Laboratory of Organic Analytical Chemistry
Wadsworth Center
New York State Department of Health

STANDARD OPERATING PROCEDURE

ANALYSIS OF PERFLUOROOCTANE SULFONATE OR OTHER SIMILAR PERFLUORINATED COMPOUNDS IN SERUM USING HPLC-ELECTROSPRAY/TANDEM MASS SPECTROMETRY

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APPROVAL PAGE

Revisions to an existing SOP, addition of a SOP change form, or preparation of a new SOP must be reviewed, approved, and signed by the following:

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Supervisor Review By: _____ Date: _____

Reviewed By: _____ Date: _____
(QA Coordinator)

DEFINITIONS AND ACRONYMS

PFOS perfluorooctane sulfonate (anion of potassium salt), $C_8F_{17}SO_3^-$

PFOA perfluorooctanoic acid, $C_7F_{15}COO^-$

PFBS perfluorobutane sulfonate, $C_4F_9SO_3^-$

PFHxS perfluorohexane sulfonate, $C_6F_{13}SO_3^-$

PFDS perfluorodecane sulfonate, $C_{10}F_{21}SO_3^-$

PFHpA perfluoroheptanoic acid, $C_6F_{13}COO^-$

PFNA perfluorononanoic acid, C_8F_{17} COO^-

PFDA perfluorodecanoic acid, C_9F_{19} COO^-

PFUnDA perfluoroundecanoic acid, $C_{10}F_{21}$ COO^-

PFDoDA perfluorododecanoic acid, $C_{11}F_{23}$ COO^-

PFOSA perfluorooctane sulfonylamide, $C_8F_{17}SO_2NH_2$

HPLC High Performance Liquid Chromatography

ESI/MS/MS Electrospray/tandem Mass spectrometer

PFCs Perfluorinated Compounds

1.0 PURPOSE

This method describes the procedure for extracting perfluorooctane sulfonate (PFOS) or other fluorochemical surfactants from serum, or other bodily fluids, using an ion pairing reagent and methyl-tert-butyl ether (MTBE). In this method, eleven fluorochemicals can be extracted and analyzed and they are: perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), PFOS, perfluorodecane sulfonate (PFDS), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA) and perfluorooctane sulfonylamide (PFOSA), and labeled surrogate/internal standards. A strong mineral acid or alkali is added to the sample to free bound fluorocarbons from the biological matrix and extracted into a solvent. The sample extracts are analyzed by HPLC equipped with an electrospray (ESI) tandem mass spectrometry (MS/MS) detector by multiple reaction monitoring (MRM).

2.0 SCOPE AND APPLICATION

This method is for the extraction of PFOS or other fluorochemicals from serum and other bodily fluids. The extracted fluorochemicals are quantified by HPLC-ES/MS-MS.

3.0 SAFETY CONSIDERATIONS

All safety considerations will be in accordance with Wadsworth-LOAC procedures and with the requirements of the Wadsworth Center Safety Manual. These requirements include:

Personnel protective equipment (PPE) consisting of lab coats, safety glasses, and latex gloves will be worn at all times when handling samples.

All personnel using such equipment must be aware of the hazards and must operate equipment according to the manufacturer’s safety procedures.

Organic solvents are used for rinsing equipment, glassware cleaning, and in the extraction phase of the method. These solvents represent a potential hazard to personnel in the laboratory. Care must be taken to minimize exposure in accordance to institutional guidelines.

4.0 EQUIPMENT, MATERIALS, AND REAGENTS

*Note: Avoid materials, solvents and reagents that contact with **teflon**. Teflon contains some polymers that might interfere with PFOS/PFC analysis.*

4.1 Equipment and Materials

The following equipment is used while performing this method. Equivalent equipment is acceptable.

4.1.1 Sample storage and extraction

- Balance (sensitivity to 0.100 g)
- Freezer @ -20^{o} C (For sample storage)
- Alconox detergent
- Vortex mixer
- Centrifuge
- Shaker, Eberbach or equivalent
- Nitrogen Evaporator, Organomation
- Eppendorf or equivalent pipettes
- Timer
- 15 ml Polypropylene (PP) Centrifuge Tubes
- 5 ml polypropylene tubes
- Oxford Dispenser

4.1.2 Sample Clean-up (if necessary)

- Disposable plastic 3cc syringes
- 0.2 µm, 25 mm nylon filters with leur lock,
- Autosampler vials with caps
- Syringes (capable of dilivering 5 µl to 50 µl)

Note: Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-Q water. Rinse syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

