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Water quality — Determination of perfluoroalkyl and polyfluoroalkyl substances (PFAS) in water — Method using solid phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS)

Qualité de l'eau — Détermination des substances d'alkyle perfluorés et polyfluorés (SPFA) dans l'eau — Méthode par extraction en phase solide et chromatographie liquide et spectrométrie de masse en tandem (CL-SM/SM)

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Water quality — Determination of perfluoroalkyl and polyfluoroalkyl substances (PFAS) in water — Method using solid phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS)

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably qualified staff.

1 Scope

This document specifies a method for the determination of selected perfluoroalkyl and polyfluoroalkyl substances (PFAS) in non-filtrated waters, for example drinking water, natural water (fresh water and sea water) and waste water containing less than 2 g/l solid particulate material (SPM) using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The compounds monitored by this method are typically the linear isomers. The group of compounds determined by this method are representative of a wide variety of PFAS. The analytes specified in Table 1 can be determined by this method. The list can be modified depending on the purpose for which the method is intended. The lower application range of this method can vary depending on the sensitivity of the equipment used and the matrix of the sample. For most compounds to which this document applies ≥0,2 ng/l as limit of quantification can be achieved. Actual levels can depend on the blank levels realized by individual laboratory.

The applicability of the method to further substances, not listed in Table 1, or to further types of water is not excluded, but is intended to be validated separately for each individual case.

NOTE 1 PFAS is used in this document to describe the analytes monitored. Many of the compounds in Table 1 are perfluoroalkyl and are also considered polyfluoroalkyl substances.

NOTE 2 The linear PFAS isomers are specified in this document. The branched isomers can be present in environmental samples, especially for PFOS. Annex E provides an example of an analytical approach to the chromatographic and spectroscopic separation of individual isomers.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 5667-1, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes and sampling techniques*

ISO 5667-3, *Water quality — Sampling — Part 3: Preservation and handling of water samples*

ISO 21253-1, *Water quality — Multi-compound class methods — Part 1: Criteria for the identification of target compounds by gas and liquid chromatography and mass spectrometry*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
perfluoroalkyl and polyfluoroalkyl substances
PFAS
commonly used international abbreviation for organic compounds with replacement of most or all hydrogen atoms by fluorine in the aliphatic chain structure

Note 1 to entry: The term is used in the broader sense for per- and polyfluoroalkyl substances (PFAS), and per- and polyfluorinated compounds (PFC) as well.

4 Principle

The analytes listed in Table 1 are extracted from the water sample by solid-phase extraction using a weak anion exchange sorbent followed by solvent elution and determination by liquid chromatography-tandem mass-spectrometry.

The user should be aware that each analyte has its own specific optimum conditions and therefore modification of the analyte list could require the specification of additional conditions for each additional parameter.

Table 1 — Analytes determinable by this method

Analyte	IUPAC[a] name	Formula	Abbreviation	CAS-RN[b]
Perfluoro-*n*-butanesulfonic acid	1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulfonic acid	$C_4HF_9O_3S$	PFBS	375-73-5
Perfluoro-*n*-hexanesulfonic acid	1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid	$C_6HF_{13}O_3S$	PFHxS	355-46-4
Perfluoro-*n*-heptanesulfonic acid	1,1,2,2,3,3,4,4,5,5,6,6,7,7,7-Pentadecafluoroheptane-1-sulfonic acid	$C_7HF_{15}O_3S$	PFHpS	375-92-8
Perfluoro-*n*-octanesulfonic acid	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluorooctane-1-sulfonic acid	$C_8HF_{17}O_3S$	PFOS	1763-23-1
Perfluoro-*n*-decanesulfonic acid	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Henicosafluorodecane-1-sulfonic acid	$C_{10}HF_{21}O_3S$	PFDS	335-77-3
Perfluorooctanesulfonamide	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluoro-1-octanesulfonamide	$C_8H_2F_{17}NO_2S$	FOSA	754-91-6
N-methyl perfluorooctanesulfonamide	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluoro-N-methyl-1-octanesulfonamide	$C_9H_4F_{17}NO_2S$	*N*-MeFOSA	31506-32-8
N-ethyl perfluorooctanesulfonamide	N-Ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctane-1-sulfonamide	$C_{10}H_6F_{17}NO_2S$	*N*-EtFOSA	4151-50-2
N-methyl perfluorooctanesulfonamidoacetic acid	2-[1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluorooctylsulfonyl(methyl)amino] acetic acid	$C_{11}H_6F_{17}NO_4S$	*N*-MeFOSAA	2355-31-9
N-ethyl perfluorooctanesulfonamidoacetic acid	2-[Ethyl(1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctylsulfonyl)amino] acetic acid	$C_{12}H_8F_{17}NO_4S$	*N*-EtFOSAA	2991-50-6
6:2 Fluorotelomer sulfonic acid	3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctane-1-sulfonic acid	$C_8H_5F_{13}O_3S$	6:2 FTSA	27619-97-2
8:2 Fluorotelomer sulfonic acid	3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptadecafluorodecane-1-sulfonic acid	$C_{10}H_5F_{17}O_3S$	8:2 FTSA	39108-34-4

[a] IUPAC: International Union of Pure and Applied Chemistry.

[b] CAS-RN: Chemical Abstract Services Registry Number.

Table 1 *(continued)*

Analyte	IUPAC[a] name	Formula	Abbreviation	CAS-RN[b]
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	2-(6-Chloro-1,1,2,2,3,3,4,4,5,5,6,6-dodecafluorohexoxy)-1,1,2,2-tetrafluoroethanesulfonic acid	$C_8HClF_{16}O_4S$	9Cl-PF3ONS	73606-19-6
Perfluoro-*n*-butanoic acid	2,2,3,3,4,4,4-Heptafluorobutanoic acid	$C_4HF_7O_2$	PFBA	375-22-4
Perfluoro-*n*-pentanoic acid	2,2,3,3,4,4,5,5,5-Nonafluoropentanoic acid	$C_5HF_9O_2$	PFPeA	2706-90-3
Perfluoro-*n*-hexanoic acid	2,2,3,3,4,4,5,5,6,6,6-Undecafluorohexanoic acid	$C_6HF_{11}O_2$	PFHxA	307-24-4
Perfluoro-*n*-heptanoic acid	2,2,3,3,4,4,5,5,6,6,7,7,7-Tridecafluoroheptanoic acid	$C_7HF_{13}O_2$	PFHpA	375-85-9
Perfluoro-*n*-octanoic acid	2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Pentadecafluorooctanoic acid	$C_8HF_{15}O_2$	PFOA	335-67-1
Perfluoro-*n*-nonanoic acid	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-Heptadecafluorononanoic acid	$C_9HF_{17}O_2$	PFNA	375-95-1
Perfluoro-*n*-decanoic acid	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Nonadecafluorodecanoic acid	$C_{10}HF_{19}O_2$	PFDA	335-76-2
Perfluoro-*n*-undecanoic acid	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10, 11,11,11-Henicosafluoroundecanoic acid	$C_{11}HF_{21}O_2$	PFUnDA	2058-94-8
Perfluoro-*n*-dodecanoic acid	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10, 11,11,12,12,12-Tricosafluorododecanoic acid	$C_{12}HF_{23}O_2$	PFDoDA	307-55-1
Perfluoro-*n*-tridecanoic acid	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10, 11,11,12,12,13,13,13-Pentacosafluorotridecanoic acid	$C_{13}HF_{25}O_2$	PFTrDA	72629-94-8
Perfluoro-*n*-tetradecanoic acid	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10, 11,11,12,12,13,13,14,14,14-Heptacosafluorotetradecanoic acid	$C_{14}HF_{27}O_2$	PFTeDA	376-06-7
Perfluoro-*n*-hexadecanoic acid	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10, 11,11,12,12,13,13,14,14,15,15,16,16, 16-Hentriacontafluorohexadecanoic acid	$C_{16}HF_{31}O_2$	PFHxDA	67905-19-5
Perfluoro-*n*-octadecanoic acid	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10, 11,11,12,12,13,13,14,14,15,15,16,16, 17,17,18,18,18-Pentatriacontafluorooctadecanoic acid	$C_{18}HF_{35}O_2$	PFOcDA	16517-11-6
8:2 Fluorotelomer unsaturated carboxylic acid	3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Hexadecafluorodec-2-enoic acid	$C_{10}H_2F_{16}O_2$	8:2 FTUCA	70887-84-2
8:2 Polyfluoroalkyl phosphate diester	Bis(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl) hydrogen phosphate	$C_{20}H_9F_{34}O_4P$	8:2 diPAP	678-41-1
Hexafluoropropylene oxide dimer acid	2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid	$C_6HF_{11}O_3$	HFPO-DA	13252-13-6
4,8-Dioxa-3H-perfluorononanoic acid	2,2,3-Trifluoro-3-[1,1,2,2,3,3-hexafluoro-3-(trifluoromethoxy)propoxy] propanoic acid	$C_7H_2F_{12}O_4$	DONA	919005-14-4

[a] IUPAC: International Union of Pure and Applied Chemistry.

[b] CAS-RN: Chemical Abstract Services Registry Number.

5 Interferences

5.1 Interferences with sampling and extraction

Sample bottles (7.1) shall consist of materials that do not contaminate or change the composition of the sample during sample storage. All types of fluoropolymer plastics, including polytetrafluoroethylene (PTFE) and fluoroelastomer materials, shall be avoided during sampling, sample storage and extraction.

