INTERNATIONAL STANDARD

**ISO 17993**

First edition
2002-08-15

# Water quality — Determination of 15 polycyclic aromatic hydrocarbons (PAH) in water by HPLC with fluorescence detection after liquid-liquid extraction

*Qualité de l'eau — Dosage de 15 hydrocarbures aromatiques polycycliques (HAP) dans l'eau par HPLC avec détection par fluorescence après extraction liquide-liquide*



Reference number
ISO 17993:2002(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.ch
Web www.iso.ch

Printed 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 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 17993 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Annexes A and B of this International Standard are for information only.

# Introduction

Polycyclic aromatic hydrocarbons (PAH) occur in nearly all types of waters. These compounds 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 Council Directive 98/83/EC on the quality of water intended for human consumption set the maximum acceptable level for benzo(a)pyrene at 0,010 µg/l, and for the sum of four specified PAH [benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene, indeno(1,2,3-cd)-pyrene] at 0,100 µg/l.

# Water quality — Determination of 15 polycyclic aromatic hydrocarbons (PAH) in water by HPLC with fluorescence detection after liquid-liquid extraction

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

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



## 1 Scope

This International Standard specifies a method using high performance liquid chromatography (HPLC) with fluorescence detection after liquid-liquid extraction for the determination of 15 selected PAH (see Table 1) in drinking and ground water in mass concentrations greater than 0,005 µg/l (for each single compound) and surface waters in mass concentrations above 0,01 µg/l.

This method is, with some modification, also suitable for the analysis of wastewater. This method may be applicable to other PAH, provided the method is validated for each case.

## 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 5667-2, *Water quality — Sampling — Part 2: Guidance on sampling techniques*

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

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

The PAH present in the aqueous sample are extracted from the water sample with hexane. The extract is concentrated by evaporation and the residue taken up in a solvent appropriate for HPLC analysis.

If necessary, extracts of surface water or more contaminated water samples are cleaned by chromatography over silica prior to analysis.

PAH are separated by HPLC on a suitable stationary phase using gradient elution. Identification and quantification is performed by means of fluorescence detection with wavelength programming for both the excitation and the emission wavelength.

NOTE If only a limited number of PAH are to be determined, separation can also be performed under isocratic conditions.

**Table 1 — Polycyclic aromatic hydrocarbons determinable by this method**

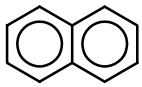
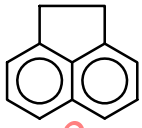
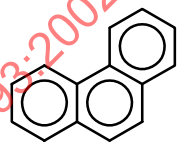
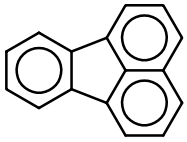
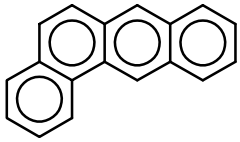
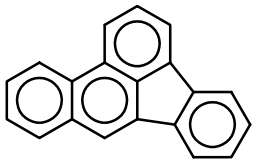
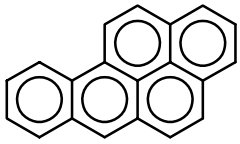
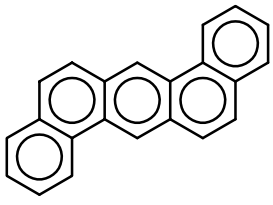
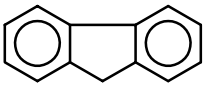
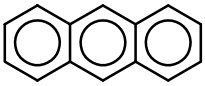
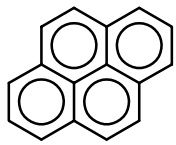
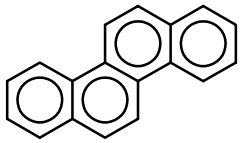
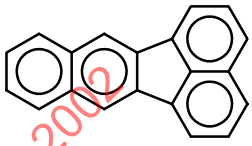
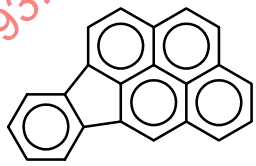
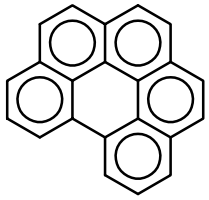
| Name | Chemical formula | Molar mass | Percentage carbon | CAS-number | Structure |
|---|---|---|---|---|---|
| Naphthalene | $C_{10}H_8$ | 128,17 g/mol | 93,75 % C | 091-20-3 |  |
| Acenaphthene | $C_{12}H_{10}$ | 154,21 g/mol | 93,05 % C | 083-32-9 |  |
| Phenanthrene | $C_{14}H_{10}$ | 178,23 g/mol | 94,05 % C | 085-01-8 |  |
| Fluoranthene | $C_{16}H_{10}$ | 202,26 g/mol | 95,0 % C | 206-44-0 |  |
| Benzo(a)anthracene | $C_{18}H_{12}$ | 228,29 g/mol | 94,45 % C | 056-55-3 |  |
| Benzo(b)fluoranthene [a] | $C_{20}H_{12}$ | 252,32 g/mol | 95,2 % C | 205-99-2 |  |
| Benzo(a)pyrene [a] | $C_{20}H_{12}$ | 252,32 g/mol | 95,2 % C | 050-32-8 |  |
| Dibenzo(a,h)anthracene | $C_{22}H_{14}$ | 278,35 g/mol | 94,7 % C | 053-70-3 |  |
| Fluorene | $C_{13}H_{10}$ | 166,22 g/mol | 93,59 % C | 086-73-7 |  |
| Anthracene | $C_{14}H_{10}$ | 178,23 g/mol | 94,05 % C | 120-12-7 |  |
| Pyrene | $C_{16}H_{10}$ | 202,26 g/mol | 95,0 % C | 129-00-0 |  |

