Nonylphenols

Experimental work including ruggedness test

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SUMMARY

The present report describes the experimental work being carried out for the development of a horizontal standard for the determination of nonylphenols in solid matrices. The work is part of two projects: Project HORIZONTAL WP 5: Organic Contaminants, and the research project HORIZONTAL-ORG WP 3.

The work is based on the recommendations in the desk study report for LAS and Nonylphenols from January 2004 /1/. In the report it was recommended that a standard method for nonylphenols should be based on the principle: an extraction of a dry or wet sample, possibly a clean-up step and a derivatization, and a measurement by GC-MS. Also it was recommended that several issues should be studied for the preparation of a draft method.

The present report describes the pre-normative experimental work and a ruggedness test carried out on the draft standard.

Before the beginning of the experimental work, the scope was discussed at several meetings in an ad-hoc group for LAS and nonylphenols and at workshop meetings in the sludge committee CEN/TC 308. It was decided that the scope shall include the matrices sludge, soil and compost (bio-waste) and the analytes 4-nonylphenol (NP), 4-nonylphenol monoethoxylate (NP1EO) and 4-nonylphenol diethoxylate (NP2EO). The three analytes are all mixtures of isomers.

In the report the following experiments are presented:

- The derivatization and GC-MS measurement
- The extraction solvent
- The extraction technique
- The pre-treatment of the sample (use of dry or wet sample)
- The clean-up procedure
- The storage and stability

Based on the experiments the following conclusions were made:

Choice of derivatization reagent and GC-MS conditions
A comparison of two derivatization reagents: Methyl-N-(trimethylsilyl)-trifluracetamide (MSTFA) and Pentafluorobenzoyl chloride (PFBCl) was conducted. An evaluation on the basis of chromatographic appearance, repeatability and limit of detection resulted in the choice of MSTFA as the most advantageous derivatization reagent.

Derivatization procedure
Changes in the initial derivatization procedure were introduced. The amount of MSTFA was reduced from 200 µl to 50 µl without loss of derivatization efficiency, and a derivatization with pure MSTFA (50 µl) followed by dissolution in 950 µl isooctane was substituted with the simultaneous addition of MSTFA and isooctane (i.e. 1 ml 5% MSTFA in isooctane).

Derivatization efficiency and time
The analysis of a MSTFA-derivatized 20 mg/l standard, recording both derivatized and un-derivatized alkylphenols, showed that the derivatization procedure is more than 95% efficient regarding the derivatization of NP and more than 99% regarding the derivatization of both NP1EO and NP2EO. This study was made with 15 minutes reaction, and based on these results the derivatization time was reduced from 30 to 15 minutes.

Choice of solvent
The extraction efficiencies of toluene, ethyl acetate, dichloromethane and acetone/pentane (1:1) were compared in several studies. The most efficient solvent was found to be acetone/pentane, whereas the extraction efficiencies of the other solvents were found to be very similar. The use of acetone/hexane-like solvent (1:1) was therefore introduced in the method.

Extraction volume
The influence of sample/solvent-ratio on extraction efficiency was tested on sludge samples by comparing extractions of 2g dm/15 ml and 1g dm/15 ml, respectively. This study did not indicate any influence of sample/solvent-volume within the tested range. Mainly for practical reasons a sample/solvent-ration of 2 g dm/20 ml acetone/hexane-like solvent was chosen.

Extraction technique
A comparison of soxhlet and reciprocating shaker based on both acetone/pentane and toluene as extraction solvents was conducted. The reciprocating shaker was found to be more efficient than soxhlet when the extractions were conducted with acetone/pentane. No unambiguous conclusion was found regarding the toluene extractions. The reciprocating shaker was consequently chosen for the method.

Extraction time
An optimal extraction of NP, NP1EO and NP2EO from freeze-dried sludge samples was achieved after 0,5 hours. Extractions conducted on field moist sludge samples resulted in an optimal extraction of NP after 2 hours. The extraction efficiency of NP1EO and NP2EO was, however, found to be slightly increasing over time. The increase in extraction efficiency was not found to necessitate an extraction time of more than 2 hours. Extraction times of 1 and 2 hours for the extraction of freeze-dried and wet samples, respectively, were consequently chosen for the method.

Oven-drying
A tendency of oven-dried samples to result in a reduction in extraction efficiency of NP was observed. However, the effect was not statistically significant due to relatively large standard deviations obtained in this study. No indications of reduced extraction efficiencies were observed for NP1EO (NP2EO < LOD). Only drying by freeze-drying is included in the method.

Freeze-drying
Freeze-drying was found to result in a significant reduction in extraction efficiencies for NP and NP1EO (NP2EO not effected), when freeze-dried sludge was extracted directly. However, the addition of water to the freeze-dried sample before extraction increased the extraction efficiency to a similar level as seen by the extraction of wet samples.

Influence of water content
A significant decrease in extraction efficiency of NP and NP1EO in sewage sludge was observed when the dry matter content was reduced to 5%. A significant effect on the extraction of NP2EO was observed when the dry matter content was reduced to 10%. The use of two internal standards (4-n-nonylphenol and 4-n-nonylphenol diethoxylate), however, compensates for the low recoveries and thus enables a correct quantification of NP, NP1EO and NP2EO, even when analysing samples of low dry matter content, possibly down to 2%. For samples with lower than 2% dry matter freeze-drying should be used.

Clean-up
An example of a clean-up method based on silica columns was tested. It was shown that a clean-up of dirty extracts can be obtained, resulting in extracts free from chromatographic interference.

Storage and stability
The stability of samples has been studied for sludge and will be presented in a separate report. The stability of NP-D4, NP, NP1EO and NP2EO and their MSTFA-derivates stored at –18°C, 4°C and 22°C was tested. An acceptable storage period of 2 weeks was observed regarding both derivatized and un-derivatized calibration standards (0,1-2,5 mg/l). The acceptable storage time of a sludge extract was found to be approximately 4 weeks. The less stable analyte was NP2EO (and MSTFA-derivates of NP2EO) in all the tested solutions.

On the basis of these conclusions, the draft method has been written. The 1st and 2nd draft was also discussed at ad-hoc group meetings, and the 3rd draft was written, dated November 2005, see Appendix 9.

Ruggedness test
The 3rd draft was subjected to a ruggedness test to examine the influence of several factors related to the extraction procedure. By the ruggedness test 10 samples were tested, each for the influence of 7 different factors.

The results of the ruggedness test was positive, since only one change in the method was found necessary: For wet soil (and compost) the amount of extracting solvent was increased in order to give a higher extraction yield. The draft method was changed accordingly and some minor adjustments were also made. The result is the 4th draft standard, now ready for consultation.

1. INTRODUCTION

The present report describes the experimental work being carried out for the development of a horizontal standard for the determination of nonylphenols in solid matrices. The work is part of two projects: Project HORIZONTAL WP 5: Organic Contaminants, and the research project HORIZONTAL-ORG WP 3.

In the desk study report for LAS and nonylphenols /1/ it was recommended that pre-normative studies should be conducted for both groups of compounds before horizontal standards could be drafted. Also recommendations were given for the issues to be included in the further work. Further suggestions were received from interested parties commenting the desk study /2/. The desk study report and the summary of comments is published at the HORIZONTAL website http://www.ecn.nl/horizontal/.

The present work has been based on the desk study and the comments given. The following recommendations were presented:

- That the method will include an extraction of a dry or wet sample, possibly a clean-up step and a derivatization, and a measurement by GC-MS
- That many issues had to be studied before a draft standard for nonylphenols could be presented

The desk study report pointed at several main issues to be studied:

- Scope – shall mono- and di-ethoxylates of nonylphenols be included
- Scope – which matrices shall be included
- Sample storage
- Use of wet and/or dry sample
- Choice of extraction solvent
- Choice of extraction technique
- The necessity of clean-up and choice of clean-up
- Choice of derivatization procedure – if derivatization shall be included

The desk study report and many of the results from the experiments have been presented and discussed by an ad-hoc group formed to facilitate such discussions. The ad-hoc group have met in conjunction with standardisation meetings in the Sludge Committee CEN/TC 308/WG 1 and the Soil Committee ISO/TC 190: In Hamburg 28 August 2003, in Copenhagen 29 January 2004, in Paris 21 September 2004, in Vienna 8 March 2005 and in Madrid 21 September 2005.

In addition, the work has been presented and discussed at the workshop in HORIZONTAL-ORG held in Paris 28 – 29 April 2005, and at a working group meeting at ISO190/SC 3 held in Tokyo 11 October 2005.

The present report describes the results from the experimental work on the issues mentioned. It also includes the results from a ruggedness test carried out on the draft standard.

2. SCOPE AND PRINCIPLE

After the publication of the desk study report the scope of the method was further discussed at several working group meetings. The conclusions of these discussions are described in this chapter.

As already described in the desk study report, the method for nonylphenols can be shortly described:

The test sample (wet or freeze-dried sample) is extracted with an organic solvent or mixture of solvents. If necessary, interfering compounds are removed from the extract by a clean-up on a suitable column. Due to the inclusion of mono- and di-ethoxylates in the scope (see sub-section 2.2) the extract is treated with a derivatization reagent to derivatize the analytes, and they are subsequently analysed 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 the MS diagnostic ions. The quantification is based on internal standard procedure.

2.1 Matrices

Since the start of the project Horizontal, the work has been closely related to the Commission's plan to write a new Sewage Sludge Directive, and it was therefore obvious that sludge must be part of the scope.

At the first enquiry a comment from AFNOR, France, stated that the standard should also include soil. This item was also discussed at several working group meetings in ISO/TC 190 (Soil Committee) as well as in the ad-hoc group for nonylphenols and LAS. It was the general opinion that soil shall also be included in the scope.

Other matrices like biowaste, sediments and selected solid wastes may also be analysed by the method. Among these, only biowaste (compost) is included in the scope, since the planned validation study will include sample(s) of compost, however, not samples of sediment and solid waste.

Therefore the standard will include sludge, soil and compost (biowaste). Other solid materials like sediment and selected solid wastes may be analysed by the method.

2.2 Analytes

At the first two ad-hoc group meetings in Hamburg, 28 August 2003, and in Copenhagen, 29 January 2004, it was discussed whether mono- and di-ethoxylates of nonylphenols (NP1EO and NP2EO) should also be included in the method in addition to the nonylphenols (NP). From several national representatives wishes were expressed to include the ethoxylates in the method, and it was therefore decided to do so.

At the ad-hoc group meeting in Vienna on 08 March 2005 it was discussed if 4-tert-octylphenol (4-(1,1,3,3-tetramethylbutyl)phenol) should also be included in the standard. This compound is mainly found in the environment as a degradation product from non-ionic detergents in the group of octylphenol polyethoxylates, similarly to nonylphenol, which also derives from non-

ionic detergents in the group of nonylphenol polyethoxylates. It was decided that 4-tert-octylphenol should not be included in the normative part of the method. However, since the compound can easily be determined by the method, it was decided to include the compound in a note in the method.

Therefore, the standard will include the four analytes shown in Table 1.

Table 1 Analytes included in the standard.

Analyte	Formula	Synonym	CAS No.
4-nonylphenol (mixture of isomers)	$C_{15}H_{24}O$	NP	104-40-5, 25154-52-3
4-nonylphenol monoethoxylate (mixture of isomers)	$C_{17}H_{28}O$	NP1EO	26027-38-3
4-nonylphenol diethoxylate (mixture of isomers)	$C_{19}H_{32}O$	NP2EO	26027-38-2
4-tert-octylphenol *	$C_{14}H_{22}O$	OP	140-66-9

* (4-(1,1,3,3-tetramethylbutyl)phenol)

For the nonylphenol and the ethoxylates the analyte is a mixture of isomers. By the GC-MS analysis about 10-14 isomers have been found, and the gas chromatogram thus shows a numbers of more or less resolved peaks.

4-tert-octylphenol is a single compound.

2.3 Internal standards

The method is based on the use of internal standard calculations. The internal standards are added to the test sample and are taken through the whole analytical procedure.

The work began using only the D_4-labelled 4-n-nonylphenol as internal standard. Later in the studies the ^{13}C-labelled 4-n-nonylphenol diethoxylate was introduced as a second internal standard (see sub-sections 4.2 and 5.2). A good alternative to D_4-labelled 4-n-nonylphenol is the ^{13}C-labelled 4-n-nonylphenol.

At the ad-hoc group meeting in Vienna on 08 March 2005 it was furthermore agreed that non-labelled compounds may be used as alternatives, if it is shown that they are not present in the sample.

Thus the standard will mention the five internal standards shown in Table 2.

Table 2 Internal standards included in the method.

Analyte	Formula	Synonym
^{13}C-labelled 4-n-nonylphenol	$C_{15}H_{24}O$	^{13}C-NP
^{13}C-labelled 4-n-nonylphenol diethoxylate	$C_{19}H_{32}O$	^{13}C-NP2EO
D_4-labelled 4-n-nonylphenol	$C_{15}H_{20}D_4O$	NP-D4
Unlabelled 4-n-nonylphenol	$C_{15}H_{24}O$	4-n-NP
Unlabelled 4-n-nonylphenol diethoxylate	$C_{19}H_{32}O$	4-n-NP2EO

3. MATERIALS/SAMPLES

Samples for the experimental work are collected from several sources. Many samples are so-called playground samples made available through Horizontal Work Package 1, other samples are natural samples collected in Denmark, and one sludge sample was taken from the Danish Eurofins proficiency-testing scheme. A list of samples is given in Table 3.

Table 3 Description of samples used in the study.

Sample ID	Sample description	Pre-treatment	C_{org} wgt. %	Dry matter %	Approx. conc., mg/kg DM NP	NP1EO	NP2EO
SO-4	Clay soil, Speyer, Germany	Ball-milled and sieved < 125 µm	1.652		1.1	1.1	0.98
SO-9	Soil, Hagen, Germany	Ball-milled and sieved < 125 µm			0.23	0.57	0.20
SO-E1	Soil enriched with sewage sludge, DHI, Hoersholm, Denmark. 921916-01	Sieved < 1 mm		68.77	2.0	0.20	0.10
SL-4	Sewage sludge, domestic, Essen, Germany (= BCR 144)	Ball-milled and sieved < 125 µm	29.035		7.6	37	29
SL-11	Sewage sludge, electronic industry, Turin, Italy	Ball-milled and sieved < 125 µm	3.177		3.2	22	18
SL-E1	Sewage sludge, VKI, Hoersholm, Denmark			Ca. 100	41	6.1	1.5
SL-E2	Sewage sludge, domestic, Vejle, Denmark. 908134			28	27	5.9	< 0.05
SL-E3	Sewage sludge, domestic, Helsingør, Denmark. 920702			24	39	5.3	0.86
SL-E4	Sewage sludge, domestic, Marselisborg, Denmark. 914822-01			29	20	9.4	1.6
CW-1	Composted garbage, Munich, Germany	Dried and ball-milled	12.122		0.30	0.19	0.18
CW-5	Compost, Fulda, Germany		11.45		0.13	0.05	< 0.05

The samples SO-4, SO-9, SL-4, SL-11, CW-1 and CW-5 are playground samples from Horizontal Work Package 1 and a general characteristic of the samples is given in two reports /3/ and /4/.

Sample SL-E1 is a freeze-dried sludge from the Danish proficiency-testing scheme.

The samples SO-E1, SL-E2, SL-E3 and SL-E4 are samples collected in Denmark for various purposes.

4. EXPERIMENTAL WORK

The experimental work that has been carried out in order to gather the necessary information to develop a Horizontal Nonylphenol standard is explained in this chapter. The results of the experimental work are described in chapter 5.

Many preliminary studies are not included in the report, however the report contains the work that is the basis for drafting the horizontal standard method for nonylphenols.

The experimental work has included studies of the following elements:

- The GC-MS measurement including the derivatization step. First the derivatization method was chosen, and subsequently the procedure was optimized.
- Extraction. Several solvents and two extraction techniques were examined and one was chosen for further work. The extraction procedure was further optimized and documented.
- Pre-treatment of the sample. The use of wet and dried sample for the analysis was compared.
- Clean-up. A sample clean-up using silica column was examined.
- Storage and stability. Stability of extracts and derivatives has been studied.
- Calibration procedure. The choice of internal standards was changed due to the results obtained.

4.1 Derivatization and GC-MS measurement

Two GC-MS methods were set up to analyse two types of alkylphenol derivates (4.1.1). The performance of the derivatization reagents, Pentafluorobenzoyl chloride (PFBCl) and Methyl-N-(trimethylsilyl)-trifluracetamide (MSTFA), were subsequently tested and compared with the purpose of selecting the most suitable derivatization reagent (4.1.2).

4.1.1 Gas Chromatographic - Mass Spectrometric (GC-MS) measurement

A GC-MS method enabling the detection of PFBCl derivates of 4-tert-octylphenol (OP), nonylphenol (NP), deuterated 4-n-nonylphenol (NP-D4), nonylphenol monoethoxylate (NP1EO) and nonylphenol diethoxylate (NP2EO) was set up according to Wahlberg et al (1990) /5/. A second GC-MS method was set up to detect MSFTA derivates of the described alkylphenols according to a draft method from Northrhine Westfalia State Environment Agency (May 2004) /6/.

The methods were set up on a GC-MS (HP 5890 GC with a HP 5973 Mass Selective Detector from Agilent) equipped with an automatic liquid sampler (7683 Agilent) and a 5% Diphenyl Methyl Siloxane capillary column (30 m x 0.25 mm ID with 0.25 μm film thickness). The GC and MS settings of the two methods are presented in Table 4. The selection of ions was based on mass spectra from GC-MS scans of alkylphenol standard solutions, see Appendix 1. For the selected target ions and qualifier ions see Table 8 in sub-section 5.1.1.

Table 4 Settings and parameters of the two GC-MS methods, “PFBCl” and “MSTFA”, developed for the analysis of PFBCl- and MSTFA-derivates of the alkylphenols.

Parameter	“PFBCl-method”	”MSTFA-method”
Injection	Pulsed Splitless	Pulsed Splitless
Injection temperature	250 °C	250 °C
Temperature program	80°C (1 min) 30°C/min to 210°C (0 min) 10°C/min to 300°C	100°C (1 min) 10°C/min to 200°C (3 min) 10°C/min to 300°C (7 min)
Injection volume	1 µl	1 µl
Carrier Gas	Helium	Helium
Electron impact ionization	70 eV	70 eV
Transfer line	280 °C	280 °C

4.1.2 Choice of derivatization reagent

A study was carried out with the use of two derivatization reagents: (MSTFA) (silylation agent) and (PFBCl) for the analysis of OP, NP, NP-D4, NP1EO and NP2EO. The study was set up to evaluate and compare the derivatization reagents, which was done by measuring repeatability, linearity and limit of detection for the two GC-MS methods described in 4.1.1.

4.1.2.1 Derivatization procedure - MSTFA

The derivatization procedure with MSFTA was based on a draft method from Northrhine Westfalia State Environment Agency (May 2004) /6/.

According to the procedure a solution (extract or standard) was evaporated until dryness and 200 µl of MSTFA was added. After derivatization an appropriate volume of toluene was added and the solution was ready for GC-MS analysis. No derivatization time or temperature was described in the draft method. Based on other literature (Thuyne & Delbeke, 2003) /7/ the derivatization was carried out for 30 minutes at room temperature.

4.1.2.2 Derivatization procedure - PFBCl

The derivatization with PFBCl was based on a derivatization procedure described by Wahlberg et al (1990) /5/.

By the procedure 1 ml of toluene or isooctane was used and 10 µl PFBCl and 5 µl pyridine was added. The solution was heated for 15 minutes at 60°C. After the derivatization 10 ml 1 M sodium hydroxide was added to destroy excess of PFBCl and the mixture was shaken for one minute. After separation of the phases the organic phase was ready for analysis on GC/MS.

4.1.2.3 Comparing MSTFA and PFBCl

Five calibration standards at a concentration of 5 mg/l, 2 mg/l, 0,5 mg/l, 0,2 mg/l and 0,05 mg/l containing the compounds OP, NP, NP-D4, NP1EO and NP2EO were prepared in isooctane with internal standard phenanthrene-D10 (0,52 mg/l). The solutions were transferred (1,0 ml) to GC-vials and derivatized with MSTFA and PFBCl according to the respective derivatization procedures and analysed according to the described GC-MS methods (4.1.1). Four replicates of two calibration standards (2 mg/l and 0,05 mg/l) were likewise derivatized and analysed to enable the calculation of repeatability and to estimate the limit of detection. The replicates were

prepared from the same solution, but derivatized separately. No effort was made to improve the derivatization procedures or GC-MS methods. All calculations were based on phenanthrene-D10 as internal standard.

4.1.3 Amount of derivatization reagent (MSTFA)

Since MSTFA was chosen for the further work, studies were undertaken for the evaluation and optimisation of the use of MSTFA.

A study was conducted to estimate the smallest necessary amount of MSTFA required for the derivatization procedure. The study was based on an extract of a sewage sludge sample (SL-E2), which was derivatized with decreasing amounts of MSTFA. The responses of the MSTFA-derivates where finally compared and evaluated.

Two samples of 60 g sludge (SL-E2) were placed in 250 ml screw cap flasks and to each sample approximately 10 µg internal standard (NP-D4) was added. The two samples were extracted with 120 ml of DCM and acetone/pentane (1:1), respectively. The samples were shaken for 2 hours on a reciprocating shaker. The extracts were transferred to 100-ml screw cap flasks and dried with anhydrous sodium sulphate. From each extract 1,0 ml of organic solvent was transferred to six GC vials, which were evaporated to dryness and derivatized with 200, 100, 50, 25, 10 or 0 ul of MSTFA. After 30 min at room temperature the samples were re-dissolved in 800 ul isooctane. The solutions were analysed according to the GC-MS method described in 4.1.1 and the areas of MSTFA-derivates of NP, NP-D4 and NP1EO (NP2EO being below limit of detection) were used to compare the efficiencies of the MSTFA.

A second study was conducted to verify the results of the first study and to test the implication of a simplified derivatization procedure. Three derivatization procedures were tested on the previously described extracts: The first procedure was the one described in 4.1.2.1, the second procedure was using 10 µl MSTFA instead of 200 µl, and the third procedure was adding 25 µl MSTFA and isooctane simultaneously (i.e. 5% MSTFA in isooctane).

The three derivatization procedures were tested on the previously described sludge extract (n = 2) and analysed according to the GC-MS procedure described in 4.1.1. The areas of MSTFA-derivates of NP, NP-d4 and NP1EO (NP2EO < LOD) were used to compare the efficiencies of the derivatization procedures.

4.1.4 Efficiency of derivatization procedure

A test of the derivatization efficiency of a 5% MSTFA-solution was conducted. The test was based on a GC-MS analysis of the alkylphenols as well as their MSTFA-derivates, hence enabling the detection of possible non-derivatized alkylphenols.

Two 20 mg/l solutions containing NP, NP1EO and NP2EO was prepared in isooctane and 5% MSTFA, respectively. The two solutions containing the non-derivatized and derivatized alkylphenols, respectively, were analysed with a GC-MS SIM method set to monitor the ions characteristic for both derivatized and non-derivatized alkylphenols (Table 5):

Table 5 Selected ions for the detection of Nonylphenol, Nonylphenol monoethoxylate and Nonylphenol diethoxylate and their MSTFA-derivates.

Compound	Target ion	Qualifier ion	Qualifier ion
NP	135	149	220

NP1EO	179	193	
NP2EO	223	237	
MSTFA-derivate of NP	207	221	179*
MSTFA-derivate of NP1EO	251	265	207*
MSTFA-derivate of NP2EO	295	309	323

* The second qualifier ion of the MSTFA-derivate of NP and NP1EO was later changed to 193 and 279, respectively.

The ion chromatograms of the target ions in Table 5 were used to identify retention times, characteristic peaks and responses of the derivatized and non-derivatized alkylphenols. The areas of the characteristic peaks (or noise when no clear peaks were found) for possible non-derivatized alkylphenols in the 5% MSTFA solution were measured and compared with the areas of the peaks of the non-derivatized alkylphenols. The calculated proportions were used as "worst case scenario"-estimations of the derivatization efficiency.

4.1.5 Time of derivatization

By preliminary experiments it was proven that 30 minutes derivatization was sufficient to obtain the equilibrium stage, and there was no difference between 30 minutes and 2 hours.

Since the reaction is not stopped by chemical means, the derivatization may continue while the sample is waiting to be injected into the GC. The stability of the derivates is therefore critical, and this has been thoroughly examined, see sub-sections 4.5.2 and 5.5.2.

In the ISO Technical Committee for Water quality ISO/TC 147 a draft method for alkylphenols has been presented in working group 17 /8/. In this working draft for water the same derivatization with MSTFA is used as in the horizontal standard, and the method for water describes a reaction time of only ½ minute. Also in this method the reaction is not stopped, so only a minimum time can be stated.

In most of the present work a minimum reaction time of 15 minutes has been used. This was also the case for the experiments of the derivatization efficiency discribed in sub-section 4.1.4 and 5.1.4.

4.2 Extraction

The extraction procedure was developed through a number of studies which were carried out in order to examine the influence of several parameters such as extraction solvent, extraction technique, extraction time, pre-treatment (use of dry or wet sample), etc.

Although the influence of several parameters often was examined simultaneously, the different parameters are described separately in the following text.

For the evaluation of recovery by the analysis of solid samples several possibilities exist. Since a 100% recovery of the analyte can often not be obtained, and since the recovery of spiked analytes are different from the recovery of the analytes already present in the sample, a procedure using spiking with standard solutions will often create too optimistic recoveries. In the present work the extraction efficiency was chosen as the main criteria by which the parameters of extraction were compared. The calculations of extraction efficiency were based on calibration with external standards or based on an internal standard added after the extraction.

4.2.1 Extraction solvent

Extractions of nonylphenol (NP), nonylphenol monoethoxylate (NP1EO) and nonylphenol diethoxylate (NP2EO) from various solid matrixes have been conducted with the purpose of comparing the extraction efficiencies of toluene, dichloromethane (DCM), ethyl acetate and acetone/pentane (1:1). The influence of parameters as extraction time, extraction technique, pre-treatment, sample-to-solvent ratio and water content on the extraction efficiency was likewise examined.

Pentane was used as a representative of an alkane based hydrocarbon solvent, called “Hexane-like solvent”.

4.2.1.1 Comparison of toluene, dichloromethane (DCM), ethyl acetate and acetone/pentane

Two studies of extraction efficiency were conducted: One study comparing toluene, DCM and acetone/pentane extractions of a freeze-dried sludge sample (SL-E1) and another study comparing toluene, ethyl acetate and acetone/pentane extractions of a freeze-dried sludge sample (SL-11).

The first study (comparing toluene, DCM and acetone/pentane) examined the influence of varying the extraction time, water and sulphuric acid content, and the second study (comparing toluene, ethyl acetate and acetone/pentane) included a comparison of the extraction techniques soxhlet and reciprocating shaking (4.2.2).

The sample (approximately 2 g) was extracted on a reciprocating shaker for 2 and 16 hours. The extractions were conducted on freeze-dried sample, freeze-dried sample added 10 or 50 ml of water and freeze-dried sample added 55 ml of 0,4 M sulphuric acid. It was, however, decided not to examine the effect of 50 ml water and 50 ml sulphuric acid when extracting with acetone/pentane.

Except for the described changes in extraction time, extraction solvent, water and sulphuric acid, the extraction procedure was conducted according to the Horizontal method sub-section 10.1.2 (Appendix 9). The final extract, however, was not washed with water as described. All calculations of concentrations were based on external standard.

The second study comparing toluene, ethyl acetate and acetone/pentane is described in 4.2.2.

4.2.1.2 Comparison of ethyl acetate, dichloromethane and acetone/pentane

A total of 12 extractions were set up to compare the extraction efficiencies of ethyl acetate, DCM and acetone/pentane. The extractions were conducted on a freeze-dried soil sample (SO-4). The extraction time was 2 hours and the chosen extraction technique was shaking. Half of the samples (6 samples) were added 5 ml of water and the other half were added 20 ml of water. One half of the samples were, furthermore, spiked with 25 µg of NP, NP1EO and NP2EO. Besides the described changes the extraction procedure was conducted according to the Horizontal standard method sub-section 10.1.2 (Appendix 9). The final extract, however, was not washed with water as described. The calculations were based on external standard.

A second set of extraction (a total of 9 extractions) was conducted to further investigate the extraction efficiencies of ethyl acetate, DCM and acetone/pentane (1:1). The extractions were conducted on a sludge sample (SL-E2), which was divided into 9 sub samples of approximately

2 g dm. The effect of a) freeze-drying, b) adding 50 ml of water and c) no pre-treatments (wet sludge) on the extraction efficiency was tested in triplicates. The samples were added 10 µg NP2EO and 2 µg of internal standard (NP-D4) before the extractions. The samples were extracted for four hours on a reciprocating shaker. After the extraction an additional internal standard (10 µg phenanthrene-D10) was added to the extracts. The extractions were, except for the described changes, conducted according to the Horizontal standard method sub-section 10.1.1 and 10.1.2 (Appendix 9). The calculations were based on phenanthrene-D10 as internal standard.