Sample bottles (7.1) shall be checked for possible background contamination before use. If background contamination is suspected or detected in sample bottles (7.1), then wash sample bottles (7.1) with water (6.1) and methanol (6.6) prior to use. To avoid cross contamination, the sample bottles (7.1) should only be used once. The use of intermediate sample tubes (7.6) and vials (7.10) should be limited in the overall process to avoid contamination of loss by sorption. To avoid losses resulting from adsorption of target analytes to the wall of sample bottle (7.1) and reservoir column (7.4), extract all of the sample from the sample bottle (7.1) and rinse the wall of sample bottle (7.1) and reservoir column (7.4) with methanol (6.6).

Commercially available adsorbent materials often vary in quality or activity. Considerable batch-to-batch differences in quality and selectivity of these materials are possible. The recovery of a single substance may also vary with respect to its concentration. Therefore, check analyte recovery periodically at different concentrations and whenever new batches/lots of reagents or labware are used (12.1).

5.2 Interferences with LC-MS/MS

Substances with similar retention times that can produce ions with similar mass to charge ratios (m/z) to those produced by the analytes of interest may interfere with the determination.

These interferences may lead to incompletely resolved signals and/or additional signals in the mass chromatograms of target substances. Depending on their levels in the sample, such substances may affect the accuracy and precision of the results. The chromatographic separation is different with the LC column (see Annex C for examples). As long as the peak of interest can be separately integrated from interferences, it may be used.

Matrix interferences may be caused by contaminants that are co-extracted from the samples. The extent of matrix interferences varies considerably, depending on the nature of the samples. In drinking water and ground water, matrix interferences are usually negligible, whereas waste water and sea water matrices can be affected by matrix interferences that lead to ionization suppression or enhancement resulting in bias or reduced sensitivity of the method. As long as the required limits of quantification can be achieved in samples, samples can be diluted to minimize matrix effects.

Interferences arising directly from analytical instruments can be significant for unmodified commercial LC systems because many parts are made of PTFE and other fluoropolymers. It is necessary to check for possible blank contamination from the individual parts, such as tubing, solvent inlet filters, valve seals and the degassing equipment, and replace these with materials such as stainless steel and polyetheretherketone (PEEK), where possible.

NOTE Background contamination can arise from within the instrument. A delay column can be attached between the solvent mixer and injection valve to chromatographically resolve these background contaminants from the instrument and/or mobile phases from the target analytes.

The LC-vial caps shall be free of fluoropolymer material. Efforts should be taken to minimize background levels in procedural blank materials such that the procedural blank, including the instrumental blank, is at least 10-fold below the reporting limit.

6 Reagents

Whenever possible, use certified or analytical-grade reagents or residue free-analytical grade reagents stored in glass or polypropylene containers with metal or polypropylene lined caps. Avoid using reagents with fluoropolymer lined caps and check contamination levels of target substances using repeated blank determinations. Carry out additional cleaning or conditioning steps to ensure background levels are minimized, if necessary.

6.1 Water, blank-value free, e.g. complying with grade 1 as specified in ISO 3696.

Purified laboratory water can be used, but should be confirmed to be free of PFAS. The quality of water is checked by the same procedure given in 9.3.

6.2 Acetic acid, $w(CH_3COOH)$ = 99,9 % mass fraction (999 g/kg).

6.3 Acetonitrile, CH_3CN.

6.4 Ammonia solution, $w(NH_3)$ = 25 % mass fraction (250 g/kg).

6.5 Ammonium acetate, $w(CH_3COONH_4)$ = 97 % mass fraction (970 g/kg).

6.6 Methanol, CH_3OH, blank-value free.

NOTE The quality of methanol is checked by evaporating 10 ml of methanol with a gentle stream of nitrogen gas (6.13) to 0,5 ml and determining levels according to this document.

6.7 Reference substances, see Table 1.

Reference substances are analytical standards used for quantitative determination of the method analytes. Use only reference substances or solutions, where the content of linear isomers is at least 95 %. Make sure that the individual reference substances do not contain detectable concentrations of other target analytes to be determined by analysing alternate lots or second sources.

NOTE Solutions of reference substances are commercially available.

6.8 Internal standard substances, see Table 3.

Internal standard substances are labelled forms of the reference substances to be used in the analytical procedure to correct for recovery due to losses of analyte or changes in analytical conditions that could result in bias. Make sure that the internal standard substances do not contain detectable concentrations of the analytes to be determined by analysing new lots using this document.

NOTE Solutions of internal standard substances are commercially available.

6.9 Preparation of the solutions

Calculate the concentration of all reference substances and internal standard solutions with regard to the anion content.

Store the solutions at (5 ± 3) °C in the dark, protected against evaporation. Bring them to room temperature prior to use (i.e. before dilution or spiking or injection).

6.9.1 Individual stock solutions of the reference substances

Stock solutions of the individual reference substances (6.7) in methanol (6.6) or acetonitrile (6.3) should be of mass concentration to enable dilution to the desired range, e.g. 50 µg/ml each.

6.9.2 Individual stock solutions of internal standard substances

Stock solutions of the individual internal standard substances (6.8) in methanol (6.6) or acetonitrile (6.3) should be of mass concentration to enable dilution to the desired range, e.g. 50 µg/ml each.

6.9.3 Native stock solution (reference substances)

Prepare a solution of the reference substances with a mass concentration of, for example, 0,1 µg/ml each.

Fill, for example, 1 ml of each solution of the individual reference substances, for example 50 µg/ml (6.9.1), into a 500 ml volumetric flask (7.7) and make the solution up to the mark with methanol (6.6).

6.9.4 Labelled stock solution (internal standard substances)

Prepare a solution of the labelled internal standard substances with a mass concentration of, for example, 0,1 µg/ml each.

Fill, for example, 1 ml of each solution of the individual internal standard substances, e.g. 50 µg/ml (6.9.2), into a 500 ml volumetric flask (7.7) and make the solution up to the mark with methanol (6.6).

6.9.5 Spiking solution (reference substances)

Prepare a solution of the reference substances with a mass concentration of, for example, 10 ng/ml each.

Fill, for example, 1 ml of the native stock solution e.g. 0,1 µg/ml (6.9.3) into a 10 ml volumetric flask (7.7) and make the solution up to the mark with methanol (6.6).

This solution is used for recovery samples (see 12.1).

6.9.6 Spiking solution (internal standard substances)

Prepare a solution of the labelled internal standard substances with a mass concentration of, for example, 10 ng/ml each.

Fill, for example, 1 ml of the labelled stock solution e.g. 0,1 µg/ml (6.9.4) into a 10 ml volumetric flask (7.7) and make the solution up to the mark with methanol (6.6).

This solution is used for water samples (see 9.1.2) and spiking recovery samples (see 12.1).

6.9.7 Reference solution

Prepare the reference solutions by setting up dilutions of the stock solutions (6.9.3, 6.9.4). Add the same amount of internal standards to each reference solution.

Prepare the reference solution, for example a solution with a mass concentration of the substances to be determined and of the internal standard substances e.g. 1 ng/ml each.

Fill, for example, 0,1 ml of native stock solution (reference substances) e.g. 0,1 µg/ml (6.9.3) and 0,1 ml of labelled stock solution (internal standard substances) e.g. 0,1 µg/ml (6.9.4) into a 10 ml volumetric flask (7.7) and make the solution up to the mark with methanol (6.6).

6.10 Acetate buffer, for solid-phase extraction, 0,025 mol/l, pH 4.

Mix, for example, 0,5 ml of acetic acid (6.2) with e.g. 349,5 ml of water (6.1). Dissolve e.g. 0,116 g of ammonium acetate (6.5) in e.g. 60 ml of water (6.1). Mix e.g. 200 ml of the diluted acetic acid with e.g. 50 ml of the ammonium acetate solution.

6.11 Ammonia/methanol solution, for solid-phase extraction, with a mass fraction of approximately 0,1 %.

Mix, for example, 0,4 ml of ammonia solution (6.4) with e.g. 99,6 ml of methanol (6.6), with a volume of (NH_3) of e.g. 0,1 %.

6.12 Solid-phase extraction material, weak anion exchanger on a copolymer-based. Suitable materials are available commercially (see Annex A).

6.13 Nitrogen, N_2, purity >99,9 %.

7 Apparatus

Equipment of which any part may come into contact with the water sample or the extract shall be free from interfering compounds.

The blank determination shall be conducted before the sampling. Clean labware and apparatus for solid-phase extraction by washing with water (6.1) and methanol (6.6) if background contamination is detected in labware and apparatus.

Equipment in contact with sample or reference solutions should be made of polypropylene or polyethylene. It was not tested except for compounds listed in Annex H for sampling whether the use of glassware may lead to adsorption of some of the analytes within the scope of the method.

7.1 Sample bottles

Narrow-neck flat-bottomed polypropylene or polyethylene bottles, normal volume 50 ml, 100 ml, 250 ml, 500 ml and 1 000 ml, with conical shoulders and screw caps.

NOTE Glass bottles can be used for compounds listed in Annex H. Glass bottles can be used for sampling provided that storage conditions of samples have been validated in each case. See Annex H for examples.

The entire sample shall be extracted and the appropriate-sized sample bottle should be used to collect the sample.

Sample bottles shall be checked for possible background contamination before use. The bottles and screw caps are washed with methanol (6.6) and dried before use in order to minimize contamination, if background contamination is detected in sample bottles.

7.2 Pipettes, with polypropylene tips.

7.3 Solid-phase extraction medias (cartridges or disks), made of inert non-leaching plastic, e.g. polypropylene or polyethylene frits.

The cartridges shall be packed with 50 mg to 1 000 mg of solid-phase extraction material (6.12) as sorbent. In general, 150 mg to 250 mg of sorbent (see Annex A) in a single cartridge is sufficient for up to 500 ml of water.

NOTE 1 The 500 mg of sorbent (see Annex A) in a single cartridge is sufficient for 1 000 ml of sea water.

