INTERNATIONAL STANDARD

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Soil quality — Determination of polynuclear aromatic hydrocarbons — Method using high-performance liquid chromatography

Qualité du sol — Dosage des hydrocarbures aromatiques polycycliques — Méthode par chromatographie en phase liquide à haute performance

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Contents

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Foreword

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

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 13877 was prepared by Technical Committee TC 190, *Soil quality*, Subcommittee SC 3, *Chemical methods and soil characteristics.*

Annex A of this International Standard is for information only.

Introduction

Polynuclear aromatic hydrocarbons (PAH) can be generated when organic matter is treated under pyrolytic conditions, meaning conditions of incomplete heating or combustion under exclusion of oxygen. In addition to PAH, other substances are formed which cover a wide range of molecular mass, some of which cannot be vaporized without decomposition.

PAH are ubiquitous in soil in a concentration range from 1 μg/kg to 10 μg/kg per single component. In soils influenced by human activities (industries, traffic, etc.) concentrations in the range from 1 mg/kg to 10 mg/kg per single component may be found. In soils from (former) industrial sites, levels of hundreds of mg/kg per single component may be detected.

PAH are neither very volatile nor very soluble in water. They will adsorb on almost any solid surface and have a very strong affinity to organic matter. Therefore PAH can be present in different distributions, e.g. in more or less homogeneous thin layers or in finely divided small particles as well as liquids.

The first situation is found for example in non- or lightly polluted areas. Depending on the type of soil, PAH can be present as a monomolecular layer on the surface of particles or, with clayey types of soil, PAH can also be found within the clay aggregates.

Frequently the second situation is found in more heavily polluted areas. PAH can be present in dust, soot or tar particles.

These differences in occurrence and concentration make it impossible, for the time being, to prepare one single method for all applications. Although high pressure liquid chromatography (HPLC) is used for the determination of PAH in extracts, different approaches for extraction and/or concentration and/or clean-up are necessary.

In this International Standard, two different methods, A and B, are described.

For non- or lightly polluted soils ("μg/kg range") it is of major importance that the extractant be able to break up the soil aggregates and allow an intensive contact between extractant and individual particles. This can be achieved by using a polar extractant, such as acetone, in combination with mechanical shaking (method A).

For more heavily polluted soils ("mg/kg range") a less polar extractant is needed for dissolving PAH from soot or tar particles. Although the highly toxic benzene still is the best extractant, the less toxic toluene is prescribed for this purpose in combination with exhaustive Soxhlet extraction (method B).

Both methods can be applied to all concentration ranges. However, applying acetone extraction for highly contaminated soils and toluene extraction for lightly contaminated soils can result in poor recoveries. Selection of the appropriate method should be based on concentrations of PAH and the expected type of adsorption or distribution within the soil.

In the literature a number of experiments has been reported using different solvents and/or extraction techniques. Solvents such as hexane, cyclohexane, methylene chloride, acetonitrile or tetrahydrofuran have been used. Other extraction techniques such as ultrasonic extraction or supercritical fluid extraction (SFE) have been applied. The results from these experiments are often comparable to those obtained by using the methods given in this International Standard. However, the use of procedures other than those described in this International Standard is not covered and their users should not refer to this International Standard.

Before commencing PAH analysis in a laboratory, it would be wise to discuss the facilities with the appropriate Health Authority. It is suggested that initial familiarization with the techniques be carried out using a non-carcinogenic PAH such as fluoranthene. General techniques for the handling of hazardous materials have been given elsewhere [1], [2], [3], [4]; these publications or their equivalent should be studied before handling PAH.

Soil quality — Determination of polynuclear aromatic hydrocarbons — Method using high-performance liquid chromatography

WARNING: Certain polynuclear aromatic hydrocarbons (PAH) are highly carcinogenic; handle with extreme care. Do not allow solid materials, solvent extracts and solutions of standard PAH to contact the body. PAH can co-distil with solvent and deposit on the outside of stoppered bottles, therefore always handle all vessels containing solutions of PAH using gloves which are solvent-resistant and preferably disposable. Vessels containing PAH solutions should be stored standing in beakers to contain any spillage in case of breakage. PAH contamination of vessels can be detected by irradiation with light of 366 nm wavelength.

