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Secretariat:

Soils, sludges and treated bio-waste — Determination of nonylphenos (NP) and nonylphenol-mono- and diethoxylates — Method by gas chromatography with mass selective detection (GC-MS)

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Contents

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Foreword

This document (BT WI) has been prepared by Technical Committee CEN/C BT 151 “Horizontal”, the secretariat of which is held by DS.

This document is a working document.

The following TC's have been involved in the preparation of the standard: CEN/TC 292 (Waste), CEN/TC 308 (Sludge Characterization) and ISO/TC 190 (Soil Quality).

This European standard is applicable and validated for several types of matrices. The table below indicates which ones.

Material	Validated	Document
Soil	☐	[reference]
Sludge	☐	
Treated bio-waste	☐	
Soil improvers	Not validated yet	
Waste		

Introduction

This document has been developed in the framework of the project 'Horizontal'. It is the result of a desk study “LAS and Nonylphenols” from January 2004 /1/ and an extensive experimental study of the method including a ruggedness test /2/. Nonylphenols (NP) are mainly found in the environment as degradation products of nonylphenol polyethoxylates (NPEO). NPEO have many uses as nonionic detergents in washing and cleaning agents.

After use NPEO are degraded by de-ethoxylation, resulting in polyethoxylates with less ethoxy-groups. Nonylphenol-diethoxylates (NP2EO), nonylphenol-monoethoxylates (NP1EO) and nonylphenols (NP) are the last three products in the degradation chain. Due to their significant presence in sewage sludge, all three components are included in the horizontal standard.

The method describes the determination of NP, NP1EO and NP2EO in sludge, soil, treated bio-waste and neighbouring fields.

After an evaluation study, in which e.g. the ruggedness of the method was studies, a European wide validation of the draft standard has taken place. The results of the desk studies as well as the evaluation and validation studies have been subject to discussions with all parties concerned in CEN. The standard is part of a modular horizontal approach in which the standard belongs to the analytical step.

Until now test methods determining properties of materials were often prepared in Technical Committees (TCs) working on specific products or specific sectors. In those test methods often steps as sampling, extraction, release or other processing, analyses, etc were included. In this approach it was necessary to develop, edit and validate similar procedural steps over and over again for every material or product. Consequently this has resulted in duplication of work. To avoid such duplication of work for parts of a testing procedure references to parts of test methods from other TCs were introduced. However the following problems are often encountered while using references in this way: 1) The referenced parts are often not edited in a way that they could easily be referred to, 2) the referenced parts are often not validated for the other type of material and 3) the updates of such test standards on products might lead to inadequate references.

In the growing amount of product and sector oriented test methods it was recognised that many steps in test procedures are or could be used in test procedures for many products, materials and sectors. It was supposed that, by careful determination of these steps and selection of specific questions within these steps, elements of the test procedure could be described in a way that can be used for all materials and products or for all materials and products with certain specifications.

Based on this hypothesis a horizontal modular approach is being investigated and developed in the project 'Horizontal'. 'Horizontal' means that the methods can be used for a wide range of materials and products with certain properties. 'Modular' means that a test standard developed in this approach concerns a specific step in assessing a property and not the whole ” chain of measurement” (from sampling to analyses). A beneficial feature of this approach is that “modules” can be replaced by better ones without jeopardizing the standard “chain”.

The use of modular horizontal standards implies the drawing of test schemes as well. Before executing a test on a certain material or product to determine certain characteristics it is necessary to draw up a protocol in which the adequate modules are selected and together form the basis for the test procedure.

The modules that relates to this standard are specified in section XX Normative references.

An overview of modules and the manner, in which modules are selected will be worked out later, at which time proper reference in this standard will be provided.

1 Scope

This European Standard describes a method for the determination of nonylphenols (NP), nonylphenol-monoethoxylates (NP1EO) and nonylphenol-diethoxylates (NP2EO) in soil, sludge and treated bio-waste using GC/MS.

This European Standard primarily describes the analysis of sludge, soil and treated bio-waste. Other solid materials like sediment and selected solid wastes may also be analysed by the method.

For sludge a limit of detection of 0,1 mg/kg and for soil and treated bio-waste 0,02 mg/kg (expressed as dry matter) may be achieved.

Matrices for which this European Standard has been validated are listed in the foreword.

Lower LOD's may be achieved by concentrating the extract by solvent evaporation.

NOTE With this method 4-tert-octylphenol can also be analysed.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, only the edition cited applies. For undated references, the latest edition of the normative document referred to applies (including any amendments).

ISO 10381-1, *Soil quality – Sampling – Part 1: Guidance on the design of sampling programmes.*

ISO 10381-2, *Soil quality – Sampling – Part 2: Guidance on sampling techniques.*

CSS99031 *Sludge, treated biowaste, and soils in the landscape – Sampling – Framework for the preparation and application of a sampling plan*

CSS99058 *Sludge, treated biowaste, and soils in the landscape – Sampling – Part 1: Guidance on selection and application of criteria for sampling under various conditions*

CSS99057 *Sludge, treated biowaste, and soils in the landscape – Sampling – Part 2: Guidance on sampling techniques*

CSS99032 *Sludge, treated biowaste, and soils in the landscape – Sampling - Part 3: Guidance on sub-sampling in the field*

CSS99059 *Sludge, treated biowaste, and soils in the landscape – Sampling – Part 4: Guidance on procedures for sample packaging, storage, preservation, transport and delivery*

CSS99060 *Sludge, treated biowaste, and soils in the landscape – Sampling – Part 5: Guidance on the process of defining the sampling plan*

EN 12880, *Characterization of sludge – Determination of dry residue and water content.*

ISO 11465:1993, *Soil quality – Determination of dry matter and water content on mass basis – Gravimetric method.*

PrEN 14346[1)] *Characterisation of waste – Calculation of dry matter by determination of dry residue and water content*.

