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Water quality — Determination of individual isomers of nonylphenol — Method using solid phase extraction (SPE) and gas chromatography/mass spectrometry (GC/MS)

Qualité de l'eau — Détermination des isomères individuels de nonylphénol — Méthode par extraction en phase solide (SPE) et chromatographie en phase gazeuse/spectrométrie de masse (GC/MS)

Reference number
ISO 24293:2009(E)

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Foreword

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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.

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ISO 24293 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Introduction

The user should be aware that particular problems could require the specifications of additional marginal conditions.

Water quality — Determination of individual isomers of nonylphenol — Method using solid phase extraction (SPE) and gas chromatography/mass spectrometry (GC/MS)

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This 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 International Standard be carried out by suitably qualified staff.

1 Scope

This International Standard specifies a method for the determination of selected individual isomers of nonylphenol in non-filtered samples of drinking water, waste water, ground water and surface water. The method is applicable in concentrations between 0,001 µg/l and 0,1 µg/l for individual isomers and from 0,01 µg/l to 0,2 µg/l for the sum of 4-nonylphenol (mixture of isomers). Depending on the matrix, the method is also applicable to waste water in concentrations between 0,1 µg/l and 50 µg/l.

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 referenced document (including any amendments) applies.

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

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

ISO 8466-1, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function*

3 Principle

Extraction of the analytes listed in Table 1 from the acidified water sample by solid phase extraction, solvent elution and determination by gas chromatography with mass spectrometric detection.

The thirteen isomers listed (eleven identified isomers and two isomers with tentative identification) constitute more than 90 % of the 4-nonylphenol isomers that are detectable in technical products and in environmental samples in general. Water samples containing more than 500 mg/l of suspended matter and waste water samples are extracted by passing 100 ml of the sample through the solid phase extraction cartridge (5.2).

Table 1 — Analytes determinable by this method

Analyte	Formula	Abbreviation
4-(2,4-Dimethylheptan-4-yl)phenol	$C_{15}H_{24}O$	NP1
4-(2,4-Dimethylheptan-2-yl)phenol	$C_{15}H_{24}O$	NP2
4-(3,6-Dimethylheptan-3-yl)phenol	$C_{15}H_{24}O$	NP3
4-(3,5-Dimethylheptan-3-yl)phenol	$C_{15}H_{24}O$	NP4[a]
4-(2,5-Dimethylheptan-2-yl)phenol	$C_{15}H_{24}O$	NP5
4-(3,5-Dimethylheptan-3-yl)phenol	$C_{15}H_{24}O$	NP6 [a]
4-(3-Ethyl-2-methylhexan-2-yl)phenol	$C_{15}H_{24}O$	NP7
4-(3,4-Dimethylheptan-4-yl)phenol[b]	$C_{15}H_{24}O$	NP8[c]
4-(3,4-Dimethylheptan-3-yl)phenol	$C_{15}H_{24}O$	NP9[e]
4-(3,4-Dimethylheptan-4-yl)phenol	$C_{15}H_{24}O$	NP10[c]
4-(2,3-Dimethylheptan-2-yl)phenol	$C_{15}H_{24}O$	NP11
4-(3-Methyloctan-3-yl)phenol	$C_{15}H_{24}O$	NP12
4-(3,4-Dimethylheptan-3-yl)phenol[d]	$C_{15}H_{24}O$	NP13[e]

[a] Possible enantiomer.

[b] Information from MAKINO et al. [6]

[c] Possible enantiomer.

[d] Information from KATASE et al. [5]

[e] Possible enantiomer.

4 Reagents

Use reagents with negligible concentrations of the compounds of interest compared with the concentrations to be determined. Verify by blank determinations and, if necessary, apply additional cleaning steps.

4.1 Water, grade 1, as specified in ISO 3696.

4.2 Acid, e.g. hydrochloric acid, w(HCl) = 37 %, or sulfuric acid, c(H_2SO_4) = 1 mol/l.

4.3 Acetone, C_3H_6O.

4.4 Methanol, CH_3OH.

4.5 Hexane, C_6H_{14}.

4.6 Sodium sulfate, anhydrous, Na_2SO_4, powdered.

4.7 Internal standard solution, 4-*n*-Nonylphenol (ring-$^{13}C_6$), C_9H_{19}-$^{13}C_6H_4$-OH solution, $\rho = 1$ ng/µl.

Weigh 10 mg of 4-*n*-nonylphenol in a 100 ml measuring flask and bring to volume with methanol (4.4). Dilute this solution with methanol in the ratio of 1:100. Acetone is not suitable for preparation of standard solution in this method. Alternative internal standards [e.g. 4-*n*-nonylphenol (deuterium label)] may be used if internal standard requirements can be met.

4.8 4-nonylphenol solution, $\rho = 1$ ng/µl (calibration standard).

Weigh 10 mg of 4-nonylphenol, $C_{15}H_{24}O$ (technical mixture of isomers), CAS No 25154-52-3, in a 100 ml measuring flask and bring to volume with hexane (4.5). Dilute this solution in the ratio of 1:100 with hexane if a calibration over the total procedure is applied.

4.9 Solid phase material, on styrene-divinylbenzene polymer basis, e.g. commercially available packing material (see Annex A).

4.10 Nitrogen, N_2, purity $\geqslant$ 99,996 % volume fraction, for drying of the sorbent packing after sample extraction and for concentration of extracts by evaporation.

4.11 Sodium thiosulfate pentahydrate, $Na_2S_2O_3 \cdot 5\ H_2O$.

4.12 Ethyl acetate, $C_4H_8O_2$.

4.13 Diethyl ether, $C_4H_{10}O$.

4.14 Corresponding internal standard solution for syringe spike, phenanthrene (d_{10}), $C_{14}D_{10}$ solution, CAS No 85-01-8, $\rho = 0{,}1$ ng/µl. Weigh 10 mg of phenanthrene (d_{10}) in a 100 ml measuring flask and bring to volume with hexane (4.5). Dilute this solution with hexane in the ratio of 1:1 000.

5 Apparatus

Equipment or parts which may come into contact with the water sample or the extract should be free from interfering compounds.

Clean all glasswares by rinsing with acetone (4.3). Avoid detergents when using a labware washing machine. Alternatively, heat all glassware, except volumetric wares, to at least 400 °C for at least 2 h prior to use.

