METHOD 550

DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN DRINKING WATER BY LIQUID-LIQUID EXTRACTION AND HPLC WITH COUPLED ULTRAVIOLET AND FLUORESCENCE DETECTION

July 1990

J.W. Hodgeson

W.J. Bashe (Technology Applications Inc.)
T.V. Baker (Technology Applications Inc.)

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

METHOD 550

DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN DRINKING WATER BY LIQUID-LIQUID EXTRACTION AND HPLC WITH COUPLED ULTRAVIOLET AND FLUORESCENCE DETECTION

1.0 SCOPE AND APPLICATION

1.1 This method describes a procedure for determination of certain polycyclic aromatic hydrocarbons (PAH) in drinking water sources and finished drinking water. The following analytes can be determined by this method:

Analyte	Chemical Abstract Services Registry Number
Acenaphthene	83-32-9
Acenaphthylene	208-96-8
Anthracene	120-12-7
Benzo(a)anthracene	56-55-3
Benzo(a)pyrene	50-32-8
Benzo(b)fluoranthene	205-99-2
Benzo(g,h,i)perylene	191-24-2
Benzo(k)fluoranthene	207-08-9
Chrysene	218-01-9
Dibenzo(a,h)anthracene	53-70-3
Fluoranthene	206-44-0
Fluorene	86-73-7
Indeno(1,2,3-cd)pyrene	193-39-05
Naphthalene	91-20-3
Phenanthrene	85-01-8
Pyrene	129-00-0

1.2 This is a high performance liquid chromatography (HPLC) method applicable to the determination of the compounds listed above. When this method is used to analyze unfamiliar samples, compound identifications should be supported by at least one qualitative technique. Method 525 provides gas chromatographic / mass spectrometer (GC / MS) conditions appropriate for the qualitative and quantitative confirmation of results for the above analytes, using the extract produced by this method.

1.3 The method detection limit[1] (MDL, defined in Section 13.0) for each analyte is listed in Table 1. The MDL for a specific matrix may differ from those listed, depending on the nature of interferences in the sample matrix.

2.0 SUMMARY OF METHOD

2.1 A measured volume of sample, approximately 1 L, is serially extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and concentrated to a volume of 1 mL. A 3.0 mL portion of acetonitrile is added to the extract and concentrated to a final volume of 0.5 mL. The extract analytes are then separated by HPLC. Ultraviolet adsorption (UV) and fluorescence detectors are used with HPLC to quantitatively measure the PAHs.

3.0 DEFINITIONS

3.1 Internal Standard -- A pure analyte(s) added to a solution in known amounts(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.

3.2 Surrogate Analyte -- A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction and is measured with the same procedures used to measure other sample components. The purpose of a surrogate analyte is to monitor method performance with each sample.

3.3 Laboratory Duplicates (LD1 and LD2) -- Two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4 Field Duplicates (FD1 and FD2) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.5 Laboratory Reagent Blank (LRB) -- An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6 Field Reagent Blank (FRB) -- Reagent water placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.7 Laboratory Fortified Blank (LFB) -- An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the method is in control, and whether the laboratory is capable of making accurate and precise measurements at the required method detection limit.

3.8 Laboratory Fortified Matrix Sample (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.9 Stock Standard Solution -- A concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.

3.10 Primary Dilution Standard Solution -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.11 Calibration Standard (CAL) -- A solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration. One of these standards, usually of middle concentration, can be used as the calibration check standard.

3.12 Quality Control Sample (QCS) -- A sample matrix containing method analytes or a solution of method analytes in a water miscible solvent which is used to fortify reagent water or environmental samples. The QCS is obtained from a source external to the laboratory, and is used to check laboratory performance with externally prepared test materials.

4.0 INTERFERENCES

4.1 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 10.2.

4.1.1 Glassware must be scrupulously cleaned[2]. 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

15-30 minutes. 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 glassware 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.

4.1.2 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.

4.2 Matrix interferences may be caused by contaminants that are coextracted 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 suggested in Section 11.1 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDLs listed in Table 1.

4.3 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 covered by this method, other PAHs may interfere.

