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

Part 2:

**Gas chromatographic-mass  
spectrometric determination of  
alkylphenols, their ethoxylates and  
bisphenol A in non-filtered samples  
following solid-phase extraction and  
derivatisation**

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

*Partie 2: Dosage par chromatographie en phase gazeuse-spectrométrie  
de masse d'alkylphénols, de leurs éthoxylates et de bisphénol A dans  
des échantillons non filtrés après extraction en phase solide et  
dérivation*



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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 18857-2 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*
- *Part 2: Gas chromatographic-mass spectrometric determination of alkylphenols, their ethoxylates and bisphenol A in non-filtered samples following solid-phase extraction and derivatisation*

## Introduction

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

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# Water quality — Determination of selected alkylphenols —

## Part 2:

# Gas chromatographic-mass spectrometric determination of alkylphenols, their ethoxylates and bisphenol A in non-filtered samples following solid-phase extraction and derivatisation

**WARNING** — Persons using this part of ISO 18857 should be familiar with normal laboratory practice. This part of ISO 18857 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 gas chromatographic-mass spectrometric (GC-MS) determination of selected alkylphenols, their ethoxylates and bisphenol A in non-filtered samples of drinking, ground, surface, and waste waters following solid-phase extraction and derivatisation.

The lower limit of the working range depends on the matrix, on the specific compound to be analysed and on the sensitivity of the mass spectrometric detection unit. The method is applicable in a working range from 0,005 µg/l to 0,2 µg/l for 4-(1,1,3,3-tetramethylbutyl)phenol (OP), and its mono- (OP<sub>1</sub>EO) and diethoxylate (OP<sub>2</sub>EO), from 0,03 µg/l to 0,2 µg/l for 4-nonylphenol (mixture of isomers) (NP), and its mono- (NP<sub>1</sub>EO) and diethoxylate (NP<sub>2</sub>EO), and from 0,05 µg/l to 0,2 µg/l for bisphenol A (BPA).

Depending on the matrix, the method is also applicable to waste water in a working range from 0,1 µg/l to 50 µg/l for OP, OP<sub>1</sub>EO, OP<sub>2</sub>EO and BPA, and from 0,5 µg/l to 50 µg/l for NP, NP<sub>1</sub>EO and NP<sub>2</sub>EO. The working ranges are based on experimental work carried out in ruggedness testing. Water samples containing suspended matter at concentrations of more than 500 mg/l and waste water samples are extracted by passing a 100 ml sample through the cartridge.

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

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 an acidified water sample by solid-phase extraction, solvent elution, derivatisation and determination by GC-MS detection.

**Table 1 — Analytes determinable by GC-MS following solid-phase extraction and derivatisation**

| Analyte   | Empirical formula                              | Abbreviation       | CAS <sup>a</sup> No.    |
|---|--|--------------------|-------------------------|
| 4-(1,1,3,3-Tetramethylbutyl)phenol                | C <sub>14</sub> H <sub>22</sub> O              | OP                 | 140-66-9                |
| 4-(1,1,3,3-Tetramethylbutyl)phenol monoethoxylate | C <sub>16</sub> H <sub>26</sub> O <sub>2</sub> | OP <sub>1</sub> EO | —                       |
| 4-(1,1,3,3-Tetramethylbutyl)phenol diethoxylate   | C <sub>18</sub> H <sub>30</sub> O <sub>3</sub> | OP <sub>2</sub> EO | —                       |
| 4-Nonylphenol (mixture of isomers)                | C <sub>15</sub> H <sub>24</sub> O              | NP                 | 84852-15-3 <sup>b</sup> |
| 4-Nonylphenol monoethoxylate (mixture of isomers) | C <sub>17</sub> H <sub>28</sub> O <sub>2</sub> | NP <sub>1</sub> EO | —                       |
| 4-Nonylphenol diethoxylate (mixture of isomers)   | C <sub>19</sub> H <sub>32</sub> O <sub>3</sub> | NP <sub>2</sub> EO | —                       |
| Bisphenol A                                       | C <sub>15</sub> H <sub>16</sub> O <sub>2</sub> | BPA                | 80-05-7                 |

<sup>a</sup> CAS: Chemical Abstracts Service.

<sup>b</sup> The commercially produced nonylphenols are predominantly 4-nonylphenol with a varied and undefined degree of branching in the alkyl groups. This mixture of isomers falls under the CAS number 84852-15-3, but CAS numbers 104-40-5 (4-nonylphenol, straight chain) and 25154-52-3 (nonylphenol, straight chain) have also been incorrectly used to denote this isomer mixture.

### 4 Interferences

#### 4.1 Sampling and extraction

Sampling containers shall consist of materials that do not change the sample when in contact with it. Avoid contact with plastics and other organic materials during sampling, sample storage or extraction.

Commercially available adsorbent materials are often of varying quality. Considerable batch-to-batch differences in quality and selectivity of this material are possible. The recovery of single substances can vary with the concentration. Therefore, check the recovery regularly at different concentrations and whenever new batches are used. Perform calibration and analysis with material from the same batch.

#### 4.2 Gas chromatography-mass spectrometry

Substances with retention times or which produce masses similar to the analytes to be determined can interfere with the determination.

These interferences may lead to incompletely resolved signals and to additional signals in the chromatographic pattern of NP, NP<sub>1</sub>EO and NP<sub>2</sub>EO. They may, depending on their magnitude, affect accuracy and precision of the analytical results, since all three analytes are determined from the sum of a cluster of eight to ten chromatographic peaks (Table 3 and Annex C). It is important that the interfering peaks are not included in the calculations.

The presence of interfering compounds can, if necessary, be detected by recording full mass spectra (range of mass fragments to monitor  $m/z = 50$  to  $m/z = 350$ ).

Matrix interferences can be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences varies considerably, depending on the nature of the sample. In drinking water and ground water, matrix interferences usually do not occur.



## 5 Reagents

The reagents shall not have blank values that would interfere with the GC-MS analysis.

Use solvents and reagents of sufficient purity, i.e. with negligibly low impurities compared with the concentration of analytes to be determined. As reagents, use, as far as available, “residual grade” or better in order to obtain clean blanks. Check blanks regularly and establish proper charge control.

**5.1 Water**, as specified in ISO 3696, grade 1, or equivalent.

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

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

**5.4 Internal standard solutions.**

Examples of suitable internal standards are given in Table 2.

Store solutions 5.4.1 and 5.4.2 in a refrigerator protected from light. Check the solutions weekly prior to use.

