LAS

Experimental work including ruggedness test
Final report

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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 Linear Alkylbenzene Sulphonates (LAS) 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 LAS should be based on the principle: an extraction of a dry sample, possibly a clean-up step and a measurement by HPLC using UV, fluorescence or MS detectors. 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).

In the report the following experiments are presented:

- The separation and detection by HPLC with fluorescence (FLD) and MS detection (MSD)
- The extraction solvent
- The extraction technique
- The pre-treatment of the sample (drying of sample)
- The clean-up procedure
- The calibration procedure

Based on the experiments the following conclusions were made:

Chromatography
Mobile phases using either ammonium acetate or sodium perchlorate as additive were compared. Based on repeatability of peak area and retention time ammonium acetate was found to be superior.

C18 and C8 HPLC columns were used. With the C18 column a separation of both isomers and homologues were obtained, with the C8 column only a separation of homologues were obtained.

Choice of solvent
The extraction efficiencies of methanol, basic methanol (0,5 M NaOH) and methanol/water (1:1) were compared in several studies. Methanol and basic methanol were found to be equally efficient and significantly better than methanol/water. As basic methanol requires a pH regulation before HPLC-analysis, the most robust and therefore the best choice of extraction solvent was found to be methanol.

Extraction technique
A comparison of reciprocating shaker, sonication in combination with reciprocating shaker, sonication alone, reflux and soxhlet based on both methanol and basic methanol as extraction solvents was conducted. Disregarding sonication all extraction techniques were found to be

equally efficient. Reciprocating shaking, being less time consuming and most easy to adapt for laboratories with many samples, was therefore chosen for the LAS Horizontal standard.

Extraction time
In parallel to the test of extraction techniques several extraction times were compared and no differences were found, which suggests that one single extraction of 30 minutes is sufficient. An additional study based on one extraction technique and three different solvents also concluded 30 minutes to be sufficient.

Pre-treatment
Freeze-drying was found to be significantly better than oven-drying regarding the recovery of LAS. Only drying by freeze-drying is therefore included in the method.

Clean-up
Two clean-up procedures on GCB and SAX columns were tested. Both clean-up procedures were found to give satisfactory recoveries, however, the removal of interfering compounds was not sufficient when detection was based on FLD. A modified GCB procedure was tested after completion of the ruggedness test. The modification resulted in a higher recovery and a cleaner extract, thereby giving a lower limit of detection.

Response factors
Pure standards of C8-, C11-, C12- and C13-LAS were analysed and compared on both FLD and MSD. A correlation between the response factors of the LAS homologues analysed on FLD and molecular weight indicated that the response factors of the LAS should be based on molar response. This difference was however only significant when comparing the C8-LAS with the other homologues, and no difference was found for the C11-, C12- and C13-LAS response factors calculated per weight. When the analysis was conducted on MSD the response factors were found to be very similar.

Purity
The purity of two technical LAS mixtures was analysed and estimated to be significantly lower than stated by the supplier.

Calibration standards
For both detectors the calibration of LAS can be based on the responses per weight, and the calibration can be based on mixtures of LAS homologues with the alkyl chain length of C10 to C14 with known or unknown distribution, however of known purity. As it may be difficult to obtain mixtures of known purity it is decided, that a mixture of the three homologues C11-, C12- and C13-LAS are used for calibration.

Ruggedness test
The 2nd draft of the LAS Horizontal Standard was subjected to a ruggedness test to examine the influence of several factors, mainly related to the extraction procedure. The ruggedness test also included an examination of other factors like HPLC column, detector and clean-up. By the ruggedness test 6 samples were tested, each for the influence of 9 factors.

The ruggedness test resulted in small adjustments of the method. The extraction time is increased from 30 to 60 minutes for soil and compost , the calibration standard is changed from the use of a commercial LAS mixture to the use of a mixture of three LAS homologues (C11, C12 and C13 LAS) of high purity (99% stated by supplier), the amount of internal standard (C8-LAS) is increased from 10 µg to 100 µg, and more information is needed about the advantages and disadvantages of the two detectors.

These changes will be incorporated in the draft horizontal standard, together with a detailed description of the clean-up which was tested after completion of the ruggedness test.

1. INTRODUCTION

The present report describes the experimental work being carried out for the development of a horizontal standard for the determination of Linear Alkylbenzene Sulphonates (LAS) 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 sample, possibly a clean-up step and a measurement by HPLC using UV, fluorescence or MS detectors
- That many issues had to be studied before a draft standard for LAS could be presented

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

- Scope – which matrices shall be included
- Sample storage
- Drying procedure
- Selection of extraction solvent
- Selection of extraction technique
- The necessity of clean-up and choice of clean-up
- Selection of HPLC-detector
- Selection of internal standard

The desk study report and results from the experimental work 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 LAS can be shortly described:

The test sample (dried sample) is extracted with methanol or 0.5M NaOH in methanol. If necessary, interfering compounds are removed from the extract by a clean-up on a suitable column. The extract is subsequently analysed by high performance liquid chromatography (HPLC) and detection by UV, fluorescence (FLD) or mass spectrometric detector (MSD).

LAS is identified from the chromatographic fingerprint of the homologues and maybe the isomers in combination with the relative retention times. 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.

The potential inclusion of soil in the scope was 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 and no 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 Analyte

The analyte is linear alkylbenzene sulfonate, sodium salt (LAS) originating from commercial products. The analyte consists of a mixture of homologues (i.e. C_{10}-LAS, C_{11}-LAS, C_{12}-LAS, C_{13}-LAS and C_{14}-LAS) with C_{11}-LAS and C_{12}-LAS as the dominant homologues. Only para-substituted LAS is included.

For each homologue many isomers are present. Theoretically C_{10}-LAS consists of 5 isomers, C_{11}-LAS of 6 isomers, C_{12}-LAS of 6 isomers, C_{13}-LAS of 7 isomers and C_{14}-LAS of 7 isomers. Thus LAS products may theoretically include up to 31 components. However, when manufacturing LAS the aromatic ring is never positioned in the terminal end of the linear alkyl. Without the 1-phenyl isomer the number of isomers is therefore reduced to 26 components /3/.

2.3 Internal standard

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

The internal standard used in the method is 4-octylbenzenesulfonate, in the report called C_8-LAS.

2.4 Calibration principle

For discussion at the ad-hoc group meeting in Madrid, 21 September 2005, a note about the calibration of LAS was prepared. The note is enclosed as Appendix 1.

The background for the discussion is, that a LAS standard substance with a well-defined composition is not available. However, it is possible to provide a LAS standard of well-defined purity.

Also the technical LAS products vary from one supplier to the other, and the LAS will undergo changes after being released into the environment.

It was concluded that the calibration must be based on the use of the LAS standards of known purity, even if the composition is not unambiguous.

Four possible calibration principles were described in the note and discussed at the ad hoc group meeting in Madrid. For both detectors used (FLD and MSD) knowledge about the responses of the single homologues was lacking, and no decision was taken at the meeting.

Further experiments were carried out in the laboratory as described in the present report, and on the basis of the results the following calibration was chosen:

The sum of areas of all LAS homologues and isomers is used for the calibration, taking into consideration the purity of the calibration standard. Also for the samples LAS is calculated from the sum of the areas. If the single homologue has to be determined, this will also be possible.

The calibration is further described in chapter 4.4.

2.5 Detectors

In the Desk Study report it was described, that three detectors were possible: UV detector, fluorescence detector (FLD) and mass spectrometric detector (MSD). However, it was also recommended, that due to the low specificity UV should not be used as the only detector.

The initial studies confirmed the limited specificity of the UV detector, and in a discussion paper prepared for the ad-hoc group for nonylphenols and LAS it was proposed not to include the UV detector in the draft standard. At the ad-hoc group meeting in Vienna 8 March 2005 this was accepted.

Therefore the work has included the use of two detectors: FLD and MSD.

3. MATERIALS/SAMPLES

Samples for the experimental work are collected from several sources. Four samples are so-called playground samples made available through Horizontal Work Package 1, two 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 1.

Table 1 Description of samples used in the study.

Sample ID	**Sample description**	**Pre-treatment**	**C_{org} wgt. %**	**Dry matter %**	**Approx. LAS conc. mg/kg DM**
SO-4	Clay soil, Speyer, Germany	Ball-milled and sieved < 125 µm	1.652		2
SO-9	Soil, Hagen, Germany	Ball-milled and sieved < 125 µm			3
SO-E2	Clay soil, Galten, Denmark	Sieved < 2 mm and freeze-dried			0
SL-11	Sewage sludge, electronic industry, Turin, Italy	Ball-milled and sieved < 125 µm	3.177		60
SL-E1	Sewage sludge, VKI, Hoersholm, Denmark	Freeze-dried		Ca. 100	1900
SL-E2	Sewage sludge, domestic, Vejle, Denmark. 908134	Freeze-dried		28	1700 *
CW-5	Compost, Fulda, Germany		11.45		7

* See text.

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

Sample SO-E2 is a natural clayish soil taken in Denmark.

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

The sample SL-E2 is a municipal sludge sample collected in Denmark. Several batches of freeze-dried sludge have been used for the experiments, and the concentration may vary from one experiment to another. However, for every experiment the same homogenized batch of freeze-dried sludge is used.

4. EXPERIMENTAL WORK

The experimental work and the results that have been gathered in order to obtain the necessary information to develop a Horizontal LAS standard is described in this chapter.

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 LAS.

The experimental work has included studies of the following elements:

- The development of a LC-FLD-MSD measurement.
- Extraction. Three solvents and four extraction techniques were examined. One extraction technique and one associated extraction solvent was chosen for further work.
- Pre-treatment of the sample. The extraction efficiency of oven- and freeze-dried sample was compared.
- Clean-up. GCB and SAX columns were tested on sludge extracts.
- Calibration procedure. Response factors of LAS-homologues were established and a calibration procedure was chosen.

4.1 Separation and detection: HPLC-FLD-MSD

The analysis of LAS was based on chromatographic separation with high performance liquid chromatography (HPLC) and detection with fluorescence detector (FLD) and mass spectrometry (MSD).

The analysis of LAS was conducted with an Agilent HP1100 HPLC system consisting of autosampler, degasser, binary pump, and column oven. Two different settings of the chromatographic system were used. One set-up using a C18 column with acetonitrile in the mobile phase resulted in isomeric separation of each LAS-homologue (chapter 4.1.1), another set-up using a C8 column with methanol in the mobile phase resulted in separation of the homologues only (4.1.2). The detection was conducted with an Agilent 1046A Programmable Fluorescence Detector (4.1.3) and an Agilent 1100 MSD (4.1.4). The FLD being a non-destructive detector enabled the simultaneous use of FLD and MSD, using the FLD as the first detector. The detectors were therefore used simultaneously when possible.

4.1.1 Isomeric separation

4.1.1.1 Experimental

The separation of the isomers and the homologues of LAS were conducted with the chromatographic conditions described in Table 2. Two different mobile phase additives (sodium perchlorate and ammonium acetate) were evaluated and compared under otherwise identical chromatographic conditions. Mobile phases based on 0.01 M ammonium acetate and 0.1 M sodium perchlorate are in Table 2 referred to as *mobile phase 1* and *mobile phase 2*, respectively.

Table 2 Chromatographic settings used for isomeric separation of LAS

Column	C18, 150 x 2,0 mm, particle size 5 µm
Mobile phase 1	A) 0.01 M ammonium acetate in water B) Acetonitrile
Mobile phase 2	A) 0.1 M sodium perchlorate ($NaClO_4$) in water B) 0.1 M sodium perchlorate in acetonitrile
Solvent gradient	t (min) : 0 → 20 → 35 → 36 → 40 → 41 → 45 %B : 45→ 55 → 65 → 95 → 95 → 45 → 45
$V_{Injection}$	25 µl
Flow	0.2 ml/min
Column temperature	30 °C

Examples of chromatograms obtained with the HPLC conditions of Table 2 are shown in Figure 1 and Figure 2. The chromatograms show the separation of each of the 5 LAS homologues (C_{10}-C_{14} LAS) into 3-4 chromatographic peaks, which represent the respective isomers of each homologue. Sodium perchlorate is not compatible with a mass spectrometer (MSD) and a fluorescence detector (FLD) is therefore used.

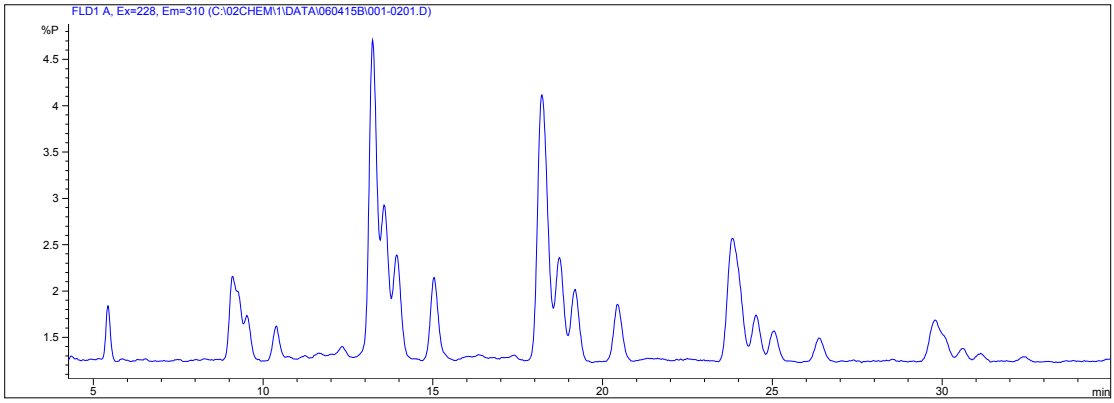

Figure 1. Chromatogram of a 5.0 mg/l C_{10}-C_{14} LAS standard using ammonium acetate in the mobile phase.

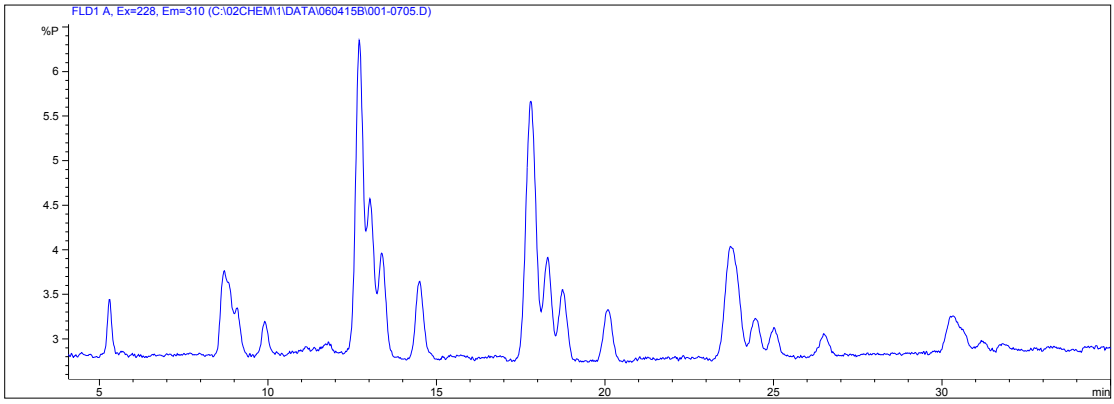

Figure 2. Chromatogram of a 5.0 mg/l C_{10}-C_{14} LAS standard using sodium perchlorate in the mobile phase.

The parameters by which the mobile phases were evaluated were: Separation of peaks, peak width, repeatability of areas and retention time and signal to noise ratio (S/N-ratio). The results are shown in chapter 4.1.1.2.

The evaluation was based on standards of a technical mixture of 0.5 mg/l and 5 mg/l C_{10}-C_{14} LAS (CAS# 69669-44-9) added 1 mg/l C_8 LAS. Each standard mixture was injected 5 times and submitted to the two chromatographic conditions described in Table 2.

4.1.1.2 Results

The chromatographic conditions resulting in isomeric separation of the LAS homologues were tested using two different mobile phases (Table 2), mobile phase 1 with ammonium acetate and mobile phase 2 with sodium perchlorate.

Mobile phase 1 and 2 were found to be equally good when comparing the peak width and the separation of homologues and isomers. The signal to noise ratios (S/N-ratio) were, however, found to be much better when based on the ammonium acetate. This difference in S/N-ratio is illustrated in Figure 3, which shows the two chromatograms of the C_{14}-homologue.

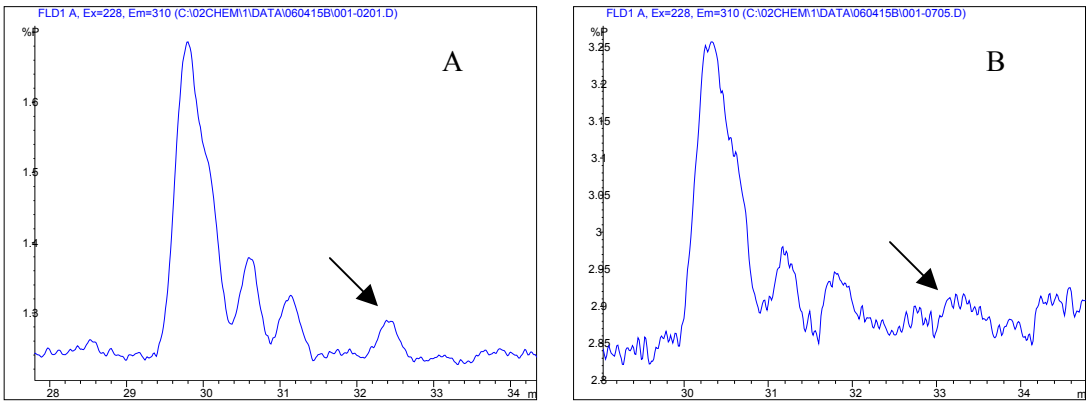

Figure 3. Chromatogram of C_{14}-LAS from a LAS standard mixture (5.0 mg/l) using A) ammonium acetate and B) sodium perchlorate in the mobile phase. The arrow indicates the 2-phenyl isomer.