**Table 1** (*continued*)

| Name | Chemical formula | Molar mass | Percentage carbon | CAS-number | Structure |
|---|---|---|---|---|---|
| Chrysene | $C_{18}H_{12}$ | 228,29 g/mol | 94,45 % C | 218-01-9 |  |
| Benzo(k)fluoranthene [a] | $C_{20}H_{12}$ | 252,32 g/mol | 95,2 % C | 207-08-9 |  |
| Indeno(1,2,3-cd)pyrene [a] | $C_{22}H_{12}$ | 276,34 g/mol | 95,6 % C | 193-39-5 |  |
| Benzo(ghi)perylene [a] | $C_{22}H_{12}$ | 276,34 g/mol | 95,6 % C | 191-24-2 |  |
| NOTE The 15 PAH selected for determination by this method correspond to those of the US EPA list with the exception of acenaphthylene. Acenaphthylene cannot be determined by this method because it is not fluorescent. | | | | | |
| [a] Compounds specified in the Council Directive 98/83/EC. | | | | | |



## 4 Interferences

### 4.1 Sampling and extraction

Use sampling containers of materials (preferably of steel or glass) 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 these materials are present, make sure that they are as short as possible. Rinse the sampling line with the water to be sampled before taking the test sample. Refer to ISO 5667-2 and ISO 5667-3 for guidance.

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

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

### 4.2 HPLC

Compounds that show either fluorescence or quenching and co-elute with the analyte PAH may interfere with the determination. These interferences may lead to incompletely resolved signals resulting in peak overlap and may, depending on their magnitude, affect accuracy and precision of the analytical results. Unsymmetrical peaks and peaks being broader than the corresponding peaks of the reference compound suggest interferences. This problem may arise for naphthalene and phenanthrene depending on the selectivity of the phases used.

Incomplete removal of the solvents used for sample pretreatment (hexane, acetone, dichloromethane) may lead to poor reproducibility of the retention times and peak broadening or double peaks especially for the 2- and 3-ring PAH.

Separation between dibenzo(ah)anthracene and indeno(1,2,3-cd)pyrene can be critical. When incomplete resolution is encountered, peak integration shall be checked and, when necessary, corrected.

Usually perylene is incompletely resolved from benzo(b)fluoranthene, but by choosing a selective wavelength (see Table A.1) the perylene peak can be suppressed.

As perylene can be detected under the conditions given in the isocratic method for the PAH, which are relevant for drinking water (see Figure A.3), it should be included in the calibration step.

## 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 fluorescence possible.

Monitor the blank to guarantee that the reagents do not contain PAH in detectable concentrations (see 8.9).

### 5.1 Solvents.

**5.1.1 Extraction solvents**, as follows:

— hexane, $C_6H_{14}$;

— other volatile solvents may be used as well, if it is proved that there is equivalent or better recovery.

**5.1.2 Extraction clean-up solvents**, as follows:

— dichloromethane, $CH_2Cl_2$ (see note);

— hexane, $C_6H_{14}$;

— *N*,*N*-dimethylformamide, $(CH_3)_2NCHO$;

— acetone, $C_3H_6O$.

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

**5.1.3 HPLC solvents**, as follows:

— acetonitrile, $CH_3CN$;

— methanol, $CH_3OH$.

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

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

**5.4 Nitrogen,** having a volume fraction of at minimum 99,999 %.

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

NOTE Prepacked silica cartridges are commercially available.

**5.6 Molecular sieve beads,** having a pore diameter of 0,4 nm and having been completely activated.

**5.7 Reference compounds,** listed in Table 1.

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

**5.8 Stock solutions.**

The solutions 5.8.1 and 5.8.2 are stable for at least a year when stored in the dark at room temperature and protected from evaporation.

**5.8.1 Single compound stock solutions,** of those listed in Table 1, diluted in acetonitrile (5.1.3) to a mass concentration of, for example, 10 µg/ml.

These solutions are used for confirmation and identification of single PAH in the chromatogram.

**5.8.2 Multiple compound stock solution,** certified, diluted in acetonitrile (5.1.3) to a mass concentration of, for example, 10 µg/ml for each individual compound.

