Global Monitoring Plan on Persistent Organic Pollutants

Protocol 1:

The Analysis of Perfluorooctane Sulfonic Acid (PFOS) in Water and Perfluorooctane Sulfonamide (FOSA) in Mothers' Milk, Human Serum and Air, and the Analysis of Some Perfluorooctane Sulfonamides (FOSAS) and Perfluorooctane Sulfonamido Ethanols (FOSES) in Air

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Procedure for the Analysis of Persistent Organic Pollutants in Environmental and Human Matrices to Implement the Global Monitoring Plan under the Stockholm Convention

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Chemicals Branch
United Nations Environment Programme (UNEP)
Division of Technology, Industry and Economics

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This document has been prepared by:

Institute for Environmental Studies
VU University
De Boelelaan 1087
NL-1081HV Amsterdam
The Netherlands

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Chemicals Branch
Division of Technology, Industry and Economics
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Protocol 1: The Analysis of Perfluorooctane Sulfonic Acid (PFOS) in Water and Perfluorooctane Sulfonamide (FOSA) in Mothers' Milk, Human Serum and Air, and the Analysis of Some Perfluorooctane Sulfonamides (FOSAS) and Perfluorooctane Sulfonamido Ethanols (FOSES) in Air

1 SCOPE

The Global Monitoring Plan of the Stockholm Convention sets a framework for the analysis of persistent organic pollutants (POPs); therein, the congeners recommended for analysis in the core matrices are listed (see chapter 2 of the "Guidance on the global monitoring plan for persistent organic pollutants", UNEP 2015a). A protocol is needed to ensure that these compounds are always analysed correctly in various laboratories and in the same way. In order to assist POPs laboratories in the analysis of POPs, Chemicals Branch of the Division of Technology, Industry and Economics (DTIE) of the United Nations Environment Programme is developing generic procedures for the analysis of initial and new POPs.

This procedure covers perfluorooctane sulfonic acid (PFOS) and perfluorooctane sulfonamide (FOSA) in water, mothers' milk, human serum and air, and the analysis of methyl perfluorooctane sulfonamide (MeFOSA), ethyl perfluorooctane sulfonamide (EtFOSA), methyl perfluorooctane sulfonamide ethanol (MeFOSE), and ethyl perfluorooctane sulfonamide ethanol (EtFOSE) in air. The present protocol describes the method for sample preparation, extraction, purification and analysis of the aforementioned perfluoroalkyl substances (PFASs) in water, mother's milk, human serum and air.

2 PRINCIPLE

All PFASs need to be released from their matrices because matrix components interfere in the final determination. Water, mothers' milk and human serum samples can be extracted with solid phase extraction (SPE) on a Wax column. Air samples (on polyurethane foams) are first extracted with Soxhlet extraction and then further purified. The instrumental analysis of the cleaned extracts of all samples is carried out by liquid chromatography and mass spectrometry (LC-MS/MS) after which all target compounds can be identified and quantified.

3 PRECAUTIONS

Before starting with the analysis and the preparation of the necessary materials, it is essential to take two precautions.

1. Several instruments such as the LC/MS system and the ultrapure water system often contain Teflon. However, Teflon contains PFASs and these cause a high background and disturb the determination of PFASs in the samples. Therefore, replace Teflon tubing in the LC and Teflon materials in the ultrapure water system or use HPLC grade water for all solutions in water. The blank contamination of solvents and materials used during the analysis must be tested to prove they do not contain any PFAS of interest.
2. The present protocol describes the analysis of PFASs. However, it is possible to change certain parameters and analytical conditions described in this protocol, while still obtaining the same results. In case of such changes, the entire method should be optimised and validated to ensure the comparability of data.