The coefficient of variation (CV) calculated from the variation in retention time and area of the five injections are shown in Table 3 and 4 for the 5.0 mg/l and 0.5 mg/l LAS-standard respectively. The integrations of which the CVs were calculated were based on the peaks of:

C_8-LAS (2-phenyl isomer)
C_{12}-LAS (sum of 3,4,5,6-phenyl isomers)
C_{12}-LAS (2-phenyl isomer)
C_{14}-LAS (sum of 3,4,5,6,7-phenyl isomers)
C_{14}-LAS (2-phenyl isomer)

For the 0.5 mg/l C_{14}-LAS homologue the calculations were, however, not conducted, as the C_{14}-LAS homologue was found to be below the limit of detection.

Table 3 The CV of retention time (r_t) and area of a 5,0 mg/l C10-C14 LAS standard.

5 mg/l	Ammonium acetate Mobile phase 1		Sodium perchlorate Mobile phase 2	
	CV (r_t), %	CV (area), %	CV (r_t), %	CV (area), %
C_8-LAS (2-phenyl isomer)	0.39	0.54	2.69	1.33
C_{12}-LAS (3-6 phenyl isomers)	0.48	0.98	3.59	0.64
C_{12}-LAS (2-phenyl isomer)	0.50	2.05	3.39	0.76
C_{14}-LAS (3-7 phenyl isomers)	0.31	2.29	2.16	4.17
C_{14}-LAS (2-ph. isomer)	0.23	16.36	2.09	14.31

Table 4 The CV of retention time (r_t) and area of a 0.5 mg/l C10-C14 LAS standard.

0.5 mg/l	Ammonium acetate Mobile phase 1		Sodium perchlorate Mobile phase 2	
	CV (r_t), %	CV (area), %	CV (r_t), %	CV (area), %
C_8-LAS (2-phenyl isomer)	0.42	1.79	4.70	4.71
C_{12}-LAS (3-6 phenyl isomers)	0.42	6.64	5.49	3.22
C_{12}-LAS (2-phenyl isomer)	0.40	5.83	5.48	11.32

The repeatability of the retention time for the LAS homologues was found to be significantly better, when the mobile phase was based on ammonium acetate instead of sodium perchlorate. The repeatability of the measured area of the LAS homologues was also found to be slightly better, when the mobile phase was based on ammonium acetate instead of sodium perchlorate.

Based on the S/N-ratio and repeatability ammonium acetate was found to be the best mobile phase additive compared to sodium perchlorate. This conclusion is based on the detection with FLD. Since sodium perchlorate is not compatible with the MSD, ammonium acetate is clearly the best choice for mobile phase.

Linearity – isomeric separation

The linearity of C_8-LAS, C_{12}-LAS and C_{10}-C_{14} LAS was examined. The quantification of C8- and C_{12}-LAS was based on C_{11}-LAS as internal standard. The quantification of C_{10}-C_{14} LAS was based on C_8-LAS as internal standard.

Three calibration curves based on the standard solutions of Table 5 were constructed. The calibration curves for the isomeric separation are presented in Figure 4.

Table 5 Concentration of calibration standards used in tests of linearity of LAS on MSD.

	Concentration (mg/l)											
C8-LAS	-	-	24,0	6,0	2,40	0,60	0,24	0,060	0,024	0,0060	0,0024	0,0
C12-LAS	-	220	44,0	11,0	4,40	1,10	0,44	0,11	0,044	0,011	0,0044	0,0
C10-C14 LAS	455	-	45,5	-	4,55	-	-	-	-	-	-	0,0

The calibration curves for the three standards (C_8-LAS, C_{12}-LAS and C_{10}-C_{14} LAS) were all found to be linear in the entire range examined (i.e. 24–0.0024 mg/l, 220–0.0044 and 455–4.55 mg/l, respectively). The chromatography of the 220 mg/l C_{12}-LAS standard was however indicating an overloaded column.

As the standard curves were found to be linear in the entire range examined, extrapolating from

the 455 mg/l C_{10}-C_{14} LAS standard it can be concluded, that a sample analysed according to the LAS Horizontal standard could contain approximately 4000 mg/kg before problems with linearity should be considered.

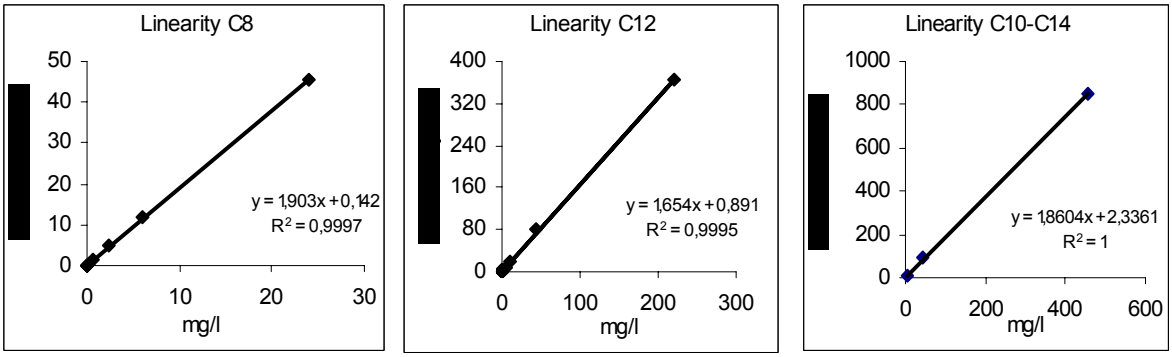

Figure 4. Linearity of C8-LAS, C12-LAS and C10-C14 LAS analysed with MSD and separated on C18-column (isomeric separation).

4.1.2 Homolog separation

The separation of the homologues of LAS was conducted with the chromatographic conditions described in Table 3.

Table 6 Chromatographic settings used for homolog separation of LAS

Column	C8, 125 x 2,0 mm, particle size 5 µm
Mobil phase	A) 0.01 M ammonium acetate in water B) Methanol
Solvent gradient	t (min) : 0 → 12 → 16 → 18 → 19 → 25 %B : 58 → 80 → 95 → 95 → 58 → 58
$V_{Injection}$	25 µl
Flow	0.2 ml/min
Column temperature	30 °C

Examples of chromatograms obtained with the HPLC conditions of Table 6 are shown in Figure 5. The chromatograms show the separation of the 5 LAS homologues (i.e. C_{10}-, C_{11}-, C_{12}-, C_{13}- and C_{14}-LAS). The detection is conducted with FLD and MSD simultaneously.

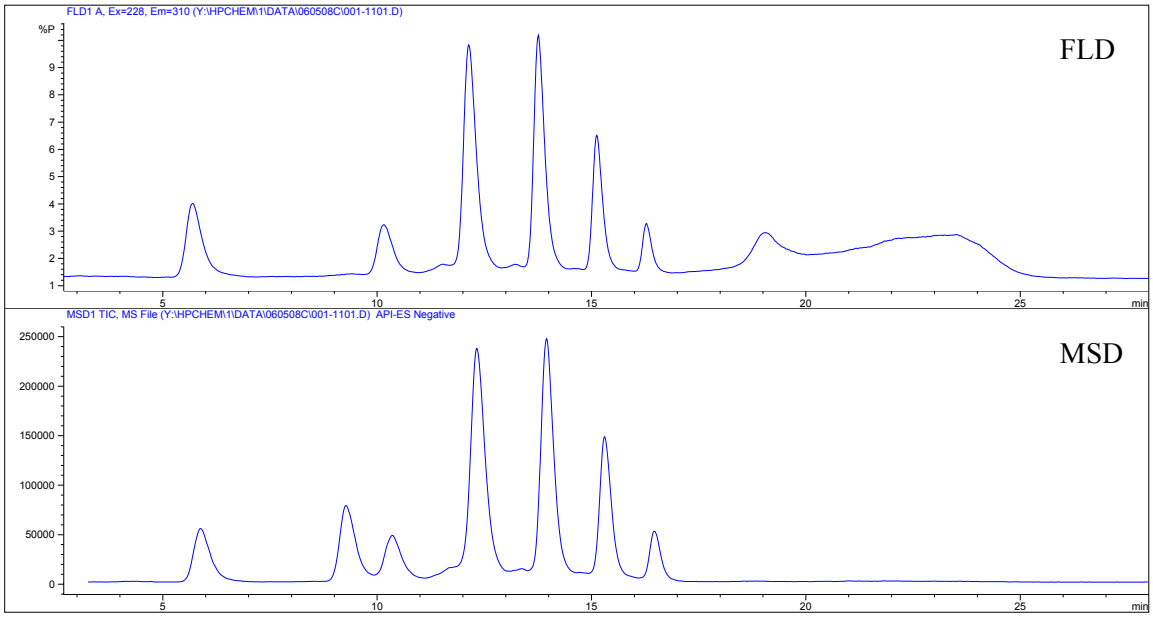

Figure 5. Chromatograms of a 5.0 mg/l C_{10}-C_{14} LAS standard with sodium laurylsulphate (rt = 9.2 min) and C_8-LAS (rt = 5.9 min) as internal standards. Detection with FLD (upper) and MSD (lower).

Linearity – homologue separation

The standard solutions of Table 5 was also subjected to analysis by homologue separation on a C8-column. The calibration curves for the homologue separation are presented in Figure 6.

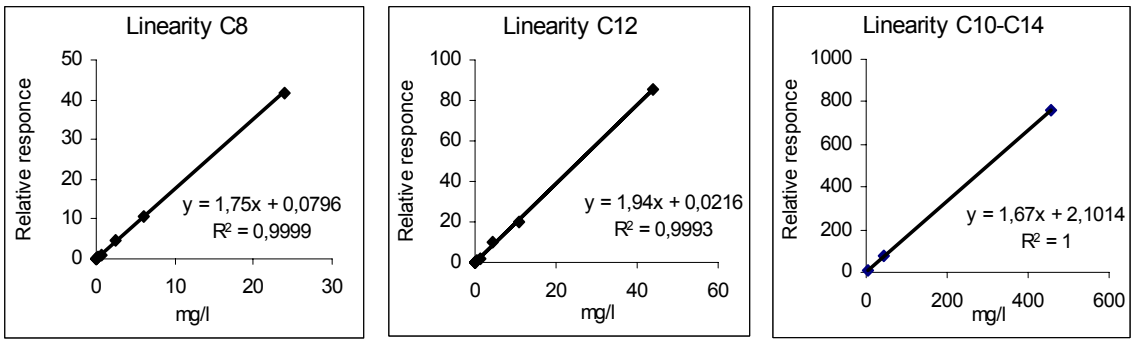

Figure 6: Linearity of C_8-LAS, C_{12}-LAS and C_{10}-C_{14} LAS analysed with MSD and separated on C_8-column (homologue separation).

C_8-LAS and C_{10}-C_{14} LAS was found to be linear in the entire range of the analysed calibration standards (i.e. 24–0.0024 mg/l and 455–4.55 mg/l, respectively). The linearity of C_{12}-LAS analysed on C8-column was however found to exclude the highest calibration standard (220 mg/l). The linearity of C_{12}-LAS was therefore found to have a range of 44–0.0044 mg/l.

4.1.3 Fluorescence detector (FLD)

A 100 mg/l LAS standard was used to find the optimal wavelengths of excitation (λ_{ex}) and emission (λ_{em}). This was done by injecting 50 µl directly to the fluorescence detector, stopping

the flow when the solution entered the photo cell and finally recording a 190 to 800 nm scan of both emission and excitation. Optima of excitation and emission wavelengths were found to be 228 and 305 nm, respectively (Figure 7). In practice this implies that the wavelengths must be selected close to these values, however, a difference of 5 nm is not critical.

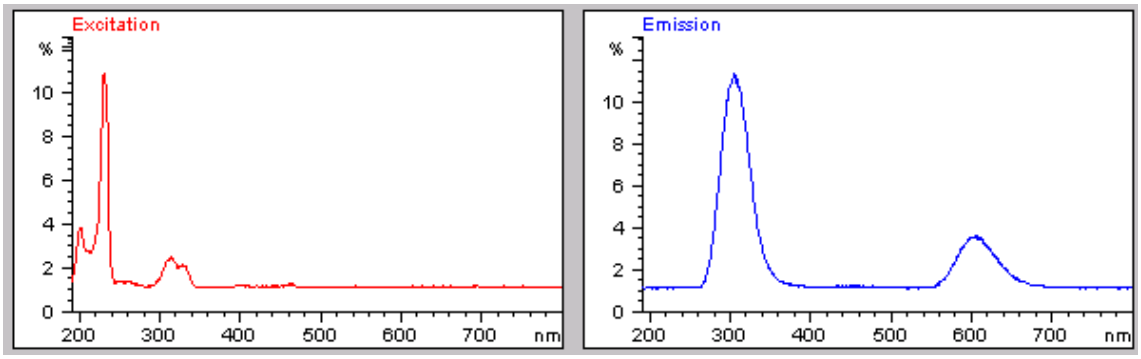

Figure 7. Fluorescence spectra of LAS, excitation (left) and emission (right).

4.1.4 Mass spectrometric detector (MSD)

The detector was set to operate in SIM mode with a specific target ion for each LAS homologue and for the internal standard C_8-LAS (Table 7). A qualifier ion was also chosen (q1 = 183 m/z). However, the fragmentation of LAS is generally low under normal circumstances ($V_{fragmentor}$: 50-110 V), and the use of a qualifier ion was found to be limited ($V_{Fragmenter}$ should be > 180 V to produce the 183 fragment of LAS).

Sodium dodecyl sulfate (sodium laurylsulfate) was used as an extra internal standard in the study of extraction efficiency, mainly for examining the extraction of the internal standard C_8-LAS, see chapter 4.2.

Table 7 Selected target ions for the LAS homologues including the internal standard.

Compound	C_8-LAS	Sodium dodecyl sulfate	C_{10}-LAS	C_{11}-LAS	$C1_2$-LAS	C_{13}-LAS	C_{14}-LAS
Ion (m/z)	269	265	297	311	325	339	353

Atmospheric pressure ionisation electrospray (API-ES) was used as inlet and the spray was set with a drying gas flow and temperature of 10 l/min and 300°C, respectively. The API-ES was used in negative mode, and the fragmentation voltage was set to 70 V.

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, etc.

The different parameters of extraction were often examined simultaneously to ease the experimental work. These experiments are described in the following text as Extraction study 1, Extraction study 2, Extraction study 3 and Extraction study 4. The results relating to the effects

of extraction solvent, extraction technique, extraction time and pre-treatment, are treated in the four chapters 4.2.2 – 4.2.5, describing the respective parameters.

4.2.1 Experimental

Extraction study 1 and 2: Extraction solvent, technique and time

Two studies were conducted on the sludge sample SL-E2 in order to test the extraction efficiencies of different solvents (methanol and alkaline methanol), extraction techniques (reciprocating shaker, sonication, combined sonication and reciprocating shaker, reflux and soxhlet) and extraction times. The tested parameters of the two studies are shown in Table 8 and 9.

Table 8 Design of *Extraction study 1* examining extraction techniques and extraction time based on extraction of SL-E2.

Extraction technique	N	m_{sludge} (g)	V_{MeOH} (ml)	Extraction time 1	Extraction time 2
Shaker	2	2	10	30 min	4 hours
Sonication	2	2	10	10 min	30 min
Sonication + shaking	2	2	10	5 min + 30 min	5 min + 4 hours
Reflux	2	6	30	1 hour	4 hours
Soxhlet	2	10	50	6 hours	20 hours

Table 9 Design of *Extraction study 2* examining extraction solvent, extraction technique and extraction time tested based on extraction of SL-E2.

Extraction technique	N	m_{sludge} (g)	V_{MeOH} (ml)	$V_{MeOH/NaOH}$ (ml)	Extraction time
Shaker	2	2	10	10	1 hour
Sonication	2	2	10	10	1 hour
Sonication + shaking	2	2	10	10	5 min + 1 hour
Reflux	2	5	25	25	1 hour
Soxhlet	2	10	50	Not conducted	Over night

The studies were performed on freeze dried sludge (SL-E2). Apart from the described changes both studies were conducted according to the LAS Horizontal standard 1st Draft. The calculations were based on either external standard or with the use of an injection standard (in this case Na-laurylsulfate, which was added after extraction).

Extraction study 3: Extraction solvent, pre-treatment and extraction time

A third study was conducted to further investigate the effect of different solvents (methanol, alkaline methanol and methanol/water), extraction time (30 and 60 min) and pre-treatment (freeze- and oven-drying). The study was designed according to Table 10.

Table 10 Design of *Extraction study 3* examining solvent, pre-treatment and extraction time. The extractions were based on SO-E2 and SL-E2 (freeze- and oven-dried).

	Extraction time	Extraction solvent	Sample types (pretreatment)	Replicates
Variable:	30 min. 60 min.	MeOH MeOH/water (1:1) MeOH/NaOH (0,5 M)	Soil Sludge A (freeze-dried) Sludge B (oven-dried)	2 2 2

In this study a spiked soil sample and an unspiked sludge sample was used. The 36 samples were extracted according to the LAS Horizontal standard, 1st Draft.

