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Techniques of Water-Resources Investigations
of the United States Geological Survey

Chapter A3

METHODS FOR THE DETERMINATION OF ORGANIC SUBSTANCES IN WATER AND FLUVIAL SEDIMENTS

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This manual is a revision of "Methods for Analysis of Organic Substances in Water," by Donald F. Goerlitz and Eugene Brown, Book 5, Chapter A3, published in 1972.

Book 5
LABORATORY ANALYSIS

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where

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

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 µL, and

V_4 = weight of sample in g expressed in mL (1.000 mL = 1.000g).

8. Report

8.1 Bottom material

8.1.1 Report concentrations of organochlorine compounds (except chlordane, perthane, toxaphene, PCB's, and PCN's) and organophosphorous insecticides in bottom material as follows: less than 0.1 µg/kg, as "less than 0.1 µg/kg" 0.1 to 1.0 µg/kg, one significant figure; 1.0 µg/kg and above, two significant figures.

8.1.2 Report concentrations of chlordane, perthane, PCB's, and PCN's in bottom materials as follows: less than 1.0 µg/kg, as "less than 1.0 µg/kg"; 1.0 µg/kg and above, two significant figures.

8.1.3 Report concentrations of toxaphene in bottom materials as follows: less than 10 µg/kg, as "less than 10 µg/kg"; 10 µg/kg and above, two significant figures.

8.2 Suspended sediment

8.2.1 Report concentrations of organochlorine compounds (except chlordane, perthane, toxaphene, PCB's, and PCN's) and organophosphorous insecticides in suspended sediment as follows: less than 0.01 µg/L as "less than 0.01 µg/L"; 0.01 to 0.10 µg/L, one significant figure; 0.1 µg/L and above, two significant figures.

8.2.2 Report concentrations of chlordane, perthane, PCB's, and PCN's in suspended sediment as follows: less than 0.1 µg/L, as "less than 0.1 µg/L"; 0.1 µg/L and above, two significant figures.

8.2.3 Report concentrations of toxaphene in suspended sediment as follows: less than 1.0 µg/L, as "less than 1.0 µg/L"; 1.0 µg/L and above, two significant figures.

9. Precision

It is estimated that the percent relative standard deviation for recoverable insecticides and PCB's from bottom material and suspended sediment will be greater than that reported for dissolved insecticides and PCB's (method O–1104–83).

Selected references

Goerlitz, D.F., and Brown, Eugene, 1972, Methods for analysis of organic substances in water: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A3, 40 p.

Goerlitz, D.F., and Law, L.M., 1971, Note on removal of sulfur interferences from sediment extracts for pesticide analysis: Bulletin of Environmental Contamination and Toxicology, v. 6, p. 9–10.

_____ 1972, Chlorinated naphthalenes in pesticide analysis: Bulletin of Environmental Contamination and Toxicology, v. 7, p. 243–251.

_____ 1974, Determination of chlorinated insecticides in suspended sediment and bottom material: Journal of the Association of Official Analytical Chemists, v. 57, p. 176–181.

Organochlorine compounds, recoverable from fish tissue, gas chromatographic (O–9104–83)

Parameter	*Code*
Aldrin	None assigned.
Chlordane	
DDD	
DDE	
DDT	
Dieldrin	
Endosulfan	
Endrin	
Polychlorinated biphenyls	
Polychlorinated naphthalenes	
Heptachlor	
Heptachlor epoxide	
Lindane	
Methoxychlor	
Mirex	
Perthane	
Toxaphene	

1. Application

This method is suitable for the determination of organochlorine compounds in fish containing at least 0.1 µg/kg of the analyte.

2. Summary of method

A homogenized sample of whole fish or fish fillet is extracted with petroleum ether to isolate the fat. The organochlorine compounds are extracted from the fat with acetonitrile. The acetonitrile extract is diluted with water and extracted with petroleum ether to partition the organochlorine compounds.

The petroleum ether extract is concentrated and purified using adsorption chromatography. Organochlorine compounds are identified and quantified by gas chromatography using electron-capture detectors.

3. Interferences

Sulfur and organosulfur compounds will interfere, but these substances can be removed by treating the final extracts with mercury.

4. Apparatus

4.1 *Alumina column*: To a 130-mm×10-mm id glass tube having a coarse-porosity fritted disc, add 1 cm anhydrous sodium sulfate, 10 cm alumina, and 1 cm anhydrous sodium sulfate.

4.2 *Blender*, 3.8-L-capacity, Waring, three-speed with stainless steel container, cover, and blade assembly, or equivalent.

4.3 *Blender*, 1.2 L-capacity, Waring, with explosion-resistant motor base, borosilicate glass containers, vinyl/plastic cover, and stainless steel blade assembly, or equivalent.

4.4 *Boiling chips*, micro, granular, Hengar H–1366C, or equivalent. Rinse with hexane, air dry, and heat overnight at 300°C.

4.5 *Concentrator, Kuderna-Danish (K-D)*, 500 mL, with 5-mL and 10-mL volumetric receivers and a one-ball Snyder column.

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

4.7 *Fish tissue preparation equipment*, consisting of a nonporous ceramic or stainless steel cutting board, a heavy-duty stainless steel knife, and a stainless steel spatula having a 25-cm blade.

4.8 *Gas chromatograph*, Tracor 560, or equivalent.

4.8.1 The following conditions are recommended:

Columns, borosilicate glass, 1.8 m×2 mm id operated at 200°C: Column packing materials are (1) 3 percent SP 2100 on 100/120 mesh Supelcoport, or equivalent; and (2) 1.5 percent SP 2250 + 1.95 percent SP 2401 on 100/120 mesh Supelcoport, or equivalent.

Detectors, electron capture, operated at 345°C.

Injection port temperature, 220°C.

Carrier gas, nitrogen or 5 percent methane in argon, flow rate 30 mL/min.

4.9 *Glass filter*, 142 mm, 0.3 μm mean pore size, Gelman, or equivalent: Prepare the filters by rinsing with acetone and hexane, evaporating the solvent, and heating overnight at 300°C.

4.10 *Silica column*: To a 10-mm×130-mm id glass tube having a sealed-in, coarse-porosity fritted disc, add 1 cm anhydrous sodium sulfate, 10 cm silica, and 1 cm anhydrous sodium sulfate.

5. Reagents

5.1 *Acetonitrile, petroleum ether saturated*: Place 800 mL acetonitrile in a 1-L separatory funnel. Add sufficient petroleum ether (about 50 mL) so that after vigorously mixing, an excess of petroleum ether is visible above the acetonitrile. Draw off and retain the lower layer.

5.2 *Alumina adsorbent*, Woelm neutral aluminum oxide, or equivalent: Prepare deactivated adsorbent by adding 8 g deionized water to 92 g alumina and shake for at least 2 h on a wrist-action shaker. The alumina is tested for required deactivation by attempting to elute the organochlorine compounds of interest from a test column according to the column fractionation scheme (table 5).

5.3 *Mercury*, metallic, reagent grade.

Table 5. Column fractionation scheme for alumina and silica columns for organochlorine insecticides, PCB's, and PCN's in fish

Alumina Column	
Fraction 1 20 mL hexane eluate	Fraction 2 20 mL hexane eluate
Aldrin	Dieldrin
Chlordane	Endrin
Heptachlor	Heptachlor epoxide
p,p–DDD	Endosulfan
p,p–DDE	
p,p–DDT	
Lindane	
Mirex	
Perthane	
PCB's	
PCN's	
Toxaphene	
Silica Column	
Fraction 1 (25 mL hexane eluate)	Fraction 2 (30 mL bezene eluate)
Aldrin	Chlordane
Mirex	p,p–DDD
PCB's	p,p–DDE
PCN's	p,p–DDT
p,p–DDE	Perthane
	Heptachlor
	Lindane
	Toxaphene

5.4 *Silica adsorbent*, Woelm silica, 70–150 mesh, or equivalent: Prepare deactivated adsorbent by adding 0.2 g deionized water to 99.8 g silica, and shake for at least 2 h on a wrist-action shaker. The silica is tested for required deactivation by attempting to reproduce the elution scheme in table 5.

5.5 *Sodium chloride*, granular, reagent grade: Heat at 300°C overnight before use.

5.6 *Sodium chloride solution*, saturated: Dissolve 360 g sodium chloride in 1,000 mL deionized water. Add additional sodium chloride in about 5 g increments, stirring well after each addition, until an excess of the salt is observed.

5.7 *Sodium hypochlorite solution*, (5 percent), Clorox bleach, or equivalent.

5.8 *Sodium sulfate*, granular, anhydrous: Heat overnight at 300°C and store in a covered beaker at 130°C.

5.9 *Solvents*, acetonitrile, benzene, hexane, iso-octane, petroleum ether, distilled in glass, pesticide analysis quality, Burdick and Jackson, or equivalent.

5.10 *Pesticide mixed standards*, analytical reference grade, EPA analytical reference standards, or equivalent: Prepare individual stock solutions by weighing about 10 mg of each compound to at least three significant figures, and quantitatively transfer each compound to a 25-mL volumetric flask. Dilute to volume with benzene and mix thoroughly. Aliquots are removed and diluted to volume with iso-octane to obtain final concentrations listed in table 6.

Table 6. Concentrations of pesticides and PCB's in mixed standard solutions used for gas chromatograph calibration of fish tissue

[Picograms per microliter]

Mixture number	Compound	High standard concentration	Low standard concentration
1	Lindane	40	20
	Heptachlor	40	20
	Aldrin	40	20
	p,p–DDE	40	20
	p,p–DDD	40	20
	Mirex	40	20
	Methoxychlor	70	35
2	Aldrin	40	20
	Heptachlor epoxide	40	20
	Dieldrin	40	20
	Endrin	40	20
3	Chlordane	200	100
4	Aldrin	40	20
	Endosulfan	40	20
	Perthane	40	20
	p,p–DDT	40	20
5	Toxaphene	600	300
6	o,p–DDE	40	20
	o,p–DDD	40	20
	o,p–DDT	40	20
7	Aroclor 1016 (a PCB)	300	150
8	Aroclor 1254 (a PCB)	400	200
9	Aroclor 1260 (a PCB)	300	150

6. Procedure

All glassware must be washed in warm detergent solution, rinsed with organic-free water, and heated at 300°C overnight. Rinse all glassware, fish-preparation equipment, and blender containers with petroleum ether before use, and air dry. After use with fish samples, the glassware should be washed immediately in a warm solution of detergent and bleach to aid in the removal of residual oils and odor. Do not use stopcock grease on ground-glass joints.

6.1 Sample preparation:

6.1.1 Scrape the scales from whole fish samples and cut off tough fins. Discard scales and fins. Chop whole fish into small pieces. If sample is a fillet, slice into small pieces.

6.1.2 Add fish pieces to a stainless steel blender container, cover, and blend until a homogeneous sample is obtained. Weigh 50 g of blended sample and place in a tared, glass blender container. Record the weight of the sample in the glass blender container to three significant figures. (Use excess sample for a duplicate, or store it frozen if a rerun is needed.)

6.2 Extraction of fat from fish samples:

6.2.1 Add 100 g anhydrous sodium sulfate to the weighed fish in the glass blender container and blend until thoroughly mixed. Scrape the sides of the container with a spatula during mixing to ensure homogeneity.

6.2.2 Add 150 mL petroleum ether to the sample in the blender container, cover, and blend at high speed for 2 min using the explosion-resistant base. (Start blending at low speed to avoid splashing and gradually increase to high). CAUTION: Petroleum ether is extremely flammable. Provide additional air circulation around the blender base to prevent solvent fumes from contacting the motor housing.

6.2.3 Slowly decant the petroleum ether extract into a Buchner funnel fitted with filter paper. Slowly filter the extract into the filtration flask using a water aspirator. (Care must be taken to prevent solids from entering the filtrate.)

6.2.4 Scrape down the walls of the blender container and break up any caked material with a spatula. Add 100 mL petroleum ether, cover, and repeat the extraction (step 6.2.2), blending for 1 min. Scrape down the walls of the blender container and blend for another minute.

6.2.5 Slowly decant the petroleum ether extract into the Buchner funnel and filter.

6.2.6 Repeat steps 6.2.4 and 6.2.5. Quantitatively transfer the remaining fish residue from the blender to the Buchner funnel using the spatula and small amounts of petroleum ether to aid in the transfer. Continue aspirating until the residue appears dry.

6.2.7 Quantitatively transfer the filtrate to a 500-mL Erlenmeyer flask. Add 2 g anhydrous sodium sulfate, cover the flask, and allow to stand for at least 1 h.

6.2.8 Weigh a 10-mL receiver for the concentration step and record its weight to two significant figures. Quantitatively transfer the extract into a K-D flask fitted with the weighed receiver. Add a boiling chip, fit the K-D flask with a Snyder column, and concentrate the extract to about 5 mL on a water bath maintained at 90°C.

6.2.9 Remove the K-D apparatus from the water bath, disconnect the receiver, and place it on an evaporative concentrator to evaporate any remaining petroleum ether. The receiver contains the fat extract.

6.2.10 Weigh the receiver, record the weight, and calculate the weight of the extract. If the extracted fat weighs more than 3 g, take 3.0 g for the liquid–liquid partitioning (step 6.3) and use the formula in calculation 7.1 to determine the final sample weight. If the weight of the extracted fat is 3 g or less, the analysis is based on the weight of the original sample (step 6.1.1).

6.3 Liquid–liquid partitioning:

6.3.1 Quantitatively transfer the extract (3 g or less) to a 125-mL separatory funnel using small amounts of petroleum ether to bring the total volume to about 15 mL.

6.3.2 Add 30-mL petroleum-ether-saturated acetonitrile to the separatory funnel. Stopper and shake vigorously for at least 1 min, venting often. Allow the layers to separate.

6.3.3 Drain the acetonitrile layer (bottom) into a 1,000-mL saturated sodium chloride solution, and add 100 mL petroleum ether. Retain the fat extract in the 125-mL funnel.

6.3.4 Repeat steps 6.3.2 and 6.3.3 twice. Combine all acetonitrile extracts in the 1,000-mL separatory funnel.

6.3.5 Stopper the 1,000-mL separatory funnel and shake vigorously for 1 min, venting often. Allow the layers to separate and drain the aqueous layer into a 1-L glass bottle. Pour the petroleum ether extract from the funnel into a 250-mL Erlenmeyer flask.

6.3.6 Return the aqueous layer to the separatory funnel, add 100 mL petroleum ether to the bottle, swirl to rinse sides, and pour into separatory funnel.

6.3.7 Repeat the extraction (step 6.3.5). Allow the layers to separate, discard the aqueous layer, and add the extract to the Erlenmeyer flask containing the first extract (step 6.3.5). If the extract is highly colored, wash it twice by extracting it with 100-mL portions of deionized water. Discard the aqueous layers after each extraction and transfer the final extract to the 250-mL Erlenmeyer flask. Add 2 g sodium sulfate to the flask, cover, and allow the extract to stand over sodium sulfate for at least 1 h.

6.4 Concentration and cleanup:

6.4.1 Quantitatively transfer the extract with hexane to a K-D flask fitted with a 5-mL volumetric receiver. Add a boiling chip, attach a Snyder column, and concentrate the extract to about 5 mL on a 95°C water bath. Remove the K-D apparatus from the water bath, allow to cool, wipe the joints with a towel, disconnect the receiver, rinse the lower joint with hexane into the receiver, and concentrate the extract on an evaporative concentrator to 2–4 mL. Rinse down the sides of the receiver during concentration with small amounts of hexane. Adjust the volume of extract in the receiver to 5.00 mL with hexane.

