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Soils sludges and treated bio-waste – Organic constituents - Nonylphenols (NP) and nonylphenol-mono- and diethoxylates by gas chromatography with mass selective detection (GC-MS)

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Foreword

This document has been prepared in the framework of the project Horizontal.

This document is a working document.

The following TC's have been involved in the preparation of the standard: CEN/TC 292, CEN/TC 308 and ISO/TC 190.

This 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 is 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/. During the development of the horizontal method, the work has been discussed by an ad-hoc group formed to facilitate such discussions. The ad-hoc group have had five meetings during the period September 2003-September 2005, all meetings held in conjunction with other standardisation committee meetings in CEN/TC 308/WG 1 and ISO/TC 190.

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.

The texts of the chapters are normative; annexes are normative or informative, as stated in the top lines of the annexes.

Soils sludges and treated bio-waste – Organic constituents - Nonylphenols (NP) and nonylphenol-mono- and diethoxylates by gas chromatography with mass selective detection (GC-MS)

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 compost using GC/MS.

The 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 0,01-0,02 mg/kg (expressed as dry matter) may be achieved.

Temporary remark: The LOD may be revised after the method validation.

Matrices for which the 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/DIS 10381-1, *Soil quality – Sampling – Part 1: Guidance on the design of sampling programmes.*

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

ISO/DIS 10381-8, *Soil quality – Sampling – Part 8: Guidance on sampling of stockpiles.*

Horizontal standard 2-2, *Sampling of sludges and treated bio-wastes.*

Horizontal standard 2-3, *Sampling of soils.*

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, *Characterisation of waste – Calculation of dry matter by determination of dry residue and water content*.

Horizontal standard 17-1 (draft), *Solid materials – Determination of dry matter and water content on a mass basis – Gravimetric method.*

ISO/DIS 14507, *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.*

Horizontal standard 33-2, *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, *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

In the context of this international standard, the analytes are nonylphenols (mixture of isomers), nonylphenol-monoethoxylates (mixture of isomers), and nonylphenol-diethoxylates (mixture of isomers).

3.2 Calibration standard

A solution prepared from stock solutions of the analytes and used to calibrate the response of the instrument with respect to analyte concentration.

3.3 Internal standard

The ^{13}C-labelled 4-n-nonylphenol and ^{13}C-labelled 4-n-nonylphenol-diethoxylate is added to the test sample before extraction. The internal standards are used to correct for losses during the analysis and are used for calculating the concentration of the analytes.

NOTE 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 standard shall not be used.

3.4 Test sample

The test sample is the 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 chapter 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 effect the sample during the contact through sampling and storage. Plastic containers may be used, if they have been proven not to significantly effect 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

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.

7.1 **Acetone,** C_3H_6O

7.2 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 chapter 6.

7.3 **Anhydrous sodium sulphate,** Na_2SO_4, powdered

Heated for at least 6 h to 550 $\pm$ 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.

7.4 Reagents for clean-up procedures

7.5 MSTFA for derivatization

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

7.6 Isooctane, C_8H_{17}, b.p. 99°C

7.7 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.

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

7.8 Operating gas for gas chromatography with MS-detector

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

7.9 Nitrogen for solvent evaporation

Nitrogen of sufficient purity.

7.10 Standards for calibration

The following standard substances are 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 must 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.

7.11 Internal standards

The following internal standard substances are 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.

7.12 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.

NOTE Store the internal standard solution in a dark place at a temperature of 4°C ± 3°C. The solution is stable for at least 2 years, provided that evaporation of solvent is negligible.

7.13 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.

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

7.14 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.

NOTE Store the calibration standard solutions in a dark place at a temperature of less than 4°C. The solutions are stable for at least 2 weeks, provided that evaporation of solvent is negligible.

8 Apparatus

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 hours at 450°C.

8.1 Standard laboratory glassware

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

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

Test tubes and vials.

8.2 Shaking device, reciprocating shaker

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

8.3 Evaporator

Rotary evaporator. Other device like turbo evaporator or Kuderna Danish may be applied.

8.4 Clean-up column

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

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

NOTE Florisil is a trade name for prepared magnesium silicate.

8.5 Freeze drying apparatus

8.6 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 - 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 must 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

Obtain representative samples in accordance with ISO 10381-1 (soil) using sampling apparatus in accordance with ISO 10381-2. Use Horizontal standard 2-2 for sampling of sludgeand biowaste.

Store the samples in a dark place at a temperature below 10°C, if possible in a refrigerator. Determine the content of dry matter in the sample in accordance with EN 12880, ISO 11465 or PrEN 14346 – Temporary remark: Horizontal standard 17-1.

