



## Guideline on the determination of Perfluoroalkylated substances (PFAS) in seawater

### 1. Background

Perfluorooctane sulphonic acid (PFOS), perfluorooctanoic acid (PFOA) and other per- and polyfluoroalkyl substances (PFASs) are considered global environmental contaminants. PFOS and PFOA are chemically and biologically inert and very stable (Poulsen et al. 2005). PFOS meets the P (persistent) and vP (very persistent) criteria due to very long half lives. PFOS is also bioaccumulative (B) and toxic (T) (OSPAR 2005). PFOA is considered as very persistent (vP) and toxic (T), but not bioaccumulative (Van der Putte et al. 2010). Both have the capacity to undergo long-range transportation.

These guidelines concentrate on the sampling, extraction and instrumental analysis of per- and polyfluoroalkyl substances (PFASs) from seawater and also address special aspects of the sampling matrix. Currently, there are no HELCOM monitoring guidelines for PFASs in biota and sediments but information can be found in Ahrens, L., Vorkamp, K., Lepom, P., Bersuder, P., Theobald, N., Ebinghaus, R., Bossi, R., Barber, J. L., McGovern, E. 2010. Determination of perfluoroalkyl compounds in water, sediment, and biota. ICES Techniques in Marine Environmental Sciences No. 48. 16 pp.

These guidelines provide advice for the analysis of *per- and polyfluoroalkyl substances* in total seawater which basically includes the following steps:

1. i) sampling,
2. ii) extraction and clean-up, and

iii) instrumental analysis and quantification  
The extraction and preconcentration of the PFASs is a crucial step in the procedure as the expected concentrations in seawater are often only in the  $\text{pg L}^{-1}$  range. Extraction and enrichment are usually conducted through solid phase extraction (SPE).

Determination is usually performed by separation with liquid chromatography (LC) and advanced mass spectrometric (MS) detection.

All steps of the procedure are susceptible to insufficient recovery and contamination. Therefore, regular quality control measures must be applied in order to monitor method performance.

These guidelines are not intended as a complete laboratory manual. If necessary, guidance should be sought from specialized laboratories. Laboratories should demonstrate validity of each methodological step. Moreover, use of an alternative method, carried out concurrently to the routine procedure, is recommended for validation.

Contracting parties should follow the HELCOM monitoring guideline but minor deviations from this are acceptable if the method achieves comparable results. Validation of the adopted method needs to be performed on the relevant matrix and concentration range e.g. by taking part in intercomparison studies or proficiency testing schemes.

## 1.1 Introduction

PFOS and PFOA are members of the larger family of per- and polyfluoroalkyl substances (PFASs). PFOS precursor can degrade to PFOS, also called PFOS-related compounds. Some 100–200 PFOS-related compounds have been identified (KEMI 2006, Buck et al 2011). Table 1 provides an overview of PFAS that are environmentally relevant in water 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 PFAS analysis.

Table 1. List of possible perfluoroalkyl substances to be monitored in water and their isotopically labelled internal standards.

