

Environmental Protection Agency

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METHOD 610—POLYNUCLEAR AROMATIC HYDROCARBONS

1. Scope and Application

1.1 This method covers the determination of certain polynuclear aromatic hydrocarbons (PAH). The following parameters can be determined by this method:

Parameter	STORET No.	CAS No.
Acenaphthene	34205	83-32-9
Acenaphthylene	34200	208-96-8
Anthracene	34220	120-12-7
Benzo(a)anthracene	34526	56-55-3
Benzo(a)pyrene	34247	50-32-8
Benzo(b)fluoranthene	34230	205-99-2
Benzo(ghi)perylene	34521	191-24-2
Benzo(k)fluoranthene	34242	207-08-9
Chrysene	34320	218-01-9
Dibenzo(a,h)anthracene	34556	53-70-3
Fluoranthene	34376	206-44-0
Fluorene	34381	86-73-7
Indeno(1,2,3-cd)pyrene	34403	193-39-5
Naphthalene	34696	91-20-3
Phenanthrene	34461	85-01-8
Pyrene	34469	129-00-0

1.2 This is a chromatographic method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. Method 625 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for many of the parameters listed above, using the extract produced by this method.

1.3 This method provides for both high performance liquid chromatographic (HPLC) and gas chromatographic (GC) approaches for the determination of PAHs. The gas chromatographic procedure does not adequately resolve the following four pairs of compounds: Anthracene and phenanthrene; chrysene and benzo(a)anthracene; benzo(b)fluoranthene and benzo(k)fluoranthene; and dibenzo(a,h)anthracene and indeno(1,2,3-cd)pyrene. Unless the purpose for the analysis can be served by reporting the sum of an unresolved pair, the liquid chromatographic approach must be used for these compounds. The liquid chromatographic method does resolve all 16 of the PAHs listed.

1.4 The method detection limit (MDL, defined in Section 15.1)¹ for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.5 The sample extraction and concentration steps in this method are essentially the same as in Methods 606, 608, 609, 611, and 612.

Thus, a single sample may be extracted to measure the parameters included in the scope of each of these methods. When clean-up is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, to apply appropriate cleanup procedures. Selection of the aliquots must be made prior to the solvent exchange steps of this method. The analyst is allowed the latitude, under Sections 12 and 13, to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters.

1.6 Any modification of this method, beyond those expressly permitted, shall be considered as a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.7 This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC and GC systems and in the interpretation of liquid and gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately 1-L, is extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and concentrated to a volume of 10 mL or less. The extract is then separated by HPLC or GC. Ultraviolet (UV) and fluorescence detectors are used with HPLC to identify and measure the PAHs. A flame ionization detector is used with GC.²

2.2 The method provides a silica gel column cleanup procedure to aid in the elimination of interferences that may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.1.3.

3.1.1 Glassware must be scrupulously cleaned.³ Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. Solvent rinsing should be followed by detergent washing with hot water, and rinses with tap water and distilled water. The glassware should then be drained dry, and heated in a muffle furnace at 400°C for 15 to 30 min. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be

substituted for the muffle furnace heating. Thorough rinsing with such solvents usually eliminates PCB interference. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

3.3 The extent of interferences that may be encountered using liquid chromatographic techniques has not been fully assessed. Although the HPLC conditions described allow for a unique resolution of the specific PAH compounds covered by this method, other PAH compounds may interfere.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method have not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified^{4,6} for the information of the analyst.

4.2 The following parameters covered by this method have been tentatively classified as known or suspected, human or mammalian carcinogens: benzo(a)anthracene, benzo(a)pyrene, and dibenzo(a,h)-anthracene. Primary standards of these toxic compounds should be prepared in a hood. A NIOSH/MESA approved toxic gas respirator should be worn when the analyst handles high concentrations of these toxic compounds.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle—1-L or 1-qt, amber glass, fitted with a screw cap lined

with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The bottle and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional)—The sampler must incorporate glass sample containers for the collection of a minimum of 250 mL of sample. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only.):

5.2.1 Separatory funnel—2-L, with Teflon stopcock.

5.2.2 Drying column—Chromatographic column, approximately 400 mm long × 19 mm ID, with coarse frit filter disc.

5.2.3 Concentrator tube, Kuderna-Danish—10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.4 Evaporative flask, Kuderna-Danish—500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.5 Snyder column, Kuderna-Danish—Three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.6 Snyder column, Kuderna-Danish—Two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.7 Vials—10 to 15-mL, amber glass, with Teflon-lined screw cap.

5.2.8 Chromatographic column—250 mm long × 10 mm ID, with coarse frit filter disc at bottom and Teflon stopcock.

5.3 Boiling chips—Approximately 10/40 mesh. Heat to 400 °C for 30 min or Soxhlet extract with methylene chloride.

5.4 Water bath—Heated, with concentric ring cover, capable of temperature control (±2 °C). The bath should be used in a hood.

