Polynuclear aromatic hydrocarbons (PNA), total recoverable, high-performance liquid chromatographic (0-3113-83)

Parameter	*Code*
Acenaphthene	34205
Anthracene	34220
Benzo(a)anthracene	34526
Benzo(g,h,i)perylene	34521
Benzo(a)pyrene	34247
Chrysene	34320
Dibenzo(a,h)anthracene	34556
Fluoranthene	34376
Fluorene	34381
Naphthalene	34696
Phenanthrene	34461
Pyrene	34469

1. Application

This method is suitable for the analysis of water and water-suspended-sediment mixtures for polynuclear aromatic hydrocarbons (PNA's) containing at least 1 ug/L of the analyte.

2. Summary of method

PNA's are extracted from water or water-suspended-sediment mixtures with methylene chloride. The extract is concentrated and subjected to high-performance liquid chromatographic (HPLC) analysis using a 10-gm reverse-phase column and a dual-channel ultraviolet detector.

3. Interferences

Any compounds that exhibit chemical and (or) physical properties similar to the compounds of interest can interfere.

4. Apparatus

4.1 *Concentrator apparatus*, Kuderna-Danish (K-D), with a 500-mL flask, a three-ball Snyder column, and a 10-mL graduated receiver tube.

4.2 *Evaporative concentrator*, Organomation N-Evap, or equivalent.

4.3 *Filtering apparatus*, Millipore, or equivalent: The filtering apparatus consists of a 250-mL reservoir with a glass frit, and a 1,000-mL receiving reservoir with FH, Millipore no. FHUP 04700 and HA, Millipore no. HAWP 40700 0.45-gm filters for the corresponding organic and aqueous solvents, or equivalent.

4.4 *Liquid chromatograph*, Waters Associates ALC/GPC 204 liquid chromatograph equipped with a dual-channel, variable-wavelength detector, a model 6000A solvent-delivery system, model WISP 710A microprocessor with a model 730 data module and a model 720 system controller, or equivalent.

4.4.1 The following conditions are recommended:

Column, reverse-phase, micro-bondapak C18-10 gm, Waters Associates, or equivalent.

Wavelengths, 254 and 313 nm.

Solvent, 40 to 80 percent acetonitrile/water, linear slope gradient at a flow rate of 1.0 mL/min.

5. Reagents

5.1 *PNA standards*, EPA analytical reference grade, or highest purity available: Use methylene chloride as a solvent to prepare stock solutions in the approximate 100-300 ng/uL concentration range. Store in the dark at 4°C.

5.2 *PNA working-standard solution*: Prepare three standard mixtures of 12 PNA's at concentrations of 1, 5, and 10 ng/uL in acetonitrile, from step 5.1.

5.3 *Sodium sulfate*, granular, anhydrous: Heat overnight at 300°C and store at 130°C.

5.4 *Solvents*, HPLC-quality acetonitrile, and methylene chloride: Filter before use with the filtering apparatus described above (step 4.3).

5.5 *Water*, organic-free.

6. Procedure

Glassware must be cleaned by washing with a hot detergent solution, rinsing with deionized water, and heating overnight at 300°C. Just prior to use, the glassware is rinsed with solvent. Stopcock grease should not be used on ground-glass joints.

6.1 Weigh the bottle containing the sample and record the weight. Pour the sample into a 1-L separatory funnel. Weigh the empty sample bottle. Calculate the net sample weight and record the value obtained to three significant figures.

6.2 Add 50 mL methylene chloride to the sample bottle, swirl to rinse the sides of the

bottle, and transfer the solvent to a separatory funnel. The Teflon-lined cap is not rinsed because of the potential for contamination from solvent that has contacted the threads and surface beneath the Teflon liner. Shake the separatory funnel vigorously for 1 min. Vent often. Allow the layers to separate and draw off the methylene chloride layer into a 250-mL
Erlenmeyer flask that contains 0.5 g anhydrous sodium sulfate.

6.3 Repeat the extraction of the water sample twice using 40 mL methylene chloride each time. Combine all organic extracts in the 250-mL Erlenmeyer flask.

6.4 Transfer the extract to a 500-mL K-D apparatus fitted with a three-ball Snyder column and a 10-mL receiver containing a micro boiling chip and 0.5 mL of acetonitrile.

6.5 Place the apparatus on a water bath at about 80^{o}C and concentrate to about 5 mL. Remove from the heat and allow to cool. Dry the joints with a towel. Rinse the lower joint with acetonitrile into the receiver.

