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Draft Procedure for Analysis of Perfluorinated Carboxylic Acids and Sulfonic Acids in Sewage Sludge and Biosolids by HPLC/MS/MS

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U.S. Environmental Protection Agency
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Introduction and Disclaimer

This document represents a draft procedure for the analysis of perfluorinated carboxylic acids and perfluorinated sulfonates in sewage sludge and biosolids, using high performance liquid chromatography (HPLC) combined with tandem mass spectrometry (MS/MS).

This draft procedure has been tested in one laboratory under contract to EPA's Engineering and Analysis Division in the Office of Water, but it is incomplete and is undergoing continued development by EPA. Further method development work and validation must take place including: MDL studies, IPR studies, and generation of QC acceptance criteria. Following those development activities, the draft procedure should be validated in multiple laboratories.

However, EPA is publicly releasing this draft procedure because of interest in methods for perfluorinated compounds as "emerging contaminants."

This procedure does not determine fluorotelomer alcohols and related precursor compounds, which can degrade to form perfluorinated carboxylic acids and sulfonic acids, and thus may contribute to the overall concentrations of perfluorinated compounds in sewage sludge and biosolids.

This procedure should be restricted to use by analysts who are experienced in HPLC-MS/MS.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Acknowledgements

This draft procedure was written by staff in EPA's Engineering and Analysis Division in the Office of Water, based on extraction, cleanup, and instrumental procedures developed at U.S. EPA's National Exposure Research Laboratory in Athens, GA (References 1 and 2) and additional supplementary procedures from EPA Method 537 (Reference 3), developed by the National Exposure Research Laboratory in Cincinnati, OH.

Initial single-laboratory testing of the procedure was performed by AXYS Analytical Services Ltd., Sydney, British Columbia, Canada, under contract to EPA. Following that testing, a version of the procedure was subjected to a limited internal Agency peer review by two researchers from EPA's National Risk Management Research Laboratory in Cincinnati, and one researcher from EPA's National Environmental Research Laboratory in Athens, GA.

EPA gratefully acknowledges the contributions of all of these organizations and individuals.

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1.0 Scope and Application

1.1 This draft procedure is intended for use by EPA in the development of standardized methods for the determination of perfluorinated carboxylic acids, perfluorinated sulfonic acids, methyl and ethyl perfluroro-octanesulfonamides, and (methyl and ethyl sulfonamido)-ethanols in sewage sludge and biosolids. The procedure employs high performance liquid chromatography (HPLC) combined with tandem mass spectrometry (MS/MS). The list of target analytes is presented in Table 1.

1.2 This procedure may be applied to various solids from sewage treatment plant operations, including sewage sludge and biosolids. Sewage sludge is the solid, semisolid, or liquid organic material that results from the treatment of domestic wastewater by municipal wastewater treatment plants. Biosolids are defined in EPA regulations at 40 CFR Part 503 as sewage sludge that has had additional processing for land application. However, EPA often uses the terms sewage sludge and biosolids interchangeably, and may do so in this procedure as well.

1.3 This draft procedure is based on extraction, cleanup, and instrumental procedures developed at U.S. EPA's National Exposure Research Laboratory in Athens, GA (References 1 and 2) and additional supplementary procedures from EPA Method 537 (Reference 3). The quality control protocols found in this draft procedure are based on existing EPA HPLC/MS/MS methods (Reference 4).

1.4 Early work on this procedure focused on analysis of the solid portion of sewage sludge samples and described discarding any supernatant aqueous liquid in the sample. Subsequent efforts suggest that the extraction procedures are capable of dealing with samples containing large amounts of water, which may better represent actual sewage sludges and biosolids from wastewater treatment operations. These capabilities will be the subject of further testing and refinement of this draft procedure by the EPA Office of Water's Engineering and Analysis Division.

1.5 This draft procedure does not determine fluorotelomer alcohols and related precursor compounds, which can degrade to form perfluorinated carboxylic acids and sulfonic acids, and thus may contribute to the overall concentrations of perfluorinated compounds in sewage sludge and biosolids.

1.6 This draft procedure should be restricted to use by analysts who are experienced in HPLC MS/MS.

1.7 *The draft procedure has not been published in 40 CFR Part 136 and is not approved for either general purpose or regulatory use*. This draft procedure has not been validated by EPA and is being used strictly for method development purposes. It is being publicly released because of interest in methods for perfluorinated compounds as "emerging contaminants."

2.0 Summary of Procedure

The general steps in this procedure are summarized in Secs. 2.1 to 2.3. A flow chart that summarizes the procedures for sample preparation, cleanup, and analysis is shown in Figure 1 at the end of the document.

2.1 Sample collection and digestion – Collect a sample of sewage sludge or biosolids sufficient to yield at least 0.5 g of wet solids. (Larger samples are recommended to ensure that they are more representative of the bulk source of the material.) Homogenize the sample and transfer a subsample containing 0.5 g of wet solids to a centrifuge tube, spike the sample with the labeled compound spiking solution, and digest the sample for 30 min with 1M NaOH by heating and ultrasonic agitation, followed by overnight incubation. Samples are neutralized with HCl and extracted with solvent.

2.2 Solvent extraction and cleanup – Extract the digested sample twice by shaking and ultrasonic agitation, using 10 mL 50:50/ACN:MeOH (v/v). Dilute the sample extract, acidify it to pH 6.5, agitate it ultrasonically, and clean up the extract using a weak anion exchange (WAX) solid-phase extraction (SPE) cartridge.

2.3 Analysis – Reconstitute the sample extract with 1 mL 0.3% NH_4OH in methanol containing the labeled injection internal standards. Analyze a 15-µL aliquot on a dedicated HPLC/MS/MS equipped with a trapping column (if needed), using negative electrospray ionization (ESI-) mode. Sample concentrations are calculated using either isotope dilution quantitation for those analytes with exact labeled analogs in the labeled compounds spiking solution, or internal standard quantitation for those analytes without an exact labeled analog. The recoveries of the labeled analogs themselves are determined by internal standard quantitation and used as a quality control check on the overall analytical process.

3.0 Definitions and Units of Measure

Definitions of terms, acronyms, abbreviations, and units of measure are given in the glossary in Sec. 19 of this document.

4.0 Interferences and Contamination

4.1 Background levels of perfluorinated chemicals must be controlled during this analysis. To determine if background concentrations of perfluorinated compounds (PFCs) are under control, analysts must be able to see a significant difference between a blank and a low-level standard. This test is discussed in Sec. 12.5. If this cannot be achieved, it may be necessary to proof reagents and equipment to find the source of contamination or to modify the tubing on the LC/MS/MS system. Modifications to LC/MS/MS systems are discussed in Sec. 6.4.5.

4.2 Containers –Aqueous solutions of PFC compounds should be stable for at least 28 days when stored in glass or high density polyethylene (HDPE) containers. Storage of aqueous solutions in polypropylene containers has resulted in significant loss of certain perfluorocarboxylic acids (C_{11} and C_{12}) after 7 days, although some researchers have suggested that stability of the perfluorocarboxylic acids may be improved through the inclusion of a substantial fraction of an organic solvent. PFC solutions in basic methanol have not been observed to degrade when in contact with any of these materials for 6 months.

4.3 All solvents and reagent water used in the analysis must be demonstrated to be free of PFC contamination and other interferences. A sample (equivalent in volume to the amount used in the procedure) from each lot number of solvent or water should be proofed before use. Concentrations of any detected target compounds are compared to the method detection limits (MDLs) and minimum levels (MLs) to determine whether they are acceptable for use. PFC-free reagent water is available (see Sec. 7.1.4), but a procedure for "polishing" deionized water is provided in Appendix 2.

4.4 All materials used in the analysis, and the entire analytical process, must be demonstrated to be free of PFCs and other interferences by running reference matrix method blanks (Sec. 10.7) initially, and with each sample batch (samples started through the extraction process on a given 12-hour shift, to a maximum of 20 samples).

4.5 False positives, false negatives, and co-extracted interferences – Interferences co-extracted from samples will vary considerably from source to source. Taurodeoxycholic Acid (TDCA) and some of its isomers, including tauroursodeoxycholic acid and taurochendeoxycholic acid, are known interferences which may overestimate or yield a false positive result for perfluoro-octanesulfonic acid (PFOS) (Ref. 5), while 5-pregnan-3,20-diol-3-sulfate and 34S-3-hydroxy-5-pregnan-20-one sulfate may interfere with perfluorohexanesulfonic acid (PFHxS) (Ref. 6). Protocols for ensuring chromatographic separation of PFOS from TDCA and for detecting interferences by monitoring secondary multiple reaction monitoring (MRM) transitions for both PFOS and PFHxS are discussed in Sec. 13.5.

4.6 Matrix Effects – Matrix effects can occur during LC/MS/MS analysis when the sample extract contains material that co-elutes with the target analytes causing enhancement or suppression of their MS/MS response, potentially impacting the reliability of data obtained for samples with complex matrices. This type of matrix effect manifests itself as either high or low labeled compound recovery (indicating that some of the labeled compounds or injection internal standards are either being enhanced or suppressed).

If the labeled standard recoveries do not meet the acceptance criteria in Table 8, then the laboratory should determine whether a matrix effect is the cause. One diagnostic test is to dilute the sample extract and reanalyze it. Dilution decreases the amount of matrix entering the MS/MS system, diminishing its effect, and therefore dilution should produce a change in labeled standard recoveries as the effect of matrix is diminished. The "method of standard additions" also can be used to diagnose matrix effects, but because it introduces even more material into the ionization chamber, it is unlikely to be useful in resolving those effects in specific samples.

When performing isotope dilution quantification, the labeled compound and its exact analogue enter the MS/MS system at the same time and are therefore similarly enhanced or suppressed. Therefore, the quantification of target compounds is not affected, even if the labeled standard recovery is outside of the specified range. For those target compounds whose concentration is determined by internal standard quantification (vs. isotope dilution), the matrix enhancement or suppression may have a different effect on the target compounds compared to the internal standard used for quantification and therefore, there can be an effect on quantified results.

There are three possible remedies for this situation: the affected target compounds could be reported from the diluted analysis, the sample extract could be subjected to further cleanup (repeat the SPE cleanup on the finished extract), or the analysis could be repeated using a smaller sample size to reduce the matrix effects.

5.0 Safety

5.1 The toxicity or carcinogenicity of each chemical used in this procedure has not been precisely determined; however, each compound should be treated as a potential health hazard. Pure standards of the compounds should be handled only by highly trained personnel thoroughly familiar with handling and cautionary procedures and the associated risks. It is recommended that the laboratory purchase dilute standard solutions of the analytes in this method. However, if primary solutions are prepared, they should be prepared in a hood, and a NIOSH/MESA approved toxic gas respirator may be necessary when high concentrations are handled.

5.2 This procedure does not address all safety issues associated with its use. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this procedure. A reference file of material safety data sheets (MSDSs) should also be made available to all personnel involved in these analyses. It is also suggested that the laboratory perform personal hygiene monitoring of each analyst who uses this procedure and that the results of this monitoring be made available to the analyst. Additional information on laboratory safety can be found in Refs. 7 to 9.

5.3 Spiking solutions or samples suspected to contain high concentrations of these compounds should be handled with care.

5.3.1 Facility – When finely divided samples (dusts, soils, dry chemicals) are handled, all operations (including removal of samples from sample containers, weighing, transferring, and mixing) should be performed in a glove box demonstrated to be leak tight or in a fume hood demonstrated to have adequate air flow. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in the case of an accident.

5.3.2 Protective equipment – Disposable plastic gloves (Latex or non-Latex [such as nitrile]), apron or lab coat, safety glasses or mask, and a glove box or fume hood should be used. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters. Eye protection (preferably full face shields) should be worn while working with exposed samples or pure analytical standards. Latex or non-Latex (such as nitrile) gloves are commonly used to reduce exposure of the hands.

5.3.3 Training – Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

5.3.4 Personal hygiene – Hands and forearms should be washed thoroughly after each operation involving high concentrations of the analytes of interest, and before breaks (coffee, lunch, and shift).

5.3.5 Confinement – Isolated work areas posted with signs, segregated glassware and tools, and plastic absorbent paper on bench tops will aid in confining contamination.

5.3.6 Waste handling – Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors and other personnel should be trained in the safe handling of waste. See Sec. 17 for additional information on waste handling and disposal.

5.4 Sewage sludge and biosolids samples may contain high concentrations of biohazards, and must be handled with gloves and opened in a hood or biological safety cabinet to prevent exposure. Laboratory staff should know and observe the safety procedures required in a microbiology laboratory that handles pathogenic organisms when handling such samples.

6.0 Equipment and Supplies

Note: *Brand names, suppliers, and part numbers are cited for illustration purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here. Demonstration of equivalent performance that meets the requirements of this procedure is the responsibility of the laboratory.*

6.1 Disposable lab equipment

6.1.1 Sample bottles and caps

6.1.1.1 Sample collection bottle – High density polyethylene (HDPE) sample bottles with polypropylene lids, Nalgene, Part No.: 69032, Lima, Ohio, USA, or equivalent.

6.1.1.2 Extract dilution bottle – HDPE bottles 125-mL Nalgene, Part No.: 332189-0004, Lima, Ohio, USA, or equivalent.

6.1.2 Centrifuge tubes

6.1.2.1 Polypropylene centrifuge tubes (15 mL) with polypropylene screw caps, Corning®, Cat. No: 430766, Corning, NY, USA, or equivalent.

6.1.2.2 Glass centrifuge tubes (15 mL), Kimble Chase, Part No.: 7379015, Vineland, NJ, USA, or equivalent.

6.1.3 Autosampler vials – Polypropylene 0.3-mL autosampler crimp-top vials (Canadian Life Sciences, Peterborough, ON, Canada, Cat. No.: 30300P-1232), or equivalent, with polypropylene snap caps (Canadian Life Sciences, Peterborough, ON, Canada. Cat. No.: 30300P-1232), or equivalent. Polypropylene vials and caps are required, but do not adequately reseal after use. Therefore, due to potential evaporation, multiple injections from the same vial are not recommended.

6.1.4 Syringes – Polypropylene 5-mL NORM-JECT syringes with Luer-slip fitting (Fisher Scientific Cat. No. 1481728), or equivalent.

6.1.5 Disposable pipets – Glass or polypropylene disposable pipets (Fisher Cat. No. 13-711-17) or equivalent.

6.1.6 Miscellaneous labware – Glass or polypropylene, as required.

6.1.7 pH paper – Whatman® Panpeha® pH indicator strips pH range 0 to 14 (Sigma-Aldrich, Cat. No.: Z134147), or equivalent.

6.1.8 Syringe filters – Acrodisc 0.45-µm, 25-mm filters with nylon membranes (Ann Arbor, MI, USA, Part No.: 4614), or equivalent.

6.2 Non-disposable lab equipment

6.2.1 Auto-pipettes – Gilson (Middleton, WI, USA) Microman® positive displacement pipettes, volume 10-, 25-, 100-, and 1000-µL with disposable tips, or equivalent.

6.2.2 Balances – An analytical balance capable of weighing 0.1 mg is used for sample weight measurements. An analytical balance capable of weighing 10 mg is used for measuring standards.

6.2.3 Equipment for concentration of extracts – Extracts should be concentrated by evaporation with nitrogen using a water bath set at 40 ºC (Meyer N-Evap, Model 111, Organomation Associates, Inc.) or equivalent.

6.2.4 Ultrasonic bath – Branson Model 5510 ultrasonic bath (Danbury, CT, USA) or equivalent.

6.2.5 Shaker – Barnstead LabQuake Tube Shaker/Rotators (Waltham, MA, USA, Cat. No.: 415110), or equivalent.

6.2.6 Bench top Centrifuge – Thermo Scientific, Sorvall Legend RT+ (Waltham, MA, USA) or equivalent.

6.2.7 Vortex mixer – Barnstead (Dubuque, IA, USA Thermolyne Model No: M16715) or equivalent.

6.3 Solid-phase extraction

6.3.1 Solid-phase extraction cartridge – Oasis weak anion exchange (WAX) extraction cartridge, 6-cc barrel size, 150-mg sorbent weight, 30-µm particle size (Waters Milford, MA, USA), or equivalent.

6.3.2 SPE extraction kit – Vacuum extraction manifold (Alltech/Grace Davison, Deerfield, IL, USA, Part No:. 210016) or equivalent, using 60-mL polypropylene reservoirs (Supelco/ Sigma Aldrich, Part No. 57022) or equivalent, with reservoir adapters (Supelco/Sigma Aldrich, Part No. 57020-U) or equivalent, and polypropylene needles (Alltech/Grace Davison, Deerfield, IL, USA, Part No. 210916) or equivalent. Either a manual or automatic vacuum manifold may be used for SPE extractions.

Caution: *Automated systems may contain parts made of polytetrafluoroethylene (PTFE). Before use, the system should be proofed to ensure it is free from contamination.*

6.3.3 Laboratory vacuum system or aspirator vacuum system – Capable of maintaining 23 in. Hg, equipped with shutoff valve and vacuum gauge.

