INTERNATIONAL STANDARD

ISO 25101

First edition
2009-03-01

Water quality — Determination of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) — Method for unfiltered samples using solid phase extraction and liquid chromatography/mass spectrometry

Qualité de l'eau — Détermination du sulfonate de perfluorooctane (PFOS) et de l'octanoate perfluoré (PFOA) — Méthode par extraction en phase solide et chromatographie liquide/spectrométrie de masse pour des échantillons non filtrés

Reference number
ISO 25101:2009(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 25101 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Water quality — Determination of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) — Method for unfiltered samples using solid phase extraction and liquid chromatography/mass spectrometry

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This standard 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 and to ensure compliance with any national regulatory conditions.

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

1 Scope

This International Standard specifies a method for the determination of the linear isomers of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) in unfiltered samples of drinking water, ground water and surface water (fresh water and sea water) using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Other isomers may be reported separately as non-linear isomers and qualified as such. The analytes specified in Table 1 can be determined by this method. The method is applicable to a concentration range of 2,0 ng/l to 10 000 ng/l for PFOS and 10 ng/l to 10 000 ng/l for PFOA. Depending on the matrix, the method may also be applicable to higher concentrations ranging from 100 ng/l to 200 000 ng/l after suitable dilution of the sample or reduction in sample size.

The user should be aware that particular problems could require the specification of additional conditions.

2 Normative references

The following referenced documents are indispensable for the application 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 3696:1987, *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 8466-1, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function*

3 Principle

The analytes listed in Table 1 are extracted from the water sample by solid-phase extraction followed by solvent elution and then determined by liquid chromatography with tandem mass-spectrometric detection.

NOTE This method is also applicable, with some limitations, to determination using high-performance liquid chromatography with single mass-spectrometric (HPLC-MS) detection (see Annex D).

Table 1 — Analytes determinable by this method

Analyte	Formula [a]	Abbreviation	CAS [b] No.
Perfluoro-*n*-octanesulfonic acid (1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-*n*-octanesulfonic acid)	$CF_3(CF_2)_7SO_3H$	PFOS	1763-23-1
Perfluoro-*n*-octanoic acid (pentadecafluoro-*n*-octanoic acid)	$CF_3(CF_2)_6COOH$	PFOA [c]	335-67-1

[a] The anion is the analyte.

[b] CAS = Chemical Abstract System.

[c] PFOA includes the acid and its salts.

4 Interferences

4.1 Interferences with sampling and extraction

Sampling containers shall consist of materials that do not change the composition of the sample during sample storage. All types of fluoropolymer plastics, including polytetrafluoroethene (PTFE) and fluoroelastomer materials, shall be avoided during sampling, sample storage and extraction. Glassware shall be avoided for sampling due to potential analyte loss due to adsorption. Sample containers shall be rinsed thoroughly with water (5.1) and methanol (5.5) prior to use. Sample containers shall be checked for possible background contamination before use.

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

4.2 Interferences with HPLC-MS/MS

Substances with similar retention times and producing ions similar to those produced by the analytes of interest may interfere with the determination.

These interferences may lead to incompletely resolved signals or additional signals in the chromatographic pattern of target analytes, or both. Depending on their levels in the sample, such substances may affect the accuracy and precision of the results.

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 wastewater and sea water matrices can be affected by matrix interferences that lead to ionization suppression or enhancement.

Interferences from instruments are significant for normal HPLC systems because many parts are made of PTFE and other fluoropolymers. It is necessary to check for possible blank contamination from 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. The HPLC-vial caps should preferably be free of fluoropolymer material. The procedural blank including the instrumental blank should preferably be at least 10-fold less than the expected concentrations in real samples.

5 Reagents

Use certified or analytical-grade reagents and check contamination levels of target compounds by blank determinations. If necessary, carry out additional cleaning steps to ensure background levels are minimized.

