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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

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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

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Foreword

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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 7981-1 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

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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*

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— *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.

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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	

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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.

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Monitor the blank to guarantee that the reagents do not contain PAH in detectable concentrations (see Clause 11).

4.1 Solvents

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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).

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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).

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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.

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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.

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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.

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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.

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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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