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Method for the Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Sediment

Standard Operating Procedure of the
TRACE ORGANIC ANALYTICAL LABORATORY (TOAL)

by

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This method is used to determine the presence and amount of certain PAHs in samples of soil and sediment. The PAHs covered by this method are shown in Table I; the method was used in the following project.

"Sediment Toxicity, Contaminant Concentrations
and Benthic Community Structure
as Indicators of Sediment Quality in the St Louis River:
A Test of EMAP Concepts Applied to A Great Lakes Area of Concern"

This was known at the "R-EMAP project", and the Principal Investigator is C. Richards. The PAH analysis was done on samples collected in 1995 and 1996.

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1. SUMMARY OF METHOD
    1.1 PAHs are extracted by the Soxhlet method. Silica gel is used for cleanup.
    1.2 Analysis is by gas chromatography/mass spectrometry (GC/MS). Parameters are found in Table II.
    1.3 Quantitation is by the internal standard method. The Appendix contains information on standards used and their concentrations.

2. HANDLING OF SPECIMENS
    2.1. Wet samples are received, given a unique identifying number and immediately recorded in the samples received book. The containers of sediment are labeled with that number and placed in a refrigerator in a locked room.

3. APPARATUS AND REAGENTS
    3.1. Soxhlet extractor, paper or glass thimble, flat bottom 500mL flask, condenser and heating mantle.
    3.2. Steam bath fitted with concentric rings in a hood.
    3.3. Kuderna-Danish apparatus including a 15mL receiver tube and 3-ball Snyder column.
    3.4. Teflon boiling chips - solvent extracted.
    3.5. Apparatus for determining percent dry weight
        3.5.1. Drying oven.
        3.5.2. Dessicator.
        3.5.3. Porcelain crucibles.
    3.6. Analytical balance – reading to 0.0001g.
    3.7. Miscellaneous supplies - Scoopulas, beakers (250mL), funnels, forceps.
    3.8. Syringes - microliter for spiking.
    3.9. Hot water bath with nitrogen for concentrating extracts.
    3.10. Chromatographic column with teflon plug, 250mm x 10.5mm i.d., with 200mL reservoir at top.
    3.11. 10g copper beads per sample activated with 15% nitric acid, rinsed ten times with deionized water, five times with acetone, two times with dichloromethane (DCM) and two times with hexane.
    3.12. Hexane, dichloromethane, acetone, iso-octane, methanol (MeOH), toluene, benzene (Fisher Optima), and carbon disulfide (CS_2).
    3.13. Sodium sulfate: granular, anhydrous, trace analysis quality (Fisher Scientific), baked at 650°C for 4 hours.
    3.14. Glass wool, Soxhlet extracted with methanol for eight hours.
    3.15. Silica gel (Aldrich), 70-270 mesh, 60A, Soxhlet extracted for eight hours with methanol, activated for 18 hours at 225°C, cooled and deactivated to 1% water by adding 1mL of deionized water to 100g.
    3.16. Standards: The perdeuterated PAH analogs, used for surrogate and instrument internal standards, are obtained from Cambridge Isotope Laboratory in neat form at 98% purity. The calibration standards were received from Ultra Scientific. The sixteen PAHs are in a mixture in a 1:1 dichloromethane/benzene solution, A2LA certified. Perylene was received in neat form at 99% purity.

