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**Leather — Determination of
ethoxylated alkylphenols —**

**Part 2:
Indirect method**

*Cuir — Détermination chimique 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 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 18218-2 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.

ISO 18218 consists of the following parts, under the general title *Leather — Determination of ethoxylated alkylphenols*:

- Part 1: Direct method
- Part 2: Indirect method

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 has to 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 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].*

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 analysing 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 part of ISO 18218 is 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 part of ISO 18218 is 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 part of ISO 18218 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, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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 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 ^a 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

^a CAS = Chemical Abstract Service.

Table 1 (continued)

Analyte	Empirical formula	Abbreviation	CAS ^a no.
Octylphenol ethoxylate	C ₈ H ₁₇ -C ₆ H ₄ -(OC ₂ H ₄) _n OH (n≈10)	OPEO ₁₀	9002-93-1

^a 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 [7.2](#)).

4 Apparatus and materials

Normal laboratory apparatus and, in particular, the following:

- 4.1 **Analytical balance**, weighing to an accuracy of 0,1 mg.
- 4.2 **Ultrasonic bath**, (40 ± 2) kHz, with thermostat capable of maintaining a temperature of (50 ± 5) °C.
- 4.3 **Separating funnels**, 150 ml.
- 4.4 **Rotary evaporator**, with thermostat and vacuum system.
- 4.5 **Membrane filter**, polyamide, 0,45 µm.
- 4.6 **HPLC**, equipped with diode array detector (DAD) or fluorescence detector (FLD).
- 4.7 **GC**, equipped with mass selective detector (MSD).
- 4.8 **Filter paper**, fast, quantitative.

5 Chemicals

Unless otherwise stated, analytical grade chemicals shall be used.

- 5.1 **Acetonitrile**, for HPLC.
- 5.2 **n-Hexane**.
NOTE Iso-hexane can also be used.
- 5.3 **Aluminium triiodide**, commercially available, or prepared according to [Annex A](#).
- 5.4 **Sulfuric acid solution**, 0,5 mol/L.
- 5.5 **Sodium thiosulfate solution**, saturated at room temperature.
- 5.6 **Anhydrous magnesium sulfate** (MgSO₄), for analysis.
- 5.7 **Anhydrous sodium sulfate** (Na₂SO₄), 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.

- 5.8 **Sodium chloride solution**, saturated at room temperature.

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

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

5.11 OPEO (in [Table 1](#)) **solution**, for calibration, 2 000 mg/L in acetonitrile. Dilute this solution with acetonitrile ([5.1](#)) if a calibration is applied.

5.12 NPEO (in [Table 1](#)) **solution**, for calibration, 4 000 mg/L in acetonitrile. Dilute this solution with acetonitrile ([5.1](#)) if a calibration is applied.

5.13 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 ([5.1](#)) if the internal calibration curve is applied.

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

6 Sampling and sample preparation

6.1 Preparation of leather samples

6.1.1 Sampling and preparation of samples

If possible, sample the leather according to ISO 2418. Grind or cut the leather in accordance with ISO 4044.

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.

6.1.2 Sample extraction

Approximately 2,5 g of the leather sample ([6.1.1](#)) is accurately weighed to 10 mg into an Erlenmeyer flask, mix with approximately 3 g of Na₂SO₄ ([5.7](#)). Then add a (50,0 ± 1) ml aliquot of acetonitrile ([5.1](#)) into the flask, which is then closed with a stopper.

For GC-MS analysis, an internal standard shall be used. A 100 µl aliquot of 4n-NP solution ([5.13](#)) is added into the flask and results in the final concentration of 2,0 mg/l.

Put the flask into an ultrasonic water bath ([4.2](#)) 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 ([4.8](#)) to remove leather and salt particles. Collect at least 30 ml of the filtrate for the analysis as in [6.4](#) and [6.5](#).

6.2 Preparation of leather process auxiliary samples

Approximately, 0,5 g of leather auxiliary sample is accurately weighed to 10 mg into a flask, carefully mixed with approximately 2 g of MgSO₄ ([5.6](#)). Then use acetonitrile ([5.1](#)), 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. A 100 µl aliquot of 4n-NP solution ([5.13](#)) is added into the flask and results in the final concentration of 2,0 mg/l.

