination is an issue, FTPs potentially will play a role in the contamination. Our test spike and recoveries of PFASs from soils containing FTPs were very low and unacceptable. In response to these poor recoveries, we tried numerous alternatives including: i) six serial extractions with 60/40 ACN/H_2O in a row, ii) lowering the solvent dipole with butanol, iii) increasing the solvent dipole with propylene carbonate, and iv) pre-oxidizing the soil with permanganate. None of this yielded acceptable recoveries – we were stymied. At about this time, researchers investigating mobilization of PFASs from a fluorotelomer stationary phase in a liquid chromatograph reported that 90/10 ACN/H_2O liberated PFASs more effectively than any other solvent they tried, including pure ACN. We tried this solvent on soils containing FTPs and achieved very good recoveries, including with perfluorododecanoic acid (C12), which is among the most resistant of PFASs to extraction from soil and sediment.

The method we developed for testing soils containing FTPs was complicated by including the objective of quantitating uncharged PFASs, such as fluorotelomer alcohols (FTOHs) and fluorotelomer acrylates (FTAcs). So in a later study of charged PFASs in global soils, we simplified the soil/FTP method for charged

PFASs only, and used the method on >60 soils collected from around the world, for which we uniformly achieved acceptable recoveries of spiked PFOA (Rankin et al. 2015). This resulting method is described below.

2. *Summary of Method*

All extraction apparatus are washed with Optima-grade methanol (MeOH). Process blanks and spiked samples are included in the extraction procedure. Soil is air-dried sufficiently to pass through a 2-mm stainless-steel sieve. An aliquot of sieved soil is weighed then dried at 105 C to obtain remaining moisture content. Triplicate 1 g aliquots of soil are placed in polycarbonate centrifuge tubes with matching caps. Mass-labeled PFOA is added to the soil as a recovery standard. Base and water is added to the soil and allowed to react for 30 min. Then acid is added to neutralize the base and ACN is added to achieve ~90/10 ACN/ H_2O by volume. The choice of 90/10 ACN/H_2O is a critical component for the objective of exhaustive PFAS extraction (Washington et al. 2014). The tubes are capped, vortexed until visually homogenous, sonicated for 1 hr, then mixed on a rotisserie spit overnight. Choice of rotisserie mixing is critical – shaker table mixing can compact subsurface, high-clay soils, diminishing recovery (Washington et al. 2010). In the morning, tubes are centrifuged and the liquid decanted to 12 mL disposable glass vials. Three mL of 90/10 ACN/H_2O are added to the tubes, the extraction process repeated and the supernatant combined with that of the first extraction round. Serial extraction can be repeated if soils are expected to be heavily laden with PFASs (Figure 1). Then the combined extracts are subjected to a cleanup step to reduce matrix noise (Washington) – we find liquid-liquid to be simple and effective.

3. *Definitions/Acronyms*

FTP: Fluorotelomer-based polymer
FTOH: Fluorotelomer alcohol
FTAc: Fluorotelomer acrylate
NPW: Nanopure water
PNPW: Polished Nanopure water
PFAS: Per- and Polyfluoroalkyl substance
PFDA: Perfluorodecanoic acid
PFDoDA: Perfluorododecanoic acid
PFOA: Perfluorooctanoic acid
SPE: solid phase extraction

4. *Health and Safety Warnings*

Perfluorinated substances are potentially carcinogenic and perfluorooctanoic acid has been documented to contribute to enhanced risk of birth defects. Work in hoods with standard personnel protection: acetonitrile resistant gloves and safety glasses.

5. *Cautions/Interferences*

Clean-room techniques should be employed to minimize cross-contamination between samples. Multiple samples of soils, soil extracts, and clean-up analytical samples are employed to optimize statistical reliability. Work surfaces are cleaned regularly with methanol-saturated KimWipes.

6. *Personnel Qualifications/Responsibilities*

Personnel should be trained in extraction and analytical techniques by SOP steward, including practices such as using a suite of challenge analyses that have been previously analyzed and have demonstrated reliability.

7. *Equipment and Supplies*

Thoroughly clean glassware, using Nanopure water and methanol.