NOTE 2 The stationary phase can be modified if analytes are not recovered quantitatively (for example neutral substances such as FOSA, *N*-MeFOSA and *N*-EtFOSA) using solid-phase extraction material (6.12) for example strongly hydrophilic reversed -phase copolymer or silica-based.

7.4 Reservoir column, normal volume 60 ml, with adaptor for cartridges (7.3), made of polypropylene or polyethylene.

7.5 Vacuum or pressure assembly, for the extraction step.

7.6 Sample tubes, made of, for example, polypropylene or polyethylene, for collecting and concentrating the eluate, e.g. test tube, nominal volume of 15 ml.

7.7 Volumetric flasks, with inert stoppers, made of polypropylene or polyethylene not containing fluoropolymer materials, normal volume 10 ml, 50 ml, 100 ml and 500 ml.

7.8 Graduated cylinder, normal volume 50 ml, 100 ml and 500 ml.

7.9 Evaporation assembly, using a nitrogen (6.13) stream passing through a stainless-steel needle.

7.10 Vials, made of polypropylene or polyethylene not containing fluoropolymer materials, capacity e.g. 1,5 ml, depending on the auto-sampler, with e.g. polyethylene snap-on caps.

7.11 Liquid chromatograph, temperature-controlled and with all necessary accessories, including gases, LC columns (see Annex B), injector and tandem mass spectrometer (7.12).

7.12 Tandem mass spectrometer, with an ion source capable of generating ions for the analytes of interests [e.g. electrospray ionization (ESI)] and capable of determining the *m/z* values of selected precursor ions and product ions of the target substances listed in Table 2 and Table 3.

7.13 Analytical balance, capable of weighting to the nearest 0,1 g.

7.14 Centrifuge, capable of 3 000 rpm.

7.15 pH indicator paper.

8 Sampling

Take, preserve and handle samples as specified in ISO 5667-1 and ISO 5667-3.

For sampling, use thoroughly cleaned sample bottles (7.1). Fill the bottle with the water to be sampled.

Store samples in a refrigerator at (5 ± 3) °C and analyse within four weeks.

PFAS compounds with 11 or more carbon atoms may fall out of solution during storage. 8:2 FTUCA in sea water samples is not stable for four weeks. Sample storage conditions should be checked to confirm maximum sample storage times. A storage study should be conducted during the method validation stage for all analytes routinely determined. The entire sample shall be extracted. If the entire sample is analysed and the sample bottle is rinsed with solvent, the longer chain compounds should be quantitatively recovered.

9 Procedure

9.1 Solid-phase extraction

9.1.1 General

In general, in this procedure, samples are analysed without pre-treatment. Before starting the analysis, the sample and internal standard substances (6.9.6) shall have time to equilibrate to room temperature before analysis.

NOTE Annex F and Annex G provide examples of sample preparation for rapid methods without sample extraction by solid-phase extraction in 9.1, but these procedures do not have sufficient data for method validation.

9.1.2 Sample preparation

Weigh the sample bottle with its original cap and water sample, to the nearest 1 g or mark the line on the sample bottle (7.1) with the sample volume.

The entire sample shall be extracted. The water sample which is collected into the sample bottle (7.1) shall not be separated into new sample bottles to avoid losses of target analytes due to sorption to the sample bottle (7.1).

The pH value of the sample shall be adjusted to the pH value of 3 with acetic acid (6.2) or ammonia solution (6.4) by pH indicator paper (7.15), if necessary.

NOTE Low recoveries of internal standard substances (6.9.6) can be improved by adjusting the pH value to 3, especially for short chain PFAS such as PFBA in a sea water sample.

Add the spiking solution containing the internal standard substances (6.9.6) to the water sample in the sample bottle (adding e.g. 100 µl of each, actual amount can be adjusted depending on the sample matrix) and mix thoroughly by shaking.

If the solid-phase extraction cartridge becomes clogged due to large amounts of suspended particulate in the sample, it may be possible to carry out the operation in Annex D or to divide the sample between two cartridges and pool the extracts. There may be a risk of increased blank level, which shall be checked for.

9.1.3 Conditioning of the solid-phase extraction material

The following procedure describes that used for commercially available 6 ml copolymer cartridges packed with 150 mg of sorbent sandwiched between two polyethylene frits.

Wash the cartridge in the following sequence with 4 ml of ammonia/methanol solution (6.11), 4 ml of methanol (6.6) and lastly 4 ml of water (6.1) prior to use. Make sure that the sorbent packing in the cartridge does not run dry. Retain the water in the cartridge (with the water level just above the packing) to keep the sorbent activated.

NOTE The solvent and water volumes used for conditioning depend on the amount the solid phase material used (for examples see Annex A).

9.1.4 Sample extraction

Start the extraction immediately after conditioning the sorbent packing. Make sure that no air bubbles are trapped in the sorbent bed when changing from conditioning to extraction. Do not let the sorbent material in the cartridge go dry and ensure it is immersed in water at all times.

Let sample (see 9.1.2) run through the cartridge, conditioned as specified in 9.1.3, at a rate of one drop per second (3 ml/min to 6 ml/min). Regulate the flow rate by changing the vacuum or the pressure (7.5), respectively.

Collect the sample, using a reservoir column (7.4) connected to the cartridge (7.3) with an adaptor.

Extract the entire sample in the sample bottle (7.1), to avoid losses resulting from adsorption to the wall of sample bottle (7.1).

Rinse the wall of sample bottle (7.1) and reservoir column (7.4) with a volume of methanol (6.6) which corresponds to at least 0,5 % of original sample volume. This aliquot of methanol is collected and used as elution solvent for sample extraction (see 9.1.5).

NOTE In the case of the longer chain PFAS such as PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxDA and PFOcDA, loss can result from adsorption to the sample bottle (7.1) and reservoir column (7.4). Losses due to sorption to the sample bottle can be reduced by rinsing the sample bottle with methanol.

Measure the volume (in millilitres) of the water used in the extraction by reweighting the empty sample bottle with its original cap and calculate the net mass of sample, to the nearest 1 g, from the difference in weight (see 9.1.2). Assuming a density of 1 g/ml, the value of the net mass (in grams) is equivalent to the volume (in millilitres) of the water used in the extraction. Alternatively, add water (6.1) to the empty sample bottle up to the mark (see 9.1.2), and measure the water volume using a graduated cylinder (7.8). This volume is equivalent to the volume (in millilitres) of the original water sample.

9.1.5 Elution

Add 4 ml of water (6.1) and 4 ml of acetate buffer solution (6.10) to the cartridge and discard the eluate.

The water (6.1) volume may be increased to remove interferences in the cartridge, if necessary. Low recoveries of internal standard substances (see 12.2) can be improved by increasing the amount of water (6.1), especially for short chain PFAS such as PFBA in sea water samples.

Centrifuge (7.14) the cartridge at 1 500 *g* for about 2 min or apply a vacuum to completely remove the residual solution from the cartridge. Then elute the target substances with 4 ml of methanol (6.6), followed by 4 ml of 0,1 % ammonia/methanol (6.11) at a rate of one drop per second and collect into the sample tube (7.6), separately.

NOTE 1 The solvent and water volumes used for washing and for the elution of the analytes depend on the used mass of the solid phase material (for examples see Annex A).

NOTE 2 Methanol which is used to rinse the walls of sample bottle (7.1) and reservoir column (7.4) (see 9.1.4) is used as the elution solution.

NOTE 3 Neutral substances such as FOSA, *N*-MeFOSA and *N*-EtFOSA elute with methanol (6.6). Anionic substances such as PFOS and PFOA elute with ammonia/methanol (6.11).

Evaporate the eluate with a gentle stream of nitrogen gas (6.13) to a final volume of e.g. 1 ml. The extract is now ready for LC-MS/MS analysis. The final extract volume may be adjusted by dilution with methanol, depending on the expected concentrations of the target substances in the sample. The concentration of the sample should be adjusted (by dilution or concentration) so that the concentrations of the target substances lie within the calibration range of the instrument. Store the extract at (5 ± 3) °C in the dark until analysis.

9.2 LC-MS/MS operating conditions

Optimize the operating conditions of the LC-MS/MS system in the electrospray ionization (ESI) negative mode in accordance with the manufacturer's instructions. The appropriate LC gradient programme for the mobile phase is determined experimentally during method development and validation. For optimum sensitivity, selected ions for MS/MS transitions are listed in Table 2 and Table 3. An example of typical operating conditions is given in Annex C.

NOTE Usually, optimum conditions for chromatography and detection are achieved with water (6.1) and methanol (6.6) in the presence of ammonium acetate (6.5) and, if appropriate, acetonitrile (6.3).

Table 2 — Selected diagnostic ions used in the determination (target substance)

Analyte	Selected diagnostic ions *m/z*		
	Precursor M_1[a]	Quantifier M_2[a]	Qualifier M_3[a]
PFBS	299	80	99
PFHxS	399	80	99
PFHpS	449	80	99
PFOS	499	80	99
PFDS	599	80	99
FOSA	498	78	169
N-MeFOSA	512	169	219
N-EtFOSA	526	169	219
N-MeFOSAA	570	419	512
N-EtFOSAA	584	419	526
6:2 FTSA	427	407	81
8:2 FTSA	527	507	81
9Cl-PF3ONS	531	351	83
PFBA	213	169	

[a] M_1 is the precursor ion used to obtain the product ion. M_2 is used as the product ion for the quantitation. M_3 can be used for confirmation.

Table 2 *(continued)*

Analyte	Selected diagnostic ions *m/z*		
	Precursor M_1[a]	Quantifier M_2[a]	Qualifier M_3[a]
PFPeA	263	219	69
PFHxA	313	269	119
PFHpA	363	319	169
PFOA	413	369	169
PFNA	463	419	219
PFDA	513	469	219
PFUnDA	563	519	269
PFDoDA	613	569	269
PFTrDA	663	619	269
PFTeDA	713	669	369
PFHxDA	813	769	369
PFOcDA	913	869	369
8:2 FTUCA	457	393	343
8:2 diPAP	989	97	543
HFPO-DA	329	169	285
DONA	377	251	85

[a] M_1 is the precursor ion used to obtain the product ion. M_2 is used as the product ion for the quantitation. M_3 can be used for confirmation.

