INTERNATIONAL STANDARD

ISO 13859

First edition
2014-02-01

Soil quality — Determination of polycyclic aromatic hydrocarbons (PAH) by gas chromatography (GC) and high performance liquid chromatography (HPLC)

Qualité du sol — Détermination des hydrocarbures aromatiques polycycliques (HAP) par chromatographie en phase gazeuse (CPG) et chromatographie liquide à haute performance (CLHP)

Reference number
ISO 13859:2014(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 190, *Soil quality*, Subcommittee SC 3, *Chemical methods and soil characteristics*.

Introduction

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 preceding steps for sampling, pretreatment, extraction, clean-up by measurement of specific PAH by means of gas chromatography in combination with mass spectrometric detection (GC-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.

It is to be underlined that the target contamination level of PAH can lie in the range of about 0,01 mg/kg per individual PAH (agricultural soil and sediment) to about 200 mg/kg and higher (e.g. contaminated soil at coking plant sites or waste). The use of internal and injection standards is described in order to have an internal check on execution of the extraction and clean-up procedure. The method is as far as possible in agreement with the method described for PCBs (see EN 16167).

This International Standard is the result of a desk study "Horizontal International Standard for determination of PAH in sludge, soil, and biowaste" in the project "Horizontal" and aims at evaluating the latest developments in assessing PAH in sludge, soil, treated biowaste, and neighbouring fields. After an evaluation study, in which the ruggedness of the method was studied, a European-wide validation of the draft standard has taken place. The results of the desk studies as well as the evaluation and validation studies have been subject to discussions with all parties concerned in CEN.

This International Standard is applicable and validated for several types of matrices as indicated in Table 1 (see also Annex A for the results of the validation).

Table 1 — Matrices for which this International Standard is applicable and validated

Matrix	Materials used for validation
Sludge	Municipal sludge
Biowaste	Fresh compost

Soil quality — Determination of polycyclic aromatic hydrocarbons (PAH) by gas chromatography (GC) and high performance liquid chromatography (HPLC)

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

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

1 Scope

This International Standard specifies the quantitative determination of 16 PAH (see Table 2) in sludge, soil, and treated biowaste using GC-MS and HPLC-UV-DAD/FLD covering a wide range of PAH contamination levels (see also Annex B).

When using fluorescence detection, acenaphthylene cannot be measured.

Table 2 — Polycyclic aromatic hydrocarbons which can be analysed using this International Standard

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] 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.

Typically, a lower limit of application of 0,01 mg/kg (expressed as dry matter) can be ensured for each individual PAH. This depends on instrument and sample.

Sludge, soil, and treated biowaste can differ in properties and also in the expected contamination levels of PAH and presence of interfering substances. These differences make it impossible to describe one general procedure. This International Standard contains decision tables based on the properties of the sample and the extraction and clean-up procedure to be used. Two general lines are followed, an agitation procedure (shaking) or use of Soxhlet/pressurized liquid extraction.

NOTE Other PAH compounds can also be analysed with this method, provided suitability has been proven.

2 Normative references

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

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

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

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

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

ISO 18512, *Soil quality — Guidance on long and short term storage of soil samples*

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

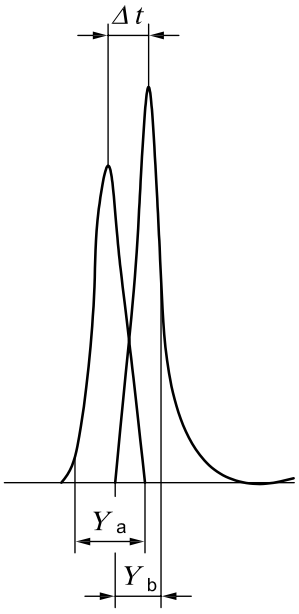
3 Terms and definitions

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

3.1
critical pair
pair of congeners that will be separated to a predefined degree (e. g. $R = 0,5$) to ensure chromatographic separation meets minimum quality criteria

[SOURCE: EN 15308:2008, 3.6]

Note 1 to entry: See Figure 1.



Key

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

4 Principle

After pretreatment to reduce the moisture content and to increase the homogeneity (see 9.2), the test sample is extracted with a solvent.

The extract is concentrated and interfering compounds are removed by a clean-up method suitable for the specific matrix. The eluate is concentrated. For HPLC analysis, the concentrated eluate is taken up in an appropriate less volatile water miscible polar solvent and the non-polar eluate residue is removed.

The extract is analysed by GC-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 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 external standard solutions. The efficiency of the procedure depends on the composition of the matrix that is investigated.

5 Interferences

5.1 Interference with sampling and extraction

Use sampling containers of materials (preferably of steel, aluminium, or glass) that do not change 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 can 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 incomplete 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. Unsymmetrical 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 (e. g. 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. Triphenylene cannot be completely separated from benz(*a*)anthracene and chrysene. In this case it shall be stated in the report.

5.3 Interferences with the HPLC

Substances that show either fluorescence or quenching and co-elute with the PAH to be determined can interfere 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-ring and 3-ring PAH. Extracts shall be diluted sufficiently with acetonitrile for the HPLC analysis, otherwise the detection of naphthalene and 3-ring PAH can be interfered by a broad toluene peak.

Separation between dibenz(*a,h*)anthracene and indeno(1,2,3-cd)pyrene can be critical. When incomplete resolution is encountered, peak integration shall be checked and, when necessary, corrected.

Usually perylene is incompletely resolved from 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.

PAH can co-distil with solvent and become deposited outside of stoppered bottles. All containers containing solutions of PAH in solvent shall therefore always be handled using gloves which are solvent resistant and preferably disposable.

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

Vessels containing PAH solutions should be stored standing in beakers to contain any spillage in the case of breakage.

Solid PAH are the most dangerous and give rise to a dust hazard due to their crystals becoming electrostatically charged. These materials shall only be handled where proper facilities are available (e. g. adequate fume hoods, protective clothing, dust masks). It is strongly advised that standard solutions are prepared centrally in suitably equipped laboratories or purchased from suppliers specialized in their preparation.

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

National regulations shall 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 shall be less than 50 % of the lowest reporting limit.

