PD ISO/TS 13896:2012

BSI Standards Publication

Soil quality — Determination of linear alkylbenzene sulfonate (LAS) — Method by HPLC with fluorescence detection (LC-FLD) and mass selective detection (LC-MSD)

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National foreword

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A list of organizations represented on this committee can be obtained on request to its secretary.

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Soil quality — Determination of linear alkylbenzene sulfonate (LAS) — Method by HPLC with fluorescence detection (LC-FLD) and mass selective detection (LC-MSD)

Qualité du sol — Détermination des sulfonates d'alkyl benzène linéaires (SAL) — Méthode par chromatographie liquide à haute performance (CLHP) avec détection par fluorescence (CL-DFL) et détection sélective de la masse (CL-DSM)

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Foreword

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The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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

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Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO/TS 13896 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 3, *Chemical methods and soil characteristics*.

Introduction

The anionic surfactant LAS (Linear Alkylbenzene Sulfonate) is found in the environment due to its use 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 sludge of municipal origin. By the use of sludge for soil improvement, LAS can end up in 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 Technical Specification is applicable and validated for several types of matrices as indicated in Table 1 (see also Annex A for the results of the validation).

Table 1 — Matrices for which this Technical Specification is applicable and validated

Matrix	**Materials used for validation**
Sludge	Municipal sewage sludge
Biowaste	Fresh compost
Soil	Sludge amended soil

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WARNING — Persons using this Technical Specification should be familiar with usual laboratory practice. This Technical Specification does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted according to this Technical Specification be carried out by suitably trained staff.

1 Scope

This Technical Specification specifies a method for the determination of linear alkylbenzene sulfonate (LAS) in sludge, treated biowaste and soil using high-performance liquid chromatography (HPLC) with a fluorescence detector (FLD) or a mass selective detector (MSD).

This Technical Specification specifies the determination of the sum of LAS. Under the conditions specified in this Technical Specification, typically a limit of detection of 20 mg/kg (expressed as dry matter) for sludge and of 0,2 mg/kg to 0,5 mg/kg for soil and treated biowaste may be achieved.

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

NOTE The single LAS homologues C_{10} to C_{14} can be determined by this Technical Specification.

2 Normative references

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

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

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

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

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

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
analyte
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 four to six isomers depending on the length of the alkyl group

NOTE The dominant homologues in detergents and environmental samples are C_{11}-LAS and C_{12}-LAS. C_{10} to C_{14} refers to the chain length of the linear alkyl group.

4 Principle

After pretreatment, the test sample 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 analysed by high-performance liquid chromatography (HPLC) on a C_8- or C_{18}-column and detection by fluorescence (FLD) or mass spectrometry (MS).

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 the use of MS detection the relative intensities of two diagnostic ions may also be used for the identification (optional).

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

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 2.

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 HPLC-FLD and HPLC-MS

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 B.

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 not be 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 usually 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.

Table 2 — Choice of analytical procedure

Matrix	FLD		MS	
	C_8-column	C_{18}-column	C_8-column	C_{18}-column
Sludge	No	Yes	Yes	Yes
Soil	No	(Yes) [a]	Yes	Yes
Treated biowaste	No	(Yes) [a]	Yes	Yes

[a] For FLD, the limit of detection will generally be inadequate for this type of matrix.

6 Reagents

6.1 General

Use only reagents of recognized analytical grade, unless otherwise specified.

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

6.2 Methanol, CH_3OH; HPLC grade.

6.3 Acetonitrile, C_2H_3N; HPLC grade.

6.4 Ammonium acetate, $c(CH_3COO^- NH_4^+)$ = 0,01 mol/l.

6.5 Mobile phases for HPLC

6.5.1 For isomeric separation on C_{18}-column

— Mobile phase A: 0,01 mol/l ammonium acetate (6.4);

— Mobile phase B: Acetonitrile (6.3).

6.5.2 For homologue separation on C_8-column

— Mobile phase A: 0,01 mol/l ammonium acetate (6.4);

— Mobile phase B: Methanol (6.2).

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, CH_3OH

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

6.6.2.1 GCB column

6.6.2.2 Hydrochloric acid, HCl

6.6.2.3 Tetramethylammonium hydroxide, $C_4H_{13}NO$ (CAS-RN 10424-65-4[1)]) pentahydrate.

6.6.2.4 Formic acid, HCOOH

6.6.2.5 Dichloromethane, CH_2Cl_2

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1) CAS-RN Chemical Abstracts Service Registry Number.