5.5 Balance—Analytical, capable of accurately weighing 0.0001 g.

5.6 High performance liquid chromatograph (HPLC)—An analytical system complete with column supplies, high pressure syringes, detectors, and compatible strip-chart recorder. A data system is recommended for measuring peak areas and retention times.

5.6.1 Gradient pumping system—Constant flow.

5.6.2 Reverse phase column—HC-ODS Sil-X, 5 micron particle diameter, in a 25 cm x 2.6 mm ID stainless steel column (Perkin Elmer No. 089-0716 or equivalent). This column was used to develop the method performance statements in Section 15. Guidelines for the use of alternate column packings are provided in Section 12.2.

5.6.3 Detectors—Fluorescence and/or UV detectors. The fluorescence detector is used for excitation at 280 nm and emission greater than 389 nm cutoff (Corning 3-75 or equivalent). Fluorometers should have dispersive optics for excitation and can utilize either filter or dispersive optics at the emission detector. The UV detector is used at 254 nm and should be coupled to the fluorescence detector. These detectors were used to develop the method performance statements in Section 15. Guidelines for the use of alternate detectors are provided in Section 12.2.

5.7 Gas chromatograph—An analytical system complete with temperature programmable gas chromatograph suitable for on-column or splitless injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.7.1 Column—1.8 m long x 2 mm ID glass, packed with 3% OV-17 on Chromosorb W-AW-DCMS (100/120 mesh) or equivalent. This column was used to develop the retention time data in Table 2. Guidelines for the use of alternate column packings are provided in Section 13.3.

5.7.2 Detector—Flame ionization detector. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope (Section 1.1), excluding the four pairs of unresolved compounds listed in Section 1.3. Guidelines for the use of alternate detectors are provided in Section 13.3.

6. Reagents

6.1 Reagent water—Reagent water is defined as a water in which an interferent is not observed at the MDL of the parameters of interest.

6.2 Sodium thiosulfate—(ACS) Granular.

6.3 Cyclohexane, methanol, acetone, methylene chloride, pentane—Pesticide quality or equivalent.

6.4 Acetonitrile—HPLC quality, distilled in glass.

6.5 Sodium sulfate—(ACS) Granular, anhydrous. Purify by heating at 400 °C for 4 h in a shallow tray.

6.6 Silica gel—100/200 mesh, desiccant, Davison, grade-923 or equivalent. Before use, activate for at least 16 h at 130 °C in a shallow glass tray, loosely covered with foil.

6.7 Stock standard solutions (1.00 µg/µL)—Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in acetonitrile and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.7.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4 °C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.7.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicates a problem.

6.8 Quality control check sample concentrate—See Section 8.2.1.

7. Calibration

7.1 Establish liquid or gas chromatographic operating conditions equivalent to those given in Table 1 or 2. The chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with acetonitrile. One of the external standards should be at a concentration near, but above, the MDL (Table 1) and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.2.2 Using injections of 5 to 25 µL for HPLC and 2 to 5 µL for GC, analyze each calibration standard according to Section 12 or 13, as appropriate. Tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (<10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.3 Internal standard calibration procedure—To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the

compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with acetonitrile. One of the standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.3.2 Using injections of 5 to 25 μL for HPLC and 2 to 5 μL for GC, analyze each calibration standard according to Section 12 or 13, as appropriate. Tabulate peak height or area responses against concentration for each compound and internal standard. Calculate response factors (RF) for each compound using Equation 1.

$$\text{RF} = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

Equation 1

where:

A_s =Response for the parameter to be measured.

A_{is} =Response for the internal standard.

C_{is} =Concentration of the internal standard ($\mu\text{g/L}$).

C_s =Concentration of the parameter to be measured ($\mu\text{g/L}$).

If the RF value over the working range is a constant (<10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is} , vs. RF.

7.4 The working calibration curve, calibration factor, or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 15\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.5 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and an ongoing analysis of spiked samples to evaluate and document data quality. The laboratory must maintain records to document the quality of data that is generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method. When results of sample spikes indicate atypical method performance, a quality control check standard must be analyzed to confirm that the measurements were performed in an in-control mode of operation.

8.1.1 The analyst must make an initial, one-time, demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of advances that are occurring in chromatography, the analyst is permitted certain options (detailed in Sections 10.4, 11.1, 12.2, and 13.3) to improve the separations or lower the cost of measurements. Each time such a modification is made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 Before processing any samples the analyst must analyze a reagent water blank to demonstrate that interferences from the analytical system and glassware are under control. Each time a set of samples is extracted or reagents are changed a reagent water blank must be processed as a safeguard against laboratory contamination.

8.1.4 The laboratory must, on an ongoing basis, spike and analyze a minimum of 10% of all samples to monitor and evaluate laboratory data quality. This procedure is described in Section 8.3.

8.1.5 The laboratory must, on an ongoing basis, demonstrate through the analyses of quality control check standards that the operation of the measurement system is in control. This procedure is described in Section 8.4. The frequency of the check standard analyses is equivalent to 10% of all samples analyzed but may be reduced if spike recoveries from samples (Section 8.3) meet all specified quality control criteria.

