HORIZONTAL

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Ad-hoc group PAH/PCB

Soils, sludges and treated bio-waste – Organic constituents – Polycyclic aromatic hydrocarbons(PAH) by gas chromatography(GC) and high performance liquid chromatography(HPLC)

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Horizontal PAH-standard

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Horizontal PAH-standard

Foreword

The European project HORIZONTAL is focussed on the standardisation of test procedures in environmental samples. Several studies have been started to elaborate the possibility of horizontal standardisation on specific subjects. One of the subjects is the horizontal standardisation of polycyclic aromatic hydrocarbons (PAH) as described in this standard. PAH are subject of standardisation in CEN 292 (waste), CEN 308 (sludge) and ISO TC190 (soil). This PAH-standard has been developed in co-operation with experts from CEN 308, CEN 292 and ISO/TC 190.

This standard has been validated for the matrices given below

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

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Introduction

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous because of the fact that 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) are regularly monitored and controlled. At present determination of PAH are carried out in these matrices in most of the routine laboratories following the preceding steps 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 by HPLC in combination with UV-DAD- or fluorescence-detection(HPLC-UV-DAD/FLD). Therefore these two methods are included in this horizontal standard. It is to be underlined that the target contamination level of PAH can lay in the range of about 0,01 mg/kg per individual PAH (agricultural soil and sediment) to about 200 mg/kg and higher(contaminated soil at coking plant and waste). 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 PCBs.

The texts of the chapter 1-11 are normative; annexes are normative or informative as stated in the top lines of the annexes.

Soils, sludges and treated bio-waste – Organic constituents – Polycyclic aromatic hydrocarbons(PAH) by gas chromatography(GC) and high performance liquid chromatography(HPLC)

1   Scope

This European Standard specifies the quantitative determination of 16 polycyclic aromatic hydrocarbons (PAH)-see table1 in soil, sludge, sediments, suspended solids and treated bio-waste using GC/MS and HPLC-UV-DAD/FLD covering a wide range of PAH contamination levels (see also annex A). When using fluorescence detection acenaphthylene cannot be measured. The GC-MS part of this standard is identical to WI 00292049 and is therefore applicable to waste.

The limit of detection is dependent 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) may be ensured for each individual PAH. This is instrument and sample depenent.

Waste, sludge and soil may differ in properties and also in the expected contamination levels of PAHs and presence of interfering substances. These differences make it impossible to describe one general procedure. This standard contains decision tables based on the properties of the sample and the extraction and clean up procedure to be used. Two general lines will be followed, an agitation procedure (shaking) or use of Soxhlet/pressurised liquid extraction. The choice of 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 relativelyinsoluble in the usual unpolar solvents such as petroleum ether and other hydrocarbons the choice of the solvents has to be made in accordance with the expected contamination level.

This method is 'performance based'. It is permitted to modify the method to overcome interferences not foreseen in this standard, provided that all performance criteria mentioned in this standard are met.

Note: With this method also other PAH compounds can be analysed provided suitability is proven.

2   Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

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

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

ISO 14507, *Soil quality – Guidance for sample pretreatment for the determination of organic contaminants in soil.*

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ISO/DIS 10381-8:2003, *Soil quality - Sampling - Part 8: Guidance on sampling of stockpiles*.

ISO/DIS 16720:2003, *Soil quality - Pretreatment of samples by freeze-drying for subsequent analysis*.

ISO/CD xxxxx, *Soil quality - Sample pretreatment of large samples* (in preparation; at present: doc. 190/3/9 N 092)

prEN 14346:2004 , *Characterisation of waste - Calculation of dry matter by determination of dry residue and water content*

ISO 13877:2003, *Soil quality - Determination of polynuclear aromatic hydrocarbons (PAH) - Method using high-performance liquid chromatography*

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

3 Terms and definitions

3.1 analyte

In the context of this standard, these are polycyclic aromatic hydrocarbons also known as polynuclear aromatic hydrocarbon (PAH) compounds with 2 to 6 condensed aromatic rings.

3.2 calibration standard

A solution prepared from stock solutions of native PAH and used to calibrate the response of the instrument with respect to analyte concentration

3.3 internal standard

The addition of deuterated or $^{13}C_{12}$-labelled PAH standards corresponding to the native to be analysed PAH for GC-MS . A relative response factor (RRF) of the native compound relative to the isotope labelled compound is used for calibration and quantification. Recoveries of these standards are also calculated and used to check the performance of the procedure. For the HPLC method, an external calibration is used and hence no internal standard is required for addition to the sample. For this method a recovery control can be made by addition of a suitable deuterated PAH or a native PAH not mentioned in the scope e.g. 6-methylchrysene, which is not interfering with the target analytes.

3.4 injection standard

A deuterated or a $^{13}C_{12}$-labelled PAH not mentioned in the scope and not added as internal standard, is added to the extract before injection into the GC, to monitor variability of the instrument response, the so called injection standard. The recovery of the internal standards through out the whole method can be calculated by the related response of the internal standard to the injection standard.

3.5 Critical pair

a pair of congeners that must be separated to a predefined resolution to ensure chromatographic separation meets minimum quality criteria

3.6 Limit of detection (EURACHEM Guide- Fitness for purpose of analytical methods)

The lowest content that can be measured with reasonable statistical certainty

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Remarks: It was deeply discussed within the experts who had attended the TF151/TG3 Meetings and it was resolved that the definition of limit of detection is to be given according to the CEN rules, if there is any. Otherwise a definition is to be given, which is to be stated in all the Horizontal standards.

