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Soils, sludges and treated bio-waste — Determination of LAS — Method by HPLC with fluorescence detection (LC-FLD) and mass selective detection (LC-MSD)

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Foreword

This document (BT/TF 151 WI CSS99041) has been prepared by Technical Committee CEN/TC BT “Horizontal”, the secretariat of which is held by DS.

This document is a working document.

The following TC's have been involved in the preparation of the standard: CEN/TC 292 (Waste), CEN/TC 308 (Sludge Characterization) and ISO/TC 190 (Soil Quality). This European Standard is applicable and validated for several types of matrices. The table below indicates which ones.

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

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Introduction

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.

This standard is developed in the European project 'HORIZONTAL'. It is the result of a desk study "Horizontal European standard for determination of LAS in sludge, soil and biowaste" which aimed at evaluating the latest developments in assessing LAS in sludge, soil, treated biowaste. After an evaluation study, in which e.g. the ruggedness of the method was studies, a European wide validation of the draft standard has taken place. The results of the desk studies as well as the evaluation and validation studies have been subject to discussions with all parties concerned in CEN. The standard is part of a modular horizontal approach in which the standard belongs to the analytical step.

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

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

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

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

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

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

1 Scope

This European Standard (CSS99041) describes a method for the determination of Linear Alkylbenzene Sulphonate (LAS) in soil, sludge and treated bio-waste using HPLC with a fluorescence detector or a mass selective detector.

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

This European Standard describes the determination of the sum of LAS. Under the conditions specified in this European Standard, typically a limit of detection of 20 mg/kg (expressed as dry matter) for sludge and of 0,2 – 0,5mg/kg for soil and treated bio-waste may be achieved.

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

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

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

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

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

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

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

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

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

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

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

1) Under preparation

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

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

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

4 Principle

After pre-treatment according to the methods referred to in Clause 9, the test sample (freeze-dried) is extracted by shaking 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 it has been proven that they do not 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 determines 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 can best be detected when a C_{18} column is used for the LC analysis, due to the partial separation of the isomers. The C_{18} column is mandatory 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.

Depending on the type of matrices from which LAS is extracted different analytical pathways can be applied. An overview of the analytical procedure for the matrix of interest is shown in Table 1.

Table 1 - Choice of analytical procedure

	FLD		MSD	
	C8-column	C18-column	C8-column	C18-column
Sludge	No	Yes	Yes	Yes
Soil	No	(Yes)[a)]	Yes	Yes
Treated Bio-waste	No	(Yes)[a)]	Yes	Yes
[a)] For FLD the LOD will generally be inadequate for this type of matrix.				

6 Reagents

6.1 General

All reagents shall be of recognised analytical grade.

The purity of the reagents used shall be checked by running a blank determination as described in 9.4. 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.

6.2 Methanol, CH_3OH

HPLC-grade.

6.3 Acetonitrile, C_2H_3N

HPLC-grade.

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

6.5 Mobile phases for HPLC

For isomeric separation on C_{18} column:

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

For homologue separation on C_8 column:

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

6.6 Reagents for clean-up procedures

6.6.1 Clean-up procedure based on Strong anion exchange (SAX)

6.6.1.1 SAX column

6.6.1.2 Acetic acid (CH_3COOH)

6.6.1.3 Hydrochloric acid (HCl)

6.6.1.4 Methanol

6.6.2 Clean-up procedure based on Graphitised carbon black (GCB)

6.6.2.1 GCB column

6.6.2.2 Hydrochloric acid (HCl)

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

6.6.2.4 Formic acid (HCOOH)

6.6.2.5 Dichloromethane

6.6.2.6 Methanol

6.7 Nitrogen for solvent evaporation

Nitrogen of sufficient purity.

6.8 Standards for calibration

6.8.1 C_{11} LAS, Sodium Linear Undecylbenzene Sulfonate $C_{17}H_{27}SO_3Na$, 99%

6.8.2 C_{12} LAS, Sodium Linear Dodecylbenzene Sulfonate $C_{18}H_{29}SO_3Na$, 99% (CAS# 2211-98-5)

6.8.3 C_{13} LAS, Sodium Linear Tridecylbenzene Sulfonate $C_{19}H_{31}SO_3Na$, 99%

6.8.4 C_{10}-C_{14} LAS mixture of homologues and isomers, highest possible purity, (CAS # 69669-44-9, CAS # 25155-30-0)

6.9 Internal standard, C_8-LAS

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

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

6.10 Internal standard solution

Prepare internal standard solution by dilution to about 1 000 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.