4 MATERIALS AND REAGENTS

4.1 Materials

High Density Polyethylene bottle (100 and 500 mL)

Plastic pipettes

Polypropylene tubes (15 mL)

Micro tubes (1.5 mL)

Crimpcap polypropylene vial (700 µL)

Seal, silver aluminum 11 mm, PTFE/Rubber Liner

Capper/Decapper

Ultrasonic bath

Vacuum desiccator

Passive sampler

Balance (precision 0.01 g)

Pipettes (50, 100 and 200 µL)

Centrifuge

Oven (37 °C)

SPE device (rinse with methanol and water prior to use)

pH meter

Vacuum pump

Water bath (50 °C)

Whirlmixer

LC-MS/MS (LC-QQQ). Electrospray source (ESI) with negative polarity

FluoroSEP-RP Octyl column, 15 cm x 2.1 mm, 5 µm particle size, ES Industries (132211-FO)

2 x Symmetry columns C_{18}, 20 mm x 3.9 mm, 5 µm particle size, Waters (WAT054225)

Symmetry column C_{18}, 50 mm x 2.1 mm, 5 µm particle size, Waters (18600206)

4.2 Reagents

Polyurethane foam (PUF) disk, 14 cm x 1.35 cm, surface area 365 cm^2, mass 4.40 g, volume 207 cm^3, Tisch Environmental, Cleves, OH

Aceton, Ultraresi, J.T. Baker (9254)

Petroleum ether, J.T. Baker

Methanol, HPLC gradient grade, J.T.Baker (8402)

Internal standard ($^{13}C_4$ PFOS + $^{18}O_2$ FOSA + $^{2}H_3$ MeFOSA + $^{2}H_5$ EtFOSA + $^{2}H_7$ MeFOSE + $^{2}H_9$ EtFOSE) in methanol (100 ng/mL)

Internal standard ($^{13}C_4$ PFOS + $^{18}O_2$ FOSA) in methanol (100 ng/mL)

50% Formic acid in water

SPE Cartridge, Oasis WAX 6cc, Waters 186002493

Ammonia 25% p.a. purity

0.1% NH_4OH in methanol; add 400 µL ammonia to 100 mL methanol

2 % NH_4OH in methanol; add 8 mL ammonia to 92 mL methanol

HPLC water, HPLC analyzed, J.T. Baker (4218), or MilliQ purity

Acetic acid 100 % pro analysis (p.a.) purity

Ammonium acetate p.a. purity

25 mM ammonium acetate; add 190 mg ammonium acetate to 100 mL water and adjust the pH to pH=4 with acetic acid

Nitrogen gas. Purity 5.0

Injection standard (1) ($^{13}C_8$ PFOS) in methanol/water (1:1, v/v) (150 ng/mL)

Injection standard (2) ($^{13}C_8$ PFOS) in methanol/water (1:1, v/v) (50 ng/mL)

Injection standard (3) ($^{13}C_8$ PFOS) in methanol/water (1:1, v/v) (25 ng/mL)

Ammonium formate, (>99%), Fluka (09735)

Ammonium formate buffer 5 mM: Dissolve 315 mg ammonium formate in 1 L HPLC water. Filter prior to use.

PFAS calibration solutions (0.05, 0.25, 0.5, 5, 50, 100 ng/mL) in methanol/water (1:1, v/v)

5 METHOD

5.1 Sample preparation

5.1.1 Air

For air sampling, polyurethane foam (PUF) disk is used.

5.1.1.1 Preparation of the PUF

- Cleaning of a PUF:
  - If necessary, wash the PUF in water;
    - Perform a Soxhlet extraction on the PUF with acetone (24 h), followed by petroleum ether (24 h)
  - Dry the PUF in a desiccator (24 h).

5.1.1.2 Air sampling

Place a PUF in a passive sampler for three months at an outdoor sampling location.

5.1.1.3 Sample preparation

- Take the PUF out of the sampler;
- Add 150 µL Internal standard (I.S.) to the PUF.

5.1.1.4 Procedural blank

Prepare a PUF as described above without the exposure time during the sampling.