8.1.6 The laboratory must maintain performance records to document the quality of data that is generated. This procedure is described in Section 8.5.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 A quality control (QC) check sample concentrate is required containing each parameter of interest at the following concentrations in acetonitrile: 100 $\mu\text{g/mL}$ of any

of the six early-eluting PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, and anthracene); 5 µg/mL of benzo(k)fluoranthene; and 10 µg/mL of any of the other PAHs. The QC check sample concentrate must be obtained from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory in Cincinnati, Ohio, if available. If not available from that source, the QC check sample concentrate must be obtained from another external source. If not available from either source above, the QC check sample concentrate must be prepared by the laboratory using stock standards prepared independently from those used for calibration.

8.2.2 Using a pipet, prepare QC check samples at the test concentrations shown in Table 3 by adding 1.00 mL of QC check sample concentrate to each of four 1-L aliquots of reagent water.

8.2.3 Analyze the well-mixed QC check samples according to the method beginning in Section 10.

8.2.4 Calculate the average recovery (\bar{X}) in µg/L, and the standard deviation of the recovery (s) in µg/L, for each parameter using the four results.

8.2.5 For each parameter compare s and \bar{X} with the corresponding acceptance criteria for precision and accuracy, respectively, found in Table 3. If s and \bar{X} for all parameters of interest meet the acceptance criteria, the system performance is acceptable and analysis of actual samples can begin. If any individual s exceeds the precision limit or any individual \bar{X} falls outside the range for accuracy, the system performance is unacceptable for that parameter.

NOTE: The large number of parameters in Table 3 present a substantial probability that one or more will fail at least one of the acceptance criteria when all parameters are analyzed.

8.2.6 When one or more of the parameters tested fail at least one of the acceptance criteria, the analyst must proceed according to Section 8.2.6.1 or 8.2.6.2.

8.2.6.1 Locate and correct the source of the problem and repeat the test for all parameters of interest beginning with Section 8.2.2.

8.2.6.2 Beginning with Section 8.2.2, repeat the test only for those parameters that failed to meet criteria. Repeated failure, however, will confirm a general problem with the measurement system. If this occurs, locate and correct the source of the problem and repeat the test for all compounds of interest beginning with Section 8.2.2.

8.3 The laboratory must, on an ongoing basis, spike at least 10% of the samples from each sample site being monitored to assess accuracy. For laboratories analyzing one to ten samples per month, at least one spiked sample per month is required.

8.3.1 The concentration of the spike in the sample should be determined as follows:

8.3.1.1 If, as in compliance monitoring, the concentration of a specific parameter in the sample is being checked against a regulatory concentration limit, the spike should be at that limit or 1 to 5 times higher than the background concentration determined in Section 8.3.2, whichever concentration would be larger.

8.3.1.2 If the concentration of a specific parameter in the sample is not being checked against a limit specific to that parameter, the spike should be at the test concentration in Section 8.2.2 or 1 to 5 times higher than the background concentration determined in Section 8.3.2, whichever concentration would be larger.

8.3.1.3 If it is impractical to determine background levels before spiking (e.g., maximum holding times will be exceeded), the spike concentration should be (1) the regulatory concentration limit, if any; or, if none, (2) the larger of either 5 times higher than the expected background concentration or the test concentration in Section 8.2.2.

8.3.2 Analyze one sample aliquot to determine the background concentration (B) of each parameter. If necessary, prepare a new QC check sample concentrate (Section 8.2.1) appropriate for the background concentrations in the sample. Spike a second sample aliquot with 1.0 mL of the QC check sample concentrate and analyze it to determine the concentration after spiking (A) of each parameter. Calculate each percent recovery (P) as $100(A-B)/T$, where T is the known true value of the spike.

8.3.3 Compare the percent recovery (P) for each parameter with the corresponding QC acceptance criteria found in Table 3. These acceptance criteria were calculated to include an allowance for error in measurement of both the background and spike concentrations, assuming a spike to background ratio of 5:1. This error will be accounted for to the extent that the analyst's spike to background ratio approaches 5:1.⁷ If spiking was performed at a concentration lower than the test concentration in Section 8.2.2, the analyst must use either the QC acceptance criteria in Table 3, or optional QC acceptance criteria calculated for the specific spike concentration. To calculate optional acceptance criteria for the recovery of a parameter: (1) Calculate accuracy (\bar{X}') using the equation in Table 4, substituting the spike concentration (T) for C; (2) calculate overall precision (S') using the equation in Table 4, substituting \bar{X}' for \bar{X} ; (3) calculate the range for recovery at the spike concentration as $(100 \bar{X}'/T) \pm 2.44(100 S'/T)\%$.⁷

8.3.4 If any individual P falls outside the designated range for recovery, that parameter has failed the acceptance criteria. A check standard containing each parameter

that failed the criteria must be analyzed as described in Section 8.4.

8.4 If any parameter fails the acceptance criteria for recovery in Section 8.3, a QC check standard containing each parameter that failed must be prepared and analyzed.

