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Determination of perfluoroalkyl compounds in water, sediment, and biota

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ICES International Council for the Exploration of the Sea

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Contents

Abstract

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This document provides advice on the analysis of polyfluoroalkyl compounds (PFCs) in samples of water, sediment, and biota. The analysis of PFCs in these matrices generally includes extraction with organic solvents, clean-up, and liquid chromatography (LC) with mass spectrometric (MS) detection. This document provides an overview of environmentally relevant PFCs and information on the currently applied techniques for the analysis of these PFCs, including sampling, pretreatment, extraction, clean-up, instrumental analysis, quantification and quality assurance, and quality control.

Keywords: polyfluoroalkyl compounds, PFCs, water, sediment, biota, sample pretreatment, extraction, clean-up, calibration, liquid chromatography, mass spectrometry.

1 Introduction

Polyfluoroalkyl compounds (PFCs) are man-made chemicals that have been in use since the 1950s and are ubiquitous in the environment (Giesy and Kannan, 2001). They are widely used as processing additives during fluoropolymer production and as surfactants in consumer applications, including surface coatings for carpets, furniture, and paper products. They are also components in breathable waterproof fabrics, firefighting foams, and insulators for electric wires (Kissa, 2001). They can be released into the environment through their production, use, and disposal.

These compounds have a hydrophilic functional group and a hydrophobic, fully fluorinated carbon chain of varying length. The perfluoroalkyl acids have moderate water solubilities and low pKa values, and they are therefore dissociated at environmentally relevant pH values (Kissa, 2001). Perfluoroalkyl sulfonamides (PFOSA) and fluorotelomer alcohols (FTOHs) are neutral compounds, with a moderate vapour pressure, that are possibly precursors to perfluoroalkyl acids, such as perfluorooctane sulfonate (PFOS) and perfluorooctanate (PFOA; Ellis *et al.*, 2004).

The objective of this document is to provide advice on the analysis of PFCs in water, sediment, and biota. The detection and quantification of PFCs at ppb to ppt levels is particularly challenging because of the high risk of contamination during sample handling, storage, preparation, and instrumental analysis. Various methods are applied to determine PFCs in water, sediment, and biota, and they generally comprise extraction with polar solvents, clean-up steps, and liquid chromatography (LC) coupled with mass spectrometric (MS) detection. The International Organization for Standardization (ISO) has issued a standard procedure for the determination of PFOS and PFOA in unfiltered water samples (ISO, 2009). However, because this procedure has a limit quantification of 10 ng l^{-1}, it may not be applicable to the analysis of PFCs in seawater samples, which normally contain PFCs at pg l^{-1} levels. Furthermore, no standard procedure is available for the analysis of PFCs in sediment and biota samples. This document fills these gaps and provides advice on analytical methodologies applicable to the analysis of PFCs in water as well as in sediment and biota.

2 Analytes

Table 1 provides an overview of PFCs that are environmentally relevant and provides information on chemical names, acronyms, formula, and Chemical Abstracts Service (CAS) numbers, as well as suggestions for suitable isotopically labelled internal standards for use in PFC analysis. The individual compounds belong to the following compound groups: perfluoroalkyl sulfonates (PFSAs), perfluoroalkyl carboxylates (PFCAs), perfluoroalkyl sulfonamides, and perfluoroalkyl sulfonamidoacetic acids. For monitoring purposes, the chemicals PFOS and PFOA are considered to be the most important PFCs. Although most studies have focused on PFOS and PFOA, it is suggested that PFCs with longer and shorter carbon chain lengths should also be included in the analysis. Long-chain PFCs (≥C8) should be included because of their potential to bioaccumulate and to adsorb onto sediment. Perfluorobutane sulfonate (PFBS) and perfluorobutanate (PFBA), the short-chain substitutes of PFOS and PFOA, should preferentially be monitored in water as they may bioaccumulate to a lesser extent. However, PFBS may also be included in biota monitoring, provided that there is no clear scientific evidence of its significant bioaccumulation.