6.4 LC/MS/MS system – The analytical instrumentation used should meet the following requirements:

6.4.1 HPLC system – The HPLC/MS/MS system used must have a high-pressure inlet, must have a post-column pump for admission of calibrant during mass spectrometry calibration and optimization, must be capable of multi-segment gradient separation, producing the separations for the analytical runs detailed in Table 2 under the instrument

conditions detailed in Table 5, and meeting other HPLC requirements in Sec. 12. This system must also be equipped with a 50-µL loop capable of using 'partial loop with needle overfill' mode.

6.4.2 Columns – Injections are introduced into a 50-µL loop using 'partial loop with needle overfill' mode connected to a C18 guard cartridge, followed by an analytical column (PN 186000404, Waters Xterra MS C18 3.5 µm, 2.1 x 100 mm column) or equivalent. Alternative columns may be used as long as they provide comparable chromatography to that described in Table 2.

6.4.3 MS/MS system – The MS/MS system must be capable of negative electrospray ionization (ESI-) under the conditions in Table 5, producing unique product ions for analytes within specified retention time segments. A minimum of 10 scans across the chromatographic peak of the lowest concentration standard is required to ensure adequate precision (Waters Quattro Ultima tandem mass spectrometer) or equivalent. The system should be able to resolve native and labeled compounds with a mass difference of two.

6.4.4 Data system and other software – All system operations should be controlled by appropriate system software (Waters MassLynx 4.1 and QuanLynx 4.1), or equivalent. The software should be interfaced to the HPLC/MS/MS to control the LC gradient and other LC and MS/MS operating conditions, and to acquire, store, reduce and output HPLC/MS/MS data. The software must be able to identify a compound by retention time and precursor-product m/z, allow integration of an ion abundance of any specific ion within specified time or scan number limits, be able to quantify the compound using relative responses and response factors, or linear or quadratic multi-point weighted regressions, by isotope dilution and internal standard quantitation techniques.

6.4.5 Elimination of PFC background – Background levels of perfluorinated chemicals must be controlled to ensure that they do not interfere with this analysis. To determine if background levels of PFCs interfere with this analysis, the background test in Sec. 12.5 must be conducted.

If instrument background levels of PFCs are found (see diagnostic test in Sec. 12.5), they must be eliminated before analyses proceed. The following instrumentation modifications may be required:

- substitution of tubing with PEEK™ tubing,
- replacement of PTFE solvent frits with stainless steel frits,
- inserting a trap column (PN WAT200650, Symmetry C18, 3.5 µm, 2.1 x 50 mm column) or equivalent, as part of the solvent manager at the most down-gradient point in the water-eluent line immediately before the solvent mixing cell, and
- injection of sufficient blanks to cleanse the system (3 to 5 blanks may be required).

Additionally, to minimize buildup of PFCs during mobile phase equilibration and keep background level constant, the time the system sits at initial conditions should be kept constant and as short as possible, but should also assure reproducible retention times in continuing calibration verifications. Prior to daily use, flush the LC column with elution solvents before initiating a sequence. It may also be necessary to flush other LC components such as syringes and other system components.

7.0 Reagents

7.1 Solvents - Each lot of solvent must be demonstrated to be free from contamination on a routine basis.

7.1.1 Trace-grade methanol (BDH, VWR International, West Chester, PA, USA) or equivalent.

7.1.2 HPLC-grade acetonitrile (VeX Chem, Aurora, ON, Canada) or equivalent.

7.1.3 Plasma-grade reagent water (Fisher Chemicals) or equivalent, for use in the preparation of standards and samples.

7.1.4 HPLC-grade water (Fisher Chemicals) or equivalent, for use as the LC mobile phase.

7.2 Gases

7.2.1 Argon, used as the collision gas. Ultra high purity (Alphagaz 1, Air Liquide Canada, or Airgas, Radnor, PA) or equivalent. Argon gas used should meet or exceed instrument manufacturer's specifications. Nitrogen may be used as the collision gas, provided that sufficient sensitivity can be achieved.

7.2.2 Nitrogen, ultra high purity or from a nitrogen generator. Nitrogen may be used as a carrier gas and as a nebulizer gas in aerosol generation in ESI liquid spray (Alphagaz 1, Air Liquide Canada, or Airgas, Radnor, PA) or equivalent. Nitrogen also is used to concentrate sample extracts (Ultra High Purity) or equivalent. Nitrogen gas used should meet or exceed instrument manufacturer's specifications.

7.3 Other reagents

7.3.1 Certified ACS grade sodium hydroxide (Fisher Chemical) or equivalent.

7.3.2 Ultra pure hydrochloric acid (Seastar Chemicals Inc., Sidney, BC, Canada) or equivalent.

7.3.3 Glacial acetic acid, HPLC grade (Fisher Chemical, Fair Lawn, NJ, USA) or equivalent.

7.3.4 Ammonium hydroxide – (Fisher Chemicals, certified ACS+ grade, 30% in water, Fair Lawn, NJ, USA) is used as received.

7.3.5 Formic acid – (Alfa Aesar, greater than 96% purity, Ward Mill, MA, USA,) is used as received.

7.3.6 Ammonium acetate, purity >98%. (Sigma Chemicals) or equivalent.

7.3.7 Reference matrices – Reference matrices must be free from contamination. It may be difficult to obtain a sewage sludge or biosolids sample that does not have detectable concentrations of one or more of the native PFCs covered by this procedure. Therefore, organic-component-rich, commercially available top soil may be used as a reference matrix if testing demonstrates that it does not contain the native PFCs. Other reference matrices will be evaluated.

7.4 Preparation of reagents

7.4.1 Pretreatment reagent (1 M NaOH) – prepared by adding 40 g of NaOH to 1 L of reagent water.

7.4.2 Neutralization reagent (1M HCl) – prepared by dilution of 83.5 mL of concentrated hydrochloric acid (HCl) to 1 L with plasma-grade reagent water.

7.4.3 Extraction solvent (50:50/ACN:MeOH [v/v]) – prepared by mixing 500 mL of methanol (MeOH) and 500 mL of Acetonitrile.

7.4.4 Acetic acid (3%, v/v) – prepared by dissolving 30 mL of glacial acetic acid in 1 L of reagent water.

7.4.5 SPE reagents

7.4.5.1 Basic methanol (0.3% NH_4OH v/v in methanol) – prepared by mixing 30 mL of ammonium hydroxide with 1 L of methanol.

7.4.5.2 Formic acid, 0.1 M – prepared by dissolving 4.8 g formic acid (96%) in 1 L of reagent water.

7.4.5.3 Methanol (20%): formic acid 0.1M, (80%) – prepared by mixing 200 mL of methanol with 800 mL of 0.1 M formic acid in reagent water.

7.4.5.4 Aqueous ammonium hydroxide (0.3% v/v) – prepared by adding 1 mL, of 30% ammonium hydroxide to 99 mL of reagent water.

7.5 Preparation of LC mobile phases and wash solutions

7.5.1 Aqueous mobile phase – 12.1 mM ammonium acetate in 0.1% acetic acid (aqueous) is prepared by adding 4 g of ammonium acetate and 4 mL of acetic acid to 4 L of HPLC-grade water.

7.5.2 Organic mobile phase – 90% acetonitrile/10% HPLC water is prepared by adding 400 mL of HPLC-grade water to 3600 mL of acetonitrile.

7.5.3. Seal wash solution – 10% acetonitrile/90% HPLC water is prepared by adding 400 mL of acetonitrile to 3600 mL HPLC-grade water.

7.5.4 Needle wash solution – methanol is used as received.

7.5.5 Purge solvent – HPLC-grade water is used as received, or aqueous mobile phase may be used.

8.0 Standards

8.1 Sources of standards

The standards used and suggested suppliers are listed in Table 9. Standards are used as received. If the stated chemical purity is 98 % or greater, the weight may be used without correction to

calculate the concentration of the standard. All solution concentrations and calculated results are reported in terms of the acid form. Where the obtained standards are salts of the analyte compounds, a salt-to-parent conversion factor must be applied to the concentration.

8.2 Validation

Before preparation of mixed standards, each individual standard is validated by analysis to confirm its identity and the absence of impurities. A combined working level solution containing native (unlabeled) standards, labeled standards, and labeled injection standards was prepared and analyzed to demonstrate accurate quantification against the calibration standards. A combined solution of labeled standards and labeled injection standards was prepared and analyzed to demonstrate cleanliness of these solutions.

8.3 Stock solutions

Native (unlabeled) perfluoroheptanesulfonic acid (PFHpS) and all individual labeled compounds, except perfluoro-n-[1,2-$^{13}C_9$]undecanoic acid, were purchased as solutions in methanol. Individual native alky 1-octanesulfonamide and (1-octanesulfonamido)-ethanol compounds (N-Me/Et-FOSA/Es) were purchased as solutions in 90% nonane/10% toluene. Individual natives for all compounds except PFHpS and perfluoro-n-[1,2-$^{13}C_9$]undecanoic acid were prepared from solids.

Individual standards stock solutions should be prepared in methanol, except perfluorotetra-decanoic acid (PFTeDA), which is prepared in acetonitrile (ACN). See Appendix 1 for details on standards preparation.

Mixed standards stocks and working level solutions should be prepared in 60:40 ACN:H_2O or basic methanol in glass, Class A volumetric flasks. 60:40 ACN:H_2O should be prepared from Optima grade ACN and PFC-free 18-MΩ water.

Mixed standards stocks and working level solutions for N-Me/Et-FOSA/Es should be prepared in methanol.

Prepare the following mixed stock standards. See Appendix 1 for preparation details.

8.3.1 Native stock mix (containing all native perfluorocarboxylic acids, perfluorosulfonic acids, and PFOSA)

8.3.2 Stock labeled standard mix

8.3.3 Stock labeled injection standard

8.4 Working-level native standards

8.4.1 Working-level native (unlabeled; authentic) compound spiking solution – This solution is spiked into calibration standards, IPR, OPR, and matrix spike samples. Prepare target analyte native compounds at the concentrations shown in Table 7 (and detailed in Appendix 1) in basic methanol. 40 µL of the native compound spiking solution is added to each OPR, IPR or matrix spike sample before digestion. See Sec. 11.3.5.

8.4.2 Low-level native standard – This solution is spiked into calibration standards, IPR, OPR, MDL, and matrix spike samples. Prepare target analytes at the concentrations shown in Table 7 (and detailed in Appendix 1) in basic methanol.

8.4.3 Working-level native standard for N-Me/Et-FOSA/Es – This solution is spiked into calibration standards, IPR, OPR, MDL, and matrix spike samples. Prepare target analyte native compounds at the concentrations shown in Table 7 (and detailed in Appendix 1) in methanol containing 10% propan-2-ol. A 16-µL aliquot of this solution is added to each OPR, IPR, or matrix spike sample before digestion. See Sec. 11.3.5.

Note: *Larger volumes of the solutions in Secs. 8.4.1 to 8.4.3 may be used, provided that the concentrations are adjusted accordingly.*

8.5 Labeled internal standards

Labeled internal standard solution (sometimes called the labeled compound spiking solution) – This solution is spiked directly into samples prior to extraction. Labeled compounds are used to quantify unlabeled target compounds and perform recovery correction. Prepare the labeled compounds at the concentrations shown in Table 7 (and detailed in Appendix 1) in basic methanol. A 100-µL aliquot of this solution is added to each sample before extraction. See Sec. 11.3.6.

8.6 Labeled injection standards

Labeled injection standard solution – this solution contains the labeled compounds that are used to quantify all of the other labeled compounds in the labeled internal standard solution (Sec. 8.5), and is added to the final sample extract prior to instrumental analysis. Prepare the labeled compounds used as injection standards at the concentrations shown in Table 7 (and detailed in Appendix 1) in basic methanol. A 12.5-µL aliquot of this solution is added to each extract in preparation for LC/MS/MS analysis. See Sec. 11.6.3.

Note: *A larger volume of this solution may be used, provided that the concentration is adjusted accordingly.*

8.7 Calibration standards

Combine and dilute the solutions in Secs. 8.4, 8.5, and 8.6 in basic methanol to produce the calibration solutions at the levels shown in Table 6 or, if available, purchase prepared standards for calibration solutions. These solutions are used for initial calibration of the analytical system (Sec. 13.1). The CS-4 standard is used for ongoing calibration verification (Sec. 13.3).

8.8 Storage

Standards are stored in glass in the dark at 4 °C. Longer-term storage stability is to be determined, but stability for 6 months has been observed. Place a mark on the vial or ampule at the level of the solution so that solvent loss by evaporation can be detected. Alternatively, weigh the vial or ampule before storage, record the mass, and reweigh the vial or ampule before the next use.

8.9 Stability

Shelf life of purchased solutions in methanol is determined by the supplier. Stability of diluted solutions and working solutions remains to be determined.

Perfluorocarboxylic acid standards in methanol solution may undergo esterification to the methyl esters. Most purchased perfluorocarboxylic acid standard solutions were received in methanol containing 4 mole equivalents of NaOH. Basic methanol (Sec. 7.4.5.1), rather than straight methanol, is used for all standard dilutions to avoid this potential problem.

9.0 Sample Collection, Preservation, Storage, and Holding Times

9.1 Sample collection – Collect samples in amber high density polyethylene (HDPE) containers with propylene caps/lids, following conventional sampling practices designed to obtain a sample that is representative of the material of interest. Lids and other materials containing PTFE must be avoided, due to possible leaching of fluorinated materials.

9.2 Collect a sample of sewage sludge or biosolids sufficient to yield at least 0.5 g of wet solids for analysis, plus enough sample to allow the determination of % solids determination (Sec. 11.2) and to provide volume for QC samples (Sec. 10.5). Larger samples are recommended to ensure that they are more representative of the bulk source of the material.

9.3 Holding times – EPA has not yet conducted a formal holding time study and will conduct one after the procedure is finalized. Until that time, default holding times that begin at the time of sample collection are as follows:

9.3.1 Begin sample extraction within 60 days of collection (to be validated).

9.3.2 Analyze extracts within 30 days of extraction (to be validated).

9.3.3 Store all samples and extracts at less than 4 ºC in HDPE containers

10.0 Quality Control (QC)

10.1 Each laboratory that uses this draft procedure is required to operate a formal quality assurance program. The minimum requirements of this program consist of initial and ongoing quality control samples. Initial quality control samples include: an initial precision and recovery (IPR) study described in Sec. 10.2, an MDL study described in Sec. 10.3, and a reporting limit sample (RLS) described in Sec. 10.4.

Ongoing quality control samples (Secs. 10.5 – 10.8) include calibration verification (CALVER) standards at the beginning of every shift, analysis of ongoing calibration verification standards with every sample batch, and analysis of a method blank with every sample batch.

Laboratory performance is compared to the draft performance criteria (Table 8) to determine if the results of analyses meet the performance characteristics of the procedure. (The performance criteria will be revised by EPA as the procedure is finalized.)

Additionally, the test for determination of PFC backgrounds in Sec. 12.5 must be performed on every instrument to determine the modifications to instrument plumbing that are required.

10.2 Initial precision and recovery (IPR) – To establish the ability to generate acceptable precision and recovery in reference matrices, that the analytical system is performing properly, and that the laboratory may perform the procedure, the laboratory must perform the following operations.

10.2.1 Spike four aliquots of the reference matrix (Sec. 7.3.7) with 40 µL of the working level native standard (Sec. 8.4.1), 16 µL of working-level native standard solution for N-Me/Et-FOSA/Es (Sec. 8.4.3), and 100 µL of labeled internal standard solution (see Sec. 8.5), and analyze each aliquot according to the procedures in Secs. 11 through 14. All processing steps that are used for samples, including preparation, extraction, and cleanup (Sec. 11), must be included in this test.

10.2.2 Using results of the set of four analyses, compute the average percent recovery (X) of each compound in each extract and the relative standard deviation (RSD) of the recovery for each compound, by isotope dilution for compounds with a labeled analog, and by injection internal standard for compounds without a labeled analog and for the labeled compounds.

10.2.3 For each native and labeled compound, compare RSD and X with the corresponding limits for initial precision and recovery in Table 8. If RSD and X for all compounds meet the acceptance criteria, then system performance is acceptable and analysis of blanks and samples may begin.

Note: *EPA has not yet developed formal acceptance criteria for this procedure. Therefore, use the draft criteria in Table 8 as guidance. If more than one target compound fails the IPR recovery test, examine the system to determine the cause and repeat the test.*

10.3 Method detection limit (MDL) study – Determine the MDL in accordance with the procedures described in 40 CFR Part 136, prior to the analysis of field samples.

10.4 Reporting limit sample (RLS) – Use 0.5 gram of the soil reference matrix (Sec. 7.3.7) to prepare the RLS. Spike this aliquot with 10 µL of the low-level native standard (Sec. 8.4.2) that is equivalent to the concentration in the lowest (CS-1) calibration standard. Analyze this sample as an unknown in the sample batch. Analyze the RLS immediately prior to analysis of the OPR, and samples from the same batch.

Note: *The RLS is not required during routine sample analysis after method development has been completed and single-lab validation has occurred.*

10.5 Matrix spike samples are used to assess performance of the procedure on the biosolids being analyzed. The laboratory must determine the recovery of both labeled and native compounds spiked into a biosolids matrix. By preparing two such samples, i.e., a matrix spike and a matrix spike duplicate, the laboratory can also assess precision of the procedure in routine application.