4. QUALITY ASSURANCE/QUALITY CONTROL
    4.1. A method blank and fortified matrix or reference material is included with each set of nine samples. One of the sediment samples is run in duplicate to test precision. All of these QA samples are treated identically to the sediment samples being analyzed.
        4.1.1. The method blank consists of 70g of sodium sulfate. This blank gives level of contamination of glassware and reagents.
        4.1.2. The fortified matrix sample consists of a "clean" sediment sample that has been previously analyzed for PAHs so that quantity present is known. In addition, the sample is spiked with x ppm of each of the PAHs of interest. The percent recovery (%R) of the PAHs is calculated and checked for acceptable limits. (x is chosen on the basis of the anticipated levels of PAHs in the samples.)
        4.1.3. The reference material is a freeze-dried sediment sample (HS-5) obtained from the National Research Council of Canada. This sample contains 16 PAHs in various concentrations. It is used in alternate sample sets with the fortified matrix.
        4.1.4. Two portions of one sample are prepared as separate samples to test precision. The relative percent difference (R%D) is determined by the difference in the measured concentration of each PAH divided by the average of the two results times 100.
        4.1.5. All samples, including the QA samples, are spiked with a known amount of surrogate internal standard (see The Appendix) and recoveries calculated. Recovery must fall within 40-120% limits. Samples, whose surrogate standard recoveries do not fall within those limits, are flagged.

5. PROCEDURE
    5.1. Soxhlet extractors, flat bottom flasks along with the paper or glass thimble, are assembled and extracted for four hours with extraction solvent before sediment samples are extracted. All other glassware is rinsed with acetone, DCM and hexane before use.
    5.2. Extraction
        5.2.1. Sample handling - any water layer on the sediment sample is decanted and discarded. The sample is mixed thoroughly. Any foreign objects such as sticks, leaves and rocks are discarded. Approximately 20g of wet sediment are weighed in a tared glass beaker and the weight is recorded. 70g of sodium sulfate is added to the beaker and mixed well. Sediment is allowed to dry with frequent stirring. A portion of ~1g is added to a dried and preweighed porcelain crucible to determine % dry weight (see 5.4).
        5.2.2. After the sediment sample has dried sufficiently, it is transferred to the extracted thimble. If a glass thimble is used, 0.5cm of silica gel is placed in the bottom followed by 1cm sodium sulfate, followed by the sediment. All are spiked with the appropriate standards (see The Appendix). One cm of sodium sulfate is added on top. The method blank is prepared by adding 70g of sodium sulfate to a thimble.
        5.2.3. Approximately, 250mL of acetone are added to the flat bottom flask along with several boiling beads. The thimble is placed in the Soxhlet using a long forceps. The Soxhlet is attached to the flat bottom flask. The unique identifying label from the beaker is transferred to the flask. The beaker is rinsed with acetone, which is then added to the top of the thimble.
        5.2.4. The apparatus is attached to the condenser on a refluxing bank positioning the flask in the heating mantle. Cold water and heat are turned on and sample is extracted for 4 hours.
        5.2.5. The sample extract is allowed to cool, the solvent is drained from the Sohxlet body

into the flask, which is then disconnected. The acetone extract is quantitatively transferred to a K-D apparatus.

5.2.6. Two hundred and fifty milliliters of a 3:1 solution (v/v) of methanol/toluene is added to the flat bottom flask along with fresh boiling chips, the Sohxlet reattached and sample put back on the heat to extract for 18 hours. This extract is then transferred, quantitatively, to the same K-D as the acetone extract, which has been reduced to ~ 5mL.

5.3. Reduction

5.3.1. A boiling bead is added to the K-D along with a few milliliters of iso-octane as a keeper. A Snyder column is attached. The K-D is placed on a cork ring on the steam bath and the solvent is evaporated until the sample is reduced to approximately 5mL.

5.3.2. The K-D is removed from the steam bath, swirling to wash down the sides and set in a rack to cool.

5.3.3. The Snyder column is removed and the lower tube is separated from the K-D, rinsing the ground glass joint with appropriate solvent into the lower tube. The K-D is retained; it is to be used after drying the extract with sodium sulfate. The 5mL of extract is transferred to a funnel, stoppered with glass wool, containing sodium sulfate and washed through with ~ 150mL 3:1 methanol/toluene into the same K-D. It is again reduced on the steam bath and treated as above.

5.3.4. The lower tube is placed in a hot water bath (50-60°C) and a stream of nitrogen is positioned over the surface of the solvent, barely riffling the surface. The extract is reduced to 0.5mL.