5.1 Narrow-neck flat-bottomed glass bottles, conical shoulders, preferably brown glass, of capacity 1 000 ml, with glass stoppers or with PTFE-lined screw caps (PTFE = polytetrafluoroethene).

Keep samples away from light if brown glass bottles are not available. The bottle and cap liner or glass stopper should be rinsed with acetone (4.3) and dried before use in order to minimize contamination.

5.2 Solid phase extraction cartridges, inert non-leaching plastic, e.g. polypropene or glass.

The cartridges should be packed with a minimum of 200 mg of sorbent (4.9). The commercially available disk type may be used provided there is enough information available concerning the sample volume and the required quantity of elution solvent. These cartridges are used for extraction.

5.3 Vacuum or pressure assembly, for the extraction step.

5.4 Volumetric flasks, with inert stopper.

5.5 Quartz wool, rinsed with acetone (4.3).

5.6 Muffle furnace, capable of being maintained at a temperature of 400 °C.

5.7 Evaporation assembly, e.g. rotary evaporator with vacuum stabilizer and water bath.

5.8 Vials, brown glass with PTFE-lined septa, capacity e.g. 1,5 ml, according to the autosampler.

5.9 Gas chromatograph/mass spectrometer. The gas chromatograph shall be temperature-programmable, with all required accessories including gases, capillary columns, capillary injector and mass spectrometric detector.

The mass spectrometer should be capable of operating over the mass range of interest and it should be equipped with a data system capable of quantifying ions using selected m/z values.

5.10 Clean up cartridge column, inert non-leaching plastic, e.g. polypropene or glass.

The cartridges should be packed with a minimum of 200 mg of sorbent (reverse phase, silica). These cartridges are used for clean up.

5.11 Flame ionization detector.

6 Sampling and sample pretreatment

Take samples as specified in ISO 5667-1.

Use carefully cleaned bottles for sampling (5.1). Fill each bottle only to its shoulder with water to be sampled (approximately 1 000 ml). In the presence of free chlorine, immediately add approximately 80 mg of sodium thiosulfate pentahydrate (4.11). Other non-interfering substances may be used for dechlorination as well (e.g. sodium sulfite). Acidify the samples with acid (4.2) to pH 3,5.

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

7 Procedures

7.1 Solid phase extraction

7.1.1 General

In general, samples are examined without pretreatment; in other words, suspended solids are not removed prior to analysis. Before starting the analysis, homogenize the samples. If blocking of the cartridge packing is likely to occur, use a filter aid, e.g. quartz wool (5.5).

7.1.2 Conditioning of the solid phase material

The following procedures are described for commercially available 6 ml polypropylene cartridges (5.2) packed with 200 mg of sorbent (4.9) sandwiched between two polyethylene frits. The manufacturer's guidance for other materials of the SPE cartridge shall be preferred.

Rinse the cartridge with two 10 ml aliquots of acetone (4.3) and let the cartridge drain dry after the first rinsing. Before the acetone level of the second aliquot falls below the top edge of the packing, add 10 ml of water (4.1), acidified with acid (4.2) to pH 3,5, to the cartridge, and make sure that the sorbent packing in the cartridge does not run dry. Retain the water in the cartridge (water level just above the packing) to keep the sorbent activated.

7.1.3 Sample extraction

Start the extraction immediately after conditioning. Make sure that no air bubbles are trapped in the sorbent bed when changing from conditioning to extraction. Maintain the sorbent material in the cartridge immersed in water at all times.

Add the internal standard solution (4.7), in a known amount (e.g. 50 µl) dependent on the sample matrix, to the water sample (e.g. 1 000 ml) in the sample bottle and mix thoroughly. Let this sample run through the cartridge, conditioned as specified in 7.1.2, at a flow rate of 5 ml/min to 10 ml/min. Extract samples containing more than 500 mg/l of suspended matter and waste water samples by passing a 100 ml sample through the cartridge. Rinse the cartridge with 10 ml of water (4.1), acidified with acid (4.2) to pH 3,5.

Remove the residual water in the sorbent packing by passing nitrogen through the cartridge at a flow rate of about 500 ml/min for about 1 h.

NOTE Depending on the colour of the moist adsorbent, the end of the removal of water from the cartridge can be recognized by the change of colour of sorbent material. The colour of the moist adsorbent is brown; the dry material is light orange. The end of the removal of water from the cartridge can usually be recognized by brightening of the sorbent packing.

Reweigh the empty sample bottle with its original cap or stopper and calculate the net weight of sample by difference to the nearest 1 g. For an assumed density of 1 g/ml, this net weight (in grams) is equivalent to the volume (in millilitres) of water extracted. The amount of the added volume of acid (4.2) to acidify the sample is negligible.

7.1.4 Elution

Add 1 ml of acetone (4.3) to the completely dried cartridge, allow to equilibrate for e.g. 10 min and elute through the cartridge, followed by adding five 1 ml aliquots of acetone (4.3) to the cartridge, but do not allow the acetone to elute below the top of the sorbent packing during the elution steps.

7.2 Clean up

Concentrate the eluate using a gentle stream of nitrogen to almost dryness. Add 1 ml of hexane (4.5) and transfer all into a clean up cartridge column (5.10). In general, 500 mg of silica in the cartridge requires the following extraction procedure. Wash with 10 ml of ethyl acetate (4.12) and subsequently with 15 ml of hexane. Add the sample and immediately elute with 15 ml of hexane followed by 10 ml of 30 % of diethyl ether (4.13) in hexane.

To confirm elution profiles of 4-nonylphenol, carry out a separation test using 4-nonylphenol for each batch of cartridges before analysis. Evaporate the cleaned extract using the evaporation device, concentrate the extract to a volume of approximately 2 ml and spike 50 µl to 100 µl of corresponding internal standard substance for syringe spike (4.14) into the extract, then subsequently concentrate the extract further to a volume of 50 µl to 100 µl using a gentle flow of nitrogen. Transfer the extract to a suitable vial.

7.3 GC/MS operating conditions

Optimize the operating conditions of the GC/MS system in electron ionization mode in accordance to the manufacturer's instructions. Determine the appropriate GC oven temperature programme experimentally during method development and validation. For the sake of sensitivity, selected ions (Table 2) are detected. An example of operating conditions is given in Annex C.

7.4 Blank determination

Treat the blank in exactly the same way as the sample, but replace the sample by the appropriate amount of pure water (4.1).

