CEN/TC 223/WG 4 "Analytical methods"
WG Secretariat: **ASI**
Convenor: **Baumgarten Andreas Mr**

WI 00223116_draft PAH´s TC223WG04_2022-02-09_clean_HT

Document type	Related content	Document date	Expected action
Project / Draft		2022-03-24	**INFO**

Description

Please find enclosed the current draft on WI 00223116, Compost and digestate – Determination of polycyclic aromatic hydrocarbons (PAH) by gas chromatography (GC) and high performance liquid chromatography (HPLC).

This draft version includes amendments made by Hamid Tbal in track changes mode after the last meeting of CEN/TC 223/WG 4 on 2022-03-09.

CEN/TC 223

Date: 2022-02

prEN 00223116:2021

Secretariat: NEN

Compost and digestate – Determination of polycyclic aromatic hydrocarbons (PAH) by gas chromatography (GC) and high performance liquid chromatography (HPLC)

Kompost und Gärreste — Bestimmung von polycyclischen aromatischen Kohlenwasserstoffen (PAK) mittels Gaschromatographie (GC) und Hochleistungs-Flüssigkeitschromatographie (HPLC)

Compost et digestat— Dosage des hydrocarbures aromatiques polycycliques (HAP) par chromatographie en phase gazeuse (CPG) et chromatographie liquide à haute performance (CLHP)

ICS:

Contents

Page

European foreword ... 3
Introduction ... 3
1 Scope ... 5
2 Normative references ... 5
3 Terms and definitions ... 6
4 Principle ... 7
5 Interferences ... 8
6 Safety remarks ... 8
7 Reagents ... 9
8 Apparatus ... 13
9 Sample storage and preservation ... 15
10 Procedure ... 15
11 Performance characteristics ... 28
12 Precision ... 28
13 Test report ... 28
Annex A (informative) Repeatability and reproducibility data ... 29
A.1 Materials used in the interlaboratory comparison study ... 29
A.2 Interlaboratory comparison results ... 29
Annex B (informative) Examples of instrumental conditions and chromatograms ... 33
B.1 Measurement of PAH with GC-MS ... 33
B.2 Measurement of PAH with HPLC fluorescence ... 39
B.3 Example for measurement conditions of PAH with GC-MS/MS ... 45
Bibliography ... 47

European foreword

This document (prEN 00223116:2021) has been prepared by Technical Committee CEN/TC 223 "Soil improvers and growing media", the secretariat of which is held by NEN, in collaboration with the Technical Committee CEN/TC 444 "Environmental characterization of solid matrices"

This document is a working document.

This document has been prepared under a standardisation request given to CEN by the European Commission and the European Free Trade Association.

Introduction

This document is applicable for compost and digestate.

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous because they are released in appreciable quantities every year into the environment through the combustion of organic matters such as coal, fuel oils, petrol, wood, refuse and plant materials. Since some of these PAH compounds are carcinogenic or mutagenic, their presence in the environment (air, water, soil, sediment and waste) is regularly monitored and controlled. At present determination of PAH is carried out in these matrices in most of the routine laboratories following the prescribed steps specified for sampling, pre-treatment, extraction, clean-up by measurement of specific PAH by means of gas chromatography in combination with mass spectrometric detection (GC-MS or GC/MS/MS) or by high performance liquid chromatography (HPLC) in combination with UV-DAD- or fluorescence detection (HPLC-UV-DAD/FLD). Both the GC-MS and the HPLC methods are included in this horizontal standard.

This document was developed in accordance with FprEN 17503:2020.

Considered the different matrices and possible interfering compounds, this document does not contain one single possible way of working. Several choices are possible, relating to clean-up. Quantification with both GC-MS-detection and HPLC-DAD-UV/FLD is possible. ~~Four~~ Three different extraction procedures are described and three clean-up procedures. The use of internal and injection standards is described in order to have an internal check on choice of the extraction and clean-up procedure. The method is as far as possible in agreement with the method described for polychlorinated biphenyls (PCB) (EN 17322) [3]. ~~It has been tested for ruggedness.~~

WARNING — Persons using this document shall be familiar with usual laboratory practice. This document 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.

WARNING — It is absolutely essential that tests conducted according to this document be carried out by suitably trained staff.

1 Scope

This document specifies different methods for quantitative determination of 16 polycyclic aromatic hydrocarbons (PAH) (see Table 1) in compost or digestate, using GC-MS, GCMS/MS or HPLC-UV-DAD/FLD covering a wide range of PAH contamination levels (see Table 1)

When using fluorescence detection, acenaphthylene cannot be measured.

Table 1 —Target analytes of this document

Target analyte	CAS-RN[a]
Naphthalene	91–20–3
Acenaphthene	83–32–9
Acenaphthylene	208–96–8
Fluorene	86–73–7
Anthracene	120–12–7
Phenanthrene	85–01–8
Fluoranthene	206–44–0
Pyrene	129–00–0
Benz[*a*]anthracene	56–55–3
Chrysene	218–01–9
Benzo[*b*]fluoranthene	205–99–2
Benzo[*k*]fluoranthene	207–08–9
Benzo[*a*]pyrene	50–32–8
Indeno[*1,2,3-cd*]pyrene	193–39–5
Dibenz[*a,h*]anthracene	53–70–3
Benzo[*ghi*]perylene	191–24–2

[a] CAS-RN Chemical Abstracts Service Registry Number.

The limit of detection depends on the determinants, the equipment used, the quality of chemicals used for the extraction of the sample and the clean-up of the extract.

This document contains decision tables based on the properties of the sample and the extraction and clean-up procedure to be used.

The method can be applied to the analysis of other PAH not specified in the scope, provided suitability is proven by proper in-house validation experiments.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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.

EN ISO 5667-15, *Water quality - Sampling — Part 15: Guidance on the preservation and handling of sludge and sediment samples (ISO 5667-15)*

EN ISO 16720, *Soil quality — Pretreatment of samples by freeze-drying for subsequent analysis (ISO 16720)*

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

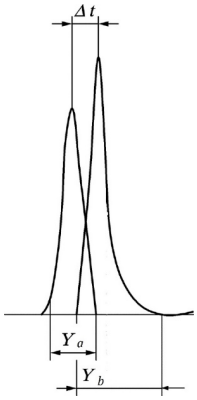
EN 15934, *Sludge, treated biowaste, soil and waste — Calculation of dry matter fraction after determination of dry residue or water content*

EN 13040-1:—[1], *Soil improvers and growing media – Sample preparation – Part 1: Sample preparation for chemical and physical tests, determination of dry matter content, moisture content and laboratory bulk density*

3 Terms and definitions

For the purposes of this document, the following terms and definitions given in CEN/TS 00223137 apply.

3.1
critical pair
pair of PAH that shall be separated to a predefined degree to ensure chromatographic separation meets minimum quality criteria



$$R = 2 \times \frac{\Delta t}{Y_a + Y_b} \quad (1)$$

Where:

R peak separation

Δt difference in retention times of the two peaks *a* and *b* in seconds (s)

Y_a peak width at the base of peak a in seconds (s)

Y_b peak width at the base of peak b in seconds (s)

Figure 1 — Example of a chromatogram of a critical pair

[1] Under preparation. Stage at the time of publication: prEN 13040-1:20**.

3.2
internal standard
compounds added in a known amount to the sample from the beginning of the protocol and enabling analytical coverage throughout the procedure, and that is used to correct for losses during sample preparation and analysis by accounting for all-system matrix effects (recoveries, ionization effect, variability of the detector response of the instrument for example)

Note 1 to entry: isotopically labelled mostly deuterated PAH or native PAH unlikely to be present in the sample

SOURCE : EN ISO 21253-2:2019(en), 3.10

3.3
injection standard
standard mixture added to a sample before injection into the GC-MS apparatus, to monitor variability of instrument response and to calculate internal standard recovery

SOURCE: ISO 28540:2011, 3.4

3.4
extraction standard
PAH that is unlikely to be present in samples added to the sample prior to extraction, used for checking the extraction efficiency and not used for quantification purposes

4 Principle

Due to the multi matrix character of this document, different procedures for different steps (modules) are allowed. Which modules should be used depends on the sample. A recommendation is given in this European Standard. Performance criteria are described and it is the responsibility of the laboratories applying this European Standard to show that these criteria are met. Using of spiking internal standards allows an overall check on the efficiency of a specific combination of modules for a specific sample. But the use of internal standards does not necessarily give the information regarding the extensive extraction efficiency of the native PAH bonded to the matrix.

After pre-treatment, the sample is extracted with a suitable solvent.

The extract is concentrated by evaporation. If necessary, interfering compounds are removed by a clean-up method suitable for the specific matrix, after the concentration step.

If a solvent exchange for HPLC analysis is necessary, the concentrated extract is taken up in an appropriate less volatile water miscible polar solvent and the non-polar extract residue is removed under a gentle flux of inert gas.

The extract is analysed by GC-MS or GC/MS/MS using a capillary column with a stationary phase of low polarity or by HPLC-UV-DAD/FLD with an appropriate reversed phase column.

PAH are identified and quantified with GC-MS or GC/MSMS by comparison of relative retention times and relative peak heights (or peak areas) with respect to internal standards added, and with HPLC by using the corresponding variables of the extraction standard solutions. The efficiency of the procedure depends on the composition of the matrix that is investigated.

5 Interferences

5.1 Interference with containers

Use sample containers of materials (preferably of steel, aluminium or glass) that do not affect the sample during the contact time. Avoid plastics and other organic materials during sampling, sample storage or extraction. Keep the samples from direct sunlight and prolonged exposure to light.

During storage of the samples, losses of PAH may occur due to adsorption on the walls of the containers. The extent of the losses depends on the storage time.

5.2 Interference with GC-MS

Substances that co-elute with the target PAH can interfere with the determination. These interferences can lead to incompletely resolved signals and can, depending on their magnitude, affect accuracy and precision of the analytical results. Peak overlap does not allow an interpretation of the result. Asymmetrical peaks and peaks broader than the corresponding peaks of the reference substance suggest interferences.

Chromatographic separation between dibenz[*a,h*]anthracene and indeno[*1,2,3-cd*]pyrene are mostly critical. Due to their molecular mass differences, quantification can be made by mass selective detection. When incomplete resolution is encountered, peak integration shall be checked and, if necessary, corrected.

Sufficient ~~resolution~~ separation (e.g. R= 0,8) between the peaks of benzo[*b*]fluoranthene and benzo[*k*]fluoranthene as well as of benzo[*a*]pyrene and benzo[*e*]pyrene shall be set as quality criteria for the capillary column. Benzo[*b*]fluoranthene and benzo[*j*]fluoranthene cannot be separated. It can happen that the signal of triphenylene is not completely separated from the signals of benz[*a*]anthracene and chrysene. In this case it shall be stated in the report.

5.3 Interference with the HPLC

Substances that show either fluorescence or quenching and co-elute with the PAHs to be determined can interfere with the determination. These interferences can lead to incompletely resolved signals and can, depending on their magnitude, affect accuracy and precision of the analytical results. Peak overlap does not allow an interpretation of the result. Asymmetrical peaks and peaks being broader than the corresponding peaks of the reference substance suggest interferences. This problem can arise for naphthalene and phenanthrene depending on the selectivity of the phases used.