- 2mm stainless steel sieve for preparing soil samples
- 16 mL polypropylene copolymer (PPCO) centrifuge tubes with size 18 PPCO caps
- Nanopure water filtration system using deionized water as input
- Oasis 35 cc HLB cartridge for polishing Nanopure water, with dedicated glassware system (2L volumetric source; 2L flask with vacuum side arm receiver)
- Dedicated 1L glass containers for polished water storage and for 60/40 acetonitrile/polished water mixtures used in sample preparation
- Solid-phase extraction system adapted to evaporate samples to dryness prior to ion-pairing cleanup
- Vortex mixer
- High RPM centrifuge to fit 16 mL tubes.

8. *Reagents and Standards*

A. Polished Nanopure Water (PNPW)

1. To polish water, i.e., purge PFCAs, use glassware system dedicated to water polishing.
2. Pass 2L 18MΩ (Nanopure) water through a 35cc "Oasis HLB" cartridge (use the same cartridge no more than 3 times).
3. Store polished NPW in dedicated 1L containers.

B. Polished Tetrabutylammonium (TBA) Mix (Ion Pairing Reagent)

1. Prepare 0.50M Tetrabutylammonium Hydrogen Sulfate (TBAHS) in 18MΩ Nanopure water. Formula weight of TBAHS is 339.54 g/mol. Dissolve 169.77 g TBAHS in Nanopure water and dilute to 1000 mL in volumetric flask (adjust all masses according to need for number of samples).

2. Prepare 0.25M Na_2CO_3 in 18MΩ Nanopure water. Formula weight of anhydrous Na_2CO_3 is 105.99 g/mol. Dissolve 26.50 g anhydrous Na_2CO_3 in Nanopure water and dilute to 1000 mL in volumetric flask (adjust all masses according to need for number of samples).
3. Add 2.0 parts of the 0.25M Na_2CO_3 solution to 1.0 part 0.50M TBAHS solution, mixing slowly to avoid spillage due to CO_2 generation.
4. Place a 500mL Nalgene waste collection bottle in the reservoir of a Waters or comparable solid-phase extraction (SPE) vacuum system.
5. Mount a 35cc HLB cartridge on the port above the Nalgene bottle.
6. Flush with 50mL NPW and 50mL methanol, HPLC grade.
7. Replace the waste Nalgene bottle with a methanol-washed Nalgene bottle; and discard the waste.
8. Pass the TBA Mix in part 8.B.3 through the cartridge until desired volume has been polished; cap and label polished TBA mix.
9. Flush cartridge with 50mL methanol (MeOH) per steps 8.B.4 and 8.B.6. Store in labelled Ziploc bag for further use in polishing this reagent mix only.

C. $^{13}C_8$-PFOA (M8C8) Extraction Recovery Spike Solution

1. Prepare from Cambridge Isotope Laboratories Certified Stock Solution in 60/40 (v/v) ACN/polished water to give a concentration of ~100 ng M8C8 per gram of solution.

D. $^{13}C_9$-PFNA (M9C9) Cleanup Recovery Spike Solution

1. Prepare from Wellington Certified Stock Solution in 60/40 (v/v) ACN/polished water to give a concentration of ~15 ng M9C9 per gram of solution.

E. $^{13}C_4$-PFOA (MC8) (and other mass-labelled perfluorinated compounds) mixed Internal Standard Solution (designated MMX)

1. Prepare from Cambridge Isotope Laboratories or Wellington Certified Stock Solutions in 60/40 (v/v) ACN/PNPW to give concentrations of ~100 pg/g for each mass-labeled internal standard per gram of solution. Internal standards chosen to match as many individual PFAAs as possible, enabling individual isotopic dilution quantitation for each analyte.

F. 2.0 M NaOH Solution and 2.0M HCl Solution

1. Prepare from concentrated stock solutions using polished NPW. Formula weight of NaOH is 40.0 g/mol and purchased concentrated HCl is usually 17 M. To prepare 100 mL of 2.0 M NaOH, dissolve 8.0 g NaOH to a small amount of Nanopure water and dilute to 100 mL in volumetric flask. To prepare 100 mL of 2.0 M HCl add 11.76 mL concentrated HCl to ~50 mL Nanopure water in a 100 mL volumetric flask, mix, and dilute to 100 mL with Nanopure water.

