



BSI Standards Publication

**Determination of extractable  
perfluorooctanesulphonate  
(PFOS) in coated and  
impregnated solid articles,  
liquids and fire fighting foams  
— Method for sampling,  
extraction and analysis by LC-  
qMS or LC-tandem/MS**

**National foreword**

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A list of organizations represented on this committee can be obtained on request to its secretary.

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**CEN/TS 15968**

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English Version

Determination of extractable perfluorooctanesulphonate (PFOS)  
in coated and impregnated solid articles, liquids and fire fighting  
foams - Method for sampling, extraction and analysis by LC-  
qMS or LC-tandem/MS

Détermination du sulfonate de perfluorooctane (SPFO)  
extractible dans des articles solides couchés et imprégnés,  
des liquides et des mousses anti-incendie - Méthode  
d'échantillonnage, d'extraction et d'analyse par LC-MS ou  
LC-MS/MS

Bestimmung von extrahierbarem Perfluorooctansulfonat  
(PFOS) in beschichteten und imprägnierten  
Feststoffartikeln, Flüssigkeiten und Feuerlöschschäumen -  
Verfahren zur Probenahme, Extraktion und Analyse mittels  
LC-MS oder LC-MS

This Technical Specification (CEN/TS) was approved by CEN on 25 October 2009 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

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EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: Avenue Marnix 17, B-1000 Brussels

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## **Foreword**

This document (CEN/TS 15968:2010) has been prepared by Technical Committee CEN/TC 382 “Project Committee - PFOS”, the secretariat of which is held by NEN.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association and supports essential requirements of REACH (1907/2006), annex XVII, designation 53 [1].

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to announce this Technical Specification: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and the United Kingdom.

## Introduction

For implementation of REACH (1907/2006), annex XVII, designation 53 [1] the European Commission asked CEN to develop an European Technical Specification with test methods for measuring levels of PFOS containing compounds in preparations, semi-finished products and articles including textile and coated materials.

REACH (1907/2006), annex XVII, designation 53 [1] states that the substances that are under the Directive are perfluorooctanesulfonates (PFOS), that means substances with the formula  $C_8F_{17}SO_2X$  (where  $X=OH$ , Metal salt (O-M<sup>+</sup>), halide, amide and other derivatives including polymers).

## 1 Scope

This Technical Specification describes the determination of perfluorooctanesulfonate (PFOS) in concentrated extracts from coated and impregnated solid articles, liquids and fire extinguishing foams using high performance liquid chromatography-tandem mass spectrometry (LC-tandemMS) or quadrupole mass spectrometry (LC-qMS).

The method is applicable for a concentration range for PFOS in the extract solution of 0,5 µg/l to 50 µg/l.

**WARNING — Persons using this Technical Specification should be familiar with normal laboratory practice. This Technical Specification 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 Technical Specification be carried out by suitably qualified staff.**

## 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.

EN 20187, *Paper, board and pulps — Standard atmosphere for conditioning and testing and procedure for monitoring the atmosphere and conditioning of samples (ISO 187:1990)*

EN ISO 2419, *Leather — Physical and mechanical tests — Sample preparation and conditioning (ISO 2419:2006)*

EN ISO 3696:1995, *Water for analytical laboratory use — Specification and test methods (ISO 3696:1987)*

EN ISO 8130-9, *Coating powders — Part 9: Sampling (ISO 8130-9:1992)*

EN ISO 15194, *In vitro diagnostic medical devices — Measurement of quantities in samples of biological origin — Requirements for certified reference materials and the content of supporting documentation (ISO 15194:2009)*

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 Terms and definitions

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

### 3.1

#### **analyte**

substance or chemical constituent that is subjected to measurement

### 3.2

#### **blank matrix**

matrix which is largely identical with the analytical one, but does not contain the analyte or contains it in low and known content

NOTE Blanks are used to check the analytical process.



### 3.3

#### **constituents**

all pure chemical materials and substances of which a material is composed

### 3.4

#### **extract**

concentrated preparation of the analytes isolated from the treated material

### 3.5

#### **foams**

fire extinguishing foams

### 3.6

#### **overall treated materials**

materials with a seamless finish

### 3.7

#### **internal standard**

compound different from the analyte, present in the sample with known content or added to the sample, simultaneously detected with the analyte, with physical and chemical properties as similar as possible to the analyte

### 3.8

#### **reference material**

##### **RM**

material, sufficiently homogeneous and stable regarding one or more properties, used in calibration, assignment of a value to another material, or quality assurance (conform EN ISO 15194)

### 3.9

#### **coated materials**

materials that are treated with chemicals to provide them with water and soil repellent properties; or treated for aesthetic or decorative reasons

## 4 Symbols and abbreviations

LC-qMS            liquid chromatography (LC) coupled with quadrupole mass spectrometry (qMS)

LC-tandemMS    liquid chromatography (LC) coupled with tandem mass spectrometry (tandemMS)

LC-TOFMS        liquid chromatography (LC) coupled with time of flight mass spectrometer (TOFMS)

## 5 Principle

The analytes listed in Table 1, after extraction with methanol, are determined by liquid chromatography with tandem mass spectrometric detection (LC-tandemMS) or liquid chromatography mass spectrometric detection (LC-qMS). The list of analytes is not comprehensive for all possible PFOS derivatives.