7.5 Identification

Identify the sample component by matching both the retention times and relative intensities of the diagnostic ions (Table 2) of sample components and calibration standard (4.8). It is a critical issue to identify individual isomers in order to obtain a similar chromatogram to Annex C that enables accurate identification. Old column material and inadequate temperature control may result in shifting of retention time between isomers. Reliable measurements of the thirteen peaks can be enabled by using isomer-specific single ion monitoring. Because

of the absence of all of the fragment ions, 4-*n*-NP is not suitable. Major ions obtained from 4-*n*-NP are 107 and 220; detectable ions are obtained for 121, 135 and 149. It is difficult to use 4-*n*-NP to obtain ions 163 and 191. It is necessary to use a specific pair of ions (target M_1 and qualifier M_2 in Table 2) for the quantification of each resolved peak.

The target compound is present (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 ± 0,2 % (or a maximum of ± 6 s) in the chromatogram of the latest calibration standard, measured under identical conditions;

— the selected diagnostic ions (see Table 2) are present at the substance specific retention time;

— the relative intensities of all selected diagnostic ions measured in the sample do not deviate by more than ± (0,1 Q + 10) % from the relative intensities determined in the external standard solution. (Q is the relative intensity of the diagnostic ion in the external standard solution.)

Table 2 — Selected diagnostic ions for identification and quantification

No	Analyte	Abbreviation	Selected diagnostic ions	
			Target M_1 [a]	Qualifier M_2 [a]
1	4-(2,4-Dimethylheptan-4-yl)phenol	NP1	121	163
2	4-(2,4-Dimethylheptan-2-yl)phenol	NP2	135	220
3	4-(3,6-Dimethylheptan-3-yl)phenol	NP3	135	107 or 121
4	4-(3,5-Dimethylheptan-3-yl)phenol	NP4 [b]	149	191
5	4-(2,5-Dimethylheptan-2-yl)phenol	NP5	135	163
6	4-(3,5-Dimethylheptan-3-yl)phenol	NP6 [b]	149	191
7	4-(3-Ethyl-2-methylhexan-2-yl)phenol	NP7	135	220
8	4-(3,4-Dimethylheptan-4-yl)phenol[c]	NP8[d]	163	121
9	4-(3,4-Dimethylheptan-3-yl)phenol	NP9[f]	149	107
10	4-(3,4-Dimethylheptan-4-yl)phenol	NP10[d]	163	121
11	4-(2,3-Dimethylheptan-2-yl)phenol	NP11	135	220
12	4-(3-Methyloctan-3-yl)phenol	NP12	191	163
13	4-(3,4-Dimethylheptan-3-yl)phenol[e]	NP13[f]	135	107
	4-*n*-Nonylphenol (ring-$^{13}C_6$)	—	113	—

[a] M_1 is used for quantification; M_2 may be used for identification.

[b] Possible enantiomer.

[c] Information from MAKINO et al. [6]

[d] Possible enantiomer.

[e] Information from KATASE et al. [5]

[f] Possible enantiomer.

8 Calibration

8.1 General requirements

For practical reasons, the calibration is based on a solution containing the calibration standard (4.8).

Ensure that 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 for 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 means of a two-point calibration is sufficient.

For routine analysis, only a calibration over the total procedure with internal standards (including extraction, concentration, derivatisation and GC/MS-steps) shall be applied. As the calibration is performed over the total procedure with internal standard, determination of the recoveries is not necessary.

Table 3 gives an explanation of the subscripts used in the equations and in the following text.

Table 3 — Explanation of subscripts

Subscript	Meaning
i	Identity of the substance
e	Calibration step
I	Identity of internal standard
g	Overall procedure

8.2 Calibration over the total procedure with internal standard

When using the internal standard (4.7), the determination of the concentration is independent of possible errors made during injection. Apart from this, errors caused by sample losses during distinct steps of sample pretreatment or the difficult adjustment for a (low) sample volume can be avoided. Additionally, the concentration determination is independent from matrix effects in the sample, provided that the recoveries of the substances analysed and the corresponding internal standard are approximately the same.

Prior to analysis, add the internal standard (4.7), in a known amount dependent on the sample matrix, to the water sample. The mass concentration ρ_I should be the same for calibration and sample measurement.

For calibration over the whole procedure, add aliquots of calibration solutions (4.8) to each water sample (i.e. 1 000 ml) and add the internal standard (4.7) always in the same concentration to each water sample (i.e. 1 000 ml).

Pretreat and analyse the samples as specified in Clause 7.

Use the same solvent composition and internal standard concentrations for the working standard solutions and the extracts.

Plot the values of the ratio y_{ieg} / y_{Ieg} (peak areas, peak heights or integration units) for each substance i on the ordinate and the associated ratio of the mass concentration ρ_{ieg} / ρ_{Ieg} on the abscissa.

Establish the linear regression function using the corresponding pairs of values y_{ieg} / y_{leg} and ρ_{ieg} / ρ_{leg} of the measured series in accordance with Equation (1):

$$\frac{y_{ieg}}{y_{leg}} = a_{igl} \frac{\rho_{ieg}}{\rho_{leg}} + b_{igl} \quad (1)$$

where

y_{ieg} is the dependent variable corresponding to the measured response, expressed in units depending on the analytical method, e.g. area value, for a given ρ_{ieg} of substance i in the calibration;

y_{leg} is the dependent variable corresponding to the measured response, expressed in units depending on the analytical method, e.g. area value, for a given ρ_{leg} of the internal standard I in the calibration;

ρ_{ieg} is the independent variable corresponding to the mass concentration, expressed in micrograms per litre, of substance i in the calibration solution;

ρ_{leg} is the independent variable corresponding to the mass concentration, in micrograms per litre, of the internal standard I;

a_{igl} is the slope of the calibration curve from y_{ieg}/y_{leg} as a function of the mass concentration ratio ρ_{ieg}/ρ_{leg}, often called the response factor;

b_{igl} is the ordinate intercept of the calibration.

9 Calculation

9.1 Calculation of contribution of individual isomers of nonylphenol in technical mixture

It is necessary to confirm the composition ratio of the respective isomers of 4-nonylphenol standard product because isomer composition in technical mixture may be variable. Use 4-nonylphenol solution (4.8) to calculate the composition of individual isomers in the technical mixture. Inject 1 µl of the standard solution to GC-flame ionization detector (FID) (5.11) and obtain a chromatogram of each peak according to Annex D. Identify thirteen isomers according to the method described in Annex D. Calculate the contribution percentage, w, of individual isomers in accordance with Equation (2).

$$w_i = A_i / A_t \cdot 100 \quad (2)$$

where

w_i is the contribution percentage (%) of individual isomers;

A_t is the sum of area count of thirteen isomers;

A_i is the area count of individual isomers.

