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**Water quality — Determination of  
selected alkylphenols —**

**Part 1:**

**Method for non-filtered samples using  
liquid-liquid extraction and gas  
chromatography with mass selective  
detection**

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*Qualité de l'eau — Dosage d'alkylphénols sélectionnés —*

*ISO 18857-1:2005*

*Partie 1: Méthode pour échantillons non filtrés par extraction en phase  
liquide-liquide et chromatographie en phase gazeuse avec détection  
sélective de masse*



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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 18857-1 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

ISO 18857 consists of the following parts, under the general title *Water quality — Determination of selected alkylphenols*:

- *Part 1: Method for non-filtered samples using liquid-liquid extraction and gas chromatography with mass selective detection*  
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- *Part 2: Method for filtered samples using solid phase extraction and gas chromatography with mass selective detection*

# Water quality — Determination of selected alkylphenols —

## Part 1:

## Method for non-filtered samples using liquid-liquid extraction and gas chromatography with mass selective detection

**WARNING** — Persons using this International Standard should be familiar with normal laboratory practice. This International Standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

**IMPORTANT** — It is absolutely essential that tests conducted in accordance with this part of ISO 18857 be carried out by suitably qualified staff.

### 1 Scope

This part of ISO 18857 specifies a method for the determination of 4-nonylphenol (mixture of isomers) and 4-(1,1,3,3-tetramethylbutyl)phenol in non-filtered samples of drinking water, ground water and surface water. The method is applicable in a concentration range from 0,005 µg/l to 0,2 µg/l for 4-(1,1,3,3-tetramethylbutyl)phenol and from 0,02 µg/l to 0,2 µg/l for 4-nonylphenol (mixture of isomers). Depending on the matrix, the method is also applicable to waste water containing the analyzed compounds in the concentration range from 0,1 µg/l to 50 µg/l. Higher concentrations can be measured after appropriate dilution of the sample.

### 2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the reference document (including any amendments) applies.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 5667-1, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes*

ISO 5667-2, *Water quality — Sampling — Part 2: Guidance on sampling techniques*

ISO 5667-3, *Water quality — Sampling — Part 3: Guidance on the preservation and handling of water samples*

### 3 Principle

The compounds 4-(1,1,3,3-tetramethylbutyl)phenol and 4-nonylphenol (mixture of isomers) are extracted from the acidified water sample with toluene. The extract is cleaned, if necessary, with silica and the alkylphenols are separated by gas chromatography using capillary columns. The alkylphenols are identified by mass spectrometry and quantified using an internal standard over the total procedure. The response factor using 4-(1,1,3,3-tetramethylbutyl)phenol and a technical mixture of isomers of 4-nonylphenol is determined daily.

## 4 Interferences

The extent and the importance of interferences are sample-dependent.

There are many sources of sample contamination, including contamination of reagents during storage, contamination of equipment reused in the sequential extraction of samples and standards and carryover contamination from the septum seal on a sample bottle or vial and GC septa. Organic polymers can form alkyl phenols thus leading to elevated values. Avoid direct contact with plastics material as these can contain alkylphenols that can contaminate the sample.

## 5 Reagents

Use reagents with negligibly low concentrations of target alkyl phenols compared with the concentration to be determined and verify by blank determinations.

**5.1 Water**, grade 1, as specified in ISO 3696:1987.

**5.2 Acid**, e.g. hydrochloric acid,  $c(\text{HCl}) = 37\%$ , or sulfuric acid,  $c(\text{H}_2\text{SO}_4) = 1 \text{ mol/l}$ .

**5.3 Silica**, medium pore size 6 nm (60 Å), grain-size 0,063 mm to 0,2 mm (230 mesh to 70 mesh).

Purify about 100 g of silica in a quartz beaker (6.5) by heating to  $550\text{ }^\circ\text{C} \pm 20\text{ }^\circ\text{C}$  in a muffle furnace (6.6) for at least 10 h. Let the silica cool to about  $100\text{ }^\circ\text{C}$  and transfer it to a wide-necked glass bottle. Let it cool to room temperature in a desiccator. Add water (5.1) to the silica to produce a concentration of about 3 % mass fraction. Homogenize on a shaking device for 2 h. Store tightly sealed.

**5.4 Hexane**,  $\text{C}_6\text{H}_{14}$ .

**5.5 Toluene**,  $\text{C}_7\text{H}_8$ .

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Other extracting solvents may be used if equivalent recoveries can be achieved (see Annex C).