6.4.2 Prepare an alumina column for the alumina fractionation, referring to table 5 for the fractionation scheme. Elute the column with 30 mL hexane to remove contaminants. Discard the eluate. Quantitatively transfer the extract from step 6.4.1 to the top of the column and elute using 45 mL hexane (the column holdup is about 5 mL). Collect the first 20 mL (fraction 1) and the second 20 mL (fraction 2) in graduated centrifuge tubes. Reduce the second alumina fraction to 1.0 mL on the evaporative concentrator and analyze by gas chromatography. Treat the first alumina fraction as described in step 6.4.3.

6.4.3 Prepare a silica column for the silica fractionation, referring to table 5. Elute the column with 30 mL hexane and discard the eluate. Reduce the volume of the first alumina fraction to about 0.5 mL on the evaporative concentrator and quantitatively transfer it to the top of the silica column. Add hexane to the top of the column and collect 25 mL of eluate (fraction 1) in a graduated centrifuge tube. As the last of the hexane enters the top sodium sulfate layer, add benzene to the top of the column and collect 30 mL of eluate (fraction 2) in a graduated centrifuge tube. Reduce the volume of each of these two fractions to 1.0 mL on the evaporative concentrator, and analyze each by gas chromatography.

Removal of sulfur from the first alumina and first silica fractions is accomplished by adding several drops of mercury and shaking for at least 1 min. The addition of mercury is continued until no further reaction occurs, as evidenced by blackening of the mercury.

6.5 Sample analysis:

6.5.1 Prepare gas chromatograph calibration curves daily with the mixed standards listed in table 6. Operating conditions must be identical to those used for sample analysis. Calibrate both analytical columns. Record the volume of the standard injected and the retention time and integrated peak area of each component in the standard.

6.5.2 Inject an aliquot of sample extract (from steps 6.4.2 and 6.4.3) into the gas chromatograph. Record the volume injected. Identify peaks by retention time. The identification must be made on both analytical columns. Record the retention time and integrated area of any identified peak. Dilute any extract containing an identifiable component above the highest standard.

7. Calculations

7.1 Determine the equivalent subsample weight from the following equation:

$$W = \frac{W_1 \times W_3}{W_2},$$

($W_1 = W_2$ when W_2 is equal to or less than 3 g)

where

W = equivalent subsample weight, in g,
W_1 = weight of fat taken for cleanup, in g,
W_2 = total weight of extracted fat, in g, and
W_3 = weight of original fish subsample, in g.

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

$$RF = \frac{A_1}{C_s \times V_1},$$

where

RF = response factor of identified component in calibration standard, in area/pg,
C_s = concentration of standard, in pg/μL (step 5.10),
V_1 = volume of standard injected, in μL (step 6.5.1), and
A_1 = integrated peak area of identified component in calibration standard (step 6.5.1).

7.3 Calculate the concentration of each identified component (see step 6.5.2) in the original fish sample from the equation

$$\text{Concentration } (\mu g/L) = \frac{A_2 \times V_2}{V_3 \times W \times RF},$$

where

RF = response factor of identified calibration standard component, in area/pg (step 7.2),
A_2 = integrated peak area of identified sample component (step 6.5.2),
V_2 = final volume of sample extract, in mL (step 6.4.3),
V_3 = volume of sample extract injected, in μL (step 6.5.2), and
W = equivalent subsample weight of fish, in g (calculation 7.1).

8. Report

8.1 Report concentrations of organochlorine compounds (except chlordane, perthane, and toxaphene) in fish samples as follows: less than 0.1 μg/kg, as "less than 0.1 μg/kg"; 0.1 to 1.0 μg/kg, one decimal; 1.0 μg/kg and above, two significant figures.

8.2 Report concentrations of chlordane, perthane, PCB's, and PCN's in fish samples as follows: less than 1.0 μg/kg, as "less than 1.0 μg/kg"; 1.0 μg/kg and above, two significant figures.

8.3 Report concentrations of toxaphene in fish samples as follows: less than 10 μg/kg, as "less than 10 μg/kg"; 10 μg/kg and above, two significant figures.

9. Precision

Precision data are not available.

Selected references

Goerlitz, D.F., and Law, L.M., 1971, Note on removal of sulfur interferences from sediment extracts for pesticide analysis: Bulletin of Environmental Contamination and Toxicology, v. 6, p. 1–10.

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Chlorophenoxy acids, total recoverable (O–3105–83) and dissolved (O-1105-83), gas chromatographic

Parameter	*Code* *Total recoverable*	*Dissolved*
2,4-D	39730	39732
2,4-DP	82183	82356
Silvex	39760	39762
2,4,5-T	39740	39742

1. Application

This method is suitable for the determination of chlorophenoxy acid herbicides, and their esters and salts, in water and water–suspended-sediment mixtures containing at least 0.01 µg/L of the analyte.

2. Summary of method

Chlorophenoxy acid herbicides and their esters are extracted with either diethyl or methyl t-butyl ether from an acidified water sample. The extracted herbicides are hydrolyzed to the free acids which are converted to their methyl esters with boron trifluoride-methanol and purified using adsorption chromatography. The methyl esters are determined by gas chromatography using electron capture detectors.

3. Interferences

Halogenated organic acids, and their salts and esters, may cause interference.

4. Apparatus

4.1 *Boiling chips*, granules, micro, Hengar, H–1366C, or equivalent: Rinse with hexane, air dry, and heat at 300°C overnight.

4.2 *Centrifuge tube*, 5 mL, Pyrex, graduated, with ground-glass stopper.

4.3 *Concentrator, Kuderna-Danish (K-D)*, 125-mL flask and 5.0-mL receiver, one-ball Snyder column, and modified micro-Snyder column, Kontes 569251, or equivalent.

4.4 *Florisil column*, a disposable glass pipet with glass-wool plug: Fill to a depth of 1.5 cm with florisil adsorbent, followed by 2 cm sodium sulfate.

4.5 *Gas chromatograph*, Tracor Model 550, or equivalent.

4.5.1 The following conditions are recommended:

Columns, borosilicate glass, 1.8 m×2 mm id (inside diameter), operated at 180°C: Column packing materials are (1) 3 percent SP 2100 on 100/120 mesh Supelcoport; and (2) 3 percent SP 2250 on 100/120 mesh Supelcoport, or equivalent.

Detector, dual electron capture operated at 350°C.

Injection port temperature, 200°C.

Carrier gas, nitrogen, flow rate 20 mL/min.

4.6 *Glass filters*, 142 mm, 0.3 µm mean pore size, Gelman, or equivalent: Prepare the filters by rinsing with acetone and hexane, evaporating the solvent, and heating overnight at 300°C.

4.7 *Glass wool*, fine, rinsed with hexane, air dried, and heated at 300°C overnight.

4.8 *Sandbath*, Tecam, or equivalent.

5. Reagents

5.1 *Boron trifluoride-methanol esterification reagent*, 14 percent BF_3; (weight/volume; w/v) in methanol, Applied Science Labs, or equivalent.

5.2 *Florisil adsorbent*, commercially activated at 650°C, washed with hexane, allowed to air dry, and stored at 130°C in a glass-stoppered flask: Prior to use, the florisil is deactivated by adding 10 percent water by weight and shaking for at least 2 h

on a wrist-action shaker. The florisil is then tested for activity by attempting to elute the herbicides of interest with benzene from a test column. If the test compounds do not elute within 2.0 mL, further deactivation is required until the desired results are obtained.

5.3 *Potassium hydroxide solution*, 37 percent (w/v): Dissolve 78 g KOH reagent-grade pellets in 200 mL deionized water. Reflux for 8 h.

5.4 *Sodium sulfate, acidified*: Prepare a slurry of sodium sulfate with enough diethyl ether to cover the crystals, and acidify to pH 2 or less by adding a few milliliters of concentrated sulfuric acid. Determine the pH by transferring a small portion of the slurry to a beaker and removing the ether by evaporation. Add a few drops of deionized water to the crystals and measure the pH with pH paper. Allow to air dry overnight. Store in a covered Pyrex beaker or flask at 130°C.

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

5.6 *Sodium sulfate solution*, 5 percent (w/v): Dissolve 50 g neutral sodium sulfate in deionized water and dilute to 1 L.

5.7 *Solvents*, benzene, unpreserved diethyl ether or methyl t-butyl ether, and iso-octane, distilled in glass, pesticide analysis quality, Burdick and Jackson, or equivalent: Diethyl ether preserved with ethanol cannot be used in this procedure because it results in the formation of extraneous ethyl esters.

5.8 *Standards, methyl esters of chlorophenoxy acid herbicides*, EPA analytical reference grade or equivalent: Prepare a stock solution by weighing about 10 mg of compound to at least three significant figures and transfer to a 25-mL volumetric flask. Dilute to volume with benzene and mix thoroughly. Prepare a series of mixed-compound standards by volumetric dilution with iso-octane, as described in table 7.

5.9 *Sulfuric acid*, concentrated (sp. gr. 1.84), Mallinckrodt analytical reagent, A.C.S. grade, or equivalent.

5.10 *Sulfuric acid*, (1 + 3): Prepare by adding 1 part concentrated sulfuric acid to 3 parts deionized water. Store in a refrigerator at 4°C.

5.11 *Water*, deionized, organic-free.

Table 7. Concentration of herbicides in mixed standard solutions used for gas chromatograph calibration of water and water-suspended sediment

[Picograms per microliter]

Herbicide	High standard concentration	Low standard concentration
2,4-DP	100	50
2,4-D	100	50
2,4,5-T	40	20
Silvex	40	20

6. Procedure

All glassware must be washed in warm detergent solution, rinsed with organic-free water, and heated at 300°C overnight. Prior to use, all glassware is rinsed with the solvent it will contact. Stopcock grease should not be used on ground-glass joints.

For the determination of dissolved components, filter the sample through a glass filter to remove the suspended material. Pour the filtrate into the original sample bottle and continue with the procedure.

6.1 Immediately upon receipt of a sample in the laboratory, it must be acidified to pH 2 or lower with concentrated sulfuric acid and stored at 4°C.

6.2 A blank must accompany each group of samples. For each sample, rinse a 1,000-mL separatory funnel and a 250-mL Erlenmeyer flask with ether.

6.3 Weigh the sample bottle plus sample and record the weight to three significant figures.

6.4 Pour the sample into the separatory funnel and allow the bottle to drain completely. Weigh the empty bottle and record the weight to three significant figures. Calculate and record the sample weight.

6.5 Add 150 mL ether to the sample bottle, rinse the sides thoroughly, and pour the solvent into the separatory funnel. The Teflon-lined cap is not rinsed because of the potential for contamination from solvent that has contacted the threads and the surface beneath the Teflon liner. Shake the funnel vigorously for at least 1 min, venting often. Allow the layers to separate and drain the aqueous layer. Pour the ether extract into the Erlenmeyer flask. Extract the sample twice more, using 50 mL ether each time, and collect the extracts in the Erlenmeyer flask.

6.6 Add 15 mL distilled water, 2.0 mL 37 percent KOH, and a boiling chip to the extract. Fit the flask with a Snyder column and heat the assembly on a steam bath for a total of 90 min, during which time the ether will evaporate and the herbicide esters are hydrolyzed.

6.7 Remove the assembly from the water bath, allow to cool, and quantitatively transfer the water to a 125-mL separatory funnel. Extract the basic solution with 20 mL ether and discard the ether layer; repeat twice with 10 mL ether and discard the ether layers. The herbicide potassium salts remain in the aqueous phase. Add 2 mL sulfuric acid (1 + 3) to the contents of the funnel to bring the pH to 2 or below, and extract the aqueous phase with 20 mL ether; repeat twice with 10 mL ether to extract the herbicides in their acid forms. Collect the ether extracts in a 125-mL Erlenmeyer flask containing about 0.5 g acidified sodium sulfate. Cover the flask with foil and set aside for at least 1 h, or store in a refrigerator until analysis can continue.

6.8 Quantitatively transfer the ether extract into a K-D apparatus fitted with a 5-mL volumetric receiver. add 1 mL benzene and a boiling chip. Concentrate the extract to about 0.5 mL on a fluidized sandbath heated to 60–70°C. Under no circumstances should the extract be allowed to evaporate completely to dryness. Clear sand from the glass joint before opening. (Use a water bath at 80°C for methyl t-butyl ether extracts.) Rinse the bottom joint with benzene into the receiver.

6.9 After the benzene solution in the receiver has cooled, add 0.5 mL boron trifluoride-methanol reagent. The modified Snyder column is used as an air-cooled condenser, and the contents of the receiver are held at 50°C for 30 min in a sandbath. Cool the reaction mixture to room temperature and add sodium sulfate solution until the benzene-aqueous solution interface is observed in the restricted neck of the receiver. Stopper the receiver, shake vigorously for 1 min, and allow to stand for at least 1 h for phase separation. Loosen the stopper after shaking.

6.10 Transfer the benzene layer from the receiver to a florisil column. Elute with benzene until a total of 2.0 mL of benzene has been collected in a graduated centrifuge tube. Analyze the eluate by gas chromatography.

6.11 Prepare gas chromatograph calibration curves daily with the mixed standards listed in table 7. Operating conditions must be identical to those used for sample analysis. Calibrate both analytical columns. Record the volume of the standard injected and the retention time and integrated peak area of each component in the standard.

6.12 Inject an aliquot of sample extract into the gas chromatograph. Record the volume injected. Identify peaks by retention time. The identification must be made on both analytical columns. Record the retention time and integrated area of any identified peak. Dilute any extract containing an identifiable component above the highest standard.

7. Calculations

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

$$RF = \frac{A_1}{C_s \times V_1},$$

where

RF = response factor of identified component in calibration standard, in area/pg,
C_s = concentration of standard, in pg/ μL, (step 5.8,
V_1 = volume of standard injected, in μL (step 6.11), and
A_1 = integrated peak area of an identified component in calibration standard (step 6.11).

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

$$\text{Concentration } (\mu g/L) = \frac{A_2 \times V_2}{V_3 \times W \times RF},$$

where

RF = response factor of identified component in sample, in area/pg,
A_2 = integrated peak area of identified component,
V_2 = final volume of sample extract, in mL,
V_3 = volume of sample extract injected, in μL, and
W = weight of sample in g, expressed in mL (1.000 mL = 1.000 g).

7.3 Calculate the free chlorophenoxy acid concentration:

$$\text{Concentration of acid } (\mu g/L) = C \times f,$$

where

C = concentration of methyl ester (calculation 7.2), and

$$f = \frac{\text{molecular weight of acid}}{\text{molecular weight of methyl ester}}.$$

8. Report

Report chlorophenoxy acid herbicide concentrations as follows: less than 0.01 μg/L, as "less than

0.01 µg/L"; 0.01 µg/L to 0.10 µg/L, one significant figure; 0.10 µg/L and above, two significant figures.

9. Precision

9.1 Precision for dissolved chlorophenoxy acids in deionized water for 35 replicates using diethyl ether is as follows:

Compound	Spiked concentration (µg/L)	Mean concentration determined (µg/L)	Relative standard deviation (percent)
2,4-D	0.10	0.075	10.0
Silvex	.048	.036	11.6
2,4,5-T	.058	.045	12.2

9.2 Precision for dissolved chlorophenoxy acids in deionized water for 35 replicates using methyl t-butyl ether is as follows:

Compound	Spiked concentration (µg/L)	Mean concentration determined (µg/L)	Relative standard deviation (percent)
2,4-D	0.10	0.083	10.1
Silvex	.048	.040	10.4
2,4,5-T	.056	.049	10.0

9.3 It is estimated that the percent relative standard deviation for total recoverable chlorophenoxy acids will be greater than that reported for dissolved chlorophenoxy acids.

Selected references

Goerlitz, D.F., and Brown, Eugene, 1972, Methods for analysis of organic substances in water: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A3, 40 p.

Goerlitz, D.F., and Lamar, W.L., 1967, Determination of phenoxy acid herbicides in water by electron-capture and microcoulometric gas chromatography: U.S. Geological Survey Water-Supply Paper 1817-C, 21 p.