NOTE Freeze-dried samples, if kept sealed, may be stored for a longer period at room temperature (approx. 1 month). Hygroscopic dried sludge may to preserved by mixing with anhydrous sodium sulphate.

9.2 Sample pre-treatment

Samples shall be pre-treated as soon as possible after sampling. Samples shall be stored in the dark at a temperature of 4°C ± 3°C no longer than 7 days.

Methods for pre-treatment of solid samples to be used for the analysis of organic contaminants are described in a separate standard, Horizontal standard 33-2. This 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.

Some sludge and sediment samples may have a high amount of water, which results in low recoveries and higher limits of detection, when extracted as wet samples. In a note a filtration procedure is described for samples with a low content of dry matter.

Table 1 — Pretreatment methods used prior to nonylphenol analysis.

Sludge dm > 2%	Sludge dm < 2%	Soil	Treated bio-waste	Sediment dm > 10%	Sediment dm < 10%
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)

dm: dry matter.

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

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

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

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

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

10 Procedure

10.1 Extraction

Four extraction methods are described – one for extraction of wet sludge samples, one for extraction of freeze-dried sludge samples, one for extraction of wet samples of soil, sediment and treated bio-waste, and one for extraction of freeze-dried samples of soil, sediment and treated bio-waste.

10.1.1 Extraction 1 – Wet sludge samples

Wet sludge samples are extracted as follows:

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

b) Add 100 µl of internal standard solution (7.12) equal to 2 µg of 4-n-NP and 10 µg of 4-n-NP2EO.

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

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

e) Shake for at least 2 hours with 250 $\pm$ 20 strokes per minute.

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 glass tube and dry the extract by adding anhydrous sodium sulphate.

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

10.1.2 Extraction 2 – Freeze-dried sludge samples

Freeze dried sludge samples are extracted as follows:

a) Take 2-3 g of test sample and place it in a 100 ml screw cap flask with teflon seal.

b) Add 100 µl of internal standard solution (7.12) 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, close the screw cap and shake thoroughly by hand.

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

f) Shake for at least 1 hour with 250 ± 20 strokes per minute.

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 glass tube and dry the extract by adding anhydrous sodium sulphate.

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

10.1.3 Extraction 3 – 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 and 40 g of test sample (depending on dry matter content) and place it in a 100 ml screw cap flask with teflon seal. The sample shall contain between 10 and 20 g dry matter.

b) Add 100 µl of internal standard solution (7.12) 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 to the test sample, close the screw cap and shake thoroughly by hand.

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

f) Shake for at least 2 hours with 250 ± 20 strokes per minute.

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 glass tube and dry the extract by adding anhydrous sodium sulphate.

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

10.1.4 Extraction 4 – Freeze-dried soil, sediment and treated bio-waste samples

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

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

b) Add 100 µl of internal standard solution (7.12) equal to 2 µg of 4-n-NP and 10 µg of 4-n-NP2EO.

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

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

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

f) Shake for at least 2 hours with 250 ± 20 strokes per minute.

g) Transfer the organic phase to a 100 - 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 glass tube and dry the extract by adding anhydrous sodium sulphate.

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

The extracts can be stored in a refrigerator (4°C ± 3°C) and are stable for at least 1 month.

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.

NOTE 2 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 standard shall be proven.

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 or by the use of a gentle stream of nitrogen at room temperature. Since the internal standard 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 has to be used if compounds are present that can interfere with the analytes or the internal standard 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 (8.4). 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 are 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.

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

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

d) Wait 15 minutes 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.

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

The derivates can be stored in a refrigerator (4°C ± 3°C) and are stable for at least 2 weeks.

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

NOTE 2 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.

10.5 Blank

Perform a blank determination following the procedure as described. 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 (8.6) according to the instrument manufacturer's manual. The separation of nonylphenols must fulfil the requirements described in 8.6.

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	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

* 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

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 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.

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. This is described in 11.1.

For NP, NP1EO and NP2EO the areas are determined as the sum of the peak areas of the isomeric mixture. This is described in chapter 11.

10.7.1 Initial calibration

Inject at least 5 standard solutions with concentrations from 0,01 mg/l to 5 mg/l (7.14) 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 description in ISO 8466-1. This standard for linear calibration gives acceptance and rejection criteria for linearity.

Note It is allowed to use non-linear calibration using all 5 standards. In that case, all 5 standards shall be used for recalibration and not only the 2 standards described below.

