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Textiles — Method for the detection and determination of alkylphenol ethoxylates (APEO) —

Part 1: Method using HPLC-MS

Textiles — Méthode de détection et de détermination des alkylphénols éthoxylés (APEO) —

Partie 1: Méthode utilisant la CLHP-SM

Reference number
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Foreword

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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).

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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 18254-1 was prepared by the European Committee for Standardization (CEN) in collaboration with ISO Technical Committee ISO/TC 38, *Textiles*, and Technical Committee CEN/TC 248, *Textiles and textile products* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

ISO 18254 consists of the following parts under the general title *Textiles — Method for the detection and determination of alkylphenol ethoxylates (APEO)*:

— *Part 1: Method using HPLC-MS*

The following part is under preparation:

— *Part 2; Method using NPLC*

Introduction

Alkylphenol ethoxylates (APEOs) are high-value products commonly used in industrial and consumer detergents and cleaners, some plastics and many industrial applications. Their “down the drain” uses may result in their presence in wastewater streams and receiving water bodies. Human exposure to APEO can occur through diverse sources such as environmental, food, or skin contact. Considering their toxicity on several animal species, minimization of exposure to APEO is recognized as important to the preservation of human health.

Nonylphenol ethoxylates belong to the non-ionic surfactant category and are of particular concern. The biodegradation of nonylphenol ethoxylate releases the branched nonylphenol, which is difficult to biodegrade. Nonylphenol is a substance having endocrine disruptive properties that can have serious effects on aquatic and many other organisms. For this reason, the release of nonylphenol ethoxylate into the environment should be avoided.

Chemical products containing nonylphenol and/or nonylphenol ethoxylates in concentrations equal to or greater than 0,1 % are restricted within the EU for specific uses, among others, the processing of leather and textiles, industrial, and institutional cleaning.

This restriction is part of the entry 46 of Annex XVII of the REACH regulation EU 1907/2006, which repealed the former Directive 2003/53/EC.

The current restriction is due to be widened to apply to textile products that can be washed in water. A limit value of 0,01 % (100 ppm) is expected.

Textiles — Method for the detection and determination of alkylphenol ethoxylates (APEO) —

Part 1: Method using HPLC-MS

SAFETY PRECAUTIONS — It is the user's responsibility to use safe and proper techniques in handling materials in this test method. Consult manufacturers for specific details such as material safety, data sheets, and other recommendations. Good laboratory practice should be followed. Users should comply with any national and local safety regulations.

1 Scope

This part of ISO 18254 describes analyses that are used to detect extractable alkylphenol ethoxylates (nonylphenol ethoxylates and octylphenol ethoxylates) in textile products. This document provides a method that uses Liquid Chromatograph (LC) with Mass Spectrometry (MS) system to detect and quantify alkylphenol ethoxylates of defined ethoxylate chain length.

2 Principle

The textile sample is cut into small pieces, transferred to a vial, and extracted with methanol using ultrasound. The extract is filtered and not subjected to any additional cleaning. Subsequently, the methanol extract is analysed by Liquid Chromatography (LC) with Mass Spectrometry (MS).

3 Reagents

During the analysis, unless otherwise stated, only reagents of recognized analytical grade shall be used.

NOTE OPEO and NPEO are available currently as technical grade.

3.1 Solvents, of quality for HPLC analysis

3.2 Octylphenol ethoxylates, (Triton®[1)] X-100), (OPEOs) CAS no. 9002-93-1, Sigma-Aldrich® Part number T9284 (see Note in 3.3).

3.3 Nonylphenol ethoxylates, (IGEPAL®[2)] CO-630), (NPEOs) CAS no. 68412-54-4, Sigma-Aldrich® Part number 542334 (see Note).

NOTE The mentioned brand names in 3.2 and 3.3 are given to improve the comparability of the test results amongst laboratories. Using another batch or another supplier could lead to different results.

3.4 Methanol.

3.5 Acetonitrile (ACN).

1) Triton® 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.

2) IGEPAL® 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.

3.6 HPLC grade water.

3.7 Formic acid, volume fraction of 30 %.

3.8 Ammonium acetate.

3.9 10 mM ammonium acetate, pH 3,6.

3.9.1 0,771 g of ammonium acetate is dissolved in 900 ml of water (3.6).

3.9.2 10 ml of ACN (3.5) is added and mixed well.

3.9.3 The pH is adjusted to 3,6 with volume fraction of 30 % formic acid and made up to the mark with water (3.6) in a 1 l volumetric flask.

3.9.4 The buffered solution should be filtered before use.

4 Apparatus

4.1 Apparatus and auxiliaries for preparing the sample

4.1.1 Standard laboratory equipment.

4.1.2 Analytical balance, resolution at 0,01 g, for test specimen preparation.

4.1.3 Analytical balance, resolution at 0,001 g, for standard preparation.

4.1.4 Glass container with screw top, 40 ml has been found suitable.

4.1.5 Ultrasonic water bath, to be set up at 70 °C ± 5 °C.

4.1.6 Membrane filter, with 0,45 µm pore size.

4.1.7 Glass vial, with septum cap (HPLC vial).

4.1.8 pH meter, resolution of 0,1 pH.