Chlorophenoxy acids, recoverable from bottom material (O-5105-83) and recoverable from suspended sediment (O-7105-83), gas chromatographic

Parameter	Code: Recoverable from bottom material	Code: Recoverable from suspended sediment
2,4-D	39731	39733
2,4-DP	34609	34608
Silvex	39761	39763
2,4,5-T	39741	39743

1. Application

This method is suitable for the determination of chlorophenoxy acid herbicides, and their esters and salts, in bottom material and in suspended sediment isolated from water containing at least 0.1 µg/kg and 0.01 µg/L of the analyte, respectively.

2. Summary of method

Chlorophenoxy acid herbicides and their esters are extracted with either diethyl or methyl t-butyl ether from an acidified slurry of bottom material or suspended sediment and water. The extracted herbicides are hydrolyzed to the free acids, which are converted to their methyl esters with boron trifluoride-methanol and purified using adsorption chromatography. The methyl esters are determined by gas chromatography using electron-capture detectors.

3. Interferences

Halogenated organic acids, and their salts and esters, may cause interference.

4. Apparatus

4.1 *Boiling chips*, granules, micro, Hengar H-1366C, or equivalent: Rinse with hexane, air dry, and heat at 300°C overnight.

4.2 *Centrifuge tube*, 10-mL, Pyrex, graduated, with ground-glass stopper.

4.3 *Concentrator, Kuderna-Danish (K-D)*, 125-mL flask, 5.0-mL volumetric receiver, one-ball Snyder column, and modified micro-Snyder column, Kontes 569251 or equivalent.

4.4 *Florisil column*: To a 130 mm×10 mm id (inside diameter) glass tube having a coarse-porosity fritted disc, add 3 cm florisil and 1 cm anhydrous sodium sulfate.

4.5 *Gas chromatograph*, Tracor model 550, or equivalent.

4.5.1 The following conditions are recommended:

Columns, borosilicate glass, 1.8 m×2 mm id operated at 180°C: Column packing materials are (1) 3 percent SP 2100 on 100/120 mesh Supelcoport; and (2) 3 percent SP 2250 on 100/120 mesh Supelcoport, or equivalent.

Detector, dual electron capture operated at 350°C.

Injection port temperature, 200°C.

Carrier gas, nitrogen, flow rate 20 mL/min.

4.6 *Glass filters*, 142 mm, 0.3 µm mean pore size, Gelman, or equivalent: Prepare the filters by rinsing with acetone and hexane, evaporating the solvent, and heating overnight at 300°C.

4.7 *Glass wool*, fine, rinsed with hexane, air dried, and heated at 300°C overnight.

4.8 *Oven*, Precision model 18EGE, or equivalent, capable of maintaining 130°C.

4.9 *Sandbath*, Tecam, or equivalent.

4.10 *Shaker*, wrist-action, Burrell, or equivalent.

5. Reagents

5.1 *Boron trifluoride-methanol esterification reagent*, 14 percent BF_3 (weight/volume; w/v) in methanol, Applied Science Labs, or equivalent.

5.2 *Florisil adsorbent*, commercially activated at 650°C, washed with hexane, allowed to air dry, and stored at 130°C in a glass-stoppered flask: Prior to use, the florisil is deactivated by adding 10 percent water by weight and shaking for at least 2 h on a wrist-action shaker. The florisil is then tested for activity by attempting to elute the herbicides of interest with benzene from a test column. If the test compounds do not elute within 10.0 mL, further deactivation is required.

5.3 *Hydrochloric acid*, concentrated (sp. gr. 1.19), analytical reagent, A.C.S. grade.

5.4 *Postassium hydroxide solution*, 37 percent (w/v): Dissolve 78 g KOH reagent-grade pellets in 200 mL deionized water. Reflux for 8 h.

5.5 *Sodium sulfate, acidified*: Prepare a slurry of sodium sulfate with enough diethyl ether to cover the crystals and acidify to pH 2 or less by adding a few mL of concentrated sulfuric acid. Determine the pH by transferring a small portion of the slurry to a beaker, removing the ether by evaporation, adding a few drops of deionized water to the crystals, and measuring the pH with pH paper. Allow to air dry overnight. Store in a covered Pyrex beaker or flask at 130°C.

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

5.7 *Sodium sulfate solution*, 5 percent (w/v): Dissolve 50 g neutral sodium sulfate in deionized water and dilute to 1 L.

5.8 *Solvents*, acetone, benzene, unpreserved diethyl ether or methyl t-butyl ether, and iso-octane: Diethyl ether preserved with ethanol cannot be used in this procedure because it results in the formation of extraneous ethyl esters.

5.9 *Standards, methyl esters of chlorophenoxy acid herbicides*, EPA analytical reference grade, or equivalent: Prepare a stock solution by weighing about 10 mg of compound to at least three significant figures and transfer to a 25 mL volumetric flask. Dilute to volume with benzene and mix thoroughly. Prepare a series of mixed-compound standards by volumetric dilution with iso-octane as described in table 8.

Table 8. Concentration of herbicides in mixed standard solutions used for gas chromatograph calibration of bottom material

[Picograms per microliter]

Herbicide	High standard concentration	Low standard concentration
2,4-DP	100	50
2,4-D	100	50
2,4,5-T	40	20
Silvex	40	20

5.10 *Sulfuric acid*, concentrated (sp. gr. 1.84), Mallinckrodt analytical reagent, A.C.S. grade, or equivalent.

5.11 *Sulfuric acid*, (1 + 3): Prepare by adding one part concentrated sulfuric acid to three parts deionized water. Store in a refrigerator at 4°C.

5.12 *Water*, deionized, organic-free.

6. Procedure

All glassware must be washed in warm detergent solution, rinsed with organic-free water, and heated at 300°C overnight. Prior to use, all glassware is rinsed with the solvent it will contact. Stopcock grease should not be used on ground-glass joints.

For bottom-material samples, begin at step 6.1.

For water–suspended-sediment mixtures, first determine the weight of the water, then filter the sample using a glass fiber filter to isolate the suspended sediment. Use the filter and the retained sediment to begin the procedure at step 6.2.

6.1 Moisture determination:

6.1.1 Decant excess water from the bottom material. Use a spatula to thoroughly mix the moist solid. Weigh 10 g of solid into a tared weighing dish. Record the weight to three significant figures.

6.1.2 Place the tared dish containing the sample in an oven at 130°C overnight. Remove from oven, allow to cool, weigh, and record the weight to three significant figures.

6.2 Add either the filter from the suspended-sediment filtration or the calculated amount of bottom material (not more than 100 g) to a 500-mL Erlenmeyer flask with a ground-glass stopper. Stir the sample and slowly add deionized water until the

mixture has the consistency of paste or until water begins to separate from the solid. Acidify the slurry to pH 2 or below by the dropwise addition of concentrated hydrochloric acid. Use pH paper to determine the pH. Periodically check the pH, adding more acid, if necessary, to maintain the pH at 2 or below.

6.3 Measure 20 mL acetone into the Erlenmeyer flask containing the acidified sample and stopper securely. Mix the contents of the flask for 20 min using the wrist-action shaker. Add 80 mL ether and shake again for 10 min. Decant the extract into a 1-L separatory funnel containing 400 mL of 5 percent sodium sulfate solution. Add 20 mL acetone to the Erlenmeyer flask and shake 20 min. Again, add 80 mL ether, shake 10 min, and decant the acetone-ether extract into the same separatory funnel. Repeat the process as in the second extraction one more time, and collect the acetone-ether extract in the separatory funnel containing the sodium sulfate solution.

6.4 Gently mix the contents of the separatory funnel for about 1 min. Allow the layers to separate. Discard the aqueous layer and collect the extract in a 500-mL Erlenmeyer flask.

6.5 Add 2 mL of 37 percent KOH and 30 mL distilled water to the extract in the 500-mL Erlenmeyer flask. Add a boiling chip and fit the flask with a one-ball Snyder column. Evaporate the ether on a water bath at 80°C in a hood, and continue the heating for a total of 90 min.

6.6 Remove the assembly from the water bath and allow it to cool. Remove the Snyder column. Quantitatively transfer the water to a 125-mL separatory funnel. Extract the basic solution once with 40 mL ether and discard the ether layer; repeat twice with 20 mL ether and discard the ether layers. The herbicides remain in the aqueous phase as their potassium salts. Add 5 mL (1 + 3) sulfuric acid to the contents of the funnel to lower the pH to 2 or below (measure with pH paper), and extract the aqueous phase with 40 mL ether; repeat twice with 20 mL ether. Collect the ether extracts in a 125-mL Erlenmeyer flask containing about 0.5 g acidified anhydrous sodium sulfate. Stopper the flask and set aside for at least 2 h or store in a refrigerator until analysis can continue.

6.7 Quantitatively transfer the ether extract into the K-D apparatus fitted with a 5-mL volumetric receiver. Add 1.0 mL benzene and a boiling chip. Concentrate the extract to about 0.5 mL on a fluidized sandbath heated to 60°–70°C. Under no circumstances allow the extract to evaporate completely to dryness. Clear sand from the glass joint before opening. Rinse the bottom joint with benzene into the receiver.

6.8 After the benzene solution in the receiver has cooled, add 0.5 mL boron trifluoride-methanol reagent. The modified Snyder column is used as an air-cooled condenser, and the contents of the receiver are held at 50°C for 30 min in a sandbath. Cool the reaction mixture to room temperature and add sodium sulfate solution until the benzene-aqueous solution interface is observed in the restricted neck of the receiver. Stopper the receiver, shake vigorously for 1 min, and allow to stand for at least 1 h for phase separation. Loosen the stopper after shaking.

6.9 Transfer the benzene layer from the receiver to a florisil column. Elute with benzene and collect 10.0 mL in a graduated receiver. Analyze the eluate by gas chromatography.

6.10 Prepare gas chromatograph calibration curves daily with the mixed standards shown in table 8. Operating conditions must be identical to those used for sample analysis. Calibrate both analytical columns. Record the volume of the standard injected and the retention time and integrated peak area of each component in the standard.

6.11 Inject an aliquot of sample extract into the gas chromatograph. Record the volume injected. Identify peaks by retention time. The identification must be made on both analytical columns. Record the retention time and integrated area of any identified peak. Dilute any extract containing an identifiable component above the highest standard.

7. Calculations

7.1 Calculate the weight required for a dry weight equivalent of 50 g:

$$\text{Wet weight} = \frac{W_1}{W_2} \times 50 \text{ g},$$

where

wet weight = amount of sample to be taken for extraction, in g,

W_1 = wet weight of sample, in g, and

W_2 = dry weight of sample, in g.

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

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

where

RF = response factor of identified component in calibration standard, in area/pg,
C_s = concentration of standard, in pg/μL,
V_1 = volume of standard injected, in μL, and
A_1 = integrated peak area of identified component in calibration standard.

7.3 Calculate the concentration of each identified component in the original bottom-material sample from the equation

$$\text{Concentration (μg/kg)} = \frac{A_2 \times V_2}{V_3 \times W \times RF},$$

where

RF = response factor of identified calibration standard component, in area/pg,
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 μL, and
W = dry weight equivalent of sample, in g.

7.4 Calculate the concentration of each identified component in the original suspended sediment from the following equation:

$$\text{Concentration (μg/L)} = \frac{A_2 \times V_2}{V_3 \times V_4 \times RF},$$

where

RF = response factor of identified calibration standard component, in area/pg,
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 μL, and
V_4 = weight of sample in g, expressed in mL (1.000 mL = 1.000 g).

7.5 Calculate the free phenoxy acid concentrations using the following equation:

$$\text{Concentration of acid (μg/L or μg/kg)} = C \times f,$$

where

C = concentration of methyl ester determined in calculation 7.3 or 7.4, and

$$f = \frac{\text{molecular weight of acid}}{\text{molecular weight of methyl ester}}.$$

8. Report

8.1 Report chlorophenoxy acid herbicide concentrations in bottom materials as follows: less than 0.10 μg/kg, as "less than 0.10 μg/kg"; 0.10 μg/kg and above, two significant figures.

8.2 Report chlorophenoxy acid herbicide concentrations in suspended materials as follows: less than 0.01 μg/L, as "less than 0.01 μg/L"; 0.01 to 0.10 μg/L, two decimals; 0.10 μg/L and above, two significant figures.

9. Precision

It is estimated that the percent relative standard deviation for recoverable chlorophenoxy acids from bottom material and suspended sediment will be greater than that reported for dissolved chlorophenoxy acids (method O–1105).

Selected references

American Public Health Association, 1981, Standard methods for the examination of water and wastewater (15th ed.): Washington, American Public Health Association, Inc., 1,134 p.

American Society for Testing and Materials, 1983, Annual Book of ASTM Standards, Sect. 11, v. 11.01: Philadelphia, American Society for Testing and Materials, 752 p.

Goerlitz, D.F., and Brown, Eugene, 1972, Methods for analysis of organic substances in water: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A3, 40 p.

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Triazines, total recoverable, gas chromatographic (O–3106–83)

Parameter	*Code*
Ametryn	82184
Atrazine	39630
Cyanazine	81757
Prometon	39056
Prometryn	39057
Propazine	39024
Simazine	39055
Simetryn	39054
Alachlor	None assigned.
Trifluralin	None assigned.

1. Application

This method is suitable for the determination of triazine herbicides, alachlor, and trifluralin in water and water–suspended-sediment mixtures containing at least 0.1 μg/L of each constituent.

2. Summary of method

Triazine herbicides are extracted from water with methylene chloride following adjustment to pH 7 to 9. Optional adsorption chromatography on alumina is used for the elimination of most nonpesticide interferences. Identification is made by selective gas chromatographic separation through the use of two or more dissimilar column packing materials using a nitrogen specific detector.

3. Interferences

Solvents, reagents, glassware, and other sample-processing hardware may yield discrete artifacts or elevated baselines which may cause misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free of interferences under the conditions of the analysis. Specific selection of reagents and purification of solvents in an all-glass system is required. Glassware should be cleaned by washing with hot detergent solution, rinsing with organic-free water, and heating overnight at 300°C.

4. Apparatus

4.1 *Alumina column*: To a 15-cm-long disposable Pasteur pipet, add a glass-wool plug, anhydrous sodium sulfate to a depth of 10 cm, 3 cm alumina, and 0.5 cm anhydrous sodium sulfate. Tap the column gently to promote settling to a uniform bed.

4.2 *Boiling chips*, granular, micro, Hengar H-1366C, or equivalent: Rinse with hexane, air dry, and heat at 300°C overnight.

4.3 *Concentrator apparatus*, Kuderna-Danish (K-D), with a 500-mL flask, a 10.0-mL receiver, and a three-ball Snyder column.

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

4.5 *Gas chromatograph*, Hewlett-Packard model 5880A/Tracor model 560, or equivalent: Instrument must incorporate a glass-lined injection port and a glass column.

4.5.1 The following conditions are recommended:

Columns, borosilicate glass, 1.8 m×2 mm id (inside diameter) rendered inert by treatment with silanizing agent: Column packing materials are (1) 1 percent OV–101 on 100/120 mesh UltraBond 20 M or equivalent, operated at an oven temperature of 175°C; and (2) 100/120 mesh UltraBond PEGS, or equivalent, operated at an oven temperature of 200°C.

Detector, alkali flame ionization (N/P), operated at 300°C.

Injection port temperature, 200°C.

Carrier gas, helium, flow rate 35 mL/min.

Detector flows, hydrogen at 3.2 mL/min and air at 100 mL/min.

5. Reagents

5.1 *Alumina*, Woelm W 200, neutral, activity I: Prepare activity V (16 percent deactivation) by mixing 100 g activity I with 19 mL water. Mix on wrist-action shaker for 2 h and let stand overnight in a sealed container. Prepare fresh weekly.

5.2 *Borosilicate glass wool*, filtering grade, prewashed with hexane and heated overnight at 300°C.

