INTERNATIONAL STANDARD

ISO 7981-2

First edition
2005-06-15

Water quality — Determination of polycyclic aromatic hydrocarbons (PAH) —

Part 2: Determination of six PAH by high-performance liquid chromatography with fluorescence detection after liquid-liquid extraction

Qualité de l'eau — Détermination des hydrocarbures aromatiques polycycliques (HAP) —

Partie 2: Dosage de six HAP par chromatographie de haute performance en phase liquide avec détection fluorimétrique à la suite d'une extraction liquide-liquide

Reference number
ISO 7981-2:2005(E)

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Published in Switzerland

Contents

Page

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 2.

The main task of technical committees is to prepare International Standards. 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.

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

ISO 7981-2 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

ISO 7981 consists of the following parts, under the general title *Water quality — Determination of polycyclic aromatic hydrocarbons (PAH)*:

— *Part 1: Determination of six PAH by high-performance thin-layer chromatography with fluorescence detection after liquid-liquid extraction*

— *Part 2: Determination of six PAH by high-performance liquid chromatography with fluorescence detection after liquid-liquid extraction*

Introduction

Polycyclic aromatic hydrocarbons (PAH) are present in nearly all types of waters. These substances are adsorbed on solids (sediments, suspended matter) as well as dissolved in the liquid phase.

Some PAH are known or suspected to cause cancer. The maximum acceptable levels of PAH in waters intended for human consumption are given in European Legislation [1] [2] [3] [4].

The sum of the mass concentrations of the six PAH specified in this part of ISO 7981 usually is about 0,01 µg/l to 0,05 µg/l in ground water, up to 1 µg/l in surface water, and up to 1 000 µg/l in waste water.

Water quality — Determination of polycyclic aromatic hydrocarbons (PAH) —

Part 2:
Determination of six PAH by high-performance liquid chromatography with fluorescence detection after liquid-liquid extraction

WARNING — Some compounds being measured are presumed to be carcinogenic. Acetonitrile and hexane are harmful.

Persons using this part of ISO 7981 should be familiar with normal laboratory practise. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this part of ISO 7981 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 part of ISO 7981 be carried out by suitably trained staff.

1 Scope

This part of ISO 7981 specifies the determination of six selected PAH in drinking, mineral and table waters and ground and surface waters in mass concentrations above 0,005 µg/l, by high-performance liquid chromatography with fluorescence detection after liquid-liquid extraction. The six PAH are: fluoranthene, benzo[*b*]fluoranthene, benzo[*a*]pyrene, benzo[*k*]fluoranthene, indeno[1,2,3-*cd*]pyrene, and benzo[*ghi*]perylene (see Table 1).

With some modification, this method is also applicable for the analysis of moderately polluted waste waters.

2 Normative references

The following referenced documents are indispensable for the application 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.

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*

3 Principle

Since PAH can to a large extent be adsorbed on particulate matter, the whole sample is analysed.

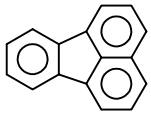
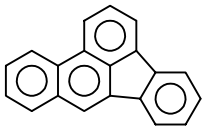
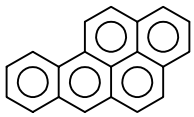
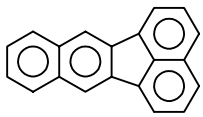
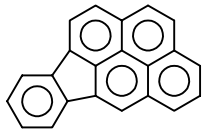
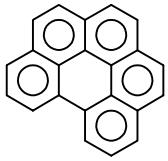
NOTE For the analysis of surface water, a differentiation between dissolved and undissolved PAH can be desirable, but this is not relevant for drinking water.

PAH are extracted from the water sample by liquid-liquid extraction. The extract is evaporated to dryness and the residue is taken up in a solvent and analysed.

Extracts of surface waters and other contaminated water samples should be cleaned on silica (8.4) prior to analysis.

PAH are then separated by high performance liquid chromatography (HPLC) on suitable stationary phases under isocratic conditions, identified and quantified by means of fluorescence detection at a constant combination of excitation and emission wavelengths.

Table 1 — Polycyclic aromatic hydrocarbons determinable by this method

Name	Chemical formula	Molar mass g/mol	Carbon fraction	CAS-number	Structure
Fluoranthene	$C_{16}H_{10}$	202,26	95,0	206-44-0	
Benzo[*b*]fluoranthene	$C_{20}H_{12}$	252,32	95,2	205-99-2	
Benzo[*a*]pyrene	$C_{20}H_{12}$	252,32	95,2	50-32-8	
Benzo[*k*]fluoranthene	$C_{20}H_{12}$	252,32	95,2	207-08-9	
Indeno[1,2,3-*cd*]pyrene	$C_{22}H_{12}$	276,34	95,6	193-39-5	
Benzo[*ghi*]perylene	$C_{22}H_{12}$	276,34	95,6	191-24-2	

4 Interferences

4.1 Interferences with sampling and extraction

Use sampling containers made of materials (preferably of glass or steel) that do not affect the sample during the contact time. Avoid plastics and other organic materials during sampling, sample storage or extraction.

If automatic samplers are used, avoid the use of silicone or rubber material for the tubes. If present, make sure that the tubes are as short as possible. Rinse the sampling line with the water to be sampled before the test sample is taken. ISO 5667-2 and ISO 5667-3 can be used for guidance.