Store the internal standard solution in a dark place at a temperature of 4°C ± 3°C. The solution is stable for at least two years.

6.11 Stock solutions

Prepare individual stock solutions of 1 000 ml/l – 5 000 mg/l in methanol (6.2), either from solid standard substances or from solutions with a certified concentration. Prepare stock solutions of C_{11} LAS (6.8.1), C_{12} LAS (6.8.2) and C_{13} LAS (6.8.3).

Prepare a calibration mixture by mixing stock solutions of C_{11} LAS, C_{12} LAS and C_{13} LAS obtaining equal concentration of each homologue.

Prepare a stock solution of C_{10} – C_{14} LAS mixture (6.8.4) of 1 000 mg/l – 5 000 mg/l in methanol. This solution is only for identification.

Store the stock solutions and the calibration mixture in a dark place at a temperature of 4°C ± 3°C. The solutions are stable for at least two years.

6.12 Calibration standard solutions

Calibration standard solutions are prepared from the calibration mixture by diluting with a 1:1 mixture of methanol (6.2) and 0,01 M ammonium acetate (6.5). The calibration range is different for sludge and for soil/sediment/treated bio-waste.

6.12.1 Sludge

For sludge samples the calibration standards are made to concentrations from 5 mg/l to 500 mg/l. The internal standard solution (6.10) is added to a concentration of 10 mg/l.

6.12.2 Soil, sediment and treated bio-waste

For samples of soil, sediment or treated bio-waste the calibration standards are made to concentrations from 0,05 mg/l to 5 mg/l. The internal standard solution (6.10) is added to a concentration of 1 mg/l.

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

NOTE A diluted C_{10} – C_{14} mixture is prepared for the identification of the C_{10} and C_{14} homologues, which are not present in the calibration mixture.

7 Apparatus

7.1 General

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

7.2 Standard laboratory glassware

7.2.1 Screw cap glass flask with PTFE seal. Volume 20 ml and 100 ml.

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

7.2.3 Test tubes and vials.

7.3 Shaking device

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

7.4 Evaporator

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

7.5 Freeze drying apparatus

7.6 (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 excitation wavelength of 230 nm and emission wavelength of 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 shall fulfil the following requirements: The five homologues C_{10} – C_{14} shall all be separated to baseline.

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

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

8 Sampling and sample pretreatment

8.1 Sampling and sample storage

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

Store the samples in a dark place at a temperature below 10 °C, if possible in a refrigerator. Determine the content of dry matter in the sample in according to CSS99022, EN 12880, ISO 11465 or PrEN 14346.

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

8.2 Sample pre-treatment

Methods for pre-treatment of solid samples to be used for the analysis of organic contaminants are described in a separate standard (CSS99034). This 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.

9 Procedure

9.1 Extraction

The following two extraction methods are described :

— extraction of sludge samples

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

All types of solids (sludge, soil, sediments and treated bio-waste) are dried and extracted with methanol (6.2).

9.1.1 Extraction of dried sludge

Dried sludge samples are extracted as follows:

a) Weigh accurately 2 g -3 g (with two decimals) of test sample and place it in a screw cap flask (7.2.1) (20 ml - 100 ml) with PTFE seal.

b) Add 100 µl of internal standard solution (6.10) equal to 100 µg of internal standard (6.9).

c) Add 10 ml of methanol (6.2), close the screw cap and place the flask on the horizontal shaker (7.3). The flask shall be placed in horizontal position.

d) Shake for at least 30 min with (250 ± 20) strokes per min.

e) Wait for sample to settle, then transfer 500 µl of extract to a vial (7.2.3) and add 500 µl of 0.01M ammonium acetate (mobile phase A) (6.5).

The extract is now ready for analysis by LC.

By high LAS concentrations less sample can be taken into analysis.