10.5.1 Spike two 0.5-g aliquots of a sewage sludge or biosolids sample with 40 µL of the working-level native standard (Sec. 8.4.1), 16 µL of the working-level native standard solution for N-Me/Et-FOSA/Es (Sec. 8.4.3), and 100 µL of labeled internal standard solution (Sec. 8.5) and mix thoroughly. Analyze both matrix spike samples according to the procedures in Secs. 11 through 14.

10.5.2 Compute the recovery of the labeled compounds using the internal standard method (quantify using the injection internal standard).

10.5.3 It may be necessary to spike the native compounds at concentrations that will allow meaningful recovery data to be obtained, e.g., 3 to 5 times the background levels in the unspiked sample. Thus, it may be advisable to spike new aliquots of a sample that has already been analyzed.

10.5.4 Report the background concentrations of native compounds, and recoveries and spiking concentrations for both the native and labeled compounds. Compare the recoveries to the draft acceptance criteria in Table 8.

10.5.5 Calculate and report the relative percent difference (RPD) of the recoveries of the native analytes in the matrix spike and matrix spike duplicate samples. Compare the RPDs to the draft acceptance criteria in Table 8.

10.6 Calibration verification

Note: *Initial calibration is discussed in Sec. 13.1 and must be performed prior to analysis of samples.*

10.6.1 Frequency – Ongoing calibration verification consists of the analysis of bracketing calibration verification samples, one at the beginning of every 12-hour shift and another at the end of every batch or 12-hour shift, whichever is more frequent.

10.6.2 In addition, a calibration verification sample is required during the middle of every sample batch larger than 10 samples. (Sample batches may be no larger than 20 samples.)

10.6.3 The standards used for calibration, calibration verification, and for initial and ongoing precision and recovery should be identical, so that the most precise results will be obtained. The CS-4 calibration standard is used for calibration verification samples.

10.6.4 Inject the VER (CS-4) standard (Table 6) using the analysis procedure in Sec. 14.

Note: *The requirements in Sec. 15 must be met when analyzing ongoing calibration verification samples.*

10.7 Method blank – A method blank is analyzed with each sample batch to demonstrate freedom from contamination. Use a 0.5-g aliquot of the reference matrix (Sec. 7.3.7) to prepare a method blank. Extract the sample following the procedure described in Sec. 11. The method blank should be analyzed immediately after the OPR sample. If native compounds will be carried from the OPR into the method blank, analyze one or more aliquots of solvent between the OPR and the method blank.

Note: *If aliquots of solvent must be analyzed, record how many, and update the analytical sequence in Sec. 14 of this draft procedure to reflect the use of solvent blanks.*

Alternatively, analyze the method blank *before* the OPR sample, and analyze one or more use solvent blanks after the OPR to prevent carryover from the OPR into the first sample.

10.8 Ongoing precision and recovery (OPR) sample – The laboratory must, on an ongoing basis, demonstrate through analysis of the ongoing precision and recovery standard that the analytical system is in control. Use a 0.5-gram aliquot of the soil reference matrix (Sec. 7.3.7) as the matrix for the OPR. Spike this sample with 40 µL of the working-level native standard (Sec. 8.4.1), 16 uL of the working-level native standard for N-Me/Et-FOSA/Es (Sec. 8.4.3), and 100 µL of the

labeled internal standard solution (Sec. 8.5). The aliquot of native standard spiked is equivalent to the concentration in the CS-3 calibration standard. Extract the sample following the procedure described in Sec. 11. Analyze the extracts of the OPR aliquot prior to analysis of samples from the associated batch. Compare the results to the acceptance criteria in Table 8.

Note: *EPA has not yet developed formal acceptance criteria for this procedure. Therefore, use the draft criteria in Table 8 as guidance. If more than one target compound fails the OPR test, examine the system to determine the cause and repeat the test.*

11.0 Preparation, Extraction, and Cleanup of Field Samples and QC Samples

11.1 Sample preparation

Note: *As noted in Sec. 1.4, early work on this procedure focused on analysis of the solid portion of sewage sludge samples and described discarding any supernatant aqueous liquid in the sample. EPA believes that this approach was intended to provide a consistent mass of solids from a bulk sample that could be used for testing the many extraction conditions studied by Yoo et al, 2009. At present, EPA believes that the extraction procedures are capable of dealing with samples containing large amounts of water, which may better represent actual sewage sludges and biosolids from wastewater treatment operations.*

Homogenize the entire sample in the original sample container, by shaking samples that are pourable liquids, or by stirring solids in their original container with a clean spatula, glass stirring rod, or other suitable implement. Once homogenized, remove an aliquot of the sample to determine the percent solids content of the sample using the procedure in Sec. 11.2. Remove a second aliquot of the sample for analysis, as described in Sec. 11.3.

11.2 Determination of solids content

The percent solids of sewage sludge and biosolids will vary depending on the source of the sample and the treatment processes applied. The solids content of the bulk sample is determined from a subsample that is used only for the solids determination. Separate procedures are used for the solids determination, based on the nature of the sample, as described below.

11.2.1 Single-phase solid samples and multi-phase samples in which the main phase is not aqueous

11.2.1.1 Using a solvent-rinsed spatula, transfer a 1-gram subsample of the homogenized sample into tared weighing boat. Record weight of the subsample to three significant figures. (If there is not sufficient mass of the original sewage sludge and biosolid sample, a smaller subsample may be used for the solids determination.)

11.2.1.2 Dry the subsample for a minimum of 12 hours in a drying oven set at 110 ± 5 º C, and cool in a desiccator.

11.2.1.3 Weigh the dried aliquot and calculate percent solids as follows:

$$\% \text{ Solids} = \frac{\text{Weight of sample aliquot after drying (g)}}{\text{Weight of sample aliquot before drying (g)}} x\ 100$$

11.2.2 Multi-phase biosolids samples consisting of mainly an aqueous phase

11.2.2.1 Dry a GF/A filter and weigh to three significant figures. Mix the bulk sample in the original container (e.g., cap the bottle and shake) and take a 10.0 ± 0.2 mL aliquot. Filter that aliquot through the filter. Dry the filter in an oven for a minimum of 12 hours at 110 ± 5 ºC and cool in a desiccator.

11.2.2.2 Weigh the filter and calculate percent solids as follows:

$$\% \text{ Solids} = \frac{\text{Weight of sample aliquot after drying (g) - weight of filter (g)}}{10\text{ g}} x\ 100$$

11.3 Sample digestion

Each sample batch to be digested and extracted during the same 12-hour shift consists of a maximum of 20 field samples, plus one method blank, and one OPR sample. A reference matrix (e.g., reference soil) known to be free (below background levels) of the target analytes is used as the matrix for the method blank and OPR sample (Sec. 7.3.7).

11.3.1 Place a 0.5-g (wet weight) subsample of thoroughly homogenized sample into a 15-mL polypropylene centrifuge tube.

11.3.2 Place a 0.5-g (wet weight) aliquot of the reference soil into a 15-mL polypropylene centrifuge tube. This sample is used as the method blank.

11.3.3 Place another 0.5-g (wet weight) aliquot of the reference soil into a 15-mL polypropylene centrifuge tube. This sample is used as the OPR sample.

11.3.4 If matrix spike samples are to be analyzed, prepare those aliquots as described in Sec. 10.5 and spike them with the native analytes at 3 – 5 times the background concentrations.

11.3.5 Spike all of the QC samples (OPR, IPR, matrix spike samples) with 40 µL of the working-level native standard solution (Sec. 8.4.1) and 16 µL of the working-level native standard solution for N-Me/Et-FOSA/Es (Sec. 8.4.3).

Note: *Larger volumes of these solutions may be used, provided that the concentrations are adjusted accordingly.*

11.3.6 Spike all samples and QC samples with 100 µL of the labeled internal standard solution (Sec. 8.5). The volume spiked will yield a sample extract with labeled compounds present at a concentration equivalent to the native compounds in the mid-level calibration standard.

11.3.7 Add 0.5 mL of 1 M NaOH solution to each sample and QC sample. If a sample is very dry, add enough NaOH to wet and cover the sample. Record volume of NaOH used.

11.3.8 Sonicate the samples in a heated water bath at 40 ºC for 30 min, followed by incubation overnight (12 h) at ambient temperature.

Note: *Mixing the sample by sonication is described in Ref. 1. EPA has not tested whether or not this step is essential, but may do so in the future.*

11.3.9 After the incubation period, neutralize the NaOH by adding an equivalent number of moles of the 1 M HCl solution (Sec. 7.4.2), allow the mixture to react for 1 min, and vortex.

Note: *Some biosolids are treated with lime and will require additional HCl to neutralize them. If the samples are known or suspected to involve lime treatment, then use a clean glass stirring rod to remove a small amount of the supernatant from the incubated sample and test the pH with a wide-range pH paper. Do NOT dip the pH paper into the sample. If the pH of the incubated sample is not 7.0 ± 0.5, add additional HCl, vortex, and retest the pH until the sample is neutral.*

11.4 Sample extraction

11.4.1 Add 10 mL of 50:50/ACN:MeOH (v/v) to the sample in the centrifuge tube.

11.4.2 Shake the mixture for 1 h on a tube shaker (Sec. 6.2.5) at a moderate speed.

11.4.3 Centrifuge the sample at a speed of 3000 x g for 20 min.

11.4.4 Decant the 10 mL supernatant into a 250-mL HDPE bottle containing 180 mL of plasma-grade reagent water (Sec. 7.1.3).

11.4.5 Repeat the extraction (Secs. 11.4.1 to 11.4.4) one more time and add the extract to the 250-mL HDPE bottle.

11.4.6 After the second extraction, check that the pH of the diluted extract is 6.5 ± 0.5. If required, adjust the pH with 3% (v/v) acetic acid in reagent water or 0.3% (v/v) aqueous ammonium hydroxide (Sec. 7.4.5.4).

11.4.7 Sonicate the mixture for 30 min. The extract is ready for cleanup.

11.5 Extract cleanup

All sample extracts are subjected to cleanup using an SPE cartridge, as described below.

11.5.1 Prepare the SPE extraction manifold, reservoirs, SPE adapters, and SPE needles. Use SPE cartridges that pass the SPE cartridge performance check described in Sec. 11.7.

11.5.2 Label each Oasis WAX SPE cartridge with the sample ID and place the cartridge on the extraction manifold.

11.5.3 Condition each cartridge with 5 mL of 0.3% NH_4OH in methanol, followed by 5 mL of 0.1M formic acid in reagent water. Discard eluants.

11.5.4 Equilibrate the cartridge with 5 mL of plasma-grade reagent water (Sec. 7.1.3). Discard the eluant.

11.5.5 Load the sample onto the cartridge drop-wise (~5 mL/min).

11.5.6 Wash the sample with 5 mL of 20% MeOH in 80% 0.1M formic acid in reagent water, followed by 2 mL of 0.3% (v/v) NH_4OH in reagent water. Discard the eluants.

Note: *Other wash solvents have been reported in the literature and may provide acceptable results.*

11.5.7 Dry the cartridge by pulling air under vacuum for 5 min.

11.5.8 Elute the cartridge into a 15-mL clean glass centrifuge tube with 4 mL 0.3% NH_4OH (v/v) in MeOH.

11.6 Extract concentration

11.6.1 Reduce the extract to about 50 µL using a gentle stream of nitrogen and a water bath set at 40 ºC.

11.6.2 Reconstitute the extract with 938 µL of 0.3% NH_4OH (v/v) in MeOH and vortex to mix.

11.6.3 Spike the extract with 12.5 µL of the injection internal standard solution (Sec. 8.6), vortex to mix, and filter the sample extract through a syringe filter (Sec. 6.1.8) into a clean centrifuge tube. The final extract volume is 1.0 mL.

Note: *Other volumes and solvents may be used in Secs. 11.6.1 to 11.6.3, provided that the mass of the injection internal standard is equal to the mass in the calibration standards and the final extract volume is the same for all sample extracts. However, avoid taking the sample extract to dryness in Sec. 11.6.1, because it may result in loss of short-chain analytes such as PFBA.*

11.6.4 Vortex the extract and transfer 300 µL of the final extract to a polypropylene LC/MS/MS auto-sampler vial (Sec. 6.1.3) for analysis. Cap the centrifuge tube containing the remaining 700 µL and store at 4 ºC for backup. Place a mark on the tube at the level of the solution so that solvent loss by evaporation can be detected. Alternatively, weigh the tube before storage, record the mass, and reweigh the tube before any subsequent analysis of the stored extract.

11.7 SPE cartridge performance check

In order to be used for cleanup of sample extracts, the performance of the WAX SPE cartridges must be checked at least once for each manufacturer's lot of cartridges. This performance check is accomplished by processing a spiked reagent water sample through the extraction procedure and analyzing the extract. Labeled compounds are not added to these check samples before extraction because the recovery correction inherent in isotope dilution will mask problems with the cartridges. Cartridge performance is acceptable if the recoveries of the native analytes are within the QC acceptance criteria for the OPR in Table 8. Perform this cartridge check as outlined below.

Note: *This check is performed whenever a new lot number of cartridges is purchased.*

11.7.1 Spike 100 mL reagent water with 40 µL of the working-level native standard solution (Sec. 8.4.1) and 16 µL of the working-level native standard solution for N-Me/Et-FOSA/Es (Sec. 8.4.3). Do NOT spike the labeled internal standard compounds.

11.7.2 Process the solution through the SPE cleanup procedure in Sec. 11.5.

11.7.3 After cleanup, spike the solution with the labeled internal standard solution (Sec. 8.5) and complete the analysis as per Sec. 14.

11.7.4 Recovery of the native compounds must be within the QC acceptance criteria for the OPR in Table 8. If the compounds are not recovered in this range, adjust the elution volumes or reject the cartridge batch.

12.0 LC/MS/MS Set Up and Calibration

Samples are analyzed on a high performance liquid chromatograph coupled to a triple quadrupole mass spectrometer (LC/MS/MS), or equivalent, equipped with an electrospray ionization (ESI) source. The tandem MS systems operate at a nominal resolution of 1 amu. The LC/MS/MS is run in the negative ion electrospray (ESI-) mode using multiple reaction monitoring (MRM). Data acquisition and quantification are performed by recording the peak areas of the applicable parent ion /daughter ion transitions. The instrument manufacturer's software is used to acquire data and calculate results using isotope dilution and internal standard quantitation (Sec. 15.2).

Once the mass spectrometer has been optimized and the LC/MS/MS operating conditions for the targeted compounds have been established, the same conditions must be used for the analysis of all standards, blanks, IPR and OPR standards, field samples, and QC samples.

12.1 Establishing LC/MS/MS Operating Conditions

Prior to any analyses, optimize the following instrumental conditions: mass calibration, MRM acquisition parameters, scans per peak, chromatographic resolution, retention time calibration, sensitivity and instrument background elimination. Example analyte-specific instrumental source parameters for PFCs analysis are found in Table 3, but actual tuning parameters are instrument-specific and should be optimized according to manufacturer's specifications.

12.2 Mass calibration

The mass spectrometer system must undergo mass calibration according to manufacturer's specifications to ensure accurate assignments of m/z values by the instrument. Mass calibration is performed at least annually, after performing major maintenance, or as required to maintain routine instrument sensitivity and stability performance. The reference calibrant used is a 50:50 isopropanol:water (IPA:water) solution containing sodium cesium iodide (NaCsI), which is infused directly into the electrospray source during calibration. Mass calibration is performed at instrumental settings corresponding to a nominal unit mass resolution, but mass resolution is not directly measured.

In the absence of manufacturer-specific instructions and acceptance criteria, the following procedure may be used.

12.2.1 Use a NaCsI calibration solution (in 50:50 IPA:water) containing 2 µg/µL sodium iodide and 50 ng/µL cesium iodide.

12.2.2 Use a syringe pump to infuse the calibration solution as a stable aerosol spray at 10 µL/min directly into the ESI-MS/MS source.

12.2.3 Scan the MS/MS over the mass range from 20 to 2000 Daltons. Adjust the source parameters to optimize peak intensity and shape across the mass range. The exact m/z values for NaCsI calibration are contained as a reference file on the instrument data system and are:

Calibration Masses (Daltons - m/z)		
22.9898	922.3552	1971.6149
132.9054	1072.2494	2121.5091
172.8840	1222.1437	2271.4033
322.7782	1372.0379	2421.2976
472.6725	1521.9321	2571.1918
622.5667	1671.8264	2721.0861
772.4610	1821.7206	2870.9803

During the mass calibration, examine the instrument parameters to ensure detection of the specified ions.

Mass calibration is judged on the basis of the presence or absence of the exact calibration masses, e.g., a limit on the number of masses that are "missed." Repeat the test if more than two masses are missed.

12.3 MRM acquisition parameters

During method setup, the mass spectrometer response must be separately optimized for each target compound, using a solution containing only the compound of interest. These parameters are then used for analysis of all standards and samples.