Table 1. Full names, acronyms, formulas, Chemical Abstracts Service (CAS) numbers, and environmental relevance of native and isotopically labelled PFCs relevant to water, sediment, and biota analysis.

				Environmental relevance		
Analyte	**Acronym**	**Formula**	**CAS-number**	**Water**	**Sediment**	**Biota**
Perfluorobutanate	PFBA	$C_3F_7COO^-$	375-22-4	x		
Perfluoropentanate	PFPA	$C_4F_9COO^-$	2706-90-3	x		
Perfluorohexanate	PFHxA	$C_5F_{11}COO^-$	307-24-4	x		
Perfluoroheptanate	PFHpA	$C_6F_{13}COO^-$	375-85-9	x		
Perfluorooctanate	PFOA	$C_7F_{15}COO^-$	335-67-1	x	x	x
Perfluorononanate	PFNA	$C_8F_{17}COO^-$	375-95-1	x	x	x
Perfluorodecanate	PFDA	$C_9F_{19}COO^-$	335-76-2	x	x	x
Perfluoroundecanate	PFUnDA	$C_{10}F_{21}COO^-$	2058-94-8		x	x
Perfluorododecanate	PFDoDA	$C_{11}F_{23}COO^-$	307-55-1		x	x
Perfluorotridecanate	PFTriDA	$C_{15}F_{25}COO^-$	72629-94-8		x	x
Perfluorotetradecanate	PFTeDA	$C_{13}F_{27}COO^-$	376-06-7		x	x
Perfluorohexadecanate	PFHxDA	$C_{15}F_{31}COO^-$	67905-19-5			
Perfluorooctadecanate	PFOcDA	$C_{17}F_{35}COO^-$	n.a.			
Perfluorobutane sulfonate	PFBS	$C_4F_9SO_2O^-$	29420-49-3	x	x	x
Perfluorohexane sulfonate	PFHxS	$C_6F_{13}SO_2O^-$	3871-99-6 (potassium salt)	x	x	x
Perfluorooctane sulfonate	PFOS	$C_8F_{17}SO_2O^-$	2795-39-3	x	x	x
Perfluorodecane sulfonate	PFDS	$C_{10}F_{21}SO_2O^-$	13419-61-9 (sodium salt)		x	x
6:2 fluorotelomer sulfonate	6:2 FtS (THPFOS)	$C_6F_{13}C_2H_4SO_3^-$	27619-97-2	x		
Perfluorooctane sulfonamide	PFOSA	$C_8F_{17}SO_2NH_2$	754-91-6	x	x	x
N-ethylperfluoro-1-octanesulfonamidoacetic acid	N-EtFOSAA	$C_8F_{17}SO_2N(C_2H_5)CH_2CO_2H$	n.a.		x	
Internal Standards						
Perfluoro-n-(1,2,3,4-$^{13}C_4$)butanate	[$^{13}C_4$]-PFBA	(2,3,4-$^{13}C_3$)$F_7{}^{13}COO^-$	n.a.			
Perfluoro-n-(1,2-$^{13}C_2$)hexanate	[$^{13}C_2$]-PFHxA	C_4F_9(2-^{13}C)$F_2{}^{13}COO^-$	n.a.			
Perfluoro-n-(1,2,3,4-$^{13}C_4$)octanate	[$^{13}C_4$]-PFOA	C_4F_9(2,3,4-$^{13}C_3$)$F_6{}^{13}COO^-$	n.a.			
Perfluoro-n-(1,2,3,4,5,6,7,8-$^{13}C_8$)octanate	[$^{13}C_8$]-PFOA	(2,3,4,5,6,7,8-$^{13}C_7$)$F_{15}{}^{13}COO^-$	n.a.			
Perfluoro-n-(1,2,3,4,5-$^{13}C_5$)nonanate	[$^{13}C_5$]-PFNA	C_4F_9(2,3,4,5-$^{13}C_4$)$F_8{}^{13}COO^-$	n.a.			
Perfluoro-n-(1,2-$^{13}C_2$)decanate	[$^{13}C_2$]-PFDA	$C_8F_{17}{}^{13}CF_2{}^{13}COO^-$	n.a.			
Perfluoro-n-(1,2-$^{13}C_2$)undecanate	[$^{13}C_2$]-PFUnDA	$C_9F_{19}{}^{13}CF_2{}^{13}COO^-$	n.a.			
Perfluoro-n-(1,2-$^{13}C_2$)dodecanate	[$^{13}C_2$]-PFDoDA	$C_{10}F_{21}{}^{13}CF_2{}^{13}COO^-$	n.a.			
Perfluoro-1-hexane($^{18}O_2$)sulfonate	[$^{18}O_2$]-PFHxS	$C_6F_{13}S(^{18}O_2]O^-$	n.a.			

