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IULTCS IUC 28-1

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Leather — Determination of ethoxylated alkylphenols (APEO) —

Part 1: Direct method

Cuir — Détermination des alkylphénols éthoxylés (APEO) —

Partie 1: Méthode directe

Reference numbers
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Contents

Page

Foreword

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

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 18218-1:2015), which has been technically revised.

The main changes are as follows:

— a new Clause 3 has been added and subsequent clauses renumbered;

— Clauses 6 and 8 have been technically revised;

— the NOTE in 7.1 of the previous edition has been deleted;

— a new calculation procedure in 8.4 has been added;

— Annex A has been technically revised;

— a new Annex B has been added.

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, 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[4] restricted the sale and use of nonylphenol and nonylphenol ethoxylate in product preparations for industries with discharges to wastewater. 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).[3]

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

(C_9 alkyl chain, branched or linear)–Ph–$[OCH_2CH_2]_n$–OH

where Ph = phenyl, $n \geq 1$.

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]."[5]

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 document 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 liquid chromatography (LC) with triple quadrupole mass spectrometer (MS/MS) to identify the nonylphenol ethoxylate and octylphenol ethoxylate.

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

Leather — Determination of ethoxylated alkylphenols (APEO) —

Part 1: Direct method

1 Scope

This document is a method for determining ethoxylated alkylphenols (APEO) [nonyphenol ethoxylate ($NPEO_n$, where $2 \leq n \leq 16$) and octylphenol ethoxylate ($OPEO_n$, where $2 \leq n \leq 16$)] in leather. This direct method is especially suitable when a larger number of leather samples are to be checked for the presence of ethoxylated alkylphenols.

This method requires the use of liquid chromatography (LC) with a triple quadrupole mass spectrometer (MS/MS) to identify and quantify the ethoxylated alkylphenols.

NOTE 1 In the leather industry, the most commonly used 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 This document 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 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*

3 Terms and definitions

No terms and definitions are listed in this document.

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

— ISO Online browsing platform: available at www.iso.org/obp

— IEC Electropedia: available at www.electropedia.org/

4 Principle

The leather sample is extracted with methanol using an ultrasonic bath. Subsequently, an aliquot of the solution can, after filtering, be directly analysed without further cleaning of the sample using LC with a MS/MS detector.

5 Apparatus

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

5.1 Ultrasonic bath, with controllable heating capable of maintaining a temperature of (60 ± 5) °C.

5.2 Glass vial with a screw cap, for example, 22 ml is suitable.

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

5.4 Membrane filter, for example, pore size 0,2 µm, for use with a syringe (5.3).

5.5 Volumetric flasks, 10 ml, 100 ml and 1 000 ml.

5.6 Analytical balance, weighing to 1 mg.

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

5.8 LC vial with cap.

5.9 Instrumental equipment, high-performance LC with MS/MS and electrospray ionization (ESI).

6 Reagents

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

6.1 Methanol, LC-MS grade.

6.2 Nonylphenol ethoxylate, $NPEO_n$ where n = 9 to 10, CAS Registry Number®[1] 68412-54-4, Sigma-Aldrich® Product No. 542334 (IGEPAL® CO-630)[2], technical grade.

6.3 Octylphenol ethoxylate, $OPEO_n$ where n = 9 to 10, CAS Registry Number 9036-19-5, Sigma-Aldrich® Product No. 93443 (Triton™ X-100)[3], technical grade.

NOTE In 6.2 and 6.3 the brand name is given to improve the comparability of test results among laboratories. The commercial nonylphenol or octylphenol ethoxylate contains groups of ethoxylates of nonylphenol or 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.

6.4 Stock solution of nonylphenol ethoxylate, β = 250 µg/ml

Weigh 25 mg of the nonylphenol ethoxylate (6.2) into a 100 ml volumetric flask (5.5), dissolve it in methanol (6.1) and fill up to the mark with methanol.

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1) Chemical Abstracts Service (CAS) Registry Number® is a trademark of the American Chemical Society (ACS). 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.

2) Sigma-Aldrich® Product No. 542334 (IGEPAL® CO-630) is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

3) Sigma-Aldrich® Product No. 93443 (Triton™ X-100) is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

6.5 Stock solutions of octylphenol ethoxylate, β = 250 µg/ml

Weigh 25 mg of the octylphenol ethoxylate (6.3) into a 100 ml volumetric flask (5.5), dissolve it in methanol (6.1) and fill up to the mark with methanol.

6.6 Standard solution of nonylphenol ethoxylate, β = 25 µg/ml

Transfer 1,0 ml of the stock solution (6.4) to a 10 ml volumetric flask (5.5) and fill up to the mark with methanol (6.1).

6.7 Standard solution of octylphenol ethoxylate, β = 25 µg/ml

Transfer 1,0 ml of the stock solution (6.5) to a 10 ml volumetric flask (5.5) and fill up to the mark with methanol (6.1).

6.8 Calibration solutions of nonylphenol ethoxylate and octylphenol ethoxylate

Prepare at least four calibration solutions using the respective standard solutions (6.6 and 6.7). Table 1 gives an example of the calibration solutions prepared in a 10 ml volumetric flask.

