INTERNATIONAL STANDARD

ISO 7981-1

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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

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

Partie 1: Dosage de six HAP par chromatographie de haute performance sur couche mince avec détection fluorimétrique à la suite d'une extraction liquide-liquide

Reference number
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Foreword

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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 7981-1 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*

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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 normally is about 0,01 µg/l to 0,05 µg/l in ground water and up to 1 µg/l in surface water.

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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

WARNING — Some substances 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 water by high-performance thin-layer 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).

A screening method (method A) is described to exclude those samples containing less than 20 % of the limit values given in References [1], [2], [3] and [4].

A quantitative method (method B) is also described, with a working range of 40 ng/l to 240 ng/l (sum of 6 PAH). Higher concentrations can be determined by using a smaller aliquot of the sample.

With some modifications, this method is also applicable for the analysis of ground waters and moderately polluted surface waters.

2 Principle

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

NOTE For the analysis of surface water, a differentiation between dissolved and undissolved PAH may 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 prior to analysis (7.4).

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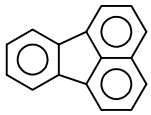
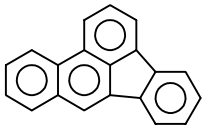
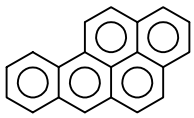
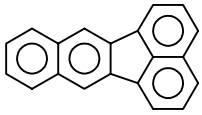
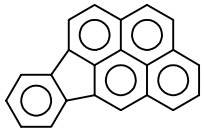
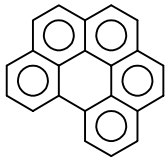
PAH are then separated by high-performance thin-layer chromatography (HPTLC) on appropriate stationary phases and detected either visually or by *in situ* fluorescence measurement at constant or differing wavelength combinations.

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	

3 Interferences

3.1 Interferences with screening method (method A)

Other compounds and/or impurities can interfere in the screening method, thus the use of spectroscopic identification is recommended in order to lower the occurrence of false positives in screening test samples.

It may be necessary to clean coloured extracts or test samples known to contain other organic substances on silica prior to analysis (7.4).

3.2 Interferences with sampling and extraction

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

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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.

3.3 Interferences with HPTLC

Substances that exhibit 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 the accuracy and precision of the analytical results. Band overlap will make an interpretation of the result impossible. Unsymmetrical bands and bands broader than the corresponding bands of the reference substance suggest interferences.

The identity and purity of the bands can be checked by recording the excitation and emission spectra.

4 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 11).

4.1 Solvents

4.1.1 Extraction and clean-up solvents

4.1.1.1 **Cyclohexane**, C_6H_{12}

4.1.1.2 **Hexane**, C_6H_{14}

4.1.1.3 **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.

4.1.2 HPTLC solvents

4.1.2.1 **Methanol**, CH_3OH

4.1.2.2 **Acetonitrile**, CH_3CN

4.1.2.3 **2,4-dimethyl-3-oxypentane** (diisopropyl ether), $C_6H_{14}O$

4.1.2.4 **2-propanol (isopropanol)**, C_3H_7OH

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

4.3 **Sodium chloride**, NaCl

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

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

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

NOTE Prepacked silica cartridges are commercially available.

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

4.8 Caffeine, $C_8H_{10}N_4O_2$

4.9 Liquid paraffin

4.10 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.

4.11 Single-substance stock solutions, of those listed in Table 1, diluted in cyclohexane (4.1.1.1) or methanol (4.1.2.1) to a mass concentration of, for example, 10 µg/ml.

4.12 Multiple-substance stock solution, preferably certified, diluted in cyclohexane (4.1.1.1) or methanol (4.1.2.1) to a mass concentration of, for example, 10 µg/ml for fluoranthene and 2 µg/ml for the other reference substances (4.10).

4.13 Calibration solutions

Transfer 20 µl, 40 µl, 60 µl, 80 µl, 100 µl and 120 µl of the stock solution (4.12) into a graduated 10 ml flask (5.14) and make up to volume with cyclohexane (4.1.1.1) or methanol (4.1.2.1).

1 ml of this reference solution contains 20 ng, 40 ng, 60 ng, 80 ng, 100 ng and 120 ng of fluoranthene and 4 ng, 8 ng, 12 ng, 16 ng, 20 ng and 24 ng of the other reference substances (4.10).

NOTE The solutions 4.11 to 4.13 are stable for at least one year when stored in the dark at room temperature and protected from evaporation.

5 Apparatus

Use standard laboratory apparatus, cleaned to eliminate all interferences.

