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Soil quality — Determination of nonylphenols (NP) and nonylphenol-mono- and diethoxylates — Method by gas chromatography with mass selective detection (GC-MS)

Qualité du sol — Détermination des nonyphénols (NP) et des mono- et di-éthoxylates de nonylphénol — Méthode par chromatographie en phase gazeuse avec détection sélective de masse (CPG-SM)

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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.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of document:

— an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;

— an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

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/TS 13907 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 3, *Chemical methods and soil characteristics*.

Introduction

Nonylphenols (NP) are mainly found in the environment as degradation products of nonylphenol polyethoxylates (NPEO). NPEO have many applications as non-ionic detergents in washing and cleaning agents.

After use, NPEO are degraded by de-ethoxylation, resulting in polyethoxylates with fewer 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 this Technical Specification.

This Technical Specification is applicable for several types of matrices and validated for municipal sewage sludge (see also Annex A for the results of the validation).

Soil quality — Determination of nonylphenols (NP) and nonylphenol-mono- and diethoxylates — Method by gas chromatography with mass selective detection (GC-MS)

WARNING — Persons using this Technical Specification should be familiar with usual laboratory practice. This Technical Specification 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 according to this Technical Specification be carried out by suitably trained staff.

1 Scope

This Technical Specification specifies a method for the determination of nonylphenols (NP), nonylphenol-monoethoxylates (NP1EO) and nonylphenol-diethoxylates (NP2EO) in sludge, treated biowaste and soil using GC-MS.

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

Lower limits of detection may be achieved by concentrating the extract by solvent evaporation.

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

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

ISO 14507, *Soil quality — Pretreatment of samples for determination of organic contaminants*

ISO 16720, *Soil quality — Pretreatment of samples by freeze-drying for subsequent analysis*

ISO 22892, *Soil quality — Guidelines for the identification of target compounds by gas chromatography and mass spectrometry*

3 Principle

After pretreatment, the test sample is extracted by shaking 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 analysed by gas chromatography and mass selective detection (GC-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 an 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.

4 Interferences

4.1 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.

4.2 Interferences by GC-MS

Substances that co-elute with NP, NP1EO or NP2EO and give the same ion(s) may interfere with the determination. This may have a large influence on the result, since all three analytes are determined from the sum of a cluster of five to nine chromatographic peaks. It is essential 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 usually 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.

5 Reagents

5.1 General

All reagents shall be of recognized analytical grade.

The purity of the reagents used shall be checked by running a blank determination as described in 8.5.

5.2 Acetone, C_3H_6O.

5.3 Hexane-like solvent

Any aliphatic hydrocarbon solvent with a boiling point or boiling range between 34 °C and 100 °C may be applied.

5.4 Anhydrous sodium sulfate, 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 sulfate shall be kept carefully sealed.

5.5 *N*-methyl-*N*-(trimethylsilyl)-trifluoracetamide, $C_6H_{12}F_3NOSi$, (MSTFA), CAS-RN[1)] 24589-78-4, for derivatization.

5.6 Isooctane, C_8H_{17}, boiling point 99 °C.

5.7 Derivatization solution, 5 % MSTFA (5.5) in isooctane (volume fraction).

Dissolve e.g. 1 ml of MSTFA (5.5) in isooctane (5.6) in a 20 ml volumetric flask and make up to volume with isooctane (5.6).

1) CAS-RN Chemical Abstracts Service Registry Number.

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

5.8 Operating gas for gas chromatography with MS-detector

Helium of sufficient purity and in accordance with the manufacturer's specification.

5.9 **Nitrogen,** N2, for solvent evaporation.

Nitrogen of sufficient purity.

5.10 Standards for calibration

The following standard substances shall be used:

— 4-nonylphenols (NP), mixture of isomers, CAS-RN 84852-15-3;

— 4-nonylphenol monoethoxylates (NP1EO), mixture of isomers, CAS-RN 26027-38-3;

— 4-nonylphenol diethoxylates (NP2EO), mixture of isomers, CAS-RN 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 stored in a freezer at a temperature of (–18 ± 3) °C.

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

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

5.11 Internal standards

The following internal standard substances may 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 shall be stored in a freezer at a temperature of (–18 ± 3) °C.

D4-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 standards shall not be used.

5.12 Internal standard solution

Prepare an internal standard solution with the two internal standards in isooctane (5.6). 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.