4.2.1.3 Comparison of dichloromethane (DCM) and acetone/pentane

A study was conducted to compare the extraction efficiencies of DCM and acetone/pentane (1:1). In this study the extractions were conducted on a sludge sample (SL-E2) with 28% dry matter which was divided into three sub samples. The first sub-sample was oven-dried, to the second sample was added an additional amount of water and the third sub-sample was without any pre-treatment (Table 6). Approximately 2 grams of dry sludge was used.

Table 6 Conditions of the DCM- acetone/pentane-extraction of SL-E2.

Parameter	Variable 1	Variable 2	Variable 3
Solvent	DCM	Acetone/Pentane (1:1)	-
Extraction time	2 hours	20 hours	-
$V_{solvent}$	15 ml	30 ml	-
Pre-treatment	Oven-dried	None	50 ml of water
pH regulation*	H_2SO_4	None	NaOH

* Sulphuric acid and sodium hydroxide were only applied in the 20-hour extraction.

Except for the described changes the extractions were conducted according to the Horizontal NP-method sub-sections 10.1.1 and 10.1.2 (Appendix 9). The concentrations calculations were based on external standard.

4.2.2 Extraction technique

A sludge sample (SL-11) was used to compare the extraction efficiency of Soxhlet and a reciprocating shaker. The soxhlet extractions were conducted with acetone/pentane and toluene. The extractions with reciprocating shaking were conducted with toluene, ethyl acetate and acetone/pentane.

4.2.2.1 Soxhlet-procedure

Approximately 4 x 5 g of freeze-dried sludge was transferred to four thimbles (22 x 80 mm) and one empty thimble was used for the blank determination. The Soxhlet equipment was cleaned with pentane (2 cycles of extraction) and the solvent was discarded before the sample was added. A volume of 100 ml extraction solvent was used and the samples were extracted for 5 hours (equivalent to approximately 100 cycles of extraction). The internal standards NP-D4 and 4-n-NP2EO were added to the samples before the extractions (2 µg of each) and the recovery was later calculated. The samples were extracted with acetone/pentane (1:1) and toluene in duplicates (n = 2). After the extraction the solvent was evaporated until dryness on a rotary evaporator and re-dissolved in 10 ml 5% MSTFA in isooctane. An additional internal standard (2,5 µg of OP) was added to the extract and used for quantification of the alkylphenols.

4.2.2.2 Shaking-procedure

Approximately 6 x 2,5 g of freeze-dried sludge was transferred to six 100-ml screw cap flasks. One screw cap flask was kept free of sample for the blank determination. A volume of 20 ml extraction solvent was added to the samples and the samples were extracted for 2 hours. Ethyl acetate was tested in addition to toluene and acetone/pentane. The internal standards NP-D4 and 4-n-NP2EO were added to the samples before the extraction (2 µg of each) and the recovery was later calculated. The samples were extracted with acetone/pentane (1:1), ethyl acetate and toluene in duplicates (n = 2). After the extraction OP was added as an additional internal standard (equal to soxhlet extracts) and the extracts were further derivatized and analysed according to the NP horizontal standard described in Appendix 9.

4.2.3 Extraction time

The investigation of extraction time was based on two sludge samples, SL-E2 and SL-4. The samples were extracted for ½, 1, 2, 3, 4, 6 and 20 hours. The extractions were done in duplicates.

The extraction and subsequent analysis was carried out according to the Horizontal standard method (Appendix 9). Each sample was additionally spiked with 100 µl of 100 ppm NP2EO and 100 µl phenanthrene-D10. The spike with NP2EO was done prior to the extraction to compensate for a low content of NP2EO, and the spike with phenanthrene-D10 was done after the extraction. All calculations were based on phenanthrene-D10 as internal standard.

4.3 Pre-treatment

From the beginning of the project it was decided that the horizontal standard, if possible, should allow the use of wet as well as dried (probably freeze-dried) samples. The reason for this being, that different European countries have different routines for handling the samples.

Therefore the extraction efficiency was compared for wet, freeze-dried and oven-dried samples. Two separate studies were carried out. The addition of water to a sample was not regarded as pre-treatment but as a part of the method, and is therefore described in 4.2.4.

4.3.1 Oven-drying

The first study was conducted to compare the extraction efficiency for a sludge sample SL-E2 subjected to either freeze-drying or no pre-treatment. Various parameters effecting extraction efficiency were also tested, they are, however, not described here (se 4.2.1.3).

4.3.2 Freeze-drying

A second study (previously described in 4.2.1.2) was conducted to compare the extraction of freeze-dried and wet sludge (SL-E2). The freeze-drying was conducted according to ISO/FDIS 16720:2003 /9/. The differences in extraction efficiencies of the three solvents ethyl acetate, DCM, and acetone/pentane (1:1) were, among other things, also tested (see sub-section 4.2.1.2 for details). Extracts of all three solvents were analysed to examine the effect on extraction efficiency of the two means of pre-treatment. Each parameter was tested in triplicates (n=3).

4.3.3 Addition of water to freeze-dried sludge

Based on previous studies it was decided to test whether the addition of water to freeze-dried sludge would increase the extraction efficiency of acetone/pentane. A large amount of sludge sample (SL-E2) was freeze-dried and from this 9 sub-samples of approximately 2 g were transferred to 100-ml screw cap flasks. Standard addition of 100 µl of a 100 mg/l NP2EO was applied to the samples. Dry matter percentages of approximately 100%, 30% and 5% was obtained by adding 0, 6 and 40 ml of water prior to the extraction. After the extraction 100 µl of 100 mg/l phenanthrene-D10 was added to the organic phase in the 100-ml screw cap flasks. The subsequent analysis were carried out according to the Horizontal standard method (Appendix 9).

4.3.4 Influence of water content

Preliminary studies had shown, that the alkylphenols may be poorly recovered when the water content was too high. Especially the diethoxylates could be lost in the extraction, probably due to their high water solubility.

A study was therefore conducted to examine the effect of water on the extraction of NP, NP-D4, NP1EO and NP2EO from sludge. For this purpose subsamples of approximately 10 g wet sludge (SL-E2) was transferred to 250 ml flasks, added 200, 50, 20, 10, 5 and 0 ml of water, thereby obtaining dry matter percentages of 1%, 5%, 10%, 15%, 20% and 28%. NP2EO and NP-D4 was added to the samples before the addition of water. NP2EO was added due to a low content (below LOD) in the sludge. Each sample was additionally added 10 µg phenanthrene-D10 after the extraction. The treatments were made in triplicates.

The results of the above-described study initiated a new investigation on the influence of water on extraction efficiency. This study had two purposes: a) to test the use of ^{13}C-labeled 4-n-NP2EO as internal standard for NP2EO and b) to increase the extraction efficiency by filtering sludge with a dry matter content of 5% and below.

The study was conducted by transferring approximately 10 g sludge (SL-E2) to 250 ml flasks, subsequently adding 200, 100, 50, 20 and 0 ml of water, thereby obtaining dry matter percentages of 1%, 2%, 5%, 10% and 28%. The samples with 10 and 28% dm were prepared in triplicates, whereas six replicates were prepared for the samples with 1, 2 and 5% dm. The compounds NP2EO, NP-D4 and ^{13}C-labeled 4-n-NP2EO was added to the samples before the addition of water. After approximately half an hour of shaking, three (of the six) replicates of the sludge samples with dry matter content 1-5% were filtered through a GF/C-filter (1,2 µm, Whatman). The filtering process proceeded until the dry matter content was above 10%. The filtered samples (with filters) and un-filtered samples were subsequently extracted and further analysed according to the Horizontal standard method (appendix 9). The calculations were based on phenanthrene-D10 as internal standard.

4.4 Clean-up methods

A total of four clean-up studies were conducted to test the ability of solid phase extraction (SPE) columns to clean up extracts containing NP, NP-D4, NP1EO, NP2EO and 4-n-NP2EO. The investigations were based on standard solutions and sludge extracts and the clean-up was conducted on a 500 mg silica column (Bond Elut LRL-SI). After the addition of standards or sample extracts to the column solvents with increasing polarity were added, and the elution pattern of the analytes and internal standards were determined by analysis of the fractions collected from the column. Table 7 shows the applied in the four clean-up studies.

Table 7 Description of the clean-up studies.

	Samples			
	1. Study	*2. Study*	*3. Study*	*4. Study*
Samples Solvents	Standard 2,5	Standard 2,5 SL-E4	SL-E4	SL-E4 SL-E3 SL-11
Pentane	4 ml	-	2 x 2 ml	3 x 3 ml
Pentane/DCM (1:1)	4 ml	2 x 2 ml	-	-
DCM	2 x 2 ml	-	-	-
DCM/acetone (3:1)*	2 x 2 ml	3 x 2 ml	3 x 2 ml	2 x 3 ml
DCM/acetone (1:1)	2 x 2 ml	2 ml	2 ml	-
Acetone	4 ml	2 ml	2 ml	6 ml

* Prepared by mixing 3 volumes of DCM with 1 volume of acetone.

First clean-up study:
In this initial study the clean-up of a 2,5 mg/l standard solution was examined on a 500 mg silica column. The concentration of internal standards was 0,2 mg/l. Pentane (5 ml) was added to two SPE-columns in order to activate the silica surface and remove air bobbles from the column. The pentane was eluted until reaching the top of the column and 1 ml of the standard solution was applied (n = 2). Once more the solution was eluted until reaching the top of the column and the eluent was collected. This procedure of adding solvent and collecting the eluent was repeated with 4 ml pentane, 4 ml pentane/DCM (1:1), 2 x 2 ml DCM, 2 x 2 ml DCM/acetone (3:1), 2 x 2 ml DCM/acetone (1:1), and 4 ml acetone (the first two fractions were collected as one). All 9 fractions were added 25 µl of a 25 mg/l additional internal standard (OP), derivatized and analysed according to the Horizontal standard method (Appendix 9). The calculation of recoveries was based on OP as internal standard, mainly to compensate for the variation in volume.

Second clean-up study:
The clean-up procedure was changed and tested on 1 ml of a standard solution and 1 ml of a sludge extract (SL-E4). The columns were eluted with 3 x 2 ml pentane/DCM (1:1), 3 x 2 ml DCM/acetone (3:1), 2 ml DCM/acetone (1:1) and 2 ml acetone. The additional internal standard (OP) was added, and the analysis of the 8 fractions and the calculations were conducted as in the first clean-up study.

Third clean-up study:
Based on the obtained results, the clean-up procedure was changed according to Table 8. After application of the sludge extract (SL-E4) the silica column was eluted with 2 x 2 ml pentane, 3 x 2 ml DCM/acetone (3:1), 2 ml DCM/acetone (1:1) and 2 ml acetone. The additional internal standard (OP) was added, and the analysis of the 7 fractions and the calculations were conducted as in the first clean-up study.

Fourth clean-up study:
A final test of the clean-up procedure was conducted. In this study extracts of three sludge samples (SL-E4, SL-E3 and SL-11) were applied to three columns. The columns were eluted with 3 x 3 ml pentane, 2 x 3 ml DCM/acetone (3:1) and 3 ml acetone. The additional internal standard (OP) was added, and the analysis of the 6 fractions and the calculations were conducted as in the first clean-up study.

4.5 Storage and stability

As part of the work stability studies were carried out for the samples as well as for the standard solutions before and after derivatization.

4.5.1 Samples

Stability studies have been carried out to investigate the degradation of nonylphenol polyethoxylates in sewage sludge. This will be published in a separate report.

4.5.2 Solutions and extracts

The stability of NP, NP1EO and NP2EO and their MSTFA-derivates was tested at three different temperatures: a) 22°C ± 3°C (room temperature), b) 4°C ± 3°C (refrigerator) and c) -18°C ± 3°C (freezer). The stability was tested on two calibration standards (0,1 mg/l and 2,5 mg/l) and one extract of a sludge sample (SL-E2). All three solutions were tested as the alkylphenols and as the derivatized alkylphenols. The stability of NP-D4, NP, NP1EO and NP2EO was measured in all solutions.

Approximately 40 g of sludge was added 1000 µl of 20 mg/l internal standard (NP-D4) and 400 µl of 100 mg/l NP2EO. The sludge was extracted on a reciprocating shaker for 2 hours with 300 ml of acetone/isooctane (1:1). After the extraction the extract was transferred into two 50-ml volumetric flasks to which 2,5 ml MSTFA and 2,5 ml isooctane was added respectively. Calibration standards of 0,1 mg/l and 2,5 mg/l were prepared in the same way.

The six solutions were transferred (1,0 ml) to 50 GC-vials and stored a) at room temperature, b) in the refrigerator or c) in the freezer and analysed after 0, 1, 2, 4, 8, 16, 38 and 80 days of storage.

Before each analysis the vials were added 50 µl of a 40 mg/l internal standard solution containing OP. To the un-derivatized solutions 25 µl MSTFA was furthermore added. The calculation of concentrations was based on OP.

5. RESULTS AND DISCUSSION

The results are presented in this chapter in the same order as in chapter 4. To facilitate the reading the same subdivision and headlines are used in chapter 5 as in chapter 4.

5.1 Derivatization and GC-MS measurement

The derivatizations and GC-MS measurements were examined in a combined study, since they are so close related.

5.1.1 Gas Chromatographic - Mass Spectrometric (GC-MS) method

The GC-MS methods described in Table 4 in sub-section 4.1.1 was initially set to record a scan of two solutions containing the MSTFA- and PFBCl-derivates of OP, NP, NP1EO and NP2EO (Figures 1 and 2). The selected ion monitoring (SIM) GC-MS method was created subsequently (4.1.1).

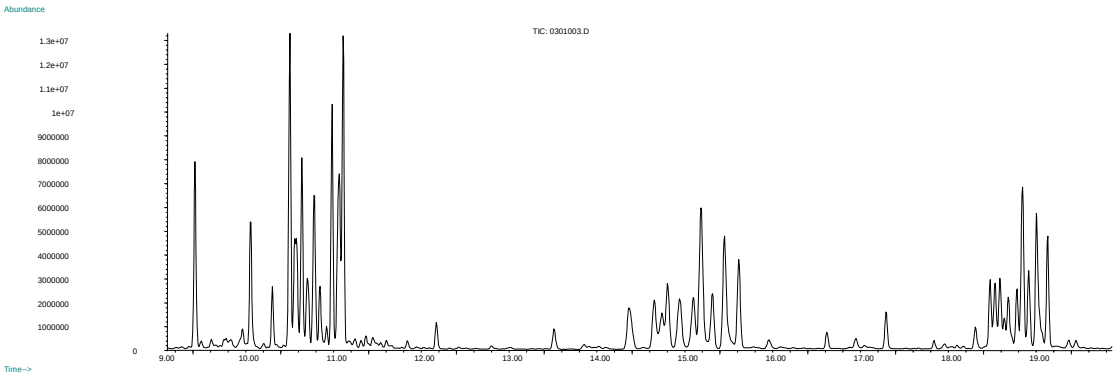

Figure 1. A total ion chromatogram (TIC) of MSTFA derivates of OP, NP, NP1EO and NP2EO obtained from a GC-MS scan.

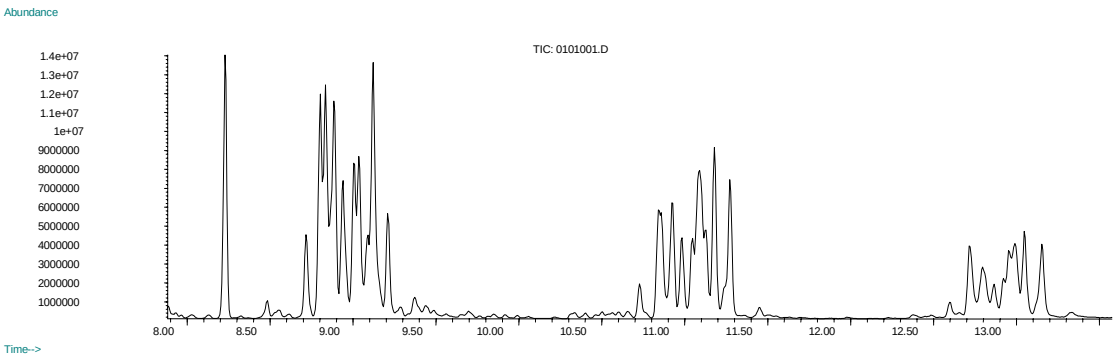

Figure 2. A total ion chromatogram (TIC) of PFBCl derivates of OP, NP, NP1EO and NP2EO obtained from a GC-MS scan.

Mass spectra obtained from the chromatograms in Figures 1 and 2 were used to select the target and qualifier ions of OP, NP, NP1EO and NP2EO. The mass spectra are shown in Appendix 1 and the selected ions are shown in Table 8.

Table 8 Selected target and qualifier ions of PFBCl- and MSTFA-derivates of OP, NP, NP-D4, NP1EO, NP2EO and phenanthrene-D10.

	PFBCl-derivate		MSTFA-derivate	
Compounds	Target ion	Qualifier ion(s)	Target ion	Qualifier ion(s)
OP	195	329	207	191
NP	195	329, 343, 301	207	221,179
NP1EO	239	195, 373	251	265, 207
NP2EO	417	195, 431	295	309, 207
NP-D4	195		183	
Phenanthrene-D10*	188		188	

* Phenanthrene-D10 is not derivatized and is used for calculation of recovery etc.

5.1.2 Choice of derivatization reagent

For the evaluation and comparison of the two derivatization reagents the following parameters were chosen: The chromatographic pattern and background, linearity, repeatability, and limit of detection. All measurements in this chapter are made by use of the SIM mode.

5.1.2.1 Chromatography

The target ions of Table 8 were used for the Selected Ion Monitoring GC-MS methods described in 4.1.2. The ion chromatograms of NP, NP1EO and NP2EO in 2 mg/l and 0.05 mg/l standards were extracted and used to evaluate the performance of the derivatization reagents, see Figures 3-6.

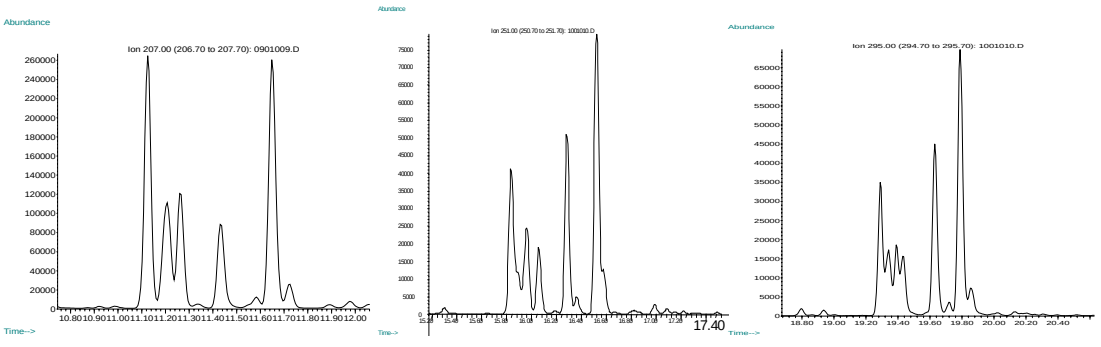

Figure 3. The extracted ion chromatograms of a 2 mg/l standard containing MSTFA-derivates of: a) NP, b) NP1EO and c) NP2EO.

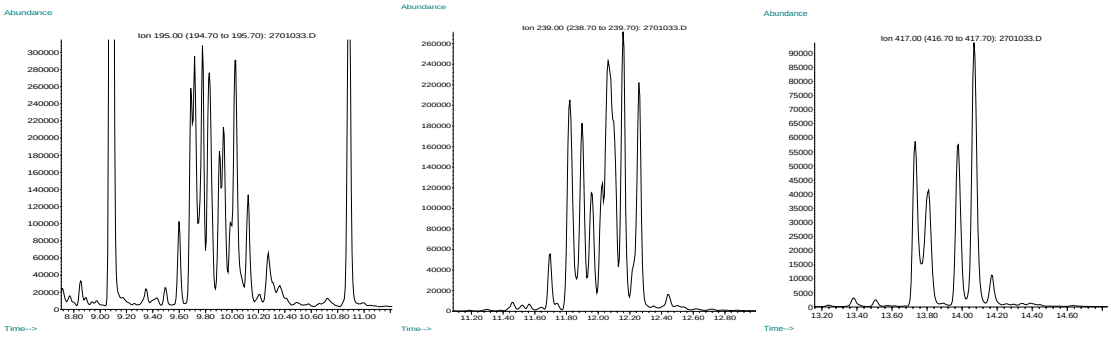

Figure 4. Extracted ion chromatograms of a 2 mg/l standard containing PFBCl-derivates of: a) NP, b) NP1EO and c) NP2EO.

When comparing the ion chromatograms of the 2 mg/l standard (Figures 3 and 4) the responses of the two derivates of NP and NP2EO are rather similar, whereas the response of the PFBCl-derivates of NP1EO are significantly higher than the respective MSTFA-derivates. The chromatograms of the 0.05 mg/l standard (Figures 5 and 6) show the same relations between the MSTFA- and PFBCl derivates of NP, however, for NP1EO and NP2EO the PFBCl derivate has a relatively lower response. This is further described in sub-section 5.1.2.2.

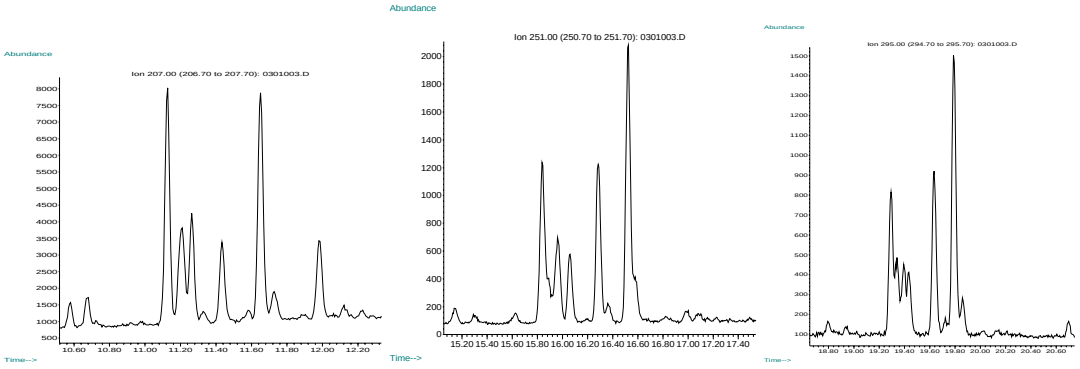

Figure 5. Extracted ion chromatograms of a 0.05 mg/l standard containing MSTFA derivates of: a) NP, b) NP1EO and c) NP2EO.

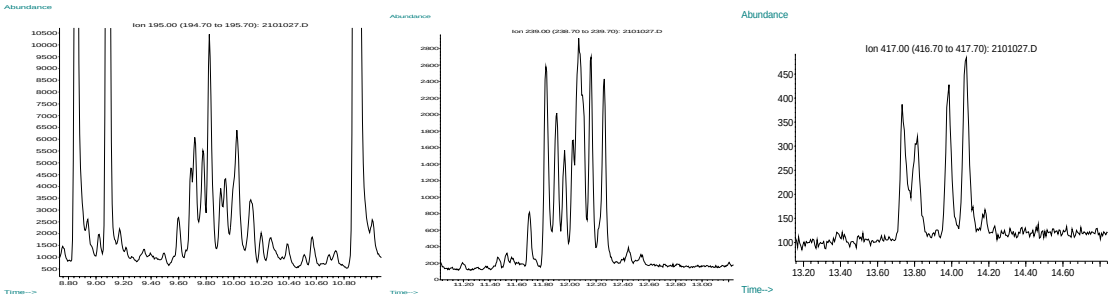

Figure 6. Extracted ion chromatograms of a 0.05 mg/l standard containing PFBCl derivates of: a) NP, b) NP1EO and c) NP2EO.

More important, however, is the presence of interfering peaks and the signal-to-noise (S/N) ratio. In Figures 5 and 6 some foreign peaks are seen in the NP window for PFBCl derivates, however, they are not interfering with the NP mixture. The signal-to-noise (S/N)-ratios of the

MSTFA-derivates of NP and NP2EO is higher than the respective PFBCl-derivates, whereas the S/N-ratios of the MSTFA- and PFBCl-derivates of NP1EO is similar.

5.1.2.2 Linearity

Calibration curves were produced based on five standards containing OP, NP, NP1EO and NP2EO and they are presented in Figure 7. Equations and linearity (measured as the regression coefficient r^2) of the calibration curves are shown in Table 9.

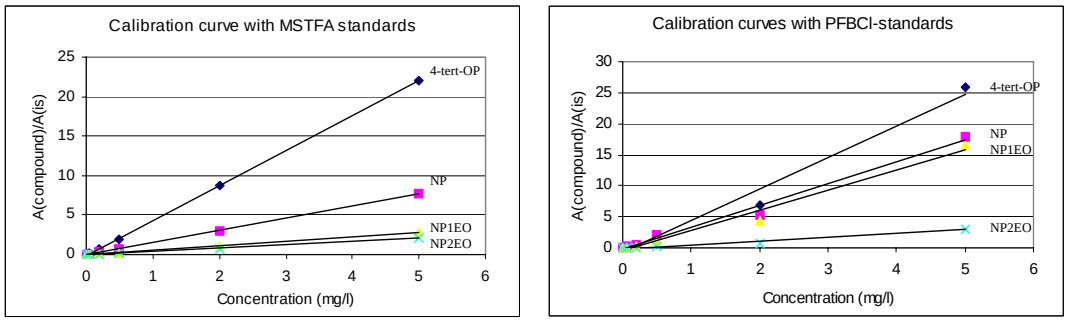

Figure 7. Calibration curves of OP, NP, NP1EO and NP2EO derivatized with a) MSTFA and b) PFBCl.

The slopes of the calibration curves reveal a higher response of the PFBCl derivates of NP and NP1EO compared to the respective MSTFA derivates. The slopes of the calibration curves of OP and NP2EO indicate, however, no difference in response obtained by the two types of derivatization. The linearity (r^2) is highest when the derivatization is conducted with MSTFA (Table 9).

Table 9 Equations and linearity (r^2) of the calibration curves of MSTFA- and PFBCl-derivates of OP, NP, NP1EO and NP2EO.

	MSTFA-derivate		PFBCl -derivate	
Compounds	Equation	r^2	Equation	r^2
OP	y = 4.41x – 0.0945	0.9999	y = 5.09x – 0.700	0.9819
NP	y = 1.55x – 0.0299	0.9997	y = 3.51x – 0.200	0.9859
NP1EO	y = 0.552x – 0.0235	0.9995	y = 3.27x + 0.0482	0.9837
NP2EO	y = 0.412x – 0.0341	0.9982	y = 0.607x – 0.117	0.9775

5.1.2.3 Standard deviation

The standard deviation (*s*) of four calibration standards at two levels (2 mg/l and 0.05 mg/l) was calculated. The standard derivation was based on standards of the same solution, which was derivatized separately as described in sub-section 4.1.2. Repeatability was calculated for OP, NP, NP1EO and NP2EO, see Table 10.

Table 10 The standard derivation of the MSTFA- and PFBCl-derivates of OP, NP, NP1EO and NP2EO (n = 4).

	Standard derivation (mg/l)

Compound	MSTFA derivates		PFBCl derivates	
	0.05 mg/l	2 mg/l	0.05 mg/l	2 mg/l
OP	0.00010	0.0082	0.0050	0.077
NP	0.0029	0.0087	0.0031*	0.18
NP1EO	0.0016	0.013	0.00087	0.016
NP2EO	0.0033	0.012	0.0028	0.060

* One outlier was removed, which resulted in a reduction in standard derivation from 0.012 to 0.0031 mg/l.

The repeatabilities shown in Table 10 only cover the derivatization and GC-MS step of the method. The MSTFA derivates show an equal or better repeatability than the PFBCl derivates.

5.1.2.4 Limit of detection

Estimates of the limit of detection (LOD) of the two methods were calculated from the standard deviation of a low standard according to the equation

$$LOD\ (mg/l) = t_{0.995}(f) \times s_{0.05\ mg/l} \approx 5 \times s_{0.05\ mg/l}$$

The estimated limits of detection for the derivatized alkylphenols are shown in Table 11. The estimates show very similar LODs of the two types of derivates.

Table 11 Limit of detection estimated for the MSTFA and PFBCl derivates of OP, NP,NP1EO and NP2EO (n = 4).

	Limit of detection (mg/l)*	
Compound	MSTFA	PFBCl
OP	0.00051	0.025
NP	0.014	0.015
NP1EO	0.0078	0.0044
NP2EO	0.017	0.014

* The limit of detection.

5.1.2.5. Choice of reagent

Based on the relatively poor chromatography of the PFBCl-derivates (NP and NP2EO-derivates) and the higher standard derivation of both a high and a low standard (Table 10) it was decided not further to investigate the derivatization with PFBCl.

5.1.3 Amount of derivatization reagent (MSTFA)

The effect of reducing the quantity of derivatization reagents was tested in two studies. The first study measured the responses of NP-D4, NP and NP1EO in two sludge-extracts derivatized with 200, 100, 50, 25 or 10 µl of MSTFA. The results are presented in Figure 8, which shows the responses of the compounds calculated in percentage of the average response of the five MSTFA-derivations. The derivatizations were conducted on both a DCM and an acetone/pentane-extract of a sludge sample (NOVANA 2204).