**Table 1 — Analytes determinable by this method**

Compound name	Chemical formula	Acronym	CAS No.
Perfluorooctane sulphonic acid	C8F17SO3H	PFOS	1763-23-1
Perfluorooctane sulphonamide	C8F17SO2NH2	PFOSA	754-91-6
N-Methyl-heptadecafluorooctane sulphonamide	C8F17SO2NH(CH3)	N-Me-FOSA	N.A. <sup>a</sup>
N-Ethyl-heptadecafluorooctane sulphonamide	C8F17SO2NH(C2H5)	N-Et-FOSA	N.A. <sup>a</sup>
N-Methyl-heptadecafluorooctane sulphonamidoethanol	C8F17SO2N(CH3)CH2CH2OH	N-Me-FOSE alcohol	24448-09-7
N-Ethyl-heptadecafluorooctane sulphonamidoethanol	C8F17SO2N(C2H5)CH2CH2OH	N-Et-FOSE alcohol	1691-99-2
<p>NOTE PFOS may be present in its salt form e.g. potassium salt (CAS 2795-39-3), lithium salt (CAS 29457-72-5), ammonium salt (CAS 29081-56-9) and diethanolamine salt (CAS 70225-39-5). Others may exist. The method described in this protocol is valid for all these different chemical forms.</p> <p><sup>a</sup> N.A.: Not available.</p>			

This Technical Specification describes the method for determining the PFOS concentration in the following steps:

- a) sampling (see Clause 7);
- b) extraction with methanol (see Clause 9);
- c) clean up of the methanol solution (see 9.2);
- d) analysis by LC-qMS or LC-tandemMS (see Clause 11);
- e) calculation of the results (see Clause 13).

## 6 Apparatus and Reagents

### 6.1 General

Sample containers shall be rinsed thoroughly with water (0) and methanol (0) prior to use. Sample containers shall be checked for possible background contamination before use.

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

Clean all lab-ware and accessible parts of apparatus for extraction by rinsing with methanol (0).

## 6.2 Equipment

Use equipment free from glass (e.g. PP, PE):

- cutting die or template and cutting tool to measure and cut area materials (textiles, leather, paper, etc.). Mill to grind granular bulk solids;
- 125 µm sieve;
- volumetric flasks, with inert stopper;
- vials, polypropylene or polyethylene;
- ultrasonic bath, equipped with adjustable and stable bath temperature (20 °C to 70 °C);
- concentration equipment (e.g. hydrophilic lipophilic base, SPE, weak anion exchange resin or rotavapor);
- high performance liquid chromatograph, temperature-controlled and with all required accessories including gases, LC columns, injector and a mass spectrometric detector either tandemMS or qMS. Alternative methods e.g. LC-/TOFMS are discussed in Annex A.

## 6.3 Reagents for extraction

**6.3.1 Methanol**, CH<sub>3</sub>OH (ultra pure).

## 6.4 Reagents for LC and MS

**6.4.1 Water**, complying with at least grade 3 as specified in EN ISO 3696:1995, also applicable for blank determinations.

**6.4.2 Acetic Acid**,  $w(\text{CH}_3\text{COOH}) \geq 99,9 \%$ .

**6.4.3 Ammonium acetate**,  $w(\text{CH}_3\text{COONH}_4) \geq 97 \%$ .

**6.4.4 Formic Acid**,  $w(\text{HCOOH}) \geq 99 \%$ .

**6.4.5 Methanol**, CH<sub>3</sub>OH (HPLC grade).

### 6.4.6 Gases for MS.

- Nitrogen, GC grade 5.0 or better;
- Collision gas (e.g. Argon);
- Helium, GC grade 5.0 or better.

### 6.4.7 Reference solutions.

- <sup>13</sup>C<sub>x</sub>-PFOS (e.g. [F(CF<sub>2</sub>)<sub>8</sub>SO<sub>3</sub><sup>-</sup>H<sup>+</sup>]<sup>-</sup>, 1,2,3,4-<sup>13</sup>C<sub>4</sub>); or
- <sup>18</sup>O<sub>x</sub>-PFOS (e.g. [F(CF<sub>2</sub>)<sub>8</sub>SO<sub>3</sub><sup>-</sup>H<sup>+</sup>]<sup>-</sup>, <sup>18</sup>O<sub>2</sub>).

In addition, when other types of labelled internal standards become available, they may be used.

Solutions of the reference compounds are available commercially. They should be diluted to required concentrations.

If reference compounds are obtained as neat, weigh 100 mg of each standard separately into a 100 ml volumetric flask and make up to the mark with methanol. Dilute this solution with methanol at a ratio 1:1 000.

The impurity levels of the internal standards should be determined prior to the use of new of every new lot.

**6.4.8 Range of calibration solutions**, in concentration range 0,5 µg/l to 50 µg/l.

## **7 Sampling**

### **7.1 General**

The chosen sample should be representative for the lot it is taken from. This document describes two different ways of reporting results which require two different ways of sample collection. One way is based on the content per area (mass/area). The other way is based on content per mass (mass/percentage).

### **7.2 Conditioning samples**

Materials and samples shall be stored preferably at 4 °C and in clean containers, see 6.2, before testing.

Conditioning of leather shall be done according to EN ISO 2419.

Conditioning of paper shall be done according to EN 20187.

### **7.3 Sampling solids**

#### **7.3.1 Coated materials like paper, textile, leather, carpets, clothes and footwear**

If applicable take a sample of at least 200 cm<sup>2</sup> using a cutting die or any other suitable tool and template; weigh the sample to make sure results can be reported as well in percentage/weight as in micrograms per square metre.

NOTE Make sure that the sample is not stretched (be aware that crinkled materials can easily have a much higher surface area).

If the material is not suitable to provide area-based samples, take a sample by weight, using at least 2 g of material.

