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**Leather — Determination of
ethoxylated alkylphenols —**

**Part 2:
Indirect method**

Cuir — Détermination des alkylphénols éthoxylés —

Partie 2: Méthode indirecte
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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.

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*, the secretariat of which is held by UNI, in accordance with the agreement on technical co-operation 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.

This second edition cancels and replaces the first edition (ISO 18218-2:2015), which has been technically revised as follows:

- [6.14](#) and [6.15](#) have been added;
- [7.4](#), [7.5](#) and [7.6](#) have been technically revised;
- [8.1](#) has been revised by including a reference to [6.14](#).

A list of all parts in the ISO 18218 series can be found on the ISO website.

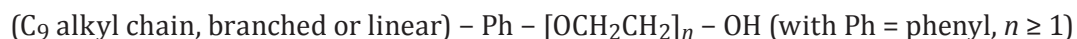
Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Nonylphenol ethoxylate belongs to the non-ionic surfactants. The biodegradation of nonylphenol ethoxylate releases the persistent pollutant 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 shall 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 to or higher 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:



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].'

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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.

ISO 18218-1 is a method that directly determines the ethoxylated alkylphenol. It is an efficient procedure for the analysis 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.

This document specifies 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.

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Leather — Determination of ethoxylated alkylphenols —

Part 2: Indirect method

1 Scope

This document specifies a method for determining alkylphenols (nonylphenol and octylphenol) and alkylphenol ethoxylates (nonylphenol ethoxylate and octylphenol ethoxylate) in leather and process auxiliaries. The analysis is based on high-performance liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS).

The analysis of the alkylphenol ethoxylate is made by cleaving the alkylphenol ethoxylate and measuring the released alkylphenol.

NOTE ISO 18218-1 and this document 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 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 and mechanical and fastness tests — Sampling location*

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

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

3 Terms and definitions

No terms and definitions are listed in this document.

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

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

4 Principle

Leather samples are extracted with acetonitrile using an ultrasonic bath and the nonylphenol (NP) and/or octylphenol (OP) in the extract is quantitatively determined by HPLC or GC-MS.

The leather process auxiliaries are dissolved in acetonitrile and the NP and/or OP in the solution is quantitatively determined by HPLC or GC-MS.

The nonylphenol ethoxylate (NPEO) and octylphenol ethoxylate (OPEO) in the extract or solution are first converted into NP and OP, using aluminium triiodide as cleavage agent, and the NP and OP are determined by HPLC or GC-MS. The contents of NPEO and OPEO are then calculated by normalizing to

NPEO₉ and OPEO₁₀, respectively. Examples of the four analytes used for the determination are shown in [Table 1](#).

Table 1 — Analytes determinable by this method

Analyte	Empirical formula	Abbreviation	CAS no.
4-nonylphenol (mixture of isomers)	C ₉ H ₁₉ -C ₆ H ₄ -OH	NP	84852-15-3
4-tert-octylphenol	C ₈ H ₁₇ -C ₆ H ₄ -OH	OP	140-66-9
Nonylphenol ethoxylate	C ₉ H ₁₉ -C ₆ H ₄ -(OC ₂ H ₄) _n OH (n≈9)	NPEO ₉	9016-45-9
Octylphenol ethoxylate	C ₈ H ₁₇ -C ₆ H ₄ -(OC ₂ H ₄) _n OH (n≈10)	OPEO ₁₀	9002-93-1
Key			
CAS: chemical abstract service			

NOTE There are many CAS numbers for the nonylphenol ethoxylates and octylphenol ethoxylates. The CAS numbers in [Table 1](#) are for the normalized structures used in the external calibration (see [8.2](#)).

5 Apparatus and materials

Normal laboratory apparatus and, in particular, the following:

- 5.1 **Analytical balance**, weighing to an accuracy of 0,1 mg.
- 5.2 **Ultrasonic bath**, (40 ± 2) kHz, with thermostat capable of maintaining a temperature of (50 ± 5) °C.
- 5.3 **Separating funnels**, 150 ml.
- 5.4 **Rotary evaporator**, with thermostat and vacuum system.
- 5.5 **Membrane filter**, polyamide, 0,45 µm.
- 5.6 **HPLC**, equipped with diode array detector (DAD) or fluorescence detector (FLD).
- 5.7 **GC**, equipped with mass selective detector (MSD).
- 5.8 **Filter paper**, fast, quantitative.

6 Chemicals

Unless otherwise stated, analytical grade chemicals shall be used.

- 6.1 **Acetonitrile**, for HPLC.
- 6.2 **n-Hexane**.

NOTE Iso-hexane can also be used.

- 6.3 **Aluminium triiodide**, commercially available, or prepared according to [Annex A](#).
- 6.4 **Sulfuric acid solution**, 0,5 mol/l.
- 6.5 **Sodium thiosulfate solution**, saturated at room temperature.