4.4 Matrix interferences have been found for benzo(a)anthracene, benzo(a)pyrene and benzo(g,h,i)perylene. The nature of the interferences has not been fully assessed.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has 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 for the information of the analyst.[3-5]

5.2 The following analytes 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

NOISH / MESA approved toxic gas respirator should be worn when the analyst handles high concentrations of these toxic compounds.

6. APPARATUS AND EQUIPMENT (All specifications are suggested. Catalog numbers are included for illustration only).

6.1 SAMPLING EQUIPMENT, for discrete or composite sampling.

6.1.1 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.

6.2 GLASSWARE

6.2.1 Separatory funnels - 2 L, with Teflon stopcock, 125 mL, with Teflon stopcock.

6.2.2 Drying column - Chromatographic column, approximately 250 mm long x 19 mm ID, with coarse frit filter disc.

6.2.3 Concentrator tube, Kuderna-Danish - 10 mL, graduated Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

6.2.4 Evaporative flask, Kuderna-Danish - 500 mL Attach to concentrator tube with springs.

6.2.5 Synder column, Kuderna-Danish - Three-ball macro

6.2.6 Vials - 10 to 15 mL, amberglass, with Teflon-lined screw cap.

6.2.7 Boiling chips - carborundum, #12 granules Heat at 400°C for 30 minutes prior to use. Cool and store in dessicator.

6.3 Evaporation Equipment

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

6.3.2 Nitrogen evaporation manifold -- Twelve-port (Organomation, N-EVAP, Model III or Equivalent.)

6.4 Balance -- Analytical, capable of accurately weighing 0.0001g.

6.5 High Performance Liquid Chromatograph -- An analytical system complete with liquid pumping system, column supplies, temperature controlled column oven, injector, detectors, and a compatible strip-chart recorder. A data system is highly recommended for measuring peak areas and retention times.

6.5.1 Gradient pumping system -- Constant flow.

6.5.2 Analytical reverse-phase column -- Supelco LC-PAH, 5 micron particle diameter, in a 25 cm x 4.6 mm ID stainless steel column or equivalent. This column was used to develop the method performance statements in Section 13.0.

6.5.3 Detectors -- Fluorescence and UV detectors. The fluorescence detector is used for excitation at 280 nm and emission greater than 389 nm cut-off (Schoeffel FS970 or equivalent). Fluorometer 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 (Waters Assoc. Model 450) and should be coupled to the fluorescence detector. These detectors were used to develop the method performance statements in Section 13.0.

7.0 REAGENTS AND CONSUMABLE MATERIALS

7.1 Reagent Water -- Reagent water is defined as a water in which an interferant is not observed at the MDL of the analytes of interest. Prepare reagent water by passing tap water through a filter bed containing about 0.5 kg of activated carbon, or by using a water purification system. Store in clean bottles with Teflon lined screw caps.

7.2 Sodium Thiosulfate -- ACS, granular.

7.3 Methylene Chloride -- Pesticide quality or equivalent.

7.4 Acetonitrile -- HPLC quality, distilled in glass.

7.5 Sodium Sulfate -- ACS, granular, anhydrous. Purify by heating at 400°C for four hours in a shallow tray.

7.6 Stock Standard Solutions (1.00 µg/µL) -- Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

7.6.1 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 at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Certified, commercially prepared stock standards can be used at any concentration.

7.6.2 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.

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

7.7 Laboratory Control Sample Concentrate -- See Section 10.3.1.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Grab samples must be collected in glass containers. Conventional sampling practices should be followed, except that the bottle must not be pre-rinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program.

8.2 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 100 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. Field test kits are available for this purpose. Adjust the pH of the sample to <2 with 6N HCl to inhibit biological activity.

8.3 All samples must be extracted within seven days of collection and completely analyzed within 30 days of extraction.[6] Polycyclic aromatic hydrocarbons are known to be light sensitive. Therefore sample extracts and standards should be stored in amber vials in the dark in a refrigerator or freezer in order to minimize photolytic decomposition.

9.0 CALIBRATION

9.1 Use liquid chromatographic operating conditions given in Table 1. The chromatographic system can be calibrated using the external standard technique (Section 9.2) or the internal standard technique (Section 9.3.)