				Environmental relevance		
Analyte	**Acronym**	**Formula**	**CAS-number**	**Water**	**Sediment**	**Biota**
Perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	[$^{13}C_4$]-PFOS	C_4F_9(1,2,3,4-$^{13}C_4$)$F_8SO_2O^-$	n.a.			
Perfluoro-1-(1,2,3,4,5,6,7,8-$^{13}C_8$)octanesulfonate	[$^{13}C_8$]-PFOS	(1,2,3,4,5,6,7,8-$^{13}C_8$)$F_{17}SO_2O^-$	n.a.			
Perfluoro-1-(1,2,3,4,5,6,7,8-$^{13}C_8$)octanesulfonamide	[$^{13}C_8$]-PFOSA	(1,2,3,4,5,6,7,8-$^{13}C_8$)$F_{17}SO_2NH_2$	n.a.			
N-deuterioethylperfluoro-1-octanesulfonamidoacetic acid	d_5-N-EtFOSAA	$C_8F_{17}SO_2N(C_2D_5)CH_2CO_2H$	n.a.			

The use of isotopically labelled analogues of the target analytes as internal standards for the quantification of PFCs is strongly recommended. Other PFC groups recently detected in environmental samples, such as FTOHs, fluorotelomer saturated and unsaturated carboxylates, perfluoroalkyl sulfonamidoethanols, and polyfluoroalkyl phosphate surfactants, may be considered when extending the scope of analysis (D'eon and Mabury, 2007; Ahrens *et al*., 2009a; Mahmoud *et al*., 2009).

3 Sampling, pretreatment, and storage

3.1 Risk of contamination

The sampling and processing of the samples should be carried out by trained personnel who are aware of the risk of contamination of samples posed by incorrect handling. A variety of laboratory items widely used in analytical laboratories may contain fluoropolymers, such as polytetrafluoroethylene (PTFE; Yamashita *et al*., 2004), and can cause contamination of samples with PFCs. Materials and clothes that contain, or may adsorb, fluorinated compounds must be avoided during sampling and sample processing. In particular, the containers or bags that come into direct contact with the sample must not contain any fluorinated polymers (e.g. PTFE). Containers and equipment made of polypropylene, glass, or stainless steel should be used. However, even glass and polypropylene sampling and storage containers should be carefully checked for PFCs and cleaned before use because blank contamination caused by adsorption of PFCs onto these materials has been reported (Martin *et al*., 2004a; Taniyasu *et al*., 2005). Every material that may come into contact with the sample must be free of fluorinated compounds. Materials used in the analysis of PFCs should be cleaned with polar solvents, such as methanol, covered with solvent-rinsed aluminium foil to keep out any dust, and tested for blank contamination. The highest contamination risk was observed for PFOA and perfluorononate (PFNA; Theobald *et al*., 2007). In order to minimize the risk of sample contamination, sample treatment and processing should be carried out on a clean bench or in a clean room containing no fluorinated compounds (e.g. PTFE).