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

Dispose of solvent solutions containing PAH in a manner approved for disposal of toxic wastes.

1 Scope

This International Standard describes two methods for quantitative determination of polynuclear aromatic hydrocarbons (PAH) in soil.

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 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 8466-2, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 2: Calibration strategy for non-linear second order calibration function*.

ISO 10381-5, *Soil quality — Sampling — Part 5: Guidance on the procedure for investigation of soil contamination on urban and industrial sites.*

ISO 11464, *Soil quality — Pretreatment of samples for physico-chemical analysis*.

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 the determination of organic contaminants.*

3 Principle

Soil is extracted with acetone without drying (method A) or extracted with toluene after drying (method B).

NOTE 1 When applying method B, loss of naphthalene can occur while air-drying soil.

Analysis of the extract is carried out by high performance liquid chromatography (HPLC), using ultraviolet or fluorimetric detectors with variable excitation and emission wavelengths. Quantitative evaluation is carried out by recording detector signals (area or height) by the external standard method.

16 compounds, listed in 5.11 (the so-called EPA-priority pollutants PAH [5]), can be determined. For other PAH, the validity of the method has yet to be proven.

NOTE 2 Acenaphthylene cannot be measured using fluorimetric detection.

4 Apparatus

4.1 General

4.1.1 Analytical balance, with an accuracy of 0,01 g.

4.1.2 Analytical balance, with an accuracy of 0,01 mg.

NOTE This balance should be used only for preparation of standards.

4.1.3 Drying cabinet in accordance with ISO 11465.

4.2 Apparatus for sample preparation (Method A)

4.2.1 Conical flask of 500 ml capacity.

4.2.2 Separating funnel of 1000 ml capacity.

4.2.3 Shaking machine, horizontal movement with up to 200 shakes per minute.

4.2.4 Concentration apparatus, for example Kuderna-Danish or rotary apparatus.

4.2.5 Water bath, with a temperature range up to 100 °C.

4.2.6 Chromatography column for clean-up, internal diameter 1 cm.

4.3 Apparatus for sample preparation (Method B)

4.3.1 Soxhlet, approx. 30 ml, with paper or glass fibre thimbles, 100 ml round-bottom flask and reflux condenser.

NOTE If blank values cannot be excluded, thimbles should be cleaned with toluene before use.

4.3.2 Measuring flasks, 50 ml and 100 ml nominal volumes.

4.2 Apparatus for analysis

4.4.1 High performance liquid chromatograph, equipped according to requirements with either an ultraviolet detection system (with variable wavelength) or a fluorimetric detection system (with free choice of excitation and emission wavelengths). If fluorimetric detection is used, degassing (removal of oxygen) of mobile phase, e.g. by helium, is necessary.

4.4.2 Separation column with guard column, with reversed-phase materials for PAH analysis.

NOTE In soil analysis, utilization of a guard column helps to improve the durability of the analytical column.

4.4.3 Recording device, computer integrator with printer/plotter. Measuring range according to HPLC output signal, preferably with monitor interpretation and the possibility of subsequent baseline correction.

5 Reagents

5.1 General reagents

Chemicals fulfilling the requirements for residue analysis and which do not contain PAH are suitable for sample preparation. Periodic blank value determinations shall be carried out to verify the purity of chemicals, generally after introduction of new stock batches. Batches of solvents containing PAH either shall be substituted by ones free from PAH, or shall be purified by distillation (e.g. over a Vigreux column of 1 m length).

5.2 Acetone, p.a. grade or residue analysis grade (A).

5.3 Petroleum ether (boiling range 40 °C to 60 °C), p.a. grade or residue analysis grade (A).

5.4 Toluene, p.a. grade or residue analysis grade (B).

5.5 Sodium sulfate (anhydrous), p.a. grade.

5.6 Aluminium oxide, basic or neutral, specific surface area 200 m^2/g, activity super I according to Brockmann.

5.7 Methanol or acetonitrile, HPLC purity grade.

5.8 Water.

5.8.1 For extraction (A): **double-distilled or deionized**.

5.8.2 For HPLC mobile phase: **ultra-pure water** (HPLC purity grade).

5.9 Helium, of suitable purity for degasification of solvents (see 4.4.1).

5.10 Nitrogen, of suitable purity of volume reduction.

NOTE It should be verified that no impurities are introduced, e.g. by plastics pipe installations.