12.3.1 Using a post-column pump, infuse a solution mixture of methanol and mobile phase containing approximately 1 ppm of the compound of interest directly into the ESI-MS/MS source. This solution is prepared by adding mobile phase to a 1 ppm methanol solution of the compound being tested in the ratio of 1 part mobile phase/2 parts test solution.

12.3.2 For each compound, optimize the sensitivity to the specified parent-daughter transitions (see Table 3) by adjusting the collision energy and cone voltage.

12.3.3 The optimum parameters are compound specific and a set of single settings cannot be used for all target compounds in the analysis. Use the optimized settings for the analysis of all standards and samples.

12.4 Chromatographic separation

Establish liquid chromatography conditions suitable for the separation of the target compounds. To achieve the retention times in Table 2, the HPLC should be operated according to the parameters in Table 5. A C18 analytical column is used. The exact gradient is optimized for the chromatography system in use, but the conditions in Table 5 can be used as guidelines. The chromatographic separation should ensure that there is adequate resolution of target compounds from potentially interfering substances (Sec. 13.5). Note that the LC gradient selected will affect the chromatographic resolution of linear and branched PFC compounds. After the LC column conditions and gradient have been determined, they should be used for all analyses.

12.5 Instrument background determination

To determine if background concentrations of PFCs significantly impact this analysis, a 40 part per trillion (ppt) standard containing all of the target analytes in 0.3% NH_4OH (v/v) in methanol must be analyzed three times, with alternating instrument solvent (methanol) blank measurements. If the peak area from the standards is not greater than that of the solvent blank using a Student's t-test (95% confidence), then it may be necessary to modify the "plumbing" of the analytical system as outlined in Sec. 6.4.5. This test should be performed prior to any analysis, at least annually, and after major instrument maintenance.

12.6 Establishing retention time windows

Analyze individual solutions of the each of the target compounds using the LC gradient and acquisition parameters determined above. Analyze a mixed solution of all target compounds to confirm their separation and identification. A total ion chromatogram (TIC) indicating the separation of the target analytes is shown in Figure 2, as an example.

12.7 Analytical data acquisition program

All of the information is now in place to finalize the analytical acquisition routine to be used for calibration of instrument response and analysis of all samples. The acquisition program will monitor each of the MRM ions listed in Table 3 at its optimum cone voltage and collision energy, as determined in Sec. 12.3, and in the appropriate retention time window established in Table 2.

12.8 Instrument sensitivity

Prior to commencement of any analysis, and at least once every 24 hours during extended runs, determine that the instrument is meeting the sensitivity specifications. Analyze the lowest concentration calibration solution (CS-1) using the acquisition program described in Sec. 12.7. Ensure that all compounds are detected with S/N > 3. If sensitivity is inadequate, perform system cleaning and maintenance and repeat the test. If calibration verification can not be established after this maintenance, a new initial calibration (Sec. 13.2) is required.

13.0 Instrument Quality Control

The mass spectrometer is optimized and the LC/MS/MS operating conditions are established for the target compounds as described in Sec. 12. The same conditions must be used for the analysis of all standards, blanks, IPR and OPR standards, and samples.

13.1 Initial calibration

Initial calibration (ICAL) must be performed prior to analysis of any field sample or QC sample. Approaches to quantitation vary depending on the compound. Some compounds are quantified using isotope dilution (for those target compounds with exact labeled analogs) and others are quantified using internal standard (those without an exact labeled analog). Additionally, labeled compounds are quantified using the internal standard approach. These two approaches are discussed in Secs. 13.2.1 and 13.2.2, respectively. The requirements for initial calibration are outlined in Sec. 13.2, and Table 8. After the initial calibration, analyze an initial calibration blank and an initial calibration verification (CALVER) standard.

13.2 Calibration by isotope dilution and internal standard

Calibrations should be constructed using regressions of untransformed data and plotting normalized relative response versus the absolute concentration of analyte. Normalized relative response is defined as:

$$NRR = \frac{A_n}{A_l} \times C_l$$

where:

NRR = Normalized relative response
A_n = Area of the peak for the native (unlabeled) analyte
A_l = Area of the peak for the specified labeled compound
C_l = Concentration of the specified labeled compound (in pg/mL)

Efforts to date indicate that a linear equation may be used for all of the analytes except: perfluorobutanesulfonic acid (PFBS), perfluoro-n-heptane sulfonic acid (PFHpS), perfluorooctane sulfonamide (PFOSA), and those compounds containing more than 10 carbons ($C_{11} - C_{14}$). For those analytes, the response is better fit (higher r^2) with a quadratic equation.

For either a linear regression or a quadratic fit, weight the equation using the inverse of the concentration (e.g., 1/x), and do not force the calibration through the origin.

13.2.1 Calibration by isotope dilution

Isotope dilution is used for calibration of each native compound for which an exact labeled analog is available (Table 3). An 8-point initial calibration is prepared for each native compound. The calibration solutions are listed in Table 6.

13.2.1.1 To calibrate the analytical system by isotope dilution, inject the CS-1 through CS-8 calibration solutions (Sec. 8.7 and Table 6). Use an injection volume of 15 µL.

13.2.1.2 For each compound determined by isotope dilution, compute its normalized relative response (NRR) over the calibration range. Determine the NRR of each compound using the area responses of the product m/z values specified in Table 3. Use the labeled compounds listed in the tables as the quantitation reference and the product m/z values of these labeled compounds for quantitation. Determine the calibration equation for each compound by regressing the NRR against the native compound concentration to produce a calibration weighted inversely proportional to concentration (i.e., a 1/x weighted linear regression). Select the calibration equation (linear or quadratic) that provides the best fits to the data, as indicated by the coefficient of determination (r^2). Examine the residuals of the regression (i.e., the distance between the observed response for each calibration standard and the response predicted by the regression) to determine if they are randomly distributed (i.e., the regression line passes between the calibration points, and not above or below all of the points). Whichever calibration is selected, r^2 must be at least 0.99. Use the resultant equation to calculate the concentration of analyte in each sample or CALVER.

13.2.1.3 Some of the labeled compounds may contain native analogs as impurities. If the labeled compounds are present at a constant concentration in calibration and other solutions (including sample extracts), a constant background response will be added to the response from analysis of calibration and other solutions. A calibration not forced through zero will accommodate any such residual.

13.2.2 Calibration by internal standard

Internal standard calibration is applied to the determination of the native compounds that do not have exact labeled analogs, and that are not being quantified by isotope dilution. Internal standard calibration is also used to quantify the labeled compounds themselves. The internal standard approach utilizes the injection internal standard (IIS) that is added to the extract after extraction and cleanup and prior to injection into the instrument as the quantitation reference. The reference compound for each native and/or labeled compound is listed in Table 3. For the labeled compounds, calibration is performed at a single concentration, using data from the 8 points in the calibration (all of which contain the labeled compounds at the same concentration).

13.2.2.1 To calibrate the system for native compounds for which isotope dilution is not being performed, use the data from the 8-point calibration and the labeled injection internal standard.

13.2.2.2 For each compound determined by internal standard quantitation, its NRR is computed over the calibration range. Determine the NRR of each compound using the area responses of the product m/z's specified in Table 3. Use the labeled IIS compounds listed in the tables as the quantitation reference and the product m/z's of these labeled compounds for quantitation. Determine the calibration equation for each compound by regressing the NRR against the native compound concentration to produce a calibration weighted inversely proportional to concentration (i.e., a 1/x weighted linear regression). Do not force through zero (Sec. 13.2.1.3). Select the calibration equation (linear or quadratic) that provides the best fits to the data, as indicated by the coefficient of determination (r^2). Examine the residuals of the regression (i.e., the distance between the observed response for each calibration standard and the response predicted by the regression) to determine if they are randomly distributed (i.e., the regression line passes between the calibration points, and not above or below all of the points). Whichever calibration is selected, r^2 must be at least 0.99. Use the resultant equation to calculate the concentration of analyte in each sample or CALVER.

13.2.2.3 For each labeled compound, regress its NRR against the concentration of the labeled compound using the labeled IIS as the quantitation reference as indicated in Table 3. The labeled compounds and the labeled injection internal standards are in each calibration solution at a constant concentration. The regression will simplify to a single-point calibration because the concentrations are constant.

13.2.3 Quantification of the labeled injection internal standard concentration

The injection internal standard (IIS) itself is quantified by external standard. Monitoring of the recovery of the IIS is used as a diagnostic test for matrix effects during the LC analysis (Sec. 4.6).

13.3 Calibration verification

Calibration verification (VER) requires the analysis of bracketing calibration verification samples, one at the beginning of every 12-hour shift, and another after the analysis of every 10 samples, or at the end of each 12-hour shift, whichever is more frequent. The standards used for calibration, calibration verification, and for initial and ongoing precision and recovery should be identical, so that the most precise results will be obtained. The CS-4 calibration standard is used for calibration verification samples. Inject the CS-4 calibration standard (Table 7) using the analysis procedure in Sec. 14. The requirements in Sec. 10.8, and Table 8 must be met when analyzing calibration verification samples.

13.4 Quantitation of linear and branched isomers

Some PFCs consist of linear and branched isomers, depending on manufacturing processes (Ref. 5). There are reports that during MS/MS analysis, the linear and branched isomers ionize with different efficiencies, complicating the quantification of PFC compounds (Ref. 3). The LC analysis performed by this procedure results in partial chromatographic resolution of the isomers. Additionally, the composition of standards may differ by vendor, and differ from the distribution of the PFCs in environmental samples. Therefore, peak integration in samples should ensure that the PFC target peaks include the linear and branched isomers as a single total response.

13.5 Co-extracted interferences

Interferences co-extracted from samples will vary considerably from source to source. Taurodeoxychloic Acid (TDCA) is a known interference which may lead to an overestimate or yield a false positive result for PFOS (Ref. 5) while 5-pregnan-3,20-diol-3-sulfate and 34S-3-hydroxy-5-pregnan-20-one sulfate may interfere with PFHxS (Ref. 6). The 499 > 80 transition is prominent in all TDCA isomers and in PFOS. However, the 499 > 99 transition for PFOS is not affected by the TDCA. In the absence of chromatographic separation of TDCA from PFOS, the 499 > 80 transition will result in significant bias in PFOS concentrations. Therefore, both transitions must be monitored for PFOS and results must agree within 20%, to ensure accurate quantification of PFOS. Similarly, analysis for PFHxS can be biased by co-eluting interferences. In this case, the 399 > 80 and the 399 > 99 transitions may both be affected, and therefore, a third transition, 399 > 119, also must be monitored to demonstrate that there is not a bias from co-eluting interferences.

14.0 Instrumental Analysis

Once the operating conditions have been established and the instrument tuned (Sec. 12), inject a 15-µL aliquot of sample extract into a 50-uL loop, using partial-loop-with-needle-overfill mode onto a trapping column (if needed). Start the gradient according to the parameters found in Table 5. Start data collection 1 to 2 minutes prior to elution of the first analyte. Monitor the product m/z's for each native and labeled analyte throughout its retention time window. Stop data

collection after elution of the last analyte in each group. Return the gradient to the initial mixture for analysis of the next sample extract or standard.

A typical instrument analysis sequence is as follows:

- 1-2 Instrument blanks (more may be included)
- 8 Initial calibration standards
- 2 Instrument blanks
- Opening calibration verification standard
- OPR sample
- 1-2 Instrument blanks
- Method blank
- Up to 6 field samples
- Bracketing calibration verification standard (every 10th injection)
- Up to 10 field samples
- Closing calibration verification standard (every 10th injection, which may serve as the opening VER for the next cycle)
- OPR sample, etc.

15.0 Qualitative Identification and Quantitation

15.1 Qualitative identification – The following requirements must be met in all samples for a compound to be identified.

15.1.1 Signal to noise requirements – The LC peak representing the quantitation m/z of each native compound in the upper 7 ICAL standards must be present with a S/N of at least 10, and with a S/N of at least 3 for the lowest standard (CS-1). The LC peak representing each labeled compound quantitation m/z in the CALVER standard and in extracts from all other samples must be 10 or greater. If these requirements are not met in ICAL or CALVER samples, the LC/MS/MS system must be adjusted or recalibrated until these requirements can be met. If these requirements are not met in samples, the CALVER test should be repeated.

15.1.2 Relative intensity – The ion intensity of each monitored ion transition in the ICAL should be recorded for use in qualitative identification for all other samples. The monitored ion transitions are found in Table 3. These parameters may be instrument specific. If alternate transitions are monitored for diagnostic purposes (called confirmatory transitions), the following requirements should be met:

1) The molecular ion shall preferably be the precursor of one of the selected diagnostic transitions (the molecular ion, characteristic adducts of the molecular ion, characteristic fragment ions and all their isotope ions),

2) Diagnostic transitions preferably should not originate from the same part of the molecule as that for the quantitation transition, and

3) The signal-to-noise ratio for each diagnostic transition must be greater than or equal to 3.

Comparison of the ratio of the confirmation transition response to the quantitation transition response in a sample to the ratio in the most recent CALVER can assist in confirmation of the presence or absence of a target compound in samples. The relative ion ratio requirements are described below.

15.1.3 Relative ion intensity ratio requirement – Depending on the relative intensity of the confirmatory m/z to the quantitation m/z, the following requirements must be met to provide confirmation of the presence of the target analyte:

Relative intensity of confirmation transition to quantitative transition in most recent CALVER	Agreement of ratio of confirmation transition to quantitative transition in sample relative to same ratio in most recent CALVER
>50 %	±20%
20 to 50 %	±25%
10 to 20 %	±30%
<10 %	±50%

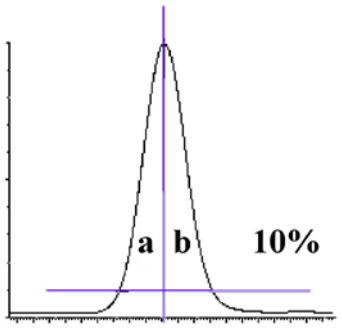
Note: *If the results for any of the qualitative identification criteria above are ambiguous, or if false negatives or false positives occur, the laboratory should consult EPA to determine corrective action and next steps during method development.*

15.1.4 Retention time window requirement – There are two retention time requirements:

1) the retention times of the native and labeled compounds in the initial calibration must be stored in the system for verification of the retention time window requirement during analysis of all subsequent samples. Data acquired for all subsequent samples should be within the required retention time windows. If this is not the first time an initial calibration is being performed, retention times from this calibration should be checked against those from previous calibrations to determine if the separation of target analytes is being affected.

2) the relative retention times for native compounds and the labeled compound for which each native is being quantified must fall within a certain range of each other. Typical retention times, relative retention times and retention time windows are listed in Table 2.

15.1.5 Peak asymmetry factor – During initial calibration and calibration verification, the peak asymmetry factor must be calculated for all analytes using the following figure and equation:



$A_s = b/a$

where:
A_s = peak asymmetry factor
b = width of the back half of the peak measured (at 10% peak height) from the trailing edge of the peak to a line dropped perpendicularly from the peak apex, and
a = the width of the front half of the peak measured (at 10% peak height) from the leading edge of the peak to a line dropped perpendicularly from the apex.

Calculated peak asymmetry factors for the first two eluting peaks in the CS-4 standard of the ICAL must fall within 0.8 to 1.5.

If this criterion cannot be achieved, the LC mobile phase conditions need to be modified. This criterion must be met each time a new calibration curve is generated.

15.2 Quantitation

15.2.1 Isotope dilution quantitation

Using the most recent multi-point calibration (Sec. 13.2.1), calculate native and labeled compound concentration in the extract. Do not use calibration verification data to quantify analytes.

15.2.2 Internal standard quantitation and labeled compound recovery

Compute the concentration of each native compound in the extract that does not have an exact labeled analog and each labeled compound by internal standard, using the weighted regression established in Secs. 13.2.2.2 and 13.2.2.3, respectively.

Using the concentration in the extract determined above, compute the percent recovery of each labeled compound using the following equation:

$$\text{Recovery (\%)} = \frac{\text{Concentration found (ng/mL)}}{\text{Concentration spiked (ng/mL)}} \text{ x } 100$$

15.2.3 The concentration of a native compound in the solid sample is computed using the concentration of the compound in the extract, and the wet weight of the solids, and the percent solids, as follows:

$$\text{Concentration in solid sample (ng/kg)} = \frac{C_{ex}\, V_{ex}}{W_s\ (\%\,\text{solids})}$$

where:
C_{ex} = Concentration of the compound in the extract in ng/mL
V_{ex} = Extract volume in mL
W_s = Sample weight (wet weight) in kg
% solids = Percent solids determined in Sec. 11.2

If desired, divide the concentration by 1000 to convert ng/kg (ppt) to µg/kg (ppb).

15.3 Reporting results

Unless otherwise specified, report results in ng/kg (parts-per-trillion) to three significant figures, based on the dry weight of the sample. Also report the percent solids so that the result may be converted to wet-weight units by the end user.

Results for a compound in a sample that has been diluted must be reported at the least dilute level at which the area at the quantitation m/z is within the calibration range. Results should be flagged to indicate that they are from a diluted analysis.