**5.6 Sodium sulfate**,  $\text{Na}_2\text{SO}_4$ , anhydrous, powdered.

**5.7 Acetone**,  $\text{C}_3\text{H}_6\text{O}$ .

**5.8 4-*n*-nonylphenol(ring- $^{13}\text{C}_6$ ) solution**,  $\text{C}_9\text{H}_{19}\text{[}^{13}\text{C}_6\text{]H}_4\text{-OH}$ , 1 ng/μl, used as an internal standard.

Weigh 10 mg of 4-*n*-nonylphenol(ring- $^{13}\text{C}_6$ ) in a 100 ml measuring flask and bring to volume with acetone (5.7). Dilute this solution with acetone (5.7) in the ratio 1:100.

Alternative internal standards (e.g. 4-*n*-nonylphenol) that meet the internal standard requirements are acceptable. An internal standard should have the following characteristics: It should be stable and should not interfere with the analyte. It should be as similar in structure to the analyte as possible, to ensure that it will show the same properties relative to, for example, adsorption on glass surfaces, extraction, concentration as the analyte. It should not be present in the sample matrix.

**5.9 4-nonylphenol solution**, 1 ng/μl, used as a calibration standard.

Weigh 10 mg of 4-nonylphenol,  $\text{C}_{15}\text{H}_{24}\text{O}$  (technical mixture of isomers), CAS No 84852-15-3, in a 100 ml measuring flask and bring to volume with toluene (5.5). Dilute this solution in the ratio 1:100 with toluene (5.5) or acetone (5.7) if a calibration over the total procedure (9.3, 9.4) is applied.

**5.10 4-(1,1,3,3-tetramethylbutyl)phenol solution**, 1 ng/μl, used as a calibration standard.

Weigh 10 mg of 4-(1,1,3,3-tetramethylbutyl)phenol, C<sub>14</sub>H<sub>22</sub>O, CAS No 140-66-9, in a 100 ml measuring flask and bring to volume with toluene (5.5). Dilute this solution in the ratio 1:100 with toluene (5.5) or acetone (5.7) if a calibration over the total procedure (9.3, 9.4) is applied.

Store solutions 5.8, 5.9 and 5.10 in the refrigerator protected from light. Check the solutions weekly prior to use.

NOTE The solutions 5.8, 5.9 and 5.10 are commercially available.

**5.11 Nitrogen**, N<sub>2</sub>, purity ≥ 99,996 %.

## 6 Apparatus

Clean all glassware by rinsing with acetone (5.7). Avoid detergents when using a labware-washing machine. Alternatively, prior to use, heat all glassware, except volumetric ware, to at least 250 °C for a minimum of 2 h.

The given volumes are chosen corresponding to the described extraction volume of 1 000 ml. Smaller volumes are possible.

Usual laboratory equipment, and the following.

**6.1 Shaking device.**

**6.2 Flat-bottomed glass bottles**, 1 000 ml, preferably of brown glass, with straight shoulders and a glass stopper or polytetrafluoroethene- (PTFE-) lined screw cap.

The sampling bottle shall allow direct extraction from the bottle.

**6.3 Quartz wool**, rinsed with hexane (5.4).

**6.4 Clean up column**, inner diameter 8 mm, length 120 mm, with glass or PTFE stopcocks.

**6.5 Quartz beaker**, 100 ml.

**6.6 Muffle furnace**, capable of maintaining a temperature of at least 600 °C.

**6.7 Evaporation device, rotary evaporator, turbo-evaporator or vacuum concentration device.**

**6.8 Separator**, for an example see Annex D, or another suitable device for phase separation.

**6.9 Drying column**, chromatographic column, length 600 mm, inner diameter 30 mm (or other convenient size), with coarse frit filter disk.

**6.10 Tapered flask**, 100 ml.

**6.11 Vials**, of brown glass, with a capacity of, for example 1,5 ml, compatible with the autosampler.

**6.12 Gas chromatograph**, temperature-programmable, with all required accessories, including gases, capillary columns, capillary injector and mass spectrometric detector.

The mass spectrometer should be capable of operating across the mass range of interest and incorporate a data system capable of quantifying ions using selected *m/z* values.

## 7 Sampling and sample pretreatment

Take samples in accordance with ISO 5667-1, ISO 5667-2 and ISO 5667-3.

For sampling, use bottles (6.2), and acidify the sample with acid (5.2) to pH 2.

Do not fill the sample bottles completely (e.g. fill them to the shoulder) in order to allow the addition of the extracting agent.