4.2 Reagents

- Milli-Q Water
- Sodium Bicarbonate ($NaHCO_3$), J.T.Baker
- Sodium Carbonate (Na_2CO_3), J.T.Baker
- Tetrabutylammonium Hydrogen Sulfate (TBAHS), J.T.Baker
- Methyl-tert-butyl ether (MTBE), J.T. Baker, glass distilled or HPLC grade
- Methanol, J.T. Baker, glass distilled or HPLC grade
- Fluorochemical standard solution (stock)
    - PFBS (3M Specialty Chemical Division), 20 μg/mL
    - PFHxS (3M Specialty Chemical Division), 20 μg/mL
    - PFOS (TCI America), 20 μg/mL
    - PFDS (Wellington), 5 μg/mL
    - PFOSA (3M Specialty Chemical Division), 20 μg/mL
    - PFHpA (Flurochem), 20 μg/mL
    - PFOA (TCI America), 20 μg/mL
    - PFNA (Research Chem Ltd.), 20 μg/mL
    - PFDA (Flurochem), 20 μg/mL
    - PFUnDA (Aldrich), 25 μg/mL
    - PFDoDA (Aldrich), 25 μg/mL
    - Other fluorochemicals, as appropriate

4.3 Standards preparation

4.3.1 Secondary standard preparation: mix above standards and methanol into a pp-tube as listed below, the final solution contains 1 μg/mL of each compound listed above.

- PFBS (3M Specialty Chemical Division), 20 μg/mL, 200 μL
- PFHxS (3M Specialty Chemical Division), 20 μg/mL, 200 μL
- PFOS (TCI America), 20 μg/mL, 200 μL
- PFDS (Wellington), 5 μg/mL, 800 μL
- PFOSA (3M Specialty Chemical Division), 20 μg/mL, 200 μL
- PFHpA (Flurochem), 20 μg/mL, 200 μL
- PFOA (TCI America), 20 μg/mL, 200 μL
- PFNA (Research Chem Ltd.), 20 μg/mL, 200 μL
- PFDA (Flurochem), 20 μg/mL, 200 μL
- PFUnDA (Aldrich), 25 μg/mL, 160 μL
- PFDoDA (Aldrich), 25 μg/mL, 160 μL
- MeOH, 1280 μL

4.3.2 Working standard preparation: mix 9 mL of methanol and 1 mL of the secondary dilution standard into a 15 mL pp-tube. Final solution contains 100 ng/mL of each compound listed above.

4.4 Internal (^{13}C-labeled) standard preparation

Mix 1 mL of 500 ng/mL $^{13}C_4$-PFOS (Wellington Laboratories, Guelph, Ontario, Canada), 1 mL of 500 ng/mL $^{13}C_4$-PFOA (Wellington Laboratories, Guelph, Ontario, Canada), 1 mL of 500 ng/mL $^{13}C_2$-PFNA (3M Company, St. Paul, MN), 1 mL of 500 ng/mL $^{13}C_2$-PFDA (3M Company, St. Paul, MN) and 6 mL of MeOH into a 15 mL pp-tube. The final solution contains 50 ng/mL of each internal standard listed above.

4.5 Reagent preparation

Note: When preparing larger or smaller volumes than what is listed in reagent, standard, or surrogate preparation, adjust accordingly.

4.5.1 10N sodium hydroxide (NaOH): Weigh 200 g NaOH and transfer into a 1000 mL beaker containing 500 mL Milli-Q water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.

4.5.2 1N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 mL of 10 N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q water. Store in a 125 mL Nalgene bottle.

4.5.3 0.5 M tetrabutylammonium hydrogen sulfate (TBAHS): Weigh 169 g of TBA into a 1 L volumetric containing 500 mL Milli-Q water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH. Dilute to volume with Milli-Q water. Store in a 1 L Nalgene bottle. Check pH before use. Adjust to pH=10 using 1 N NaOH solution.

4.5.4 0.25 M sodium carbonate/sodium bicarbonate buffer (Na_2CO_3/$NaHCO_3$): Weigh 26.5 g of sodium carbonate and 21 g of sodium bicarbonate into a 1 L volumetric flask and bring to volume with Milli-Q water. Store in a 1 L Nalgene bottle.

4.6 Standard storage and use conditions

Store spiking solutions at 4°C in sealed polypropylene tubes.

Note: Analysts must allow all spiking solutions to equilibrate to room temperature before use.

Note: Each time a vial containing small volumes of solutions is warmed to room temperature and opened, a small volume of solvent in the vial headspace evaporates, significantly affecting concentration. Solutions should be stored with the smallest possible headspace, and opening vials should be minimized. Also, the solution must be at room temperature before use.