Clean all glassware, for example by rinsing with detergent and hot water, and dry 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.

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

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

5.3 Measuring cylinders, nominal capacities 10 ml, 25 ml and 1 000 ml.

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

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

5.6 Reduction flask, nominal capacity 50 ml (see Figure C.1).

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

5.8 Pasteur pipettes

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

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

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

5.12 Polypropene or glass cartridges, filled with at least 0,5 g silica (4.6).

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

5.14 Graduated flasks, nominal capacity 10 ml, 20 ml, 100 ml and 250 ml.

5.15 High-performance thin-layer precoated plates, e.g. silica 60, preferably with fluorescence indicator without concentrating zone.

For caffeine impregnation of the silica plates, dip the precoated plates by means of a mechanical dipping device during 4 s into a solution containing 4 g caffeine (4.8) in 96 g dichloromethane (4.1.1.3). Dry the plates for 30 min at 110 °C, and store in a desiccator until use.

Prior to use, clean the pretreated plate by blank chromatography to the upper edge, dry for 30 min at 110 °C, and store in a desiccator until use.

NOTE These plates are commercially available.

5.16 High-performance thin-layer precoated plates, e.g. RP-18, preferably with fluorescence indicator and without concentrating zone.

5.17 Development chamber, for low consumption of mobile phase, suitable for trace analysis.

5.18 Automatic dosing and application device, suitable for spot and band applications, or **microlitre syringes**.

5.19 UV lamp, operating at 366 nm.

5.20 TLC scanner, for the direct fluorimetric measurement.

6 Sampling

When sampling drinking water from a tap of the water supply, collect the test sample prior to sterilizing the tap for bacteriological sampling.

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

Collect the test sample in brown glass bottles (5.1) of known mass. Dechlorinate water samples containing chlorine by immediately adding approximately 50 mg of sodium thiosulfate (4.2).

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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. If the complete analysis cannot be performed within 24 h, the following procedure shall be performed within this time limit. If necessary remove part of the homogenized sample from the sampling bottle until a sample volume of about 1 000 ml ± 10 ml remains, and determine the volume of the test sample by weighing the bottle. Add 25 ml of cyclohexane (4.1.1.1) and shake well. The pretreated test sample may be stored for 72 h at about + 4 °C, protected from light.

7 Procedure

7.1 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 test 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 (4.3) to improve the extraction efficiency. Add 25 ml of cyclohexane (4.1.1.1) and mix. Keep the test sample in a cool and dark place until the extraction is carried out.

Add a stirring rod and put the lid on the bottle. Then thoroughly mix the test sample using the magnetic stirrer (5.2) at maximum setting (1 000 min^{-1}) for 60 min. Transfer the test sample to a separating funnel (5.4) and allow the phases to separate for at least 5 min.

For the extraction of waste water and other water samples with high concentrations of PAH, only 10 ml to 100 ml of the homogeneous test sample should be transferred to a 250 ml graduated flask (5.14) and diluted with water to 200 ml. After adding 20 g of sodium chloride (4.3) and 25 ml of cyclohexane (4.1.1.1), proceed as described above.

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

If a stable emulsion is formed during the extraction process, collect it in a centrifuge tube (5.7) and centrifuge it for 10 min at about 3 000 min^{-1}.

Transfer the aqueous phase into the sample bottle (5.1) and collect the cyclohexane extract in an conical flask (5.5). Dry the extract in accordance with 7.2.

7.2 Drying of the extract

Rinse the separating funnel with 10 ml of cyclohexane (4.1.1.1) and add the cyclohexane to the total extract.

Dry the extract with sodium sulfate (4.4) for at least 30 min, swirling the vessel frequently.

Decant the dry extract into a reduction flask (5.6). Rinse the conical flask (5.5) twice with 5 ml of cyclohexane (4.1.1.1) and add to the same reduction flask.

7.3 Enrichment

Evaporate the filtered cyclohexane extract until it fills only the tapered tip of the reduction flask (5.6) (approximately 500 µl), with the evaporation assembly (5.9), 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 (5.10).

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If the extract is colourless or precleaned according to 7.4, evaporate the remaining cyclohexane with the blow-down assembly (5.11) using nitrogen (4.5) until incipient dryness. Dissolve the dry residue in 40 µl (for spot application) to 120 µl (for band application) of cyclohexane (4.1.1.1) or methanol (4.1.2.1).

If necessary, pre-clean the extract in accordance with 7.4.

7.4 Clean-up of the extract

For clean-up of the extract, use columns or cartridges (5.12) containing at least 0,5 g of silica (4.6). 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 (4.7).

