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**Leather — Per- and polyfluoroalkyl
substances —**

**Part 1:
Determination of non-volatile
compounds by extraction method
using liquid chromatography**

Cuir — Substances perfluoroalkylées et polyfluoroalkylées —

*Partie 1: Détermination des composés non volatils par une méthode
d'extraction utilisant la chromatographie en phase liquide*

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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.

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).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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.

IULTCS, originally formed in 1897, is a world-wide organization of professional leather societies to further the advancement of leather science and technology. IULTCS has three Commissions, which are responsible for establishing international methods for the sampling and testing of leather. ISO recognizes IULTCS as an international standardizing body for the preparation of test methods for leather.

This document was prepared by the Chemical Test Commission of the International Union of Leather Technologists and Chemists Societies (IUC Commission, IULTCS), in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 289, *Leather*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO 23702-1:2018), which has been technically revised.

The main changes are as follows:

- to clarify the relevant organic fluorine compounds, the title has been modified;
- the Introduction, Scope and [Clauses 3](#) to [10](#) have been editorially and technically modified;
- the previous Clause 7 has been split into two separate clauses, [Clause 7](#), “Sampling”, and [Clause 8](#), “Procedure”;
- the previous Clause 8 is now [Clause 9](#), “Expression of results” and includes the previous Clause 9, “Precision”, as [9.4](#);
- a new [Annex A](#), listing the category of application of the per- and polyfluoroalkyl substances (PFAS), has been inserted and the subsequent annexes relettered accordingly;
- [Annexes B](#) and [C](#) have been technically modified and are now lists of “PFAS regulated substances” and “PFAS non-regulated substances”, respectively;

- a new [Annex D](#), listing the usable ions and possible quantification limits for PFAS analysis by LC-MS/MS, has been inserted and the subsequent annexes relettered accordingly;
- [Annex E](#) has been technically modified.

A list of all parts in the ISO 23702 series can be found on the ISO website.

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.

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Introduction

The per- and polyfluoroalkyl substances (PFAS) consists of a large group of surface active compounds. The most well-known are perfluorooctanoic sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). [Table A.1](#) presents PFAS substance categories and applications.

Perfluorooctanoic sulfonic acid (PFOS) is classified as persistent, bio-accumulative and toxic (PBT). PFOS and its salts are restricted and regulated in many countries regarding their marketing and use (see References [4] and [7]).

Perfluorooctanoic acid (PFOA) and its salts and related substances are suspected of having a similar risk profile to PFOS and are also restricted and regulated in many countries regarding their marketing and use (see Reference [4]).

Furthermore, restrictions on perfluorocarboxylic acids containing 9 to 14 carbon atoms in the chain (C₉-C₁₄ PFCAs), their salts and C₉-C₁₄ PFCA-related substances are restricted in some countries (see References [5] and [8]).

A number of long-chain per- and polyfluoroalkyl compounds have been included in the EU Candidate List of Substances of Very High Concern (SVHC), which is available at: <https://echa.europa.eu/candidate-list-table>.^[6]

The regulatory thresholds for restricted per- and polyfluoroalkyl compounds limit their use to a level below which they cannot be meaningfully used. The thresholds must take into consideration the possible presence of unavoidable impurities and unintentional trace contaminants.

The long-chain, fully fluorinated anions are non-volatile. They are heat-stable and resistant to breaking down in the environment. The per- and polyfluoroalkyl compounds have been widely used in many industries, including in oil-, soil- and water-repellent finishes for textiles, leather products, paper, furniture and carpets.

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Leather — Per- and polyfluoroalkyl substances —

Part 1:

Determination of non-volatile compounds by extraction method using liquid chromatography

WARNING — The use of this document involves hazardous materials. It does not purport to address all of the safety or environmental problems associated with its use. It is the responsibility of users of this document to take appropriate measures to ensure the safety and health of personnel and the environment prior to application of this document and to fulfil the relevant requirements for this purpose.

1 Scope

This document specifies a test method for detection and quantification of extractable non-volatile per- and polyfluoroalkyl substances (PFAS) in leather and coated leather by solvent extraction and liquid chromatography coupled with mass spectrometry.

