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

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Foreword

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

This document is a working document.

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

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

Material	Validated	Document
Soil	☐	[reference]
Sludge	☐	
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

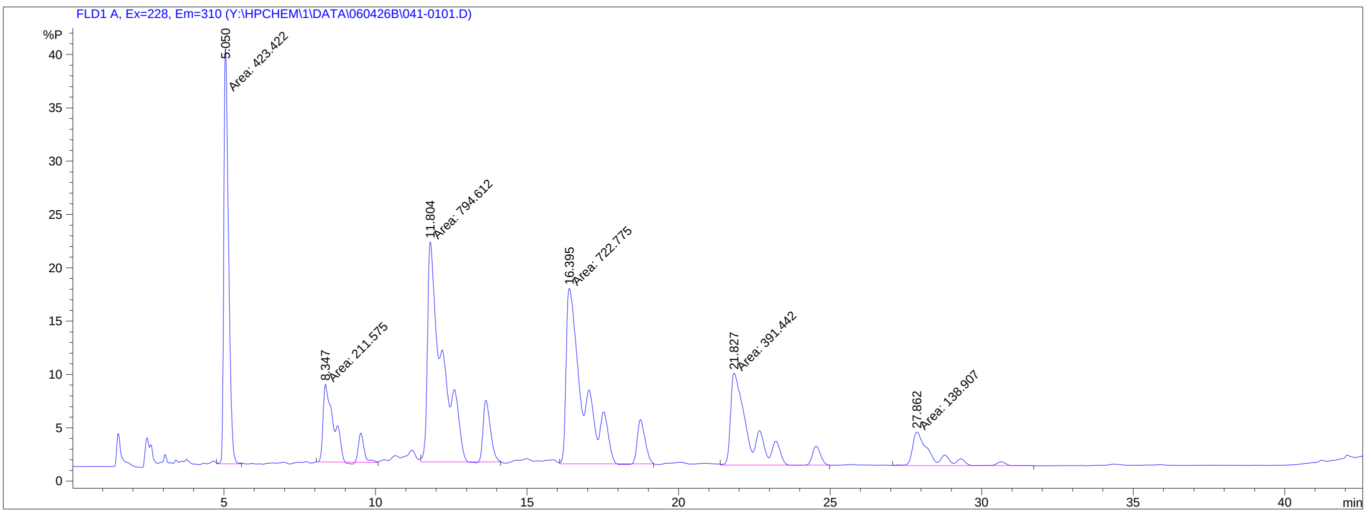
Solvent gradient:

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

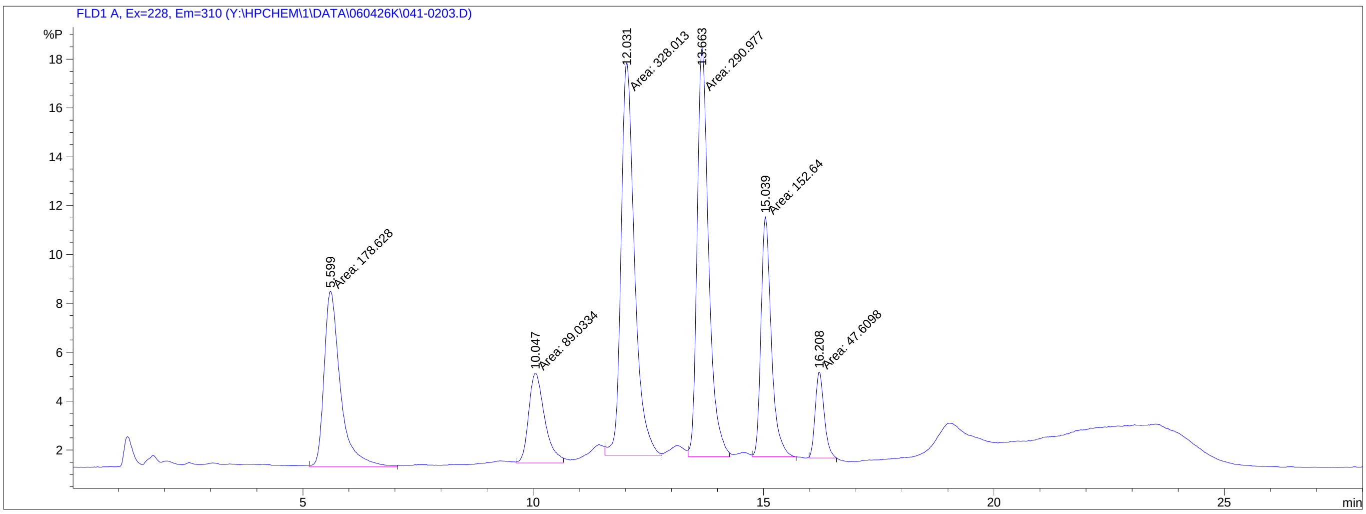
Solvent gradient:

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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