Transfer the concentrated extract (7.3) with a Pasteur pipette (5.8) onto the hexane-covered silica and allow to soak almost completely into the silica. Collect the eluate in a glass vial (5.13).

Rinse the reduction flask with 500 µl of hexane (4.1.1.2), add this solution to the column and allow to soak almost completely into the silica.

Elute the PAH with a mixture of dichloromethane (4.1.1.3)/hexane (4.1.1.2) 1:1 volume fraction, and evaporate the eluate until it fills only the tapered tip of the reduction flask (5.6) (approximately 500 µl), with the evaporation assembly (5.9), followed by the blow-down assembly (5.11) using nitrogen (4.5) until incipient dryness. Dissolve the residue in 40 µl or 120 µl of cyclohexane (4.1.1.1) or methanol (4.1.2.1).

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) for the elution of the PAH.

7.5 High-performance thin-layer chromatography

7.5.1 Application of the extracts

On one HPTLC plate, several samples may be analysed simultaneously, together with two or more reference solutions of varying concentration. If a considerable number of samples has to be analysed, apply the extracts on both ends of the HPTLC plate, provided the plate is developed in a horizontal development chamber.

Apply an aliquot of the total extract (7.3) either by means of the automated volume dosing device (5.18) or by hand as spots or bands. For screening purposes, apply about half of the extract as a spot. If using band application, apply 7 mm bands with 3 mm intervals.

7.5.2 Screening method (method A)

7.5.2.1 General

The screening method is a preliminary examination which is meant to exclude samples containing less than 20 % of the maximum acceptable levels (total concentration 30 ng/l to 40 ng/l).

Choose one of the following procedures for the screening test:

a) separation on caffeine impregnated silica plates (7.5.2.2);

b) separation at room temperature on HPTLC-RP-18 material (7.5.2.3).

Procedure a), if performed at –20 °C, is also recommended as a quantitative procedure. This method shows a linear correlation between peak height and mass applied in the range of 2 ng to 12 ng for fluoranthene and 0,4 ng to 2,4 ng for the other PAH. If an aliquot of 10 % of the concentrated extract of a 1 000 ml water sample is applied on the HPTLC plate, the concentration of fluoranthene can be reliably determined in the range of 20 ng/l to 120 ng/l and the other PAH in the range of 4 ng/l to 24 ng/l.

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7.5.2.2 Separation on caffeine-impregnated silica plates at room temperature

Condition the plates after application of the extracts for 30 min at room temperature over water in a conditioning chamber. Immediately develop the chromatogram vertically in a trough chamber or horizontally in a horizontal development chamber.

As mobile phase, use 2,4-dimethyl-3-oxypentane (4.1.2.3)/hexane (4.1.1.2), 4:1 volume fraction.

Using a trough chamber, the run time for a migration distance of 6,5 cm is about 25 min. In a horizontal development chamber, the run time for a migration distance of 6,5 cm is about 15 min, and for 4,5 cm about 10 min.

Dry the plate for 2 min in a stream of air at ambient temperature, then dip it for 2 s into a solution of liquid paraffin (4.9)/hexane (4.1.1.2) 1:2 volume fraction to stabilize and increase the fluorescence intensity (by a factor of 5 for benzo[*a*]pyrene and benzo[*ghi*]perylene and by a factor of 2 for the other substances), then dry again for 2 min.

7.5.2.3 Separation on HPTLC-RP-18 plates

Develop the chromatogram vertically in a trough chamber or horizontally in a horizontal development chamber (5.17).

As mobile phase, use acetonitrile (4.1.2.2)/2-propanol (4.1.2.4)/methanol (4.1.2.1), 1:2:1 volume fraction. Dry the chromatogram during 2 min in a stream of air at ambient temperature.

Using a trough chamber, the run time for a migration distance of 6,5 cm is 20 min to 25 min. In a horizontal development chamber, the run time for a migration distance of 6,5 cm is about 20 min, and for 4,5 cm about 15 min (without chamber saturation).

NOTE A better separation can be achieved by working at a lower temperature.

7.5.3 Separation on caffeine-impregnated silica plates at –20 °C in the freezer (method B)

Precool the plates at –20 °C for 20 min, then develop them using dichloromethane (4.1.1.3) as mobile phase at –20 °C.

Using a trough chamber, the run time for a migration distance of 6,5 cm is about 20 min. In a horizontal development chamber, the run time for a migration distance of 6,5 cm is about 17 min, and for 4,5 cm about 12 min.