7.2 Reagents for extraction

7.2.1 **Acetone (2-propanone)**, C_3H_6O.

7.2.2 **Petroleum ether**, boiling range 40 °C to 60 °C.

NOTE Hexane-like solvents with a boiling range between 30 °C and 69 °C are allowed.

7.2.3 **Toluene**, C_7H_8.

7.2.4 **Anhydrous 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, anhydrous.

7.3 Reagents for clean-up

7.3.1 Clean-up using aluminium oxide

7.3.1.1 Aluminium oxide, Al_2O_3, basic or neutral, specific surface 200 m^2/g, activity Super I according to Brockmann.

NOTE 1 Hexane-like solvents with a boiling range between 30 °C and 69 °C are allowed.

NOTE 2 Brockman Activity Scale is a measure of the percentage of water added to the adsorbent based upon weight/weight relationships between water and the adsorbent. Grade I corresponds to 0 % water added.[14] [15]

7.3.1.2 Deactivated aluminium oxide, deactivated with approximately 10 % water.

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.

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

7.3.2 Clean-up 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, heated for at least 3 h at 450 °C, cooled down in a desiccator, and stored containing magnesium perchlorate or a suitable drying agent. Before use, heat at least for 5 h at 130 °C in a drying oven. Allow cooling in a desiccator and add 10 % water (mass fraction) in a flask. Shake for 5 min intensively until

all lumps have disappeared, and then for 2 h in a shaking device (8.1.2). Store the deactivated silica gel in the absence of air; use it for maximum of two weeks.

Silica gel 60 is stable for at most two weeks.

7.3.3 Clean-up using gel permeation chromatography (GPC)

7.3.3.1 Bio-Beads®[1] S-X3.

7.3.3.2 Ethyl acetate, $C_4H_8O_2$.

7.3.3.3 Cyclohexane, C_6H_{12}.

7.3.3.4 Spherical, porous styrene divinylbenzene resin.

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) 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.

7.3.4 Clean-up using liquid-liquid partition/DMF/cyclohexane

7.3.4.1 Dimethylformamide (DMF), C_3H_7NO.

7.3.4.2 Dimethylformamide:water, **9:1**.

7.4 Reagents for chromatographic analysis

7.4.1 GC-analysis

Carrier gas for GC-MS: helium or hydrogen of high purity and in accordance with the manufacturer's specifications.

7.4.2 HPLC analysis

7.4.2.1 Mobile phase.

7.4.2.2 Acetonitrile, CH_3CN or methanol, CH_3OH, HPLC purity grade.

7.4.2.3 Ultra-pure water, HPLC purity grade.

7.4.2.4 Helium, He, of suitable purity for degasification of solvents.

1) Bio-Beads© 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 ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

7.5 Standards

7.5.1 Reference substances, internal standards

Choose the internal standards substances whose physical and chemical properties (such as extraction behaviour, retention time) are similar to those of the compounds to be analysed. A minimum of five labelled internal standards shall be used as internal standards for the GC-MS method for evaluation of results. Verify the stability of the internal standards regularly. Table 3 contains native and deuterated PAH to be used for calibration of specific analytes.

Table 3 — Native PAH and deuterated PAH

PAH reference substances		Internal standard substances deuterated PAH
Naphthalene	(CAS-RN 91-20-3)	Naphthalene-d8
Acenaphthene	(CAS-RN 83-32-9)	Acenaphthene-d10
Acenaphthylene	(CAS-RN 208-96-8)	Acenaphthylene-d8
Fluorene	(CAS-RN 86-73-7)	Fluorene-d10
Anthracene	(CAS-RN 120-12-7)	Anthracene-d10
Phenanthrene	(CAS-RN 85-01-8)	Phenanthrene-d10
Fluoranthene	(CAS-RN 206-44-0)	Fluoranthene-d10
Pyrene	(CAS-RN 129-00-0)	Pyrene-d10
Benz(*a*)anthracene	(CAS-RN 56-55-3)	Benz(*a*)anthracene-d12
Chrysene	(CAS-RN 218-01-9)	Chrysene-d12
Benzo(*b*)fluoranthene	(CAS-RN 205-99-2)	Benzo(*b*)fluoranthene-d12
Benzo(*k*)fluoranthene	(CAS-RN 207-08-9)	Benzo(*k*)fluoranthene-d12
Benzo(*a*)pyrene	(CAS-RN 50-32-8)	Benzo(*a*)pyrene-d12
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
Dibenz(*a,h*)anthracene	(CAS-RN 53-70-3)	Dibenz(*a,h*)anthracene-d14
Benzo(*ghi*)perylene	(CAS-RN 191-24-2)	Benzo(*ghi*)perylene-d12

[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 standard.

NOTE 2 Certified solutions of PAH, and single solid PAH substances with certified purity are available from a limited number of suppliers e. g. Institute for Reference Materials and Measurements (IRMM) B-2440 Geel, Belgium; National Institute of Science and Technology; Office of Standard Ref. Data, Washington DC 20 234 U.S.A; or from other commercial providers.

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

7.5.2 Injection standard

7.5.2.1 GC-MS

A deuterated PAH such as 1-Methylnaphthalene-d10, Triphenylene-d12, and Perylene-d12 shall be added to the final extract before GC-MS injection to check the recovery of the deuterated internal standards.

7.5.2.2 HPLC

For this method, a recovery control shall be made by addition, and which is not interfering with the target analytes, of a suitable native PAH not mentioned in the scope, e. g. 6-methylchrysene, to the sample before extraction. The recovery range for this control should be between 70 % and 110 %.

7.6 Preparation of standard solutions

7.6.1 General

Commercially available, preferably certified standard solutions should be used due to the dangerous nature of the substances. Avoid skin contact.

7.6.2 Standard solutions for HPLC analysis

7.6.2.1 Single substance stock solutions

Prepare solutions of the single substances (see Table 3) in acetonitrile (7.4.2.2) to achieve a mass concentration of 10 µg/ml. These solutions are used for confirmation and identification of single PAH in the chromatogram.