**Table 2 — Internal standards**

| No. | Name   | Abbreviation                              | CAS No.    |
|-----|--|---|------------|
| 1   | 4-(1,1,3,3-Tetramethylbutyl)phenol (ring- $^{13}\text{C}_6$ )                | OP- $^{13}\text{C}_6$                     | —          |
| 2   | 4-(1,1,3,3-Tetramethylbutyl)phenol monoethoxylate (ring- $^{13}\text{C}_6$ ) | OP <sub>1</sub> EO- $^{13}\text{C}_6$     | —          |
| 3   | 4-(1,1,3,3-Tetramethylbutyl)phenol diethoxylate (ring- $^{13}\text{C}_6$ )   | OP <sub>2</sub> EO- $^{13}\text{C}_6$     | —          |
| 4   | 4-(3,6-Dimethyl-3-heptyl)phenol (ring- $^{13}\text{C}_6$ )                   | 363 NP- $^{13}\text{C}_6$                 | —          |
| 5   | 4-(3,6-Dimethyl-3-heptyl)phenol monoethoxylate (ring- $^{13}\text{C}_6$ )    | 363 NP <sub>1</sub> EO- $^{13}\text{C}_6$ | —          |
| 6   | 4-(3,6-Dimethyl-3-heptyl)phenol diethoxylate (ring- $^{13}\text{C}_6$ )      | 363 NP <sub>2</sub> EO- $^{13}\text{C}_6$ | —          |
| 7   | Bisphenol A-d16  | BPA-d16                                   | 96210-87-6 |

**5.4.1 Internal standard stock solution.**

Use commercially available internal standard solutions or prepare a solution as follows.

Weigh 10 mg of each internal standard (Table 2) separately in a 100 ml one-mark volumetric flask and make up to the mark with acetone (5.3) to give a concentration of each internal standard of 100 ng/μl.

**5.4.2 Internal standard working solution.**

Dilute the solution (5.4.1) with acetone (5.3) 1→100 to give a final concentration of each internal standard of 1 ng/μl.

## 5.5 Solutions of reference standards of the analytes listed in Table 1.

Store solutions 5.5.1 and 5.5.2 in a refrigerator protected from light. Check the solutions weekly prior to use.

### 5.5.1 Reference standard stock solution.

Use commercially available reference standard solutions or prepare a solution as follows.

Weigh 10 mg of each reference substance separately in a 100 ml volumetric flask and bring to volume with acetone (5.3) to give a concentration of each reference standard of 100 ng/μl.

### 5.5.2 Reference standard working solution.

Dilute the solution (5.5.1) 1→100 with acetone (5.3) to give a final concentration of each reference substance of 1 ng/μl.

## 5.6 2,2,2-Trifluoro-*N*-methyl-*N*-(trimethylsilyl)acetamide (MSTFA), C<sub>6</sub>H<sub>12</sub>F<sub>3</sub>NOSi.

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

**5.8 Sand**, e.g. sea sand, acid washed and calcinated for analysis, particle size 0,1 mm to 0,3 mm.

**5.9 Nitrogen**, N<sub>2</sub>, purity ≥ 99,996 % volume fraction, for drying of the sorbent packing after sample extraction and for concentration by evaporation.

**5.10 Sodium thiosulfate pentahydrate**, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> · 5 H<sub>2</sub>O.

## 6 Apparatus

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

Clean all glassware by rinsing with acetone (5.3). Avoid detergents when using a labware washing machine. Alternatively, prior to use, heat all glassware, except volumetric ware, to at least 200 °C for a minimum of 2 h.

Usual laboratory equipment, and in particular the following.

**6.1 Narrow-neck flat bottomed glass bottles**, conical shoulders, preferably brown glass, 1 000 ml, with glass stoppers or with PTFE-lined screw caps (PTFE = polytetrafluoroethene). Protect samples from light if brown glass bottles are not available. Wash the bottle, cap liner or glass stopper, then rinse with acetone (5.3), and dry before use in order to minimise contamination.

**6.2 Solid-phase extraction cartridges**, of inert non-leaching plastic, e.g. polypropene, or glass. The cartridges should be packed with a minimum of 200 mg of sorbent (5.7).

**6.3 Vacuum or pressure assembly**, for the extraction step.

**6.4 One-mark volumetric flasks**, ISO 1042<sup>[1]</sup> class A, with inert stoppers.

**6.5 Quartz wool**, rinsed with acetone.

**6.6 Pear-shaped flask**, 10 ml, with inert stopper.

**6.7 Evaporation assembly**, e.g. rotary evaporator with vacuum stabiliser and water bath.

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

**6.9 Stainless steel cocks**, with stainless steel cone.

**6.10 Gas chromatograph**, temperature-programmable and with all required accessories including gases, capillary columns, capillary injector and mass spectrometer detector. The mass spectrometer should be capable of operating in electron impact mode over the mass range of interest and incorporate a data system capable of quantifying ions using selected  $m/z$  values (selected ion monitoring).

## 7 Sampling and sample pretreatment

Take samples as specified in ISO 5667-1 and ISO 5667-3.

For sampling, use carefully cleaned bottles (6.1). Fill bottles only to the shoulder, in which case the sample volume is approximately 1 l. This volume is completely used for extraction (8.1). If the presence of free chlorine is suspected, immediately add approximately 80 mg of sodium thiosulfate (5.10). Other non-interfering substances may be used for dechlorination as well (e.g. sodium sulfite). Acidify the sample with acid (5.2) to pH ( $2 \pm 0,2$ ).

If necessary, store the sample in a refrigerator ( $2\text{ }^{\circ}\text{C}$  to  $5\text{ }^{\circ}\text{C}$ ) and analyse as soon as possible, but not later than 2 weeks after sampling.

Weigh the sample bottle with its contents to the nearest 1 g and record the mass for subsequent sample volume determination (8.1.2).

## 8 Procedure

### 8.1 Solid-phase extraction

In general, samples are examined without pretreatment, which means that suspended solids are not removed prior to analysis. Before starting the analysis, homogenise the sample. In cases where blocking of the cartridge packing is likely to occur, use some filter aid, e.g. quartz wool (6.5) or sand (5.8) e.g. 0,5 cm bed thickness.

#### 8.1.1 Conditioning of the solid-phase material

The following procedure is described for commercially available 6 ml polypropene cartridges packed with 200 mg of sorbent sandwiched between two polyethylene frits.

Rinse the cartridge with two 10 ml aliquots of acetone (5.3). Allow the first aliquot to drain from the cartridge. Before the acetone level of the second aliquot falls below the top edge of the packing, add 10 ml of water (5.1), acidified with acid (5.2) to pH ( $2 \pm 0,2$ ), to the cartridge, and make sure that the sorbent packing in the cartridge does not run dry, e.g. by using a stainless steel cock (6.9). Retain the water in the cartridge (water level just above the packing) to keep the sorbent activated.