Dry the plate for 2 min in a stream of air at ambient temperature, then dip it for 2 s into a solution of liquid paraffin (4.9)/hexane (4.1.1.2) 1:2 volume fraction to stabilize and increase the fluorescence intensity (by a factor of 5 for benzo[*a*]pyrene and benzo[*ghi*]perylene and by a factor of 2 for the other substances), then dry again for 2 min.

8 Evaluation

8.1 Visual evaluation of the screening method

For R_f values for the PAH under various chromatographic conditions see Annex A.

Dry the plate for 2 min in a stream of air at ambient temperature, then place the plate under a UV lamp (5.19) (λ = 366 nm) and evaluate the fluorescence intensity of the sample by comparison with the standard solutions by allocating the zones by colour, R_f value and relative position to the reference chromatogram.

NOTE On RP-18-plates, only 3 zones can be recognized (see Figure 2).

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It is not necessary to determine the substance-specific spectra of the individual spots.

If 1 000 ml water have been extracted, the residue after evaporation was dissolved in 40 µl solvent, and an aliquot of 20 µl applied, and the chromatogram fluorescence is nowhere stronger than the standard (sum of PAH applied 10 ng), the result is:

— sum of polycyclic aromatic hydrocarbons < 20 ng/l (no further action required).

If visually the sample chromatogram shows stronger fluorescence than the standard chromatogram, quantitative measurements are necessary.

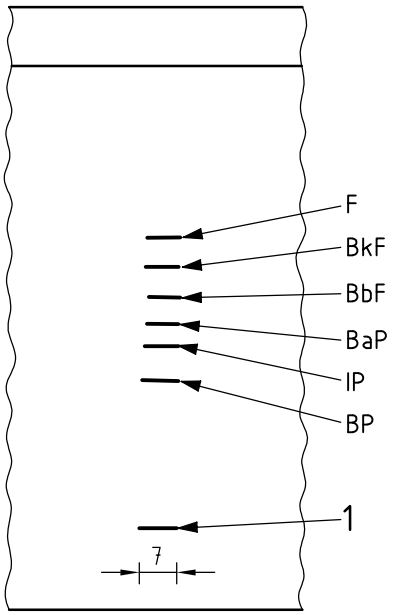
8.2 Fluorescence densitometric evaluation

Evaluate the chromatogram by either measuring the peak height or the peak area at an excitation wavelength of 366 nm and a fluorescence wavelength of 430 nm (edge filter), using the calibration function established on the same kind of plate.

If applying spots, the slit of the scanner (5.20) shall be broader than the largest zone of the chromatogram in the X-direction. If applying bands, the recommended slit width in the x-direction is 1/2 to 2/3 of the band length. In the Y-direction, the slit should not be smaller than 0,3 mm.

Figures 1, 2 and 3 show position, fluorescence colours and fluorescence position curves of the six PAH (10 ng for fluoranthene and 2 ng for the other PAH). Annex B gives information about spectroscopic identification.

Dimensions in millimetres



Key

1 start

Fluorescence colours

F light blue
BkF dark blue
BbF blue
BaP violet
IP light yellow
BP violet

Figure 1 — Chromatogram of the 6 PAH on a caffeine-impregnated silica plate, developed at –20 °C

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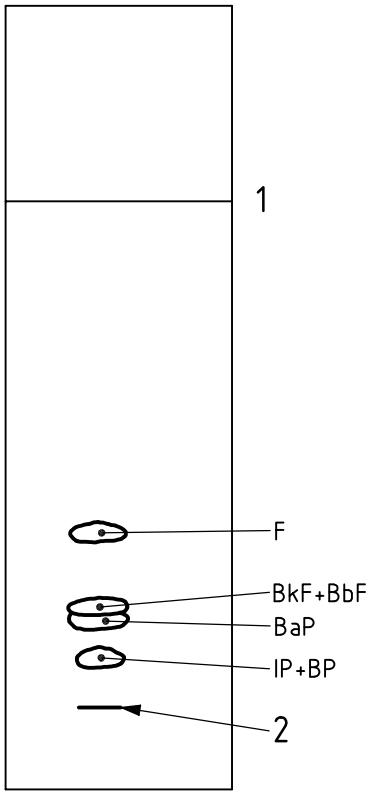