**5.9 Reference solutions.**

Prepare at least five calibration solutions by appropriate dilution of the stock solution (5.8.2), using methanol (5.1.3) or acetonitrile (5.1.3) as the solvent. The choice of the solvent depends on the composition of the HPLC mobile phase.

Transfer, for example, 50 µl of the stock solution into a 5 ml volumetric flask and make up to the mark with acetonitrile. One microlitre of this reference solution contains 100 pg of the respective individual compounds.

These solutions remain stable for at least a year when stored in the dark at room temperature and protected from evaporation. To ensure their stability, run a quality control check regularly on the reference solutions.

Checking the mass concentration of the PAH in the stock solution is only possible by comparison with an independent, preferably certified, standard solution.

## 6 Apparatus

Standard laboratory glassware cleaned to eliminate all interferences. All glassware can be cleaned, 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.

Do not use glassware that has been in contact with wastewater samples or samples with high PAH concentrations for drinking water analysis.

**6.1 Brown glass bottles**, narrow-necked, flat-bottomed, 1 000 ml, with glass stopper, preferably of known mass.

**6.2 Magnetic stirrer**, with stirring bars, glass or polytetrafluoroethene (PTFE) coated, for stirring the solvent used for extraction.

**6.3 Separating funnel**, of 1 000 ml capacity, with PTFE stopcock and glass stopper.

**6.4 Conical flasks**, of 100 ml and 250 ml capacity, with glass stopper.

**6.5 Microlitre syringes**, of 500 µl and 1 000 µl capacity.

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

**6.7 Centrifuge with rotor**, for centrifuge tubes with tapered bottoms of 50 ml capacity (see Figure B.2).

**6.8 Pasteur pipettes.**

**6.9 Evaporation assembly**, for example a rotary evaporator with a vacuum stabilizer and a water bath.

**6.10 Shaking apparatus**, with adjustable rotational speed.

**6.11 Microfilter,** with a solvent-resistant hydrophilic membrane and a pore size of 0,45 µm.

**6.12 Glass autosampler vials,** of approximately 2 ml capacity, with an inert cap, e.g. PTFE coated septum.

**6.13 Polypropene or glass cartridges,** filled with at least 0,5 g of silica (see 5.5).

NOTE These cartridges are commercially available.

**6.14 Glass vials**, e.g. centrifuge tubes, graduated (scale division 0,1 ml), nominal capacity 10 ml, with glass stoppers.

**6.15 High performance liquid chromatograph** (HPLC), with fluorescence detector and data evaluation system, including:

**6.15.1 Degassing assembly**, e.g. for degassing with vacuum or helium.

**6.15.2 Analytical pumps**, capable of binary gradient elution.

**6.15.3 Column thermostat**, capable of keeping the temperature constant to within ± 0,5 °C.

**6.15.4 Fluorescence detector**, capable of programming at least six pairs of wavelengths, including damping/amplification, preferably equipped with monochromator(s).

**6.16 Analytical separation column,** meeting the separation requirements given in 8.5.2 (for examples see annex A).

## 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.

Collect the test sample in a brown glass bottle (6.1). Dechlorinate water test 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 protect it 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, perform the following procedure within this time limit. 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 test sample by weighing the bottle, add 25 ml of hexane (5.1.2) and shake well. The pretreated test sample may be stored for 72 h at about + 4 °C, protected from light.

## 8 Procedure

### 8.1 Extraction

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 25 ml of hexane (5.1.2) and mix. Other volatile solvents may be used for extraction, provided they give equal or better recovery.

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 (6.3) and allow the phases to separate for at least 5 min. The separation of hexane from water can also be carried out using a microseparator (an example is given in Figure B.3).

If a stable emulsion forms during the extraction process, collect it in a centrifuge tube (6.14) and centrifuge it for 10 min at about 3 000 $min^{-1}$. Remove the separated water with a Pasteur pipette (6.8), transfer the extract to a 100 ml conical flask (6.4). Dry extract according to 8.2.

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 are transferred to a 250 ml conical flask (6.4) with a pipette and diluted with water to 200 ml. After adding 25 ml of hexane (5.1.2) proceed as described above.

## 8.2 Drying of the extract

Transfer the hexane layer obtained according to 8.1 into a 100 ml conical flask (6.4). Rinse the funnel or centrifuge tube with 5 ml of hexane (5.1.2) and add it to the total extract.

Dry the extract with sodium sulfate (5.3) for at least 30 min. Swirl the vessel frequently.

Decant the dry extract into a reduction flask (6.6). Rinse the conical flask twice with 5 ml of hexane (5.1.2) and add to the same reduction flask.

## 8.3 Enrichment

Evaporate the dried hexane extract obtained according to 8.2 until it fills only the tapered tip of the reduction flask (approximately 2 ml), with e.g. a rotary evaporator (6.9), with a bath temperature of 30 °C and slowly decreasing the pressure to 200 hPa.

Do not evaporate the extracts to dryness, as losses of the 2- or 3-ring compounds may occur.