This document, taking into account the three-dimensional distribution of the fibres within leather, makes the evaluation of the PFAS with respect to the mass.

PFAS substances categories and applications are listed in [Annex A, Table A.1](#). Classes of PFAS regulated compounds listed in [Annex B, Table B.1](#), include acids, telomers, sulfonates and sulphonamide alcohols. Classes of other non-regulated compounds that can be determined by this document are listed in [Annex C, Table C.1](#).

NOTE 1 By applying the method specified in this document, the concentration of free fluorotelomer alcohols (FTOH) in a sample cannot be correctly quantified if perfluoropolymers that release FTOH due to transesterification with the extraction solvent methanol are present in the sample.

NOTE 2 Some regulations (e.g. Reference [4]) also restrict perfluoropolymers having a linear or branched perfluoroheptyl group with the moiety $(C_7F_{15})C$ as one of the structural elements that can degrade to PFOA, e.g. polymers containing 2-perfluorooctylethanol (8:2 FTOH, CAS Registry Number[®] 678-39-7) bonded as esters. To determine whether these perfluoropolymers are intentionally present, it could be necessary to introduce an alkaline hydrolysis method to remove the 8:2 FTOH side-chain from the polymer. In addition, other FTOH, e.g. 6:2 FTOH or 10:2 FTOH, will be released from relevant perfluoropolymers by alkaline hydrolysis.^[3]

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 2418, *Leather — Chemical, physical, mechanical and fastness tests — Position and preparation of specimens for testing*

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

ISO 4044, *Leather — Chemical tests — Preparation of chemical test samples*

EN 15987, *Leather — Terminology — Key definitions for the leather trade*.

1) CAS Registry Number[®] (CAS RN[®]) is a trademark of CAS corporation. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 15987 apply.

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

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

4 Principle

The PFAS substances listed in [Annexes B](#) and [C](#) are extracted in an ultrasonic bath with methanol at 60 °C; the extract is analysed by high-performance liquid chromatograph with a tandem mass spectrometric detector (LC-MS/MS).

5 Reagents

Unless otherwise specified, all reagents shall be of a recognized analytical grade.

5.1 Water, grade 1 (according to ISO 3696) or LC-MS grade.

5.2 Methanol, CAS RN® 67-56-1, LC-MS grade.

5.3 Ammonium acetate, CAS RN® 631-61-8.

5.4 Stock solutions of reference compounds, purity > 95 % for the pure substance.

Solutions of the reference compounds listed in [Annex B](#) and [Annex C](#) are available commercially. They should be diluted to the required concentrations. If reference compounds are obtained pure, for example, weigh 100 mg of each standard separately into a 100 ml volumetric flask and fill up to the mark with methanol ([5.2](#)). Dilute this solution with methanol ([5.2](#)) at a ratio of 1:1 000 to prepare a 1 000 µg/l stock solution.

5.5 Target compound solutions.

Prepare a 25 µg/l solution of each target compound by diluting the 1 000 µg/l reference compound stock solutions ([5.4](#)) with methanol ([5.2](#))

For the preparation of the target compound solution, certified solutions are commercially available. The purity level and the solvent shall be checked in order to be in accordance with this document.

5.6 Internal standard.

5.6.1 Internal standard solution I (2 000 µg/l) (used for extraction).

At least two suitable internal standards for perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) shall be used. The impurity level of the internal standard should be determined prior to the use of every new lot.

Suitable mass-labelled internal standards are:

- ¹³C_x-PFOA (e.g. perfluoro[1,2,3,4-¹³C₄]-octanoic-acid, CAS RN® 960315-48-4);
- ¹³C_x-PFOS (e.g. sodium perfluoro-1-[1,2,3,4-¹³C₄]-octanesulfonate, CAS RN® 960315-53-1).

Prepare a 2 000 µg/l solution of the internal standard by diluting the commercial solution with methanol (5.2).