#### **7.3.2 Non-coated materials**

Powders, granulates and other bulk materials shall be sampled according to EN ISO 8130-9.

For any other material an appropriate representative amount of the product shall be cut off, see 7.3.1.

### **7.4 Sampling products that have separate distinct parts**

Products consisting of separate distinctive parts shall be dismantled and each part shall be treated according to 7.3.1.

### **7.5 Sampling liquids**

The samples for testing shall be taken and delivered by the supplier using a method which will provide a representative sample of the liquid to be tested.

## 8 Sample pre-treatment

Sample materials need to be ground as appropriate to ensure an efficient extraction process.

Leather materials shall be cut in pieces of maximum 25 mm<sup>2</sup>.

Textile and paper shall be cut into pieces of maximum 1 cm<sup>2</sup> before methanol is added.

For grinding of polymers and granulates it is recommended to use EN ISO 6427 [2] or ISO 9113 [3].

## 9 Extraction

### 9.1 Extraction method for textile, fabrics, leather and paper

- a) Place the sample in a closed container to avoid loss of solvent through evaporation.
- b) Add a suitable quantity of labelled internal standard solution.
- c) Equilibrate the sample for at least 1 h.
- d) Add 50 ml methanol or as much as is needed to cover the treated material.
- e) Place in an ultrasonic bath for 2 h at 60 °C.

### 9.2 Clean-up method for textile, fabrics, leather and paper

Co-extracted materials can interfere in the determination by LC-tandemMS and LC-qMS. These can include e.g. natural lipids from leather. Their influence should be minimised to obtain accurate results.

Clean up of the methanol extract and preparation of the test:

- a) Concentrate the extract by a factor of 10 and use a clean-up if necessary.

NOTE Active carbon clean-up and/or solid phase extraction (SPE) clean-up may be used.

- b) Transfer a known volume into a suitable LC sampling vial and perform LC-qMS analysis according to Clause 11.
- c) If necessary dilute the original solution further and repeat the analysis.

### 9.3 Dilution method for aqueous fire extinguishing foams

The sample should be equilibrated at room temperature (20 ± 5) °C before any solutions or analyses are performed. Homogenise the sample and make up dilutions as follows:

- a) Pre-dilute the sample in separate steps with purified water or methanol (1:10 v/v, 1:100 v/v, 1:1 000 v/v) and mix sufficiently (e.g. 500 µl sample into 4 500 µl diluent).
- b) Ensure that there is no phase-separation in the sample.
- c) If necessary centrifuge the sample to precipitate non soluble particles.
- d) Dilute the pre-diluted sample 1:10 with water or methanol and an appropriate volume of internal standard solution (e.g. 100 µl diluted sample, 100 µl reference solution, 800 µl water or methanol) to have in every dilution (1:10 v/v, 1:100 v/v, 1:1 000 v/v) the same concentration of the internal standard.

- e) Transfer an aliquot to a LC vial and analyse the sample. If high PFOS concentrations are expected, begin with the highest dilution (1:10 000 v/v) to avoid a carry over.

NOTE 1 The dilution error introduced by this method should be much smaller than the analytical error involved in the method. As a general rule the dilution error should be less than 1 %.

NOTE 2 If it is possible and necessary a dilution of 1:10 v/v or a direct injection of the sample with internal standard solution is allowed.

#### **9.4 Extraction method for other liquids**

There is no best practice method available for the extraction of other liquids which cannot be extracted with SPE. The method in 9.3 may be used as an example for the extraction of other liquids.

NOTE ISO 25101 [4] describes a method for the determination of PFOS in unfiltered samples of drinking water, ground water and surface water.

### **10 Interference with LC-tandemMS**

Substances with similar retention times and similar fragment masses when compared with the analytes of interest can interfere with the determination.

These interferences can lead to incompletely resolved signals and additional signals in the chromatographic pattern of target analytes. They can, depending on their levels in the sample, affect the accuracy and precision of the analytical results.

Matrix interferences can 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.

### **11 Analysis**

#### **11.1 LC-tandemMS operating conditions**

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

#### **11.2 Blank determination**

Treat the blank in exactly the same way as the samples, except that the analyte is absent. After a maximum of ten samples of one batch at least one blank should be analysed.

#### **11.3 Quality Control samples**

Analyse the Quality Control (QC) samples (procedural blank and spiked matrix sample) for each batch of samples to ensure accuracy and reliability of the analytical process. Samples spiked with internal standard solutions should be analysed according to 9.1.

#### **11.4 Identification**

For confirmation of the identity of the analytes, the detection method should comply with the European Commission Decision 2002/657/EC [5]. PFOS and related compounds fall under group B of the Annex 1 of the above-mentioned EC Decision, which concerns veterinary drugs and contaminants. Hence, the number of

identification points required would be 3, and therefore LC-tandemMS (triple quad) using two transition products, or LC-qMS (single quad) using three ions typically qualify for the confirmation of PFOS, see also Annex C.

NOTE 1 The determination of the accurate mass (e.g. with Time of Flight MS) can be used for a confirmation of a positive result.

Identify the sample component by matching both retention times and relative intensities of the monitored ions (Table 2) of the sample components and the reference substances.

The target compound is present (identified) in the sample if the relative or the absolute sample component retention time measured in the mass chromatogram matches the relative or the absolute retention time of the authentic compound within  $\pm 0,2\%$  in the chromatogram of the latest calibration standard, measured under identical conditions.