9. *Procedures*

SOIL SAMPLE EXTRACTION

A. Prepared 2mm Sieved Soil Sample

1. If necessary for handling, air dry bulk sample in hood in methanol-washed stainless-steel or glass container to a moisture content level which will enable the soil to be easily sieved – generally in the range of 20% water content.
2. Using all methanol-washed equipment, sieve using a 2mm stainless steel sieve, forcing soil as needed with large rubber stopper or nitrile-gloved hand. Store sieved soil in methanol-washed 500mL Nalgene bottle.
3. Weigh three ~1-5 gram aliquots to preweighed weigh boats; vacuum dry over Drierite for 18 hours and weigh again.
4. Repeat step A.3 as needed until constant weight is obtained. Calculate percent moisture of soil.
5. To prepare extraction sample:
    a. pass entire aliquot through 12-in diameter 2mm sieve;
    b. cone and quarter in sieve pan using large spatula;
    c. remove three quarters and sieve to a second pan; return remainder to original container;
    d. cone and quarter in sieve pan using large spatula;
    e. repeat steps c and d until size of aliquot is reduced to four grams;
    f. cone and quarter final aliquot and charge to extraction tubes in part B.

B. Prepare Spiked Soil Samples

1. Charge 1g-dry weight equivalent of soil to pre-weighed (tube and cap) MeOH- or MTBE-washed, 16-mL polypropylene copolymer (PPCO) centrifuge tubes with size-18 PPCO caps. Re-weigh and record weight in data table.
2. Add 50uL 100 ng/g M8C8 spike solution to provide a loading of ~4 ng M8C8 per gram of dry soil. Reweigh.
3. Add 200uL 2.0M NaOH and allow to react for 30 min.
4. Add PNPW to achieve a total water content of 0.4g, compensating for soil moisture and water added in steps B.3 and C.2. Reweigh. Let stand for at least 30 min before proceeding to step C.1.

C. Extract Spiked Soil Samples

1. Add 3.6mL ACN to yield a 90:10 by-volume solution of ACN:H_2O. Reweigh.
2. Add 200uL of 2.0M HCl to neutralize NaOH added in B.3. Reweigh.
3. Vortex until homogeneous appearance, sonicate for 60 min using ice to maintain lower bath temperature, transfer to Labquake rotisserie for 15-24 hours;
4. Centrifuge in Sorvall at 5,000 rpm and 18 to 22 °C for 20 min. Reweigh to capture solvent losses due to evaporation.
5. Decant liquid to 12mL preweighed (with top) glass vial. Reweigh both centrifuge tube and 12mL vial (with caps).
6. Add 3.0 mL 90:10 ACN/PNPW to centrifuge tube. Vortex, sonicate 60 min in ice, rotate or shake for 15-24 hours, centrifuge and reweigh.
7. Decant liquid to 12mL glass vial, combining with previous extracts, and reweigh vial.
8. Repeat steps C.5 and C.6 for a total of 2 (low level PFAS) to 4 (high level) extractions.
9. Evaporate contents of 12mL vial to dryness in SPE assembly, using nylon filters and 5-7 psi vacuum.

D. Cleanup Extract using Ion Pairing

1. Add 4 mL TBA Mix to dried extract from C.9. Vortex. Reweigh.
2. Add 5 mL methyl-tert-butyl-ether (MTBE). Vortex. Reweigh.
3. Freeze. Transfer MTBE to tared 12 mL glass vial. Reweigh.
4. For high levels of PFASs repeat D2 and D3 and combine MTBE fractions
5. Evaporate MTBE to dryness in SPE apparatus. Reweigh.

6. Reconstitute with 2mL 60/40 ACN/H2O containing mass-labeled internal matrix standards (MMX). Reweigh.

E. Analyze samples on Waters Triple-Quad LC/MS/MS

10. *Data and Records Management*

Analytical data generated from samples is supplied with the instrument. It is the primary system for data analysis and storage. Data records are downloaded to jump-drives for continued analysis and statistical manipulation in Excel and standard statistical programs. A written log of activity on the LCMSMS is maintained for QA purposes.

11. *Quality Assurance/Quality Control*

Certified internal standards are used in all samples. These are purchased from Wellington Laboratories. Multiple samples are prepared for each soil sample and for each extraction/cleanup.