5.0 SAMPLE HANDLING

In the laboratory, all samples must be kept frozen (-20°C) until grinding and/or sample extraction. A sample track sheet or laboratory notebook is used to locate samples, processing date and so on.

6.0 QUALITY CONTROLS

For each set of 30 to 40 samples (depending on the sample size and available sample volume and project goals), there will be a minimum quality control samples that includes a solvent blank, duplicate method blanks, and duplicate matrix spikes.

6.1 Method Blank

The method blank is extracted with the samples to monitor for any interference that may have been introduced to the sample during sample preparation. Blanks include:

6.1.1 A 1.0 ml of Milli-Q water is used as a solvent blank.

6.1.2 Extract two 1 mL samples following this procedure and use as matrix blanks. There should be matrix blanks for each matrix or sample type used in the analysis.

6.2 Surrogate Spike

6.2.1 All samples, blanks and matrix spike samples will be fortified with mass labeled internal standard before solvent extraction begins.

6.2.2 Surrogate spike samples are needed to monitor the quantitative transfer of the organic compounds of interest throughout sample preparation to the LC-MS/MS detector.

6.3 Matrix Spike/Matrix Spike Duplicate

6.3.1 Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction for each matrix that is to be evaluated, especially interference and ionization suppression due to matrix effects. The spiked compounds are used to monitor sample matrix effects that could interfere with the analytes of interest.

6.3.2 Prepare each spike using a sample chosen by the analyst. This is usually a control for samples received from a laboratory toxicity test.

6.3.3 Expected concentrations for spiking should fall in the mid-range of the initial calibration curve. Additional spikes may be included and may fall in the low-range of the initial calibration curve.

6.3.4 Prepare one matrix spike and matrix spike duplicate per 40 samples, with a minimum of two matrix spikes per batch.

7.0 SAMPLE EXTRACTION

7.1 Obtain frozen samples and allow thawing at room temperature.

7.2 Vortex mix for 15 seconds, then transfer 1.0 mL or appropriate volume to a 15 mL polypropylene centrifuge tube.

7.3 Return unused samples to freezer after the volume for extraction has been removed.

7.4 Record the initial volume on the lab notebook.

7.5 Label the tube with the study number, sample ID, date and analyst initials.

7.6 Spike all samples, including blanks, ready for extraction with 100 μL of surrogate standards as described in **4.4.**

7.7 Matrix spike samples preparation: spike matrix samples with 100 μL of native standards as described in **4.3.2.**

7.8 Vortex to mix the samples for 15 seconds.

7.9 To each sample, add 2 mL of 0.25 M sodium carbonate/sodium bicarbonate buffer, vortex to mix the samples for 15 seconds.

7.10 Check to ensure that 0.5 M TBA reagent is at pH 10. If not, adjust accordingly. To each sample, add 1 mL of 0.5 M TBA, vortex to mix the samples for 15 seconds.

7.11 Using an Oxford Dispenser, add 5 mL of MTBE.

7.12 Cap each sample and place on an orbital shaker at a setting of 300 rpm, for 40 minutes.

7.13 Centrifuge for 5 minutes at a setting of 3500 rpm, until the liquid layers are well separated.

7.14 Label a fresh 15 mL pp-tube with the same information as in **7.5**.

7.15 Remove 4.5 mL of the organic layer to the clean 15 mL pp-tube.

7.16 Repeat extraction as indicated on **7.11, 7.12 and 7.13** using 3 mL MTBE.

7.17 Remove the organic layer to the clean pp-tube as in **7.15**, and combine the two layers.

7.18 Put each sample under nitrogen evaporator until near dryness (takes approximately 2 hours).

7.19 Add exactly 1.0 mL of methanol to each centrifuge tube using a graduated pipette.

7.20 Vortex mix for 60 seconds.

7.21 When necessary, attach a 0.2 μm nylon mesh filter to a 3 cc syringe and transfer the sample extract to this syringe. Filter into a 1.5 mL amber glass autosampler vial.

7.22 Label the autosampler vials with the sample ID, extraction date and initial of the analyst.

7.23 Cap and store the extracts in the refrigerator until analysis.

7.24 Complete the lab note with sufficient experiment information.

8.0 DATA ANALYSIS AND CALCULATIONS

8.1 Instrumental analysis

Concentrations of 11 PFCs including PFBS, PFHxS, PFOS, PFDS, PFOSA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, and PFDoDA were detected and quantified on an Agilent 1100 Series high performance liquid chromatography (HPLC), coupled with an Applied Biosystems API 2000 electrospray triple-quadrupole mass spectrometer (ESI-MS/MS). Aliquots of ten microliters were injected into a Betasil C18 (100 × 2.1 mm) column with a (20 × 2.1 mm) guard column, both with a 5 μm particle size (Thermo Electron Corporation, Waltham, MA) at a flow rate of 300 μL min^{-1}. The mobile phase consisted of 2mM ammonium acetate and methanol. The gradient elution started at 10% methanol and increased to 100% after 10 min; it was held at 100% for 2 min, and then reverted to 10% methanol. The separation of target compounds is shown in Figure 1.