#### 8.1.2 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 mixture (5.4.2) well below the surface of the water sample (Clause 7) in the sample bottle [50  $\mu\text{l}$  to 200  $\mu\text{l}$  of the prepared mixture (5.4) dependent on the sample matrix] and mix thoroughly. Allow this sample to run through the cartridge (8.1.1) at a flow rate between 5 ml/min and 10 ml/min. Water samples containing suspended matter at a concentration of more than 500 mg/l and waste water samples are extracted by passing a 100 ml sample through the cartridge. Rinse the cartridge with 10 ml of water (5.1), acidified with acid (5.2) to pH ( $2 \pm 0,2$ ).

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

NOTE The end of the removal of water from the cartridge can usually be recognised by the change in colour of the adsorbent. The colour of the moist adsorbent is brown, the dry material is light orange.

Reweigh the empty sample bottle with its original cap or stopper and calculate the net mass of the sample by difference to the nearest 1 g. For an assumed density of 1 g/ml, this net mass, in grams, is equivalent to the volume, in millilitres, of water extracted. The volume of the acid (5.2) added to acidify the sample is negligible.

### 8.1.3 Elution

Add 1 ml of acetone (5.3) to the completely dried cartridge, allow to equilibrate for e.g. 10 min and elute the cartridge. Then, add five further 1 ml aliquots of acetone (5.3) to the cartridge, but do not allow the acetone to elute below the top of the sorbent packing during the elution steps.

After these elutions, draw the remaining solvent through the cartridge in order to collect the remaining drops. Collect all eluates in a pear-shaped flask (6.6). Evaporate the eluates to dryness under a moderate stream of nitrogen. Dissolve the residue by adding 200  $\mu$ l of acetone (5.3) and continue with derivatisation (8.2).

## 8.2 Derivatisation

Add 25  $\mu$ l of MSTFA (5.6) to the sample extract solution (8.1.3) and mix gently. Allow the mixture to react at room temperature for at least 30 s. Transfer the solution of the derivatised analytes and internal standards to a suitable vial (6.8). This acetone solution may be stored for 1 week. Afterwards, decomposition of the derivatives may take place. The decomposition can be reversed by addition of another portion of MSTFA.

## 8.3 GC-MS operating conditions

Optimise the operating conditions of the GC-MS system in electron ionisation 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, selected ions (Table 3) are detected. The electron energy is set at 70 eV. An example of operating conditions is given in Annex C.

## 8.4 Blank determination

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

## 8.5 Identification

Determine the intensities,  $I_1$ ,  $I_2$ ,  $I_3$ , of the selected diagnostic ions,  $M_1$ ,  $M_2$ ,  $M_3$  (see Table 3), in a reference standard working solution (5.5.2) as the peak areas of the ions in the corresponding extracted ion current chromatograms. Calculate the relative intensities as the ratio of the peak areas determined to the peak area of the most intensive diagnostic ion.

Identify the sample component by matching both retention times and relative intensities of the diagnostic ions (Table 3) of sample components and reference substances (5.5).

The target compound is present (is identified) in the sample if:

- the sample component retention time,  $t_{\text{ret}}$ , measured in the selected ion current chromatogram matches the retention time of the authentic compound within a limit deviation of  $t_{\text{ret}} \pm 0,02$  min in the chromatogram of the latest calibration standard, measured under identical conditions;
- the three selected diagnostic ions (see Table 3) 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  $\pm (0,1I + 10)$  % from the relative intensities determined in the reference standard working solution (5.5.2) [ $I$  is the relative intensity of the diagnostic ion in the reference standard working solution (5.5.2)].

Table 3 — Selected diagnostic ions for identification and quantification

| No.   | Analyte<br>[trimethylsilyl (TMS) derivative]   | Abbreviation  | Selected diagnostic ions              |  |  | ISTD for<br>analyte<br>No. |
|---|--|---|---------------------------------------|--|--|----------------------------|
|   |  |   | Target<br>M <sub>1</sub> <sup>a</sup> | Qualifier<br>M <sub>2</sub> <sup>a</sup> | Qualifier<br>M <sub>3</sub> <sup>a</sup> |                            |
| 1   | 4-(1,1,3,3-Tetramethylbutyl)phenol   | OP-TMS  | 207                                   | 278                                      | 208                                      | —                          |
| 2   | 4-(1,1,3,3-Tetramethylbutyl)phenol monoethoxylate  | OP <sub>1</sub> EO-TMS                                    | 251                                   | 322                                      | 252                                      | —                          |
| 3   | 4-(1,1,3,3-Tetramethylbutyl)phenol diethoxylate  | OP <sub>2</sub> EO-TMS                                    | 295                                   | 366                                      | 296                                      | —                          |
| 4   | 4-Nonylphenol (mixture of isomers)   | NP-TMS  | 207                                   | 221                                      | 193, 179                                 | —                          |
| 5   | 4-Nonylphenol monoethoxylate (mixture of isomers)  | NP <sub>2</sub> EO-TMS                                    | 251                                   | 265                                      | 279, 307                                 | —                          |
| 6   | 4-Nonylphenol diethoxylate (mixture of isomers)  | NP <sub>2</sub> EO-TMS                                    | 295                                   | 309                                      | 323, 351                                 | —                          |
| 7   | Bisphenol A  | BPA-TMS   | 357                                   | 358                                      | 372                                      | —                          |
| 8   | 4-(1,1,3,3-Tetramethylbutyl)phenol (ring- <sup>13</sup> C <sub>6</sub> ) <sup>b</sup>                | OP- <sup>13</sup> C <sub>6</sub> -TMS                     | 213                                   | 284                                      | 214                                      | 1                          |
| 9   | 4-(1,1,3,3-Tetramethylbutyl)phenol monoethoxylate (ring- <sup>13</sup> C <sub>6</sub> ) <sup>b</sup> | OP <sub>1</sub> EO- <sup>13</sup> C <sub>6</sub> -TMS     | 257                                   | 328                                      | 213                                      | 2                          |
| 10  | 4-(1,1,3,3-Tetramethylbutyl)phenol diethoxylate (ring- <sup>13</sup> C <sub>6</sub> ) <sup>b</sup>   | OP <sub>2</sub> EO- <sup>13</sup> C <sub>6</sub> -TMS     | 301                                   | 372                                      | 213                                      | 3                          |
| 11  | 4-(3,6-Dimethyl-3-heptyl)phenol (ring- <sup>13</sup> C <sub>6</sub> ) <sup>b</sup>                   | 363 NP- <sup>13</sup> C <sub>6</sub> -TMS                 | 227                                   | 269                                      | 298                                      | 4                          |
| 12  | 4-(3,6-Dimethyl-3-heptyl)phenol monoethoxylate (ring- <sup>13</sup> C <sub>6</sub> ) <sup>b</sup>    | 363 NP <sub>1</sub> EO- <sup>13</sup> C <sub>6</sub> -TMS | 271                                   | 313                                      | 342                                      | 5                          |
| 13  | 4-(3,6-Dimethyl-3-heptyl)phenol diethoxylate (ring- <sup>13</sup> C <sub>6</sub> ) <sup>b</sup>      | 363 NP <sub>2</sub> EO- <sup>13</sup> C <sub>6</sub> -TMS | 315                                   | 357                                      | 386                                      | 6                          |
| 14  | Bisphenol A-d16 <sup>b</sup>   | BPA-d16-TMS   | 368                                   | 369                                      | 386                                      | 7                          |
| <sup>a</sup> M <sub>1</sub> is used for quantification, M <sub>2</sub> and M <sub>3</sub> may be used for identification. |  |   |                                       |  |  |                            |
| <sup>b</sup> Internal standard (ISTD).  |  |   |                                       |  |  |                            |

## EXAMPLE

Three selected diagnostic ions have the following relative intensities: 100 %, 50 % and 15 %.