1) Under preparation

CSS99022, *Solid materials – Determination of dry matter and water content on a mass basis –Gravimetric method.*

ISO/DIS 14507[1)], *Soil quality – Guidance for sample pre-treatment for the determination of organic contaminants in soil.*

ISO/DIS 16720:2003, *Soil quality – Pre-treatment of samples by freeze-drying for subsequent analysis.*

CSS99034, *Solid materials – Pre-treatment for organic characterisation.*

ISO/FDIS 18857-1, *Water quality – Determination of selected alkylphenols – Part 1: Method for nonfiltered samples using liquid extraction and gas chromatography with mass selective detection.*

ISO/WD 18857-2, *Water quality – Determination of selected alkylphenols, alkylphenol ethoxylates and bisphenol A – Part 1: Method for non-filtered samples using solid-phase extraction and gas chromatography with mass selective detection after derivatisation.*

ISO/FDIS 22982:2004[1)], *Soil quality –Guidelines for the identification of target compounds by gas chromatography and mass spectrometry*

ISO 8466-1, *Water quality – Calibration and evaluation of analytical methods and estimation of performance characteristics.*

3 Terms and definitions

3.1
analyte
nonylphenols (mixture of isomers), nonylphenol-monoethoxylates (mixture of isomers), and nonylphenol-diethoxylates (mixture of isomers)

3.2
calibration standard
solution prepared from stock solutions of the analytes and used to calibrate the response of the instrument with respect to analyte concentration

3.3
test sample
sample after pre-treatment such as homogenisation, grinding, sieving, drying, etc. The test sample is ready for the chemical analysis

4 Principle

After pre-treatment according to methods referred to in clause 9, the test sample (wet or freeze-dried sample) is extracted by shaking the sample with a mixture of acetone and petroleum ether (1:1). If necessary interfering compounds are removed from the extract by a clean-up on a suitable column.

The extract is treated with N-methyl-N-(trimethylsilyl)-trifluoracetamide (MSTFA) reagent for the derivatization (silylation) of the analytes, and subsequently analyzed by gas chromatography and detection by mass spectrometry (MS).

Nonylphenols and nonylphenol-mono- and diethoxylates are identified from the GC-fingerprint, the relative retention times and the relative intensities of two diagnostic ions. The quantification is based on internal standard procedure. The internal standards (^{13}C-labelled 4-n-NP and ^{13}C-labelled 4-n-NP2EO) are taken through the whole analytical procedure.

5 Interferences

5.1 Interferences from sampling

Use sampling containers of materials (preferably glass or steel) that do not significantly affect the sample during the contact through sampling and storage. Plastic containers may be used, if it has been proven that they do not significantly affect the sample.

5.2 Interferences by GC-MS

Substances that co-elute with NP, NP1EO or NP2EO and give the same ion(s) may interfere in the determination. This may have a large influence on the result, since all 3 analytes are determined from the sum of a cluster of 5-9 chromatographic peaks. It is important, that the interfering peaks are not included in the calculations. A peak is excluded, if the retention times are not the same as expected from the calibration standard, and if the relative peak areas from the two diagnostic ions differ more than 30% from the same peak in the calibration standard. Interfering peaks may normally be spotted by comparing the fingerprints of the sample with the fingerprints of the calibration standard, although the isomer-distribution in the environmental samples may differ from the distribution in the calibration standard.

6 Hazards

The solvent hexane is neuro-toxic and its use is not recommended.

7 Reagents

7.1 General

All reagents shall be of recognised analytical grade.

The purity of the reagents used shall be checked by running a blank determination as described in 10.5. If the blank value is unreasonably high, i.e. more than 10 % of the lowest value of interest, find the cause through a step-by-step examination of the whole procedure.

7.2 Acetone, C_3H_6O

7.3 Hexane-like solvent

Any aliphatic hydrocarbon solvent with a boiling point or boiling range between 34 °C and 100 °C may be applied. For safety, see clause 6.

7.4 Anhydrous sodium sulphate, Na_2SO_4, powdered

Heated for at least 6 h to (550 ± 20) °C, cooled to about 200 °C in the furnace and then to ambient temperature in a desiccator containing magnesium perchlorate or a suitable alternative. The anhydrous sodium sulphate shall be kept carefully sealed.

7.5 Reagents for clean-up procedures

7.6 MSTFA for derivatization

N-methyl-N-(trimethylsilyl)-trifluoracetamide, $C_6H_{12}F_3NOSi$, CAS # 24589-78-4

7.7 Isooctane, C_8H_{17}, b.p. 99°C

7.8 Derivatization solution, 5% MSTFA in isooctane (vol/vol)

Dissolve e.g. 1 ml of MSTFA in isooctane in a 20 ml volumetric flask and make up to volume with isooctane.

Store the derivatization solution in a dark place at a temperature of (4 ± 3) °C. The solutions are stable for at least 2 months.

7.9 Operating gas for gas chromatography with MS-detector

Helium of sufficient purity and in accordance with manufacture´s specification.

7.10 Nitrogen for solvent evaporation

Nitrogen of sufficient purity.

7.11 Standards for calibration

The following standard substances shall be used:

- 4-Nonylphenols (NP), mixture of isomers, CAS # 84852-15-3
- 4-Nonylphenol monoethoxylates (NP1EO), mixture of isomers, CAS # 26027-38-3
- 4-Nonylphenol diethoxylates (NP2EO), mixture of isomers, CAS # 20427-84-3

The two nonylphenol ethoxylates may contain small amounts of other ethoxylates. It is important to check the purity of all the standards used for calibration.

The standards may be taken from pure compounds or from solutions with a guaranteed concentration.

The standards shall be kept in a freezer at a temperature of –18°C ± 3°C.

NOTE 1: If 4-tert-octylphenol is included: 4-(1,1,3,3-tetramethylbutyl)phenol, CAS # 140-66-9.

NOTE 2: For NP, NP1EO and NP2EO conflicting information about CAS numbers may be found.

7.12 Internal standards

The following internal standard substances shall be used:

- ^{13}C-labelled 4-n-nonylphenol (4-n-NP), C_9H_{19}-[$^{13}C_6$]H_4-OH
- ^{13}C-labelled 4-n-nonylphenol-diethoxylate (4-n-NP2EO)

The internal standards must be kept in a freezer at a temperature of –18°C ± 3°C.