5.1 Water, complying with at least grade 3 as specified in ISO 3696:1987.

5.2 Acetic acid, $w(CH_3COOH) = 99{,}9$ % mass fraction.

5.3 Ammonia solution, $w(NH_3) = 25$ % mass fraction.

5.4 Ammonium acetate, $w(CH_3COONH_4) = 97$ % mass fraction.

5.5 Methanol (CH_3OH), HPLC grade.

5.6 Internal-standard solutions:

1,2,3,4-$^{13}C_4$-PFOA, $\rho = 1$ ng/µl.

1,2,3,4-$^{13}C_4$-PFOS, $\rho = 1$ ng/µl.

Solutions of the internal standards are available commercially. They shall be diluted to the required concentrations. If the standards are obtained as pure compounds, weigh 10 mg of each standard into a separate 100 ml volumetric flask and make up to the mark with methanol (5.5). Dilute the solution thus obtained initially by a factor of 100 with methanol (5.5).

Other internal standards, e.g. $^{13}C_5$-PFNA [perfluoro-*n*-nonanoic acid, $CF_3(CF_2)_6COOH$)], that meet the internal-standard requirements are acceptable for use. However, the purity of some of these commercially available standards is not adequate and, if such standards are used, the purity shall be determined in the laboratory. Analysis of impurities in standards shall be carried out prior to using new batches of standards.

5.7 Solutions of reference compounds of the analytes listed in Table 1, 0,1 ng/µl, used as calibration standards.

Weigh 10 mg of each reference compound into a separate 100 ml volumetric flask and make up to the mark with methanol (5.5). Dilute this solution serially with methanol (5.5) to give an overall dilution of 1:1 000. Standards may also be obtained as solutions if commercially available and diluted to the required concentration.

Store solutions 5.6 and 5.7 at a temperature of (4 ± 2) °C and bring them to room temperature prior to use (i.e. before dilution or spiking or injection).

5.8 Acetate buffer, 0,025 mol/l, pH 4.

Mix 0,5 ml of acetic acid (5.2) with 349,5 ml of water (5.1). Dissolve 0,116 g of ammonium acetate (5.4) in 60 ml of water (5.1). Mix 200 ml of the diluted acetic acid with 50 ml of the ammonium acetate solution.

5.9 Ammonia/methanol solution, $w = 0{,}1$ % mass fraction.

Mix 0,4 ml of 25 % ammonia solution (5.3), with 99,6 ml of methanol (5.5).

5.10 Solid-phase extraction material, copolymer-based. Suitable materials are available commercially (see Annex A).

5.11 Nitrogen (N_2), purity > 99,996 %.

5.12 Sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$).

6 Apparatus

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

Clean all labware and apparatus for solid-phase extraction by rinsing with water (5.1) and methanol (5.5).

6.1 Narrow-neck flat-bottomed polypropene bottles, capacity 1 000 ml, with conical shoulders and screw caps.

The bottles and screw caps shall be washed, rinsed with methanol (5.5) and dried before use in order to minimize contamination.

6.2 Solid-phase extraction cartridges, made of inert non-leaching plastic, e.g. polypropene.

The cartridges shall be packed with a minimum of 150 mg of solid-phase extraction material (5.10) as sorbent. In general, 150 mg to 250 mg of sorbent (Annex A) in a single cartridge is sufficient for up to 1 000 ml of water.

6.3 Vacuum or pressure assembly, for the extraction step.

6.4 Volumetric flasks, with inert stoppers.

6.5 Graduated cylinder, capacity 500 ml.

6.6 Evaporation assembly, using a nitrogen (5.11) stream passing through a stainless-steel needle.

6.7 Vials, made of polypropene or polyethylene not containing fluoropolymer materials, capacity e.g. 1,5 ml, depending on the auto-sampler.

6.8 High-performance liquid chromatograph, temperature-controlled and with all necessary accessories, including gases, HPLC columns, injector and tandem mass spectrometer (6.9).

6.9 Tandem mass spectrometer, capable of determining the m/z values of selected precursor ions and product ions of the target compounds listed in Table 2.