9.2 Calculation of relative response factor of individual isomers of nonylphenol

Prepare a calibration curve encompassing the concentration range for each of the isomers to be determined. Plot the relative response factor (F_R) (^{13}C-4-nonylphenol to individual isomers) versus concentration in standard solutions or compute using a linear regression. Determine the relative response in accordance with Equation (3). Employ at least five calibration points.

$$F_{\mathrm{R}} = (A_{\mathrm{st}} / A_{\mathrm{is}}) \cdot m_{\mathrm{is}} / (m_{\mathrm{s}} \cdot w_{\mathrm{i}}) \quad (3)$$

where

w_{i} is the contribution percentage (%) of individual isomers;

A_{st} is the area count of individual isomers;

A_{is} is the area count of ^{13}C-4-nonylphenol;

m_{is} is the mass of ^{13}C-4-nonylphenol, in micrograms, µg;

m_{s} is the mass of 4-nonylphenol, in micrograms, µg.

9.3 Quantification of individual isomers of nonylphenol using relative response factor

Calculate the concentration of individual isomers of nonylphenol in accordance with Equation (4).

$$\rho = (A_{\mathrm{s}} \cdot m_{\mathrm{13C\text{-}4}}) / (A'_{\mathrm{is}} \cdot f_{\mathrm{R}} \cdot V_{\mathrm{e}}) \quad (4)$$

where

ρ is the concentration of individual isomers, in micrograms per litre, µg/l;

A_{s} is the area count of individual isomers;

A'_{is} is the area count of ^{13}C-4-nonylphenol;

$m_{\mathrm{13C\text{-}4}}$ is the mass of ^{13}C-4-nonylphenol, in micrograms, µg;

V_{e} is the volume of water sample, in litres, l;

f_{R} is the response factor.

9.4 Calculation of internal standard recovery

The recovery ratio of internal standard shall be calculated from Equation (5) by using the ratio between the peak area of internal standard against that of the corresponding internal standard for the syringe spike and by using the corresponding relative response factor ($F_{\mathrm{R,rs}}$). See Equation (6).

$$R_{\mathrm{rec}} = (A'_{\mathrm{is}} \cdot m'_{\mathrm{ris}} \cdot 100) / (A'_{\mathrm{ris}} \cdot f_{\mathrm{R,rs}} \cdot m'_{\mathrm{is}}) \quad (5)$$

where

R_{rec} is the percentage of internal standard recovery;

A'_{ris} is the area count of corresponding internal standard for syringe spike, phenantherene-d_{10};

m'_{is} is the mass of spiked internal standard, phenantherene-d_{10}, in micrograms, µg;

m'_{ris} is the mass of spiked corresponding internal standard for syringe spike, phenantherene-d_{10}, in micrograms, µg;

$f_{\mathrm{R,rs}}$ is the response factor of corresponding internal standard for syringe spike.

$$f_{R,rs} = (A_{is}/A_{ris}) \cdot (m_{ris}/m_{is}) \quad (6)$$

where

A_{ris} is the area count of corresponding internal standard for syringe spike, phenantherene-d_{10};

m_{ris} is the mass of corresponding internal standard for syringe spike, phenantherene-d_{10}, in micrograms, µg.

10 Expression of results

Report the results of compounds listed in Table 1 in micrograms per litre, µg/l, to two significant figures as x,x µg/l. Results of branched isomers may be reported, but shall be identified as such.

11 Test report

The test report shall include at least the following information:

a) a reference to this International Standard (ISO 24293);

b) identification of the sample;

c) the sample storage and pretreatment protocol;

d) the results obtained for the individual compounds, expressed in accordance with Clause 10;

e) details of any deviation from the procedure specified and of all circumstances that may have influenced the results;

f) the date of the analysis.

Annex A
(informative)

Sorbent example

Table A.1 provides information on a suitable sorbent for the solid phase extraction of analytes.

Table A.1 — Example of a sorbent suitable for solid phase extraction of analytes

Sorbent	Product name (supplier) [1)]	Amount of sorbent in cartridge
Styrene-divinyl benzene copolymer	SDB 1 (Mallinckrodt Baker) SDB-RPS (disk type, Empore)	200 mg or more 47 mm id or 90 mm id

Sorbents of other suppliers may be applicable, but they have not been evaluated for these uses.

1) This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

Annex B
(informative)

Suitable capillary column

The following capillary column is suitable:

(5 %-phenyl)-methylpolysiloxane phase, non-polar, bonded and cross-linked, low bleed (e.g. DB-5, Agilent).[2)]

Other capillary columns may be suitable, but they have not been evaluated for these uses. Comparable separation of individual isomers, as presented in Figure C.1, is desirable.

2) DB-5 is the trade name of a product supplied by Agilent Technologies. This information is given for the convenience of users of this International Standard 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.

Annex C
(informative)