The manufacture of PFOS results in a product that generally contains both the linear and the branched isomers. Therefore PFOS when analysed does not appear as a single peak, but rather as a number of peaks in the mass chromatograms. Branched isomers can co-elute as a single peak, but the linear (n-octyl) isomer can be separated from others using specific chromatography columns (Annex A). Because the availability of standards of the branched isomers is rather limited, the quantification of all isomers should be based on the response factor of the linear isomer. A similar approach is applicable to other PFOS compounds (e.g. PFOSA, PFOSE).

NOTE 2 Normally the branched isomers elute before the linear isomer.

**Table 2 — Selected diagnostic ions for quantification**

No	Analyte	Abbreviation	Selected diagnostic ions Primary Product Qualifier		
			M <sub>1</sub> <sup>a</sup>	M <sub>2</sub> <sup>a</sup>	M <sub>3</sub> <sup>a</sup>
1	n-octanesulphonic acid; 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-	PFOS	498,93	79,96	98,96
2	n-octanesulphonic acid; 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8 -heptadecafluoro- (1,2,3,4- <sup>13</sup> C <sub>4</sub> ) <sup>b</sup>	<sup>13</sup> C <sub>4</sub> -PFOS	502,94	79,96	98,96
3	Perfluorooctane sulphonate	PFOS	498,93	79,96	98,96
4	Perfluorooctane Sulphonamide	PFOSA	499,14	77,96	168,99
5	N-Methyl-heptadecafluorooctane Sulphonamide	N-Me-FOSA	511,96	168,99	482,93
6	N-Ethyl-heptadecafluorooctane Sulphonamide	N-Et-FOSA	525,98	168,99	482,93
7	N-Methyl-heptadecafluorooctane Sulphonamidoethanol	N-Me-FOSE	555,99	526,98	482,93
8	N-Ethyl-heptadecafluorooctane sulphonamidoethanol	N-Et-FOSE	570,00	541,00	482,93
M <sub>1</sub> <sup>a</sup> is the molecular weight of the parent ion. M <sub>2</sub> <sup>a</sup> is the molecular weight of the most abundant ion daughter ion. M <sub>3</sub> <sup>a</sup> is the molecular weight of the second most abundant daughter ion.					

## 12 System suitability test

### 12.1 Calibration

#### 12.1.1 General requirements

The calibration is based on solutions containing the analytes of interest and reference compounds (Table 2).

Ensure there is a linear dependence between signal and concentration. The working range will be from 0,5 µg/l to 50 µg/l for each compound (see 0). Determine the linear working range using at least five measurements at different concentrations conform ISO 8466-1.

The calibration curve for a substance is valid only for the measured concentration range. Additionally, the calibration curve depends on the condition of the instrument which shall be checked regularly. For routine analysis, a check (i.e. continuing calibration check) of the calibration curve by means of two-point calibration is sufficient.

For routine analysis, a single calibration over the total analytical run with labelled reference solutions shall be applied. As the calibration is performed over the total procedure with reference solutions, determination of the recoveries is not necessary, but should be recorded for quality control purposes.

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

**Table 3 — Explanation of subscripts**

Subscript	Meaning
i	Identity of the substance
e	Calibration step
l	Identity of labelled reference solution
g	Overall procedure

#### 12.1.2 Calibration over the total procedure with reference compounds

Calibrations shall be done using the linear isomer.

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

Plot the values of the ratio  $y_{ieg}$  and  $y_{leg}$  (peak areas, peaks heights or integration units) for each substance  $j$  or  $l$  on the ordinate and the associated concentration  $\rho_{ieg}$  or  $\rho_{leg}$  in micrograms per litre on the abscissa.

Establish the linear regression function using the corresponding pairs of values  $y$  and  $\rho$  of the measured series in accordance with Equations (1) or (2):

$$y_{ieg} = a_{ig} * \rho_{ieg} + b_{ig} \quad (1)$$

$$y_{leg} = a_{lg} * \rho_{leg} + b_{lg} \quad (2)$$

where

$y_{ieg}$  is the dependent variable corresponding to the measured response, expressed in units depending on the analytical method, e.g. area value, for a given  $\rho_{ieg}$  of substance  $i$  in the calibration;



$y_{ieg}$  is the dependent variable corresponding to the measured response, expressed in units depending on the analytical method, e.g. area value, for a given  $\rho_{ieg}$  of the labelled reference solution  $l$  in the calibration;

$\rho_{ieg}$  is the independent variable corresponding to the mass concentration, expressed in nanograms per millilitre, of substance  $i$  in the calibration solution;

$\rho_{ieg}$  is the independent variable corresponding to the mass concentration, expressed in nanograms per millilitre, of the labelled reference solution  $l$  in the calibration solution;

$a_{ig}$  is the slope of the calibration curve of substance  $l$  from  $y_{ieg}$  as a function of the mass concentration ratio  $\rho_{ieg} / l_{ieg}$ , often called the response factor;

$b_{ig}$  is the ordinate intercept of the calibration curve of substance  $i$ ;

$a_{ig}$  is the slope of the calibration curve of reference standard from  $y_{ieg}$  as a function of the mass concentration  $\rho_{ieg}$ , often called the response factor;

$b_{ig}$  is the ordinate intercept of the calibration curve of reference standard.

## 12.2 Recovery

The recovery of labelled reference compound shall be in the range from 70 % to 125 % for the sample to be considered valid.

## 12.3 Blank measurement

The procedural blank shall be at least threefold less than the limit of quantification of the method.