Keep the samples from direct sunlight and prolonged exposure to light.

During storage of the test sample, losses of PAH can occur due to adsorption on the walls of the containers. The extent of the losses depends on the storage time.

4.2 Interferences with the HPLC

Substances that show either fluorescence or quenching and co-elute with the PAH 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 overlaps will prevent the measurement of peak height and/or area. Unsymmetrical peaks and peaks broader than the respective peaks of the reference substance suggest interferences.

5 Reagents

Use only reagents of recognized analytical grade (e.g. “for residue analysis” or “for HPLC analysis”) as far as available, and only distilled water or water of equivalent purity showing the lowest possible fluorescence.

Monitor the blank to guarantee that the reagents do not contain PAH in detectable concentrations (see Clause 12).

5.1 Solvents

5.1.1 Solvents for extraction and clean-up of the extract

5.1.1.1 **Cyclohexane**, C_6H_{12}

5.1.1.2 **Hexane**, C_6H_{14}

5.1.1.3 **Dichloromethane**, CH_2Cl_2

Other volatile solvents may be used as well, if it is proved that the recovery is equivalent or better.

NOTE Dichloromethane often contains stabilizers, e.g. ethanol or amylene. Stabilizers can influence the elution strength of the eluent. Without stabilizer, free radicals might develop. This can lead to degradation of PAH. The presence of hydrogen chloride indicates the presence of radicals. Hydrogen chloride can be determined by extracting dichloromethane with water and measuring the pH value.

5.1.2 HPLC solvents

5.1.2.1 **Methanol**, CH_3OH

5.1.2.2 **Acetonitrile**, CH_3CN

5.1.2.3 **Tetrahydrofuran**, C_4H_8O, without stabilizer

NOTE Tetrahydrofuran can contain peroxides. Although peroxides have not yet shown to cause any interference with the HPLC determination, it is preferred to use batches with low peroxide content (regularly checked using test rods). It is of advantage to use small packages.

5.2 **Sodium thiosulfate pentahydrate**, $Na_2S_2O_3 \cdot 5H_2O$

5.3 **Sodium chloride**, NaCl

5.4 **Sodium sulfate**, Na_2SO_4, anhydrous, precleaned by heating to 500 °C.

5.5 **Nitrogen**, having a purity (volume fraction) of at least 99,999 %.

5.6 Helium, having a purity (volume fraction) of at least 99,999 %.

5.7 Silica, with an average particle size approximately 40 µm and stored in a desiccator to ensure maximum activity.

NOTE Prepacked silica cartridges are commercially available.

5.8 Molecular sieve beads, pore size 0,4 nm.

5.9 Reference substances (see Table 1)

Because of the dangerous nature of the substances to be used, it is highly recommended to use commercially available, preferably certified, standard solutions. Avoid skin contact.

5.10 Single-substance stock solutions, of those listed in Table 1, diluted in acetonitrile (5.1.2.2) to a mass concentration of, for example, 10 µg/ml.

5.11 Multiple-substance stock solution, preferably certified, diluted in acetonitrile (5.1.2.2) to a mass concentration of, for example, 10 µg/ml for each individual compound.

5.12 Calibration solutions

Prepare at least five calibration solutions by appropriate dilution of the stock solution (5.11), using methanol (5.1.2.1) or acetonitrile (5.1.2.2) as solvent. The choice of solvent depends on the composition of the mobile phase.

For example, using 50 µl of the stock solution (5.11) in a graduated 10 ml flask (6.16), make up to volume with acetonitrile (5.1.2.2) or methanol (5.1.2.1). 1 µl of this reference solution contains 50 pg of the respective individual substance.

NOTE The solutions 5.10 to 5.12 are stable for at least one year when stored in the dark at room temperature and protected from evaporation.

6 Apparatus

Standard laboratory equipment cleaned to eliminate all interferences.

Clean all glassware, for example, by rinsing with detergent and hot water, and drying for about 15 min to 30 min at about 120 °C. After cooling, rinse with acetone, seal the glassware and store in a clean environment.

Glassware that has been in contact with waste water samples or samples with high PAH concentrations shall not be re-used for drinking water analysis.

6.1 Brown glass bottles, narrow-necked, flat-bottomed, nominal capacity 1 000 ml, with solid glass stopper.

6.2 Magnetic stirrer with stirring rods, PTFE coated, kept under cyclohexane, with a maximal rotational frequency of 1 000 min^{-1}.

6.3 Measuring cylinder, nominal capacity 10 ml, 25 ml and 1 000 ml.

6.4 Separating funnel, nominal capacity 1 000 ml, with PTFE stopcock, kept under cyclohexane, and glass stopper, e.g. a Squibb funnel.

6.5 Conical flask, nominal capacity 100 ml, with glass stopper.

6.6 Reduction flask, nominal capacity 100 ml (see Figure B.1).

6.7 Centrifuge with rotor, with a rotational frequency of about 3 000 min^{-1} and with centrifuge tubes with tapered bottom, nominal capacity 50 ml (see Figure B.2).