NOTE: The frequency for the required analysis of a QC check standard will depend upon the number of parameters being simultaneously tested, the complexity of the sample matrix, and the performance of the laboratory. If the entire list of parameters in Table 3 must be measured in the sample in Section 8.3, the probability that the analysis of a QC check standard will be required is high. In this case the QC check standard should be routinely analyzed with the spike sample.

8.4.1 Prepare the QC check standard by adding 1.0 mL of QC check sample concentrate (Section 8.2.1 or 8.3.2) to 1 L of reagent water. The QC check standard needs only to contain the parameters that failed criteria in the test in Section 8.3.

8.4.2 Analyze the QC check standard to determine the concentration measured (A) of each parameter. Calculate each percent recovery (P_s) as $100 (A/T)\%$, where T is the true value of the standard concentration.

8.4.3 Compare the percent recovery (P_s) for each parameter with the corresponding QC acceptance criteria found in Table 3. Only parameters that failed the test in Section 8.3 need to be compared with these criteria. If the recovery of any such parameter falls outside the designated range, the laboratory performance for that parameter is judged to be out of control, and the problem must be immediately identified and corrected. The analytical result for that parameter in the unspiked sample is suspect and may not be reported for regulatory compliance purposes.

8.5 As part of the QC program for the laboratory, method accuracy for wastewater samples must be assessed and records must be maintained. After the analysis of five spiked wastewater samples as in Section 8.3, calculate the average percent recovery (\bar{P}) and the standard deviation of the percent recovery (s_p). Express the accuracy assessment as a percent recovery interval from $\bar{P}-2s_p$ to $\bar{P}+2s_p$. If $\bar{P}=90\%$ and $s_p=10\%$, for example, the accuracy interval is expressed as 70–110%. Update the accuracy assessment for each parameter on a regular basis (e.g. after each five to ten new accuracy measurements).

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to assess the precision of the environmental measurements. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass

spectrometer must be used. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices⁸ should be followed, except that the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon tubing and other potential sources of contamination.

9.2 All samples must be iced or refrigerated at 4°C from the time of collection until extraction. PAHs are known to be light sensitive; therefore, samples, extracts, and standards should be stored in amber or foil-wrapped bottles in order to minimize photolytic decomposition. Fill the sample bottles and, if residual chlorine is present, add 80 mg of sodium thiosulfate per liter of sample and mix well. EPA Methods 330.4 and 330.5 may be used for measurement of residual chlorine.⁹ Field test kits are available for this purpose.

9.3 All samples must be extracted within 7 days of collection and completely analyzed within 40 days of extraction.²

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel.

10.2 Add 60 mL of methylene chloride to the sample bottle, seal, and shake 30 s to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 min. with periodic venting to release excess 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 volume 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, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D concentrator if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a solvent-rinsed drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.6 Add one or two clean boiling chips to the evaporative 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 hot water bath (60 to 65 °C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. 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 with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.7 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extract will be stored longer than two days, it should be transferred to a Teflon-sealed screw-cap vial and protected from light. If the sample extract requires no further cleanup, proceed with gas or liquid chromatographic analysis (Section 12 or 13). If the sample requires further cleanup, proceed to Section 11.

10.8 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. If particular circumstances demand the use of a cleanup procedure, the analyst may use the procedure below or any other appropriate procedure. However, the analyst first must demonstrate that the requirements of Section 8.2 can be met using the methods as revised to incorporate the cleanup procedure.

11.2 Before the silica gel cleanup technique can be utilized, the extract solvent must be exchanged to cyclohexane. Add 1 to 10 mL of the sample extract (in methylene

chloride) and a boiling chip to a clean K-D concentrator tube. Add 4 mL of cyclohexane and attach a two-ball micro-Snyder column. Prewet the column by adding 0.5 mL of methylene chloride to the top. Place the micro-K-D apparatus on a boiling (100 °C) water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete concentration in 5 to 10 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 the liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a minimum amount of cyclohexane. Adjust the extract volume to about 2 mL.

11.3 Silica gel column cleanup for PAHs:

11.3.1 Prepare a slurry of 10 g of activated silica gel in methylene chloride and place this into a 10-mm ID chromatographic column. Tap the column to settle the silica gel and elute the methylene chloride. Add 1 to 2 cm of anhydrous sodium sulfate to the top of the silica gel.

11.3.2 Preelute the column with 40 mL of pentane. The rate for all elutions should be about 2 mL/min. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, transfer the 2-mL cyclohexane sample extract onto the column using an additional 2 mL cyclohexane to complete the transfer. Just prior to exposure of the sodium sulfate layer to the air, add 25 mL of pentane and continue the elution of the column. Discard this pentane eluate.

11.3.3 Next, elute the column with 25 mL of methylene chloride/pentane (4+6)(V/V) into a 500-mL K-D flask equipped with a 10-mL concentrator tube. Concentrate the collected fraction to less than 10 mL as in Section 10.6. When the apparatus is cool, remove the Snyder column and rinse the flask and its lower joint with pentane. Proceed with HPLC or GC analysis.