Project-specific reporting requirements may apply, including reporting results based on the volume of the original sample, e.g., ng/L or other weight/volume units

16.0 Method performance

This procedure is still under development. Preliminary method performance information can be found in Tables 4, 8, and 10. Additional performance data will be added by EPA as they are developed.

17.0 Pollution prevention and waste management

17.1 The laboratory is responsible for complying with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance is also required with any sewage discharge permits and regulations. An overview of requirements can be found in Reference 12.

17.2 Samples at pH <2, or pH >12 are hazardous and must be neutralized before being poured down a drain, or must be handled as hazardous waste.

17.3 Standards should be prepared in volumes consistent with laboratory use to minimize the disposal of excess volumes of expired standards. For information about pollution prevention that may be applied to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Governmental Relations and Science Policy, 1155 16th Street NW, Washington DC 20036.

17.4 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

18.0 References

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12. "Environmental Management Guide for Small Laboratories" USEPA Office of the Administrator, Washington, DC, EPA 233-B-00-001, May 2000.

19.0 Glossary

These definitions and purposes are specific to this method but have been conformed to common usage to the extent possible.

Symbols

º C	degrees Celsius
µL	microliter
µm	micrometer
<	less than
>	greater than
%	percent

Abbreviations (in alphabetical order)

cm	centimeter
ESI-	Negative Electrospray Ionization
g	gram
h	hour
ID	inside diameter

in.	inch
L	liter
M	molecular ion
m	meter
mg	milligram
min	minute
mL	milliliter
mm	millimeter
m/z	mass-to-charge ratio
N	normal; gram molecular weight of solute divided by hydrogen equivalent of solute, per liter of solution
NRR	Normalized relative response
OD	outside diameter
pg	picogram
ppb	part-per-billion
ppm	part-per-million
ppq	part-per-quadrillion
ppt	part-per-trillion
psig	pounds-per-square inch gauge
v/v	volume per unit volume
w/v	weight per unit volume

Definitions and acronyms (in alphabetical order)

Analyte –A perfluorinated compound tested for by this method. The analytes are listed in Table 1.

Calibration standard (CAL) – A solution prepared from a secondary standard and/or stock solution and used to calibrate the response of the HPLC/MS/MS instrument. Referred to as CS-1, CS-2, CS-3, CS-4, CS-5, CS-6.

Calibration verification standard (CALVER) – A calibration standard close to the mid-point calibration standard that is used to verify calibration.

HPLC – High performance liquid chromatograph or high performance liquid chromatography

ICAL – Initial calibration

Internal standard quantitation – A means of determining the concentration of (1) a naturally occurring (native) compound by reference to a compound other than its labeled analog and (2) a labeled compound by reference to another labeled compound.

IPR – Initial precision and recovery; four aliquots of a reference matrix spiked with the analytes of interest and labeled compounds and analyzed to establish the ability of the laboratory to generate acceptable precision and recovery. An IPR is performed prior to the first time this method is used and any time the method or instrumentation is modified.

Isotope dilution quantitation – A means of determining a naturally occurring (native) compound by reference to the same compound in which one or more atoms has been isotopically enriched. In this method, labeled compounds are enriched with deuterium to produce ^{2}H-labeled analogs or carbon-13 to produce ^{13}C-labeled analogs. The labeled analogs are spiked into each sample to allow identification and correction of the concentration of the native compounds in the extraction, cleanup and the analytical process.

Labeled compound, or labeled analog – A molecule in which one or more of the atoms is isotopically enriched, thereby increasing the mass of the molecule. As used in this procedure, all isotopic labels are stable (non-radioactive).

Labeled injection standard – A labeled compound used as a reference for quantitation of other labeled compounds and for quantitation of a native compound for which there is not an exact labeled analog. This compound is spiked into the sample extract prior to instrumental analysis.

Labeled internal standard – A labeled compound used as a reference for quantitation of native compounds. This compound is spiked into the sample prior to extraction.

Method blank – An aliquot of a reference matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with samples. The method blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

Method detection limit (MDL) – The minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte (see 40 CFR 136, appendix B).

Minimum level (ML) – The greater of a multiple of the MDL or the lowest calibration point (see 68 FR 11790, March 12, 2003).

MS – Mass spectrometer or mass spectrometry

Native compound – A molecule in which the atoms all have naturally occurring isotopic abundances

OPR – Ongoing precision and recovery standard (OPR); an aliquot of a reference matrix spiked with known quantities of analytes. Also known as a "laboratory control sample" (LCS). The OPR is analyzed exactly like a sample. Its purpose is to assure that the results produced by the laboratory remain within the limits specified in this method for precision and recovery.

Reagent water – water demonstrated to be free from the analytes of interest and potentially interfering substances at the method detection limit for the analyte.

Relative standard deviation (RSD) – The standard deviation times 100, divided by the mean. Also termed the "coefficient of variation."

Relative percent difference (RPD) – The absolute difference between two values, divided by the mean of the two values. Used to compare results when there is no true value.

Signal-to-noise ratio (S/N) – The height of the signal as measured from the mean (average) of the noise to the peak maximum divided by the width of the noise.

SPE – Solid-phase extraction; an extraction technique in which an analyte is extracted from an aqueous solution by passage over or through a material capable of reversibly adsorbing the analyte. Also termed liquid-solid extraction. SPE is used in this procedure as a cleanup technique.

20.0 Tables and figures

Table 1. Names and CAS Registry numbers for PFCs determined by isotope dilution and internal standard

Compound	CAS Number	Formula	Acronym
Native PFCs			
Perfluoorobutanoic acid	375-22-4	$CF_3(CF_2)_2COOH$	PFBA
Perfluoropentanoic acid	2706-90-3	$CF_3(CF_2)3COOH$	PFPA
Perfluoro-n-hexanoic acid	307-24-4	$CF_3(CF_2)_4COOH$	PFHxA (C6)
Perfluoro-n-heptanoic acid	375-85-9	$CF_3(CF_2)_5COOH$	PFHpA (C7)
Perfluoro-n-octanoic acid	335-67-1	$CF_3(CF_2)_6COOH$	PFOA (C8)
Perfluoro-n-nonanoic acid	375-95-1	$CF_3(CF_2)_7COOH$	PFNA (C9)
Perfluoro-n-decanoic acid	335-76-2	$CF_3(CF_2)_8COOH$	PFDA (C10)
Perfluoro-n-undecanoic acid	2058-94-8	$CF_3(CF_2)_9COOH$	PFUnDA (C11)
Perfluoro-n-dodecanoic acid	307-55-1	$CF_3(CF_2)_{10}COOH$	PFDoDA (C12)
Perfluoro-n-tridecanoic acid	72629-94-8	$CF_3(CF_2)_{11}COOH$	PFTriDA (C13)
Perfluoro-n-tetradecanoic acid	376-06-7	$CF_3(CF_2)_{12}COOH$	PFTeDA (C14)
Perfluorobutanesulfonic acid	375-73-5	$CF_3(CF_2)_3SO_3H$	PFBS (S4)
Perfluorohexanesulfonic acid	355-46-4	$CF_3(CF_2)_5SO_3H$	PFHxS (S6)
Perfluoro-n-heptanesulfonic acid	375-92-8	$CF_3(CF_2)_6SO_3H$	PFHpS (S7)
Perfluorooctanesulfonic acid	1763-23-1	$CF_3(CF_2)_7SO_3H$	PFOS (S8)
Perfluorooctane sulfonamide	754-91-6	$CF_3(CF_2)_6SO_2NH_2$	PFOSA (S8)
N-methylperfluoro-1-octanesulfonamide	31506-32-8	$CF_3(CF_2)_7SO_2N.H.CH_3$	N-MeFOSA
N-ethylperfluoro-1-octanesulfonamide	4151-50-2	$CF_3(CF_2)_7SO_2N.H.C_2H_5$	N-EtFOSA
2-(N-methylperfluoro-1-octanesulfonamido)-ethanol	24448-09-7	$CF_3(CF_2)_7SO_2N.\ CH_3.C_2H_4OH$	N-MeFOSE
2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol	1691-99-2	$CF_3(CF_2)_7SO_2N.\ C_2H_5.C_2H_4OH$	N-EtFOSE
Labeled Internal Standards			
Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid		$^{13}CF_3(^{13}CF_2)_2{}^{13}COOH$	[$^{13}C_4$]PFBA-(MPFBA)
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid		$CF_3(CF_2)_3(^{13}CF_2)^{13}COOH$	[$^{13}C_2$]PFHxA-(MPFHxA)
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid		$CF_3(CF_2)_3(^{13}CF_2)_3{}^{13}COOH$	[$^{13}C_4$]PFOA-(M4PFOA)
Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid		$CF_3(CF_2)_3(^{13}CF_2)_4{}^{13}COOH$	[$^{13}C_5$]PFNA-(MPFNA)
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid		$CF_3(CF_2)_7(^{13}CF_2)^{13}COOH$	[$^{13}C_2$]PFDA-(M2PFDA)
Perfluoro-n-[1,2,3,4,5,6,7,8,9-$^{13}C_9$]decanoic acid		$CF_3(\ ^{13}CF_2)_8{}^{13}COOH$	[$^{13}C_9$]PFDA-(M9PFDA)
Perfluoro-n-[1,2-$^{13}C_2$]undecanoic acid		$CF_3(CF_2)_8(^{13}CF_2)^{13}COOH$	[$^{13}C_2$]PFUnDA-(M2PFUnDA)
Perfluoro-n-[2,3,4,5,6,7,8,9,10-$^{13}C_9$] undecanoic acid		$CF_3(^{13}CF_2)_9COOH$	[$^{13}C_9$]PFUnDA-(M9PFUnDA)
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid		$CF_3(CF_2)_9(^{13}CF_2)^{13}COOH$	[$^{13}C_2$]PFDoDA-MPFDoDA)
Perfluoro-1-[1,2-$^{18}O_2$]-hexanesulfonic acid		$CF_3(CF_2)_5S^{18}O_2H$	[$^{18}O_2$]PFOS-(MPFHxS)
Perfluoro-n-[1,2,3,4-$^{13}C_4$]-octanesulfonic acid		$CF_3(CF_2)_3(^{13}CF_2)_4SO_3H$	[$^{13}C_4$]PFOS-(MPFOS)
2-(N-deuteriomethylperfluoro-1-octane sulfonamido)-1,1,2,2-tetradeuterioethanol		$CF_3(CF_2)_7SO_2N.\ CD_3.C_2D_4OH$	d_7-N-MeFOSE
Injection Internal Standards			
Perfluoro-n-[1,2,3,4,5,6,7,8-$^{13}C_8$]octanoic acid		$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$	[$^{13}C_8$]PFOA-(M8PFOA)
2H-Perfluoro-[1,2-$^{13}C_2$]-2-decenoic acid		$CF_3(CF_2)_6CF^{13}CH^{13}COOH$	[$^{13}C_2$]PFOUEA-MPFOUEA)

Table 2. Typical retention times (RT), relative retention times (RRT), and RT windows

Compound	Mean RT in Calibration Standard[1]	Standard Deviation of RT	Mean RT in Biosolids[2]	Standard Deviation of RT	RT Window	Mean RRT in a Calibration Standard[1]	Standard Deviation of RRT	Mean RRT in Biosolids[2]	Standard deviation RRT	RRT Window
Native PFCs										
Perfluoorobutanoic acid	5.22	0.025	5.24	0.036	± 0.3	1.000	0.0026	1.008	0.0116	± 0.05
Perfluoropentanoic acid	6.36	0.016	6.37	0.016	± 0.1	0.725	0.0018	0.723	0.0018	± 0.05
Perfluoro-n-hexanoic acid	7.06	0.016	7.07	0.015	± 0.1	1.001	0.0018	1.000	0.0000	± 0.01
Perfluoro-n-heptanoic acid	7.81	0.014	7.82	0.020	± 0.1	0.891	0.0015	0.887	0.0022	± 0.05
Perfluoro-n-octanoic acid	8.78	0.026	8.80	0.016	± 0.1	1.000	0.0000	1.000	0.0000	± 0.01
Perfluoro-n-nonanoic acid	10.01	0.036	10.05	0.048	± 0.5	1.001	0.0027	1.002	0.0039	± 0.01
Perfluoro-n-decanoic acid	11.50	0.026	11.48	0.040	± 0.5	1.002	0.0017	1.000	0.0000	± 0.01
Perfluoro-n-undecanoic acid	13.16	0.030	13.02	0.113	± 0.5	1.001	0.0013	1.001	0.0014	± 0.01
Perfluoro-n-dodecanoic acid	15.12	0.032	14.99	0.168	± 0.5	1.000	0.0009	1.001	0.0010	± 0.01
Perfluoro-n-tridecanoic acid	17.48	0.038	17.46	0.073	± 0.5	1.993	0.0043	1.981	0.0083	± 0.05
Perfluoro-n-tetradecanoic acid	20.56	0.072	20.64	0.100	± 0.5	2.344	0.0082	2.343	0.0114	± 0.05
Perfluorobutanesulfonic acid	7.20	0.018	7.21	0.016	± 0.1	0.821	0.0020	0.819	0.0018	± 0.05
Perfluoro-n-hexane sulfonic acid	9.25	0.019	9.25	0.016	± 0.1	1.001	0.0020	1.000	0.0000	± 0.01
Perfluoro-n-heptane sulfonic acid	10.61	0.032	10.65	0.029	± 0.5	1.210	0.0037	1.209	0.0032	± 0.05
Perfluoro-n-octanesulfonic acid	12.20	0.047	12.15	0.073	± 0.5	1.002	0.0017	1.001	0.0013	± 0.01
Perfluorooctane sulfonamide	17.56	0.032	17.52	0.064	± 0.5	2.002	0.0036	1.988	0.0073	± 0.05
N-methylperfluoro-1-octanesulfonamide	23.15	0.063	23.07	0.096	± 0.5	2.640	0.0072	2.619	0.0109	± 0.05
N-ethylperfluoro-1-octanesulfonamide	24.91	0.039	24.95	0.031	± 0.5	2.841	0.0045	2.832	0.0036	± 0.05
2-(N-methylperfluoro-1-octane sulfonamido)-ethanol	21.53	0.057	21.36	0.127	± 0.5	1.009	0.0024	1.005	0.0022	± 0.01
2-(N-ethylperfluoro-1-octane sulfonamido)-ethanol	24.27	0.034	24.22	0.070	± 0.5	2.767	0.0039	2.749	0.0080	± 0.05
Mass-labeled PFCs										
Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid	5.22	0.028	5.20	0.084	±0.3	0.595	0.0032	0.590	0.0096	± 0.05
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	7.05	0.011	7.07	0.015	± 0.1	0.804	0.0012	0.802	0.0018	± 0.05
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	8.78	0.026	8.80	0.016	± 0.1	1.001	0.0029	0.999	0.0019	± 0.01
Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid	9.99	0.038	10.03	0.036	± 0.1	1.139	0.0043	1.138	0.0041	± 0.05
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	11.49	0.033	11.48	0.040	± 0.5	1.310	0.0037	1.303	0.0045	± 0.05
Perfluoro-n-[1,2,3,4,5,6,7,8,9-$^{13}C_9$]decanoic acid	11.48	0.034	11.48	0.040	± 0.5	1.309	0.0039	1.303	0.0045	± 0.05
Perfluoro-n-[1,2-$^{13}C_2$]undecanoic acid	13.15	0.029	13.06	0.069	± 0.5	1.499	0.0033	1.482	0.0079	± 0.05

Table 2. Typical retention times (RT), relative retention times (RRT), and RT windows

Compound	Mean RT in Calibration Standard[1]	Standard Deviation of RT	Mean RT in Biosolids[2]	Standard Deviation of RT	RT Window	Mean RRT in a Calibration Standard[1]	Standard Deviation of RRT	Mean RRT in Biosolids[2]	Standard deviation RRT	RRT Window
Perfluoro-n-[2,3,4,5,6,7,8,9,10-$^{13}C_9$]undecanoic acid	13.15	0.031	13.01	0.124	± 0.5	1.499	0.0035	1.477	0.0141	± 0.05
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	15.11	0.025	14.98	0.162	± 0.5	1.723	0.0029	1.700	0.0184	± 0.05
Perfluoro-1-[1,2-$^{18}O_2$]-hexanesulfonic acid	9.24	0.021	9.25	0.016	± 0.1	1.054	0.0024	1.050	0.0019	± 0.05
Perfluoro-n-[1,2,3,4-$^{13}C_4$]-octanesulfonate	12.18	0.032	12.14	0.083	± 0.5	1.389	0.0036	1.378	0.0095	± 0.05
2-(N-deuteriomethylperfluoro-1-octane sulfonamido)-1,1,2,2-tetradeuterioethanol	21.34	0.071	21.26	0.094	± 0.5	2.433	0.0081	2.414	0.0106	± 0.05
Injection Internal Standards (compound added after extraction, but prior to injection)										
Perfluoro-n-[1,2,3,4,5,6,7,8-$^{13}C_8$]octanoic acid	8.78	0.026	8.80	0.016	± 0.1	--	--	--	--	--
2H-Perfluoro-[1,2-$^{13}C_2$]-2-decenoic acid	9.64	0.037	9.65	0.016	± 0.1	--	--	--	--	--