The soil sample was prepared by transferring approximately 200 g of oven dried (60 C) soil to a 1000-ml round bottom flask. Acetone was added and the suspended soil was then spiked with 750 µl of a 5305 mg/l solution, thus ending up with a concentration of 19.9 mg/kg. The acetone was evaporated at low temperature until dryness. The soil was then placed in a fume hood over night (room temp) to further remove residues of acetone.

Calibration is based on the sum of the response of each homologue LAS. An average the respons factors of 5 calibration standards is used. All calculations were based on external standard.

Extraction study 4: Extraction solvent

An addition study - *Extraction study 4* - was conducted to compare the extraction efficiency of methanol and methanol/NaOH. This study was based on two successive extractions of 2 g of a freeze-dried sludge sample (SL-E2). This double extraction was conducted to estimate the extraction efficiency of the two extraction solvents (n = 2). This procedure was also used to estimate the recovery of LAS.

Table 11 Design of *Extraction study 4* examining the effect of solvents on extraction efficiency of sludge sample SL-E2.

Fixed parameters of extraction	Extraction Solvent	Sample
Extr. time: 30 min. Extr. technique: Reciprocating shaker. Replicates: 2	MeOH MeOH/water (1:1) MeOH/NaOH (0.5 M)	Sludge (SL-E2)

Approximately 2 g of sludge sample was extracted with 10,0 ml extraction solvent. After 30 min of extraction the samples and solvent was separated. The solvent was analysed and the remaining sample was extracted a second time. The volume of solvent, which could not be separated from the sample, was quantified before the second extraction. The sample extracts were diluted 10 times in ammonium acetate/MeOH (1:1) with 1% acetic acid before analysis.

Results from all the extraction studies are presented in Appendix 2.

4.2.2 Results - Extraction solvent

Extraction study 2, 3 and 4 all involved extractions with 2 or 3 of the solvents including: a) methanol, b) alkaline methanol (methanol with 0.5 M NaOH) and c) methanol/water (1:1).

Extraction study 2 was conducted with methanol and alkaline methanol. The results of extraction study 2 were difficult to interpret due to chromatographic problems with the alkaline extracts. Initially the extracts were analysed directly without any adjustment of pH in the alkaline extracts, which resulted in a change in retention time and possibly also a change in the response of LAS. The extracts were therefore neutralised with acetic acid and analysed again. The results showed that methanol and alkaline methanol were equally good (see Figure 11), but due to the high uncertainty introduced by the described chromatographic problems no conclusion was made from this study regarding the choice of extraction solvent.

Extraction study 3 enabled the comparison of the 3 solvents: methanol, alkaline methanol and methanol/water. Figure 8 shows the results of the extractions of SO-E2 (spiked) and SL-E2 (freeze- and oven-dried). The examination of extraction time (see 4.2.4) revealed no difference between the two tested extraction times and the samples were therefore treated as replicates.

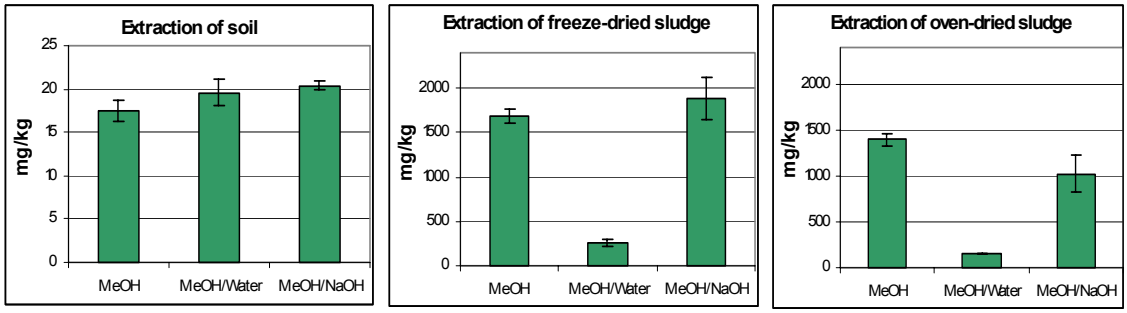

Figure 8. The extraction of SO-E2 and SL-E2 (freeze- and oven-dried) with MeOH, MeOH/NaOH and MeOH/water (n = 4).

The extraction efficiency of methanol, methanol/water and alkaline methanol was found to be similar when the extraction was conducted on the spiked soil. When the extraction was conducted on sludge methanol and alkaline methanol was found to be equally good. The extraction efficiency of methanol/water was however found to be significantly lower than methanol and alkaline methanol. This difference was also found when calculations were based on internal standard (C_8-LAS).

Extraction study 4 further investigated the extraction efficiencies of methanol and alkaline methanol. This study was based on two successive extractions of SL-E2.

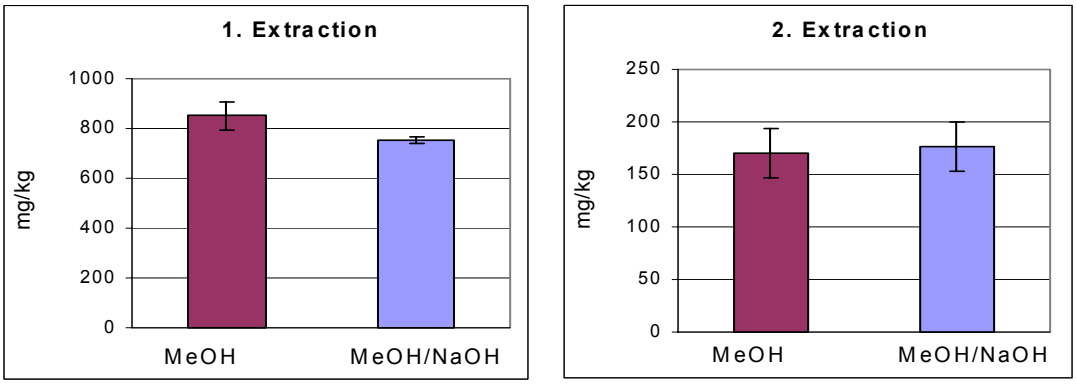

Figure 9. The extraction of freeze-dried sludge (SL-E2) with MeOH and MeOH/NaOH (n = 2). The extractions were preformed two times on each sample.

The results (Figure 9) show no significant differences in the extraction efficiencies of the methanol and methanol/NaOH extractions (p = 0.05). There is, however, a tendency of the methanol extractions to be more efficient than the basic methanol extractions.

Setting the extraction efficiency of LAS of the first and second extraction to 100% results in an estimated recovery of the first extraction to be approximately 80% for both solvents. Based on this calculation the recovery of the internal standard (C_8-LAS) of the first extraction was estimated to 85%. The actual recovery (%) of the internal standard (C_8-LAS) was found to be 70 ± 11% in the first extraction.

It is concluded that one extraction is sufficient, due to the ability of the internal standard to compensate for the minor lack in extraction efficiency.

From the obtained results it is not critical whether methanol or basic methanol is used for the extraction of LAS. However, the practical analysis by HPLC is much easier using methanol without base, since it is not necessary to include a pH regulation of the extract before injection into the HPLC system. Therefore methanol is preferred as extraction solvent.

4.2.3 Results - Extraction technique

Extraction study 1 and 2 involved tests of the extraction techniques: a) Reciprocating shaker, b) Sonication followed by reciprocating shaker, c) Sonication, d) Reflux and e) Soxhlet.

The results of the two studies concerning extraction techniques are shown in Figure 10 and 11 and in Appendix 2A and 2B.

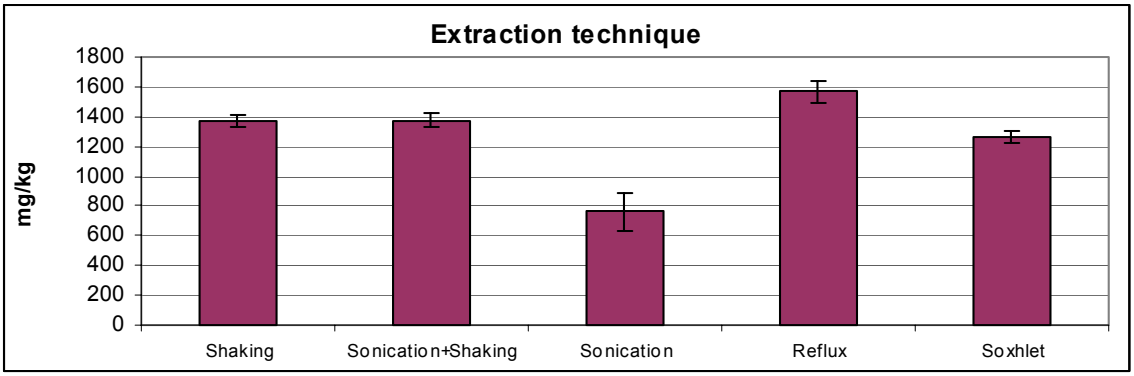

Figure 10. The extraction of SL-E2 with methanol using reciprocating shaker, reciprocating with sonication, sonication, reflux and soxhlet, results from *Extraction study 1*.

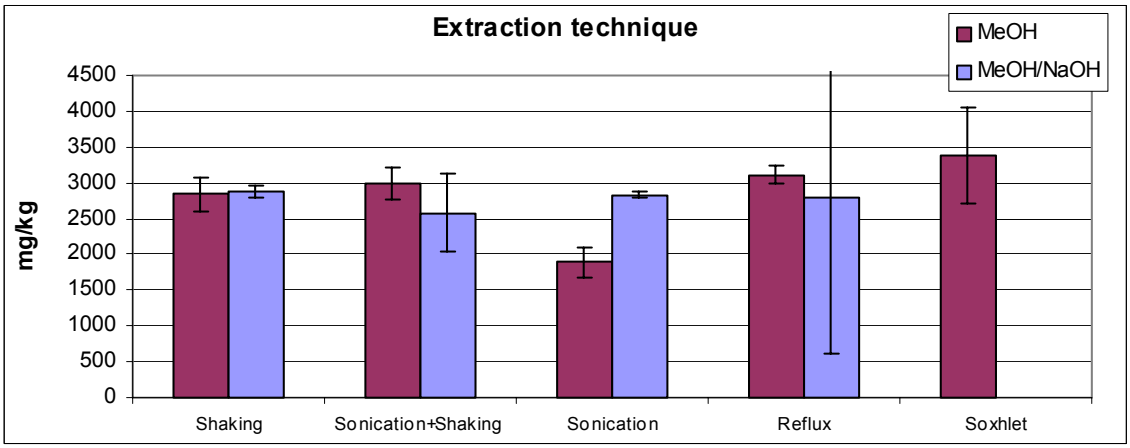

Figure 11. The extraction of SL-E2 with methanol and alkaline methanol using reciprocating shaker, reciprocating with sonication, sonication, reflux and soxhlet, results from *Extraction study 2*.

Most of the examined techniques were found to be very similar in extraction efficiency. Sonication with methanol was the only extraction technique that was found to be less effective. For sonication the presence of NaOH in the methanol was found to significantly increase the efficiency. However, due to the difficulties with standardisation of the sonication treatment, this technique is not found applicable in a future horizontal standard.

The extraction techniques reflux and soxhlet, which are based on extraction with boiling solvent, was not found to be significantly more efficient than extractions based on shaking.

Comparing the extraction techniques shaking, reflux and soxhlet, the shaking technique was preferred, since it is equally efficient and less comprehensive regarding laboratory equipment and workload.

4.2.4 Results - Extraction time

Extraction study 1 and *Extraction study 3* included tests of the extraction time:

In *Extraction study 1* the extraction time was found to introduce a significant difference when the extractions were based on sonication, however, the short extraction time was as low as 10 minutes. By comparing the other extraction techniques (shaking, reflux and soxhlet) the long extraction times (4 – 20 hours, depending on technique) were found to result a 3 % higher

recovery than the extractions with short extraction time (30 min – 6 hours, depending on extraction technique). This difference was however not significant (paired t-test, p = 0,05).

The difference in extraction efficiency of the 30 and 60 minute extractions of *Extraction study 3* was tested. Statistical analysis (paired t-test) shows that a 30-minute extraction and a 60-minute extraction are equally efficient. The test found the 60 minute extraction to give a 0,5% higher recovery than the 30 minute extraction (n = 9). If this difference was to become significant (p = 0.05), the CV% of the method should be less than 0.7%.

The similarity of the extractions is also shown in Figure 12, where the results of the 30 minute extractions are plotted against the 60 minute extractions. The slope (α = 1.01) and the linearity (r^2=0.990) of the line in Figure 12 also show the equality of the two extraction times.

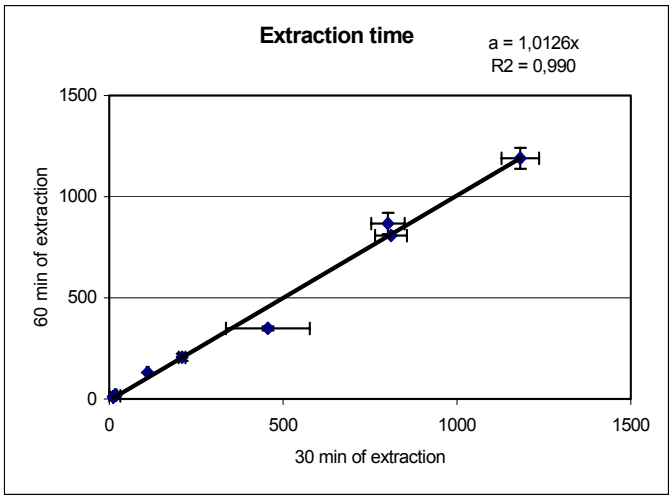

Figure 12. Concentration of samples extracted on a reciprocating shaker for 30 minutes plotted against concentrations of samples extracted for 60 min (n=2).

From these results the extraction time was set to 30 minutes in the 2nd draft standard.

In the conclusions from the ruggedness test in chapter 6.4 the extraction time for soil and compost samples is increased from 30 to 60 minutes.

4.2.5 Results - Pre-treatment

In Extraction study 3 the effect of pre-treatment was examined. The study was, along with other parameters of extraction, designed to measure the influence of freeze-drying and oven-drying on the extraction efficiency of sludge (SL-E2).

Based on 12 extractions of freeze-dried and 12 extractions of oven-dried sludge, freeze-drying was found to be significantly better (paired t-test, p = 0,01) than oven-drying (calculation based on external standard). The internal standard (C_8-LAS) was almost un-effected by the type of pre-treatment and could therefore not be used to correct for the difference.

It is therefore recommended, that freeze-drying is the only drying method for the pre-treatment of samples for LAS analysis.

4.3 Clean-up methods

4.3.1 Experimental

The performance of two clean-up procedures was tested on LAS standard solutions and sample extracts. The procedures are described in Table 12.

Two commercial pre-packed SPE columns were tested: Graphitised Carbon Black (GCB) and Strong Anion Exchange (SAX). The GCB columns were purchased at Supelco (500 mg Supelclean ENVI-Carb) and the SAX columns were purchased at Analytical Instruments (500 mg Bond Elut, SAX).

The washing and elution procedures were taken from the literature, the GCB column from /6/ and the SAX column from /7/.

Table 12 Description of the clean-up procedures.

Column	**GCB (500 mg)**	**SAX (500 mg)**
Column preparation	5 ml mobile phase 2 ml MeOH 30 ml 0,1 M HCl 2 ml water	5 ml MeOH 10 ml water
Sample application	1 ml sample in MeOH	1 ml sample in MeOH
Column wash	5 ml water 1 ml MeOH 10 ml DCM/MeOH (80:20) with 15 mM formic acid	5 ml 2% acetic acid in MeOH 5 ml MeOH
Elution	10 ml DCM/MeOH (80:20) with 5 mM TMAH*	15 ml 2 M HCl:MeOH (1:1)

*) Tetramethylammonium hydroxide (CAS# 10424-65-4)

The solvents applied during column preparation, sample application, column wash and elution were collected and analysed as separate fractions. Initially the clean-up procedures were tested on 1 ml of 100 mg/l LAS standard (n = 2). The clean-up procedure was afterwards tested on extracts of SL-E1, SL-E2 and SL-E2 diluted by a factor of ten (n = 1). Modifications of the GCB clean-up procedure were also tested. These modifications are shown in Table 13.

The fractions collected and analysed is described in Table 13. These fractions correspond to the clean-up steps *column wash* and *elution* described in Table 12, using a further subdivision of the washing step and elution step.

Table 13 Description of the collected fractions.