<b>ANALYTE</b>	<b>ACRONYM</b>	<b>FORMULA</b>	<b>CAS-NUMBER</b>
Perfluorobutanate	PFBA	$C_3F_7COO-$	375-22-4
Perfluoropentanate	PFPA	$C_4F_9COO-$	2706-90-3
Perfluorohexanate	PFHxA	$C_5F_{11}COO-$	307-24-4
Perfluoroheptanate	PFHpA	$C_6F_{13}COO-$	375-85-9
Perfluorooctanate	PFOA	$C_7F_{15}COO-$	335-67-1
Perfluorononanate	PFNA	$C_8F_{17}COO-$	375-95-1
Perfluorodecanate	PFDA	$C_9F_{19}COO-$	335-76-2
Perfluorobutane sulfonate	PFBS	$C_4F_9SO_2O-$	29420-49-3
Perfluorohexane sulfonate	PFHxS	$C_6F_{13}SO_2O-$	3871-99-6 (potassium salt)
Perfluorooctane sulfonate	PFOS	$C_8F_{17}SO_2O-$	2795-39-3 (potassium salt)
6:2 fluorotelomer sulfonate	6:2 FTSA (THPFOS)	$C_6F_{13}C_2H_4SO_3-$	27619-97-2
Perfluorooctane sulfonamide	FOSA	$C_8F_{17}SO_2NH_2$	754-91-6
N-ethylperfluoro-1-octanesulfonamidoacetic acid	N-EtFOSAA	$C_8F_{17}SO_2N(C_2)CH_2CO_2H$	2991-50-6
<b>INTERNAL STANDARDS</b>			
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-1-hexane( $^{18}O_2$ )sulfonate	$[^{18}O_2]$ -PFHxS	$C_6F_{13}S(^{18}O_2)O-$	n.a.
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.
6:2 fluorotelomer sulfonate (1,2- $^{13}C_2$ )	$[^{13}C_2]$ -6:2 FTSA	$(1,2-^{13}C_2)C_6H_4F_{13}SO_3-$	n.a.
Perfluoro-1-(1,2,3,4,5,6,7,8- $^{13}C_8$ )octanesulfonamide	$[^{13}C_8]$ -FOSA	$(1,2,3,4,5,6,7,8-^{13}C_8)F_{17}SO_2NH_2$	n.a.

<i>N-deuterioethylperfluoro-1-octanesulfonamidoacetic acid</i>	<i>d5-N-EtFOSAA</i>	<i>C<sub>8</sub>F<sub>17</sub>SO<sub>2</sub>N(C<sub>2</sub>D<sub>5</sub>)CH<sub>2</sub>CO<sub>2</sub>H</i>	<i>n.a.</i>
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The individual compounds belong to the following compound groups: perfluoroalkyl sulfonates (PFASs), perfluoroalkyl carboxylates (PFCAs), perfluorooctane sulfonamides (FOSAs), and perfluorooctane sulfonamidoacetic acids (FOSAAs). For monitoring purposes, the chemicals PFOS, PFOA, C4-C12 PFCAs, C4, C6, C8 PFASs and FOSA are considered to be the most important PFASs. Although most studies have focused on PFOS and PFOA, it is suggested that PFAS with longer and shorter carbon chain lengths should also be included in the analysis. Long-chain PFAS ( $\geq$  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.

The presence of PFAS in seawater samples provides information on the contaminant load of parts of the Baltic Sea. At the moment monitoring is coordinated nationally. Poland, Lithuania and Germany monitor PFOS concentrations in seawater in their national monitoring program.

## 1.2 Purpose and aims

The aim of monitoring PFASs is to identify their spatial variations and temporal trends in seawater.

## 2. Monitoring methods

### 2.1 Monitoring features

The main focus of monitoring is surface waters (< 5 meters) of coastal and open sea waters.

### 2.2 Time and area

Monitoring covers the entire Baltic area, and is performed throughout the year.

### 2.3 Monitoring procedure

#### 2.3.1 Monitoring strategy

The analysis of PFAS in seawater generally includes solid-phase extraction (SPE) and liquid chromatography (LC) with mass spectrometric (MS) detection.

#### 2.3.2 Sampling method and equipment

When analyzing PFASs from biologically active samples, it is highly recommended to extract the water sample as soon as possible after sampling without further manipulation, e.g. seawater samples should not be transferred into further containers before extraction. It is recommended to take, preserve and handle samples as specified in ISO 5667-1.