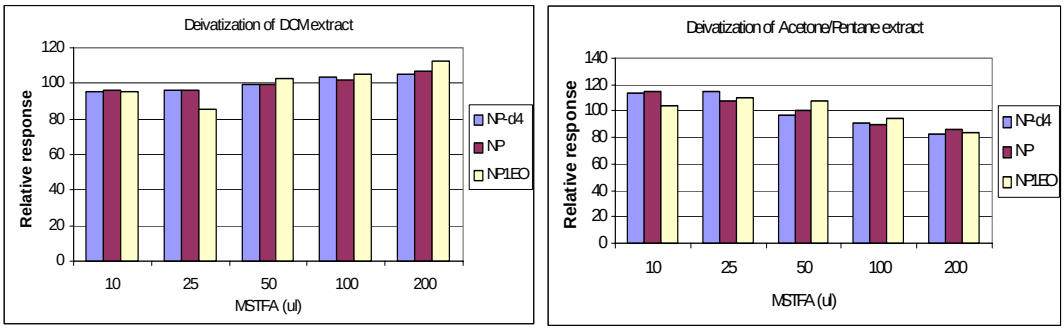

Figure 8. The responses (%) of NP-d4, NP and NP1EO treated with varying amounts of MSTFA (n = 1). The responses are calculated relative to the average response of the five treated extracts.

A slight increase in response was observed for the DCM-extract, when the amount of MSTFA was increased. For the acetone/pentane-extracts, however, the response was found to decrease with increasing amount of MSTFA. This decrease in response was, however, caused by decreasing response during the GC-MS analysis and was also observed for the calibration standards measured during the GC-MS run. The changes were not caused by a change in the derivatization yield, and it was concluded that no reduction in derivatization efficiency was observed.

Based on the results of Figure 8 a second study of derivatization efficiency was conducted (4.1.3). Sludge extracts were derivatized according to sub-section 4.1.2.1 with 200 or 10 ul of MSTFA. In addition the effect of adding MSTFA and isooctane simultaneously was also tested. This was done by adding 1 ml of a solution of 5% MSTFA in isooctane.

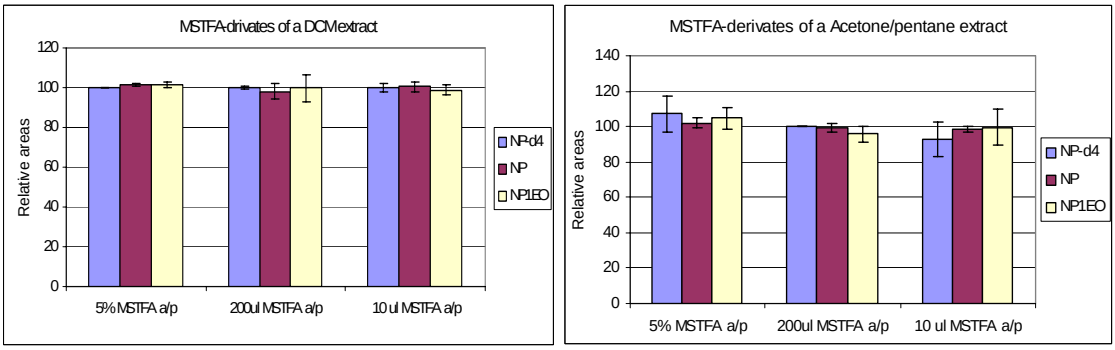

Figure 9. The responses (%) of NP-D4, NP and NP1EO in a) DCM-extract and b) Acetone/Pentane-extract submitted to different derivation procedure. The responses are calculated relative to the average response of the differently treated extracts. The error bars indicate standard derivation of the replicates (n = 2).

The results are presented in Figure 9, and the results did not show any significant difference in derivatization efficiency for the three different ways of adding the MSTFA. Although 10 µl MSTFA was found to be sufficientt in this study, other heavily polluted extracts may require a larger quantity for complete derivatization, and it was therefore decided to use 50 µl of MSTFA. To facilitate the procedure it was furthermore decided to add MSTFA and isooctane simultaneously. The derivatization procedure was therefore changed, so that 1 ml of 5% MSTFA in isooctane is added instead of the previously used derivatization with 200 µl MSTFA followed by the addition of 800 µl isooctane.

5.1.4 Efficiency of derivatization procedure

The derivatization efficiency was determined by comparing the un-derivatized and the

derivatized alkylphenols after derivatization with 5% MSTFA in an isooctane solution.

Extracted ion chromatograms of NP, NP1EO and NP2EO (135, 179, 223) and MSTFA-derivates of NP, NP1EO and NP2EO (207, 251, 295) were used to calculate the derivatization efficiency of MSTFA. A 20 mg/l solution of MSTFA-derivatized alkylphenols were analysed. The ions characteristic for the un-derivatized alkylphenols were subsequently extracted and compared to extracted ion chromatograms of a 20 mg/l solution containing un-derivatized alkylphenols. The derivatization efficiency was here determined as the remaining fraction of un-derivatized alkylphenols after the derivatization. In practice is was calculated as the fraction of un-derivatized alkylphenol residues in a 5% MSTFA solution relative to the same un-derivatized alkylphenols in an isooctane solution.

Derivatization efficiency of NP:
The GC did not completely separate NP and the derivates of NP and the ions characteristic for NP are also found to be characteristic for the NP-derivate. It is therefore difficult to differentiate between fragment-ions from NP-derivates and from un-derivatized NP. However by comparing the pattern of peaks of NP and NP-derivates, no peaks in the MSTFA-solution indicate the presence of un-derivatized nonylphenol (Figure 10).

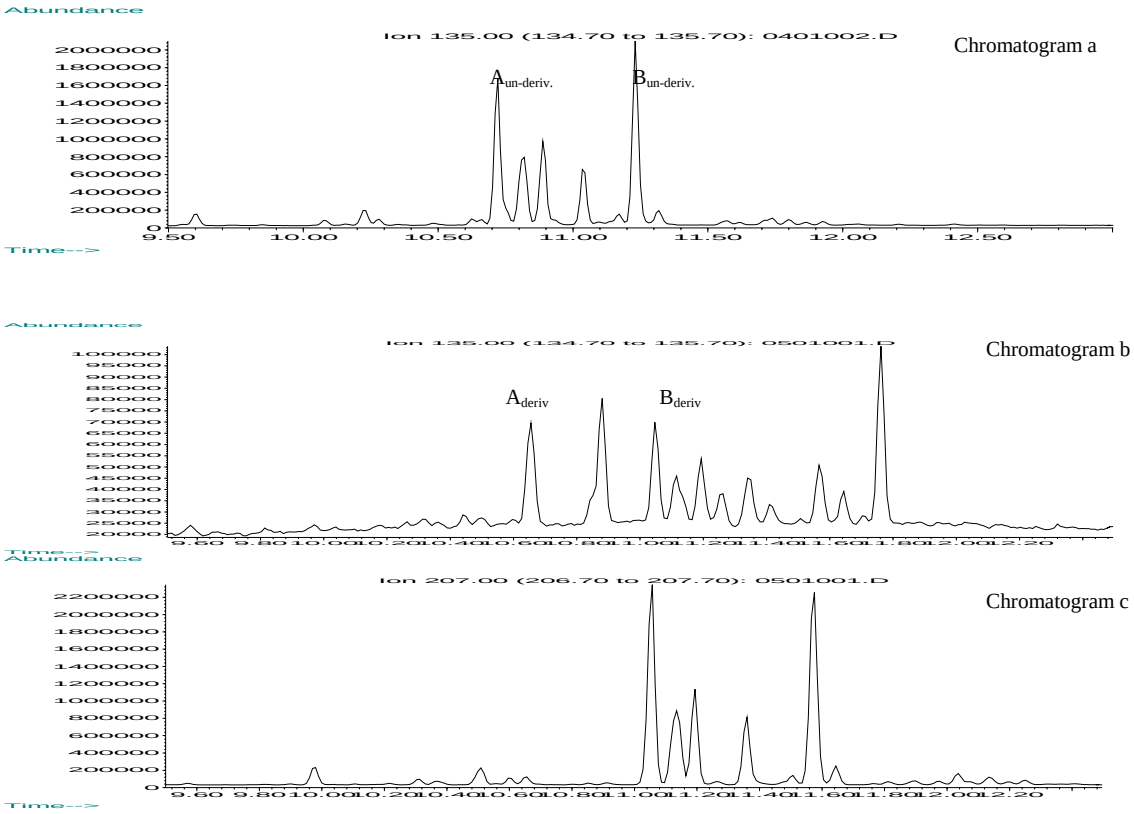

Figure 10: Chromatograms of a) a 20 mg/l un-derivatized NP (ion 135), b) a 20 mg/l derivatized NP (ion 135) and c) a 20 mg/l derivatized NP (ion 207).

A “worst case scenario”-calculation of the derivation efficiency is estimated by measuring the response of two peaks characteristic for un-derivatized NP ($A_{un\text{-}deriv.}$ and $B_{un\text{-}deriv.}$ in chromatogram *a* Figure 10) and calculating the response relatively to the response of corresponding peaks/background of the derivatized NP with the same retention time ($A_{deriv.}$ and $B_{deriv.}$ in chromatogram *b*, Figure 10). The calculated ratios ($A_{un\text{-}deriv}/A_{deriv.}$ and $B_{un\text{-}deriv}/B_{deriv)}$ are calculated and used as an estimate of the derivatization efficiency.

Fraction of un-derivatized NP: $\frac{\text{MSTFA}-\text{derivate}_{\text{ion135}}\ ^{*}}{\text{Derivatized}_{\text{ion135}}} =$

$$\frac{A_{un-deriv.}}{A_{deriv}} = \frac{1{,}4\cdot 10^{6}}{28\cdot 10^{6}} = 4{,}8\% \quad \text{and} \quad \frac{B_{un-deriv.}}{B_{deriv}} = \frac{0{,}71\cdot 10^{6}}{32\cdot 10^{6}} = 2{,}2\%$$

*) Un-derivatized ≈ Noise, fragment ions of derivatized NP and actual un-derivatized NP.

Derivatization efficiency of NP1EO:
NP1EO and the derivate of NP1EO are completely separated and there are no indications of un-derivatized NP1EO (Figure 11).

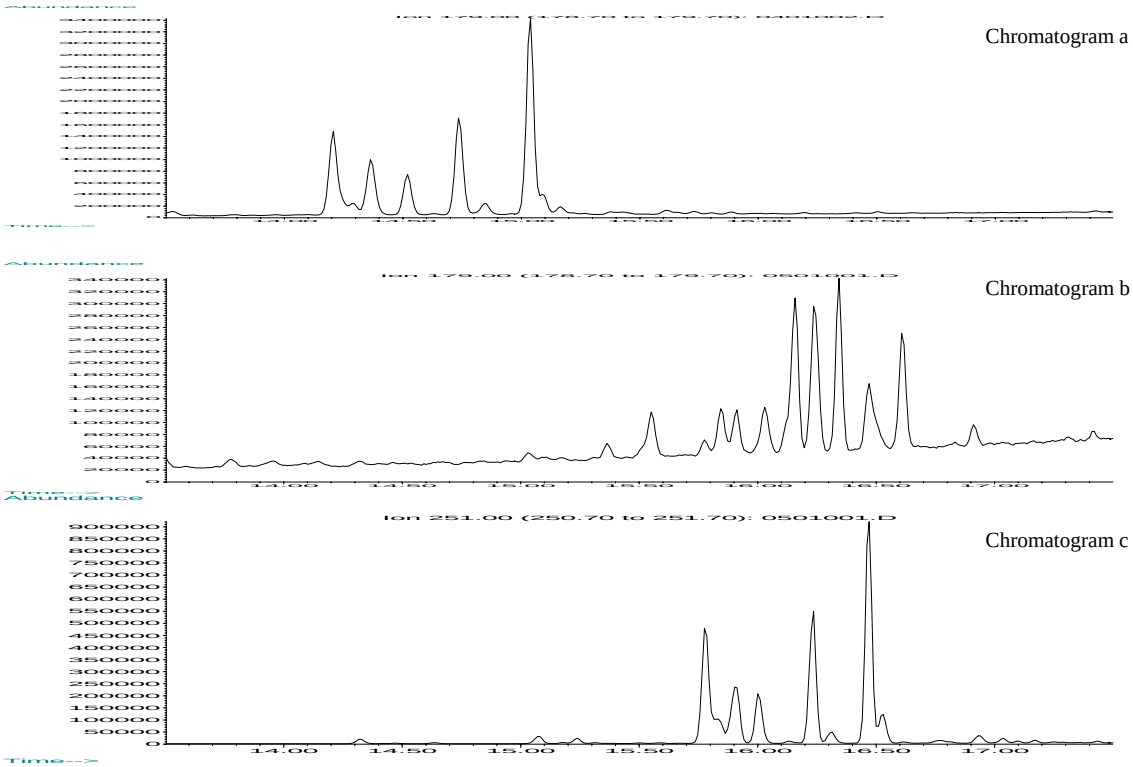

Figure 11: Chromatograms of a) a 20 mg/l un-derivatized NP1EO (ion 179), b) a 20 mg/l derivatized NP1EO (ion 179) and c) a 20 mg/l derivatized NP1EO (ion 251).

The response of possible un-derivatized NP1EO including background noise (chromatogram b, Figure 11) present in the MSTFA solution was measured and calculated relatively to the response of the actual un-derivatized NP1EO (chromatogram a, Figure 11).

Fraction of un-derivatized NP1EO: $\frac{MSTFA-derivate_{ion179}\ ^{*}}{Derivatized_{ion179}} = \frac{1{,}8\cdot 10^{6}}{230\cdot 10^{6}} = 0{,}78\%$

*) The response of un-derivatized NP1EO and/or background noise.

Derivatization efficiency of NP2EO:
NP2EO and the MSTFA-derivate of NP2EO are completely separated and there are no

indications of un-derivatized NP2EO (Figure 12).

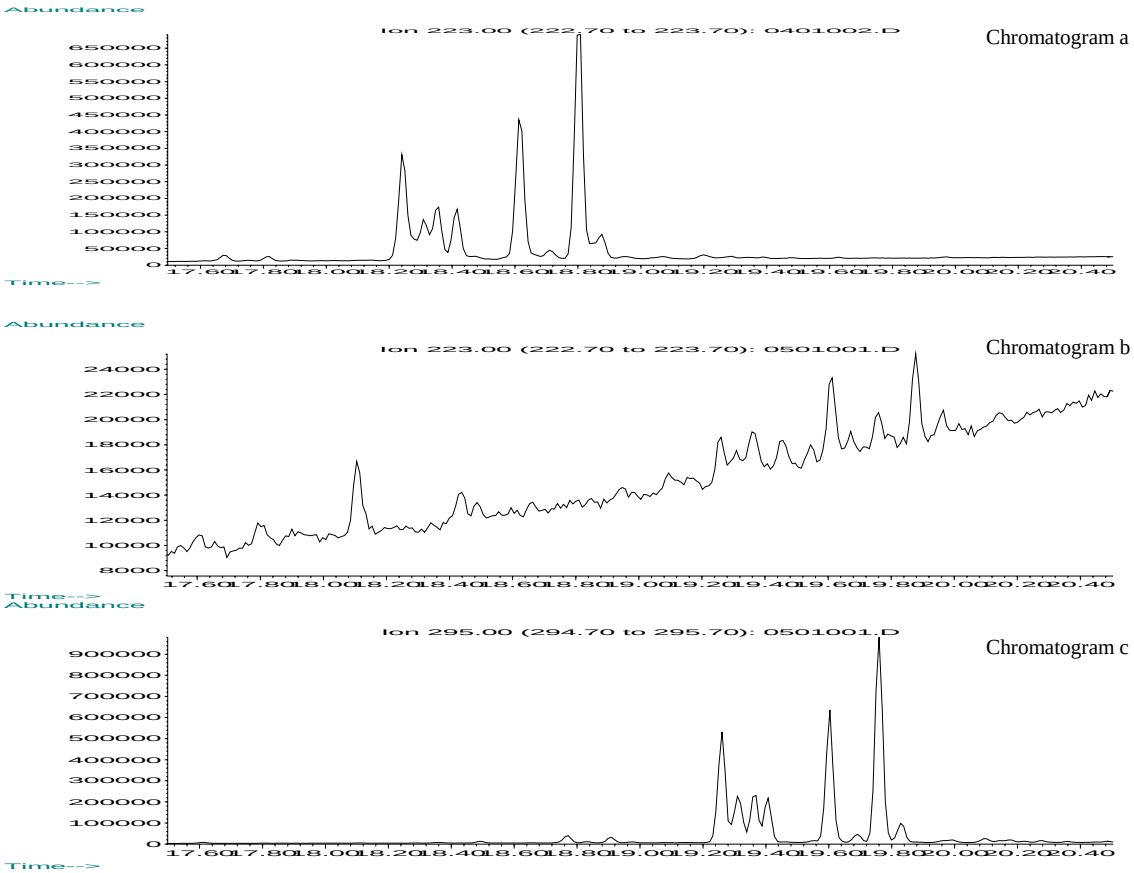

Figure 12: Chromatograms of a) a 20 mg/l un-derivatized NP2EO (ion 223), b) a 20 mg/l derivatized NP2EO (ion 223) and c) a 20 mg/l derivatized NP2EO (ion 295).

The area of the noise, which could be caused by un-derivatized NP2EO is calculated below.

Fraction of un-derivatized NP2EO: $\frac{MSTFA-derivate_{ion223}}{Derivatized_{ion223}}^{*} = \frac{0{,}23 \cdot 10^6}{40 \cdot 10^6} = 0{,}70\%$

*) The response of un-derivatized NP2EO and/or background noise.

The derivatization efficiency of NP, NP1EO and NP2EO was thus estimated to be >95%, 99% and 99%, respectively. This study supports the continuous use of MSTFA as the derivatization reagent of choice.

5.1.5 Time of derivatization

In sub-section 4.1.5 it was described, that the derivatization time was set to a minimum of 15 minutes in the horizontal method, and the background for the decision was given.

The experiments of the derivatization efficiency discribed in sub-sections 4.1.4 and 5.1.4 has proven, that the efficiency was high, with near 100% reaction, within 15 minutes. It was therefore decided to continue with 15 minutes as the minimum reaction time.

5.2 Extraction

As already mentioned in sub-section 4.2 it is generally not possible to obtain a 100% recovery by the extraction of organic pollutants from solid samples. By the evaluation of extraction parameters such as solvents and techniques is was chosen to use real samples – preferably without spike - and to use the extraction yield as the main criteria when comparing the parameters.

5.2.1 Extraction solvent

5.2.1.1 Comparison of toluene, dichloromethane (DCM), ethyl acetate and acetone/pentane

The first study was conducted to compare the extraction efficiencies of toluene, dichloromethane (DCM) and acetone/pentane (1:1) on a freeze-dried sludge sample (SL-E1). The effect of extraction time (2 and 16 hours), dry matter content (addition of 0, 10 and 50 ml water) and pH (addition of sulphuric acid) was also examined in this study (se 4.2.1.1). The results are shown in Appendix 2A.

The use of 2 hours and 16 hours extractions did not result in a significant difference in the concentrations (Paired t-test, p = 0.05), and the extractions at 2 and 16 hours were therefore regarded as replicates in the following description of the results.

The concentrations of NP, NP1EO and NP2EO from each treatment (n = 2) are summarized in Figure 13. The figure shows the extraction efficiency of the three solvents toluene, DCM and acetone/pentane, where the notations *a*, *b* and *c* refers to the addition of 0 ml, 10 ml and 50 ml of water and *d* refers to the addition of 50 ml 0.4 M sulphuric acid to the freeze-dried sludge sample. The concentrations in Figure 13 are only relative, due to a mistake in the preparation of standards.

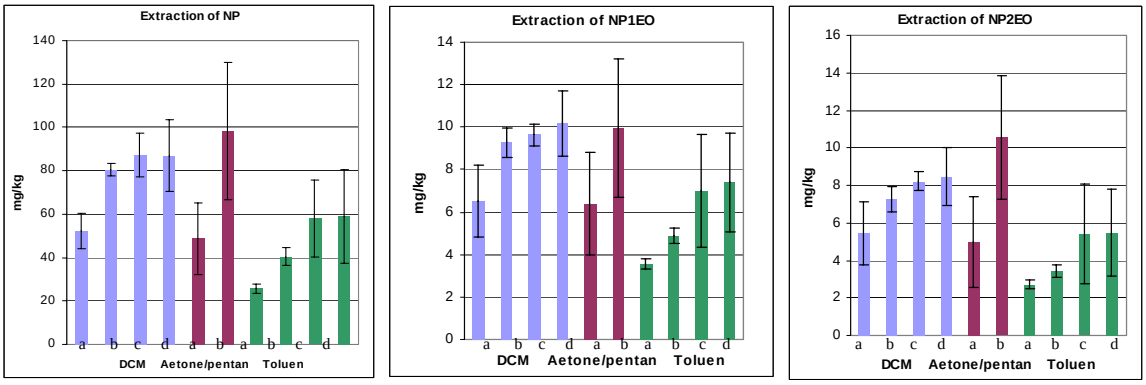

Figure 13: Concentration (mg/kg) of NP, NP1EO and NP2EO in DCM-, acetone/pentane- and toluene-extracts of a sludge sample (SL-E1). The dry samples were added a) 0 ml of water, b) 10 ml of water, c) 50 ml of water, d) 50 ml of 0.4 M sulphuric acid prior to the extractions. The error bars indicate standard deviation (n = 2).

Water was found to have a significant effect on the extraction of NP, NP1EO and NP2EO from the freeze-dried sludge sample extracted with acetone/pentane (1:1). This effect was also found in the DCM and toluene extractions of NP (Figure 13). A tendency of a positive effect of water on the extraction of NP1EO and NP2EO with DCM and toluene was also observed; however, this difference was not significant.

By treating the wet sludge samples (i.e. samples added water or sulphuric acid) as replicates the differences of the three solvents became more clear (Table 12).

Table 12 The extraction of NP, NP1EO and NP2EO from freeze-dried sludge sample (SL-E1) with toluene, DCM and acetone/pentane (1:1). Mean concentration (mg/kg) with confidence interval is stated (p = 0.05; n = 6*).

mg/kg	Toluene	DCM	Acetone/pentane (1:1)
NP	52 ± 16	85 ± 9.8	98 ± 33
NP1EO	6.4 ± 2.1	9.7 ± 0.93	10 ± 3.4
NP2EO	4.8 ± 1.8	8.0 ± 0.99	11 ± 4.7

* No true replicates were used.

The extraction of NP, NP1EO and NP2EO using DCM were found to be significantly more efficient compared to the extractions with toluene. Although the acetone/pentane appear to be the most efficient extraction solvent the relatively large confidence interval prevented a statistical confirmation of the tendency.

A second study was conducted to compare the extraction efficiency of toluene, ethyl acetate and acetone/pentane. The sludge sample SL-11 was used, and the extraction was carried out with a reciprocating shaker. The results are presented in Appendix 3 and in Figure 14.

The results verify the results from the previous study indicating acetone/pentane as being the most efficient extraction solvent.

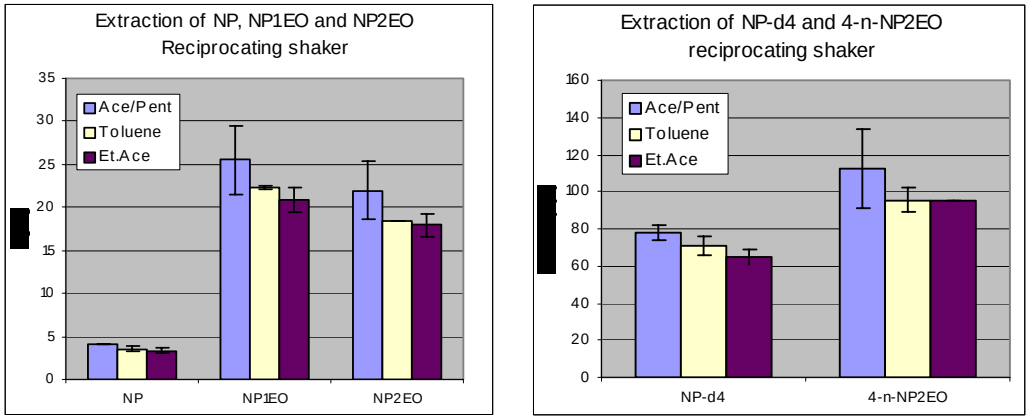

Figure 14: Concentration (mg/kg) of NP, NP1EO and NP2EO and recovery (%) of NP-D4, 4-n-NP2EO in extracts of a freeze dried sludge sample (SL11). The quantification of NP and NP-D4 was based on the internal standard (OP) and the quantification of NP1EO, 4-n-NP2EO and NP2EO was based on external standard. The error bars indicate standard deviation (n = 2).

Based on the results of this study and the results of a soxhlet extraction (see sub-sections 5.2.2), it was decided not to include toluene in the further work.

5.2.1.2 Comparison of ethyl acetate, dichloromethane (DCM) and acetone/pentane

A freeze-dried soil sample (SO-4) was extracted with DCM, acetone/pentane and ethyl acetate. The influence of water on the extraction of freeze-dried soil was found to be insignificant and the different treatments (i.e. addition of 5 or 20 ml of water) were therefore used as replicates.

The results from the extractions of the soil sample (spiked and un-spiked) are shown in Appendix 2B and Figure 15.

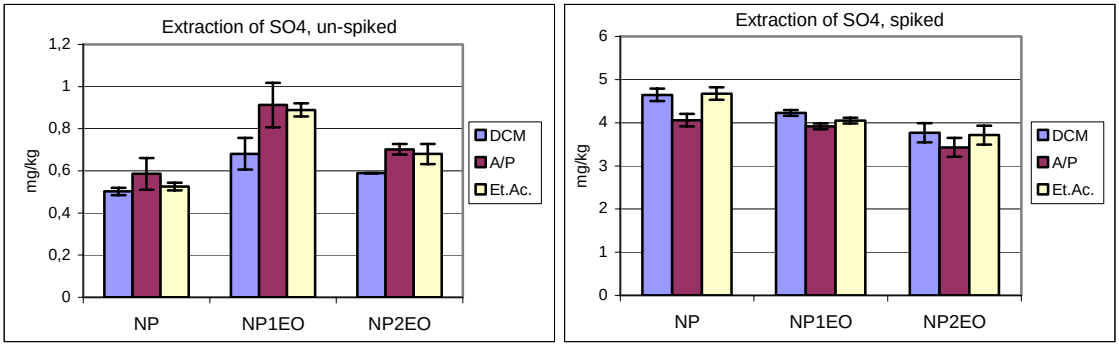

Figure 15. The extraction of NP, NP1EO and NP2EO with DCM, acetone/pentane (A/P) and ethyl acetate from a) an un-spiked soil sample (SO-4) and b) a spiked soil sample (SO-4). The quantification was based on external standard. The error bars indicate standard deviation (n = 2).

No significant differences of the three solvents were observed when comparing the extraction of NP, NP1EO and NP2EO from freeze-dried soil (SO-4).

The extraction efficiencies of the three solvents DCM, acetone/pentane and ethyl acetate were furthermore tested on a sludge sample (SL-E2). The effects of pre-treatment/dry matter percentage were also tested by extracting the sludge as either: a) wet and not pre-treated (28% dm), b) freeze-dried (100% dm) and c) diluted (5% dm). The results are shown in Appendix 2C and in Figure 16.

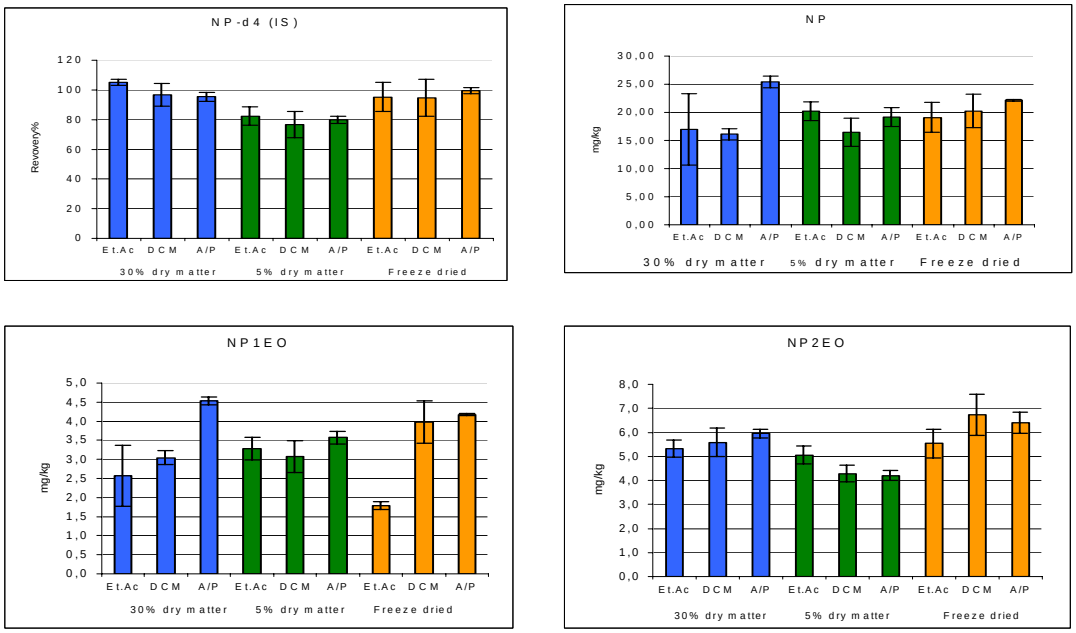

Fig. 16. The concentration of NP-D4, NP, NP1EO and NP2EO in extracts of not pre-treated sludge (28% dm), diluted sludge (5% dm) and freeze dried sludge (100% dm) extracted with ethyl acetate, DCM and acetone/pentane (A/P). Error bars indicate standard deviations of average concentration (n = 3).