Table 3. Analytes, ions, quantification references, and instrument conditions

Compound	Precursor Ion (m/z)	Quant Ion (m/z)	Precursor formula	Primary Quant Ion formula	Cone Voltage (V)	Collision (eV)	2nd Qual Ion Mass (m/z)	2nd Qual Ion formula	Collision (eV)	Quant by:	Quantitation Reference
Native PFCs											
Perfluoorobutanoic acid	213	169	$[CF_3(CF_2)_2CO_2]^-$	$[CF_3(CF_2)_2]^-$	27	8				ID	$^{13}CF_3(^{13}CF_2)_2{}^{13}COOH$
Perfluoropentanoic acid	263	219	$[CF_3(CF_2)_3CO_2]^-$	$[CF_3(CF_2)_3]^-$	27	8				IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
Perfluoro-n-hexanoic acid	313	269	$[CF_3(CF_2)_4CO_2]^-$	$[CF_3(CF_2)_4]^-$	27	20	119	$[CF_3CF_2]^-$	8	ID	$CF_3(CF_2)_3(^{13}CF_2){}^{13}COOH$
Perfluoro-n-heptanoic acid	363	319	$[CF_3(CF_2)_5CO_2]^-$	$[CF_3(CF_2)_5]^-$	27	12	169	$[CF_3(CF_2)_2]^-$	8	IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
Perfluoro-n-octanoic acid	413	369	$[CF_3(CF_2)_6CO_2]^-$	$[CF_3(CF_2)_6]^-$	19	12	169	$[CF_3(CF_2)_2]^-$	12	ID	$CF_3(CF_2)_3(^{13}CF_2)_3$ $^{13}COOH$
Perfluoro-n-nonanoic acid	463	419	$[CF_3(CF_2)_7CO_2]^-$	$[CF_3(CF_2)_7]^-$	20	13	219	$[CF_3(CF_2)_3]^-$	12	ID	$CF_3(CF_2)_3(^{13}CF_2)_4$ $^{13}COOH$
Perfluoro-n-decanoic acid	513	469	$[CF_3(CF_2)_8CO_2]^-$	$[CF_3(CF_2)_8]^-$	21	11	219	$[CF_3(CF_2)_3]^-$	13	ID	$CF_3(^{13}CF_2)_8{}^{13}COOH$
Perfluoro-n-undecanoic acid	563	519	$[CF_3(CF_2)_9CO_2]^-$	$[CF_3(CF_2)_9]^-$	21	15	269	$[CF_3(CF_2)_4]^-$	12	ID	$CF_3(^{13}CF_2)_9COOH$
Perfluoro-n-dodecanoic acid	613	569	$[CF_3(CF_2)_{10}CO_2]^-$	$[CF_3(CF_2)_{10}]^-$	22	15	319	$[CF_3(CF_2)_5]^-$	12	ID	$CF_3(CF_2)_9(^{13}CF_2){}^{13}COOH$
Perfluoro-n-tridecanoic acid	663	619	$[CF_3(CF_2)_{11}CO_2]^-$	$[CF_3(CF_2)_{11}]^-$	20	17	319	$[CF_3(CF_2)_5]^-$	13	IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
Perfluoro-n-tetradecanoic acid	713	669	$[CF_3(CF_2)_{12}CO_2]^-$	$[CF_3(CF_2)_{12}]^-$	27	21	319	$[CF_3(CF_2)_5]^-$	11	IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
Perfluorobutanesulfonic acid	299	80	$[CF_3(CF_2)_3SO_3]^-$	$[SO_3]^-$	70	40	99	$[FSO_3]^-$	35	IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
Perfluoro-n-hexane sulfonic acid [1]	399	80	$[CF_3(CF_2)_5SO_3]^-$	$[SO_3]^-$	30	45	99	$[FSO_3]^-$	40	ID	$CF_3(CF_2)_5S(^{18}O)_2OH$
Perfluoro-n-heptane sulfonic acid	449	80	$[CF_3(CF_2)_6SO_3]^-$	$[SO_3]^-$	50	39	99	$[FSO_3]^-$	38	IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
Perfluoro-n-octanesulfonic acid	499	80	$[CF_3(CF_2)_7SO_3]^-$	$[SO_3]^-$	80	45	99	$[FSO_3]^-$	40	ID	$CF_3(CF_2)_3(^{13}CF_2)_4SO_3H$
Perfluorooctane sulfonamide	498	78	$[CF_3(CF_2)_7SO_2NH]^-$	$[SO_2N]^-$	80	40	478	$[(CF_2)_8SO_2N]^-$	16	IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
N-methylperfluoro-1-octanesulfonamide	512	169	$[CF_3(CF_2)_7SO_2N(CH_3)]^-$	$[CF_3(CF_2)_2]^-$	27	45				IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
N-ethylperfluoro-1-octanesulfonamide	526	169	$[CF_3(CF_2)_7SO_2N(C_2H_5)]^-$	$[CF_3(CF_2)_2]^-$	27	45				IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
2-(N-methylperfluoro-1-octanesulfonamido)-ethanol	616	59	$[CF_3(CF_2)_7SO_2N(CH_3)C_2H_4OH{\cdot}CH_3CO_2]^-$	$[CH_3CO_2]^-$	27	45				ID	$CF_3(CF_2)_7SO_2N$ $(CD_3)C_2D_4OH{\cdot}CH_3COOH$
2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol	630	59	$[CF_3(CF_2)_7SO_2N(C_2H_5)C_2H_4OH{\cdot}CH_3CO_2]^-$	$[CH_3CO_2]^-$	27	45				IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$

Table 3. Analytes, ions, quantification references, and instrument conditions

Compound	Precursor Ion (m/z)	Quant Ion (m/z)	Precursor formula	Primary Quant Ion formula	Cone Voltage (V)	Collision (eV)	2nd Qual Ion Mass (m/z)	2nd Qual Ion formula	Collision (eV)	Quant by:	Quantitation Reference
Mass-labeled PFCs											
Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid	217	172	$[^{13}CF_3(^{13}CF_2)_2\ ^{13}CO_2]^-$	$[^{13}CF_3(^{13}CF_2)_2]^-$	27	8				IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	315	270	$[CF_3(CF_2)_3(^{13}CF_2)\ ^{13}CO_2]^-$	$[CF_3(CF_2)_3(^{13}CF_2)\ ]^-$	27	8				IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	417	372	$[CF_3(CF_2)_3\ (^{13}CF_2)_3{}^{13}CO_2]^-$	$[CF_3(CF_2)_3\ (^{13}CF_2)_3]^-$	21	12				IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid	468	423	$[CF_3(CF_2)_3\ (^{13}CF_2)_4{}^{13}CO_2]^-$	$[CF_3(CF_2)_3(^{13}CF_2)_4]^-$	20	12				IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	515	470	$[CF_3(CF_2)_7(^{13}CF_2)\ ^{13}CO_2]^-$	$[CF_3(CF_2)_2(^{13}CF_2)\ ]^-$	21	12				IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
Perfluoro-n-[1,2,3,4,5,6,7,8,9-$^{13}C_9$]decanoic acid	522	477	$[CF_3(\ ^{13}CF_2)_8\ ^{13}CO_2]^-$	$[CF_3(\ ^{13}CF_2)_8]^-$	20	12				IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
Perfluoro-n-[1,2-$^{13}C_2$]undecanoic acid	565	520	$[CF_3(CF_2)_8(^{13}CF_2)\ ^{13}CO_2]^-$	$[CF_3(CF_2)_8(^{13}CF_2)\ ]^-$	20	12				IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
Perfluoro-n-[2,3,4,5,6,7,8,9,10-$^{13}C_9$]undecanoic acid	572	528	$[CF_3(\ ^{13}CF_2)_9CO_2]^-$	$[CF_3(\ ^{13}CF_2)_9]^-$	20	12				IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	615	570	$[CF_3(CF_2)_9\ (\ ^{13}CF_2)^{13}CO_2]^-$	$[CF_3(CF_2)_9\ (\ ^{13}CF_2)\ ]^-$	22	12				IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
Perfluoro-1-[1,2-$^{18}O_2$]-hexanesulfonic acid	403	84	$[CF_3(CF_2)_5\ S(^{18}O)_2O]^-$	$[S(^{18}O)_2O]^-$	30	45	103	$[FS(^{18}O)_2\ O]^-$	45	IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
Perfluoro-n-[1,2,3,4-$^{13}C_4$]-octanesulfonate	503	80	$[CF_3(CF_2)_3\ (^{13}CF_2)_4SO_3]^-$	$[SO_3]^-$	40	45	99	$[FSO_3]^-$	40	IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
2-(N-deuteriomethylperfluoro-1-octanesulfonamido)-1,1,2,2-tetradeuterioethanol	623	59	$[CF_3(CF_2)_7SO_2N(CD_3)C_2D_4OH{\cdot}CH_3CO_2]^-$	$[CH_3CO_2]^-$	27	45				IS	$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$
Injection Internal Standards (compound added after extraction, but prior to injection)											
Perfluoro-n-[1,2,3,4,5,6,7,8-$^{13}C_8$]octanoic acid	421	376	$[^{13}CF_3(\ ^{13}CF_2)_6\ ^{13}CO_2]^-$	$[^{13}CF_3(\ ^{3}CF_2)_6]^-$	21	12				ES	
2H-Perfluoro-[1,2-$^{13}C_2$]-2-decenoic acid	459	394	$[CF_3(CF_2)_6\ CF^{13}CH^{13}CO_2]^-$	$[CF_3(CF_2)_3\ (^{13}CF_2)_3]^-$	21	11				ES	

[1] This analyte has a third ion that can be used for qualitative identification. The m/z of that ion is 119, the formula is CF3CF2-, and the collision energy is 30 eV.

Table 4. Method detection limits, minimum levels of quantitation, and provisional health advisory

Compound	MDL (ng/g)	ML (ng/g)	OW Provisional Health Advisory
Perfluoorobutanoic acid	0.125	0.250	
Perfluoropentanoic acid	0.185	0.250	
Perfluoro-n-hexanoic acid	0.136	0.250	
Perfluoro-n-heptanoic acid	0.054	0.250	
Perfluoro-n-octanoic acid	0.085	0.250	0.4 ppb
Perfluoro-n-nonanoic acid	0.127	0.250	
Perfluoro-n-decanoic acid	0.084	0.250	
Perfluoro-n-undecanoic acid	0.080	0.250	
Perfluoro-n-dodecanoic acid	0.067	0.250	
Perfluoro-n-tridecanoic acid	0.067	0.250	
Perfluoro-n-tetradecanoic acid	0.028	0.250	
Perfluorobutanesulfonic acid	0.115	0.500	
Perfluorohexanesulfonic acid	0.268	0.500	
Perfluoroheptanesulfonic acid	0.291	0.500	
Perfluorooctanesulfonic acid	0.208	0.500	0.2 ppb
Perfluorooctane sulfonamide	0.040	0.250	
N-methylperfluoro-1-octanesulfonamide	3.65	10.0	
N-ethylperfluoro-1-octanesulfonamide	3.05	10.0	
2-(N-methylperfluoro-1-octanesulfonamido)-ethanol	7.65	5.00	
2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol	1.88	5.00	

Table 5. Instrument conditions

Instruments		Waters Acquity high performance liquid chromatography (HPLC) or equivalent			
		Waters Quattro Ultima tandem mass spectrometer or equivalent			
Trapping cartridge		NA			
HPLC Column		Waters Xtera C18MS analytical column, 100 mm length, 2.1 mm ID, 3.5 µm particle size, or equivalent			
Ionization		Negative Ion Electrospray			
Acquisition		MRM mode, unit resolution			
Injection Volume		15 µL			
Final Extract Volume		1 mL			
HPLC Gradient Program		**HPLC Flow rate**	**Gradient Curve**		
Time (min)	**Flow mixture**	**(mL/min)**		**HPLC conditions**	
0.00	A=15% B=85%	0.150	1	Column Temp (°C)	40
1.00	A=15% B=85%	0.150	1	Max Pressure (bar)	345
5.00	A=50% B=50%	0.200	4		
20.00	A=65% B=35%	0.200	4		
23.00	A=100% B=0%	0.200	4		
26.00	A=100% B=0%	0.200	4	**MS Conditions**	
26.50	A=15% B=85%	0.200	2	Source Temp (°C)	120
30.00	A=15% B=85%	0.200	2	Desolvation Temp (ºC)	325
32.00	A=15% B=85%	0.150	2	Capillary voltage (kV)	3.50

Solvent A = 90% ACN:10% water (organic phase)

Solvent B = 12.1 mM ammonium acetate and 0.1% acetic acid in water (aqueous phase)

Table 6. Concentrations of calibration standards (CS) in pg/mL

	CS-1	CS-2	CS-3	CS-4 (VER)	CS-5	CS-6	CS-7	CS-8
Native Analytes								
Perfluoorobutanoic acid	125	312.5	1,250	5,000	25,000	50,000	125,000	312,500
Perfluoropentanoic acid	125	312.5	1,250	5,000	25,000	50,000	125,000	312,500
Perfluoro-n-hexanoic acid	125	312.5	1,250	5,000	25,000	50,000	125,000	312,500
Perfluoro-n-heptanoic acid	125	312.5	1,250	5,000	25,000	50,000	125,000	312,500
Perfluoro-n-octanoic acid	125	312.5	1,250	5,000	25,000	50,000	125,000	312,500
Perfluoro-n-nonanoic acid	125	312.5	1,250	5,000	25,000	50,000	125,000	312,500
Perfluoro-n-decanoic acid	125	312.5	1,250	5,000	25,000	50,000	125,000	312,500
Perfluoro-n-undecanoic acid	125	312.5	1,250	5,000	25,000	50,000	125,000	312,500
Perfluoro-n-dodecanoic acid	125	312.5	1,250	5,000	25,000	50,000	125,000	312,500
Perfluoro-n-tridecanoic acid	125	312.5	1,250	5,000	25,000	50,000	125,000	312,500
Perfluoro-n-tetradecanoic acid	125	312.5	1,250	5,000	25,000	50,000	125,000	312,500
Perfluorobutanesulfonic acid	250	625	2,500	10,000	50,000	100,000	250,000	625,000
Perfluorohexanesulfonic acid	250	625	2,500	10,000	50,000	100,000	250,000	625,000
Perfluoro-n-heptanesulfonic acid	250	625	2,500	10,000	50,000	100,000	250,000	625,000
Perfluorooctanesulfonic acid	250	625	2,500	10,000	50,000	100,000	250,000	625,000
Perfluorooctane sulfonamide	125	312.5	1,250	5,000	25,000	50,000	125,000	312,500
N-methylperfluoro-1-octanesulfonamide	5,000	10,000	20,000	40,000	80,000	160,000	320,000	--
N-ethylperfluoro-1-octanesulfonamide	5,000	10,000	20,000	40,000	80,000	160,000	320,000	--
2-(N-methylperfluoro-1-octanesulfonamido)-ethanol	2,500	5,000	10,000	20,000	40,000	80,000	160,000	--
2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol	2,500	5,000	10,000	20,000	40,000	80,000	160,000	--
Labeled Internal Standards								
Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
Perfluoro-n-[1,2,3,4,5,6,7,8,9-$^{13}C_9$]decanoic acid	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
Perfluoro-n-[1,2-$^{13}C_2$]undecanoic acid	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
Perfluoro-n-[2,3,4,5,6,7,8,9,10-$^{13}C_9$]undecanoic acid	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
Perfluoro-1-[1,2-$^{18}O_2$]-hexanesulfonic acid	4,270	4,270	4,270	4,270	4,270	4,270	4,270	4,270
Perfluoro-n-[1,2,3,4-$^{13}C_4$]-octanesulfonic acid	4,310	4,310	4,310	4,310	4,310	4,310	4,310	4,310
2-(N-deuteriomethylperfluoro-1-octanesulfonamido)-1,1,2,2-tetradeuterioethanol	20,000	20,000	20,000	20,000	20,000	20,000	20,000	
Internal Injection Standards								
Perfluoro-n-[1,2,3,4,5,6,7,8-$^{13}C_8$]octanoic acid	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
2H-Perfluoro-[1,2-$^{13}C_2$]-2-decenoic acid	2,500	2,500	2,500	2,500	2,500	2,500	2,500	2,500

[1] Calibration standards are stored in ~ 92% MeOH, up to 2.75% Nonane, 2% Propan-2-ol, 1%MeCN, 1% H2O, up to 0.3% Toluene, 0.3% NH_4OH solution.