Incomplete removal of the solvents used for sample extraction and clean-up can lead to poor reproducibility of the retention times and wider peaks or double peaks especially for the 2- and 3-ring PAHs. Extracts shall be diluted sufficiently with acetonitrile for the HPLC-analysis, otherwise the detection of naphthalene and 3-ring PAH can be interfered with by a broad toluene peak.

Usually the signal of perylene is incompletely resolved from the signal of benzo[*b*]fluoranthene, but by choosing a selective wavelength, the perylene peak can be suppressed.

6 Safety remarks

Certain PAH are highly carcinogenic and shall be handled with extreme care. Avoid contact with solid materials, solvent extracts and solutions of standard PAH. It is strongly advised that standard solutions are prepared centrally in suitably equipped laboratories or are purchased from suppliers specialized in their preparation.

PAH contamination of vessels may be detected by irradiation with 366 nm UV-light.

Solvent solutions containing PAH shall be disposed of in a manner approved for disposal of toxic wastes.

For the handling of hexane precautions shall be taken because of its neurotoxic properties.

National regulations should be followed with respect to all hazards associated with this method.

7 Reagents

7.1 General

All reagents shall be of recognized analytical grade. The purity of the reagents used shall be checked by running a blank test as described in 10.1. The blank result shall be less than 50 % of the lowest reporting limit for each PAH.

7.2 Reagents for extraction

7.2.1 Acetone, (2-propanone), $(CH_3)_2CO$.

7.2.2 Toluene, C_7H_8.

7.2.3 Petroleum ether, boiling range 40 °C to 60 °C.

Hexane-like solvents with a boiling range between 40 °C and 98 °C are allowed.

7.2.4 Sodium sulfate, Na_2SO_4.

The anhydrous sodium sulfate shall be kept carefully sealed.

7.2.5 Distilled water or water of equivalent quality, H_2O.

7.2.6 Sodium chloride, NaCl

7.2.7 Keeper substance (high boiling compound, e.g. octane, nonane)

7.3 Reagents for clean-up

7.3.1 Clean-up "A" using aluminium oxide

7.3.1.1 Aluminium oxide, Al_2O_3

Basic or neutral, specific surface 200 m^2/g, activity Super I [10~~1~~].

7.3.1.2 Deactivated aluminium oxide

Deactivated with water by approximately 100 g/kg.

Add approximately 10 g of water (7.2.5) to 90 g of aluminium oxide (7.3.1.1). Shake until all lumps have disappeared. Allow the aluminium oxide to condition before use for some 16 h, sealed from the air, use it for maximum of two weeks.

The activity depends on the water content. It can necessary to adjust the water content.

NOTE ~~2~~ Commercially available aluminium oxides with 100 g/kg mass fraction water can also be used.

7.3.2 Clean-up "B" using silica gel 60 for column chromatography

7.3.2.1 Silica gel 60, particle size 63 µm to 200 µm.

7.3.2.2 Silica gel 60, water content (mass fraction) $w(H_2O)$ = 10 %.

Silica gel 60 (7.3.2.1), heated for at least 3 h at 450 °C, cooled down and stored in a desiccator containing magnesium perchlorate or a suitable drying agent. Before use, heat for at least for 5 h at 130 °C in a drying oven. Then allow cooling in a desiccator. Put the silica gel in a stopperad flask and add 10 % water (7.2.5) (mass fraction), shake for 5 min intensively until all lumps have disappeared and then for 2 h in a shaking device (8.1.3). Store the deactivated silica gel in the absence of air, use it for a maximum of two weeks.

7.3.3 Clean-up "C" using gel permeation chromatography (GPC)

7.3.3.1 Bio-Beads® S-X3[2]

7.3.3.2 Ethyl acetate, $C_4H_8O_2$

7.3.3.3 Cyclohexane, C_6H_{12}

Preparation of GPC, for example: Put 50 g Bio-Beads® S-X3 (7.3.3.1) into a 500 ml Erlenmeyer flask and add 300 ml elution mixture made up of cyclohexane (7.3.3.3) and ethyl acetate (7.3.3.2) 1 : 1 (volume fraction) in order to allow the beads to swell. After swirling for a short time until no lumps are left, maintain the flask closed for 24 h. Drain the slurry into the chromatography tube for GPC. After approximately three days, push in the plungers of the column so that a filling level of approximately 35 cm is obtained. To further compress the gel, pump approximately 2 l of elution mixture through the column at a flow rate of 5 ml · min^{-1} and push in the plungers to obtain a filling level of approximately 33 cm.

7.4 Reagents for chromatographic analysis

7.4.1 GC-Analysis

7.4.1.1 Carrier gas for GC-MS

Operating gases of high purity and in accordance with the manufacturer's specifications.

7.4.2 HPLC-analysis

7.4.2.1 Acetonitrile (CH_3CN) or **methanol (CH_3OH),** HPLC purity grade

7.4.2.2 Ultra-pure water, HPLC purity grade.

7.4.2.3 Helium, He, of suitable purity for degasification of solvents

7.5 Standards

7.5.1 General

Choose the internal and/or extraction standards whose physical and chemical properties (such as extraction behaviour, retention time) are similar to those of the compounds to be analysed. For GC-MS a

[2] Bio-Beads® is an example of a suitable product available commercially. This information is given for convenience of users of this European Standard and does not constitute an endorsement by CEN of this product. Equivalent products can be used if they can be shown to lead to the same results.

minimum of four deuterated internal standards shall be used as internal standards for evaluation of results.

7.5.2 Calibration substances and internal standards

Table 2 contains native and deuterated PAH to be used for calibration. Verify the stability of the internal standards regularly.

Table 2 — Native PAH and deuterated PAH

PAH reference substances		Internal standard substances (deuterated PAHs)	
Naphthalene	(CAS-RN 91-20-3)	Naphthalene-d8	(CAS-RN 1146-65-2)
Acenaphthene	(CAS-RN 83-32-9)	Acenaphthene-d10	(CAS-RN 15067-26-2)
Acenaphthylene	(CAS-RN 208-96-8)	Acenaphthylene-d8	(CAS-RN 93951-97-4)
Fluorene	(CAS-RN 86-73-7)	Fluorene-d10	(CAS-RN 81103-79-9)
Anthracene	(CAS-RN 120-12-7)	Anthracene-d10	(CAS-RN 1719-06-8)
Phenanthrene	(CAS-RN 85-01-8)	Phenanthrene-d10	(CAS-RN 1517-22-2)
Fluoranthene	(CAS-RN 206-44-0)	Fluoranthene-d10	(CAS-RN 93951-69-0)
Pyrene	(CAS-RN 129-00-0)	Pyrene-d10	(CAS-RN 1718- 52–1)
Benz[*a*]anthracene	(CAS-RN 56-55-3)	Benz[*a*]anthracene-d12	(CAS-RN 1718-53-2)
Chrysene	(CAS-RN 218-01-9)	Chrysene-d12	(CAS-RN 1719-03-5)
Benzo[*b*]fluoranthene	(CAS-RN 205-99-2)	Benzo[*b*]fluoranthene-d12	(CAS-RN 93951-98-5)
Benzo[*k*]fluoranthene	(CAS-RN 207-08-9)	Benzo[*k*]fluoranthene-d12	(CAS-RN 93952-01-3)
Benzo[*j*]fluoranthene[a]	(CAS-RN 205–82–3)		
Benzo[*a*]pyrene	(CAS-RN 50-32-8)	Benzo[*a*]pyrene-d12	(CAS-RN 63466-71-7)
Benzo[*e*]pyrene[a]	(CAS-RN 192-97-2)		
Indeno[*1,2,3-cd*]pyrene	(CAS-RN 193-39-5)	Indeno*[1,2,3-cd*]pyrene-d12	(CAS-RN 203578-33-0)
Dibenz[*a,h*]anthracene	(CAS-RN 53-70-3)	Dibenz[*a,h*]anthracene -d14	(CAS-RN 13250-98-1)
Benzo[*ghi*]perylene	(CAS-RN 191-24-2)	Benzo[*ghi*]perylene-d12	(CAS-RN 93951-66-7)

[a] Not part of 16 target analytes, but applicable for resolution check for the separation with benzo[*a*]pyrene for GC measurements

NOTE 1 $^{13}C_{12}$-labelled PAH standards can also be used as internal standards.

NOTE 2 Certified solutions of PAH, and single solid PAH substances with certified purity are available from a limited number of suppliers.

When highly contaminated samples are analysed, an aliquot of the extract is often used for further clean-up. This makes the costs of analyses caused by the use of deuterated standard very high. In these cases, it is allowed to add the internal standard in two steps:

- Step 1 addition of unlabelled internal standards to the sample.
- Step 2 addition of deuterated compounds to the aliquot of the extract used for clean-up.

For HPLC 6-methylchrysene, 1-methylnaphthalene or other alkylated PAH which are not present in the sample and which are sufficient separated from the target PAH can be used as internal or extraction standard.

7.5.3 Injection standard

If required for GC-MS a deuterated PAH such as 1-methylnaphthalene-d10, triphenylene-d12 and perylene-d12 can be added to the final extract before GC-MS injection to verify the recovery of the deuterated internal standards.

7.6 Preparation of standard solutions

7.6.1 General

The procedure for the preparation of standard solutions for GC-MS and HPLC is the same only different solvents are used.

Because of the dangerous nature of the substances to be used, commercially available - preferably certified - standard solutions or mixed standard solutions are preferred. It is very strongly recommended to avoid skin contact.

The working standard solutions shall be in the same solvent as the extract.

Store the primary and diluted standard solutions in a dark place at a temperature of (5 ± 3) °C. The solutions are stable for at least one year, provided that evaporation of solvent is negligible.

PAH to analyse present in mixed standard solutions should be separated by the chromatographic columns used (see 3.1, 5.2, 5.3).

7.6.2 Preparation of calibration standard solutions for GC-MS

Prepare individual concentrated primary standard solutions of about 0,4 mg/ml in hexane-like solvent (7.2.3) by weighing approximately 10 mg of each of the calibration standards (7.5.2 Table 2 left column) to the nearest 0,1 mg and dissolving them in 25 ml of hexane-like solvent.

Combine small quantities (2 ml to 10 ml) of these individual primary standard solutions into a mixed standard solution of PAH.

7.6.3 Preparation of internal standard solution for GC-MS

Prepare a concentrated primary internal standard solution, containing at least four different components (7.5.2 Table 2 right column), of about 0,4 mg/ml in one of the solvents used for extraction (~~Table 3~~adequate solvents are specified in 10.2.2, 10.2.3 and 10.2.4~~ specifies the adequate solvents~~) by weighing approximately 10 mg of each of the chosen internal standards to the nearest 0,1 mg and dissolving them in 25 ml of extraction solvent or hexane-like solvent. Prepare from this a secondary internal standard solution with such a concentration that the added amount gives a peak with measurable peak area or peak height in the chromatogram (at least 10 times the detection limit).