NOTE D_4-labelled 4-n-nonylphenol or 4-n-nonylphenol (non labelled) may be used as an alternative internal standard to ^{13}C-labelled 4-n-nonylphenol. 4-n-nonylphenol-diethoxylate (non labelled) may be used as an alternative internal standard to ^{13}C-labelled 4-n-nonylphenol-diethoxylate. Non-labelled compounds may only be used if it is shown, that they are not present in the sample. For ion trap MS deuterated internal standard shall not be used.

7.13 Internal standard solution

Prepare internal standard solution with the two internal standards in isooctane. The concentrations are 20 mg/l for 4-n-NP and 100 mg/l for 4-n-NP2EO.

It is essential, that the same internal standard solution is used for calibration standard solutions and for samples, blank and internal quality control samples.

Store the internal standard solution in a dark place at a temperature of (4 ± 3) °C. The solution is stable for at least two years, provided that evaporation of solvent is negligible.

7.14 Stock solutions

Prepare individual stock solutions of about 100 mg/l in isooctane, either from solid standard substances or from solutions with a guaranteed concentration.

Store the stock solutions in a dark place at a temperature of less than 4°C. The solutions are stable for at least two years, provided that evaporation of solvent is negligible.

7.15 Calibration standard solutions

A mixed calibration standard solution is prepared from the stock solutions by diluting the stock solutions with isooctane. Internal standard solution is added to a concentration of 0,2 mg/l for 4-n-NP and 1,0 mg/l for 4-n-NP2EO. The calibration standards are made to concentrations from 0,01 mg/l to 5 mg/l.

Store the calibration standard solutions in a dark place at a temperature of less than 4°C. The solutions are stable for at least two weeks, provided that evaporation of solvent is negligible.

8 Apparatus

8.1 General

All equipment that gets into contact with the sample or extract shall be free from nonylphenols and nonylphenol ethoxylates. Glassware may be cleaned by heating, at least for 2 h at 450°C.

8.2 Standard laboratory glassware

8.2.1 Screw cap glass flask with teflon seal. Volume 100 ml and 250 ml.

8.2.2 Round-bottomed flasks. Volume 100 ml and 250 ml.

8.2.3 Test tubes and vials.

8.3 Shaking device, reciprocating shaker

With horizontal movement (up to at least 250 strokes per min).

8.4 Evaporator

Rotary evaporator. Other device like turbo evaporator or Kuderna Danish[2)] may be applied.

8.5 Clean-up column

Silica column. Commercial columns or freshly prepared columns may be used.

Alternative materials like aluminium oxide or Florisil[3)] may be used, provided that a sufficient recovery of the analytes have been proven.

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2) Kuderna Danish is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

3) Florisil is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

8.6 Freeze drying apparatus

8.7 Gas chromatograph with mass selective detector

Equipped with a capillary column: 5% phenyl-methyl silicone stationary phase coated onto fused silica or an equivalent chemically bonded phase. The dimensions should be sufficient to separate the nonylphenols as described below. In general column length should be 25 m - 50 m. An example of a column is given in Annex A.

The first two peaks in the SIM chromatogram of the nonylphenols are selected as critical pairs for the quality criteria for the chromatographic system. The resolution shall be sufficiently high, so that the first two peaks in nonylphenols are baseline separated when measured at ion 207, see table 2.

9 Sampling and sample pretreatment

9.1 Sampling and sample storage

Sampling shall be carried out in accordance with sampling standards CSS99031-32 and 99057-60.

Store the samples in a dark place in a freezer or in a refrigerator. The samples can be stored up to 3 weeks in a freezer at a temperature of –18°C ± 3°C or up to 7 days in the refrigerator at 4°C ± 3°C.

Determine the content of dry matter in the sample in accordance with EN 12880, ISO 11465 or PrEN 14346.

NOTE Sludge samples with unusually high amounts of nonylphenol polyethoxylates (NPPEO) relative to the analytes can only be stored 7 days in the freezer (–18°C ± 3°C).

9.2 Sample pre-treatment

Methods for pre-treatment of solid samples to be used for the analysis of organic contaminants are described in a separate standard CSS99034. This European Standard describes procedures for the preparation of the test sample from the laboratory sample.

Different pre-treatment procedures are used for the different matrices. This is presented in Table 1.

Table 1 — Pre-treatment methods used prior to nonylphenol analysis.

Sludge dry matter > 2%[a)]	Sludge dry matter < 2%[b)]	Soil[c)]	Treated bio-waste[c)]	Sediment dry matter > 10%[d)]	Sediment dry matter < 10%[e)]
No drying		No drying	No drying	No drying	
Freeze drying (ISO/DIS 16720)	Freeze drying (ISO/DIS 16720)	Freeze drying (ISO/DIS 16720)	Freeze drying (ISO/DIS 16720)	Freeze drying (ISO/DIS 16720)	Freeze drying (ISO/DIS 16720)

[a] Sludge samples with more than 2% dry matter can be analysed as wet samples, or they can be analysed after freeze-drying.

[b] Sludge samples with less than 2% dry matter can only be analysed after freeze-drying.

[c] Soil and treated bio-waste samples can be analysed as wet samples (field-moist samples), or they can be analysed after freeze-drying.

[d] Sediment samples with more than 10% dry matter can be analysed as wet samples, or they can be analysed after freeze-drying.

[e] Sediment samples with less than 10% dry matter can only be analysed after freeze-drying.

10 Procedure

10.1 Extraction

The following four extraction methods are described :

— extraction of wet sludge samples,

— extraction of freeze-dried sludge samples,

— extraction of wet samples of soil, sediment and treated bio-waste,

— extraction of freeze-dried samples of soil, sediment and treated bio-waste.