3.2 Water

Generally, water samples should be processed as quickly as possible after sampling. The use of polypropylene sampling containers is recommended because PFCs may be adsorbed onto glass surfaces. A filtration step may be required for water samples with a high content of suspended particulate matter in order to avoid blocking of the solid-phase extraction (SPE) cartridges. In addition, separate analyses of dissolved and particle bond PFCs can give information on the partitioning behaviour of PFCs (Ahrens *et al*., 2009b). However, during filtration, PFCs may be adsorbed onto the filtration equipment, and dissolved PFCs may be adsorbed onto the filter material (e.g. glassfibre filter or syringe nylon membrane filter). The filtration equipment may be a source of contamination (Ahrens *et al*., 2009b; Arp and Goss, 2009). To avoid adsorption of PFCs, the sample container or filtration equipment must be rinsed with a polar solvent, such as methanol.

When extraction cannot be done within a few days after sampling, water samples should be stored at −20°C, because biotransformation may occur in biologically active samples (Huset *et al*., 2008), and be analysed within two weeks (ISO, 2009).

3.3 Sediment

It is advisable to determine the water content and to dry samples before extraction. There are several techniques for drying the samples, such as freeze-drying, air-drying at room temperature, and oven-drying. For each technique, it is important to check and confirm that the PFC concentrations are not changed by degradation, volatilization, or contamination. With freeze-drying, losses through evaporation are diminished by keeping the temperature in the evaporation chamber below 0°C. Air-drying to constant weight at room temperature should be done on a clean bench. However, PFCs may be lost by volatilization, depending on temperature and drying time.

Before taking a subsample for analysis, samples should be homogenized and stored in closed containers at a temperature below −20°C until sample extraction.

Total organic carbon (TOC) should be determined for characterization of the sediment and for possible use as a normalization parameter.

3.4 Biota

3.4.1 Species and tissue for analysis

The PFCs can bind to the plasma protein serum albumin and accumulate in blood and organs (Jones *et al*., 2003). Accumulation in the marine foodweb starts from the bottom of the food chain, with invertebrates such as zooplankton and molluscs, followed by crustaceans, and then fish (Van de Vijver *et al*., 2003). The highest PFC concentrations have been found in blood and internal organs (e.g. liver, kidney) of top predators, such as marine mammals (Martin *et al*., 2004b; Ahrens *et al*., 2009c) and fish-eating birds (Kannan *et al*., 2001). Blood samples are recommended for living marine mammals and egg samples for seabirds. In marine mammals, the patterns and distribution of PFCs may change after death; therefore, if cadavers are used for monitoring purposes, care should be taken when interpreting results.

Fish species widely used for monitoring of organic contaminants are also suitable for PFC analysis. A list of species is provided in the Joint Assessment and Monitoring Programme (JAMP) Guidelines for Monitoring Contaminants in Biota (OSPAR, 1999). Liver is the first choice of tissue for monitoring purposes. However, PFCs have also been detected in other organs (e.g. blood and muscle).

For temporal and spatial trend analysis, it is important to identify possible sources of PFCs and to evaluate the effects of emission control and reduction measures, and of restrictions of the production and use of PFCs, on environmental concentrations.

3.4.2 Sampling, transportation, and storage

The dissection of biota and the collection of eggs and blood must be carried out by trained personnel because incorrect handling of biota samples can result in sample contamination. After collection, samples should be stored in closed containers at a temperature below −20°C until sample preparation. Handling time at room temperature should be minimal in order to prevent the possible degradation of precursors to PFCAs and PFSAs (Rhoads *et al*., 2008).

3.4.3 Homogenization

An Ultra-Turrax disperser (IKA, Staufen, Germany) with non-fluorinated plastic dispersing components (e.g. polycarbonate and polysulfone) is recommended in order to obtain a homogeneous sample free of contamination (Ahrens *et al*., 2009c).

Depending on matrix and expected concentrations, an appropriate sample amount is weighed in polypropylene tubes for the extraction.