9.1.2 Extraction of dried soil, sediment and treated bio-waste

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

a) Weigh accurately 10 g - 15 g (with two decimals) of test sample and place it in a 100 ml screw cap flask (7.2.1) with PTFE seal.

b) Add 50 µl of internal standard solution (6.10) equal to 50 µg of internal standard (6.9).

c) Add 50 ml of methanol (6.2), close the screw cap and place the flask on the horizontal shaker (7.3). The flask shall be placed in horizontal position.

d) Shake for at least 60 min with (250 $\pm$ 20) strokes per min.

e) Wait for sample to settle, then transfer 500 µl of extract to a vial (7.2.3) and add 500 µl of 0,01 M ammonium acetate (mobile phase A) (6.4 and 6.5)

The extract is now ready for analysis by LC.

The extracts can be stored in a refrigerator (4 $\pm$ 3) °C.

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 European Standard shall be proven.

9.2 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 (7.4) or by the use of a gentle stream of nitrogen at room temperature. Since the internal standard (6.9) 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.

9.3 Clean-up (optional)

Clean-up shall be used if compounds are present that can interfere with the analytes or the internal standard (6.9) 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 (6.6.1.1 or 6.6.2.1) 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 %.

Two columns are described for clean-up. Descriptions of the clean-up procedures are given in Annex B.

9.4 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.

9.5 HPLC-analysis

Two types of columns (C_8 and C_{18}) and many HPLC-conditions may be used. The choice of column and detector depends on the matrix to be analysed (See table 1 in 5.1). By the use of FLD only C_{18} columns are allowed, by MSD both columns are allowed. Examples are described in Annex A.

Optimise the HPLC-system including the detector according to the instrument manufacturer's manual. The separation of LAS-homologues/isomers shall fulfil the requirements described in 7.6.

9.5.1 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

9.5.2 Mass selective detection

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

The ions used by the LC-MS analysis are described in Table 2.

Table 2 — 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.

9.6 Calibration and analysis of samples

9.6.1 General

Two types of calibration are used : the initial calibration (9.6.2) and the recalibration, which is carried out daily (9.6.3).

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. See 10.1. For LAS the area is determined as the sum of the peak areas of mixtures of homologues (from C_{10} to C_{14}) and mixtures of isomers. This is the case for measurements with both detectors.

9.6.2 Initial calibration

Inject at least five standard solutions with concentrations within the working range described in 6.12. For sludge the working range is from 5 mg/l to 500 mg/l. For soil, sediment and treated bio-waste the working range is from 0,05 mg/l to 5 mg/l. The actual working range may however differ depending 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 International Standard provides acceptance and rejection criteria for linearity.

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

9.6.3 Recalibration

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

9.6.4 Analysis of samples and identification

Inject the extracts of samples and blanks obtained from the extraction in 9.1, from 9.2 (concentration) or from 9.3 (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 Clause 5.

Use ISO/FDIS 22982 for identification of LAS.

The C_{10} – C_{14} LAS mixture is only used to determine the retention times of C_{10} LAS and C_{14} LAS, which are not present in the calibration standard.

If the concentration of LAS is out of the calibration range (higher than the upper calibration limit), the analysis shall be repeated with a smaller amount of sample.

10 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, 100 µg for extraction method 9.1.1 and 50 µg for extraction method 9.1.2. The recovery of the internal standard shall be 60 % - 120 % to ensure an acceptable quality of the performed analysis. This may be checked against the internal standard in the calibration standard solutions.

10.1 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 micrograms per millilitres (µg/ml)

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

b is the intercept of the calibration curve with the ordinate

10.2 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 milligrams per kilogram (mg/kg) dry matter

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

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

b is the intercept of the calibration curve with the ordinate

s is the slope of the calibration function

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

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

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

V is the volume of methanol used for extraction of the test sample, in millilitres (ml).

