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

**Part 1:  
Method using HPLC-MS**

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*Textiles — Méthode de détection et de détermination des alkylphénols  
éthoxylés (APEO) —  
Partie 1: Méthode utilisant la CLHP-SM*

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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](http://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](http://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 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.

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# 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<sup>®</sup> X-100), (OPEOs) CAS no. 9002-93-1, Sigma-Aldrich<sup>®</sup> Part number T9284 (see Note in 3.3).

**3.3 Nonylphenol ethoxylates**, (IGEPAL<sup>®</sup> CO-630), (NPEOs) CAS no. 68412-54-4, Sigma-Aldrich<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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.

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**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.

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## 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.

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

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).

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

where

$C_{\text{std}}$  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).