Table 7. Detailed concentrations of actual working level standards (pg/mL)

Working-level Standards (pg/mL)		
Native standards	**Working level**	**Low level**
Perfluoorobutanoic acid	125,000	12,500
Perfluoropentanoic acid	125,000	12,500
Perfluoro-n-hexanoic acid	125,000	12,500
Perfluoro-n-heptanoic acid	125,000	12,500
Perfluoro-n-octanoic acid	125,000	12,500
Perfluoro-n-nonanoic acid	125,000	12,500
Perfluoro-n-decanoic acid	125,000	12,500
Perfluoro-n-undecanoic acid	125,000	12,500
Perfluoro-n-dodecanoic acid	125,000	12,500
Perfluoro-n-tridecanoic acid	125,000	12,500
Perfluoro-n-tetradecanoic acid	125,000	12,500
Perfluorobutanesulfonic acid	250,000	25,000
Perfluorohexanesulfonic acid	250,000	25,000
Perfluoro-n-heptanesulfonic acid	250,000	25,000
Perfluorooctanesulfonic acid	250,000	25,000
Perfluorooctane sulfonamide	125,000	12,500
N-Me/Et-FOSA/Es Natives (NAT2)		
N-methylperfluoro-1-octanesulfonamide	2,500,000	
N-ethylperfluoro-1-octanesulfonamide	2,500,000	
2-(N-methylperfluoro-1-octanesulfonamido)-ethanol	1,250,000	
2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol	1,250,000	
Labeled Internal Standard Solution		
Mass Labeled PFCs		
Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid	30,000	
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	30,000	
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	30,000	
Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid	30,000	
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	30,000	
Perfluoro-n-[1,2-$^{13}C_9$]decanoic acid	30,000	
Perfluoro-n-[1,2-$^{13}C_2$]undecanoic acid	30,000	
Perfluoro-n-[1,2-$^{13}C_9$]undecanoic acid	30,000	
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	30,000	
Perfluoro-1-[1,2-$^{18}O_2$]-hexanesulfonic acid	42,700	
Perfluoro-n-[1,2,3,4-$^{13}C_4$]-octanesulfonic acid	43,100	
2-(N-deuteriomethylperfluoro-1-octanesulfonamido)-1,1,2,2-tetradeuterioethanol	200,000	
Labeled Injection Standards		
Perfluoro-n-[1,2,3,4,5,6,7,8-$^{13}C_8$]octanoic acid	12,000	
2H-Perfluoro-[1,2-$^{13}C_2$]-2-decenoic acid	10,000	

Table 8. QC acceptance criteria for analytes, labeled compounds and internal standards in VER, IPR, OPR, matrix spikes, and samples

Compound	Quant	ICAL and CALVER Recovery (%)			IPR Recovery (%)			OPR Recovery (%)		RLS Recovery (%)		Recovery in Matrix Spikes and Samples		
		Low	High	RSD	Low	High	RSD	Low	High	Low	High	Low	High	RPD
Native PFCs														
Perfluoorobutanoic acid	ID	70	130	20	70	130	20	70	130	50	200	50	150	30
Perfluoropentanoic acid	IS	70	130	20	70	130	20	70	130	50	200	50	150	30
Perfluoro-n-hexanoic acid	ID	70	130	20	70	130	20	70	130	50	150	50	150	30
Perfluoro-n-heptanoic acid	IS	70	130	20	70	130	20	70	130	50	150	50	150	30
Perfluoro-n-octanoic acid	ID	70	130	20	70	130	20	70	130	50	150	50	150	30
Perfluoro-n-nonanoic acid	ID	70	130	20	50	150	30	50	150	50	150	50	150	30
Perfluoro-n-decanoic acid	ID	70	130	20	70	130	20	70	130	50	150	50	150	30
Perfluoro-n-undecanoic acid	ID	70	130	20	70	130	20	70	130	50	150	50	150	30
Perfluoro-n-dodecanoic acid	ID	70	130	20	70	130	20	70	130	50	150	50	150	30
Perfluoro-n-tridecanoic acid	IS	70	130	20	20	130	30	20	130	50	150	50	150	30
Perfluoro-n-tetradecanoic acid	IS	70	130	20	20	130	30	20	130	50	150	50	150	30
Perfluorobutanesulfonic acid	IS	70	130	20	60	130	20	60	130	50	150	50	150	30
Perfluorohexanesulfonic acid	IS	70	130	20	70	130	20	70	130	50	150	50	150	30
Perfluoroheptanesulfonic acid	ID	70	130	20	70	130	20	70	130	50	150	50	150	30
Perfluorooctanesulfonic acid	ID	70	130	20	70	130	20	70	130	50	150	50	150	30
Perfluorooctane sulfonamide	IS	70	130	20	20	130	30	20	130	50	150	50	150	30
N-methylperfluoro-1-octanesulfonamide	IS	70	130	20	5	130	20	5	130	10	150	5	150	40
N-ethylperfluoro-1-octanesulfonamide	IS	70	130	20	5	130	40	5	130	10	150	5	150	40
2-(N-methylperfluoro-1-octanesulfonamido)-ethanol	ID	70	130	20	40	130	30	40	130	50	150	50	150	30
2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol	IS	70	130	20	10	130	40	10	130	25	150	10	150	40
Labeled Internal Standards: Compounds added before extraction														
Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid	IS	70	130	20	60	130	20	60	130	60	130	60	130	20
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	IS	70	130	20	70	130	20	70	130	70	130	70	130	20
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	IS	70	130	20	60	130	20	60	130	60	130	60	130	20
Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid	IS	70	130	20	50	130	25	50	130	50	130	50	130	25
Perfluoro-n-[1,2,3,4,5,6,7,8,9-$^{13}C_9$]decanoic acid	IS	70	130	20	60	130	20	60	130	60	130	60	130	20
Perfluoro-n-[1,2,3,4,5,6,7,8,9-$^{13}C_9$]undecanoic acid	IS	70	130	20	60	130	20	60	130	60	130	60	130	20
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	IS	70	130	20	30	130	20	30	130	30	130	30	130	20
Perfluoro-n-[1,2,3,4-$^{13}C_4$]-octanesulfonic acid	IS	70	130	20	60	130	20	60	130	60	130	60	130	20
Perfluoro-1-[$^{18}O_2$]-hexanesulfonic acid	IS	70	130	20	60	130	20	60	130	60	130	60	130	20
2-(N-deuteriomethylperfluoro-1-octane sulfonamido)-1,1,2,2-tetradeuterioethanol	IS	70	130	20	5	130	70	5	130	5	130	5	130	70
Injection Internal Standard: Compound added after extraction, but prior to injection														
Perfluoro-n-[1,2,3,4,5,6,7,8-$^{13}C_8$]octanoic acid	ES	80	120	10	80	120	10	80	120	80	120	80	120	10

Quantitation methods: ID = Isotope dilution IS = Internal standard ES = External standard

Table 9. Sources used for standards, materials, and equipment during method development[1]

Native Compounds	Vendor	Part Number	Shelf Life
Perfluoorobutanoic acid	Sigma-Aldrich	164194	To be determined
Perfluoropentanoic acid	Sigma-Aldrich	77285	To be determined
Perfluoro-n-hexanoic acid	Sigma-Aldrich	29226	To be determined
Perfluoro-n-heptanoic acid	Sigma-Aldrich	34,204-1	To be determined
Perfluoro-n-octanoic acid	Sigma-Aldrich	17,146-8	To be determined
Perfluoro-n-nonanoic acid	Sigma-Aldrich	77284	To be determined
Perfluoro-n-decanoic acid	Sigma-Aldrich	17,774-1	To be determined
Perfluoro-n-undecanoic acid	Sigma-Aldrich	446777	To be determined
Perfluoro-n-dodecanoic acid	Sigma-Aldrich	40,644-9	To be determined
Perfluoro-n-tridecanoic acid	Sigma-Aldrich	654973	To be determined
Perfluoro-n-tetradecanoic acid	Sigma-Aldrich	446785	To be determined
Perfluorobutanesulfonic acid (Tetrabutylammonium salt)	Sigma-Aldrich	86909	To be determined
Perfluorohexanesulfonic acid (Potassium salt)	Sigma-Aldrich	50929	To be determined
Perfluoro-n-heptanesulfonic acid (Sodium salt)	Wellington Labs	L-PFHpS	To be determined
Perfluorooctanesulfonic acid (Potassium salt)	Sigma-Aldrich	77282	To be determined
Perfluorooctane sulfonamide	Chiron	2043.8	To be determined
N-methylperfluoro-1-octanesulfonamide	Wellington Labs	N-MeFOSA	To be determined
N-ethylperfluoro-1-octanesulfonamide	Wellington Labs	N-EtFOSA	To be determined
2-(N-methylperfluoro-1-octanesulfonamido)-ethanol	Wellington Labs	N-MeFOSE	To be determined
2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol	Wellington Labs	N-EtFOSE	To be determined
Labeled Internal Standards			
Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid	Wellington Labs	MPFBA	To be determined
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	Wellington Labs	MPFHxA	To be determined
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	Wellington Labs	MPFOA	To be determined
Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid	Wellington Labs	MPFNA	To be determined
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	Wellington Labs	MPFDA	To be determined
Perfluoro-n-[1,2,3,4,5,6,7,8,9-$^{13}C_9$]decanoic acid	CIL	CLM-8172-S	To be determined
Perfluoro-n-[1,2-$^{13}C_2$]undecanoic acid	Wellington Labs	MPFUdA	To be determined
Perfluoro-n-[2,3,4,5,6,7,8,9,10-$^{13}C_9$]undecanoic acid	CIL	CLM-8240	To be determined
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	Wellington Labs	MPFDoA	To be determined
Perfluoro-1-[1,2-$^{18}O_2$]-hexanesulfonic acid	Wellington Labs	MPFHxS	To be determined
Perfluoro-n-[1,2,3,4-$^{13}C_4$]-octanesulfonic acid	Wellington Labs	MPFOS	To be determined
2-(N-deuteriomethylperfluoro-1-octanesulfonamido)-1,1,2,2-tetradeuterioethanol	Wellington Labs	d7-N-MeFOSE-M	To be determined
Injection Internal Standards			
Perfluoro-n-[1,2,3,4,5,6,7,8-$^{13}C_8$]octanoic acid	CIL	CLM-8005-S	To be determined
2H-Perfluoro-[1,2-$^{13}C_2$]-2-decenoic acid	Wellington Labs	MFOUEA	To be determined

[1] Provided for informational purposes only. Part numbers subject to change. Other suppliers may have equivalent materials.

Table 10. Performance data from single-laboratory validation

Compound	Transition	Solid IPR, n=4			Sludge, n=6		
		Mean % Rec	Standard Deviation	%RSD	Mean % Rec	Standard Deviation	%RSD
Native PFCs							
Perfluorobutanoic acid	Primary	105	1.61	1.54	95.8	9.55	9.97
Perfluoropentanoic acid	Primary	114	4.46	3.81	56.4	31.4	55.6
Perfluoro-n-hexanoic acid	Primary	116	2.28	1.98	115	9.83	8.52
Perfluoro-n-heptanoic acid	Primary	95.6	3.25	3.40	91.1	16.3	17.9
Perfluoro-n-octanoic acid	Primary	108	5.59	5.20	111	7.57	6.83
Perfluoro-n-octanoic acid	Secondary	109	8.65	7.96	119	14.9	12.6
Perfluoro-n-nonanoic acid	Primary	102	13.1	12.7	97.3	16.8	17.2
Perfluoro-n-decanoic acid	Primary	101	2.37	2.34	111	8.41	7.59
Perfluoro-n-undecanoic acid	Primary	101	2.20	2.18	104	7.35	7.09
Perfluoro-n-dodecanoic acid	Primary	106	2.64	2.48	109	6.50	5.96
Perfluoro-n-tridecanoic acid	Primary	41.3	2.41	5.83	126	29.1	23.1
Perfluoro-n-tetradecanoic acid	Primary	48.6	4.52	9.30	131	16.1	12.2
Perfluorobutanesulfonic acid	Primary	84.0	5.09	6.07	69.0	17.1	24.7
Perfluorobutanesulfonic acid	Secondary	86.3	5.55	6.43	69.9	18.8	26.9
Perfluoro-n-hexane sulfonate	Primary	101	2.18	2.16	105	11.0	10.5
Perfluoro-n-hexane sulfonate	Secondary	105	4.18	4.00	107	11.5	10.8
Perfluoro-n-heptane sulfonate	Primary	83.7	14.6	17.4	90.2	7.72	8.57
Perfluoro-n-heptane sulfonate	Secondary	93.5	4.95	5.30	92.7	5.28	5.70
Perfluoro-n-octanesulfonate	Primary	98.1	5.64	5.75	97.3	8.36	8.59
Perfluoro-n-octanesulfonate	Secondary	102	4.30	4.21	99.6	7.32	7.35
Perfluorooctane sulfonamide	Primary	39.5	3.69	9.33	50.7	3.63	7.15
N-methylperfluoro-1-octane sulfonamide	Primary	9.33	0.542	5.81	48.8	13.3	27.2
N-ethylperfluoro-1-octanesulfonamide	Primary	10.1	0.939	9.31	14.6	3.75	25.7
2-(N-methylperfluoro-1-octane sulfonamido)-ethanol	Primary	87.6	10.6	12.1	96.9	8.20	8.46
2-(N-ethylperfluoro-1-octane sulfonamido)-ethanol	Primary	21.8	1.37	6.27	50.3	13.6	27.0
Labeled Internal Standards							
Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid	Primary	95.2	6.84	7.19	81.6	37.5	45.9
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	Primary	98.9	2.55	2.58	72.0	18.2	25.3
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	Primary	90.4	6.62	7.32	96.1	5.03	5.23
Perfluoro-n-[1,2,3,4,5-$^{13}C_5$] nonanoic acid	Primary	85.6	8.35	9.75	92.5	8.80	9.52
Perfluoro-n-[1,2,3,4,5,6,7,8,9-$^{13}C_9$] decanoic acid	Primary	85.2	4.61	5.41	107	11.1	10.4
Perfluoro-n-[2,3,4,5,6,7,8,9,10-$^{13}C_9$] undecanoic acid	Primary	76.7	0.853	1.11	80.3	28.9	36.0
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	Primary	53.5	3.50	6.54	109	13.1	12.0
Perfluoro-n-[1,2,3,4-$^{13}C_4$]-octane sulfonic acid	Primary	76.9	4.82	6.27	78.8	7.97	10.1
Perfluoro-1-[1,2-$^{18}O_2$]-hexane sulfonic acid	Primary	76.8	3.33	4.33	79.4	7.73	9.74
2-(N-deuteriomethylperfluoro-1-octanesulfonamido)-1,1,2,2-tetradeuterioethanol	Primary	18.3	3.32	18.2	45.5	13.0	28.6
Injection Internal Standards							
Perfluoro-n-[1,2,3,4,5,6,7,8-$^{13}C_8$] octanoic acid	Primary	108	11.0	10.2	84.5	7.42	8.79