12. High Performance Liquid Chromatography

12.1 To the extract in the concentrator tube, add 4 mL of acetonitrile and a new boiling chip, then attach a two-ball micro-Snyder column. Concentrate the solvent as in Section 10.6, except set the water bath at 95 to 100 °C. When the apparatus is cool, remove the micro-Snyder column and rinse its lower joint into the concentrator tube with about 0.2 mL of acetonitrile. Adjust the extract volume to 1.0 mL.

12.2 Table 1 summarizes the recommended operating conditions for the HPLC. Included in this table are retention times, capacity factors, and MDL that can be achieved under

these conditions. The UV detector is recommended for the determination of naphthalene, acenaphthylene, acenaphthene, and fluorene and the fluorescence detector is recommended for the remaining PAHs. Examples of the separations achieved by this HPLC column are shown in Figures 1 and 2. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.

12.3 Calibrate the system daily as described in Section 7.

12.4 If the internal standard calibration procedure is being used, the internal standard must be added to the sample extract and mixed thoroughly immediately before injection into the instrument.

12.5 Inject 5 to 25 μ L of the sample extract or standard into the HPLC using a high pressure syringe or a constant volume sample injection loop. Record the volume injected to the nearest 0.1 μ L, and the resulting peak size in area or peak height units. Re-equilibrate the HPLC column at the initial gradient conditions for at least 10 min between injections.

12.6 Identify the parameters in the sample by comparing the retention time of the peaks in the sample chromatogram with those of the peaks in standard chromatograms. The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.7 If the response for a peak exceeds the working range of the system, dilute the extract with acetonitrile and reanalyze.

12.8 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Gas Chromatography

13.1 The packed column GC procedure will not resolve certain isomeric pairs as indicated in Section 1.3 and Table 2. The liquid chromatographic procedure (Section 12) must be used for these parameters.

13.2 To achieve maximum sensitivity with this method, the extract must be concentrated to 1.0 mL. Add a clean boiling chip to the methylene chloride extract in the concentrator tube. Attach a two-ball micro-Snyder column. Prewet the micro-Snyder column by adding about 0.5 mL of methylene chloride to the top. Place the micro-K-D apparatus on a hot water bath (60 to 65 °C) so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 5 to 10 min. At the proper rate

of distillation the balls will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a minimum amount of methylene chloride. Adjust the final volume to 1.0 mL and stopper the concentrator tube.

13.3 Table 2 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are retention times that were obtained under these conditions. An example of the separations achieved by this column is shown in Figure 3. Other packed or capillary (open-tubular) columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.

13.4 Calibrate the gas chromatographic system daily as described in Section 7.

13.5 If the internal standard calibration procedure is being used, the internal standard must be added to the sample extract and mixed thoroughly immediately before injection into the gas chromatograph.

13.6 Inject 2 to 5 μ L of the sample extract or standard into the gas chromatograph using the solvent-flush technique.¹⁰ Smaller (1.0 μ L) volumes may be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 μ L, and the resulting peak size in area or peak height units.

13.7 Identify the parameters in the sample by comparing the retention times of the peaks in the sample chromatogram with those of the peaks in standard chromatograms. The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

13.8 If the response for a peak exceeds the working range of the system, dilute the extract and reanalyze.

13.9 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

14. Calculations

14.1 Determine the concentration of individual compounds in the sample.

14.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor determined in Section 7.2.2. The concentration in the sample can be calculated from Equation 2.

$$\text{Concentration } (\mu\text{g/L}) = \frac{(A)(V_t)}{(V_i)(V_s)}$$

Equation 2

where:

A=Amount of material injected (ng).

V_i=Volume of extract injected (μL).V_t=Volume of total extract (μL).V_s=Volume of water extracted (mL).

13.1.2 If the internal standard calibration procedure is used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 and Equation 3.

$$\text{Concentration } (\mu\text{g/L}) = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

Equation 3

where:

A_s=Response for the parameter to be measured.A_{is}=Response for the internal standard.I_s=Amount of internal standard added to each extract (μg).V_o=Volume of water extracted (L).

14.2 Report results in μg/L without correction for recovery data. All QC data obtained should be reported with the sample results.

15. Method Performance

15.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.¹ The MDL concentrations listed in Table 1 were obtained using reagent water.¹¹ Similar results were achieved using representative wastewaters. MDL for the GC approach were not determined. The MDL actually achieved in a given analysis will vary depending on instrument sensitivity and matrix effects.

15.2 This method has been tested for linearity of spike recovery from reagent water and has been demonstrated to be applicable over the concentration range from 8 × MDL to 800 × MDL¹¹ with the following exception: benzo(ghi)perylene recovery at 80 × and 800 × MDL were low (35% and 45%, respectively).

15.3 This method was tested by 16 laboratories using reagent water, drinking water, surface water, and three industrial wastewaters spiked at six concentrations over the range 0.1 to 425 μg/L.¹² Single operator precision, overall precision, and method accuracy were found to be directly related to the concentration of the parameter and essentially independent of the sample matrix.

Linear equations to describe these relationships are presented in Table 4.