Column	**GCB**	**GCB modified**	**SAX**
Samples / Fractions	100 mg/l LAS standard SL-E1 SL-E2 SL-E2 diluted x 10	100 mg/l LAS standard SO-4 SO-9	100 mg/l LAS standard SL-E1 SL-E2 SL-E2 diluted x 10
Sample application + 1st Wash	1 ml sample in MeOH + 5 ml water 1 ml MeOH	1 ml sample in MeOH + 5 ml water 5 ml MeOH	1 ml sample in MeOH + 5 ml 2% acetic acid in MeOH
2nd Wash	10 ml DCM/MeOH (80:20) with 15 mM formic acid	10 ml DCM/MeOH (80:20) with 15 mM formic acid	5 ml MeOH
3rd Wash	-	10 ml DCM with 15 mM formic acid	-
4nd Wash	-	10 ml DCM	-
1st Elution	5 ml DCM/MeOH (80:20) with 5 mM TMAH	10 ml DCM/MeOH (80:20) with 5 mM TMAH	7,5 ml 2 M HCl:MeOH (1:1)
2nd Elution	5 ml DCM/MeOH (80:20) with 5 mM TMAH	10 ml DCM/MeOH (80:20) with 5 mM TMAH	7,5 ml 2 M HCl:MeOH (1:1)

Based on the results of the clean-up of extracts of SL-E1 and SL-E2 (and the clean-up of SO-4 and SO-9, described in the ruggedness test), the clean-up procedure for GCB was modified and tested on two soil samples; SO-4 and SO-9. The modification was consisting of the inclusion of two additional washing steps and an increase in volume of the first elution step. The two additional washing steps, "3rd Wash" and "4th Wash", was conducted with 10 ml DCM with 15 mM formic acid and 10 ml pure DCM, respectively. The 1st and 2nd elution step was changed by increasing the volume from 5 ml to 10 ml.

4.3.2 Results

The concentration of LAS in the different fractions of Table 13 was measured and calculated as percentage of total collected LAS. The results are shown in Table 14.

Table 14 Results from the clean-up studies.

Fractions	Samples	GCB Recovery of LAS	GCB modified Recovery of LAS	SAX Recovery of LAS
Sample application + 1st Wash	100 mg/l	0 % (<LOD)	0 % (<LOD)	0 % (<LOD)
	SL-E1	0 % (<LOD)		0 % (<LOD)
	SL-E2	0 % (<LOD)		0 % (<LOD)
	SL-E2**	0 % (<LOD)		0 % (<LOD)
	SO-4	.	0 % (<LOD)	
	SO-9		0 % (<LOD)	
2nd Wash	100 mg/l	0 % (<LOD)*	0 % (<LOD)	0 % (<LOD)*
	SL-E1	0 % (<LOD)		0 % (<LOD)
	SL-E2	0 % (<LOD)		0 % (<LOD)
	SL-E2**	0 % (<LOD)		0 % (<LOD)
	SO-4		0 % (<LOD)	
	SO-9		0 % (<LOD)	
3ed Wash	100 mg/l		0 % (<LOD)	
	SL-E1			
	SL-E2			
	SL-E2**			
	SO-4		0 % (<LOD)	
	SO-9		0 % (<LOD)	
4th Wash	100 mg/l		0 % (<LOD)	
	SL-E1			
	SL-E2			
	SL-E2**			
	SO-4		0 % (<LOD)	
	SO-9		0 % (<LOD)	
1st Elution	100 mg/l	99 % ± 0.13 %*	99 %#	99.8 %*
	SL-E1	23%		95 %
	SL-E2	5.2 %		96.3 %
	SL-E2**	78%		97.4 %
	SO-4		98 %#	
	SO-9		96 %#	
2nd Elution	100 mg/l	0.79 % ± 0.13 %*	1 %#	0.2 %*
	SL-E1	77%		5.0 %
	SL-E2	94.8 %		3.7 %
	SL-E2 **	22%		2.6%
	SO-4		2 %#	
	SO-9		4 %#	

* Based on two determinations (n = 2).
** Extract from SL-E2 diluted 10 times.
# Recovery calculation based on external standard.

Graphitised Carbon Black (GCB)

The clean up procedure based on GCB columns was found to give good results. Initially no residues of LAS were found in solvents collected from the washing steps. Whether the elution of the GCB columns is efficient is however uncertain. The main fraction of LAS from the sludge extracts SL-E1 and SL-E2 was found to elute in the 2nd elution step, which lead to the possibility that the clean-up columns could contain un-eluted LAS. By comparing the chromatograms of SL-E1 and SL-E2 with and without clean-up, the total recoveries (i.e. the sum of LAS collected from 1st and 2nd elution) were estimated to approximately 80% and 100%,

respectively (based on external standard calculation).

The modification of the GCB clean-up procedure (i.e. additional washing steps and increased volumes of eluting solvents) was found to improve the recoveries measured in the 1st elution step (through increase of solvent volume). The signal/noise-ratios were also found to be significantly improved when comparing the extracts of SO-4 and SO-9 with and without clean-up (Table 15).

Table 15 The signal to noise ratios measured on chromatograms of soil samples, SO-4 and SO-9, with and without clean-up. S/N-ratios have been calculated for both FLD and MS.

Signal/Noise-ratio*	FLD		MS	
Sample (Concentration)	No clean-up	Clean-up	No clean-up	Clean-up
SO-4 (2 mg/kg)	10-15**	30	65	140
SO-9 (3 mg/kg)	10-15**	40	80	200

* Signal/Noise-ratio is based on the C12-LAS homologue.

** Quantification of signal and noise was uncertain due to interfering compounds.

The signal/noise-ratios of Table 15 show that the clean-up procedure improved the signal/noise-ratio by a factor of 2-3 for SO-4 and 3-4 for SO-9. By extrapolating the S/N-ratio of Table 15 to an accept value of 3 (i.e. a value at which the S/N-ratio could define the LOD) the detection limits of detection can be estimated. The LODs of the FLD is thus estimated to 0.9 and 0.2 mg/kg for the un-cleaned and cleaned extract, respectively. Similarly the LODs of the MS are estimated to 0.1 and 0.04 mg/kg, respectively.

The chromatographic improvement caused by the modified GCB clean-up procedure is furthermore shown in Figure 13-16.

Figure 13 and 14 show the chromatograms of the un-cleaned and cleaned extract of soil sample SO-4 analysed on FLD. The presence of multiple interfering compounds is shown in Figure 13. The interferences are removed by the clean-up (Figure 14) and the use of a clean-up method is therefore suggested when FLD is used for the analysis of soil samples.

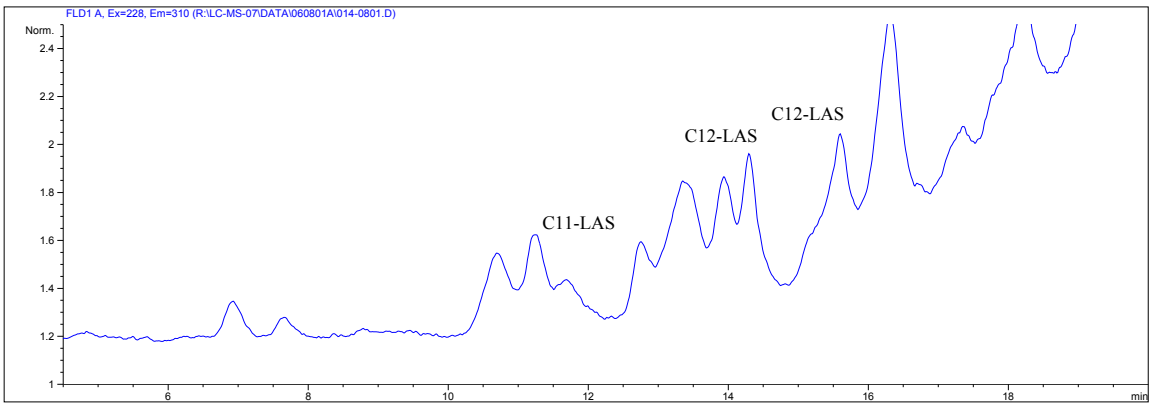

Figure 13. Extract of soil sample SO-4 before clean-up. Analysed with FLD.

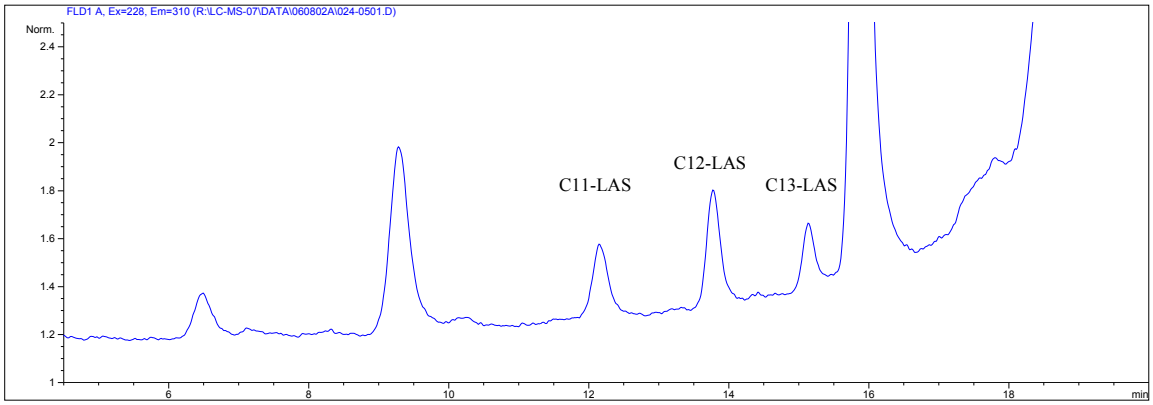

Figure 14. Extract of soil sample SO-4 after clean-up. Analysed with FLD.

Figure 15 and 16 show the chromatograms of the un-cleaned and cleaned extract of SO-4 analysed on MS. The presence of interfering compounds is minimal as shown in Figure 15. The effect of clean-up is therefore not as significant as for FLD. There is however a reduction in backgound noise which improves the S/N-ratio (see Table 15) and the clean-up furthermore reduces the wearing of the HPLC-MS system.

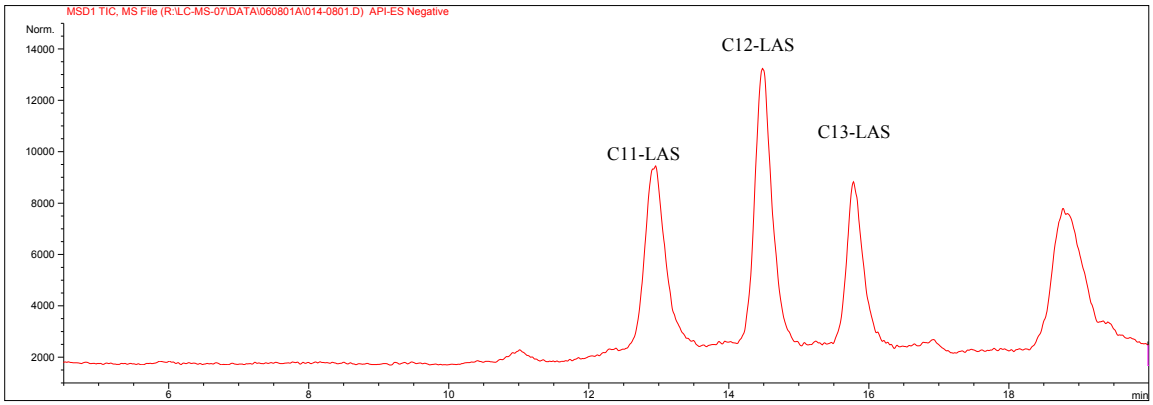

Figure 15. Extract of soil sample SO-4 before clean-up. Analysed with MSD.

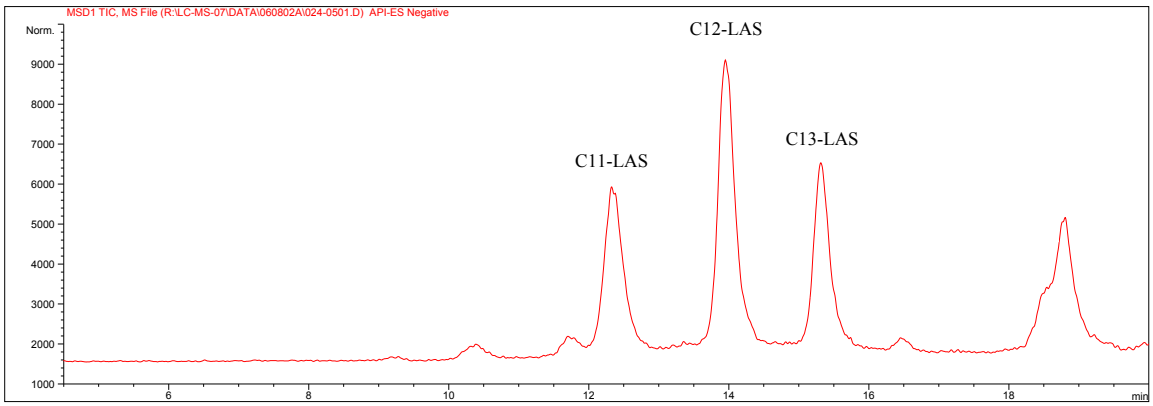

Figure 16. Extract of soil sample SO-4 after clean-up. Analysed with MSD.

Strong Anion Exchange (SAX)

The clean up procedure based on SAX columns was also found to give good results. No residues of LAS were found in solvents collected from the washing steps and LAS was almost completely eluted after applying the first 7.5 ml of solvent.

Proposed clean-up method

Based on the high recoveries and effective removal of interfering compounds the modified GCB described in Table 13 (i.e. GCB mod) is suggested as the most suitable clean-up method.
The analysis of collected fractions (1st, 2nd, 3rd and 4th Wash and 1st and 2nd Elution) showed that the 3rd Wash and 2nd elution of the GCB column have very little effect. Therefore, the two steps are not included. The proposed clean-up method therefore consist of:

- Column preparation: 5 ml mobile phase
  2 ml MeOH
  30 ml 0,1 M HCl
- Sample application: 1 ml MeOH extract
- Wash: 5 ml water
  5 ml MeOH
  10 ml DCM/MeOH (80:20) with 15 mM formic acid
  10 DCM
- Elution: 10 ml DCM/MeOH (80:20) with 5 mM TMAH
- Solvent transfer: Evaporate until dryness
  and re-dissolve in MeOH/mobile phase (50:50)

This proposed clean-up method based on GCB is also included in the 3rd draft of LAS Horizontal Standard.

4.4 Calibration

As earlier mentioned a note about the calibration of LAS has been prepared for discussion at an ad-hoc group meeting. The note gives the background for calibration of LAS and describes the possible solutions. Based on the note and the discussions at the ad-hoc group meeting the experiments were planned.

The note is enclosed as Appendix 1.

The calibration of LAS can be based on at least four different procedures as described in the note in Appendix 1. The validity of the chosen calibration procedure depends on:

- The uncertainty of the purity of the chosen LAS standard.
- The relations between the response factors (R_f) of the C_{10}, C_{11}, C_{12}, C_{13} and C_{14} LAS homologue.
- The relations between the response factors (R_f) of the isomers of each homologue.

To examine these issues a series of LAS standards of different purities and different distributions of homologues and isomers were analysed (Table 16).

Table 16 LAS compounds used for calculating response factors and purities.

Chemical name	Abbreviation	CAS #	Purity	Ref*
4-Octylbenzenesulfonic acid, sodium salt	C_8-LAS	6149-03-7	97%	1
Sodium Linear Undecylbenzene Sulfonate	C_{11}-LAS	-	99%	2
Sodium Linear Dodecylbenzene Sulfonate	C_{12}-LAS	2211-98-5	99%	2
Sodium Linear Tridecylbenzene Sulfonate	C_{13}-LAS	-	99%	2
Sodium Linear Dodecylbenzene Sulfonate	C_{10}-C_{13} LAS	25155-30-0	80%	1
Dodecylbenzene Sulfonate acid, sodium salt	C_{10}-C_{14} LAS	69669-44-9	62%	3

* Purchased at: 1) Sigma Aldrich, 2) Wako Chemicals GmbH and 3) TCI.

The standards referred to as C_8-LAS, C_{11}-LAS, C_{12}-LAS and C_{13}-LAS in Table 16 consists of single homologues of LAS. The single C_{11}-, C_{12}-, and C_{13}-LAS homologues however contain several (5-6) isomers.

4.4.1 Response factors of LAS homologues

First solutions of each single LAS homologue were analysed separately, mainly to rule out the presence of additional LAS homologues. The chromatograms are presented in Figure 17-20.

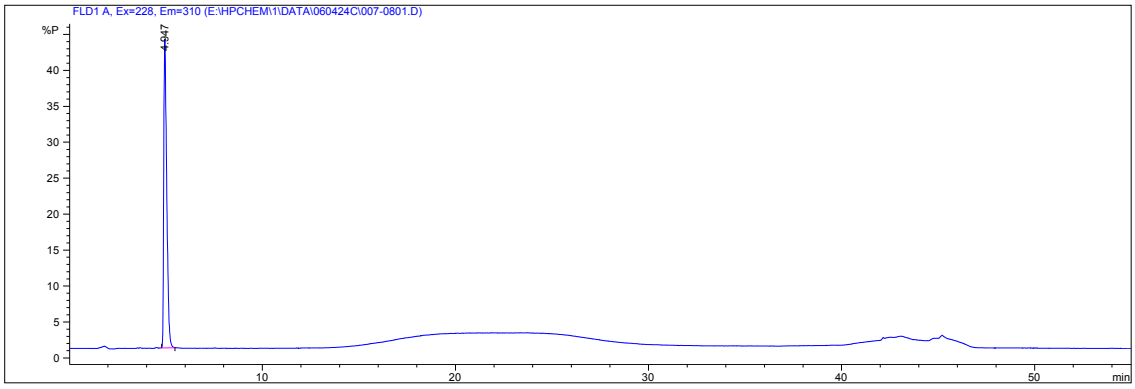

Figure 17. Chromatogram of a 10 mg/l C_8-LAS internal standard. Detection with FLD.