5.4. Determination of percent moisture

5.4.1. A ceramic crucible is dried overnight in a 105°C oven, placed in a dessicator to cool and weighed.

5.4.2. A minimum of 1g of the original sample is placed in the crucible and weight is recorded to 0.0001g. This weight minus the weight of the empty container gives the wet sediment weight.

5.4.3. The crucible and sediment is placed in the oven at 105°C to dry to a constant weight. This is usually accomplished overnight or within 24 hours.

5.4.4. The sample is removed from the oven to a dessicator to cool to room temperature (1/2 hour) before weighing.

5.4.5. The weight of the dry sediment plus the crucible minus the weight of the crucible gives the dry sediment weight.

5.4.6. Percent dry weight is calculated by dividing the dry sediment weight by the wet sediment weight and the result is multiplied by100.

5.5. Cleanup

5.5.1. Chromatographic columns (10.5mm i.d.) are rinsed with acetone, methylene chloride and hexane. About 1cm of glass wool is inserted and packed into the bottom. 2.1g deactivated silica gel (see 3.15) is added to the column with tapping to pack it. 1-2cm of sodium sulfate is added to the top followed by a plug of glass wool. Five centimeters of copper beads (see 3.11) are added on top to remove sulfur if present in the sample and the column is washed with approximately 50mL of hexane. When the surface of the sodium sulfate is just about to be exposed the stopcock is shut.

5.5.2. The reduced sample is transferred quantitatively to the silica column with three 1mL

rinses of hexane. A K-D is positioned below the column and the stopcock opened. When the sodium sulfate is just about to be exposed, the reservoir is rinsed with 5mL of hexane. Again when the sodium sulfate is about to be exposed, 60mL of 15% (v:v) DCM/ hexane is added to elute the sample.

5.5.3. After all of the solvent with the sample has run through, the K-D is placed on the steam bath and the volume reduced as before. The lower tube is removed to the hot water bath and solvent reduced to less than 5mL.

5.6. Preparation for analysis

5.6.1. The reduced sample is then transferred to a 10mL volumetric flask with three rinses of hexane. Before volume is made up to 10mL, 10µl of the instrument internal standard, PAHIIS#A, are added. The amount of internal standard added to the extract must be equivalent to the amount in the GC/MS calibration standards. Volume is made up to 10mL with hexane, mixed well and the total volume is transferred to a 12mL amber vial with teflon-lined lid. The vial is refrigerated until injection into the GC/MS.

6. ANALYSIS

6.1. The capillary column is installed in the gas chromatograph and the MSD is pumped down. Following the manufacturer's directions, the system is checked for leaks. When it has been determined that the system is leak-free, the oven temperature is raised. The gas flow rate is set for linear velocity desired. The MSD is tuned either manually or with the standard automatic tune program. Method *PAHSIM.m* is loaded. In this method, the target analytes' molecular ions are scanned in groups along with their confirmation ion (see Table I).

Group I - naphthalene, acenaphthylene, acenaphthalene and fluorene
Group 2 - phenanthrene, phenanthrene-d_{10}, anthracene and anthracene-d_{10}
Group 3 - fluoranthene, pyrene-d_{10}, pyrene, benz(a)anthracene and chrysene
Group 4 - benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, perylene-d_{12}, and perylene
Group 5 - indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene

GC/MSD parameters are listed below in Table II.