6.6 Anhydrous magnesium sulfate (MgSO_4), for analysis.

6.7 Anhydrous sodium sulfate (Na_2SO_4), as desiccant for analysis. If not already an anhydrous powder, it can be treated at 800 °C for 4 h, store dry.

NOTE Other suitable desiccants can be used.

6.8 Sodium chloride solution, saturated at room temperature.

6.9 NP (in [Table 1](#)) **solution**, for calibration, 1 000 mg/l in n-hexane.

6.10 OP (in [Table 1](#)) **solution**, for calibration, 1 000 mg/l in n-hexane.

6.11 OPEO (in [Table 1](#)) **solution**, for calibration, 2 000 mg/l in acetonitrile. Dilute this solution with acetonitrile ([6.1](#)) if a calibration is applied.

6.12 NPEO (in [Table 1](#)) **solution**, for calibration, 4 000 mg/l in acetonitrile. Dilute this solution with acetonitrile ([6.1](#)) if a calibration is applied.

6.13 4n-nonylphenol (4n-NP, CAS no. 104-40-5) **solution**, 1 000 mg/l in acetonitrile. The 4n-NP can be used as an internal standard for GC-MS analysis. Dilute this solution with acetonitrile ([6.1](#)) if the internal calibration curve is applied.

6.14 4n-nonylphenol (4n-NP, CAS no. 104-40-5) **solution**, 1 000 mg/l in n-hexane. The 4n-NP can be used as an internal standard for GC-MS analysis. Dilute this solution with in n-hexane ([6.2](#)) if the internal calibration curve is applied.

6.15 Distilled or deionised water, according to ISO 3696:1987, Grade 3.

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7 Sampling and sample preparation

7.1 Preparation of leather samples

7.1.1 Sampling and preparation of samples

Sample the leather according to ISO 2418. If sampling in accordance with ISO 2418 is not possible (e.g. leathers from finished products like shoes, garments), details about the sampling shall be given in the test report.

Prepare the leather sample in accordance with ISO 4044.

7.1.2 Sample extraction

Accurately weigh to 10 mg approximately 2,5 g of the leather sample ([7.1.1](#)) into an Erlenmeyer flask, then mix with approximately 3 g of Na_2SO_4 ([6.7](#)). Add a (50,0 ± 1) ml aliquot of acetonitrile ([6.1](#)) into the flask, then close with a stopper.

For GC-MS analysis, an internal standard shall be used. Add a 100 µl aliquot of 4n-NP solution ([6.13](#)) to the flask to achieve the final concentration of 2,0 mg/l.

Put the flask into an ultrasonic water bath ([5.2](#)) and extract at (50 ± 5) °C for (60 ± 5) min. Then cool the flask to room temperature.

Filter the extracts through a fast, quantitative filter paper ([5.8](#)) to remove leather and salt particles. Collect at least 30 ml of the filtrate for the analysis as in [7.4](#) and [7.5](#).

7.2 Preparation of leather process auxiliary samples

Accurately weigh to 10 mg approximately 0,5 g of leather auxiliary sample into a flask, carefully mixed with approximately 2 g of MgSO₄ (6.6). Then use acetonitrile (6.1), 3 × 7 ml (approximately), to dissolve the sample by stirring with a glass rod. Filter the extracts through a quantitative filter paper. If the extracts contain insoluble material, centrifuge the extract. Collect the extracts in a 50 ml volumetric flask and fill to 50,0 ml with acetonitrile.

For GC-MS analysis, an internal standard shall be used. Add a 100 µl aliquot of 4n-NP solution (6.13) to the flask to achieve the final concentration of 2,0 mg/l.

7.3 Blank determination

Treat the blank in exactly the same way as the sample, but replace the sample with the appropriate amount of acetonitrile.

7.4 Determination of OP and NP

For HPLC analysis, use the sample extracts, either (7.1.2) or (7.2), directly after filtering through a polyamide membrane (5.5).

For GC-MS analysis, add 10,0 ml of the sample extract, either (7.1.2) or (7.2), to a separating funnel (5.3). Subsequently, add approximately 20 ml of water (6.15) and 1 ml of sulfuric acid solution (6.4). Extract the mixture with 2 × 20 ml (approximately) of n-hexane (6.2), separate, and collect the organic phase. After that, wash the n-hexane extracts with approximately 30 ml of water, remove the aqueous layer, and dehydrate the organic layer with approximately 5 g of Na₂SO₄ (6.7). Remove the organic solvent by rotary evaporator (5.4) at approximately 50 °C. Redissolve the residues in (10,0 ± 0,1) ml of n-hexane (6.2) and the solution is then ready for GC-MS analysis after filtering through a polyamide membrane (5.5).

If the organic phase cannot separate freely in the funnel after treating with n-hexane, add approximately 30 ml of saturated sodium chloride (6.8) to the funnel, then shake the mixture for approximately 30 s and stand until separated.