Table 3 — Selected diagnostic ions used in the determination (internal standard) and corresponding analyte

Analyte	Corresponding analyte	Selected diagnostic ions *m/z*		
		Precursor M_1[a]	Quantifier M_2[a]	Qualifier M_3[a]
$^{13}C_3$-PFBS	PFBS	302	80	99
$^{13}C_3$-PFHxS	PFHxS	402	80	99
$^{18}O_2$-PFHxS	PFHxS	403	84	103
$^{13}C_4$-PFOS	PFOS/PFDS/9Cl-PF3ONS	503	80	99
$^{13}C_8$-PFOS	PFOS/PFDS/9Cl-PF3ONS	507	80	99
$^{13}C_8$-FOSA	FOSA	506	78	172
d_3-*N*-MeFOSA	*N*-MeFOSA	515	169	219
d_5-*N*-EtFOSA	*N*-EtFOSA	531	169	219
d_3-*N*-MeFOSAA	*N*-MeFOSAA	573	419	515
d_5-*N*-EtFOSAA	*N*-EtFOSAA	589	419	531
$^{13}C_2$-6:2 FTSA	6:2 FTSA	429	409	81
$^{13}C_2$-8:2 FTSA	8:2 FTSA	529	509	81
$^{13}C_4$-PFBA	PFBA	217	172	
$^{13}C_5$-PFPeA	PFPeA	268	223	
$^{13}C_2$-PFHxA	PFHxA	315	270	119
$^{13}C_5$-PFHxA	PFHxA	318	273	120
$^{13}C_4$-PFHpA	PFHpA/DONA	367	322	169
$^{13}C_4$-PFOA	PFOA	417	372	169

[a] M_1 is the precursor ion used to obtain the product ion. M_2 is used as the product ion for the quantitation. M_3 can be used for confirmation.

Table 3 *(continued)*

Analyte	Corresponding analyte	Selected diagnostic ions *m/z*		
		Precursor M_1[a]	Quantifier M_2[a]	Qualifier M_3[a]
$^{13}C_8$-PFOA	PFOA	421	376	172
$^{13}C_5$-PFNA	PFNA	468	423	219
$^{13}C_9$-PFNA	PFNA	472	427	223
$^{13}C_2$-PFDA	PFDA	515	470	219
$^{13}C_6$-PFDA	PFDA	519	474	219
$^{13}C_2$-PFUnDA	PFUnDA	565	520	269
$^{13}C_7$-PFUnDA	PFUnDA	570	525	269
$^{13}C_2$-PFDoDA	PFDoDA/PFTrDA	615	570	269
$^{13}C_2$-PFTeDA	PFTeDA	715	670	369
$^{13}C_2$-PFHxDA	PFHxDA/PFOcDA	815	770	369
$^{13}C_2$-8:2 FTUCA	8:2 FTUCA	459	394	344
$^{13}C_4$-8:2 diPAP	8:2 diPAP	993	97	545
$^{13}C_3$- HFPO-DA	HFPO-DA	332	169	287

[a] M_1 is the precursor ion used to obtain the product ion. M_2 is used as the product ion for the quantitation. M_3 can be used for confirmation.

9.3 Blank determination

Treat the blank in exactly the same manner as the samples, except that the sample is replaced by the appropriate amount of pure water (6.1). Procedural blanks should be analysed with each batch of samples (a maximum of 20 samples).

In case of blank values exceeding 10 % of the lowest concentration level in the samples, clarify the reason by systematic examinations (i.e. root cause analysis) to eliminate the source of contamination.

NOTE Background contamination can arise from in the instrument. A delay column can be attached between the solvent mixer and injection valve to chromatographically resolve these background contaminants from the instrument and/or mobile phases from the target analytes.

9.4 Identification

Determine the optimum settings for ionization under the specified chromatographic conditions for each substance (Table 2 and Table 3), in the negative mode, where the ions of the type $[M–H]^-$ are formed.

NOTE 1 The peaks of short chain of PFAS such as PFBA may not be well retained on the octadecyl silyl (ODS) LC column. The mix mode ion exchange LC column can be used for short chain PFAS which has superior retention properties, especially for short chain PFAS. For examples see Annex C.2.

Choose the substance-specific settings such that two product ions are obtained per substance, if possible. Identification using only one product ion may lead to erroneous results, if there are co-elution of interferences to target analytes. The two or more different LC columns may be used (for examples see Annex B and Annex C) if only one product ion is selected.

NOTE 2 PFBA yields just one product ion. In the case of PFPeA and PFHxA, the intensity of the second product ion is usually not sufficient to support positive results.

Each peak should be registered with a minimum of ten data points across the peak.

Peak identification is by comparison of the retention times and relative signal intensities observed for the ions monitored (see Table 2 and Table 3) in the samples compared with those observed for the reference substances.

The retention time for a target substance peak shall agree with the retention time observed for the target substance in the reference solution (6.9.7) within an acceptable tolerance. This applies to comparison by either absolute or relative retention time. The absolute or relative retention time measured in the sample shall meet the criteria in ISO 21253-1. The relative abundance of diagnostic ions observed for samples shall meet the criteria in ISO 21253-1.

The branched isomers may be present in environmental samples, especially for PFOS. Incomplete separation of the branched isomers from the linear isomer can lead to co-elution, which may interfere with the analysis. The linear isomer can be separated from the others by using specific chromatography columns (see Annex B) and by optimizing the chromatographic conditions. The linear isomers of PFAS are the target substances of this method, and separation of the linear isomer from the other, branched, isomers shall be demonstrated.

NOTE 3 Example of analysis of individual branched isomers of PFAS is described in Annex E.

10 Calibration

10.1 General requirements

For practical reasons, calibration uses a solution containing the native analytes of interest and labelled internal standards (see Table 2 and Table 3).

Ensure there is a linear dependence between signal and concentration. Determine the linear working range using at least five measurements at different concentrations (see ISO 8466-1), e.g. 0,002 ng/ml, 0,01 ng/ml, 0,05 ng/ml, 0,2 ng/ml, 1 ng/ml and 5 ng/ml as reference substances (6.9.7).

The calibration function for a compound is valid only for the measured concentration range. Additionally, the calibration function depends on the condition of the instrument, which shall be checked regularly. For routine analysis, a regular check of the calibration function by means of a two-point continuing calibration verification is sufficient.

As far as the establishment of the calibration functions is concerned, two methods are described:

a) calibration using an external standard (see 10.2);

b) calibration using an internal standard (see 10.3).

NOTE Internal standard calibration is preferred when the internal standards are available.

Table 4 gives an explanation of the subscripts used in the following formulae and text.

Table 4 — Explanation of subscripts

Subscript	Meaning
i	Identity of the target substance
e	Calibration
I	Identity of the internal standard
g	Overall procedure

10.2 Calibration using an external standard

Perform this type of calibration in all cases, as it is required for the determination of recovery for this standard as well as for optimizing individual working steps of sample processing.

For establishing the calibration, inject reference solutions containing all substances to be determined (6.9.7).

Use the same solvent composition for the reference solutions (6.9.7) and for the extracts.

Prepare a plot of the results, plotting the values y_{ie} (using peak areas, peak heights or integration units) for each target substance as the ordinate and the associated mass concentrations ρ_{ie} as the abscissa.

NOTE The use of peak heights may not result in accurate determination of concentration if peak shapes are not Gaussian or tailing.

Determine the linear regression function using the corresponding pairs of values y_{ie} and ρ_{ie} in the measurement series in accordance with Formula (1):

$$y_{ie} = a_{ie} \cdot \rho_{ie} + b_{ie} \quad (1)$$

where

y_{ie} is the measured response (dependent variable) of target substance i in the calibration as function of ρ_{ie}, expressed in units which will depend on the method of measurement used, e.g. area;

ρ_{ie} is the mass concentration (independent variable) of target substance i in the calibration solution, expressed in nanograms per litre, ng/l;

a_{ie} is the slope of the calibration curve of y_{ie} as a function of the mass concentration ρ_{ie}, often called the response factor;

b_{ie} is the ordinate intercept of the calibration curve.

10.3 Calibration using an internal standard

When using labelled internal standards, the determination of the concentration is corrected for errors made during injection. Also, errors caused by sample losses during particular steps of sample pre-treatment or the adjustment of the final sample extract volume, as well as by matrix effects in the sample, are minimized.

Use internal standards compounds that are equal to substances under investigation which are labelled with different isotopes, e.g. deuterated or ^{13}C-labelled compounds (see Table 3).

Analytes for which no isotopically labelled compounds are available may be evaluated using other internal standards, if they have been tested and documented that the analyte recovery calculated from spiked matrix samples of the specific examined sample types is within the same range as the recovery of the selected internal standard. If no internal standards are available for any specific analytes, use the external standard calibration procedure (see 10.2).

NOTE Using an internal standard that is different from the analyte of interest can result in a significant bias for the determination of concentration. During specific sample pre-treatment steps, the adjustment of the final sample extract volume, and/or matrix effects in the sample, an internal standard that is different from the analyte of interest will act differently than the target substance to be determined.

Add the internal standards prior to extraction of the target substances from the samples. The spiking solution of internal standard (6.9.6) e.g. 100 µl, where the mass is known, to be added depends on the sample volume and on the expected concentration of the target substances in the sample.

For establishing the calibration, inject reference solutions containing all substances to be determined and the internal standards (6.9.7).