4 Analysis

Samples should be spiked with internal standards (see Table 1) before extraction, at concentrations close to the environmental level, in order to correct for losses during extraction, extract clean-up and concentration, and for matrix effects during analysis. After spiking and before extraction, sediment and biota samples should be left to equilibrate for ca. 1 h at ~4°C.

4.1 Blank control

Within each sample batch, a method blank should be analysed. If measurable blanks occur, the analytical instrumentation and every sample preparation step must be checked for contamination and appropriate measures taken before continuation of analysis. Solvents, including ultrapure water and the internal standard spiking solution, should be of high purity and must be tested for contamination prior to use. Commonly used water-purification units may contain wetted fluorinated components, thereby producing PFC-contaminated water (Yamashita *et al*., 2004). Moreover, PTFE-free septa made, for example, of silicon polymers and aluminium (e.g. Barrier septa, Supelco Inc., Bellefonte, PA), should be used.

4.2 Extraction and clean-up

4.2.1 Water

The most commonly applied method for the extraction of PFCs from aqueous samples is SPE (Moody and Field, 1999). The SPE method was further optimized by Taniyasu and co-workers to determine a wide range of PFCs, including short- and long-chain PFCs (Taniyasu *et al*., 2005, 2008; ISO, 2009). The ISO standard 25101 (ISO, 2009; Taniyasu *et al*., 2008) recommends the use of Oasis WAX (Weak Anion-eXchange) cartridges (Waters Corporation, Milford, MA, 150 mg, 6 ml, 30 μm) for SPE extraction. Briefly, after preconditioning with 4 ml of ammonium hydroxide in methanol, 4 ml of methanol, then 4 ml of ultrapure water, cartridges are loaded with the samples (100–1000 ml) at a flow rate of approximately 1 drop sec^{-1}. A pH adjustment is usually not necessary for water samples before extraction, but it may improve the recoveries for some PFCs, depending on the matrices and target compounds (Van Leeuwen *et al*., 2006). The cartridges are then washed with 4 ml of 25 mM ammonium acetate buffer (pH 4) in ultrapure water and dried by centrifugation at 3000 rpm for 2 min.

The PFCs are then eluted from the cartridges in two fractions. The first fraction is obtained with 4 ml of methanol and contains the neutral PFCs, whereas the second fraction is obtained with 4 ml of 0.1% ammonium hydroxide in methanol and contains the ionic PFCs. Alternatively, large-volume injection can be used to analyse PFCs directly without sample pretreatment (Schultz *et al*., 2006) if concentrations in the samples are high enough.

Generally, no further clean-up is required and both fractions may be analysed directly or after being concentrated to ~1 ml. If the sample matrix affects ionization yield (enhancement/suppression) in electrospray ionization–tandem mass spectrometry (ESI-MS/MS), a clean-up of the extracts may be necessary. An appropriate clean-up method has been described by Powley *et al*. (2005) using

graphitized carbon adsorbent (see Section 4.2.2). The extraction of the particulate phase should be performed according to the extraction of sediment (see Section 4.2.2)

4.2.2 Sediment

Four methods for the extraction of PFCs from sediments have been described in the scientific literature.

1) a wrist-action shaker operated at maximum deflection, using methanol, followed by a graphitized carbon adsorbent clean-up (Powley *et al*., 2005)

2) an acetic acid wash, followed by repeated extraction with methanol/1% acetic acid in water (90:10, v/v) in a heated (60°C) sonication bath and subsequent clean-up using C_{18} cartridges (Higgins *et al*., 2005)

3) pressurized fluid extraction with acetone/methanol (25:75, v/v) at 100°C followed by headspace solid-phase microextraction (Alzaga *et al*., 2005))

4) sonication with acetonitrile/water (60:40, v/v) and ion pairing clean-up (Washington *et al*., 2008)

To ensure complete extraction of PFCs from sediments, samples should be extracted at least three times, each time with an amount of solvent corresponding to ten times the sample weight. The three extracts are combined for clean-up.