4.2 Chromatographic equipment

4.2.1 High-performance Liquid Chromatography (LC) with Mass Spectrometry (MS) and Electro Spray Ionization (ESI).

4.2.2 Reverse phase column with guard column.

5 Procedure

5.1 Standard preparation

Stock solutions of a) OPEO and b) NPEO are prepared in methanol containing 1 000 mg/l OPEO (3.2) and NPEO (3.3).

5.2 Sample preparation

Cut the textile sample into pieces of approximately 5 mm × 5 mm and mix them homogeneously.

Prepare approximately 1 g of the cut textile, weigh it to the nearest 10 mg, and then place it into the glass container (extraction vessel).

Pipette 20 ml of methanol into the glass container (extraction vessel).

Place the glass container (extraction vessel) into an ultrasonic bath at 70 °C for (60 ± 5) min.

Afterwards, let the extract cool down to room temperature.

Filter about 1 ml of the extraction solution into a HPLC vial using a disposable syringe equipped with a membrane filter.

5.3 Analysis

The detection and quantification of defined alkylphenol ethoxylates is conducted using LC/MS with gradient elution and ESI mass spectrometer.

Congeners with 2 to 16 ethoxylate groups shall be used for quantification.

Guidelines for suitable chromatographic conditions are given in Annex A for LC/MS and in Annex B for LC/MS/MS.

6 Calculation of results

6.1 Determination of the *R* value for each congener of APEO

Calibrate the mass fraction (R) for each APEO congener and calculate the concentration of each APEO isomer as follows.

$$\text{A mass fraction in each APEO } (n),\ R\ (\%) = \frac{\text{AO}}{\text{AOT}} \times 100 \tag{1}$$

where

AO is the area response of each APEO congener;

AOT is the total sum of APEO area responses (from APEO 2 to APEO 16).

$$\text{Exact concentration in each APEO } (n),\ \text{Exact concentration (mg/l)} = \frac{R \times C_{\text{std}}}{100}\ \text{mg/l} \tag{2}$$

where

C_{std} is the concentration of APEO standard mix solution of each working solution.

6.2 Calibration curve

Calibration curves with standards of a) OPEO and b) NPEO at 0,5 mg/l, 2 mg/l, and 5 mg/l are prepared with at least three calibration points.

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

For quantification, the calibration curve shall have a correlation coefficient greater than 0,990 (R^2 greater than 0,995).

6.3 Calculation of concentration of APEO result

The APEO level in the textile sample is calculated according to Formula (3).

$$\text{NPEOs in sample, NPEOs (mg/kg)} = \left(\sum \frac{C \times V}{M} \right) \times DF \quad (3)$$

where

C is the total sum of concentration of each NPEO isomer (mg/l), with: $C = \sum \text{NPEO}_i \times R_i$;

i represents the ethoxylate chain length;

R_i is the percentage of "i" in a standard solution;

V is the extraction volume according to 5.1;

M is the mass of the textile specimen, in grams;

DF is the dilution factor.

$$\text{OPEOs in sample, OPEOs (mg/kg)} = \left(\sum \frac{C \times V}{M} \right) \times DF \quad (4)$$

where

C is the total sum of concentration of each OPEO isomer (mg/l), with: $C = \sum \text{OPEO}_i \times R_i$;

i represents the ethoxylate chain length;

R_i is the percentage of "i" in a standard solution;

V is the extraction volume according to 5.1;

M is the mass of the textile specimen, in grams;

DF is the dilution factor.

If a result is below 50 mg/kg, then report it as "less than 50 mg/kg".

7 Test report

The test report shall include the following particulars:

a) a reference to this part of ISO 18254, i.e. ISO 18254-1:2016;

b) the kind, origin, and designation of the specimen (partial specimen, if applicable);

c) the detection method and quantification method, including the number (e.g. EO_2 to EO_{16}) and name (e.g. NPEO and OPEO) of all the target analytes determined and of all the standards used;

d) the results as sum of NPEOs and sum of OPEOs in mg/kg;

e) any deviation from the given procedure.

Annex A
(informative)

Examples of chromatographic method – LC/MS

A.1 Identification with the LC/MS method

A.1.1 Chromatographic conditions for the LC/MS method

— Mobile Phase: Ammonium acetate 10 mM pH 3,6 / acetonitrile (ACN)

— Gradient:

  — 0 min to 1 min 40 % to 60 % ACN

  — 1 min to 5 min 60 % to 98 % ACN

  — 5 min to 10 min 98 % ACN

— Flow: 300 µl/min

— Injection: 5 µl

— Temperature column oven: 40°C

— Run injection: Each batch consists of an initial three-point calibration, and then a blank check and a Quality Control (QC) check for every 20 samples or less.

