INTERNATIONAL STANDARD

ISO 7981-2

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

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

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

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

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Foreword

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

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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

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

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

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

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

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

Introduction

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

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

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

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

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

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

Persons using this part of ISO 7981 should be familiar with normal laboratory practise. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this part of ISO 7981 to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted according to this part of ISO 7981 be carried out by suitably trained staff.

1 Scope

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

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

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

3 Principle

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

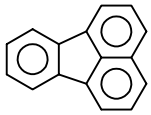
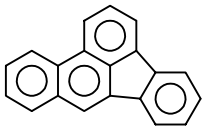
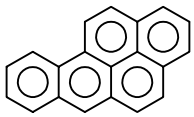
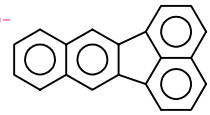
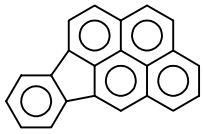
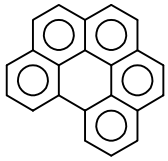
NOTE For the analysis of surface water, a differentiation between dissolved and undissolved PAH can be desirable, but this is not relevant for drinking water.

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

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

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

Table 1 — Polycyclic aromatic hydrocarbons determinable by this method

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

4 Interferences

4.1 Interferences with sampling and extraction

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

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

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

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

4.2 Interferences with the HPLC

Substances that show either fluorescence or quenching and co-elute with the PAH to be determined can interfere with the determination. These interferences can lead to incompletely resolved signals and can, depending on their magnitude, affect accuracy and precision of the analytical results. Peak overlaps will prevent the measurement of peak height and/or area. Unsymmetrical peaks and peaks broader than the respective peaks of the reference substance suggest interferences.

5 Reagents

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

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

5.1 Solvents

5.1.1 Solvents for extraction and clean-up of the extract

5.1.1.1 Cyclohexane, C_6H_{12}

5.1.1.2 Hexane, C_6H_{14}

5.1.1.3 Dichloromethane, CH_2Cl_2

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

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

5.1.2 HPLC solvents

5.1.2.1 Methanol, CH_3OH

5.1.2.2 Acetonitrile, CH_3CN

5.1.2.3 Tetrahydrofuran, C_4H_8O, without stabilizer

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

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

5.3 Sodium chloride, NaCl

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

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

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

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

NOTE Prepacked silica cartridges are commercially available.

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

5.9 Reference substances (see Table 1)

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

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

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

5.12 Calibration solutions

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

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

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

6 Apparatus

Standard laboratory equipment cleaned to eliminate all interferences.

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

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

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

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

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

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

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

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

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

6.8 Pasteur pipettes

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

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

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

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

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

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

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

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

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

- degassing assembly, e.g. for degassing with vacuum or helium;
- low pulsating analytical pump;
- manual or automatic sample applicator;

- column thermostat, capable of keeping the temperature constant to within ± 0,5 °C;
- fluorescence detector, preferably equipped with a monochromator on either the excitation and emission sides, or with a filter (8.5.2);
- analytical separation column, e.g. a column with length up to 250 mm, internal diameter 2 mm to 4,6 mm, packed with particle size 3 µm to 5 µm material, capable of near baseline separation (at least as good as in Figure A.1) of the PAH to a large extent.

7 Sampling

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

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

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

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