6.8 Pasteur pipettes

6.9 Evaporation assembly, such as a rotary evaporator with vacuum stabilizer and water bath.

6.10 Shaking apparatus, with adjustable rotational speed, suitable for test tubes.

6.11 Blow-down assembly, nitrogen pressure cylinder with pressure-reducing valve and needle valve for fine adjustment.

6.12 Microfilter, with solvent-resistant membrane, pore size 0,45 µm.

6.13 Autosampler vials, capacity approximately 2 ml, with inert filler cap, e.g. PTFE coated septum.

6.14 Polypropene or glass cartridges, filled with at least 0,5 g silica (5.7).

6.15 Glass vials, e.g. centrifuge tubes, nominal capacity 10 ml, with glass stoppers.

6.16 Graduated flasks, nominal capacities 10 ml, 100 ml and 250 ml.

6.17 High-performance liquid chromatograph, with fluorescence detector and data evaluation system, including:

— degassing assembly, e.g. for degassing with vacuum or helium;

— low pulsating analytical pump;

— manual or automatic sample applicator;

— column thermostat, capable of keeping the temperature constant to within ± 0,5 °C;

— fluorescence detector, preferably equipped with a monochromator on either the excitation and emission sides, or with a filter (8.5.2);

— analytical separation column, e.g. a column with length up to 250 mm, internal diameter 2 mm to 4,6 mm, packed with particle size 3 µm to 5 µm material, capable of near baseline separation (at least as good as in Figure A.1) of the PAH to a large extent.

7 Sampling

When sampling drinking water from a tap of the water supply, collect the test sample before the tap is sterilized for bacteriological sampling.

Plastics materials – with the exception of polytetrafluorethene (PTFE) – should not be used during sampling and sample treatment, as losses can occur due to adsorption of PAH on the material. Take care during handling of the test samples to keep them from direct sunlight, as PAH can decompose.

Collect the test sample in brown glass bottles (6.1) of known mass. Dechlorinate water samples containing chlorine by immediately adding approximately 50 mg of sodium thiosulfate (5.2).

Fill the bottle to the shoulder (approximately 1 000 ml) and store the test sample at about + 4 °C and protected from light until the extraction is carried out. Ensure that the extraction is carried out within 24 h after sampling in order to avoid losses due to adsorption. When the complete analysis cannot be performed within 24 h, the following procedure shall be performed within this time limit. If necessary remove a part of the sample from the sampling bottle until a sample volume of about 1 000 ml ± 10 ml remains, and determine the volume of the sample by weighing the bottle, add 25 ml of cyclohexane (5.1.1.1) and shake well. The pretreated sample can be stored for 72 h at about + 4 °C, protected from light.

8 Procedure

8.1 Extraction

Take care during the handling of the samples to keep them from direct sunlight, as PAH can decompose.

Homogenize the test sample, e.g. with a magnetic stirrer. Remove a part of the sample from the sampling bottle until a test sample volume of about 1 000 ml ± 10 ml remains, and determine the volume of the test sample by weighing the bottle.

Add 20 g of sodium chloride (5.3) to improve the extraction efficiency. Add 25 ml of cyclohexane (5.1.1.1) and mix. Keep the test sample in a cool and dark place until the extraction is carried out.

Add a stirring bar and put the lid on the bottle. Then thoroughly mix the test sample using the magnetic stirrer (6.2) at maximum setting (1 000 min^{-1}) for 60 min. Transfer the test sample to a separating funnel (6.4) and allow the phases to separate for at least 5 min.

The extraction procedure can also be carried out using a microseparator (see Figure B.3).

If a stable emulsion is formed during the extraction process, collect it in a centrifuge tube (see Figure B.2) and centrifuge it for 10 min at about 3 000 min^{-1} (6.7).

Transfer the aqueous phase into the sample bottle (6.1) and collect the cyclohexane extract in a 100 ml conical flask (6.5). Dry the extract in accordance with 8.2.

8.2 Drying of the extract

Rinse the separating funnel (6.4) with 10 ml of cyclohexane (5.1.1.1) and add the cyclohexane to the total extract.

Dry the extract with sodium sulfate (5.4) for at least 30 min, swirling the vessel frequently.

Decant the dry extract into a reduction flask (6.6). Rinse the conical flask (6.5) twice with 5 ml of cyclohexane (5.1.1.1) and add to the same reduction flask.

8.3 Enrichment

Evaporate the dried cyclohexane extract until it fills only the tapered tip of the reduction flask (6.6) (approximately 500 µl), with the evaporation assembly (6.9), e.g. the rotary evaporator at 120 hPa and 30 °C.

Dissolve any residues that might have been deposited on the glass wall by shaking the extract using the shaking apparatus (6.10).

If the sample is mineral water or drinking water, evaporate the remaining cyclohexane until incipient dryeness with the blow-down assembly (6.11) using nitrogen (5.5). Dissolve the dry residue either in methanol (5.1.2.1) or in acetonitrile (5.1.2.2), respectively, depending on the HPLC mobile phase to be used. Use a defined volume of the solvent, e.g. 1 ml.

Ensure complete dissolution of the PAH for example by standing for a period of about 15 min.

Transfer the enriched sample, if necessary after filtration through a microfilter (6.12), into an autosampler vial (6.13). Keep the sample in a cool and dark place until the analysis is carried out.

Clean extracts from surface waters and other contaminated water samples in accordance with 8.4.