The maximum allowed deviation for  $I_2$  and  $I_3$  in the sample is ( $I_1$  is by definition 100 % in both the sample and reference standard):

$I_2: \pm (0,1 \times 50 + 10) \% = \pm 15 \%$ ; relative intensity in the sample shall lie between 35 % and 65 %;

$I_3: \pm (0,1 \times 15 + 10) \% = \pm 11,5 \%$ ; relative intensity in the sample shall lie between 3,5 % and 26,5 %;

and if the peak pattern of NP, NP<sub>1</sub>EO, and NP<sub>2</sub>EO in the sample chromatogram is to a wide extent in agreement with the peak pattern of NP, NP<sub>1</sub>EO, and NP<sub>2</sub>EO (5.5) used as calibration standards, measured under identical conditions (to indicate the presence of the technical products NP, NP<sub>1</sub>EO and NP<sub>2</sub>EO). The relation between the individual peaks may differ in samples and standards.

**NOTE** The reference standards used, NP, NP<sub>1</sub>EO and NP<sub>2</sub>EO, are technical products. Therefore, mass spectrometry shows an isomer pattern that usually consists of eight to ten main peaks (see Annex C). The isomer pattern can differ from batch to batch. Due to the different composition of the technical products, a fixed ratio of the relative intensities of the selected diagnostic ions is not obtained for the whole peak pattern.

## 9 Calibration and analysis of samples

### 9.1 General requirements

For practical reasons, the calibration is based on a solution containing reference substances (5.5) and internal standards (5.4) derivatised according to 8.2.

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 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.

### 9.2 Calibration with internal standards

The use of an internal standard for the determination of the concentration minimises both possible errors made during injection and by sample losses during sample pre-treatment steps, as well as differences in the final sample extract volumes and changes in recoveries caused by matrix effects. This calculation is usually available as an option in the quantitation programs of most manufacturers' data analysis software.

Additionally, it is possible to estimate the recovery for the total procedure for each sample, if the area values from the internal standard in the calibration solutions are compared to the area values obtained from the extract. To achieve this, it is essential that the final volumes be nearly the same.

Adjust the concentration range for calibration according to the sensitivity of the equipment used and the range of determinations required. Choose the same concentrations of the internal standards (5.4.2) in the different reference standard working solutions (5.5.2) for calibration.

Establish the linear function of the pairs of values  $y_i/y_{is,i}$  and  $\rho_i/\rho_{is,i}$  of the measured series using Equation (1):

$$\frac{y_i}{y_{is,i}} = a_i \frac{\rho_i}{\rho_{is,i}} + b_i \quad (1)$$

where

- $y_i$  is the measured response, as an area value, of substance  $i$ ;
- $\rho_i$  is the mass concentration, in nanograms per millilitre, of substance  $i$  (external standard), in the working standard solution;
- $a_i$  is the slope of the calibration function, expressed as the area value times millilitres per nanogram, of substance  $i$ ;
- $b_i$  is the ordinate intercept, as an area value, of the calibration curve;
- $y_{is,i}$  is the measured response, as an area value, of the internal standard for the substance  $i$ ;
- $\rho_{is,i}$  is the mass concentration, in nanograms per millilitre, of the internal standard, for the substance  $i$  in the working standard solution.

For all calibrations, the relative area values are used, i.e. the area for the analyte to the area for the internal standard.

### 9.3 Quantitation with the internal standard

Add a known amount of the internal standards (5.4.2) to the water sample (Clause 7) prior to extraction. The mass concentration of the internal standard,  $\rho_{is,i}$ , in the final volume of extract shall be nearly the same for calibration and sample measurement. Use the same solvent composition for the working standard solutions (5.5.1 and 5.5.2) and the extracts.

Pre-treat and analyse the samples as specified. Inject identical volumes of the sample extracts as injected for calibration solutions.

For NP, NP<sub>1</sub>EO and NP<sub>2</sub>EO, the areas are determined as the sum of the peak areas of the isomer mixture. Substances that co-elute with NP, NP<sub>1</sub>EO and NP<sub>2</sub>EO and give the same ion(s) can interfere in the determination. This can have a large influence on the result. Ensure that interfering peaks are excluded from the sum of the areas. Include only those peaks from the sample attributable to the multicomponent analyte.

Calculate the mass concentration,  $\rho_{i,sam}$ , of the substance using Equation (2).

$$\rho_{i,sam} = \left[ \frac{(y_{i,sam} / y_{is,i,sam}) - b_i}{a_i} \right] \rho_{is,i,sam} = \left( \frac{\rho_{i,sam \text{ extr}}}{\rho_{is,i,sam \text{ extr}}} \right) \rho_{is,i,sam} \quad (2)$$

where

|                                |  |
|--------------------------------|--|
| $y_{i,sam}$                    | is the measured response, as a peak area, of the substance $i$ in the water sample extract;  |
| $y_{is,i,sam}$                 | is the measured response, as a peak area, of the internal standard, for substance $i$ in the water sample extract;   |
| $\rho_{i,sam \text{ extr}}$    | is the mass concentration, in nanograms per millilitre, of the substance $i$ in the water sample extract usually calculated by the software;   |
| $\rho_{is,i,sam \text{ extr}}$ | is the mass concentration, in nanograms per millilitre, of the internal standard, for substance $i$ , in the water sample extract usually reported by the software;  |
| $\rho_{i,sam}$                 | is the mass concentration, in nanograms per millilitre or micrograms per litre, of the substance $i$ in the water sample;  |
| $\rho_{is,i,sam}$              | is the mass concentration, in nanograms per millilitre, of the internal standard, for substance $i$ , in the water sample usually calculated from the amount of the internal standard added to the volume of the water sample; |
| $a_i$                          | see Equation (1);  |
| $b_i$                          | see Equation (1).  |

## 10 Expression of results

For drinking water and surface water, report the results, in nanograms per litre, to two significant figures. For waste water, report the results in micrograms per litre, as follows:

- < 1 µg/l: one significant figure;
- ≥ 1 µg/l: two significant figures (at most).