## 13 Calculation

### 13.1 Calibration curve for quantification

Quantify the samples by using the unextracted external calibration curve. Only the calibration procedure should be standardised by selecting some calibration curve points, but not the concentration of the standard solutions. Concentrations used in the calibration curve shall bracket the concentrations in samples. Discard, if necessary, high or low points on the curve to provide a better linear fit over the curve range most appropriate to the set of samples being analysed. Standards shall be injected regularly to check for the stability and linearity of LC. Perform a new calibration curve if the conditions of LC change or the continuing calibration check samples deviate by more than 20 % from the original calibration curve.

All the branched isomers have to be calculated using the response factors of the linear isomers.

When spiking with labelled reference solutions, the determination of the concentration is independent of possible errors made during injection. Also, errors caused by sample losses during distinct steps of sample pre-treatment or the adjustment of final sample extract volume as well as matrix effects in the sample are accounted for.

The concentration  $\rho$  [ $\mu\text{g}/\text{l}$ ] of labelled compound in the extract should be lower or equivalent to the maximum allowable concentration on sample for compliance.

### 13.2 Calculation of results after calibration with reference compounds

Calculate the mass concentration,  $\rho_{ig}$ , of the substance  $i$  in accordance with Equation (3) after solving Equation (1).

$$\rho_{ig} = \frac{y_{ig} - b_{ig}}{a_{ig}} \quad (3)$$

Calculate the mass concentration,  $\rho_{lg}$ , of the reference standard  $l$  in accordance with Equation (4) after solving Equation (2).

$$\rho_{lg} = \frac{y_{lg} - b_{lg}}{a_{lg}} \quad (4)$$

where

$y_{ig}$  is the measured value, expressed in units depending on the analytical method, e.g. value, of the substance  $i$  in the sample;

$y_{lg}$  is the measured value, expressed in units depending on the analytical method, e.g. area value, of the labelled reference solution  $l$  in the sample;

$\rho_{ig}$  is the mass concentration, expressed in nanograms per millilitre, of the substance  $i$  in the sample;

$\rho_{lg}$  is the mass concentration, expressed in nanograms per millilitre, of the labelled reference standard  $l$  in the sample.

Calculate the nominal concentration  $\rho_{ln}$  in micrograms per litre of the reference standards in the final sample solution starting from the spiked amount.

Calculate the recovery of reference standards for each sample solution in accordance with the Equation (5):

$$\%R_{lg} = \frac{\rho_{lg}}{\rho_{ln}} \times 100 \quad (5)$$

where

$\%R_{lg}$  is the percent recovery of the reference standard in the sample solution.

Verify that each  $\%R_{lg}$  is inside the acceptable range (70 % to 125 % see 12.2).

If the  $\%R_{lg}$  is not acceptable, the measurement should be considered non valid; otherwise apply the  $\%R_{lg}$  to the measured concentration  $\rho_{kg}$  of the corresponding non labelled compound in accordance with the Equation (6):

$$\rho_{kf} = \frac{\rho_{kg}}{\%R_{lg}} \times 100 \quad (6)$$

where

$\rho_{kf}$  is the non labelled compound  $k$  recovery corrected concentration on the sample final solution in nanograms per millilitre;

$\rho_{kg}$  is the non labelled compound  $k$ , corresponding to  $l$ , calculated from Equation (3).

The correction for the recovery should not be applied to the compounds other than the corresponding non-labelled one.

Starting from the  $\rho_{kf}$  or  $\rho_{lg}$  concentrations in micrograms per litre calculate the concentration on the starting sample, by applying the dilution and/or concentration and/or portion factors used for the extraction procedure.

The concentration of the compound  $i$  on the sample  ${}^iC_{\text{measured}}$  should be calculated and reported as:

- micrograms per square metre for coated materials; and
- percent by weight for all other types of sample.

### 13.3 Calculation of results to PFOS equivalents

The sample may contain several PFOS related species and they shall all be taken into account for calculating the total PFOS concentration. The molecular weights of the various PFOS related species in a sample can cover a wide range of values. The aggregation of the amounts of the different species is therefore achieved as follows:

The measured concentration of each extractable PFOS related compound should be expressed as the corresponding concentration of PFOS acid ( $C_8F_{17}SO_3H$ ) scaling on a molar basis in accordance with the Equation (7):

$${}^iC_{[PFOS,acid]} = \frac{{}^iC_{\text{measured}} \times [PFOS,acid] MW}{{}^iMW} \quad (7)$$

where

- ${}^{\text{comp}i}C_{\text{measured}}$  is the measured concentration of the PFOS related compound  $i$ ;
- ${}^{\text{comp}i}MW$  is the molecular weight of the PFOS related compound  $i$ ;
- $[PFOS,acid]MW$  is the molecular weight of the PFOS acid (i.e. 500,13);
- ${}^{\text{comp}i}C_{[PFOS\_acid]}$  is the requested concentration of the compound  $i$  expressed as PFOS acid.

The total PFOS concentration is the sum of the concentration of all the measured species expressed as PFOS acid in accordance with the Equation (8):

$$C_{PFOS} = \sum_i {}^iC_{[PFOS,acid]} \quad (8)$$

## 14 Expression of results

Report the results of compounds listed in Table 1 in micrograms per square metre for coated materials and in percent by weight for the other types of samples to two significant figures.

- a) Report each result in two significant figures. Report the result of each of the compounds found, listed in Table 1, including:
  - 1) the compound measured concentration in:
    - i) micrograms per square metre of analysed sample for coated materials;
    - ii) % by weight of analysed sample for the other type of samples;

- 2) the PFOS acid equivalent concentration calculated for the compound in:
  - i) micrograms per square metre of analysed sample for coated samples;
  - ii) % of analysed sample for the other type of samples.
- b) Report the sum of the PFOS acid equivalent of all the measured compounds and the related maximum allowed concentration as reported in the EU directive.
- c) Report a statement of compliance, if it is the case, following the rules of Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC [6] concerning the performance of analytical methods and the interpretation of results.