8.4 Clean-up of the extract

For clean-up of the extract, use columns or cartridges (6.14) containing at least 0,5 g of silica (5.7). Clean the silica in the column or in the cartridge by rinsing with five bed volumes of dichloromethane, followed by conditioning with the same volume of hexane.

Dry the solvents used for cleaning the extract by applying molecular sieve (5.8). The silica should have its maximum activity.

Transfer the concentrated extract (8.3) with a Pasteur pipette (6.8) onto the hexane-covered silica and allow to soak almost completely into the silica. Collect the eluate in a glass vial (6.15).

Rinse the reduction flask with 500 µl of hexane (5.1.1.2), add this solution onto the column and allow to soak almost completely into the silica.

Elute the PAH with a mixture of dichloromethane (5.1.1.3)/hexane (5.1.1.2) 1:1 volume fraction.

NOTE Commercially available cartridges containing 0,5 g of silica require a volume of at least 3 ml of the mixture of dichloromethane/hexane 1:1 volume fraction for the elution of the PAH.

Evaporate the solvent remained with the blow-down assembly (6.11) using nitrogen (5.5) until incipient dryness.

Dissolve the dry residue either in methanol (5.1.2.1) or in acetonitrile (5.1.2.2), respectively, depending on the HPLC mobile phase to be used. Use a defined volume of the solvent, e.g. 1 ml.

Ensure complete dissolution of the PAH, for example, by standing for a period of about 15 min.

Transfer the enriched sample, if necessary after filtration through a microfilter (6.12), into an autosampler vial (6.13). Keep the sample in a cool and dark place until the analysis is carried out.

8.5 High-performance liquid chromatography

8.5.1 Chromatographic separation

Adjust the high-performance liquid chromatograph according to the manufacturer's instructions. Regularly check baseline noise and baseline drift against the specifications guaranteed by the manufacturer. If the results of these tests do not meet the specified values, detect and eliminate the causes.

Condition the separation column (6.17). Optimize the separation isocratically with solvent (5.1), and, if necessary, add water, depending on the condition and the selectivity of the stationary phase. The PAH should be separated as completely as possible (see Figure A.1).

Perylene [which, although it does not belong to the PAH to be determined, is often present in natural samples, and will be determined under the prevailing conditions of detection (8.5.2)], should be included in the calibration step.

NOTE Some stationary phases allow an improvement of the separation between benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene by optimizing the temperature of the column. Thermostating the separation column at room temperature has proved advantageous.

The maximum sample volume injected onto the HPLC column depends on the stationary phase used and the internal diameter of the separation column. A sample volume should be chosen that will not give additional band broadening. For example, when using a column with an internal diameter of 4 mm, the volume should be about 20 µl.

8.5.2 Detection

Use a fluorescence detector (6.17) for the detection of the PAH. If it is supplied by a monochromator, choose excitation and emission wavelengths to optimize the sensitivity and selectivity of the PAH detection. An excitation wavelength of 365 nm (bandwidth of about 25 nm) and an emission wavelength of 450 nm (bandwidth of about 50 nm) is recommended as a compromise between sensitivity and selectivity.

If using a filter fluorimeter, the filter characteristics should, as far as possible, be identical with the conditions of detection for monochromator instruments: excitation at 365 nm (e.g. interference filter); emission at 415 nm to 420 nm (edge filter).

A scanning fluorescence spectrometer may also be used. In that case the excitation and emission wavelengths can be used to optimize the sensitivity and selectivity of detection for each of the PAH.

Dissolved oxygen in the eluent can reduce the fluorescence signal, hence variations in the oxygen concentrations affect the reproducibility of the measurement. Therefore the oxygen content of the eluent should be kept as low and constant as possible by degassing the eluent using, for example, helium (5.6) or vacuum.

8.5.3 Identification of individual compounds

If there is no peak at the characteristic retention time, and the chromatogram is normal in all other aspects, assume the compound not detected.

Assume the presence of an individual compound to be detected, if the retention time of the substance in the chromatogram of the sample agrees with the retention time in the chromatogram obtained from a reference substance (5.9) in a reference solution, measured under the same conditions (tolerance $\pm$ 1 %, max. 10 s).

NOTE The verification of a positive result can be obtained, if required, using different methods:

a) by comparison of the excitation and emission spectrum of the substance in the sample, which has been allocated by its retention time, and the spectrum of the reference substance, taken under the same conditions;

b) by repetition of the chromatography using a stationary phase of different selectivity and/or an eluent of different composition (see Figure A.1);

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

9 Calibration

9.1 General

For calibration a distinction is made between initial calibration, routine calibration and checking of the validity of the calibration curve. Initial calibration determines the working range and the linearity of the calibration function as specified in ISO 8466-1. Perform this calibration when the apparatus is used for the first time.

In the next step, establish the final working range and perform the routine calibration. Repeat this calibration after maintenance (e.g. replacement of the column), after repair of the HPLC system, and in case the system has not been in use for a long time, or if the validity criteria cannot be met. Check the validity of the routine calibration with each series of test samples to be analysed.

9.2 Initial calibration

Establish the preliminary working range by analysing at least five dilutions of the calibration solutions (5.12). Test for linearity as specified in ISO 8466-1.

9.3 Routine calibration

After examining the final working range, analyse a minimum of five dilutions of the calibration solutions (5.12). Calculate a calibration function by linear regression analysis of the corrected peak areas. The actual sensitivity of the method can be estimated from the calculated regression function.