Dissolve any residues that may be deposited on the glass wall by shaking the extract using the shaking apparatus (6.10).

Clean extracts of wastewater samples and other samples of unknown origin using the method given in 8.4.

Add 250 µl of *N*,*N*-dimethylformamide (5.1.2) to the concentrated extract and homogenize the mixture with 500 µl of acetone (5.1.2). Dichloromethane (5.1.2) may also be used instead of acetone for homogenization after the addition of dimethylformamide.

Remove the hexane and the acetone completely by a gentle stream of nitrogen (5.4), so as to reduce the volume of the extract to between 200 µl and 250 µl. The enriched extract should not contain residues of hexane or acetone, because the presence of these solvents in the measuring solution leads to interferences with the HPLC (see 4.2).

Dilute the extract to a known volume (e.g. 2 ml) with the same solvent that has been used for the preparation of the reference solutions (5.9). The volume fraction of dimethylformamide should not exceed 20 % in order to avoid peak broadening in the chromatogram.

Transfer the enriched test sample, if necessary after filtration through a microfilter (6.11), into a glass sample vial (6.12). Keep the extract in a cool and dark place until the analysis is carried out.

## 8.4 Clean-up of the extract

To clean the extract, use columns [Pasteur pipettes (6.8)] or cartridges (6.13) containing at least 0,5 g of silica (5.5). Wash the silica in the column or in the cartridge by rinsing it with five bed volumes of a mixture of dichloromethane/hexane (1:1), followed by conditioning with the same volume of hexane (5.1.2) and do not allow to dry.

Dry the solvents used for cleaning the extract using activated molecular sieve beads (5.6).

Concentrate the hexane extract (8.3) further to a volume of 500 µl ± 50 µl by gently blowing a stream of nitrogen (5.4) over the extract.

Transfer the concentrated extract using a Pasteur pipette (6.8) onto the hexane-covered silica and allow the extract to permeate almost completely into the silica. Collect the eluate in a glass vial (6.14).

Rinse the reduction flask with 500 µl of hexane (5.1.2), and add this solution onto the column and allow it to permeate almost completely into the silica.

Elute the PAH from the column with a mixture of dichloromethane/hexane (1:1).

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.

Other combinations of eluting solvents may be used, provided they lead to equal or better recovery.

Add 250 µl of *N*,*N*-dimethylformamide (5.1.2) to the eluate, homogenize it by shaking, and concentrate it (see 8.3) to between 200 µl and 250 µl, for example first using a rotary evaporator (6.9) to about 2 ml, then using a stream of nitrogen (5.4).

Dilute the extract to a known volume (e.g. 2 ml) with the same solvent that has been used for the preparation of the reference solutions (5.9).

## 8.5 HPLC operating conditions

### 8.5.1 General

Adjust the HPLC system 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.

### 8.5.2 Chromatographic separation

Use a column and chromatographic conditions which allow efficient separation. For a choice of columns and the corresponding gradients see annex A.

Determine the resolution, $R_{A,B}$, between two chromatographic peaks, for compounds A and B, using equation (1).

$$R_{A,B} = \frac{2(t_{r,B} - t_{r,A})}{w_{b,A} + w_{b,B}} \quad (1)$$

where

$t_{r,A}$, $t_{r,B}$ are the respective retention times, expressed in minutes, of compounds A and B ($t_{r,B} > t_{r,A}$);

$w_{b,A}$, $w_{b,B}$ are the respective base widths, expressed in minutes, of compounds A and B.

Take care that a resolution of at least $R_{ac,fl} = 1$ is obtained for the separation of acenaphthene (ac) from fluorene (fl) and at least $R_{A,B} = 1,5$ for the others. A resolution of at least 2,5 shall be obtained between chromatographic peaks where the wavelength and/or damping is changed.

Perform the separation at constant temperature (± 0,5 °C) to improve the repeatability.

The separation of benzo(ghi)perylene and indeno(1,2,3-cd)pyrene can be improved by optimizing the column temperature.

The maximum injection volume depends on the inner diameter of the separation column. It should be chosen so that band broadening is minimized (typically 10 µl injection volume for a column inner diameter of 3 mm).

It is also possible to separate the six PAH specified in the EU drinking water guideline using isocratic elution (see Figure A.3).

### 8.5.3 Detection

Use a fluorescence detector and choose the appropriate excitation and emission wavelengths with regard to sensitivity and selectivity. A typical wavelength programme is given in Table A.1.

During wavelength programming baseline disturbance should be avoided. Therefore, only make any programming changes if the resolution between peaks is at least 2,5 (see 8.5.2).

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

A change of wavelength should be made at times when the fluorescence is low. At high fluorescence, wavelength changes can lead to a displacement of the baseline. Readjusting the baseline after changing the wavelength may interfere with integration and hence with quantification.

It may be necessary to change the wavelengths and the damping simultaneously to obtain constant peak heights. The damping conditions are part of the detection criteria and should not be changed after calibration. If damping is low, the resultant increase in noise should not impair signal integration.