5.3 *Potassium hydroxide*, 37 percent (weight/volume; w/v) aqueous solution prepared from reagent-grade KOH and reagent water.

5.4 *Sodium sulfate*, granular, anhydrous, heated overnight at 300°C and stored at 130°C.

5.5 *Solvents*, benzene, ethyl ether, hexane, and methylene chloride, pesticide residue quality, distilled in glass.

5.6 *Sulfuric acid*, 25 percent (volume/volume; v/v) prepared from high-purity concentrated H_2SO_4 (sp. gr. 1.84) and reagent water.

5.7 *Triazine standards*, EPA analytical reference grade or highest purity available: Dissolve 5 mg of standard in benzene in a 50-mL volumetric flask, dilute to volume, and mix. Dilute stock standard with hexane to working concentration shown in table 9.

6. Procedure

6.1 Rinse all glassware with methylene chloride before using. Do not use stopcock grease on ground-glass joints.

6.2 Weigh the sample and capped bottle to the nearest 0.1 g and record the weight.

Table 9. Concentration of triazines in mixed standard solutions used for gas chromatograph calibration

[Picograms per microliter]

Mixture number	Triazines	Low standard	Medium standard	High standard
1 -------	Atrazine	500	1000	5000
	Propazine	500	1000	5000
	Simazine	500	1000	5000
2 -------	Ametryn	500	1000	5000
	Prometon	500	1000	5000
	Prometryn	500	1000	5000
	Simetryn	500	1000	5000

6.3 Transfer the sample to a 2,000-mL separatory funnel using a stainless steel powder funnel.

6.4 Weigh the empty sample bottle with cap to the nearest 0.1 g and calculate the sample weight.

6.5 Dissolve 5 g sodium chloride in sample and adjust the pH to 7 to 9 using the potassium hydroxide solution or sulfuric acid solution as necessary.

6.6 Add 75 mL methylene chloride to sample bottle, swirl, and transfer to separatory funnel. Allow to drain, rinsing the walls of the bottle.

6.7 Insert the glass stopper and shake the funnel vigorously for 1 min. Vent the system several times during this initial shakeout.

6.8 Let stand undisturbed while layers clarify, and draw off the methylene chloride into a 250-mL glass-stoppered Erlenmeyer flask.

NOTE: Most water–suspended-sediment mixtures will require special treatment. If emulsions occur, add sufficient volume of hexane (50–75 mL) to float the extract. Shake the separatory funnel again to mix the solvents, and allow the layers to separate. Draw off the sample into the sample bottle. The remaining emulsion can be eliminated by vigorous shaking (CAUTION: Vent often). Decant the extract into a 250-mL glass-stoppered Erlenmeyer flask and return the sample to the separatory funnel. Proceed to step 6.9.

6.9 Repeat the extraction with two additional 50-mL portions of methylene chloride, collecting the organic layers in the flask.

6.10 Add 5 gm anhydrous sodium sulfate, stopper, and shake.

6.11 Quantitatively transfer the combined extracts to the K-D apparatus, add approximately 3 mL hexane as a keeper, add a boiling chip, and concentrate to 3–5 mL in a water bath maintained at 80°C.

6.12 Continue evaporation to approximately 0.5 mL in a warm water bath under nitrogen or helium stream using the N-Evap apparatus. Wash the walls of the tube with 5 mL hexane. Concentrate to 0.5 mL and wash the walls again with 5 mL hexane. Concentrate to 0.5 mL.

6.13 Bring the volume to 1 mL with hexane, and proceed to alumina column cleanup, if necessary.

6.13.1 Alumina column cleanup:

6.13.1.1 Quantitatively transfer the extract to the column using hexane. Elute the column with hexane until 6 mL has been eluted, and discard.

6.13.1.2 When the last of the hexane has just entered the sodium sulfate, elute the column with hexane-ethyl ether (2:1) until a 4-mL fraction has been collected.

6.13.1.3 Reduce the volume to 1.0 mL under a nitrogen or helium stream and proceed to gas chromatographic analysis.

6.14 Frequently use standard triazine mixtures to demonstrate the effectiveness of the alumina in characterizing the eluate composition and providing quantitative recovery.

6.15 Prepare gas chromatograph calibration curves daily with the mixed standards in table 9. Operating conditions must be identical to those used for sample analysis. Calibrate both analytical columns. Record the volume of the standard injected and the retention time and peak area of each component in the standard.

6.16 Chromatograph and identify the triazines by comparing retention time with standards (3-percent window for identification) using at least two of the dissimilar column packings described.

7. Calculations

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

$$RF = \frac{A_1}{C_s \times V_1},$$

where

RF = response factor of identified component in calibration standard, in area/pg,
C_s = concentration of standard, in pg/μL,
V_1 = volume of standard injected, in μL, and
A_1 = 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

$$\text{Concentration } (\mu g/L) = \frac{A_2 \times V_2}{V_3 \times W \times RF},$$

where

RF = response factor of identified calibration standard component, in area/pg,
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 μL, and

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

8. Report

Report concentrations of total recoverable triazines, alachlor, and trifluralin as follows: less than 0.1 µg/L, as "less than 0.1 µg/L"; 0.1 to 1.0 µg/L, one decimal; 1.0 µg/L and above, two significant figures.

9. Precision

9.1 Recovery and precision (seven replicates) for total recoverable triazines in tapwater are as follows:

Compound	Amount spiked (µg)	Mean percent recovery	Standard deviation	Relative standard deviation (percent)
Prometon	0.5	85.5	10.9	12.8
	1.0	94.4	6.60	6.99
	5.0	87.0	5.92	6.81
Propazine	.5	82.3	11.6	14.1
	1.0	97.0	4.90	5.05
	5.0	91.7	5.74	6.26
Atrazine	.5	85.1	11.8	13.9
	1.0	97.3	4.35	4.47
	5.0	87.1	5.26	6.04
Prometryn	.5	87.3	9.98	11.4
	1.0	98.3	3.97	4.04
	5.0	81.5	4.50	5.53
Simazine	.5	81.0	12.0	14.8
	1.0	96.6	8.68	8.99
	5.0	61.8	9.41	15.2
Ametryn	.5	89.6	10.5	11.7
	1.0	97.2	4.52	4.65
	5.0	91.4	5.70	6.24
Simetryn	.5	87.7	1.01	11.5
	1.0	92.2	6.42	6.97
	5.0	88.3	7.38	8.36

9.2 Recovery and precision (10 replicates) for total recoverable triazines from a natural surface water are as follows:

Compound	Amount spiked (µg)	Mean percent recovery	Standard deviation	Relative standard deviation (percent)
Prometon	0.5	95.1	5.83	6.13
	1.0	96.9	2.08	2.15
	5.0	84.6	4.58	5.41
Propazine	.5	96.2	5.46	5.68
	1.0	100.9	2.06	2.05
	5.0	87.6	4.62	5.28
Atrazine	.5	96.0	7.34	7.65
	1.0	98.1	2.11	2.15
	5.0	85.5	5.14	6.01
Prometryn	.5	98.0	5.09	5.20
	1.0	77.9	1.67	2.15
	5.0	83.5	3.82	4.57
Simazine	.5	96.9	4.90	5.05
	1.0	98.8	4.91	4.97
	5.0	82.8	7.69	9.29
Ametryn	.5	101.1	8.82	8.73
	1.0	101.5	3.83	3.77
	5.0	87.8	4.03	4.58
Simetryn	.5	98.7	7.17	7.27
	1.0	94.7	2.08	2.19
	5.0	86.4	4.23	4.90

Carbamate pesticides, total recoverable, high-performance liquid chromatographic (O-3107-83)

Parameter	Code
Aldicarb	None assigned.
Carbaryl	39750
Carbofuran	None assigned.
3-Hydroxycarbofuran	None assigned.
Methomyl	39051
1-Naphthol	None assigned.
Propham	39052

1. Application

This method is suitable for the determination of carbamate pesticides in water or water-suspended-sediment samples containing at least 2 µg/L of the analyte.

2. Summary of method

The carbamates are extracted from water or water-suspended-sediment mixtures with methylene chloride. The extract is concentrated and analyzed by high-performance liquid chromatography (HPLC) using a C_{18} reverse phase column and a dual-channel variable-wavelength ultraviolet detector.

3. Interferences

Compounds that exhibit chemical and physical properties similar to the compounds of interest can interfere.

4. Apparatus

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

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

4.3 *Filters*, 0.5 µm millipore FHUP, catalog no. 04700, or equivalent, and 0.45 µm millipore HAWP, catalog no. 04700, 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, a model 660 solvent flow programmer, a model WISP 710A microprocessor, and a data module, or equivalent.

4.4.1 The following conditions are recommended:

Columns, Waters Associates Radial Compression Module with a Radial PAK reverse-phase cartridge (octadecylsilane permanently bonded to unmodified silica), 10 µm particle size, or equivalent.

Wavelengths, 254 and 280 nm.

Solvent, 45 percent water and 55 percent methanol at a flow rate of 1.0 mL/min isocratic.

4.5 *Solvent clarification kit*, Waters Associates 85113, or equivalent.

5. Reagents

5.1 *Carbamate and metabolite standards*, EPA analytical reference grade or highest purity available: Prepare by dissolving 5 mg of standard in acetonitrile in a 50-mL volumetric flask, dilute to volume, and mix. Dilute this stock solution with acetone to the working concentrations listed in table 10.

Table 10. Concentration of carbamates in mixed standard solutions used for liquid chromatograph calibration

[Nanograms per microliter]

Carbamate	Low standard	Medium standard	High standard
Propham	9	18	36
Methomyl	2	4	8
Carbaryl	2	4	8

5.2 *Phosphate buffer*, pH 7.5: Mix 50 mL of 0.1 *M* KH_2PO_4 with 41 mL of 0.1 *M* NaOH and dilute to 100 mL with deionized water.

5.3 *Potassium dihydrogen phosphate*, 0.1 *M*: Dissolve 13.6 g KH_2PO_4 in deionized water and dilute to 1 L.

5.4 *Sodium hydroxide*, 0.1 *M*: Dissolve 4 g NaOH pellets in deionized water and dilute to 1 L.

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

5.6 *Solvents*, HPLC quality, acetonitrile, methanol, tetrahydrofuran, and pesticide grade methylene chloride.

5.7 *Water*, organic-free.

6. Procedure

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

6.1 Weigh the sample bottle plus the sample and record the weight. Pour the sample into a 1,000 mL-separatory funnel, add 10 mL phosphate buffer, and shake until well mixed. Weigh the empty sample bottle. Calculate the net sample weight and record the value obtained to three significant figures.

6.2 Add 75 mL methylene chloride to the sample bottle, swirl to rinse the sides of the bottle, and transfer the solvent to the 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 layer to separate and draw off the methylene chloride layer into a 250-mL Erlenmeyer flask that contains 1 g anhydrous sodium sulfate.

6.3 Repeat the extraction of the water sample two more times, using 50 mL methylene chloride each time. Combine all the organic extracts in the 250-mL Erlenmeyer flask containing the first extract.

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 4 mL acetonitrile.

6.5 Place the apparatus on a hot-water bath (75–85°C) until the volume is reduced to about 4 mL. Remove from the heat and allow to cool. Wipe the joints with a towel. Rinse the bottom joint with acetonitrile into the receiver.

6.6 Further reduce the volume of solvent to about 1 mL on an evaporative concentrator with the water bath at 35°C. Rinse down the sides of the tube with 1 mL acetonitrile and concentrate to a final volume of 1.0 mL. Stopper until chromatographic analysis can begin.

6.7 Prepare the solvents for the mobile phase by filtering, using the solvent clarification kit and HAWP filters for water and FHUP filters for the organic solvents.

6.8 Prepare liquid chromatograph calibration curves daily by injecting the reference standards listed in table 10. Operating conditions need to be

identical to those used for sample analysis in step 6.9. Record the volume of the standard injected and the retention time and integrated peak area of each component in the standard.

6.9 Inject an aliquot of sample extract into the calibrated liquid chromatograph. Record the volume injected. Identify the peaks by retention time. Confirmation is obtained by measuring the peak area at the two different wavelenghths (254 and 280 nm) 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.

7. Calculations

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

$$RF = \frac{A_1}{C_s \times V_1},$$

where

RF = response factor of identified component in calibration standard, in area/ng,
C_s = concentration of standard component, in ng/μL,
V_1 = volume of standard injected, in μL, and
A_1 = integrated peak area of identified component in calibration standard.

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

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

where

RF = response factor of identified calibration standard, 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 μL, and
W = weight of sample in g, expressed in mL (1.000 mL = 1.000 g).

8. Report

Report concentrations of carbamates in water or water–suspended-sediment mixtures as follows: less than 2 μg/L, as "less than 2.0 μg/L"; 2.0 μg/L and above, two significant figures.

9. Precision

Single-operator precision was determined by spiking surface-water samples at four concentrations and four replicates performed on different days. Results are as follows:

Compound	Concentration spiked (μg/L)	Mean concentration recovered (μg/L)	Relative standard deviation (percent)
Methomyl	2.51	2.13	20
	5.02	3.80	11
	10.0	7.47	9.4
	20.1	15.5	7.3
Carbaryl	2.36	2.33	5.7
	4.72	4.58	4.8
	9.44	9.18	5.5
	18.9	18.6	3.4
Propham	7.05	5.68	13
	14.1	11.2	8.0
	28.2	22.2	7.7
	56.4	43.0	8.2

Selected references

Goerlitz, D.F., and Brown, Eugene, 1972, Methods for analysis of organic substances in water: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A3, 40 p.

Sparacine, C.M., and Hines, J.W., 1976, High-performance liquid chromatography of carbamate pesticides: Journal of Chromatography Science, v. 14, p. 549–555.

Oil and grease, extractable, extraction-gravimetric (O-3108-83)

Parameter	Code
Oil and grease, total (mg/L as oil and grease)	00556

1. Application

This method is suitable for the determination of oil and grease in water–suspended-sediment mixtures containing at least 1 mg/L of the analyte.

2. Summary of method

2.1 A sample is extracted twice with trichlorotrifluoroethane and the extract is evaporated at 20°C to leave a nonvolatile residue whose weight represents an estimate of the extractable organic matter in the sample.

2.2 The procedure approximates the determination of oils and grease in water, and is similar to Method 2778–70 described by the American Society for Testing and Materials (1982) and to Method 137, Oil and Grease, described by the American Public

Health Association and others (1981) in "Standard Methods for the Examination of Water and Wastewater."

3. Interferences

Organic solvents vary considerably in their ability to dissolve oil substances and other organic matter. Any method used to obtain an estimate of the amount of extractable organic matter must, of necessity, be highly empirical. Close attention to all operations of the analytical procedure is required to obtain reproducible results.

4. Apparatus

4.1 *Dish*, aluminum foil, 110 mm in diameter, 100-mL capacity.

4.2 *Funnel*, separatory, pear-shaped, 2-L capacity (Corning 6404, or equivalent).

5. Reagents

5.1 *Sodium sulfate*, anhydrous, granular.

5.2 *Sulfuric acid*, concentrated (sp. gr. 1.84).

5.3 *Sulfuric acid*, (1 + 1): Slowly and cautiously, with constant stirring and cooling, add 100 mL concentrated H_2SO_4 to 100 mL demineralized water.

5.4 *Trichlorotrifluoroethane solvent*, 1,1,2-Trichlorotrifluoroethane, b.p. 47.6°C, reagent grade.

6. Procedure

6.1 Collect approximately 900 mL of sample in a 1-L glass bottle.

6.2 Transfer the entire contents of the glass bottle to a 1,000-mL graduated cylinder. Record the volume. Prepare a 900-mL demineralized water blank and carry it through the sample-analysis procedure. Subtract the residual weight (blank) from the sample extract residual weight. If the weight of the blank exceeds 4.0 mg, a new bottle of solvent must be obtained to provide a blank of 4.0 mg or less.