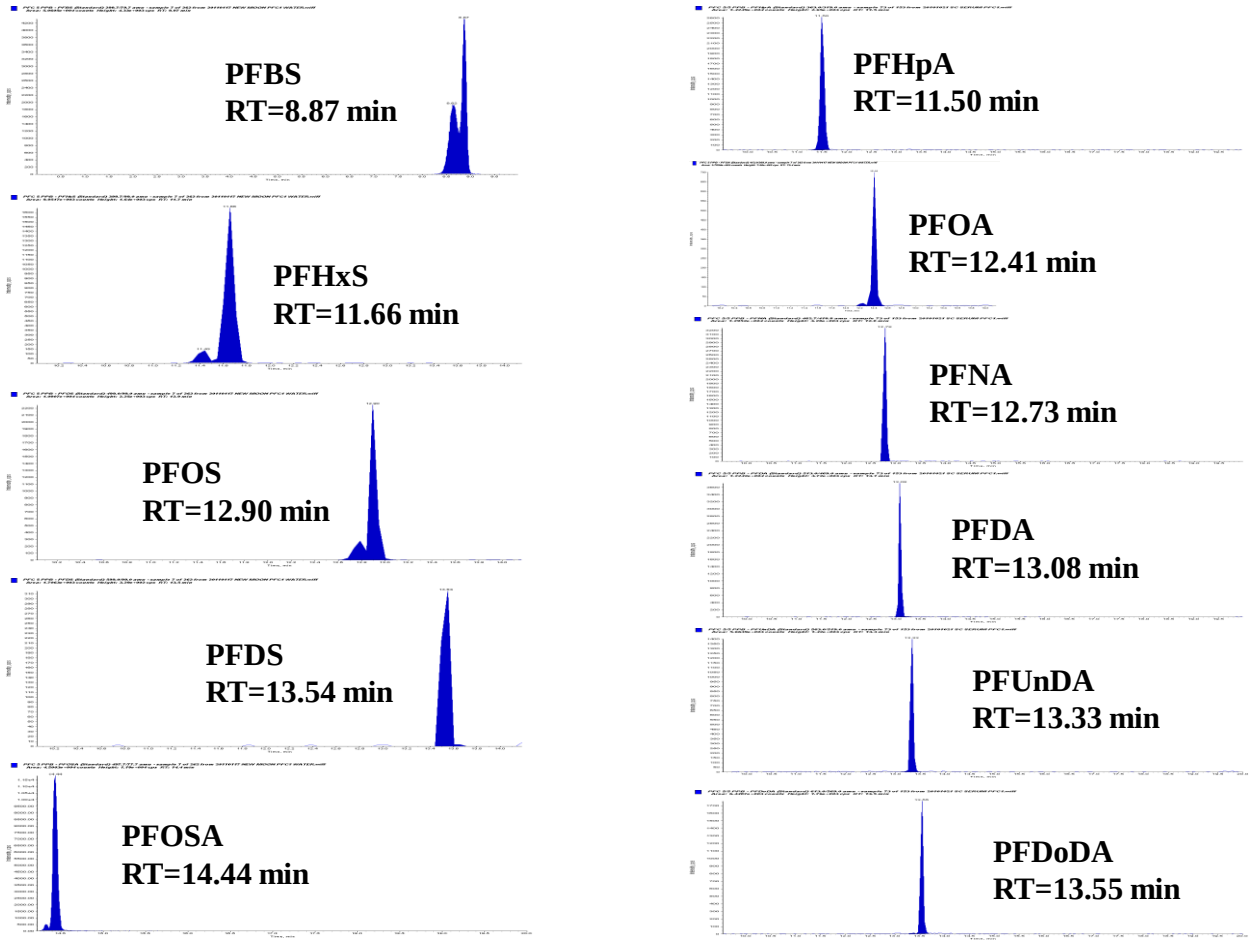

Figure 1. Separation of target PFCs using Betasil C18 (100 × 2.1 mm) column.