11 Precision data

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

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

The reproducibility limit provides a determination of the differences (positive and negative) that can be found (with a 95 % statistical confidence) between a single test result obtained by a laboratory using its own facilities and another test result obtained by another laboratory using its own facilities, both test results being obtained under the following conditions : The tests are performed in accordance with all the requirements of the present standard and the two laboratory samples are obtained from the same primary field sample and prepared under identical procedures. Conversely, the repeatability limit refers to measurements obtained from the same laboratory, all other conditions being identical. The reproducibility limit and the repeatability limit do not cover sampling but cover all activities carried out on the laboratory sample including its preparation from the primary field sample.		
Results of the validation of the Determination of LAS by HPLC with fluorescence detection (LC_FLD) and mass selective detection (LCMSD) in soil, sludge and treated biowaste	Typical value %	Observed range %
Repeatability limit, r	9	*9 - 30*
Reproducibility limit, R	125 #	125 -200 #

Further evaluation may lead to changes in these numbers

NOTE 1. The above results refer to the difference that may be found between two test results performed on two laboratory samples obtained under the same conditions. In the case when reference is made to the dispersion of the

values that could reasonably be attributed to the parameter being measured, the above typical reproducibility values and observed reproducibility ranges should be divided by √2 to obtain the corresponding typical dispersion limit and its observed range. In the example of LAS in Sludge 1 the result and its dispersion limit is 1769 ± 1644 mg/kg (2 * sR = 93 % of 1769). This means that with a 95 % statistical confidence, the values reasonably attributable to the measured parameter are larger than 125 mg/kg and lower than 3414 mg/kg.

NOTE 2. The repeatability limit (r) and the reproducibility limit (R) as given in Table C.2 (Annex C) and in this table are indicative values of the attainable precision if the Determination of LAS by HPLC with fluorescence detection (LC_FLD) and mass selective detection (LCMSD) is performed in accordance with this standard [CSS99041].

NOTE 3 A limited number of materials and parameters were tested. Consequently, for other materials and parameters, performance characteristics may fall outside the limits as derived from the validation of the the Determination of LAS by HPLC with fluorescence detection (LC_FLD) and mass selective detection (LCMSD) in soil, sludge and treated biowaste.

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

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 European Standard ;

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

d) any details not specified in this European 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 (other column dimensions such as 150 x 4,6 mm have also proven adequate).

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

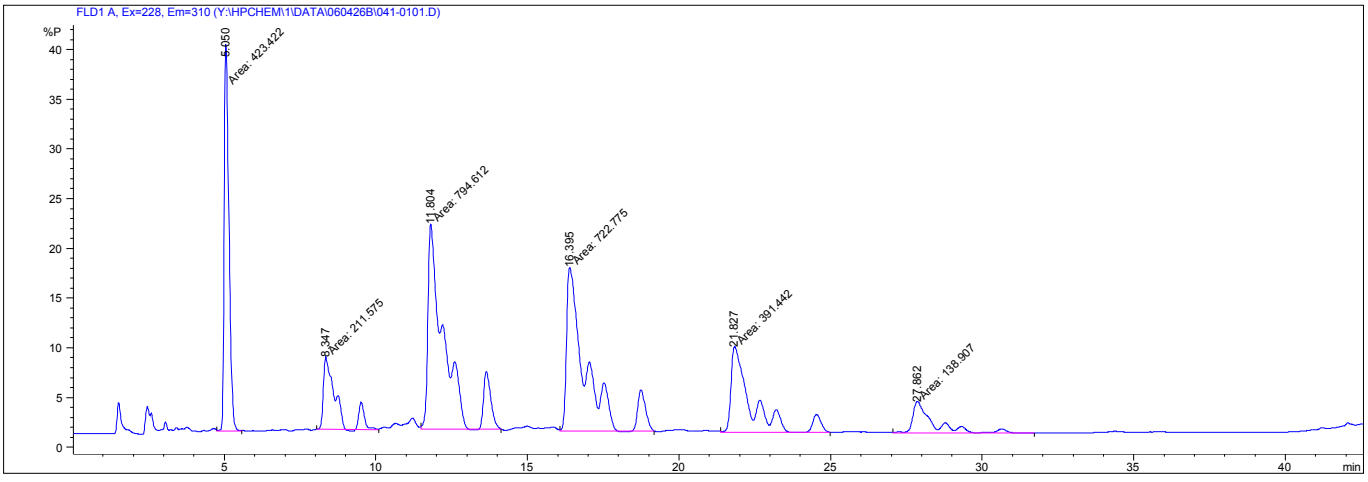
Solvent gradient:

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 (other column dimensions such as 125 x 4,6 mm have also proven adequate).

.