5.11 Reference materials

NOTE Reference materials and standard solutions of PAH are available from a limited number of suppliers.[1)]

No.	Substance	CAS No.
1	Naphthalene	91-20-3
2	Acenaphthylene	208-96-8
3	Acenaphthene	83-32-9
4	Fluorene	86-73-7
5	Phenanthrene	85-01-8
6	Anthracene	120-12-7
7	Fluoranthene	206-44-0
8	Pyrene	129-00-0
9	Benz[a]anthracene	56-55-3
10	Chrysene	218-01-9
11	Benzo[b]fluoranthene	205-99-2
12	Benzo[k]fluoranthene	207-08-9
13	Benzo[a]pyrene	50-32-8
14	Dibenz[ah]anthracene	53-70-3
15	Benzo[ghi]perylene	191-24-2
16	Indeno[1,2,3-cd]pyrene	193-39-5

5.12 Standard solutions

WARNING: Do NOT undertake the preparation of PAH standards from the solid materials unless the correct safety equipment is available. Use proper handling techniques.

Weigh approximately 5 mg of each of the reference materials (5.11) with an accuracy of 0,01 mg and place into a 100 ml measuring flask. Fill with methanol or acetonitrile (5.7) up to the mark. From this stock solution take 1 ml by using calibrated pipettes with an accuracy of 1 % and introduce into an ampoule which can be sealed by melting. After cooling with liquid nitrogen, seal the ampoule by melting. Store the ampoule in a sparkproof refrigerator; contents are stable under these conditions for at least one year.

For use as a calibration solution, transfer the contents of an ampoule into a 100 ml measuring flask and make up with acetonitrile (method A) or toluene (method B). If very low concentrations of PAH in soil are expected, and if using a fluorimetric detector, prepare the calibration solution with acetonitrile and dilute further at a ratio of 1 : 10.

NOTE It is advisable to use commercially available standards unless the laboratory has great experience in handling hazardous materials.

5.13 Commercially prepared standards

Stock solutions in e.g. acetonitrile are available commercially. Their use is strongly recommended.

To prepare the calibration solution, dilute the stock solution in accordance with 5.12.

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1) Certified standards are supplied by:

Institute for Reference Materials and Measurement (IRMM), Retiesweg, B-2440 Geel, Belgium.

National Institute for Science and Technology, Office of Standard Ref. Data, Washington D.C. 20234 USA.

Other suitable sources may be available; this information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of the suppliers named.

6 Sampling, sample conservation and pretreatment

Sampling shall be carried out in accordance with ISO 10381-5 in cooperation with the analytical laboratory. Store soil in containers which do not influence the PAH content of the samples, e.g. glass or metal. The sample mass shall be about 500 g to 1000 g.

NOTE 1 A detailed sampling report should be provided with the samples.

Detailed information on sample conservation and pretreatment can be taken from ISO 14507.

Stones and other material of diameter larger than 10 mm and obviously not contaminated should be separated, weighed and the result of weighing recorded. Large particles with expected contamination shall be reduced in size and analysed separately or together with the finer sample material. In the test report, specify the procedure followed to ascertain the original condition of the sample and specify to what part the analytical result relates.

To minimize degradation, keep samples in the dark and preferably cool (i.e. 4 °C ± 2 °C).

NOTE 2 Samples should be analysed as quickly as possible. Microbial degradation can occur, especially when investigating agricultural soils.

If using method B, dry the sample before analysis in air in accordance with ISO 11464. If the soils tend to agglomerate during drying, crush them again in a mortar.

Sieve the sample to a particle size of 2 mm in accordance with ISO 11464. Determine and record the percentage mass fraction of diameters < 2 mm and > 2 mm.

NOTE 3 Use of a sieving apparatus is advantageous.

Take a subsample from the fraction < 2 mm in accordance with ISO 11464.