7 Sampling and sample pretreatment

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

For sampling, use thoroughly cleaned bottles (6.1). Fill the bottle only to the shoulder with the water to be sampled (approximately 1 000 ml). In the presence of free chlorine, immediately add approximately 80 mg of sodium thiosulfate pentahydrate (5.12) or another suitable dechlorinating agent (e.g. sodium sulfite).

Store samples in a refrigerator at (4 ± 2) °C and analyse within two weeks. If the sample cannot be analysed within two weeks of sampling, the sample may be frozen until analysis but its stability shall be checked during storage, if necessary.

8 Procedure

8.1 Solid-phase extraction

8.1.1 General

In general, in this procedure samples are analysed without pretreatment, i.e. suspended solids are not removed prior to analysis. Before starting the analysis, homogenize the sample by shaking.

8.1.2 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.

Rinse the cartridge, in the following sequence, with 4 ml of ammonia/methanol solution (5.9), 4 ml of methanol (5.5) and lastly 4 ml of water (5.1) 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.

8.1.3 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. Maintain the sorbent material in the cartridge immersed in water at all times.

Add the internal-standard solutions (5.6) to e.g. 500 ml of the water sample in the sample bottle (adding e.g. 100 µl of each, depending on the sample matrix) and mix thoroughly by shaking. Let this sample run through the cartridge, conditioned as specified in 8.1.2, at a rate of one drop per second (3 ml/min to 6 ml/min). With water samples containing more than 500 mg/l of suspended matter, carry out the extraction by passing 100 ml of sample through the cartridge. Remove residual water in the sorbent packing by applying a vacuum to the cartridge for 30 s. If the period of vacuum application is not enough to remove the water, repeat the vacuum application several times, but not for more than 2 min because overuse of vacuum may lead to loss of target compounds.

Reweigh 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. 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.

8.1.4 Elution

Add 4 ml of acetate buffer solution (5.8) to the dried cartridge and discard the eluate. Centrifuge the cartridge at 1 500g for about 2 min or apply a vacuum to remove completely the residual solution from the cartridge. Then elute the target analytes with 4 ml of methanol (5.5), followed by 4 ml of 0,1 % ammonia/methanol (5.9) at a rate of one drop per second. Evaporate the eluate with a gentle stream of nitrogen gas (5.11) to a final volume of 500 µl. The extract is now ready for HPLC-MS/MS analysis. The final extract volume may be adjusted by dilution with methanol, depending on the concentrations of the target analytes in the sample. The concentration of the sample should preferably be adjusted (by dilution or concentration) so that the concentrations of the target analytes lies within the calibration range of the instrument.

8.2 HPLC-MS/MS operating conditions

Optimize the operating conditions of the HPLC-MS/MS system in the electrospray ionization (ESI) negative mode in accordance with the manufacturer’s instructions. The appropriate HPLC 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. An example of typical operating conditions is given in Annex C.

8.3 Blank determination

Treat the blank in exactly the same manner as the samples, except that the sample is replaced by the appropriate amount of water (5.1). Procedural blanks should preferably be analysed with each batch of samples (a maximum of 10 samples).

8.4 Quality control samples

Analyse the quality control samples (procedural blank and spiked matrix sample) for each batch of samples to ensure accuracy and reliability of the analytical process. Spike samples with solutions of reference compounds (5.7), using volumes identical to those of the samples being analysed, and process them using the same procedure used for real samples.

8.5 Identification

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

The retention time for a target compound peak shall agree with the retention time observed for the reference compound to within 0,5 % when measured under identical conditions. This applies to comparison by either absolute or relative retention time. The relative abundance of diagnostic ions observed for samples shall match the abundance observed for reference compounds to within 25 %. It is important that both of the above criteria be satisfied in order to confirm the presence of a target compound.