Key

1 front
2 start

Fluorescence colours

F light blue
BkF dark blue
BbF blue
BaP violet
IP light yellow
BP violet

Figure 2 — Chromatogram of the six PAH on an RP-18 plate

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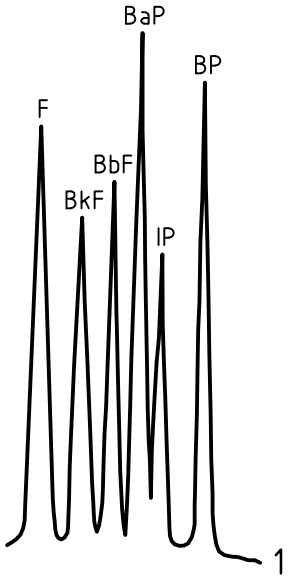

Key

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

Figure 3 — Fluorescence position curve of the six PAH on a caffeine-impregnated silica plate, developed at –20 °C

9 Calibration

9.1 General

Establish a calibration function by analysing all substances in Table 1. To achieve this, the R_f-values of the individual substances shall be known beforehand or determined by analysing appropriate dilutions of the single-substance stock solutions (4.11) under specified chromatographic conditions.

Adjust the analytical procedure so as to obtain a linear calibration function for each individual substance. Choose a calibration range adapted to the analytical problem.

If there is no experience with this method, establish the precision data according to 9.2 and 9.3:

a) calibration of the HPTLC step only, using an external standard (9.2);

b) calibration of the total method, including the extraction, using an external standard (9.3).

The procedure described in 9.2 is recommended for routine purposes, to be carried out on every HPTLC plate.

The calibration function is only valid for the range chosen and the HPTLC plate concerned, and is verified with two points on every plate.

Table 2 explains the indices that are used in the following text.

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Table 2 — Indices used in this part of ISO 7981

Index	Designation
i	identity of the substance
e	value measured at calibration
t	total method

9.2 Calibration of the HPTLC step using an external standard

Establish a calibration function for each determinand using at least five different concentrations, by applying different volumes of the multiple substance stock solution (4.12) diluted 1:5 volume fraction. Recommended volumes are: 1 µl, 2 µl, 3 µl, 4 µl, 5 µl and 6 µl, corresponding to 2 ng, 4 ng, 6 ng, 8 ng, 10 ng and 12 ng for fluoranthene and 0,4 ng, 0,8 ng, 1 ng, 2 ng, 1 ng, 6 ng, 2,0 ng and 2,4 ng for the other substances. The sum of the PAH applied is therefore 4 ng, 8 ng, 12 ng, 16 ng, 20 ng and 24 ng.

Plot the measured values y_{ie} against the mass $\rho_{A,ie}$.

Calculate the linear calibration function using Equation (1):

$$y_{ie} = \left(a_i \cdot \rho_{A,ie}\right) + b_i \tag{1}$$

where

y_{ie} is the measured value of determinand i, as e.g. peak height or peak area;

a_i is the slope of the calibration function of determinand i, in peak height or peak area per nanogram /chromatogram zone;

$\rho_{A,ie}$ is the mass of determinand i per area applied onto the HPTLC plate, in nanogram/chromatogram zone;

b_i is the intercept of the calibration function of determinand i with the ordinate, as e.g. peak height or peak area.

9.3 Calibration of the total method using an external standard

Add 1 ml of each of the calibration solutions (4.13) to 1 000 ml of water, and extract and enrich in accordance with Clause 7.

Calculate the linear calibration function using Equation (2):

$$y_{ie\mathrm{t}} = \left(a_{i\mathrm{t}} \cdot \rho_{ie\mathrm{t}}\right) + b_{i\mathrm{t}} \tag{2}$$

where

$y_{ie\mathrm{t}}$ is the measured value of determinand i, as e.g. peak height or peak area;

$a_{i\mathrm{t}}$ is the slope of the calibration function of determinand i;

$\rho_{ie\mathrm{t}}$ is the mass concentration of determinand i in the calibration solution, in nanograms per litre;

$b_{i\mathrm{t}}$ is the intercept of the calibration function of determinand i with the ordinate, as e.g. peak height or peak area.

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10 Determination of the recovery

Determine the mean recovery $\overline{\eta_i}$ of the determinand i using Equations (3) and (4):

$$\eta_{i,N} = \frac{\rho_{i,N_f}}{\rho_{i,N_e}} \tag{3}$$

$$\overline{\eta_i} = \frac{\sum_{N=1}^{n} \eta_{i,N}}{n} \tag{4}$$

where

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

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

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

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

n is the number of concentration levels.

Use the value obtained to correct the result, with recovery data distributed equidistantly over the working range.

With the extraction procedure as described in Clause 7, high recoveries are usually obtained. Inconsistent recoveries indicate matrix effects and/or problems with the extraction procedure.

11 Blank measurements

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 7.

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.