NOTE: Calibration standard solutions must be prepared such that no unresolved analytes are mixed together. Special care must be taken so that analyte concentrations in standard solutions are not so high as to cause peak fusing or overlap.

9.2 External Standard Calibration Procedure

9.2.1 Prepare calibration standards at a minimum of three (recommend five) concentration levels for each analyte by adding volumes of one or more primary dilution standard solutions (Section 3.10) 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 bracket the expected range of concentrations found in real samples or should define the working range of the detector.

9.2.2 Using injections of 5-100 µL, analyze each calibration standard according to Section 11.3. Tabulate peak area or height 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.

9.3 Internal Standard (IS) 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.

9.3.1 Prepare calibration standards at a minimum of three (recommend five) concentration levels for each analyte of interest by adding volumes of one or more primary dilution standard solutions (Section 3.10) to a volumetric flask. To each calibration standard, add a known 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 bracket the analyte concentrations found in the sample concentrates or should define the working range of the detector.

9.3.2 Using injections of 5-100 µL analyze each calibration standard according to Section 11.3. Tabulate peak height or area responses against concentration for each compound and internal standard. Calculate response factor (RF) for each compound using Equation 1.

Equation 1

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

where: A_s = Response for the analyte to be measured.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard (µg/L).
C_s = Concentration of the analyte to be measured (µg/L).

If RF value over the working range is constant (<10% RSD), the RF can be assured 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. C_s/C_{is}.

9.4 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 analyte varies from the predicted response by more than ±20%, the test must be repeated using fresh calibration standard. If the fresh calibration standard also deviates by more ±20%, a new calibration curve must be prepared for that compound.

9.4.1 Daily calibration requirements using the external standard calibration procedure are a minimum of two calibration check standards, one at the beginning and one at the end of the analysis day. These check standards should be at two different concentration levels to verify the calibration curve. For extended periods of analysis (greater than eight hours), it is strongly recommended that check standards be interspersed with samples at regular intervals during the course of the analysis.

9.4.2 Minimum daily calibration requirements using the internal standard calibration procedure consist of initial analyses of a calibration check standard followed by verification of the internal standard response of each sample applying criterion described in Section 10.4.

9.5 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 reagents.

10.0 QUALITY CONTROL

10.1 Each laboratory that uses this method is required to operate a formal quality control (QC) program. The minimum QC requirements are initial demonstration of laboratory capability, analysis of laboratory reagent blanks, laboratory fortified blanks, laboratory fortified matrix samples and QC

samples. The laboratory must maintain records to document the quality of the data generated. Additional quality control practices are recommended.

10.2 Laboratory Reagent Blanks (LRB) -- Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is analyzed or reagents are changed, a LRB must be analyzed. For this method, the LRB is filtered reagent water. If within the retention time window (Section 11.4.2) of an analyte of interest, the LRB produces a peak that interferes with analyte determination, determine the source of contamination and eliminate the interference before processing samples.

10.3 Initial Demonstration of Capability

10.3.1 Select a representative spike concentration (about 10 times MDL) for each analyte. Prepare a laboratory control sample concentrate (in acetonitrile) from the stock standard solution containing each analyte at 1000 times the selected concentration. Using a pipet, add 1.00 mL of the concentrate to each of at least four 1 L aliquots of reagent water and analyze each aliquot according to procedures beginning in Section 11.2.

10.3.2 For each analyte, the recovery value must for at least three out of four consecutively analyzed samples fall in the range of R ±30% (or within R ±3 S_r, if broader) using the values for R and $_rS$ for reagent water in Table 2 (Fortification Level 1). The relative standard deviation of the mean recovery measured in Section 10.3.1 should be ≤30% or $3S_r$ (whichever is greater), using the values for S_r (Level 1) in Table 2. For those compounds that meet the acceptance criteria, performance is acceptable and sample analysis may begin. For those compounds that fail these criteria, initial demonstration of capability must be repeated.

10.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples by a new, unfamiliar method prior to evidencing a basic level of skill at performing the technique. It is expected that as laboratory personnel gain experience with this method the quality of the data will improve beyond the requirements stated in Section 10.3.2.