5.1.2 Water

5.1.2.1 Water sampling

The water sampling aspects are described in "PFOAS analysis in water for the Global Monitoring Plan of the Stockholm Convention" from UNEP GMP working group (UNEP 2015b).

5.1.2.2 Sample preparation

- As soon as the sample arrives to the analytical laboratory internal standards (IS) should be added to compensate for absorbance to laboratory equipment. See further section 4.2 for the addition of standards to samples. The sample (incl. IS) should have time to equilibrate before analysis.
- Keep the water samples (500 mL) in a high density polyethylene (HDPE) in the fridge or freezer (-20 °C) and defrost them the day before analysis;
- Shake the water rigorously before subsamples are taken out;
- Weigh 100 mL of water sample in a HDPE bottle (100 mL);

5.1.2.3 Procedural blank

Prepare a procedural blank sample as described above in sample description but using ultra clean (MilliQ) water as sample substitute.

5.1.3 Mother's milk and human serum

5.1.3.1 Human sampling

Follow the UNEP/WHO protocol for sampling of human milk 'UNEP-coordinated Survey of Mothers' Milk for Persistent Organic Pollutants' (http://www.unep.org/chemicalsandwaste/portals/9/POPs/docs/Mothers%20milk%20guide%20POPs.pdf)

5.1.3.2 Sample preparation

- Homogenise the samples (50 mL) manually by shaking for 1 min;
- Weigh 1 mL of milk sample, or 0.5 mL serum sample in a PP tube (15 mL);
- Add 50 µL I.S. (4.2);
- Add 2 mL 50% formic acid and shake manually;
- Place the sample in an ultrasonic bath for 15 min;
- Centrifuge for 15 min at 3,000 rpm;
- Place the samples in an oven at 37 °C for 30 min.

5.1.3.3 Procedural blank

Prepare a procedural blank sample as described above in sample description but using ultra clean (MilliQ) water as sample substitute.

5.2 Sample extraction

5.2.1 Air

- Perform a Soxhlet extraction with methanol (12 h);
- Concentrate the extract to 1 mL by using either a rotary evaporator or Kuderna-Danish;
- Filter the extract through a 0.2 µm glass hydrophilic polypropylene (GHP) filter into a polypropylene LC vial;
- Concentrate to 200 µL under a gentle stream of nitrogen;
- Add 100 µL injection standard 1 (see section 4.2);
- Add 300 µL 2mM ammonium acetate and shake manually;
- Analyze with LC-MS/MS (see Chapter 6).

5.2.2 Water, mother's milk and human serum

- Solid phase extraction (SPE) is used for the extraction of water, mothers' milk and serum. Install an SPE cartridge on the SPE device.
- Condition the SPE cartridge by adding the solutions from Table 1 to the cartridge one after the other as soon as the previous solution has sunk into the cartridge (don't use the vacuum pump in this step);

Table 1: Solutions used for conditioning the SPE cartridge

	Water		Milk and serum	
	Amount (mL)	Concentration (%)	Amount (mL)	Concentration (%)
NH_4OH in methanol	4	0.1	2	2
Methanol	4		2	
Milli-Q H_2O	4		2	

- Add the sample extract to the SPE column (flow rate max. 1 drop/second);
- Wash the SPE cartridge by adding the solutions from Table 2.

Table 2: Solutions used for washing the SPE cartridge

	Water Amount (mL)	Milk and serum Amount (mL)
25 mM ammonium acetate (adjusted to pH=4 with acetic acid)	4	2
40% methanol in water		2

- Dry the cartridge by switching on the vacuum pump. Elute the PFASs from the SPE cartridge by adding the solutions from Table 3.

