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Surface active agents — Detergents — Determination of alkylphenol ethoxylates

Agents de surface — Détergents — Dosage des alkylphénols éthoxylés

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Foreword

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This document was prepared by Technical Committee ISO/TC 91, *Surface active agents*.

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.

Surface active agents — Detergents — Determination of alkylphenol ethoxylates

1 Scope

This document provides a method for the determination of alkylphenol ethoxylates (APEOs) in surfactants using high performance liquid chromatography (HPLC) and detected with diode array detector (DAD) or fluorescence detector (FLD).

This method is appropriate for the detection and quantification of APEOs in surfactants.

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 607, *Surface active agents and detergents — Methods of sample division*

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

The sample is weighed into a vial and extracted in methanol using an ultrasonic bath. Subsequently the extract is filtered and analysed using high performance chromatography system with diode array detector (DAD) or fluorescence detector (FLD). Quantification is achieved by external quantification method and using the relationship chromatographic peak through its unique its retention time.

5 Reagents and materials

During the analysis, use only reagents of recognized analytical grade and the water of quality for HPLC analysis.

5.1 Methanol, HPLC Grade.

5.2 Octylphenol ethoxylates (OP_nEO, $2 \leq n \leq 12$), for example, Triton X-100[1)], CAS no. 9002-93-1, Sigma-Aldrich Part Number T9284.

1) Triton X-1000 ®, CAS no. 9002-93-1, Sigma Aldrich Part Number T9284 is an example of products. 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.

5.3 Nonylphenol ethoxylates (NP_nEO, $3 \leq n \leq 18$), for example, IGEPAL CO-630[2], CAS no. 9016-45-9, Sigma-Aldrich Part Number 542334.

NOTE There is a possibility that reagents of the grade of OPEO and NPEO are not available.

6 Apparatus

Normal laboratory apparatus and, in particular, the following:

6.1 Analytical balance, weighing to an accuracy of 0,1 mg.

6.2 Ultrasonic bath, 40 kHz, with thermostat.

6.3 Membrane filter, polyamide, 0,45 µm.

6.4 Volumetric flasks, 10 ml, 50 and 100 ml capacity.

6.5 High-performance Liquid Chromatography (HPLC) with DAD or FLD detector.

6.6 Reverse phase column with guard column, a stainless-steel column 150 mm and 2,1 mm in internal diameter filled with 3,5 µm ODS (C_{18}) as stationary phases.

7 Procedure

7.1 Standard preparation

7.1.1 Prepare 1 000 mg/l stock solutions of each APEOs from standard materials or certified solutions in methanol. 100 mg of the OP_nEO (5.2) and NP_nEO (5.3) are dissolved in different 100 ml volumetric flasks with methanol and filled up to the mark respectively.

Depending on the stock concentrations prepared, the solubility at that concentration will have to be ensured.

7.1.2 Prepare calibration curve for samples of each APEO stock standard solution at required concentrations by adding aliquots of stock solutions to a 100 ml volumetric flask and then diluted with methanol to the desired calibration level. If lower reporting limit is required, lower concentration of calibration standard shall be prepared to cover the reporting limit. Each calibration curve is prepared using the respective stock solution. The preparation of the Level 5 standard can be accomplished using different volumes and concentrations of stock solutions as is accustomed in the individual laboratory.

The calibration vials shall be used within 24 h to ensure optimum results. Stock calibration standards are routinely replaced every 14 days if not previously discarded for quality control failure.

7.2 Sample preparation

The laboratory samples shall be prepared and stored in accordance with ISO 607.

The test samples can be in liquid, paste or solid form. Liquefaction and homogenization can be necessary for paste and solid substances. Liquefaction can be accelerated by gentle warming (below 50 °C).

2) IGEPAL CO-630 ®, CAS no. 9016-45-9, Sigma-Aldrich Part Number 542334 is an example of products. 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.

Weigh approximately 1,0 g (accurate to 0,1 mg) of the test sample to 50 ml volumetric flasks and make up to volume with methanol. Put the flask into an ultrasonic water bath and extract at least 30 min. Then filter the extracts through a 0,45 µm nylon filter disc for analysis.

7.3 Analysis

7.3.1 Chromatographic condition

As the HPLC equipment system of the laboratories can vary, no general valid instructions can be provided for the chromatographic analysis. The following parameters are provided as an example.