Materials and clothes that contain, or may adsorb, fluorinated compounds, such as polytetrafluoroethylene (PTFE), must be avoided during sampling and sample processing. In particular, the containers that come into direct contact with the sample must not contain any fluorinated polymers (e.g. PTFE). Containers and equipment made of polypropylene (according to ISO 25101:2009), polyethylene, glass, or stainless steel should be used. However, especially glass and polypropylene sampling and storage containers should be carefully checked for PFAS, as longer chained PFASs tend to sorp to glass walls, and cleaned before use (e.g. the sample container or filtration equipment must be rinsed with a polar solvent, such as methanol and dried before use) in order to minimize contamination. Every material that may come into contact with the sample

must be free of fluorinated compounds. The highest contamination risk was observed for PFOA and perfluorononate (PFNA; Theobald *et al.*, 2007).

### 2.3.3 Sample handling and analysis

For the extraction of total water samples (sum of dissolved and particulate phase), no pre-treatment is necessary. But in order to avoid blocking of the solid-phase extraction (SPE) cartridges for water samples with a high content of suspended particulate matter, a filtration step might be necessary. Filtration can be done on GF/F glass fiber filter or with syringe nylon membrane filter. As flat-bed filters have a very limited capacity, the use of coiled glass fiber filter is recommended for volumes larger than 10 l and water samples with high amounts of suspended matter. A pump is necessary to force the water through the filter. Due to the additional steps, this operation affords a number of additional quality control measures (adsorption losses, contamination problems) as the filtration equipment may be a source of contamination (Ahrens *et al.*, 2009b; Arp and Goss, 2009).

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 and concentration, and for matrix effects during analysis.

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

#### Blanks and contamination

Care should be taken to avoid contaminations during sampling, extraction and analysis.

Concentrations of PFAS in seawater are very low. 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) and it is strongly recommended to pretreat all used glassware and adsorption material.

A blank should be analysed within each sample batch. 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. Reporting of samples with measurable blanks should be considered carefully.

#### Extraction and clean-up

Large-volume injection can be used to analyse PFAS directly without sample pretreatment (Schultz *et al.*, 2006) if concentrations in the samples are high enough with the advantage that no further clean-up is required and eluates may be analysed directly or after being concentrated to ~ 1 ml.

The most commonly applied method for the extraction of PFAS from seawater samples with low concentration is SPE (Moody and Field, 1999, Taniyasu *et al.*, 2008; ICES, 2010). A pH adjustment is usually not necessary for water samples before extraction, but it may improve the recoveries for some PFAS, depending on the matrices and target compounds (Van Leeuwen *et al.*, 2006). For SPE extraction the use of Oasis WAX (Weak Anion eXchange) cartridges (Waters Corporation, Milford, MA) is recommended, according to the ISO standard 25101 (ISO, 2009;) and ICES, 2010. For large volumes (2-10 l) StrataX 33u Polymeric Reversed Phase cartridges (Phenomenex, 1 g/ 12 ml Giga Tubes) are recommended.

## 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 PFAS analysis (Berger and Haukas, 2005). Tandem MS and QTOF-MS have the advantage of providing low signal-to-noise ratio and high selectivity on low concentrations.

## Chromatographic determination

The C 8 or C 18 reversed-phase columns may be used for the LC separation of PFAS. 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. 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.

For solvents, use of PFAS trapping column is highly recommended as it delays analyte peaks originating from eluents (Stone *et al.* 2010).

## Mass spectrometry

The most widely used technique for detection of PFAS in tandem MS (MS/MS) operated is 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 triple quadrupole MS is usually approximately one order of magnitude higher than that of QTOF-MS or QTOF MS/MS (Berger *et al.*, 2004).

## 3. Data reporting and storage

Data is reported annually to the HELCOM COMBINE database, hosted by ICES. Data must be flagged as for HELCOM Combine.

## 4. Quality control

### 4.1 Quality control of methods

#### Quality assurance

A number of measures should be taken to ensure sufficient quality of the analysis. Six main areas can be identified:

- a) extraction efficiency and clean-up;
- b) calibrant and calibration;
- c) system performance;

- d) long-term stability;
- e) internal standards; and
- f) frequent participation in interlaboratory proficiency testing schemes and analysis of certified reference materials (CRM). At the moment only proficiency testing schemes for PFAS in sediment biota, or drinking water are offered, but not for seawater. Reference materials are available for PFAS in water and fish.