5.13 Stock solutions

Prepare individual stock solutions of about 100 mg/l in isooctane (5.6), either from solid standard substances or from solutions with a certified concentration.

Store the stock solutions in a dark place at a temperature of (4 ± 3) °C. The solutions are stable for at least 2 years, provided that evaporation of solvent is negligible.

5.14 Calibration standard solutions

A mixed calibration standard solution is prepared from the stock solutions (5.13) by diluting the stock solutions with isooctane (5.6). Internal standard solution (5.12) 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 (4 ± 3) °C. The solutions are stable for at least two weeks, provided that evaporation of solvent is negligible.

6 Apparatus

6.1 General

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

6.2 Usual laboratory glassware

6.2.1 Screw-cap glass flask with polytetrafluoroethylene (PTFE) seal; volume 100 ml and 250 ml.

6.2.2 Round-bottom flasks, volume 100 ml and 250 ml.

6.2.3 Test tubes and vials

6.3 Shaking device

Reciprocating shaker, with horizontal movement (up to at least 250 strokes per minute).

6.4 Evaporator

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

6.5 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 has been proven.

6.6 Freeze-drying apparatus

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

3) Florisil® is a trade name for a prepared diatomaceous substance, mainly consisting of anhydrous magnesium silicate. This information is given for the convenience of users of this Technical Specification and does not constitute an endorsement by ISO of this product.

6.7 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 to 50 m. An example of a column is given in Annex B.

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.

7 Sample storage and sample pretreatment

7.1 Sample storage

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

Determine the content of dry matter in the sample according to ISO 11465.

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

7.2 Sample pretreatment

Pretreat the samples according to ISO 14507 if not otherwise specified.

Different pretreatment procedures are used for the different matrices. This is presented in Table 1.

Table 1 — Pretreatment methods used prior to nonylphenol analysis

Matrix	Freeze drying (ISO 16720)	No drying
Sludge dry matter > 2 %[a]	x	x
Sludge dry matter < 2 %[b]	x	
Soil[c]	x	x
Treated biowaste	x	x

[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.

8 Procedure

8.1 Extraction

8.1.1 General

The wash of organic phase (extraction solvent) with water may be carried out directly in the extraction flask with the sample present.

Other extraction techniques than described in this Technical Specification, like ultrasonic extraction, microwave or pressurized liquid extraction may be suitable. If other extraction techniques are applied, the comparability to the method described in this Technical Specification shall be proven.

8.1.2 Extraction of wet sludge samples

Wet sludge samples are extracted as follows:

— Weigh between 10 g and 50 g of the test sample (depending on dry matter content) and place it in a 100 ml screw-cap flask (6.2.1). The sample should preferably contain between 2 g and 3 g of dry matter.

— Add 100 µl of internal standard solution (5.12) equal to 2 µg of 4-*n*-NP and 10 µg of 4-*n*-NP2EO.

— Add 10 ml of acetone (5.2), close the screw cap and shake thoroughly by hand.

— Add 10 ml of hexane-like solvent (5.3), close the screw cap again and place the flask on the shaking device (6.3). The flask shall be placed in a horizontal position with the movement along the flask.

— Shake for at least 2 h with (250 ± 20) strokes per minute.

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

— Add water and shake to wash the extract. Use 5 ml of water per ml hexane-like solvent (5.3).

— Transfer the extract (enough for the subsequent analysis) to a test tube (6.2.3) and dry the extract by adding anhydrous sodium sulfate (5.4).

The extract is now ready for further treatment described in 8.2 to 8.4.

8.1.3 Extraction of freeze-dried sludge samples

Freeze-dried sludge samples are extracted as follows:

— Weigh 2 g to 3 g of the test sample and place it in a 100 ml screw-cap flask (6.2.1).

— Add 100 µl of internal standard solution (5.12) equal to 2 µg of 4-*n*-NP and 10 µg of 4-*n*-NP2EO.

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

— Add 10 ml of acetone (5.2), close the screw cap and shake thoroughly by hand.

— Add 10 ml of hexane-like solvent (5.3), close the screw cap again and place the flask on a reciprocating shaker (6.3). The flask shall be placed in a horizontal position with the movement along the flask.

— Shake for at least 2 h with (250 ± 20) strokes per minute.

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

— Add water and shake to wash the extract. Use 5 ml of water per ml hexane-like solvent (5.3).