10.1.1 Extraction of wet sludge samples

Wet sludge samples are extracted as follows:

a) Take between 10 g and 50 g of test sample (depending on dry matter content) and place it in a 100 ml screw cap flask (8.2.1) with teflon seal. The sample should preferably contain between 2 g and 3 g dry matter.

b) Add 100 µl of internal standard solution (7.13) equal to 2 µg of 4-n-NP and 10 µg of 4-n-NP2EO.

c) Add 10 ml of acetone (7.2), close the screw cap and shake thoroughly by hand.

d) Add 10 ml of hexane-like solvent (7.3), close the screw cap again and place the flask on a reciprocating shaker (8.3). The flask shall be placed in horizontal position with the movement along the flask.

e) Shake for at least 2 h with (250 $\pm$ 20) strokes per min.

f) Transfer the organic phase to another 100 ml flask. If an emulsion is present, this shall be included.

g) Add water and shake to wash the extract. Use 5 ml of water per ml hexane-like solvent.

h) Transfer the extract (enough for the subsequent analysis) to a test tube and dry the extract by adding anhydrous sodium sulphate (7.4).

The extract is now ready for further treatment described in 10.2 - 10.4.

10.1.2 Extraction of freeze-dried sludge samples

Freeze dried sludge samples are extracted as follows:

a) Take 2 g -3 g of test sample and place it in a 100 ml screw cap flask (8.2.1) with Teflon®[4)] seal.

b) Add 100 µl of internal standard solution (7.13) equal to 2 µg of 4-n-NP and 10 µg of 4-n-NP2EO.

c) Add 5 ml of water (approximately 2 ml per g of dry sample), and shake the sample by hand.

d) Add 10 ml of acetone (7.2), close the screw cap and shake thoroughly by hand.

e) Add 10 ml of hexane-like solvent (7.3), close the screw cap again and place the flask on a reciprocating shaker (8.3). The flask shall be placed in horizontal position with the movement along the flask.

f) Shake for at least 1 h with (250 ± 20) strokes per min.

g) Transfer the organic phase to another flask. If an emulsion is present, this shall be included.

h) Add water and shake to wash the extract. Use 5 ml of water per ml hexane-like solvent.

i) Transfer the extract (enough for the subsequent analysis) to a test tube (8.2.3) and dry the extract by adding anhydrous sodium sulphate.

The extract is now ready for further treatment described in 10.2 - 10.4.

10.1.3 Extraction for soil, sediment and treated bio-waste samples

Soil, sediment and treated bio-waste samples are normally extracted wet without drying the sample before extraction. These samples are extracted as follows:

a) Take between 20 g and 40 g of test sample (depending on dry matter content) and place it in a 100 ml screw cap flask (8.2.1) with Teflon® seal. The sample shall contain between 10 g and 20 g dry matter.

b) Add 100 µl of internal standard solution (7.13) equal to 2 µg of 4-n-NP and 10 µg of 4-n-NP2EO.

c) Add 10 ml of water and shake the sample by hand.

d) Add 30 ml of acetone (7.2) to the test sample, close the screw cap and shake thoroughly by hand.

e) Add 30 ml of hexane-like solvent (7.3), close the screw cap again and place the flask on a reciprocating shaker (8.3). The flask shall be placed in horizontal position with the movement along the flask.

f) Shake for at least 2 h with (250 ± 20) strokes per min.

g) Transfer the organic phase to a 250 ml flask. If an emulsion is present, this shall be included.

h) Add water and shake to wash the extract. Use 5 ml of water per ml hexane-like solvent.

i) Transfer the extract (enough for the subsequent analysis) to a test tube (8.2.3) and dry the extract by adding anhydrous sodium sulphate (7.4).

The extract is now ready for further treatment described in 10.2 - 10.4.

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4) Instead of "Teflon®" write "polytetrafluoroethylene (PTFE).

10.1.4 Extraction Freeze-dried soil, sediment and treated bio-waste samples

Freeze-dried soil, sediment and treated bio-waste samples are extracted as follows:

a) Take 10 g - 20 g of test sample and place it in a 100 ml screw cap flask (8.2.1)with Teflon® seal.

b) Add 100 µl of internal standard solution (7.13) equal to 2 µg of 4-n-NP and 10 µg of 4-n-NP2EO.

c) Add 10 ml – 20 ml of water (approximately 1 ml per g of dry sample), and shake the sample by hand.

d) Add 20 ml of acetone (7.2), close the screw cap and shake thoroughly by hand.

e) Add 20 ml of hexane-like solvent (7.3), close the screw cap again and place the flask on a reciprocating shaker (8.3). The flask shall be placed in horizontal position with the movement along the flask.

f) Shake for at least 2 hours with (250 $\pm$ 20) strokes per min.

g) Transfer the organic phase to a 100 ml - 250 ml flask. If an emulsion is present, this shall be included.

h) Add water and shake to wash the extract. Use 5 ml of water per ml hexane-like solvent.

i) Transfer the extract (enough for the subsequent analysis) to a test tube (8.2.3) and dry the extract by adding anhydrous sodium sulphate (7.4).

The extract is now ready for further treatment described in 10.2 - 10.4.

The extracts can be stored in a refrigerator (4 $\pm$ 3)°C and are stable for at least one month.

Other extraction techniques, like ultrasonic extraction, microwave or pressurised liquid extraction may be suitable. However if using other extraction techniques the comparability to the method described in this European Standard shall be proven.

NOTE 1 To simplify the procedure the wash of organic phase (extraction solvent) with water may be carried out directly in the extraction flask with the sample present.

10.2 Concentration (optional)

In most cases concentration of the extract is not necessary. However if lower detection limits are needed this can be achieved by evaporation of the solvent.

Concentrate the extract on a rotary evaporator (8.4) or by the use of a gentle stream of nitrogen at room temperature. Since the internal standard (7.12) is used for the calculations, it is not necessary to know the exact volumes. If necessary, the amount of internal standard added to the sample can be reduced relative to the concentration factor to keep the concentration of internal standard at the same level in the GC-MS analysis.

NOTE Other inert gases can be used instead of nitrogen.

10.3 Clean-up (optional)

Clean-up shall be used if compounds are present that can interfere with the analytes or the internal standard (7.12) in the gas chromatogram, or if those compounds can influence the GC-procedure (i.e. contamination of the detection system). If no or negligible interfering substances are present, no clean-up is necessary.