10.3.4 The analyst is permitted to modify HPLC columns, HPLC conditions, or detectors to improve separations or lower analytical costs. Each time such method modifications are made, the analyst must repeat the procedures in Section 10.3.

10.4 Assessing the Internal Standard -- When using the IS calibration procedure, the analyst is expected to monitor the IS response (peak area or peak height) of all samples during each analysis day. The IS response for any sample

chromatogram should not deviate from the daily mean IS response by more than 30%.

10.4.1 If a deviation of >30% is encountered for a sample, re-inject the extract.

10.4.1.1 If acceptable IS response is achieved for the re-injected extract, then report the results for that sample.

10.4.1.2 If a deviation of >30% is obtained for the re-injected extract, analysis of the sample should be repeated beginning with Section 11.2, provided the sample is still available. Otherwise, report results obtained from the re-injected extract, but annotate as suspect.

10.4.2 If consecutive samples fail the IS response acceptance criterion, immediately analyze a calibration check standard.

10.4.2.1 If the check standard provides a response factor (RF) within 20% of the predicted value, then follow procedures itemized in Section 10.4.1 for each sample failing the IS response criterion.

10.4.2.2. If the check standard provides a response factor (RF) which deviates more than 20% of the predicted value, then the analyst must recalibrate, as specified in Section 9.3.

10.5 Laboratory Fortified Blank (LFB)

10.5.1 The laboratory must analyze at least one LFB per sample set (all samples prepared for analysis within a 24-hour period). The fortified concentration of each analyte in the LFB should be at least 10 times the MDL. Calculate accuracy as percent recovery (R). If the recovery of any analyte falls outside the control limits (See Section 10.5.2), that analyte is judged out of control, and the source of the problem must be identified and resolved before continuing analyses.

10.5.2 Until sufficient LFB data become available, usually a minimum of results from 20-30 analyses, the laboratory must assess its performance against the control limits described in Section 10.3.2. When sufficient laboratory performance data becomes available, develop control limits from the mean percent recovery (R) and standard deviation (S) of the percent recovery. These data are used to establish upper and lower control limits as follows:

UPPER CONTROL LIMIT = R + 3S
LOWER CONTROL LIMIT = R - 3S

After each group of 5-10 new recovery measurements, control limits should be recalculated using only the most recent 20-30 data points.

10.6 Laboratory Fortified Matrix Sample

10.6.1 The laboratory must add a known fortified concentration to a minimum of 10% of the routine samples or one fortified sample per set, whichever is greater. The fortified concentration should not be less than the background concentration of the original sample. Ideally, the fortified concentration should be the same as that used for the LFB (Section 10.5). Over time, samples from all routine sample sources should be fortified.

10.6.2 Calculate the percent recovery (R) for each analyte, corrected for background concentrations measured in the original sample, and compare these values to the control limits established in Section 10.5.2 from the analyses of LFBs.

10.6.3 If the recovery of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Section 10.5), the recovery problem encountered with the dosed sample is judged to be matrix related, not system related. The result for that analyte in the original sample must be labelled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

10.7 Quality Control Samples (QCS) -- Each quarter the laboratory should analyze one or more QCS (if available). If criteria provided with the QCS are not met, corrective action should be taken and documented.

10.8 The laboratory may adopt additional quality control 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. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation and storage.

11.0 PROCEDURE

11.1 Sample Cleanup -- Cleanup procedures may not be necessary for a relatively clean sample matrix. If particular circumstances demand the use of a cleanup procedure, the analyst first must demonstrate that the requirements of Section 10.5 can be met using the method as revised to incorporate the cleanup procedure. EPA Method 610 describes one possible cleanup procedure for this analyte list.

11.2 Sample Extraction -- Liquid-Liquid Extraction

11.2.1 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.

11.2.2 Add 60 mL of methylene chloride to the sample bottle, seal, and shake for 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layers, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250 mL Erlenmeyer flask.

11.2.3 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.

11.2.4 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 10.6 are met.

11.2.5 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 20-30 mL of methylene chloride to complete the quantitative transfer.

11.2.6 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 (60-65°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 15-20 minutes. 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 apparatus volume of liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

11.2.7 Remove the Synder column and rinse the flask and its lower joint into the concentrator tube with 1-2 mL of methylene chloride. A 5 mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated (4°C) 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.