7.6.2.2 Multiple substance stock solution

Prepare solutions of the reference substances (see Table 3) in acetonitrile (7.4.2.2) to achieve a mass concentration of the respective individual substance of 10 µg/ml.

Solutions according to 7.6.2.1 and 7.6.2.2 are stable for at least one year when stored in the dark at room temperature and protected from evaporation.

7.6.2.3 Calibration solutions

Prepare at least five calibration solutions by appropriate dilution of the stock solution (7.6.2.1 or 7.6.2.2), using acetonitrile (7.4.2.2) or methanol (7.4.2.2) as solvent. The choice of solvent depends on the composition of the mobile phase.

Transfer 50 µl of the stock solution into a graduated 5-ml flask and fill up to the mark with acetonitrile. 1 µl of this reference solution contains 100 pg of the respective individual substances.

Check the stability of the reference solutions regularly.

Check the mass concentration of the PAH in the stock solution by comparison with an independent, preferably certified, standard solution.

7.6.3 Standard solutions for GC-MS analysis

7.6.3.1 Single substance stock solution

Prepare solutions of the single substances of native and deuterated PAH (see Table 3) in toluene (7.2.3) or cyclohexane (7.3.3.3) to achieve a mass concentration of 10 µg/ml. These solutions are used for confirmation and identification of single PAH in the chromatogram.

The single substance stock solutions shall be stored in a dark place at about –15 °C to –18 °C. Store the diluted standard solutions at (5 ± 3) °C protected from light and evaporation. The solutions are stable for about one year.

7.6.3.2 Multiple substance stock solution of native PAH

Dilute the solution of the reference substances, i. e. native PAH (see Table 3) in toluene (7.2.3) or cyclohexane (7.3.3.3), to achieve a mass concentration of the respective individual substance, i. e. 10 µg/ml.

7.6.3.3 Multiple substance stock solution of deuterated or labelled PAH (internal standard)

Multiple deuterated or labelled PAH standards for use as internal standard, also available as mixtures in suitable solvent, can be diluted to the same mass concentration, i. e. 10 µg/ml for each individual deuterated standard.

7.6.4 Calibration standard solutions

Prepare a series of calibration standard solutions (at least five) over a suitable range by transferring different volumes of the multiple substance stock solution of native PAH standards (7.6.3.2) and a constant volume of the internal standard solution (7.6.3.3) into a volumetric flask and fill up with cyclohexane (7.3.3.3).

7.7 Preparation of internal standard solutions

Multiple substance stock solution of deuterated or labelled PAH (7.6.3.3) can be used for spiking of the sample before extraction. The use of the spiked internal standards shall be adjusted so that their concentration in the final extract for GC-MS determination is the same as in the calibration solutions (e. g. 100 pg/µl). For HPLC analysis, where external calibration is applied, a PAH which is not interfering with the target PAH, e. g. 6-methylchrysene, is added to the sample before extraction to check for the recovery of this substance throughout the whole procedure.

7.8 Preparation of injection standard solution

This is needed to check the recovery of the deuterated internal standards.

A single substance stock solution (7.6.3.1), e. g. deuterated benzo(*e*)pyrene, which is not interfering with the target analyte, can be used.

NOTE A deuterated or a $^{13}C_{12}$-labelled PAH not mentioned in Table 3 is added before injection into the GC-system to monitor variability of the instrument response. The recovery of the internal standards throughout the whole method can be calculated by the related response of the internal standard to the injection standard. Add such an amount to give a peak with measurable peak area or peak surface in the chromatogram (at least 10 times the detection limit).

8 Apparatus

8.1 Extraction and clean-up procedures, usual laboratory glassware.

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

8.1.1 Sample bottles, made of glass, stainless steel, or aluminium, with glass stopper or screw top and polytetrafluoroethylene (PTFE) seal of appropriate volume.

NOTE Glass is not appropriate for sludge samples.

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

8.1.2 Shaking device, with horizontal movement (200 strokes to 300 strokes per minute).

8.1.3 Water bath, adjustable up to 100 °C.

8.1.4 Separating funnels, of appropriate volume.

8.1.5 Conical flasks, of appropriate volume.

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

8.1.7 Concentrator, Kuderna Danish type.

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

8.1.8 Boiling chips, glass or porcelain beads.

8.1.9 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.

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

8.1.11 Chromatography tubes, chromatography column of glass, with 5 mm to 10 mm inside diameter length, e. g. 600 mm.

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

8.2.1 Capillary columns, each comprising a 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 below. In general, column length should be at least 30 m,with internal diameter of 0,25 mm and film thickness of 0,2 µm.

Sufficient resolution (0,8) between the chromatographic peaks of critical pairs as 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.

8.3 High performance liquid chromatograph, HPLC system, equipped according to requirements with ultraviolet (UV) and a fluorescence detection (FLD) system and a data evaluation system.

This includes the following:

— 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, preferably equipped with monochromator(s);

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

8.3.1 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. This applies in particular to the examination of microbiologically active solids.

If necessary, sludge samples shall be stored according to ISO 5667-15.

Dried samples can be stored at room temperature in a dark place up to one month. Soil samples shall be stored according to ISO 18512.

9.2 Sample pretreatment

Pretreat samples according to ISO 14507, if not otherwise specified, and considering the specific drying procedures as specified in Table 4 to obtain a test sample.

Pretreatment is necessary to reduce the moisture content to enable extraction of the PAH and to increase the homogeneity.

Complete drying of the sample is essential if Soxhlet is used for extraction and to increase the homogeneity.

Complete drying is also recommended if the sample shall be stored for a long period.

If necessary, increase the amount of solid matter of the sample by centrifugation and/or filtration (see Notes 1 and 2).

NOTE 1 Centrifugation is possible for several samples, but not practicable with material having approximately the same density of water.

NOTE 2 Filtration is possible, but the handling of some samples can cause problems due to blockages, too high water content, or extraction of target compound with filter paper.

Table 4 — Drying techniques for samples of different matrices for subsequent analyses of PAH

Matrix	Drying technique		
	Freeze drying (ISO 16720)	Na_2SO_4	No drying
Sludge	x[a]	x[b]	
Biowaste (compost, mixed waste)	x	x	x
Soil (e. g. sand, clay)	x	x	x

[a] Loss of volatile PAH is possible.