6.2. Four calibration standards are run before and after each sample set of 12 samples. The calibration standards are examined for acceptable performance. Once acceptable performance has been met, the calibration curves for each surrogate and target analyte are updated. The calibration curves are used for quantification of the target analytes in the sample extracts. Standards' compositions are found in the Appendix. The curves are generated using linear regression with the intercept set at zero (0,0) and each point given equal weight. The general linear regression equation is:

Response Ratio = (m * Amount Ratio) + b

where

Response Ratio = (Area of analyte in calibration standard)/(Area of internal standard in calibration standard)

Amount Ratio = (Concentration of analyte in calibration standard)/(Concentration of internal standard in calibration standard)

m = slope of the regression line

b = intercept of the regression line (in this case, zero)

6.3. GC/MS Analysis

6.3.1. The sample extracts, already spiked with internal standard, are removed from the refrigerator and allowed to return to room temperature. Just before injection, each extract is sonicated for 8 - 10 minutes.

6.3.2. One microliter of the extract is injected into the GC under the same conditions as used for the calibration standards.

6.3.3. Data are acquired using selective ion monitoring (SIM).

6.3.4. Samples are recapped and returned to the refrigerator

6.4. GC/MS Data Analysis

6.4.1. Data file acquired is loaded into DATA ANALYSIS program. The software is used to integrate the peaks, check qualifier ions for confirmation, determine areas of peaks and quantify the concentration of the target analyte using the internal standard. An example of the calculation is listed below.

$$C_S = \{[(A_S/A_{IS}) - b]/m\} * C_{IS}$$

where:

C_S = Concentration of target analyte or surrogate in sample extract

C_{IS} = Concentration of internal standard in sample extract

A_S = Area of target analyte or surrogate in sample extract

A_{IS} = Area of internal standard in sample extract

b = y intercept from analyte standard curve regression equation

m = slope from analyte standard curve regression equation

6.4.2. The concentration of analyte in the sample is then determined by accounting for the sample extract volume and the volume or mass of the original sample extracted. An example is below.

$$C_{target} = (C_S * V_{ext})/Sample_{amt}$$

where:

C_{target} = final concentration of target analyte

C_S = concentration of target analyte in sample extract

V_{ext} = volume of final extract

$Sample_{amt}$ = volume of water sample extracted or mass of tissue or sediment extracted

Sediment analyte concentrations are reported on a dry weight basis.

6.4.3. The percent recovery for each surrogate standard is determined for all the samples as shown below.

$$\% rec_{sur} = (obs_{sur})/(act_{sur}) * 100$$

where:

$\% rec_{sur}$ = surrogate percent recovery

obs_{sur} = observed concentration of surrogate

act_{sur} = actual concentration of surrogate spike

6.4.4. Target analyte concentrations are corrected for recovery based on surrogate recoveries for the sample analyzed. Anthracene-d_{10} is used for analytes naphthalene through anthracene, pyrene-d_{10} for fluoranthene through chrysene and perylene-d_{12} for benzo(b)fluoranthene through benzo(g,h,i) perylene. An example calculation follows.

$$C_{target,corr} = (C_{target} * 100)/\%rec_{sur}$$

where:

$C_{target,corr}$ = recovery corrected target analyte concentration

C_{target} = final concentration of target analyte in sample

$\%rec_{sur}$ = surrogate percent recovery

7. REFERENCES

7.1. EPA Test Methods for Evaluating Solid Waste (SW 846), U.S. EPA Office of Solid Waste and Emergency Response, Washington, D.C. 20460 (1986 with 1987 revisions).

7.2. Environmental Research Lab/EPA Duluth, MN. Personal Communications, B. Butterworth & P. Kosian to I Moser.

Table I. PAHs analysed with this Method

ANALYTE	CAS Number	Molecular and Confirmation ion
Naphthalene	91-20-3	128, 129
Acenaphthylene	208-96-8	152, 151
Acenaphthene	83-32-9	154, 153
Fluorene	86-73-7	166, 165
Phenanthrene	85-01-8	178, 176
Anthracene	120-12-7	178, 176
Fluoranthene	206-44-0	202, 200
Pyrene	129-00-0	202, 200
Benz(a)anthracene	56-55-3	228, 226
Chrysene	218-01-9	228, 226
Benzo(b)fluoranthene	205-99-2	252, 253
Benzo(k)fluoranthene	207-08-9	252, 253
Benzo(a)pyrene	50-32-8	252, 253
Perylene	198-55-0	252, 253
Indeno(1,2,3-cd)pyrene	193-39-5	276, 138
Dibenzo(a,h)anthracene	53-70-3	278, 139
Benzo(g,h,i)perylene	191-26-2	276, 138