The signal response for the sample extracts should be within the concentration ranges of the calibration curves. If not, then the extract solutions shall be diluted accordingly with acetonitrile for HPLC analysis or n-hexane for GC-MS analysis.

7.5 Determination of OPEO and NPEO

Prepare aluminium triiodide (6.3) in acetonitrile for the cleavage of NPEO and OPEO according to Annex A.

Aluminium triiodide is extremely air and water sensitive. If commercial aluminium triiodide (6.3) is used, it can be dissolved in carbon disulfide at a concentration of approximately 0,1 g/ml. Pipette 10 ml of the solution into a flask and remove the solvent by heating before adding the sample extracts.

Add a 10,0 ml ± 0,1 ml aliquot of the sample extracts (7.1.2 or 7.2) into the flask containing approximately 1 g of aluminium triiodide, and continue refluxing at (90 ± 2) °C for (30 ± 5) min.

Take out the flask and slowly add water (6.15) dropwise until the reaction subsides. Dilute the contents with approximately 20 ml of water (6.15) and cool to room temperature.

Add the mixture to a separating funnel (5.3), rinse the flask with approximately 20 ml of n-hexane (6.2), and transfer the organic solution to the funnel. Then add approximately 1 ml of sulfuric acid solution (6.4) and shake. Collect the organic phase and extract the aqueous phase with another 20 ml of n-hexane. Combine all the organic phase. Subsequently, add approximately 2 ml of sodium thiosulfate solution (6.5) and shake until the pink colour (from iodine) disappears. Wash the organic phase with approximately 30 ml of water (6.15), remove the aqueous layer and dehydrate the organic layer with approximately 4 g of Na₂SO₄ (6.7). Remove the organic solvent by rotary evaporator at approximately 50 °C.

If the organic phase cannot separate freely in the funnel after treating with n-hexane, add approximately 30 ml of saturated sodium chloride (6.8) to the funnel, then shake the mixture for approximately 30 s and stand until separated.

For HPLC analysis, redissolve the residues in $(10,0 \pm 0,1)$ ml of acetonitrile (6.1) and filter through a polyamide membrane (5.5).

For GC-MS analysis, redissolve the residues in $(10,0 \pm 0,1)$ ml of n-hexane (6.2) and filter through a polyamide membrane (5.5).

The signal response for the sample extracts should be within the concentration ranges of the calibration curves. If not, then the extract solutions shall be diluted accordingly with acetonitrile for HPLC analysis or n-hexane for GC-MS analysis.

7.6 Chromatographic analysis

Detection of NP and OP can be performed using the chromatographic techniques HPLC (5.6) or GC-MS (5.7). Other validated methods may be used. The quantification is performed by means of HPLC or GC-MS. Where gas chromatography is used, an appropriate internal standard (6.13) or (6.14) shall be used.

Examples of suitable operating parameters and of the chromatographic analysis for NP and OP are listed in Annex B for HPLC and Annex C for GC-MS. The diagnostic ions for the identification and quantification are listed in Table C.1. Figures B.1 and B.2 show HPLC chromatograms and Figures C.1 and C.2 show GC-MS chromatograms.

7.7 Evaluation

For NP and OP, the amounts are usually calculated by means of a software program based on their peak areas and calibration curves. For NPEO and OPEO, the amounts are calculated based on the peak areas of the yielded NP and OP, as well as their calibration curves.

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8 Calibration

8.1 Calibration for OP and NP

The external calibration curves for NP and OP are prepared by directly measuring five levels of increasing concentrations of NP and OP standards in the range 1 mg/l to 20 mg/l. For example, standards of 1 mg/l, 5 mg/l, 10 mg/l, 15 mg/l and 20 mg/l.

For GC-MS, each standard contains a 100 µl aliquot of the 4n-NP internal standard solution (6.14) with a constant 4n-NP concentration of 2,0 mg/l. For internal calibration curves, plots are made by measuring five levels of increasing concentration of NP and OP standards in the range 1 mg/l to 20 mg/l with a constant 4n-NP concentration as the internal standard.

8.2 Calibration for OPEO and NPEO

For the external calibration curves of NPEO and OPEO, 10,0 ml of acetonitrile spiked with NPEO₉ and OPEO₁₀ (listed in Table 1) standards are prepared in the range 2 mg/l to 50 mg/l. For example, standards of 2 mg/l, 10 mg/l, 20 mg/l, 30 mg/l and 50 mg/l.

For GC-MS, each standard contains a 100 µl aliquot of the 4n-NP internal standard solution (6.13) with a constant 4n-NP concentration of 2,0 mg/l.

The solutions are treated as specified in 7.2 to 7.5. The external calibration curves are made by plotting five pairs of the given amounts of NPEO₉ and OPEO₁₀ and the signal response of the yielded NP and OP. The internal calibration curves are made by measuring the five levels of increasing concentrations of NPEO₉ and OPEO₁₀ and the signal response of the yielded NP and OP with a constant 4n-NP concentration of 2,0 mg/l as internal standard.