If the two-step procedure for GC-MS is used, make two different internal standard solutions, one containing the non-labelled compounds. At least two unlabelled compounds shall be used in the first internal standard solution and at least four deuterated compounds in the second solution.

7.6.4 Preparation of injection standard solution for GC-MS

Prepare a concentrated primary injection standard solution, containing at least one compound (7.5.3), of about 0,4 mg/ml in an appropriate solvent as the one used for the extract, by weighing approximately 10 mg of the chosen injection standards to the nearest 0,1 mg and dissolving it in 25 ml. Prepare from this a secondary injection standard solution with such a concentration that the added amount gives a

peak with measurable peak area or peak height in the chromatogram (at least 10 times the detection limit).

7.6.5 Preparation of calibration standard solutions for HPLC

Prepare individual concentrated primary standard solutions of about 0,4 mg/ml in acetonitrile (7.4.2.1) by weighing approximately 10 mg of each of the calibration standards (7.5.2 Table 2 left column) to the nearest 0,1 mg and dissolving them in 25 ml of acetonitrile.

Combine small quantities (2 ml to 10 ml) of these individual primary standard solutions into a mixed standard solution of PAH.

7.6.6 Preparation of extraction standard solution for HPLC

Prepare a concentrated primary extraction standard solution, containing one or more alkylated PAH e.g. 6-methylchrysene, 1-methylnaphthalene of about 0,4 mg/ml in acetonitrile (7.4.2.1) by weighing approximately 10 mg of each of the chosen standards to the nearest 0,1 mg and dissolving them in 25 ml of acetonitrile. Prepare from this a secondary extraction standard solution with such a concentration that the added amount gives a peak with measurable peak area or peak height in the chromatogram (at least 10 times the detection limit).

8 Apparatus

8.1 Extraction and clean-up procedures

8.1.1 General

Usual laboratory glassware.

All glassware and material that comes into contact with the sample or extract shall be thoroughly cleaned.

8.1.2 Sample bottles, made of glass, stainless steel, aluminium, perfluoroalkoxy or fluorinated ethylene propylene with glass stopper or screw top and polytetrafluoroethylene (PTFE) seal of appropriate volume.

WARNING — For safety reasons, biologically active digestate samples shall not be stored in a sealed container.

8.1.3 Shaking device, with horizontal movement (200 strokes to 300 strokes per min) or an end-over-end shaking device.

8.1.4 Water bath, adjustable up to 100 °C

8.1.5 Separating funnels of appropriate volume

8.1.6 Conical flasks of appropriate volume

8.1.7 Soxhlet extraction apparatus, consisting of round bottom flask, e.g. 100 ml, Soxhlet extractors and Soxhlet thimbles, e.g. 27 mm × 100 mm, vertical condensers, e.g. 300 mm, heating device

8.1.8 Concentrator, Kuderna Danish type

Other evaporators, e.g. a rotary evaporator, may be used if found to be equally suitable.

8.1.9 Boiling chips, glass or porcelain beads.

8.1.10 Quartz wool or silanized glass wool

WARNING — Working with quartz wool imposes a risk to health through the release of fine quartz particles. Inhalation of these should be prevented by using a fume cupboard and wearing a dust mask.

8.1.11 Calibrated test tubes, with a nominal capacity of 10 ml to 15 ml and ground glass stopper.

8.1.12 Chromatography tubes (Chromatography column of glass, 5 mm to 10 mm inside diameter, length e.g. 600 mm)

8.1.13 Sonication apparatus

8.1.14 Pressurized Liquid Extraction equipment (PLE), combining elevated temperature and pressure with liquid solvents to achieve extraction of the analytes from the solid matrix

8.1.15 Gel permeation chromatograph, kind of liquid chromatograph which separates molecules on the basis of their size by using columns packed with very small, porous particles

8.2 Gas chromatograph

8.2.1 General

Gas chromatograph equipped with a non-discriminating injection system, capillary column and a mass spectrometric detector (GC-MS or GC/MS/MS).

8.2.2 Capillary columns

Low to medium capillary columns such as 5 % phenyl-methyl silicone stationary phase coated onto fused silica capillary column or an equivalent chemically bonded phase column.

Their dimensions should be sufficient to separate the critical pairs mentioned in Clause 5.2. In general column length is typically 25 m to 60 m, internal diameter 0,18 mm to 0,325 mm and film thickness 0,1 µm to 0,5 µm

8.3 High-performance liquid chromatograph

8.3.1 General

A HPLC system equipped according to requirements with ultraviolet (UV) and a fluorescence detection (FLD) system and a data evaluation system, including:

— degassing assembly, e.g. for degassing with vacuum or helium;

— analytical pumps, capable of binary gradient elution;

— column thermostat, capable of maintaining the temperature constant to within ± 0,5 °C;

— fluorescence detector capable of programming at least six pairs of wavelengths, including damping/amplification, equipped with monochromator(s);

— UV detector (with variable wavelength) or diode array.

8.3.2 Analytical separation column

A reversed phase HPLC column meeting the separation requirements described in Annex B.

9 Sample storage and preservation

9.1 Sample storage

The samples shall be analysed as soon as possible after sampling.

If necessary, digestate samples shall be stored in accordance with EN ISO 5667-15.

Dried samples can be stored at room temperature in a dark place up to one month.

During storage of the samples, losses of PAH can occur due to adsorption to the walls of the containers. The extent of the losses depend on the storage time.

9.2 Sample pre-treatment

Suitable pre-treatment of samples according to EN 16179 (compost) [4], if not otherwise specified, and considering the specific drying procedures as specified in Table 3 to obtain a test sample.

Complete drying of the sample is essential if Soxhlet or PLE is used for extraction. Complete drying is also recommended if the sample shall be stored for a long period.

Table 3 — Drying techniques for samples of different matrices for subsequent analyses of PAH

Matrix	Drying technique			
	Freeze drying (EN ISO 16720)	Na_2SO_4	Drying T < 40°C	No drying
Digestate	x[a]	x[b]	x[a]	
Compost	x[a]	x	x[a]	x

[a] Loss of naphthalene is possible.

[b] Na_2SO_4 can be used for the preservation of hygroscopic dried ~~sludge~~ digestate.

To achieve a homogeneous and representative test portion, one or more particle size reduction steps can be needed. The choice of the technique depends on the nature of the sample and on the particle size needed. Typically, particle size reduction is a multi-step operation that implies the use of a sequence of different techniques like crushing, cutting or grinding.

Grinding of plastic-rich samples ~~which have a plastic or paste-like consistency~~ requires embrittlement with liquid nitrogen and particle size reduction to less than 0,5 mm, e.g. by using an ultra-centrifugal mill.

10 Procedure

10.1 Blank test

Perform a blank test following the applied procedure (selected extraction and clean-up procedure) using the same amount of reagents that are used for the pre-treatment, extraction, clean-up and analysis of a sample. Analyse the blank immediately prior to analysis of the samples to demonstrate sufficient freedom from contamination. The blank shall be less than 50 % of the lowest reporting limit.

10.2 Extraction

10.2.1 General

Depending on the test sample (matrix and moisture content), choose a suitable extraction procedure (see 10.2.2.2, 10.2.3 and 10.2.4). The choice of the extraction solvent is more crucial than the procedure

and the extraction devices itself for the extraction of PAH from the matrices. Since some of the target PAHs are relatively insoluble in the usual non-polar solvents such as hexane-like solvent, ether and other hydrocarbons, the choice of the solvents shall be made in accordance with the expected contamination level.
Extraction procedure 1 (10.2.2) is recommended if it is important to break up aggregates in the sample to reach the PAHs. With wet samples this procedure shall be applied in order to remove the water.
If dissolving of the PAHs is the most important step (highly contaminated matrices and organic matter rich materials) and the sample is dry, extraction procedure 2 (10.2.3) using Soxhlet or ectraction procedure 3 (10.2.4) using PLE is recommended.

~~The extraction is applicable to freeze dried samples and field moist samples with dry matter content > 75 %.~~

The extraction procedure described in this European Standard is able to extract up to 20 g of dry sample, the minimum weight to be extracted is 1g. If the test sample has a low density (i.e. some matrices) or the sample is homogeneous, depending on the expected PAH content and on the homogeneity of the sample, less sample can be used. In general, the following amounts can be used: 10 g to 20 g of digestate or 5 g to 20 g of compost. The amount of sample shall be weighed with an accuracy of at least 1 %.

10.2.2 Extraction procedure~~-~~: acetone/hexane-like solvent and agitation or sonication

This extraction procedure is applicable to freeze dried samples and field moist samples with dry matter content > 75 %.

Place the sample in a bottle (8.1.2). Add a definite volume of the secondary internal standard solution (7.6.3 or 7.6.6). Add 50 ml of acetone (7.2.1) to the test sample and extract by shaking thoroughly with the shaking device (8.1.3) or sonication for 30 min to break up aggregates. Then add 50 ml of petroleum ether or hexane-like solvent (7.2.3) and shake again or sonicate thoroughly at least for 1h. Use a horizontal shaking device or end-over-end shaking (8.1.3) and have the solvent movement in the sample bottle as long as possible (horizontal position). After the solids have been settled decant the supernatant. Wash the solid phase with 50 ml of petroleum ether or hexane-like solvent (7.2.3) and decant again. Collect the extracts in a separating funnel (8.1.5) and remove the acetone by shaking twice with 400 ml of water (7.2.5). Dry the extract over sodium sulfate (7.2.4). Rinse the sodium sulfate with petroleum ether or hexane-like solvent (7.2.3) and add the rinsing to the extract.

Kommentiert [BA1]: Ev. Longer extraction time due to hugh organic matter content – Alexander will check (6 or 16h)

NOTE 1 Tap water has shown to be applicable for removal of the acetone because target compounds are not present.

In case of ~~waste~~ material with a high proportion of plastic, ~~e.g. plastic shredder,~~ extraction with an acetone containing solvent leads to a high amount of co-extractives. In these cases, extraction should be performed twice with 50 ml hexane-like solvent (7.2.3).

~~If the sample contains water up to 25 %, the same procedure can be used.~~ If the water content of the sample is greater than 25 % this procedure is less effective and the amount of acetone shall be increased. The ratio acetone: water should be at least 9:1. The ratio acetone: hexane-like solvent should be kept constant 1:2.

NOTE 2 In matrices with a high organic matter content longer extraction procedures can be necessary. Extraction procedure 2 (10.2.3) can be preferable for these samples.

10.2.3 Extraction procedure 2: Soxhlet extraction

This extraction procedure is applicable to dried samples
Place the dried test sample (9.2) in the extraction thimble (8.1.7). Add the definite amount of the secondary internal standard solution (7.6.3 or 7.6.6) and approximately 70 ml of the extraction solvent (7.2.2 or 7.2.3) to the extraction vessel. Extract the sample with the Soxhlet extraction apparatus (8.1.8). The duration of the extraction should be calculated with a minimum of 100 extraction cycles.