The efficiencies of the solvents were generally very similar regarding the extraction of the alkylphenols. The extraction of NP and NP1EO from wet sludge (28% dm) was, however, found to be significantly more efficient with acetone/pentane (1:1) than with DCM ($p = 0.05$). A tendency of acetone/pentane being more efficient than ethyl acetate was also seen; however, the

difference was not statistically significant. There was found no explanation of the low concentration of NP1EO found in the ethyl acetate extracts of the freeze-dried sludge. As this result was inconsistent with the other observations it was not taken into consideration when evaluating the solvents.

Based on the extractions of SO-4, SL-11, and SL-E2 with ethyl acetate, DCM and acetone/pentane (1:1), no solvent unambiguously appeared to qualify as the most efficient. It was, however, decided to continue the work with only two solvents. Acetone/pentane was prevailingly found to be better or equally as good as ethyl acetate and DCM regarding the extraction of the alkylphenols. Generally, the results of the ethyl acetate- and DCM-extractions were, however, found to be very similar.

Ethyl acetate was rejected for two main reasons: The relatively high solubility in water (which would acquire additional drying) and the relatively high boiling point (which would make it more difficult to evaporate the extract prior to derivatization). Therefore, the further investigations only include DCM and acetone/pentane.

5.2.1.3. Comparison of dichloromethane and acetone/pentane

Since none of the 60 DCM- and acetone/pentane extractions have been extracted under the same conditions (i.e. due to variations in time of extraction, solvent type, solvent volume, pre-treatment of sample, the use of acid/base), no replicates are available (4.2.1.3). However, by grouping different parameters (i.e. type of solvent, volume of solvent, time of extraction etc.) and disregarding the possible differences, the influences of the chosen parameters could compared.

The results are shown in Appendix 2D and in Figure 17.

A paired t-test was performed to test the differences statistically, and the extraction time was found to introduce a significant difference ($p = 0.05$) in extraction efficiency (Figure 17). The comparisons of the solvents were therefore conducted for 2 and 20 hours separately.

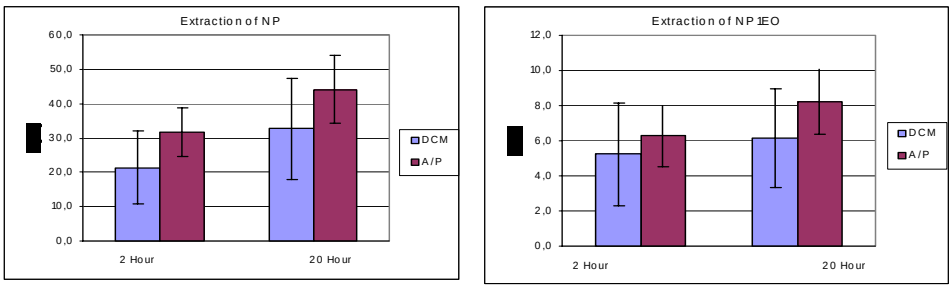

Figure 17. The extraction of a) NP and b) NP1EO from a sludge sample (SL-E2) conducted with DCM and acetone/pentane (1:1) for 2 and 20 hours.

The relatively large standard deviations of Figure 17 could be caused by the varying conditions of which the samples have been extracted. A paired t-test was performed but it was not possible to conclude which solvent was the most efficient. There was, however, a tendency for acetone/pentane to be more efficient than DCM. The difference in extraction efficiency between the two solvents was clearer in the 2-hour extractions. It was also found that the standard deviations were smallest when the sludge was extracted with acetone/pentane, which furthermore indicates that the acetone/pentane extractions are the most efficient.

Acetone/pentane (or hexane-like solvent) was therefore selected for the method.

5.2.1.4. Solvent volume

The extractions conducted with 15 and 30 ml of acetone/pentane in the experiments described above were also compared. The results are shown in Appendix 2D and in Figure 18. No significant effect was found. By grouping the data differently (for instance comparing the 15 and 30 ml of each of the solvents individually), the differences could be compared again, but no effects of the volume emerged (data not shown).

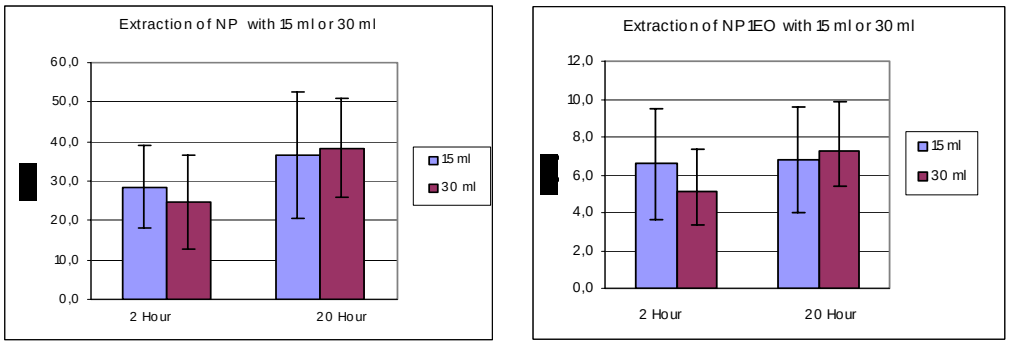

Figure 18. Extraction of a) NP and b) NP1EO from a sludge sample (SL-E2) conducted with 15 and 30 ml of solvent for 2 and 20 hours.

In the practical laboratory work it was, however, much more difficult to separate the necessary fraction of pentane from wet sludge samples when the extraction were conducted with only 15 ml solvent compared to 30 ml.

A volume of 20 ml acetone/pentane (1:1) was therefore found to be suitable.

5.2.2 Extraction technique

The alkylphenols NP, NP1EO and NP2EO were extracted from a freeze-dried sludge sample (SL-11) using both soxhlet and reciprocating shaking as extraction techniques. The comparison of the techniques was based on the extraction with acetone/pentane (1:1) and toluene. The quantification of NP was based on an internal standard (OP) added to the extract, whereas the quantification of NP1EO and NP2EO was based on external standardization. The difference in quantification was necessary due to a loss of OP and NP (and NP-D4) during the evaporation of the toluene extract. The less volatile compounds NP1EO and NP2EO were not affected by evaporation and the quantification was therefore based on external standard. None of the acetone/pentane extracts were affected by evaporation.

The results are presented in Appendix 3 and in Figure 19.

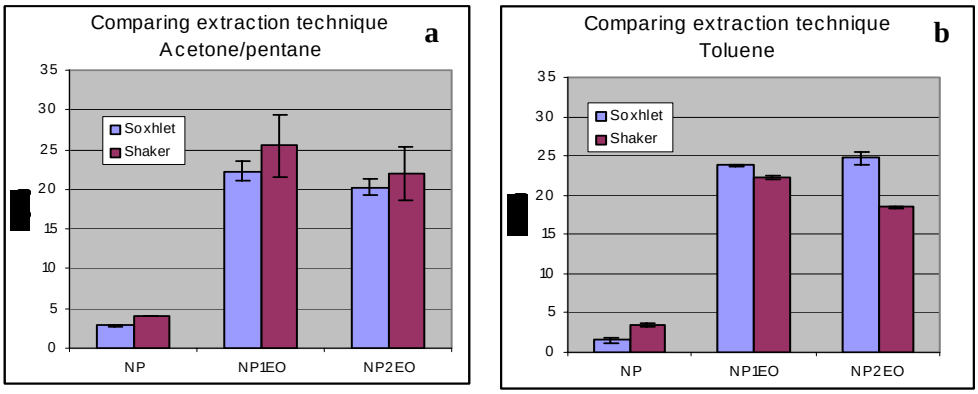

Figure 19. Extraction of NP, NP1EO and NP2EO from SL-11 with soxhlet and reciprocation shaking. The extractions were conducted with a) acetone/pentane and b) toluene. The error bars indicate the standard derivation (n = 2).

The acetone/pentane extraction of NP was found to be significantly more efficient when using a reciprocating shaker compared to an extraction conducted with soxhlet (Figure 19 a). The acetone/pentane extractions of NP1EO and NP2EO were not significantly different, but the shaking technique appeared to be slightly more efficient. The differences observed were independent of the quantification method.

The toluene extractions of NP showed that the reciprocation shaking was significantly more efficient than the extractions conducted with soxhlet, whereas the opposite was observed with the extraction of NP1EO and NP2EO (Figure 19 b).

Comparing the extraction efficiencies of extraction techniques *and* solvents, the reciprocating shaker with acetone/pentane was found to provide the highest concentrations of NP and NP1EO. The highest concentrations of NP2EO were observed when the extraction was based on soxhlet and toluene.

5.2.3 Extraction time

From previous studies no significant difference was found between 2 and 16 hours of extraction regarding the extraction of NP, NP1EO and NP2EO from freeze-dried sludge (SL-E1) (see 5.2.1.1).

The correlation between extraction time and extraction efficiency was investigated in a more detailed study on two sewage sludges: a freeze-dried sludge (SL-4) and a wet sludge (SL-E2) (4.2.3). In this study the extractions were conducted for ½, 1, 2, 3, 4, 6 and 20 hours, and the analyses covered the alkylphenols NP-D4, NP, NP1EO and NP2EO.

The results for the freeze-dried sludge are presented in Appendix 4 and in Figure 20. The pooled standard deviation from the seven double determinations was used to determine the confidence intervals shown in the figure (p = 0.05, n = 7).

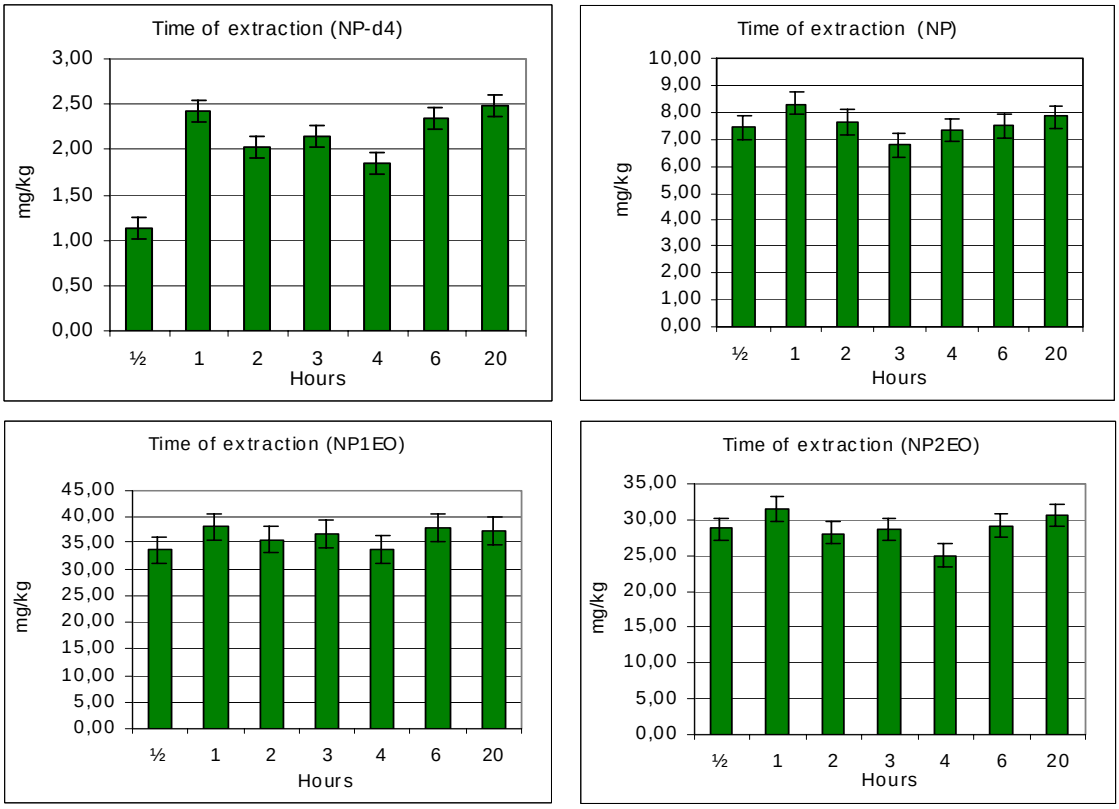

Figure 20. Concentration of NP-D4, NP, NP1EO and NP2EO extracted from freeze-dried sludge (SL-4). The quantification was based on phenanthrene-D10 as internal standard.

All four compounds appear to be relatively easily extracted from freeze-dried sludge. The concentrations of NP, NP1EO and NP2EO in the extracts were found to be stable already after half an hour of extraction. There was neither any statistical difference between the ½-hour and 20-hour extraction of NP, NP1EO and NP2EO nor any tendency suggesting an increase in extraction efficiency over time. The concentration of NP-D4, however, was not found to be stable after half an hour extraction. This compound was added to the sample only minutes before the extraction and should therefore be easily extracted compared to the other alkylphenols. Although no explanation has been found, the result is not regarded as significant.

One hour of extraction appeared to be sufficient for the extraction of NP-D4, NP, NP1EO and NP2EO from freeze-dried sludge. An extraction time of one hour was therefore implemented in the Horizontal standard method (Appendix 9).

The correlation between extraction time and extraction efficiency was also tested on a not pre-treated sludge. This wet sludge (SL-E2) was extracted in parallel to the previous described sludge. The results are shown in Appendix 4 and displayed graphically in Figure 21. The average standard deviation of the seven double determinations was used to determine the confidence intervals shown in the figure ($p = 0.05$, $n = 7$).

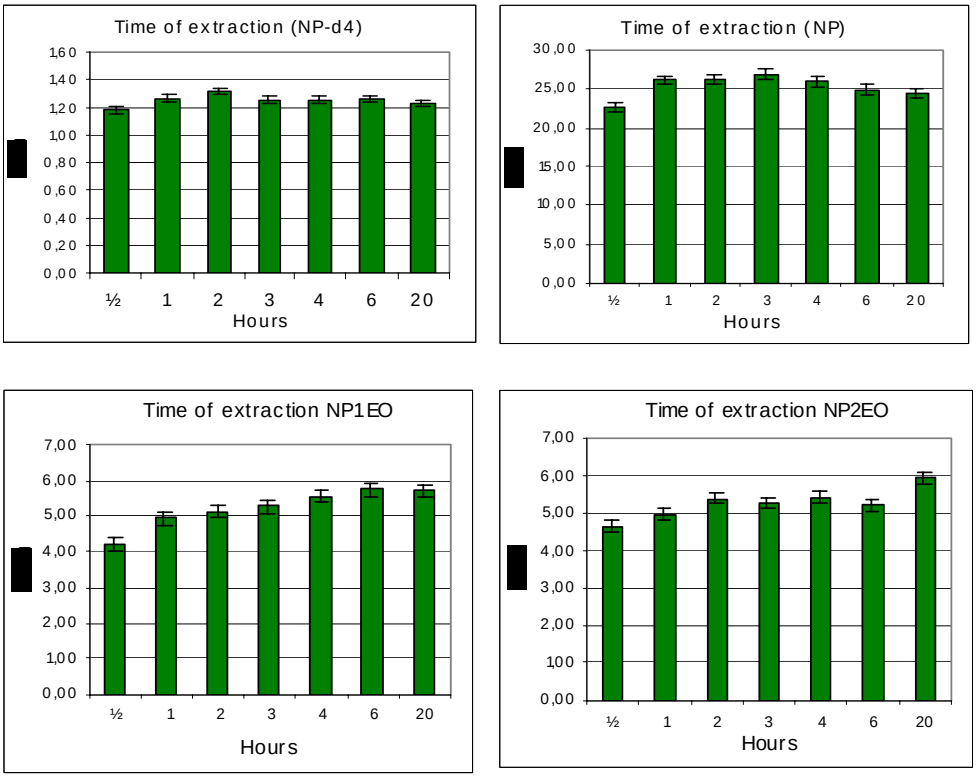

Figure 21. Concentration of NP-D4, NP, NP1EO and NP2EO extracted from wet sludge (SL-E2). The quantification was based on phenanthrene-D10 as internal standard.

NP was fully extracted from the sludge sample after 1 hour of extraction. The concentration of NP-D4 was statistically found to be stabile after one hour. The extraction efficiency of NP and NP-D4 was generally very similar, which confirmed the use of NP-D4 as internal standard.

A slight increase in extraction efficiency of NP1EO and NP2EO was observed during the 20 hours of extraction. The increase in concentration of NP1EO appeared to be stable after 4 hours of extraction, whereas the level of NP2EO was found to be almost steady in the extracts of the 2-, 3-, 4,- and 6-hour extractions. The concentration after 20 hours of extraction was, however, found to be significantly higher than the previous extractions of NP2EO. Given that no increase in concentration was observed in the interval between the first and the sixth hour of extraction and that NP2EO was added to the sample just before the extraction, it was decided that the results of the 20-hours extraction could be disregarded.

Based on the results of this study and previous results it was decided that wet sludge samples shall be extracted for a minimum of 2 hours.

5.3 Pre-treatment

Two types of pre-treatment were compared to the analysis of wet sludge without pre-treatment: oven-drying and freeze-drying.

5.3.1 Oven-drying

The study comparing the extraction of NP and NP1EO from oven-dried and wet, not pre-treated sludge (SL-E2) was in addition submitted to variations in solvent, extraction time, pH and solvent volume (4.2.1.3). The average concentration of NP and NP1EO determined after extraction of oven-dried and wet sludge samples was calculated (by external standard) for both the 2- and 20-hour extractions. The average concentrations were, as described, based on extracts of samples submitted to different extraction procedures, and no replicates were therefore present. The oven-dried and wet samples were submitted to identical variations in extraction procedures and any possible effect was therefore expected to equally affect the two types of pre-treated samples. The standard deviation was on the other hand expected to be relatively high.

The results are shown in Appendix 2D and in Figure 22.

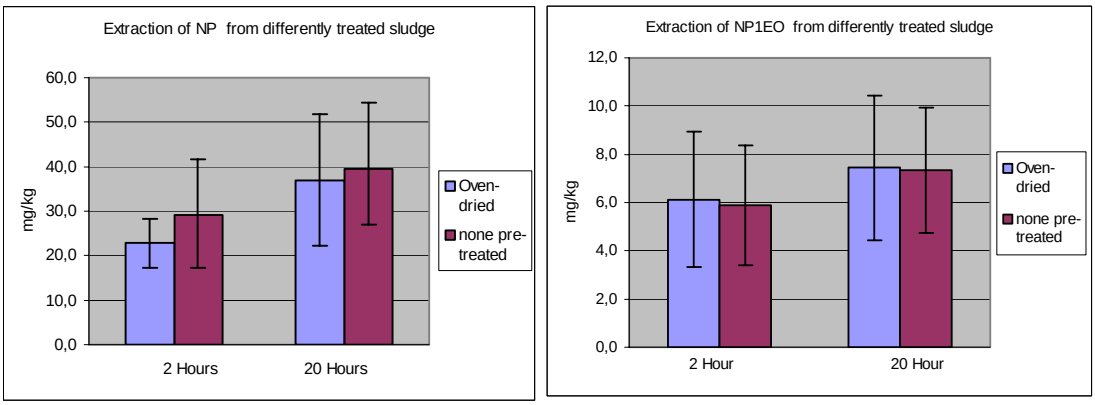

Figure 22. The average concentration of a) NP and b) NP1EO extracted from sludge samples (SL-E2) submitted to oven-drying or no pre-treatment.

No significant difference was observed when comparing the extractions of NP and NP1EO from oven-dried and wet, not pre-treated sludge sample. There was, however, a tendency that the 2-hour extractions of NP from oven-dried sludge were slightly less efficient compared to the wet sludge.

The extraction of oven-dried and freeze-dried sludge was furthermore compared in the ruggedness test, where the sludge sample SL-E2 was submitted to both pre-treatments. No significant difference was found; however, the freeze-dried sludge resulted in 6-7% higher results of NP and NP1EO than the oven-dried sludge.

5.3.2 Freeze-drying

The effect of freeze-drying on the extraction efficiency was tested in different studies. The study described in 4.3.2 was testing many parameters and among these also comparing the effect of freeze-drying and the type of solvent.

The results are shown in Appendix 2C and in Figure 23.

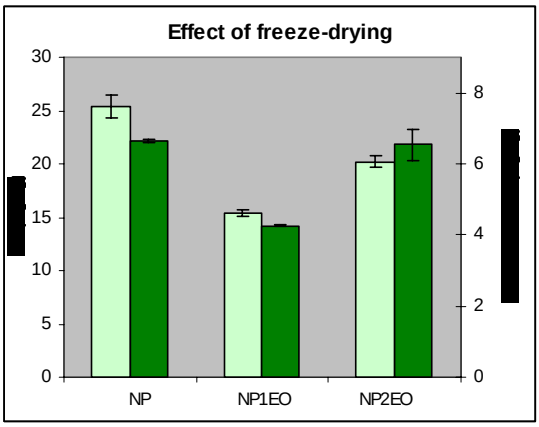

Figure 23. The concentration of NP, NP1EO and NP2EO extracted from sludge samples (SL-E2) submitted to no pre-treatment (light column) and freeze-drying (dark column).

Focusing on the acetone/pentane-extracts of freeze-dried and wet, not pre-treated sludge samples in Figure 23, it was found that the extraction of NP and NP1EO was more efficient from wet sludge than from freeze-dried sludge. The extraction of NP-D4 and NP2EO (added to the samples prior to the extraction) was, however, found to be equally efficient. The extractions conducted with DCM and ethyl acetate were generally found to be slightly more efficient when carried out on freeze-dried sludge.

5.3.3 Addition of water to freeze-dried sludge

Since the extraction efficiency was found to decrease, when freeze-dried samples was extracted with acetone/pentane, this item was further examined (4.3.3). To enhance the extraction efficiency of freeze-dried samples water was added prior to the extraction and the effect of the added water was measured. The study was set up to imitate the acetone/pentane extractions described in 4.2.1.2 by extracting sludge samples of 5, 30 and 100% dry matter. The samples of this study were, however, all freeze-dried and the dry matter percentages were obtained by adding an adequate amount of water.

The results are shown in Appendix 5A and in Figure 24.

The results obtained were very similar to the results of the acetone/pentane extractions described in 5.2.1.2 (also testing the effect of acetone/pentane, DCM and ethyl acetate). The highest concentrations were obtained when extracting the 30% dm sludge samples and the lowest concentrations were found in 5% dm sludge samples. The addition of water was therefore found to compensate the decrease in extraction efficiency observed for freeze-dried samples. When added in larger amounts, however, water was found to reduce the extraction efficiency of NP2EO. The effect of water on extraction efficiency was further investigated in 5.3.4.

Based on this study and on previous results, it was decided that water should be added to freeze-dried (and oven-dried) samples prior to extraction.

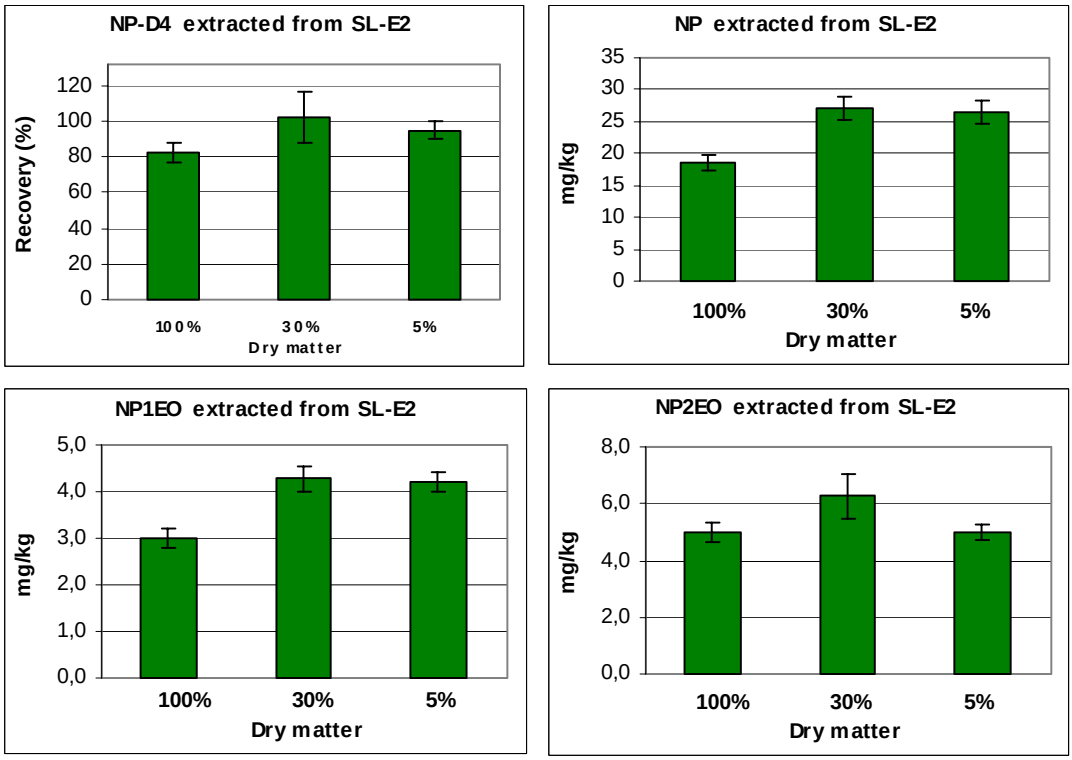

Figure 24. Concentration of a) NP-D4, b) NP, c) NP1EO and d) NP2EO in extracts of freeze-dried sludge samples added varying amounts of water.

5.3.4 Influence of water content

The effect of water was tested several times during the preliminary studies of extraction efficiency. The positive effects of the water added to freeze-dried sludge were shown in sub-section 5.2.1.1. Negative effects of water, however, were also observed when the dry matter content was low (5.2.1.2).

A more detailed study was therefore conducted (4.2.4) to examine the effect of water. The study was set up to compare the extraction efficiencies of sludge with varying dry matter (dm) contents (1%, 5%, 10%, 15%, 20% and 28%). The variation in dry matter was obtained by adding different amounts of water to the sample (SL-E2). The results are shown in Appendix 5B and Figure 25.

The asterisks above the columns indicate that the extraction efficiencies differ significantly from the extraction of the sludge with 30% dry matter. The standard derivation of the triple determinations was used to calculate the confidence levels shown in the figure ($p = 0.05$, $n = 3$).

A decrease in dry matter from 30% to 1 and 5% was found to significantly decrease the recovery of all compounds (NP-D4, NP, NP1EO and NP2EO). A decrease in dry matter from 30% to 10% was furthermore found to significantly decrease the recovery of NP2EO.

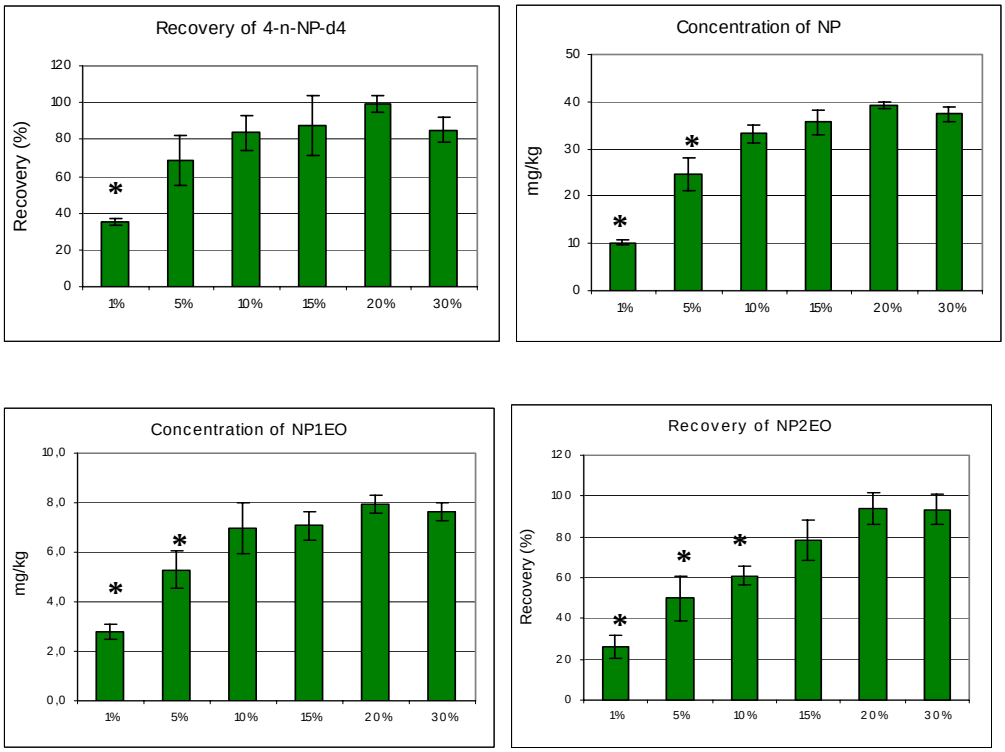

Figure 25. Concentration and recovery (%) of NP-D4, NP, NP1EO and NP2EO extracted from SL-E2. An asterisk above the column indicates significant (p = 0,05) decrease in extraction efficiency (n = 3).