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

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 pretreatment, 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 Table 5). 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 PAH are relatively insoluble in the usual non-polar solvents, such as petroleum ether and other hydrocarbons, the choice of the solvents shall be made in accordance with the expected contamination level.

Extraction procedure 1 (see 10.2.2) is recommended if it is important to break up aggregates in the sample to reach the PAH. This is especially important with soil samples containing clay particles. With wet samples, these procedures shall be applied in order to remove the water.

If dissolving of the PAHs is the most important step (waste and highly contaminated soil and organic matter rich materials) and the sample is dry, extraction procedure 2 (see 10.2.3) using Soxhlet is recommended. For sludges, it has been shown that Soxhlet or pressurized liquid extraction is applicable. However, a general rule cannot be given, because samples can contain all aggregates, organic matter, and (plastic) waste.

Other extraction procedures, e. g. ultrasonic extraction, microwave, or pressurized extraction can be used provided

— the laboratory can show that the extraction efficiency is equivalent to one of the extraction procedures 1 (see 10.2.2) or 2 (see 10.2.3) as described in this International 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 11.

NOTE For application of this International Standard for some types of waste, addition of acetone with Soxhlet extraction has been shown to be effective.

Extraction procedures described in this International Standard are able to extract up to 20 g of dry sample. 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 soil; 2 g to 10 g of sewage sludge, 5 g to 20 g of compost, or 2 g to 20 g of (bio)waste. The amount of sample shall be weighed with an accuracy of at least 1 %.

Table 5 — Extraction procedure to be used for different matrices

Moisture status of the test sample	Matrix	Extraction solvent	Extraction technique	Extraction procedure	Remark
Dry	Soil-like material, sludge, biowaste, compost	Acetone/ petroleum ether	Agitation	Extraction procedure 1 (see 10.2.2)	Also applicable for field moist samples with dry matter content > 75 %
	Highly contaminated soil, sludge, biowaste, compost	Toluene	Soxhlet, pressurized liquid extraction	Extraction procedure 2 (see 10.2.3)	
Wet	Soil-like material, biowaste compost, sludge	Acetone/ petroleum ether NaCl	Agitation	Extraction procedure 3 (see 10.2.4)	Limitations for the amount of water in the sample are given

10.2.2 Extraction procedure 1: acetone/petroleum ether and agitation

For GC-MS analysis with internal calibration, add a definite volume of the internal standard solution. Add 50 ml of acetone (7.2.1) to the test sample and extract by shaking thoroughly with the shaking device (8.1.2) for 30 min to break up aggregates. Add 50 ml of petroleum ether (7.2.2) and shake again

thoroughly at least for 12 h. After the solids have been settled, decant the supernatant. Wash the solid phase with 50 ml of petroleum ether (7.2.2) and decant again. Collect the extracts in a separating funnel (8.1.4) and remove the acetone by shaking twice with 400 ml of water. Dry the extract over anhydrous sodium sulfate (7.2.4). Rinse the sodium sulfate with petroleum ether (7.2.2) and add the rinsing to the extract.

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

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: petroleum ether should be kept constant to 2:1.

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

For HPLC analysis where external calibration is used, only the recovery standard, such as 6-methyl chrysene, should be added to the sample before extraction.

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

10.2.3 Extraction procedure 2: Soxhlet extraction (dry samples)

For subsequent GC-MS analysis with internal calibration, add a definite volume of the internal standard solution. Extract the sample with approximately 70 ml of toluene (7.2.3) using Soxhlet extraction apparatus (8.1.6). The duration of the extraction should be calculated with a minimum of 100 extraction cycles.

For subsequent HPLC analysis, only a recovery standard solution is added to the sample before extraction.

NOTE Pressurized liquid extraction (PLE) can also be used.

10.2.4 Extraction procedure 3: acetone/petroleum ether/sodium chloride and agitation

Take an amount of the wet sample and put it into a 1 000-ml screw-cap glass jar or into a 1 000-ml Erlenmeyer flask.

For subsequent GC-MS analysis with internal calibration, add a definite volume of the internal standard spiking standard solution. The deuterated substances added shall have such a quantity that their concentrations in the final extract fall under the working range of the measurement method.

For subsequent HPLC analysis, only a recovery standard solution is added to the sample before extraction.

If the sample is dry, add 50 ml water. For moist samples, the water quantity to be added is calculated using Formula (1):

$$m_w = 50 - \frac{m_E \cdot m_{H_2O}}{100} \quad (1)$$

where

m_w is the mass of water to be added, expressed in grams (g);

m_E is the mass of the sifted sample, expressed in grams (g);

m_{H_2O} is the water content of the sample, determined according to ISO 11465, expressed in percent mass (%).

Add 40 g sodium chloride (7.2.6), 100 ml acetone (7.2.1), and 50 ml petroleum ether (7.2.2) to the moistened preparations, close the container, and shake it with a shaking device (8.1.2) for at least 12 h.

The organic phase shall be separated, if necessary, using a centrifuge with sealable centrifuge cups. Acetone and polar compounds shall be removed from the organic phase by shaking twice with 150 ml water each in a separation funnel.

10.3 Concentration or dilution

10.3.1 General

According to the expected contamination level, the extract can be concentrated or diluted with an appropriate solvent for subsequent analysis when a clean-up step is not required. If appropriate, concentrate the extract to approximately 10 ml by evaporation using a concentrator (8.1.7). Transfer the concentrated extract to a calibrated test tube and concentrate to 1 ml using a gentle stream of nitrogen or another inert gas at room temperature.

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

Do not evaporate the extracts to dryness, as losses of the 2-ring or 3-ring compounds can occur. A small amount (e. g. 100 µl) of a high-boiling solvent should be added as keeper for the GC-MS analysis (see 10.6).

When clean-up is not required, bring the extract to a definite volume and add a deuterated PAH to the final extract as an injection standard for the recovery check of the internal standards prior to GC-MS injection (see 10.5).