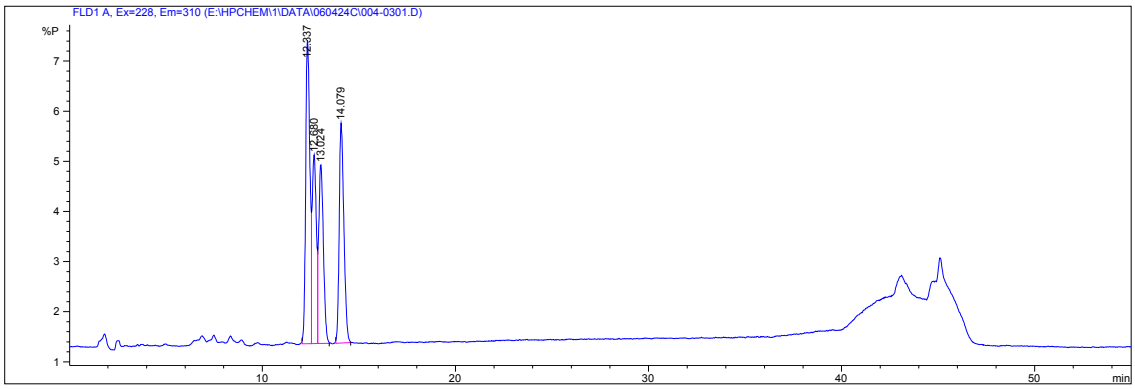

Figure 18. Chromatogram of a 12.5 mg/l C_{11}-LAS standard. Detection with FLD.

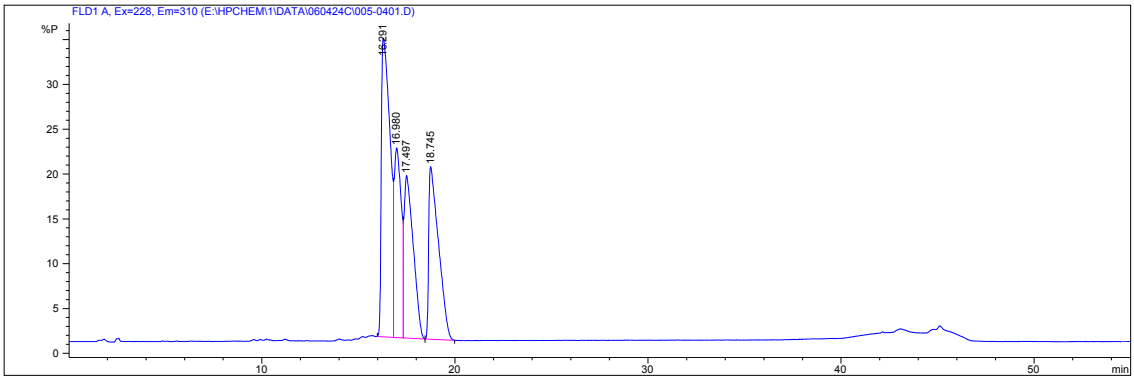

Figure 19. Chromatogram of a 50 mg/l C_{12}-LAS standard. Detection with FLD.

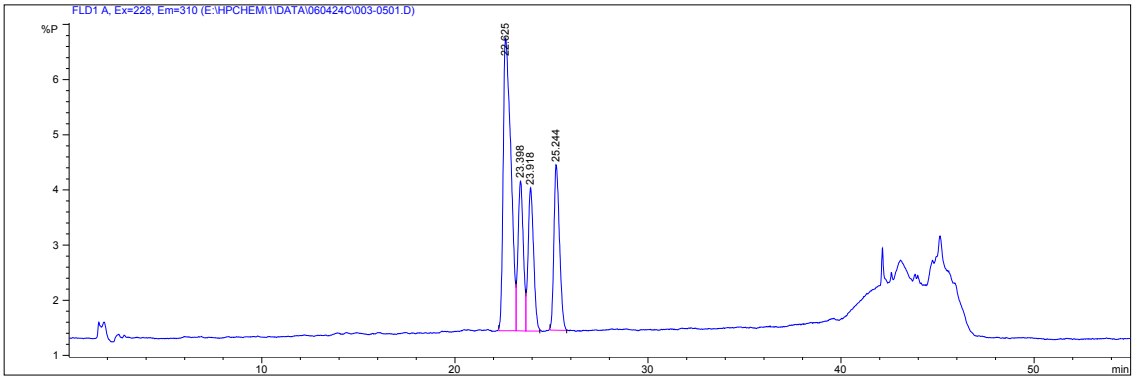

Figure 20. Chromatogram of a 12.5 mg/l C_{13}-LAS standard. Detection with FLD.

Response factors of each homologue were subsequently determined through the analysis of each single homologue at two concentration levels (12.5 mg/l and 1.25 mg/l). The analyses were conducted according to the two methods described in chapter 4.1.1, i.e. with isomeric separation and with homologue separation

The response factors (RF) were calculated as the response/concentration-ratio of C_8-, C_{11}-, C_{12}- and C_{13}-LAS homologue. To simplify the comparing of response factors obtained with different detectors and chromatography, the RFs were calculated relative (%) to the average RF (i.e. normalized).

The response factors were calculated for both detectors. The response factors were furthermore calculated for both chromatographic methods, on a C18 column for isomeric separation (Table 17) and on a C8 column for homologue separation (Table 18).

Table 17 Response factors (RF) of the C_8-, C_{11}-, C_{12}- and C_{13}-LAS homologues measured with FLD and MSD. Chromatography with isomeric separation.

	RF_{FLD} (area • L/mg)*	RF_{FLD} (area • L/mol)*	RF_{MSD} $V_{frag} = 180^{\#}$ (area • L/mg)*	RF_{MSD} $V_{frag} = 70^{\#}$ (area • L/mg)*
C_8-LAS	120 ± 3.4	105 ± 3.0	77 ± 3.3	103 ± 6.2
C_{11}-LAS	95 ± 1.5	96 ± 1.5	104 ± 1.2	101 ± 4.2
C_{12}-LAS	92 ± 1.2	97 ± 1.3	107 ± 1.3	96 ± 6.1
C_{13}-LAS	93 ± 1.9	102 ± 1.1	112 ± 0.9	-

*) The average response factors of the four LAS homologues (average of C8-, C11-, C12- and C13-LAS) were set to 100% and each RF were calculated relatively to this average. Mean ± standard deviation.
#) The fragmentor voltage of the MSD.

Table 18 Response factors (RF) of the C_8-, C_{11}-, C_{12}- and C_{13}-LAS homologues measured with FLD and MSD. Chromatography with homologue separation.

	RF_{FLD} (area • L/mg)*	RF_{FLD} (area • L/mol)*	RF_{MSD} (area • L/mg)* $V_{frag} = 70^{\#}$
C_8-LAS	123	107	98
C_{11}-LAS	95	96	99
C_{12}-LAS	91	96	100
C_{13}-LAS	92	101	104

*) The average response factors of the four LAS homologues (average of C8-, C11-, C12- and C13-LAS) were set to 100% and each RF were calculated relatively to this average.
#) The fragmentor voltage of the MSD.

Fluorescence detector:
The response factors obtained by FLD were found to be independent of the chromatographic method.

The RF of each homologue was found to be very similar when the calculations are based on the molar response factors (area • L/mol). The C_8-LAS homologue gives the best indication of this coherence. As the fluorescence detection is measuring the aromatic part of LAS, the same molar response for each homologue (and isomer) would therefore be expected.

However, when only C_{11}-C_{13} LAS is evaluated the response factor per weight is also very similar.

Mass spectrometric detector:
When MSD is used as detection method the fragmentor voltage was found to influence the relative respons of the LAS homologues. When a relatively low fragmentor voltage of the MSD was used, the response factors of the homologues were found to be similar. The influence of chromatography on the relative response factors was not found to be significant. The difference between the single peak of C_8-LAS and the more complex "landscape" of multiple peaks (the isomer mixtures of the C_{11}-, C_{12}- and C_{13} homologues), may however effect the relation between the response factors.

The use of C_8-LAS as calibration standard is therefore not advisable when the chromatography is based on isomeric separation. This possibility was presented as solution 4 in the LAS note in Appendix 1.

Also for the MSD the responses of the C_{11}, C_{12} and C_{13} homologues were found to be very similar.

Conclusion:
For both detectors the calibration of LAS can be based on the responses per weight, and the calibration can be based on mixtures of LAS homologues of known or unknown distribution.

4.4.2 Purity of LAS standards

When the response factors of the individual LAS homologues (C_8-, C_{11}-, C_{12}- and C_{13}-LAS) were established, a determination of the purity of the two LAS mixtures (CAS # 25155-30-0 and CAS # 69669-44-9) was conducted, see Table 16.

This quantification was based on external standardization (n = 2) using solutions consisting of: 1) a mixture of the four individual LAS homologues (used as calibration standards), and 2) the two LAS mixtures. Two stock solutions were prepared for each of the LAS mixture by

weighing approximately 50 mg in 10 ml MeOH (i.e. 5000 mg/l). This was done to minimise the uncertainty introduced when preparing the stock solutions.

The individual LAS homologues was analysed in concentrations of 10 mg/l (i.e. total LAS concentration of 40 mg/l). The concentrations of the technical homologue mixtures were also prepared to be approximately 40 mg/l.

Chromatograms of the homologue mixture and the two LAS mixtures are presented in Figure 21-23.

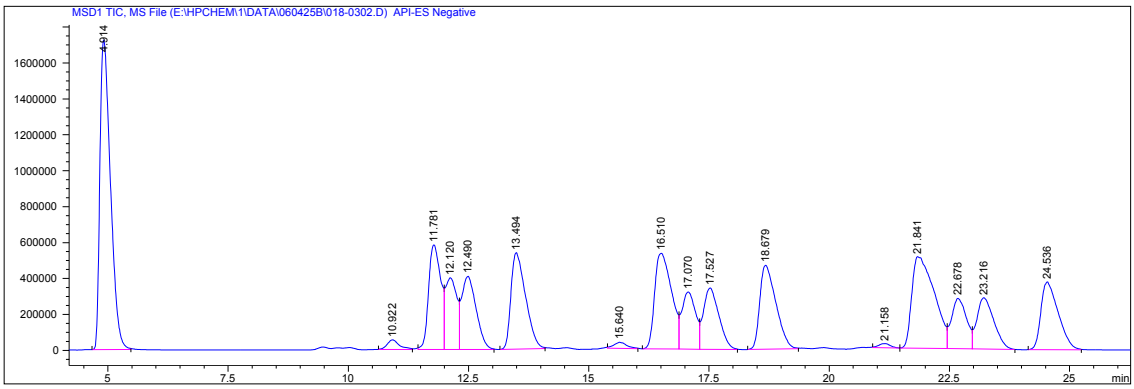

Figure 21. Chromatogram of a mixture of C_8-LAS, C_{11}-LAS, C_{12}-LAS and C_{13}-LAS, each 12.5 mg/l. Detection with MSD.

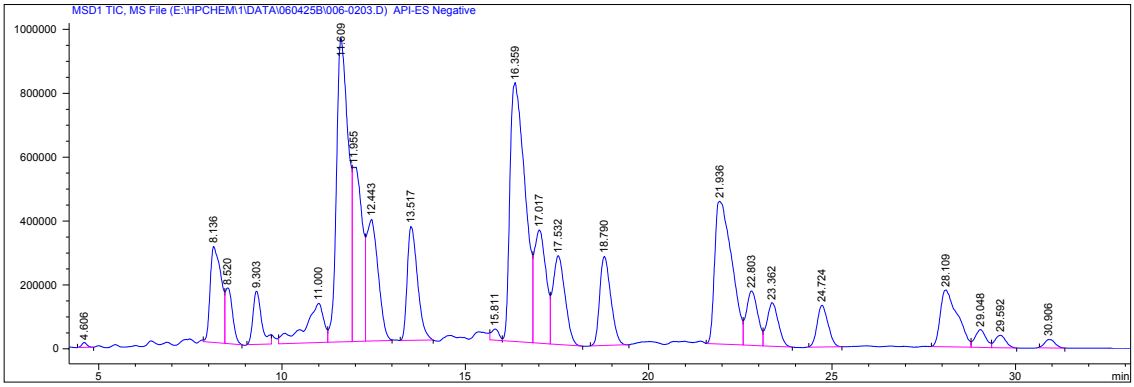

Figure 22. Chromatogram of the C_{10}-C_{14} technical LAS mixture, 62%. Concentration 55 mg/l. Detection with MSD.

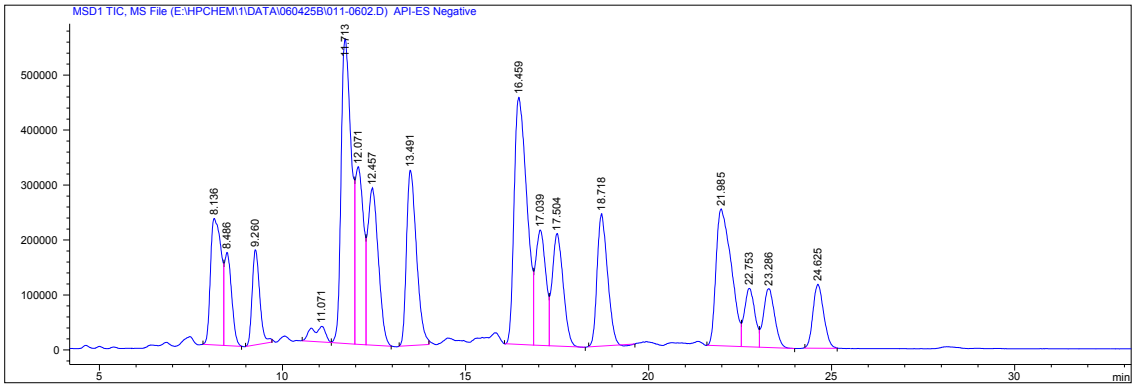

Figure 23. Chromatogram of the C_{10}-C_{13} technical LAS mixture, 80%. Concentration 40 mg/l. Detection with MSD.

Based on the response factors of the single LAS homologues described above, the purities of the two commercially available technical mixtures of LAS were calculated (Table 19).

Table 19 Purity of technical LAS mixtures.

	CAS #	Stated purity *	Calculated purity **
C_{10}-C_{13} LAS	25155-30-0	80%	57 ± 4.1%
C_{10}-C_{14} LAS	69669-44-9	62%	50 ± 2.7%

* From supplier.

** Mean ± standard deviation.

These few results show that the purity stated by the supplier may be unreliable. For the two technical LAS mixtures the purity is determined to be 20-30% lower than stated by the supplier. As the calculation is based on the assumption, that the single homologue standards are 99% pure, the real purity of the two mixtures can not be higher than calculated.

It may be possible to obtain more reliable LAS standards than these results indicate, and the second draft of the method is written with a mixture of homologues and isomers for calibration. This second draft has also been subjected to the ruggedness test described in chapter 6.

However, due to the observed uncertainty of the commercial LAS mixtures (see Table 19), it is recommended, that mixtures are not used as primary standards. As shown in Table 16, single homologues of very high purity can now be obtained for C_{11}, C_{12} and C_{13} LAS, and these were found to give very similar response (per weight), when measured by FLD as well as by MSD.

It is therefore decided, that a mixture of the three homologues are used for calibration, and that the draft method shall be revised accordingly. Technical mixtures of LAS may also be used, if the actual purity is measured against the single LAS homologues and found sufficiently high (≥98%). This will be stated as a note in the draft method.

5. CONCLUSIONS

Based on the studies and results presented in this report a number of conclusions were reached. On the basis of these conclusions a 2nd draft of the method have been written. The 2nd draft was subsequently subjected to a ruggedness test, which is described in chapter 6.

Measurement

Chromatography
Mobile phases using either ammonium acetate or sodium perchlorate as additive were compared. Based on repeatability of peak area and retention time ammonium acetate was found to be superior.

C18 and C8 HPLC columns were used. With the C18 column a separation of both isomers and homologues were obtained, with the C8 column only a separation of homologues were obtained.

Extraction

Choice of solvent
The extraction efficiencies of methanol, basic methanol (0.5 M NaOH) and methanol/water (1:1) were compared in several studies. Methanol and basic methanol were found to be equally efficient and significantly better than methanol/water. As basic methanol requires a pH regulation before HPLC-analysis, the most robust and therefore the best choice of extraction solvent was found to be methanol.

Extraction technique
A comparison of reciprocating shaker, sonication in combination with reciprocating shaker, sonication alone, reflux and soxhlet based on both methanol and basic methanol as extraction solvents was conducted. Disregarding sonication all extraction techniques were found to be equally efficient. Reciprocating shaking, being less time consuming and most easy to adapt for laboratories with many samples, was therefore chosen for the LAS Horizontal standard.

Extraction time
In parallel to the test of extraction techniques several extraction times were compared and no differences were found, which suggests that one single extraction of 30 minutes is sufficient. An additional study based on one extraction technique and three different solvents also concluded 30 minutes to be sufficient.

Pre-treatment
Freeze-drying was found to be significantly better than oven-drying regarding the recovery of LAS. Only drying by freeze-drying is therefore included in the method.

Clean-up

Two clean-up procedures on GCB and SAX columns were tested. Both clean-up procedures were found to give satisfactory recoveries, however, the removal of interfering compounds was not sufficient when detection was based on FLD. A modified GCB procedure was tested after completion of the ruggedness test. The modification resulted in a higher recovery and a cleaner extract, thereby giving a lower limit of detection.