3.7   Resolution

Difference in retention times of the two peaks a and b, which constitute the critical pair, divided by the arithmetic mean of the peak widths at base of a and b, calculated as:

$$\frac{2 \, \Delta t}{Y_a + Y_b}$$

where Δt is the difference in retention times of the two peaks a and b (sec), which constitute the critical pair and Y_a and Y_b are the peak widths at base of a and b (sec), respectively, as indicated in figure below :
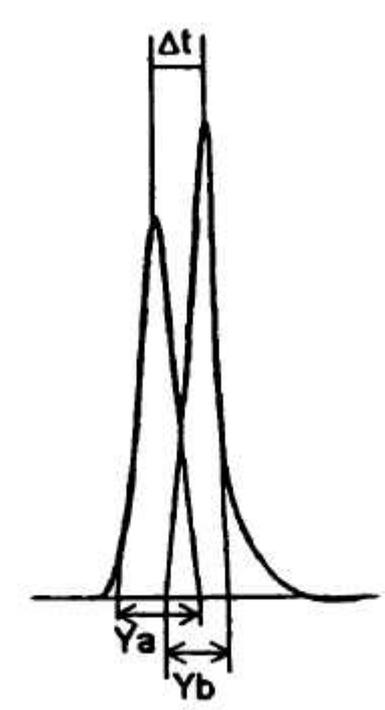
4   Principle

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

After pretreatment according to methods referred to in 8.1, the test sample is extracted with a suitable solvent.

The extract is concentrated; 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 unpolar eluate residue has to be removed.

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

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PAH are identified and quantified with GC-MS by comparison of relative retention times and relative peak heights (or peak areas) with respect to internals 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. Internal standards are to be used to have a check on the pretreatment, extraction and clean-up procedures. Recoveries of these standards should be 70 -110%. If the recovery is lower the method has to be modified using other modules described in this standard.

Note: Some samples may require multiple clean-up in that case lower recoveries are accepted (see also 10.6.9)

Temporally remark: the recovery of 70% may change after the validation study.

5 Interferences and hazards

5.1 Interferences

5.1.1 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 may occur due to adsorption on the walls of the containers. The extent of the losses depends on the storage time.

5.1.2 Interference with GC-MS

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

Chromatographic separation between dibenzo(ah)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, when necessary, corrected. Sufficient resolution (e.g. 0,8) between the peaks of benzo(b)fluoranthen and benzo(k)fluoranthen as well as of benzo (a) pyrene and benzo(e)pyrene is to be set as quality criteria for the capillary column. Benzo(b)fluoranthen and benzo(j)fluoranthen cannot be separated. Triphenylene may not be completely separated from benzo(a)anthracene and chrysene. In this case it has to be stated in the report

5.1.3 Interferences with the HPLC

Substances that show either fluorescence or quenching and co-elute with the PAHs to be determined may interfere with the determination. These interferences may lead to incompletely resolved signals and may, depending on their magnitude, affect accuracy and precision of the analytical results. Peak overlap will not allow an interpretation of the result. Unsymmetrical peaks and peaks being broader than the corresponding peaks of the reference substance suggest interferences. This problem may 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 (hexane, acetone, dichloromethane) may lead to poor reproducibility of the retention times and wider peaks or double peaks especially for the 2- and 3-ring PAHs. Extracts are to be diluted sufficiently with acetonitrile for the HPLC-analysis, otherwise the detection of naphthalene and 3ring-PAH can be interfered by a broad toluene peak.

Separation between dibenzo(ah)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.

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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 must be handled with extreme care. Contact of solid materials, solvent extracts and solutions of standard PAH with the body must not be allowed to occur. PAH may co-distill with solvent and become deposited outside of stoppered bottles, all containers containing solutions of PAH in solvent must therefore always be handled using gloves which are solvent resistant and preferably disposable. PAH contamination of vessels may detected by irradiation wit 366 nm U.V. light. Vessels containing PAH solutions should be stored standing in beakers to contain any spillage in the case of breakage.

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

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

In handling hexane precautions have to taken because of the neurotoxic properties of hexane.

Anyone dealing with waste and sludge analysis has to be aware of the typical risks of that kind of material irrespective of the parameter to be determined. Waste and sludge samples may contain hazardous (e. g. toxic, reactive, flammable, infectious) substances, which can be liable to biological and/or chemical reaction. Consequently it is recommended that these samples should be handled with special care. The gases, which may be produced by microbiological or chemical activity, are potentially flammable and will pressurise sealed bottles. Bursting bottles are likely to result in hazardous shrapnel, dust and/or aerosol. National regulations should be followed with respect to all hazards associated with this method.

7   Reagents

7.1 General

All reagents shall be of recognised analytical grade. The purity of the reagents used shall be checked by running a blank determination as described in 8.1. If the blank value is unreasonably high, i.e. more than half of the lowest reported value, , find the cause through a step by step examination of the whole procedure.

7.2 Reagents for extraction procedures

7.2.1 Acetone, C_2H_4O

7.2.2 Petroleum ether (Boiling range 40 °C to 60 °C)

Note: Hexane-like solvents with a boiling range between 30°C and 68 °C are allowed to use

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, anhydrous, NaCl

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7.3   Reagents for clean-up procedures

7.3.1 Clean-up – Aluminium oxide

7.3.1.1 Aluminium oxide

Basic or neutral, specific surface 200 m^2/g, activity Super I according to Brockmann.

7.3.1.2   Deactivated aluminium oxide

Deactivated with 10 % water.

To 90 g of aluminium oxide (7.3.1.1) add 10 g of water. 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 2 weeks.

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

7.3.2 Clean up - 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(H2O) = 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. Then allow to cool in a desiccator and add 10 % water (*w/w*) in a flask. Shake for 5 min intensively by hand until all lumps have disappeared and then for 2 h in a shaking machine. Store the desactivated silica gel in the absence of air, use it for maximum of 2 weeks.

7.3.3 Clean up - Gel permeation chromatography

7.3.3.1 Bio beads SX-3

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 into a 500ml Erlenmeyer flask and add 300 ml elution mixture made up of cyclohexane and ethyl acetate 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 3 days, push in the plungers of the column so that a filling level of approx. 35 cm is obtained; to further compress the gel, pump approximately 2 l 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 approx. 33 cm.