6.3 Transfer the sample from the graduated cylinder to a 2-L separatory funnel, and add 5 mL sulfuric acid (1 + 1). Shake to mix thoroughly.

6.4 Rinse the glass bottle with 25 mL trichlorotrifluoroethane solvent and transfer the solvent to the graduated cylinder. Rinse the graduate and transfer the solvent to the separatory funnel. Shake vigorously for 2 min, stopping to vent the pressure as necessary.

6.5 Allow the layers to separate and then draw off the solvent and filter it through a small amount of anhydrous Na_2SO_4 placed on a small filter paper (Whatman No. 40, or equivalent) in a funnel. Collect the filtrate in a tared aluminum-foil dish.

6.6 Repeat steps 6.4 and 6.5, filtering the solvent through the same funnel and adding the filtrate to that already collected in the aluminum-foil dish.

6.7 Wash the filter paper with three 5-mL portions of solvent, collecting all washings in the aluminum-foil dish.

6.8 Evaporate the solvent collected in the aluminum-foil dish at room temperature (20°C) in a well-ventilated fume hood.

6.9 Rinse the inside of the aluminum-foil dish with demineralized water to remove traces of sulfuric acid. Dry the dish in a desiccator to remove water.

6.10 Weigh the residue in the dish after the water has evaporated.

7. Calculations

Determine the mg/L extractable organic matter in the samples as follows:

$$\text{Organic matter, extractable, mg/L} = \frac{R_x - R_b}{\text{mL sample}} \times 1{,}000,$$

where

R_x = weight of extracted residue, in mg, and

R_b = weight of solvent residue (blank), in mg.

8. Report

Report organic matter, extractable, water–suspended-sediment, concentrations as follows: less than 10 mg/L, nearest mg/L; 10 mg/L and above, two significant figures.

9. Precision

Precision data cannot be given for this determination because of the variable nature of the materials being extracted.

Selected references

American Public Health Association, 1981, Standard methods for the examination of water and wastewater (15th ed.): Washington D.C., American Public Health Association, Inc., p. 461.

American Society for Testing and Materials, 1982, Annual Book of ASTM Standards, Part 31, Water: Philadelphia, American Society for Testing and Materials, p. 627.

Oil and grease, extractable from bottom material, extraction-gravimetric (O-5108-83)

Parameter	*Code*
Oil and grease, recoverable from bottom material (mg/kg as oil and grease)	00557

1. Application

1.1 This method is suitable for the determination of oil and grease in air-dried bottom materials containing at least 1,000 mg/kg.

1.2 This method may be used for the determination of oil and grease in wet bottom materials if the appropriate moisture correction is applied.

2. Summary of method

A sample is extracted twice with trichlorotrifluoroethane and the extract is evaporated at 20°C to leave a nonvolatile residue whose weight represents an estimate of the extractable organic matter in the sample.

3. Interferences

Organic solvents vary considerably in their ability to dissolve oil substances and other organic matter. Any method used to obtain an estimate of the amount of extractable organic matter must, of necessity, be highly empirical. Close attention to all operations of the analytical procedure are required to obtain reproducible results.

4. Apparatus

4.1 *Dish*, aluminum foil, 110 mm in diameter, 100-mL capacity.

4.2 *Funnel*, separatory, pear-shaped, 2-L capacity (Corning 6404, or equivalent).

5. Reagents

5.1 *Sodium sulfate*, anhydrous, granular.

5.2 *Sulfuric acid*, concentrated (sp. gr. 1.84).

5.3 *Sulfuric acid*, (1 + 1): Slowly and cautiously, with constant stirring and cooling, add 100 mL concentrated H_2SO_4 to 100 mL demineralized water.

5.4 *Trichlorotrifluoroethane solvent*, 1,1,2-Trichlorotrifluoroethane, b.p. 47.6°C, reagent grade.

6. Procedure

6.1 Weigh, to the nearest mg, approximately 1 g of air-dried sample material. Alternatively, a wet sample may be weighed if a correction is made for moisture content.

6.2 Quantitatively transfer the weighed sample to a 2-L-capacity separatory funnel. Add approximately 900 mL demineralized water and shake to mix. Prepare a 900-mL demineralized water blank and carry it through the sample-analysis procedure. Subtract the residual weight (blank) from the sample extract residual weight. If the weight of the blank exceeds 4.0 mg, a new bottle of solvent must be obtained to provide a blank of 4.0 mg or less.

6.3 Add 5 mL sulfuric acid (1 + 1). Shake to mix thoroughly.

6.4 Add 25 mL trichlorotrifluoroethane and shake vigorously for 2 min, stopping to vent the pressure as necessary.

6.5 Allow the layers to separate and then draw off the solvent and filter it through a small amount of anhydrous Na_2SO_4 placed on a small filter paper (Whatman No. 40, or equivalent) in a funnel. Collect the filtrate in a tared aluminum-foil dish.

6.6 Repeat steps 6.4 and 6.5, filtering the solvent through the same funnel and adding the filtrate to that already collected in the aluminum-foil dish.

6.7 Wash the filter paper with three 5-mL portions of solvent, collecting all washings in the aluminum-foil dish.

6.8 Evaporate the solvent collected in the aluminum-foil dish at room temperature (20°C) in a well-ventilated fume hood.

6.9 Rinse the inside of the aluminum-foil dish with demineralized water to remove traces of sulfuric acid. Dry the dish in a desiccator to remove water.

6.10 Weigh the residue remaining in the dish after the water has evaporated.

7. Calculations

7.1 Determine the mg/kg extractable organic matter in the air-dried sample as follows:

$$\text{Organic matter, extractable, mg/kg} = \frac{R_x - R_b}{\text{sample weight in g}} \times 1{,}000,$$

where

R_x = weight of extracted residue, in mg, and

R_b = weight of solvent residue (blank), in mg.

NOTE: If wet bottom-material sample is used in preference to air-dried sample, a factor correcting for moisture content must be applied to above equation.

8. Report

8.1 Report organic matter, extractable, air-dried bottom material, concentrations as follows: less than 10,000 mg/kg, nearest 1,000 mg/kg; 10,000 mg/kg and above, two significant figures.

9. Precision

Precision data cannot be given for this determination because of the variable nature of the materials being extracted.

Fuel oils, light, total recoverable, gas chromatographic (O-3109-83)

Parameter *Code*

Fuel oils, light, total recoverable (mg/L) - - - - None assigned.

1. Application

This method is suitable for the determination of light fuel oils (C_{10}–C_{22}) in water or water–suspended-sediment mixtures at concentrations of 0.1 mg/L and above.

2. Summary of method

A water or water–suspended-sediment sample containing light fuel oils (diesel oils) is acidified and extracted with hexane. The extract is concentrated and analyzed by temperature-programmed gas chromatography using a flame-ionization detector. These oils possess a characteristic envelope which appears during the temperature-programmed run. This envelope, composed of the various hydrocarbon peaks, approximates the boiling range profile of the oil. The area of the envelope is reproducible and is the basis for quantitation. Characterization is based on comparison of the peaks in the residue chromatogram with those of a known oil sample.

3. Interferences

Any compound having chemical and physical properties similar to an analyte of interest may interfere.

4. Apparatus

4.1 *Boiling chip*, micro, granules, Hengar H-1366C, or equivalent.

4.2 *Concentrator apparatus*, Kuderna-Danish (K-D) type, with 10.0- and 4.0-mL receivers, 200- and 500-mL flasks, and a one-ball Snyder column.

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

4.4 *Gas chromatograph*, Hewlett-Packard 5710, or equivalent.

4.4.1 The following conditions are recommended:

Columns, borosilicate glass, 1.8 m×2 mm id (inside diameter). Column packing is 5 percent OV-101 on 100/120 mesh Gas Chrom Q, or equivalent. A capillary column operated under appropriate conditions may also be used.

Temperature program, 3-min postinjection hold, 100°C to 230°C at 8°C/min, hold for 2 min, end of cycle.

Detector, flame-ionization operated at 250°C.

Injection port temperature, 210°C.

Carrier gas, helium, flow rate 30 mL/min.

5. Reagents

5.1 *Fuel oil standard*: A sample of the oil being determined must be available from the manufacturer or other suitable source. The Environmental Protection Agency Environmental Monitoring and Support Laboratory in Cincinnati, Ohio, has several standard oil references. Weigh about 10 mg of standard to at least three significant figures and quantitatively transfer it to a 25-mL volumetric flask. Dilute to volume with iso-octane. Prepare standards in iso-octane at concentrations of 100, 1,000, and 10,000 ng/μL.

5.2 *Sodium sulfate*, granular, anhydrous: The sodium sulfate should be heated at 300°C overnight and stored in a stoppered, glass container.

5.3 *Solvents*, acetone and hexane, pesticide residue quality, distilled in glass, Burdick and Jackson, or equivalent.

5.4 *Sulfuric acid*, concentrated (sp. gr. 1.84).

5.5 *Sulfuric acid*, 2 *N*: Add 53 mL concentrated H_2SO_4 slowly to 500 mL water. Cool and dilute to 1 L.

5.6 *Water*, reagent grade, organic-free.

6. Procedure

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

6.1 Refrigerate the sample until extraction. The extraction must be performed within 24 to 48 h after receipt to minimize oil degradation.

6.2 Weigh the bottle containing the sample and record the weight. Pour the sample into a 1-L

separatory funnel and weigh the empty sample bottle. Calculate the net sample weight and record the value obtained to three significant figures. Use 2 N sulfuric acid to adjust the pH to 1 to 2.

6.3 Add 20 mL hexane to the sample bottle, swirl, and pour into a separatory funnel. Allow bottle to drain.

6.4 Shake the funnel vigorously for 1 min, venting often to relieve pressure. Decant the solvent layer into a 500-mL Erlenmeyer flask.

6.5 Repeat steps 6.3 and 6.4 twice, for a total of three extractions.

6.6 Add about 5 g anhydrous sodium sulfate to the Erlenmeyer flask containing the extracts and allow to stand covered for at least 4 h.

6.7 Quantitatively transfer the extract to a K-D apparatus. Add a boiling chip, and concentrate on a water bath to about 5 mL. Remove the apparatus and dry the joints with a towel. Rinse the lower joint with hexane into the receiver.

6.8 Continue concentration on an evaporative concentrator until the volume is reduced to about 0.5 mL. Wash the walls of the receiver with small portions of hexane during concentration and dilute to 1.0 mL with hexane.

6.9 Prepare gas chromatograph calibration curves daily by injecting the series of reference standards. Operating conditions must be identical for samples and standards. Record the volume of the standard injected.

6.10 Inject an aliquot of sample extract into the gas chromatograph. Record the volume injected. Dilute any extract containing fuel oil in a concentration greater than the highest standard.

6.11 Determine the area under the envelope of the fuel oil standard. Choose the beginning and ending peaks so that a reproducible area will be measured when quantitating different standards and samples. Use the equations is section 7 to calculate the concentration of fuel oil in the sample.

7. Calculations

7.1 Calculate the response factor for the calibration standard:

$$RF = \frac{A_1}{V_1 \times C_s},$$

where

RF = response factor of fuel oil in calibration standard, in area/ng,
C_s = concentration of standard, in ng/μL,
V_1 = volume of standard injected, in μL, and
A_1 = integrated peak area of calibration standard.

7.2 Calculate the concentration of fuel oil in the original water sample from the equation

$$\text{Concentration (mg/L)} = \frac{A_2 \times V_2}{V_3 \times W \times RF},$$

where

RF = response factor of fuel oil in calibration standard, in area/ng,
A_2 = integrated peak area of sample,
V_2 = final volume of sample extract, in mL,
V_3 = volume of sample extract injected, in μL, and
W = weight of sample in g, expressed in mL (1.000 mL = 1.000 g).

8. Report

Report concentrations of fuel oil in water or water–suspended-sediment mixtures as follows: less than 0.02 mg/L, as "less than 0.02 mg/L"; 0.02 to 0.1 mg/L, two decimals, 0.1 mg/L and above, two significant figures.

9. Precision

Single-operator precision data for No. 2 diesel fuel spiked into distilled and natural water samples are as follows:

Concentration of spiked fuel oil sample (mg/L)	*Number of replicates*		*Mean concentration determined (mg/L)*		*Relative standard deviation (percent)*	
	Distilled water	*Natural water*	*Distilled water*	*Natural water*	*Distilled water*	*Natural water*
0.085	5	5	0.064	0.058	28	22
.85	5	5	.68	.67	4.8	10
8.5	5	4	7.0	6.8	1.7	7.3

Phenols, total recoverable, colorimetric, 4-aminoantipyrine (O-3110-83)

Parameter	*Code*
Phenols, total recoverable (μg/L as phenol)	32730

1. Application

This method may be used to analyze water or water–suspended-sediment mixtures containing at least 1.0 μg/L of phenolic material.

2. Summary of method

Steam-distillable phenols react with 4-aminoantipyrine at pH 10.0 ± 0.2 in the presence of potassium ferricyanide to form a colored antipyrine

dye. This dye is extracted from aqueous solution with chloroform, and the absorbance is measured at 460 nm. This method is similar in principle to, but different in detail from ASTM Method D 1783–80 (American Society for Testing and Materials, 1982).

3. Interferences.

Phenol has been selected as the standard for reference. Substituted phenols may produce less color than phenol. The concentration of phenols determined by this method represents the minimum concentration of phenolic compounds present in the sample.

Certain bacteria, oxidizing and reducing substances, and highly alkaline waste waters may interfere with this method. Information for removal of major interferences may be found in ASTM Method D 1783–80.

4. Apparatus

4.1 *Distillation apparatus*, all glass, consisting of a 1-L Pyrex distilling apparatus and a water-cooled condenser (Corning 3360, or equivalent).

4.2 *Funnels*, Buchner type with fritted-glass disk (15-mL Corning 36060, or equivalent).

4.3 *Photometer*, spectrophotometer or filter photometer operating at 460 nm, and accommodating cells having light paths of 1.0 and 10 cm.

4.4 *Funnel*, Separatory, pear-shaped, 1,000 mL (Corning 6404, or equivalent).

5. Reagents

All reagents must be prepared with phenol-free distilled water. Deionized water is usually not satisfactory.

5.1 *Aminoantipyrine solution*, 2 g/100 mL: Dissolve 2.0 g of 4-aminoantipyrine in distilled water and dilute to 100 mL. This solution is not stable and must be prepared each day.

5.2 *Ammonium chloride solution*, 20 g/L: Dissolve 20 g reagent-grade ammonium chloride in water and dilute to 1 L.

5.3 *Ammonium hydroxide*, concentrated (sp. gr. 0.90), reagent grade.

5.4 *Chloroform*, spectrophotometric grade.

5.5 *Copper sulfate solution*, 100 g/L: Dissolve 100 g of $CuSO_4 \cdot 5H_2O$ in water and dilute to 1 L.

5.6 *Phenol standard solution*, 1.00 mL = 1.00 mg phenol: Dissolve 1.00 g analytical reagent phenol in 1,000 mL freshly boiled and cooled distilled water. Solution is stable for 1 mo.

5.7 *Phosphoric acid solution*: Dilute 10 mL of 85 percent H_3PO_4 to 100 mL with phenol-free water.

5.8 *Potassium ferricyanide solution*, 8 g/100 mL: Dissolve 8.0 g of $K_3Fe(CN)_6$ in water, dilute to 100 mL, and filter. This solution is not stable and must be prepared each day.

5.9 *Sodium sulfate*, anhydrous, granular, ACS reagent grade.

6. Procedure

Samples should be protected from light and analyzed as soon as possible. The analyst is referred to "Standard Methods for the Examination of Water and Wastewater," 15th edition (American Public Health Association 1981) for the analysis of very alkaline or highly polluted water.