If soils have a high content of technological substrate (e.g. ashes, bricks) or if these substrates have to be analysed, crush the whole sample to < 2 mm. This procedure is usually chosen when soils from abandoned industrial sites have to be investigated.

7 Procedure

7.1 Extraction Method A: non- or lightly polluted soil

7.1.1 Extraction procedure

Weigh about 20 g of soil (4.2.1) to the nearest 0,1 g into a conical flask of 500 ml capacity. Add 200 ml of acetone and close the container. Shake vigorously on a mechanical shaker (4.2.3) for 15 min.

Add 100 ml of petroleum ether to the mixture of soil and acetone and shake for another 15 min.

After settling, decant the supernatant into a separating funnel of 1000 ml capacity (4.2.2). Remove acetone and polar compounds from the supernatant by shaking twice with 800 ml water each. Discard the water.

Dry the remaining extract over anhydrous sodium sulfate, transfer the dried extract into a concentrator (4.2.4) and reduce volume to about 10 ml. During this stage the temperature of the water bath should be in the range 40 °C to 60 °C. Concentrate at room temperature with a gentle stream of nitrogen to about 1 ml.

7.1.2 Clean-up procedure

Depending on the purpose of investigation and the required limit of detection, a clean-up may be necessary. If required, proceed as follows:

Dry aluminium oxide (5.6) overnight at 150 °C. Cool in a desiccator and add 11 g of water per 89 g of alumina. Transfer into a tightly closed bottle. Shake until all lumps have been dispersed and let the alumina equilibrate for at least 16 h before use.

Check the activity of the prepared aluminium oxide regularly by clean-up of a standard solution.

Prepare an adsorption column by weighing (2,0 ± 0,1) g of deactivated alumina into a chromatographic tube (4.2.6) fitted with a small plug of glass wool at the bottom. Add about 1 cm of anhydrous sodium sulfate to the top of the column.

NOTE 1 Commercially available disposable columns may also be used if found to be equally suitable.

Transfer the concentrated extract to the top of the column with a Pasteur pipette. Rinse the concentrator tube twice with 1 ml of petroleum ether and add the rinsings to the column. Elute with 8 ml of petroleum ether and collect the eluate in a pointed, calibrated test tube, e.g. a Kuderna-Danish concentrator tube. Add about 0,8 ml of acetonitrile 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.

NOTE 2 When desired, 20 µl of an internal standard solution may be added at this stage. The internal standard is only used for verification of the retention time. A suitable internal standard solution is prepared by dissolving 6-methylchrysene into acetonitrile to a concentration of 15 µg/ml.

7.2 Extraction Method B: polluted soils

Weigh 15 g to 30 g of air-dried soil to the nearest 0,1 g into a thimble and extract in a Soxhlet apparatus (4.3.1) for 4 h to 8 h using 50 ml to 100 ml of toluene.

Determine the actual time for extraction for each individual case. In particular, soils with larger amounts of fine-grain material and high contamination need longer times for extraction.

Place the extract in a measuring flask, fill with toluene to 50 ml or 100 ml and take an aliquot for analysis.

7.3 HPLC analysis

7.3.1 General considerations

The operating conditions for HPLC analysis can be varied over a wide range. To give advice to the user of this International Standard, two examples for HPLC analysis are presented in 7.3.2. However, the appropriate operating conditions have to be chosen according to the equipment and column used.

Chromatograms of real samples show more peaks than those of PAH alone. Therefore optimization of the chromatographic conditions should be based not only on the standard solution.

NOTE In general, at least 25 min are required to obtain an appropriate separation of the PAH and other components in the sample.

Before using new columns, check the separation and order of elution on a calibration mixture.

7.3.2 Examples of HPLC operating conditions

7.3.2.1 General

Two examples of operating conditions are presented, one for detection using ultraviolet (diode array) and one for detection using fluorimetry, under identical conditions of the separating system.

The following parameters have been found advantageous in soil analysis:

Injector:	autoinjector
Injection volume:	10 µl
Column:	250 mm × 4,6 mm reversed-phase C18 material for PAH analysis, 5 µm particle size
Mobile phase:	methanol/water

Flowrate:	1 ml/min
Gradient:	50 % methanol, in 20 min to 100 %, then 10 min isocratic
Recording device:	laboratory data system.