As ionization enhancement/suppression often occurs in ESI-MS/MS when analysing complex sample matrices, a clean-up of the extracts may be necessary. Different methods can be used, either separately or in combination, depending on the characteristics of the sediment, the extraction solvent, and the concentration level.

An appropriate clean-up method is described by Powley *et al*. (2005). Briefly, 25 mg of ENVI-Carb (Supelco, Bellefonte, PA, 100 $m^2 g^{-1}$, 120/400 mesh) and 50 µl acetic acid are added to a small polypropylene tube. The extract is concentrated to 1 ml and transferred to this tube. The suspension is mixed vigorously and centrifuged, and 0.5 ml of the supernatant is transferred to another flask.

Another appropriate clean-up method is based on SPE (Taniyasu *et al*., 2008). Briefly, the extract is diluted in ultrapure water to obtain a solution with less than 5% sample extract. The aqueous sample extract is then extracted by SPE using Oasis WAX cartridges (see Section 4.2.1).

Additional clean-up may be required, depending on sample type and concentration levels (Higgins *et al*., 2005; Washington *et al*., 2008).

4.2.3 Biota

Three methods are commonly used for the extraction of PFCs from biota samples.

1) ion pair extraction with tetrabutylammonium (TBA) and the extraction solvent methyl *tert*-butyl ether (MTBE; Hansen *et al*., 2001)

2) ultrasonic extraction (UE) with subsequent clean-up (Powley *et al*., 2005)

3) alkaline digestion followed by SPE on Oasis WAX cartridges (So *et al*., 2006)

The UE method is further described here because of its ease of handling and good recoveries, but the other two methods constitute effective alternatives. The UE method includes a minimum of three repeated extractions using acetonitrile or methanol, each with a tenfold solvent of the sample amount and 30 min extraction time. The three extracts are combined for clean-up.

Following extraction, a clean-up of the extracts is necessary to prevent matrix effects when carrying out instrumental analysis. Gel permeation chromatography (GPC) for lipid removal is not advisable because lipids are poorly separated from some target compounds (with chain lengths ≥C_8). Silica can be used for lipid removal, but may lead to losses of PFOSA (Van Leeuwen and De Boer, 2007). Lipids can be removed from methanol or acetonitrile extracts by precipitation at −20°C (Theobald *et al.*, 2007). The extract is then centrifuged for 1 min and the supernatant is decanted into a clean vial. After lipid removal, additional clean-up steps may be required, depending on sample type and analyte concentrations (as described by Powley *et al.*, 2005). Different methods can be used, either separately or in combination, depending on the extraction solvent and the expected concentration level in the sample (see Section 4.2.2), but the use of ENVI-Carb graphitized carbon as a dispersive sorbent is recommended (Powley *at al.*, 2005; Van Leeuwen *et al.*, 2009).

4.3 Pre-concentration

Sample extracts should be concentrated in order to meet the required detection limits. Concentration techniques at low temperature (<40°C) and controlled pressure conditions are preferred in order to avoid losses of volatile PFCs. Evaporation to dryness should be avoided.

An injection standard, preferably an isotopically labelled PFC (e.g. [$^{13}C_8$] PFOS, [$^{13}C_8$] PFOA), may be added to the final extract for correction of the injection volumes and calculation of the recoveries of the isotopically labelled internal standard.

The solvent composition of the final extract should correspond to the mobile phase of the LC method in order to obtain a satisfactory peak shape of the compounds, in particular of short-chain PFCs eluting early from the column. Unless the samples are analysed immediately, the vials should be kept at a temperature below 4°C. If glass vials are used, PFCs can be adsorbed onto the glass surface with solvents containing a greater amount of water. It is therefore advisable to use polypropylene vials.