By calculating the concentration of NP, NP1EO and NP2EO relative to NP-D4 the recovery of NP and NP1EO were corrected up to about 80-90% for the extractions with 5% dm. For the extractions with 1% dm the correction was lower (70-90%). For NP2EO the correction was even lower by NP-D4, only 65% for extractions with 1-10% dm. The probable reason is the larger water solubility of NP2EO, which results in lower recoveries with large water contents.

Therefore, the use of an additional internal standard (4-n-NP2EO) was tested in order to improve the robustness of the method. Similar to the previously described study, sludge samples of 1, 2, 5, 10 and 30 % were extracted and the results were evaluated with the main purposes of testing the use of 4-n-NP2EO as internal standard and improving the extraction efficiency of samples of low dry matter (1-5%). By filtering the samples the extraction efficiencies were increased and the negative effect of water could thus be avoided.

However, the procedure was found to be too time consuming (approximately 45 minutes per sample) and it was decided not to include filtering as part of the NP-Horizontal method.

The results of the no-filtered samples are shown in Appendix 5C and in Figure 26.

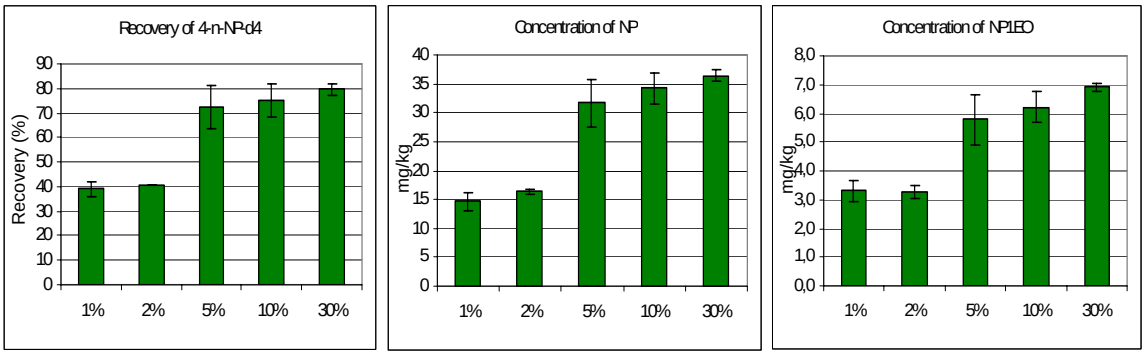

Figure 26.A. The recoveries of NP-D4, NP and NP1EO from sludge samples (SL-E2) with varying content of dry matter.

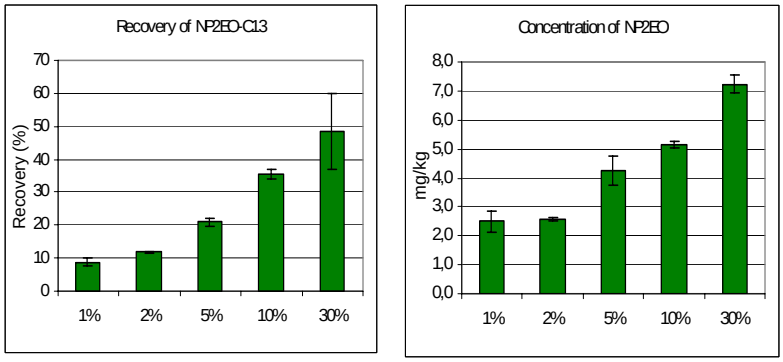

Figure 26.B. The recoveries of ^{13}C- NP2EO and NP2EO from sludge samples (SL-E2) with varying content of dry matter.

By calculating the concentration of NP and NP1EO relative to NP-D4 the results of the 1 and 2% dm sludge extractions were compensated approximately 80 and 90%, respectively. The concentrations of NP and NP1EO in the 5-10% dm-extracts were fully compensated.

The recovery of 4-n-NP2EO and NP2EO in the differently diluted sludge samples was found to be very similar. The linear alkylphenol, 4-n-NP2EO, was therefore found to be suitable as internal standard for NP2EO. The recoveries of the two diethoxylates in the most diluted sludge (1% dm) were, however, found to be somewhat different resulting in an overestimation of NP2EO. This difference in recovery could be an indication of an increase in uncertainty when extractions are conducted on sludge samples with a very low dry matter content.

Although some of the experiments gave results with high uncertainty, the overall conclusion is that by the use of two internal standards satisfactory results van be obtained for sludge samples with dry matter content above 2%.

It was decided to include 4-n-NP2EO or ^{13}C- NP2EO as internal standard. The costs of using ^{13}C- NP2EO were, however, found to be very high (maybe 4-5 Euro per sample) and the use of the unlabelled 4-n-NP2EO was therefore preferred.

5.4 Clean-up methods

For the large majority of samples it will not be necessary to include a clean-up step in the analysis. However, for special samples, mostly special sludges, a GC-MS measurement without clean-up can result in chromatograms with interfering peaks or a large background, resulting in low selectivity and a high limit of detection. In such cases a clean-up step can be beneficial.

A clean up procedure based on the chromatographic performance of silica columns was tested. The objective of the clean-up studies was to separate interfering compounds and the alkylphenols in three fractions. One fraction consisting of compounds eluting prior to the alkylphenols, a second fraction consisting of collectively eluted alkylphenols and a third fraction of compounds being retained by the column.

The clean-up was evaluated by the elution pattern of the alkylphenols and by comparison of the GC-MS chromatograms before and after clean-up.

First clean-up study:
First a 2.5 mg/l standard was used to identify the solvents capable of washing the silica column and subsequently eluting the alkylphenols from the column. The results are shown in Appendix 6 and in Figure 27. The results showed that pentane and pentane/DCM (1:1) enable the column to be washed without eluting the alkylphenols, whereas the solvents DCM and DCM/acetone were found to elute the alkylphenols in three separate fractions. The results also showed that the fraction eluted with DCM/acetone (1:1) was free of alkylphenols.

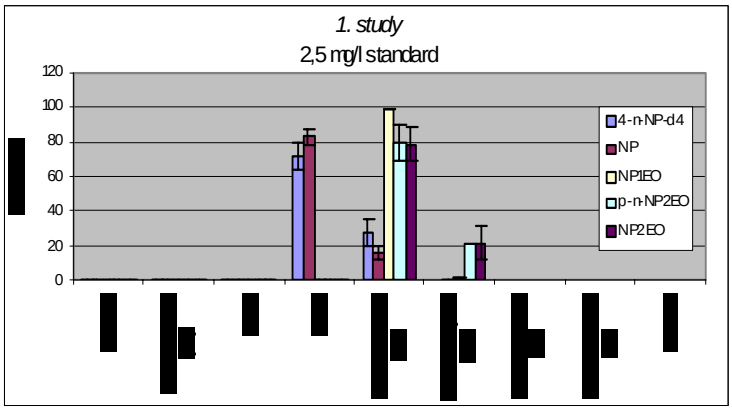

Figure 27. A standard (2.5 mg/l) containing NP-D4, NP, NP1EO, NP2EO and 4-n-NP2EO applied to a 500 mg silica column and eluted with solvent of increasing polarity.

Second clean-up study:
Based on the results of the first study, the clean-up procedure was modified (Table 8) and the clean-up was tested on a 2.5 mg/l standard and an extract of SL-E4. The initial wash with pentane was substituted with an additional wash with pentane/DCM (1:1) and the polarity of the alkylphenol eluting solvents was reduced to consist of only DCM/acetone (3:1). The results are shown in Appendix 6 and in Figure 28.

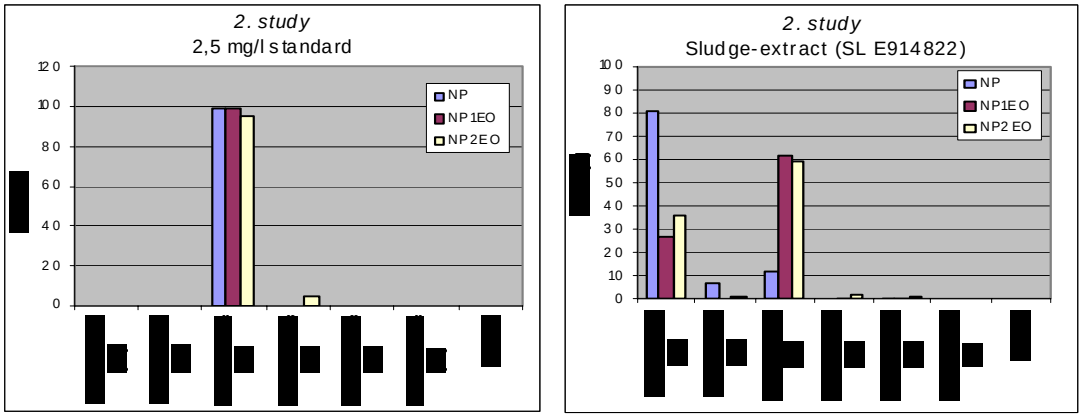

Figure 28. A standard (2.5 mg/l) and a sludge extract (SL-E4) containing NP, NP1EO and NP2EO applied to a 500 mg silica column and eluted with solvent of increasing polarity.

More than 95% of the alkylphenols of the 2.5 mg/l standard was found to elute when applying 2 ml DCM/acetone (3:1) as expected from the previous study. This was, however, not the case with the extract of SL-E4. The initially applied 2 ml pentane/DCM (1:1) was found to elute most of the NP and large fractions of NP1EO and NP2EO. To test if the capacity of the 500 mg silica material was exceeded, the study was repeated with 500 and 250 µl extract instead of 1000 µl. These clean-up tests were, however, not different from the results of Figure 28 (data not shown). The polarity of the pentane/DCM (1:1) was therefore found to be too high to be used in a washing step as it was intended.

Third clean-up study:
The composition of the solvents applied to the silica column was therefore changed again. To increase the difference in polarity of the initial washing solvent and the solvent eluting the alkylphenols, the pentane/DCM (1:1) mixture was changed to pure pentane. The results are shown in Appendix 6 and in Figure 29. The modified clean-up method was found to enable the elution of a disposable pentane fraction and collecting the main part of NP, NP1EO and NP2EO in 2 ml DCM/acetone (3:1).

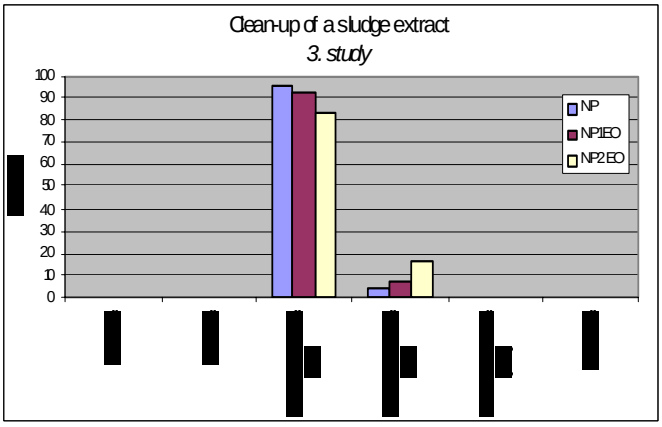

Figure 29. A sludge extract (SL-E4) containing NP, NP1EO and NP2EO applied to a 500 mg silica column and eluted with solvent of increasing polarity.

Fourth clean-up study:
The results from the third study were found to be satisfactory and the clean-up procedure was tested on two additional extracts (SL-11 and SL-E3) and repeated on the extract from SL-E4. The amount of pentane used in the initial wash was increased to evaluate the robustness of the procedure. The volume of DCM/acetone was also increased to collect the alkylphenols in a single fraction, if possible. The results are shown in Appendix 6 and in Figure 30.

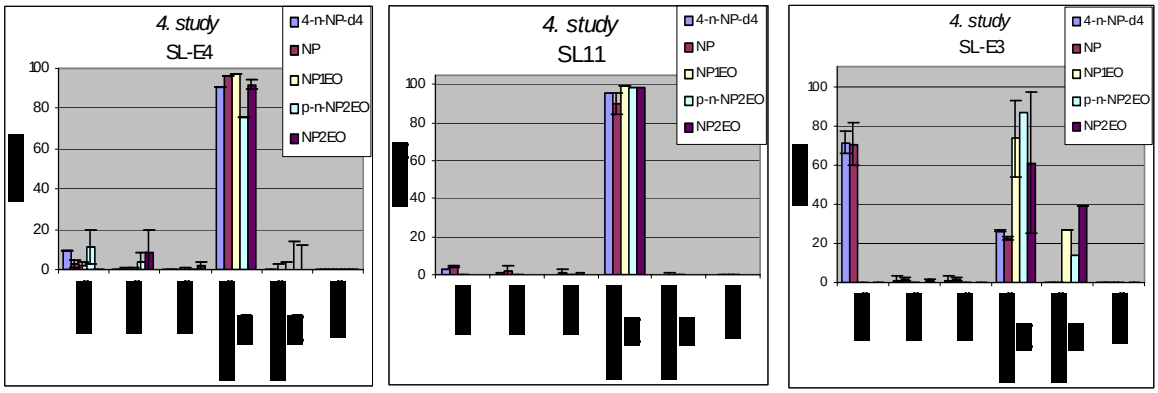

Figure 30. Extracts of a) SL-E4, b) SL-11 and c) SL-E3 applied to a 500 mg silica column and eluted with solvent of increasing polarity.

For the extracts from SL-E4 and SL-11 a successful clean-up of all five alkylphenols was achieved. However, for the sludge sample SL-E3 a relatively large fraction of NP-D4 and NP (approximately 70%) was eluted by the initial pentane wash. The reason for this is not known. The retention of NP1EO and NP2EO was, however, unaffected and these compounds could therefore be separated from the interfering compounds.

From the analyses of a large number of samples it was the experience, that interference by GC-MS was mainly found for NP1EO and NP2EO. Especially for NP2EO interferences could be important, as was the case with the sludge sample SL-E3. The clean-up method was found suitable for all three samples.

The effect of the clean-up can be shown by comparing the chromatograms of NP2EO from extracts of SL-E4 before and after a clean-up step (Figure 31). The identification and especially the quantification of NP2EO were significantly improved when a clean-up procedure was performed.

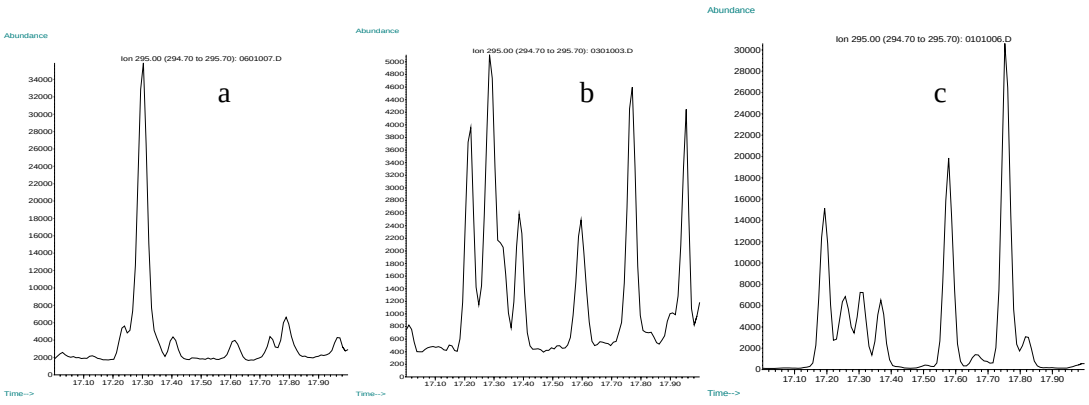

Figure 31. Chromatograms of NP2EO obtained from extract of SL-E4 a) without clean-up and b) with clean-up. For comparison the chromatogram of c) 0.5 mg/l NP2EO standard was also shown. The arrows of chromatogram a) indicate the occurrence of a major and minor interfering compound.

Based on the extracts of SL-E4, SL-1, SL-E3 and SL-E1 a clean-up step was not found to have any significant influence on the identification and/or quantification of NP and NP1EO.

5.5 Storage and stability

As already described in sub-section 4.5, storage and stability studies were carried out for the samples as well as for the standard solutions before and after derivatization.

5.5.1 Samples

Stability studies have been carried out to investigate the degradation of nonylphenol polyethoxylates in sewage sludge. This will be published in a separate report.

5.5.2 Solutions and extracts

The stability of NP-D4, NP, NP1EO and NP2EO and their MSTFA-derivates stored at 22 ± 3°C, 4 ± 3°C and –18 ± 3°C was measured. The stability of the compounds was tested in six different solutions, including derivatized and un-derivatized standards of 0.1 and 2.5 mg/l and derivatized and un-derivatized extracts of the sludge sample SL-E2. A description of the solutions is given in Table 13.

Table 13 Description of solutions used in stability tests.

Abbreviation	Description	Derivatized during storage	Concentration (mg/l)			
			NP-D4	NP	NP1EO	NP2EO
A	0.1 mg/l Standard	No	0.04	0.1	0.1	0.1
B	0.1 mg/l Standard	Yes	0.04	0.1	0.1	0.1
C	2.5 mg/l Standard	No	1.0	2.5	2.5	2.5
D	2.5 mg/l Standard	Yes	1.0	2.5	2.5	2.5
E	Extract of SL-E2	No	0.15	2.1	0.41	0.2
F	Extract of SL-E2	Yes	0.15	2.1	0.41	0.2

The stability of the MSTFA-derivates of the alkylphenols and the un-derivatized alkylphenols were tested by periodical analysis of the solutions. The results are presented in Appendix 7.

The degradation of the derivatized and un-derivatized 0.1 mg/l standards (solution A and B) was found to be very similar (Figures 32 and 33). Assuming the degradation rate of the alkylphenols and their MSTFA-derivates to be first-order (the correlation between concentration and time was generally best, when described as first-order reactions compared to zero-order and second-order reactions), half-lives ($t_{½}$) of approximately 150, 200 and 100 days were observed for NP-D4, NP and NP1EO, respectively, when stored at -18°C and 4°C. The half-lives for NP2EO and its derivate were, however, found to be significantly lower (approximately 45 days). Storing the 0.1 mg/l standards at 22°C was also found to significantly reduce the stability of the alkylphenols. When stored at 22°C, half-lives of approximately 30, 20 and 15 days were calculated for NP, NP1EO and NP2EO, respectively (based on first-order kinetics).

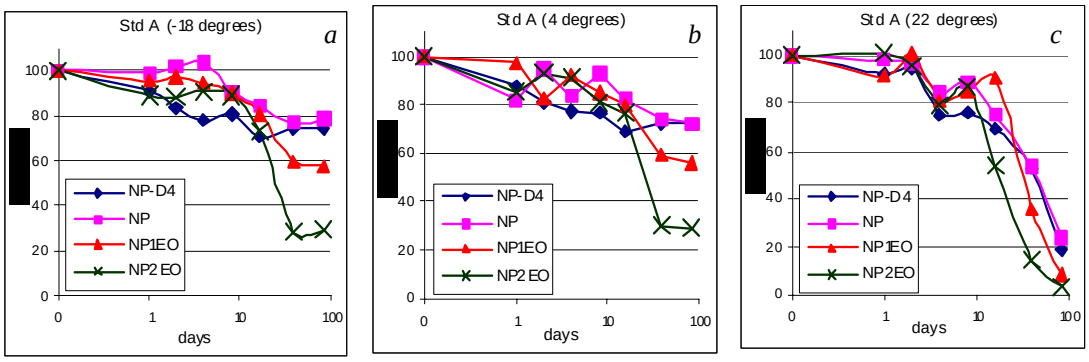

Figure 32. The stability of Solution A (0.1 mg/l un-derivatized standard) stored at: *a*) -18 ± 3°C, *b*) 4 ± 3°C and *c*) 22 ± 3°C.

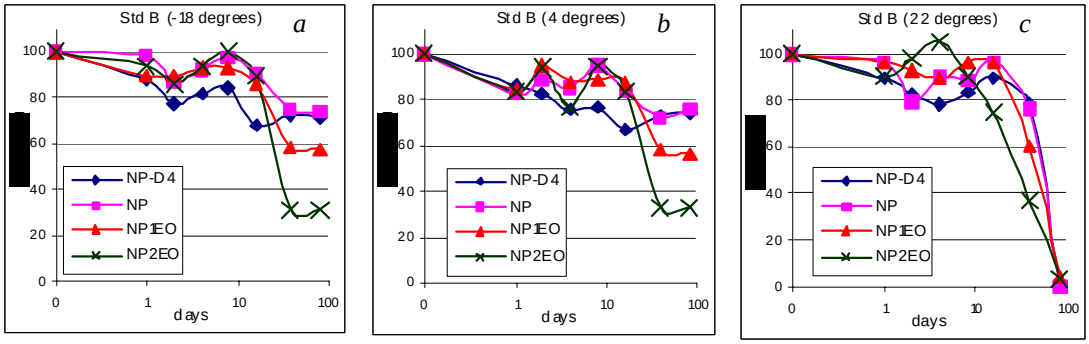

Figure 33. The stability of Solution B (0.1 mg/l MSTFA-derivatized standard) stored at: *a*) -18 ± 3°C, *b*) 4 ± 3°C and *c*) 22 ± 3°C.

The stability tests were, as described, also conducted on a 2.5 mg/l standard of both derivatized and un-derivatized alkylphenols (Figures 34 and 35). The patterns observed with the solutions C and D were similar to the above described stabilities of the 0.1 mg/l standards (A and B). The derivatization was not found to influence the stability of the compounds when comparing the two solutions (C and D) stored at -18°C and 4°C. However, when comparing the stability of mainly NP-D4 and NP stored at 22°C, solution C (which was derivatized only hours before the analysis) was found to be more stable than solution D (which was derivatized at day 0).

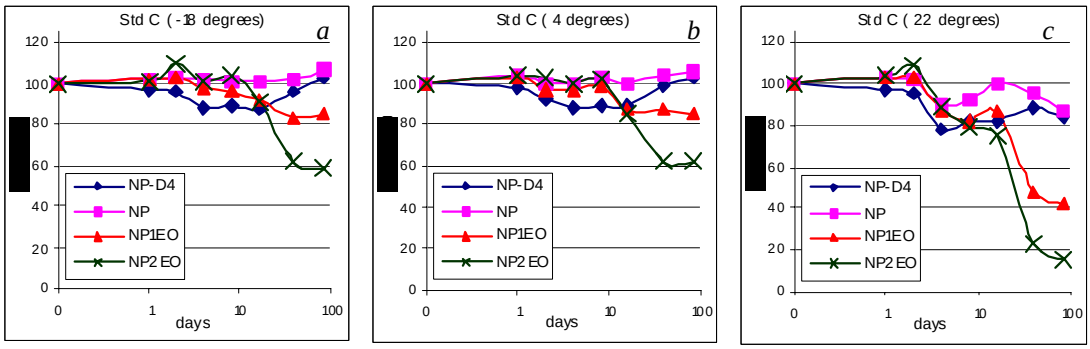

Figure 34. The stability of Solution C (2.5 mg/l un-derivatized standard) stored at: *a*) -18 ± 3°C, *b*) 4 ± 3°C and *c*) 22 ± 3°C.

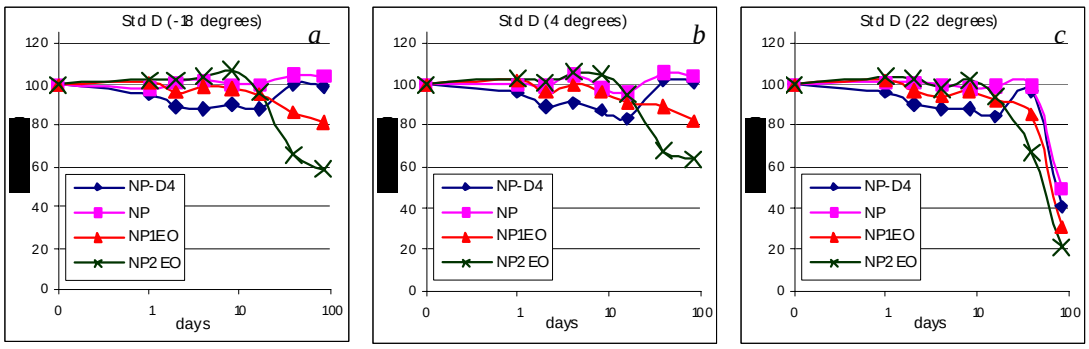

Figure 35. The stability of Solution D (2.5 mg/l MSTFA-derivatized standard) stored at: *a*) –18 ± 3 °C, *b*) 4 ± 3°C and *c*) 22 ± 3°C.

NP-D4 and NP and their derivates did not appear to be submitted to any significant degradation and calculation of half-lives was therefore not possible. Based on first-order degradation-rates half-lives of NP1EO and NP2EO stored at -18°C and 4°C were calculated to be more than 250 and 90 days, respectively. As previously shown for the 0.1 mg/l standards, NP2EO in the 2.5 mg/l standards NP2EO was found to be less stable than the other alkylphenols. Storing the derivatized standard (solution D) at 22°C resulted in half-lives for NP, NP1EO and NP2EO of approximately 85, 50 and 35 days, whereas the half-lives for the un-derivatized standards (solution C) were found to be slightly higher.

The stability of a sludge extract (SL-E2) was also tested (Figures 36 and 37). The stability of the alkylphenols and their MSTFA-derivates were, as previously observed, found to be very similar. Storage at -18°C or 4°C resulted in a high stability of NP-D4, NP, NP1EO and to some extent NP2EO. The concentration of NP-D4, NP and NP1EO appeared unchanged throughout the study, whereas half-lives for NP2EO could be calculated to approximately 110 days (first-order kinetics).

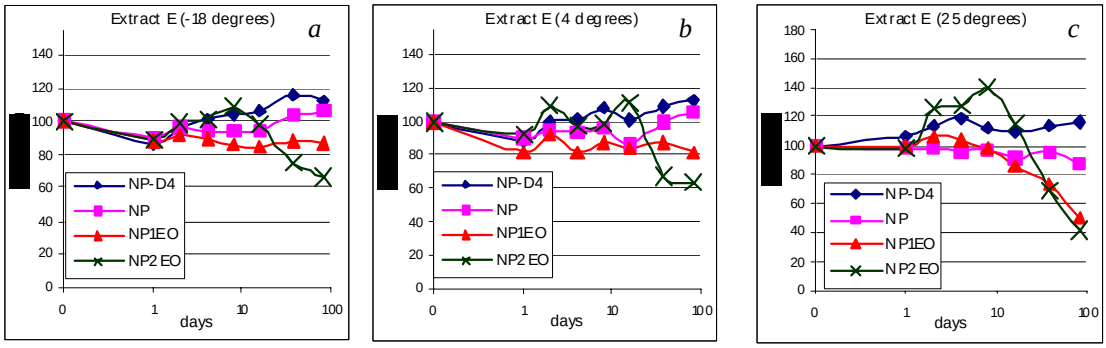

Figure 36. The stability of Solution E (un-derivatized extract of SL-E2) stored at: *a*) -18 ± 3°C, *b*) 4 ± 3°C and *c*) 22 ± 3°C.

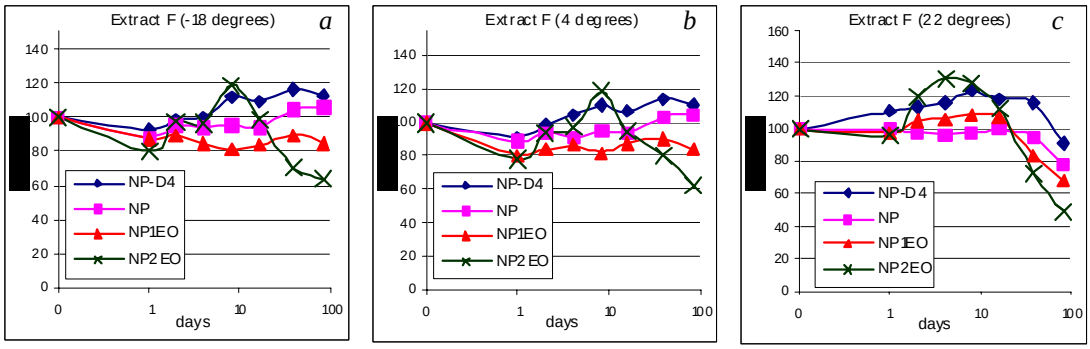

Figure 37. The stability of Solution E (MSTFA-derivatized extract of SL-E2) stored at: *a*) -18 ± 3°C, *b*) 4 ± 3°C and *c*) 22 ± 3°C.