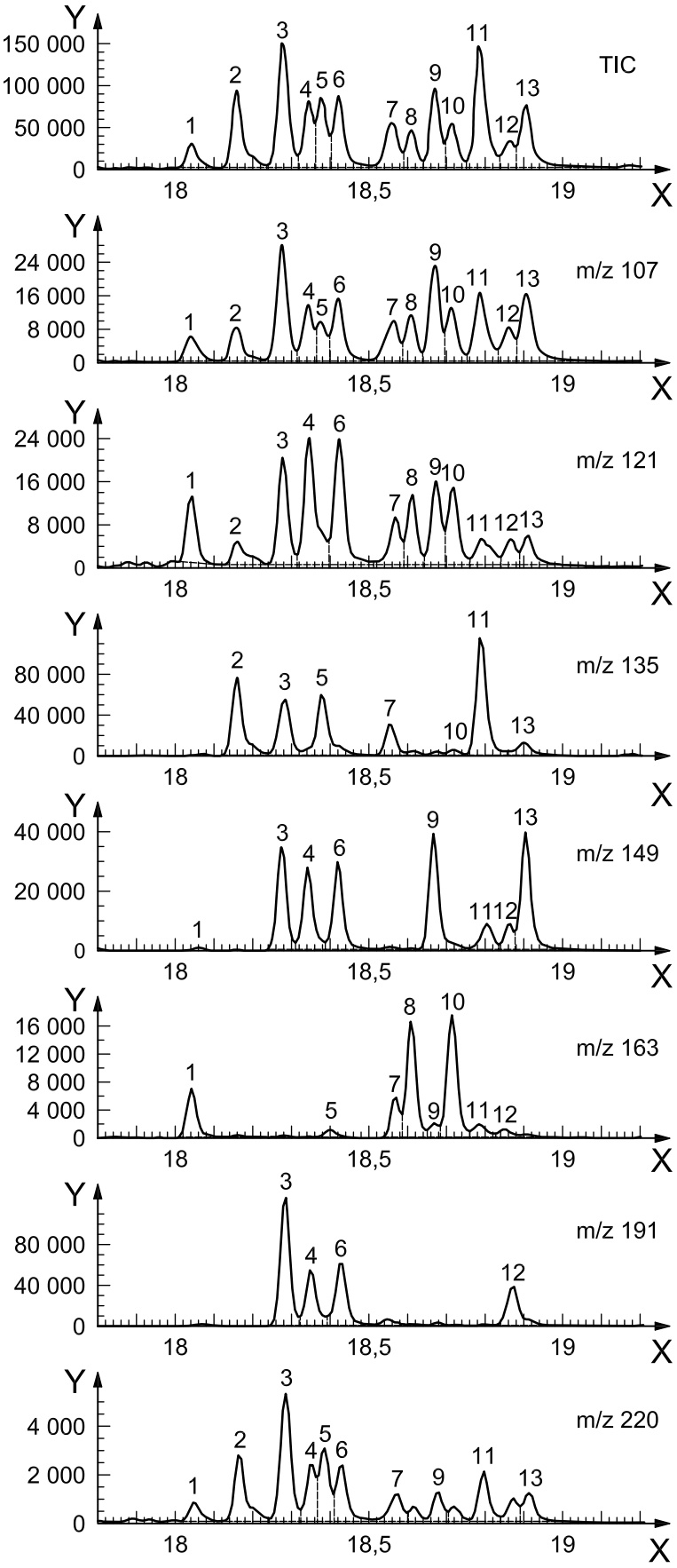
Examples of chromatograms

GC conditions for Figures C.1 and C.2:

Injection:	splitless	
Injector temperature:	250 °C	
Injection volume:	1 µl to 2 µl	
Transfer line temperature:	280 °C	
Flow rate:	1 ml/min to 1,5 ml/min	
Carrier gas:	helium, pre-pressure 69 kPa (10 psi)	
Capillary column:	stationary phase:	DB-5
	length:	30 m
	inner diameter:	0,25 mm
	film thickness:	0,25 µm
Temperature programme:	at 50 °C for 4 min, then to 280 °C at 8 °C/min, 5 min	

MS conditions for Figures C.1 and C.2:

Type:	quadrupole	
Ionization:	EI 70 eV	
Mode:	SIM	
Temperatures:	MS source:	230 °C
	MS quadrupole:	150 °C



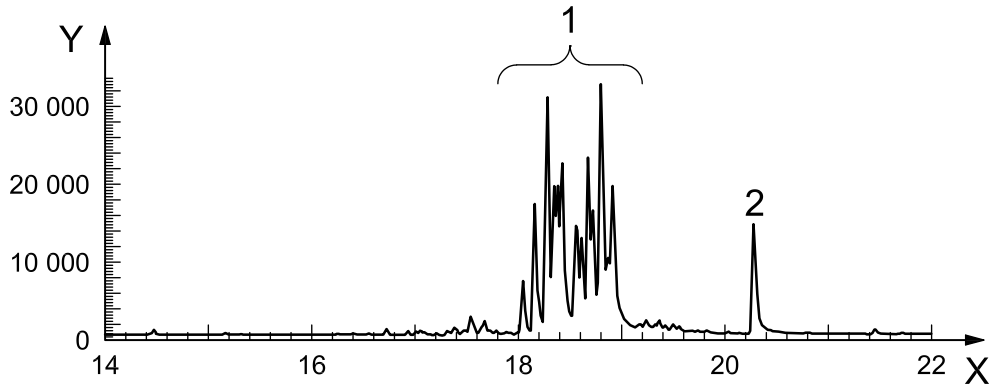
Key

X retention time, min

Y abundance

TIC total ion current

Figure C.1 — Chromatogram of a calibration standard and identification of individual isomers



Key

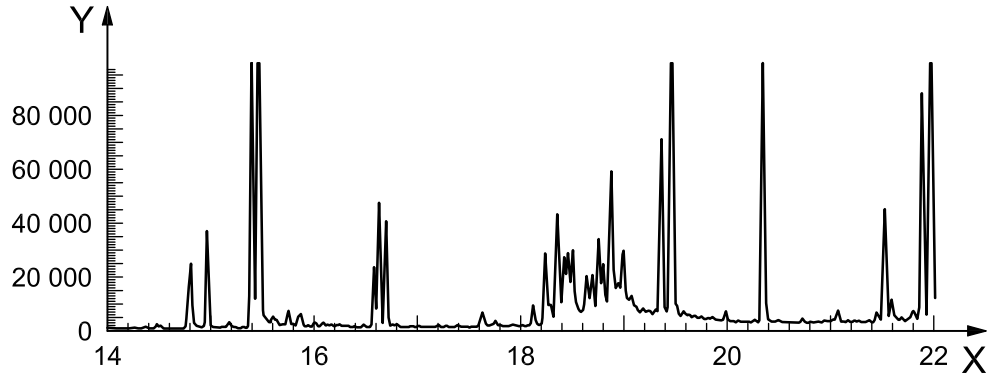
1 4-NP

2 labeled *p*-*n*-NP

X retention time, min

Y abundance

a) Calibration

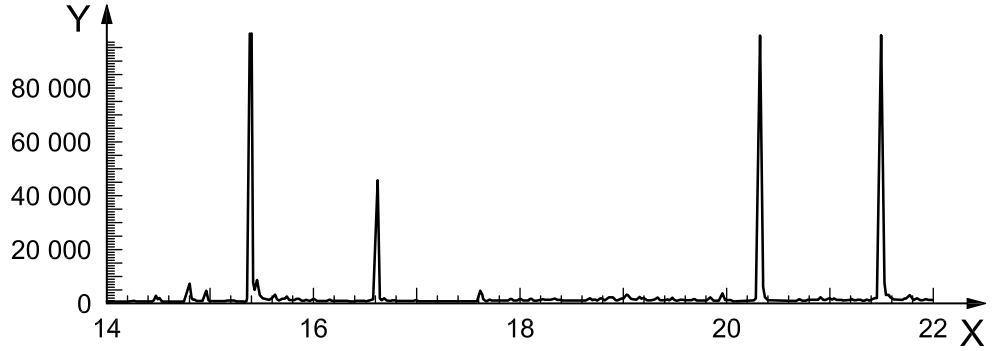


Key

X retention time

Y abundance

b) River water extract



Key

X retention time

Y abundance

c) Cartridge blank

Figure C.2 — Total ion chromatogram of calibration, river water extract and cartridge blank

Annex D
(informative)

Example of FID chromatogram and composition ratio (%) of isomers in 4-nonylphenol standard

It is necessary to confirm the composition ratio of the respective isomers of 4-nonylphenol standard product because the isomer composition in different technical mixtures may be variable.