12 Calculation

Calculate the mass concentration ρ_i of determinand i in the water sample according to Equation (5):

$$\rho_i = \frac{(y_i - b_i) \cdot V_{\mathrm{E}}}{a_i \cdot V_{\mathrm{s}} \cdot V_{\mathrm{a}} \cdot \overline{\eta_i}} \tag{5}$$

where

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

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

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

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V_E is the extract volume, from which injection was made, in microlitres, µl;

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

V_s is the sample volume, in millilitres, ml;

V_a is the volume of the extract applied to the HPTLC plate, in microlitres, µl;

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

13 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 $\times$ 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 micrograms per litre (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 micrograms per litre, 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 micrograms per litre, as specified above.

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14 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 7981-1:2005);

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 13; and

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

15 Accuracy

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

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Annex A
(informative)

R_f values of the six PAH under various chromatographic conditions

Table A.1 — R_f values of the six PAH under various chromatographic conditions

	Benzo[*ghi*]-perylene	Indeno-[1,2,3-*cd*]-pyrene	Benzo[*a*]-pyrene	Benzo[*b*]-fluor-anthene	Benzo[*k*]-pyrene	Fluor-anthene
RP 18 vertical 6 cm room temperature	70 to 75	70 to 75	75 to 80	75 to 80	80 to 85	80 to 85
RP 18 horizontal 6 cm room temperature	70 to 75	70 to 75	75 to 80	75 to 80	75 to 80	80 to 85
silica caffeine concentration zone vertical 6,5 cm room temperature	15 to 20	20 to 25	15 to 30	30 to 35	35 to 40	50 to 55
silica caffeine concentration zone horizontal 6,5 cm room temperature	5 to 10	10 to 15	15 to 20	20 to 25	25 to 30	35 to 40
silica caffeine concentration zone vertical 6,5 cm –20 °C	20 to 25	30 to 35	35 to 40	40 to 45	45 to 50	55 to 60
silica caffeine concentration zone horizontal 6,5 cm –20 °C	20 to 25	30 to 35	40 to 45	35 to 40	45 to 50	50 to 55
silica caffeine concentration zone horizontal 4,5 cm –20 °C	25 to 30	33 to 40	45 to 50	40 to 45	50 to 55	60 to 65
silica caffeine no concentration zone horizontal 6,5 cm –20 °C	10 to 15	20 to 25	30 to 35	25 to 35	40 to 45	50 to 55

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Annex B
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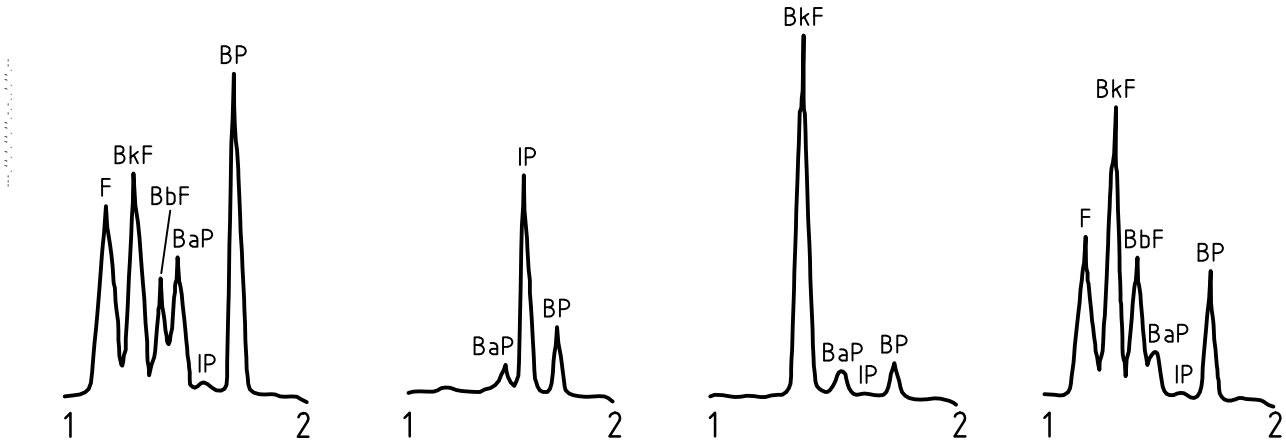
Spectroscopic identification



a) λ_{exc} = 365 nm, λ_{fl} = 436 nm

b) λ_{exc} = 436 nm, λ_{fl} = 578 nm

c) λ_{exc} = 405 nm, λ_{fl} = 436 nm

d) λ_{exc} = 334 nm, λ_{fl} = 436 nm

Key

1	front
2	start

Fluorescence colours

F	light blue
BkF	dark blue
BbF	blue
BaP	violet
IP	light yellow
BP	violet

λ_{exc}	excitation wavelength;
λ_{fl}	fluorescence wavelength.