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

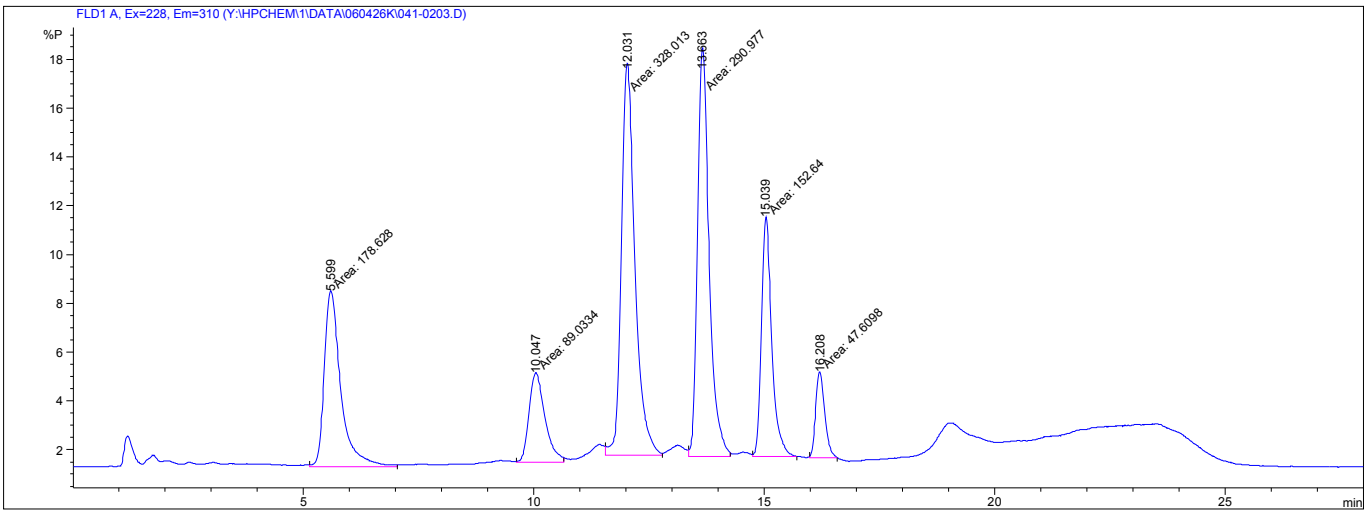
Solvent gradient:

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:


FLD1 A, Ex=228, Em=310 (Y:\HPCHEM\1\DATA\060426K\041-0203.D)
%P
18
16
14
12
10
8
6
4
2
5.599
Area: 178.628
10.047
Area: 89.0334
12.031
Area: 328.013
13.663
Area: 290.977
15.039
Area: 152.64
16.208
Area: 47.6098
5
10
15
20
25
min

Annex B (informative)

Example of Clean-up procedure

Clean-up procedure based on graphitised carbon black (GCB) columns:

Type of column:	500 mg pre-packed GCB solid phase extraction (SPE) column.
1. Column preparation:	5 ml mobile phase
	2 ml methanol
	30 ml 0,1 M hydrochloride acid
2. Sample application:	1 ml MeOH extract
3. Wash:	5 ml water
	5 ml methanol
	10 ml dichloromethane/methanol (80:20) with 15 mM formic acid
	10 ml dichloromethane
4. Elution:	10 ml dichloromethane/methanol (80:20) with 5 mM tetramethyl-ammoniumhydroxide
5. Solvent transfer:	Evaporate until dryness and re-dissolve in methanol/mobile phase (1:1).

Clean-up procedure based on strong anion exchange (SAX) columns:

Type of column:	500 mg pre-packed SAX solid phase extraction (SPE) column.
1. Column preparation:	5 ml methanol
	10 ml water
2. Sample application:	1 ml MeOH extract
3. Wash:	5 ml 2% acetic acid in methanol
	5 ml methanol
4. Elution:	15 ml 2 M hydrochloride acid:methanol (1:1)
5. Solvent transfer:	Neutralize with ammonium acetate, evaporate until dryness and re-dissolve in methanol/mobile phase (1:1).