— Transfer the extract (enough for the subsequent analysis) to a test tube (6.2.3) and dry the extract by adding anhydrous sodium sulfate (5.4).

The extract is now ready for further treatment described in 8.2 to 8.4.

8.1.4 Extraction of soil and treated biowaste samples

Soil and treated biowaste samples are usually extracted wet without drying the sample before extraction. These samples are extracted as follows:

— Weigh between 20 g and 40 g of the test sample (depending on dry matter content) and place it in a 100 ml screw-cap flask (6.2.1). The sample shall contain between 10 g and 20 g dry matter.

— Add 100 µl of internal standard solution (5.12) equal to 2 µg of 4-*n*-NP and 10 µg of 4-*n*-NP2EO.

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

— Add 30 ml of acetone (5.2) to the test sample, close the screw cap and shake thoroughly by hand.

— Add 30 ml of hexane-like solvent (5.3), close the screw cap again and place the flask on a reciprocating shaker (6.3). The flask shall be placed in horizontal position with the movement along the flask.

— Shake for at least 2 h with (250 ± 20) strokes per minute.

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

— Add water and shake to wash the extract. Use 5 ml of water per millilitre of hexane-like solvent (5.3).

— Transfer the extract (enough for the subsequent analysis) to a test tube (6.2.3) and dry the extract by adding anhydrous sodium sulfate (5.4).

The extract is now ready for further treatment described in 8.2 to 8.4.

8.1.5 Extraction of freeze-dried soil and treated biowaste samples

Freeze-dried soil and treated biowaste samples are extracted as follows:

— Weigh 10 g to 20 g of the test sample and place it in a 100 ml screw-cap flask (6.2.1).

— Add 100 µl of internal standard solution (5.12) equal to 2 µg of 4-*n*-NP and 10 µg of 4-*n*-NP2EO.

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

— Add 20 ml of acetone (5.2), close the screw cap and shake thoroughly by hand.

— Add 20 ml of hexane-like solvent (5.3), close the screw cap again and place the flask on a reciprocating shaker (6.3). The flask shall be placed in horizontal position with the movement along the flask.

— Shake for at least 2 h with (250 ± 20) strokes per minute.

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

— Add water and shake to wash the extract. Use 5 ml of water per ml hexane-like solvent (5.3).

— Transfer the extract (enough for the subsequent analysis) to a test tube (6.2.3) and dry the extract by adding anhydrous sodium sulfate (5.4).

The extract is now ready for further treatment described in 8.2 to 8.4.

8.2 Concentration (optional)

In most cases concentration of the extract is not necessary. If lower detection limits are required, this can be achieved by evaporation of the solvent.

Concentrate the extract on a rotary evaporator (6.4) or by the use of a gentle stream of nitrogen at room temperature. Since the internal standard (5.12) is used for the calculations, it is not necessary to know the exact volumes. If necessary, the amount of the internal standard added to the sample can be reduced relative to the concentration factor to keep the concentration of the internal standard at the same level in the GC-MS analysis.

NOTE Other inert gases can be used instead of nitrogen.

8.3 Clean-up (optional)

Clean-up shall be used if compounds are present that can interfere with the analytes or the internal standard (5.12) 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, clean-up is not necessary.

Add 1 ml of extract to the clean-up column (6.5). Elute the column with a solvent, e.g. a mixture of hexane-like solvent (5.3) and acetone (5.2). Evaporate the eluted solvent to about 1 ml. The cleaned extract is now ready for derivatization described in 8.4.

Before use the column shall be tested to ensure that the analytes are recovered in the collected fraction. The criterion 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.

8.4 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:

— Transfer 1,0 ml of extract to a GC vial (6.2.3).

— Evaporate the solvent slowly (room temperature) until dryness under a gentle stream of nitrogen (5.9).

— Add 1,0 ml of 5 % MSTFA (5.5) in isooctane (5.6), close the vial and shake for dissolution.

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

— If the solution is not clear, transfer the isooctane solution to a new GC vial (6.2.3). 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 The derivatization is sensitive to the amount of water in the extract, therefore anhydrous sodium sulfate (5.4) is used for drying the extract.

8.5 Blank test

Perform a blank determination in accordance with the procedure described in 8.1 to 8.4. 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.

