INTERNATIONAL STANDARD

ISO 18218-1

IULTCS/IUC 28-1

First edition
2015-06-15

Leather — Determination of ethoxylated alkylphenols —

Part 1: Direct method

Cuir — Détermination des alkylphénols éthoxylés —

Partie 1: Méthode directe

Reference numbers
ISO 18218-1:2015(E)
IULTCS/IUC 28-1:2015(E)

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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 on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

ISO 18218-1 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, the secretariat of which is held by UNI, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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.

ISO 18218 consists of the following parts, under the general title *Leather — Determination of ethoxylated alkylphenols*:

— *Part 1: Direct method*

— *Part 2: Indirect method*

Introduction

Nonylphenol ethoxylate belongs to the non-ionic surfactants. The biodegradation of nonylphenol ethoxylate releases the persistent pollutant, the branched nonylphenol. Nonylphenol is a hormonal acting substance that is toxic for waterborne organisms and many other organisms. For this reason the release of nonylphenol ethoxylate into the environment should be avoided.

In 2003 the European Directive 2003/53/EC restricted the sale and use of nonylphenol and nonylphenol ethoxylate in product preparations for industries with discharges to waste water. Preparations containing concentrations equal or higher than than 0,1 % of nonylphenol ethoxylate or nonylphenol were forbidden. This Directive is included as part of the EU Regulation 1907/2006 (REACH).

No detailed composition of the chemical substance nonylphenol ethoxylate can be given, it is assigned the general structural formula:

$$\left(C_9 \text{ alkyl chain, branched or linear}\right) - \text{Ph} - \left[OCH_2CH_2\right]_n - \text{OH} \quad \left(\text{with Ph} = \text{phenyl}, n \geq 1\right).$$

To cover the group of ethoxylates of 4-nonylphenol, branched and linear, the European Chemical Agency (ECHA) has assigned the substance the following definition: *4-nonylphenol, branched and linear, ethoxylated [substances with a linear and/or branched alkyl chain with a carbon number of 9 covalently bound in position 4 to phenol, ethoxylated covering UVCB and well-defined substances, polymers and homologues, which include any of the individual isomers and/or combinations thereof].*

In the leather industry nonylphenol ethoxylate and octylphenol ethoxylate surfactants have been used. However, the water insoluble substances, nonylphenol and octylphenol, have not been used. For this reason two different analytical procedures have been prepared for analysing leather samples.

This part of ISO 18218 is a method that directly determines the ethoxylated alkylphenol. It is an efficient procedure for the analysing of a larger number of leather samples. This procedure requires HPLC with triple quadrupole mass spectrometer (MSMS) to identify the nonylphenol ethoxylate and octylphenol ethoxylate.

ISO 18218-2 is a procedure for analysing the alkylphenol. The ethoxylated alkylphenol is cleaved to form the alkylphenol, which is identified using high-performance liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS) equipment. This method can also be used to indirectly determine the alkylphenol ethoxylate content in leather and process auxiliaries.

Leather — Determination of ethoxylated alkylphenols —

Part 1:
Direct method

1 Scope

This part of ISO 18218 is a method for determining ethoxylated alkylphenols (nonylphenol ethoxylate [$NPEO_n$ with $1 \leq n \leq 16$] and octylphenol ethoxylate [$OPEO_n$ with $1 \leq n \leq 16$]) in leather. This direct method is especially suitable where a larger number of leather samples are to be checked for the presence of ethoxylated alkylphenols.

This method requires the use of high-performance liquid chromatography (HPLC) with triple quadrupole mass spectrometer (MSMS) to identify and quantify the ethoxylated alkylphenols.

NOTE 1 In the leather industry, the most commonly used commercial ethoxylated alkylphenol is the NPEO with an average of 9 EO. It has an optimum cloud point in water for the typical leather processing temperatures of 40 °C to 55 °C.

NOTE 2 ISO 18218-1 and ISO 18218-2 use different solvents for the extraction of the ethoxylated alkylphenols from leather. Consequently, the two analytical methods are expected to give similar trends but not necessarily the same absolute result for the ethoxylated alkylphenol content in leather.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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 and mechanical and fastness tests — Sampling location*

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

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

3 Principle

The leather sample is extracted in methanol using an ultrasonic bath. Subsequently, an aliquot of the solution can, after filtering, be directly analysed without further cleaning of the sample using high-performance liquid chromatography (HPLC) with a triple quadrupole mass spectrometer (MSMS).

4 Apparatus and materials

Normal laboratory apparatus and, in particular, the following:

4.1 Ultrasonic bath, with controllable heating capable of maintaining a temperature of (70 ± 5) °C.

4.2 Glass container with a screw cap (22 ml has been found suitable).

4.3 Polypropylene or **polyethylene syringe**, 2 ml.

4.4 Syringe membrane filters, pore size 0,2 µm, for use with syringe (4.3).

4.5 Volumetric flasks, 10 ml and 100 ml.

4.6 Analytical balance, weighing to 1 mg.

4.7 Pipettes, various sizes, 1 ml to 5 ml.

4.8 Instrumental equipment, high-performance liquid chromatograph with gradient elution and triple quadrupole mass spectrometer (HPLC-MSMS).

5 Chemicals

If not otherwise defined, analytical reagent grade chemicals shall be used.

5.1 Methanol.

5.2 Nonylphenol ethoxylate, $NPEO_n$ with n = 9 – 10, CAS No. 68412-54-4, Sigma-Aldrich® Product No. T9284 (IGEPAL® CO-630)[1)], technical grade.