A slight increase in the concentration of NP2EO was observed after a few days. This increase was mainly found in the solutions stored in 22°C. This increase could be an indication of degradation of larger NP ethoxylates present in the extract.

By the introduction of an internal standard similar to NP2EO (for example 4-n-NP2EO), the storage time of extracts could possibly be prolonged.

A more detailed description of the calculated half-lives of the un-derivatized and derivatized solutions were given in Table 14 and Table 15, respectively. From the tables an evident correlation between temperature and degradation rates was shown. No significant difference was found, however, when comparing the degradation rates of the solutions (standards or extracts) stored at -18°C and 4°C, respectively. The calculated half-lives furthermore outlined the respective stabilities of NP, NP1EO and NP2EO to be decreasing with increasing amount of ethoxylate groups. Comparing the results of the two tables the similarity of the stability of the derivatized and un-derivatized also becomes evident.

Table 14 Half-lives ($t_{½}$) and associated correlation coefficient (r^2) of NP, NP1EO and NP2EO measured in 0.1 mg/l standard, 2.5 mg/l standard and an extract of SL-E2. Calculations based on 1-order kinetics.

Un-derivatized		0.1 mg/l Solution A		2.5 mg/l Solution C		Extract of SL-E2 Solution E	
Compound	T/°C	$t_{½}$/day	(r^2)	$t_{½}$/day	(r^2)	$t_{½}$/day	(r^2)
NP	-18 ± 3°C	198	(0.658)	Stable	-	Stable	-
	4 ± 3°C	210	(0.599)	Stable	-	Stable	-
	22 ± 3°C	40	(0.993)	462	(0.421)	495	(0.709)
NP1EO	-18 ± 3°C	94	(0.862)	277	(0.721)	Stable	-
	4 ± 3°C	98	(0.838)	315	(0.651)	Stable	-
	22 ± 3°C	23	(0.969)	59	(0.872)	75	(0.987)
NP2EO	-18 ± 3°C	41	(0.822)	85	(0.852)	126	(0.793)
	4 ± 3°C	42	(0.842)	95	(0.825)	106	(0.721)
	22 ± 3°C	16	(0.988)	27	(0.921)	53	(0.837)

Table 15 Half-lives ($t_{½}$) and associated correlation coefficient (r^2) of NP, NP1EO and NP2EO-derivates (MSTFA) measured in 0.1 mg/l standard, 2.5 mg/l standard and an extract of SL-E2. Calculations based on 1-order kinetics.

Un-derivatized		0.1 mg/l Solution A		2.5 mg/l Solution C		Extract of SL-E2 Solution E	
Compound	T/°C	$t_{½}$/day	(r^2)	$t_{½}$/day	(r^2)	$t_{½}$/day	(r^2)
NP	-18 ± 3°C	192	(0.724)	Stable	-	Stable	-
	4 ± 3°C	257	(0.494)	Stable	-	Stable	-
	22 ± 3°C	13	(0.820)	86	(0.814)	257	(0.849)
NP1EO	-18 ± 3°C	97	(0.832)	257	(0.930)	Stable	-
	4 ± 3°C	99	(0.802)	301	(0.883)	Stable	-
	22 ± 3°C	19	(0.891)	51	(0.885)	128	(0.864)
NP2EO	-18 ± 3°C	42	(0.804)	89	(0.881)	117	(0.613)
	4 ± 3°C	47	(0.785)	100	(0.868)	131	(0.587)
	22 ± 3°C	16	(0.960)	36	(0.950)	64	(0.801)

The stability of NP2EO appeared to limit the acceptable storage time of calibration standards in the concentration interval 0.1-2.5 mg/l to approximately two weeks. Not surprisingly the standards should be kept at -18°C or 4°C. It was furthermore shown that the alkylphenols can be stored both as un-derivatized and derivatized.

Extrapolating from the results of the stability tests conducted on extracts of SL-E2, storage of extracts was, in general, estimated to be 30 days at -18°C or 4°C.

6. CONCLUSIONS

Based on the studies and results presented in this report a number of conclusions were reached. On the basis of these conclusions three drafts of the method have been written, and the 3rd draft was subjected to the ruggedness test described in chapter 7.

Derivatization

Choice of derivatization reagent and GC-MS conditions
A comparison of two derivatization reagents: Methyl-N-(trimethylsilyl)-trifluracetamide (MSTFA) and Pentafluorobenzoyl chloride (PFBCl) was conducted. An evaluation on the basis of chromatographic appearance, repeatability and limit of detection resulted in the choice of MSTFA as the most advantageous derivatization reagent.

Derivatization procedure
Changes in the initial derivatization procedure were introduced. The amount of MSTFA was reduced from 200 µl to 50 µl without loss of derivatization efficiency, and a derivatization with pure MSTFA (50 µl) followed by dissolution in 950 µl isooctane was substituted with the simultaneous addition of MSTFA and isooctane (i.e. 1 ml 5% MSTFA in isooctane).

Derivatization efficiency and time
The analysis of a MSTFA-derivatized 20 mg/l standard, recording both derivatized and un-derivatized alkylphenols, showed that the derivatization procedure is more than 95% efficient regarding the derivatization of NP and more than 99% regarding the derivatization of both NP1EO and NP2EO. This study was made with 15 minutes reaction, and based on these results the derivatization time was reduced from 30 to 15 minutes.

Extraction

Choice of solvent
The extraction efficiencies of toluene, ethyl acetate, dichloromethane and acetone/pentane (1:1) were compared in several studies. The most efficient solvent was found to be acetone/pentane, whereas the extraction efficiencies of the other solvents were found to be very similar. The use of acetone/hexane-like solvent (1:1) was therefore introduced in the method.

Extraction volume
The influence of sample/solvent-ratio on extraction efficiency was tested on sludge samples by comparing extractions based on 1 and 2 gram dm per 15 ml solvent. This study did not indicate any influence of sample/solvent-volume within the tested range. Mainly for practical reasons a sample/solvent-ratio of 2 g dm/20 ml acetone/hexane-like solvent was chosen.

Extraction technique
A comparison of soxhlet and reciprocating shaker based on both acetone/pentane and toluene as extraction solvents was conducted. The reciprocating shaker was found to be more efficient than soxhlet when the extractions were conducted with acetone/pentane. No unambiguous conclusion was found regarding the toluene extractions. The reciprocating shaker was consequently chosen for the method.

Extraction time
An optimal extraction of NP, NP1EO and NP2EO from freeze-dried sludge samples was achieved after 0.5 hours. Extractions conducted on field moist sludge samples resulted in an optimal extraction of NP after 2 hours. The extraction efficiency of NP1EO and NP2EO was,

however, found to be slightly increasing over time. The increase in extraction efficiency was not found to necessitate an extraction time of more than 2 hours. Extraction times of 1 and 2 hours regarding the extraction of freeze-dried and wet samples, respectively, were consequently chosen for the method.

Pre-treatment

Oven-drying
A tendency of oven-dried samples to result in a reduction in extraction efficiency of NP was observed. However, the effect was not statistically significant due to relatively large standard deviations obtained in this study. No indications of reduced extraction efficiencies were observed for NP1EO (NP2EO < DL). Only drying by freeze-drying is included in the method.

Freeze-drying
Freeze-drying was found to result in a significant reduction in extraction efficiencies for NP and NP1EO (NP2EO not effected), when freeze-dried sludge was extracted directly. However, the addition of water to the freeze-dried sample before extraction increased the extraction efficiency to a similar level as seen by the extraction of wet samples.

Influence of water content
A significant decrease in extraction efficiency of NP and NP1EO in sewage sludge was observed when the dry matter content was reduced to 5%. A significant effect on the extraction of NP2EO was observed when the dry matter content was reduced to 10%. The use of two internal standards (4-n-nonylphenol and 4-n-nonylphenol diethoxylate), however, compensates for the low recoveries and thus enables a correct quantification of NP, NP1EO and NP2EO, even when analysing samples of low dry matter content, possibly down to 2%. For samples with less than 2% dry matter, freeze-drying should be used.

Clean-up

An example of a clean-up method based on silica columns was tested. It was shown that a clean-up of relatively dirty extracts can be obtained, resulting in extracts free from chromatographic interference.

Stability of solutions

The stability of samples has been studied for sludge and will be presented in a separate report.

The stability of NP-D4, NP, NP1EO and NP2EO and their MSTFA-derivates stored at –18°C, 4°C and 22°C was tested. An acceptable storage period of 2 weeks was observed regarding both derivatized and un-derivatized calibration standards (0.1-2.5 mg/l). The acceptable storage time of a sludge extract was found to be approximately 30 days. The less stable analyte was NP2EO (and MSTFA-derivate of NP2EO) in all the tested solutions.

7. RUGGEDNESS TEST

Based on the results obtained from the pre-normative work with NP, NP1EO and NP2EO described in the previous chapters, the 2nd draft of the NP Horizontal Standard was completed. A few comments were received during the ad-hoc group meeting in Madrid 21 September 2005, and a 3rd draft was written (Appendix 9).

The ruggedness of the 3rd draft of the method was subsequently tested. The results of the test and the consequences are presented in this chapter, and the draft method was revised according to the results from the ruggedness test.

7.1 Materials

The samples chosen for the ruggedness tests are shown in Table 16. The samples were selected to represent sludge, soil and compost samples. The ruggedness of the method was also tested on samples of different pre-treatment (wet, freeze-dried and oven-dried).

Table 16 Samples used in the ruggedness tests of the NP Horizontal Standard.

Sample ID	Sample description	Pre-treatment	Extraction procedure*	NP mg/kg dm	NP1EO mg/kg dm	NP2EO mg/kg dm
SL-E4	Sewage sludge, domestic, Helsingør, Denmark. 920702	None	1	20	9.4	1.6
SL-E1	Sewage sludge, VKI, Hoersholm, Denmark	Freeze-dried	2	41	6.1	1.5
SL-E2	Sewage sludge, domestic, Vejle, Denmark. 908134	Freeze-dried	2	27	5.9	< 0.05
SL-E2	Sewage sludge, domestic, Vejle, Denmark. 908134	Oven-dried	2	27	5.9	< 0.05
SL-11	Sewage sludge, electronic industry, Turin, Italy	Freeze-dried	2	3.2	22	18
SO-E1	Soil enriched with sewage sludge, DHI, Hoersholm, Denmark. 921916-01	None	3	0.23	0.57	0.20
SO-4	Clay soil, Speyer, Germany	Dried	4	2.0	0.20	0.10
SO-9	Soil, Hagen, Germany	Dried	4	1.1	1.1	0.98
CW-1	Composted garbage, Munich, Germany	Dried	4	0.30	0.19	0.18
CW-5	Compost, Fulda, Germany	Dried	4	0.13	0.05	< 0.05

* Four different extraction procedures are included in the NP Horizontal standard, sub-section 10.1, see Appendix 9. The number (1-4) refers to the procedure of which the sample was extracted.

A more detailed description of the samples is given in chapter 3, Table 3.

7.2 Experimental

7.2.1 Design of ruggedness test

The ruggedness test was conducted according to a multifactorial experimental design described by Plackett & Burman /10/. The experimental set up allows simultaneous variation of a relatively large number of experimental conditions, requiring only a relatively small number of samples. The ruggedness test conducted during the evaluation of this method was based on eight analysis of one sample, which enabled the determination of the effect of seven experimental factors (Table 17). The effect of each factor was measured at two levels (+ and -) representing the extremes of which the analytical method could be subjected.

Table 17 Multifactorial design of which the ruggedness tests were conducted.

Experimental factor	Sample no. and level of factor (+/-)							
	1	2	3	4	5	6	7	8
A	+	+	+	+	-	-	-	-
B	+	+	-	-	+	+	-	-
C	+	-	+	-	+	-	+	-
D	+	+	-	-	-	-	+	+
E	+	-	+	-	-	+	-	+
F	+	-	-	+	+	-	-	+
G	+	-	-	+	-	+	+	-

The effects of each factor were measured by calculating the average result of the samples denoted a plus and subtracting the average results of the samples denoted a minus. The effect of "A" was for instance measured by subtracting the average of the results of sample 5, 6, 7 and 8 from the average of the results of sample 1, 2, 3 and 4.

7.2.2 Factors and levels of ruggedness test

Variations in some parts of the analytical method (such as variations in the GC-MS settings) were expected to equally affect the response of both extract and calibration standard. In this case a possible variation was not expected to affect the ruggedness of the method and were therefore not tested. The main effects on the method were expected to arise from the extraction procedures and the selection of experimental factors was therefore based on this part of the analytical procedure.

Due to the use of the method for different sample matrices and for wet and dried samples, four slightly different extractions were used in the NP horizontal standard, as previously described. The seven factors, or parameters, on which effects of variations were tested, were therefore depending on the sample. The experimental factors included in the ruggedness tests were:

A The velocity of the reciprocating shaker

B Shaking of the sample-acetone mixture prior to the addition of hexane-like solvent

C_{SL} The sample/solvent-ratio used in the extraction of sludge

$C_{SO,CW}$ The sample/solvent-ratio used in the extraction of soil and compost

D To perform the wash of extract with water (to get rid of the acetone) in a separate flask or directly in the extraction flask (with sample present)

E The type of hexane-like solvent used for the extraction

F1 The amount of water used for the wash of extracts (sludge samples)

F2 The amount of water used for the wash of extracts (soil and compost samples)

G The use of anhydrous sodium sulphate for drying the extract

H1 The amount of water added to freeze-dried samples prior to the extraction

H2 The amount of water added to freeze-dried samples prior to the extraction (the volume was increased based on the results of the first ruggedness tests)

I Extraction time

To measure the influence of the described experimental factors, a "minimum"- and "maximum"-value of each factor was selected (Table 18). This range, limited by the minimum- and maximum-value, was used to test the influence of the applied methodical changes.

Table 18 The selected values of the two-level experimental factors.

Factor	**Description of factor**	**"Minimum" value (-)**	**"Maximum" value (+)**	**Samples tested**
A	Velocity of reciprocating shaker	230 strokes pr min.	270 strokes pr min.	SL-E4, SL-E1, SL-E2*, SL-11, SO-E1, SO-4, CW-5
B	Shaking of sample-acetone mixture	No	Yes	SO-9, CW-1
C1	Sample/solvent-ratio	2 g dm/10 ml	3 g dm/10 ml	SL-E4, SL-E1, SL-E2*, SL-11
C2	Sample/solvent-ratio	10 g dm/20 ml	20 g dm/20 ml	SO-E1, SO-4, SO-9, CW-1, CW-5
D	Transfer extract to new flask	No	Yes	All‡
E	Type of solvent	Pentane	Isooctane	All‡
F1	Washing of sludge-extracts	40 ml	60 ml	SL-E4, SL-E1, SL-E2*, SL-11
F2	Washing of soil and compost-extracts	80 ml	120 ml	SO-E1, SO-4, SO-9, CW-1, CW-5
G	Addition of Na_2SO_4	No	Yes	All‡
H1	Addition of water prior to extraction	0 ml/g dm	1 ml/g dm	CW-1, CW-5
H2	Addition of water prior to extraction	1 ml/g dm	2 ml/g dm	SL-E1, SL-E2*, SL-11, SO-4, SO-9
I	Time of extraction	2 hours	3 hours	SL-E4, SO-E1

‡ "All" refers to all samples on which the ruggedness test was conducted (se Table 16).
* Both freeze- and oven-dried SL E2 were tested.

In each test on a sample, seven of the twelve factors (A-I) of Table 18 were selected and subsequently submitted to the multifactorial design described in Table 17. The factors tested on

each sample are shown in Table 18. The factors of Table 18 are connected to the factors of Table 17, so that the seven factors selected from Table 18 were selected in chronological order (from A to I), so that they represent the respective seven factors of Table 17 (A-G). Using SL-E4 as an example, the factors A, B, C, D, E, F and G of Table 17 were substituted with A, C1, D, E, F1, G and I of Table 18 when the ruggedness test were conducted.

Apart from the described analytical variations, the ruggedness test was conducted according to NP-Horizontal standard (Appendix 9).

7.3 Results

The diagrams below include the results of the 10 ruggedness tests. The height of each coloured column indicates the magnitude of the effect (% relative difference) introduced by each factor (A-I) on each sample (Table 20). To distinguish actual effects of a tested variation from the uncertainty of the method, different conditions were evaluated by: a) the relative difference of a sample should be significant compared to the variability of the method, b) the effects should be uniformly distributed as either positive or negative, and c) the effect should be reasonable (for example should longer extraction times not reduce the extraction efficiency).

The results are presented in Appendix 8.

7.3.1 Extraction of wet and freeze-dried sludge

The ruggedness tests conducted on wet (SL-E4), oven-dried (SL-E2 (OD)) and freeze-dried sludge samples (SL-E2 (FD), SL11 and SL-E1) where submitted to almost identical variations and the results were therefore shown together in Figures 32-34. The effects of the analysis of NP, NP1EO and NP2EO are presented separately. The extractions were conducted according to the extraction procedure described in the Horizontal standard sub-sections 10.1.1 and 10.1.2 concerning the extraction of wet sludge and freeze-dried sludge, respectively.

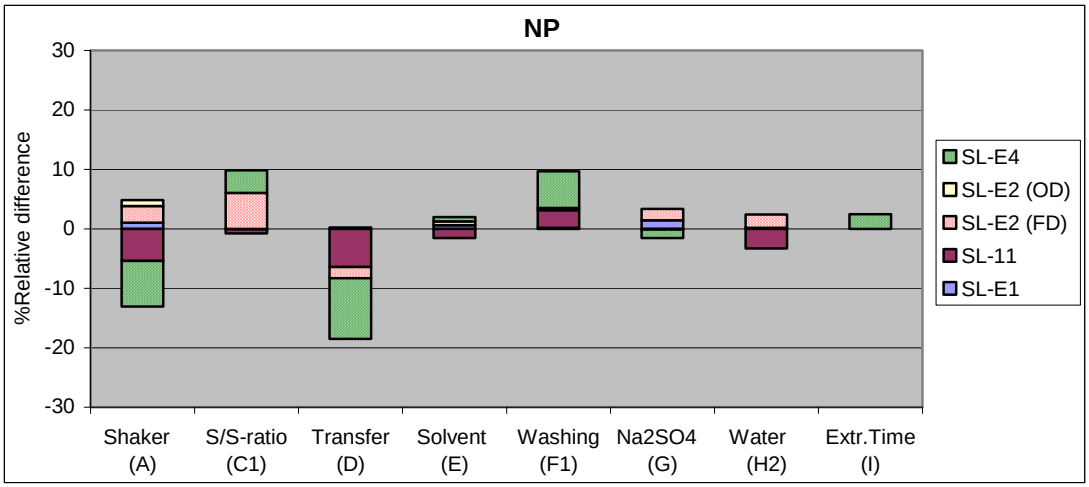

Figure 38. The relative difference (%) in concentration of NP caused by the variation of the described parameters.

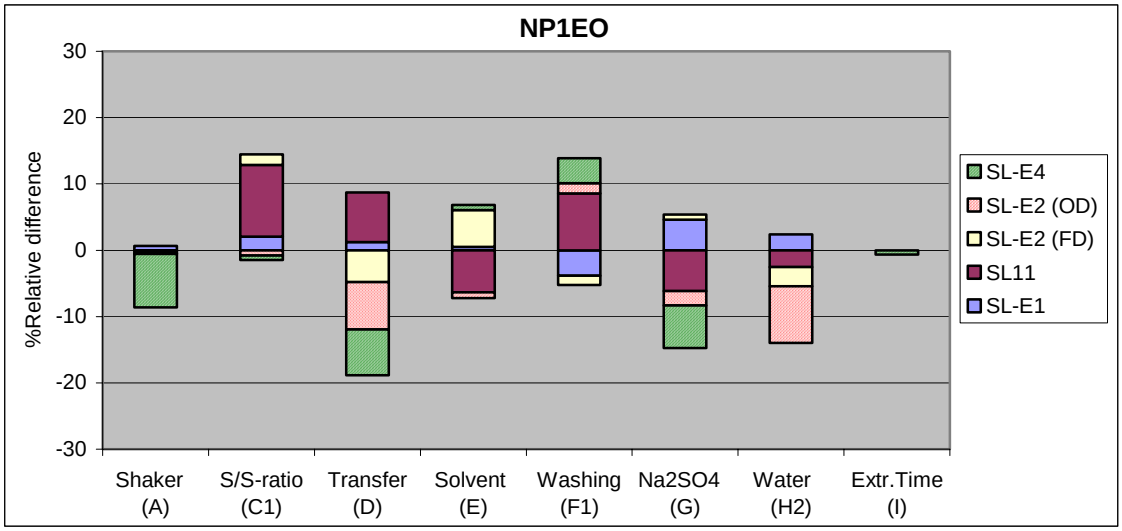

Figure 39. The relative difference (%) in concentration of NP1EO caused by the variation of the described parameters.

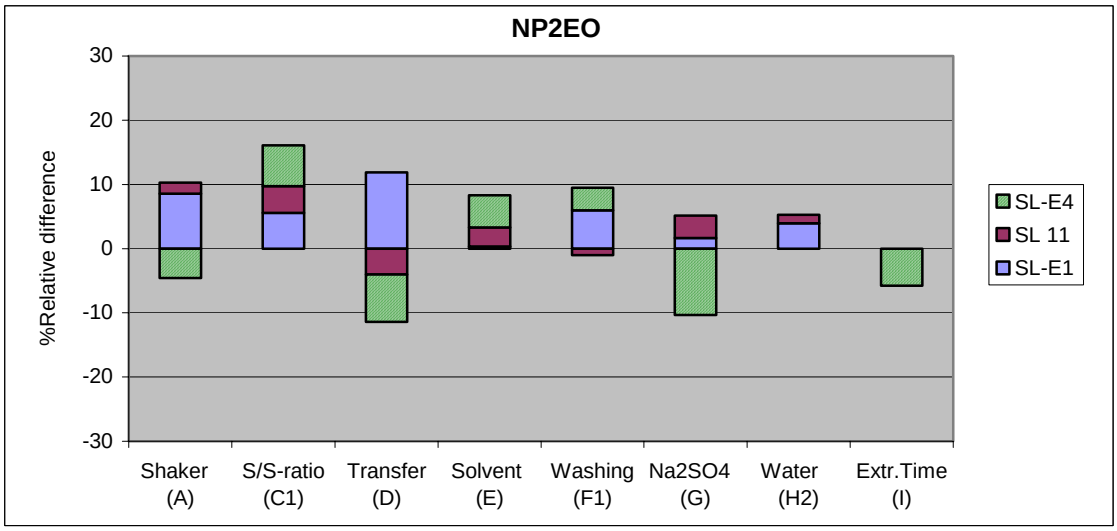

Figure 40. The relative difference (%) in concentration of NP2EO caused by the variation of the described parameters. The concentrations of NP2EO in oven- and freeze-dried SL-E2 were below detection limit and were therefore not included.

7.3.2 Extraction of wet and freeze-dried soil and compost

The results of the ruggedness tests conducted on freeze-dried soil (SO-4 and SO-9) and compost (CW-1 and CW-5) are displayed together with the results of ruggedness test conducted on wet soil (SO-E1).

The effects of the analysis of NP, NP1EO and NP2EO are presented separately in Figures 35-37. The extractions were conducted according to the extraction procedure described in the Horizontal standard sub-sections 10.1.3 and 10.1.4 concerning the extraction of wet soil or compost and freeze-dried soil or compost, respectively.

The relative difference introduced by adding 0 ml/g dm and 1 ml/g dm of water (i.e. factor H1)

to the freeze-dried samples CW-1 and CW-2 were in total above 40% regarding both NP and NP1EO. To avoid the scale of the y-axis being undesirably large (hence diminishing a visual evaluation of the other the results), these results were not included in the figures.

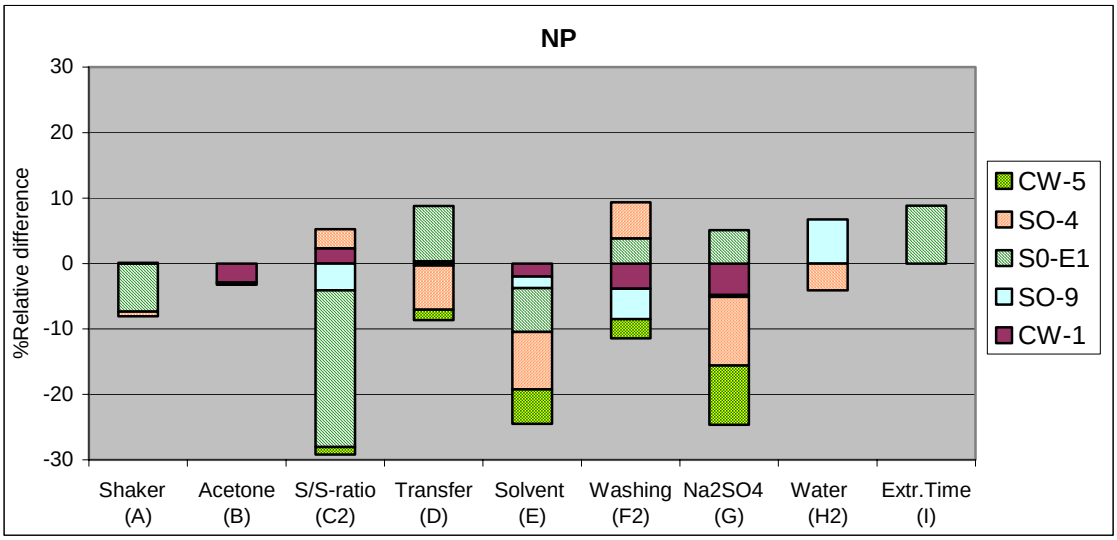

Figure 41. The relative difference (%) in concentration of NP caused by the variation of the described parameters.

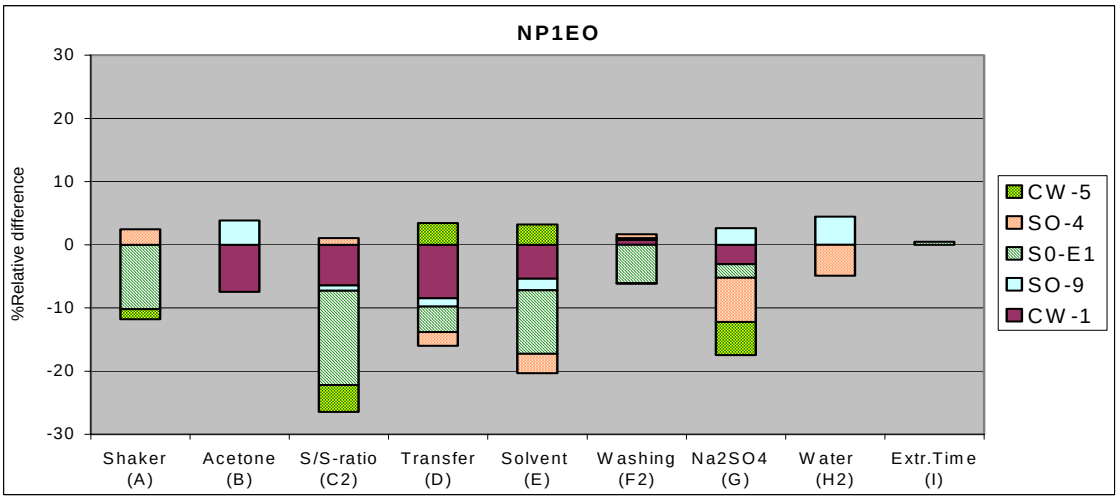

Figure 42. The relative difference (%) in concentration of NP caused by the variation of the described parameters.

The internal standard of NP2EO described in NP-Horizontal standard was not used in the analysis of SO-4 and the results were therefore not presented in Figure 43. The concentration of NP2EO in CW-5 was below the detection limit.

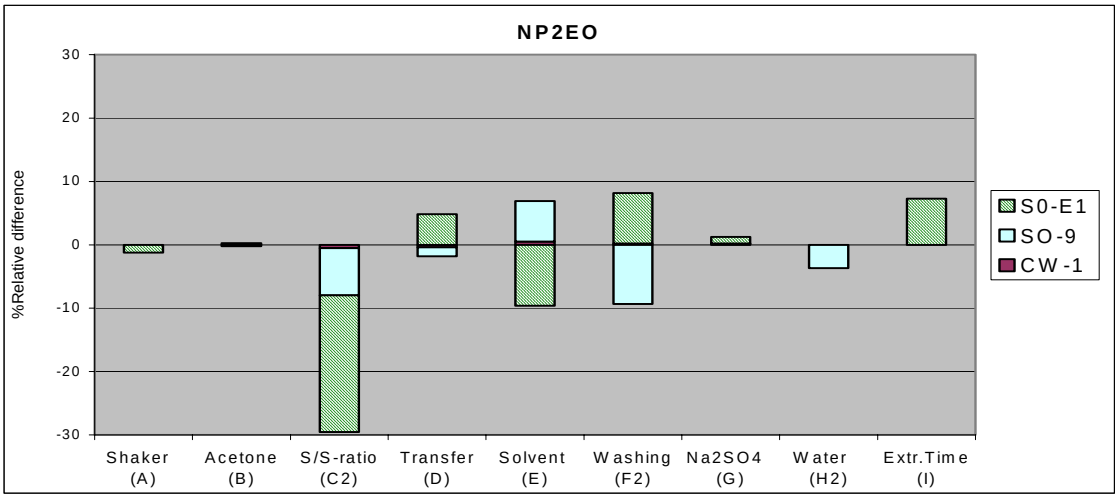

Figure 43. The relative difference (%) in concentration of NP caused by the variation of the described parameters.