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

7.3.4.1 Dimethylformamide, C_3H_7NO

7.3.4.1.1 Dimethylformamide/water: 9:1

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7.4 For chromatographic analysis

7.4.1 Gas chromatographic analysis

Carrier gas for gas chromatography - MS, helium or hydrogen of high purity and in accordance with manufacturer's specifications

7.4.2 For HPLC-Analysis

7.4.2.1 mobile phase

7.4.2.2 acetonitrile, CH_3CN or methanol, CH_4O , HPLC purity grade.

7.4.2.3 ultra-pure water is required (HPLC purity grade)

7.4.2.4 Helium, of suitable purity for degasification of solvents

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 5 internal standards, e.g. 5 deuterated PAH should be used as internal standards for the GC-MS method for evaluation of results. Verify the stability of the internal standards regularly. Table 1 contains native and a number of deuterated PAH to be used for calibration of specific analyte.

Note 1: $^{13}C_{12}$-labelled PAH standards can be used also 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 D.C. 20 234 U.S.A or from other commercial providers

When highly contaminated samples are analysed, often an aliquot of the extract is used for further clean-up. This will make the costs of analyses caused by the use of labelled standard very high. In these cases it is allowed to add the internal standard in two steps. Step 1 addition of unlabeled internal standards to the sample. Step 2 addition of labelled congeners to the aliquot of the extract used for clean-up.

Table 1 — Native PAH and deuterated PAH

PAH reference substances		Internal standard substances deuterated PAHs
Naphthalene	(CAS No. 91-20-3)	<u>Naphthalene-D-$_8$</u>
Acenaphthene	(CAS No.83-32-9)	Acenaphthene-D-$_{10}$
Acenaphthylene	(CAS No.208-96-8)	Acenaphthylene-D-$_8$
Fluorene	(CAS No.86-73-7)	Fluorene-D-$_{10}$
Anthracene	(CAS No.120-12-7)	Anthracene-D-$_{10}$

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Phenanthrene	(CAS No. 85-01-8)	Phenanthrene- D-$_{10}$
Fluoranthene	(CAS No.206-44-0)	Fluoranthene-D-$_{10}$
Pyrene	(CAS No.129-00-0)	Pyrene-D-$_{10}$
Benz(a)anthracene	(CAS No.56-55-3)	Benz(a)anthracene-D-$_{12}$
Chrysene	(CAS.No.218-01-9)	Chrysene-D-$_{12}$
Benzo(b)fluoranthene	(CAS No. 205-99-2)	Benzo(b)fluoranthene-D-$_{12}$
Benzo(k)fluoranthene	(CAS No.207-08-9)	Benzo(k)fluoranthene-D-$_{12}$
Benzo(a)pyrene	(CAS No.50-32-8)	Benzo(a)pyrene-D-$_{12}$
Benzo(e)pyrene*	(CAS No. 192-97-2)	
Indeno(1,2,3-cd)pyrene	(CAS No.193-39-5)	Indeno(1,2,3-cd)pyrene-D-$_{12}$
Dibenz(ah)anthracene	(CAS No.53-70-3)	Dibenz(ah)anthracene- D-$_{14}$
Benzo(ghi)perylene	(CAS No.191-24-2)	Benzo(ghi)perylene-D-$_{12}$

* Not part of 16 target analytes, but only for resolution check for the separation with benzo(a)pyrene for GC measurements

7.5.2 Injection standard

7.5.2.1 GC-MS

A deuterated PAH as 1-Methylnaphthalene- D$_{10}$, Triphenylene-D-$_{12}$ and Perylene-D-$_{12}$ 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 of a suitable native PAH not mentioned in the scope e.g. 6-methylchrysene to the sample before extraction, which is not interfering with the target analytes.

7.6 Preparation of standard solutions

7.6.1 Preparation of calibration standard solutions of PAH

Because of the dangerous nature of the substances to be used, commercially available - preferably certified - standard solutions should be used. Avoid skin contact.

7.6.2 for HPLC-Analysis

7.6.2.1 Single substance stock solutions

Solutions of the single substances (see Table 1) in acetonitrile (7.5.1.1) mass concentration e.g. 10 μg/ml. These solutions are used for confirmation and identification of single PAHs in the chromatogram.

7.6.2.2 Multiple substance stock solution

(Certified) solution of the reference substances (see Table 1) in acetonitrile (7.5.1.1),mass concentration of the respective individual substance e.g.10 μg/ml.

The solutions 7.6.2.1 to 7.6.2.2 are stable for at least a year when stored in dark at room temperature and protected from evaporation.

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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 methanol (7.4.2.2) or acetonitrile (7.4.2.2) as solvent. The choice of solvent depends on the composition of the mobile phase.

Transfer e.g. 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 by measurement within the laboratory's internal quality assurance system.

Checking the mass concentration of the PAH in the stock solution is only possible by comparison with an independent, preferably certified, standard solution.

7.7.3 for GC-MS Analysis

7.7.3.1 Single substance stock solution

Solutions of the single substances of native and deuterated PAH (see Table 1) in toluene or cyclohexane (7.2.2.) mass concentration e.g. 10 µg/ml. These solutions are used for confirmation and identification of single PAHs in the chromatogram.

NOTE   The single substance stock solutions are to be stored in a dark place at about –15 °C to –18 °C. Store the diluted standard solutions at about 4 °C protected from light and evaporation. They are stable for about 1 year.

7.7.3.2 Multiple substance stock solution of native PAH

  Dilute the(certified) solution of the reference substances i.e. native PAH (see table 1) in toluene or cyclohexane (7.2.2.), to mass concentration of the respective individual substance e.g. 10 µg/ml.