## 11 Test report

The test report shall contain at least the following information:

- a) the test method used, together with a reference to this part of ISO 18857;
- b) all the information required for the complete identification of the sample;
- c) the content of the analytes, expressed in accordance with Clause 10;
- d) all operating details not specified in this part of ISO 18857, or regarded as optional, together with details of any incident that may have influenced the result(s).



## Annex A (informative)

### Example of a sorbent

See Table A.1.

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

| Sorbent   | Product name (supplier) <sup>a</sup> |
|---|--------------------------------------|
| Styrene-divinyl benzene copolymer   | SDB 1 (Mallinckrodt Baker)           |
| <sup>a</sup> This information is given for the convenience of users of this part of ISO 18857 and does not constitute an endorsement by ISO of this product and supplier. |                                      |

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

## Annex B (informative)

### Suitable capillary columns

- DB-1701<sup>1)</sup>**: (14 %-Cyanopropyl-phenyl)-methylpolysiloxane phase, low- to mid-polarity, bonded and cross-linked, low bleed
- DB-5<sup>1)</sup>**: (5 %-Phenyl)-methylpolysiloxane phase, non-polar, bonded and cross-linked, low bleed
- HP-Ultra1<sup>1)</sup>**: 100 % Dimethylpolysiloxane phase, non-polar, bonded and cross-linked, low bleed

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1) Examples of suitable products available commercially. This information is given for the convenience of users of this part of ISO 18857 and do not constitute an endorsement by ISO of these products.

## Annex C (informative)

### Examples of chromatograms

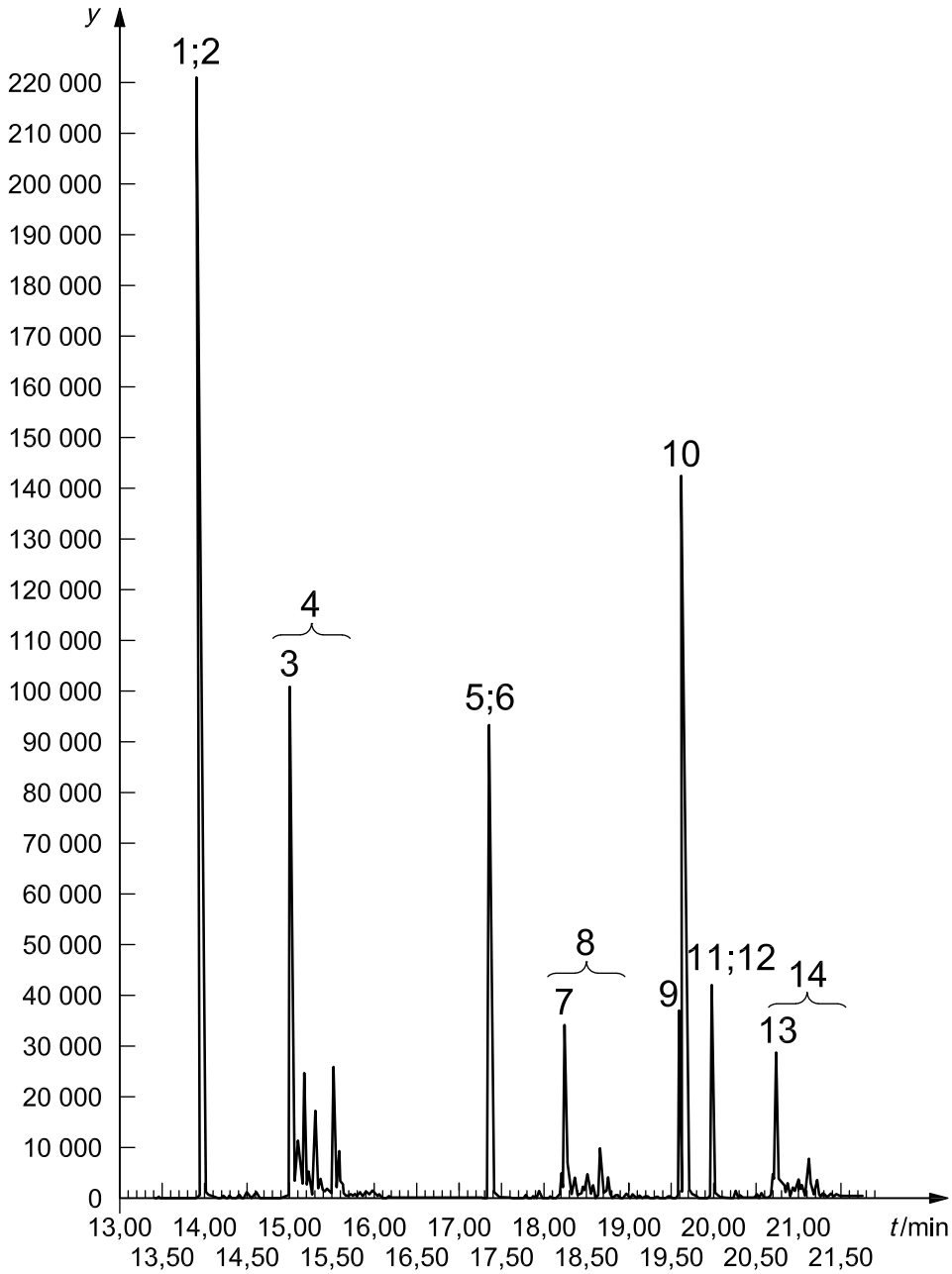
#### GC conditions for Figures C.1, C.2 and C.3

|                            |   |                       |
|----------------------------|---|-----------------------|
| injection:                 | splitless                                       |                       |
| injector temperature:      | 250 °C  |                       |
| injection volume:          | 1 µl  |                       |
| transfer line temperature: | 280 °C  |                       |
| flow rate:                 | 1 ml/min  |                       |
| carrier gas:               | helium, pre-pressure 77 kPa (11 psi)            |                       |
| capillary column:          | stationary phase:                               | VF-5ms <sup>2</sup> ) |
|                            | length:   | 30 m                  |
|                            | inner diameter:                                 | 0,25 mm               |
|                            | film thickness:                                 | 0,25 µm               |
| temperature programme      | at 60 °C for 1 min, then to 280 °C at 10 °C/min |                       |

#### MS conditions for Figures C.1, C.2 and C.3

|               |  |        |
|---------------|--|--------|
| type:         | quadrupole                                 |        |
| ionisation:   | EI 70 eV                                   |        |
| mode:         | selected ion monitoring (SIM), see Table 3 |        |
| temperatures: | MS source:                                 | 230 °C |
|               | MS quadrupole:                             | 150 °C |

2) Example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 18857 and does not constitute an endorsement by ISO of this product.