D.1 Gas chromatograph.

Optimize the operating conditions of the GC system with Hydrogen Flame Ionization Detector (FID) in accordance with the manufacturer's instructions. The appropriate GC oven temperature programme is determined experimentally during method development and validation. An example of operating conditions is given below.

D.1.1 Detector, Hydrogen Flame Ionization Detector (FID).

D.1.2 Carrier gas, Helium, 99,999 9% or more of volume fraction, linear velocity: 20 cm/s to 40 cm/s.

D.1.3 Sample injection and injection port temperature, splitless (non-dividing introduction method); temperature at the sample introduction point: 260 °C.

D.1.4 Detector, temperature 300 °C.

D.1.5 Temperature programme, 50 °C to 280 °C.

50 °C 4 min

→ 170 °C at 8 °C/min

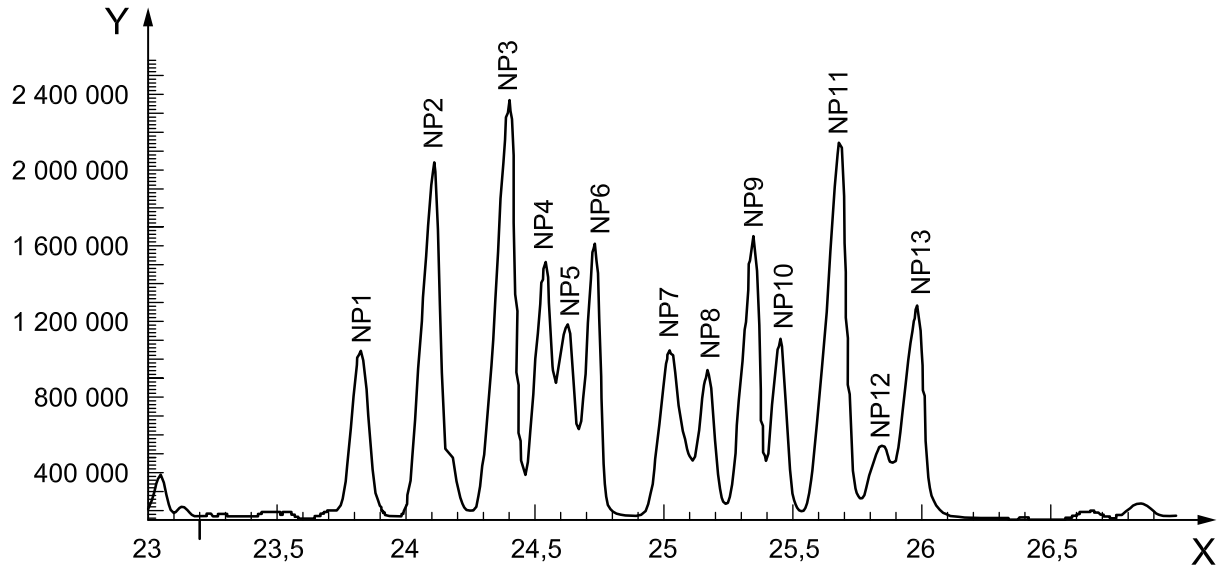
170 °C 10 min

→ 280 °C at 20 °C/min

280 °C 5 min.

D.1.6 Capillary column

Stationary phase:	DB-5
Length:	30 m
Inner diameter:	0,25 mm
Film thickness:	0,25 µm



Key

X retention time, min

Y abundance

Figure D.1 — Example of FID chromatogram of 4-nonylphenol standard (500 ng)

Table D.1 — Isomer compositions (%) of nonylphenol in seven different nonylphenol standards from several suppliers

Parameter	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	$\bar{X}$	CV %
NP1	5,3	5,5	3,9	4,8	6,2	5,6	5,1	5,1	15
NP2	12,7	12,4	16,1	12,4	10,7	11,7	12,0	12,6	13
NP3	14,2	17,5	18,6	18,4	13,7	16,4	19,4	16,9	13
NP4	7,0	6,6	6,8	6,5	6,9	6,9	6,6	6,8	2,8
NP5	6,1	7,5	9,3	7,5	5,8	7,2	7,9	7,3	16
NP6	6,9	6,9	6,9	6,6	6,8	6,8	6,5	6,8	2,4
NP7	6,5	6,7	5,8	6,8	7,2	6,7	7,3	6,7	7,4
NP8	4,5	3,6	2,1	3,4	4,7	4,0	3,1	3,6	24
NP9	9,3	7,8	5,5	7,6	9,2	8,3	6,7	7,7	17
NP10	4,8	4,0	3,0	3,9	5,2	4,4	3,7	4,1	18
NP11	13,4	12,3	14,8	12,4	13,7	12,8	11,9	13,0	7,7
NP12	2,9	5,4	3,8	4,0	2,7	3,0	4,4	3,7	26
NP13	6,5	3,8	3,5	5,7	7,2	6,2	5,5	5,5	25

$\bar{X}$ is the mean of values;

CV is the coefficient of variation.

Annex E
(informative)

Method performance data

An international interlaboratory trial was performed in August 2008. Seventeen laboratories from three countries took part (China: 1; Japan: 15; the United States of America: 1). Individual isomers of nonylphenol were analysed in

— two concentrations matrix river water (sample 1, sample 2),

— two concentrations matrix waste water (sample 3, sample 4), and

— standard in hexane solution (sample 5).

The performance data are summarized in Tables E.1 to E.5.