10.7.2 Recalibration

Inject at least two calibration standards (after derivatization) with concentrations of 20 $\pm$ 10 % and 80 $\pm$ 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 has to be established according to 10.7.1.

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 chapter 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 minutes 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

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

11.1 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 µg/ml

$\rho_{is,c}$ is the mass concentration of internal standard in the calibration standard solution in 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

11.2 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 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

d_s is the dry matter content of the test sample in g/g

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

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

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

12 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 international standard;

c) the contents of the analytes in 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

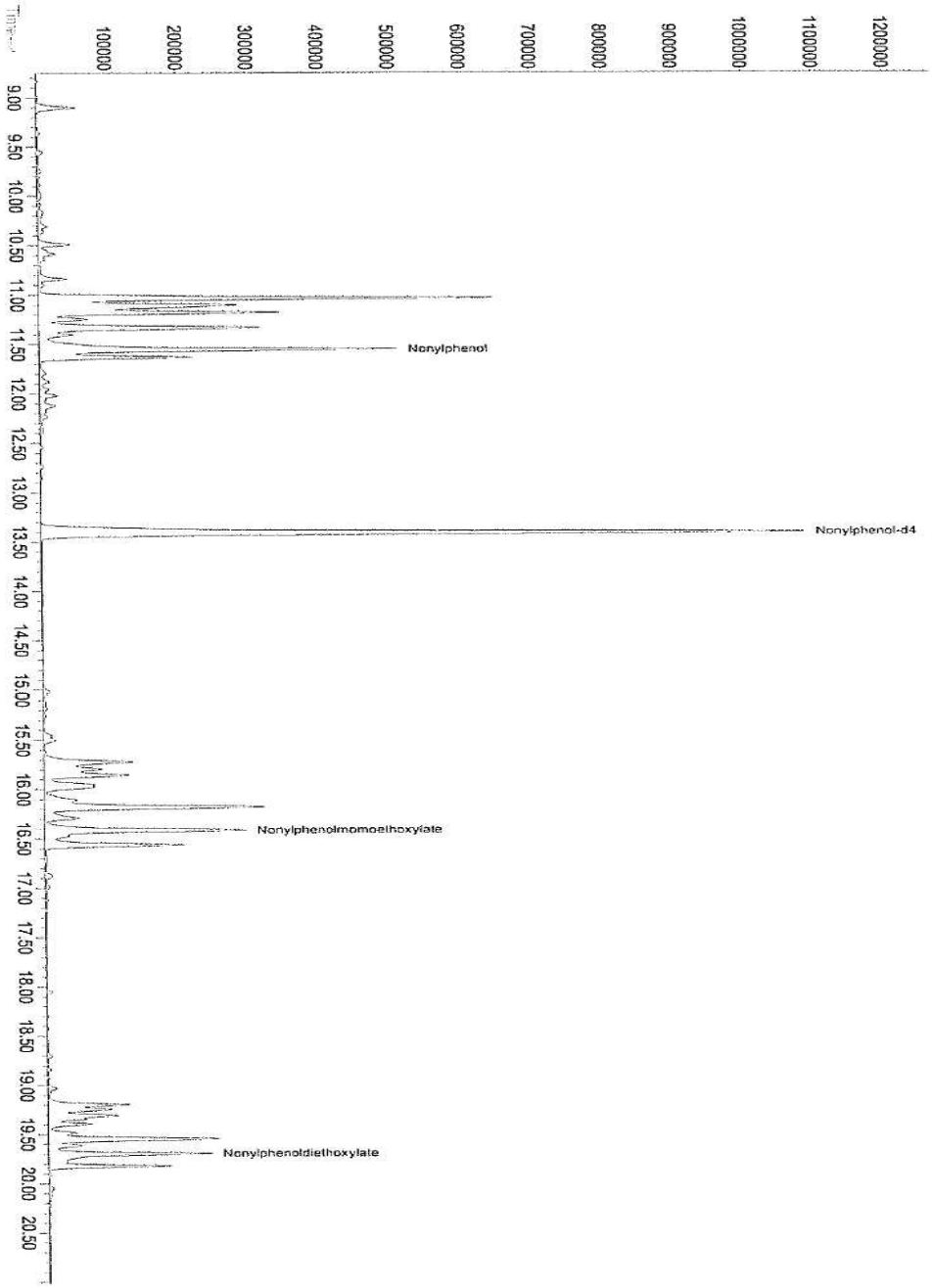
GC-conditions:

Separation column:	5% phenyl methyl siloxane, film thickness o,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)

Validation

Annex C (informative)

Information on project Horizontal and WPxx

Bibliography

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