For the analysis of sludge samples a clean-up is necessary for special types of sludge.

Add 1 ml of extract to the clean-up column (8.5). Elute the column with a solvent, e.g. a mixture of hexane-like solvent and acetone. Evaporate the eluted solvent to about 1 ml. The cleaned extract is now ready for derivatization described in 10.4.

Before use the column shall be tested to ensure that the analytes are recovered in the collected fraction. The criteria for the clean-up is, that the recovery for both internal standards is more than 80 %.

NOTE The sample matrix may influence the elution of the analytes and internal standards from the column, and the recovery should therefore be checked on the actual sample. This can be done by adding an additional standard to the extract after clean-up. If e.g. phenanthrene-D10 is added, the recoveries of the two internal standards can be calculated and thereby used as recovery standards.

10.4 Derivatization

The derivatization shall be carried out on the extract without clean-up or on the extract after a clean-up.

A fraction (always 1,0 ml) of the extract is treated as follows:

a) Transfer 1,0 ml of extract to a GC vial (8.2.3).

b) Evaporate the solvent slowly (room temperature) until dryness under a gentle stream of nitrogen (7.10).

c) Add 1,0 ml of 5 % MSTFA in isooctane (7.8), close the vial and shake for dissolution.

d) Wait 15 min for the reaction to occur (room temperature).

e) If the solution is not clear transfer the isooctane solution to a new GC vial. Avoid particles in the solution.

The extract is now ready for analysis by GC-MS.

The derivates can be stored in a refrigerator at a temperature of (4 ± 3) °C and are stable for at least two weeks.

If isooctane is used as extraction solvent, evaporation of the solvent can be omitted. The MSTFA can be added as 50 µl pure MSTFA instead of adding the 5 % solution of MSTFA. The calibration standards shall be treated like the samples.

NOTE 1 The derivatization is sensitive to the amount of water in the extract, therefore anhydrous sodium sulphate (7.4) is used for drying the extract.

10.5 Blank

Perform a blank determination in accordance with the following procedure. Prepare the blank exactly as by the analysis of the sample, including the clean-up if the clean-up has been used for the samples.

The blank value shall be lower than 50 % of the lowest reporting limit.

10.6 GC-MS analysis

Optimize the gas chromatograph and mass selective detector (8.7) according to the instrument manufacturer's manual. The separation of nonylphenols shall fulfil the requirements described in 8.5 .Many columns and GC-conditions may be used. An example is described in Annex A.

The detection is done by Electron Impact Ionization (EI) 70 eV. In Table 2 the ions used for the analysis are shown.

Table 2 — Diagnostic ions used by the GC-MS analysis

No.	Analyte (MSTFA derivative)	Selected diagnostic ions				Internal standard for analyte No.
		Abbreviation	Target ion M_1 [1)]	Qualifier ion M_2	Qualifier ion M_3	
1	Nonylphenol	NP	207	221	193	
2	Nonylphenol monoethoxylate	NP1EO	251	265	279	
3	Nonylphenol diethoxylate	NP2EO	295	309	323	
4	^{13}C-labelled 4-n-nonylphenol	^{13}C-4-n-NP	185			1,2
5	^{13}C-labelled 4-n-nonylphenol diethoxylate	^{13}C –4-n-NP2EO	252			3
6	D4-labelled 4-n-nonylphenol	4-n-NP-D4	183			1,2
7	Unlabelled 4-n-nonylphenol	4-n-NP	179			1,2
8	Unlabelled 4-n-nonylphenol diethoxylate	4-n-NP2EO	246			3

[1)] M_1 is used for quantification, M_2 and M_3 is used for identification

The GC-MS analysis of samples is described in 10.7.3.

10.7 Calibration and analysis of samples

10.7.1 Initial calibration

Two types of calibration are used: the initial calibration (10.7.1) and the recalibration, which is carried out daily (10.7.2).

The initial calibration is used to establish the linear working range of the calibration curve. This calibration is performed when the method is used for the first time and after maintenance and/or repair of the equipment.

The recalibration checks the validity of the linear working range of the initial calibration curve and is performed before each series of samples.

For all calibrations the relative areas are used, i.e. the area for the analyte relative to the area for the internal standard. (see 11.2). For NP, NP1EO and NP2EO the areas are determined as the sum of the peak areas of the isomeric mixture. See Clause 11. Initial calibration

Inject at least five standard solutions with concentrations from 0,01 mg/l to 5 mg/l (7.15) and include a solvent blank. Before injection 1 ml of the standard solution is treated (derivatized) as described in 10.4. Identify the peaks and prepare a calibration curve for each analyte.

Evaluation of the calibration curve shall be done according to the procedure described in ISO 8466-1. This International Standard provides acceptance and rejection criteria for linearity.

It is allowed to use non-linear calibration using all five standards. In that case, all five standards shall be used for recalibration and not only the two standards described below.

10.7.2 Recalibration

Inject at least two calibration standard solutions (7.15) (after derivatization) with concentrations of (20 ± 10) % and (80 ± 10) % of the established linear range and calculate the straight line from these measurements. If the straight line falls within the 95 % confidence limits of the initial calibration line, the initial calibration line is assumed to be valid. If not, a new calibration line shall be established according to 10.7.2.

10.7.3 Analysis of samples and identification

Inject the extracts of samples and blanks obtained from the derivatization in 10.4.

The identification of NP, NP1EO and NP2EO is based on three parameters:

— The peak pattern of the chromatogram, i.e. the fingerprint, although the relation between the individual peaks may differ in samples and standards

— The retention times of the individual peaks

— The relation between peak areas of the qualifier ions and the target ion

From the identification select the peaks to be included in the sum area. Peaks not found in the calibration standard is not included. See about interferences in clause 5.

Use ISO/FDIS 22982 for identification of the analytes.

If the concentration of one of the analytes is out of the calibration range (higher than the upper calibration limit), the final extract is diluted with 5 % MSTFA in isooctane (7.7). Wait minimum 15 min for the reaction to occur and inject again. A 10 times dilution of the extract is allowed. The linearity of the internal standard shall be checked.