10.3.2 For HPLC analysis

When clean-up is not required, the solvent of the final extract [from extraction procedure 1 (see 10.2.2) and 2 (see 10.2.3)] shall be changed from petroleum ether to a solvent such as acetonitrile (7.4.2.2) which is compatible with the mobile phase of the HPLC system. For example, add 0,8 ml acetonitrile (7.4.2.2) to an aliquot of 1 ml of petroleum ether (7.2.2) and concentrate at room temperature with a gentle stream of nitrogen until all petroleum ether has been removed, i. e. until the volume of 0,8 ml has been reached. Add acetonitrile (7.4.2.2) 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.

NOTE For extraction procedure 3 (see 10.2.4), the final extract of toluene can be diluted with acetonitrile (7.4.2.2) in accordance with the expected contamination level prior to HPLC analysis.

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 injector system, column, and detection). If no or negligible interfering substances are present, clean-up is not necessary. Depending on the substances to be removed, Table 6 can be used for the selection of clean-up methods.

Table 6 — Clean-up methods

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

Before application of the clean-up to real samples, the laboratory shall ensure that recoveries for a standard after use of the clean-up are at least 80 % for all relevant PAH (including spiking and internal standards).

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 can 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 spiking and internal standards).

The extract or an aliquot obtained in 10.3 or in a previous clean-up step 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 (8.1.9) in the chromatography tube (8.1.11) and packing it dry with 2,0 g ± 0,1 g of aluminium oxide (7.3.1.1).

Before use, the elution pattern of each series of aluminium oxide columns and the necessary elution volume should be verified using a standard solution of PAH.

Transfer the extract to the dry packed adsorption column with a pipette. Rinse the test tube twice with 1 ml of petroleum ether (7.2.2) and transfer the rinsing to the column with the same pipette as soon as the liquid level reaches the upper side of the column packing. Elute with approximately 10 ml of petroleum ether (7.2.2).

Keeper substance (see 10.3.1) is added to the eluate, and then the eluate is reduced to approximately 0,5 ml at a maximum temperature of 40 °C using a suitable enrichment device.

NOTE Commercially available disposable columns can be used alternatively if found equally suitable.

10.4.3 Clean-up B – silica gel

Pack glass wool and 10 g silica gel into the glass column for clean-up. Add a layer of 1 cm of anhydrous sodium sulfate (7.2.4) and condition with 20 ml of the solvent mixture for silica gel (7.3.2.2) used to dissolve the residue. Put the sample into the column when the level of the solvent mixture has drained to approximately 0,5 cm above the column packing.

The reduced extract, eluate, or aliquot obtained in the previous clean-up step shall be quantitatively transferred to the column.

Elution is performed using a total of 100 ml solvent mixture for the silica gel column (7.3.2.2). Keeper substance (see 10.3.1) is added to the eluate, and then the eluate is concentrated by removing the solvent.

10.4.4 Clean-up C – gel permeation chromatography (styrene divinylbenzene resin)

The extract obtained is carefully reduced under a gentle nitrogen flow. The residue is immediately dissolved in 15 ml solvent mixture (7.3.3.4). Put 5 ml of the dissolved residue 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 are recommended values and should regularly be verified using the multiple substance stock solution (7.6.2.2).

Keeper substance (see 10.3.1) 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.

10.4.5 Clean-up D – DMF/cyclohexane partitioning for aliphatic hydrocarbons removal

Extracts of samples containing a high amount of aliphatic compounds need additional clean-up by dimethylformamide/cyclohexane partitioning, especially for GC-MS measurement.

Transfer an aliquot of the extract (e. g. 1 ml) to a separatory funnel (8.1.4) with a volume of 100 ml containing 10 ml of DMF/water 9:1 (7.3.4.2) and remove the aliphatic hydrocarbons by extraction with 10 ml cyclohexane (7.3.3.3). Repeat twice. Transfer the DMF/water 9:1 phase to a separatory funnel with a volume of 500 ml, add 100 ml of pure water and extract the PAH with 10 ml of cyclohexane (7.3.3.3). Repeat once. Combine the cyclohexane extracts. Keeper substance (see 10.3.1) is added to the extracts, and then the extract volume is reduced to approximately 0,5 ml using a suitable enrichment device.

10.5 Addition of the injection standard

Add an appropriate amount of the injection standard to the extract obtained after clean-up. This shall be in line with the concentration of the calibration standard. Record the final volume *V*.

10.6 Gas chromatographic analysis (GC)

10.6.1 Gas chromatographic analysis with mass spectrometric detection

10.6.1.1 Settings of the gas chromatograph

Set the gas chromatograph in such a way that sufficient separation of the PAH is achieved. Optimize the gas chromatograph starting from the following typical conditions:

— separation column: capillary column, e. g. DB5 MS 30 m, film thickness 0,25 µm, 0,25 mm internal diameter (see 8.2.2);

— oven temperature program: 60 °C, 2 min;
30 °C/min to 120 °C/min;
5 °C/min to 300 °C/min;
300 °C, 15 min;

— injector temperature: 260 °C;

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

— carrier gas: helium 0,8 ml/min to 1 ml/min.

10.6.1.2 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 following mass numbers (see Table 7) can be used for the quantitative analysis in selected ion monitoring mode.

Table 7 — Diagnostic ions to be used for identification using GC-MS according to ISO 22892

Compound	CAS-RN	Diagnostic ion 1 *m/z*	Diagnostic ion 2 *m/z*	Diagnostic ion 3 *m/z*
Naphthalene	91-20-3	128 (100)	102 (11)	-
Acenaphthene	83-32-9	154 (70)	153 (100)	76 (10)
Acenaphthylene	208-96-8	152 (100)	150 (3)	[76] (0)
Fluorene	86-73-7	166 (81)	165 (100)	[139] (4)
Anthracene	120-12-7	178 (100)	152 (12)	[76] (0)
Phenanthrene	85-01-8	178 (100)	152 (9)	[76] (3)
Fluoranthene	206-44-0	202 (100)	200 (31)	[100] (3)
Pyrene	129-00-0	202 (100)	200 (2)	[101] (4)
Benz(*a*)anthracene	56-55-3	228 (100)	226 (3)	[114] (2)
Chrysene	218-01-9	228 (100)	226 (6)	[113] (4)
Benzo(*b*)fluoranthene	205-99-2	252 (100)	250 (22)	126 (5)
Benzo(*k*)fluoranthene	207-08-9	252 (100)	250 (22)	126 (5)
Benzo(*a*)pyrene	50-32-8	252 (100)	250 (18)	[113] (11)
Indeno(1,2,3-cd)pyrene	193-39-5	276 (100)	138 (12)	[274] (4)
Dibenz(*a,h*)anthracene	53-70-3	278 (100)	139 (9)	[276] (5)
Benzo(*ghi*)perylene	191-24-2	276 (100)	138 (12)	[274] (4)

10.6.2 Calibration of the method using an internal standard

10.6.2.1 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 standard.