### 8.5.4 Identification of individual compounds

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

Assume that an individual compound is present if the retention time of the compound in the test sample chromatogram coincides with the retention time of a reference compound in the reference chromatogram measured under the same conditions (tolerance ± 1 %, maximum 10 s).

A positive result may be verified, if required, using one of the following methods:

— by comparing the excitation and emission spectrums of the compound in the test sample with those of the reference compound having the coinciding retention time taken under the same conditions;

— at higher concentrations, by using a second detector known not to cause interference by broadening of the fluorescence peaks, for example using a diode array detector and comparing absorption spectrums of the test sample and reference peaks;

— by applying an independent method, e.g. gas chromatography.

## 8.6 Calibration

### 8.6.1 General

A distinction is made between the initial calibration, the routine calibration and a routine check to determine if the calibration curve is still valid. The initial calibration is performed to determine the working range and the linearity of the calibration function in accordance with ISO 8466-1, specifically when the HPLC apparatus is used for the first time.

Subsequently, the final working range is established and routine calibration performed. It is necessary to 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 period of time, or if the validity criteria of the routine check cannot be met.

The validity of the initial calibration is made by running a routine check with each series of test samples to be analysed.

### 8.6.2 Initial calibration

Establish the preliminary working range by analysing at least five dilutions of the calibration standard mixture (5.9). Test for linearity in accordance with ISO 8466-1.

### 8.6.3 Routine calibration

After establishing the final working range, analyse a minimum of five dilutions of the standard calibration mixture (5.9). Calculate a calibration function by linear regression analysis of the corrected peak areas. The actual sensitivity of the method may be estimated from the calculated regression function.

### 8.6.4 Routine check

Make a routine check to establish whether the calibration function is still valid by analysing one standard solution after every ten test samples for each batch of test samples. The concentration of this standard solution shall lie between 40 % and 80 % of the working range. If the individual results do not deviate by more than 10 % of the working calibration line, assume the calibration function to be valid. If not, recalibrate the system in accordance with 8.6.3

## 8.7 Determination

Stabilize the HPLC system before analysing the test samples and adjust the wavelength programme in relation to the retention times found.

NOTE Reproducible retention times are usually obtained after two or three injections of a reference solution (5.9).

Analyse the test sample, the calibration solutions and the blank using the HPLC.

Ensure that the peaks of each test sample are integrated correctly and make any necessary corrections.

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

## 8.8 Recovery

Spike 1 000 ml water with, for example 2 ml of the reference solutions prepared according to 5.9 and proceed as described in 8.1 to 8.5.

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

Determine the mean recovery of the analyte $i$, $\overline{\eta_i}$, using equations (2) and (3):

$$\eta_{i,N} = \frac{\rho_{i,N\,\mathrm{cal}}}{\rho_{i,N\,\mathrm{sp}}} \tag{2}$$

$$\overline{\eta_i} = \frac{\sum_{N=1}^{n} \eta_{i,N}}{n} \tag{3}$$

where

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

$\rho_{i,N\text{cal}}$ is the measured mass concentration, expressed in micrograms per litre, of analyte $i$ at the concentration level $N$, and calculated using the calibration function;

$\rho_{i,N\text{sp}}$ is the spiked mass concentration, expressed in micrograms per litre, of analyte $i$ at the concentration level $N$;

$n$ is the number of concentration levels.

Mean recoveries of 95 % are usually obtained for the extraction method described in 8.1 to 8.5. If this applies, the correction factor for the recovery, $\overline{\eta_i}$, can be omitted (see clause 9).

### 8.9 Blank measurement

Monitor the reagents and the correct operation of the instruments by regularly performed blank measurements, analysing 1 000 ml of PAH-free water treated as described in 8.1 to 8.5.

If any analytes are found in the blanks, investigate the cause and eliminate any sources of contamination.

## 9 Calculation

Calculate the mass concentration, expressed in micrograms per litre, of analyte $i$, $\rho_i$, in the water sample using equation (4):

$$\rho_i = \frac{(y_i - b_i) \cdot V_\text{e}}{a_i \cdot V_\text{s} \cdot \overline{\eta_i}} \tag{4}$$

where

$y_i$ is the measured value of the analyte $i$, for example expressed as peak area,;

$a_i$ is the slope of the calibration function of the analyte $i$, also called compound-specific response factor, for example expressed as peak area/(pg/µl);

$b_i$ is the intercept of the calibration function with the ordinate, for example expressed as peak area;

$V_\text{e}$ is the volume, expressed in millilitres, of the extractant from which the injection was made;

$V_\text{s}$ is the volume, expressed in millilitres, of the test sample;

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

Report the mass concentration, expressed in micrograms per litre, of PAH to no more than two significant figures. For mass concentrations $<$ 0,01 µg/l, round them up to the nearest 0,001 µg/l. Examples for rounding results are given in Table 2.

## 10 Precision

Statistical data obtained from results of an interlaboratory trial carried out in Germany in 1996 are given in Tables 3 and 4.