11.2.8 Evaporate the extract with a gentle stream of N_2 flow to a volume of 1.0 mL. Add 3.0 mL of acetonitrile (MeCN) and concentrate with the N_2 flow to a final volume of 0.5 mL. 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 liquid chromatographic analysis (Section 11.3).

11.2.9 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.

11.3 Sample Analysis

11.3.1 Table 1 summarizes the recommended operating conditions for the HPLC. Included in this table are retention times and MDLs that can be achieved under these conditions. The UV detector is recommended for the determination of naphthalene, acenaphthylene, acenaphthene and fluorene. The fluorescence detector is recommended for the remaining PAHs. An example for the separation achieved by this HPLC column is shown in Figure 1. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of Section 10.5 are met.

11.3.2 Calibrate the system daily as described in Section 9.0.

11.3.3 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.

11.3.4 Inject 5-100 µL 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 µL, 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 minutes between injections.

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

11.4 Identification of Analytes

11.4.1 Identify a sample component by comparison of its retention time to the retention time in reference chromatogram. If the retention time of an unknown compound corresponds, within limits, to the retention time of a standard compound, then identification is considered positive.

11.4.2 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 can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.4.3 Identification requires expert judgement when sample components are not resolved chromatographically, that is, when GC peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima). Any time doubt exists over the identification of a peak in a chromatogram, appropriate confirmatory techniques need to be employed such as use of an alternative detector which operates on a chemical/physical principle different from that originally used, e.g., mass spectrometry, or the use of a second chromatography column.

12.0 CALCULATIONS

12.1 Determine the concentration of individual compounds in the sample as follows.

12.1.1 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 9.2.2. The concentration in the sample can be calculated from Equation 2.

Equation 2

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

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 extraction (mL).

12.1.2 If the internal standard calibration procedure is used, calculate the concentration in the sample using the response factor (RF) determined in Section 9.3.2 and Equation 3.

Equation 3

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

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).

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

13.0 METHOD PERFORMANCE

13.1 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. The MDL is equal to the level calculated by multiplying the standard deviation of N replicate measurements times the students' t test critical value for a 99% confidence level at N-1 degrees of freedom.

13.2 In a single laboratory, analyte recoveries from reagent water were determined at two concentration levels. Results were used to determine analyte MDLs and demonstrate method range. Analytes were divided into three spiking sets: compounds measured by UV detection (UV) and two groups of compounds measured by fluorescence detection (FD-A and FD-B), and analyzed separately. MDL values are given in Table 1. Precision and accuracy data obtained for the two concentration levels in reagent water are presented in Table 2.

13.3 In a single laboratory, analyte recoveries from dechlorinated tap water were determined at one concentration level. Results were used to demonstrate method performance capabilities for a finished drinking water matrix. As with Section 13.2, analytes were grouped into three spiking sets (UV, FD-A and FD-B). Precision and accuracy results for the dechlorinated tap water are shown in Table 3.

14.0 REFERENCES

1. Glaser, J.A., Foerst, D.L., McKee, G.D., Qwave S.A., and Budde, W.L. "Trace Analysis of Wastewaters", Environ. Sci. Technol. 15, 1426, 1981.

2. ASTM Annual Book of Standards, Part 31, D3694. "Standard Practices for Preparation of Sample Containers and for Preservation of Organic Constituents", American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.

3. "Carcinogens - Working with Carcinogens", Department of Health, Education and Welfare. Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August 1977.

4. "OSHA Safety and Health Standards, General Industry", (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised 1976).

5. "Safety in Academic Chemistry Laboratories", American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

6. "Determination of Polynuclear Aromatic Hydrocarbons in Industrial and Municipal Wastewaters", EPA-600/4-82-025, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, September 1982.