*a) Extraction efficiency and clean-up*

The use of internal standards is recommended in order to take any losses or matrix effects into account. For the determination of the recovery rates of the clean-up and concentration steps, it is recommended to pass a standard solution through the entire procedure. If major losses have occurred, the results should not be reported.

*b) Calibrant and calibration*

Calibration solutions should be stored in ampoules at a cool, dark place. Weight loss during storage should be recorded for all standards. Risk of contamination during the storage must be monitored and suitable ampoules used.

For PFAS determination calibration solutions from certified PFAS solutions should be used. When using a PFOS standard solution of mixed isomers, it should be determined whether the nominal concentration corresponds to the linear PFOS or to the total amount of isomers (Kaupmees & Rebane, 2017).

#### System performance

The performance of the LC system can be monitored through regularly analyzing the resolution of two closely eluting compounds. A decrease in resolution indicates deteriorating LC conditions.

The signal-to-noise ratio of a low-concentration standard gives information on the condition of the detector. For example, a dirty MS-source can be recognized by the presence of a higher background signal, together with a reduced signal-to-noise ratio.

*c) Long-term stability*

One laboratory reference sample should be included in each series of samples. A quality control chart should be recorded for selected PFAS compounds. If warning limits are exceeded, the method should be checked for possible errors and the obtained sample results should not be reported.

*d) Internal standards*

Internal standards should be added to all standards and samples either in a fixed volume or by weight and should not interfere with the target analytes.

A number of mass-labelled PFAS compounds were proven to be suitable for LC-MS analysis (Table 1).

## 4.2 Quality control of data and reporting

Measurement uncertainty should be estimated using ISO 11352:2012 or according to the Nordtest report TR537. Estimation should be based on within-laboratory reproducibility, data from proficiency tests, IRM, and, when available, CRM.

Data must be flagged if normal QA routines or recommended storage conditions cannot be followed.

## 5. Contacts and references

### 5.1 Contact persons

Berit Brockmeyer, Federal Maritime and Hydrographic Agency (BSH), Germany

### 5.2 References

Ahrens, L., Plassmann, M., Xie, Z., and Ebinghaus, R. 2009. Determination of polyfluoroalkyl compounds in water and suspended particulate matter in the River Elbe and North Sea, Germany. *Frontiers of Environmental Science and Engineering in China*, 3: 152 – 170.

Arp, H. P. H., and Goss, K-U. 2009. Gas/particle partitioning behavior of perfluorocarboxylic acids with terrestrial aerosols. *Environmental Science and Technology*, 43: 8542 – 8547.

Berger, U., and Haukas, M. 2005. Validation of a screening method based on liquid chromatography coupled to high-resolution mass spectrometry for analysis of perfluoroalkylated substances in biota. *Journal of Chromatography A*, 1081: 210 – 217.

Berger, U., Langlois, I., Oehme, M., and Kallenborn, R. 2004. Comparison of three types of mass spectrometer for high-performance liquid chromatography/mass spectrometry analysis of perfluoroalkylated substances and fluorotelomer alcohols. *European Journal of Mass Spectrometry*, 10: 579 – 588.

Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., de Voogt, P., Jensen, A.A., Kannan, K., Mabury, S.A., van Leeuwen, S.P.J 2011. Perfluoroalkyl and Polyfluoroalkyl Substances in the Environment: Terminology, Classification, and Origins. *Integr Environ Assess Manag*. 2011 Oct; 7(4): 513–541.

Huset, C. A., Chiaia, A. C., Barfosky, D. F., Jonkers, N., Kohler, H-P. E., Ort, C., Giger, W., Field, J. A. 2008. Occurrence and mass flows of fluorochemicals in the Glatt Valley watershed, Switzerland. *Environmental Science and Technology*, 42: 6369 – 6377.

