SLOVENSKI STANDARD
oSIST prEN 16181:2017

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Blato, obdelani biološki odpadki in tla - Določevanje policikličnih aromatskih ogljikovodikov (PAH) s plinsko kromatografijo (GC) in s tekočinsko kromatografijo visoke ločljivosti (HPLC)

Soil, treated biowaste and sludge - Determination of polycyclic aromatic hydrocarbons (PAH) by gas chromatography (GC) and high performance liquid chromatography (HPLC)

Boden, behandelter Bioabfall und Schlamm - Bestimmung von polycyclischen aromatischen Kohlenwasserstoffen (PAK) mittels Gaschromatographie (GC) und Hochleistungs-Flüssigkeitschromatographie (HPLC)

Sols, biodéchets traités et boues - Dosage des hydrocarbures aromatiques polycycliques (HAP) par chromatographie en phase gazeuse et chromatographie liquide à haute performance

Ta slovenski standard je istoveten z: **prEN 16181**

ICS:

13.030.20	Tekoči odpadki. Blato	Liquid wastes. Sludge
13.080.10	Kemijske značilnosti tal	Chemical characteristics of soils

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Soil, treated biowaste and sludge - Determination of polycyclic aromatic hydrocarbons (PAH) by gas chromatography (GC) and high performance liquid chromatography (HPLC)

Schlamm, behandelter Bioabfall und Boden - Bestimmung von polycyclischen aromatischen Kohlenwasserstoffen (PAK) mittels Gaschromatographie (GC) und Hochleistungs-Flüssigkeitschromatographie (HPLC)

This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 444.

If this draft becomes a European Standard, CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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European foreword

This document (prEN 16181:2017) has been prepared by Technical Committee CEN/TC 444 “Test methods for environmental characterization of solid matrices”, the secretariat of which is held by NEN.

This document is currently submitted to the CEN Enquiry.

This document will supersede CEN/TS 16181:2013.

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

The preparation of this document by CEN is based on a mandate by the European Commission (Mandate M/330), which assigned the development of standards on sampling and analytical methods for hygienic and biological parameters as well as inorganic and organic determinants, aiming to make these standards applicable to sludge, treated biowaste and soil as far as this is technically feasible.

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 document is the result of a desk study “Horizontal European 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 European 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 European Standard is applicable and validated

Matrix	Materials used for validation
Sludge	Municipal sludge
Biowaste	Fresh compost
Soil	Sandy soil

WARNING — Persons using this European Standard should be familiar with usual laboratory practice. This European 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 European Standard be carried out by suitably trained staff.

1 Scope

This European Standard specifies the quantitative determination of 16 polycyclic aromatic hydrocarbons (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 European 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 CAS-RN Chemical Abstracts Service Registry Number.	

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

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 PAHs and presence of interfering substances. These differences make it impossible to describe one general procedure. This European 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 are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

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

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

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

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

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

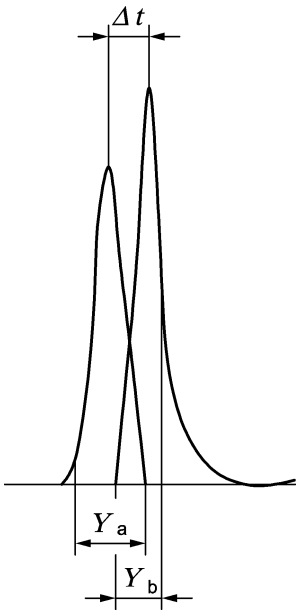
3 Terms and definitions

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

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

[SOURCE: EN 15308:2016, 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

3.2
internal standard
isotopically labelled PAH or PAH unlikely to be present in the sample, added to samples prior to extraction, against which the concentrations of native substances are calculated

3.3
injection standard
PAH that is unlikely to be present in samples added to the sample extract before injection into the gas chromatograph, to monitor variability of instrument response and the recovery of the internal standards

[SOURCE: EN 15527:2008, 3.5, modified – the wording of the definition was revised.]

3.4
Surrogate
PAH that is unlikely to be present in samples added to the sample prior to analysis and used to correct for losses of the PAH analytes during sample extraction or clean-up

4 Principle

Due to the multi matrix (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 European Standard. Performance criteria are described and it is the responsibility of the laboratories applying this European Standard to show that these criteria are met. Using of spiking 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 the information upon the extensive extraction efficiency of the native PAH bonded to the matrix.

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

5.2 Interference with GC-MS

Substances that co-elute with the target PAH may interfere with the determination. These interferences may lead to incomplete resolved signals and may, 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 dibenzo[*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.

A chromatographic resolution of $R > 0,8$ is required for benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and benzo[*k*]fluoranthene ($m/z = 252$). If the criterion is not met for benzo[*j*]fluoranthene, the result shall be reported as the sum of the coeluted PAH.

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

Usually 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 may 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 may 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 are 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

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.

7.1 Reagents for extraction:

7.1.1 Acetone, C_3H_6O.

7.1.2 Hexane-like solvent, boiling range 40 °C to 60 °C.

NOTE Hexane-like solvents with a boiling range between 40 °C and 89 °C are allowed.

7.1.3 Toluene, C_7H_8.

7.1.4 Anhydrous sodium sulfate, Na_2SO_4.

The anhydrous sodium sulfate shall be kept carefully sealed.

7.1.5 Distilled water or water of equivalent quality, H_2O.

7.1.6 Sodium chloride, NaCl, anhydrous.

7.2 Reagents for clean-up:

7.2.1 Clean-up using aluminium oxide:

7.2.1.1 Aluminium oxide, Al_2O_3.

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

NOTE 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. See [15], [16].

7.2.1.2 Deactivated aluminium oxide.

Deactivated with approximately 10 % water.

Add approximately 10 g of water (7.1.5) to 90 g of aluminium oxide (7.2.1.1). 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 might be necessary to adjust the water content.

7.2.2 Clean-up using silica gel 60 for column chromatography:

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

7.2.2.2 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. Then 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 a maximum of two weeks.

Silica gel 60 is stable for at most 2 weeks.

7.2.3 Clean-up using gel permeation chromatography (GPC):

7.2.3.1 Bio-Beads® S-X3[1)]**.**

7.2.3.2 Ethyl acetate, $C_4H_8O_2$.

7.2.3.3 Cyclohexane, C_6H_{12}.

7.2.3.4 Spherical, porous styrene divinylbenzene resin:

Preparation of GPC, for example: Put 50 g Bio-Beads® S-X3 (7.2.3.1) into a 500 ml Erlenmeyer flask and add 300 ml elution mixture made up of cyclohexane (7.2.3.3) and ethyl acetate (7.2.3.2) 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.

7.2.4 Clean-up using liquid-liquid partition/DMF/cyclohexane:

7.2.4.1 Dimethylformamide (DMF), C_3H_7NO.

7.2.4.2 Dimethylformamide:water, 9:1.

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