Use the same solvent composition and internal standard concentrations for the reference solutions (6.9.7) and for the extracts.

Prepare a plot of the results, plotting the values of the ratio y_{ie}/y_{Iie} (see below) (using peak areas, peak heights or integration units) for each target substance as the ordinate and the associated ratio of the mass concentrations ρ_{ie}/ρ_{Iie} as the abscissa.

Determine the linear regression function using the corresponding pairs of values of y_{ie}/y_{Iie} and ρ_{ie}/ρ_{Iie} in the measurement series in accordance with Formula (2):

$$\frac{y_{ie}}{y_{Iie}} = a_{iIe}\frac{\rho_{ie}}{\rho_{Iie}} + b_{iIe} \tag{2}$$

where

y_{ie} is the measured response (dependent variable) of target substance *i* in the calibration as a function of ρ_{ie}, expressed in units which will depend on the method of measurement used, e.g. area;

y_{Iie} is the measured response of internal standard I of target substance *i* in the calibration as a function of ρ_{Iie}, expressed in units which will depend on the method of measurement used, e.g. area;

ρ_{ie} is the mass concentration (independent variable) of target substance *i* in the calibration solution, expressed in nanograms per litre, ng/l;

ρ_{Iie} is the mass concentration of internal standard I of target substance *i* in the calibration solution, expressed in nanograms per litre, ng/l;

a_{iIe} is the slope of the calibration curve of y_{ie}/y_{Iie} as a function of the mass concentration ratio ρ_{ie}/ρ_{Iie}, often called the response factor;

b_{iIe} is the ordinate intercept of the calibration curve.

11 Calculation

11.1 Use of a calibration curve to determine concentration

Determine the result for each sample using the calibration curve as described in Clause 10. The same procedure shall always be used for calibration, but the concentrations of the internal standard solutions used will depend on the samples being analysed. The concentrations used to prepare the calibration curve shall exceed the concentration range expected in the samples. Discard, if necessary, high or low points on the curve to provide a better linear fit over the concentration range most appropriate to the set of samples being analysed. Choose the one concentration of the reference solution (6.9.7) as quality control solution, inject quality control or calibration solutions at least every ten injections to check the stability and linearity of the LC-MS/MS curve. Prepare a new calibration curve if the LC-MS/MS conditions change or if the results of a calibration check using quality control/calibration solutions differ by more than 20 % from the original calibration curve.

11.2 Calculation of concentration using calibration with external standards

Calculate the mass concentration ρ_{ig} of target substance *i* in accordance with Formula (3) after solving Formula (1).

$$\rho_{ig} = \left(y_{ig} - b_{ie}\right)\cdot\frac{1}{a_{ie}} \tag{3}$$

where

y_{ig} is the measured response for target substance *i* in the sample, expressed in units which will depend on the method of measurement used, e.g. area;

ρ_{ig} is the mass concentration of target substance *i* in the sample, expressed in nanograms per litre, ng/l;

b_{ie} is the ordinate intercept of the calibration curve;

a_{ie} is the slope of the calibration curve of y_{ie} as a function of the mass concentration ρ_{ie}, often called the response factor.

11.3 Calculation of concentration using calibration with internal standards

Calculate the mass concentration ρ_{ig} of target compound *i* in accordance with Formula (4) after solving Formula (2).

$$\rho_{ig} = \left(\frac{y_{ig}}{y_{Iig}} - b_{iIe} \right) \cdot \frac{\rho_{Ii}}{a_{iIe}} \tag{4}$$

where

y_{ig} is the measured response for target substance *i* in the sample, expressed in units which will depend on the method of measurement used, e.g. area;

y_{Iig} is the measured response for internal standard I of target substance *i* in the sample, expressed in units which will depend on the method of measurement used, e.g. area;

ρ_{ig} is the mass concentration of target substance *i* in the sample, expressed in nanograms per litre, ng/l;

ρ_{Ii} is the predefined mass concentration of internal standard I of target substance *i* in the spiked
sample, expressed in nanograms per litre, ng/l;

b_{iIe} is the ordinate intercept of the calibration curve;

a_{iIe} is the slope of the calibration curve of y_{ie}/y_{Iie} as a function of the mass concentration ratio ρ_{ie}/ρ_{Iie}, often called the response factor.

11.4 Treatment of results outside the calibration range

If the concentrations of the target substances in the sample lie outside the range of the calibration curve, prepare a new calibration curve that matches the concentration range of the sample extracts.

12 Determination of analyte recovery

12.1 Recovery

For optimizing the method steps including extraction and sample preparation, determine the recovery for each target substance *i* at different concentration levels. Also, determine the recovery of the internal standards. The recovery at one concentration level should be analysed with each batch of samples (a maximum of 20 samples).

Spike e.g. 500 ml of water (6.1) with a spiking solution of reference substances (6.9.5) e.g. 100 µl and spiking solution of internal substances (6.9.6) e.g. 100 µl, process and analyse it in the same way as an actual water sample.

Calculate the recovery of reference substance using Formula (5):

$$w_i = \frac{\rho_{ig}}{\rho_i} \times 100 \quad (5)$$

where

w_i is the percent recovery of reference substance *i* from the spiked sample;

ρ_{ig} is the mass concentration of target substance *i* in the sample, expressed in nanograms per litre, ng/l;

ρ_i is the predefined mass concentration of target substance *i* in the spiked sample, expressed in nanograms per litre, ng/l.

The recovery of the reference substance calculated using Formula (5) shall be between 70 % and 125 % to be considered acceptable.

12.2 Recovery of internal standards

Calculate the recovery of internal standards using Formula (6):

$$w_{Ii} = \frac{\rho_{Iig}}{\rho_{Ii}} \times 100 \quad (6)$$

where

w_{Ii} is the percent recovery of internal standard I of target substance *i* from the spiked sample;

ρ_{Iig} is the determined mass concentration of internal standard I of target substance *i* in the spiked sample, expressed in nanograms per litre, ng/l;

ρ_{Ii} is the predefined mass concentration of internal standard I of target substance *i* in the spiked sample, expressed in nanograms per litre, ng/l.

The recovery of the internal standard calculated using Formula (6) shall be between 70 % and 125 % to be considered acceptable.

13 Expression of results

Report the results for the target substances listed in Table 1, in nanograms per litre, ng/l, to two significant figures. Results should be reported as the anion concentration. Results should not be corrected by blanks. Results for branched isomers and additional compounds may be reported, but shall be identified as such.

14 Test report

The test report shall contain at least the following information:

a) the test method used, together with a reference to this document, i.e. ISO 21675:2019;

b) all information necessary for the complete identification of the water sample;

c) the results obtained for the individual substances, expressed in accordance with Clause 11;

d) any deviation from this method and report of circumstances that may have affected the results;

e) the date of the analysis.

The method performance of this document is presented in Annex I.

Annex A
(informative)

Examples of suitable sorbents

Solid-phase adsorbent of performance at least equivalent to those given in Table A.1 should be used. When methanol (6.6) is used as an elution solvent, the final solution should be an alkaline methanol solution (e.g. prepared by dissolving 2 mg of sodium hydroxide in 100 ml of methanol) to prevent the esterification of PFOA. When aqueous ammonium methanol is used, it is already alkaline and therefore this step is not required.

NOTE The solid-phase absorbents given in Table A.1 (in alphabetical order) have already confirmed their applicability of this document.

This annex does not recommend the usage of any specific product.

Table A.1 — Examples of sorbents and conditions suitable for solid-phase extraction

Product name (supplier)[a]	Amount of sorbents and particle size	Sample pH	Conditioning solvents	Washing solvents	Elution solvents
Oasis®WAX[a] (Waters)	150 mg, 30 µm	3	4 ml of 0,1 % ammonia in methanol, 4 ml of methanol, 4 ml of water	4 ml of 25 mmol/l acetate buffer (pH 4), 4 ml of water	4 ml of methanol, 4 ml of 0,1 % ammonia in methanol
Oasis®WAX[a] (Waters)	500 mg, 30 µm	3	10 ml of 0,1 % ammonia in methanol, 10 ml of methanol, 4 ml of water	10 ml of 25 mmol/l acetate buffer (pH4), 50 ml of water	10 ml of methanol, 10 ml of 0,1 % ammonia in methanol
Presep®PFC-II[a] (WAKO)	60 mg, 30 µm	3	4 ml of 0,1 % ammonia in methanol, 4 ml of methanol, 4 ml of water	4 ml of water	4 ml of methanol, 4 ml of 0,1 % ammonia in methanol

[a] Oasis®WAX and Presep®PFC-II are examples of suitable products which are commercially available. Sorbents from other suppliers may be suitable, but they have not been evaluated for use in this method. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

Annex B
(informative)

Examples of suitable LC columns

This annex does not recommend the usage of any specific product.

A LC column of performance at least equivalent to those given in Table B.1 shall be used.

NOTE Examples of LC columns given in Table B.1 (listed in alphabetical order) are suitable for use with this document.