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

Analyte	Sample Set	Retention Time (min)	Method Detection Limit (µg/L)[a]	Method Detection Limit Fortification Level (µg/L)
Naphthalene	UV	12.5	3.3	10.0
Acenaphthylene	UV	13.8	2.3	10.0
Acenaphthene	UV	15.4	3.0	10.0
Fluorene	UV	15.6	0.25	1.00
Phenanthrene	FD-B	16.8	0.162	0.500
Anthracene	FD-A	17.6	0.079	0.625
Fluoranthene	FD-B	18.7	0.026	0.025
Pyrene	FD-A	19.4	0.126	0.625
Benzo(a)anthracene	FD-B	21.9	0.002	0.010
Chrysene	FD-A	22.3	0.063	0.625
Benzo(b)fluoranthene	FD-B	24.2	0.003	0.010
Benzo(k)fluoranthene	FD-A	25.0	0.002	0.0125
Benzo(a)pyrene	FD-B	26.0	0.029	0.050
Dibenzo(a,h)anthracene	FD-B	27.1	0.019	0.125
Benzo(g,h,i)perylene	FD-B	27.8	0.014	0.050
Indeno(1,2,3-cd)pyrene	FD-A	28.3	0.011	0.125

HPLC column conditions: Reverse-phase LC-PAH, 5 micron particle size, in a 25 cm x 4.6 mm ID stainless steel column. Isocratic elution for two minutes using acetonitrile/water (3.5:6.5), then linear gradient elution to 100% acetonitrile over 22 minutes at 2.0 mL/min. flow rate.

[a]The MDL for naphthalene, acenaphthylene, acenaphthene, and fluorene were determined using a UV detector. All others were determined using a fluorescence detector.

TABLE 2. SINGLE-LABORATORY ACCURACY AND PRECISION FROM SEVEN REPLICATE ANALYSES OF FORTIFIED REAGENT WATER

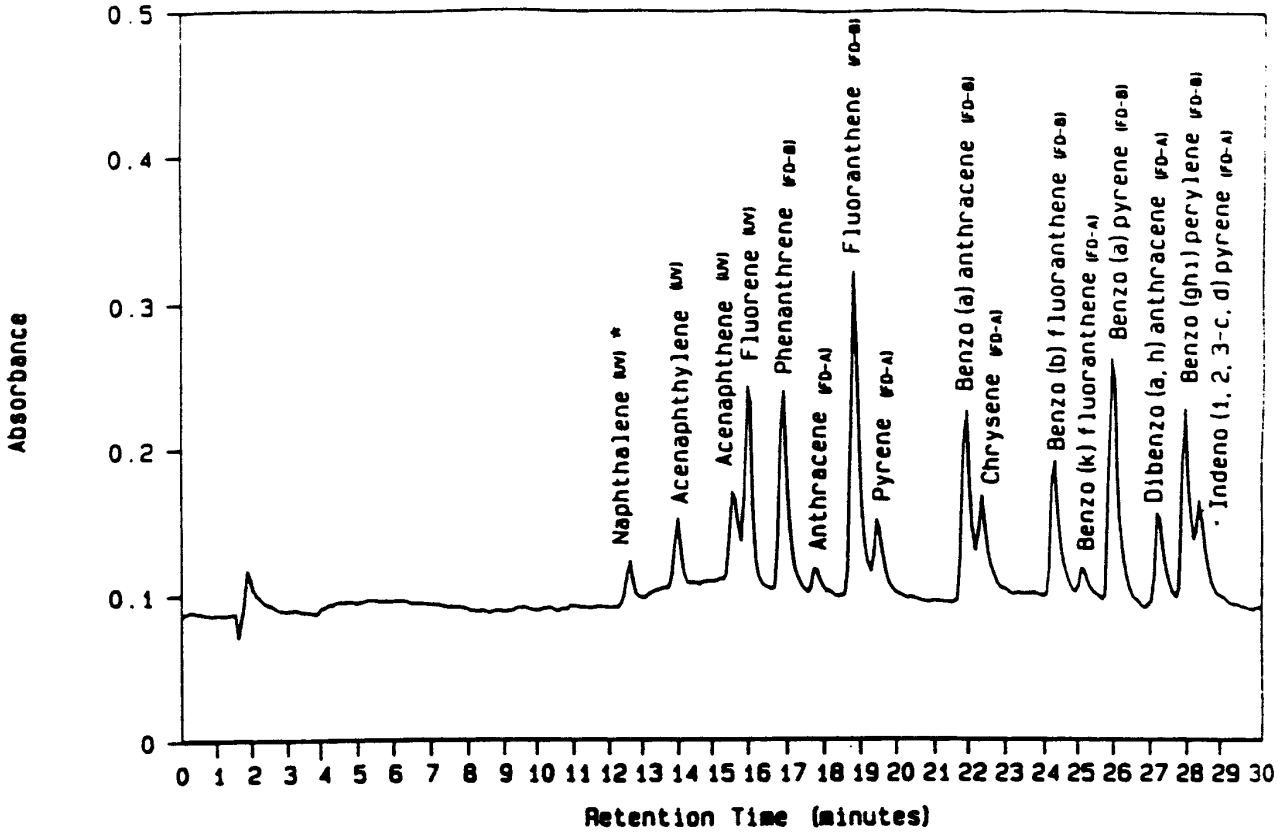
Analyte	Concentration Level 1 (µg/L)	R[a]	S_r[b]	Concentration Level 2[c] (µg/L)	R	S_r
Naphthalene	10.0	96.0	10.5	2.0	83.3	17.4
Acenaphthylene	10.0	95.5	7.0	2.0	98.5	15.2
Acenaphthene	10.0	94.5	9.5	2.0	47.3	6.2
Fluorene	1.0	91.0	8.0	0.2	92.0	7.8
Phenanthrene	0.5	72.5	10.3	0.1	69.5	17.0
Anthracene	0.625	89.6	4.0	0.125	74.4	13.2
Fluoranthene	0.025	113	33.2	0.005	140	50.0
Pyrene	0.625	93.6	6.4	0.125	82.4	9.6
Benzo(a)anthracene	0.01	99.0	10.5	0.002	50.0	10.0
Chrysene	0.625	94.4	3.2	0.125	94.0	11.6
Benzo(b)fluoranthene	0.01	99.0	10.5	0.002	50.0	25.0
Benzo(k)fluoranthene	0.0125	77.6	6.0	0.0025	100	40.0
Benzo(a)pyrene	0.05	85.7	18.3	0.01	41.7	16.7
Dibenzo(a,h)anthracene	0.125	81.6	4.8	0.025	78.0	12.0
Benzo(g,h,i)perylene	0.05	108	9.0	0.01	50.0	10.0
Indeno(1,2,3-cd)pyrene	0.125	72.4	2.8	0.025	66.0	8.0

