



Designation: D 4657 – 92 (Reapproved 1998)

An American National Standard

Standard Test Method for Polynuclear Aromatic Hydrocarbons in Water¹

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1. Scope

1.1 This test method covers the determination of certain polynuclear aromatic hydrocarbons (PAHs) in water and wastewater. The following compounds may be determined by this test method: acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(ghi)perylene, benzo(k)fluoranthene, chrysene, dibenzo(ah)anthracene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene, and pyrene. Additional PAHs may also be determined; however, the analyst should demonstrate that the test method is in fact applicable to the specific PAH(s) of interest before applying it to sample analysis. This test method has high sensitivity for the compounds of interest. It is limited to use by analysts familiar with high-performance liquid chromatography (HPLC) or working under close supervision of such persons.

1.2 This test method is applicable to the determination of the compounds in 1.1 in water and wastewater. This test method has been successfully used with distilled water, tap water, surface water, and the following wastewaters: effluent from an oil refinery, blast furnace, and combined coke oven and blast furnace. It is the user's responsibility to ensure the validity of this test method for waters of untested matrices. It presupposes a high expectation of finding the specific compounds of interest. If the user is attempting to screen samples for any or all of the compounds above, component identities should be verified by using two different types of reverse phase HPLC columns, both ultraviolet and fluorescence detection, or gas chromatography/mass spectrometry-spectroscopy screening procedures, or both.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* Specific hazards statements are given in Section 9.

¹ This test method is under the jurisdiction of ASTM Committee D-19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

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2. Referenced Documents

2.1 ASTM Standards:

- D 1129 Terminology Relating to Water²
- D 1192 Specification for Equipment for Sampling Water and Steam in Closed Conduits²
- D 1193 Specification for Reagent Water²
- D 1253 Test Method for Residual Chlorine in Water²
- D 3370 Practices for Sampling Water from Closed Conduits²
- D 3856 Guide for Good Laboratory Practices in Laboratories Engaged in Sampling and Analysis of Water²
- D 4210 Practice for Intralaboratory Quality Control Procedures and a Discussion on Reporting Low-Level Data²

3. Terminology

3.1 *Definitions*—For definitions of terms used in this test method, refer to Terminology D 1129.

4. Summary of Test Method

4.1 This test method consists of the extraction of 1 L of water with methylene chloride. This extract is then reduced in volume using Kuderna-Danish (K-D) evaporation followed by column chromatography on silica gel. The appropriate fraction from the silica gel chromatography containing the PAHs is again reduced in volume using K-D evaporation and is solvent exchanged with acetonitrile to an exact volume of 1 mL. This concentrated extract is then analyzed for PAH using high-performance liquid chromatography (HPLC).

4.2 The HPLC analysis utilizes reverse phase chromatography using a combination of isocratic and gradient elution. Acetonitrile and water are used as the mobile phase on a Perkin Elmer PAH/10 reverse phase column.³ Other C-18 reverse phase columns may be used, provided that they yield adequate resolution of the PAHs of interest. Fluorescence or ultraviolet detection depending on the needs is used to monitor the PAH of interest; quantitation is by peak area integration or peak height measurement. Results are reported in micrograms per litre.

² *Annual Book of ASTM Standards*, Vol 11.01.

³ Perkin-Elmer PAH/10 reverse phase column is available from Perkin-Elmer Corporation, 761 Main Ave., Norwalk, CT 06859.

4.3 If interferences are encountered, this test method provides a selected general purpose cleanup procedure to aid the analyst in their elimination.

5. Significance and Use

5.1 Prominent among the group of compounds found in various water supplies and considered to be potential health risks are the polynuclear aromatic hydrocarbons (PAHs). These compounds have been found to occur naturally in water as a result of pyrolytic processes in the environment. Other sources include automobile exhaust, runoff from highways, and municipal and industrial discharges. Several of these compounds have been determined to be at least weak carcinogens even at very low concentrations. For this reason, a method for the detection and quantitation of these compounds in the water environment is necessary.