A.1.2 Device parameters for the LC/MS method

Example: Suitable parameters for quadrupole mass-spectrometer

— Polarity: Positive API-ES

— Mode: SCAN mode

— Mass range: 100 amu to 1 100 amu

— Fragmentor: 80 V

— EMV and Threshold: 150

— Dry gas temperature: 300 °C

— Nebulizer pressure: 2,4 bar (35 psi)

— Gas flow: 10 l/min

— Capillary voltage 3 000 V

Table A.1 — Characteristic masses for quantification

NPEO congeners	Target mass, m/z [M + NH4]	OPEO congeners	Target mass, m/z [M + NH4]
NPEO 16	942	OPEO 16	928
NPEO 15	898	OPEO 15	884
NPEO 14	854	OPEO 14	840
NPEO 13	810	OPEO 13	796
NPEO 12	766	OPEO 12	752
NPEO 11	722	OPEO 11	708
NPEO 10	678	OPEO 10	664
NPEO 9	634	OPEO 9	620
NPEO 8	590	OPEO 8	576
NPEO 7	546	OPEO 7	532
NPEO 6	502	OPEO 6	488
NPEO 5	458	OPEO 5	444
NPEO 4	414	OPEO 4	400
NPEO 3	370	OPEO 3	356
NPEO 2	326	OPEO 2	312

A.2 Reliability of the detection method

The following data have been obtained by correlation study from December (2010) to January (2011) performed by 12 laboratories, related to alkylphenol ethoxylates (APEO) in a blue denim fabric, carrying out the extraction at 40 °C.

All laboratories gave consistent results. No outlier was observed in this exercise.

Table A.2 — APEO content in a blue denim fabric

Laboratory	Amount (mg/kg)
A	940
B	937
C	969
D	1 006
E	1 068
F	1 050
G	804
H	811
I	828
J	1 030
K	1 028
L	979
Mean	**954**
Reproducibility standard deviation *R* limit	 94 262

Based on the algorithm A, as described in ISO 13528:2005 [3)], the results are:

Result	Amount (mg/kg)
“robust” mean	974
“robust” std deviation	101
uncertainty type u_X	44

3) ISO 13528:2005 has been revised by ISO 13528:2015.

Annex B
(informative)

Examples of chromatographic method – LC/MS/MS

B.1 Chromatographic conditions for the LC/MS/MS method

Table B.1 — Chromatographic conditions

Eluent 1:	Ammonium acetate 10 mM pH 3,6
Eluent 2:	Acetonitrile (ACN)
Column:	Hypersil Gold (2,1 × 100 mm) or equivalent ones of other manufacturers
Flow rate:	250 µl/min
Gradient **Time program**	— 0 min to 1 min 70 % ACN — 1 min to 5 min 95 % ACN — 5 min to 7 min 70 % ACN
Column temperature	40 °C
Injection volume:	5 µl
Detection:	Four tandem type pile pole or ion trap mass detector SRM (Selected Reaction Monitoring) method Product ion mass spectrum
Ionizing:	ESI electro spray ionizing method and positive/negative ion detection
Capillary voltage:	3 000 V
Temperature of spray:	100 °C
Spray gas:	Nitrogen
Spray energy:	30 eV

Table B.2 — Characteristic masses for quantification

NPEO congeners	Q1 (m/z)	Q3 (m/z)	Collision energy (eV)	OPEO congeners	Q1 (m/z)	Q3 (m/z)	Collision energy (eV)
NPEO 15	898	133	33	OPEO 15	884	133	33
NPEO 14	854	133	32	OPEO 14	840	133	33
NPEO 13	810	133	31	OPEO 13	796	133	31
NPEO 12	766	133	30	OPEO 12	752	133	30
NPEO 11	722	133	28	OPEO 11	708	133	30
NPEO 10	678	133	29	OPEO 10	664	133	30
NPEO 9	634	133	26	OPEO 9	620	133	25
NPEO 8	590	133	26	OPEO 8	576	560	23
NPEO 7	546	133	22	OPEO 7	532	515	22
NPEO 6	502	485	20	OPEO 6	488	359	19
NPEO 5	458	441	17	OPEO 5	444	315	17

Table B.2 *(continued)*

NPEO congeners	Q1 (m/z)	Q3 (m/z)	Collision energy (eV)	OPEO congeners	Q1 (m/z)	Q3 (m/z)	Collision energy (eV)
NPEO 4	414	271	13	OPEO 4	400	271	14
NPEO 3	370	227	13	OPEO 3	356	227	12
NPEO 2	326	183	11	OPEO 2	312	183	12

Bibliography

[1] ISO 13528:2005, *Statistical methods for use in proficiency testing by interlaboratory comparisons*

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