6.1 Measure a volume of sample containing less than 50 µg phenol (500 mL maximum) into a beaker. If less than 500 mL of sample is used, dilute sample with distilled water to 500 mL. Determine the pH and adjust to below 4.0, if necessary. (Add 5.0 mL copper sulfate solution if it was not added at sampling.) Transfer the solution to the distillation apparatus, add boiling stones, and distill. Collect 450 mL distillate and stop. Add 50 mL distilled water to the residue and proceed with distillation until 500 mL of distillate is collected.

6.2 Prepare a 500-mL distilled-water blank. Also prepare 500-mL standards containing 5, 10, 20, 30, 40, and 50 µg phenol, using the standard phenol solution.

6.3 Treat the sample, blank, and standards as follows: Add 1.0 mL ammonium chloride solution and 1.0 mL ammonium hydroxide, mix, and adjust the pH to 10.0 ± 0.2 with concentrated ammonium hydroxide. Add 3.00 mL aminoantipyrine solution and mix. Add 3.00 mL potassium ferricyanide solution and again mix. Allow the color to develop for 3 min. A clear to light-yellow solution should result.

6.4 Add 25.0 mL chloroform for 1-and 5-cm cells and 50.0 mL chloroform for 10-cm cells. Shake the separatory funnel vigorously for 1 min. Allow the layers to separate and repeat the shaking to achieve a higher recovery.

6.5 After the layers have separated, draw off the lower chloroform layer and filter through a 5-g layer of anhydrous sodium sulfate, using a sintered-glass funnel, directly into an appropriate absorption cell. Avoid working in a draft so as to reduce evaporation of the solvent.

6.6 Measure the absorbance of the sample and standards against the blank at 460 nm. Prepare a calibration curve plotting absorbance versus µg of phenol.

7. Calculations

Calculate the phenolic content in the sample, in µg/L, as follows:

$$\text{Phenolic material (µg/L)} = \frac{\text{µg phenol in sample} \times 1{,}000}{\text{mL original sample}}.$$

8. Report

Report concentrations of phenolic material as follows: less than 1 µg/L, as "less than 1 µg/L"; 1 to 100 µg/L, nearest µg/L; 100 µg/L and above, two significant figures.

9. Precision

Single-operator precision (10 replicates) for phenol spiked into distilled water is as follows:

Phenol concentration (µg/L)	*Mean found (µg/L)*	*Relative standard deviation (percent)*
4.0	4.5	12
40	37	5.5

Selected references

American Public Health Association, 1981, Standard methods for the examination of water and wastewater (15th ed.): Washington D.C., American Public Health Association, Inc., 1,134 p.

American Society for Testing and Materials, 1982, Annual book of ASTM standards, Part 31, Water: Philadelphia, American Society for Testing and Materials, p. 789.

Goerlitz, D.F., and Brown, Eugene, 1972, Methods for analysis of organic substances in water: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A3, 40 p.

Methylene blue active substances, total recoverable, colorimetric (O-3111-83)

Parameter	*Code*
Methylene blue active substances, total recoverable (mg/L as MBAS)	38260

1. Application

This method is applicable to the analysis of water or water–suspended-sediment mixtures containing at least 0.01 mg/L methylene blue active substances (MBAS) relative to linear alkyl sulfonate (LAS) standard.

2. Summary of method

Methylene blue reacts with anionic surfactants, both alkyl benzene sulfonates (ABS) and LAS, to form a blue-colored dye complex. The complex is extracted with chloroform, and the methylene blue active substances are determined spectrophotometrically. This method is similar in substance to the MBAS method in "Standard Methods for the Examination of Water and Wastewater," 15th edition (American Public Health Association, 1981).

3. Interferences

Phenols, proteins, and inorganic chloride, cyanate, nitrate, and thiocyanate will complex methylene blue and give a positive interference. With LAS concentrations from 0.0 to 0.1 mg/L, tests have shown no interference from the following individual constituents: 10 mg/L nitrite, 25 mg/L nitrate, 5 mg/L phenol, and 1 mg/L hydrogen sulfide. Organic amines cause low results.

4. Apparatus

4.1 *Spectrophotometer*, for use at 635 nm.

4.2 Refer to the manufacturer's manual to optimize instrument.

5. Reagents

5.1 *Chloroform*, spectrophotometric grade.

5.2 *Detergent*, primary stock standard, 1 mL = 1 mg LAS: Use reference LAS acid. An ampoule of LAS acid may be obtained from the Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH 45268. The ampoule has a shelf life of 2 yr when stored unopened in a cool, dark location. The LAS ampoule is labeled in percent active LAS (weight/volume). Weigh an amount of LAS acid equal to 1,000 g LAS on a 100 percent basis and dilute with demineralized water to 1,000 mL. To obtain the amount of active LAS, divide 1.000 g by the percent active stated on the ampoule label. For example, if the LAS solution is stated as 5.69 percent active, multiply 1.000 by 100 and divide by 5.69. The result, 17.575g, is the amount of LAS solution to be weighed out and diluted with demineralized water to 1,000 mL.

5.3 *Detergent working standard I*, 1.00 mL = 0.01 mg LAS: Dilute 10 mL of primary stock

standard to 1,000 mL with demineralized water. This has a shelf life of 1 mo when refrigerated.

5.4 *Detergent working standard II*, 1.00 mL = 0.001 mg LAS: Dilute 100 mL of working standard I to 1,000 mL with demineralized water. Prepare fresh daily.

5.5 *Methylene blue reagent*: Dissolve 0.35 g methylene blue in 0.01 *N* H_2SO_4 and dilute to 1 L with 0.01 *N* H_2SO_4.

5.6 *Phenolphthalein solution*: Dissolve 2.5 g phenolphthalein in 250 mL of 95 percent ethyl alcohol and add 250 mL deionized water. Then add 0.02 *N* NaOH dropwise until a faint pink color appears.

5.7 *Sodium hydroxide solution*, 0.5 *N*: Dissolve 10 g NaOH in deionized H_2O and dilute to 500 mL.

5.8 *Sulfuric acid solution*, 5 *N*: Cautiously, mix with cooling, 138 mL concentrated H_2SO_4 (sp. gr. 1.84) in 500 mL deionized water and dilute to 1,000 mL with deionized water.

5.9 *Sulfuric acid solution*, 0.1 *N*: Cautiously, mix 3 mL concentrated H_2SO_4 (sp. gr. 1.84) with deionized water and dilute to 1 L.

6. Procedure

All glassware must be rinsed with dilute HCl and deionized water immediately before use.

6.1 Pipet a volume of sample (100 mL maximum) containing less than 0.10 mg MBAS into a separatory funnel. Prepare a blank and standards in the same manner.

6.2 Add 2 drops of phenolphthalein solution to the samples and standards and adjust the pH to near neutral by the dropwise addition of 0.5 *N* NaOH or 0.1 *N* H_2SO_4. To the blank, standards, and samples add 1.0 mL of 5 *N* H_2SO_4, mix, and then add 5.0 mL methylene blue solution. Mix thoroughly.

6.3 Add 25.0 mL chloroform and shake the contents of the funnel for 30 s. Allow the layers to separate.

6.4 Drain off the lower chloroform layer into a 1.0-cm cell and measure the absorbance of the sample and standards against the blank at 635 nm.

7. Calculations

Determine the amount of detergent contained in the sample, minus the blank, from the analytical curve. Calculate the amount of MBAS in the sample using the following equation:

$$\text{mg/L MBAS (as LAS)} = \frac{W \times 1000}{S},$$

where

W = mg MBAS obtained from calibration curve minus mg blank MBAS, and

S = mL of sample.

8. Report

Report MBAS concentrations as follows: less than 0.01 mg/L, as "less than 0.01 mg/L"; 0.01 to 1.0 mg/L, two decimals; 1.0 mg/L and above, two significant figures.

9. Precision

Deviation of ±10 percent may be expected in the range of 1 to 5 mg/L, in surface water.

Selected references

American Public Health Association, 1981, Standard methods for the examination of water and wastewater (15th ed.): Washington, American Public Health Association, Inc., 1,134 p.

Goerlitz, D.F., and Brown, Eugene, 1972, Methods for analysis of organic substances in water: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A3, 40 p.

TNT, RDX, and picric acid, total recoverable, high-performance liquid chromatographic (O-3112-83)

Parameter	*Code*
TNT	81360
RDX	81364
Picric acid	82340

1. Application

This method is suitable for the determination of 1,3,5-trinitro-1,3,5-triaza-cyclohexane (RDX), 2,4,6-trinitrophenol (picric acid), and 2,4,6-trinitrotoluene (TNT) in water or water–suspended-sediment mixtures containing at least 2 µg/L of analyte.

2. Summary of method

RDX, picric acid, and TNT are extracted from water or water–suspended-sediment mixtures with methylene chloride. The extract is concentrated and subjected to high-performance liquid chromatography (HPLC) analysis using a reverse-phase column and a dual-wavelength ultraviolet detector.

3. Interferences

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

4. Apparatus

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

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

4.3 *Filters*, 0.5 μm millipore FHUP, catalog no. 04700, or equivalent, and 0.45 μm millipore HAWP, catalog no. 04700, 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, a model 660 solvent-flow programmer, a model WISP 710A microprocessor, and a data module, or equivalent.

4.4.1 The following conditions are recommended:

Column, 300 mm×3.9 mm id (inside diameter) stainless steel packed with 10 μm particle size reverse-phase material: Waters Associates *u*–Bonapak C_{18} packing, or equivalent.

Wavelengths, 254 and 356 nm.

Solvent, 36 percent solution A (step 5.2.1) and 64 percent solution B (step 5.2.2) at a flow rate of 1.10 mL/min isocratic.

4.5 *Solvent clarification kit*, Waters Associates 85113, or equivalent.

4.6 *Shaker*, mechanical, wrist-action.

5. Reagents

5.1 *Hydrochloric acid*, concentrated (sp. gr. 1.19), reagent grade.

5.2 *Mobile phase solutions*: Prepare the ion pairing, tetrabutylammonium-phosphate solutions for the mobile phase using Waters Associates PIC Reagent A, or equivalent.

5.2.1 *Solution A*: Add one bottle of PIC reagent A to 1,000 mL organic-free water. Stir for 5 min and then filter through a 0.45-μm filter, type HAWP for aqueous solvents.

5.2.2 *Solution B*: Add one bottle of PIC reagent A to 1,000 mL acetonitrile. Stir for 5 min and then filter through a 0.45-μm filter, type FHUP for organic solvents.

5.3 *RDX, picric acid, and TNT standards*, analytical reference grade or highest purity available: These may be obtained from chemical specialty suppliers or from military sources. Weigh about 10 mg to three significant figures and dilute to 25 mL with acetonitrile. Prepare standards at 1, 5, and 10 ng/μL in acetonitrile.

5.4 *Sodium chloride*, anhydrous, granular: Prepare by heating at 300°C overnight.

5.5 *Solvents*, acetone, diethylether (unpreserved) methylene chloride, pesticide residue quality, distilled in glass, Burdick and Jackson, or equivalent: HPLC-grade acetonitrile.

5.6 *Water*, organic-free.

6. Procedure

6.1 Pour 500 mL water into a 1-L separatory funnel. Do not use stopcock grease on ground-glass joints.

6.2 Add 3 mL concentrated HCl and 85 g NaCl to the sample contained in the separatory funnel and shake until the salt is dissolved.

6.3 Add 75 mL methylene chloride to the separatory funnel. Stopper and shake for 1 min. Vent the pressure often. Allow the layers to separate and draw off the methylene chloride layer into a 250-mL Erlenmeyer flask.

6.4 Repeat the extraction twice using 50 mL methylene chloride each time. Combine the extracts in the 250-mL Erlenmeyer flask.

6.5 Quantitatively transfer the extract to a 500-mL K-D apparatus fitted with a three-ball Snyder column and a 20-mL receiver. Add a micro boiling chip and 4 mL acetonitrile.

6.6 Place the K-D apparatus on a hot-water bath (approximately 85°C). Reduce the volume of the extract to about 4 mL. Remove the K-D apparatus and allow it to cool. Wipe the joints with a towel. Rinse the lower joint with acetonitrile into the receiver.

6.7 Use the evaporative concentrator to reduce the volume of solvent to 0.8–0.9 mL by directing a stream of nitrogen onto the surface of the extract in the receiver in a water bath at 35°C.

6.8 Proceed to HPLC analysis.

6.9 The chromatographic system should be evaluated each day to determine retention volume and detector response for RDX, picric acid, and TNT. Prepare calibration curves daily by injecting 10 μL of a standard mixture at three concentrations (see step 5.3). Record the volume of the standard injected and the retention time and integrated peak area of each component in the standard. The operating conditions used for calibration must be identical to those used for sample analysis (step 6.10).

6.10 Add 0.1 mL PIC reagent A to the sample extract, mix, and make up to a volume of 1.0 mL; allow the extract to stand 10 min, and then inject 10 µL 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 the peak area ratio of the standard. Dilute any extract containing an identifiable component above the highest standards.

7. Calculations

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

$$RF = \frac{A_1}{C_s \times V_1},$$

where

RF = response factor of identified component in calibration standard, in area/ng,
C_s = concentration of standard component, in ng/µL,
V_1 = volume of standard injected, in µL, and
A_1 = 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

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

where

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 µL, and
W = weight of sample in g, expressed in mL (1.000 mL = 1.000 g).

8. Report

Report concentrations of TNT, RDX, and picric acid in water or water–suspended-sediment mixtures as follows: less than 2 µg/L, as "less than 2 µg/L"; 2.0 µg/L and above, two significant figures.

9. Precision

Single-operator precision for eight replicates, expressed in terms of percent relative standard deviation, is as follows:

Compound	Concentration spiked (µg/L)	Mean concentration recovered (µg/L)	Relative standard deviation (percent)
RDX	2.0	2.2	37
	4.0	3.9	20
	8.0	7.9	12
	16	16	11
Picric acid	2.0	2.2	10
	4.0	4.0	10
	8.0	7.6	4
	16	16	5
TNT	2.0	1.7	23
	4.0	3.0	17
	8.0	6.8	10
	16	14	11

Selected references

Goerlitz, D.F., and Law, L.M., 1975, Gas chromatographic method for the analysis of TNT and RDX explosives contaminating water and soil-core material: U.S. Geological Survey Open-File Report 75–182, 21 p.

——— 1979b, Direct analyses of RDX and TNT in water by high-performance liquid chromatograph: U.S. Geological Survey Open File Report 79–916, 11 p.

Goerlitz, D.F., 1979a, Analysis of picric acid in water by high-performance liquid chromatography: U.S. Geological Survey Open File Report 79–207, 7 p.

Polynuclear aromatic hydrocarbons (PNA), total recoverable, high-performance liquid chromatographic (O–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 µg/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-μm 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-μm 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 C_{18}–10 μm, 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/μL 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/μL 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°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 inject-

ed. 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:

$$RF = \frac{A_1}{C_s \times V_1},$$

where

RF = response factor of identified component in calibration standard, in area/ng,
C_s = concentration of standard component, in ng/μL,
V_1 = volume of standard injected, in μL, and
A_1 = 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

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

where

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 μL, 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 μg/L, as "less than 1 μg/L"; 1 to 10 μg/L, one significant figure; 10 μg/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 (μg/L)*	*Mean concentration recovered (μg/L)*	*Relative standard deviation (percent)*
Naphthalene	1.3	0.94	6.9
	2.6	1.41	20
	5.1	2.4	24
Fluorene	.64	.47	12
	1.3	.94	7.8
	2.6	1.6	14
Acenaphthene	2.8	2.01	8.5
	5.50	3.6	13
	11.	6.5	18
Phenanthrene	.21	.15	8.3
	.42	.36	6.3
	.84	.66	8.5
Anthracene	.052	.038	3.9
	.10	.076	5.1
	.21	.142	7.4
Pyrene	.32	.28	2.8
	.64	.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	.43	.39	3.0
	.85	.80	3.4
	1.7	1.6	5.5
Benzo(a)pyrene	.15	.13	4.8
	.29	.29	7.5
	.58	.55	5.5
Dibenz(a,h)anthracene	.13	.12	6.9
	.26	.28	7.4
	.51	.50	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	.64	.57	5.1
	1.3	1.3	5.2
	2.6	2.3	7.2

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Keith, L.H., and Telliard, W.A., 1979, Priority pollutants: Environmental Science and Technology, v. 13, p. 416–423.