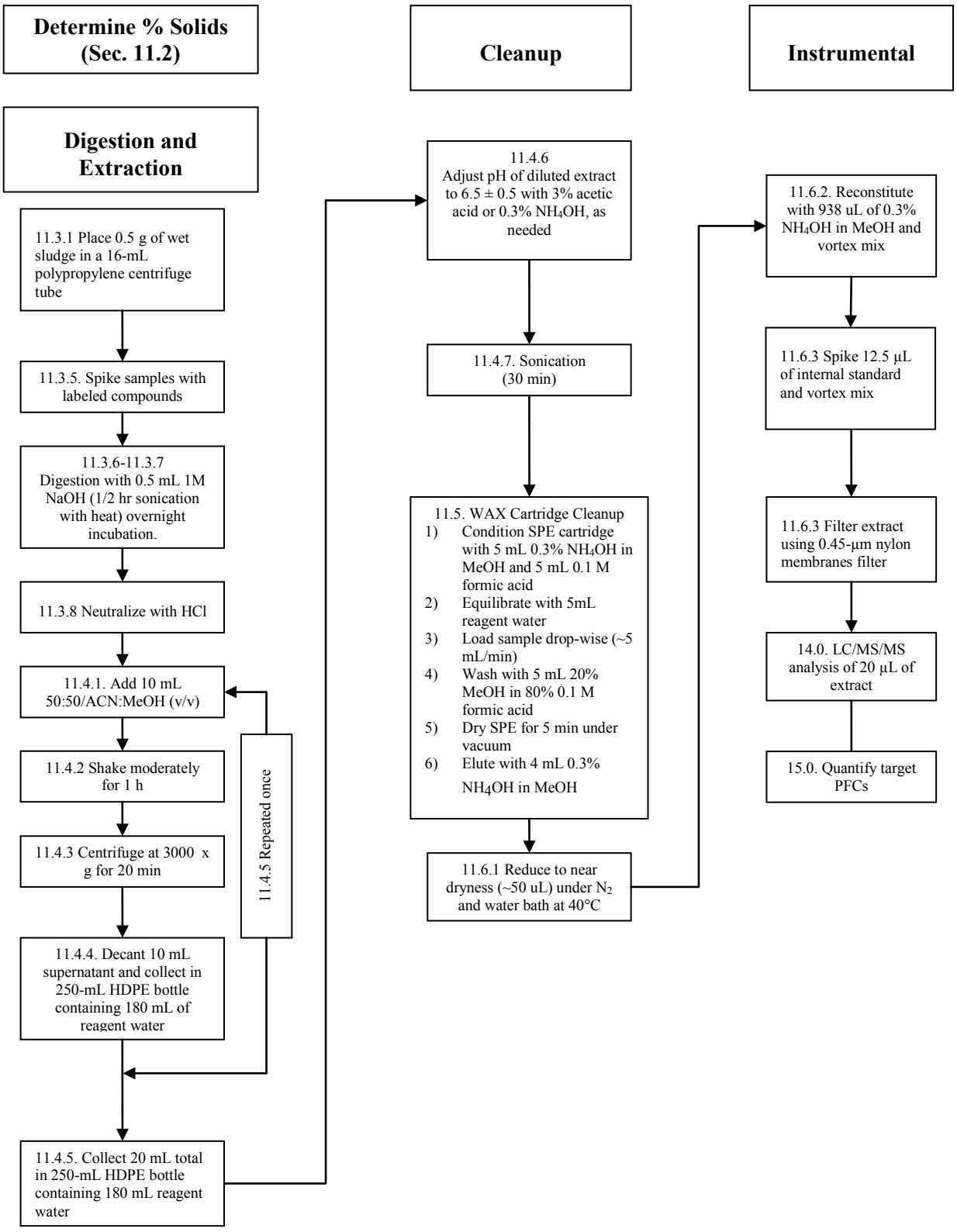

Figure 1. Flow chart for determination of PFCs by LC/MS/MS

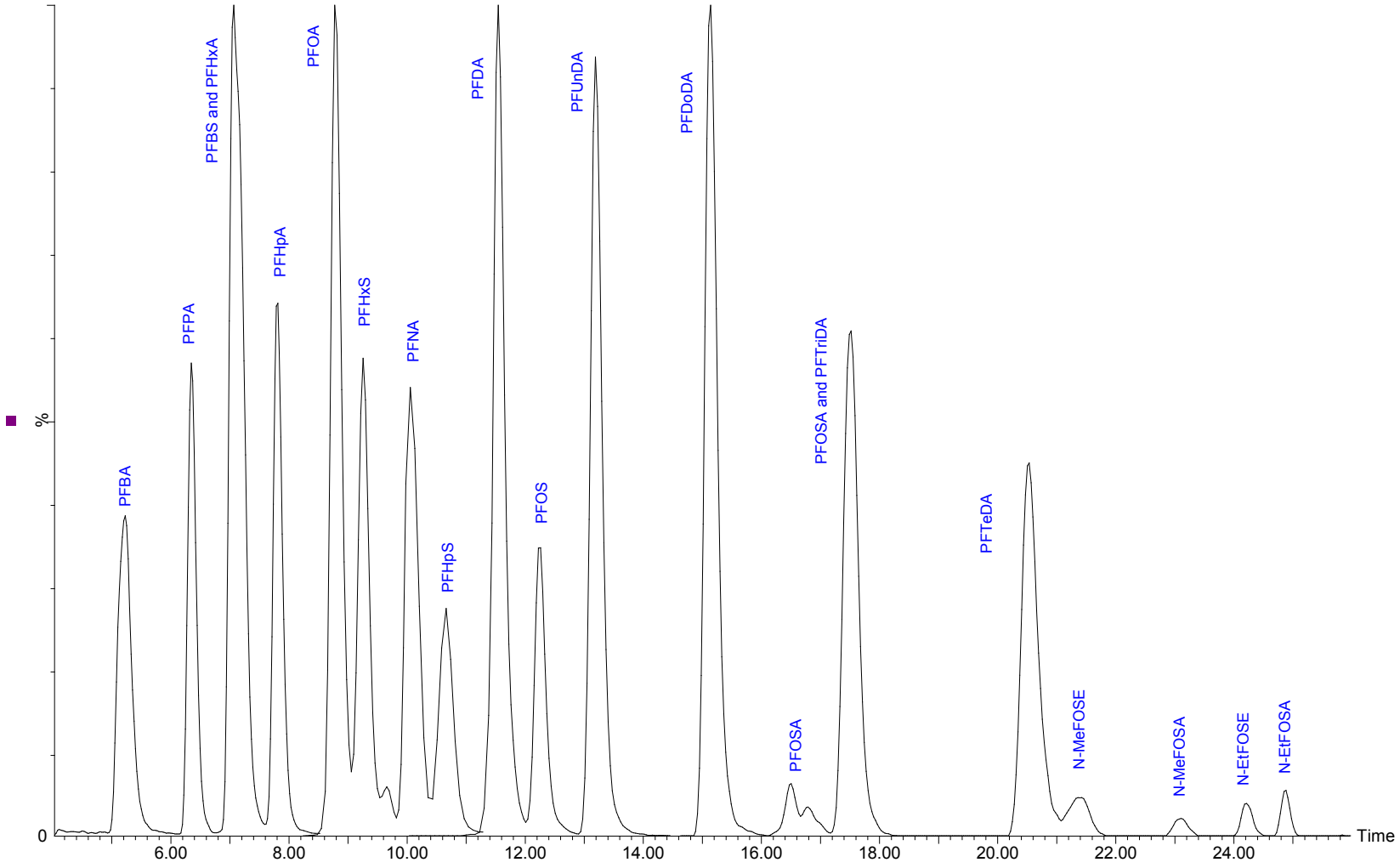

Figure 2. Example Chromatogram for CS-4 Calibration Standard

APPENDIX 1

Details on Preparation of Standards

Appendix 1. Details on Preparation of Standards

Individual PFCs are used to make standard solutions. From solids – native PFBS, PFHxS, and PFOS were obtained as sodium, potassium or tetrabutylammonium salts – all others were acids.
[$^{13}C_9$]PFUnDA was obtained as a solid.

Native PFHpS and labeled compounds except [$^{13}C_9$]PFUnDA, were obtained as 50µg/mL solutions in methanol; 1.2 mL ampule. The PFHpS was a solution of its sodium salt.
Native N-Me/Et-FOSA/Es were obtained as 50µg/mL solutions in 90% Nonane/ 10% Toluene.

1. Use Fisher brand, Plasma grade water (has 18 MΩ specification and a bottle was proofed for PFC compounds of interest).

2. Prepare 60/40 (v/v) acetonitrile/ plasma grade water mix and 99% methanol / 0.7% water / 0.3% NH_4OH (basic methanol) mixes and store in dedicated 3X methanol-washed 1L HDPE solvent bottles.

3. Prepare stock solutions of individual native compounds in methanol at ~ 20-60 mg/mL by weighing 20 – 60 mg into a 10 mL glass grade A volumetric flask using a 4 place balance, then diluting to mark with methanol. For carboxylic acids, 4 mole equivalents of NaOH in methanol was added to reduce esterification in accordance with purchased standards from Wellington. The same procedure was used for [$^{13}C_9$]PFUnDA except 2 mg in a 5 mL flask was used. PFTeDA is much less soluble than the other compounds so a solution is prepared by weighing 10 mg into a 50 mL volumetric flask and diluting with acetonitrile without NaOH added.
   Note that PFTeDA, as well as being difficult to dissolve may also be much more likely to stick to glass, so the flask used to prepare it should receive more extensive cleaning after use than other glassware. Sonication for 20 minutes in basic Methanol followed by regular cleaning is recommended. Similar precautions for PFTriDA are also recommended.

4. Clean glass syringes by rinsing with 20X Toluene, 20X Hexane then sonicating for 20 minutes in 80:20 Toluene/Acetone then rinsing with 3X basic Methanol , 3X Toluene, 3X Hexane.
   Clean class A volumetric flasks rinsing with 3X Toluene, 3X Hexane then sonicating for 20 minutes in 80:20 Toluene/Acetone, then rinsing with 3X Toluene, 3X Hexane, 3X Dichloromethane, then a final rinse before use with the dilution solvent being used for the standard.

   Prepare Stock Mass-Labeled internal standard mix – **does not include injection internal standard compounds**.

   Calculate the volumes required of each labeled standard to give a concentration of 600 ng/mL for each carboxylic acid and 900 ng/mL for each sulfonic acid.
   Using a cleaned glass syringe that is reserved for mass labeled standards, transfer and combine the required measured quantities of each labeled standard into a cleaned volumetric flask. Dilute to the mark with the prepared 60:40 acetonitrile/water mix.

   Prepare Labeled Internal Standard Solution (LINT)
   - 4.1 Objective: prepare a solution of 30-45 pg/µL for the labeled carboxylic and sulfonic acids, plus 200 pg/µL of d_7-N-MeFOSE, in basic methanol.
   - 4.2 Using cleaned syringes and volumetric flasks transfer a portion of the Stock Mass-Labeled internal standard mix so that it will be diluted 20X, plus a portion of the 50 µg/mL d_7-N-MeFOSE so that it will be diluted 200X and dilute to mark with the prepared basic methanol mix.

Prepare Stock Mass-Labeled Injection Internal Standard.
(Can be used as Injection Internal Standard or to prepare a more diluted INJ.)

Calculate the volumes required of each labeled standard to give a concentration of 240 ng/mL $^{13}C_8$-Perfluorooctanoic acid and 200 ng/mL ^{2}H-Perfluoro-[1,2-$^{13}C_2$]-2-decenoic acid.
Using a cleaned glass syringe that is reserved for mass labeled standards, transfer and combine the required measured quantities of each labeled standard into the cleaned volumetric flask. Dilute to the mark with the prepared 60:40 acetonitrile/water mix.

Prepare Mass-Labeled injection standard

4.3 Objective: prepare a solution of 10-12 ng/mL in basic methanol.

4.4 Using cleaned syringes and volumetric flasks transfer a portion of the Stock Mass-Labeled internal standard mix and dilute to mark with the prepared basic methanol mix so that it is diluted 20X.

Prepare Intermediate Stock Mix of native PF Carboxylic and Sulfonic acids

Calculate the volumes required of each native stock standard to give a concentration of 50 µg/mL for each carboxylic acid and 100 µg/mL for each sulfonic acid.
Using a cleaned glass syringe that is reserved for native standards, transfer and combine the required measured quantities of each individual native standard into the cleaned volumetric flask.
Dilute to the mark with the prepared 60:40 acetonitrile/water mix.

5. Prepare Full Native Stock Mix containing all native PF Carboxylic and Sulfonic acids

Calculate the volumes required of the intermediate Stock Mix standard and native PFHpS solution to give a concentration of 2.5 µg/mL for each carboxylic acid and 5 µg/mL for each sulfonic acid.
Using a cleaned glass syringe that is reserved for native standards, transfer and combine the required measured quantities of each standard into the cleaned volumetric flask.
Dilute to the mark with the prepared basic methanol mix.

Prepare working level native standard of PF Carboxylic and Sulfonic acids

5.1. Objective: prepare a solution of 125-250 ng/mL in basic methanol.

5.2. Using appropriate cleaned syringes and volumetric flasks, transfer a portion of the Full Native Stock Mix and dilute to mark with the prepared basic methanol mix so that it is diluted 20X.

Prepare working-level native standard of N-Me/Et-FOSA/Es

5.3. Objective: prepare a solution of 1250 ng/mL of the FOSEs and 2500 ng/mL of the FOSAs in methanol, plus 10% propan-2-ol which is required to allow full mixing of the nonane and methanol.

5.4. Using appropriate cleaned syringes and volumetric flasks, transfer and combine the required portion of each individual 50 µg/mL standard, add propan-2-ol so that it is 10% of the flask volume and dilute to mark with methanol.

Prepare low level native standard for low level spiking and Calibration standard preparation

5.5. Objective: prepare a solution of 12.5-25 ng/mL in basic methanol.

5.6. Using appropriate cleaned syringes and volumetric flasks, transfer a portion of the Full Native Stock Mix and dilute to mark with the prepared basic methanol mix so that it is diluted 200X.

6. Prepare Calibration Standards (see Table 6 in the body of the procedure)

Label baked disposable 12mL glass vials.
Using disposable tip pipettors, transfer a 200µL portion of the Labeled Internal Standard Solution and 25 µL of the Stock Mass-Labeled injection internal standard to each vial.
Using disposable tip pipettors, transfer portion of the full Native Stock Mix, working level native standard or low level native standard to give levels of native compounds matching those shown in Table 7.

Using disposable tip pipettors, add basic methanol to dilute each Calibration Standard to 2.0 mL. Cap and mix thoroughly.

For Matrix Matched Calibration standards, only 1 mL of each CAL was prepared, so volumes of standards used were halved and 0.5 mL of soil extract (containing the equivalent of 0.5 g of clean soil in 0.5 mL basic Methanol) was added before diluting each Calibration Standard to 1.0 mL with basic methanol.

Table of Calibration Standards

STD ID	Internal Standard Solution (LINT) µL	Stock Mass-Labeled injection internal standard mix (INJ) µL	Low level native standard µL	Working level native standard (NAT) µL	Full Native Stock Mix µL	N-Me/Et-FOSA/Es native standard (NAT2) µL	Final Volume µL
CS-1	200	25	20			4	2000
CS-2	200	25	50			8	2000
CS-3	200	25		20		16	2000
CS-4	200	25		80		32	2000
CS-5	200	25			20	64	2000
CS-6	200	25			40	128	2000
CS-7	200	25			100	256	2000
CS-8	200	25			250		2000

APPENDIX 2

Procedure for Polishing Deionized Water

Appendix 2. Procedure for Polishing Deionized Water

Laboratory produced de-ionized water can be purified to produce PFC-free water for use in this analysis. Polished water is used for preparation of strong needle wash solutions, seal wash solutions, 60:40 ACN:H_2O standards solutions LC aqueous mobile phase and during the extraction and cleanup procedure.

1.0 Equipment for polishing water

1.1 Cartridge for polishing 18MΩ water – Waters Oasis 35cc (6g) HLB Extraction Cartridge.

1.2 Glassware for polishing 18MΩ water – Fisher Scientific, 2000 mL Kimax Brand Volumetric Flask (or equivalent), Fisher Scientific: 2000 mL Pyrex Filtering Flask (or equivalent) and Fisher Scientific Glass Magnetic Stir Bar 1.5" x 3/8" (or equivalent). All glassware should be washed 3 times using PFC free methanol.

2.0 Procedure for Polishing Water

2.1 Use glassware dedicated to water polishing (See Sec. 1.2 for specified glassware).

2.2 Pass 2L of 18MΩ (nanopure or equivalent) water through a 60 cc "Oasis HLB" cartridge. This cartridge should be used no more than 3 times.

Polished **nanopure** water should be stored in dedicated 1-L HDPE containers.

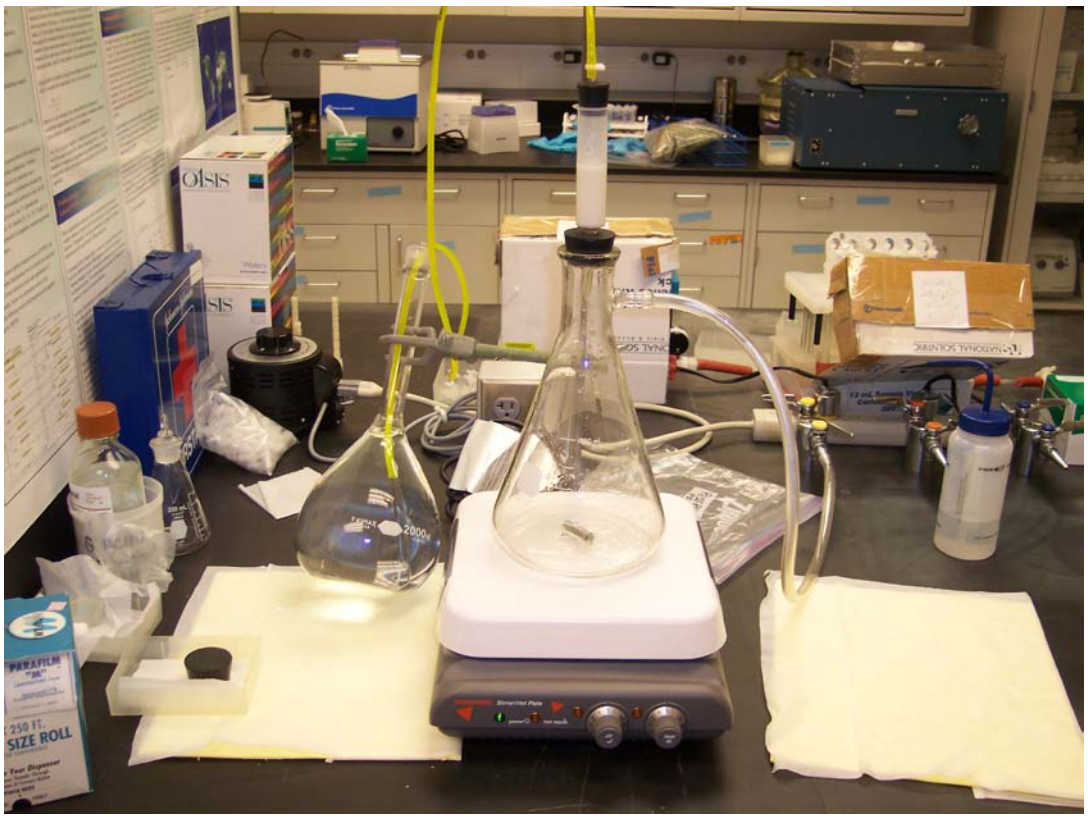

Figure 1. Setup for Water Polishing