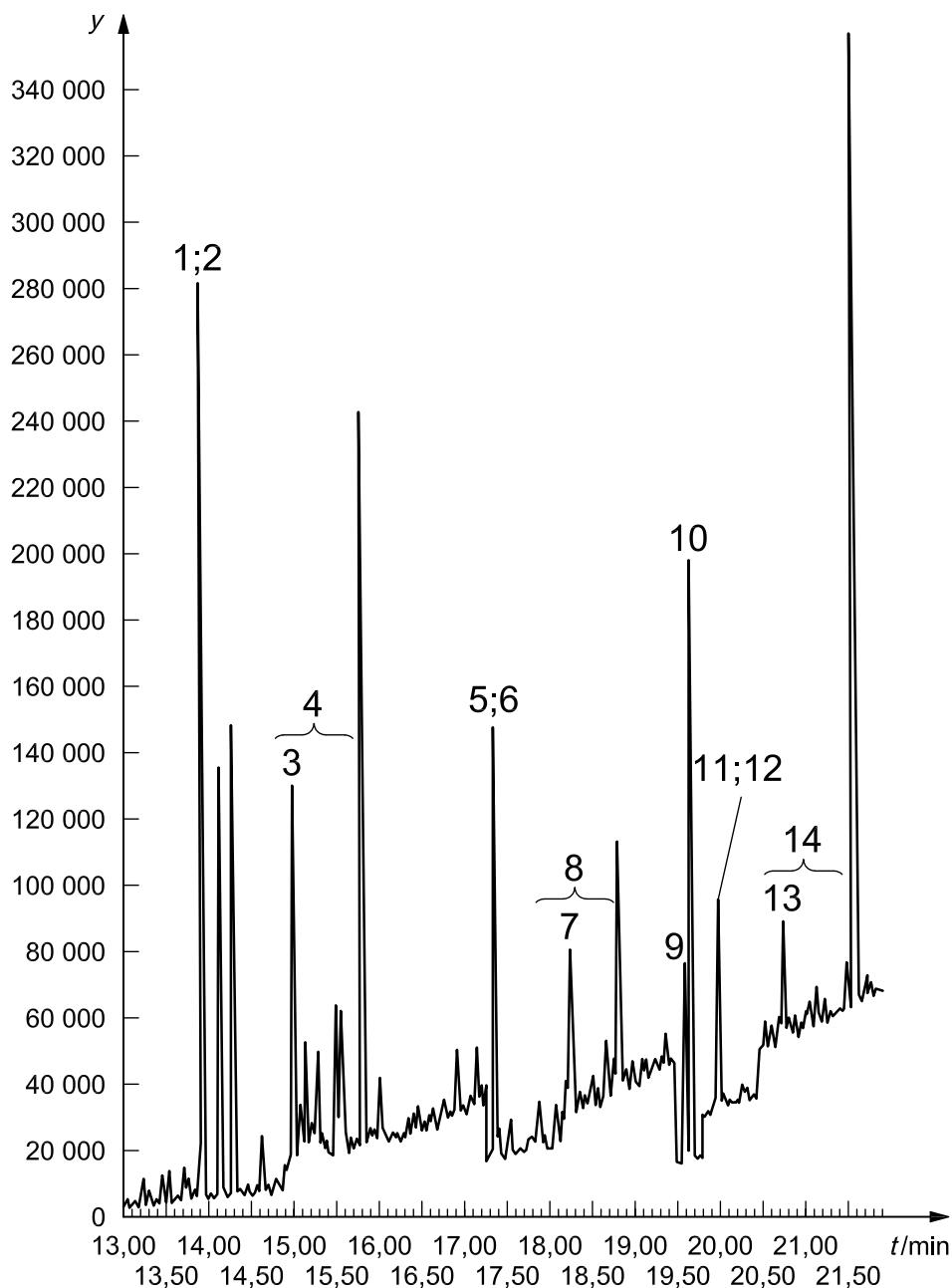


**Key** (see also Table 3)

- |  |   |
|--|---|
| 1 4-(1,1,3,3-tetramethylbutyl)phenol (OP-TMS)  | 9 bisphenol A-d16 (BPA-d16-TMS)   |
| 2 4-(1,1,3,3-tetramethylbutyl)phenol (ring- <sup>13</sup> C <sub>6</sub> ) (OP- <sup>13</sup> C <sub>6</sub> -TMS)                                 | 10 bisphenol A (BPA-TMS)  |
| 3 4-(3,6-dimethyl-3-heptyl)phenol (ring- <sup>13</sup> C <sub>6</sub> ) (363 NP- <sup>13</sup> C <sub>6</sub> -TMS)                                | 11 4-(1,1,3,3-tetramethylbutyl)phenol diethoxylate (OP <sub>2</sub> EO-TMS)   |
| 4 4-nonylphenol (mixture of isomers) (NP-TMS)  | 12 4-(1,1,3,3-tetramethylbutyl)phenol diethoxylate (ring- <sup>13</sup> C <sub>6</sub> ) (OP <sub>2</sub> EO- <sup>13</sup> C <sub>6</sub> -TMS)  |
| 5 4-(1,1,3,3-tetramethylbutyl)phenol monoethoxylate (OP <sub>1</sub> EO-TMS)   | 13 4-(3,6-dimethyl-3-heptyl)phenol diethoxylate (ring- <sup>13</sup> C <sub>6</sub> ) (363 NP <sub>2</sub> EO- <sup>13</sup> C <sub>6</sub> -TMS) |
| 6 4-(1,1,3,3-tetramethylbutyl)phenol monoethoxylate (ring- <sup>13</sup> C <sub>6</sub> ) (OP <sub>1</sub> EO- <sup>13</sup> C <sub>6</sub> -TMS)  | 14 4-nonylphenol diethoxylate (mixture of isomers) (NP <sub>2</sub> EO-TMS)   |
| 7 4-(3,6-dimethyl-3-heptyl)phenol monoethoxylate (ring- <sup>13</sup> C <sub>6</sub> ) (363 NP <sub>1</sub> EO- <sup>13</sup> C <sub>6</sub> -TMS) |   |
| 8 4-nonylphenol monoethoxylate (mixture of isomers) (NP <sub>1</sub> EO-TMS)   | <i>t</i> time   |
|  | <i>y</i> abundance  |

NOTE All analytes are analysed as trimethylsilyl (TMS) derivatives.