6.3 Blank determination

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

6.4 Determination of OP and NP

For HPLC analysis, use the sample extracts, either (6.1.2) or (6.2), directly after filtering through a polyamide membrane (4.5).

For GC-MS analysis, add 10,0 ml of the sample extract, either (6.1.2) or (6.2), to a separating funnel (4.3). Subsequently, add approximately 20 ml of water (5.14) and 1 ml of sulfuric acid solution (5.4). Extract the mixture with 2×20 ml (approximately) of n-hexane (5.2), 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 Na_2SO_4 (5.7). Remove the organic solvent by rotary evaporator (4.4) at approximately 50 °C. Redissolve the residues in $10,0 \text{ ml} \pm 0,1 \text{ ml}$ of n-hexane (5.2) and the solution is ready for GC-MS analysis after filtering through a polyamide membrane (4.5).

NOTE If the organic phase cannot separate freely in the funnel after treating with n-hexane, add approximately 30 ml of saturated sodium chloride (5.8) 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 are to be diluted accordingly with acetonitrile for HPLC analysis or n-hexane for GC-MS analysis.

6.5 Determination of OPEO and NPEO

Prepare aluminium triiodide (5.3) in acetonitrile for the cleavage of NPEO and OPEO according to Annex A.

NOTE Aluminium triiodide is extremely air and water sensitive. If commercial aluminium triiodide (5.3) 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 $10,0 \text{ ml} \pm 0,1 \text{ ml}$ aliquot of the sample extracts (6.1.2 or 6.2) into the flask containing approximately 1 g of aluminium triiodide, and continue refluxing at $(90 \pm 2) \text{ }^\circ\text{C}$ for $(30 \pm 5) \text{ min}$.

Take out the flask and slowly add water (5.14) dropwise until the reaction subsides. Dilute the contents with approximately 20 ml of water (5.14) and cool to room temperature.

Add the mixture to a separating funnel (4.3), rinse the flask with approximately 20 ml of n-hexane (5.2), and transfer the organic solution to the funnel. Then add approximately 1 ml of sulfuric acid solution (5.4) 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 (5.5) and shake until the pink colour (from iodine) disappears. Wash the organic phase with approximately 30 ml of water (5.14), remove the aqueous layer, and dehydrate the organic layer with approximately 4 g of Na_2SO_4 (5.7). Remove the organic solvent by rotary evaporator at approximately 50 °C.

NOTE If the organic phase cannot separate freely in the funnel after treating with n-hexane, add approximately 30 ml of saturated sodium chloride (5.8) to the funnel, then shake the mixture for approximately 30 s and stand until separated.

For HPLC analysis, redissolve the residues in $(10,0 \pm 0,1) \text{ ml}$ of acetonitrile (5.1) and filter through a polyamide membrane (4.5).

For GC-MS analysis, redissolve the residues in $(10,0 \pm 0,1) \text{ ml}$ of n-hexane (5.2) and filter through a polyamide membrane (4.5).

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

6.6 Chromatographic analysis

Detection of NP and OP can be performed using the chromatographic techniques HPLC (4.6) or GC-MS (4.7). 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 (5.13) shall be used.

Operating parameters and examples of the chromatographic analysis for NP and OP are listed in Annex B and Annex C.

6.7 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.

7 Calibration

7.1 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 (5.13) 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 from 1 mg/l to 20 mg/l with a constant 4n-NP concentration as the internal standard.

7.2 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 (5.13) with a constant 4n-NP concentration of 2,0 mg/l.

The solutions are treated as specified in 6.2 to 6.5. 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.

8 Calculation

8.1 Calculation of OP and NP

Calculate the concentration of OP and NP by using the external standard according to Formula (1).

$$w_{AP} = \frac{A_{AP1}}{\rho} \times \frac{V}{m_E} \quad (1)$$

If the internal standard was used, the calculation is according to Formula (2).

$$w_{AP} = \frac{A_{AP1}}{A_{ISTD}} \times \frac{1}{\rho} \times \frac{V}{m_E} \quad (2)$$

where

w_{AP} is the mass portion of NP or OP in the specimen, in mg/kg;

ρ is the slope of the calibration curve;

A_{AP1} is the area response of NP or OP in the specimen solution;

A_{ISTD} is the area of the internal standard in the specimen solution;

V is the volume the specimen is made up, in ml;

m_E is the weight of the leather specimen or leather process auxiliary, in g.