NOTE 1 If the sample is hygroscopic and is not dried just before analysis, add Na2SO4 to the sample to get a free-flowing material.
NOTE 2 A mixture of acetone/petroleum ether in combination with Soxhlet is also allowed for extraction provided that the boiling points are as similar as possible to ensure the same composition of the extraction mixture in the liquid and gaseous phase.

10.2.4 Extraction procedure 3: Pressurized liquid extraction (PLE)

This extraction procedure is applicable to dried samples
The apparatus (8.1.14) is prepared according to the instructions of the manufacturer. The volume of the PLE-cell chosen is dependent upon the level of contamination and the volume of the sample to be analysed. Place a cellulose filter at the bottom of the cell and add the sample or a mixture of the sample with diatomaceous earth or sea sand in the extraction cell. Add the definite amount of the secondary internal standard solution (7.6.3 or 7.6.6). If necessary add further diatomaceous earth or sea sand so that the cell is completely filled. Close the cell tightly and follow the manufacturer instructions. In

dependence of the moisture content choose hexane-like solvent (7.2.3) or a mixture of hexane-like solvent (7.2.3) and acetone (7.2.1).
Example for setting The PLE equipement :

- Temperature 100°C;
- Pressure 10^7 Pa (100 bar);
- Static phase 10 min;
- Number of cycles 2;
- Flush 20 ml (4 min).

10.2.5 Extraction procedure 3: acetone/hexane-like solvent/sodium chloride and agitation

This extraction procedure is applicable to wet, dry and moist samples.
Take an amount of the wet sample and put it into a 1 l sample bottle (8.1.2). Add the definite amount of the secondary internal standard solution (7.6.3 or 7.6.6). If the sample is dry, add 50 ml water. For moist samples, the water quantity to be added is calculated using Formula (2):

$$m_w = 50 - \frac{m_E * m_{H2O}}{100} \qquad (2)$$

Where
m_w is the mass of the water to be added, expressed in grams (g);
m_E is the mass of the sifted sample, expressed in grams (g);
m_{H2O} is the water content of the sample determined according to EN 15934, expressed in percent mass (%).
Add 40 g sodium chloride (7.2.6), 100 ml acetone (7.2.1) and 50 ml petroleum ether or hexane-like solvent (7.2.3) to the moistened preparations, close the container and shake it with a shaking device (8.1.3) for at least 12 h.
The organic phase shall be separated, if necessary, using a centrifuge with sealable centrifuge cups. Collect the extract in a separating funnel of 1 l capacity. Dry the extract over sodium sulfate (7.2.4) and transfer the dried extract to the concentrator (8.1.8). Rinse the sodium sulfate with petroleum ether or hexane-like solvent (7.2.3) and add the rinsing to the extract.

The definite amount of the internal standard added in all extraction procedures shall have such a quantity that their concentrations in the final extract are within the working range of the detection method. Typically, the concentration of the individual internal standards in the final extract is 0,1 µg/ml. To 'wet' the complete sample, a minimum amount of 100 µl of internal standard is recommended.

Other extraction procedures, e.g. microwave may be used provided:

— the laboratory can show that the extraction efficiency is equivalent to one of the extraction procedures 1, 2 or 3 as described in this European Standard, or

— the sample requires another approach as shown by the laboratory and the results of the procedure are in agreement with the performance criteria described in Clause 10.6.6 and 11.

~~NOTE 2 In matrices with a high organic matter content (e.g. some sludge) longer extraction procedures can be necessary. Extraction procedure 2 (10.2.3) can be preferable for these samples.~~

Kommentiert [BA2]: See comment above regarding 1h extraction time)

10.3 Concentration

10.3.1 General

If necessary, concentrate the extract to approximately 10 ml by evaporation using a concentrator (8.1.8). If necessary add a boiling chip (8.1.9). Transfer the concentrated extract to a calibrated test tube (8.1.11) and concentrate up to 1 ml using a gentle stream of nitrogen or another inert gas at room temperature. If reduced below 1 ml make back up to this volume with appropriate solvent.

NOTE Too high temperatures and a too high flow of nitrogen can result in losses of the more volatile PAH.

To prevent losses of the most volatile PAHs evaporation to dryness must be avoided. It is advisable to add a small amount (one drop) of keeper substance (7.2.7).

In heavily contaminated samples, an aliquot is used for further clean-up. Establish the fraction *f* of the extract used for further clean-up. If non-labelled PAHs have been used as extraction standard added to the sample, add a definite amount of the internal standard solution containing deuterated PAHs (7.6.3).

When clean-up is not required, bring the extract to a definite volume.

10.3.2 For HPLC analysis

When clean-up is not required, the solvent of the final extract shall be changed from hexane-like solvent to a solvent such as acetonitrile or methanol (7.4.2.1) which is compatible with the mobile phase of the HPLC system. For example, add 0,8 ml acetonitrile to an aliquot of 1 ml of the concentrated extract and concentrate at room temperature with a gentle stream of nitrogen until all hexane-like solvent has been removed, i.e. until the volume of 0,8 ml has been reached. Add acetonitrile up to the mark of 1,0 ml for subsequent HPLC analysis.

The enriched extract should not contain residues of acetone, because the presence of this solvent in the measuring solution leads to interferences with the HPLC.

If toluene is used for extraction the final extract of toluene can be diluted with acetonitrile or methanol (7.4.2.1).

10.4 Clean-up of the extract

10.4.1 General

Clean-up shall be used if compounds are present that can interfere with the PAH of interest in the GC-MS chromatogram respectively HPLC chromatogram or if they can influence the measurement procedure (i.e. contamination of the chromatographic system). If no or negligible interfering substances are present, no clean-up is necessary. Depending on the substances to be removed, Table 5 can be used.

When highly contaminated samples are analysed, an aliquot of the extract is often used for a further clean-up.

Table 5 — Clean-up methods

Method	Clean-up	For removal of	Suitable for	Remarks
Clean-up A	Aluminium oxide	Polar compounds	GC-MS and HPLC	Difficult to adjust water content and keep it constant
Clean-up B	Silica	Polar compounds	GC-MS and HPLC	
Clean-up C	Gel permeation	Large molecular compounds, lipids and oils	GC-MS	
NOTE Addition of keeper during final concentration after clean-up.				

NOTE PAH in contrary to PCB are not very stable and persistent, therefore extreme clean-up conditions (e.g. sulfuric acid) are not applicable.

Other clean-up procedures such as polymer-based solid extraction or other materials such as modified silica gel may also be used, provided they remove the interfering peaks in the chromatogram and recoveries after use of the clean-up are at least 80 % for all relevant PAH (including internal standards).

The extract or an aliquot obtained in 10.3 shall be quantitatively transferred to the clean-up system.

10.4.2 Clean-up A – Aluminium oxide

Prepare an adsorption column by placing a small plug of quartz wool (8.1.10) in the chromatography tube (8.1.12) and packing it dry with (2,0 g ± 0,1) g of aluminium oxide (7.3.1.2). If necessary, add a layer of sodium sulfate (7.2.4).

Apply the extract to the dry packed adsorption column with a pipette. Rinse the test tube twice with 1 ml of petroleum ether or hexane-like solvent (7.2.3) and quantitatively transfer the rinsing to the column as soon as the liquid level reaches the upper side of the column packing. Elute with approximately 20 ml of petroleum ether or hexane-like solvent (7.2.3). Collect the entire eluate.

Keeper substance (7.2.7) is added to the eluate, and then the eluate is reduced to the desired volume (see 10.3).

If a new batch of aluminium oxide is used, the solvent volume to eluate the specified PAH completely from the column shall be determined using a proper PAH standard solution.

NOTE Commercially available disposable columns can be used alternatively, if found equally suitable. A column is suitable if the performance of the method is in agreement with 5.2, 10.6.6 and 11.

10.4.3 Clean-up B – Silica gel

Put glass wool (8.1.10) and 10 g silica gel (7.3.2.2) into the chromatographic tube (8.1.12). Then add a layer of sodium sulfate (7.2.4) and condition with 20 ml petroleum ether or hexane-like solvent (7.2.3). Apply the extract to the column when the level of the solvent is drained to approximately 0,5 cm above the column packing.

Elution is performed using a total of 100 ml petroleum ether or hexane-like solvent (7.2.3). Keeper substance (7.2.7) is added to the eluate, and then the eluate is concentrated to the desired volume (see 10.3).

10.4.4 Clean-up C – Gel permeation chromatography

The extract obtained is carefully reduced under a gentle nitrogen flow. The residue is immediately dissolved in 5 ml solvent mixture [ethyl acetate (7.3.3.2) and cyclohexane (7.3.3.3) (1:1 volume fraction)] and is then put into the GPC column.

The typical GPC system-settings are given below:

— flow rate: 5 ml/min;

— volume of the sample loop: 5 ml;

— first fraction: 120 ml (24 min);

— PAH elution: 155 ml (31 min);

— last fraction: 20 ml (4 min).

The elution volumes of the first fraction, eluate and last fraction shall be considered recommended values and shall regularly be verified by means of the multi-component PAH-standard solution.

Keeper substance (7.2.7) is added to the eluate, and then the eluate is reduced to the desired volume (see 10.3).

NOTE During use of the gel permeation column a small shift in volume to be collected can occur. This is visible in a decrease of recoveries of the internal standards. If this occurs, readjustment of the sampled volume can be necessary.

10.5 Addition of the injection standard

If addition of an injection standard is necessary, add an appropriate amount of the secondary injection standard solution (7.6.4) to the extract prior to injection (this amount shall be in line with the concentration of the calibration standard). Record the final volume *V*.

10.6 GC-MS analysis

10.6.1 Settings of the gas chromatograph

Operating conditions should be set for optimum separation of the PAHs (see 5.2). Optimize the gas chromatograph starting from the following conditions:

— Separation column: Capillary column (see 8.2.2);

— Oven temperature program: 60 °C, 2 min;
30 °C/min to 120 °C;
5 °C/min to 310 °C;
310 °C, 6 min;

— Injector temperature: 250 °C;

— Splitless injection: 1 µl, keep the split 1,8 min closed;

— Carrier gas: Helium 0,8 ml/min to 1 ml/min.

10.6.2 Mass spectrometric (MS) conditions

Tune the mass spectrometer in accordance with the manufacturer's instructions. Chromatograms are recorded in full scan or selected ion monitoring/recording mode (SIM/SIR). The ions to be selected are given in Table 6.