Calibration

Response factors
Pure standards of C_8-, C_{11}-, C_{12}- and C_{13}-LAS were analysed and compared on both FLD and MSD. A correlation between the response factors of the LAS homologues analysed on FLD and molecular weight indicated that the response factors of the LAS should be based on molar response. This difference was however only significant when comparing the C_8-LAS with the other homologues, and no difference was found for the C_{11}-, C_{12}- and C_{13}-LAS response factors calculated per weight. When the analysis was conducted on MSD the response factors were found to be very similar.

Purity
The purity of two technical LAS mixtures was analysed and estimated to be significantly lower than stated by the supplier.

Calibration standards
For both detectors the calibration of LAS can be based on the responses per weight, and the calibration can be based on mixtures of LAS homologues with the alkyl chain length of C_{10} to C_{14} with known or unknown distribution, however of known and high purity. As it may be difficult to obtain mixtures of known purity it is suggested, that a mixture of the three homologues C_{11}-, C_{12}- and C_{13}-LAS are used for calibration.

6. RUGGEDNESS TEST

A 1st draft of the LAS Horizontal Standard was completed for discussion at the ad-hoc group meeting in Madrid 21 September 2005. Based on the results obtained from the pre-normative work with LAS described in the previous chapters, and a few comments from the meeting, a 2nd draft was written (Appendix 4).

The ruggedness of the 2nd draft of the method was subsequently tested. The results of the test and the consequences are presented in this chapter, and the draft method is revised according to the results from the ruggedness test.

6.1 Materials

The samples chosen for the ruggedness tests are shown in Table 20. The samples were selected to represent sludge, soil and compost samples.

Table 20 Samples used in the ruggedness tests of the LAS Horizontal Standard.

Sample ID	Sample description	Pre-treatment	Extraction procedure*	LAS mg/kg dm
SL-E1	Sewage sludge, VKI, Hoersholm, Denmark	Freeze-dried	1	1900
SL-E2	Sewage sludge, domestic, Vejle, Denmark. 908134	Freeze-dried	1	1700
SL-11	Sewage sludge, electronic industry, Turin, Italy	Freeze-dried	1	57
SO-4	Clay soil, Speyer, Germany	Dried	2	2.2
SO-9	Soil, Hagen, Germany	Dried	2	3.4
CW-5	Compost, Fulda, Germany	Dried	2	7.2

* Extraction procedure 1 and 2 refers to the two extraction procedures which are included in the LAS Horizontal standard, sub-section 10.1.1 and 10.1.2, see Appendix 4.

A more detailed description of the samples is given in chapter 3, Table 1.

6.2 Experimental

6.2.1 Design of ruggedness test

The ruggedness test was conducted according to a multifactorial experimental design described by Plackett & Burman /9/. 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 21). The effect of each factor was measured at two levels (+ and -) representing the extremes of which the analytical method could be subjected.

Table 21 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 the experimental factor 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.

6.2.2 Factors and levels of ruggedness test

The main effects on the method were expected to arise from the extraction procedures and the selection of experimental factors was therefore concentrated on this part of the analytical procedure. Although no effects on the ruggedness of the method were expected to arise from variations in chromatographic and detector settings (as calibration standards and samples are expected to be equally effected), one factor in HPLC was also examined.

The method was tested for variations in:

A Sample/solvent-ratio used by the extraction.
B Extraction solvent
C Position of sample glass on reciprocating shaker
D Activity (velocity) of reciprocating shaker
E Extraction time
F Use of additive to sample extracts and calibration standards
G None

The factor G (“None”) was included in the ruggedness test to establish a value representing the general uncertainty of the method submitted to the variations of factor A-F.

To measure the influence of the described experimental factors, a minimum and maximum value of each factor was selected (Table 22). This range, limited by the minimum- and maximum-value, was used to test the influence of the applied methodical changes.

In parallel to the multifactorial setup of the ruggedness test described in Table 21, the variation of three additional factors H, I and J was also examined. These factors were the choice of HPLC column, choice of detector and the use of clean-up. The extracts of sample 1-8 of Table 21 were re-used to test the possible variations for these factors.

Table 22 The selected values of the two-level experimental factors.

Factor	Description of factor	"Minimum" value (-)	"Maximum" value (+)	Samples tested
A_{SL}	Sample/solvent-ratio	2 g dm/10 ml	3 g dm/10 ml	SL-E1, SL-E2, SL-11
$A_{SO,CW}$	Sample/solvent-ratio	10 g dm/50 ml	15 g dm/50 ml	SO-4, SO-9, CW-5
B	Extraction solvent	MeOH	MeOH:water (100:4)	All‡
C	Position of glass on reciprocating shaker	Horizontal	Vertical	All‡
D	Activity of reciprocating shaker	230 strokes pr min.	270 strokes pr min.	All‡
E	Time of extraction	30 min	60 min	All‡
F	Additive to extracts and calibration standards#	Sodium Dodecylhydrogen Sulfate	Ammonium Acetate	All‡
G	None	-	-	All‡
H	Type of HPLC-column	C8	C18	All‡
I	Detector	FLD	MSD	All‡
J	Clean-up	Yes	No	SL-11, SO-4, SO-9

‡ "All" refers to all six samples that were included in the ruggedness test (se Table 20).
# 0.05 M Sodium dodecylhydrogen sulfate or 0.01 M ammonium acetate was added to extracts and calibration standards before analysis (1:1).

The effects of the variations introduced by the factors A-G were examined by the multifactorial design described in Table 21. The effects of the factors H, I and J were evaluated by comparing the results obtained with the two "values" used (Table 22).

Apart from the described analytical variations, the ruggedness test was conducted according to the LAS Horizontal standard (Appendix 4). The analysis of the samples used to examine the experimental factors A-G were based on the HPLC-MSD settings described in Annex A of the standard (i.e. homologue separation with C8-column and detection with MSD).

6.3 Results

The diagrams below include the results of the 6 ruggedness tests. The height of each column indicates the magnitude of the effect (% relative difference) introduced by each factor (A-H) on each sample (Table 22). To distinguish actual effects of a tested variation from the uncertainty of the method, different conditions were evaluated by:

- The relative difference of a sample should be significant compared to the uncertainty of the method, as determined by the factor G
- The effects should be uniformly distributed as either positive or negative
- The effect should be realistic (for example should longer extraction times not reduce the extraction efficiency).

The results are presented in Appendix 3.

6.3.1 Extraction of LAS in sludge

The results of ruggedness tests based on the sludge samples SL-E1, SL-E2 and SL-11 are shown in Figure 24. The effects of the tested variations were in general found to be very low. By comparing the influence of the factors A-F (se Table 22) to the factor G, which refers to "none" variation, very few factors appeared to have an effect.

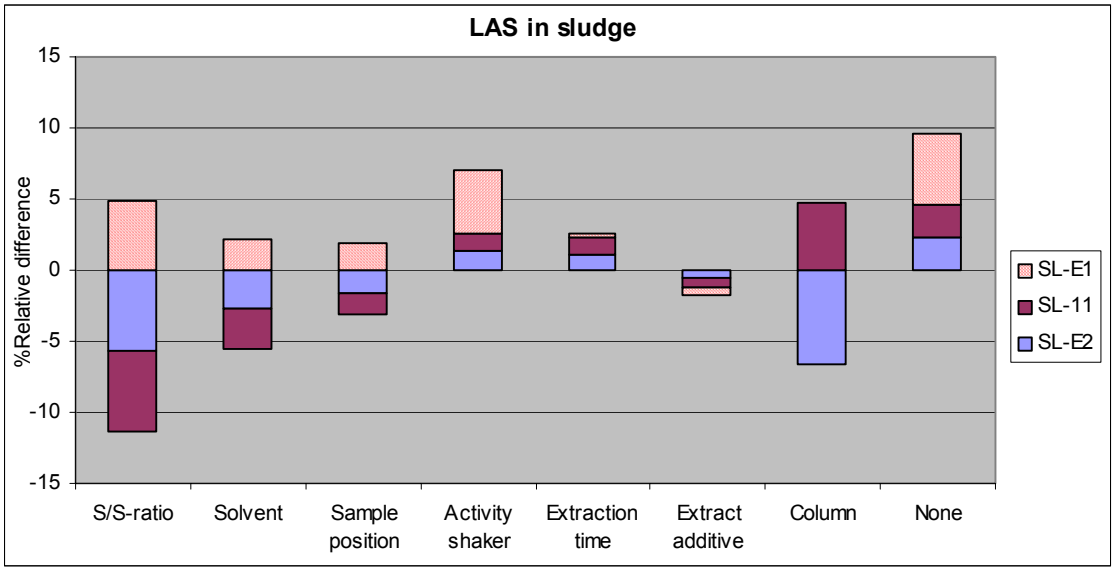

Figure 24. The relative difference (%) in concentration of LAS caused by the variation of the described parameters.

6.3.2 Extraction of LAS in soil and compost

The results of ruggedness tests based on the soil and compost samples SO-4, SO-9 and CW-5 are shown in Figure 25. The effects of the tested variations were in general found to be relatively low. By comparing the influence of the factors A-F (se Table 22) to the factor G, which refers to "none" variation, very few factors appeared to have an effect.

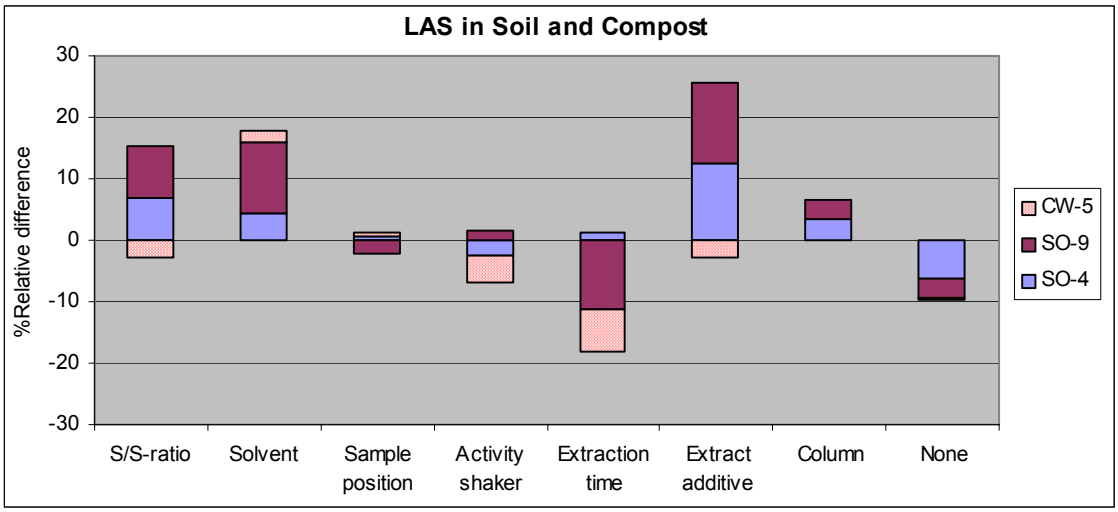

Figure 25. The relative difference (%) in concentration of LAS caused by the variation of the described parameters.

Evaluating the sample/solvent-ratio (A), solvent type (B) and time of extraction (E) collectively indicate that the extraction of LAS from soil and compost under some circumstances may be submitted to reduced extraction efficiency.

6.3.3 The influence of the tested factors

The sample/solvent ratio (A):
A slight effect was found when the extraction was conducted on soil/compost, whereas no effect was found when the extraction was conducted on sludge.

Extraction solvent (B):
The effect of added water to methanol was found to be low (1-10%) but uniform, as 5 out of 6 samples were found to be extracted most efficiently with methanol without water.

Position of sample glass on reciprocating shaker (C):
No effect on extraction efficiency was indicated, when the extraction was conducted with the sampler glass placed in vertical or horizontal position on the reciprocating shaker.

Activity (velocity) of reciprocating shaker (D):
By comparing the columns representing the effect of the activity of the reciprocating shaker in Figure 24 and 25 with columns representing "none" effect (G), the activity was not found to have any effect.

Extraction time (E):
The extraction of LAS from soil and compost was found to slightly increase, when the extraction time was doubled (from 30 to 60 min). When the extraction was conducted on sludge no effect was found.

Use of additive to sample extracts and calibration standards (F):
The use of sodium dodecylhydrogen sulfate as additive to the sample extracts (and calibration standards) was found to interfere with the chromatography of C_8-, C_{10}-, C_{11}- and C_{12}-LAS when the separation was done on a C18-column. When the separation of LAS was done on a C8-column the chromatography of only the C_8- and C_{10}-LAS was influenced. The use of

dodecylhydrogen sulfate was furthermore found to suppress the response of C_{10}-LAS when the MSD was used. This observed effect on the chromatography and on the detection was found to slightly reduce the response of C_8- and C_{10}-LAS, and this may explain the calculated difference shown in Figure 25.

6.3.4 Other parameters

HPLC-Column (H):
When the analysis of LAS was conducted on a C8- and C18-column respectively, no difference in concentration was found. It is therefore concluded, that the changes in the chromatographic conditions have the same influence on the calibration standards and sample extracts, so that the choice of HPLC conditions are of no significance.

Detector (I):
The soil, compost and sludge samples SO-4, SO-9, CW-5, SL-11, SL-E1 and SL-E2 were analysed with both FLD and MSD. The chromatographic separation was furthermore done with both C8- and C18-column for all samples.

Table 23 shows the performance of the two detectors regarding the analysis of the samples used in the ruggedness test.

Table 23 The performance of FLD and MSD based on analysis of the samples SO-4, SO-9, CW-5, SL-11, SL-E1 and SL-E2. The symbols v and % and refers to a successful and non-successful completion of the analysis.

	FLD		MSD	
Sample ID	C8-column	C18-column	C8-column	C18-column
SL-E1	v	v	v	v
SL-E2	v	v	v	v
SL-11	%	%	v	v
SO-4	(v)[#]	(v)[#]	v	v
SO-9	%	%	v	v
CW-5	%	-	v	v

- Not analysed.
[#] Close to LOD

The analysis based on MSD was for all samples found to give satisfactory results. This was found to be the case for both the C8- and the C18-column.

The use of FLD was found to be less sensitive and less specific than the MSD. Particularly the analysis of soil and compost was found to be problematic, due to the lower limit of detection (LOD) required.

The analysis of the sewage sludge samples SL-E1 and SL-E2 was performed successfully, also by the FLD. The industrial sludge sample SL-11, however, showed chromatographic interferences when detected by FLD, and LAS could not be determined by FLD. The samples SO-9 and CW-5 also showed interference from the matrix when measured by FLD, and LAS could only be detected with MSD.

The response of the internal standard C8-LAS was found to be too low, and an increase in the added amount of internal standard is therefore required. This is especially the case for the use of FLD. An increase from 10 µg C_8-LAS to 100 µg pr. sample is suggested.

From the comparison of the two detectors some recommendations can be stated:

- The FLD is valid for the analysis of sewage sludge from most waste water treatment plants, as only a high LOD is required, typically 20-50 mg/kg dm.
- When FLD is used, the C18-column is the best choice, as the isomeric pattern can be used for the identification of LAS, thereby increasing the selectivity of the FLD.
- Special sludges may be better analysed by MSD.
- The MSD is the best choice for the analysis of soil and compost due to the lower LOD and higher selectivity obtained by MSD compared to FLD.

Clean-up:

In order to examine the interference on the FLD a clean-up of the extracts of SO-4, SO-9 ad SL-11 was performed according to the procedures described in chapter 4.3. The clean-up procedure on GCB-column was tested on all three samples and the clean-up procedure on SAX-column was tested on extracts of SL-11. The effect of clean-up was however found to be little, as no significant reduction in interferences, level of baseline or improvement of signal/noise ratio was obtained.

After completion of the robustness test further studies were conducted on the clean-up, as described in chapter 4.3. Using a modified clean-up on GCB-column it was possible to obtain a higher recovery and a cleaner extract, resulting in a lower limit of detection. This was the case for both detectors, although the biggest improvement was seen for the FLD.

Performance parameters:

Limit of detection (LOD)

The ruggedness test was based on 8 extractions on each of 6 samples. Although the 8 extractions were conducted under different conditions (Table 21 and 22) the results were used to estimate the LOD of the method. The estimation of LOD was calculated from the standard deviation of the 8 extractions of SL-11. LOD was calculated as approximately S_w multiplied by a factor of 3.5. Since the conditions were not true repeatability conditions, the calculation will result in an overestimation of LOD, especially for the samples with higher amounts of LAS compared to the LOD.

LODs were calculated for SL-11, SO-4, SO-9 and CW-5 (Table 24).

Table 24 Standard deviation (s_w), coefficient of variation (CV%) and limit of detection (LOD), calculated from the results of the ruggedness test.

	SL-11	SO-4	SO-9	CW-5
sw (mg/kg)	2,4	0,070	0,20	0,21
CV%	6,0	6,9	11	5,7
LOD (mg/kg)	8,5	0,24	0,71	0,73

Satisfactory results were found regarding the LOD of sludge. A calculated LOD of 8.5 mg/kg was found to be below the LOD of 20 mg/kg, which was described in the draft standard. For soil and compost the LAS Horizontal standard states a LOD of 0,2 mg/kg. This LOD was not

reached in the case of SO-4, SO-9 and CW-5. The LOD is however overestimated, and it is expected to be lower when the standard deviation is calculated from true repeatability conditions.

Based on the improvements introduced by the clean-up procedure (chapter 4.3), the limit of detection is furthermore expected to decrease a factor of 2-3. The use of a clean-up step therefore enables the method to reach a LOD of approximately 0,2 mg/kg or lower for soil samples.