Table E.1 — Performance data for river water with low concentration native standard spiked (sample 1)

Parameter	l	l_o	n	n_o %	$\bar{\bar{X}}$ µg/l	s_R µg/l	CV_R %	s_r µg/l	CV_r %
NP1	14	6	24	43	0,031 0	0,003 9	12,7	0,002 3	7,1
NP2	15	6	27	40	0,077 1	0,023 6	30,6	0,005 9	8,0
NP3	15	6	27	40	0,090 8	0,023 9	26,3	0,010 4	10,7
NP4	14	6	24	43	0,042 1	0,009 8	23,2	0,004 6	10,5
NP5	15	6	27	40	0,033 9	0,008 8	26,0	0,002 9	9,1
NP6	14	6	24	43	0,045 5	0,009 7	21,4	0,007 7	14,8
NP7	14	6	24	43	0,033 9	0,008 3	24,4	0,003 9	11,9
NP8	14	6	24	43	0,024 3	0,006 0	24,8	0,001 9	7,4
NP9	15	6	27	40	0,054 9	0,017 2	31,2	0,004 6	8,5
NP10	14	6	24	43	0,028 0	0,006 7	23,8	0,003 0	10,2
NP11	15	6	27	40	0,086 0	0,025 5	29,7	0,005 8	6,3
NP12	11	4	21	32	0,017 0	0,000 6	35,4	0,002 2	11,1
NP13	14	6	24	43	0,037 7	0,005 3	14,2	0,006 3	16,3

Explanation of symbols

l	is the number of laboratories
l_o	is the number of outlier laboratories
n	is the number of analytical values
n_o	is the percentage of outlier analytical values
$\bar{\bar{X}}$	is the mean of values after outlier rejection
s_R	is the reproducibility standard deviation
CV_R	is the reproducibility coefficient of variation
s_r	is the repeatability standard deviation
CV_r	is the repeatability coefficient of variation

Table E.2 — Performance data for river water with high concentration native standard spiked (sample 2)

Parameter	l	l_o	n	n_o %	$\overline{\overline{X}}$ µg/l	s_R µg/l	CV_R %	s_r µg/l	CV_r %
NP1	15	4	33	27	0,133	0,037	27,4	0,010	7,8
NP2	15	4	33	27	0,328	0,103	31,5	0,024	7,8
NP3	15	4	33	27	0,398	0,101	25,4	0,035	8,9
NP4	15	4	33	27	0,187	0,041	22,1	0,023	12,6
NP5	15	4	33	27	0,150	0,035	23,3	0,012	8,2
NP6	15	4	33	27	0,187	0,049	25,9	0,019	10,1
NP7	15	4	33	27	0,158	0,053	33,7	0,012	8,8
NP8	15	4	33	27	0,116	0,070	60,2	0,010	9,9
NP9	15	4	33	27	0,236	0,077	32,6	0,023	10,0
NP10	15	4	33	27	0,134	0,046	34,4	0,012	9,6
NP11	15	4	33	27	0,356	0,085	23,9	0,029	8,2
NP12	12	2	30	17	0,073	0,029	39,3	0,012	19,7
NP13	13	3	30	23	0,177	0,032	18,1	0,020	11,8
For an explanation of the symbols, see Table E.1.									

Table E.3 — Performance data for waste water with low concentration native standard spiked (sample 3)

Parameter	l	l_o	n	n_o %	$\overline{\overline{X}}$ µg/l	s_R µg/l	CV_R %	s_r µg/l	CV_r %
NP1	14	5	27	36	0,137 3	0,048 6	35,4	0,010 1	6,1
NP2	14	5	27	36	0,382 7	0,174 2	45,5	0,054 3	10,2
NP3	14	5	27	36	0,405 7	0,212 9	52,5	0,043 0	8,4
NP4	14	5	26	37	0,185 1	0,113 5	61,3	0,035 3	10,8
NP5	14	5	27	36	0,152 2	0,077 3	50,8	0,010 3	4,7
NP6	14	5	27	36	0,186 5	0,088 7	47,6	0,022 6	8,2
NP7	13	5	24	38	0,181 2	0,093 2	51,5	0,016 5	6,9
NP8	14	5	27	34	0,191 0	0,286 6	150,1	0,033 5	9,4
NP9	14	5	27	36	0,235 0	0,103 1	43,9	0,012 3	4,4
NP10	13	5	24	38	0,152 5	0,090 4	59,3	0,019 8	8,3
NP11	13	5	24	38	0,387 4	0,167 1	43,1	0,034 2	6,9
NP12	10	3	21	30	0,217 3	0,278 4	128,1	0,076 9	21,6
NP13	12	4	24	33	0,232 5	0,125 6	54,0	0,038 8	17,3
For an explanation of the symbols, see Table E.1.									

Table E.4 — Performance data for waste water with high concentration native standard spiked (sample 4)

Parameter	l	l_o	n	n_o %	$\overline{\overline{X}}$ µg/l	s_R µg/l	CV_R %	s_r µg/l	CV_r %
NP1	14	4	30	29	0,641	0,240	37,5	0,098	11,6
NP2	14	4	30	29	1,411	0,377	26,7	0,142	9,2
NP3	14	4	30	29	1,621	0,590	36,4	0,145	8,2
NP4	14	4	30	29	0,752	0,277	36,9	0,076	9,2
NP5	14	4	30	29	0,620	0,174	28,1	0,066	9,3
NP6	14	4	30	29	0,783	0,240	30,6	0,067	7,3
NP7	14	4	30	29	0760	0,197	25,9	0,092	11,1
NP8	14	4	30	29	0,567	0,362	63,8	0,088	10,3
NP9	14	4	30	29	0,951	0,285	29,9	0,095	9,3
NP10	14	4	30	29	0,547	0,169	31,0	0,054	9,3
NP11	14	4	30	29	1,473	0,494	33,5	0,145	9,2
NP12	12	3	27	25	0,407	0,275	67,6	0,045	12,6
NP13	13	4	27	31	0,957	0,271	28,3	0,166	14,8
For an explanation of the symbols, see Table E.1.									