6.6.2.6 Methanol, CH_3OH

6.7 Nitrogen, N_2, for solvent evaporation of sufficient purity.

6.8 Standards for calibration

6.8.1 General

The standards shall be stored in a freezer at a temperature of (–18 ± 3) °C.

6.8.2 C_{11}-LAS, sodium linear undecylbenzene sulfonate, $C_{17}H_{27}SO_3Na$; 99 %.

6.8.3 C_{12}-LAS, sodium linear dodecylbenzene sulfonate, $C_{18}H_{29}SO_3Na$; 99 % (CAS-RN 2211-98-5).

6.8.4 C_{13}-LAS, sodium linear tridecylbenzene sulfonate, $C_{19}H_{31}SO_3Na$; 99 %.

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

6.9 Internal standard, C_8-LAS

Octylbenzene sulfonic acid, sodium salt $C_{14}H_{21}SO_3Na$ (CAS-RN 6149-03-7).

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

6.10 Internal standard solution

Prepare the internal standard solution of the internal standard (6.9) by dilution to about 1 000 mg/l in methanol (6.2).

It is essential that the same internal standard solution be used for calibration standard solutions and for samples, blank tests and internal quality control samples.

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

6.11 Stock solutions

Prepare individual stock solutions of 1 000 mg/l to 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 (6.8.2), C_{12}-LAS (6.8.3) and C_{13}-LAS (6.8.4).

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

Prepare a stock solution of C_{10} to C_{14}-LAS mixture (6.8.5) of 1 000 mg/l to 5 000 mg/l in methanol (6.2). This solution is only for identification.

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

6.12 Calibration standard solutions

6.12.1 General

Calibration standard solutions are prepared from the calibration mixture (6.11) by diluting with a 1:1 mixture of methanol (6.2) and ammonium acetate (6.4).

The calibration range is different for sludge (see 6.12.2) and for soil or treated biowaste (see 6.12.3).

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

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

6.12.2 Sludge samples

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

6.12.3 Soil and treated biowaste samples

For samples of soil or treated biowaste, the calibration standards are prepared for 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.

7 Apparatus

7.1 General

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

7.2 Usual laboratory glassware

7.2.1 Screw-cap glass flask with polytetrafluoroethylene (PTFE) seal; volume 20 ml and 100 ml.

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

7.2.3 Test tubes and vials

7.3 Shaking device

Reciprocating shaker with horizontal movement [suitable for (250 ± 20) strokes per minute].

7.4 Evaporator

Rotary evaporator, turbo evaporator or Kuderna Danish[2)].

7.5 Freeze-drying apparatus

7.6 High-performance liquid chromatograph with fluorescence or mass selective detector

The HPLC system is equipped with a C_8- or C_{18}-reverse phase chromatographic column. The dimensions should be sufficient to separate the LAS as described below.

NOTE Two examples for HPLC-columns are given in Annex B.

The fluorescence detector shall be suitable 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 atmospheric pressure ionization electro-spray (API-ES) interface. Use the negative ion mode.

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

The separation of LAS homologues shall fulfil the following requirements: The five homologues C_{10} to 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.

8 Sample storage and sample pretreatment

8.1 Sample storage

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 according to ISO 11465.

NOTE 1 Freeze-dried samples, if kept sealed, may be stored for a longer period at room temperature (approximately one month).

NOTE 2 Hygroscopic dried sludge may be preserved by mixing with anhydrous sodium sulfate.

8.2 Sample pretreatment

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

All samples shall be dried, preferably by freeze drying. Conventional drying at 60 °C can be used, but this can result in crusty hard samples that are difficult to homogenize.

9 Procedure

9.1 Extraction

9.1.1 Extraction of dried sludge

— Weigh accurately 2 g to 3 g (with two decimals) of the test sample and place it in a screw-cap flask (7.2.1).

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

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

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

— Wait for sample to settle, then transfer 500 µl of the extract to a vial (7.2.3) and add 500 µl ammonium acetate (mobile phase A) (6.5.1).

The extract is now ready for analysis.

In the case of high LAS concentrations less of the test sample can be taken for analysis.