8.2 Calculation of OPEO and NPEO

Calculate the concentration of NPEO and OPEO by using the external standard according to Formula (3).

$$w_{APEO} = \frac{(A_{AP2} - A_{AP1})}{\rho'} \times \frac{V'}{m_E} \quad (3)$$

If the internal standard was used, the calculation is according to Formula (4).

$$w_{APEO} = \frac{(A_{AP2} - A_{AP1})}{A'_{ISTD}} \times \frac{1}{\rho'} \times \frac{V'}{m_E} \quad (4)$$

where

w_{APEO} is the mass portion of NPEO or OPEO in the specimen, in mg/kg;

A_{AP2} is the area of NP or OP in the specimen solution of the cleaved sample NPEO and OPEO;

A_{AP1} is the area of NP or OP in the specimen solution of the uncleaved sample [see Formula (1)];

ρ' is the slope of the calibration curve;

V' is the volume the specimen is made up, in ml;

m_E is the weight of the leather specimen or leather process auxiliary, in g [see Formula (1)];

A'_{ISTD} is the area of the internal standard in the specimen solution.

NOTE It is noted that the NP and OP is stable during the cleavage process. Thus, the area response (A_{AP1}) of NP and OP in the sample extracts contributed to the area response (A_{AP2}) of the isolated total NP + OP from the cleavage reaction because the sample extracts are directly submitted for cleavage without removing the free NP and OP. Accordingly, ($A_{AP2} - A_{AP1}$) is used when calculating the contents of NP and OP yielded from the NPEO and OPEO.

9 Test report

The test report shall include at least the following information:

- a) reference to this part of ISO 18218, i.e. ISO 18218-2;
- b) either the type, origin, and designation of the analysed leather sample and the sampling method used or the name and origin of the process auxiliary;
- c) the analytical procedure and instrument used;
- d) the analytical results for the OP, NP, OPEO, and NPEO contents, as well as the sum of the four results;
- e) any deviations from the analytical procedure, particularly any additional steps performed;
- f) the date of the test.

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Annex A (informative)

Preparation of aluminium triiodide

A.1 Reagents

A.1.1 Acetonitrile, for HPLC.

A.1.2 Aluminium, with purity more than 99,9 %.

A.1.3 Iodine, with purity more than 99,8 %.

A.2 Apparatus

A.2.1 Analytical balance, weighing to an accuracy of 0,01 g.

A.2.2 Distillation flask with flat bottom, 100 ml.

A.2.3 Oil bath or other suitable heating mantel with thermostat control, ± 1 °C.

A.2.4 Condenser tube, Allihn or Graham, matching the distillation flask neck ([A.2.2](#)).

A.3 Procedure

- 1) All the glassware, such as flask and condenser tube, shall be water-free and rinsed with acetonitrile ([A.1.1](#)) prior to use.
- 2) Weight 3,2 g iodine ([A.1.3](#)) and 0,4 g aluminium ([A.1.2](#)) into a 100 ml flask ([A.2.2](#)), pipette 10 ml of acetonitrile ([A.1.1](#)) to the flask, and shake the flask slightly to mix the contents.
- 3) Put the flask in an oil bath ([A.2.3](#)) and fit a condenser tube ([A.2.4](#)).
- 4) Heat the flask at 90 °C under reflux condition until the iodine colour disappears (approximately 2 h), yielding aluminium triiodide (white precipitate), which is ready for use.