 7.7.3.3 Multiple substance stock solution of deuterated or labelled PAH

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 e.g. 10 µg/ml for each individual deuterated standard.

7.7.4 Calibration standard solutions

Prepare a series of calibration standard solutions (at least 5) over a suitable range by transferring different volumes of the multiple substance stock solution of native PAH standards (7.7.3.2) and a constant volume of the internal standard solution (7.7.3.3) into a volumetric flask and fill up to the mark with cyclohexane. [h1]

7.8 Preparation of internal standard solutions

Multiple substance stock solution of deuterated or labelled PAH (7.7.3.3) can be used for spiking to the sample before extraction. The amount of the spiking internal standards has to be adjusted so that their concentration in the final extract for GC-MS is the same as that in the calibration solutions (e.g. 100pg /µl). In 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 through out the whole procedure.

7.9 Preparation of injection standard solution

This is needed to check the recovery of the deuterated internal standards. A single substance stock solution (7.7.3.1) e.g. deuterated benzo(e)pyrene which is not interfering with the target analyte can be used.

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Horizontal PAH-standard

A deuterated or a $^{13}C_{12}$-labelled PAH not mentioned in the scope is added before injection into the GC, to monitor variability of the instrument response, the so called injection standard. The recovery of the internal standards through out 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

Customary laboratory glassware.

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

8.1.1. Sample bottles

 Glas or stainless steel or aluminium bottles with glas stopper or screw top and polytetrafluoroethene seal (PTFE). Size in agreement with the amount of sample taken.

NOTE   Glass is not appropriate for sludges.

8.1.2 Shaking device

With horizontal movement (200 to 300 strokes a 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.100ml, Soxhlet extractors and Soxhlet thimbles e.g. 27mm*x100mm, vertical condensers e.g. 300mm, heating device

8.1.7 Concentrator

 Kuderna Danish type (see figure D.1).

Note: Other evaporators, e.g. a rotary evaporator, may 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

NOTE   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 capacity of 10 ml to 15 ml and ground glass stopper.

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8.1.11 Chromatography tubes

Chromatography column of glass, 5 mm-10 mm inside diameter, length e.g. 600 mm

See figure D.2.

8.2 Gas chromatograph

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. Internal diameter 0,25 mm and film thickness 0,2 µm.

Sufficient resolution (0,8) between the chromatographic peaks of critical pairs as benzo(b)fluoranthen and benzo(k)fluoranthen as well as of benzo(a)pyrene and benzo(e)pyrene is to be set as quality criteria for the capillary column.

8.3 High performance liquid chromatograph

A HPLC- system equipped according to requirements with either an ultraviolet or a fluorimetric detection 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 keeping the temperature constant to within ± 0,5 °C;

⎯ fluorescence detector capable of programming at least 6 pairs of wavelengths, including damping/amplification, preferably equipped with monochromator(s)

⎯ UV detector(with variable wave length) or Diode Array

8.3.1 Analytical separation column,

A reversed phase HPLC column meeting the separation requirements given in xxx x (examples see Annex)

9 Sampling and preservation of samples

9.1 Sampling

Obtain representative samples using sampling apparatus in accordance with suitable European or International standards. In principle, the samples shall be analyzed as soon as possible after sampling. This applies in particular to the examination of microbiologically active solids

Samples shall be stored on a cool (< 10 °C) dark place. Samples can be stored for one week at a temperature between 0 and < 10 °C and for a year at temperatures < -18 °C. Dried samples can be stored at room temperature in a dark place.

9.2 Sample preservation and pretreatment

Samples shall be pretreated according the mentioned International Standards using the following scheme to obtain a test sample. Pretreatment is necessary to reduce the moisture content to enable extraction of the PCBs and to increase the homogeneity. Complete drying of the sample is essential if Soxhlet is used for extraction or to increase the homogeneity. Complete drying is also recommended if the sample shall be stored for a long period.

Following scheme will be removed if the Horizontal pre-treatment standard is available

(Bulk) sample

↓

Homogenization and subdividing (Method for bulk samples)

↓

Laboratory sample

↓

If necessary, increasing solid matter (centrifugation[a)], filtration[b)])

↓

Drying

Sludge[4)]	Sediment	**Suspended solids**	**Soil (e.g. clay, sandy)**	Waste (compost, bio-waste, mixed waste	**Waste (shredder/plastics materials**
Freeze drying[c)] (ISO/DIS 16720	Freeze drying (ISO/DIS 16720) No drying[f)]	Freeze drying (ISO/DIS 16720)	Na_2SO_4 [d)] Freeze drying (ISO/DIS 16720) No drying	Na_2SO_4 Freeze drying (ISO/DIS 16720) No drying	Air drying [e)]

↓

Grinding (ISO 14507)/sieving (homogenisation, subdividing)

↓

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Test sample

(Dry or high solid matter content >)

a) Centrifugation   Possible with several samples, but not practicable with material having approximately the   same density of water (i.e. sludges)

b) Filtration   Possible, but no good handling with several samples, blockages, water content to high, Extraction of target compound with filter paper

c) Freeze drying   For samples like sludges, sediments and suspended soil with higher water content >50%. Loss of volatile PAHs is possible.

d) Na_2SO4 can be used for preservation of the hygroscopic dried sludge

e) Air drying   Not allowed for PAH, because they are degradable under aerobic conditions and when volatile

PAH are to be analysed.

f) No drying   If water content enables direct extraction according to 10.2.1

Note   If it is possible to take a representative wet sample, the following procedure can be used to make a dry sample. Mix in a mortar ca 10 g of sample, weighed to the nearest 0.01 g, with one or more equivalents of Na_2SO_4, weighed to the nearest 0.01 g, so that a homogenous dry mass is obtained. Use this whole sample for further analysis;

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

NOTE   It is permissible for dried samples, if kept sealed, to be stored for a longer period at room temperature (approx. 1 month).