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

9.1.2 Extraction of dried soil, sediment and treated biowaste

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

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

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

— Shake for at least 60 min with (250 ± 20) strokes per minute.

— Wait for sample to settle, then transfer 500 µl of the extract to a vial (7.2.3) and add 500 µl ammonium acetate (mobile phase A) (6.5.1).

The extract is now ready for analysis.

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

NOTE Other extraction techniques, like ultrasonic extraction, Soxhlet, reflux, microwave or pressurized liquid extraction may be suitable. However, if using other extraction techniques, the comparability to the method described in this Technical Specification should be proven.

9.2 Concentration (optional)

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

Concentrate the extract on an 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 the internal standard added to the sample can be reduced relative to the concentration factor to keep the concentration of the internal standard at the same level in the 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, clean-up is not necessary.

The selectivity of the MS is higher than of the FLD, and the clean-up is therefore mostly used for the FLD. For the MS, 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 criterion for the clean-up is that the recovery for LAS is higher than 80 %.

Descriptions of the clean-up procedures are given in Annex C.

9.4 Blank test

Perform a blank determination in accordance with the procedure described in 9.1 to 9.4. Prepare the blank exactly as by the analysis of the sample, including the clean-up if the clean-up has been used for the samples.

The blank value shall be lower than 50 % of the lowest reporting limit.

9.5 HPLC analysis

9.5.1 General

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 2). Using FLD, only C_{18}-columns are allowed; using MS, both columns are allowed. Examples are described in Annex B.

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

9.5.2 Fluorescence detection

Optimize the fluorescence detector according to the manufacturer's instructions.

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

The following wavelengths are used:

— Excitation: 230 nm;

— Emission: 310 nm.

9.5.3 Mass selective detection

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

The diagnostic ions used by the HPLC-MS analysis are specified in Table 3.

Table 3 — Diagnostic ions used for the HPLC-MS analysis

Analyte	Abbreviation	Target ion
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 to C_{14} indicate the length of the alkyl chain.

9.6 Calibration

9.6.1 General

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

For all calibrations the relative areas are used, i.e. the area for the analyte relative to the area for the internal standard (see 10.2). 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

The initial calibration serves to establish the linear working range of the calibration curve. This calibration is performed when the method is used for the first time and after maintenance and/or repair of the equipment.

Inject at least five standard solutions with concentrations within the working range described in 6.12.2 and 6.12.3. The actual working range may however differ depending on the matrix and the values of interest. This shall be stated in the test report. 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 ISO 8466-1. ISO 8466-1 provides acceptance and rejection criteria for linearity.

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

9.6.3 Verification of calibration

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

Inject at least two calibration standard solutions 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 according to 9.1, 9.2 (concentration) or 9.3 (clean-up).

Identify LAS 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 in the case of 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 Clause 5 for interferences.

Use ISO 22892 for the identification of LAS.

The C_{10}- to 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 outside 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

10.1 General

For 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 % to 120 % to ensure

an acceptable quality of the performed analysis. This may be checked against the internal standard in the calibration standard solutions.

10.2 Calibration

Obtain a calibration curve from the chromatograms of the calibration standards by plotting the ratio of the mass concentrations against the ratio of the peak areas using Formula (1):

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

where

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

$A_{\mathrm{is,c}}$ is the response of internal standard in the calibration standard (peak area);

s is the slope of the calibration function;

ρ_{c} is the mass concentration of analyte in the calibration standard solution, expressed in micrograms per millilitre (µg/ml);

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

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

10.3 Calculation

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

$$w_{\mathrm{s}} = \frac{\left(\frac{A_{\mathrm{s}}}{A_{\mathrm{is,s}}}\right) - b}{s \cdot m \cdot d_{\mathrm{s}}} \cdot \rho_{\mathrm{is,s}} \cdot V \tag{2}$$

where

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

A_S is the response of analyte in the sample (sum of peak areas);

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

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

s is the slope of the calibration function;

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

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

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

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

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

11 Precision

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

12 Test report

The test report shall contain at least the following information:

a) a reference to this Technical Specification (ISO/TS 13896);

b) complete identification of the sample;

c) expression of results, according to 10.3;

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

Annex A
(informative)

Repeatability and reproducibility data

A.1 Materials used in the interlaboratory comparison study

The interlaboratory comparison for determination of LAS by HPLC with fluorescence detection (FLD) and mass selective detection (MSD) in sludge, treated biowaste and soil was carried out by six European laboratories on three materials. Detailed information can be found in the final report on the interlaboratory comparison study mentioned in References [2] and [3].