Table 3: Solutions used for extracting PFASs from the SPE cartridge

	Water		Milk and serum	
	Amount (mL)	Concentration (%)	Amount (mL)	Concentration (%)
Methanol	4			
NH_4OH in methanol	4	0.1	1	2

- Evaporate the extract to dryness in a water bath of 50 °C with a gentle stream of nitrogen;
  - Reconstitute the milk extract with 100 µL ammonium acetate solution and 100 µL injection standard 2 (see section 4.2);
  - Reconstitute the water extract and the serum extract with 200 µL injection standard 3 (see section 4.2);
- Shake the extract manually for 1 min;
- Centrifuge for 10 min at 3,000 rpm;
- Transfer the supernatant into a polypropylene LC vial and analyze with LC-MS/MS (see section 7).

6 INSTRUMENTAL ANALYSES

Please note that the gradient and mass spectrometer (MS) settings are dependent of the LC-MS/MS system and on the type of columns used. Those settings should be optimized for the in-house instruments and columns. The linear and branched PFOS isomers should be separated for individual quantification (Figure 1). Note that the ratio of linear vs branched isomers differs significantly between samples and in Figure 1 is only one example.

- Install the analytical column (see section 4.1) and the guard column (see section 4.1) in the HPLC;
- Install an extra column (50 mm) (see section 4.1) and a guard column (see section 4.1) between the LC pump and the injector, to prevent interference of PFASs, originating from the LC system, with the target compounds;

Purge all the mobile phase solvents through the system (the gradient is given in

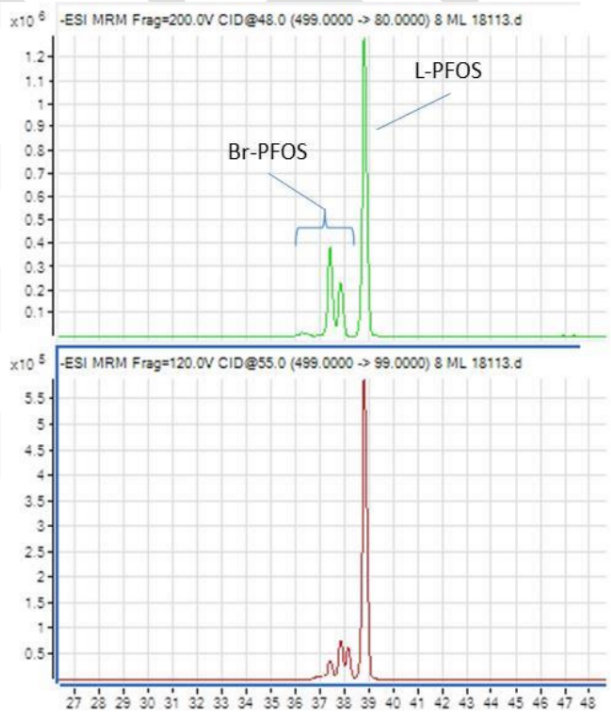

Figure 1: Chromatogram showing the separation of linear and branched PFOS in water (surface water sample from The Netherlands)

- Table 4);
- Start the pump with 65% ammonium formate and 35% methanol;

- Put all the extracts, blanks, and calibration solutions in the tray of the autosampler;
- Make a sequence in the computer (the mass settings for PFAS detection and quantification are given in Table 5). Analyse the samples, the calibration solutions, the blank and the reference material in random order;
- Inject a calibration solution after the pump has been running for at least 30 min;
- Check the performance of the LC-MS/MS by comparing the results (retention times and peak intensities) of the injected calibration solution with earlier results;
- Start the sequence.