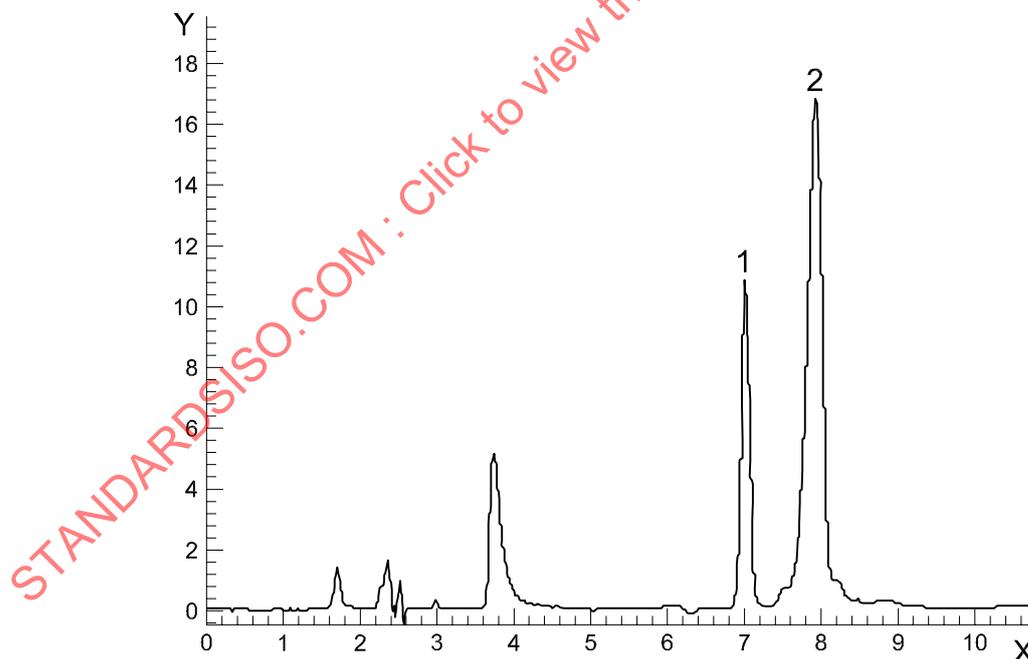
Annex B (informative)

Example of HPLC chromatograms

B.1 HPLC conditions

As the instrumental equipment of the laboratories may vary, no generally applicable instructions can be provided for chromatographic analysis. The following parameters have been successfully tested and used.

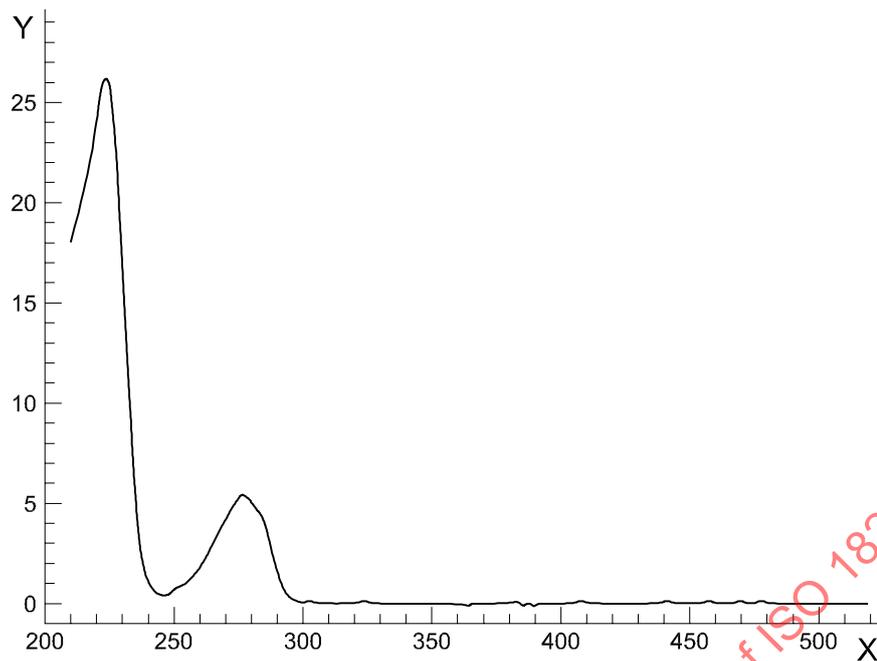
- Stationary phase: C₁₈ reverse phase
- Mobile phase: 70 % methanol/30 % water
- Flow rate: 1,0 mL/min
- Column temperature: 35 °C
- Injection volume: 10,0 µL
- Detection: DAD or FLD, spectrograph
- Quantification: for DAD at 225 nm, for FLD with Ex = 230 nm and Em = 296 nm



Key

- X time, min
Y absorbance unit, mAU
1 4-tert-octylphenol (OP), 7,015 min
2 4-nonylphenol (NP), 7,931 min

Figure B.1 — Chromatogram of NP and OP in acetonitrile (HPLC-DAD)



Key

X wavelength, nm

Y absorbance unit, mAU

Figure B.2 — HPLC/DAD — UV-VIS Spectrum of alkylphenols

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