— Column: StableBond SB-C18, 150 mm × 2,1 mm (i.d.) or equivalent

— Stationary phase: C18 reverse phase, 3,5 µm

— Eluent 1: Methanol

— Eluent 2: Water

— Gradient elution: see Table 1

— Flow rate: 0,3 ml/min

— Column temperature: 40 °C

— Injection: 10,0 µl

— Detection: FLD or DAD, spectrograph

— Quantification: for DAD at 225 nm
for FLD with Ex = 230 nm and Em = 296 nm

Table 1 — Gradient elution for HPLC

Time min	Eluent 1 %	Eluent 2 %
0,00	85	15
20,00	98	2
25,00	98	2
26,00	85	15
30,00	85	15

7.3.2 Qualitative determination

Injection volumes of all calibration standards and extraction solutions at concentration gradient are made at 10,0 µl volume by autosampler under selected conditions. An aliquot of the extraction solution is automatically ejected onto a reverse phase column (6.6) for sample component separation. Under the chromatographic conditions given, the detection and identification of the chromatogram peaks can be identified by comparison with the retention times of the substances contained in the chromatogram of all the pure substances.

7.3.3 Calibration curve

Prepare a 5-point calibration curve by plotting the mass concentration of alkylphenol ethoxylates as abscissa and peak area for the vertical axis, using the standard curve samples determine the concentration. For quantification, the calibration curve shall have a correlation coefficient greater than 0,990 (R^2 greater than 0,980).

The Limit of quantitation (LOQ) of this method for OP_nEO (5.2) and NP_nEO (5.3) are 25 mg/kg and 10 mg/kg respectively. Observed detection limits may vary among different samples, depending upon the nature of interferences in the sample matrix and the characteristics of the specific instrumentation used.

7.3.4 Quantitative determination

Test the samples by chromatography procedure and calculate the analyte level in sample based on the concentration measured according to the corresponding linear regression equations and peak areas. For quantitative analysis of the analytes, the target compounds are identified by comparison of retention times in the sample to those of the standards.

Using the calibration solution, establish a standard calibration curve. External calibration curves are used to calculate the amount of each individual target compound. The response value of sample solution should be within the linear range of the instrument detected. It is possible to dilute with methanol when sample concentration falls outside the calibration range.

The quantification limit of an individual analytical procedure is based on signal-to-noise approach with the signal to noise ratio (S/N) of 3 and 10 for LOD and LOQ respectively.

8 Expression of results

8.1 Calculation

The determination of the alkylphenol ethoxylates in the extracted solution takes place using calibration graphs that will be defined through the peak areas of the analysed calibration solutions. The content of alkylphenol ethoxylates is calculated as mass portion, w, in milligram per kilogram (mg/kg) of the sample according to the following formula:

$$w_i = \frac{A_i \rho_s V}{A_s m}$$

where

w_i mass portion of alkylphenol ethoxylates in the samples, in mg/kg;

ρ_s concentration of alkylphenol ethoxylates in the calibration solution, in mg/l;

A_i area response of alkylphenol ethoxylates in the sample solution;

A_s area response of alkylphenol ethoxylates in the calibration solution;

V volume the sample is made up, in ml;

m weight of the sample in gram, in g.

8.2 Evaluation

In order to be certain that analytical values obtained using this test method are valid and accurate within the confidence limits of the test, the following procedures shall be followed when performing the test method.

Methodological evaluation could be done by test method verification and validation of all analytical process within quality assurance. To ensure that the test method is in control, regularly analyse a blank matrix solution or a matrix spike sample with a known concentration of OP_nEO and NP_nEO for acceptable performance assessment of the analytical results.

Using the calibration standard solutions prepared by sequential dilution of solutions according to 7.1.2, add a known concentration of OP_nEO and NP_nEO to the blank matrix solution that does not include

the analytes or a sample with known concentration of the analytes when blank matrix sample is not available, and follow the procedure described in Clauses 7 – 8.1. The acceptable target recoveries of this method should be not less than 90 %.

8.3 Precision

8.3.1 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will not exceed the repeatability limit, *r*, in more than 5 % of cases (See Table C.1 and Table C.2).

8.3.2 Reproducibility limit

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories by different operators using different equipment, will not exceed the reproducibility limit, *R*, in more than 5 % of cases.

9 Test report

The test report shall include the following information:

a) all information necessary for the complete identification of the sample;

b) the method used;

c) the results obtained and the used determination procedure;