To calculate the mass per unit area,  $M$ , in grams per square metre, Equation (9) can be used (according to EN 12127):

$$M = \frac{m \times 10\,000}{A} \quad (9)$$

where

- $M$  is the mass per unit area, in grams per square metre;
- $m$  is the mass of a relaxed test specimen conditioned or oven-dry, in grams;
- $A$  is the area of the same test specimen, in square centimetres.

Using the determined mass per unit area, PFOS results calculated as  $\mu\text{g/g}$  can be transformed in  $\mu\text{g/m}^2$  according to Equation (10):

$$c_A = c_m \times M \quad (10)$$

where

- $c_A$  is the area related result, in micrograms per square metre;
- $c_m$  is the mass related result, in micrograms per gram;
- $M$  is the mass per unit area, in grams per square metre.

## 15 Test report

The report shall contain at least the following information:

- a) identity of the sample;
- b) the method used, by reference to this Technical Specification, i.e. CEN/TS 15968;
- c) identification and quantification of individual components;
- d) expression of results according to Clause 14;
- e) recoveries of the labelled reference compounds;
- f) any deviation from this procedure and all circumstances that may have influenced the result;

- g) report the limit of quantification (LOQ) and the limit of detection (LOD);
- h) report the sample intake (in grams or square metres).

## Annex A (informative)

### LC-QMS and LC-TOFMS

#### A.1 General

Liquid chromatography with single quad mass spectrometry (LC-QMS) can only be used if additional clean up steps have been taken and the user has demonstrated that the level of uncertainty obtained by the LC-QMS method is equivalent to the uncertainty obtained with the LC-tandemMS method.

#### A.2 Example for operating conditions for LC-tandemMS

**Table A.1 — LC-tandemMS operating conditions**

<b>LC conditions</b>		
injection volume:	10 µl	
flow rate:	0,3 ml/min	
Mobile phase:	(A) 9:1 2mM ammonium acetate aqueous solution/methanol	
	(B) methanol	
guard column:	stationary phase:	Zorbax XDB-C8
	length:	12,5 mm
	inner diameter:	2,1 mm
	particle size:	5 µm
main column:	stationary phase:	Betasil C18
	length:	50 mm
	inner diameter:	2,1 mm
	particle size:	5 µm
column temperature:	30 °C	
gradient program (B %)	30 % at 0,0 min, increase to 85 % at 18 min and keep at the level until 20 min before reversion to original conditions.	
<b>MS conditions</b>		
type:	Quadrupole	

ionization:	ESI negative	
mode:	MRM	
capillary voltage	1 kV	
temperatures:	MS source:	120 °C
	desolvation temperature	450 °C
Flow	Cone gas flow	60 l/h
	desolvation gas flow	740 l/h
cone voltage	for PFOS	90 V
collision energy	for PFOS	35 eV

### A.3 Example for operating conditions for Time-of-Flight MS (TOF-MS)

Aliquots (25 µl to 50 µl) are injected automatically on a HPLC coupled to ESI time-of-flight-high-resolution MS in the negative ion mode (HPLC-Q-ToF-HRMS ESI). PFCs can be separated on an ACE C18 column (150 – 2,1 mm, 3 µm particle size) using a gradient of 200 µl/min methanol and water (both with 2 mM NH<sub>4</sub>OAc). The initial mobile phase condition was 50:50 methanol/water, followed by a 5 min ramp increase to 85:15, a 5 min hold at 85:15, a 0,5 min ramp to 99:1, and hold until reverting to initial condition after min 15. Full scan (*m/z* 165-720) high resolution mass spectra were monitored throughout the chromatograms.

**Table A.2 — Time-of-Flight MS (TOF-MS) operating conditions**

HPLC system	
HPLC column (type and manufacture)	C18
HPLC column (dimensions)	150 mm × 2,1 mm
Pre-column (type, manufacture)	C 18
Pre-column (dimensions)	10 mm × 2,1 mm
Column temperature (°C)	Ambient
Flow rate (µl/min)	200
Injection volume (µl)	50
Splitted flow prior to MS? (split ratio if applicable)	n.a.
Mobile phase A	Methanol with 2 mM NH <sub>4</sub> OAc
Mobile phase B	HLB-cleaned MiliQ-water with 2 mM NH <sub>4</sub> OAc
Mobile phase C (if applicable)	n.a.
Gradient	

t = 0 min	50%A, 50%B
t = 5 min	85%A, 15%B
t = 10 min	85%A, 15%B
t = 11 min	99%A, 1%B
t = 20 min	99%A, 1%B
t = 21 min	50%A, 50%B
t = 28 min	50%A, 50%B
Precautions should be taken to prevent HPLC system blank peaks	
Column preceding sample injection loop	
PTFE parts replaced by e.g. PEEK	
MS-TOF system	
Type	Time-of-flight MS, high resolution
Interface type (ESI or other)	ESI
Spray direction (orthogonal, z-spray, direct or other)	z-spray
Ionisation	Negative
Source temp. [°C]:	125
Desolvation gas type and flow	Nitrogen (600 l/h)
Desolvation temp. [°C]:	400
Capillary voltage (kV)	3
Cone gas type and flow	nitrogen set 50
Nebuliser gas type and flow	nitrogen set maximum
Ions	
Full scan screening in high resolution	Cone Voltage (V)
PFOS	50