5.6.2 Internal standard solution II (100 µg/l) (used for preparation of calibration solutions).

Prepare a 100 µg/l solution of the internal standard by diluting the commercial solution with methanol (5.2).

5.7 Preparation of calibration solutions.

Materials and liquids shall be stored at 4 °C and in clean containers.

Prepare suitable calibration solutions using methanol (5.2), target compound solutions (5.5) and the internal standard solution II (5.6.2). At least five calibration solutions shall be prepared with a concentration range to match the limits given. For example, prepare according to the volumes given in Table 1 in a 1 000 µl flask.

Table 1 — Example of calibration solutions

Concentration (µg/l)	0,25	0,5	1	2,5	5	10
Volume methanol (µl)	940	930	910	850	750	550
Volume target compound solution at 25 µg/l (µl)	10	20	40	100	200	400
Volume internal standard solution II at 100 µg/l (µl)	50	50	50	50	50	50

5.8 Eluent for the LC-MS/MS.

10 mM ammonium acetate solution is prepared by dissolving 0,771 g of ammonium acetate (5.3) in 1 000 ml deionized water (5.1).

6 Apparatus

The usual laboratory apparatus and, in particular, the following shall be used.

Equipment or any accessible part of it that could come into contact with the sample or the extract shall be free from interfering compounds, see Annex F.

Use equipment free from all types of fluoropolymer plastics, including polytetrafluoroethene (PTFE) and glassware. For example, use equipment made of polypropylene (PP) or polyethylene (PE).

Sample containers shall be rinsed thoroughly with water (5.1) and methanol (5.2) and checked for possible background contamination before use.

- 6.1 Suitable device with a **sharp blade** to cut leather sample.
- 6.2 **Volumetric flasks**, PP or PE, with inert stopper may be used.
- 6.3 **Extraction vials**, suitable PP or PE vials, volume at least 20 ml and able to be used in a centrifuge.
- 6.4 **Laboratory centrifuge**, suitable for the extraction vials (6.3).
- 6.5 **Ultrasonic bath**, equipped with adjustable bath temperature control, up to at least 60 °C.
- 6.6 **Analytical balance**, weighing up to 0,001 g.

6.7 High-performance liquid chromatograph coupled with a tandem mass spectrometric detector, (LC-MS/MS), free from all types of fluoropolymer plastics, including polytetrafluoroethene (PTFE).

6.8 Membrane filter equipment and polyamide or polypropylene membrane filter, for example 0,22 µm pore size.

7 Sampling

The chosen leather piece should be representative of the lot it is taken from. Cut a test specimen in accordance with ISO 2418. If cutting a test specimen in accordance with ISO 2418 is not possible (e.g. for leathers from finished products such as shoes or garments), details about the selection of the test specimen shall be given in the report. When testing leather products that have separate distinct parts, the product shall be taken apart and each part shall be analysed separately.

In the case of coated leather, separate, if possible, the coating from the leather substrate. The leather substrate shall be analysed according to the procedure in this document. If separation of the coating from the leather cannot be carried out, the entire article shall be analysed according to this procedure.

NOTE The coating can be analysed according to CEN/TS 15968.

Take a leather test piece by mass using ≥ 1 g of leather. The results shall be reported in units of mg/kg.

Cut (6.1) the leather into small pieces according to the method specified in ISO 4044.

8 Procedure

Accurately weigh $1,0 \text{ g} \pm 0,1 \text{ g}$ of the leather pieces with the analytical balance (6.6) into an appropriate extraction vial (6.3). Record the mass of the leather test sample, *m*.

Add 10 ml methanol (5.2) and 50 µl of the internal standard solution I (5.6.1) to the extraction vial containing the leather pieces. Extract the test specimen in an ultrasonic bath (6.5) at a temperature of $(60 \pm 5) \text{ }^\circ\text{C}$ for (120 ± 5) min. Let the solution cool down to room temperature and pipette 0,5 ml of the residual extract plus 0,5 ml of water in the HPLC-vial and seal with a cap for LC-MS/MS analysis. If necessary, the extracted solution can be filtrated with a membrane filter (6.8) or centrifuged before taking the 0,5 ml aliquot.