Mixtures of PFOSs may contain branched isomers. Incomplete separation of the branched isomers from the linear (*n*-octyl) isomer can lead to co-elution, which may interfere with the analysis. The linear (*n*-octyl) isomer can be separated from the others by using specific chromatography columns (see Annex B) and by optimizing the chromatographic conditions. The linear isomer of PFOS is the target compound of this method, and separation of the linear isomer from the other, branched, isomers shall be demonstrated.

Table 2 — Selected diagnostic ions used in the determination

No.	Analyte	Abbreviation	Selected diagnostic ions *m/z*		
			Precursor M_1 [a]	Product M_2 [a]	Qualifier M_3 [a]
1	Perfluoro-*n*-octanesulfonic acid	PFOS	499	80	99
2	Perfluoro-*n*-octanoic acid	PFOA	413	369	169
3	1,2,3,4-$^{13}C_4$-Perfluoro-*n*-octanesulfonic acid [b]	$^{13}C_4$-PFOS	503	80	99
4	1,2,3,4-$^{13}C_4$-Perfluoro-*n*-octanoic acid [b]	$^{13}C_4$-PFOA	417	372	169

[a] M_2 is used as the product ion for the determination. M_3 may be used for identification. M_1 is the precursor ion used to obtain the product ion.

[b] Internal standard.

9 Calibration

9.1 General requirements

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

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).

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 two-point calibration is sufficient.

For routine analysis, a single calibration shall be carried out with internal standards over the complete analytical procedure. As the calibration is performed over the complete procedure with internal standards, determination of the recoveries is not necessary, but should preferably be recorded for quality control purposes.

Table 3 gives an explanation of the subscripts used in the following equations and text.

Table 3 — Explanation of subscripts

Subscript	Meaning
i	Indicates the identity of the target compound
e	Indicates calibration
I	Indicates the identity of the internal standard
g	Indicates the overall procedure

9.2 Calibration over the complete procedure with internal standards

When using labelled internal standards, the determination of the concentration is independent of any 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. The recovery of the internal standard calculated using Equation (1) shall be between 70 % and 125 % for the internal-standard batch to be considered acceptable.

Add the internal standards prior to extraction of the target compounds from the samples. The mass of internal standard to be added depends on the sample volume and on the expected concentration of the target compounds in the sample. The mass concentration ρ_I of the internal standard shall be the same for calibration and for sample measurement.

$$w_{\text{rec}} = \frac{\rho_{I\text{g}} \cdot V_{\text{sam}}}{\rho_I \cdot V_I} \cdot 100 \qquad (1)$$

where

w_{rec} is the percent recovery of internal standard I from the spiked sample;

$\rho_{I\text{g}}$ is the mass concentration of internal standard I in the spiked sample, expressed in nanograms per litre;

ρ_I is the mass concentration of internal standard I in the internal-standard solution used to spike the sample, expressed in nanograms per litre;

V_{sam} is the volume of the sample, expressed in litres;

V_I is the volume of the internal-standard solution added to the sample, expressed in litres.

Use the same solvent composition and internal-standard concentrations for the working standard solutions and for the extracts.

Prepare a plot of the results, plotting the values of the ratio $y_{i\text{e}}/y_{I\text{e}}$ (see below) (using peak areas, peak heights or integration units) for each target compound as the ordinate and the associated ratio of the mass concentrations $\rho_{i\text{e}}/\rho_{I\text{e}}$ as the abscissa.

Determine the linear regression function using the corresponding pairs of values of $y_{i\text{e}}/y_{I\text{e}}$ and $\rho_{i\text{e}}/\rho_{I\text{e}}$ in the measurement series in accordance with Equation (2):

$$\frac{y_{i\text{e}}}{y_{I\text{e}}} = a_{iI\text{e}} \frac{\rho_{i\text{e}}}{\rho_{I\text{e}}} + b_{iI\text{e}} \qquad (2)$$

where

$y_{i\text{e}}$ is a dependent variable corresponding to the measured response, expressed in units which will depend on the method of measurement used, e.g. area, for a given value of $\rho_{i\text{e}}$ of target compound i in the calibration;