Figure B.1 — Selective detection of the six PAH

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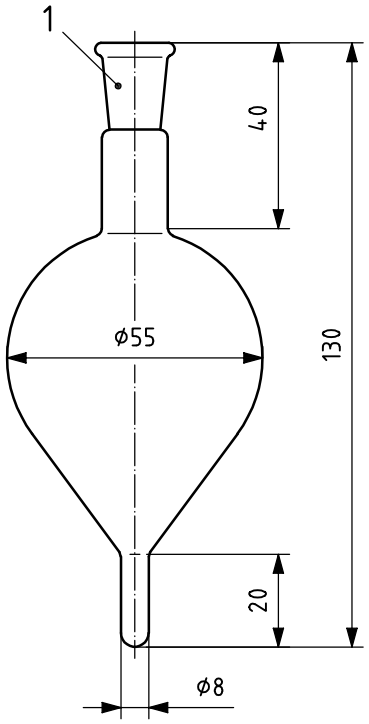
Annex C
(informative)

Examples for the construction of special apparatus

Dimensions in millimetres



Key
1 ground stopper

Figure C.1 — Reduction flask (50 ml)

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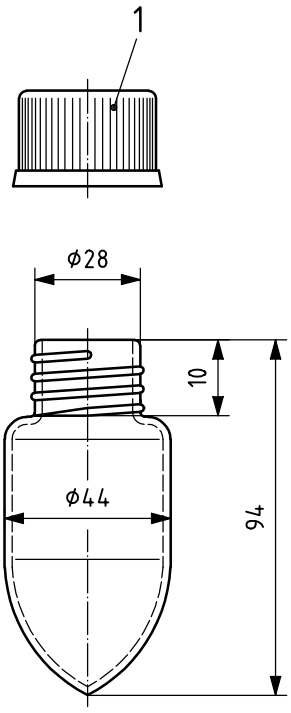
Dimensions in millimetres



Key

1 PTFE screw cap

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

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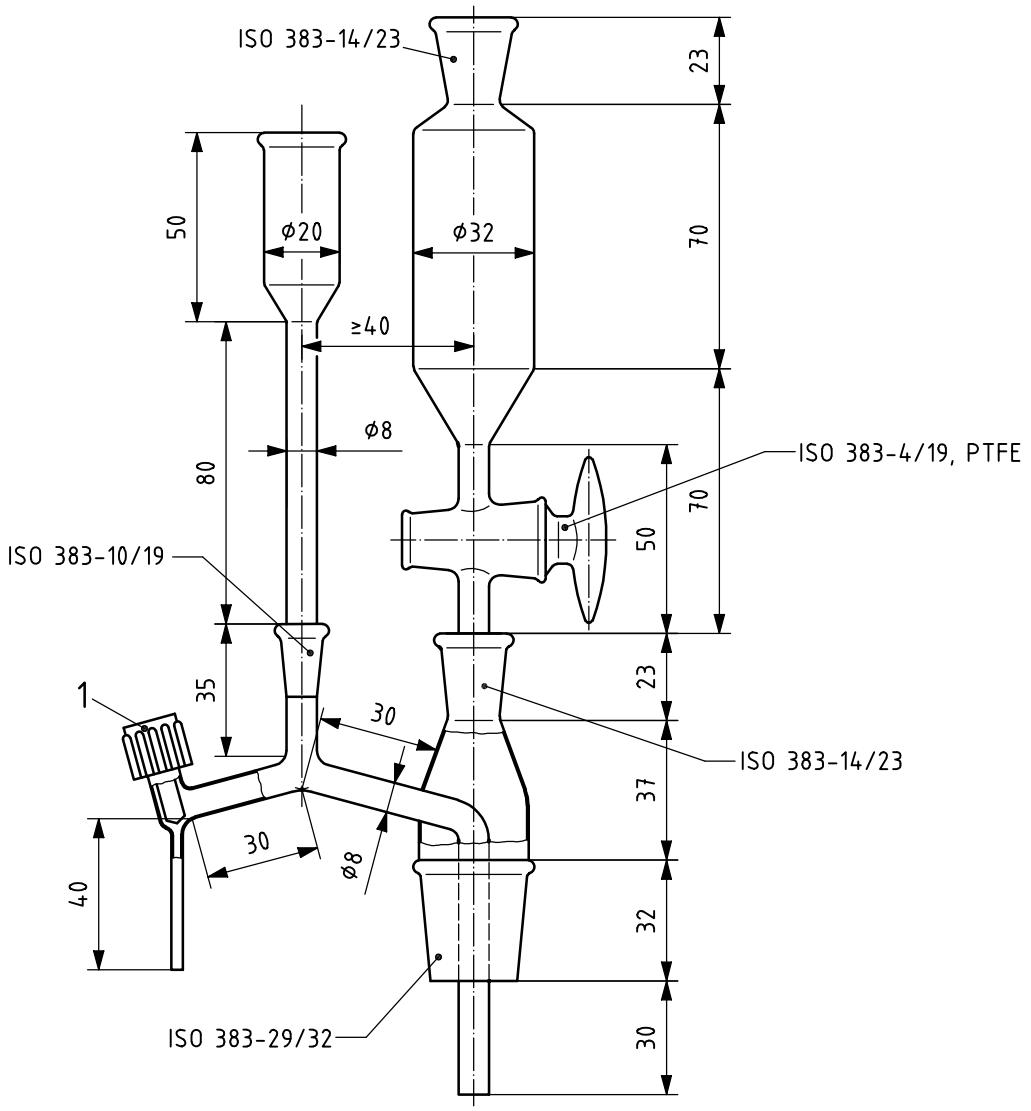
Dimensions in millimetres