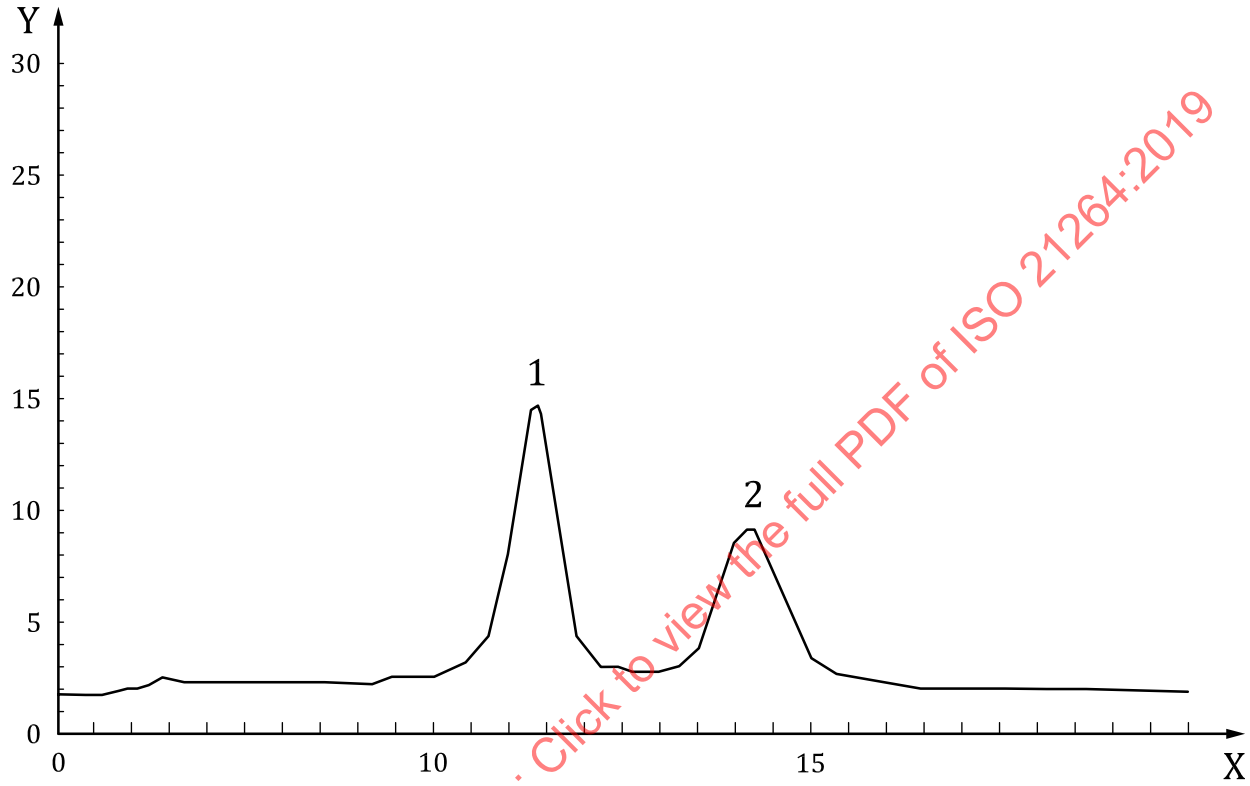
d) the results calculated according to Clause 8;

e) any details not specified this method or which are optional, as well as any factor which may have affected the results;

f) type and date of sampling, reference analyst, notebook reference number, date of the analysis, and date of the report.

Annex A
(informative)

Chromatogram of alkylphenol ethoxylates (APEOs)



Key

X retention time, minutes
Y detector response
1 peak of octylphenol ethoxylates (OPEO)
2 peak of nonylphenol ethoxylates (NPEO)

Figure A.1 — Chromatogram of OPEO and NPEO from HPLC

Annex B
(informative)

Identification test

B.1 Preliminary comment

Although the HPLC method could be successfully used to detect positive alkylphenol ethoxylates samples, the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method could also be considered for accurate confirmation of positive APEO samples with reagents in corresponding specifications, which could improve the analytical sensitivities detection limits. These two techniques can also confirm each other and this comparison is very important when low content of substances is analysed.

B.2 LC-MS/MS conditions

B.2.1 LC conditions

— Column: ACQUITY UPLC®BEH[3)], 50 mm × 2,1 mm i.d. or equivalent

— Stationary phase: C18 reverse phase, 1,7 µm

— Eluent phase: Acetonitrile + water (75 + 25, V/V)

— Flow rate: 0,3 ml/min

— Column temperature: 40 °C

— Injection: 5,0 µl

B.2.2 MS conditions

— Detection: Triple quadrupole mass spectrometer

— Ionization: ESI

— Capillary voltage: 3,0 KV

— Screening type: Multiple reaction monitoring (MRM) in positive ion mode

— Spray-gas: Nitrogen

— Inert gas: Argon

— Ion source temperature: 110 °C

— Drying gas temperature: 380 °C

— Drying gas flow: 8,0 l/min

— Cone Gas Flow: 50 l/h

— Mass range: 100 amu to 1 200 amu

3) ACQUITY UPLC®BEH is a trademark of Waters Corporation. 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.