[a]R = Mean Recovery, %.
[b]S_r = Relative Standard Deviation of R, %.
[c]Spike Level 2 = Concentration for analytes which yield a signal-to-noise ratio of approximately 10 in the extract (25 µL injection).

TABLE 3. SINGLE-LABORATORY ACCURACY AND PRECISION FROM NINE REPLICATE ANALYSES OF FORTIFIED TAP WATER[a]

Analyte	Fortified Concentration Level (µg/L)	Relative Accuracy (Recovery) %	Relative Standard Deviation %
Naphthalene	10.0	76.0	5.4
Acenaphthylene	10.0	71.4	11.1
Acenaphthene	10.0	76.6	9.9
Fluorene	1.0	89.4	5.5
Phenanthrene	0.5	77.4	5.6
Anthracene	0.625	97.0	6.3
Fluoranthene	0.025	103.0	8.3
Pyrene	0.625	86.0	10.1
Benzo(a)anthracene	0.01	91.3	10.5
Chrysene	0.625	91.1	10.9
Benzo(b)fluoranthene	0.006	74.7	4.5
Benzo(k)fluoranthene	0.0125	101.0	12.0
Benzo(a)pyrene	0.05	87.0	5.9
Dibenzo(a,h)anthracene	0.125	94.2	10.1
Benzo(g,h,i)perylene	0.05	86.0	9.5
Indeno(1,2,3-cd)pyrene	0.125	100.0	12.3

[a]Tap water was dechlorinated with sodium thiosulfate, according to the method (100 mg/L), upon collection prior to spiking with analytes.



*(UV), (FD-A)and (FD-B) indicate analysis sets. See Section 15

Figure 1. PAH, HPLC chromatogram using UV detection. Chromatographic conditions are as stated in Table 1.