Table 6 — Diagnostic ions for PAHs to be used with GC-MS

Compound	CAS-RN	Parent ion m/z	Diagnostic ion 1 m/z	Diagnostic ion 2 m/z
Naphthalene	91–20–3	128 (100)	102 (11)	-
Naphthalene-d8	1146–65–2	136 (100)	108 (12)	137 (10)
Acenaphthene	83–32–9	154 (70)	153 (100)	76 (10)
Acenaphthene-d10	15067–26–2	162 (100)	164 (96)	80 (27)
Acenaphthylene	208–96–8	152 (100)	150 (3)	76 (0)
Acenaphthylene-d8	93951–97–4	162 (100)	164 (96)	80 (27)
Fluorene	86–73–7	166 (81)	165 (100)	139 (4)
Fluorene-d10	81103–79–9	176 (100)	174 (89)	
Anthracene	120–12–7	178 (100)	152 (12)	76 (0)
Anthracene-d10	1719–06–8	188 (100)	189 (14)	94 (9)
Phenanthrene	85–01–8	178 (100)	152 (9)	76 (3)
Phenanthrene-d10	1517–22–2	188 (100)	189 (14)	160 (12)
Fluoranthene	206–44–0	202 (100)	200 (31)	100 (3)
Fluoranthene-d10	93951–69–0	212 (100)	213 (17)	
Pyrene	129–00–0	202 (100)	200 (2)	101 (4)
Pyrene-d10	1718–52–1	212 (100)	213 (17)	
Benz[*a*]anthracene	56–55–3	228 (100)	226 (3)	114 (2)
Benz[*a*]anthracene-d12	1718–53–2	240 (100)	241 (19)	
Chrysene	218–01–9	228 (100)	226 (6)	113 (4)
Chrysene-d12	1719–03–5	240 (100)	236 (25)	241 (17)
Benzo[*b*]fluoranthene	205–99–2	252 (100)	250 (22)	126 (5)
Benzo[*b*]fluoranthene-d12	93951–98–5	264 (100)	260 (19)	
Benzo[*k*]fluoranthene	207–08–9	252 (100)	250 (22)	126 (5)
Benzo[*k*]fluoranthene-d12	93952–01–3	264 (100)	260 (19)	
Benzo[*a*]pyrene	50–32–8	252 (100)	250 (18)	113 (11)
Benzo[*a*]pyrene-d12	63466–71–7	264 (100)	260 (20)	
Indeno[*1,2,3-cd*]pyrene	193–39–5	276 (100)	138 (12)	274 (4)
Indeno[*1,2,3-cd*]pyrene-d12	203578–33–0	288 (100)	284 (19)	
Dibenz[*a,h*]anthracene	53–70–3	278 (100)	139 (9)	276 (5)
Dibenz[*a,h*]anthracene-d14	13250–98–1	292 (100)	279 (23)	
Benzo[*ghi*]perylene	191–24–2	276 (100)	138 (12)	274 (4)

Compound	CAS-RN	Parent ion m/z	Diagnostic ion 1 m/z	Diagnostic ion 2 m/z
Benzo[*ghi*]perylene-d12	93951–66–7	288 (100)	284 (19)	
m – mass of ion *z – charge number of ion* *numbers in brackets are the relative signal intensities*				

It is also possible to use GC-MS/MS. An example for measurement conditions is given in Annex B.3.

10.6.3 Calibration of the method using an internal standard

10.6.3.1 General

This is an independent method for the determination of 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 recovery of the compounds to be analysed is about equal to that of the internal standard.

Add a specific mass of the internal standard (7.6.3) and injection standard (if necessary 7.6.4) to dilutions of the mixed calibration solution (7.5.2). The mass concentration of both standards shall be the same for all calibration solutions and comparable with the concentration of both standards in the final extract. Run the GC-MS or GC-MS-MS analysis with the calibration solutions, prepared as described in 7.6. Calculate the relative response ratio for the native PAH and the deuterated PAH after obtaining a calibration curve by plotting the ratio of the mass concentrations against the ratio of the peak areas (or peak heights) using Formula (3):

$$\frac{A_{\mathrm{n}}}{A_{\mathrm{d}}} = s \cdot \frac{\rho_{\mathrm{n}}}{\rho_{\mathrm{d}}} + b \qquad (3)$$

where

A_{n} is the measured response of the native PAH, e.g. peak area;

A_{d} is the measured response of the deuterated PAH, e.g. peak area;

s is the slope of the calibration function;

ρ_{n} is the mass concentration of the native PAH in the calibration solution, expressed in micrograms per litre (µg/l);

ρ_{d} is the mass concentration of the deuterated PAH in the calibration solution, expressed in micrograms per litre (µg/l);

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

Two types of calibration are distinguished: the initial calibration (10.6.3.2) and the daily calibration (validity check of the initial calibration); the last one is called calibration verification (10.6.3.3.)

Nonlinear calibration methods may be applied e.g. ISO 8466-2 [6~~7~~].

10.6.3.2 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 (e.g. changing the analytical column, cleaning the MS-system) and/or repair of the equipment.

Take a gas chromatogram of a series of at least five standard solutions with equidistant concentrations, including the solvent blank. Identify the peaks using MS or gas chromatograms of the individual compounds. Prepare a calibration graph for each compound.

Check for linearity according to ISO 8466-1 [7~~8~~].

Nonlinear calibration using all five standards is allowed. In that case, the same five standards shall be used for recalibration.

10.6.3.3 Calibration verification

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

For every batch of samples, 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. If the straight line falls within ± 10 % of the reference values of the initial calibration line, the initial calibration line is assumed to be valid. If not, a new calibration line shall be established according to 10.6.3.2.

10.6.4 Measurement

With the aid of the absolute retention times, identify the peaks to be used to calculate the relative retention times. Use the internal standard as close as possible to the PAH-peak to be quantified. For the other relevant peaks in the gas chromatograms, determine the relative retention times.

If the concentration is above the level for proper identification or quantification, a diluted extract shall be injected for proper identification or quantification of the relevant PAHs or re-extract the sample using a lower amount of sample.

If as a result of dilution, the internal standard is outside the linear range, Formula (5) in 10.6.6 does not give the proper quantification and the deviation from linearity shall be taken into account.

10.6.5 Identification

Apply EN ISO 22892 for identification of PAH. EN ISO 22892 describes chromatographic- and MS-criteria necessary for proper identification. Use the diagnostic ions as given in Table 7. Quantifier ions as given in table B.4 (annexe B) can be used for GC/MS/MS.

10.6.6 Check on method performance

Because this document allows using different modules, comparing the measured response of the internal standards and injection standards in both the injected performance standard solution and the injected sample is a check on the performance of the total procedure.

Use for this analysis:

— the same final volume,

— the same definite volume of internal standard, and

— the same definite volume of injection standard,

as used for the samples.

This is the performance standard.

The performance standard may be one of the calibration standards, provided that the ratio of the volumes (internal standard/injection standard) used is the same.

Calculate for each internal standard the ratio between sample and performance standard solution using the closest injection standard with Formula (4).

$$U = \frac{A_1(S)}{A_2(S)} \times \frac{A_2(ps)}{A_1(ps)} \times 100 \quad (4)$$

where

U	is the recovery rate, expressed in percent (%);
A_1	is the measured response of the deuterated PAH internal standard, e.g. peak area;
A_2	is the measured response of the deuterated PAH injection standard, e.g. peak area;
ps	is the performance standard;
S	is the sample.

The recovery of the internal standard should be at least 50 %.

If the two-step procedure for addition of the internal standard has been used, calculate the extraction ratio between the non-labelled PAH added to the sample and the deuterated PAH to the extract using Formula (5):

$$E = \frac{A_{1,mean}(S) \times f}{A_3} \times \frac{A_3(ps)}{A_{1,mean}(ps)} \times 100 \quad (5)$$

where

E	is the extraction recovery rate in percent (%);
$A_{1,mean}$	is the average measured response of the deuterated PAH internal standard, e.g. peak area;
A_3	is the measured response of the non-labelled PAH internal standard, e.g. peak area;
f	is the fraction of the original extract used for clean-up;
ps	is the performance standard;
S	is the sample.

The values calculated for the concentrations of native PAHs in the sample are only considered to be acceptable if the recoveries of the internal standards fulfil the requirement described before. In other cases, perform a re-test or report the values as indicative.

10.6.7 Calculation

Calculate the mass content of the individual PAH from the multipoint calibration of the total method by using Formula (6):

$$w_n = \frac{(A_n / A_d) - b}{s \cdot m \cdot d_s} \cdot \rho_d \cdot f_e \cdot f_t \cdot V \cdot 100 \quad (6)$$

where

w_n	is the content of the individual PAH found in the sample, expressed in milligrams per kilogram (mg/kg) based on dry matter content;
A_d	is the measured response of the deuterated PAH in the sample extract;

A_n is the measured response of the native PAH in the sample extract;

ρ_d is the mass concentration of the deuterated PAH in the sample extract, expressed in micrograms per millilitre (µg/ml);

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

d_s is the dry matter fraction in the field moist sample, determined according to EN 15934 ~~[6]~~, expressed in percent mass fraction;

fe is the ratio of the total organic solvent volume used for extraction to that of the aliquot used for the analysis; f = 1 if the whole extract is used;

ft is the addition factor;

V is the volume of the final solution, expressed in millilitres (ml);

s is the slope of the recalibration function;

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

The result shall be expressed in milligrams per kilogram (mg/kg) dry matter and rounded to two significant figures.

10.7 High-performance liquid chromatographic analysis (HPLC)

10.7.1 General

Adjust the HPLC system according to the manufacturer's instructions. Regularly check baseline noise and baseline drift against the specifications given by the manufacturer. If the results of these tests do not meet the specified values, detect and eliminate the reasons.

10.7.2 Setting the HPLC

Set the HPLC (8.3) in such a way that sufficient separation of the PAHs is achieved (see 7.5.2). Optimize the HPLC starting from the following conditions:

- Separation column: Reversed phase column;
- Solvent A: Acetonitrile;
- Solvent B: Water;
- Gradient: 0 to 5 min – A: 50 % and B: 50 %;
  5 to 25 min; A: 50 to 90 % and B: 50 to 10 %;
  25 to 45 min; A: 90 % and B: 10 %;
- Injection volume: 5 µl;
- Oven temperature: 25°C.

NOTE Examples of chromatograms with instrumental conditions are given in Annex B.

10.7.3 Detection

10.7.3.1 General

Ultraviolet detectors, fluorescence detectors or a combination of both, offsetting the disadvantages of the relevant detectors, are suitable for detection.

10.7.3.2 Ultraviolet detector

Preferably, a diode array detector should be used, but comparable results can also be achieved by means of a variable-wavelength ultraviolet detector.

Diode array detectors allow for comparing the spectrum of the sample substance and the reference substance.

NOTE The advantage of ultraviolet detectors is that they have a bigger linear range than fluorescence detectors. Their disadvantage is that sensitivity and selectivity is lower in comparison to fluorescence detectors.

10.7.3.3 Fluorescence detector

If applied, use a fluorescence detector, which is capable of free selection of excitation and emission wavelengths and which is adjustable during chromatographic separation.

For detection choose the appropriate excitation and emission wavelengths with regard to sensitivity and selectivity.

During wavelength programming, baseline disturbance should be avoided.

Dissolved oxygen in the eluent can reduce the fluorescence signal. The eluent should be degassed using e.g. helium or vacuum.

10.7.3.4 Identification of individual compounds

An individual compound is assumed to be present if the retention time of the substance in the chromatogram of the sample agrees with the retention time in the chromatogram obtained from a reference substance in a reference solution, measured under the same conditions (tolerance ± 1 %, max. 10 s).