Concentration ranges for the calibration standards are subject to change depending on the need of each laboratory and equipment used.

Table 1 — Example of calibration solutions

Concentration (µg/ml)	**0,5**	**1,0**	**1,5**	**2,0**	**2,5**
Volume methanol (ml)	9,8	9,6	9,4	9,2	9,0
Volume standard solutions (6.6 or 6.7) at 25 µg/ml (ml)	0,2	0,4	0,6	0,8	1,0

6.9 Ammonium acetate

6.10 5 mM ammonium acetate

Dissolve 0,386 g ammonium acetate (6.9) in water (6.11) in a 1 000 ml volumetric flask (5.5). Fill the flask up to the mark with water (6.11).

6.11 Water, grade 3 according to ISO 3696.

7 Sampling

Cut a test specimen in accordance with ISO 2418. If cutting a test specimen according to ISO 2418 is not possible (e.g. in the case of leather from finished products such as shoes or clothing), details of the selection of the test specimen shall be given in the test report. Glue residuals shall be mechanically removed from leather pieces.

The leather test specimen shall be cut into small pieces or ground according to ISO 4044.

8 Sample preparation and analysis

8.1 Extraction

Weigh approximately 1 g of the leather pieces accurately to 10 mg in a glass vial (5.2). Add 20 ml methanol (6.1), close the vial and extract the sample at (60 ± 5) °C for (60 ± 5) min in an ultrasonic bath (5.1).

8.2 Analysis

After cooling down to room temperature, remove an aliquot of the extraction solution using a disposable syringe (5.3) and transfer into a LC vial (5.8) using a membrane filter (5.4). The aliquot is now ready for the LC analysis.

The detection of the alkylphenol ethoxylate is made using an LC with gradient elution and MS/MS (5.9). Congeners with 2 to 16 ethoxylate groups shall be used for quantification. Guidelines for suitable chromatographic conditions are given in Annex A.

8.3 Calibration

The calibration solutions (6.8) shall be transferred to an LC vial (5.8) and analysed along with each batch of test samples.

8.4 Calculation

8.4.1 Determination of the ratio of each APEO congener

Calculate the ratio of each APEO congener, R (%), according to Formula (1), using selected ion monitoring (SIM) data.

$$R = \frac{A_{(n)}}{A_{\text{sum}}} \times 100 \tag{1}$$

where

$A_{(n)}$ is the SIM area of each APEO congener;

A_{sum} is the sum of the SIM areas from all APEO congeners.

The calculation according to 8.4.1 shall be made for each new batch of the reference substances (6.2 and 6.3) and shall be done in the SIM mode. See Annex B, Tables B.1 to B.3, for the characteristic masses of the APEO congeners.

NOTE An accurate distribution of the APEO congeners cannot be received from multiple reaction monitoring (MRM) data because the APEO fragmentation efficiencies are highly dependent on the number of ethoxylate groups.

8.4.2 Determination of the real concentration of each APEO congener in the calibration standards

Calculate the real concentration of each APEO congener in the calibration standards (6.8) in µg/ml according to Formula (2).

$$c_{\text{stdAPEO}(n)} = \frac{R \times c_{\text{std}}}{100} \tag{2}$$

where

R is the ratio of each APEO congener, in %;

c_{std} is the overall concentration of the respective calibration standard, in µg/ml.

8.4.3 Calibration graph

The calibration graphs are prepared with the help of the area of each APEO congener (y-axis) and the calculated real concentration of the respective calibration standard (x-axis) (8.4.2). For each

APEO congener, a separate calibration graph is necessary. The determination of the area of the daily calibration (6.8) and the area of the daily APEO samples shall be done in MRM mode.

8.4.4 Calculation of the APEO concentration

The NPEO content in the leather sample, s_{NPEO}, in mg/kg is calculated according to Formula (3).

$$s_{NPEO} = \frac{c \times V}{m} \times F_d \quad (3)$$

where

c is the total sum of the concentrations of each NPEO congener in the sample (µg/ml) with $\sum_{n=2}^{16} c_{NPEO(n)}$;

n is the ethoxylate chain length;

$c_{NPEO(n)}$ is the concentration of the NPEO congener with n ethoxylate groups in the extract;

V is the extraction volume according to 8.1, in ml;

m is the mass of the leather sample, in g;

F_d is the dilution factor (if used).

The OPEO content in the leather sample, s_{OPEO}, in mg/kg is calculated according to Formula (4).

$$s_{OPEO} = \frac{c \times V}{m} \times F_d \quad (4)$$

where

c is the total sum of concentration of each OPEO congener in the sample (µg/ml),

with: $c = \sum_{n=2}^{16} c_{OPEO(n)}$;

n is the number of ethoxylate groups;

$c_{OPEO(n)}$ is the concentration of the OPEO congener with n ethoxylate groups in the extract;

V is the extraction volume according to 8.1, in ml;

m is the mass of the leather sample, in g;

F_d is the dilution factor (if used).