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TABLE 1—HIGH PERFORMANCE LIQUID CHROMATOGRAPHY CONDITIONS AND METHOD DETECTION LIMITS

Parameter	Retention time (min)	Column capacity factor (k')	Method detection limit (µg/L) ^a
Naphthalene	16.6	12.2	1.8
Acenaphthylene	18.5	13.7	2.3
Acenaphthene	20.5	15.2	1.8
Fluorene	21.2	15.8	0.21
Phenanthrene	22.1	16.6	0.64
Anthracene	23.4	17.6	0.66
Fluoranthene	24.5	18.5	0.21
Pyrene	25.4	19.1	0.27
Benzo(a)anthracene	28.5	21.6	0.013
Chrysene	29.3	22.2	0.15
Benzo(b)fluoranthene	31.6	24.0	0.018
Benzo(k)fluoranthene	32.9	25.1	0.017
Benzo(a)pyrene	33.9	25.9	0.023
Dibenzo(a,h)anthracene	35.7	27.4	0.030
Benzo(ghi)perylene	36.3	27.8	0.076
Indeno(1,2,3-cd)pyrene	37.4	28.7	0.043

AAAHPLC column conditions: Reverse phase HC-ODS Sil-X, 5 micron particle size, in a 25 cm × 2.6 mm ID stainless steel column. Isocratic elution for 5 min. using acetonitrile/water (4+6), then linear gradient elution to 100% acetonitrile over 25 min. at 0.5 mL/min flow rate. If columns having other internal diameters are used, the flow rate should be adjusted to maintain a linear velocity of 2 mm/sec.

^aThe MDL for naphthalene, acenaphthylene, acenaphthene, and fluorene were determined using a UV detector. All others were determined using a fluorescence detector.

TABLE 2—GAS CHROMATOGRAPHIC CONDITIONS AND RETENTION TIMES

Parameter	Retention time (min)
Naphthalene	4.5
Acenaphthylene	10.4
Acenaphthene	10.8
Fluorene	12.6
Phenanthrene	15.9
Anthracene	15.9
Fluoranthene	19.8
Pyrene	20.6
Benzo(a)anthracene	24.7
Chrysene	24.7
Benzo(b)fluoranthene	28.0

TABLE 2—GAS CHROMATOGRAPHIC CONDITIONS AND RETENTION TIMES—Continued

Parameter	Retention time (min)
Benzo(k)fluoranthene	28.0
Benzo(a)pyrene	29.4
Dibenzo(a,h)anthracene	36.2
Indeno(1,2,3-cd)pyrene	36.2
Benzo(ghi)perylene	38.6

GC Column conditions: Chromosorb W-AW-DCMS (100/120 mesh) coated with 3% OV-17 packed in a 1.8 x 2 mm ID glass column with nitrogen carrier gas at 40 mL/min. flow rate. Column temperature was held at 100°C for 4 min., then programmed at 8°C/min. to a final hold at 280°C.

TABLE 3—QC ACCEPTANCE CRITERIA—METHOD 610

Parameter	Test conc. (µg/L)	Limit for s (µg/L)	Range for \bar{X} (µg/L)	Range for P, P _s (%)
Acenaphthene	100	40.3	D-105.7	D-124
Acenaphthylene	100	45.1	22.1–112.1	D-139
Anthracene	100	28.7	11.2–112.3	D-126
Benzo(a)anthracene	10	4.0	3.1–11.6	12–135
Benzo(a)pyrene	10	4.0	0.2–11.0	D-128
Benzo(b)fluor-anthene	10	3.1	1.8–13.8	6–150
Benzo(ghi)perylene	10	2.3	D-10.7	D-116
Benzo(k)fluor-anthene	5	2.5	D-7.0	D-159
Chrysene	10	4.2	D-17.5	D-199
Dibenzo(a,h)an-thracene	10	2.0	0.3–10.0	D-110
Fluoranthene	10	3.0	2.7–11.1	14–123
Fluorene	100	43.0	D-119	D-142
Indeno(1,2,3-cd)pyrene	10	3.0	1.2–10.0	D-116
Naphthalene	100	40.7	21.5–100.0	D-122
Phenanthrene	100	37.7	8.4–133.7	D-155
Pyrene	10	3.4	1.4–12.1	D-140

s=Standard deviation of four recovery measurements, in µg/L (Section 8.2.4).

\bar{X} =Average recovery for four recovery measurements, in µg/L (Section 8.2.4).

P, P_s=Percent recovery measured (Section 8.3.2, Section 8.4.2).

D=Detected; result must be greater than zero.

NOTE: These criteria are based directly upon the method performance data in Table 4. Where necessary, the limits for recovery have been broadened to assure applicability of the limits to concentrations below those used to develop Table 4.