7.3.3 The influence of the tested factors

The effect of shaking velocity (A):
The majority of the samples were less than 5% affected by the velocity of the reciprocating shaker and the effects were distributed more or less equally around zero. The tested variation in velocity of the reciprocating shaker was thus not found to introduce any difference in the extraction of NP, NP1EO and NP2EO.

The effect of shaking the acetone-sample mixture prior to the extraction (B):
The effect of this change in extraction procedure was tested on two samples (SO9 and CW1). No indication of influence was observed on any of the alkylphenols.

The effect of the sample/solvent-ratio (C):
The extraction of NP, NP1EO and NP2EO from wet soil (SO-E1) appeared to be more efficient when conducted on the smallest sample/solvent-ratio (Figures 41-43). This tendency was not observed with the extraction of the other samples (i.e. wet and freeze-dried sludge and freeze-dried soil and compost).

The effect of transferring the extract to a new flask for wash with water (D):
The quantification of the alkylphenols were, in the majority of the sludge samples, found to result in slightly higher concentrations when the water was added to the sample-extract mixture, instead of transferring the extract to a new flask for wash with water. When the extractions were conducted on soil and compost no effect was found. The effect of the washing procedures was also evaluated by comparing the chromatograms for NP, NP1EO and NP2EO of extracts submitted to the respective treatments. The comparisons, which were based on sludge extracts, did not reveal any significant chromatographic differences, such as poor resolution, increased background noise or interfering compounds.

The effect of solvent type (E):
The use of pentane and isooctane, respectively, did not introduce any difference in the extraction of NP, NP1EO and NP2EO.

The effect of the amount of water used in the washing of extracts (F):

The variation in the amount of water (i.e. ± 20%) used for washing the extracts did not cause any effects on the results.

The effect of drying the extract with anhydrous sodium sulphate (G):
The quantification of NP and NP1EO in the soil and compost samples where slightly positively affected by the addition of sodium sulphate. The data were not sufficient to evaluate the effect on the quantification of NP2EO. The influence of sodium sulphate of sludge extracts appeared to be random.

The amount of water added to freeze-dried samples prior to the extraction (H):
Initially the influence of this factor (H1) was tested by comparing extractions of freeze-dried samples without water (0 ml) and freeze-dried samples with water (1 ml/g dm). This was found to introduce a relative difference of more than 40% (the effect on CW-1 and CW-5 in total), which confirmed the results of earlier studies (5.3.3). The succeeding ruggedness tests where therefore changed to compare samples added water to 1 ml/g dm and 2 ml/g dm (H2). No indications of differences between the samples added 1 or 2 ml/g dm were found.

Effect of extraction time (I):
The extraction time was only tested on two samples (SL-E4 and SO-E1) and no effects were observed.

7.4 Consequences of ruggedness tests

The ruggedness testing of the 3rd draft of the NP Horizontal Standard resulted in some adjustments of sub-sections 10.1.1 – 10.1.4, i.e. the description of the four extraction procedures concerning the extraction of wet sludge, freeze-dried sludge, wet soil/compost and freeze-dried soil/compost, respectively. With these changes the draft standard was written, ready for the 2nd consultation in the preparation of horizontal standards.

The consequences of the ruggedness test on the method are:

A) The minimum velocity of the reciprocating shaker is stated as 230 strokes per minute.
B) Although no effect of the initial shaking of acetone and sample was observed, it was decided not to leave out this procedure. The reason was the limited number of samples tested.
C) The description of sample/solvent ratio was kept unchanged regarding the extraction procedure of wet sludge, freeze-dried sludge and freeze-dried soil/compost (i.e. extraction procedure 1, 2 and 4). For wet soil, however, a decrease in sample/solvent ratio was found to be necessary. The total applied volume of acetone and hexane-like solvent was consequently changed from 40 ml to 60 ml (i.e. 30 ml acetone and 30 ml hexane-like solvent).
D) Based on the results of the ruggedness tests conducted on sludge samples, and in order to obtain a simplification of the method, a defined quantity of water may be added directly to the extract-sample mixture instead of transferring the extract to the water.
E) No additional limitations were introduced regarding the choice of hexane-like solvent.
F) No changes were introduced to the method regarding the description of the applied amount of water used in washing procedure of the extract.
G) The use of anhydrous sodium sulphate for drying the extract was maintained, mostly to reduce the risk of water deactivating the derivatization reagent (MSTFA).
H) The description of the applied amount of water added to freeze-dried samples was kept unchanged.

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APPENDIX 1 Mass spectra of derivatized alkylphenols

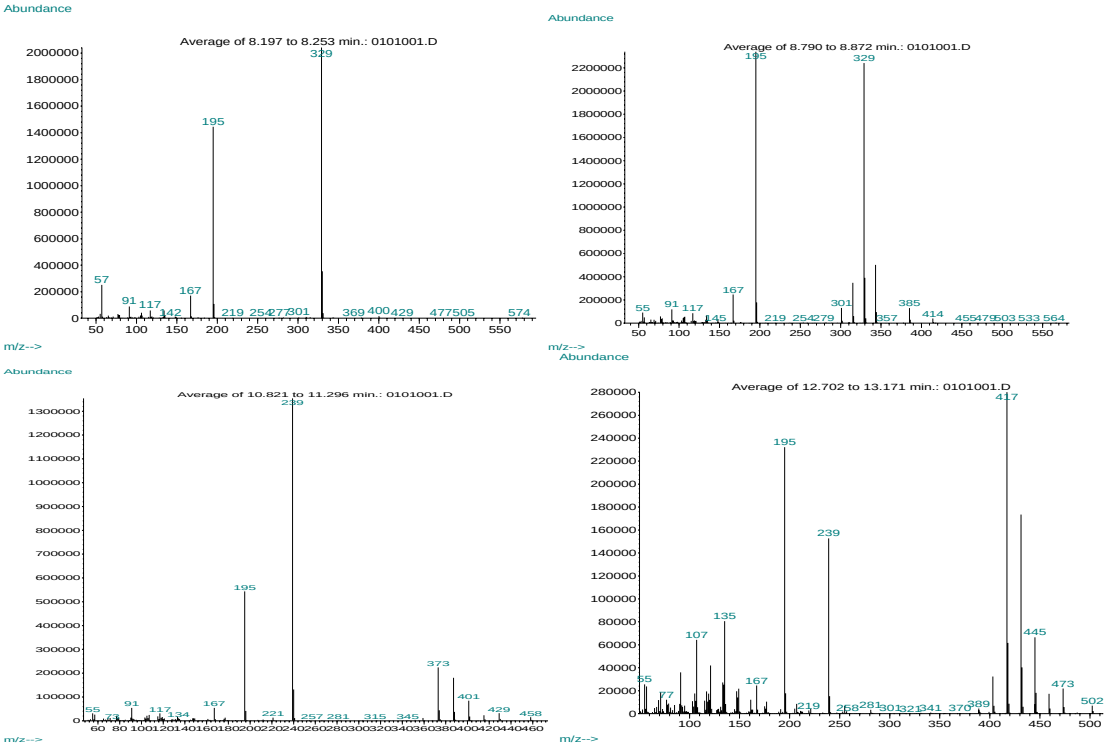

Figure A1.1: Mass spectra of PFBCl-derivate of a) OP, b) NP, c) NP1EO and d) NP2EO.

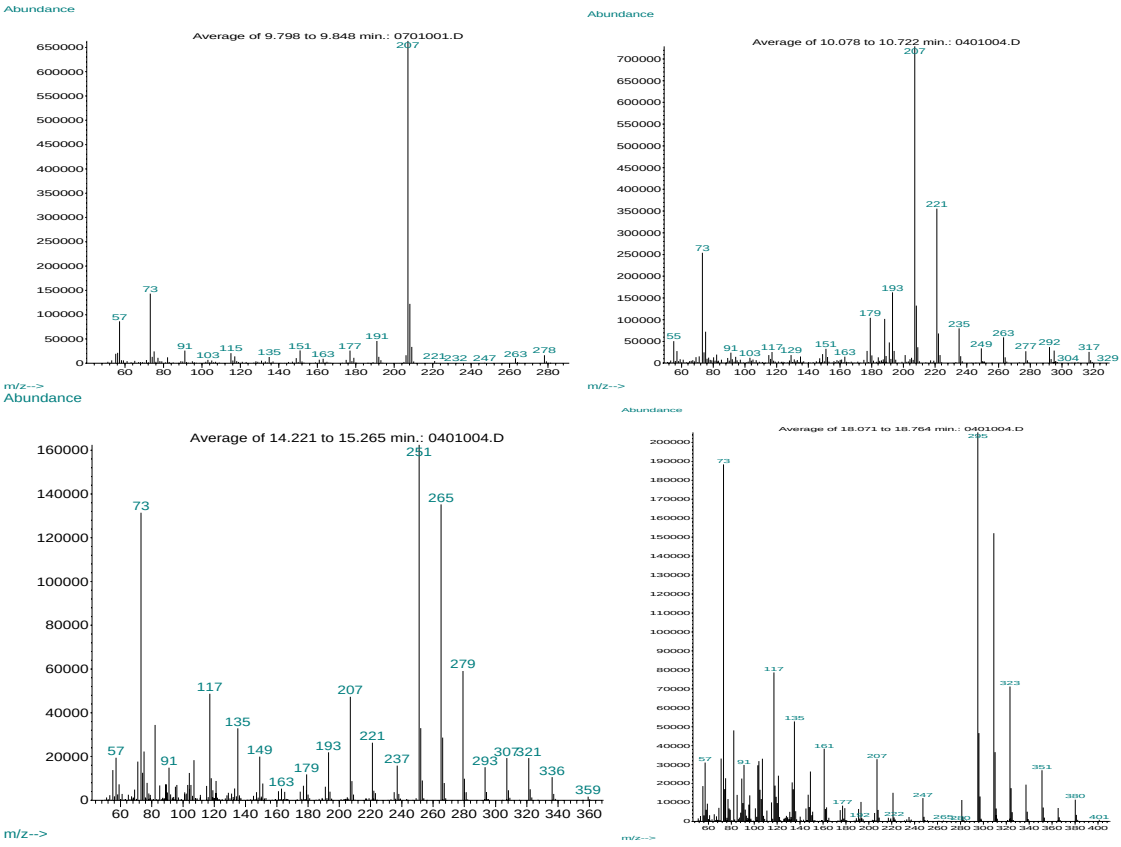

Figure A1.2: Mass spectra of MSTFA-derivate of a) OP, b) NP, c) NP1EO and d) NP2EO.

APPENDIX 2 Results – Extraction solvent

Appendix 2A Extraction of freeze-dried sludge (SL-E1) comparing the extraction efficiencies of toluene, DCM and acetone/pentane (described in 5.2.1.1).

Extracted with	Time of ext.	NP (mg/kg)	NP1EO (mg/kg)	NP2EO (mg/kg)
DCM + 0ml water	2 H	46.6	5.33	4.50
DCM +10ml water	2 H	78.5	8.80	7.28
DCM + 50ml water	2 H	80.0	9.28	7.66
DCM + 50mlwater + H2SO4	2 H	98.5	11.3	9.50
Acetone/pentane + 0ml water	2 H	37.0	4.69	4.12
Acetone/pentane + 10ml water	2 H	121	12.3	13.7
Toluene + 0ml water	2 H	27.2	3.72	2.96
Toluene + 10ml water	2 H	43.5	5.13	3.69
Toluene + 50ml water	2 H	45.4	5.13	3.91
Toluene + 50mlwater + H2SO4	2 H	43.6	5.78	3.81
DCM + 0ml water	16 H	58.0	7.70	6.36
DCM +10ml water	16 H	82.7	9.77	7.24
DCM + 50ml water	16 H	94.2	9.97	8.78
DCM + 50mlwater +H2SO4	16 H	75.2	9.10	7.42
Acetone/pentane + 0ml water	16 H	60.4	8.08	5.83
Acetone/pentane + 10ml water	16 H	75.8	7.64	7.39
Toluene + 0ml water	16 H	24.2	3.37	2.46
Toluene + 10ml water	16 H	37.5	4.64	3.17
Toluene + 50ml water	16 H	70.4	8.89	6.86

Appendix 2B Extraction of spiked and un-spiked freeze-dried soil (SO-4) comparing the extraction efficiencies of DCM, acetone/pentane and ethyl acetate (described in 5.2.1.2).

Solvent	Spiked	NP (mg/kg)	NP1EO (mg/kg)	NP2EO (mg/kg)
DCM	No	0.476	0.575	0.563
	No	0.528	0.787	4.069
	Yes	4.54	4.10	3.72
	Yes	4.75	4.36	3.82
Acetone/Pentane	No	0.692	1.016	0.786
	No	0.480	0.809	0.618
	Yes	3.96	3.88	3.39
	Yes	4.16	3.95	3.47
Ethyl acetate	No	0.525	0.854	0.613
	No	0.525	0.924	0.748
	Yes	4.83	3.81	3.41
	Yes	4.52	4.28	4.01

Appendix 2C Extraction of differently (pre-)treated sludge (SL-E2) comparing the extraction efficiencies of DCM. ethyl acetate (Et.ac.)and acetone/pentane (a/p) (described in 5.2.1.2).

Extraction #	Treatment	Solvent	GF% IS2 (NP-d4)	NP mg/kg	NP1EO mg/kg	NP2EO mg/kg
1	0 ml	Et.ac	107	6.33	1.28	4.47
2	0 ml	Et.ac	103	22.4	3.49	5.82
3	0 ml	Et.ac	106	11.5	2.07	5.24
4	0 ml	DCM	88.3	15.0	2.90	4.90
5	0 ml	DCM	100	16.5	2.99	5.97
6	0 ml	DCM	103	16.8	3.24	5.89
7	0 ml	a/p	98.2	26.2	4.64	6.10
8	0 ml	a/p	92.3	24.2	4.51	5.75
9	0 ml	a/p	95.7	25.8	4.45	6.00
10	60 ml	Et.ac	81.0	19.8	3.11	4.88
11	60 ml	Et.ac	77.0	18.8	3.12	4.80
12	60 ml	Et.ac	89.3	22.0	3.63	5.48
13	60 ml	DCM	77.1	16.6	3.04	4.21
14	60 ml	DCM	67.5	13.8	2.68	3.97
15	60 ml	DCM	85.5	18.8	3.52	4.67
16	60 ml	a/p	82.8	21.1	3.75	4.42
17	60 ml	a/p	78.0	18.3	3.47	4.01
18	60 ml	a/p	78.8	18.1	3.48	4.19
19	Freeze	Et.ac	104	21.5	1.88	6.17
20	Freeze	Et.ac	84.9	16.3	1.79	5.00
21	Freeze	Et.ac	96.6	19.6	1.67	5.45
22	Freeze	DCM	109	23.6	4.61	7.69
23	Freeze	DCM	87.2	18.4	3.60	6.07
24	Freeze	DCM	87.5	18.6	3.71	6.44
25	Freeze	a/p	102	22.3	4.20	6.80
26	Freeze	a/p	100	22.0	4.14	5.94
27	Freeze	a/p	97.5	22.2	4.18	6.47

Appendix 2D Results of study comparing acetone/pentane and DCM (5.2.1.3), pre-treatment (5.3.1) and solvent volume (5.2.1.4).

Conditions of extraction								Concentration		
Extraction No.	Time of extraction (h)	Type of solvent	$V_{solvent}$ (ml)	Oven-dried	Water added	H_2SO_4 added	NaOH added	NP (mg/kg)	NP1EO (mg/kg)	NP2EO (mg/kg)
1	2	DCM	15	x				27.1	11.4	-
2	2	DCM	15					17.2	4.32	-
3	2	DCM	15		x			4.47	1.28	-
4	2	DCM	30	x				27.9	7.26	-
5	2	DCM	30					7.28	1.82	-
6	2	DCM	30		x			16.7	3.42	-
7	2	DCM	15	x		x		13.5	3.74	-
8	2	DCM	15			x		37.6	6.27	-
9	2	DCM	15		x	x		1.50	0.36	-
10	2	DCM	30	x		x		19.1	4.58	-
11	2	DCM	30			x		24.7	4.78	-
12	2	DCM	30		x	x		6.04	1.26	-
13	2	A/P	15	x				25.7	5.16	-
14	2	A/P	15					29.7	5.46	-
15	2	A/P	15		x			29.8	5.80	-
16	2	A/P	30	x				23.6	4.69	-
17	2	A/P	30					35.4	6.86	-
18	2	A/P	30		x			12.3	2.48	-
19	2	A/P	15	x		x		1.51	0.71	-
20	2	A/P	15			x		46.5	10.4	-
21	2	A/P	15		x	x		0.0	0.00	-
22	2	A/P	30	x		x		0.82	0.63	-
23	2	A/P	30			x		35.6	7.29	-
24	2	A/P	30		x	x		34.2	5.88	-
25	20	DCM	15	x				27.9	5.35	-
26	20	DCM	15					4.35	0.79	-
27	20	DCM	15		x			6.65	1.31	-
28	20	DCM	30	x				31.2	6.44	-
29	20	DCM	30					16.3	2.88	-
30	20	DCM	30		x			25.7	4.62	-
31	20	DCM	15	x		x		24.4	5.32	-
32	20	DCM	15			x		13.8	2.63	-
33	20	DCM	15		x	x		35.4	5.86	-
34	20	DCM	30	x		x		36.2	7.40	-
35	20	DCM	30			x		43.0	7.98	-
36	20	DCM	30		x	x		28.5	4.46	-
37	20	DCM	15	x			x	43.2	8.89	-
38	20	DCM	15				x	43.4	7.79	-
39	20	DCM	15		x		x	50.8	9.39	-
40	20	DCM	30	x			x	40.4	8.99	-
41	20	DCM	30				x	50.7	9.21	-
42	20	DCM	30		x		x	11.4	2.03	-
43	20	A/P	15	x			x	56.2	10.6	-
44	20	A/P	15				x	56.4	9.53	-
45	20	A/P	15		x		x	26.7	5.50	-
46	20	A/P	30	x			x	55.7	11.6	-
47	20	A/P	30				x	43.7	7.85	-
48	20	A/P	30		x		x	20.1	3.60	-
49	20	A/P	15	x				34.7	6.61	-
50	20	A/P	15					51.3	9.23	-
51	20	A/P	15		x			42.1	7.65	-
52	20	A/P	30	x				31.7	5.98	-
53	20	A/P	30					36.0	6.97	-
54	20	A/P	30		x			23.0	4.22	-
55	20	A/P	15	x		x		5.22	3.15	-
56	20	A/P	15			x		0.04	0.00	-
57	20	A/P	15		x	x		1.58	0.35	-
58	20	A/P	30	x		x		51.5	9.98	-
59	20	A/P	30			x		46.6	8.15	
60	20	A/P	30		x	x		105	17.0	-

APPENDIX 3 Results – Extraction technique

Results of study comparing soxhlet and reciprocating shaking (5.2.2) and solvents (5.2.1.1).

		Concentration (mg/kg)				
Extraction technique	Solvent	NP-D4	NP	NP1EO	4-n-NP2EO	NP2EO
Sohxlet	Acetone/pentane	60.6 55.1	2.92 2.72	23.2 21.4	102 95.5	21.0 19.5
	Toluene	24.8 15.0	1.88 1.13	23.6 23.9	97.7 95.5	24.4 25.2
Reciprocating shaking	Acetone/pentane	75.0 80.6	4.05 4.06	28.3 22.7	127 97.7	24.4 19.6
	Toluene	74.1 67.2	3.34 3.73	22.5 22.1	100 90.9	18.4 18.5
	Ethyl acetate	61.5 67.5	3.17 3.54	21.8 19.9	95.5 95.5	18.9 17.0

APPENDIX 4 Results – Extraction time

	Concentration of alkylphenols in SL-E2 (mg/kg)				Concentration of alkylphenols in SL-4 (mg/kg)			
Time of extraction (hours)	NP-d4	NP	NP1EO	NP2EO	NP-d4	NP	NP1EO	NP2EO
½ h	1.16	22.3	4.09	4.78	1.17	7.79	35.3	30.0
½ h	1.20	22.8	4.33	4.49	1.08	7.06	32.1	27.5
1 h	1.25	25.8	4.83	4.89	2.35	8.36	38.0	31.7
1 h	1.29	26.4	5.06	5.00	2.49	8.33	37.9	31.4
2 h	1.34	26.4	5.05	5.40	2.01	7.27	33.5	26.5
2 h	1.30	26.1	5.21	5.38	2.05	8.04	37.7	29.8
3 h	1.28	27.7	5.33	5.45	2.26	6.07	37.9	29.8
3 h	1.23	26.0	5.20	5.09	2.04	7.51	35.7	27.6
4 h	1.25	25.7	5.47	5.46	1.68	7.37	29.9	22.6
4 h	1.26	26.2	5.63	5.40	2.02	7.31	37.6	27.3
6 h	1.25	25.7	5.31	4.98	2.41	7.36	39.8	29.1
6 h	1.28	25.8	5.51	5.23	2.29	7.63	35.9	29.3
20 h	1.23	24.2	5.99	5.79	2.41	8.00	37.8	31.2
20 h	1.24	24.5	5.45	6.06	2.55	7.66	37.2	30.1

APPENDIX 5 Results – Pre-treatment

Appendix 5A. Extraction of freeze-dried sludge (SL-E2) added 0, 6 and 40 ml of water (5.3.3).

			Concentration/recovery			
No. Ext.	Water added	Solvent	NP-D4 (recovery%)	NP (mg/kg)	NP1EO (mg/kg)	NP2EO (mg/kg)
1	0 ml	a/p	83.2	18.9	3.08	5.10
2	0 ml	a/p	77.3	17.2	2.74	4.59
3	0 ml	a/p	87.7	19.5	3.16	5.22
4	6 ml	a/p	99.8	27.6	4.20	6.25
5	6 ml	a/p	118	28.8	4.57	7.02
6	6 ml	a/p	89.8	25.2	4.04	5.50
7	40 ml	a/p	101	28.4	4.44	5.20
8	40 ml	a/p	92.0	26.0	4.21	5.04
9	40 ml	a/p	91.6	24.8	4.00	4.72

Appendix 5B. Concentrations and recoveries of NP-D4, NP, NP1EO and NP2EO in sludge samples (SL-E2) added 0, 20, 50, 100 and 200 ml of water (5.3.4).

				Concentrations			
Name	Dm (%)	m,sample (g)	V,water (ml)	NP-D4 (mg/kg)	NP (mg/kg)	NP1EO (mg/kg)	NP2EO (mg/kg)
A 1.5%	1.33	9.92	200	0.335	10.8	2.83	2.82
B 1.5%	1.38	10.30	200	0.336	10.1	2.57	2.04
C 1.5%	1.37	10.23	200	0.349	10.7	2.50	2.24
A 5.0%	5.02	10.82	50	0.577	22.0	4.38	3.93
B 5.0%	4.93	10.59	50	0.686	25.5	5.20	5.20
C 5.0%	4.52	9.54	50	0.880	29.3	6.19	6.45
A 10%	9.63	10.37	20	0.821	32.8	8.32	5.94
B 10%	9.92	10.85	20	0.797	32.7	6.64	6.25
C 10%	9.19	9.67	20	0.971	35.8	6.98	6.60
A 15%	13.63	9.35	10	1.047	38.2	7.65	8.77
B 15%	14.30	10.29	10	0.868	35.8	7.09	7.89
C 15%	14.77	11.00	10	0.720	32.8	6.47	6.81
A 20%	18.67	9.80	5	1.02	39.4	8.19	9.41
B 20%	18.45	9.46	5	0.999	39.3	7.74	9.24
C 20%	18.71	9.86	5	1.03	40.0	8.32	10.4
A 30%	28.20	10.95	0	0.845	36.5	7.54	9.07
B 30%	28.20	10.51	0	0.869	37.5	7.53	9.83
C 30%	28.20	9.87	0	0.928	39.7	8.16	10.06

Appendix 5C. Concentrations and recoveries of NP-D4, NP, NP1EO and NP2EO in sludge samples (SL-E2) added 0, 20, 50, 100 and 200 ml of water (5.3.4).

				Recovery/Concentration				
Name	dm%	m,sample (g)	V,water (ml)	NP-D4 (Recovery%)	NP (mg/kg)	NP1EO (mg/kg)	NP2EO-c13 (Recovery%)	NP2EO (mg/kg)
A 1%	1.12	11.12	270	36.6	13.5	3.16	7.8	2.4
B 1%	1.15	11.45	270	42.4	16.4	3.72	10	2.9
C 1%	1.16	11.61	270	38.5	14.1	3.02	8.8	2.2
A 2%	2.06	10.24	130	40.4	16.9	3.53	12	2.6
B 2%	2.37	11.92	130	40.4	15.9	3.12	12	2.6
C 2%	2.34	11.74	130	40.4	16.1	3.17	12	2.5
A 5.0%	5.17	10.10	45	73.2	32.3	5.90	21	4.4
B 5.0%	5.85	11.78	45	63.5	27.3	4.88	20	3.7
C 5.0%	5.26	10.33	45	80.9	35.5	6.59	22	4.7
A 10%	11.75	12.15	17	69.3	32.4	5.87	37	5.0
B 10%	11.77	12.18	17	73.2	33.0	5.94	36	5.2
C 10%	10.81	10.56	17	82.8	37.4	6.85	34	5.2
A 28%	28.20	10.56	0	80.9	37.3	6.95	36	7.3
B 28%	28.20	12.60	0	77.0	35.4	6.75	59	7.5
C 28%	28.20	11.55	0	80.9	36.6	7.00	51	6.9

APPENDIX 6 Results – Clean-up methods

Appendix 6: The concentrations of NP-D4, NP, NP1EO, p-n-NP2EO and NP2EO (mg/l) obtained in study 1 by eluting 1 ml of a 2,5 mg/l standard solution through a silica column with the described solvents.

Study 1 (2,5 mg/l standard)

		(mg/l)	4-n-NP-d4	NP	NP1EO	p-n-NP2EO	NP2EO
Pentane	4 ml	1.a	-	-	-	-	-
		1.b	-	-	-	-	-
Pentane/DCM (1:1)	4 ml	2.a	-	-	-	-	-
		2.b	-	-	-	-	-
DCM	2 ml	3.a	-	0,0050	-	-	-
		3.b	-	0,0033	-	-	-
DCM	2 ml	4.a	0,21	1,3	-	-	-
		4.b	0,16	1,1	-	-	-
DCM/Acetone (3:1)	2 ml	5.a	0,060	0,19	1,4	0,17	1,1
(3:1 = 3 ml DCM/ 1 ml Acetone)		5.b	0,080	0,25	1,6	0,16	1,1
DCM/Acetone (3:1)	2 ml	6.a	-	-	0,0	0,03	0,19
		6.b	-	0,0050	0,0	0,06	0,42
DCM/Acetone (1:1)	2 ml	7.a	-	-	-	-	-
		7.b	-	-	-	-	-
DCM/Acetone (1:1)	2 ml	8.a	-	-	-	-	-
		8.b	-	0,0054	-	-	-
Acetone	4 ml	9.a	-	-	-	-	-
		9.b	-	-	-	-	-

Appendix 6: The concentrations of NP, NP1EO and NP2EO (mg/l) obtained in study 2 by eluting 1 ml of a 2,5 mg/l standard solution through a silica column with the described solvents.

Study 2 (2,5 mg/l standard)

		(mg/l)	NP	NP1EO	NP2EO
Pentane/DCM (1:1)	2 ml	1. Std	0,010	-	-
Pentane/DCM (1:1)	2 ml	2. Std	-	-	-
DCM/Acetone (3:1)	2 ml	3. Std	2,4	2,5	2,1
DCM/Acetone (3:1) (3:1 = 3 ml DCM/ 1 ml Acetone)	2 ml	4. Std	0,0053	0,0079	0,11
DCM/Acetone (3:1)	2 ml	5. Std	0,0055	-	-
DCM/Acetone (1:1)	2 ml	6. Std	-	-	-
Acetone	2 ml	7. Std	-	-	-

Appendix 6: The concentrations of NP-D4, NP, NP1EO, p-n-NP2EO and NP2EO (mg/l) obtained in study 2 by eluting 1 ml of an extract (SL-E4) through a silica column with the described solvents**.**

Study 2 (SL-E4)

		(mg/l)	4-n-NP-d4	NP	NP1EO	p-n-NP2EO	NP2EO
Pentane/DCM (1:1)	2 ml	1	1,1	3,0	0,48	-	0,21
Pentane/DCM (1:1)	2 ml	2	0,081	0,25	0,18	0,0094	0,0071
DCM/Acetone (3:1)	2 ml	3	0,21	0,43	1,1	1,9	0,35
DCM/Acetone (3:1) (3:1 = 3 ml DCM/ 1 ml Acetone)	2 ml	4	-	0,0088	0,010	0,086	0,015
DCM/Acetone (3:1)	2 ml	5	-	0,014	0,0047	-	0,0071
DCM/Acetone (1:1)	2 ml	6	-	0,0077	-	-	0,0056
Acetone	2 ml	7	-	-	-	-	-

Appendix 6:
The concentrations of NP, NP1EO and NP2EO (mg/l) obtained in study 3 by eluting 1 ml of an extract (SL-E4) through a silica column with the described solvents.