Annex C (informative)

Repeatability and reproducibility data

Repeatability and reproducibility data

C.1 Performance characteristics

C.1.1 Objective of the interlaboratory comparison

In a European wide interlaboratory comparison study according to ISO 5725-2, the performance characteristics of the standard “Determination of LAS by HPLC with fluorescence detection (LC_FLD) and mass selective detection (LCMSD) in soil, sludge and treated biowaste” were established.

C.1.2 Materials used in the interlaboratory comparison study

The interlaboratory comparison of determination of LAS by HPLC with fluorescence detection (LC_FLD) and mass selective detection (LCMSD) in soil, sludge and treated biowaste was carried out with 6 European laboratories on 3 materials. The materials selected for the interlaboratory comparison were chosen to represent soil, sludge and biowaste as broad as possible, because the standard will find general application across different types of soil and soil related materials. (detailed information can be found in the final report on the Interlaboratory comparison study mentioned in the Bibliography).

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

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

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

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

The materials examined cover all the grain size classes to which the the determination of LAS by HPLC with fluorescence detection (LC_FLD) and mass selective detection (LCMSD) in soil, sludge and treated biowaste applies: very fine grained materials (like sludge: 0 µm to about 125 µm) and fine-grained materials (soil and compost: 0 mm to 4 mm).

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

Table C.C.1 — Material types tested and components analysed in the interlaboratory comparison of the method for the determination of LAS by HPLC with fluorescence detection (LC_FLD) and mass selective detection (LCMSD) in soil, sludge and treated biowaste.

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

C.1.3 Interlaboratory comparison results

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

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

its own facilities and testing laboratory samples obtained from the same primary field sample and prepared under identical procedures.

The repeatability limit was calculated using the relationship : $r_{test} = f \cdot \sqrt{2} \cdot s_{r,test}$ with the critical range factor f = 2.

For instance, the repeatability limit around a measurement result of 1000 mg LAS /kg is ± 86 mg LAS /kg (i.e ± 9 % of 1000)

NOTE The above relationship refers to the difference that may be found between two measurement results performed each on two laboratory samples obtained under the same conditions. The value f = 2 used in the factor $f \cdot \sqrt{2}$ corresponds to the theoretical factor of 1,96 for a pure normal distribution at 95 % statistical confidence. Also, this value f = 2 corresponds to the usual value k = 2 of the coverage factor recommended in the Guide to the expression of Uncertainty in Measurement (GUM). However it may be necessary to use a larger value for f in situation as described in clause 11.

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

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

For instance, the reproducibility limit around a measurement result 1000 mg LAS/kg is ± 1550 mg LAS /kg (i.e ± 155 % of 1000).

NOTE The above relationship refers to the difference that may be found between two measurement results performed each on two laboratory samples obtained under the same conditions. The value f = 2 used in the factor f ·

√2 corresponds to the theoretical factor of 1,96 for a pure normal distribution at 95 % statistical confidence. Also, this value f = 2 corresponds to the usual value k = 2 of the coverage factor recommended in the Guide to the expression of Uncertainty in Measurement (GUM). In the case when reference is made to the dispersion of the values that could reasonably be attributed to the parameter being measured, the dispersion limit is equal to $k \cdot s_R$ with the usual value k = 2, resulting in a dispersion limit lower than the reproducibility limit (i.e. a ratio of √2). However it may be necessary to use a larger value $f \cdot \sqrt{2}$ (or k) in situation as described in clause 11 .

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

Table C.C.2 — Results of the interlaboratory comparison studies of the Determination of LAS by HPLC with fluorescence detection (LC_FLD) and mass selective detection (LCMSD) in soil, sludge and treated biowaste. All concentrations in mg/kg.

Matrix	Parameter	Mean	sr	sR	r	R	p	Outliers	Total number of data	No of LOD	Avg	Std	Var %
Sludge 1	LAS Total	1769	3.07%	46.5%	151.9	2302	5	1	20	0	2432	1780	73
Compost 1	LAS Total	2.8	12.4%	55.4%	0.98	4.38	4	0	16	4	2.82	1.53	54
Soil 3	LAS Total	24.5	1.85%	78.6%	1.26	53.80	4	2	13	0	29.0	24.2	83

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

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

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

Bibliography

1. Gro Fremmersvik and Nis Hansen. Desk Study – LAS and Nonylphenols. January 2004. http://www.ecn.nl/horizontal/.

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