NOTE The brand name is given to improve the comparability of test results amongst laboratories. The commercial nonylphenol ethoxylate contains groups of ethoxylates of nonylphenol with linear and branched structures, so use of another reference can lead to different results. Only technical grade references are currently available from laboratory chemical suppliers.

5.3 Octylphenol ethoxylate, $OPEO_n$ with n = 9 – 10, CAS No. 9002-93-1, Sigma-Aldrich® Product No. 542334 (Triton™ X-100)[1)], technical grade.

NOTE The brand name is given to improve the comparability of test results amongst laboratories. The commercial octylphenol ethoxylate contains groups of ethoxylates of octylphenol with linear and branched structures, so use of another reference can lead to different results. Only technical grade references are currently available from laboratory chemical suppliers.

5.4 Stock solution of nonylphenol ethoxylate and octylphenol ethoxylate, ρ = 250 µg/ml.

25 mg of the respective alkylphenol ethoxylate (5.2 and 5.3) are dissolved in different 100 ml volumetric flasks (4.5) with methanol (5.1) and filled up to the mark.

5.5 Calibration solutions of nonylphenol ethoxylate and octylphenol ethoxylate

Four calibration solutions of ρ = 2,5 µg/ml, ρ = 5 µg/ml and ρ = 10 µg/ml and ρ = 50 µg/ml for each alkylphenol ethoxylate are prepared using the respective stock solution (5.4).

5.6 Formic acid.

5.7 Water, deionised or distilled water, grade 3 according to ISO 3696.

6 Sampling

The sampling shall be made according to ISO 2418. If a sampling according to ISO 2418 is not possible (e.g. in case of leather from finished products like shoes, clothing, etc.) the details of the sampling shall be given in the test report. Glue residuals shall be mechanically removed from leather samples.

1) Sigma-Aldrich® Product No. T9284 (IGEPAL® CO-630) and Sigma-Aldrich® Product No. 542334 (Triton™ X-100) 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 or IDF of these products.

The leather samples shall be cut into small pieces or ground according to ISO 4044. The dimensions of the pieces shall not be larger than 2 to 3 mm in size.

7 Sample preparation and analysis

7.1 Extraction

Approximately 1 g of the leather sample is weighed accurately to 10 mg in a screw-top glass container (4.2). 10 ml methanol (5.1) is added, the container closed and the sample extracted for (60 ± 5) min in an ultrasonic bath (4.1) at (70 ± 5) °C.

NOTE This method can also be used for analysing leather auxiliaries by the addition of the auxiliary to the screw-top container (4.2) instead of the leather sample. The auxiliary ought to be soluble in methanol or, if not, then the extraction solution ought to be centrifuged to give a clear extract.

7.2 Analysis

After cooling down to room temperature, an aliquot of the extraction solution is removed using a disposable syringe (4.3) and transferred into an HPLC sample vial using a syringe filter (4.4). The aliquot is now ready for the HPLC analysis.

The detection of the alkylphenol ethoxylate is made using an HPLC with gradient elution and triple quadrupole mass spectrometer (4.8). Guidelines for suitable chromatographic conditions are given in Annex A.

7.3 Calibration

The four calibration solutions (5.5) shall be transferred to an HPLC vial and analysed along with each batch of test samples. The peak areas of the four analysed calibration solutions (5.5) are used to prepare a calibration graph.

7.4 Calculation

The content of alkylphenol ethoxylates is calculated as mass portion, w, in milligrams per kilogram (mg/kg) of the leather sample according to the following formula:

$$w = \frac{\left(A_s - b\right) \times V}{a \times m_E} \quad (1)$$

where

A_S is the peak area of alkylphenol ethoxylate in the extraction solution;

b is the intercept of the calibration graph;

a is the slope of the calibration graph;

V is the final volume used according to 7.1 in millilitres (ml); here 10 ml;

m_E is the weight of the leather sample in grams (g).

8 Test report

The test report shall include at least the following information:

a) a reference to this part of ISO 18218, i.e. ISO 18218-1;

b) type, origin, and denomination of the sample (aliquot, as far as relevant);

c) date of receipt and date of examination;

d) sampling method;

e) detection method and method of the quantitative determination;

f) results, stated as content of alkylphenol ethoxylates in milligrams per kilogram (mg/kg) and the limit of detection.

Annex A
(informative)

Chromatographic analysis operating parameters

A.1 Preliminary comment

As the HPLC equipment (4.8) of the laboratories can vary, no general valid instructions can be provided for the chromatographic analysis. The following parameters have been successfully tested and used.

A.2 High-Performance Liquid Chromatography (HPLC) and Triple Quadrupole Mass Spectrometer (MSMS) operating parameters

Eluent 1: 1 ml formic acid in 1 000 ml water, 0,1 %

Eluent 2: Methanol

Stationary phase: Pursuit® XRs C18[2)] (3 µm), (150 × 2,0) mm

Guard Column: MetaGuard™ 2.0 Pursuit® XRs C18[2)] (3 µm)

Volume flow: 180 µl/min

Gradient: See Table A.1

Table A.1 — Gradient Program

Time min	Eluent 1 %	Eluent 2 %
0	40	60
12	0	100
17	0	100
18	40	60
22	40	60

Column temperature: 40 °C

Injection volume: 1 µl to 10,0 µl (depending on chromatographic method)

Detection: Triple quadrupole mass spectrometer

Spray gas: Nitrogen

Inert gas: Argon

Ionization: API Electrospray positive, fragmentor 100 V

Quantification: TIC sum of the scanned ions

2) Pursuit® XRs C18 and MetaGuard™ 2.0 Pursuit® XRs 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 or IDF of these products.