TABLE 4—METHOD ACCURACY AND PRECISION AS FUNCTIONS OF CONCENTRATION—METHOD 610

Parameter	Accuracy, as recovery, X' ($\mu\text{g/L}$)	Single analyst precision, s_r' ($\mu\text{g/L}$)	Overall precision, S' ($\mu\text{g/L}$)
Acenaphthene	$0.52C + 0.54$	$0.39\bar{X} + 0.76$	$0.53\bar{X} + 1.32$
Acenaphthylene	$0.69C - 1.89$	$0.36\bar{X} + 0.29$	$0.42\bar{X} + 0.52$
Anthracene	$0.63C - 1.26$	$0.23\bar{X} + 1.16$	$0.41\bar{X} + 0.45$
Benzo(a)anthracene	$0.73C + 0.05$	$0.28\bar{X} + 0.04$	$0.34\bar{X} + 0.02$
Benzo(a)pyrene	$0.56C + 0.01$	$0.38\bar{X} - 0.01$	$0.53\bar{X} - 0.01$
Benzo(b)fluoranthene	$0.78C + 0.01$	$0.21\bar{X} + 0.01$	$0.38\bar{X} - 0.00$
Benzo(ghi)perylene	$0.44C + 0.30$	$0.25\bar{X} + 0.04$	$0.58\bar{X} + 0.10$
Benzo(k)fluoranthene	$0.59C + 0.00$	$0.44\bar{X} - 0.00$	$0.69\bar{X} + 0.01$
Chrysene	$0.77C - 0.18$	$0.32\bar{X} - 0.18$	$0.66\bar{X} - 0.22$
Dibenzo(a,h)anthracene	$0.41C + 0.11$	$0.24\bar{X} + 0.02$	$0.45\bar{X} + 0.03$
Fluoranthene	$0.68C + 0.07$	$0.22\bar{X} + 0.06$	$0.32\bar{X} + 0.03$
Fluorene	$0.56C - 0.52$	$0.44\bar{X} - 1.12$	$0.63\bar{X} - 0.65$
Indeno(1,2,3-cd)pyrene	$0.54C + 0.06$	$0.29\bar{X} + 0.02$	$0.42\bar{X} + 0.01$
Naphthalene	$0.57C - 0.70$	$0.39\bar{X} - 0.18$	$0.41\bar{X} + 0.74$
Phenanthrene	$0.72C - 0.95$	$0.29\bar{X} + 0.05$	$0.47\bar{X} - 0.25$
Pyrene	$0.69C - 0.12$	$0.25\bar{X} + 0.14$	$0.42\bar{X} - 0.00$

X' =Expected recovery for one or more measurements of a sample containing a concentration of C , in $\mu\text{g/L}$.

s_r' =Expected single analyst standard deviation of measurements at an average concentration found of \bar{X} , in $\mu\text{g/L}$.

S' =Expected interlaboratory standard deviation of measurements at an average concentration found of \bar{X} , in $\mu\text{g/L}$.

C =True value for the concentration, in $\mu\text{g/L}$.

\bar{X} =Average recovery found for measurements of samples containing a concentration of C , in $\mu\text{g/L}$.

COLUMN: HC-ODS SIL-X
MOBILE PHASE: 40% TO 100% ACETONITRILE IN WATER
DETECTOR: ULTRAVIOLET AT 254nm

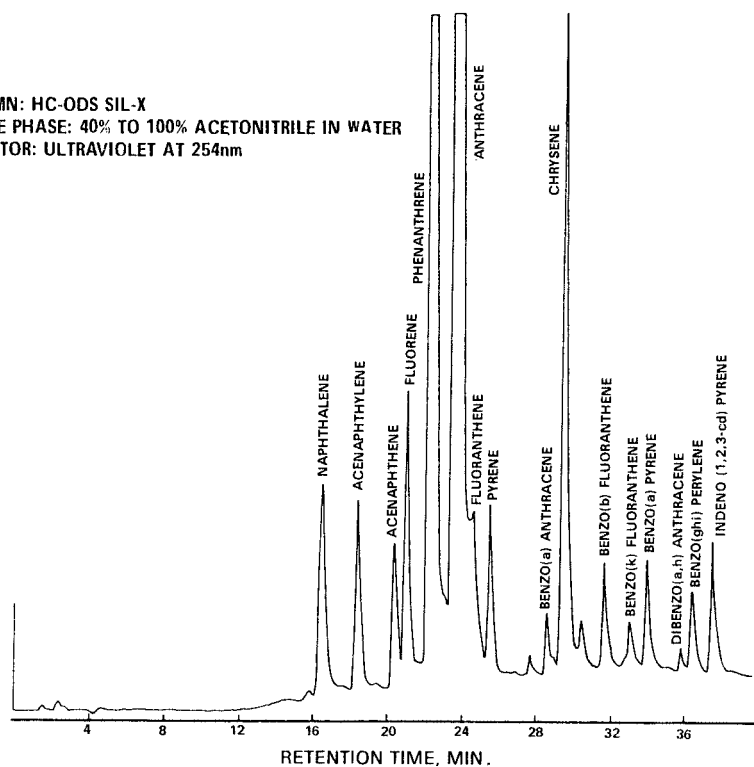


Figure 1. Liquid chromatogram of polynuclear aromatic hydrocarbons.