10 Procedure

10.1 Blank

Perform a blank determination following paragraphs for the used procedure (selected extraction and clean-up) 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 (50% of the lowest reported value).

10.2 Extraction

10.2.1 General

Depending on the test sample,(origin and moisture content), choose a suitable extraction method. Method 1 is recommended if it is important to break up aggregates in the sample to reach the PAHs . This is especially important with soil samples containing clay particles. With wet samples these methods shall be applied in order to eliminate the presence of water. If solving of the PAHs is the most important step (waste and highly contaminated soil and organic rich materials) and the sample is dry, method 2 using Soxhlet is recommended.For sludges, it has been shown that soxhlet or pressurised liquid extraction (PLE) is applicable. However, A general rule cannot be given, because samples may contain all; aggregates, organic matter and (plastic) waste.

This standard allows other extraction procedures providing:

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Horizontal PAH-standard

- The laboratory can show that the extraction is shown to be equivalent to one of the procedures 1 and 2 as described in this 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 as described in 10.7.5 or 10.8.5

Note For application of this standard for some wastes, addition of acetone with soxhlet extraction has been shown to be effective .

Extraction procedures described in this standard are able to extract up to 20 g of dry sample. If the test sample has a low density (i.e. some wastes) 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 - 20g of soil; 2 - 10 g of sewage sludge, 5 - 20 g of compost or 2 – 20 g of (bio-)waste. The amount of sample has to be weighed with an accuracy of at least 1%.

	Sample contains	Extractant	Equipment	Procedure	remarks
Dry test sample	Soil-like materials, sludge, sediments, biowaste, compost	Acetone/petr oleum ether	agitation	Extraction 1	
	Highly contaminated soil and waste, sludge, biowaste, compost, suspendes solids	Toluene	Soxhlet, Pressurised Liquid Extration	Extraction 2	
Wet test sample	Soil-like material/sedi ments biowaste compost	Acetone/petr oleum ether NaCl	agitation	Extraction 3	Limitations for the amount of water in the sample are given.

Note Annex B List the applicability of the extraction procedure for other organic contaminants

10.2.2 Extraction 1: samples using acetone/petroleum ether

Take 2-20 g dry test sample and place it in a stopperd flask. For GC-MS Analysis with internal calibration add a definite volume of the Internal standard solution. Add 50 ml of acetone to the test sample and extract by shaking thoroughly to break up the soil aggregates for 30 minutes. Then add 50 ml of petroleum ether and shake again

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thoroughly at least for 12 hours Use a horizontal shaking device and have the solvent movement in the shaking bottle in horizontal position. After the solid have been settled decant the supernatant wash the solid phase with 50 ml of petroleum ether and decant again. Collect the extracts in a separating funnel (8.1.1.4) and remove the acetone by shaking twice with 400 ml of water. Dry the extract over anhydrous sodium sulfate . Rinse the sodium sulfate with petroleum ether and add the rinsings to the extract.

Note 1   If the laboratory can prove sufficient extraction in a shorter time than 12 hours, this is acceptable.

Note 2   Tap water has shown to be applicable for removal of the acetone in several laboratories, because target compounds are not present.

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

Note 4   Other extraction techniques, like ultrasonic extraction, microwave or pressurised extraction may be suitable. However if using other extraction techniques the comparability to the method described in this standard has to be proven.

Note 5   The amount X of the internal standard added in all extraction procedures shall have such a quantity that their concentrations in the final extract fall under the working range of the measurement method. Typically the concentration of the individual internal standards in the final extract will be 0,1 µg/ml. I order to 'wet' the complete sample, a minimum amount of 100 µl of internal standard is recommended

Note 6   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 7   In matrice with a high organic matter content (i.e. some sludges) longer extraction procedures can be necessary, Extraction procedure 2 (10.2.3) may be preferred for these samples

10.2.3 Extraction 2: dry samples using Soxhlet / Pressurised liquid extraction

Take 2g-20 g dry test sample and place it the extraction thimble. 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 the solvent (toluene) using soxhlet extraction apparatus. 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

10.2.4 Extraction 3 of wet samples acetone/petroleum ether/Sodium chloride

Take an amount of sample equivalent to 2g to 20g of the dry sample and put it into a 1000 ml screw-cap glass jar or into a 1000 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 according to equation(1):

$$m_{\text{w}} = 50 - \frac{m_{\text{E}} \cdot m_{\text{H}_2\text{O}}}{100} \qquad (1):$$

m_{w} is the water to be added in grams

m_{E} is quantity of the sifted soil sample in grams

$m_{\text{H}_2\text{O}}$ is the water content of the sample according to pr EN 14346 in percent mass, (%)

Add 40 g sodium chloride, 100 ml acetone and 50 ml petroleum ether to the moistened preparations, close the container and shake it by means of a shaker for at least 12 h.

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The organic phase is to be separated, if necessary, using a centrifuge with seal able centrifuge cups. Acetone and polar compounds are to be removed from the organic phase by shaking twice with 150 ml water each in a separatory funnel.

10.3 Concentration or dilution

10.3.1 General

According to the expected contamination level the extract can be concentrated or diluted with appropriate solvent for subsequent analysis when clean up step is not required. If appropriate concentrate the extract to approximately10 ml by evaporation. Transfer the concentrated extract to a calibrated test tube and concentrate to 1 ml using a gentle stream of nitrogen or other inert gas at room temperature.

Note   Too high temperatures and a too high flow of nitrogen may result in loss of the more volatile PAH.