4.4 Instrumental analysis

Liquid chromatography coupled with a tandem mass spectrometer and interfaced with an electrospray ionization source in negative-ion mode (LC/(−)ESI-MS/MS; Hansen *et al.*, 2001) and LC coupled with an ESI quadrupole time-of-flight mass spectrometer (LC/ESI-QTOF-MS) have both been used for PFC analysis (Berger and Haukas, 2005). Tandem MS and QTOF-MS have the advantage of providing low signal-to-noise ratio and high selectivity. Alternatively, gas chromatography coupled with mass spectrometry (GC/MS), with derivatization of the ionic PFCs, can be used, which has the advantage of resolving isomers (Chu & Letcher, 2009).

4.4.1 Liquid chromatography

The C_8 or C_{18} reversed-phase columns may be used for the LC separation of PFCs. The use of a guard column is recommended in order to maintain chromatographic performance and extend the lifetime of the chromatographic column. To overcome separation problems (e.g. co-eluting matrix compounds), it may be helpful to use reversed-phase columns with polar groups instead of C_8 or C_{18} columns (Caliebe, 2007). Mixtures of water and either methanol or acetonitrile can be used as the mobile phase, in each case with 2–10 mM ammonium acetate as an ionization aid. Gradients ranging from 10% to 100% methanol or acetonitrile are required for the separation of the compounds listed in Table 1. To ensure stability of retention times, the use of a temperature-controlled column oven is recommended.

Modifications of the instrument may be necessary to minimize contact with fluorine-containing materials (Yamashita *et al*., 2004). For example, tubing, filters for the mobile-phase solvents, and degassers that contain PTFE may be sources of contamination. A scavenger cartridge (e.g. the Phenomenex Luna 3 µm C18(2) column, 300 × 4.6 mm) can be installed between the pump and injector to trap contaminants originating from the degasser, connecting tubes, and mobile-phase solvents.

4.4.2 Detection methods

The most widely used technique for detection of PFCs is tandem MS (MS/MS) operated in multiple reaction monitoring (MRM) mode. Mass spectrometry parameters, such as collision energy, clustering potential, and cone voltage, must be optimized for each individual compound and each instrument. The sensitivity of MS/MS is usually approximately one order of magnitude higher than that of QTOF-MS (Berger *et al*., 2004).

5 Calibration and quantification

5.1 Standards

The use of commercially available standards with a purity of >99% is recommended. The purity of standards should be verified because impurities from the same homologue group and isomers can occur.

Suggestions for isotopically labelled internal standards are given in Table 1. The use of internal standards for PFC analysis is strongly recommended in order to compensate for signal enhancement/suppression or losses during sample preparation. Methods using external calibration should be avoided. The internal standards and injection standard must be added before sample extraction and instrumental analysis, respectively. If possible, the corresponding isotopically labelled internal standard should be used for each target analyte. If an isotopically labelled standard is not available, an internal standard with physico-chemical characteristics and recovery rates similar to that of the target compound may be used, but matrix suppression/enhancement effects should first be checked in LC-ESI-MS/MS.

5.2 Calibration

The calibration curves must include the internal standard and injection standard in the same range as the spike level for the samples. Linearity must be checked for the calibration range, and the correlation coefficient (r) should be >0.99. The lower end of the linear range is determined by the quantification limits and the blank levels. The blank response should be lower than 20% of the limit of quantification. A multilevel calibration should have at least five calibration levels. In the case of matrix effects, the method of standard addition is a valid approach, albeit time-consuming, because it involves dividing and spiking sample extracts with at least three levels of calibration standards.

5.3 Quantification

Every detection and quantification must comply with defined quality criteria. If possible, two mass transitions should be recorded for each target analyte: one for quantification (quantifier) and one for identification (qualifier). The abundance ratio of these two masses in the sample is compared with that of the calibration standards

obtained under identical chromatographic conditions. A substance is considered to be identified if the retention time and abundance ratio of the two masses in the sample are within the specified tolerance limits obtained for a standard solution under identical conditions.

For quantification, the signal-to-noise ratio for the LC peak must be at least 10 for all target compounds. The peak height of the target compound should exceed the measured blank by a factor of at least five to nine times the standard deviation of the blanks.