12. *References*

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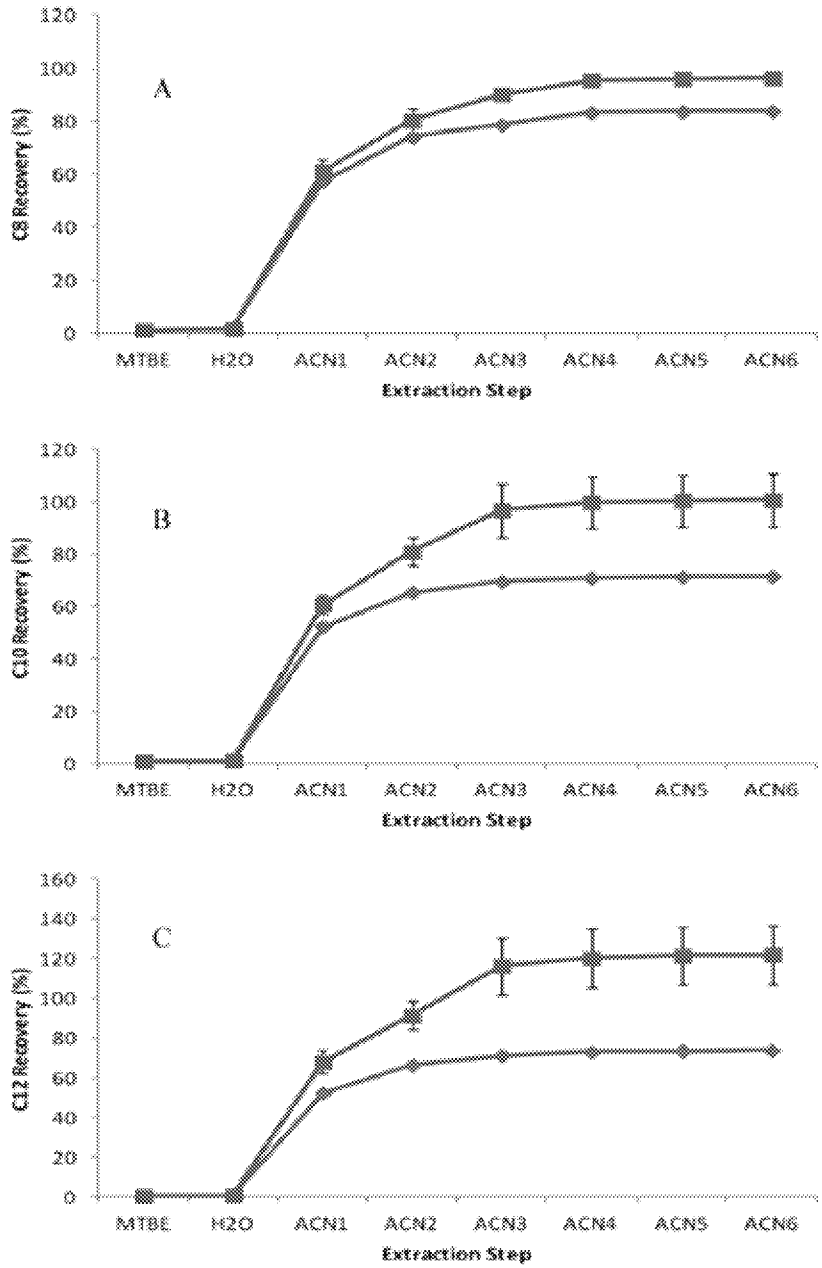

Figure 1: Recovery of PFOA (C8; a), PFDA (C10; b), and PFDoDA (C12; c) from a microcosm of soil and a fluorotelomer-based polymer (Washington et al. 2014). The first steps, MTBE and H2O, were for extraction of fluorotelomer alcohols from the polymer, followed by 90/10 ACN/H_2O, with two ion-pairing cleanups (cleanup 1 is blue, 2 is red) of each extraction step to recover the spiked analytes with four ACN extraction steps. C10 and C12 are reported here from external calibrations. Error bars are cumulative 1 SD (n = 3 microcosms) for the second ion-pairing cleanup. Spiking levels were 80-120 ng/g soil, at least 600x that of typical background soils (Rankin et al. 2015).