Add a specific mass of the internal standard (10 µg) to the test sample as to the calibration solutions. The mass concentration of the standard shall be the same for calibration and analysis. Run the GC-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 (2):

$$\frac{A_n}{A_d} = s \cdot \frac{\rho_n}{\rho_d} + b \qquad (2)$$

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 (see 10.6.2.2) and the calibration verification (see 10.6.2.3). The calibration verification is also known as daily calibration and serves as a validity check of the initial calibration.

Nonlinear calibration methods can be applied.

10.6.2.2 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 gas chromatogram of a series of at least five standard solutions with equidistant concentrations including the solvent blank. Identify the peaks using chromatograms in Annex B, and if necessary the chromatograms of the individual compounds. Prepare a calibration graph for each compound.

Check for linearity according to ISO 8466-1.

Choose as a working standard the calibration solution with the concentration closest to the middle of the linear range. When the range of the samples is lower than the linear range found, it is permissible for a working standard with a lower concentration to be chosen, corresponding to the middle of the sample range.

It is allowed to use nonlinear calibration using all five standards. In that case, the same five standards shall be used for recalibration and not the selection of two described below (see 10.6.2.3).

10.6.2.3 Calibration verification

The calibration verification checks the validity of the linear working range of the initial calibration curve and is performed before 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 10.6.2.2.

10.6.3 Measurement

Measure the gas chromatograms of the extracts obtained according to 10.4. With the aid of the absolute retention times, identify the peaks to be used to calculate the relative retention times. Use the 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.

10.6.4 Identification

Apply ISO 22892 for identification of PAH.

10.6.5 Check on method performance

Because this International Standard 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.

NOTE The performance standard can 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 (3).

$$U = \frac{A_1(S)}{A_2(S)} \times \frac{A_2(ps)}{A_1(ps)} \times 100 \quad (3)$$

where

U is the recovery rate, expressed in percent (%);

A_1 is the measured response of the PAH internal standard, e. g. peak area;

A_2 is the measured response of the PAH injection standard, e. g. peak area;

ps is the performance standard;

S is the sample.

The average ratio in the sample shall be at least 75 % of the ratio in the standard. The ratio for an individual PAH should be at least 60 %. If this is not the case, the analyses shall be repeated using modules more suitable for the sample.

If multiple clean-up is necessary, lower ratios can be found, because with each clean-up step losses are accepted by this International Standard. Lower ratios are acceptable if this can be explained by the accepted losses in each clean-up step. The minimum ratio shall be 50 %.

10.6.6 Calculation

Calculate the mass content of the individual PAH from the multipoint calibration of the total method by using Formula (4):

$$w_n = \frac{(A_n / A_d) - b}{s \cdot m \cdot d_s} \cdot \rho_d \cdot V \quad (4)$$

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_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 ISO 11465, expressed in percent (%);

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 Chromatographic separation

Use a column and chromatographic conditions which allow efficient separation of the PAH stated in the scope. For a choice of columns and the corresponding gradients, see Annex B.

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. Changes shall therefore only be made at a minimum resolution of R = 2,5.

Dissolved oxygen in the eluent can reduce the fluorescence signal. Hence, variations in the oxygen concentrations affect the reproducibility. The oxygen content of the eluent should be kept as low and constant as possible by degassing the eluent using e. g. helium or vacuum.

A change of wavelength should be made at times when the fluorescence is low. At high fluorescence values, the wavelength change leads to a displacement of the baseline. Readjusting the baseline after a change of wavelength can interfere with the integration, and hence with the quantification.

To achieve constant peak heights, it can be necessary to change wavelengths and damping at the same time. The damping conditions are part of the detection criteria and cannot be changed after calibration. If damping is low, the resultant increase in noise should not impair the integration.

10.7.3.4 Identification of individual compounds

If there is no peak at the characteristic retention time, and the chromatogram is normal in all other aspects, assume that the compound is not present.

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 can 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

For calibration, a distinction is made between initial calibration, working calibration, and checking of the validity of the calibration curve. Initial calibration determines the working range and the linearity of the calibration function according to ISO 8466-1. Perform this calibration when the apparatus is used for the first time.

In the next step, establish the final working range and perform the routine calibration. Repeat this calibration after maintenance (e. g. replacement of the column), after repair of the HPLC system, in case the system has not been in use for a longer period of time, or if the validity criteria cannot be met. Check the validity of the initial calibration with each series of samples to be analysed.

10.7.4.2 Initial calibration

Establish the preliminary working range by analysing at least five dilutions of the calibration standard mixture (7.6.2.3). Test for linearity in accordance with ISO 8466-1.

10.7.4.3 Routine calibration

After examining the final working range, analyse a minimum of five dilutions of the standard calibration solution. Calculate a calibration function by linear regression analysis of the corrected peak areas. The actual sensitivity of the method can be estimated from the calculated regression function.

10.7.4.4 Check of the validity of the calibration function

Check the validity of the calibration function from the routine calibration with each batch of samples by analysis of one standard solution after every 10 samples. The concentration of this standard solution shall be between 20 % and 80 % of the working range. Make sure that the individual results do not deviate by more than 10 % of the working calibration line. If this criterion is met, assume the calibration to be valid. If not, recalibrate in accordance with 10.7.4.3.

10.7.5 Measurement of samples

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.

Measure the sample, the calibration solutions, and the blank.

Ensure that the peaks of each sample are being integrated correctly and correct, if necessary.

