INTERNATIONAL STANDARD

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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)

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Foreword

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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).

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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*

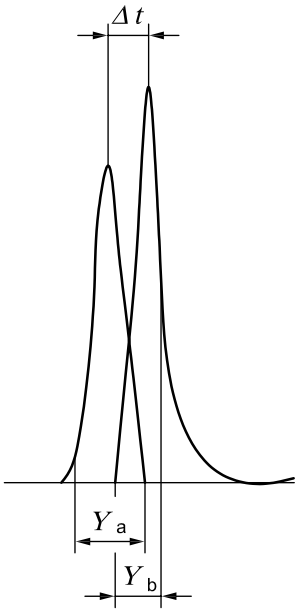
3 Terms and definitions

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.