**Table 2 — Examples for expression of results**

| Measured value<br>µg/l | Result recorded<br>µg/l |
|---|---|
| 13,54 | 14 |
| 1,354 | 1,4 |
| 0,135 4 | 0,14 |
| 0,013 5 | 0,014 |
| 0,008 5 | 0,009 |

**Table 3 — Statistical data for the HPLC determination of a certified PAH-standard**

| Compound | $l$ | $n$ | $o$<br>% | $\rho_{exp}$<br>pg/µl | $\bar{\rho}$<br>pg/µl | $\eta$<br>% | $s_r$<br>pg/µl | Repeatability CV<br>% | $s_R$<br>pg/µl | Reproducibility CV<br>% |
|---|---|---|---|---|---|---|---|---|---|---|
| Naphthalene | 34 | 136 | 0 | 50 | 49,49 | 99,0 | 1,392 | 2,8 | 3,391 | 6,85 |
| Acenaphthene | 34 | 136 | 0 | 25 | 24,44 | 97,8 | 0,750 | 3,1 | 2,227 | 9,11 |
| Fluorene | 34 | 136 | 0 | 40 | 38,30 | 95,7 | 0,952 | 2,5 | 3,133 | 8,18 |
| Phenanthrene | 32 | 128 | 5,88 | 30 | 29,35 | 97,8 | 0,785 | 2,7 | 1,394 | 4,75 |
| Anthracene | 34 | 136 | 0 | 25 | 24,82 | 99,3 | 0,753 | 3,0 | 1,712 | 6,90 |
| Fluoranthene | 34 | 136 | 0 | 40 | 39,78 | 99,4 | 1,138 | 2,9 | 2,486 | 6,25 |
| Pyrene | 34 | 135 | 0,74 | 40 | 39,59 | 99,0 | 1,194 | 3,0 | 3,061 | 7,73 |
| Benzo(a)anthracene | 34 | 135 | 0,74 | 10 | 9,76 | 97,6 | 0,349 | 3,6 | 0,910 | 9,33 |
| Chrysene | 33 | 132 | 2,94 | 20 | 19,77 | 98,9 | 0,635 | 3,2 | 1,241 | 6,27 |
| Benzo(b)fluoranthene | 34 | 135 | 0,74 | 25 | 24,41 | 97,6 | 0,577 | 2,4 | 1,168 | 4,79 |
| Benzo(k)fluoranthene | 34 | 136 | 0 | 10 | 9,57 | 95,7 | 0,187 | 2,0 | 0,734 | 7,67 |
| Benzo(a)pyrene | 34 | 136 | 0 | 20 | 18,74 | 93,7 | 0,523 | 2,8 | 1,227 | 6,49 |
| Dibenzo(ah)anthracene | 33 | 132 | 2,94 | 40 | 38,41 | 96,0 | 0,889 | 2,3 | 2,042 | 5,32 |
| Benzo(ghi)perylene | 34 | 136 | 0 | 25 | 23,74 | 95,0 | 0,811 | 3,4 | 1,749 | 7,32 |
| Indeno(1,2,3-cd)pyrene | 34 | 136 | 0 | 25 | 24,37 | 97,5 | 1,210 | 5,0 | 2,278 | 9,35 |