6.4 Consequences of ruggedness tests

The ruggedness testing of the 2nd draft of the LAS Horizontal Standard resulted in adjustments of the method in the extraction step, the calibration and the amount of internal standard. These changes will be incorporated in the draft horizontal standard.

The consequences of the ruggedness test on the method are:

- The description of sample/solvent ratio is kept unchanged for both sludge and soil/compost. The tendency of lower extraction efficiency for soil is compensated by the increased extraction time, see below.
- The minimum velocity of the reciprocating shaker is stated as 230 strokes per minute.
- The extraction time is increased from 30 to 60 minutes for soil and compost (method section 10.1.2). For sludge no change is made.
- The addition of ammonium acetate to extracts and calibration standards is kept unchanged.
- The calibration standard is changed. The use of a commercial LAS mixture is replaced by the use of a mixture of three LAS homologues (C_{11}, C_{12} and C_{13} LAS) of high purity (99% stated by supplier).
- The amount of internal standard (C_8-LAS) is increased for all samples. The amount of added internal standard is changed from 10 µg to 100 µg.
- More information about the clean-up is included, and a detailed description of the GCB clean-up is given.
- More information about the advantages and disadvantages of the two detectors is needed in the method. This will be added in the method, probably as a note.

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APPENDIX 1 Note on the calibration of LAS, September 2005

HORIZONTAL

September 2005

Ad-hoc group LAS/nonylphenols

Calibration of LAS in the Horizontal standard

Note

for discussion in connection with the CEN TC 308/WG 1 meeting
in Madrid on 21 September 2005

by

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Calibration of LAS in the Horizontal standard

1. Introduction

During the work with the preparation of a horizontal standard for LAS in solid matrices it has been demonstrated, that the calibration in LAS measurements is a very complicated matter. One reason is, that it is difficult to find a well-defined standard substance for LAS. Another reason is, that the chemical composition of LAS may change after the emission of LAS into the environment, meaning that the calibration standard and the LAS from the natural samples may have a different composition.

The matter will be further described in this note, and proposals will be given to find a solution. The note will be discussed at the next ad hoc group meeting in Madrid in September 2005, so that a decision hopefully can be taken shortly after the meeting.

2. Background

The analyte LAS is linear alkylbenzene sulfonic acid, sodium salt. The European technical LAS products consist of a mixture of the homologues C_{10}-LAS, C_{11}-LAS, C_{12}-LAS, C_{13}-LAS and C_{14}-LAS with C_{11}-LAS and C_{12}-LAS as the dominant homologues. Only para-substituted LAS is present.

For each homologue many isomers are present. Theoretically C_{10}-LAS consists of 5 isomers, C_{11}-LAS of 6 isomers, C_{12}-LAS of 6 isomers, C_{13}-LAS of 7 isomers and C_{14}-LAS of 7 isomers. Thus LAS products may theoretically include up to 31 components. However, when manufacturing LAS the aromatic ring is never positioned in the terminal end of the linear alkyl. Without the 1-phenyl isomer the number of isomers is therefore reduced to 26 components (Cook A.M & Hrsak D, 2000).

By the method total LAS must be determined. The LC-analysis are separating the homologues clearly from each other on the chromatographic column, and they are determined separately. Depending on the choice of mobile phases (and column) in the LC-analysis the isomers may be more or less separated from each other. The result for total LAS is influenced by several factors:

- The detector response for the homologues may differ
- The detector response for the isomers of the single homologue may also differ
- The composition of LAS in the environmental samples may differ from the composition of the analytical LAS-standard

This raises several questions:

- Can we obtain a standard chemical with a well-defined LAS composition?
- Can we obtain a standard of high purity and/or well-known purity?
- How similar is the composition of LAS in environmental samples and the composition of LAS in the LAS-standard – as for the distribution of homologues as well as the isomers?

The answers to these questions are of great importance for the selection of the calibration procedure in the Horizontal standard.

3. Commercial LAS standards

A wide range of LAS standards exist on the market, some commercially available and some only as “special made”. Table 3.1 shows many of the LAS standards found at catalogs of chemical suppliers (as Tokyo Kasei Organic Chemicals (TCI), Sigma-Aldrich (S&A), BuyReagents (BR) and ABCR) and LAS standards found in the literature in general.

Table 3.1: CAS No., name, range of homologues, purity of different commercially available LAS standards.

CAS #	Name	Mixture of	Purity	Ref
69669-44-9	Sodium Dodecylbenzenesulfonate	C10-C14	~60%	TCI
25155-30-0	Sodium Dodecylbenzenesulfonate	C10-C14	80%	S&A/ABCR
6149-03-7	4-Octylbenzenesulfonic acid sodium salt	C8	97%	S&A
2211-98-5	p-n-Dodecylbenzenesulfonic acid sodium salt	?		BR
85117-50-6	Monoalkylbenzenesulfonic acid, sodium salt	C10-C14	85%	
68411-30-3	Sodium Dodecylbenzenesulfonate	C10-C13		
90194-50-6	Monoalkylbenzenesulfonic acid, sodium salt	C10-C13		
1322-98-1	Decylbenzene sulfonic acid, sodium salt	?		
26248-24-8	Tridecylbenzene sulfonic acid, sodium salt	?		
27636-75-5	Undecylbenzene sulfonic acid, sodium salt	?		

As shown in table 3.1 no LAS of a high purity has been found. It is possible to buy a 96% LAS acid, however not the LAS itself.

Table 3.1 also shows that no pure LAS homologue seems to be commercially available (besides C8-LAS).

The distribution of the LAS homologues are occasionally described, but it has not been possible to get information on whether this distribution is based on concentration or on detector response (which detector?).

A wide range of CAS numbers of magnesium, calcium and ammonium salts of the linear alkylbenzene sulfuric acid and the acids are also found in the literature, but no pure standards have been found here either.

According to the HERA Project (Human and Environmental Risk Assessment) (www.heraproject.com) LAS with CAS # 68411-30-3 represent > 98% of the European marked. A LAS standard with a similar/equal distribution of homologues (and isomers) could therefore be preferable.

On the market it does not seem possible to find a LAS standard with a well-defined composition and a well-known and high purity. As long as the relative responses of the homologues are not known a good standard may be a standard with a similar distribution to that in the environmental samples.

4. Changes in the composition of LAS products after use

When LAS is being emitted into the environment after the use in cleaning or washing products, changes in the compositon of LAS may occur if the isomers and homologues have different properties. This may be differences in sorption to surfaces and solid material giving different partitioning in the environment; or it may be differences in biodegradability.

The LAS homologues with long alkyl chain length are found to be easier biodegradable than the short-chain homologues. Sorption is found to be directly proportional to the chain length and toxicity inversely proportional. As a consequence of these properties a relative increase of the short-chain LAS homologues may be seen in the environment.

Studies measuring the distribution of the different isomers of one LAS-homologue in water, sediment and aquatic organisms showed that the distribution in water and sediment was very similar, whereas the distribution in the organisms was very different (Morrall S.W. et al, 2000).

5. Discussion

As it has been described it will most probably not be possible to obtain a LAS standard substance with a well-defined composition, and the preparation of a calibration standard is not part of the preparation of the Horizontal standard for LAS.

However it is possible to provide a LAS standard of well-defined purity. It is therefore concluded, that the calibration shall be based on the use of these LAS standards, even if the composition is not unambiguous.

Therefore it is concluded that the calibration must be based on the best possible approximation.

To decide how the calibration will be described in the horizontal standard it is important to look at the LC-detectors to be used.

In the Horizontal standard for LAS the final measurement will be performed by LC with fluorescense or mass spectrometric detector (LC-FLD, LC-MSD).

The response from the **fluorescense detector** is caused by the presence of the benzene ring in the molecule of LAS. Therefore it is most likely that the response for LAS will depend on the molecular weight and that the molecular response will be constant. Based on this assumption the responses of the homologues is inversely proportional to the molecular weights of the homologues. As for the isomers, the response of the isomers for a certain homologue will be the same.

For the **mass spectrometric detector** the response will depend on many factors, which is very difficult to control. The direct response from the MSD may be constant on a molecular basis, but it can be expected that factors like the molecular size, the charge of the molecule, the volatility may influence the proportion that is being transferred to the detector and ionised.

As a consequence the calibration of the FLD may be based on the assumption of a constant molecular response. For the homologues the assumption can be confirmed, if highly purified homologues were available. However this is hardly the case. Similarly for the isomers, the assumption can only be confirmed by the use of highly purified isomers, which have not been found so far.

Whether the same assumption is reasonable for the MSD can to some extent be evaluated by the simultaneous analysis by FLD and MSD. These experiments are currently being carried out.

If it is decided, that the calibration shall be based on a constant molecular response, the responses from the homologues will have to be recalculated, using a correction by the molecular weights. These are listed in table 5.1, calculated on the basis of the weight of the anions.

Table 5.1: Factors to normalize response for the homologues to C_{12}-LAS.

Homologue	C_8-LAS	C_{10}-LAS	C_{11}-LAS	C_{12}-LAS	C_{13}-LAS	C_{14}-LAS
M.W.	269	297	311	325	339	353
Normalization factor		0.914	0.957	1.000	1.043	1.086

6. Solutions

Based on our description of the problem we can point at four possible solutions, which are listed below. All the solutions are based on the assumption, that the isomers have the same response.

The four possible solutions are:

#	Calibration	Calculation of samples
1	Responses are determined for each of the 5 homologues. The concentration of each homologue must be known.	The 5 homologues are calculated individually and summed to give the total content of LAS.
2	Responses are determined for each of the 5 homologues and normalized to C_{12}-LAS, using the normalization factors in table 5.1. The concentration of total LAS is used, since the concentration of the individual homologue is not known.	The 5 homologues are calculated individually, again using the normalization factors. The total LAS is calculated as the average value.
3	The response is only determined for total LAS, using the sum of areas of all the homologues.	Total LAS is calculated from the sum of areas. Only total LAS is determined. However the 5 homologues may be calculated separately to give an approximate concentration of the homologues.
4	No external calibration is carried out, but the C_8-LAS added to each sample is used as internal standard and for the calibration as well. The calibration is based on the assumption that the response is inversely proportional to the molecular weights of the homologues.	The 5 homologues are calculated individually from the area of the internal standard C_8-LAS added to each sample, using the relative molecular weight as a correction factor. The total content of LAS is the sum of the 5 homologues.

By the methods 1-3 C_8-LAS is used as internal standard, and the responses are calculated relative to C_8-LAS.

In all cases the results are calculated as concentrations of the sodium salt.

In chapter 5 it has been concluded, that any calibration procedure for LAS will be an approximation.

The simplest procedure is #4. It does not involve a separate calibration, and it has the highest reproducibility. However it is based on the assumption that the molecular response is constant, which has not been proven.

Contrary the most complicated procedure is #2, since it involves corrections for molecular weights by the calibration as well as by the calculation of samples. It is also difficult to understand and to communicate to users.

A less complicated procedure is #3, which is also easy to understand and to communicate to users. The results for the homologues will be less accurate, however it has been decided only to include the determination of the homologues in a technical note.

Procedure #1 cannot be recommended, since it is based on a calibration standard with a known concentration of the homologues, a standard which is not available today.

By the selection of the procedure it is important to remember, that the practical implications of the choice is not very big, since only smaller differences can be expected, using the four methods as described.

7. References

Cook A.M & Hrsak D. (2000). The complete degradation of LAS is becoming better understood with pure cultures of bacteria. The CLER Review. Vol. 6 No.1.

Morrall S.W. et al. (2000). Use of isomer distributions to characterize the environmental fate of LAS. The CLER Review. Vol. 6 No.1.

www.heraproject.com.

APPENDIX 2 Results – Extraction procedure

Appendix 2A: Results from *Extraction study 1*:

Extraction technique	time (h)	Concentration (mg/kg) A	B
Shaking	30 min	1328	1386
Shaking	4 h	1422	1362
Sonication + Shaking	5 min + 30 min	1348	1352
Sonication + Shaking	5 min + 4 h	1354	1451
Sonication	10 min	677	626
Sonication	30 min	833	908
Reflux	1 h	1492	1585
Reflux	4 h	1667	1526
Soxhlet	6 h	1216	1277
Soxhlet	20 h	1307	1232

SL-E2 extracted with MeOH

Appendix 2B: Results from *Extraction study 2*:

Extraction technique	time (h)	Concentration (mg/kg) A	B	C
Shaking	1	2622	2828	3084
Shaking + Sonication	1	2797	2946	3230
Sonication	1	1729	1816	2132
Reflux	1	2984	3224	3119
Soxhlet	12	2668	3471	3988

SL-E2 extracted with MeOH

Extraction technique	time (h)	Concentration (mg/kg) A	B	C
Shaking	1	2787	2939	2895
Shaking + Sonication	1	1959	2834	2952
Sonication	1	2837	2874	2789
Reflux	1	5140	888	2332
Soxhlet	12	-	-	-

SL-E2 extracted with NaOH/MeOH

Appendix 2C: Results from *Extraction study 3*:

		Concentration (mg/kg)		
	time	SO-E2	SL-E2 (freeze-dried)	SL-E2 (oven-dried)
MeOH	30 min	15,82	1792	1358
	30 min	18,75	1627	1353
	60 min	18,18	1673	1366
	60 min	17,15	1636	1500
MeOH/Water	30 min	18,43	315	153
	30 min	19,82	276	146
	60 min	18,62	221	148
	60 min	21,55	250	158
MeOH/NaOH	30 min	20,07	2028	720
	30 min	20,26	2024	1100
	60 min	19,92	1960	1141
	60 min	21,06	1518	1117

Extraction of SO-E2 and SL-E2

Appendix 2D: Results from *Extraction study 4*:

		Concentration (mg/kg)	
Sample	Solvent	1 .Extraction	2. Extraction
SL-E2 A	MeOH	835	173
SL-E2 B	MeOH	806	146
SL-E2 C	MeOH	912	193
SL-E2 A	MeOH/NaOH	755	185
SL-E2 B	MeOH/NaOH	767	196
SL-E2 C	MeOH/NaOH	740	150

Extraction of SL-E2

APPENDIX 3 Results – Ruggedness test

Concentration (mg/kg) of LAS in samples used in ruggedness tests.

Ext no.	SL-E1	SL-E2	SL11	SO4	SO9	CW5
1	2057	1774	54,3	2,24	3,66	6,64
2	1951	1719	55,7	2,37	3,85	7,41
3	1871	1658	55,9	2,44	2,91	7,17
4	1895	1599	55,3	2,13	3,67	7,26
5	1793	1795	55,0	2,22	3,80	7,74
6	1881	1690	59,9	2,15	3,01	7,49
7	1957	1669	60,6	1,97	2,96	7,54
8	1805	1722	58,7	2,09	3,17	6,61

APPENDIX 4 Method applied in ruggedness test

HORIZONTAL – 13.0

April 2006

Second draft

Soils, sludges and treated bio-waste – Organic constituents - LAS by HPLC with fluorescence detection (LC-FLD) and mass selective detection (LC-MSD)

ICS:

Descriptors:

Document type: International Standard
Document subtype:
Document stage: (50) Approval
Document language: E

STD Version 2.0

Contents

Page

Foreword

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

This document is a working document.

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

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

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

Introduction

This document is developed in the framework of the project 'Horizontal'. It is the result of a desk study “LAS and Nonylphenols” from January 2004 /1/ and an extensive experimental study of the method including a ruggedness test /2/. During the development of the horizontal method, the work has been discussed by an ad-hoc group formed to facilitate such discussions. The ad-hoc group have had five meetings during the period September 2003-September 2005, all meetings held in conjunction with other standardisation committee meetings in CEN/TC 308/WG 1 and ISO/TC 190.

The anionic surfactant LAS (Linear Alkylbenzene Sulfonates) is found in the environment due to the use of LAS in detergents. For more than 30 years LAS has been the largest single surfactant used in detergents, and the use continues on a high level.

Although LAS is readily biodegradable during wastewater treatment, considerable amounts may still be found in sludges of municipal origin. By the use of sludge for soil enrichment LAS may end up in the agricultural soil, where a rapid biodegradation takes place.

The method describes the determination of LAS in sludge, soil, treated biowaste and neighbouring fields. LAS is the sodium salt of alkylbenzene sulfonic acids, and it consists of a mixture of the homologues C_{10}-LAS, C_{11}-LAS, C_{12}-LAS, C_{13}-LAS and C_{14}-LAS. LAS is determined as the sum of the homologues.

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

Soils, sludges and treated bio-waste – Organic constituents - LAS by HPLC with fluorescence detection (LC-FLD) and mass selective detection (LC-MSD)

1 Scope

This european standard describes a method for the determination of Linear Alkylbenzene Sulphonate (LAS) in soil, sludge and compost using HPLC with a fluorescence detector or a mass selective detector.

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.

The standard describes the determination of the sum of LAS. For sludge a limit of detection of 20 mg/kg may be achieved, and for soil and compost a limit of detection of 0,2 mg/kg may be achieved (expressed as dry matter).

Temporary remark: The exact LOD will be determined by the method validation.

Matrices for which the standard has been validated are listed in the foreword.

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

NOTE The single LAS homologues C_{10} – C_{14} can be determined by the standard.

2 Normative references

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

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

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

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

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

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

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

PrEN 14346, *Characterisation of waste – Calculation of dry matter by determination of dry residue and water content*.