**Figure C.1 — Chromatogram of a standard solution in acetone (selected ion monitoring)**

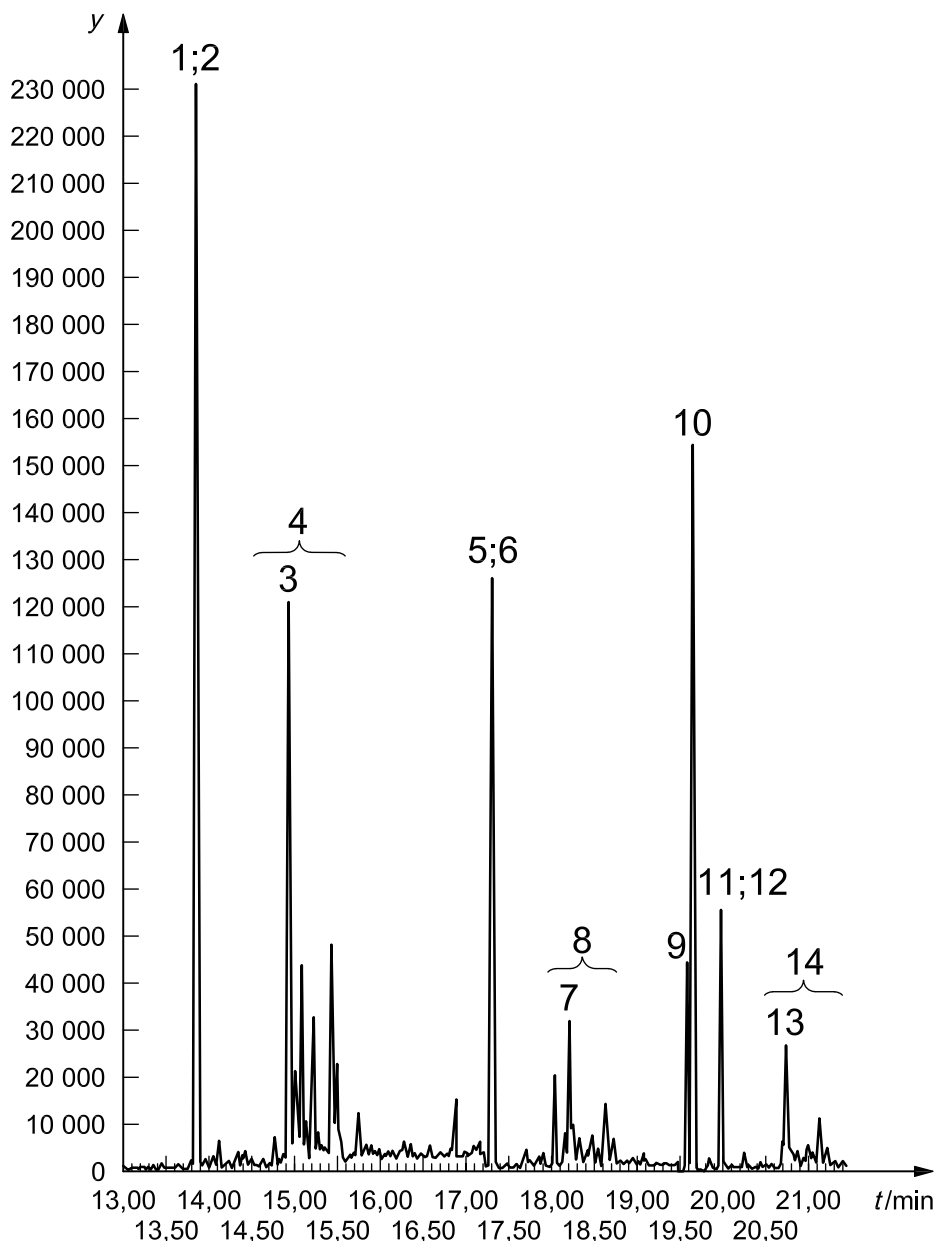


**Key** (see also Table 3)

|   |  |          |  |
|---|--|----------|--|
| 1 | 4-(1,1,3,3-tetramethylbutyl)phenol (OP-TMS)  | 9        | bisphenol A-d16 (BPA-d16-TMS)  |
| 2 | 4-(1,1,3,3-tetramethylbutyl)phenol (ring- $^{13}\text{C}_6$ ) (OP- $^{13}\text{C}_6$ -TMS)                                 | 10       | bisphenol A (BPA-TMS)  |
| 3 | 4-(3,6-dimethyl-3-heptyl)phenol (ring- $^{13}\text{C}_6$ ) (363 NP- $^{13}\text{C}_6$ -TMS)                                | 11       | 4-(1,1,3,3-tetramethylbutyl)phenol diethoxylate (OP <sub>2</sub> EO-TMS)   |
| 4 | 4-nonylphenol (mixture of isomers) (NP-TMS)  | 12       | 4-(1,1,3,3-tetramethylbutyl)phenol diethoxylate (ring- $^{13}\text{C}_6$ ) (OP <sub>2</sub> EO- $^{13}\text{C}_6$ -TMS)  |
| 5 | 4-(1,1,3,3-tetramethylbutyl)phenol monoethoxylate (OP <sub>1</sub> EO-TMS)   | 13       | 4-(3,6-dimethyl-3-heptyl)phenol diethoxylate (ring- $^{13}\text{C}_6$ ) (363 NP <sub>2</sub> EO- $^{13}\text{C}_6$ -TMS) |
| 6 | 4-(1,1,3,3-tetramethylbutyl)phenol monoethoxylate (ring- $^{13}\text{C}_6$ ) (OP <sub>1</sub> EO- $^{13}\text{C}_6$ -TMS)  | 14       | 4-nonylphenol diethoxylate (mixture of isomers) (NP <sub>2</sub> EO-TMS)   |
| 7 | 4-(3,6-dimethyl-3-heptyl)phenol monoethoxylate (ring- $^{13}\text{C}_6$ ) (363 NP <sub>1</sub> EO- $^{13}\text{C}_6$ -TMS) | <i>t</i> | time   |
| 8 | 4-nonylphenol monoethoxylate (mixture of isomers) (NP <sub>1</sub> EO-TMS)   | <i>y</i> | abundance  |