If the calculated mass concentration of a substance in the sample exceeds the calibration range, dilute the measuring sample and repeat the measurement.

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 (5):

$$w_i = \frac{A_i \cdot f_i \cdot V}{m} \tag{5}$$

where

w_i is the mass content of the substance *i* of a sample, expressed in milligrams per kilogram (mg/kg) dry matter;

A_i is the peak area or peak height of the substance *i* in the chromatogram;

f_i is the response factor of the substance *i*, in counts per microgram per millilitre (µg/ml); slope of the recalibration curve;

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

m is the mass of the sample (dry matter), expressed in grams (g).

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 lowest result. If the difference is larger than 15 %, the lowest result is reported.

11 Performance characteristics

The method is "performance-based". It is permitted to modify the method to overcome interferences not specified in this International Standard, provided that the performance criteria are met. Internal standards shall be used to check the pretreatment, extraction, and clean-up procedures. Recoveries of these standards should be 70 % to 110 %. If the recovery is outside the limits (i. e. 70 % to 110 %), the method shall be modified using other modules described in this International 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 International Standard, i.e. ISO 13859;

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 10.6.6 (GC-MS) and 10.7.6 (HPLC);

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

Annex A
(informative)

Repeatability and reproducibility data

A.1 Materials used in the interlaboratory comparison study

The interlaboratory comparison for the determination of polycyclic aromatic hydrocarbons (PAH) by gas chromatography (GC) and high performance liquid chromatography (HPLC) in sludge, treated biowaste and soil was carried out by 10 to 13 European laboratories on three materials. (Detailed information can be found in the final report on the Interlaboratory comparison study mentioned in Reference [10].)

In the interlaboratory comparison study, the following starting points were used.

The materials examined cover all the grain size classes to which the determination of polycyclic aromatic hydrocarbons (PAH) by gas chromatography (GC) and high performance liquid chromatography (HPLC) in sludge, treated biowaste and soil applies: very fine grained materials (sludge: < 125 µm) and fine-grained materials (soil and compost: < 4 mm).

Table A.1 lists the types of materials tested.

Table A.1 — Materials tested in the interlaboratory comparison for the determination of PAH by GC and HPLC in sludge, treated biowaste and soil

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

In the case of PAH, the results can be compared with validation studies carried out by CEN/TC 292 (see EN 15527) and ISO/TC 190 (see ISO 18287).

The validation has been carried out for soil also, but no results are presented because the concentrations in the test materials were lower than the limit of applicability.

A.2 Interlaboratory comparison results

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

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

Matrix	l	n	n_o	$\bar{\bar{x}}$ µg/kg	s_R µg/kg	$C_{V,R}$ %	s_r µg/kg	$C_{V,r}$ %	BD
Acenaphthene									
Sludge 1	14	44	2	87,2	55,9	64,1	13,0	14,9	4
Compost 1	7	17	2	5,5	2,0	36,8	0,5	9,6	16
Acenaphthylene									
Sludge 1	9	26	2	29,6	20,3	68,4	10,3	34,7	8
Compost 1	7	17	2	5,5	2,0	36,8	0,5	9,6	13
Anthracene									
Sludge 1	18	67	0	228	101,3	44,5	20,7	9,1	0
Compost 1	16	56	1	31,8	12,5	39,3	4,3	13,6	0
Benz(*a*)anthracene									
Sludge 1	16	60	0	977	297,2	30,4	141,6	14,5	1
Compost 1	15	60	0	369	131,8	35,7	58,6	15,9	0
Benzo(*a*)pyrene									
Sludge 1	17	58	1	820,0	304,0	37,1	64,3	7,8	0
Compost 1	15	53	1	381,1	83,1	21,8	54,0	14,2	0
Benzo(*b*)fluoranthene									
Sludge 1	16	57	1	1 274	455,2	35,7	112,1	8,8	0
Compost 1	14	55	0	544	163,1	30,0	65,3	12,0	0
Benzo(*ghi*)perylene									
Sludge 1	17	54	2	694	218,7	31,5	52,8	7,6	0
Compost 1	16	56	1	314	157,7	50,3	36,0	11,5	0
Benzo(*k*)fluoranthene									
Sludge 1	16	61	0	590	156,6	26,5	52,0	8,8	0
Compost 1	15	56	1	236	44,5	18,8	33,4	14,1	0
Chrysene									
Sludge 1	16	53	2	1 077	326,6	30,3	68,0	6,3	0
Compost 1	13	52	0	425	157,7	37,1	47,5	11,2	0
Dibenz(*a*,*h*)anthracene									
Sludge 1	16	53	2	194	80,2	41,4	14,7	7,6	0

l number of laboratories

n number of analytical results

n_o number of rejected laboratories

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

s_R reproducibility standard deviation

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

s_r repeatability standard deviation

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

BD number of measurements below detection limit

Table A.2 *(continued)*

Matrix	l	n	n_o	$\bar{\bar{x}}$ µg/kg	s_R µg/kg	$C_{V,R}$ %	s_r µg/kg	$C_{V,r}$ %	BD
Compost 1	14	43	0	74,3	28,2	38,0	10,6	14,2	1
Fluoranthene									
Sludge 1	17	60	1	2 397	417,7	17,4	135,6	5,7	0
Compost 1	14	42	0	536	154,9	28,9	56,4	10,5	0
Fluorene									
Sludge 1	17	59	1	179	50,2	28,1	11,2	6,3	0
Compost 1	11	26	1	16,8	16,8	99,6	4,5	26,5	9
Indeno(1,2,3-cd)pyrene									
Sludge 1	16	61	0	768	277,4	36,1	73,8	9,6	2
Compost 1	14	42	0	304	89,3	29,4	27,1	8,9	1
Naphthalene									
Sludge 1	15	42	3	75,6	27,0	35,7	7,7	10,1	0
Compost 1	9	15	3	7,9	2,0	25,7	0,4	5,5	10
Phenanthrene									
Sludge 1	18	58	2	1 200	357,0	29,7	60,4	5,0	0
Compost 1	16	60	0	107	35,9	33,6	10,9	10,2	0
Pyrene									
Sludge 1	17	58	1	1 579	472,2	29,9	90,0	5,7	0
Compost 1	16	60	0	448	134,5	30,0	57,5	12,8	0
Total PAH									
Sludge 1	16	59	1	12 312	3 598	29,2	782	6,4	0
Compost 1	16	61	0	3 318	1 021	30,8	712	21,5	0

l number of laboratories
n number of analytical results
n_o number of rejected laboratories
$\bar{\bar{x}}$ total mean of analytical results (without outliers)
s_R reproducibility standard deviation
$C_{V,R}$ coefficient of variation of reproducibility
s_r repeatability standard deviation
$C_{V,r}$ coefficient of variation of repeatability
BD number of measurements below detection limit