Note 1: Do not evaporate the extracts to dryness, as losses of the 2- or 3-ring compounds may occur. It is advisable to add small amount (100 microlitres) of high boiling solvent as keeper.10.3.2 for GC-MS Analysis

When clean up is not required the extract can be brought to a definite volume and a deuterated PAH is added to the final extract as an injection standard for the recovery check of the internal standards prior to GC-MS injection (see 10.3)

10.3.3 for HPLC Analysis

When clean up is not required, the solvent of the final extract (from extraction procedure 1 and 3) has to be changed from petroleum ether to a solvent such as acetonitrile which is compatible with the mobile phase of the HPLC System. Add for example 0.8 ml acetonitrile to an aliquot e.g. 1 ml of petroleum ether 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 up to the mark of 1,0 ml for subsequent HPLC analysis

Note 2: The enriched extract should not contain residues of hexane or acetone, because the presence of these solvents in the measuring solution leads to interferences with the HPLC

Note 3: For extraction procedure 3, the final extract of toluene can be diluted with acetonitrile in accordance with the expected contamination level prior to HPLC analysis.

10.4 Clean up of the extract

10.4.1 General

Clean-up has to be used if compounds are present that can interfere with the PAH of interest in the GC-MS chromatogram respectively HPLC chromatogram or if that can influence the measurement procedure (i.e. contamination of injector system, column and detection). If no or negligible interfering substances are present, no clean-up is necessary. Depending on the substances to be removed table 2 has to be used. Before application of the clean-up to real samples the laboratory has to ensure that recoveries for a standard after use of the clean-up are at least 80% for all relevant PAH (including spiking and recovery standards). One has to be aware about the fact the PAH are in contrary to the PCB not so stable and persistent so that extreme clean up conditions are not applicable.

Reference to validation reports is made in annex D.

Table 2. Possible clean-up methods

Method	Clean-up	for removal of	Special for	Validated	Remarks

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Clean-up A	Aluminium oxide	polar compounds		soil	Difficult to adjust water content and keep it constant
Clean-up B	Silica	Polar compounds		soil	
Clean-up C	Gel permeation	High molecular compounds, lipids and oils	MS		
Clean-up D,	DMF/cyclohexane	Alipahatic hydrocarbons, lipids and oils	MS		

Addition of keeper during final concentration after clean-up

Before application of the clean-up to real samples the laboratory has to ensure that recoveries after use of the clean-up are at least 80% for all relevant congeners (including internal standards).

Note Other clean-up procedures are also allowed, providing they remove the interfering peaks in the chromatogram and recoveries after use of the clean-up are at least 80% for all relevant congeners (including internal standards).

The extract obtained in 10.3 or in a previous clean-up step has to be quantitatively transferred to the clean-up system; alternatively, an aliquot may be used.

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 (7.3.1.2).

Note 1 Before use, the elution pattern of each series of aluminium oxide columns and the necessary elution volume should be verified with the aid of a standard solution of PAH

With a pipette transfer the extract to the dry packed adsorption column; rinse the test tube twice with 1 ml of petroleum ether and transfer the rinsings 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.

One drop of keeper substance is added to the eluate, and then the eluate is reduced to approximately 0.5 ml at a maximum temperature of 40 °C by means of a suitable enrichment device.

Note 2 Commercial available disposable columns may be used as an alternative if found equally suitable.

10.4.3 Clean-up B - Silica gel

Put glass wool and 10 g silica gel into the glass column for clean-up on silica gel. Then add a 1 cm layer of sodium sulfate and condition with 20 ml of the solvent mixture for silica gel (7.3.2.2) used for dissolving the residue. The sample is put into the column when the level of the solvent mixture is drained to approximately 0, 5 cm above the column packing. The reduced extract or the eluate obtained in the previous clean-up step is to be quantitatively transferred to the column; alternatively, an aliquot may be used.

Elution is performed using a total of 100 ml solvent mixture for the silica gel column. One drop of keeper substance is added to the eluate, and then the eluate is concentrated by removal of the solvent.

10.4.4 Clean-up C b- 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 for gel permeation chromatography (xx). 5 ml of the dissolved residue are put into the GPC column.

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The solvent mixture for GPC is used for elution.

Settings of the GPC system (recommended values):

Flow rate:	5	ml · min⁻¹

Flow rate: 5 $\text{ml} \cdot \text{min}^{-1}$

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 have to be regularly verified by means of the multi-component PAH standard solution.

One drop of keeper substance 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 may occur. This will be visible in a decrease of recoveries of the internal standards. If this occurs readjustment of the sampled volume may 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 dimethylsulfoxide / cyclohexane partitioning, especially for GC-MS measurement.

Transfer an aliquot of the extract (e.g.1 ml) of to a separatory funnel of 100 ml containing 10 ml of DMF/water 9:1(7.3.4.1.1) and remove the aliphatic hydrocarbons by extraction with 10 ml of cyclohexane. Repeat twice. Transfer the DMF/water 9:1 phase to a separatory funnel of 500 ml, add 100 ml of pure water and extract the PAH with 10 ml of cyclohexane. Repeat once. Combine the cyclohexane extracts. One drop of keeper substance is added to the extracts, and then the extract volume is reduced to approximately 0,5 ml by means of a suitable enrichment device.

10.5 Addition of the injection standard

Add appropriate amount of the injection standard to the extract obtained after clean-up (must be in line with the calibration standard). Note the final volume *V*.

10.6 Gas chromatographic analysis

10.6.1 Gas chromatographic analysis with mass spectrometric detection

10.6.2Setting the gas chromatograph

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

GC-conditions:

Separation column:	Capillary column, e.g. DB5 MS 30m, film thickness 0,25µm, 0,25mm i.D. (see 8.2.1)
Oven temperature program:	60 °C, 2 min 30 °C/min to 120 °C 5 °C/min to 300 °C 300 °C, 15 min
Injector temperature:	260 °C
Splitless injection:	1 µl, keep the split 1,8 min closed

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

10.6.3 Mass spectrometric (MS) conditions

The mass spectrometer is tuned 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 3) can be used for the quantitative analysis in selected ion monitoring mode.