The verification of a positive result can be obtained, using different methods:

— by comparison of the excitation and emission spectrum of the substance in the sample, which has been allocated by its retention time, and the spectrum of the reference substance, taken under the same conditions;

— at higher concentrations identification may be achieved via the absorption spectrum using a diode array detector. This second detector shall not lead to interference through broadening of the fluorescence peaks;

— by application of an independent method, e.g. gas chromatography;

— by checking results obtained at two wavelengths.

10.7.4 Calibration

10.7.4.1 General

Use external standards for calibration. Take care to inject the same volume for calibration as for the sample measurement.

10.7.4.2 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.

Take a HPLC chromatogram of a series of at least five standard solutions with equidistant concentrations, including the solvent blank. Identify the peaks in accordance to 10.7.3.4. Prepare a calibration graph for each compound.

Check for linearity according to ISO 8466-1.

10.7.4.3 Calibration verification

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

For every series of samples, 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. If the straight line falls within ± 10 % of the reference values of the initial calibration line, the initial calibration line is assumed to be valid. If not, a new calibration line shall be established according to 10.7.4.2.

10.7.5 Measurement

Equilibrate the measuring system before measuring samples and adjust the wavelength programme in relation to the retention times found.

NOTE Reproducible retention times are usually achieved after two or three injections of a reference solution.

With the aid of the retention times, identify the peaks.

Ensure that the peaks of each sample are being integrated correctly. If the concentration is above the level for proper identification or quantification, a diluted extract shall be injected for proper identification or quantification of the relevant PAHs or re-extract the sample using a lower amount of sample.

10.7.6 Calculation

Assuming the expected peak area or peak height is within the linear measuring range, the quantified result of an identified substance can be obtained using Formula (7):

$$w_n = \frac{A_n - b}{s \cdot m \cdot d_s} \cdot V \cdot f_i \cdot 100 \qquad (7)$$

where

w_n	is the content of the *individual* PAH found in the sample, expressed in milligrams per kilogram (mg/kg) on the basis of the dry matter;
A_n	is the measured response of the native PAH in the sample extract;
f_i	is the dilution factor in the HPLC analysis or other dilution steps;
V	is the volume of the extract, expressed in litre, l;
m	is the mass of the test sample used for extraction in kilograms, kg;
d_s	is the dry matter fraction in the field moist sample, determined according to EN 15934, expressed in percent mass fraction;
s	is the slope of the recalibration function;
b	Is the intercept of the recalibration curve with the ordinate.

The result shall be expressed in milligrams per kilogram (mg/kg) dry matter and rounded to two significant figures.

NOTE If two signals are used for quantification, the result is confirmed if the results do not differ more than 15 %, relative, to the lower of the two results. If the difference is larger than 15 %, the lower of the two results is reported.

11 Performance characteristics

The method is "performance based". It is permitted to modify the method to overcome interferences not specified in this European Standard, provided that the performance criteria are met. Internal standards for GC-MS and extraction standards for GC-MS and HPLC both shall be used to check the pre-treatment, extraction and clean-up procedures. Recoveries of these standards should be 50 % to 110 %. If the recovery is outside the limits (i.e. 50 % to 110 %) the method shall be modified using other modules described in this European Standard.

Some samples can require multiple clean-up in case of lower recoveries.

12 Precision

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

13 Test report

The test report shall contain at least the following information:

a) a reference to this document;

b) complete identification of the sample;

c) the extraction module, clean-up module and detection module used for the analysis;

d) the results of the determination according to 10.6 (GC-MS) and 10.7 (HPLC);

e) any details not specified in this document or which are optional, as well as any factor which may have affected the results;

f) date of receipt of the sample and date of report.

The summary of extraction, clean-up and detection procedures used for the analysis shall be documented by the laboratory and shall be made available on request.

Annex A
(informative)

Repeatability and reproducibility data

A.1 Materials used in the interlaboratory comparison study

Table A.1 lists the types of materials tested.

Table A.1 — Materials tested in the interlaboratory comparison for the determination of polycyclic aromatic hydrocarbons (PAH) by gas chromatography (GC) and high performance liquid chromatography (HPLC) in compost and digestate

Sample	Grain size	Material tested	Results
Compost	< 0,~~2~~5 mm		Table A.2
Digestate			Table A.2

A.2 Interlaboratory comparison results

The statistical evaluation was conducted according to ISO 5725-2[9~~10~~]. The average values, the repeatability standard deviation (s_r) and the reproducibility standard deviation (s_R) were obtained (Table A.2 and A.3).

Table A.2 — Results of the interlaboratory comparison studies of the determination of PAH by GC-MS and HPLC in compost and digestate

Parameter	Matrix	l_o	l	n_o	n	$\bar{x}$ mg/kg	S_R mg/kg	CV_R %	S_r mg/kg	CV_r %
Naphthalene	Compost									
	Digestate									
Acenaphthy-lene										
Acenaphthene										
Fluorene										
Phenanthrene										
Anthracene										
Fluoranthene										
Pyrene										
Benz[*a*]-anthracene										
Chrysene										
Benzo[*b*] -fluoranthene										

Parameter	Matrix	l_o	l	n_o	n	$\bar{x}$ mg/kg	S_R mg/kg	CV_R %	S_r mg/kg	CV_r %
Benzo[*k*]-fluoranthene										
Sum Benzo[*b+k*]-fluoranthene										
Benzo[*a*]-pyrene										
Benzo[*ghi*]-perylene										
Dibenz[*ah*]-anthracene										
Indeno[*1,2,3-cd*]-pyrene										
Sum 16 EPA PAH										

Parameter	Matrix	l_o	l	n_o	n	$\bar{x}$ mg/kg	S_R mg/kg	CV_R %	S_r mg/kg	CV_r %
Sum 15 EPA PAH (excl, Acenaphthy-lene)										

l_O Number of participating laboratories

l Number of laboratories after elimination of outliers

n_o Number of single measurement values

n Number of single measurement values without outliers

$\bar{x}$ Total mean (without outliers)

S_R Reproducibility standard deviation

CV_R Coefficient of variation of reproducibility

S_r Repeatability standard deviation

CV_r Coefficient of variation of repeatability

Annex B
(informative)

Examples of instrumental conditions and chromatograms

B.1 Measurement of PAH with GC-MS

GC-MS conditions:

Instrument:	— Agilent GC 7890A, Agilent MS 5975C;
Column (dimensions, phase, etc):	— Agilent CP8994VF-5ms[3];
	— 30 m;
	— 0,25 µm film thickness;
	— 0,25 mm internal diameter;
Carrier gas:	— Helium constant flow 2 ml/min;
Injection technique:	— Splitless 75 °C
Injection volume:	— 1 µl;
Temperature programme:	— Start 60 °C hold time 1,25 min;
	— 90 °C/min to 140 °C hold time 0 min;
	— 45 °C/min to 250 °C hold time 3 min;
	— 40°C/min to 325°C hold time 2,75 min.

3) DB 5ms is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can be shown to lead to the same results.

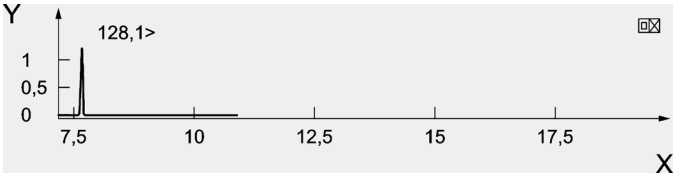
Table B.1 — SIM mass of the native and deuterated PAH

Native-PAH		SIM mass	Deuterated PAH		SIM mass
1	Naphthalene	128,1	1-D	Naphthalene d8	136,1
2	Acenaphthylene	152,1	2-D	Acenaphthylene d8	160,1
3	Acenaphthene	154,1	3-D	Acenaphthene d10	164,1
4	Fluorene	166,1	4-D	Fluorene d10	176,1
5	Phenanthrene	178,1	5-D	Phenanthrene d10	188,1
6	Anthracene	178,1	6-D	Anthracene d10	188,1
7	Fluoranthene	202,1	7-D	Fluoranthene d10	212,1
8	Pyrene	202,1	8-D	Pyrene d10	212,1
9	Benz[*a*]anthracene	228,1	9-D	Benzo[*a*]anthracene d12	240,1
10	Chrysene	228,1	10-D	Chrysene d12	240,1
11	Benzo[*b*]fluoranthene	252,1	11-D	Benzo[*b*]fluoranthene d12	264,1
12	Benzo[*k*]fluoranthene	252,1	12-D	Benzo[k]fluoranthene d12	264,1
13	Benzo[*a*]pyrene	252,1	13-D	Benzo[*a*]pyrene d12	264,1
14	Benzo[*ghi*]perylene	276,1	14-D	Benzo[*ghi*]perylene d12	288,2
15	Dibenz[*a,h*]anthracene	278,1	15-D	Dibenz[*a,h*]anthracen d14	292,2
16	Indeno[*1,2,3-cd*]pyrene	276,1	16-D	Indeno[*1,2,3-cd*]pyrene d12	288,1

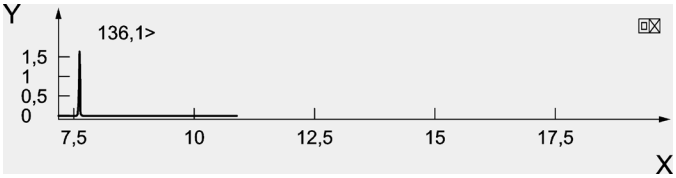
Concentration of each component in the SIM chromatogram:

PAH - Native: 570 ng/g

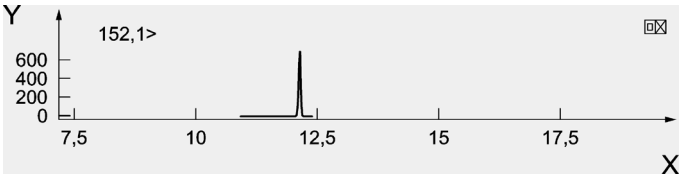
PAH – Deuterated: 468 ng/g



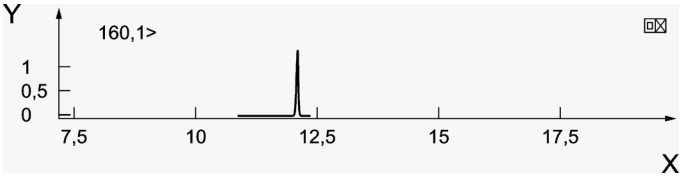
a) Naphthalene



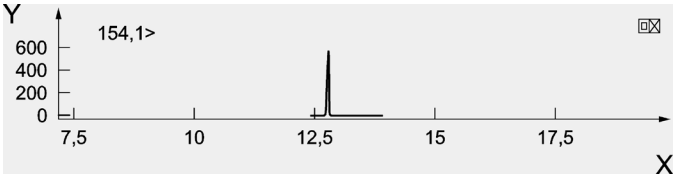
b) Naphthalene-d8



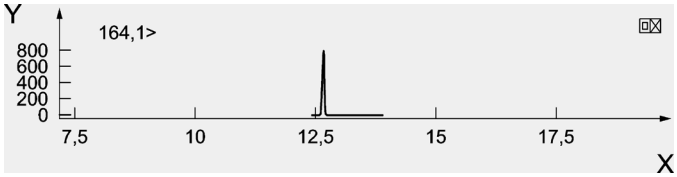
c) Acenaphthylene



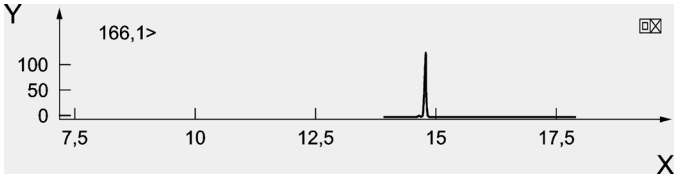
d) Acenaphthylene-d8



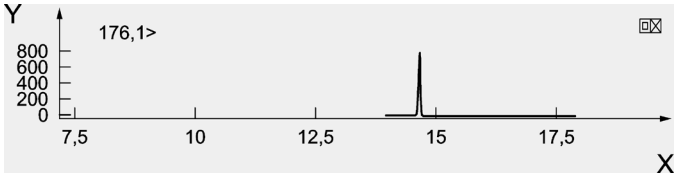
e) Acenaphthene



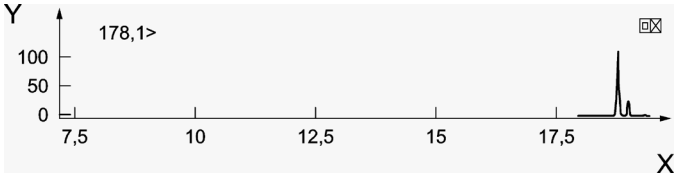
f) Acenaphthene-d10



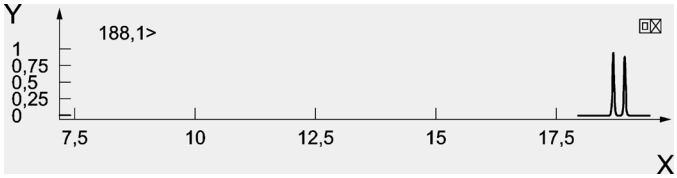
g) Fluorene



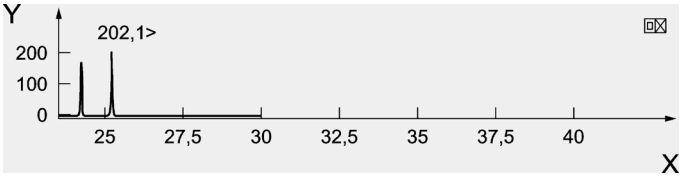
h) Fluorene-d10



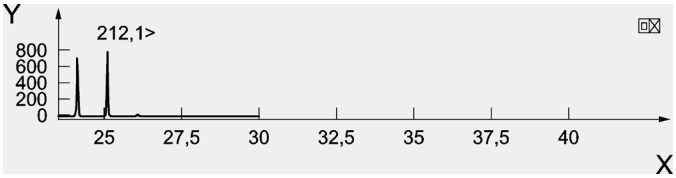
i) Phenanthrene, Anthracene



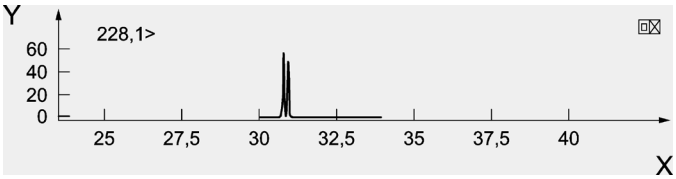
j) Phenanthrene-d10, Anthracene-d10



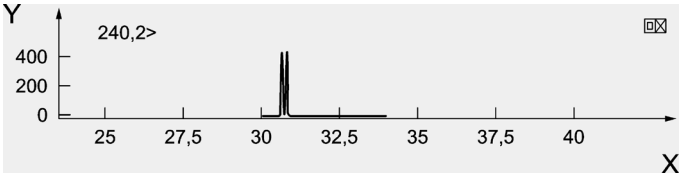
k) Fluoranthene, Pyrene



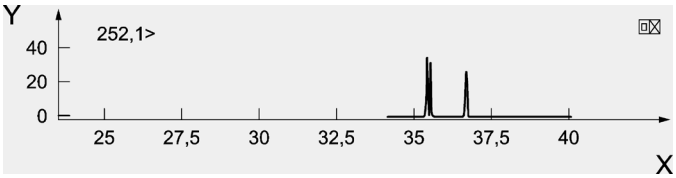
l) Fluoranthene-d10, Pyrene-d10



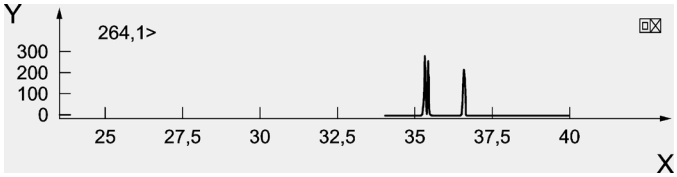
m) Benz[*a*]anthracene, Chrysene



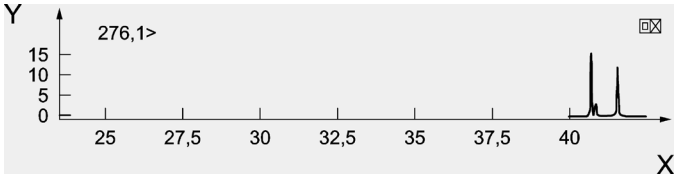
n) Benz[*a*]anthracene-d12, Chrysene-d12



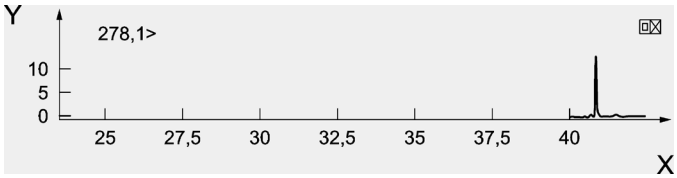
o) Benzo[*b*]fluoranthene, Benzo[*k*]fluoranthene, Benzo[*a*]pyrene



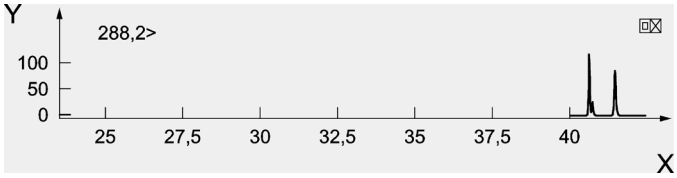
p) Benzo[*b*]fluoranthene-d12, Benzo[*k*]fluoranthene-d12, Benzo[*a*]pyrene-d12



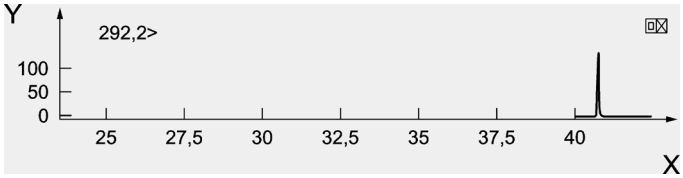
q) Indeno[*1,2,3-cd*]pyrene, Benzo[*ghi*]perylene



r) Dibenz[*a,h*]anthracene



s) Indeno[*1,2,3-cd*]pyrene-d12, Benzo[*ghi*]perylene-d12



t) Dibenz[*a,h*]anthracene-d14

X time scale

Y abundance

Figure B.1 — Example chromatograms

B.2 Measurement of PAH with HPLC fluorescence

HPLC fluorescence conditions:

Column:	SEPSERV 150 mm × 2 mm Ultrasep ES PAK 5 µm[4)]
Flow:	0,25 ml/min
Injection volume:	5 µl
Temperature programme:	25 °C

Gradient A			Gradient B		
Time min	Acetonitrile %	Water %	Time min	Methanol %	Water %
0 to 5	50	50	0 to 10	65 to 91	35 to 9
5 to 25	50 to 90	50 to 10	10 to 60	91	9
25 to 45	90	10			

Table B.2 — Key and concentrations for the target analytes in the example chromatograms (Figures B.1 to B.4) measured with HPLC-fluorescence detection

Nr	PAH Standard	µg/ml
1	Naphthalene	0,48
2	Acenaphthylene	–
3	Acenaphthene	0,50
4	Fluorene	0,11
5	Phenanthrene	0,08
6	Anthracene	0,02
7	Fluoranthene	0,18
8	Pyrene	0,20
9	Benz[*a*]anthracene	0,10
10	Chrysene	0,09
11	Benzo[*b*]fluoranthene	0,10
12	Benzo[*k*]fluoranthene	0,11

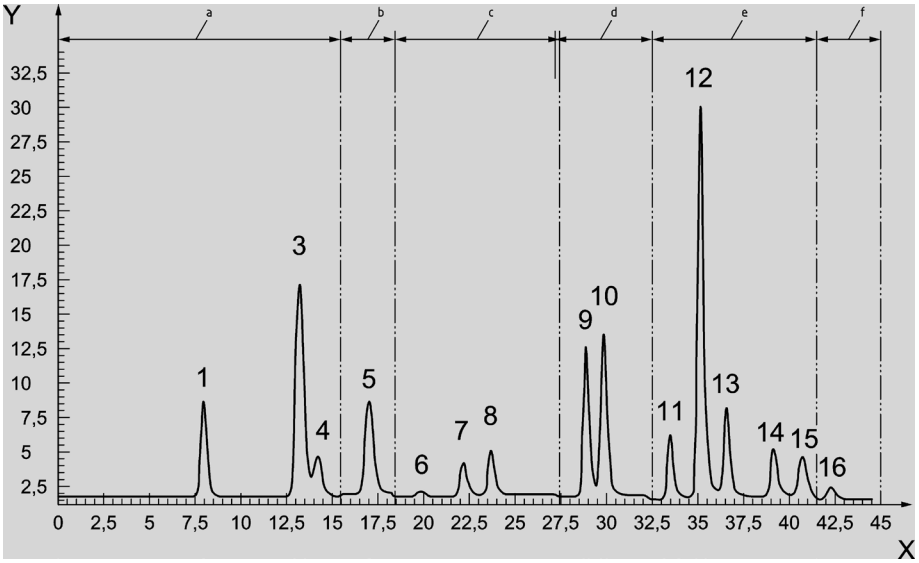
4) SEPSERV 150 mm × 2 mm Ultrasep ES PAK 5 µm is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can be shown to lead to the same results.