#### A.4 Example for operating condition for ion-trap MS

**Table A.3 — Ion-trap MS operating conditions**

<b>HPLC system</b>	
HPLC column (type and manufacture)	C18 3u
HPLC column (dimensions)	50 mm × 4,6 mm
Pre-column (type, manufacture)	n.a.
Pre-column (dimensions)	n.a.
Column temperature (°C)	40
Flow rate (µl/min)	500
Injection volume (µl)	100
Splitted flow prior to MS? (split ratio if applicable)	n.a.
Mobile phase A	water + formic acid 20 mM
Mobile phase B	Methanol
Mobile phase C (if applicable)	n.a.
Gradient	
t = 0 min	40%A, 60%B
t = 2 min	40%A, 60%B
t = 7 min	2%A, 98%B
t = 18 min	2%A, 98%B
t = 20 min	40%A, 60%B
t = 22 min	40%A, 60%B
Precautions for preventing HPLC system blank peaks	
Column preceding sample injection loop	
PTFE parts replaced by e.g. PEEK	
MS(/MS) system	
Type	Ion trap

Interface type (ESI or other)	ESI
Spray direction (orthogonal, z-spray, direct or other)	z-spray
Source temp. (°C):	230
Desolvation gas type and flow	n.a.
Desolvation temp. (°C):	n.a.
Capillary voltage (kV)	2
Cone gas type and flow	nitrogen set 15
Nebuliser gas type and flow	nitrogen set 50 & 10
Collision gas type	n.a.
Ions	
Compound specific MS-settings incl. quant. ion /transition	Cone Voltage (V)
PFOS	22

## **Annex B** (informative)

### **Sampling of liquids (example)**

Equipment: three empty and unused containers, a liquid sampler according to EN ISO 8130-9 or a siphon or similar (e.g. pipette, plunger elevator, etc.).

The recommended sample volume is 200 ml to 500 ml, depending on the necessary analysis that is to be performed.

Before the sample is taken, the stock it is taken from shall be agitated or homogenised to provide a representative sample. If this is not possible depending on the liquid layer thickness do the following:

- liquid layer thickness  $> 1$  m, take three samples (surface, middle and bottom) and combine even volumes into one sample. Homogenise the sample before further processing;
- liquid layer thickness  $> 0,2$  m and  $< 1$  m, take two samples and combine even volumes into one sample. Homogenise the sample before further processing;
- liquid layer thickness  $< 0,2$  m, take one sample.

## Annex C (informative)

### Confirmation of compound identity by MS methods

#### C.1 General

The text in Annex C has been adopted from Commission Decision 2002/657/EC [5] of 14 August 2002.

Implementing Council Directive 96/23/EC [6] concerning the performance of analytical methods and the interpretation of results (notified under document number C(2002) 3044):

"Confirmatory methods for organic residues or contaminants shall provide information on the chemical structure of the analyte. Consequently methods based only on chromatographic analysis without the use of spectrometric detection are not suitable on their own for use as confirmatory methods. However, if a single technique lacks sufficient specificity, the desired specificity shall be achieved by analytical procedures consisting of suitable combinations of clean-up, chromatographic separation(s) and spectrometric detection."

Among the methods or method combinations considered suitable for the identification of organic residues or contaminants for the substance groups indicated are mass spectrometric methods (see Table C.1):

**Table C.1 — Suitable confirmatory methods for organic residues or contaminants**

Measuring technique	Limitations
LC or GC with mass-spectrometric detection	Only if following either an on-line or an off-line chromatographic separation.  Only if full scan techniques are used or using at least three (group B) or four (group A) identification points for techniques that do not record the full mass spectra.

#### C.2 Performance criteria and other requirements for mass spectrometric detection

##### C.2.1 General

Mass spectrometric methods are suitable for consideration as confirmatory methods only following either an on-line or an off-line chromatographic separation.

##### C.2.2 Chromatographic separation

For GC-MS procedures, the gas chromatographic separation shall be carried out using capillary columns. For LCMS procedures, the chromatographic separation shall be carried out using suitable LC columns. In any case, the minimum acceptable retention time for the analyte under examination is twice the retention time corresponding to the void volume of the column. The retention time (or relative retention time) of the analyte in the test portion shall match that of the calibration standard within a specified retention time window. The retention time window shall be commensurate with the resolving power of the chromatographic system. The ratio of the chromatographic retention time of the analyte to that of the internal standard, i.e. the relative retention time of the analyte, shall correspond to that of the calibration solution at a tolerance of  $\pm 0,5$  % for GC and  $\pm 2,5$  % for LC.

### C.2.3 Mass spectrometric detection

Mass spectrometric detection shall be carried out by employing MS-techniques such as recording of full mass spectra (full scans) or selected ion monitoring (SIM), as well as MS-MS<sup>n</sup> techniques such as Selected Reaction Monitoring (SRM), or other suitable MS or MS-MS<sup>n</sup> techniques in combination with appropriate ionisation modes. In high-resolution mass spectrometry (HRMS), the resolution shall typically be greater than 10 000 for the entire mass range at 10 % valley.

Full scan: When mass spectrometric determination is performed by the recording of full scan spectra, the presence of all measured diagnostic ions (the molecular ion, characteristic adducts of the molecular ion, characteristic fragment ions and isotope ions) with a relative intensity of more than 10 % in the reference spectrum of the calibration standard is obligatory.