Table 3 — Mass numbers of polycyclic aromatic hydrocarbons

Compound	mass number amu
Naphthalene-D_8 (ISTD)	136
Naphthalene	128 (129)
Acenaphthylene	152 (151)
Acenaphthene-D_{10} (ISTD)	164
Acenaphthene	154 (153)
Fluorene	166 (165)
Phenanthrene-D_{10} (ISTD)	188
Phenanthrene	178 (179)
Anthracene-D_{10} (ISTD)	188
Anthracene	178 (89)
Fluoranthene-D_{10} (ISTD)	212
Fluoranthene	202 (101)
Pyrene	202 (101)
Benz(a)anthracene-D_{12} (ISTD)	240
Benz(a)anthracene	228 (114)
Chrysene	228 (114)
Benzo(b)fluoranthene-D_{12} (ISTD)	264
Benzo(b)fluoranthene	252 (253)
Benzo(k)fluoranthene	252 (253)
Benz(e)pyrene-D_{12} (ISTD)	264
Benzo(a)pyrene	252 (253)
Indeno(1,2,3-c,d)pyrene- D_{12} (ISTD)_	288
Indeno(1,2,3-c,d)pyrene	276 (138)
Dibenz(ah)anthracene	278 (139)
Benzo(ghi)perylene	276 (138)

List of the diagnostic ions with relative intensities with reference ISO FDIS 22892 (GC-MS identification) will be given in Annex part

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Horizontal PAH-standard

Note: Each of the PAH target analyte can be quantified by using the deuterated internal standard stated above.

Remarks: Typical examples of chromatograms with instrumental conditions will be incorporated in Annex C

10.6.4 Calibration of the method using internal standard

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 soil test sample as to the calibration solutions. The mass concentration of the standard must be the same for calibration and analysis. Run the GC-MS analysis with the calibration solutions, prepared as described in (6.6.2.3). 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 equation (2):

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

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 in micrograms per mililitre µg/ml;

ρ_d is the mass concentration of the deuterated PAH in the calibration solution micrograms per mililitre in µg/ml;

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

Two types of calibration are distinguished: the initial calibration (10.6.5) and the daily calibration (validity check of the initial calibration); the last one is called recalibration (10.6.6).

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

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

NOTE   Non linear calibration methods are allowed.

10.6.5 Initial calibration

Take a gas chromatogram of a series of at least 5 standard solutions with equidistant concentrations as given in Annex B, including the solvent blank (see Annex B). Identify the peaks; for this consult annex A and if necessary the gas chromatograms of the individual compounds. Prepare a calibration graph for each compound.

Determine the deviations between the measured values and the initial calibration line. When the deviation for the highest concentration is less than 5 %, assume linearity exists for the whole range. When this deviation is more than 5 %, decrease the range by deleting the value for the highest concentration.

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.

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Note 1   It is allowed to use non-linear calibration using all 5 standards. In that case, the same 5 standards have to be used for recalibration and not the selection of 2 described below

10.6.6 Recalibration.

For every batch of samples, inject at least two calibration standards with concentrations of $20 \pm 10\,\%$ and $80 \pm 10\,\%$ of the established linear range and calculate the straight line from these measurements. If the straight line falls within the $\pm 10\,\%$ of the reference values of the initial calibration line, the initial calibration line is assumed to be valid. If not, a new calibration line has to be established according to 9.8.1.1.

10.6.7 Measurement

Measure the gas chromatograms of the extracts obtained under 8.4. 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. Assign the name of a compound if the relative retention time differs from the relative retention time obtained under 8.5.2 by less than 0,2 %.

10.6.8 Identification

For identification of the PAHs apply ISO 22892. In this standard the chromatographic criteria and MS-criteria are described, necessary for proper identification.

10.6.9 Check on method performance

Because this 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.

Add to the volume used for analysis X µl of internal standard and Y µl of injection standard.   This is the performance standard.

Note   The performance standard can be one of the calibration standards, provided that the ratio of the volumes (X/Y) used is the same.

Calculate for each internal standard the ratio between sample and performance standard solution using the closest injection standard.

$$re\mathrm{cov}\,ery\ ratio = \frac{A_1(sample)}{A_2(sample)} \cdot \frac{A_2(p.s.)}{A_1(p.s.)}.100\% \qquad\qquad (4)$$

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

p.s. is performance standard

The average ratio in the sample must be at least 75% of the ratio in the standard. The ratio for an individual PAH should be at least 60%. If not 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 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%.

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Horizontal PAH-standard

7.1.1 Calculation

Calculate the mass content of the individual PAH from the multipoint calibration of the total method by using equation (5):

$$\omega_{\text{n}} = \frac{(A_{\text{n}} / A_{\text{d}}) - b}{s \cdot m \cdot d_{\text{s}}} \cdot \rho\partial \cdot V \qquad (5)$$

where:

ω_{n} is the content of the individual PAH found in the sample in milligrams per kilogram mg/kg on the basis of the dry substance;

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 in micrograms per millilitre µg/ml;

m is the mass of the soil test sample used for extraction in grams g;

d_{s} is the content of the dry substance in the field moist sample in mass fraction, determined according to ISO 11465 in percent %;

V is the volume of the final solution 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 mg/kg dry soil and rounded to one significant figure after the decimal point.

10.7 Liquid chromatography

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

10.7.1 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 C (Will be added)

Remarks: Typical examples of chromatograms with instrumental conditions will be incorporated in Annex C

10.7.2 Detection

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

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10.7.2.1 Ultraviolet detector

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

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

The advantage of ultra-violet detectors is that they have a bigger linear range than fluorescence detectors. Disadvantages are that their sensitivity and selectivity is lower in comparison with fluorescence detectors.

10.7.2.2 Fluorescence detector

Fluorescence detector which is capable of free selection of excitation and emission wavelengths and adjustable during chromatographic separation.