Table B.1 — Examples of suitable LC columns

Product[a]	Supplier
ACE®3 C18[a], 3 µm, 2,1 mm × 150 mm	Advanced Chromatography Technologies Ltd.
Betasil®C18[a], 5 µm, 2,1 mm × 50 mm	Thermo Electron
Luna®C18[a], 3 µm, 2,0 mm × 50 mm	Phenomenex Inc.
Shodex®RSpak JJ-50 2D[a], 2,0 mm × 150 mm	Showa Denko K.K.
Wakopac® Wakosil-II 3C18 RS[a], 3 µm, 2,0 mm × 150 mm	Wako Pure Chemical Industries Ltd.
[a] ACE®3 C18, Betasil®C18, Luna®C18, Shodex®RSpak JJ-50 2D and Wakopac® Wakosil-II 3C18 RS are examples of suitable products which are commercially available. LC columns from other suppliers may be suitable, but they have not been evaluated for use in this method. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products	

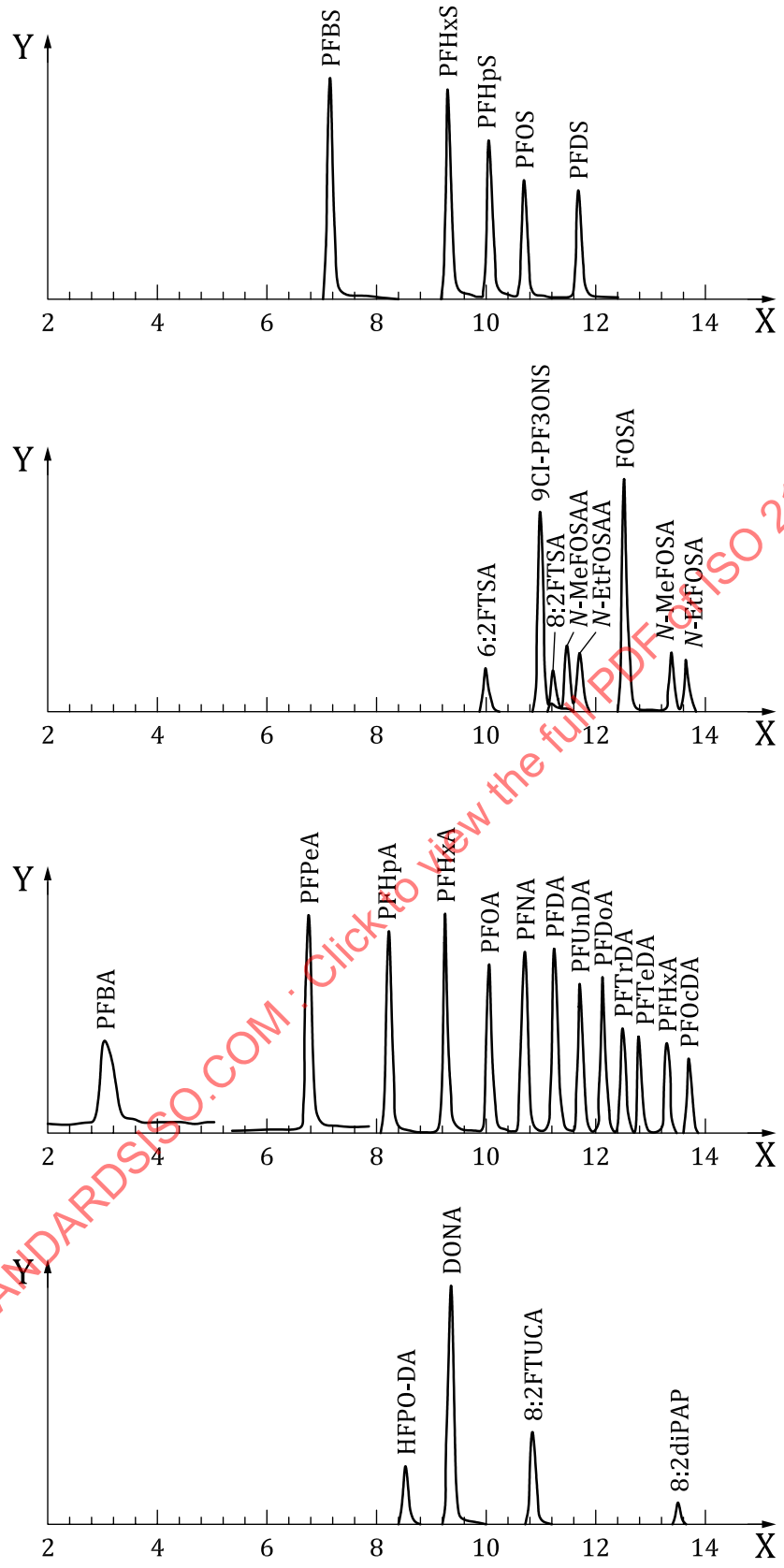
Annex C
(informative)

Examples of suitable LC-MS/MS conditions

C.1 Chromatographic conditions for the chromatograms in Figure C.1

Equipment:		Agilent® 1260a liquid chromatograph interfaced with a Sciex Triple Quad 4500[a]
LC	LC column[a]:	Main column: Betasil®C18[a], 5 µm, 2,1 mm × 50 mm
		Guard column: XDB®C8[a], 5 µm, 2,1 mm × 12,5 mm
	Injection volume:	5 µl
	Flow rate:	0,22 ml/min
	Mobile phase:	A: 9:1 2 mmol/l aqueous ammonium acetate solution in methanol
		B: Methanol
	Gradient program (% of B):	10% at 0,0 min, increase to 50 % at 2,0 min, 100 % at 10,0 min and keep at the level until 14,0 min, then revert to original condition at 15,0 min
	Column temperature:	30 °C
	Multiple draw injection program:	1. Draw 5 µl of sample 2. Draw 10 µl of 2 mM aqueous ammonium acetate solution 3. Inject
MS	Type of equipment:	Triple quadrupole
	Ionization:	ESI negative
	Mode:	Multiple reaction monitoring (MRM)
	Source temperature:	450 °C
	Ion source gas 1:	70 psi
	Ion source gas 2:	80 psi
	Curtain gas:	35 psi
	IonSpray voltage floating:	3 500 V

[a] Agilent 1260 liquid chromatograph interfaced with a Triple Quad 4500, Betasil®C18 and XDB®C8 are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.



Key

X time, min

Y intensity

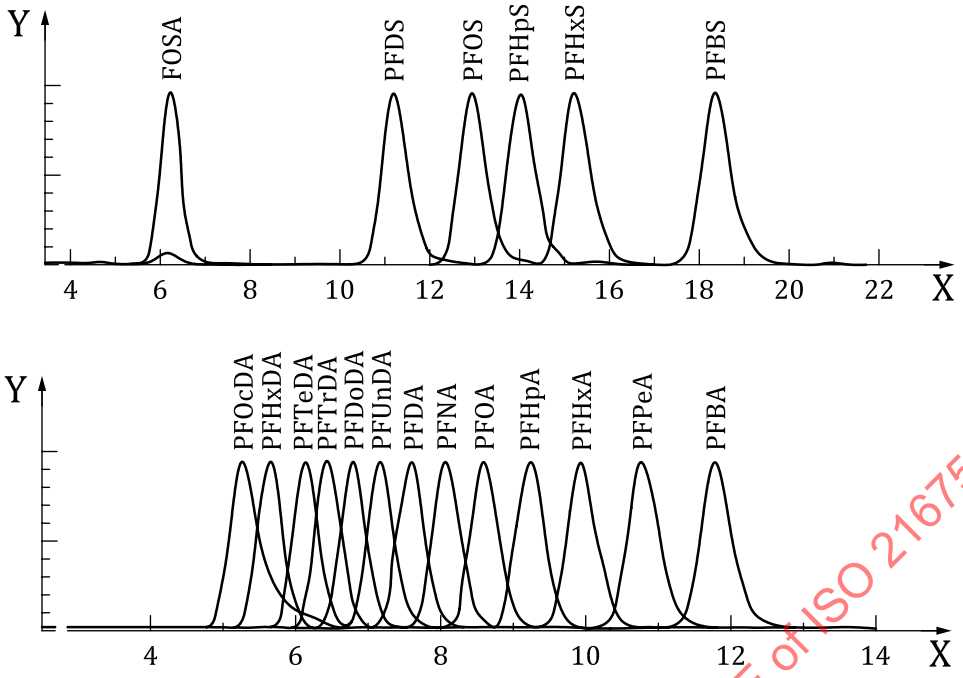
Figure C.1 — Chromatograms of PFAS, example 1

C.2 Chromatographic conditions for the chromatograms in Figure C.2

Equipment:		Agilent® HP1100[a] liquid chromatograph interfaced with a Micromass Quattro Ultima PT[a]
LC	LC column[a]:	Main column: Shodex®RSpak JJ-50 2D[a], 2,0 mm × 150 mm
		Guard column: OPTI-GUARD®mini[a], 1 mm × 15 mm
	Injection volume:	10 µl
	Flow rate:	0,3 ml/min
	Mobile phase:	A: 50 mmol/l aqueous ammonium acetate solution in methanol (pH 9)
		B: Methanol
	Gradient program (% of B):	80 % at isocratic condition
	Column temperature:	40 °C
MS	Type of equipment:	Triple quadrupole
	Ionization:	ESI negative
	Mode:	Multiple reaction monitoring (MRM)
	Capillary voltage:	1 kV
	Ion source temperature:	120 °C
	Desolvation temperature:	400 °C
	Cone gas flow rate:	60 l/h
	Desolvation gas flow rate:	650 l/h

NOTE The ammonium acetate solution (50 mmol/l) (pH 9) is obtained by preparing the ammonium acetate solution, and then adjusting it to pH 9 while adding aqueous ammonia.

[a] Agilent HP1100 liquid chromatograph interfaced with a Micromass Quattro Ultima Pt, Shodex®RSpak JJ-50 2D and OPTI-GUARD®mini are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.



Key

X time, min

Y intensity

Figure C.2 — Chromatograms of PFAS, example 2

Annex D
(normative)

Filtration and extraction of suspended matter in the sample

D.1 General

This annex specifies the method of removing the suspended matter from the sample through filtration operation and the method of extracting the target analyte remaining in suspended matter on the filter, which are applied when the sample contains a large amount of suspended matter. This method can be used if the solid-phase extraction cartridge becomes clogged due to large amounts of suspended matter in the sample or sample containing more than 2 g/l suspended matter.

Depending on local regulations, e.g. Ontario (Canada) Safe Drinking Water Act, samples may not be filtered. Analysts should align the method according to local regulatory requirements.