$l$ is the number of laboratories after elimination of outliers

$n$ is the number of results after elimination of outliers

$o$ are the outliers

$\rho_{exp}$ is the expected value

$\bar{\rho}$ is the total mean of all results free from outliers

$\eta$ is the recovery

$s_r$ is the repeatability standard deviation

$s_R$ is the reproducibility standard deviation

CV is the coefficient of variation

**Table 4 — Statistical data for drinking water spiked with low concentrations of PAH**

| Compound | $l$ | $n$ | $o$ % | $\rho_{exp}$ ng/l | $\bar{\rho}$ ng/l | $\eta$ [a] % | $s_r$ ng/l | Repeatability CV % | $s_R$ ng/l | Reproducibility CV % |
|---|---|---|---|---|---|---|---|---|---|---|
| Naphthalene | 33 | 128 | 3,03 | 60 | 52,85 | 88,1 | 7,412 | 14,0 | 15,50 | 29,33 |
| Acenaphthene | 33 | 126 | 3,82 | 30 | 24,64 | 82,1 | 2,264 | 9,2 | 5,289 | 21,47 |
| Fluorene | 33 | 128 | 3,03 | 48 | 40,81 | 85,0 | 4,139 | 10,1 | 8,771 | 21,49 |
| Phenanthrene | 31 | 119 | 8,46 | 36 | 38,95 | 108 | 3,522 | 9,0 | 7,504 | 19,26 |
| Anthracene | 33 | 124 | 0 | 30 | 26,84 | 89,5 | 1,887 | 7,0 | 4,474 | 16,67 |
| Fluoranthene | 30 | 117 | 10,0 | 48 | 46,48 | 96,8 | 2,189 | 4,7 | 4,225 | 9,09 |
| Pyrene | 33 | 127 | 0 | 48 | 45,44 | 94,7 | 3,954 | 8,7 | 7,186 | 15,81 |
| Benzo(a)anthracene | 32 | 124 | 3,13 | 12 | 11,54 | 96,1 | 1,095 | 9,5 | 2,810 | 24,35 |
| Chrysene | 32 | 122 | 6,15 | 24 | 22,20 | 92,5 | 2,069 | 9,3 | 3,743 | 16,86 |
| Benzo(b)fluoranthene | 33 | 126 | 3,08 | 30 | 27,41 | 91,3 | 2,450 | 8,9 | 4,719 | 17,22 |
| Benzo(k)fluoranthene | 32 | 123 | 3,15 | 12 | 10,87 | 90,6 | 1,148 | 10,6 | 2,382 | 21,91 |
| Benzo(a)pyrene | 33 | 126 | 3,08 | 24 | 20,43 | 85,1 | 1,912 | 9,4 | 4,170 | 20,42 |
| Dibenzo(ah)anthracene | 32 | 121 | 6,92 | 48 | 39,53 | 82,3 | 3,139 | 7,9 | 6,952 | 17,59 |
| Benzo(ghi)perylene | 32 | 122 | 6,15 | 30 | 25,21 | 84,0 | 2,765 | 11,0 | 5,941 | 23,57 |
| Indeno(1,2,3-cd)pyrene | 29 | 111 | 12,0 | 30 | 26,31 | 87,7 | 2,675 | 10,2 | 4,417 | 17,93 |

The definitions of the symbols are given in Table 3.

[a] The recoveries given in this table represent the mean recovery of the extraction step for all laboratories, as the individual results were not evaluated using correction using $\overline{\eta_i}$ .

## 11 Test report

The test report shall include the following information:

a) a reference to this International Standard, i.e. ISO 17993;

b) all details necessary for complete identification of the sample;

c) any relevant information about sampling and test sample preservation;

d) the mass concentration, calculated and expressed in accordance with clause 9, for each PAH determined;

e) any operations not included in this International Standard or which might have affected the results.

# Annex A
(informative)

# Examples of chromatographic conditions and columns

Two examples (A and B) of the HPLC chromatographic conditions, the gradient elution programme and columns under which 16 PAH are able to be separated are given in Figures A.1 and A.2. Typical chromatograms using these conditions are also shown in Figures A.1 and A.2. The wavelength programme used to obtain these chromatographic conditions and chromatograms A and B is given in Table A.1.

A third example of the HPLC chromatographic conditions, detector conditions and columns under which PAH found in drinking water can be separated and a typical chromatogram are given in Figure A.3. Isocratic conditions are used for this separation.

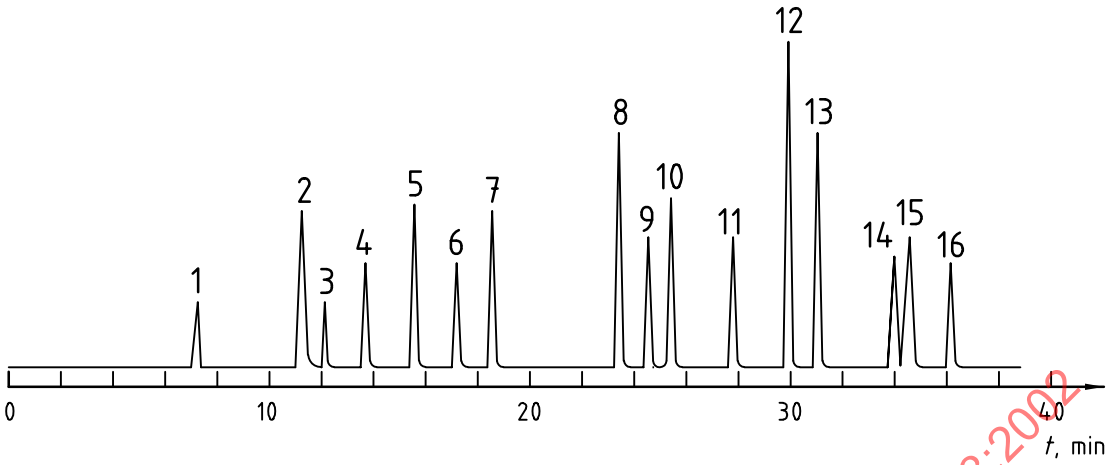
**Table A.1 — Wavelength programme for the chromatograms A and B**

| Compound | | Wavelength nm | |
|---|---|---|---|
| | | Excitation | Emission |
| 1 | Naphthalene | 275 | 350 |
| 2 | Acenaphthene | | |
| 3 | Fluorene | | |
| 4 | Phenanthrene | | |
| 5 | Anthracene | 260 | 420 |
| 6 | Fluoranthene | 270 | 440 |
| 7 | Pyrene | | |
| 8 | Benzo(a)anthracene | 260 | 420 |
| 9 | Chrysene | | |
| 10 | 6-Methylchrysene[a] | | |
| 11 | Benzo(b)fluoranthene | 290 | 430 |
| 12 | Benzo(k)fluoranthene | | |
| 13 | Benzo(a)pyrene | | |
| 14 | Dibenzo(ah)anthracene | | |
| 15 | Benzo(ghi)perylene | | |
| 16 | Indeno(1,2,3-cd)pyrene | 250 | 500 |