6. Interferences

6.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts or elevated baselines causing misinterpretation of chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis. Specific selection of reagents and the purification of solvents by distillation in all glass systems is required. Glassware should be cleaned by washing with soap and water, rinsing with tap water, reagent water (8.2), redistilled acetone, and finally with pesticide quality hexane. If the type and size of glassware permits, it is heated in a muffle furnace to approximately 400°C for 15 to 30 min. Volumetric ware should not be heated in a muffle furnace. Plastics, except TFE-fluorocarbon, can cause interference or absorption, or both, of PAHs and should be avoided.

6.2 The organic constituents in industrial effluents are often present at high concentrations and can pose great difficulty in obtaining accurate and precise measurement of PAH. The use of the fluorescence detector and the column clean-up procedure may eliminate many of these interferences.

6.3 Other PAH compounds may represent interference in certain cases. Since the PAHs listed in 1.1 represent the more commonly encountered nonalkylated PAHs, interference from isomeric, nonalkylated PAHs is minimal. Benzo(e)pyrene elutes earlier than its isomers, benzo(a)pyrene, benzo(b)fluoranthene, and benzo(k)fluoranthene and does not interfere with any compounds listed in 1.1. Alkylated PAHs will elute later than the parent, nonalkylated compound. Consequently, multi-alkylated naphthalenes (2-ring system) may coelute with fluoranthene (3-ring system). However, the use of both ultraviolet and fluorescence detection allows recognition of such interferences since widely different response ratios will be encountered for the two groups of compounds.

6.4 All reference standards must be demonstrated to be free of extraneous peaks under the conditions of analysis.

7. Apparatus

7.1 *HPLC Gradient System*, capable of constant flow.

7.2 *Reverse Phase Column*, 5- or 10- μ m, which yields resolution equivalent or better than Perkin Elmer Reverse Phase Column PAH/10, 2.6 by 250 mm, used to obtain the

precision and bias data in the test method. Equivalency is demonstrated in resolution of the analytes, and the analyst meeting or exceeding the single operator precision for the analytes of concern at known concentrations in reagent water by following procedures outlined in Section 16.

7.3 *Fluorescence Detector*, capable of excitation at 280 nm and emission at 389 nm (using a cutoff filter). Other types of fluorescence detectors (for example, grating emission monochromators) may be used. However, detection limits may vary considerably and will need to be established for the PAHs of interest in the particular application.

7.4 *Ultraviolet detector*, 254-nm with a noise specification of 2×10^{-4} AU or better.

7.5 *Chromatographic Data System*, having sufficient parameters to accurately follow a sloping baseline, or a 10-mV full-scale stripchart recorder.

7.6 *Chromatographic Column Glass*, 1 by 25 cm with TFE-fluorocarbon stopcock.

7.7 *Kuderna-Danish Evaporative Concentrator*, with the following components:

7.7.1 *Snyder Column*, three-ball.

7.7.2 *Micro Snyder Column*, two-ball.

7.7.3 *Evaporative Flask*, 500-mL.

7.7.4 *Receiver Ampule*, 10-mL.

7.7.5 *Ampule Caps*.

7.8 *Water Bath*, heated with concentric ring cover and capable of temperature control (+ 2°C).

7.9 *Graduated Cylinder*, 1000-mL.

8. Reagents and Materials

8.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society.⁴ Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Specification D 1193, Type II.

8.3 *Acetonitrile*—Spectral quality.

8.4 *Cyclohexane*—Pesticide residue quality, or equivalent.

8.5 *Methylene Chloride*—Pesticide residue quality, or equivalent.

8.6 *Pentane*—Pesticide residue quality, or equivalent.

8.7 *Silica Gel*—100/120 mesh, Davidson Grade 923. Activate in an oven at 130°C overnight in an uncapped widemouth jar. Maintain at 130°C when not in use.

8.8 *Sodium Hydroxide*.

8.9 *Sodium Sulfate*—Anhydrous.

8.10 *Sodium Thiosulfate*.

8.11 *Sulfuric Acid*, (sp gr 1.84)—Concentrated sulfuric acid (H₂SO₄).

⁴ "Reagent Chemicals, American Chemical Society Specifications," Am. Chemical Soc., Washington DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see "Analytical Standards for Laboratory Chemicals," BDH Ltd., Poole, Dorset, U.K., and the "United States Pharmacopeia."