Study 3 **SL-E4**		**(mg/l)**	NP	NP1EO	NP2EO
Pentane	2 ml	1. Std	-	-	-
Pentane	2 ml	2. Std	-	-	
DCM/Acetone (3:1)	2 ml	3. Std	2,2	0,82	0,10
DCM/Acetone (3:1) (3:1 = 3 ml DCM/ 1 ml Acetone)	2 ml	4. Std	0,11	0,066	0,020
DCM/Acetone (3:1)	2 ml	5. Std	0,0057	-	
DCM/Acetone (1:1)	2 ml	6. Std			
Acetone	2 ml	7. Std			

Appendix 6: The concentrations of NP-D4, NP, NP1EO, p-n-NP2EO and NP2EO (mg/l) obtained in study 4 by eluting 1 ml of an extract (SL-E3) through a silica column with the described solvents.

Study 4 **SL-E3**		**(mg/l)**	4-n-NP-d4	NP	NP1EO	p-n-NP2EO	NP2EO
Pentane	3 ml	1.a	0,16	0,12	-	-	-
		1.b	0,18	0,12	-	-	-
Pentane	3 ml	2.a	-	-	-	-	-
		2.b	0,0042	0,0068	-	-	-
Pentane	3 ml	3.a	-	-	-	-	-
		3.b	0,004	0,008	-	-	-
DCM/Acetone (3:1)	3 ml	4.a	0,039	0,035	0,015	0,171	0,0076
		4.b	0,094	0,041	0,019	0,216	0,012
DCM/Acetone (3:1)	3 ml	5.a	0,0028	0,0051	0,0023	-	0,0012
(3:1 = 3 ml DCM/ 1 ml Acetone)		5.b	0,0027	0,0051	0,013	0,081	0,022
DCM/Acetone (3:1)	3 ml	6.a	-	-	-	-	-
		6.b	-	-	-	-	-
Acetone	6 ml	7.a	-	-	-	-	-
		7.b	-	-	-	-	-

Appendix 6:
The concentrations of NP-D4, NP, NP1EO and NP2EO (mg/l) obtained in study 4 by eluting 1 ml of an extract (SL-11) through a silica column with the described solvents.

Study 4 **SL-11**		mg/l	4-n-NP-d4	NP	NP1EO	NP2EO
Pentane+sample	3 ml	1.a	0,0064	0,0049	-	-
		1.b	0,010	0,0065	-	-
Pentane	3 ml	2.a	-	-	-	-
		2.b	0,0051	0,0059	-	0,0034
Pentane	3 ml	3.a	-	-	-	-
		3.b	-	0,0031	-	-
DCM/Acetone (3:1)	3 ml	4.a	0,26	0,11	1,5	1,1
		4.b	0,27	0,12	1,7	1,4
DCM/Acetone (3:1)	3 ml	5.a	-	0,0022	0,0023	0,0050
(3:1 = 3 ml DCM/ 1 ml Acetone)		5.b	0,0027	0,0051	0,013	0,022
DCM/Acetone (3:1)	3 ml	6.a	-	-	-	-
		6.b	-	-	-	-
Acetone	6 ml	7.a	-	-	-	-
		7.b	-	-	-	-

Appendix 6: The concentrations of NP-D4, NP, NP1EO, p-n-NP2EO and NP2EO (mg/l) obtained in study 4 by eluting 1 ml of an extract (SL-E4) through a silica column with the described solvents.

Study 4 **SL-E4**		**(mg/l)**	4-n-NP-d4	NP	NP1EO	p-n-NP2EO	NP2EO
Pentane+sample	3 ml	1.a	0,027	0,061	0,028	0,075	-
		1.b	0,031	0,021	-	-	-
Pentane	3 ml	2.a	-	0,0073	0,0024	0,033	0,010
		2.b	-	-	-	-	-
Pentane	3 ml	3.a	-	0,0053	0,0017	0,001	0,0031
		3.b	-	-	-	-	-
DCM/Acetone (3:1)	3 ml	4.a	0,27	1,3	0,37	0,34	0,078
		4.b	0,31	1,5	0,48	-	0,060
DCM/Acetone (3:1)	3 ml	5.a	0,001	0,010	0,004	-	0,0025
(3:1 = 3 ml DCM/ 1 ml Acetone)		5.b	-	0,007	0,003	-	-
DCM/Acetone (3:1)	3 ml	6.a	-				
		6.b	-	-	-	-	-
Acetone	6 ml	7.a	-	-	-	-	-
		7.b	-	-	-	-	-

APPENDIX 7 Results – Stability of extracts

Solution	Temp	Compound	Day of analysis 0	1	2	4	8	16	38	80
Solution A 0.1 mg/l Not Derivatizised	22 ºC	NP-D4	-	0.0368	0.0377	0.0298	0.0302	0.0274	0.0211	0.00753
		NP	-	0.108	0.106	0.0927	0.0966	0.0816	0.0592	0.0263
		NP1EO	-	0.0944	0.1044	0.0840	0.0887	0.0943	0.0371	0.00921
		NP2EO	-	0.104	0.0991	0.0822	0.0901	0.0558	0.0153	0.00356
	4 ºC	NP-D4	-	0.0348	0.0322	0.0310	0.0304	0.0275	0.0288	0.0286
		NP	-	0.0889	0.103	0.0916	0.102	0.0907	0.0807	0.0787
		NP1EO	-	0.101	0.0860	0.0952	0.0881	0.0834	0.0617	0.0580
		NP2EO	-	0.0884	0.0966	0.0943	0.0835	0.0796	0.0314	0.0302
	- 18 ºC	NP-D4	-	0.0362	0.0333	0.0310	0.0320	0.0280	0.0294	0.0297
		NP	-	0.0973	0.111	0.113	0.0993	0.0925	0.0838	0.0861
		NP1EO	-	0.0985	0.101	0.0976	0.0933	0.0840	0.0620	0.600
		NP2EO	-	0.0926	0.0908	0.0937	0.0926	0.0760	0.0300	0.0302
Solution B 0.1 mg/l Derivatizised	22 ºC	NP-D4	0.0398	0.0356	0.0330	0.0310	0.0330	0.0294	0.0312	0.000481
		NP	0.109	0.105	0.0868	0.0989	0.0966	0.102	0.0839	< DL
		NP1EO	0.104	0.100	0.0966	0.0928	0.999	0.0886	0.0634	0.00469
		NP2EO	0.104	0.0779	0.101	0.108	0.0932	0.0835	0.0380	0.00314
	4 ºC	NP-D4	-	0.0340	0.0327	0.0299	0.0303	0.0266	0.0288	0.0294
		NP	-	0.0903	0.0962	0.0926	0.103	0.910	0.0788	0.0823
		NP1EO	-	0.0873	0.0978	0.0909	0.0887	0.903	0.0598	0.0589
		NP2EO	-	0.0860	0.0971	0.0789	0.0980	0.0860	0.0339	0.0345
	- 18 ºC	NP-D4	-	0.0352	0.0310	0.0326	0.0338	0.0272	0.0287	0.0285
		NP	-	0.101	0.0953	0.1014	0.107	0.0991	0.0817	0.0816
		NP1EO	-	0.0931	0.0931	0.0968	0.0966	0.0902	0.0610	0.0594
		NP2EO	-	0.0977	0.0899	0.0976	0.104	0.0936	0.0330	0.0329
Solution C 2.5 mg/l Not Derivatizised	22 ºC	NP-D4	-	1.01	0.989	0.809	0.869	0.847	0.914	0.864
		NP	-	2.42	2.41	2.10	2.26	2.35	2.24	2.04
		NP1EO	-	2.36	2.35	1.98	1.94	1.98	1.22	0.968
		NP2EO	-	2.12	2.21	1.80	1.63	1.52	0.601	0.321
	4 ºC	NP-D4	-	1.02	0.951	0.917	0.925	0.837	1.02	1.06
		NP	-	2.43	2.35	2.34	2.42	2.21	2.44	2.50
		NP1EO	-	2.35	2.21	2.22	2.25	1.98	1.98	1.94
		NP2EO	-	2.11	2.08	2.04	2.07	1.75	1.26	1.25
	- 18 ºC	NP-D4	-	0.998	0.943	0.916	0.925	0.902	0.990	1.06
		NP	-	2.39	2.34	2.39	2.36	2.37	2.39	2.50
		NP1EO	-	2.32	2.23	2.24	2.18	2.11	1.89	1.94
		NP2EO	-	2.04	2.09	2.04	2.10	1.84	1.19	1.24
Solution D 2.5 mg/l Derivatizised	22 ºC	NP-D4	1.04	0.996	0.940	0.919	0.910	0.875	1.00	0.420
		NP	2.35	2.37	2.36	2.35	2.31	2.32	2.32	1.16
		NP1EO	2.29	2.32	2.22	2.15	2.22	2.10	1.95	0.703
		NP2EO	2.03	2.10	2.09	1.99	2.07	1.91	1.36	0.437
	4 ºC	NP-D4	-	0.991	0.926	0.948	0.908	0.866	1.06	1.04
		NP	-	2.36	2.33	2.46	2.30	2.25	2.50	2.45
		NP1EO	-	2.32	2.19	2.27	2.18	2.09	2.04	1.89
		NP2EO	-	2.09	2.05	2.14	2.13	1.93	1.38	1.29
	- 18 ºC	NP-D4	-	0.981	0.919	0.915	0.928	0.910	1.03	1.02
		NP	-	2.31	2.37	2.39	2.34	2.35	2.46	2.43
		NP1EO	-	2.30	2.19	2.25	2.23	2.17	1.96	1.85
		NP2EO	-	2.07	2.07	2.11	2.16	1.94	1.32	1.20
Solution E SL-E2 Not Derivatizised	22 ºC	NP-D4	-	0.0138	0.0146	0.0153	0.0145	0.0141	0.0146	0.0149
		NP	-	1.69	1.68	1.64	1.67	1.57	1.64	1.50
		NP1EO	-	0.397	0.420	0.408	0.386	0.341	0.292	0.196
		NP2EO	-	0.199	0.257	0.261	0.284	0.235	0.140	0.086
	4 ºC	NP-D4	-	0.0115	0.0129	0.0130	0.0140	0.0131	0.0154	0.0175
		NP	-	1.54	1.63	1.60	1.64	1.48	1.72	1.82
		NP1EO	-	0.323	0.366	0.325	0.348	0.333	0.348	0.321
		NP2EO	-	0.187	0.223	0.198	0.201	0.228	0.137	0.130
	- 18 ºC	NP-D4	-	0.0113	0.0126	0.0131	0.0135	0.0138	0.0157	0.0187
		NP	-	1.56	1.66	1.62	1.63	1.63	1.79	1.82
		NP1EO	-	0.347	0.364	0.354	0.341	0.338	0.348	0.346
		NP2EO	-	0.181	0.202	0.208	0.221	0.199	0.151	0.136
Solution F SL-E2 Derivatizised	22 ºC	NP-D4	0.0129	0.0143	0.0146	0.0150	0.0160	0.0153	0.0150	0.0117
		NP	1.72	1.70	1.67	1.64	1.66	1.71	1.63	1.34
		NP1EO	0.395	0.383	0.411	0.416	0.427	0.424	0.331	0.267
		NP2EO	0.173	0.195	0.244	0.266	0.261	0.227	0.148	0.101
	4 ºC	NP-D4	-	0.0118	0.0127	0.0136	0.0128	0.0138	0.0159	0.0183
		NP	-	1.52	1.61	1.57	1.61	1.61	1.78	1.79
		NP1EO	-	0.321	0.330	0.340	0.317	0.346	0.357	0.333
		NP2EO	-	0.159	0.190	0.199	0.215	0.192	0.163	0.119
	- 18 ºC	NP-D4	-	0.0119	0.0128	0.0128	0.0143	0.0140	0.0161	0.0156
		NP	-	1.52	1.62	1.61	1.64	1.61	1.79	1.81
		NP1EO	-	0.344	0.354	0.336	0.322	0.329	0.351	0.333
		NP2EO	-	0.162	0.198	0.196	0.242	0.201	0.143	0.125

- : Not analysed

APPENDIX 8 Results – Ruggedness test

Concentration (mg/kg) of NP, NP1EO and NP2EO in samples used in ruggedness tests.

		Concentration (mg/kg)									
Sub-sample no.	Sample ID	**SL-E1**	**SL-E2 (OD)[1]**	**SL-E2 (FD)[2]**	**SL-E4**	**SL-11**	**SO-E1**	**SO-4**	**SO-9**	**CW-1**	**CW-5**
1	NP	41.2	27.4	27.4	21.2	3.21	2.34	1.00	0.237	0.349	0.151
	NP1EO	6.20	5.32	6.09	9.21	20.9	0.185	1.01	0.582	0.225	0.0498
	NP2EO	1.64	-	-	1.39	19.4	0.118	0.826	0.172	0.201	-
2	NP	40.5	25.8	27.3	17.8	3.02	1.82	1.17	0.242	0.232	0.109
	NP1EO	6.08	5.44	6.02	8.88	24.8	0.209	1.03	0.556	0.152	0.0389
	NP2EO	1.65	-	-	1.61	18.0	0.0969	0.952	0.172	0.148	-
3	NP	40.3	24.7	26.8	20.1	3.39	1.72	1.22	0.242	0.274	0.112
	NP1EO	5.62	6.00	5.80	9.86	21.8	0.179	1.18	0.545	0.176	0.0448
	NP2EO	1.47	-	-	1.64	17.1	0.0977	1.11	0.205	0.175	-
4	NP	41.0	25.1	27.0	17.1	2.98	1.75	1.07	0.258	0.339	0.165
	NP1EO	6.35	4.94	5.47	8.19	21.8	0.196	1.07	0.575	0.230	0.0514
	NP2EO	1.64	-	-	1.70	17.5	0.0931	0.85	0.192	0.200	-
5	NP	40.4	25.9	27.1	20.9	3.45	2.36	1.08	0.207	0.252	0.102
	NP1EO	6.21	5.75	6.04	9.60	27.6	0.238	1.09	0.553	0.149	0.0343
	NP2EO	1.36	-	-	1.53	17.2	0.114	1.05	0.196	0.159	-
6	NP	40.0	25.6	26.8	20.9	3.23	2.35	1.29	0.229	0.324	0.172
	NP1EO	5.92	5.11	5.47	9.84	27.6	0.238	1.14	0.551	0.194	0.0547
	NP2EO	1.67	-	-	1.60	17.2	0.123	0.951	0.190	0.190	-
7	NP	40.1	24.6	27.3	21.0	3.32	1.83	1.024	0.256	0.362	0.168
	NP1EO	5.99	5.40	6.06	10.3	17.9	0.192	1.06	0.617	0.248	0.0695
	NP2EO	1.31	-	-	1.87	17.8	0.0852	0.984	0200	0.250	-
8	NP	40.8	24.1	26.5	19.5	3.31	1.67	1.09	0.214	0.228	0.0947
	NP1EO	5.96	5.44	5.92	9.43	22.9	0.183	1.07	0.547	0.153	0.0395
	NP2EO	1.55	-	-	1.53	17.3	0.0852	1.02	0.191	0.133	-

1) Oven-dried SL-E2
2) Freeze-dried SL-E2

APPENDIX 9 Method applied in ruggedness test

HORIZONTAL

November 2005

Third draft (for ruggedness test)

Ad-hoc group LAS/nonylphenols

Horizontal standard for the determination of nonylphenols (NP) and nonylphenol-mono- and diethoxylates using gas chromatography with mass selective detection

Document type: International Standard
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STD Version 2.0

Contents

Foreword

This draft standard is developed within the project HORIZONTAL.

This document has been prepared for the ad-hoc group LAS/Nonylphenols of CEN/TC 292, CEN/TC 308 and ISO/TC 190 meeting on 21 September, 2005 in Madrid. After the meeting minor revisions in the text have been made.

This document is a working document.

Introduction

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 des-ethoxylation resulting in polyethoxylates with less ethoxy-groups. Nonylphenol-diethoxylates (NP2EO) nonylphenol-monoethoxylates (NP1EO) and nonylphenols (NP), are the 3 last products in the degradation chain. Due to their significant presence in sewage sludge, the 3 components are all included in the horizontal standard.

Horizontal standard for the determination of nonylphenols (NP) and nonylphenol mono- and diethoxylates using gas chromatography with mass selective detection

1 Scope

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

The exact LOD will be determined by the method validation. Matrices for which the standard has been validated are listed in Annex A.

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, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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.*

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*.

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.*

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/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 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. Have they been found in environmental samples?

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 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 change the sample during the contact through sampling and storage. Plastic materials may be used, if they have been proven not to change 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 great 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

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 (petroleum ether), boiling range 40 °C to 60 °C.

Any aliphatic hydrocarbon solvent with a boiling point or boiling range between 34 °C and 100 °C may be applied. The solvent hexane is neuro-toxic and is not advised to be used.

7.3 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.

7.4 Reagents for clean-up procedures

7.5 MSTFA for derivatization

Methyl-N-(trimethylsilyl)-trifluoracetamide, 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 0,5 ml of MSTFA in isooctane in a 10 ml volumetric flask and make up to volume with isooctane.

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

7.8 Operating gas for gas chromatography with MS-detector

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

7.9 Nitrogen for solvent evaporation

Nitrogen of high purity. Must be checked for purity.

7.10 Standards for calibration

The following standard substances are used:

- 4-Nonylphenols (NP), mixture of isomers, CAS # 104-40-5 and 25154-52-3 ?
- 4-Nonylphenol monoethoxylates (NP1EO), mixture of isomers, CAS # 26027-38-3 ?
- 4-Nonylphenol diethoxylates (NP2EO), mixture of isomers, CAS # 104-35-8 ?

The two nonylphenolethoxylates often contains 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 the freezer.

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

7.11 Internal standards

The following internal standard substances are used:

- ^{13}C-labelled 4-n-nonylphenol, C_9H_{19}-[$^{13}C_6$]H_4-OH, CAS # 104-40-5 ?
- ^{13}C-labelled 4-n-nonylphenol-diethoxylate (NP2EO), CAS # 20427-84-3

The internal standards must be kept in the freezer.

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 by dilution to about 20 mg/l in isooctane.

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 ± 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 4 ± 3 °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. 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 months, 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 ignition, 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

To be decided.

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 2.

The first two peaks in the SIM chromatogram of tne 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. ISO,,,,,, for waste and ISO..... for sludge

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 according to ISO 11465 or PrEN 14346.

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.

Methods for pre-treatment of solid samples to be used for the analysis of organic contaminants are described in a separate standard. 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. Therefore a special treatment is given for samples with a low content of dry matter.

Table 1 — Pretreatment methods used prior to nonylphenol analysis.

Sludge	Soil	Compost	Sediment
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)
Concentration by filtration *			Concentration by filtration *

* Part of the analytical procedure.

Sludge samples with more than 5% dry matter can be analysed as wet samples, or they can be analysed after freeze-drying.

Sludge samples with less than 5% dry matter can be analysed after filtration, or they can be analysed after freeze-drying. By the filtration the particles are concentrated in a smaller volume. The analytes are sorbed to the particles, and the smaller volume (higher dry matter) results in a higher recovery.

Soil and compost 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 be analysed after filtration, or they can 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 compost, and one for extraction of freeze-dried samples of soil, sediment and compost.

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 not contain more than 3 g dry matter.

b) Add 100 µl of internal standard solution (7.12) equal to 2 µg of each internal standard.

c) If the sludge sample contains less than 5% dry matter, concentrate the sample by filtration prior to the extraction. Filtration is done on filters allowing particles less than 1,2 µm to pass the filter (Whatman GC/F). If necessary more filters may be used. After the filtration the filters are transferred to the extraction flask and thus included in the extraction. The filter blank shall be checked.

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

e) Add 10 ml of petroleum ether, close the screw cap again and place the flask on a reciprocating shaker. The flask shall be placed in horizontal position.

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

g) Transfer the petroleum ether to another 100 ml flask. If an emulsion is present, this shall be included.

h) Add 50 ml of water and shake to wash the extract.

i) Transfer the extract (enough for the subsequent analysis) to a glass tube, if necessary dry the extract by adding anhydrous sodium sulphate.

j) The extract is now ready for the derivatization described in 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 each internal standard.

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 petroleum ether, close the screw cap again and place the flask on a reciprocating shaker. The flask shall be placed in horizontal position.

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

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

h) Add 50 ml of water and shake to wash the extract.

i) Transfer the extract (enough for the subsequent analysis) to a glass tube, if necessary dry the extract by adding anhydrous sodium sulphate.

j) The extract is now ready for the derivatization described in 10.4.

10.1.3 Extraction 3 – Soil, sediment and compost samples

Soil, sediment and compost 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 must contain between 10 and 20 g dry matter.

b) Add 100 µl of internal standard solution (7.12) equal to 2 µg of each internal standard.

c) If the sample contains less than 10% dry matter, concentrate the sample by filtration prior to the extraction. Filtration is done on filters allowing particles less than 1,2 µm to pass the filter (Whatman GC/F). If necessary more filters may be used. After the filtration the filters are transferred to the extraction flask and thus included in the extraction. The filter blank shall be checked.

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

e) Add 20 ml of acetone to the test sample, close the screw cap and shake thoroughly by hand.

f) Add 20 ml of petroleum ether, close the screw cap again and place the flask on a reciprocating shaker. The flask shall be placed in horizontal position.

g) Shake for at least 2 hours with 250 strokes per minute.

h) Transfer the petroleum ether to a 250 ml flask. If an emulsion is present, this shall be included.

i) Add 100 ml of water and shake to wash the extract.

j) Transfer the extract (enough for the subsequent analysis) to a glass tube, if necessary dry the extract by adding anhydrous sodium sulphate.

k) The extract is now ready for clean-up or the derivatization described in 10.4.

10.1.4 Extraction 4 – Freeze-dried soil, sediment and compost samples

Freeze-dried soil, sediment and compost 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 each internal standard.

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 petroleum ether, close the screw cap again and place the flask on a reciprocating shaker. The flask shall be placed in horizontal position.

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

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

h) Add 100 ml of water and shake to wash the extract.

i) Transfer the extract (enough for the subsequent analysis) to a glass tube, if necessary 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) and are stable for at least 1 month.

NOTE 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.

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 only necessary for very special sludges.

The procedure for clean-up will be described later.

10.4 Derivatization

The derivatization can 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) and are stable for at least 1 month.

NOTE 1 The derivatization is sensitive to the amount of water in the extract.

Horizontal Nonylphenol standard

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 as the samples.

10.5 Blank

Perform a blank determination following the procedure as described for the selected extraction and clean-up (optional). Prepare the blank exactly as by the analysis of a sample. .

The blank value shall not be higher than 10 % of the lowest value of interest.

10.6 GC-MS analysis

Optimize the gas chromatograph and mass spectrometric detector ( ) according to the instrument manufacturer's manual. The separation of nonylphenols must fulfil the requirements described in 8.5.

Many columns and GC-conditions are allowed to be used. An example is described in Annex B.

The detection is done by Electron Impact Ionization (EI) 70 eV. The following ions are used for the analysis:

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-n-NP	185			1,2
5	^{13}C-labelled 4-n-nonylphenol diethoxylate	^{13}C -n-NP2EO	252			3
6	D_4-labelled 4-n-nonylphenol	D_4-n-NP	183			1,2
7	Unlabelled 4-n-nonylphenol	n-NP	179			1,2

* 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 (9.7.1) and the recalibration, which is carried out daily (9.7.2).

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 (7.13) 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 ± 10 % and 80 ± 10 % of the established linear range and calculate the straight line from these measurements.

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 isooctane and injected again.

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.

Horizontal Nonylphenol standard

For all samples a specific mass of internal standard is added, 2 µg for extraction method 10.1.1 and 10.1.2, and 4 µg for extraction method 10.1.3 and 10.1.4. 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).

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

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, normally 0,2 µg/ml

V is the volume of petroleum ether 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 Description on materials for which the method is validated and also materials for which experience is present and future validation should be carried out

For the analysis of xxxxxxxxxxxx, the following relevant sample types are distinguished

- **Sludge**
  - Sewage sludge
  - Industrial sludge
- **Sediment, suspended solids**
- **Waste**
  - Soil-like waste
  - Building materials containing tar particles, creosote wood, surface treated materials
  - Mixed waste (containing different phases)
- **Soil improvers**
  - Compost (stabilized)
  - Biowaste (not stabilized) containing organic matter of natural origin
- **Soil**
  - Sandy
  - Clay
  - Organic rich

Note: Resent validation studies are available on www.....

Annex B
(informative)

Example of chromatographic conditions and chromatogram

GC-conditions:

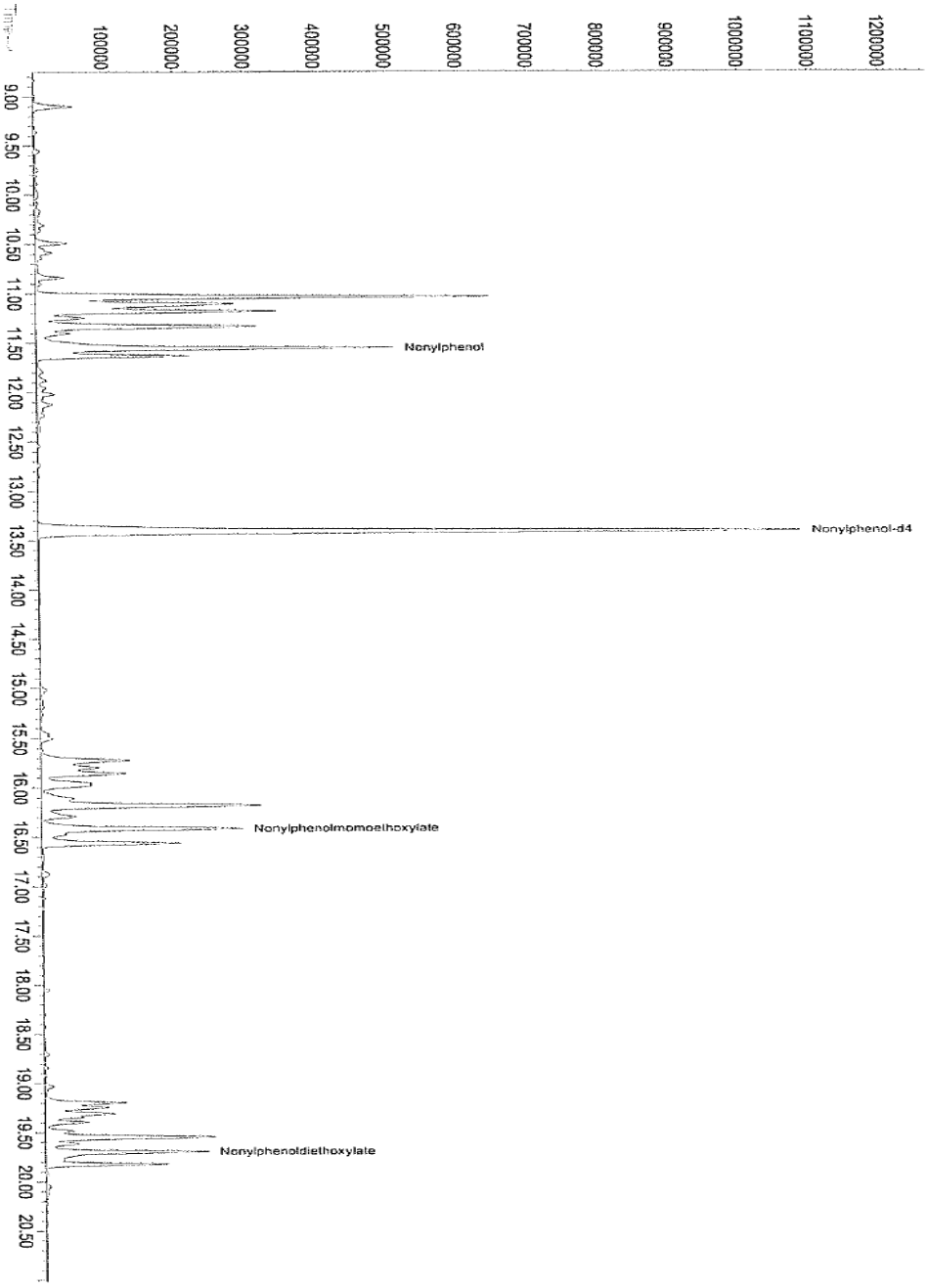
Separation column:	5% phenyl methyl siloxane, film thickness o,25 μm. length 30 m, i.d. 0,25 μm
Oven temp.:	100 °C, hold 1 min
	10 °C/min to 200 °C, hold 3 min
	10 °C/min to 300 °C, hold 7 min
Injection temp.:	250 °C
Splitless inj.:	1 μl
Carrier gas:	Helium, 0,9 ml/min

MS-conditions:

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

Horizontal Nonylphenol standard

Total ion chromatogram based on SIM analysis



Annex C

Validation results

In this annex reference is made to standards and validation reports in which parts of this Horizontal standard were validated.