Key

1 PTFE screw cock

Figure C.3 — Microseparator

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Annex D
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Accuracy

Table D.1 — Statistical data for drinking water and gravel filtrate

Sample	Compound	l	n	n_{AP} %	ρ_{exp} ng/l	$\bar{\rho}$ ng/l	η %	s_r ng/l	CV(r) %	s_R ng/l	CV(R) %
Drinking water	Fluoranthene	11	42	8,7	27,4	27,8	101,4	3,91	14,1	2,48	8,9
	Benzo[*k*]fluoranthene	11	41	8,9	6,7	6,2	92,8	1,19	19,1	0,53	8,4
	Benzo[*b*]fluoranthene	12	47	0,0	7,6	7,7	100,7	2,57	33,6	1,21	15,9
	Indeno[1,2,3-*cd*]pyrene	11	41	2,4	4,7	5,0	105,8	1,80	36,2	0,90	18,1
	Benzo[*ghi*]perylene	12	47	0,0	12,0	10,4	86,5	2,74	26,4	1,35	13,0
Gravel filtrate	Fluoranthene	12	43	2,3	136,9	123,0	89,8	20,02	16,3	7,01	5,7
	Benzo[*k*]fluoranthene	12	44	0,0	44,1	41,0	92,9	7,59	18,5	3,92	9,6
	Benzo[*a*]pyrene	12	44	0,0	21,8	17,5	80,2	4,24	24,3	2,30	13,2
	Benzo[*ghi*]perylene	12	43	0,0	52,9	48,1	90,9	9,11	19,0	7,35	15,3

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 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 D.2 — Statistical data for standards A and B

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 A	Fluoranthene	11	32	0,0	2,79	2,77	99,2	0,189	6,8	0,096	3,5
	Benzo[*k*]fluoranthene	11	32	0,0	0,88	0,84	95,5	0,075	8,9	0,035	4,2
	Benzo[*a*]pyrene	11	31	0,0	0,44	0,42	94,6	0,050	12,1	0,019	4,6
	Benzo[ghi]perylene	10	30	6,3	1,06	0,97	91,9	0,073	7,5	0,064	6,6
Standard B	Fluoranthene	11	32	0,0	3,42	3,41	99,9	0,266	7,8	0,122	3,6
	Benzo[*k*]fluoranthene	10	27	15,6	0,84	0,77	91,3	0,049	6,4	0,020	2,6
	Benzo[*b*]fluoranthene	11	32	0,0	0,95	0,97	101,6	0,144	14,9	0,028	2,9
	Indeno[1,2,3-*cd*]pyrene	11	32	0,0	0,59	0,58	97,9	0,091	15,7	0,057	9,8
	Benzo[*ghi*]perylene	11	32	0,0	1,45	1,40	96,3	0,243	17,4	0,049	3,5

Explanation of symbols see Table D.1.

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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 38407-7:2000 — *German standard methods for the examination of water, waste water and sludge — Substance group analysis (group F) — Part 7: Determination of six polycyclic aromatic hydrocarbons (PAH) in drinking and mineral water by high performance thin layer chromatography (HPTLC) (F7)*

[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*

[9] ISO 8466-1:1990, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function*

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