HPLC: HP 1046 FLD, slit; excitation: 12,5 nm; emission: 25 nm

NOTE HP 1046 FLD is an example of a suitable HPLC system with a fluorescence detector available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

[a] Additional compound




**Peak identification**

| | | | |
|---|---|---|---|
| 1 | Naphthalene | 9 | Chrysene |
| 2 | Acenaphthene | 10 | 6-Methylchrysene |
| 3 | Fluorene | 11 | Benzo(b)fluoranthene |
| 4 | Phenanthrene | 12 | Benzo(k)fluoranthene |
| 5 | Anthracene | 13 | Benzo(a)pyrene |
| 6 | Fluoranthene | 14 | Dibenzo(ah)anthracene |
| 7 | Pyrene | 15 | Benzo(ghi)perylene |
| 8 | Benzo(a)anthracene | 16 | Indeno(1,2,3-cd)pyrene |

**Operating conditions**

Injection: 5 µl calibration solution with $\rho_i$ = 100 pg/µl

Column: Bakerbond PAH 16 plus, length: 250 mm, diameter: 3 mm

Solvent flow: 0,5 ml/min

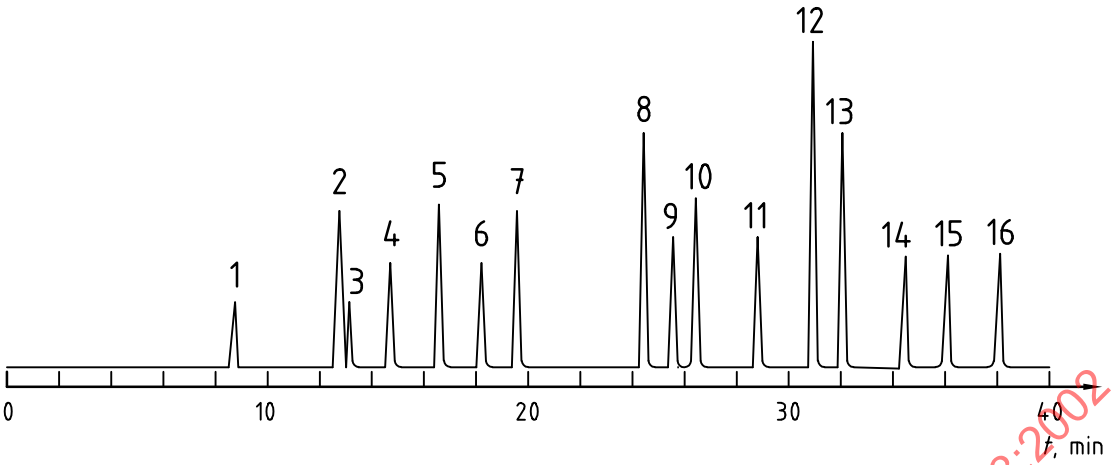
Temperature: 25 °C

Gradient elution programme:

| Time (min): | 0 | 5 | 35 | 45 |
|---|---|---|---|---|
| Volume ratio: | 50:50 | 50:50 | 100:00 | 100:00 |
| Gradient: | start conditions | isocratic | linear | isocratic |
| Eluent: | acetonitrile/water | | | |

NOTE Bakerbond PAH 16 plus is an example of a suitable column material available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

**Figure A.1 — Chromatogram A**




**Peak identification**

| | | | |
|---|---|---|---|
| 1 | Naphthalene | 9 | Chrysene |
| 2 | Acenaphthene | 10 | 6-Methylchrysene |
| 3 | Fluorene | 11 | Benzo(b)fluoranthene |
| 4 | Phenanthrene | 12 | Benzo(k)fluoranthene |
| 5 | Anthracene | 13 | Benzo(a)pyrene |
| 6 | Fluoranthene | 14 | Dibenzo(ah)anthracene |
| 7 | Pyrene | 15 | Benzo(ghi)perylene |
| 8 | Benzo(a)anthracene | 16 | Indeno(1,2,3-cd)pyrene |

**Operating conditions**

Injection: 5 µl calibration solution with $\rho_i$ = 100 pg/µl

Column: Nucleosil 5 C18 PAH, length: 250 mm, diameter: 3 mm

Solvent flow: 0,5 ml/min

Temperature: 25 °C

Gradient elution programme:

| | | | |
|---|---|---|---|
| Time (min): | 0 | 35 | 45 |
| Volume ratio: | 60:40 | 100:00 | 100:00 |
| Gradient: | start conditions | linear | isocratic |
| Eluent: | acetonitrile/water | | |

NOTE Nucleosil 5 C18 PAH is an example of a suitable column material available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

**Figure A.2 — Chromatogram B**