Table E.5 — Performance data for standard solution (sample 5)

Parameter	l	l_o	n	n_o %	$\overline{\overline{X}}$ µg/l	s_R µg/l	CV_R %	s_r µg/l	CV_r %
NP1	11	1	30	9	261	63	24,2	8,0	2,9
NP2	11	1	30	9	604	210	34,8	33,4	7,8
NP3	11	1	30	9	845	545	64,5	46,5	9,8
NP4	11	1	30	9	376	153	40,6	17,3	5,4
NP5	11	1	30	9	320	139	43,5	9,3	3,5
NP6	11	1	30	9	388	136	35,1	18,7	5,7
NP7	11	1	30	9	345	118	34,3	16,6	4,9
NP8	11	1	30	9	238	106	44,2	5,2	2,3
NP9	11	1	30	9	466	251	53,9	25,6	7,2
NP10	11	1	30	9	252	84	33,4	7,7	3,3
NP11	11	1	30	9	739	350	47,4	41,8	9,0
NP12	10	1	27	10	142	62	43,7	13,8	8,3
NP13	11	1	30	9	435	185	42,5	56,8	10,6
For an explanation of the symbols, see Table E.1.									

Annex F
(informative)

Description of the matrices of the samples used for the interlaboratory trial

F.1 General

Native standard spiked amounts for individual nonylphenol isomers were calculated based on FID analysis of 4-nonylphenol standard.

F.2 River water with low concentration

The surface river water sample for the interlaboratory trial (see Table E.1) was taken from the Edo River in Tokyo (Japan). First, the sample was homogenized by gentle stirring. Then, a low concentration of 4-nonylphenol standard in acetone solution was spiked into the river water. Individual isomers of nonylphenol were quantified (see Table F.1). The amount of suspended particle matter was 13 mg/l.

Table F.1 — Matrix of the low level of native standard spiked river water (sample 1) used for the interlaboratory trial

Parameter	Unit	Native standard spiked amount	Spiked amount + river water result	Result
NP1	µg/l	0,010 6	0,081 3	0,032 8
NP2	µg/l	0,024 7	0,129 0	0,079 2
NP3	µg/l	0,029 0	0,149 4	0,093 4
NP4	µg/l	0,014 5	0,100 0	0,044 2
NP5	µg/l	0,011 2	0,058 1	0,035 0
NP6	µg/l	0,014 2	0,092 9	0,045 7
NP7	µg/l	0,013 3	0,079 3	0,036 7
NP8	µg/l	0,008 6	0,066 1	0,026 2
NP9	µg/l	0,017 1	0,105 4	0,055 6
NP10	µg/l	0,009 5	0,099 7	0,030 3
NP11	µg/l	0,027 4	0,167 7	0,081 6
NP12	µg/l	0,005 2	0,033 1	0,017 2
NP13	µg/l	0,014 8	0,023 5	0,036 9

F.3 River water with high concentration

The surface river water sample for the interlaboratory trial (see Table E.2) was taken from the Edo River in Tokyo (Japan). First, the sample was homogenized by gentle stirring. Then, a high concentration of 4-nonylphenol standard mixed with NP10 isomer in acetone solution was spiked into the river water. Individual isomers of nonylphenol were quantified (see Table F.2).

Table F.2 — Matrix of the high level of native standard spiked river water (sample 2) used for the interlaboratory trial

Parameter	Unit	Native standard spiked amount	Spiked amount + river water result	Result
NP1	µg/l	0,053	0,123	0,124
NP2	µg/l	0,123	0,228	0,344
NP3	µg/l	0,145	0,265	0,414
NP4	µg/l	0,072	0,158	0,196
NP5	µg/l	0,056	0,103	0,159
NP6	µg/l	0,071	0,150	0,196
NP7	µg/l	0,067	0,133	0,175
NP8	µg/l	0,043	0,101	0,122
NP9	µg/l	0,085	0,174	0,244
NP10	µg/l	0,097	0,188	0,139
NP11	µg/l	0,137	0,277	0,355
NP12	µg/l	0,026	0,054	0,069
NP13	µg/l	0,074	0,083	0,183

F.4 Waste water with low concentration

The waste water sample for interlaboratory trial (see Table E.3) was taken from the waste water treatment plant in Tokyo (Japan). First, the sample was homogenized by gentle stirring. Then, a low concentration of 4-nonylphenol standard in acetone solution was spiked into the waste water. Individual isomers of nonylphenol were quantified (see Table F.3). This test sample had the highest amount of suspended particle matter, i.e. 140 mg/l.

Table F.3 — Matrix of the low level of native standard spiked waste water (sample 3) used for the interlaboratory trial

Parameter	Unit	Native standard spiked amount	Spiked amount + waste water result	Result
NP1	µg/l	0,010 6	0,071 1	0,126 8
NP2	µg/l	0,024 7	0,166 8	0,392 9
NP3	µg/l	0,029 0	0,188 8	0,358 0
NP4	µg/l	0,014 5	0,084 8	0,158
NP5	µg/l	0,011 2	0,069 7	0,136 3
NP6	µg/l	0,014 2	0,079 8	0,165 8
NP7	µg/l	0,013 3	0,071 7	0,155 9
NP8	µg/l	0,008 6	0,018 8	0,095 1
NP9	µg/l	0,017 1	0,090 1	0,210 7
NP10	µg/l	0,009 5	0,049 2	0,120 5
NP11	µg/l	0,027 4	0,170 2	0,340 6
NP12	µg/l	0,005 2	0,087 4	0,117 9
NP13	µg/l	0,014 8	0,134 9	0,259 5

F.5 Waste water with high concentration

The waste water sample for interlaboratory trial (see Table E.4) was taken from the waste water treatment plant in Tokyo (Japan). First, the sample was homogenized by gentle stirring. Then, a high concentration of 4-nonylphenol standard mixed with NP10 isomer in acetone solution was spiked into the waste water. Individual isomers of nonylphenol were quantified (see Table F.4).

Table F.4 — Matrix of the high level of native standard spiked waste water (sample 4) used for the interlaboratory trial

Parameter	unit	Native standard spiked amount	Spiked amount + waste water result	Result
NP1	µg/l	0,053	0,113	0,581
NP2	µg/l	0,123	0,266	1,358
NP3	µg/l	0,145	0,305	1,619
NP4	µg/l	0,072	0,143	0,706
NP5	µg/l	0,056	0,115	0,617
NP6	µg/l	0,071	0,136	0,686
NP7	µg/l	0,067	0,125	0,755
NP8	µg/l	0,043	0,053	0,442
NP9	µg/l	0,085	0,158	0,834
NP10	µg/l	0,097	0,137	0,537
NP11	µg/l	0,137	0,280	1,455
NP12	µg/l	0,026	0,108	0,322
NP13	µg/l	0,074	0,194	0,924

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