If necessary, store the sample in the refrigerator (2 °C to 5 °C) and analyse as soon as possible, but not later than 2 weeks after sampling.

Carry out the extraction directly from the sampling bottle. Weigh the sample bottle with its contents to the nearest 1 g and record the mass for subsequent sample volume determination (8.1).

## 8 Procedure

### 8.1 Extraction and concentration

Add 100 µl to 1 000 µl of the internal standard (5.8) to 1 000 ml of the sample in the original sample bottle.

Choose the volume of the internal standard according to the matrix-dependent extraction end volume which may vary between 100 µl and 1 000 µl. The concentration should be approximately in the middle range of the calibration, e.g. 20 ng/l for ground and surface water or 500 ng/l for wastewater.

Add 40 ml of toluene (5.5) and extract for at least 4 h using the shaking device (6.1). Make sure that the phases are well mixed.

Let the phases separate and use a separator (6.8) to collect the toluene extract.

If an emulsion forms, break the emulsion by centrifuging the extract and/or by adding sodium sulfate.

Fill the drying column (6.9) with 1 cm to 2 cm with sodium sulfate (5.6) and clean it with 10 ml of toluene (5.5); discard this toluene portion.

Subsequently let the toluene extract run through the frit into a 100 ml tapered flask (6.10).

Rinse the frit with 10 ml of toluene (5.5) and add this toluene portion to the extract.

Evaporate the extract to about 2 ml using an evaporation device (6.7).

Using a gentle nitrogen (5.11) stream, carefully evaporate, at a temperature of < 40 °C, to 50 µl to 100 µl.

If necessary, e.g. in the case of wastewater and polluted surface water, continue with the clean-up as described in 8.2.

If a clean up is not necessary, transfer the extract to a vial, rinse using a glass pipette and evaporate the extract to a suitable volume (in general, 100 µl to 500 µl) using nitrogen (5.11).

Discard the sample water and leave the empty bottle to drain for 5 min. Reweigh the empty sample bottle with the original cap and calculate the net weight of sample by difference to the nearest 1 g. This net weight (in grams) is equivalent to the volume (in millilitres) of water extracted. The volume of acid (5.2) added to acidify the sample is negligible.



## 8.2 Clean-up

### 8.2.1 Preparation of the clean up column

Fill the clean-up column (6.4), with the following, in the given sequence, and avoid trapping of air:

- quartz wool (6.3), about 0,5 cm;
- sodium sulfate (5.6), about 0,5 cm;
- prepared slurry of 2 g of silica (5.3) with hexane (5.4).

Condition the column with 5 ml of hexane (5.4).

### 8.2.2 Clean-up procedure

Dilute the extract with 1 ml of hexane (5.4) and transfer to the clean-up column.

Let the extract run through the column until the level of the extract is just above the layer of silica and then add 10 ml of hexane (5.4) and let it run through.

Elute the target compounds with 20 ml of toluene (5.5).

The toluene and hexane phases are clearly distinguishable.

Discard the hexane phase. As soon as the phase line is slightly above the stopcock, change the flask to collect the toluene extract.

Evaporate the toluene extract using the evaporation device (6.7), concentrate the extract to a volume of approximately 2 ml and subsequently concentrate the extract further to a volume of 50 µl to 100 µl using a gentle flow of nitrogen (5.11).

Transfer the extract to a suitable vial (6.11).

## 8.3 GC/MS operating conditions

Optimize the operating conditions of the GC-MS system in the electron-ionization mode in accordance with the manufacturer's instructions. The appropriate GC-oven-temperature programme is determined experimentally during method development and validation. For the sake of sensitivity, the ions listed in Table 1 are detected. The electron energy is set at 70 eV. An example of operating conditions is given in Annex B.

## 8.4 Blank determination

Treat the blank in exactly the same way as the sample, but replace the sample by the appropriate amount of pure water (5.1).

## 8.5 Identification

Identify the sample component by matching both retention times and relative intensities of the diagnostic ions of sample components and alkylphenol standards.

The target compound is present (e.g. is identified) in the sample if

- the relative or the absolute sample component retention time measured in the selected ion current chromatogram matches the relative or absolute retention time of the authentic compound within  $\pm 1$  % (or a maximum of  $\pm 6$  s) in the chromatogram of the latest calibration standard, measured under identical conditions;

- the two diagnostic ions selected,  $m/z$  135 and 107 (see Table 1), are present at the substance-specific retention time; and
- the pattern of 4-nonylphenol in the sample chromatogram is to a wide extent in agreement with the pattern of 4-nonylphenol (5.9) used as calibration standard, measured under identical conditions (to indicate the presence of the technical product 4-nonylphenol).