11 Calculation and expression of results

11.1 General

For the analytes NP, NP1EO and NP2EO the areas are determined as the sum of the peak areas of the isomeric mixture. If interfering peaks are present, these shall not be included in the sum area.

The method is based on the internal standard calculations. The method determines the mass concentrations and is not influenced by injection errors, the volume of water present in the sample or matrix effects in the sample, provided that the recovery of the analytes are about equal to that of the internal standard.

4-n-NP is used as internal standard for the calculation of NP and NP1EO. 4-n-NP2EO is used for the calculation of NP2EO.

For all samples a specific mass (2 µg of 4-n-NP and 10 µg of 4-n-NP2EO) of internal standard (7.12) is added. These masses result in the same concentration of internal standard in the sample extracts as in the calibration standard solutions (7.15) (presuming 100 % recovery of internal standard).

NOTE Recovery of the internal standards can be checked, by comparing the amount of added and found internal standard, e.g. by comparing the areas.

11.2 Calibration

From the chromatograms of the calibration standards obtain a calibration curve by plotting the ratio of the mass concentrations against the ratio of the peak areas using equation (1) :

$$\frac{A_c}{A_{is,c}} = s \cdot \frac{\rho_c}{\rho_{is,c}} + b \qquad (1)$$

where:

A_c is the response of analyte in the calibration standard = sum of peak areas

$A_{is,c}$ is the response of internal standard in the calibration standard = peak area

s is the slope of the calibration function

ρ_c is the mass concentration of analyte in the calibration standard solution in micrograms per millilitre (µg/ml)

$\rho_{is,c}$ is the mass concentration of internal standard in the calibration standard solution in micrograms per millilitre (µg/ml) = 0,2 µg/ml for 4-n-NP and 1,0 µg/ml for 4-n-NP2EO.*b* is the intercept of the calibration curve with the ordinate

11.3 Calculation

From the chromatograms of the samples and blanks calculate the mass concentrations of the analytes from the calibration curve using equation (2) :

$$\omega_s = \frac{(A_s / A_{is,s}) - b}{s \cdot m \cdot d_s} \cdot \rho_{is,s} \cdot V \qquad (2)$$

where:

ω_s is the concentration of analyte found in the sample in milligrams per kilogram (mg/kg) dry matter

A_s is the response of analyte in the sample = sum of peak areas

$A_{is,s}$ is the response of internal standard in the sample = peak area

b is the intercept of the calibration curve with the ordinate

s is the slope of the calibration function

m is the mass of the test sample used for extraction in grams (g)

d_s is the dry matter content of the test sample in gram per gram (g/g)

$\rho_{is,s}$ is the mass concentration of internal standard in the sample extract in micrograms per millilitre (µg/ml)

V is the volume of hexane-like solvent used for extraction of the test sample, in millilitres (ml)

NOTE The equations are only valid by the use of linear calibration curves.

12 Precision data

The performance characteristics of the method (Annex B) data have been evaluated. Table 3 gives the resulting typical values for repeatability and reproducibility limits as their observed ranges. The typical value is derived from the data in Table B.2 in Annex B by taking the median value and rounding the numbers.

Table 3 — Typical values and observed ranges of the repeatability and reproducibility limits

The reproducibility limit provides a determination of the differences (positive and negative) that can be found (with a 95 % statistical confidence) between a single test result obtained by a laboratory using its own facilities and another test result obtained by another laboratory using its own facilities, both test results being obtained under the following conditions : The tests are performed in accordance with all the requirements of the present standard and the two laboratory samples are obtained from the same primary field sample and prepared under identical procedures. Conversely, the repeatability limit refers to measurements obtained from the same laboratory, all other conditions being identical. The reproducibility limit and the repeatability limit do not cover sampling but cover all activities carried out on the laboratory sample including its preparation from the primary field sample.		
Results of the validation of the determination of nonylphenols (NP) and nonylphenol-mono- and diethoxylates by gas chromatography with mass selective detection (GC-MS)in soil, sludge and treated biowaste	Typical value %	Observed range %
Repeatability limit, r	28	*14 - 84*
Reproducibility limit, R	98	48 - 140

NOTE 1. The above results refer to the difference that may be found between two test results performed on two laboratory samples obtained under the same conditions. In the case when reference is made to the dispersion of the values that could reasonably be attributed to the parameter being measured, the above typical reproducibility values and observed reproducibility ranges should be divided by √2 to obtain the corresponding typical dispersion limit and its observed range. In the example of NP1 in Sludge 1 the result and its dispersion limit is 3.1± 3.8 (2 * sR = 122 % of 3.1). This means that with a 95 % statistical confidence, the values reasonably attributable to the measured parameter are larger than 0 ug/kg and lower than 6.8 ug/kg.

NOTE 2. The repeatability limit (r) and the reproducibility limit (R) as given in Table B.2 (Annex B) and in this table are indicative values of the attainable precision if the determination of nonylphenols (NP) and nonylphenol-mono- and diethoxylates by gas chromatography with mass selective detection (GC-MS) is performed in accordance with this standard [CSS99040].

NOTE 3 A limited number of materials and parameters were tested. Consequently, for other materials and parameters, performance characteristics may fall outside the limits as derived from the validation of the the

Determination of LAS by HPLC with fluorescence detection (LC_FLD) and mass selective detection (LCMSD) in soil, sludge and treated biowaste.

NOTE 4 In particular for relatively heterogeneous materials, the repeatability and the reproducibility limits may be larger than the values given in Table B.2 (Annex B) and this table.

13 Test report

The test report shall contain at least the following data:

a) the information required to identify the sample;

b) a reference to this European Standard;

c) the contents of the analytes in milligrams per kilogram (mg/kg) dry matter, with two significant figures.

d) any details not specified in this International Standard or which are optional, as well as any factor which may have affected the results.