9.4 Check of the validity of the calibration function

Check the validity of the calibration function from the routine calibration with each batch of test samples by analysis of one standard solution after every 10 test samples. The concentration of this standard solution shall be between 40 % and 80 % of the working range. Make sure that the individual results do not deviate by more than 10 % of the routine calibration line. If this criterion is met, assume the calibration to be valid. If not, recalibrate in accordance with 9.3.

10 Measurement of samples

Equilibrate the measuring system before measuring samples. Set the excitation and emission wavelengths or adjust the wavelength programme in relation to the retention times found.

NOTE Reproducible retention times are usually achieved after 2 or 3 injections of a calibration solution (5.12).

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

Ensure that the peaks of each sample are being integrated correctly, and correct if necessary.

If the calculated mass concentration of a substance in the sample exceeds the calibration range, dilute the measuring sample and repeat the measurement.

11 Determination of the recovery

Add, e.g. 2 ml of calibration solutions (5.12) to 1 000 ml water, and proceed in accordance with Clause 8.

Determine the recovery rates for surface water samples by the method of standard additions.

Determine the mean recovery $\overline{\eta_i}$ of the determinand i using Equations (1) and (2):

$$\eta_{i,N} = \frac{\rho_{i,N_f}}{\rho_{i,N_e}} \tag{1}$$

$$\overline{\eta_i} = \frac{\sum_{N=1}^{n} \eta_{i,N}}{n} \tag{2}$$

where

$\eta_{i,N}$ is the recovery of determinand i on the concentration level N;

ρ_{i,N_f} is the mass concentration of determinand i on concentration level N, calculated using the calibration function, in micrograms per litre, µg/l;

ρ_{i,N_e} is the given mass concentration of determinand i on concentration level N, in micrograms per litre, µg/l;

$\overline{\eta_i}$ is the mean recovery;

n is the number of concentration levels.

NOTE With the extraction method described in Clause 8, mean recoveries of 95 % are usually obtained. If this applies, correction for the recovery by factor $\overline{\eta_i}$ can be omitted (see Clause 13).

12 Blank measurement

Monitor the reagents and the correct operation of the instruments by performing blank measurements regularly. For this, analyse 1 000 ml of distilled water in accordance with Clause 8.

If one or more determinands are found in the blanks, perform a systematic investigation to find the cause and eliminate the source(s) of contamination.

13 Calculation

Calculate the mass concentration ρ_i of determinand i in the water sample according to Equation (3):

$$\rho_i = \frac{(y_i - b_i) \cdot V_E}{a_i \cdot V_\mathrm{s} \cdot \overline{\eta_i}} \tag{3}$$

where

ρ_i is the mass concentration of the determinand i in the water sample, in micrograms per litre, µg/l;

y_i is the measured value of the determinand i, as, e.g. peak area;

b_i is the intercept of the calibration function with the ordinate, e.g. peak area;

V_E is the extractant volume from which the injection was made, in millilitres, ml;

a_i is the slope of the calibration function of the determinand i, also called substance-specific response factor as, e.g. peak area per (picograms per microlitres);

V_s is the sample volume, in millilitres, ml;

$\overline{\eta_i}$ is the mean recovery.

14 Expression of results

Report the mass concentration of PAH in micrograms per litre, µg/l, giving two significant figures at most. Round up mass concentrations $<$ 0,01 µg/l to the nearest 0,001 µg/l.

EXAMPLE 1

	Measured value	**Result reported**
fluoranthene	0,143 µg/l	0,14 µg/l
benzo[*a*]pyrene	0,007 9 µg/l	0,008 µg/l

EXAMPLE 2

	Measured value	Result reported	Result reported (sum PAH)
fluoranthene	0,113 2 µg/l	0,11 µg/l	
benzo[*b*]fluoranthene	0,027 1 µg/l	0,027 µg/l	
benzo[*k*]fluoranthene	0,011 6 µg/l	0,012 µg/l	
benzo[*a*]pyrene	0,015 0 µg/l	0,015 µg/l	
benzo[*ghi*]perylene	0,009 7 µg/l	0,010 µg/l	
indeno[1,2,3-*cd*]pyrene	0,008 2 µg/l	0,008 µg/l	
Total concentration of the six PAH		0,182 µg/l	0,18 µg/l
Total concentration of the six PAH, calculated as carbon (C):		0,18 × 0,95 =	0,17 µg/l

If surface water is analysed in accordance with the EEC Water Regulations 1975 [2] and 1979 [3], the sum of the mass concentrations of the six PAH should be determined and the result expressed in µg/l, as specified above.

If drinking water is analysed in accordance with the EEC Drinking Water Regulation 1980 [1], the sum of the mass concentrations of the six PAH should be determined and multiplied with the factor 0,95 to obtain the carbon content. Result should be expressed in µg/l, as specified above.

If drinking water is analysed in accordance with the EC Drinking Water Regulation 1998 [4], the mass concentration of benzo[*a*]pyrene and the sum of the mass concentrations of benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene should be determined and the result expressed in µg/l, as specified above.