NOTE 1 Use of toluene as solvent leads to spreading of the bandwidth of early-eluting substances. For this reason the injection volume normally should not exceed 10 µl.

NOTE 2 For some batches of applied materials, it has been shown that under given conditions dibenz[ah]anthracene and benzo[ghi]perylene are not separated. Separation is improved by adding 10 % acetonitrile to the mobile phase.

7.3.2.2 Procedure using ultraviolet (diode array) detection

In this example a diode-array detector (DAD) is used. However, comparable results can be obtained by a conventional ultraviolet detector with variable wavelength.

Diode-array detectors offer the possibility of selecting appropriate wavelengths during the interpretation of the results, without difficulties with shifting baselines when wavelengths are changed mechanically during measurements.

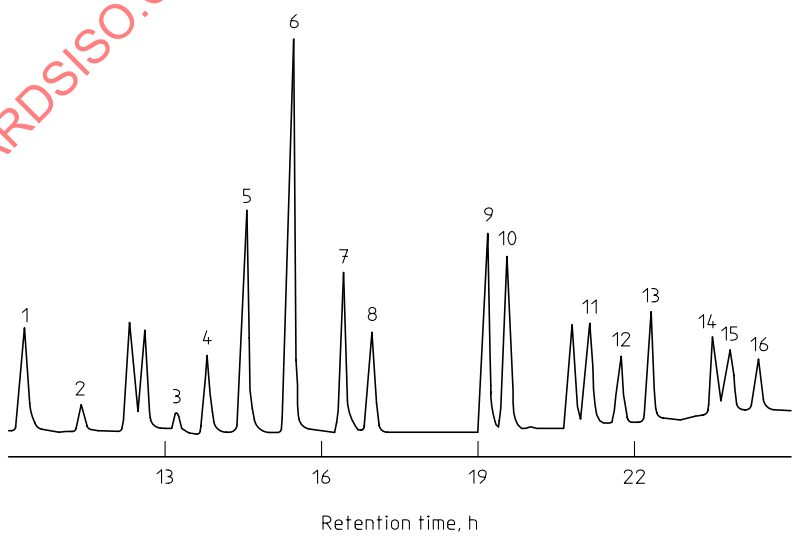
A second advantage is that with diode-array detectors the confirmation of the results can be obtained from the same measurement, e.g. by using different wavelengths and/or checking of the whole spectrum.

Detection with UV has the advantage of a wide linear range. A disadvantage is the lower sensitivity compared to the fluorimetric detector and also the lower selectivity. Diode-array detectors offer the opportunity of switching wavelengths during the measurement. This is used in the following.

Detector:	diode-array detector (UV)
Wavelength:	280 nm (switch before fluorene)
	254 nm (switch before fluoranthene)
	270 nm

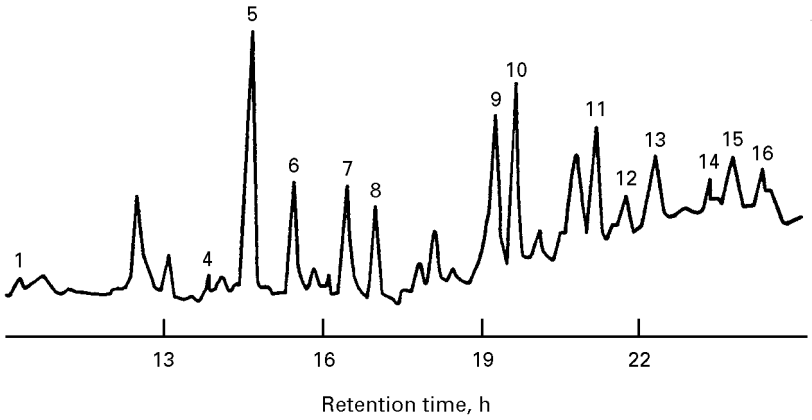
Under these conditions, all 16 components listed in 5.11 can be determined. A chromatogram of the standard solution is given in Figure 1. A chromatogram of a soil extract is given in Figure 2.