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

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

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

Horizontal standard 33-2, *Solid materials – Pre-treatment for organic characterisation.*

ISO/FDIS 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 analyte is linear alkylbenzene sulfonate, sodium salt (LAS). The analyte consists of a mixture of homologues (i.e. C_{10}-LAS, C_{11}-LAS, C_{12}-LAS, C_{13}-LAS and C_{14}-LAS) where each homologue consists of a mixture of 4-6 isomers depending on the length of the alkyl group. The dominant homologues in detergents and environmental samples are C11-LAS and C12-LAS.

NOTE C_{10}-C_{14} refers to the chain length of the linear alkyl group.

3.2 Calibration standard

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

3.3 Internal standard

The 4-octylbenzenesulfonate (C_8-LAS) is added to the test sample before extraction. The internal standard is used to correct for losses during the analysis and is used for calculating the concentration of the analytes.

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 the methods referred to in chapter 9, the test sample (freeze-dried) is extracted by shaking the sample with methanol. If necessary interfering compounds are removed from the extract by a clean-up on a suitable column.

The extract is analyzed by high performance liquid chromatography (HPLC) on a C_8- or C_{18}-column and detection by fluorescence (FLD) or mass spectrometry (MSD).

The identification is based on the retention times of the homologues and of the isomers of each homologue. Another identification point is the pattern/fingerprint of the homologues, and the isomer fingerprint of each

homologue, if a C_{18}-column is used for HPLC. By use of MS detection the relative intensities of two diagnostic ions may also be used for the identification (optional).

The quantification is based on internal standard procedure. The internal standard (C_8-LAS) is taken through the whole analytical procedure.

5 Interferences

5.1 Interferences from sampling

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

5.2 Interferences by LC-FLD and LC-MSD

The chromatographic analysis can be done on a C_8 or a C_{18} reverse phase column, and the choice of column will determine the separation obtained. On the C_8 column (with methanol in mobile phase) the LAS homologues are separated, however, there is no separation of the isomers. On the C_{18} column (with acetonitrile in mobile phase) the homologues are separated and there is a partial separation of the isomers of each homologue. This is illustrated by the chromatograms in Annex A.

The selectivity of the fluorescence as well as the mass selective detector is high, however, interference from co-eluting substances may occur. It is essential that the interfering peaks are not included in the calculations. A peak is excluded, if the retention time differs from the LAS standard mixture. Interfering peaks may best be detected when a C_{18} column is used, due to the partial separation of the isomers. The C_{18} column is recommended when fluorescence is used, due to the higher selectivity obtained. The interfering peaks can normally be detected by comparing the fingerprints of the sample with the fingerprints of the LAS standard mixture, although the isomer- and homologue-distribution in the environmental samples may differ from the distribution in the standard mixture.

The highest selectivity is obtained by the use of a C_{18} column and the MS detector. However, for most applications the separation on a C_8 column is sufficient, when MS is used. When all isomers are eluted in one peak, the integrations are less complicated, resulting in a higher precision and a lower limit of detection.

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 10.4. 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 Methanol, CH_3OH

HPLC-grade.

7.2 Acetonitrile, C_2H_3N

HPLC-grade.

7.3 Ammonium acetate, [CH_3COO^- NH_4^+]

7.4 Mobile phases for HPLC

For isomeric separation on C_{18} column:

Mobile phase A: 0.01 M ammonium acetate
Mobile phase B: acetonitrile

For homolog separation on C_8 column:

Mobile phase A: 0.01 M ammonium acetate
Mobile phase B: methanol

7.5 Reagents for clean-up procedures

7.6 Nitrogen for solvent evaporation

Nitrogen of sufficient purity.

7.7 Standards for calibration

C_{10}-C_{14} LAS mixture of homologues and isomers CAS # 69669-44-9, CAS # 25155-30-0

Standards must be of the highest possible purity.

7.8 Internal standard, C_8-LAS

Octylbenzene sulfonic acic, sodium salt $C_{14}H_{21}SO_3Na$, CAS # 6149-03-7

The internal standard must be kept in the freezer.

7.9 Internal standard solution

Prepare internal standard solution by dilution to about 100 mg/l in methanol.

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 less than 4 ± 3°C. The solution is stable for at least 2 years.

7.10 Stock solutions

Prepare individual stock solutions of 2000 - 5000 mg/l in methanol, 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.

7.11 Calibration standard solutions

Calibration standard solutions are prepared from the stock solution by diluting the stock solution with methanol. Internal standard solution is added to a concentration of 0,1-1 mg/l. The calibration standards are made to concentrations from 0,05 mg/l to 100 mg/l, the range depending on the sample matrix.

NOTE Store the calibration standard solutions in a dark place at a temperature of less than 4 ± 3 °C.

8 Apparatus

All equipment which gets into contact with the sample or extract shall be free from LAS. 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 20 ml and 100 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

Temporary remark: To be added.

8.5 Freeze drying apparatus

8.6 (High-performance) liquid chromatograph with fluorescence or mass selective detector

The HPLC system is equipped with a C8 or C18 reverse phase chromatographic column. The dimensions should be sufficient to separate the LAS as described below. Two examples of LC- columns are given in Annex A.

The fluorescence detector shall be able to measure at exitation wavelength 230 nm and emission wavelength 310 nm. If a fixed wavelength detector is used, the nearest possible wavelengths shall be used.

The mass selective detector shall be equipped with an API-ES interface (atmospheric pressure ionization electro-spray). The negative ion mode is used.

The separation of LAS homologues must fulfil the following requirements: The five homologues C_{10} – C_{14} shall all be separated to baseline.

Isomeric separation (recommended for fluorescence detection): C_{11}-LAS shall be separated into at least 4 chromatographic peaks, although these are not separated to baseline.

9 Sampling and sample pretreatment

9.1 Sampling and sample storage

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

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

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

9.2 Sample pre-treatment

Samples shall be pre-treated as soon as possible after sampling.

Methods for pre-treatment of solid samples to be used for the analysis of organic contaminants are described in a separate standard, Horizontal standard 33-2. This standard describes procedures for the preparation of the test sample from the laboratory sample.

All samples are dried, preferably by freeze-drying. Conventional drying at 60°C can be used, however, conventional drying may result in crusty hard samples that are difficult to homogenize.

10 Procedure

10.1 Extraction

Two extraction methods are described – one for extraction of sludge samples and one for extraction of samples of soil, sediment and compost.

All types of solids (sludge, soil, sediments and compost) are dried and extracted with methanol.

10.1.1 Extraction of dried sludge

Dried sludge samples are extracted as follows:

a) Take 2-3 g of test sample and place it in a screw cap flask (20-100 ml) with teflon seal.

b) Add 100 µl of internal standard solution (7.7) equal to 10 µg of internal standard.

c) Add 10 ml of methanol, close the screw cap and place the flask on the horizontal shaker. The flask shall be placed in horizontal position.

d) Shake for at least 30 min with 250 strokes per minute.

e) Transfer 500 µl of extract to a vial and add 500 µl of 0.01M ammonium acetate (mobile phase A) (7.4)

f) The extract is now ready for analysis by LC

10.1.2 Extraction of dried soil, sediment and compost

Dried soil, sediment and compost samples are extracted as follows:

a) Take 10-15 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 (7.7) equal to 10 µg of internal standard.

c) Add 50 ml of methanol, close the screw cap and place the flask on the horizontal shaker. The flask shall be placed in horizontal position.

d) Shake for at least 30 min with 250 strokes per minute.

e) Transfer 500 µl of extract to a vial and add 500 µl of 0.01M ammonium acetate (mobile phase A) (7.4)

f) The extract is now ready for analysis by LC.

The extracts can be stored in a refrigerator (4°C ± 3°C).

NOTE Other extraction techniques, like ultrasonic extraction, soxhlet, reflux, 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 the 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 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 chromatography, or if those compounds can influence the HPLC-procedure (i.e. contamination of the detection system). If no or negligible interfering substances are present, no clean-up is necessary.

The selectivity of the mass selective detector (MSD) is higher than of the fluorescence detector (FLD), and the clean-up is therefore mostly used for the FLD. For the MSD a clean-up is generally not necessary.

For sludge samples a clean-up is generally not necessary, disregarding the choice of detector.

Add a proportion of the extract to the clean-up column (8.4) and elute the column with a suitable solvent.

Before use the column shall be tested with a calibration standard to ensure that the LAS homologues are recovered in the collected fraction. The criteria for the clean-up is, that the recovery for LAS is higher than 80%.

10.4 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.

10.5 HPLC-analysis

Optimise the HPLC-system including the detector according to the instrument manufacturer's manual. The separation of LAS-homologues/isomers must fulfil the requirements described in 8.6.

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

10.5.1 Fluorescence detection

Optimise the fluorescence detector according to the instrument manufacturer's manual.

If a scanning fluorimeter is used, an initial scan of both excitation and emission wavelength are advisable if detector is not calibrated.

The following wavelengths are used:

Exitation: 230 nm

Emission: 310 nm

10.5.2 Mass selective detection

An API-ES interface set in the negative ion mode is used.

The following ions are used for the analysis:

Table 1 — Diagnostic ions used by the LC-MS analysis

Compound	**Abbreviation**	**Target ion M_1**
Analytes:		
C_{10}-Linear alkylsulfonate	C_{10}-LAS	297
C_{11}-Linear alkylsulfonate	C_{11}-LAS	311
C_{12}-Linear alkylsulfonate	C_{12}-LAS	325
C_{13}-Linear alkylsulfonate	C_{13}-LAS	339
C_{14}-Linear alkylsulfonate	C_{14}-LAS	353
Internal standard:		
C_8-Linear alkylsulfonate	C_8-LAS	269

NOTE 1 As an option the fragment ion 183 may be used as qualifier ion for the identification (for all homologues). However, for routine use the fragment ion 183 has a low abundance relative to the target ion, and a much higher fragmentor voltage is therefore required.

NOTE 2 C_8-C_{14} indicate the length of the alkyl chain.

10.6 Calibration and analysis of samples

Two types of calibration are used: the initial calibration (10.6.1) and the recalibration, which is carried out daily (10.6.2).

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 LAS the area is determined as the sum of the peak areas of both mixtures of homologues and mixtures of isomers. This is the case for measurements with both detectors.

10.6.1 Initial calibration

Inject at least 5 standard solutions with concentrations between 0,05 mg/l and 100 mg/l (7.11), the actual working range depends on the matrix and the values of interest. Include a solvent blank. Identify the peaks and add the integrated areas of the LAS peaks to give the sum area for LAS. Prepare a calibration curve.

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.

10.6.2 Recalibration

Inject at least two calibration standards with concentrations of 20 ± 10 % and 80 ± 10 % of the established linear range and calculate the straight line from these measurements.

10.6.3 Analysis of samples and identification

Inject the extracts of samples and blanks obtained from the extraction in 10.1, from 10.2 (concentration) or from 10.3 (clean-up).

The identification of LAS is using the following identification points:

- The peak pattern of the homologues, i.e. the fingerprint, although the relation between the individual peaks may differ in samples and standards
- The peak pattern of the isomers of each homologue (only if a C_{18} column is used)
- The retention times of the individual peaks
- The relation between peak areas of the qualifier ions and the target ion (only by MS-detection, optional)

From the identification select the peaks to be included in the sum area. Peaks not found in the calibration standard are 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 0.01M ammonium acetate (mobile phase A) and injected again.

11 Calculation and expression of results

For linear alkylbenzene sulphonate (LAS) the areas are determined as the sum of the peak areas of the homologue and isomeric mixtures. If interfering peaks are present, these shall not be included in the sum area.

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

For all samples a specific mass of internal standard is added, 10 µg for extraction method 10.1.1 as well as for extraction method 10.1.2

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

b is the intercept of the calibration curve with the ordinate

11.2 Calculation

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

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

where:

ω_s is the concentration of analyte found in the sample in mg/kg dry matter

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

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

b is the intercept of the calibration curve with the ordinate

s is the slope of the calibration function

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

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

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

V is the volume of petroleum ether used for extraction of the test sample, in ml

12 Test report

The test report shall contain at least the following data:

a) the information required to identify the sample;

b) a reference to this international standard;

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

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

Annex A (informative)

Example of chromatographic conditions and chromatogram

Isomeric separation of LAS

HPLC-conditions:

Separation column: Type: C18-column. Particle size: 5μm. Dimensions: 150 x 2,0 mm.

Mobile phase : A) 0,01M ammonium acetate in water, B) Acetonitrile

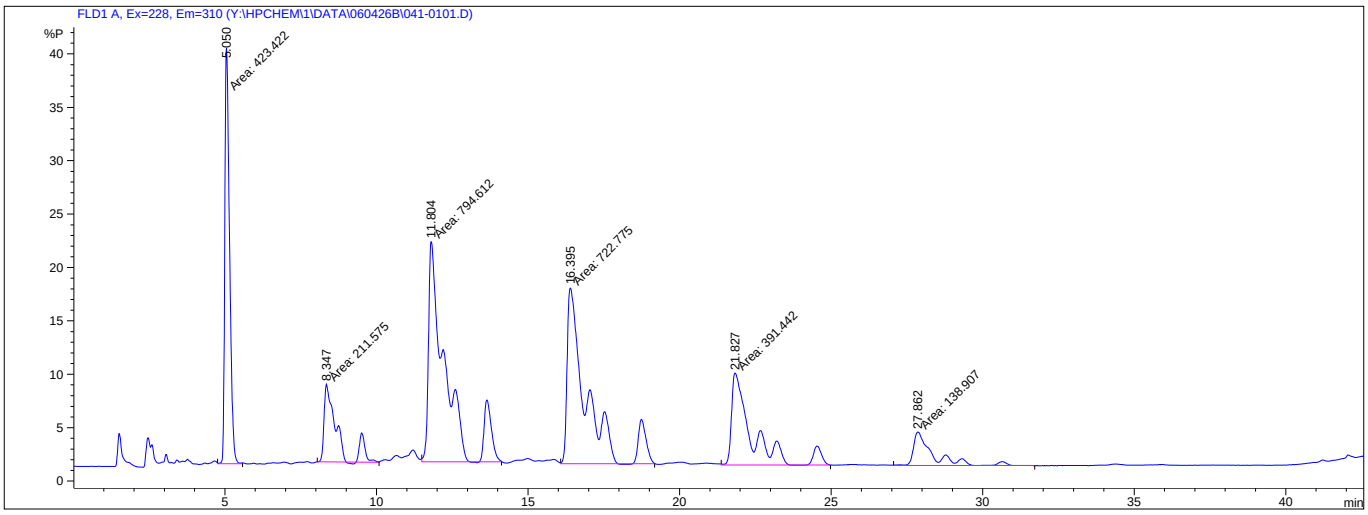
Solvent gradient:

t (min)	% B
0	45
20	55
35	65
36	95
40	95
41	45
46	45

Inj volume: 25 μl

Flow: 0,2 ml/min

Example of chromatogram obtained with fluorescence detection:



Homologue separation of LAS

HPLC-conditions:

Separation column: Type: C8-column. Particle size: 5μm. Dimensions: 125 x 2,0 mm.

Mobile phase : A) 0,01M ammonium acetate in water, B) MeOH

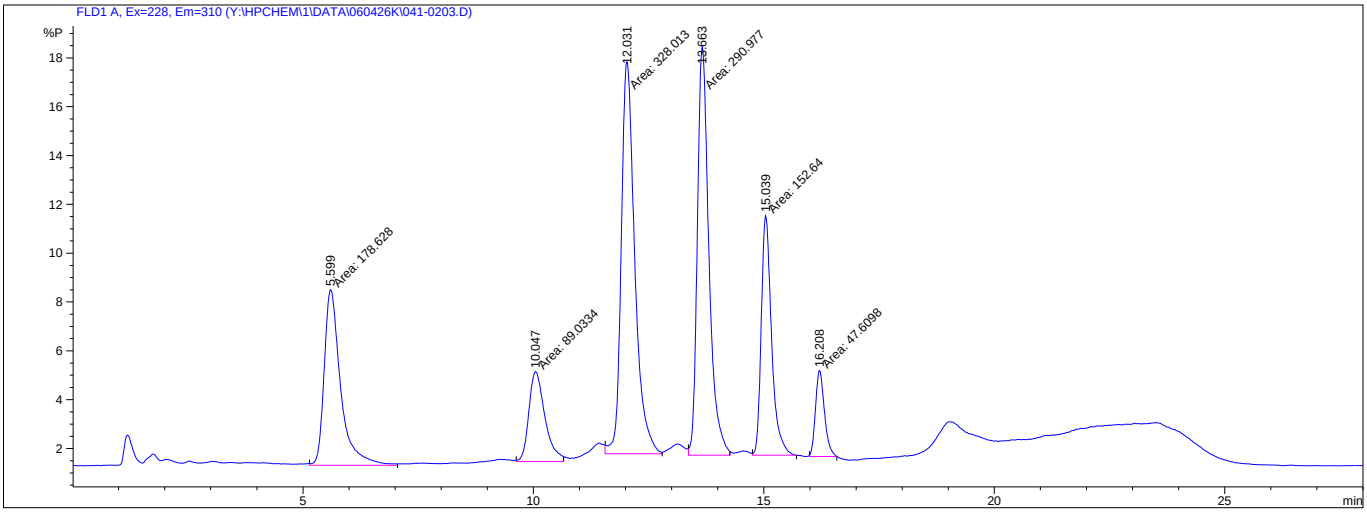
Solvent gradient:

t (min)	% B
0	58
12	80
16	95
18	95
19	58
25	58

Inj volume: 25 μl

Flow: 0,2 ml/min

Example of chromatogram obtained with fluorescence detection:



Annex B (informative)

Validation

Annex C (informative)

Information on project Horizontal and WPxx

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