15 Test report

Information on at least the following aspects of the test shall be given in the test report:

a) a reference to this part of ISO 7981 (ISO 7981-2:2005);

b) data required for identification of the sample examined;

c) relevant information about sampling and sample preservation;

d) the concentration of each of the PAH, expressed in accordance with Clause 14; and

e) all operations not prescribed in this part of ISO 7981 which might have affected the results.

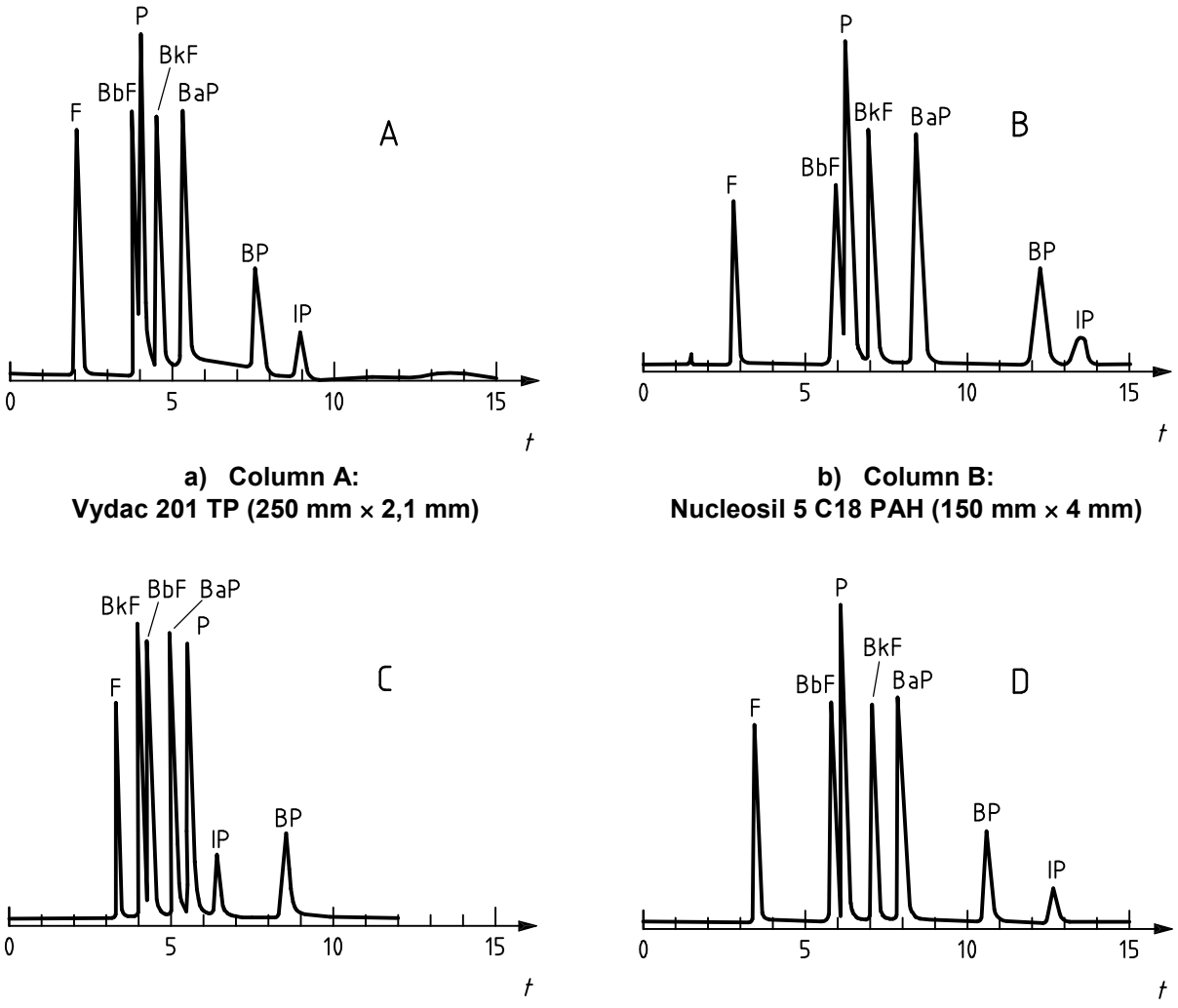
16 Accuracy

Statistical data obtained from results of an interlaboratory trial carried out in Germany [5] are given in Annex C.

Annex A
(informative)

Examples of chromatographic conditions and columns

A.1 Examples of chromatograms (see Figure A.1)



a) Column A:
Vydac 201 TP (250 mm × 2,1 mm)

b) Column B:
Nucleosil 5 C18 PAH (150 mm × 4 mm)

c) Column C:
Nucleosil 5 PAH (250 mm × 4 mm)

d) Column D:
Bakerbond PAH-16 Plus (250 mm × 3 mm)

Key

F	fluoranthene
BbF	benzo[*b*]fluoranthene
BkF	benzo[*k*]fluoranthene
BaP	benzo[*a*]pyrene
BP	benzo[*ghi*]perylene
IP	indeno[1,2,3-*cd*]pyrene
P	perylene

Figure A.1 — Isocratic separation of six PAH

A.2 Operating conditions (see Table A.1)