NOTE The standard solution in Figure 1 also contains other compounds not covered by this International Standard in detail.



NOTE For numbering of peaks refer to compounds listed in 5.11.

Figure 1 — PAH chromatogram of standard solution (DAD)



NOTE For numbering of peaks refer to compounds listed in 5.11.

Figure 2 — PAH chromatogram of soil extract (DAD)

7.3.2.3 Procedure using a fluorimetric detector

The fluorimetric detector (FLD) has the advantage of high sensitivity and selectivity. These qualities only count in case of free choice of excitation and emission wavelengths and if these can be switched during the chromatogram. A disadvantage is that at high concentrations the linear range is exceeded rather rapidly.

Operational parameters change between fields of application and between manufacturers. The following conditions have proved to be advantageous:

Detector: fluorimetric detector

Wavelengths: excitation/emission, 218 nm/332 nm

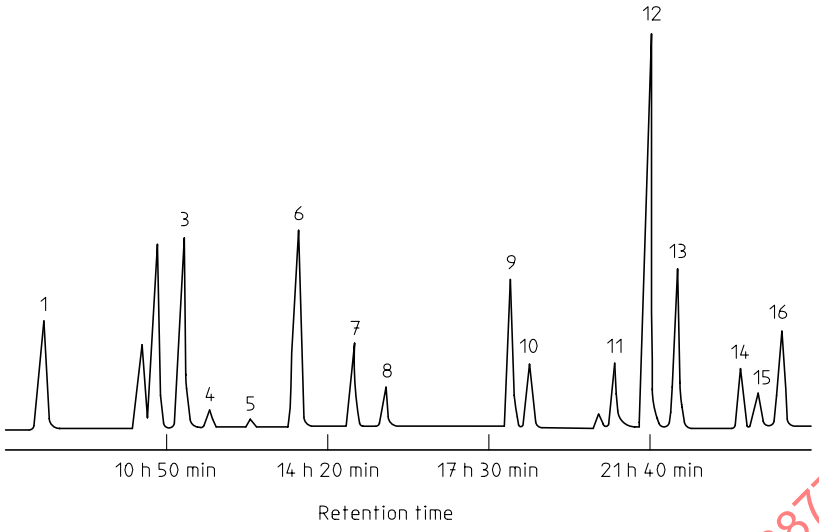
(switch before phenanthrene) 260 nm/420 nm

(switch before benzo[b]fluoranthene) 290 nm/420 nm

(switch before indeno[1,2,3-cd]pyrene) 248 nm/500 nm

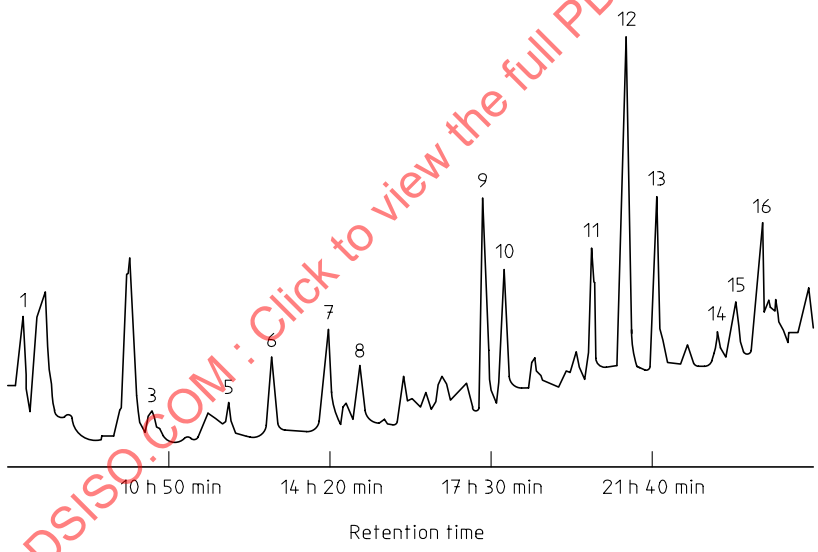
A chromatogram of the standard solution is given in Figure 3. A chromatogram of a soil extract is given in Figure 4.