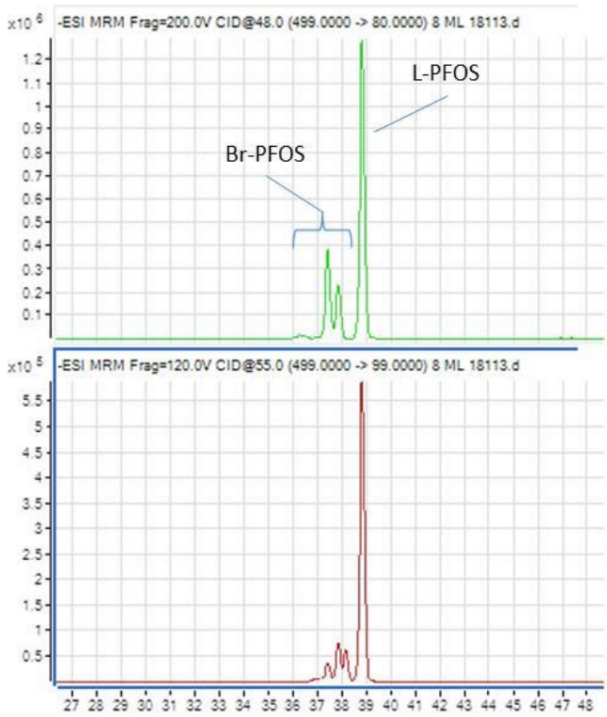

Figure 1: Chromatogram showing the separation of linear and branched PFOS in water (surface water sample from The Netherlands)

Table 4: Gradient for PFAS separation

Time (min)	Flow (µL/min)	Ammonium formate (5 mM) (%)	Methanol (%)
0	300	65	35
2	300	65	35
35	300	25	75
45	300	5	95
55	300	5	95
55.5	300	65	35
65	300	65	35

7 QUANTIFICATION

Identify the target peaks in the LC/MS chromatograms based on retention time and m/z transition. Use the peak areas of these peaks in calibration solutions to draw a calibration curve each of the target compounds. Compare the peak areas of the same peaks in the samples with those in the calibration solution and calculate the concentrations of the PFASs.

Note: PFAS concentrations should be reported on wet weight basis. However, often, results are reported on sulfonate anion basis, *i.e.*, corrected for the molecular weight of the PFOS salt. For example, the sodium salt (PFOS-Na) molecular weight is 522.11 g/mol and the M-Salt is 499.12. Hence, a correction factor of 0.96 should be applied when standard solutions are weighted and diluted.

8 QA/QC

For quality control purposes, include a blank and an internal reference material in each series of maximum twelve samples. Observe that these settings applies to both linear and branched isomers.

Table 5: Mass settings for PFAS separation

Compound		Precursor Ion (m/z)	Production (m/z)	Comment
PFOS	Target compound	499	80	Quantifier
			99	Qualifier
$^{13}C_4$ PFOS	Internal standard	503	80	Quantifier
			99	Qualifier
$^{13}C_8$ PFOS	Injection standard	507	80	Quantifier
			99	Qualifier
FOSA	Target compound	498	78	Quantifier
			169	Qualifier
$^{18}O_2$ FOSA	Internal standard	502	82	Quantifier
			169	Qualifier
MeFOSA	Target compound	512	169	Quantifier
			219	Qualifier
$^{2}H_3$ MeFOSA	Internal standard	515*	169*	Quantifier
			219*	Qualifier
EtFOSA	Target compound	526	169	Quantifier
$^{2}H_5$ EtFOSA	Internal standard	531*	169*	Quantifier
MeFOSE	Target compound	602	45	Quantifier
$^{2}H_7$ MeFOSE	Internal standard	609*	45*	Quantifier
EtFOSE	Target compound	616	45	Quantifier
$^{2}H_9$ EtFOSE	Internal standard	625*	45*	Quantifier

* Calculated, not optimized

9 REFERENCES

UNEP (2015a): Guidance on the global monitoring plan for persistent organic pollutants. UNEP/POPS/COP.7/INF/39, 26 February 2015, accessible from http://synergies.pops.int/2015COPs/MeetingDocuments/tabid/4243/language/en-US/Default.aspx

UNEP (2015b): PFAS analysis in water for the Global Monitoring Plan of the Stockholm Convention, May 2015; accessible from www.unep.org/chemicalsandwaste/