Table A.1 — Operating conditions

Column	Eluent		Flow ml/min	Temperature °C	Pressure bar
A	Acetonitrile-water	95: 5	0,4	27	85
B	Methanol-acetonitrile	20: 80	0,8	20	75
C	Methanol-tetrahydrofuran	65: 35	1,0	25	125
D	Methanol-acetonitrile	50: 50	0,5	20	45

A.3 Detection

If the fluorescence detection for the PAH is supplied by a monochromator, the folllowing excitation and emission wavelengths are recommended as a compromise between sensitivity and selectivity:

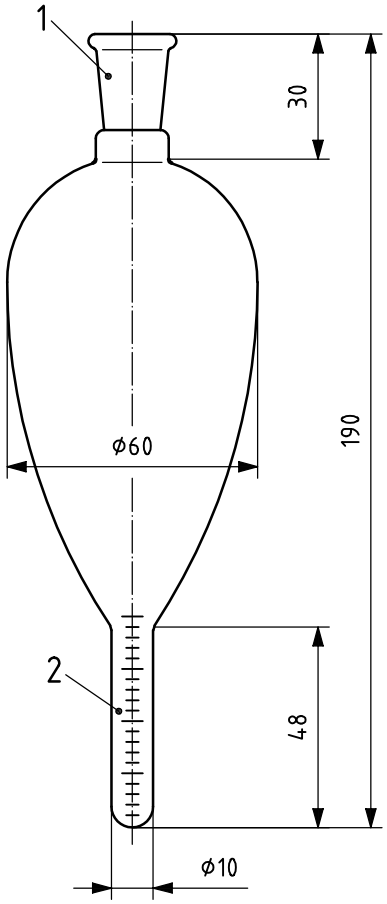
a) excitation wavelength of 365 nm (bandwidth of about 25 nm);

b) emission wavelength of 470 nm (bandwidth of about 50 nm).

Annex B
(informative)

Examples for the construction of special apparatus

Dimensions in millimetres



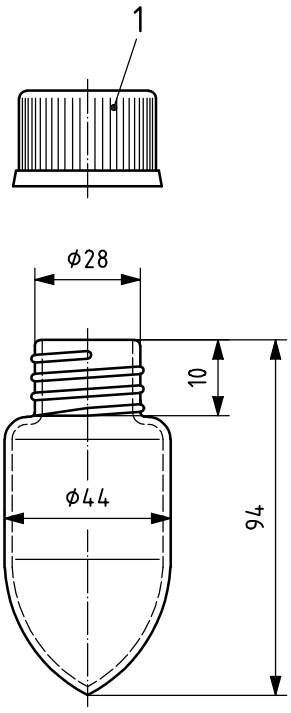
Key

1 ISO 383-14/23

2 total graduated volume of 2 ml with graduations of 0,1 ml

Figure B.1 — Reduction flask (100 ml)