NOTE The external standard 4-nonylphenol used is a technical product. Therefore, the mass spectrogram shows an isomer pattern that usually consists of 8 to 10 main peaks (see Annex B). The isomer pattern may differ from batch to batch. Due to the different composition of the technical product, a fixed ratio of the relative intensities of the diagnostic ions selected,  $m/z$  135 and 107, is not obtained for the whole peak pattern.

**Table 1 — Diagnostic ions selected for identification and quantification**

Substance	Ions
4-nonylphenol (mixture of isomers)	135, 107
4-(1,1,3,3-tetramethylbutyl)phenol	135, 107
4- <i>n</i> -nonylphenol(ring- $^{13}\text{C}_6$ )	113

## 9 Calibration

### 9.1 General requirements

For practical reasons, the calibration is based on a solution containing 4-nonylphenol (mixture of isomers) and 4-(1,1,3,3-tetramethylbutyl)phenol.

Ensure there is a linear dependence between signal and concentration.

Determine the linear working range using at least five measurements at different concentrations (see ISO 8466-1).

The calibration function for a substance is valid only within the measured concentration range. Additionally, the calibration function depends on the condition of the gas chromatograph and shall be checked regularly. For routine analysis, a check of the calibration function by measurement of two points is sufficient.

There are three different possibilities to set up the calibration function:

- calibration of the GC-MS step only (not the total procedure) with an external standard; see 9.2;
- calibration of the total procedure with an external standard (including the extraction, concentration, clean-up and GC-MS steps); see 9.3;
- calibration of the total procedure with an internal standard (including the extraction, concentration, clean-up and GC-MS steps); see 9.4.

The first two possibilities serve to estimate the recovery. For routine analysis, only the calibration of the total procedure with an internal standard shall be applied.

Table 2 gives an explanation of the subscripts used in the equations and in the following text.

**Table 2 — Explanation of subscripts**

Subscript	Meaning
<i>i</i>	identity of the substance
<i>e</i>	calibration step
<i>I</i>	identity of internal standard
<i>g</i>	overall procedure

## 9.2 Calibration of the GC-MS step only (not the total procedure) with an external standard

For both alkylphenols establish a calibration function from at least five points.

Knowledge of the retention times of the respective single substances is a prerequisite. These are evaluated with the aid of the solutions of the single substances.

Establish the calibration function by injecting the calibration solutions. For a calibration range of 0,005 µg/l to 0,2 µg/l, example mass concentrations are given in Table 3.

**Table 3 — Examples for reference solutions**

Aliquot of the diluted stock solution (1 ng/µl)	Absolute mass	Corresponding to a mass concentration (for 1 l of reference solution)
µl	µg	µg/l
5	0,005	0,005
10	0,01	0,01
20	0,02	0,02
50	0,05	0,05
75	0,075	0,075
100	0,1	0,1
200	0,2	0,2

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The injection volume in the calibration step and in the measurement shall be the same.

For a graphic presentation of the calibration curve, plot the respective measured values,  $y_{ie}$ , on the ordinate against the respective mass concentrations,  $\rho_{ie}$ , of the substance,  $i$ , on the abscissa.

The series of measured values thus obtained shall be used to establish the linear regression function in accordance with Equation (1):

$$y_{ie} = a_i \times \rho_{ie} + b_i \quad (1)$$

where

$y_{ie}$  is the dependent variable corresponding to the measured response, expressed in units dependant on the analytical method, e.g. area value, for a given  $\rho_{ie}$  of substance  $i$ ;

$\rho_{ie}$  is the independent variable corresponding to the mass concentration, expressed in micrograms per litre, of substance  $i$ , the external standard, in the working standard solution;

$a_i$  is the slope of the calibration function for substance  $i$ , expressed in unit dependent on the analytical method, e.g. area value, times the reciprocal of the concentration, expressed in litres per microgram;

$b_i$  is the ordinate intercept of the calibration curve, expressed in units dependent on the analytical method, e.g. area value.

## 9.3 Calibration of the total procedure with an external standard

To calibrate the entire procedure, add aliquots of each calibration solution (5.9 and 5.10) to 1 000 ml of water (5.1).

Treat and analyse the solution as given in Clause 8.