NOTE Acenaphthylene cannot be determined using fluorescence detectors.



NOTE For numbering of peaks refer to compounds listed in 5.11.

Figure 3 — PAH chromatogram of standard solution (FLD)



NOTE For numbering of peaks refer to compounds listed in 5.11.

Figure 4 — PAH chromatogram of a soil extract (FLD)

7.4 Set-up of calibration function and checking for confirmation of measured value

The HPLC chromatogram presents qualitative and quantitative information. A substance is characterized by its retention time. Allocation of a measuring signal (peak) to a particular substance is carried out by comparison to reference substances. Peak height or peak area is proportional to the injected mass of the substance, within the linear range.

NOTE 1 Wavelength pairs and number of switchovers are always a compromise between optimal detection and practicability. In the examples given, the number of switchovers has been kept as small as possible. For alternative pairs see annex A.

For quantitative determination of a single PAH, correct registration of the peak area is required. Incomplete separation of peaks, great asymmetry of peaks (tailing) and uneven baselines (noise, disturbing background, strong baseline drift) make correct integration difficult. In critical areas of the chromatogram, correct baseline construction by the integrator shall be observed for evaluation. Modern integrators and data systems include post-run corrections of baselines.

Peak height can also be considered for quantitative determination, especially in the case of incomplete separation of single substances. This procedure is helpful in soil analysis.

Before beginning but also during the analysis, the HPLC system shall be calibrated. Apply the calibration function in accordance with ISO 8466-1 or ISO 8466-2 by preparation and measurement of at least six dilutions from the stock solution. The resulting calibration curve shall be linear.

In daily practice, checking the calibration with one concentration is sufficient. When using an automatic sampling device, checking should be after 7 to 10 injections, while with manual injections twice a day is sufficient.

NOTE 2 If the HPLC system is not used continuously, it should be checked in terms of operating conditions before the start of calibration and analysis. Experience has shown that, depending on the applied system, the first and sometimes the second injection of the calibration solution should be rejected, so that the second or third injection can be used for checking the HPLC system.

NOTE 3 Within a series or with long operation times, drifting of retention times can appear. Modern apparatus equipped with relevant software can compensate this drift automatically by using a calibration mixture between the samples.

When analysing samples, the signals for each component should be within the linear range of the detection system used. If this is not the case, prepare dilutions of the sample extract and analyse again.

Carry out quantification using the method of external standards. On completing execution of the analytical method, chemicals, solvents and apparatus shall be checked for absence of PAH, starting with extraction of empty thimbles. Blank values shall not exceed 10 % of the lower application limit. Blank values are not considered for calculation.

NOTE 4 Attempts should be made to keep blank values much smaller than 10 % of the lower application limit.

Blank values in the range of PAH concentrations existing in real samples are not acceptable. Their source shall be determined and eliminated by checking the individual steps of the procedure.

In case of doubt, it is recommended to confirm the results obtained. This may be done by using a different wavelength for UV absorption; or a different combination of wavelengths with fluorimetric detection techniques (see annex A) based on different principles, e.g. GC-MS may also be used for confirmation.

8 Calculation

Assuming that the expected peak area or peak height lies within the linear measuring range, the quantified result of an identified substance can be obtained using the following equation.

$$C_i = \frac{A_i \cdot f_i \cdot V}{m}$$

where

C_i is the concentration of substance i of a sample, in milligrams per kilogram of dry soil;

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

f_i is the correction factor (response factor) of substance i, in micrograms per millilitre;

V is the volume of extract, in millilitres;

m is the mass of dry soil, in grams.

The result shall be expressed in milligrams per kilogram dry soil. In concentrations less than 1 mg/kg, the results shall be expressed to two decimal places, and in concentrations above that it shall be reported to three significant figures. The dry mass of soil shall be determined on a separate subsample in accordance with ISO 11465.

9 Performance characteristics

9.1 General

Soil is a complex and problematic matrix. Because of the inhomogeneous distribution of PAH and other pollutants in soil, it is rather difficult to homogenize a sample sufficiently. Normally, mechanical size reduction of the soil, especially milling, should preferably be avoided due to dangers of contamination and cross-contamination but also because of losses (through increasing temperature and surface area). In some cases reduction of size cannot be avoided.

