SLOVENSKI STANDARD
SIST EN 17503:2023

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Nadomešča:
SIST EN 15527:2009
SIST EN 16181:2018
SIST ISO 13877:1999

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

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

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

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

Ta slovenski standard je istoveten z: **EN 17503:2022**

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13.030.01	Odpadki na splošno	Wastes in general
13.080.10	Kemijske značilnosti tal	Chemical characteristics of soils
71.040.50	Fizikalnokemijske analitske metode	Physicochemical methods of analysis

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EUROPEAN STANDARD

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EN 17503

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English Version

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

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

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This European Standard was approved by CEN on 3 January 2022.

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European foreword

This document (EN 17503:2022) has been prepared by Technical Committee CEN/TC 444 “Environmental characterization of solid matrices”, the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by August 2022, and conflicting national standards shall be withdrawn at the latest by August 2022.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 15527:2008 and EN 16181:2018.

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

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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 prescribed steps specified for sampling, pre-treatment, extraction, clean-up by measurement of specific PAH by means of gas chromatography in combination with mass spectrometric detection (GC-MS) or by high performance liquid chromatography (HPLC) in combination with UV-DAD- or fluorescence detection (HPLC-UV-DAD/FLD). Both the GC-MS and the HPLC methods are included in this horizontal standard.

This document was developed by the merging of EN 16181:2018, initially elaborated as a CEN Technical Specification in the European project 'HORIZONTAL' and validated by CEN/TC 400 with the support of BAM, with EN 15527, published by CEN/TC 292.

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

This document 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 document is applicable and validated

Matrix	Materials used for validation
Soil	Sandy soil Mix of soil from an industrial area in Brandenburg, Germany and PAH-free German reference soil
Sludge	Mix of municipal waste water treatment plant sludge from the vicinity of Berlin, Germany
Biowaste	Mix of compost from the vicinity of Berlin, Germany
Waste	Contaminated soil, building debris, waste wood, roofing tar, shredder light fraction

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

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

1 Scope

This document specifies different methods for quantitative determination of 16 polycyclic aromatic hydrocarbons (PAH) (see Table 2) in soil, sludge, treated biowaste, and waste, using GC-MS or HPLC-UV-DAD/FLD covering a wide range of PAH contamination levels (see Table 2).

NOTE The method can be applied to sediments provided that validity is demonstrated by the user.

When using fluorescence detection, acenaphthylene cannot be measured.

Table 2 —Target analytes of this document

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

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

Under the conditions specified in this document, the lower limit of application from 10 µg/kg (expressed as dry matter) for soils, sludge and biowaste to 100 µg/kg (expressed as dry matter) for solid waste can be achieved. For some specific samples (e.g. bitumen) the limit of 100 µg/kg cannot be reached.

Sludge, waste and treated biowaste can differ in properties as well as in the expected contamination levels of PAH and presence of interfering substances. These differences make it impossible to describe one general procedure. This document contains decision tables based on the properties of the sample and the extraction and clean-up procedure to be used.

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

Sampling is not part of this standard. In dependence of the materials, the following standards need to be considered, e.g. EN 14899, ISO 5667-12 and EN ISO 5667-13.

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 15002, *Characterization of waste — Preparation of test portions from the laboratory sample*

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 — Part 1: Linear calibration function*

3 Terms and definitions

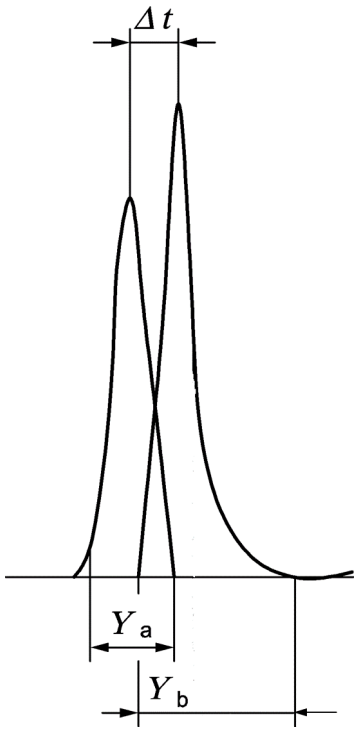
For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at https://www.electropedia.org/
- ISO Online browsing platform: available at https://www.iso.org/obp

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

EXAMPLE Figure 1 shows an example of a chromatogram of a critical pair.