The MS/MS was operated in a multiple reaction monitoring (MRM) mode; the mass transitions monitored were: 398.7 > 79.7 for PFHxS, 499 > 99 for PFOS, 503 > 99 for $^{13}C_4$-PFOS, 599 > 99 for PFDS, 497.7 > 77.7 for PFOSA, 413 > 369 for PFOA, 417 > 372 for $^{13}C_4$-PFOA, 363 > 319 for PFHpA, 462 > 419 for PFNA, 465 > 420 for $^{13}C_2$-PFNA, 513 > 469 for PFDA, 515>470 for $^{13}C_2$-PFDA, 563>519 for PFUnDA, and 613>569 for PFDoDA. When possible, multiple daughter ions were monitored for confirmation, but quantitation was based on a single product ion. A list of mass transitions of target PFCs is shown in Table 1.

Table 1. Mass transitions and compound specific parameters

Compound	Precursor ion	Product ion	Declustering potential (V)	Collision energy (eV)	Collision cell exit potential (V)

PFBS	298.7	79.7	-30	-50	-5.4
PFHxS	398.7	79.7	-60	-80	-5
PFOS	499	99	-60	-80	-5
$^{13}C_4$-PFOS	503	99	-60	-80	-5
PFHpA	363	319	-10	-15	-12
PFOA	413	369	-10	-15	-12
$^{13}C_4$-PFOA	417	372	-10	-15	-12
PFNA	463	419	-10	-15	-12
$^{13}C_2$-PFNA	465	420	-10	-15	-12
PFDA	513	469	-15	-10	-15
$^{13}C_2$-PFDA	515	470	-15	-10	-15
PFUnDA	563	519	-15	-7.5	-30
PFDoDA	613	569	-22	-15	-19

8.2. Detection limits

The limit of quantitation (LOQ) is determined based on the linear range of the calibration curve prepared at a concentration range of 0.1 to 100 ng/mL. Concentrations in samples which are at least three-fold greater than the lowest acceptable standard concentration are considered to be valid. A curve point was deemed acceptable if 1) it was back-calculated to be within 30% of the theoretical value when evaluated versus the 1/x weighted curve, and 2) the peak area of the standard was at least 3 times greater than that in the blank. Concentration/dilution factors are included in the calculation of the LOQ. The LOQ for perfluorochemicals is typically 0.1-0.5 ng/mL. A list of LOD and LOQ typically detected for PFCs is shown in Table 2.

Table 2. LOD (ng/mL) and LOQ (ng/mL) for Perfluorochemicals

ng/mL	PFBS	PFHxS	PFOS	PFDS	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFOSA
LOD	0.02	0.02	0.02	0.05	0.02	0.033	0.02	0.033	0.05	0.05	0.01
LOQ	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.05

8.3. Quality control

Recoveries of labeled internal standards $^{13}C_4$-PFOS, $^{13}C_4$-PFOA, $^{13}C_2$-PFNA, and $^{13}C_2$-PFDA should be within 100±30%. If the recoveries do not fall within this range, the sample should be analyzed again or data should be flagged. Recoveries of native standards spiked into the sample matrix should be within 100±30%. If the recoveries do not fall within this range, the sample should be analyzed again or data should be flagged. Solvents, blood collection tubes, and method and matrix blanks should be checked for the presence of the perfluorochemicals and any contamination should be reported as quality control information. The results are not corrected for the recoveries of internal standards.

9.0 RECORDS, DOCUMENTATION, AND QC REQUIREMENTS

9.1 Records and Documentation

The primary analyst shall document any anomalies and/or deviation from the specified method in a bound, serially numbered, laboratory notebook with tear-out carbon copies. All electronic files and hardcopies will be kept at the LOAC at Wadsworth Center and a duplicate copy will be kept in the Room D547, Dr. Kurunthachalam Kannan's office. The primary analyst will sign and date any forms as the analyst.

9.2 QC Requirements and Data Quality Objectives

9.2.1 Method performance

The method detection limit (MDL) and limit of quantitation (LOQ) is analyte and matrix specific. The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis

- Method blanks and matrix blanks

- Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction.

9.0 RESPONSIBILITIES

The primary analyst will complete the analysis as specified in this SOP and provide documentation of raw data and any anomalies and provide data to the data analyst who will perform data calculations.

The technical reviewer will determine if data quality objectives were met, and to notify the analyst if any problems were found.

10.0 REFERENCES

Kannan et al. Perfluorooctanesulfonate and Related Fluorochemicals in Human Blood from Several Countries, *Environmental Science and Technology*, **2004**, 38 (17), 4489-4495

Hansen et al. Compound-Specific, Quantitative Characterization of Organic Fluorochemicals in Biological Matrices, *Environmental Science and Technology*, **2001**, 35 (4), 766-770