The APEO content in leather is reported in mg/kg as the sum of the NPEO and OPEO contents determined in Formulae (3) and (4).

9 Test report

The test report shall include at least the following information:

a) a reference to this document, i.e. ISO 18218-1:2023;

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

c) the date of the test;

d) sampling method, if different to ISO 2418;

e) APEO content, stated as the sum of the NPEO and OPEO content in milligrams per kilogram (mg/kg), as determined in 8.4.4.

f) any deviation from the given procedure, including the use of other commercial standard solutions.

Annex A
(informative)

Example of chromatographic method LC-MS/MS

A.1 Preliminary comment

As the LC equipment (5.9) 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 Chromatographic conditions for the LC-MS/MS method

Eluent 1	5 mM ammonium acetate
Eluent 2	Methanol
Column	Reversed-phase column C18 (5 µm, 2,1 × 50 mm)
Guard column	Reversed-phase guard column C18, 5 µm
Flow rate	1,2 ml/min
Gradient time programme	— 0 min, 30 % MeOH — 0 min to 2 min, up to 80 % MeOH — 2 min to 3 min, up to 98 % MeOH — 3 min to 3,5 min, hold 98 % MeOH — 3,5 min to 4,5 min, down to 30 % MeOH — 4,5 min to 8 min, hold at 30 % MeOH
Column temperature	30 °C
Injection volume	20 µl
Detection	Four tandem type pile pole or ion trap mass detector Selected reaction monitoring (SRM) method Product ion mass spectrum
Ionizing	ESI (electro spray ionizing) method and positive/negative ion detection

Annex B
(informative)

Characteristic masses for quantification

Table B.1 — Characteristic masses for quantification $[M + NH_4]^+2$

NPEO congeners	Q1 m/z	Q3 m/z	OPEO congeners	Q1 m/z	Q3 m/z
NPEO 16	942	925	OPEO 16	928	911
NPEO 15	898	881	OPEO 15	884	867
NPEO 14	854	837	OPEO 14	840	823
NPEO 13	810	793	OPEO 13	796	779
NPEO 12	766	749	OPEO 12	752	735
NPEO 11	722	705	OPEO 11	708	691
NPEO 10	678	661	OPEO 10	664	647
NPEO 9	634	617	OPEO 9	620	603
NPEO 8	590	573	OPEO 8	576	559
NPEO 7	546	529	OPEO 7	532	515
NPEO 6	502	485	OPEO 6	488	471
NPEO 5	458	441	OPEO 5	444	427
NPEO 4	414	397	OPEO 4	400	383
NPEO 3	370	353	OPEO 3	356	227
NPEO 2	326	183	OPEO 2	312	183

Table B.2 — SIM masses of the APEO congeners $[M + NH_4]$

NPEO congener	SIM mass	OPEO congener	SIM mass
NPEO 2	326	OPEO 2	312
NPEO 3	370	OPEO 3	356
NPEO 4	414	OPEO 4	400
NPEO 5	458	OPEO 5	444
NPEO 6	502	OPEO 6	488
NPEO 7	546	OPEO 7	532
NPEO 8	590	OPEO 8	576
NPEO 9	634	OPEO 9	620
NPEO 10	678	OPEO 10	664
NPEO 11	722	OPEO 11	708
NPEO 12	766	OPEO 12	752
NPEO 13	810	OPEO 13	796
NPEO 14	854	OPEO 14	840
NPEO 15	898	OPEO 15	884
NPEO 16	942	OPEO 16	928

Table B.3 — Molecular weight of the APEO congeners

NPEO congener	Molecular weight	OPEO congener	Molecular weight
NPEO 2	308	OPEO 2	294
NPEO 3	352	OPEO 3	338
NPEO 4	396	OPEO 4	382
NPEO 5	440	OPEO 5	426
NPEO 6	484	OPEO 6	470
NPEO 7	528	OPEO 7	514
NPEO 8	572	OPEO 8	558
NPEO 9	616	OPEO 9	602
NPEO 10	660	OPEO 10	646
NPEO 11	704	OPEO 11	690
NPEO 12	748	OPEO 12	734
NPEO 13	792	OPEO 13	778
NPEO 14	836	OPEO 14	822
NPEO 15	880	OPEO 15	866
NPEO 16	926	OPEO 16	910

Bibliography

[1] ISO 18218-2, *Leather — Determination of ethoxylated alkylphenols — Part 2: Indirect method*

[2] ISO 18254-1, *Textiles — Method for the detection and determination of alkylphenol ethoxylates (APEO) — Part 1: Method using HPLC-MS*

[3] Regulation (EC) No 1907/2006 (REACH), Annex XVII. Available from: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:136:0003:0280:en:PDF

[4] Directive 2003/53/EC of the European Parliament and of the Council. June 2003. Available from: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32003L0053

[5] European Chemicals Agency (ECHA). Substance Information: 4-Nonylphenol, branched and linear, ethoxylated. Available from: https://echa.europa.eu/en/substance-information/-/substanceinfo/100.239.148

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