**NOTE** All analytes are analysed as trimethylsilyl (TMS) derivatives.

**Figure C.2 — Chromatogram of a river water extract in acetone (selected ion monitoring)**



**Key** (see also Table 3)

- |   |  |          |  |
|---|--|----------|--|
| 1 | 4-(1,1,3,3-tetramethylbutyl)phenol (OP-TMS)  | 9        | bisphenol A-d16 (BPA-d16-TMS)  |
| 2 | 4-(1,1,3,3-tetramethylbutyl)phenol (ring- <sup>13</sup> C <sub>6</sub> ) (OP- <sup>13</sup> C <sub>6</sub> -TMS)                                 | 10       | bisphenol A (BPA-TMS)  |
| 3 | 4-(3,6-dimethyl-3-heptyl)phenol (ring- <sup>13</sup> C <sub>6</sub> ) (363 NP- <sup>13</sup> C <sub>6</sub> -TMS)                                | 11       | 4-(1,1,3,3-tetramethylbutyl)phenol diethoxylate (OP <sub>2</sub> EO-TMS)   |
| 4 | 4-nonylphenol (mixture of isomers) (NP-TMS)  | 12       | 4-(1,1,3,3-tetramethylbutyl)phenol diethoxylate (ring- <sup>13</sup> C <sub>6</sub> ) (OP <sub>2</sub> EO- <sup>13</sup> C <sub>6</sub> -TMS)  |
| 5 | 4-(1,1,3,3-tetramethylbutyl)phenol monoethoxylate (OP <sub>1</sub> EO-TMS)   | 13       | 4-(3,6-dimethyl-3-heptyl)phenol diethoxylate (ring- <sup>13</sup> C <sub>6</sub> ) (363 NP <sub>2</sub> EO- <sup>13</sup> C <sub>6</sub> -TMS) |
| 6 | 4-(1,1,3,3-tetramethylbutyl)phenol monoethoxylate (ring- <sup>13</sup> C <sub>6</sub> ) (OP <sub>1</sub> EO- <sup>13</sup> C <sub>6</sub> -TMS)  | 14       | 4-nonylphenol diethoxylate (mixture of isomers) (NP <sub>2</sub> EO-TMS)   |
| 7 | 4-(3,6-dimethyl-3-heptyl)phenol monoethoxylate (ring- <sup>13</sup> C <sub>6</sub> ) (363 NP <sub>1</sub> EO- <sup>13</sup> C <sub>6</sub> -TMS) | <i>t</i> | time   |
| 8 | 4-nonylphenol monoethoxylate (mixture of isomers) (NP <sub>1</sub> EO-TMS)   | <i>y</i> | abundance  |

NOTE All analytes are analysed as trimethylsilyl (TMS) derivatives.

**Figure C.3 — Chromatogram of a waste water extract in acetone (selected ion monitoring)**

## Annex D (informative)

### Performance data

Results of an interlaboratory trial carried out in Germany in 2007 are given in Table D.1.

**Table D.1 — Precision data**

| Sample       | Matrix   | Parameter          | $p$ | $n$ | $n_{OP}$<br>% | $\bar{\rho}$<br>ng/l | $\rho_{ref}$<br>ng/l | $\eta$<br>% | $s_R$<br>ng/l | $C_{V,R}$<br>% | $s_r$<br>ng/l | $C_{V,r}$<br>% |
|--------------|--|--------------------|-----|-----|---------------|----------------------|----------------------|-------------|---------------|----------------|---------------|----------------|
| A            | spiked surface water                                 | OP                 | 13  | 26  | 0,0           | 53,7                 | 50,0                 | 107,3       | 10,21         | 19,0           | 1,35          | 2,5            |
|              |  | OP <sub>1</sub> EO | 11  | 22  | 15,4          | 86,2                 | 88,0                 | 98,0        | 8,59          | 10,0           | 1,76          | 2,0            |
|              |  | OP <sub>2</sub> EO | 12  | 24  | 7,7           | 68,7                 | 68,0                 | 101,0       | 10,93         | 15,9           | 2,32          | 3,4            |
|              |  | NP                 | 13  | 26  | 0,0           | 216,1                | 150,0                | 144,1       | 63,71         | 29,5           | 16,79         | 7,8            |
|              |  | NP <sub>1</sub> EO | 13  | 26  | 0,0           | 234,5                | 190,0                | 123,4       | 54,69         | 23,3           | 14,58         | 6,2            |
|              |  | NP <sub>2</sub> EO | 10  | 20  | 23,1          | 306,3                | 290,0                | 105,6       | 41,74         | 13,6           | 9,45          | 3,1            |
|              |  | BPA                | 12  | 24  | 0,0           | 82,8                 | 80,0                 | 103,4       | 19,34         | 23,4           | 3,12          | 3,8            |
|              |  |                    |     |     | %             | µg/l                 | µg/l                 | %           | µg/l          | %              | µg/l          | %              |
| B            | spiked waste water                                   | OP                 | 11  | 22  | 15,4          | 1,55                 | 1,50                 | 103,6       | 0,168         | 10,8           | 0,054         | 3,5            |
|              |  | OP <sub>1</sub> EO | 12  | 24  | 7,7           | 1,27                 | 1,30                 | 97,3        | 0,176         | 13,9           | 0,032         | 2,5            |
|              |  | OP <sub>2</sub> EO | 12  | 24  | 7,7           | 0,87                 | 0,90                 | 96,5        | 0,123         | 14,1           | 0,036         | 4,1            |
|              |  | NP                 | 12  | 24  | 7,7           | 3,80                 | 3,50                 | 108,6       | 0,639         | 16,8           | 0,209         | 5,5            |
|              |  | NP <sub>1</sub> EO | 12  | 24  | 7,7           | 3,91                 | 4,10                 | 95,4        | 0,568         | 14,5           | 0,154         | 3,9            |
|              |  | NP <sub>2</sub> EO | 13  | 26  | 0,0           | 3,84                 | 3,80                 | 101,1       | 0,843         | 21,9           | 0,163         | 4,2            |
|              |  | BPA                | 12  | 24  | 7,7           | 1,18                 | 1,20                 | 98,2        | 0,265         | 22,5           | 0,022         | 1,9            |
| $p$          | number of laboratories after outlier rejection       |                    |     |     |               |                      |                      |             |               |                |               |                |
| $n$          | number of analytical results after outlier rejection |                    |     |     |               |                      |                      |             |               |                |               |                |
| $n_{OP}$     | percentage of outliers                               |                    |     |     |               |                      |                      |             |               |                |               |                |
| $\bar{\rho}$ | total mean of all results (outliers excluded)        |                    |     |     |               |                      |                      |             |               |                |               |                |
| $\rho_{ref}$ | expected value                                       |                    |     |     |               |                      |                      |             |               |                |               |                |
| $\eta$       | recovery   |                    |     |     |               |                      |                      |             |               |                |               |                |
| $s_R$        | reproducibility standard deviation                   |                    |     |     |               |                      |                      |             |               |                |               |                |
| $C_{V,R}$    | coefficient of variation of reproducibility          |                    |     |     |               |                      |                      |             |               |                |               |                |
| $s_r$        | repeatability standard deviation                     |                    |     |     |               |                      |                      |             |               |                |               |                |
| $C_{V,r}$    | coefficient of variation of repeatability            |                    |     |     |               |                      |                      |             |               |                |               |                |

The waste water sample for the interlaboratory trial was taken from the outflow of the municipal sewage plant of the towns Adorf and Bad Elster (Saxony, Germany). The suspended solid content was 5,3 mg/l. The surface water sample was taken from the drinking water reservoir Dröda (Saxony, Germany). The suspended solid content was 0,87 mg/l.

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