9.2 Standard deviation of ultraviolet (diode array) detection

In the following subclauses, standard deviations and repeatability resulting from a measurement series covering the total procedure according to conditions set out in 7.2 (method B) and 7.3 are listed.

The sample was milled before analysis and homogenized intensively for 24 h.

Table 1 lists performance characteristics at higher concentrations, based on five determinations of the total procedure.

Standard deviations are between 4 % and 10 %.

Table 1 — Mean values, $\bar{x}$, and standard deviations, s, at concentrations between 2 mg/kg and 30 mg/kg (laboratory trial)

Compound	$\bar{x}$ mg/kg	s mg/kg	s_{rel} %
Naphthalene	not present		
Fluorene	0,5	0,02	3,7
Phenanthrene	11,2	0,38	3,4
Anthracene	2,4	0,08	3,5
Fluoranthene	26,7	0,91	3,4
Pyrene	18,2	0,70	3,8
Benz[a]anthracene	16,2	0,57	3,5
Chrysene	8,9	0,36	4,1
Benzo[b]fluoranthene	10,6	0,45	4,3
Benzo[k]fluoranthene	4,7	0,26	5,6
Benzo[a]pyrene	8,7	0,44	5,1
Dibenz[ah]anthracene	4,1	0,40	9,7
Benzo[ghi]perylene	5,1	0,48	9,5
Indeno[1,2,3-cd]pyrene	4,9	0,28	5,7

9.3 Comparison of standard deviation of UV and fluorimetric detections

Performance characteristics using method B for both detection methods for a "real" soil sample are listed in Table 2. Standard deviations for both detection methods are between 7 % and 20 % (up to 46 % for dibenz[ah]anthracene).

Table 2 — Mean values and standard deviations of application example

Compound	UV detection		Fluorescence detection	
	$\bar{x}_m$ m/kg	s_{rel} %	$\bar{x}_m$ m/kg	s_{rel} %
Naphthalene	4,1	15	5,3	19
Fluorene	—	—	0,8	10
Phenanthrene	9,0	9	11,4	10
Anthracene	2,2	9	1,8	11
Fluoranthene	11,4	10	14,4	8
Pyrene	13,5	9	13,4	9
Benz[a]anthracene	8,8	11	6,9	16
Chrysene	4,7	10	6,3	21
Benzo[b]fluoranthene	6,6	7	6,6	13
Benzo[k]fluoranthene	5,1	7	3,6	9
Benzo[a]pyrene	6,7	11	7,0	12
Dibenz[ah]anthracene [a]	1,1	46	1,7	15
Benzo[ghi]perylene	5,4	10	5,0	14
Indeno[1,2,3-cd]pyrene	4,6	11	3,7	11

[a] Dibenz[ah]anthracene is often present in only low concentrations and is not well separated from neighbouring peaks. Therefore it is recommended to use fluorometric detection at low concentration.

9.4 Limit of detection/Lower limit of application

Under conditions of the example, including extraction of 20 g soil, making up the extract to 50 ml and injection of 10 µl sample, concentrations of 0,03 mg/kg and even lower can be determined for individual compounds using a UV detector.

Under these circumstances, a lower limit of application of 0,1 mg/kg can be ensured for all compounds.

Using fluorimetric detection and appropriate choice of wavelength pairs, the lower limit of application is 0,01 mg/kg per single PAH.

NOTE 1 Because of lower concentrations on the column, higher sensitivity of the fluorimetric detector can be obtained after diluting the sample to obtain improved separation; moreover, a smaller injection volume can be used.

NOTE 2 For the compound benzo[a]pyrene it can be important to achieve quantitative results at even lower concentrations in soil. A lower limit of application of 0,02 mg/kg is under discussion. Application of fluorimetric detection therefore is strongly recommended.

NOTE 3 More sensitive detection only lowers the limit of detection, while trueness of result is not improved. In low concentration ranges, blank values and especially inhomogeneities of soil play important roles.