D.2 Description of the method

This method, which is to be performed after sample preparation as described in 9.1.2, consists of the procedure of suction-filtering the sample to remove the large amount of suspended matters present in the sample, and the procedure of extraction on the suspended matter on the filter with methanol by means of ultrasonic bath.

Recovery, as described in Clause 12, should be checked.

D.3 Reagents

The reagent used should be as follows.

D.3.1 Methanol, in accordance with 6.6.

D.4 Apparatus

The apparatus used should be as follows.

D.4.1 Filter, separation type.

D.4.2 Filtering material, nylon or glass fibre filter, 1 µm to 10 µm in pore-size.

D.4.3 Ultrasonic bath.

D.5 Procedure

The procedure shall be as follows.

a) Shake to homogenize the sample taken, disperse the suspended matters uniformly, then take the whole amount thereof and filter it under reduced pressure by using the filtering materials which has been cleaned with methanol.

b) Transfer the suspended matters retained on the filter material together with the filter into a polypropylene bottle, add 5 ml of methanol and perform the extraction procedure three times by

using an ultrasonic bath. Combine the eluates and evaporate with a gentle stream of nitrogen at a maximum temperature of 40 °C until it has concentrated to e.g. 1 ml.

c) Add the methanol concentrated solution in b) to the filtrate in a) and carry out the procedure of 9.1.3, 9.1.4 and 9.1.5. The methanol ratio to the combined samples should be less than 10 %.

Annex E
(informative)

Examples of chromatographic separation of individual linear and branched PFAS isomers

E.1 Examples for selected diagnostic ions

See Table E.1.

Table E.1 — Examples for selected diagnostic ions used in the determination for linear and branched PFOS and PFOA isomers

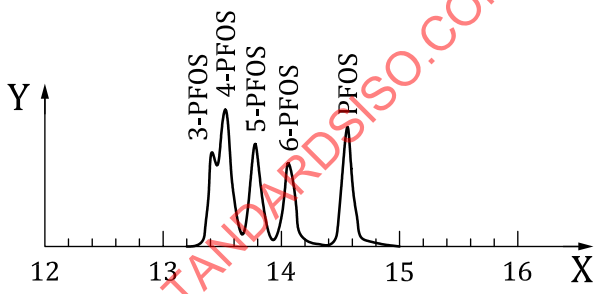
Analyte	Abbreviation	Selected diagnostic ions *m/z*		
		Precursor	Product	Qualifier
		M_1[a]	M_2[a]	M_3[a]
Perfluoro-n-octanesulfonic acid	PFOS	499	80	99
Perfluoro-1-methylheptanesulfonic acid	1-PFOS	499	419	
Perfluoro-3-methylheptanesulfonic acid	3-PFOS	499	280	
Perfluoro-4-methylheptanesulfonic acid	4-PFOS	499	230	
Perfluoro-5-methylheptanesulfonic acid	5-PFOS	499	130	280
Perfluoro-6-methylheptanesulfonic acid	6-PFOS	499	80	99
Perfluoro-n-octanoic acid	PFOA	413	369	169
Perfluoro-3-methylheptanoic acid	3-PFOA	413	369	169
Perfluoro-4-methylheptanoic acid	4-PFOA	413	119	
Perfluoro-5-methylheptanoic acid	5-PFOA	413	219	
Perfluoro-6-methylheptanoic acid	6-PFOA	413	369	169

[a] M1 is the precursor ion used to obtain the product ion. M_2 is used as the product ion for the quantitation. M_3 can be used for identification.

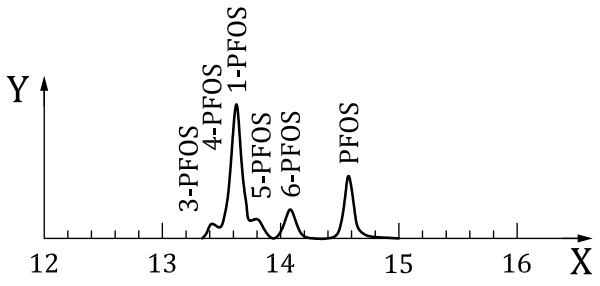
E.2 Chromatographic conditions for separation of linear and branched PFOS and PFOA isomers in Figure E.1 and Figure E.2

Equipment:		Agilent 1260[a] liquid chromatograph interfaced with a Sciex Triple Quad 4500[a]
LC	LC column[a]:	Main column: Ascentis Express F5 PFP column[a], 2,7 µm, 2,1 mm × 100 mm
		Guard column: Ascentis Express F5 PFP guard column[a], 2,7 µm, 2,1 mm ×5,0 mm
	Injection volume:	5 µl
	Flow rate:	0,25 ml/min
	Mobile phase:	A: 20 mmol/l ammonium formate/20 mmol/l formic acid in water
		B: Methanol
	Gradient program (% of B):	10 % at 0,0 min and keep at the level until 1 min, increase to 60 % at 3 min, 88 % at 20 min, 90 % at 20,5 min, then revert to original condition
	Column temperature:	30 °C
MS	Type of equipment:	Triple quadrupole
	Ionization:	ESI negative
	Mode:	Multiple reaction monitoring (MRM)
	Source temperature:	500 °C
	Ion source gas 1:	60 psi
	Ion source gas 2:	60 psi
	Curtain gas:	40 psi
	IonSpray voltage floating:	−4 500 V

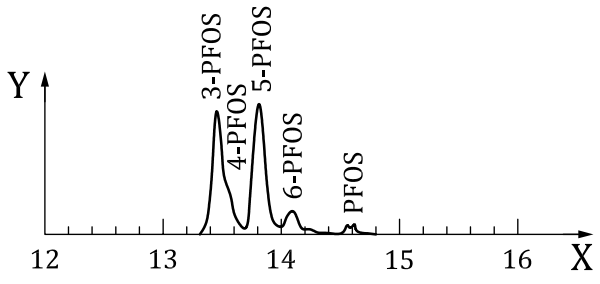
[a] Agilent 1260 liquid chromatograph interfaced with a Sciex Triple Quad 4500, Ascentis Express F5 PFP column and Ascentis Express F5 PFP guard column are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.