Annex B
(informative)

Examples of instrumental conditions and chromatograms

B.1 Measurement of PAH with GC-MS

GC-MS conditions:

- instrument:
  - GC MS 4000 Varian Ion Trap[2];
- column (dimensions, phase, etc):
  - DB 5ms[3];
  - 30 m;
  - 250 µm film thickness;
  - 250 mm internal diameter;
- carrier gas:
  - helium constant flow ml/min;
- injection technique:
  - injector 250 °C; split initial off, then after 1 min on 1: 40;
- injection volume (µl):
  - 1 µl;
- temperature programme:
  - start 80 °C;
  - 30 °C/min to 120 °C/min;
  - 5 °C/min to 310 °C/min;
  - 6 min isotherm.

2) GC MS 4000 Varian Ion Trap is an example of a suitable product which is no longer available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

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 ISO 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	Benz(*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

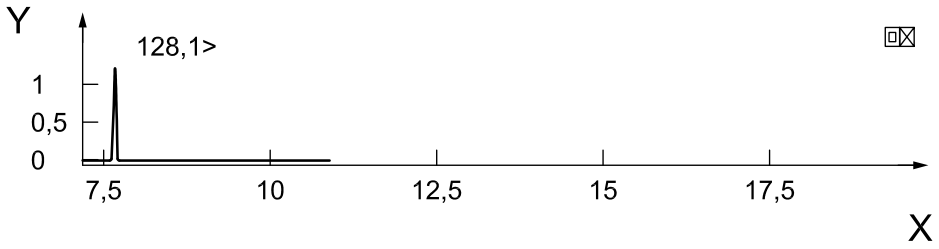
Table B.2 — Maximum absorption wavelengths and recommended/optimum combinations excitation/emission wavelengths

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]		
Fluorene	261	280/340	268/308
Pyrene	240	305/430	336/376

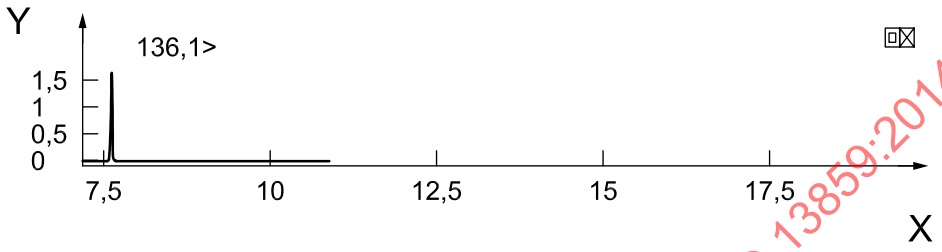
Concentration of each component in the SIM chromatogram

PAH - Native: 570 ng/g

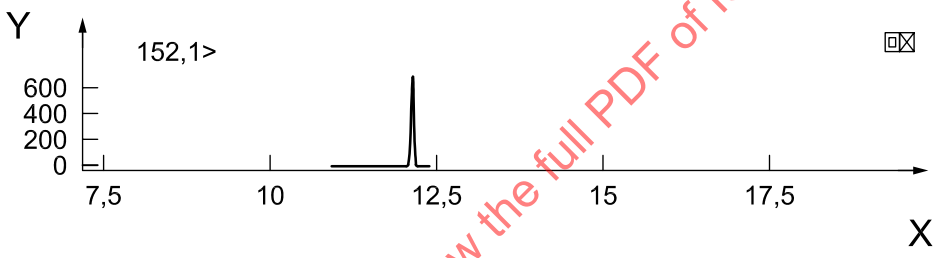
PAH - Deuterated: 468 ng/g



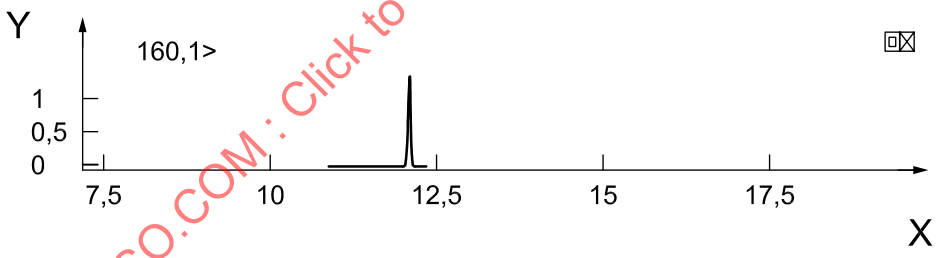
a) Naphthalene



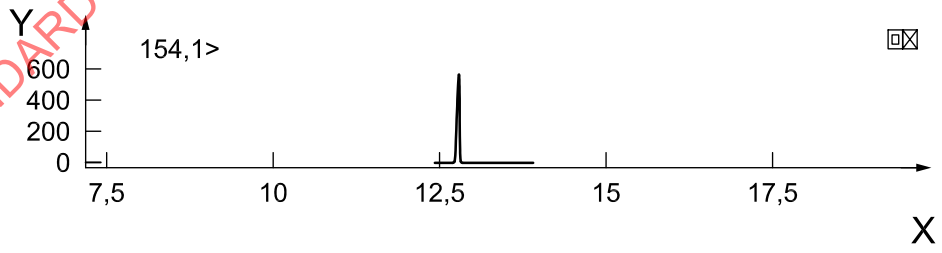
b) Naphthalene-d8



c) Acenaphthylene



d) Acenaphthylene-d8



e) Acenaphthene

Figure B.1 — *(continued)*