8.12 *PAH Standards*, analytical reference grade or highest purity available.

9. Hazards

9.1 **Precaution**—Due to the potential for detrimental health effects from handling these compounds, preparation of standards and samples must be done, using extreme care, in an appropriate hood or glove box.

9.2 **Caution**—The analyst should be aware of the fact that sunlight and certain types of fluorescent lights can cause decomposition of PAHs, so appropriate care should be taken during sample storage and preparation.

10. Sampling

10.1 Collect the sample in accordance with Specification D 1192 or Practice D 3370, as applicable.

10.2 Grab samples must be collected in glass containers with TFE-fluorocarbon lined caps. Conventional sampling practices should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program.

NOTE 1—Automatic sampling equipment must be free of plastic tubing such as PVC, and other potential sources of contamination, as determined by collecting a reagent water blank with each group of samples.

10.3 The samples must be iced or refrigerated from the time of collection until extraction. Chemical preservatives should not be used in the field unless more than 48 h will elapse before delivery to the laboratory. If the samples will not be extracted within 48 h of collection, adjust the sample to a pH range of 6.0 to 8.0 with sodium hydroxide or sulfuric acid and add 35 mg of sodium thiosulfate per part per million of free chlorine per litre. Free chlorine can be determined using Test Methods D 1253. If samples cannot be returned to the laboratory within 48 h, field measurement of free chlorine will be necessary. Test kits are commercially available for this purpose.⁵

10.4 All samples must be extracted within 7 days and completely analyzed within 30 days of collection.

11. Calibration and Standardization

11.1 Calibrate the instrument for each PAH species at four different concentration levels (in acetone or acetonitrile) using the appropriate detector settings. These concentration levels should span the detector range of interest for a particular application and should represent at least one order of magnitude from the lowest to the highest concentration injected.

11.2 Check calibration daily to establish the validity of the above curves by injection of a standard in the middle portion of the calibration range selected in 11.1.

11.3 The elution order and retention times of the various PAHs are provided in Fig. 1 and Table 1, as a guide.

12. Procedure

12.1 Sample Extraction:

12.1.1 Mark the water meniscus on the side of the sample bottle. Pour the entire sample into a 2-L separatory funnel. Check the pH with wide-range paper and adjust to within the range of 6 to 8 with sodium hydroxide or sulfuric acid. With each set of samples, 1 L aliquot of reagent water (8.2) is placed in a sampling bottle and processed as described for samples, to serve as a method blank.

12.1.2 Add 60 mL methylene chloride to the sample bottle and shake 30 s to rinse the walls. Retain the bottle for determination of sample volume in 12.1.7. Transfer the solvent into the separatory funnel, and extract the sample by shaking the funnel for 2 min with periodic venting to release vapor pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one-third the size of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

12.1.3 Add a second 60-mL volume of methylene chloride to the sample bottle and complete the extraction procedure a second time, combining the extracts in the Erlenmeyer flask.

12.1.4 Perform a third extraction in the same manner. Pour the combined extract through a drying column containing 3 to 4 in. of anhydrous sodium sulfate, and collect it in a 500-mL Kuderna-Danish (K-D) flask equipped with a 10-mL concentrator tube. Rinse the Erlenmeyer flask and column with 20 to 30 mL methylene chloride to complete the quantitative transfer.

12.1.5 Add 1 or 2 clean boiling chips to the flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a steaming hot water bath so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed in steam. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 1 mL, or when distillation ceases, remove the K-D apparatus and allow it to drain for at least 10 min while cooling. If distillation ceases prior to reaching an apparent 1 mL volume, estimate the volume remaining, after cooling, for calculation of the portion of sample to be used in 12.1.6. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately.

12.1.6 Certain highly contaminated samples may not allow concentration of the extract to 1 mL as indicated in 12.1.5. In these cases, add enough methylene chloride to redissolve the residue. Mix thoroughly and use a 5-mL aliquot of the diluted extract for further processing in 12.2. If less than 5 mL is available, record the volume used for use in 13.1. Record the volume of diluted extract (as D) in milliliters for use in 13.2.

⁵ Portable test kits for free chlorine, available from Hach Chemical Company, Loveland, CO, have been found suitable for this purpose.