Annex A (informative)

Example of chromatographic conditions and chromatogram

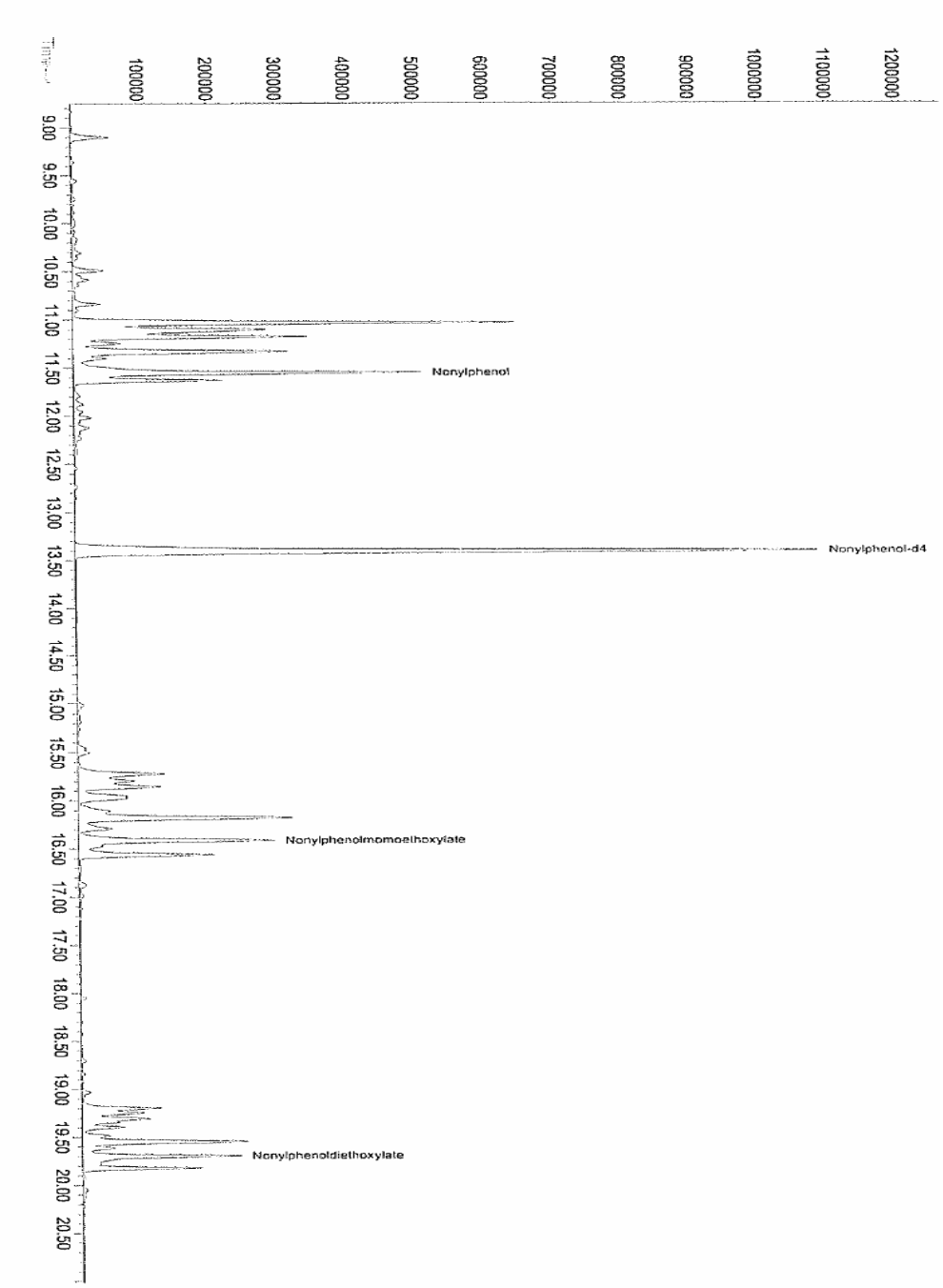
GC-conditions:

Separation column:	5% phenyl methyl siloxane, film thickness 0,25 μm. length 30 m, i.d. 0,25 μm
Oven temp.:	100 °C, hold 1 min
	10 °C/min to 200 °C, hold 3 min
	10 °C/min to 300 °C, hold 7 min
Injection temp.:	250 °C
Splitless inj.:	1 μl
Carrier gas:	Helium, 0,9 ml/min

MS-conditions:

Ionization:	Electron Impact
MS interface temp.:	280 °C
Filament on:	7 min

Total ion chromatogram based on SIM analysis



Annex B (informative)

Repeatability and reproducibility data

B.1 Performance characteristics

B.1.1 Objective of the interlaboratory comparison

In a European wide interlaboratory comparison study according to ISO 5725-2, the performance characteristics of the standard “Determination of nonylphenols (NP) and nonylphenol-mono- and diethoxylates by gas chromatography with mass selective detection (GC-MS) in soil, sludge and treated biowaste” were established.

B.1.2 Materials used in the interlaboratory comparison study

The interlaboratory comparison of determination of nonylphenols (NP) and nonylphenol-mono- and diethoxylates by gas chromatography with mass selective detection (GC-MS) in soil, sludge and treated biowaste was carried out with 5 - 6 European laboratories on 3 materials. The materials selected for the interlaboratory comparison were chosen to represent soil, sludge and biowaste as broad as possible, because the standard will find general application across different types of soil and soil related materials. (detailed information can be found in the final report on the Interlaboratory comparison study mentioned in the Bibliography).

In the interlaboratory comparison study the following starting points were used:

The laboratory samples were all taken from one large batch of the different materials according to the normal practice. The normal size reduction and the normal repeated mixing were carried out as needed to obtain representative laboratory samples from the large batch sample (ref JRC).

Note : the samples provided for the validation should not be confused with reference samples provided for certification purposes, as the performance results obtained have to be directly applicable to daily practice (less rigorous sample preparation than for a reference material).

The experimental plan was designed by project HORIZONTAL on the basis of each laboratory being given two laboratory samples of each material to be tested. This is in accordance with ISO 5725-2.