Kuratsune, M., and Hirohata, T., 1962, Decomposition of polycyclic aromatic hydrocarbons under laboratory illuminations: National Cancer Institute Monograph 9, p. 117–125.

Lee, M.L., and Wright, B.W., 1980, Capillary column gas chromatography of polycyclic aromatic compounds—A review: Journal of Chromatographic Science, v. 18, p. 345–358.

White, W.R., and Grabbe, R.R., 1981, High-performance, liquid-chromatographic method for the analysis of polynuclear aromatic hydrocarbons in water: U.S. Geological Survey Water-Resources Investigations 81–63, 13 p.

Ethylene and propane, total recoverable, gas chromatographic, purge and trap (O–3114–83)

Parameter	*Code*
Ethylene	82357
Propane	82358

1. Application

This method is suitable for the determination of ethylene and propane in water or water–suspended-sediment mixtures containing at least 0.1 µg/L of the analyte. Concentrations higher than 100 µg/L may be determined by analyzing a smaller aliquot of the sample.

2. Summary of method

A sample is injected into a purge vessel and sparged with nitrogen. The effluent gas stream is dried and passed through an alumina trap at −95°C, where ethylene and propane are adsorbed by the alumina. The trap is heated to desorb gases, which are determined by gas chromatography using a flame ionization detector.

3. Interferences

Volatile compounds that have retention times similar to ethylene or propane on the analytical column can interfere.

4. Apparatus

4.1 *Adsorption tube*, Pyrex tubing, ¼ in od (outside diameter): Bend Pyrex tubing into a U-tube approximately 8 in long by 2 in wide. Charge the tube with 5 g activated alumina held in place with plugs of glass wool.

4.2 *Cold bath*, a Dewar flask large enough to accommodate the lower half of the adsorption tube: Maintain the bath at −75 to −100°C with either dry ice/acetone or a refrigerant probe such as the Neslab CC–100, or equivalent.

4.3 *Drying tubes*, Pyrex tubing, ½ in od by 8 in long: Fill the tubes with Drierite held in place with glass-wool plugs.

4.4 *Gas chromatograph*, Tracor 560, or equivalent.

4.4.1 The following conditions are recommended:

Column, borosilicate glass, 1.8 m×2 mm id (inside diameter) operated at 90°C, packed with Porapak N, Q, or QS.

Detector, flame-ionization, operated at 250°C.

Injection port temperature, 110°C.

Carrier gas, helium, flow rate 30 mL/min.

4.5 *Gas sampling valve*, six port, Valco V-6-HPa, or equivalent.

4.6 *Hot bath*, a heating mantle for a 1-L round-bottom flask filled with sand and powered by a variable-voltage transformer: The voltage is adjusted to maintain the sand at approximately 130°C.

4.7 *Purge vessel*: See figure 3.

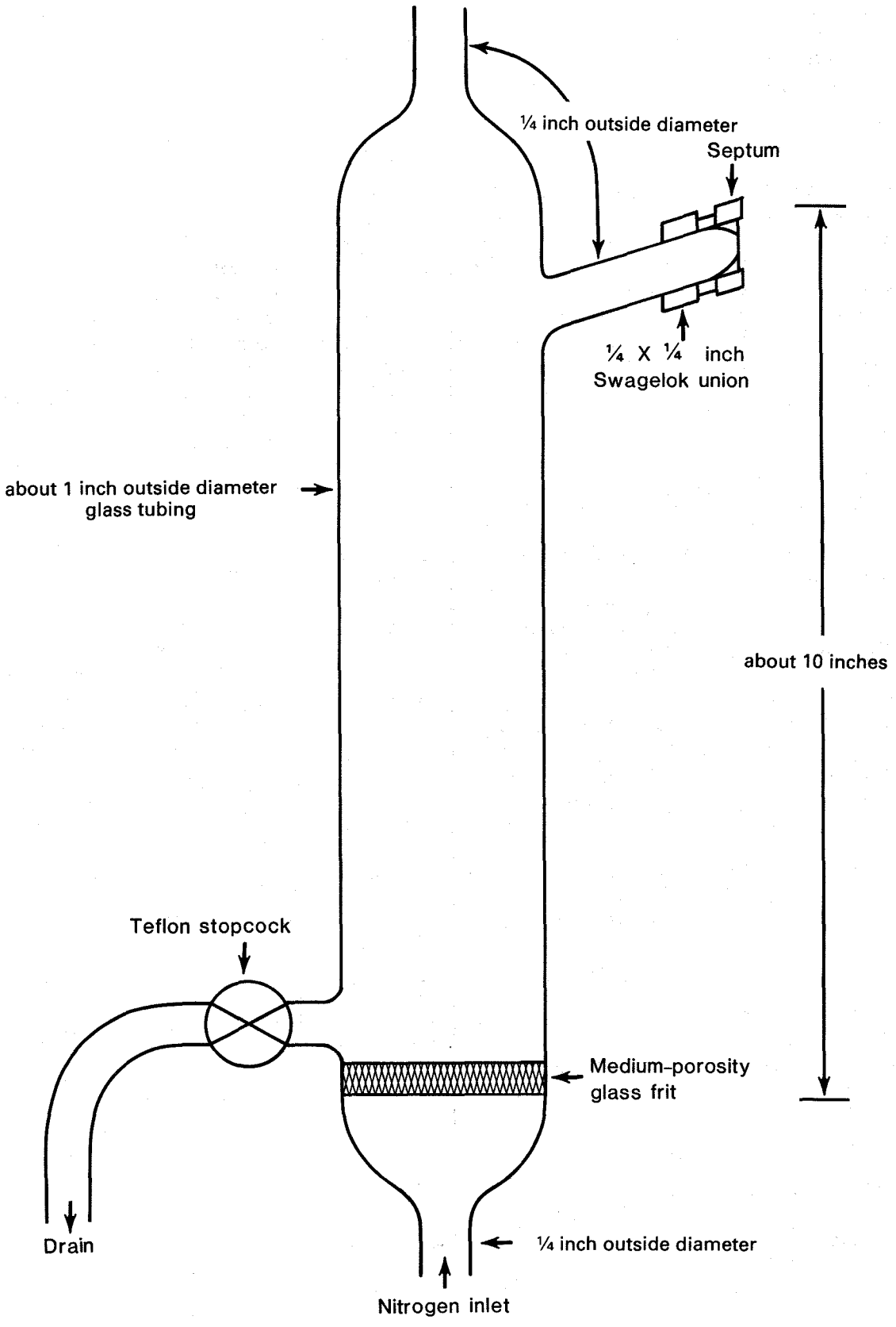
4.8 *Sample vial*, glass, 40 mL, screw cap, fitted with a Teflon-lined septum. Pierce 13075, or equivalent.

4.9 *Syringe needles*, 19 gauge, 3 in long.

4.10 *Syringes*, two syringes, 30 mL and 5 mL, glass, Luer-Lock, equipped with stopcocks, Becton-Dickinson 3152, or equivalent.

4.11 *Syringes, gas-tight*, syringes of 1.0-, 2.5-, 10-, and 50-mL capacities, Hamilton, or equivalent, used for delivery of calibration gas and equipped preferably with sideport needles.

4.12 *Teflon tubing*, ⅛ in od, used for the connecting lines.


¼ inch outside diameter
Septum
¼ X ¼ inch
Swagelok union
about 1 inch outside diameter
glass tubing
about 10 inches
Teflon stopcock
Medium-porosity
glass frit
Drain
¼ inch outside diameter
Nitrogen inlet

5. Reagents

5.1 *Alumina*, neutral aluminum oxide, activity grade I, Woelm, or equivalent.

5.2 *Calibration gas*, a certified gas mixture containing 10 ppm each of ethylene and propane in nitrogen: The regulator valve must be equipped with a stainless steel diaphragm. Equip the outlet from the regulator with an injection septum to facilitate withdrawal of aliquots of calibration gas with a gas-tight syringe.

5.3 *Drying agent*, indicating Drierite, 8-mesh: Anhydrous magnesium perchlorate (granular) is also suitable.

6. Procedure

Samples are to be collected in vials containing 1 mL formalin as a preservative and in a manner that precludes headspace formation. Samples need not be refrigerated since formalin prevents bacterial decomposition for at least 3 weeks, and probably longer. Rapid loss of ethylene and propane will occur if the preservative is omitted.

6.1 Adjust the flow of hydrogen and air to the flame-ionization detector to achieve a linear response from 1 to 100 ng of ethylene and propane.

6.2 Set the flow of nitrogen through the purging vessel at 30 to 35 mL/min. This is conveniently measured at the outlet port of the gas-sampling valve (see fig. 4).

6.3 Place the adsorption tube in the cold bath and set the gas-sampling valve as shown in figure 4.

6.4 Calibration procedure:

6.4.1 Inject an aliquot of calibration gas mixture into the purging vessel using a gas-tight syringe.

6.4.2 Start the timer and allow 12 min for complete adsorption of ethylene and propane by the alumina trap. NOTE: The minimum time required to completely strip the gases depends on the dead volume of the system (purging vessel, drying tube, adsorption tube, and connecting lines) and the flow through it.

6.4.3 Turn the gas sampling valve to the position that conducts the flow from the adsorption tube into the analytical column of the gas chromatograph. Immediately place the adsorption tube in the hot bath and begin digital peak integration. Record integrated peak areas.

6.4.4 Repeat steps 6.4.1 through 6.4.3 for as many other aliquots of calibration gas mixture as is necessary to cover the expected range of the samples.

6.5 Sample analysis:

6.5.1 Open a sample vial and fill a 30-mL glass syringe by closing the stopcock and pouring the sample gently into the barrel. Do not attempt to fill the syringe by suction.

6.5.2 Introduce 10 mL of sample into the purging vessel through the injection port.

6.5.3 Start the timer and allow the sample to be purged for 12 min (see NOTE above).

6.5.4 Turn the gas sampling valve to the position that conducts the flow from the adsorption tube into the analytical column of the gas chromatograph. Immediately place the adsorption tube in the hot bath and begin digital peak integration. Record integrated peak areas.

6.5.5 Drain the sample from the stripping chamber while gas chromatographic determination proceeds.

7. Calculations

7.1 Correction for calibration gas:

7.1.1 The calibration gas is 10 ppm by volume. This must be corrected to give the corresponding concentration by weight.

7.1.2 For 1 mL of 10 ppm calibration gas, the volume of ethylene and propane is 1.0×10^{-5} mL each.

7.1.3 Applying the ideal gas law at standard temperature and pressure (STP) where 1 mole of gas occupies 22.4 L,

$$N_2 = \frac{N_1 T_1 P_2 V_2}{T_2 P_1 V_1},$$

where

$N_1 = 1.0$ mole,
N_2 = number of moles ethylene or propane,
$T_1 = 273$°K (STP),
$P_1 = 760$ mm Hg (STP),
$V_1 = 22{,}400$ mL (volume of 1 mole gas at STP),
T_2 = actual temperature, in °K,
P_2 = actual atmosphere pressure, in mm Hg, and
$V_2 = 1.0\times10^{-5}$ mL (volume of ethylene or propane in 1.0 mL of calibration gas).

← Figure 3.—Purge vessel.

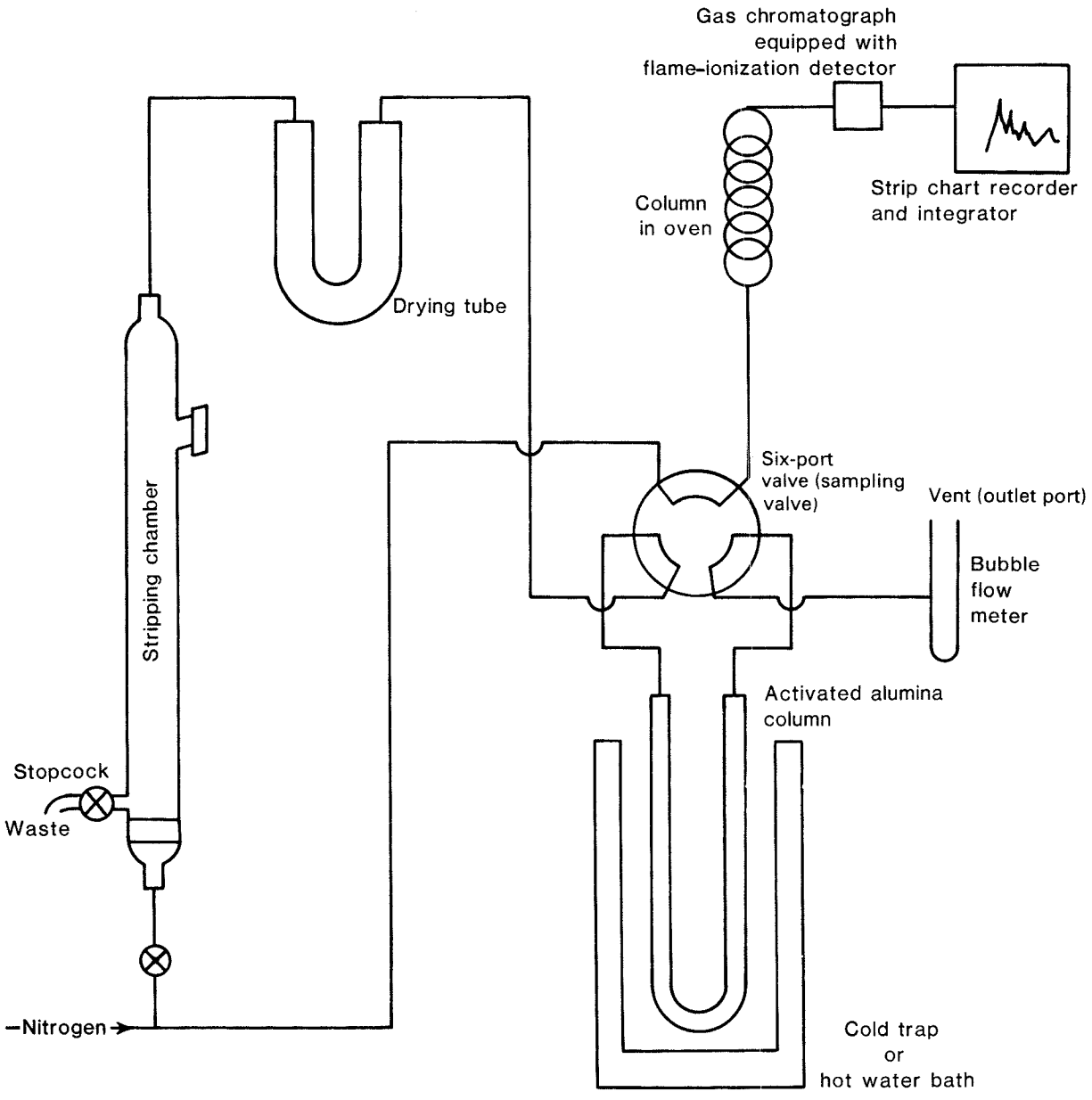


Figure 4.—Stripping and gas chromatographic system in the stripping mode.

7.2 For either ethylene or propane, calculate the concentration of ethylene or propane per mL of calibration gas from the following equation:

$$W_1 = \frac{N_1 \times MW \times 10^6}{V_3},$$

where

W_1 = concentration of ethylene or propane in calibration gas, in µg/mL,

V_3 = volume of calibration gas, in mL,

N_1 = number of moles of ethylene or propane, and

MW = molecular weight of ethylene or propane, in g/mole.