Nr	PAH Standard	µg/ml
13	Benzo[*a*]pyrene	0,12
14	Benzo[*ghi*]perylene	0,09
15	Dibenz[*a,h*]anthracene	0,09
16	Indeno[*1,2,3-cd*]pyrene	0,10
INT	6-Methylchrysene	

Table B.3 — Maximum absorption wavelengths and recommended/optimum combinations excitation/emission wavelengths

PAH	λ_{max} for UV abs. nm	Recommended $\lambda_{ex}/\lambda_{em}$ nm	Optimum $\lambda_{ex}/\lambda_{em}$ nm
Naphthalene	220	280/340	280/334
Phenanthrene	251	280/340	292/366
Anthracene	252	305/430	253/402
Fluoranthene	236	305/430	360/460
Benz[*a*]anthracene	287	305/430	288/390
Chrysene	267	305/430	268/383
Benzo[*k*]fluoranthene	307	305/430	308/414
Benzo[*a*]pyrene	296	305/430	296/408
Benzo[*ghi*]perylene	299	305/430	300/410
Indeno[*1,2,3-cd*]pyrene	250	305/500	302/506
Acenaphthene	227	280/340	292/324
Acenaphthylene	254	— [a]	— [a]
Fluorene	261	280/340	268/308
Pyrene	240	305/430	336/376

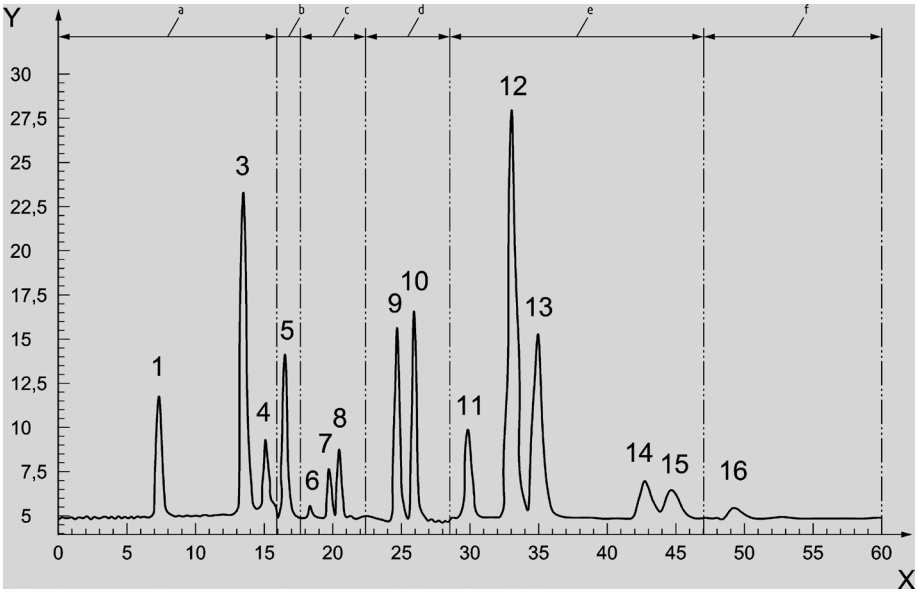
[a] Acenaphthylene cannot be measured using fluorescence detection.



X time scale

Y abundance

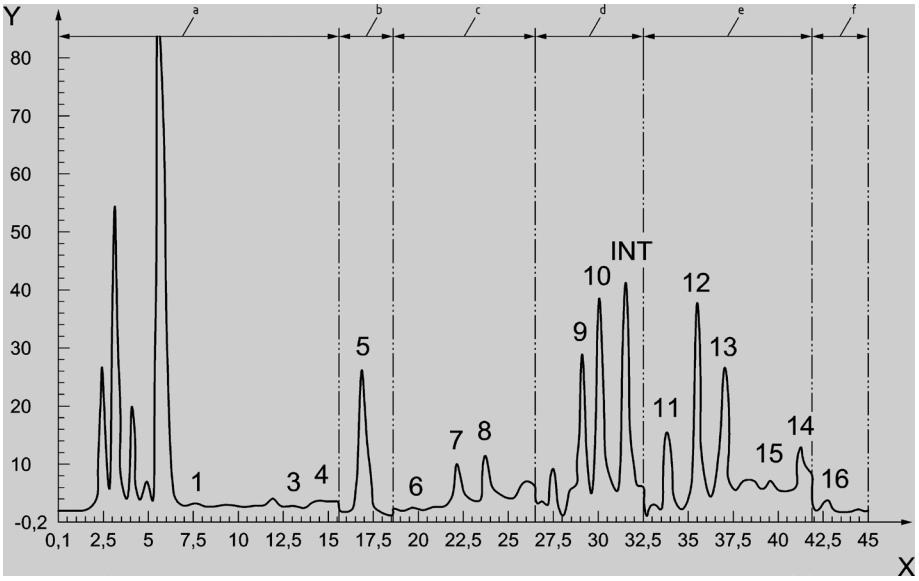
Figure B.2 — HPLC-Fluorescence chromatogram of PAH Standards – Gradient A (acetonitrile/water)



X time scale

Y abundance

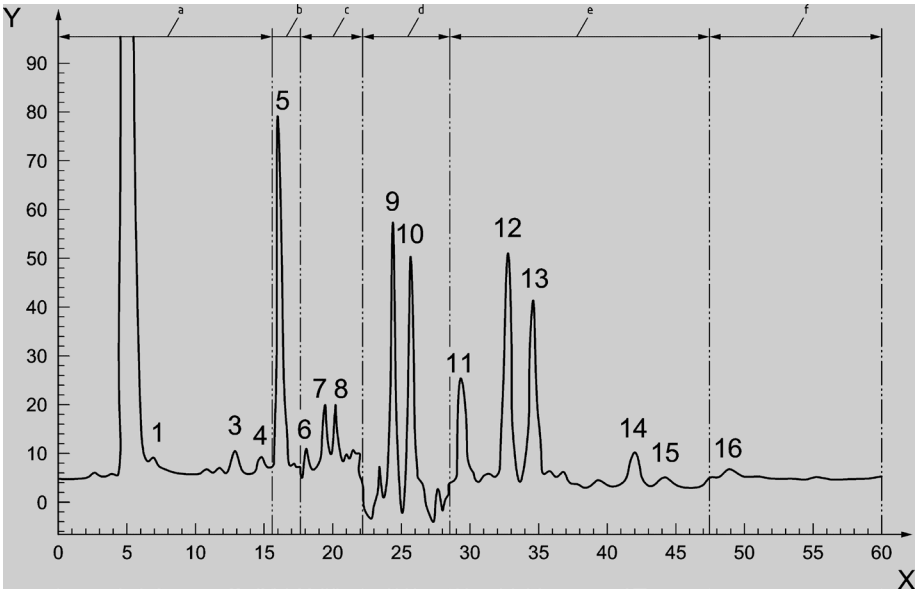
Figure B.3 — HPLC-Fluorescence chromatogram of PAH Standards – Gradient B (methanol/water)



X time scale

Y abundance

Figure B.4 — Sample Sludge – Gradient A



X time scale

Y abundance

Figure B.5 — Sample soil – Gradient B

B.3 Example for measurement conditions of PAH with GC-MS/MS

GC-MS conditions:

Instrument:	— GC-MS/MS Agilent 8890/7000D[5])
Column (dimensions, phase, etc):	— DB-EUPAH[6])
	— 15 m;
	— 0,25 µm film thickness;
	— 0,25 mm internal diameter;
Carrier gas:	— Helium constant flow, 1 ml/min;
Injection technique:	— Injector 320 °C; pulsed splitless;
Injection volume:	— 1 µl;
Temperature programme:	— Start 80 °C for 1 min;
	— 25 °C/min to 200 °C;
	— 8 °C/min to 335 °C;
	— 6 min isotherm.
Mode:	MRM
Solvent delay:	4 min
EM voltage gain mode:	10
Quad temperature:	150 °C
Source temperature:	320 °C
Transfer line temperature:	320 °C

5) GC-MS/MS Agilent 8890/7000D is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can be shown to lead to the same results.

6) DB EUPAH is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can be shown to lead to the same results.

Table B.4 — MRM transitions used for quantifier and qualifiers

Compound	RT (min)	Quantifier	CE (eV)	Qualifier	CE (eV)
Naphthalene-d8	5,041	136 → 136	19		
Naphthalene	5,067	128 → 102	22	128 → 127	20
Acenaphthylene	6,986	152 → 150	40	152 → 151	40
Acenaphthylene-d8	7,095	162 → 160	19		
Acenaphthene	7,149	154 → 152	40	153 → 152	40
Fluorene	7,858	166 → 165	30	166 → 163	34
Phenanthrene-d10	9,819	188 → 188	19		
Phenanthrene	9,879	178 → 176	34	178 → 152	30
Anthracene	9,940	178 → 176	34	178 → 152	30
Fluoranthene	12,882	202 → 200	50	202 → 201	50
Pyrene	13,692	202 → 200	50	202 → 201	30
Benz[*a*]anthracene	17,145	228 → 226	38	228 → 224	38
Chrysene-d12	17,309	240 → 236	25	118 → 116	25
Chrysene	17,400	228 → 226	38	228 → 224	38
Benzo[*b*]fluoranthene	20,379	252 → 250	42	250 → 248	40
Benzo[*k*]fluoranthene	20,445	252 → 250	42	250 → 248	40
Benzo[*a*]pyrene	21,549	252 → 250	40	250 → 248	40
Dibenz[*a,h*]anthracene	24,474	278 → 276	38	276 → 274	38
Indeno[*1,2,3-cd*]pyrene	24,504	276 → 274	42	138 → 124	30
Benzo[*ghi*]perylene	25,644	276 → 274	42	274 → 272	42
RT – retention time *CE – collision energy*					

The specified values for MRM transition are an example from publication [12~~3~~]. They can vary depending on the equipment.

Bibliography

[1] EN 16181:2018, *Soil, treated biowaste and sludge — Determination of polycyclic aromatic hydrocarbons (PAH) by gas chromatography (GC) and high performance liquid chromatography (HPLC)*

[2] EN 15527:2008, *Characterization of waste — Determination of polycyclic aromatic hydrocarbons (PAH) in waste using gas chromatography mass spectrometry (GC/MS)*

[3] EN 17322:2018, *Determination of polychlorinated biphenyls (PCB) by gas chromatography - mass selective detection (GC-MS) or electron-capture detection (GC-ECD)*

[4] EN 16179, *Sludge, treated biowaste and soil — Guidance for sample pretreatment*

[5] EN 15002, *Characterization of waste — Preparation of test portions from the laboratory sample*

~~[6] EN 15934, *Sludge, treated biowaste,soil and waste – Calculation of dry matter fraction after determination of dry residue or water content*~~

[6~~7~~] ISO 8466-2, *Water quality – Calibration and evaluation of analytical methods and estimation of performance characteristics – Part 2: Calibration strategy for non-linear second-order calibration functions*

[~~8~~7] 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*

[~~9~~8] ISO 18287, *Soil quality — Determination of polycyclic aromatic hydrocarbons (PAH) – Gas chromatographic method with mass spectrometric detection (GC-MS)*

[~~10~~9] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[10~~1~~] Brockmann H, Schrodder H, Ber. Deut. Chem. Ges. (1941), 74 p. 73

[11~~2~~] KALBE U., LEHNIK-HABRINK P., BANDOW N., SAUER A. Validation of European horizontal methods for the analysis of PAH, PCB and dioxins in sludge, treated biowaste and soil. *Environ. Sci. (Ruse)*. 2019, **31** p. 29

[12~~3~~] Andrianova A, Quimby B, Agilent Application note 5994-0499EN (2019)

[13~~4~~] CEN/TS 00223137, *Soil improvers and growing media — Terminology*