Dimensions in millimetres

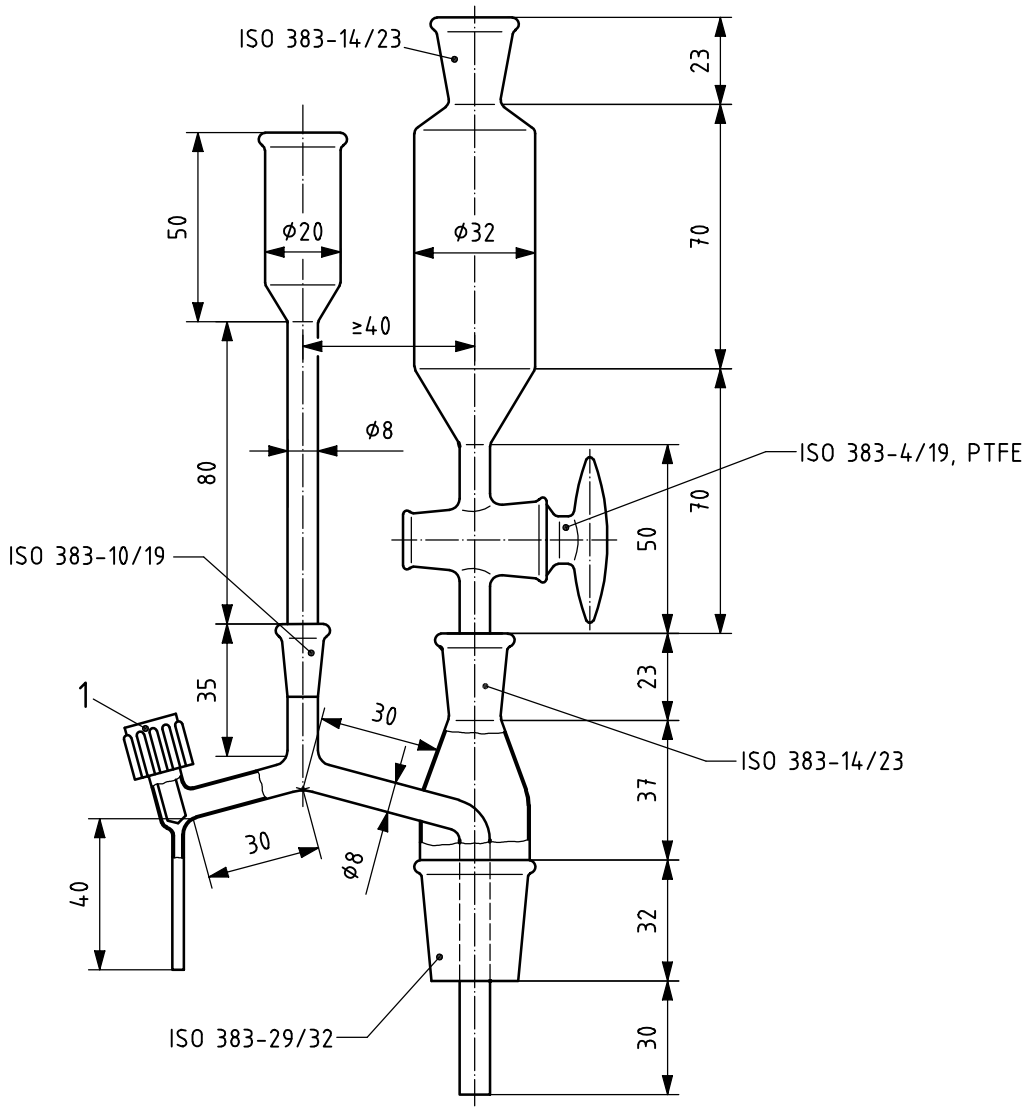


Key

1 PTFE screw cap

Figure B.2 — Centrifuge tube with tapered bottom and screw cap

Dimensions in millimetres



Key

1 PTFE screw cock

Figure B.3 — Microseparator

Annex C
(informative)

Accuracy

Table C.1 — Statistical data for reference standard

Sample	Compound	l	n	n_{AP} %	ρ_{exp} pg/µl	$\bar{\rho}$ pg/µl	η %	s_r pg/µl	CV(r) %	s_R pg/µl	CV(R) %
Standard	Fluoranthene	24	95	5,0	30,0	29,97	93,2	3,869	13,8	0,678	2,4
	Benzo[*b*]fluoranthene	23	90	6,3	30,0	27,13	90,4	3,725	13,7	0,582	2,1
	Benzo[*k*]fluoranthene	24	95	5,0	30,0	27,94	93,1	3,822	13,7	0,576	2,1
	Benzo[*a*]pyrene	23	92	8,0	30,0	27,37	91,2	4,152	15,2	0,645	2,4
	Benzo[*ghi*]perylene	24	94	6,0	30,0	27,92	93,1	5,000	17,9	1,009	3,6
	Indeno[1,2,3-*cd*]pyrene	23	92	8,0	30,0	28,08	93,6	3,417	12,2	1,279	4,6
	Sum of PAH (% C)	23	91	5,2	171	165,5	91,5	23,21	14,8	2,02	1,3

l is the number of laboratories free from outliers;
n is the number of results free from outliers;
n_{AP} is the fraction of outliers;
ρ_{exp} is the accepted reference value;
$\bar{\rho}$ is the total mean value of all results free from outliers;
η is the recovery;
s_r is the repeatability standard deviation;
CV(r) is the repeatability coefficient of variation;
s_R is the reproducibility standard deviation;
CV(R) is the reproducibility coefficient of variation.

Table C.2 — Statistical data for drinking water

Sample	Compound	l	n	n_{AP} %	ρ_{exp} pg/µl	$\bar{\rho}$ pg/µl	η %	s_r pg/µl	CV(r) %	s_R pg/µl	CV(R) %
Drinking water	Fluoranthene	23	88	9,3	20,0	19,28	96,4	3,524	18,3	1,560	8,1
	Benzo[*b*]fluoranthene	23	88	5,4	20,0	16,93	84,7	2,565	15,2	0,942	5,6
	Benzo[*k*]fluoranthene	24	92	5,2	20,0	17,31	86,6	2,965,	17,1	1,517	8,8
	Benzo[*a*]pyrene	23	89	8,2	20,0	16,81	84,0	2,824	16,8	1,686	10,0
	Benzo[*ghi*]perylene	21	80	14,0	20,0	16,52	82,6	2,242	13,6	1,187	7,2
	Indeno[1,2,3-*cd*]pyrene	23	88	9,3	20,0	16,10	80,5	3,019	18,8	1,716	10,7
	Sum of PAH (% C)	21	79	11,2	114	98,9	86,8	11,47	11,6	4,44	4,5

Explanation of symbols see Table C.1.

Bibliography

[1] Council Directive 80/778/EEC of 15 July 1980 relating to the quality of water intended for human consumption

[2] Council Directive 75/440/EEC of 16 June 1975 concerning the quality required of surface water intended for the abstraction of drinking water in the Member States

[3] Council Directive 79/869/EEC of 9 October 1979 concerning the methods of measurement and frequencies of sampling and analysis of surface water intended for the abstraction of drinking water in the Member States

[4] Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption

[5] DIN 38407-8:1995-10 — *German standard methods for the examination of water, waste water and sludge — Substance group analysis (group F) — Part 8: Determination of six polycyclic aromatic hydrocarbons (PAH) in water by high performance liquid chromatography (HPLC) with fluorescence detection (F8)*

[6] ISO 383:1976, *Laboratory glassware — Interchangeable conical ground joints*

[7] ISO 5667-2:1991, *Water quality — Sampling — Part 2: Guidance on sampling techniques*

[8] ISO 5667-3:2003, *Water quality — Sampling — Part 3: Guidance on the preservation and handling of water samples*

ICS 13.060.50

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