SIM: When mass spectrometric determination is performed by fragmentography, the molecular ion shall preferably be one of the selected diagnostic ions (the molecular ion, characteristic adducts of the molecular ion, characteristic fragment ions and all their isotope ions). The selected diagnostic ions should not exclusively originate from the same part of the molecule. The signal-to-noise ratio for each diagnostic ion shall be > 3:1.

Full scan and SIM: The relative intensities of the detected ions, expressed as a percentage of the intensity of the most intense ion or transition, shall correspond to those of the calibration standard, either from calibration standard solutions or from spiked samples, at comparable concentrations, measured under the same conditions, within the following tolerances (Table C.2 ):

**Table C.2 — Maximum permitted tolerances for relative ion intensities using a range of mass spectrometric techniques**

Relative intensity (% of base peak)	EI-GC-MS (relative)	CI-GC-MS, GC-MS <sup>n</sup> LC-QMS, LC-MS <sup>n</sup> (relative)
> 50 %	± 10 %	± 20 %
> 20 % to 50 %	± 15 %	± 25 %
> 10 % to 20 %	± 20 %	± 30 %
≤ 10 %	± 50 %	± 50 %

**Table C.3 — Relationship between a range of classes of mass fragment and identification points earned (copied from EC 2002/657)**

MS technique	Identification points earned per ion
Low resolution mass spectrometry (LR)	1,0
LR-MS <sup>n</sup> precursor ion	1,0
LR-MS <sup>n</sup> transition products	1,5
HRMS	2,0
HR-MS <sup>n</sup> precursor ion	2,0
HR-MS <sup>n</sup> transition products	2,5
<p>(1) Each ion may only be counted once.</p> <p>(2) GC-MS using electron impact ionisation is regarded as being a different technique to GC-MS using chemical ionisation.</p> <p>(3) Different analytes can be used to increase the number of identification points only if the derivatives employ different reaction chemistries.</p> <p>(4) For substances in group A of Annex I to Directive 96/23/EC, if one of the following techniques are used in the analytical procedure: HPLC coupled with full-scan diode array spectrophotometry (DAD); HPLC coupled with fluorescence detection; HPLC coupled to an immunogram; two-dimensional TLC coupled to spectrometric detection; a maximum of one identification point may be contributed, providing that the relevant criteria for these techniques are fulfilled.</p> <p>(5) Transition products include both daughter and granddaughter products.</p>	

Table C.4 (copied from Directive 2002/657/EC [5]) shows examples of the identification points earned by each technique or combination of techniques.

**Table C.4 — Examples of the number of identification points earned for a range of techniques and combinations thereof (n = an integer)**

Technique(s)	Number of ions	Identification points
GC-MS (EI or CI)	N	N
GC-MS (EI and CI)	2 (EI) + 2 (CI)	4
GC-MS (EI or CI)	2 derivatives 2 (Derivative A) + 2 (Derivative B)	4
LC-QMS	N	N
GC-MS-MS	1 precursor and 2 daughters	4
LC-MS-MS	1 precursor and 2 daughters	4
GC-MS-MS	2 precursor ions, each with 1 daughter	5
LC-MS-MS	2 precursor ions, each with 1 daughter	5
LC-MS-MS-MS	1 precursor, 1 daughter and 2 granddaughters	5,5
HRMS	N	2n
GC-MS and LC-MS	2 + 2	4
GC-MS and HRMS	2 + 1	4

PFOS and related compounds are not mentioned explicitly in Annex I, group B of [5] (source: <http://www.defra.gov.uk/animalh/by-prods/pdf/toxin.pdf>), but qualify under f) others.

Directive 96/23/EC Annex I [6] reads:

a) GROUP A – Substances having anabolic effect and unauthorised substances:

- 1) stilbenes, stilbene derivatives, and their salts and esters;
- 2) antithyroid agents;
- 3) steroids;
- 4) resorcylic acid lactones including zeranol;
- 5) beta-agonists;
- 6) compounds included in Annex IV to Council Regulation (EEC) No 2377/90 [7] of 26 June 1990;

b) GROUP B – Veterinary drugs (1) and contaminants:

- 1) antibacterial substances, including sulphonamides, quinolones;
- 2) other veterinary drugs:
  - i) anthelmintics;
  - ii) anticoccidials, including nitroimidazoles;

- iii) carbamates and pyrethroids;
  - iv) sedatives;
  - v) non-steroidal anti-inflammatory drugs (NSAIDs);
  - vi) other pharmacologically active substances;
- 3) other substances and environmental contaminants:
- i) organ chlorine compounds including PCBs;
  - ii) organ phosphorus compounds;
  - iii) chemical elements;
  - iv) mycotoxins;
  - v) dyes;
  - vi) others.



## Bibliography

- [1] 2006/122/EC: REACH (1907/2006), annex XVII, designation 53. of the European Parliament and of the Council of 12 December 2006 amending for the 30<sup>th</sup> time Council Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (perfluorooctane sulphonates)
- [2] EN ISO 6427, *Plastics — Determination of matter extractable by organic solvents (conventional methods)* (ISO 6427:1992)
- [3] ISO 9113, *Plastics Polypropylene (PP) and propylene-copolymer thermoplastics — Determination of isotactic index*
- [4] ISO 25101, *Water quality — Determination of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) — Method for unfiltered samples using solid phase extraction and liquid chromatography/mass spectrometry*
- [5] 2002/657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (notified under document number C(2002) 3044)
- [6] 96/23/EC: Council Directive of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC
- [7] Council Regulation (EEC) No 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin (OJ L 224, 18.8.1990, p. 1)
- [8] EN 12127, *Textiles — Fabrics — Determination of mass per unit area using small samples*





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