6.6 Further reduce the volume of solvent to about 1 mL on an evaporative concentrator. Rinse down the sides of the tube with 1 mL acetonitrile and concentrate to a final volume of 0.5 mL. Stopper until chromatographic analysis can begin.

6.7 Optimize the chromatographic conditions.

6.8 Prepare liquid chromatograph calibration curves daily by injecting the standards described in step 5 .2. Operating conditions must be identical to those used for sample analysis (step 6.9). Record the volume of the standard injected and the retention time and integrated peak area of each component in the standard. The calibration should be performed at the beginning and end of a run, and after every fourth sample.

6.9 Inject an aliquot of sample extract into the liquid chromatograph. Record the volume injected. Identify the peaks by retention time. Confirmation is made by measuring the peak area at two different wavelengths and comparing the ratio of the peak areas to that of the standard. Record the retention time and integrated area of any identified peak. Dilute any extract containing an identifiable component above the highest standard. The 254-nm detector does not resolve some pairs of compounds. Determination at another wavelength, 313 nm, is necessary to distinguish between these pairs. For example, fluorene and acenaphthene each absorb at 254 nm and are not separated by the column. Acenaphthene, however, absorbs at 313 nm, whereas fluorene does not. The response ratio of acenaphthene calculated at 254 nm and 313 nm is 1:1; therefore, both peaks are analyzed. Chrysene and benzo(a)anthracene are not clearly distinguishable at 254 nm, but are at 313 nm. When these compounds are determined at both wavelengths, individual contributions to peak areas can be determined and concentrations calculated.

7. Calculations

7.1 Calculate the response factor of each identified component in the calibration standard:

$$RF = \frac{A_1}{C_S x V_1},$$

Where,

RF= response factor of identified component in calibration standard, in area/ng,

C_s = concentration of standard component, in ng/uL

V_1= volume of standard injected, in uL, and

A, = integrated peak area of identified component in calibration standard.

7.2 Calculate the concentration of each identified component in the original water sample from the equation

$$\text{Concentration (pg/L)} = \frac{A_2 x V_2 x 1{,}000}{V_3 x W x RF},$$

RF= response factor of identified calibration standard component, in area/ng,

A_2 = integrated peak area of identified sample component,

V_2 = final volume of sample extract, in mL, V_3 = volume of sample extract injected, in uL, and

W= weight of sample determined in g expressed in mL (1.000 mL = 1.000 g).

8. Report

Report concentrations of individual PNA's in water or water-suspended-sediment mixtures

as follows: less than 1 ug/L, as "less than 1 ug/L"; 1 to
10 ug/L, one significant figure; 10 ug/L and greater, two significant figures.

9. Precision

Single-operator precision on seven replicates and recovery data determined by spiking water-suspended-sediment mixture samples with PNA's are as follows:

Compound	*Concentration Spiked (ug/L)*	*Mean Concentration, Recovered (ug/L)*	*Relative Standard Deviation*
Napthalene	1.3	0.94	6.9
	2.6	1.41	20
	5.1	2.4	24
Flourene	0.64	0.47	12
	1.3	0.94	7.8
	2.6	1.6	14
Acenapthene	2.8	2.01	8.5
	5.5	3.6	13
	11	6.5	18
Phenathrene	0.21	0.15	8.3
	0.42	0.36	6.3
	0.84	0.66	8.5
Anthracene	0.52	0.38	3.9
	0.1	0.76	5.1
	0.21	0.142	7.4
Pyrene	0.32	0.28	2.8
	0.64	0.58	3.4
	1.3	1.1	5.6
Fluoranthene	1.4	1.1	3.5
	2.8	2.5	5.5
	5.5	5.0	5.1
Benzo(a)anthracene	0.43	0.39	3.0
	0.85	0.8	3.4
	1.7	1.6	5.5
Benzo(a)pyrene	0.15	0.13	4.8
	0.29	0.29	7.5
	0.58	0.55	5.5
Dibenz(a,h)anthracene	0.13	0.12	6.9
	0.26	0.28	7.4
	0.51	0.5	5.0
Benzo(g,h,i)perylene	1.6	1.4	3.0
	3.2	3.2	5.8
	6.4	6.2	4.0
Chrysene	0.64	0.57	5.1
	1.3	1.3	5.2
	2.6	2.3	7.2

Selected References

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