TABLE II. Summary of GC/MS Conditions

Type of analysis:	SIM
Electron multiplier voltage:	0 relative
Cycles per second:	0.5≤cycles≤1.5
Initial temperature:	100°C, hold 2 mins.
Temperature program:	100 - 280°C @ 5°/min
Final temperature:	280°C, hold 15 mins.
Injector temperature:	300°C
Transfer line temperature:	280°C
Injector:	Grob-type, splitless
Column head pressure:	10psi
Carrier gas:	Helium at 30cm/sec
Sample volume:	1µl
Purge time:	off 0 mins; on 0.75 mins

The Appendix

STOCK CALIBRATION STANDARDS

In a 10mL volumetric flask, place 1mL of PAH mix (Ultra Scientific US-106, 16 PAHs, each at 2000μg/mL). Make up to volume with toluene for concentration of 0.2μg/μl of each. 50μl is spiked into the fortified matrix for 10μg of each PAH to test for recovery.

In a 10mL volumetric flask, place 10mg of perylene (Ultra ScientificRAH-505). Dissolve in 8mL benzene and make up to volume with toluene. 10μl is spiked into the fortified matrix for 10μg perylene to test for recovery.

STOCK SURROGATE STANDARDS

Weigh out 0.1g anthracene-d_{10} (Cambridge Isotope Lab DLM-102) and transfer to a 10mL volumetric flask. Add about 8mL of toluene and sonicate in warm water to obtain dissolution. Make up to volume with toluene after solution has returned to room temperature. Concentration is 10μg/μl. (If unable to dissolve, transfer to 50mL flask and make up to volume with toluene. Concentration is then 2μg/μl.)

Repeat in separate 10mL volumetric flask with pyrene-d_{10} (Cambridge Isotope Lab DLM-155). Concentration is 10μg/μl.

Weigh out 0.1g perylene-d_{12} (CIL DLM-366) and transfer to 50mL volumetric flask. Add 10mL carbon disulfide.
(***** Read MSDS first as this solvent is poisonous and highly flammable ****)
After perylene-d_{12} is dissolved make up to volume with benzene or toluene. Concentration is 2μg/μl.

STOCK INSTRUMENT INTERNAL STANDARD

In a 10mL volumetric flask, place 0.1g phenanthrene-d_{10} (CIL DLM-371) and dissolve in toluene. Concentration is 10μg/μl.

WORKING STANDARDS

PAHSIS#A (Surrogate Recovery Standard)

2.5mL (25mg) of each stock, anthracene-d_{10} and pyrene-d_{10}, was placed into a 25mL volumetric flask plus 12.5mL (25mg) perylene-d_{12} stock and volume made up to 25mL with hexane. Concentration is 1μg/μl of each surrogate. Spike each sample before extraction with 10μl for a final concentration of 1μg/mL in the final extract.

PAHIIS#A (Instrument internal standard)

1mL (10mg) of the stock IIS phenanthrene-d_{10} was transferred to a 10mL volumetric flask and made up to volume with hexane. Concentration is 1µg/µl. Spike 10µl into the final extract volume of 10mL for 1µg/µl.

All standards were transferred to 12mL amber vials with teflon lined lids for storage in the freezer.

Table of WORKING CALIBRATION STANDARDS

Final conc µg/mL	PAH Stock µl	Perylene stock µl	PAHSIS#A µl	PAHIIS#A µl
0.25	12.5	2.5	2.5	10
0.50	25.0	5.0	5.0	10
1.0	50.0	10.0	10.0	10
2.0	100.0	20.0	20.0	10

The End