The materials examined cover all the grain size classes to which the the determination of nonylphenols (NP) and nonylphenol-mono- and diethoxylates by gas chromatography with mass selective detection (GC-MS) in soil, sludge and treated biowaste applies: very fine grained materials (like sludge: 0 µm to about 125 µm) and fine-grained materials (soil and compost: 0 mm to 4 mm).

Table B.1 provides a list of the types of materials chosen for testing and the selected components.

Table B.B.1 — Material types tested and components analysed in the interlaboratory comparison of the method for the Determination of nonylphenols (NP) and nonylphenol-mono- and diethoxylates by gas chromatography with mass selective detection (GC-MS) in soil, sludge and treated biowaste.

Grain size class	Sample code	Material type tested	Parameters/congeners
Sludge (<0.5 mm)	Sludge 1	Sewage sludge 1 Mix 1 of municipal WWTP sludges from North Rhine Westphalia, Germany	NP, NP1, NP2
Fine grained (< 2 mm)	Compost 1	Compost 1 Fresh compost from Vienna, Austria	NP, NP1, NP2
	Soil 3	Soil 3 A sludge amended soil from Barcelona, Spain	NP, NP1, NP2

B.1.3 Interlaboratory comparison results

The statistical evaluation was conducted according to ISO 5725-2. The average values, the repeatability standard deviation (s_r) and the reproducibility standard deviation (s_R) were obtained (Table B.2).

The repeatability is determined as an interval around a measurement result (i.e. "repeatability limit"). This interval corresponds to the maximum difference that can be expected (with a 95% statistical confidence) between one test result and another, both test results being obtained under the following conditions: The tests are performed in accordance with all the requirements of the present standard by the same laboratory using its own facilities and testing laboratory samples obtained from the same primary field sample and prepared under identical procedures.

The repeatability limit was calculated using the relationship : $r_{test} = f \cdot \sqrt{2} \cdot s_{r,test}$ with the critical range factor f = 2. For instance, the repeatability limit around a measurement result of 30 ug NP /kg is ± 11.3 ug NP /kg (i.e ± 38 % of 30).

NOTE The above relationship refers to the difference that may be found between two measurement results performed each on two laboratory samples obtained under the same conditions. The value f = 2 used in the factor $f \cdot \sqrt{2}$ corresponds to the theoretical factor of 1,96 for a pure normal distribution at 95 % statistical confidence. Also, this value f = 2 corresponds to the usual value k = 2 of the coverage factor recommended in the Guide to the expression of Uncertainty in Measurement (GUM). However it may be necessary to use a larger value for f in situation as described in clause 12.

The reproducibility, like repeatability is also determined as an interval around a measurement result (i.e. "reproducibility limit"). This interval corresponds to the maximum difference that can be expected (with a 95% statistical confidence) between one test result and another test result obtained by another laboratory, both test results being obtained under the following conditions : The tests are performed in accordance with all the requirements of the present standard by two different laboratories using their own facilities and testing laboratory samples obtained from the same primary field sample and prepared under identical procedures.

The reproducibility limit was calculated using the relationship: $R = f \cdot \sqrt{2} \cdot s_R$ with the critical range factor f = 2.

For instance, the reproducibility limit around a measurement result 30 ug NP /kg is ± 46 ug NP/kg (i.e ± 155 % of 30).

NOTE The above relationship refers to the difference that may be found between two measurement results performed each on two laboratory samples obtained under the same conditions. The value f = 2 used in the factor $f \cdot \sqrt{2}$ corresponds to the theoretical factor of 1,96 for a pure normal distribution at 95 % statistical confidence. Also, this value f = 2 corresponds to the usual value k = 2 of the coverage factor recommended in the Guide to the expression

of Uncertainty in Measurement (GUM). In the case when reference is made to the dispersion of the values that could reasonably be attributed to the parameter being measured, the dispersion limit is equal to $k \cdot s_R$ with the usual value k = 2, resulting in a dispersion limit lower than the reproducibility limit (i.e. a ratio of √2). However it may be necessary to use a larger value $f \cdot \sqrt{2}$ (or k) in situation as described in clause 12 .

In case of relatively heterogeneous materials, the repeatability and the reproducibility limits may be larger than the values given in Tables B.2 (this means that the value chosen for the critical range factor f is larger than 2 as well as for the coverage factor k for dispersion). This is because the extreme results may have been obtained in accordance with the present standard and/or be caused by the variability within, or in between, the laboratory samples.

Table B.B.2 — Results of the interlaboratory comparison studies of the determination of nonylphenols (NP) and nonylphenol-mono- and diethoxylates by gas chromatography with mass selective detection (GC-MS) in soil, sludge and treated biowaste. All concentrations in ug/kg.

Matrix	Parameter	Mean	sr	sR	r	R	p	Outliers	Total number of data	No of LOD
Sludge 1	NP	31.2	13.4%	57.7%	11.7	50.43	8	2	29	0
Compost 1	NP	0.123	12.3%	39.0%	0.04	0.13	6	1	22	0
Soil 3	NP	1.81	15.0%	55.3%	0.76	2.80	7	2	28	0
Sludge 1	NP1	3.09	2.98%	61.3%	0.26	5.30	4	1	15	0
Compost 1	NP1	*0.08*		*91*			3		10	0
Soil 3	NP1	*7.85*		*186*			5		19	0
Sludge 1	NP2	1.70	7.83%	84.0%	0.37	4.01	4	1	16	0
Compost 1	NP2	*0.023*		*16*			2		8	0
Soil 3	NP2	*1.05*		*159*			4		16	0

Abbreviations: sr Repeatability standard deviation; SR Reproducibility standard deviation; r Repeatability limit (comparing two measurements); R Reproducibility limit (comparing two measurements); p Number of labs.

Note 1. In judging the results it is important to consider the concentration levels, at which measurements have been carried out. The choice was made to avoid spiking of samples. This implies that particularly in soil and compost low concentrations have been observed for some congeners and results below detection for other congeners. If measurement results are well below a possible critical level (regulation), between lab variabilities of up to 70 % may prove fit for purpose.

Note 2. The experience of labs with some of the new emerging contaminants may be limited adding to the between lab variability.

Bibliography

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