This extract is analysed by LC-MS/MS (6.7). Examples of suitable conditions for LC-MS/MS analysis are given in Annex E.

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, see Annex F.

For measuring PFAS No 29.2 HFPO-DA-X (Table B.1), a hydrolysis step is necessary. Thus, the methanol-water extract should remain for 24 h at room temperature.

If the extract is turbid, centrifuge and decant the supernatant or filter it (6.8) to a new vial (6.3) for LC-MS/MS analysis. The recovery rate of check standards in the extract should be $\geq 60 \%$ and the check standards can be used in the calculation. If $< 60 \%$ then a dilution and/or a standard addition is recommended to overcome matrix effects, with regard to ion suppression.

If dilution or repetition is needed, the methanol extract should be separated from the test specimen, otherwise inconsistent results can occur, especially for the FTOH substances.

If a concentration step is necessary, the recovery rate shall be checked to be $\geq 60 \%$. Removal of the solvent (e.g. by concentration in the rotary vacuum evaporator and evaporation to dryness) can lead to substantial losses of FTOH.

9 Expression of results

9.1 Calibration

For each of the target PFAS substances, set up individually the linear regression function by using the ratios (A_e/A_{is}) and (C_e/C_{is}) with the help of [Formula \(1\)](#):

$$\frac{A_e}{A_{is}} = a \cdot \left(\frac{C_e}{C_{is}} \right) + b \quad (1)$$

where

A_e is the peak area for the corresponding target PFAS compound;

A_{is} is the peak area for the internal standard chosen;

C_e is the concentration of the target PFAS in the calibration standard in micrograms per litre;

C_{is} is the concentration of internal standard in the calibration standard in micrograms per litre;

b is the ordinate intercept of the calibration curve of the specific PFAS;

a is the slope of the calibration curve of the specific PFAS.

9.2 Calculation of the result

The result shall be given in $\mu\text{g}/\text{kg}$.

The content of each PFAS substance is calculated as mass portion, w , in micrograms per kilogram ($\mu\text{g}/\text{kg}$) of the leather sample according to [Formula \(2\)](#):

$$w = \frac{V}{m} \cdot \left(\frac{A_s}{A_{is_sample}} - b \right) C_{is_sample} \quad (2)$$

where

A_s is the peak area of the corresponding PFAS in the extraction solution;

A_{is_sample} is the peak area of the corresponding internal standard in the extraction solution;

C_{is_sample} is the concentration of the corresponding internal standard in the extraction solution in micrograms per litre;

b is the ordinate intercept of the calibration curve of the specific PFAS determinate in [9.1](#);

a is the slope of the calibration curve of the specific PFAS determinate in [9.1](#);

V is the volume used according to [Clause 8](#) in litres;

m is the mass of the leather test sample according to [Clause 8](#) in grams.

9.3 Calculation of the results of a sum

In certain cases, a final requested result can be expressed as a sum of different PFAS substances.

All the PFAS included in the sum shall be clearly identified.

The results of the relevant identified PFAS (9.2) are added to give the result of the sum. If the result for a single PFAS is lower than the limit of quantification of the test method (9.4), this result is considered as zero and is not included in the sum.

9.4 Precision

With this method it is feasible to achieve limits of quantification (LoQ) of 2,5 µg/kg for PFOA and PFOS, see [Annex D](#) for the LoQs of other PFAS compounds.

The results of interlaboratory trials to determine the PFOS content in leather are presented in [Annex G](#).

10 Test report

The report shall contain at least the following information:

- a) identity of the sample and, if necessary, details of sampling ([Clause 7](#));
- b) the method used;
- c) a reference to this document, i.e. ISO 23702-1:2023;
- d) in the case of coated leather, whether it was possible to separate the coating from the leather and if it was tested separately;
- e) identification and quantification of individual PFAS components (9.2) expressed in µg/kg;
- f) when relevant, the results of the sum (9.3);
- g) any deviation from this procedure and all circumstances that could have influenced the result;
- h) the date of the test.

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