Some PFSAs and sulfonamides demonstrate more than one peak in the chromatogram, owing to the presence of branched isomers. The ratio of linear and branched isomers can differ between the calibration standard and environmental samples. Branched isomers should be quantified separately if calibration standards are available. If there are no proper calibration standards and the peak area of the branched isomer exceeds 10% of that of the linear isomer, it is advisable to estimate its concentration based on the response factor of the linear standard. However, the response factors of the linear and branched isomers may be different. It must be indicated with the results whether the reported concentration refers to the sum of the linear and branched isomers, or to the linear or branched isomer only.

Co-elution of matrix constituents (e.g. taurocholate bile salts) with the same transition ions as perfluorohexane sulfonate (PFHxS) and PFOS may lead to a significant bias in the quantification of these compounds in biota samples (Chan *et al.*, 2009; Lloyd *et al.*, 2009). However, bias in PFHxS and PFOS levels can be avoided by separating the interferences from the target analytes and/or by using the interference-free transition to a mass-to-charge ratio (m/z) of 119 in order to verify results obtained with the product ions at m/z 80 and 99. The PFCAs and PFSAs are almost completely dissociated in environmental matrices. If salts are used for the preparation of calibration standards, quantification results should be calculated for the corresponding acids.

6 Quality assurance and quality control

Prior to the analysis of environmental samples, the method should be subjected to a full in-house validation according to the requirements of the monitoring programme. This should include the determination of limits of detection and quantification, trueness, precision, linearity of calibration, measurement uncertainty, and robustness.

Every sample batch should include a procedural blank that has been prepared in the same way as the samples. The number of samples per batch may differ between laboratories and depends on how many samples can be processed under comparable conditions; generally, 10–12 samples per batch are recommended. If isotopically labelled internal standards are used, absolute recoveries between 50% and 150% are acceptable. In all other cases, recoveries should be between 70% and 120%. Within each sample batch, at least one sample should be extracted in duplicate, and blank samples and reference materials, such as certified reference material (CRM), laboratory reference material (LRM), and standard reference material (SRM), should be included in each sample series. The results of the reference material should be recorded and monitored in control charts. If no CRM is available, possible bias in the analytical method should be checked by the analysis of spiked laboratory control samples.

Laboratories should demonstrate their competence by participation in laboratory proficiency testing schemes relevant to the monitoring programme. Such exercises are still rarely offered by proficiency test providers, but a recent interlaboratory study, aimed at method validation, demonstrated acceptable performance of laboratories in analysing PFCs in biota and water (Van Leeuwen *et al*., 2009).

7 Data reporting

For routine analysis, the data report should be in accordance with the requirements relevant to the particular monitoring programme. For example, it should include information on sampling, sample processing, storage, and analysis. Results should be reported together with the associated measurement uncertainty.

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9 Abbreviations and technical terminology

CRM	certified reference material
ESI	electrospray ionization
FTOH(s)	fluorotelomer alcohol(s)
GC	gas chromatography
GPC	gel permeation chromatography
LC	liquid chromatography
LRM	laboratory reference material
MRM	multiple reaction monitoring
MS	mass spectrometry/spectrometer
MS/MS	tandem mass spectrometry/spectrometer
MTBE	methyl tert-butyl ether
PFBA	perfluorobutanate
PFBS	perfluorobutane sulfonate
PFC(s)	polyfluoroalkyl compound(s)
PFCA(s)	perfluoroalkyl carboxylate(s)
PFHxS	perfluorohexane sulfonate
PFNA	perfluorononanate
PFOA	perfluorooctanate
PFOS	perfluorooctane sulfonate
PFOSA	perfluorooctane sulfonamide
PFSA(s)	perfluoroalkyl sulfonate(s)
PTFE	polytetrafluoroethylene
QTOF	quadrupole time-of-flght
SPE	solid phase extraction
SRM	standard reference material
TBA	tetrabutylammonium
UE	ultrasonic extraction

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