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

where:

R peak separation

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

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

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

Figure 1 — Example of a chromatogram of a critical pair

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

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

[SOURCE: EN ISO 21253-2:2019, 3.10]

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

[SOURCE: ISO 28540:2011, 3.4]

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

3.5
sediment
solid material, both mineral and organic, deposited in the bottom of a water body

[SOURCE: ISO 5667-12:2017, 3.5]

4 Principle

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

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

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

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

The extract is analysed by GC-MS 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 extraction 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 affect the sample during the contact time. Avoid plastics and other organic materials during sampling, sample storage or extraction. Keep the samples from direct sunlight and prolonged exposure to light.

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

5.2 Interference with GC-MS

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

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

Sufficient resolution (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. It can happen that the signal of triphenylene is not completely separated from the signals of benz[*a*]anthracene and chrysene. In this case it shall be stated in the report.

5.3 Interference with the HPLC

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

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

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

6 Safety remarks

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

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

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

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

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

7 Reagents

7.1 General

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

7.2 Reagents for extraction

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

7.2.2 Toluene, C_7H_8.

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

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

7.2.4 Sodium sulfate, Na_2SO_4.

The anhydrous sodium sulfate shall be kept carefully sealed.

7.2.5 Distilled water or water of equivalent quality, H_2O.

7.2.6 Sodium chloride, NaCl.

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

7.3 Reagents for clean-up

7.3.1 Clean-up A using aluminium oxide

7.3.1.1 Aluminium oxide, Al_2O_3.

Basic or neutral, specific surface 200 m^2/g, activity Super I [9].

7.3.1.2 Deactivated aluminium oxide.

Deactivated with approximately 100 g/kg 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.

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

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

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

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

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

Silica gel 60 (7.3.2.1), heated for at least 3 h at 450 °C, cooled down and stored in a desiccator containing magnesium perchlorate or a suitable drying agent. Before use, heat for at least for 5 h at 130 °C in a drying oven. Then allow cooling in a desiccator. Put the silica gel in a stopperad flask and add 10 % water (7.2.5)

(mass fraction), shake for 5 min intensively until all lumps have disappeared and then for 2 h in a shaking device (8.1.3). Store the deactivated silica gel in the absence of air, use it for a maximum of two weeks.

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

7.3.3.1 Bio-Beads® S-X3 [1].

7.3.3.2 Ethyl acetate, $C_4H_8O_2$.

7.3.3.3 Cyclohexane, C_6H_{12}.

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

7.4 Reagents for chromatographic analysis

7.4.1 GC-Analysis

7.4.1.1 Carrier gas for GC-MS.

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

7.4.2 HPLC-analysis

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

7.4.2.2 Ultra-pure water, HPLC purity grade.

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

7.5 Standards

7.5.1 General

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

7.5.2 Calibration substances and internal standards

Table 3 contains native and deuterated PAH to be used for calibration. Verify the stability of the internal standards regularly.

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

Table 3 — Native PAH and deuterated PAH

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

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

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

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

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

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

7.5.3 Injection standard

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

7.6 Preparation of standard solutions

7.6.1 General

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

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

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

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

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

7.6.2 Preparation of calibration standard solutions for GC-MS

Prepare individual concentrated primary standard solutions of about 0,4 mg/ml in hexane-like solvent (7.2.3) by weighing approximately 10 mg of each of the calibration standards (7.5.2 Table 3 left column) to the nearest 0,1 mg and dissolving them in 25 ml of hexane-like solvent.

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

7.6.3 Preparation of internal standard solution for GC-MS

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

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

7.6.4 Preparation of injection standard solution for GC-MS

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

7.6.5 Preparation of calibration standard solutions for HPLC

Prepare individual concentrated primary standard solutions of about 0,4 mg/ml in acetonitrile (7.4.2.1) by weighing approximately 10 mg of each of the calibration standards (7.5.2 Table 3 left column) to the nearest 0,1 mg and dissolving them in 25 ml of acetonitrile.

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