Table A.1 lists the types of materials tested.

Table A.1 — Materials tested in the interlaboratory comparison for the determination of LAS by HPLC with fluorescence detection (FLD) and mass selective detection (MSD) in sludge, treated biowaste and soil

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

A.2 Interlaboratory comparison results

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

Table A.2 — Results of the interlaboratory comparison studies of the determination of LAS by HPLC with fluorescence detection (FLD) and mass selective detection (MSD) in sludge, treated biowaste and soil

Matrix	l	n	n_o	$\bar{\bar{x}}$	s_R	$C_{V,R}$	s_r	$C_{V,r}$	BD
				mg/kg	mg/kg	%	mg/kg	%	
Sludge 1	6	20	1	1 770	822	46	54	3,1	0
Compost 1	4	16	0	2,82	1,56	55	0,35	12,4	4
Soil 3	6	13	2	24,46	19,22	79	0,45	1,8	0

Explanation of symbols

l number of laboratories

n number of analytical results

n_o number of rejected laboratories

$\bar{\bar{x}}$ total mean of analytical results (without outliers)

s_R reproducibility standard deviation

$C_{V,R}$ coefficient of variation of reproducibility

s_r repeatability standard deviation

$C_{V,r}$ coefficient of variation of repeatability

BD number of measurements below detection limit

Annex B
(informative)

Examples of chromatographic conditions and chromatograms

B.1 Isomeric separation of LAS

HPLC conditions:

Separation column: C_{18}-column, particle size: 5 µm, dimensions: 150 mm × 2,0 mm;

Mobile phase: Mobile phase A: 0,01 mol/l ammonium acetate in water;

Mobile phase B: Acetonitrile;

Solvent gradient:

Table B.1

Time min	*B* %
0	45
20	55
35	65
36	95
40	95
41	45
46	45

Injection volume: 25 µl;

Flow: 0,2 ml/min.

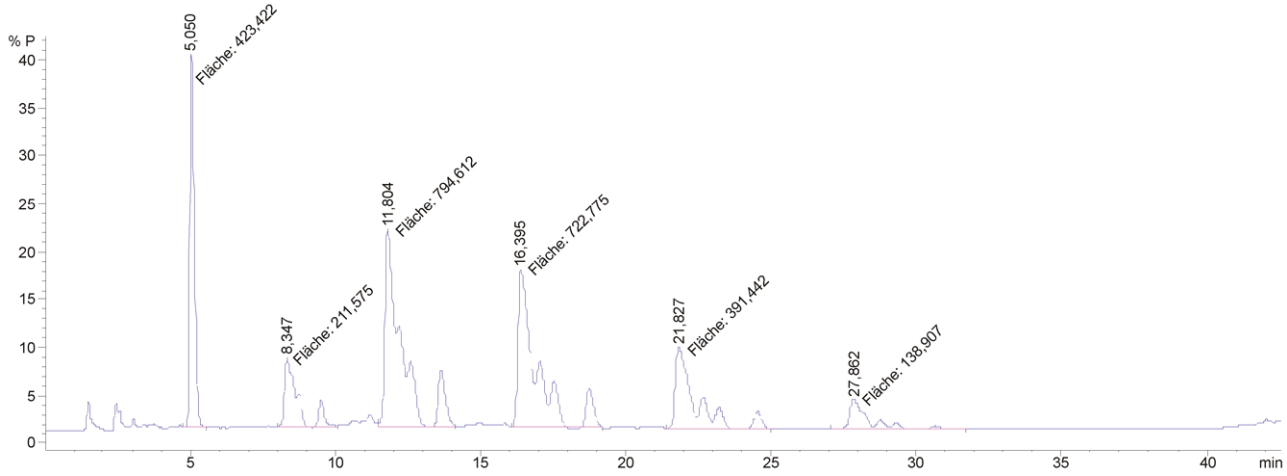

Figure B.1 — Example of chromatogram obtained with fluorescence detection

B.2 Homologue separation of LAS

HPLC conditions:

Separation column: C_8-column, particle size: 5 µm, dimensions: 125 mm × 2,0 mm;

Mobile phase: Mobile phase A: 0,01 mol/l ammonium acetate in water;

Mobile phase B: Methanol;

Solvent gradient:

Table B.2

Time min	*B* %
0	58
12	80
16	95
18	95
19	58
25	58

Injection volume: 25 µl;

Flow: 0,2 ml/min.