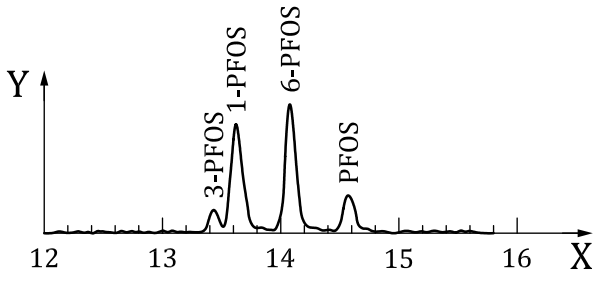
a) Precursor ion 499; product ion 80



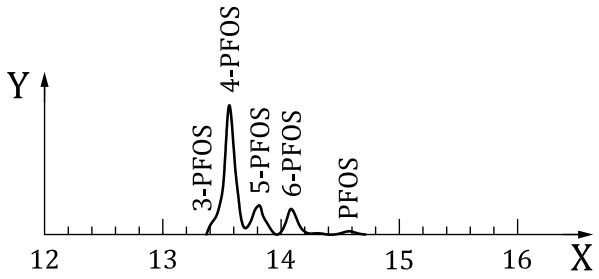
b) Precursor ion 499; production ion 99



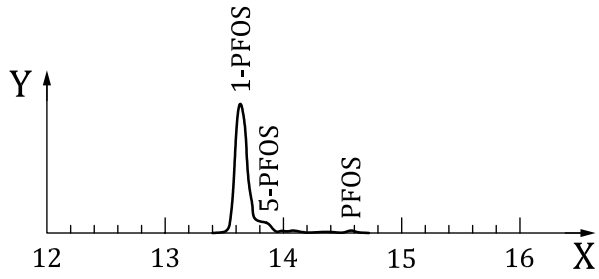
c) Precursor ion 499; product ion 130



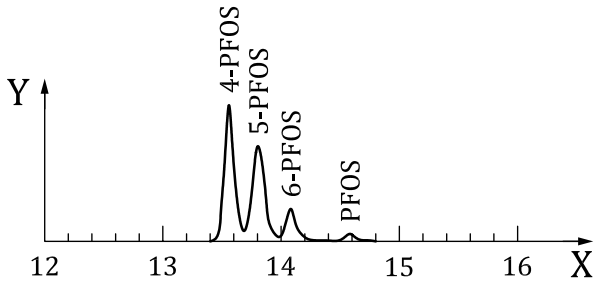
d) Precursor ion 499; product ion 169



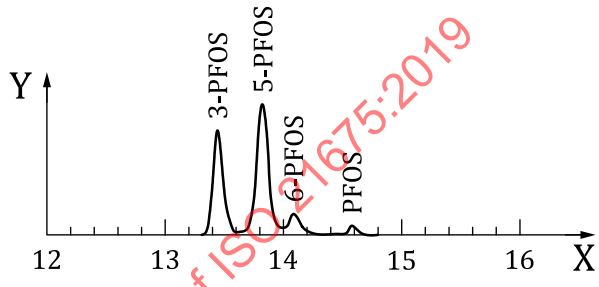
e) Precursor ion 499; product ion 180



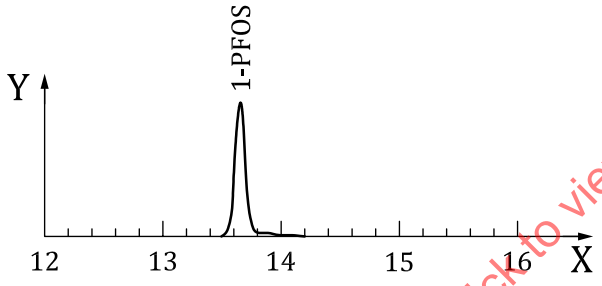
f) Precursor ion 499; product ion 219



g) Precursor ion 499; product ion 230



h) Precursor ion 499; product ion 280



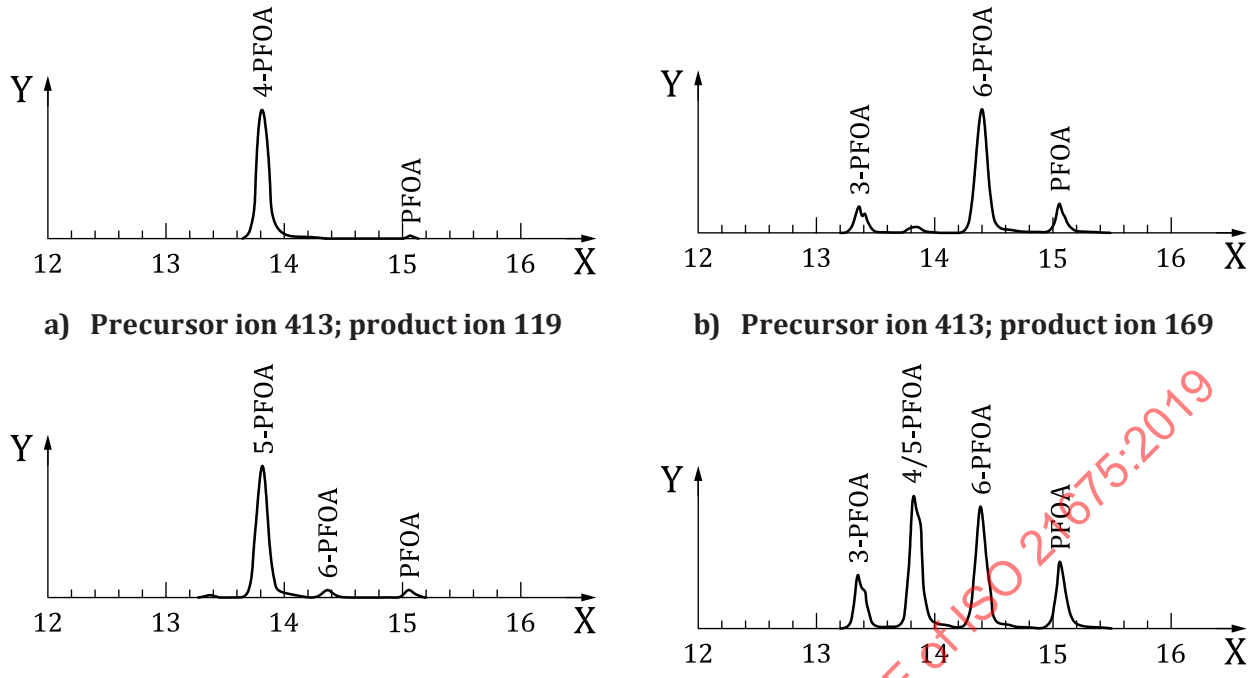
i) Precursor ion 499; product ion 419

Key

X time, min

Y intensity

Figure E.1 — Chromatograms of linear and branched PFOS isomers



a) Precursor ion 413; product ion 119

b) Precursor ion 413; product ion 169

c) Precursor ion 412; product ion 219

d) Precursor ion 412; product ion 369

Key

X time, min

Y intensity

Figure E.2 — Chromatograms of linear and branched PFOA isomers

Annex F
(informative)

Rapid method by direct injection

F.1 General

This annex gives an example of the sample preparation for a rapid method using direct injection analysis without filtration in Annex D or sample extraction by solid-phase extraction in 9.1. This procedure does not have sufficient data for method validation.

F.2 Reagents

The reagent used should be as follows.

F.2.1 Methanol, in accordance with 6.6.

F.2.2 Spiking solution (internal standard substances), in accordance with 6.9.6.

F.3 Apparatus

The apparatus used should be as follows.

F.3.1 Vials, in accordance with 7.10.

F.4 Procedure

The procedure shall be as follows.

a) The sample and spiking solution containing the internal standard substances (6.9.6) should be allowed to equilibrate to room temperature before analysis.

b) Transfer e.g. 250 µl of each sample to a vial (7.10). Then, add e.g. 50 µl of spiking solution containing the internal standard substances (6.9.6) and e.g. 200 µl of methanol (6.6) to make the final volume of e.g. 500 µl.

c) Cap each sample vial, and thoroughly mix the sample solution.

d) The concentration of the sample should preferably be adjusted by dilution so that the concentrations of the target substances lie within the calibration range of the instrument.

e) Analyse the direct injection samples using the routine method beginning with 9.2.

Annex G
(informative)

Rapid method by online solid phase extraction LC-MS/MS

G.1 General

This annex gives an example of the sample preparation for a rapid method using online solid phase extraction (OL-SPE) LC-MS/MS analysis without sample extraction by solid-phase extraction in 9.1. This procedure does not have sufficient data for method validation.

G.2 Reagents

The reagent used should be as follows.

G.2.1 Methanol, in accordance with 6.6.

G.2.2 Spiking solution (internal standard substances), in accordance with 6.9.6.

G.3 Apparatus

The apparatus used should be as follows.

G.3.1 Sample tubes, in accordance with 7.6.

G.3.2 Syringe filters, with a glass fibre filter, 1 µm in pore size, made of inert non-leaching plastic, e.g. polypropylene or polyethylene housing.

G.3.3 Syringes, made of inert non-leaching plastic, e.g. polypropylene or polyethylene housing.

G.3.4 OL-SPE columns, suitable for online solid phase extraction of the selected substances.

G.4 Procedure

The procedure shall be as follows.

a) The sample and spiking solution containing the internal standard substances (6.9.6) should be allowed to equilibrate to room temperature before analysis.

b) Shake to homogenize the sample take, disperse the suspended matters uniformly, and then take 16 ml of sample to a sample tube (7.6). Then, add 2 ml of methanol containing the internal standard substances e.g. 2 ml of spiking solution containing the internal standard substances (6.9.6) to make the final volume of 18 ml.

c) The syringe filter (G.3.2) connected to the syringe (G.3.3) is washed with methanol (6.6) before filtration. Filter the sample solution prepared in b), using a syringe filter (G.3.2) connected to the syringe (G.3.3) and collect the eluate into the sample tube (7.6). Suspended matter in the sample collected on the syringe filter (G.3.2) are extracted with twice of 1 ml of methanol (6.6) and collected eluate combined into the same sample tube (7.6).

d) The volume of eluate in c) is adjusted to the 20 ml with methanol (6.6). The resulting OL-SPE sample contains 20 % methanol.

e) e.g. 1,5 ml of OL-SPE sample in d) is injected into LC-MS/MS connected OL-SPE column (G.3.4).

f) Analyse the OL-SPE samples using the routine method beginning with 9.2. See G.5 for example for instrumental and operation conditions.

G.5 Example of instrumental conditions for analysis of PFOS and PFOA using online solid phase extraction LC-MS/MS in Table G.1

Equipment:		Standalone Waters 515 pump connected to Waters Acquity TQD tandem spectrometer[a]
LC	OL-SPE column[a]:	LiChrospher C8[a], 25 µm, 4 × 25 mm Polyclean 302H[a], 4,0 × 15 mm Upti-Trap
	LC column[a]:	X-Bridge C18[a], 2,5 µm, 2,1 mm × 50 mm
	Injection volume:	1,5 ml
	Flow rate:	0,3 ml/min (with LC separation column)
	Mobile phase for separation:	Solvent: Methanol with 20 mmol/l ammonium acetate
		Water phase: Water with 20 mmol/l ammonium acetate
	Water phase during loading:	Water with a volume fraction of 1 % formic acid
	Gradient program:	See Table G.1
	Column temperature:	40 °C
MS	Type of equipment:	Tandem quadrupole
	Ionization:	ESI negative
	Mode:	Multiple reaction monitoring (MRM)
	Capillary voltage:	3 kV
	Source temperature:	150 °C
	Desolvatation gas temperature (Nitrogen)	400 °C
	Desolvatation gas flow rate (Nitrogen):	800 l/h
	Curtain gas flow rate (Nitrogen):	50 l/h
	Reactant gas flow rate (Argon):	0,17 ml/min

[a] Standalone Waters 515 pump connected to Waters Acquity TQD tandem spectrometer, LiChrospher C8, Polyclean 302H and X-Bridge C18 are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

Table G.1 — Example for operating conditions of gradients for loading, injection, analysis and cleaning for analysis of PFOS and PFOA using online solid phase extraction LC-MS/MS

Time (min)	Water with volume fraction of 1 % formic acid	Water with 20 mmol/l ammonium acetate	Methanol with 20 mmol/l ammonium acetate	Acetonitrile	Flow rate (ml/min) (LC)	Status of OL-SPE column	Pump
0	100 %			0 %	2	1(1)	On
2	100 %			0 %	2	1(1)	On
2,1	100 %			0 %	0,3	1(1)	On
2,2		30 %	70 %	0 %	0,3	1(1)	On
2,8		30 %	70 %	0 %	0,3	2(2)	On
3,0		30 %	70 %	0 %	0,3	2(2)	Off
10,8		30 %	70 %	0 %	0,3	1(1)	On
11		30 %	70 %	0 %	0,3	1(1)	On
12				100 %	0,3	1(1)	On
12,2				100 %	2	1(1)	On
16				100 %	2	1(1)	On
20	100 %			0 %	2	1(1)	On

(1) Status 1: Loading - Serial elution of injection loop and OL-SPE column eluate is discarded.

(2) Status 2: Analysis - Elution of OL-SPE column to detector via separation column.