8.6 GC-MS analysis

Optimize the gas chromatograph and mass selective detector (6.7) according to the instrument manufacturer's manual. The separation of nonylphenols shall fulfil the requirements described in 6.5. Many columns and GC-conditions may be used. An example is described in Annex B.

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

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

No.	Analyte (MSTFA derivative)	Abbreviation	Selected diagnostic ions			Internal standard for analyte No.
			Target ion M_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
NOTE M_1 is used for quantification, M_2 and M_3 are used for identification.						

8.7 Calibration

8.7.1 General

Two types of calibration are used: the initial calibration (8.7.2) and the verification of calibration, which is carried out daily (8.7.3).

For all calibrations, the relative areas are used, i.e. the area for the analyte relative to the area for the internal standard (see 9.2). For NP, NP1EO and NP2EO, the areas are determined as the sum of the peak areas of the isomeric mixture.

8.7.2 Initial calibration

The initial calibration serves 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.

Inject at least five standard solutions with concentrations from 0,01 mg/l to 5 mg/l (5.14) and include a solvent blank. Before injection 1 ml of the standard solution is treated (derivatized) as described in 8.4. Identify the peaks and prepare a calibration curve for each analyte.

Evaluation of the calibration curve shall be done according to ISO 8466-1. ISO 8466-1 provides acceptance and rejection criteria for linearity.

Non-linear calibration may be applied. In that case, all five standards shall be used for verification of calibration.

8.7.3 Verification of calibration

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

Inject at least two calibration standard solutions (5.14) (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 8.7.2.

8.8 Analysis of samples and identification

Inject the extracts of samples and blanks obtained from the derivatization in 8.4.

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 are not included. See Clause 4 for interferences.

Use ISO 22892 for the identification of the analytes.

If the concentration of one of the analytes is outside the calibration range (higher than the upper calibration limit), the final extract is diluted with 5 % MSTFA (5.5) in isooctane (5.6). Wait at least 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.

9 Calculation and expression of results

9.1 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 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 is 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 (5.11) is added. These masses result in the same concentration of internal standard in the sample extracts as in the calibration standard solutions (5.14) (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.

9.2 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 Formula (1):

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

where

A_{c} is the response of analyte in the calibration standard (sum of peak areas);

$A_{\mathrm{is,c}}$ 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, expressed in micrograms per millilitre (µg/ml);

$\rho_{is,c}$ is the mass concentration of internal standard in the calibration standard solution, expressed in micrograms per millilitre (µg/ml);

b is the intercept of the calibration curve with the ordinate.

9.3 Calculation

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

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

where

w_s is the concentration of analyte found in the sample, expressed in milligrams per kilogram of dry matter (mg/kg);

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, expressed in grams (g);

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

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

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

The mass concentrations of the analytes are expressed in milligrams per kilogram dry matter, rounded to two significant figures.

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

10 Precision

The performance characteristics of the method data have been evaluated (see Annex A).

11 Test report

The test report shall contain at least the following information:

a) a reference to this Technical Specification (ISO/TS 13907:2012);

b) complete identification of the sample;

c) expression of results, according to 9.3;

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

Annex A
(informative)

Repeatability and reproducibility data

A.1 Materials used in the interlaboratory comparison study

The interlaboratory comparison for determination of nonylphenols (NP) and nonylphenol-mono- and diethoxylates by gas chromatography with mass selective detection (GC-MS) in sludge, treated biowaste and soil was carried out by up to 10 European laboratories on 3 materials. Detailed information can be found in the final report on the interlaboratory comparison study mentioned in Reference [5].

Table A.1 lists the types of materials tested.

Table A.1 — Materials and parameters tested in the interlaboratory comparison for the determination of nonylphenols (NP) and nonylphenol-mono- and diethoxylates by gas chromatography with mass selective detection (GC-MS) in sludge, treated biowaste and soil

Grain size	Sample	Material tested	Parameters
Sludge (<0,5 mm)	Sludge 1	Mix of municipal waste water treatment plant sludges from North Rhine Westphalia, Germany	NP, NP1, NP2
Fine grained (<2,0 mm)	Compost 1	Fresh compost from Vienna, Austria	NP, NP1, NP2
	Soil 3	Sludge amended soil from Barcelona, Spain	NP, NP1, NP2

The validation has been carried out for soil and compost also, but no results are presented because the concentrations found in the test materials were lower than the limit of applicability.

A.2 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 A.2).