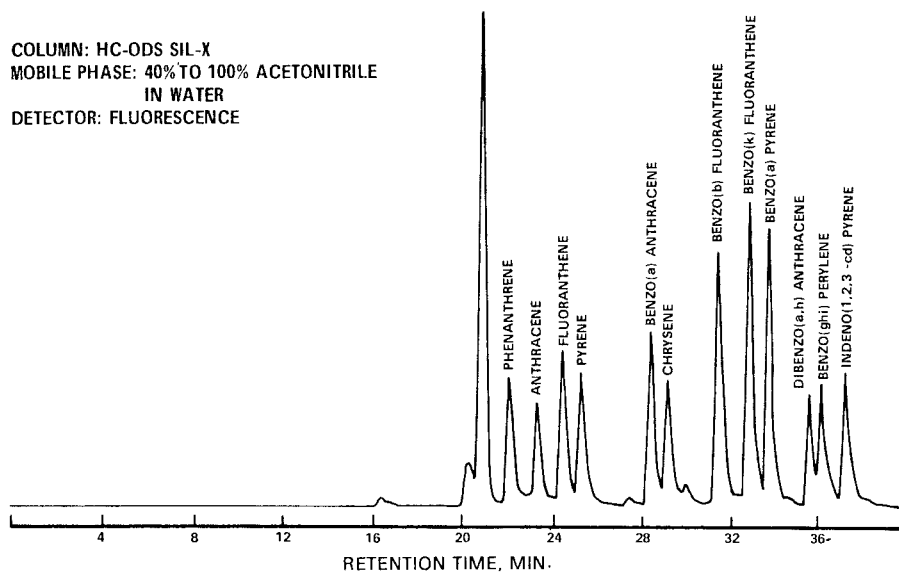


Figure 2. Liquid chromatogram of polynuclear aromatic hydrocarbons.

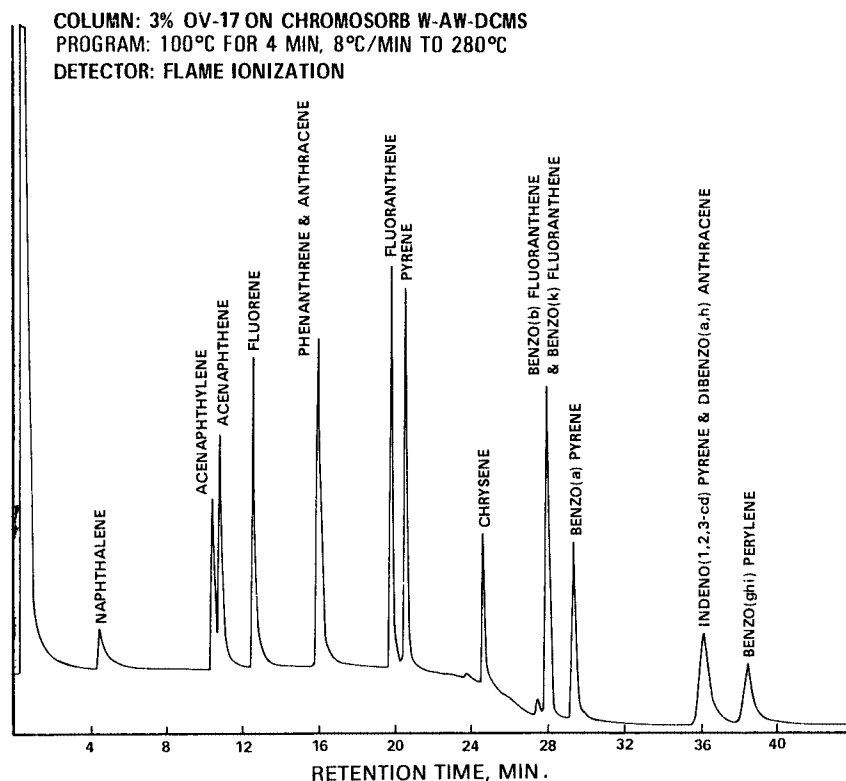


Figure 3. Gas chromatogram of polynuclear aromatic hydrocarbons.

METHOD 611—HALOETHERS

1. Scope and Application

1.1 This method covers the determination of certain haloethers. The following parameters can be determined by this method:

Parameter	STORET No.	CAS No.
Bis(2-chloroethyl) ether	34273	111-44-4
Bis(2-chloroethoxy) methane	34278	111-91-1
Bis(2-chloroisopropyl) ether	34283	108-60-1
4-Bromophenyl phenyl ether	34636	101-55-3
4-Chlorophenyl phenyl ether	34641	7005-72-3

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second

gas chromatographic column that can be used to confirm measurements made with the primary column. Method 625 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for all of the parameters listed above, using the extract produced by this method.

1.3 The method detection limit (MDL, defined in Section 14.1)¹ for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 The sample extraction and concentration steps in this method are essentially the same as in Methods 606, 608, 609, and 612. Thus, a single sample may be extracted to measure the parameters included in the scope of each of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, to apply appropriate cleanup procedures. The analyst is allowed the latitude, under Section 12, to select