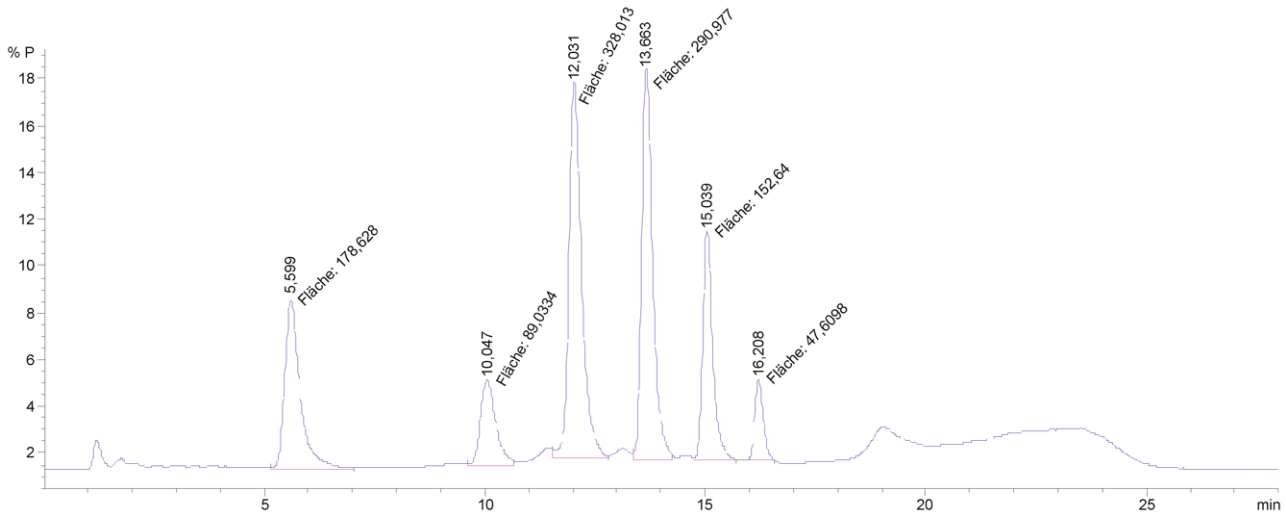

Figure B.2 — Example of chromatogram obtained with fluorescence detection

Annex C
(informative)

Examples of clean-up procedures

C.1 Clean-up procedure based on graphitized carbon black (GCB) columns

Type of column:	500 mg pre-packed GCB solid phase extraction (SPE) column (6.6.2.1);
Step 1: Column preparation:	5 ml mobile phase (6.5);
	2 ml methanol (6.2);
	30 ml 0,1 mol/l hydrochloric acid (6.6.1.3);
Step 2: Sample application:	1 ml methanol extract;
Step 3: Wash:	5 ml water (see 6.1);
	5 ml methanol (6.2);
	10 ml dichloromethane/methanol (80:20) with 15 mmol/l formic acid (6.6.2.4);
	10 ml dichloromethane (6.6.2.5);
Step 4: Elution:	10 ml dichloromethane/methanol (80:20) with 5 mmol/l tetramethyl-ammoniumhydroxide (6.6.2.3);
Step 5: Solvent transfer:	Evaporate until dryness and re-dissolve in methanol/mobile phase (1:1).

C.2 Clean-up procedure based on strong anion exchange (SAX) columns

Type of column:	500 mg pre-packed SAX solid phase extraction (SPE) column (6.6.1.1);
Step 1: Column preparation:	5 ml methanol (6.2);
	10 ml water (6.1);
Step 2: Sample application:	1 ml methanol extract;
Step 3: Wash:	5 ml 2 % acetic acid in methanol;
	5 ml methanol (6.2);
Step 4: Elution:	15 ml 2 mol/l hydrochloric acid:methanol (1:1);
Step 5: Solvent transfer:	Neutralize with ammonium acetate, evaporate until dryness and re-dissolve in methanol/mobile phase (1:1).

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