ICES - Ahrens, L., Vorkamp, K., Lepom, P., Bersuder, P., Theobald, N., Ebinghaus, R., Bossi, R., Barber, J. L., McGovern, E. 2010. Determination of perfluoroalkyl compounds in water, sediment, and biota. *ICES Techniques in Marine Environmental Sciences* No. 48. 16 pp.

ISO. 2009. Water quality – Determination of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) – Method for unfiltered samples using solid phase extraction and liquid chromatography/mass spectrometry. International Organization for Standardization, ISO 25101:2009 (TC 47/ SC 2). 19 pp.

Kaupmees, S., Rebane, R. 2017. Analysis and validation of perfluorinated compounds in water, sediment and fish with LCESI-MS/MS *International Journal of Environmental Analytical Chemistry*, Vol. 97, No. 8, 695–709 <https://doi.org/10.1080/03067319.2017.1356922>.

KEMI 2006. Perfluorinated substances and their uses in Sweden. Swedish Chemical Agency (KEMI) Report 7/06. 58 pp.

Moody, C. A., and Field, J. A. 1999. Determination of perfluorocarboxylates in groundwater impacted by fire-fighting activity. *Environmental Science and Technology*, 33: 2800 – 2806.

OSPAR 2005. OSPAR background document on perfluorooctane sulphonate. 46 pp. OSPAR Commission. Updated in 2006.

Poulsen, P., Jensen, A., Wallström, E. 2005. More environmentally friendly alternatives to PFOS-compounds and PFOA. Environmental Project No. 1013. Danish Environmental Protection Agency. 162 pp.

Schultz, M. M., Barofsky, D. F., and Field, J. A. 2006. Quantitative determination of fluorinated alkyl substances by large-volume-injection liquid chromatography tandem mass spectrometry-characterization of municipal wastewaters. *Environmental Science and Technology*, 40: 289 – 295.

Stone, P.K.W., Cote, L., Gushue, J., Letcher, R.J., Chu S. 2010. Agilent App. Note, 5990

Taniyasu, S., Kannan, K., Yeung, L. W. Y., Kwok, K. Y., Lam, P. K. S., and Yamashita, N. 2008. Analysis of trifluoroacetic acid and other short-chain perfluorinated acids (C2 – C4) in precipitation by liquid chromatography–tandem mass spectrometry: Comparison to patterns of long-chain perfluorinated acids (C5 – C18). *Analytica Chimica Acta*, 619: 221 – 230.

Theobald, N., Gerwinski, W., Caliebe, C., and Haarich, M. 2007. Development and validation of a method for the determination of polyfluorinated organic substances in sea water, sediments and biota. Occurrence of these compounds in the North and Baltic Seas. *Umweltforschungsplan des Bundesministeriums für Umwelt, Naturschutz, und Reaktorsicherheit, Forschungsbericht 202 22 213, UBA-FB 00 001409. 133 pp. (in German, with English abstract)*

Van der Putte, I., Murin, M., Van Velthoven, M., Affourtit, F. 2010. Analysis of the risks arising from the industrial use of Perfluorooctanoic acid (PFOA) and Ammonium Perfluorooctanoate (APFO) and from their use in consumer articles. Evaluation of the risk reduction measures for potential restrictions on the manufacture, placing on the market and use of PFOA and APFO. *RPS Advies B.V. 82 pp. + annexes.*

van Leeuwen, S. P. J., Kärrman, A., Van Bavel, B., De Boer, J., and Lindström, G. 2006. Struggle for quality in determination of perfluorinated contaminants in environmental and human samples. *Environmental Science and Technology*, 40: 7854 – 7860.

### 5.3 Additional literature

Ahrens, L., Vorkamp, K., Lepom, P., Bersuder, P., Theobald, N., Ebinghaus, R., Bossi, R., Barber, J. L., McGovern, E. 2010. Determination of perfluoroalkyl compounds in water, sediment, and biota. *ICES Techniques in Marine Environmental Sciences No. 48. 16 pp.*