
**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*

iTeh STANDARD REVIEW
(standards.iteh.ai)

[ISO 17993:2002](https://standards.iteh.ai/catalog/standards/sist/bb7b7db3-b461-4be1-a77a-5109cc69bb0a/iso-17993-2002)

<https://standards.iteh.ai/catalog/standards/sist/bb7b7db3-b461-4be1-a77a-5109cc69bb0a/iso-17993-2002>



PDF disclaimer

This PDF file may contain embedded typefaces. In accordance with Adobe's licensing policy, this file may be printed or viewed but shall not be edited unless the typefaces which are embedded are licensed to and installed on the computer performing the editing. In downloading this file, parties accept therein the responsibility of not infringing Adobe's licensing policy. The ISO Central Secretariat accepts no liability in this area.

Adobe is a trademark of Adobe Systems Incorporated.

Details of the software products used to create this PDF file can be found in the General Info relative to the file; the PDF-creation parameters were optimized for printing. Every care has been taken to ensure that the file is suitable for use by ISO member bodies. In the unlikely event that a problem relating to it is found, please inform the Central Secretariat at the address given below.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

ISO 17993:2002

<https://standards.iteh.ai/catalog/standards/sist/bb7b7db3-b461-4be1-a77a-5109cc69bb0a/iso-17993-2002>

© ISO 2002

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying and microfilm, without permission in writing from either ISO at the address below or ISO's member body in the country of the requester.

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	iv
Introduction.....	v
1 Scope	1
2 Normative references.....	1
3 Principle	1
4 Interferences	3
5 Reagents	4
6 Apparatus.....	5
7 Sampling	6
8 Procedure.....	6
9 Calculation	11
10 Precision	11
11 Test report.....	13
Annex A (informative) Examples of chromatographic conditions and columns	14
Annex B (informative) Examples for the construction of special apparatus.....	18

iTeh STANDARD PREVIEW
 (standards.iteh.ai)
 ISO 17993:2002
<https://standards.iteh.ai/catalog/standards/sist/bb7b7db3-b461-4be1-a77a-5109cc69bb0a/iso-17993-2002>

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.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

[ISO 17993:2002](https://standards.iteh.ai/catalog/standards/sist/bb7b7db3-b461-4be1-a77a-5109cc69bb0a/iso-17993-2002)

<https://standards.iteh.ai/catalog/standards/sist/bb7b7db3-b461-4be1-a77a-5109cc69bb0a/iso-17993-2002>

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.

iTeh STANDARD PREVIEW (standards.iteh.ai)

[ISO 17993:2002](https://standards.iteh.ai/catalog/standards/sist/bb7b7db3-b461-4be1-a77a-5109cc69bb0a/iso-17993-2002)

<https://standards.iteh.ai/catalog/standards/sist/bb7b7db3-b461-4be1-a77a-5109cc69bb0a/iso-17993-2002>

iTeh STANDARD PREVIEW
(standards.iteh.ai)

ISO 17993:2002

<https://standards.iteh.ai/catalog/standards/sist/bb7b7db3-b461-4be1-a77a-5109cc69bb0a/iso-17993-2002>

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.

[ISO 17993:2002](https://standards.iteh.ai/catalog/standards/sist/bb7b7db3-b461-4be1-a77a-5109cc69bb0a/iso-17993-2002)

<https://standards.iteh.ai/catalog/standards/sist/bb7b7db3-b461-4be1-a77a-5109cc69bb0a/iso-17993-2002>

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

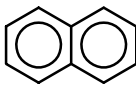
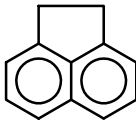
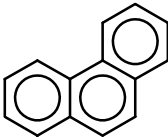
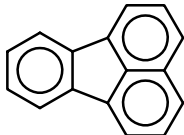
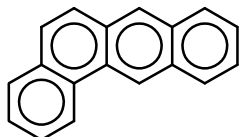
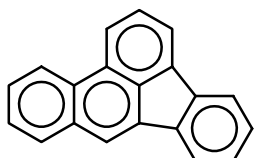
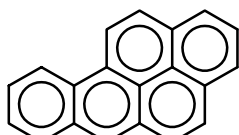
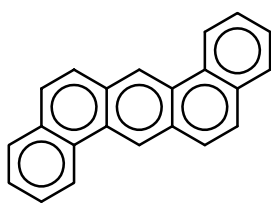
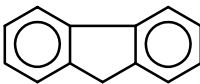
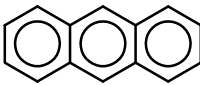
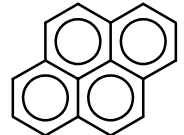
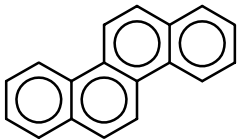
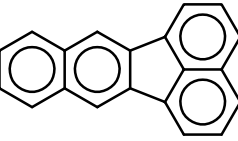
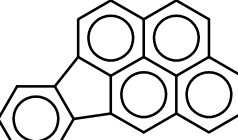
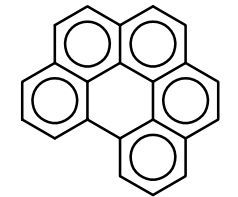
Name	Chemical formula	Molar mass	Percentage carbon	CAS-number	Structure
Naphthalene	$C_{10}H_8$	128,17 g/mol	93,75 % C	091-20-3	
Acenaphthene	$C_{12}H_{10}$	154,21 g/mol	93,05 % C	083-32-9	
Phenanthrene	$C_{14}H_{10}$	178,23 g/mol	94,05 % C	085-01-8	
Fluoranthene	$C_{16}H_{10}$	202,26 g/mol	95,0 % C	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, C₆H₁₄;
- 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: [ISO 17993:2002](https://standards.iteh.ai/catalog/standards/sist/bb7b7db3-b461-4be1-a77a-5109cc69bb0a/iso-17993-2002)

- dichloromethane, CH₂Cl₂ (see note), <https://standards.iteh.ai/catalog/standards/sist/bb7b7db3-b461-4be1-a77a-5109cc69bb0a/iso-17993-2002>
- hexane, C₆H₁₄;
- *N,N*-dimethylformamide, (CH₃)₂NCHO;
- acetone, C₃H₆O.

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, CH₃CN;
- methanol, CH₃OH.

5.2 Sodium thiosulfate pentahydrate, Na₂S₂O₃·5H₂O.

5.3 Sodium sulfate, Na₂SO₄, 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 µg 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).