For detection choose the appropriate excitation and emission wavelengths with regard to sensitivity and selectivity. A typical wavelength programme is given in annex A, table A.3.

During wavelength programming baseline disturbance should be avoided. Changes must 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.

Note 1   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 may interfere with the integration and hence with the quantification.

Note 2   To achieve constant peak heights it may be necessary to change wavelengths and damping at the same time. The damping conditions are part of the detection criteria and may not be changed after calibration. If damping is low, the resultant increase in noise should not impair the integration.

10.7.4 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 may be achieved via the absorption spectrum using a diode array detector. This second detector must not lead to interference through broadening of the fluorescence peaks;

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

Horizontal PAH-standard

10.7.5 Calibration

10.7.5.1 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, and 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.

10.7.5.2 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.

10.7.5.3 Routine calibration

After examining the final working range, analyse a minimum of five dilutions of the standard calibration mixture (xxx). 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.

10.7.5.4 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 ten samples. The concentration of this standard solution shall lie 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 10.7.5.3

10.7.5.5 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 2 or 3 injections of a reference solution (xxx).

Measure the sample, the calibration solutions and the blank in the liquid chromatograph.

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.

10.7.5.6 Calculation

Assuming the expected peak area or peak height lays within the linear measuring range the quantified result of an identified substance can be obtained following the equation (6).

$$\omega_n = \frac{Ai \bullet f_i \bullet V}{m} \qquad (6).$$

where

ω_n is the mass content of the substance i of a sample in milligrams per kilogram mg/kg (dry soil);

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

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

V =is the volume of extract, in mililitres ml;

m is the mass of soil (dry soil) in grams g.

The result shall be expressed in mg/kg dry soil. In concentrations less than 1 mg/kg the results shall be expressed with two figures after the decimal point, and in concentrations above that it shall be reported with these significant figures. The dry mass of soil shall be determined on a separate sub sample according to ISO 11465.

11 Test report

The test report shall contain at least the following data:

a)   the information required to identify the sample;

b)   a reference to this European Standard;

c)   the extraction module, clean-up module and detection module

d)   the contents of individual PAH in mg/kg on the basis of dry matter, rounded off in such a way that no more than two significant values are obtained (for instance 12; 5.5; 0.36; 0.082; 0.0069 mg/kg d.m.)

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

13. Bibliography

prEN ISO 17993:2003, *Water quality – Determination of 15 polycyclic aromatic hydrocarbons(PAH) in water by HPLC with fluorescence detection after liquid- liquid extraction*

ISO/TC 147/SC2/WG 19 N34, *Water quality – Determination of polynuclear aromatic hydrocarbons (PAH) – Part 1: Determination of 16 PAH in water by gas chromatography with mass spectrometric detection.*

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LUA NRW Merkblatt Nr. 1, 1994 *Bestimmung von polyzyklischen aromatischen Kohlenwasserstoffen in Bodenproben*

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Methods for water and associated materials, Environmental Agency, UK, 2003: *The determination of polycyclic aromatic hydrocarbons in soil by dichloromethane extraction using gas chromatography with mass spectrometric detection*

ÖNORM L1200, 2003: *Determination of polycyclic aromatic hydrocarbons (PAHs) in soils, sewage sludges and composts*

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Horizontal PAH-standard

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Annex A (Informative) Description on materials for which the method is validated and also materials for which experience is present and future validation should be carried out

For the analysis of PAH, the following relevant sample types are distinguished

- **Sludge**
    - ❑ Sewage sludge
    - ❑ Industrial sludge
- **Sediment, suspended solids**
- **Waste**
    - ❑   Soil-like waste
    - ❑   Building materials containing tar particles, creosote wood, surface treated   materials
    - ❑   , bitumen
    - ❑   Mixed waste (containing different phases)
- **Soil improvers**

    - ❑   Compost (stabilized)

    - ❑   Biowaste (not stabilized) containing organic matter of natural origin

- **Soil**

- **All type of soils**

    - ❑

    - ❑   Mineral soil

    - ❑   Organic rich soil

Note: Resent validation studies are available on www......

Annex B(informative)
Aplicability of extraction procedures for organic contaminants

	Extraction procedure 1	Extraction procedure 2	Extraction procedure 3
PAH	X	X	X
PCB	X		X

Table   Aplicacability of extraction procedures mentioned in this standard for other organic contaminants. X = aplicable

Annex C   Examples of chromatograms with instrumental conditions

Remarks: Typical examples of chromatograms with instrumental conditions will be incorporated

Annex D   Validation results

(Normative)

This method has been validated for the samples and modules described in annex A. These modules are suitable for most samples described in the scope. Because a large amount of different modules and combination of modules can be used applying this method, which are not yet validated, additional preconditions are set.

–   Follow the recommendations for selection of the different modules

–   Recovery of the internal standard as described in 10.7.5 and 10.8.5 is an essential precondition performing this analysis

–   The laboratory has to prove their proficiency according the combination of modules they are using with Certified Reference Standards that fit for purpose(that fit for purpose and/or their results in proficiency testing)

–   If the method in use is not applicable for a specific sample (low recoveries of internal standards), Validation of the new combination of modules is not necessary for this incidental case. Validation with at least 3 CRMs is necessary if this new combination is frequently used by the laboratory.

Note Suitable CRMs (State of the Art 2005) are …………………..

(Informative)

In this annex reference is made to standards and validation reports in which parts of this Horizontal standard were validated.

Extraction procedure

	Extraction	matrix	
Extraction 1		dry soil	
		Wet soil/sediment	
Extraction 2		sludge	

Clean-up procedure

	Clean-up	matrix	
Clean-up A	Aluminium oxide	soil	
Clean-up B	Silica		
Clean-up C	gelpermeation		
Clean-up D,	DMF/cyclohexane		