7.3 Calculate the response factor for the calibration curve from the following equation:

$$RF = \frac{A_1}{W_1 \times V_4},$$

where

RF = response factor of ethylene or propane, in area/µg,

A_1 = integrated peak area of standard,

V_4 = volume of calibration gas injected, in mL, and

W_1 = concentration of ethylene or propane calibration gas, in µg/mL.

7.4 The concentration of ethylene or propane in the sample is calculated from the following equation:

$$\text{Concentration (µg/L)} = \frac{A_2 \times 1{,}000}{RF \times V_5},$$

where

A_2 = integrated peak area of ethylene or propane,

RF = response factor of ethylene or propane, in area/µg, and

V_5 = volume of sample injected, in mL.

8. Report

Report concentration of ethylene and (or) propane as follows: concentrations less than 0.1 µg/L, as "less than 0.1 µg/L"; 0.1 to 1.0 µg/L, one decimal; 1.0 µg/L and above, two significant figures.

9. Precision

Solutions of ethylene and propane were prepared in deionized water and stored in sample vials (step 4.8) containing 1 mL formalin as preservative. Replicates were prepared to cover the concentration range of the method. All of the samples were analyzed within 3 weeks of preparation. Single-operator precision is as follows:

Concentration of ethylene (µg/L)	*Number of replicates*	*Standard deviation*	*Relative standard deviation (percent)*
0.072	7	0.002	2.8
.198	12	.006	3.0
1.69	11	.04	2.4
8.38	12	.10	1.2
16.0	12	.20	1.3
35.1	12	.6	1.7
82.4	11	2.1	2.5
132	11	3.0	2.3

Concentration of propane (µg/L)	*Number of replicates*	*Standard deviation*	*Relative standard deviation (percent)*
0.099	7	0.004	4.0
.257	12	.015	5.8
2.52	11	.05	2.0
12.9	12	.4	3.1
24.5	12	.4	1.6
53.5	12	.9	1.7
110	11	2.6	2.4
171	11	4.0	2.3

Selected reference

Shultz, D.J., Pankow, J.F., Tai, D.Y., Stephens, D.W., and Rathbun, R.E., 1976, Determination, storage, and preservation of low molecular weight hydrocarbon gases in aqueous solution: U.S. Geological Survey Journal of Research, v. 4, p. 247–251.

Purgeable organic compounds, total recoverable, gas chromatographic/ mass spectrometric, purge and trap (O–3115–83)

Parameter	*Code*
Chloromethane	34418
Bromomethane	34413
Vinyl chloride	39175
Chloroethane	34311
Methylene chloride	34423
1,1-Dichloroethene	34501
1,1-Dichloroethane	34496
Trans-1,2-Dichloroethene	34546
Chloroform	32106
1,2-Dichloroethane	32103
1,1,1-Trichloroethane	34506
Carbon tetrachloride	32102
Bromodichloromethane	32101
1,2-Dichloropropane	34541
Trans-1,3-Dichloropropene	34699
Trichloroethene	39180
Dibromochloromethane	32105
Benzene	34030
1,1,2-Trichloroethane	34511
Cis-1,3-Dichloropropene	34704
2-Chloroethylvinyl ether	34576
Bromoform	32104
1,1,2,2-Tetrachloroethane	34516
Tetrachloroethene	34475
Toluene	34010
Chlorobenzene	34301
Ethylbenzene	34371

1. Application

This method is suitable for the determination of purgeable organic compounds in water and water-suspended-sediment mixtures containing at least 3 µg/L of a reportable analyte.

2. Summary

A water sample is purged with helium. The purgeable organic compounds are carried with helium and trapped on a porous polymer trap. The trapped compounds are thermally desorbed into the gas chromatograph. These compounds are separated by gas chromatography (GC) and detected by mass spectrometry (MS).

3. Interferences

3.1 Any purgeable compound that elutes at a retention time similar to that of the analyte and produces an ion that is the same as the quantitation ion of the analyte is a potential interference. Common laboratory solvents such as methylene chloride, benzene, and chloroform may contaminate the sample and give erroneous results.

3.2 Special handling of samples, such as storage in a dessicator over activated charcoal, may be required to prevent contamination by common laboratory solvents.

4. Apparatus

4.1 *Gas chromatograph/mass spectrometer/data system*, Finnigan 3223, or equivalent.

4.1.1 *Gas chromatographic column*, borosilicate glass, 1.8 m×2 mm id (inside diameter) that has been deactivated and packed with 1 percent SP-1000 coated on 60/80 mesh Carbopack B, or equivalent.

4.1.2 *Gas chromatographic conditions*: GC conditions need to be optimized for each system. Use the purgeable standards (see step 5.3) to adjust conditions to obtain good peak separation in a reasonable amount of time. The following conditions should serve as a starting point for the optimization process:

Injector temperature	200°C
Carrier gas flow (He)	20 mL/min
Initial hold temperature	45°C
Initial hold time	4 min
Program rate	8°C/min
Final temperature	210°C
Final hold time	To end of data acquisition

4.1.3 *Mass spectrometer conditions*: Analyze the mass range 35–260 amu (atomic mass units) with a nominal electron energy setting of 70 eV at a scan rate sufficient to obtain a minimum of 5 scans per chromatographic peak.

4.2 *Purge and trap device*, Chemical Data Systems model 310, or equivalent: The trap is packed with the following adsorbents: 1 cm methyl silicone coated packing (3 percent SP-2100 on 80-mesh Supelcoport, or equivalent), followed by 15 cm Tenax, and ending with 8 cm silica gel (Davison grade 15), or equivalent. The silica gel may be replaced by 5 cm of Ambersorb resin (Rohm and Haas). New traps are conditioned by heating overnight at 240°C with helium flow (20 mL/min).

4.3 *Syringe*, gas-tight, 10 mL, equipped with a Teflon syringe valve and a 3-in×19-gauge needle.

5. Reagents

5.1 *BFB (4-Bromofluorobenzene) solution:* Fill a 50-mL volumetric flask to the mark with methanol and add a 1.0-μL capillary pipet filled with BFB to the volumetric flask. This solution contains 32 ng BFB per μL.

5.2 *Methanol*, pesticide analysis quality, Burdick and Jackson, or equivalent.

5.3 *Purgeable mixed standards*, EPA analytical reference grade or highest purity available: Purgeable standards may be purchased from various commercial sources or prepared from pure compounds. To prepare purgeable mixed standards, fill a 50-mL volumetric flask to the mark with methanol and, for each desired component, add a 1.0-μL capillary pipet filled with the authentic material to the volumetric flask. Mix thoroughly and store at 4°C. Calculate the concentration of each analyte from its density.

5.4 *Surrogates/internal standard solution*, bromochloromethane (EPA, or equivalent), 1-bromo-2-chloroethane (EPA, or equivalent), perdeuterobenzene (Pfaltz and Bauer, or equivalent), and fluorobenzene (the internal standard, Aldrich Chemical Co., or equivalent). Add a 1.0-μL capillary pipet filled with each component to a 50-mL volumetric flask filled to the mark with methanol. Mix thoroughly and store at 4°C. Add 5 μL to each sample, standard, and blank to monitor recovery and to provide an internal standard.

5.5 *Water*, organic-free.

6. Procedure

6.1 Condition the trap at 220°C for 10 min.

6.2 Mass spectrometer tuning:

6.2.1 Use perfluorotributylamine to tune the mass spectrometer in a manner that results in a satisfactory calibration of mass assignments as well as agreement with the criteria listed in step 6.2.2.

6.2.2 Set the MS to scan the mass range 35 to 260 amu. Set the GC column temperature to 220–230°C isothermal. Introduce 50 ng of BFB (1.6 μL of solution, step 5.1) by direct, on-column injection or by purging from 5 mL of reagent water. Obtain a background corrected mass spectrum of BFB and verify that all of the following criteria are met:

Mass	*Ion abundance criteria*
50	15 to 40 percent of mass 95
75	30 to 60 percent of mass 95
95	Base peak, 100 percent relative abundance
96	5 to 9 percent of mass 95

Mass	*Ion abundance criteria*
173	<2 percent of mass 174
174	>50 percent of mass 95
175	5 to 9 percent of mass 174
176	>95 percent but <101 percent of mass 174
177	5 to 9 percent of mass 176

6.3 Blank analysis:

6.3.1 Remove the plunger from a 10-mL syringe and attach a closed syringe valve and needle. Open the sample vial and carefully pour the water into the barrel of the syringe until it overflows. Replace the syringe plunger, open the syringe valve, and vent any air present. Adjust the plunger to the 10.0-mL mark. Add 10 μL of the surrogate/internal standard solution through the valve, close the syringe valve, and mix.

6.3.2 Transfer 5.0 mL water into a clean purge tube. Close the syringe valve and retain the second 5.0-mL aliquot of water for future analysis, if needed. The second aliquot is preserved in this manner since the integrity of the original water sample is destroyed after it is opened.

6.3.3 Purge for 11 min.

6.3.4 Immediately begin the desorb cycle and data acquisition when purging is complete.

6.3.5 Begin the temperature program of the GC oven immediately upon completion of the desorb cycle.

6.3.6 Initiate the trap bakeout when data acquisition has ended.

6.3.7 Allow the trap to cool to room temperature and return the GC oven temperature to 45 °C for the next analysis.

6.3.8 Analyze the mass spectral data for the three surrogates and the internal standard. Record the integrated area of the quantitation ion for each.

6.3.9 Examine the mass spectral data to verify that the analytical system is free from contamination.

6.4 Calibration:

6.4.1 Remove the plunger from a 10-mL syringe and attach a closed syringe valve and needle. Open the sample vial and carefully pour the organic-free water into the barrel of the syringe until it overflows. Replace the syringe plunger, open the syringe valve, and vent any air present. Adjust the plunger to the 10.0-mL mark. Add 10 μL of surrogate/internal standard solution. Also add 10 μL of purgeable standard solution. Close the syringe valve, and mix.

6.4.2 Transfer 5.0 mL water into a clean purge tube. Close the syringe valve and retain the second 5.0-mL aliquot of sample for future analysis, if needed. The second aliquot is preserved in this manner since the integrity of the original water sample is destroyed after it is opened.

6.4.3 Perform the analysis as described in steps 6.3.3 through 6.3.8.

6.4.4 Compare the recovery of the surrogates in the purgeable standard with that observed in the blank. Deviations of more than ±30 percent from theoretical are an indication of a problem (e.g., leakage in the purge and trap device) that needs to be corrected before proceeding further.

6.4.5 Process the data from the purgeable standard and record the integrated area of the quantitation ion of each component as well as its retention time.

6.4.6 Repeat steps 6.4.2 through 6.4.5 with as many other volumes of the purgeable standard solution as are necessary to define the working range of the analytical system.

6.5 Sample analysis:

6.5.1 Allow the water sample to come to room temperature.

6.5.2 Remove the plunger from a 10-mL syringe and attach a closed syringe valve and needle. Open the sample vial and carefully pour the sample into the barrel of the syringe until it overflows. Replace the syringe plunger, open the syringe valve, and vent any air present. Adjust the plunger to the 10.0-mL mark. Add 10 μL of the surrogate/internal standard solution. Close the syringe valve, and mix.

6.5.3 Transfer 5.0 mL of sample into a clean purge tube. Close the syringe valve and retain the second 5.0-mL aliquot of sample for future analysis, if needed. The second aliquot is preserved in this manner since the integrity of the original water sample is destroyed after it is opened.

6.5.4 Perform the analysis as described in steps 6.3.3 through 6.3.8.

6.5.5 Compare the recovery of the surrogates in the sample with that observed in the blank (step 6.3.8). If the recovery is not in the range of 70 to 130 percent, the sample should be reanalyzed.

6.5.6 Examine all of the mass spectral data from the sample. Identify analytes by a library search with a satisfactory match error. Positive identification is obtained when (1) the retention time is within 5 percent of the authentic material in the purgeable standard (step 6.4.5), and (2) three of the

characteristic ions of the analyte maximize within ± 1 scan of each other.

6.5.7 Integrate and record the area under the quantitation ion for each analyte identified in step 6.5.6. If the areas are greater than the calibration range of the analytical system, the sample needs to be reanalyzed using a smaller volume of sample. It may be necessary to add additional internal standard for smaller sample volumes.

6.5.8 If very high levels of analytes or contaminants are found in a sample, analyze a blank to demonstrate no carryover.

7. Calculations

7.1 External standard method:

7.1.1 Calculate a response factor for an analyte (step 6.4.5) according to the equation

$$RF = \frac{A}{C \times V},$$

where

RF = response factor of analyte, in area/ng,
C = concentration of analyte in purgeable standard, in ng/μL,
V = volume of purgeable standard analyzed, in μL, and
A = area of quantitation ion of analyte.

7.1.2 Calculate the concentration of the analyte in the original water sample from the equation

$$\text{Concentration } (\mu g/L) = \frac{A_s}{V_s \times RF},$$

where

A_s = area of quantitation ion of analyte in sample,
V_s = volume of original water sample analyzed, in mL, and
RF = response factor of analyte, in area/ng.

7.1.3 Calculate the percent recovery of each surrogate added to the water sample from the equation

$$\text{Recovery (percent)} = \frac{A_1}{A_2} \times 100,$$

where

A_1 = area of quantitation ion of surrogate in water sample, and
A_2 = area of quantitation ion of surrogate added to blank.

7.2 Internal standard method:

7.2.1 Calculate the response factor of an analyte (step 6.4.5) in the purgeable standard from the equation

$$RF = \frac{A_s}{C_s},$$

where

RF = response factor of analyte, in area/ng,
A_s = area of quantitation ion of analyte, and
C_s = amount of analyte in purgeable standard, in ng.

7.2.2 Calculate the response factor of the internal standard (step 6.4.5) in the purgeable standard from the equation

$$RFI = \frac{AI_1}{CI_1},$$

where

RFI = response factor of internal standard in purgeable standard, in area/ng,
AI_1 = area of quantitation ion of internal standard in purgeable standard, and
CI_1 = amount of internal standard in surrogate and internal standard solution, in ng.

7.2.3 Calculate a relative response factor from the equation

$$RRF = \frac{RF}{RFI},$$

where

RRF = relative response factor of analyte,
RF = response factor of analyte determined (step 7.2.1), and
RFI = response factor of analyte internal standard determined (step 7.2.2).

7.2.4 Calculate the concentration of an analyte in the original water sample from the equation

$$\text{Concentration } (\mu g/L) = \frac{A \times CI_2}{RRF \times AI_2 \times V},$$

where

A = area of quantitation ion of analyte in analyzed sample,
CI_2 = amount of internal standard in surrogate solution, in ng,
RRF = relative response factor determined (step 7.2.3),

AI_2 = area of quantitation ion of internal standard in analyzed sample (step 6.5.9), and

V = volume of original water sample analyzed, in mL.

7.2.5 Calculate the percent recovery of each surrogate added to the water sample from the equation

$$\text{Recovery (percent)} = \frac{A_1}{A_2} \times 100,$$

where

A_1 = area of quantitation ion of surrogate added to water sample, and

A_2 = area of quantitation ion of surrogate added to blank.

8. Report

Report concentrations of purgeable organic compounds in water or water–suspended-sediment mixtures as follows: less than 3 µg/L, as "less than 3 µg/L"; 3.0 µg/L and above, two significant figures.

9. Precision

Precision data are not available.

Selected references

Goerlitz, D.F., 1976, Determination of volatile organohalides in water and treated sewage effluents: U.S. Geological Survey Open-File Report 76–610, 14 p.

U.S. Environmental Protection Agency, 1979, Purgeables—Method 624: Federal Register, v. 44, no. 233, p. 69532.

Acid extractable compounds, total recoverable, gas chromatographic/ mass spectrometric (O–3117–83)

Parameter	*Code*
4-Chloro-3-methylphenol	34452
2-Chlorophenol	34586
2,4-Dichlorophenol	34601
2,4-Dimethylphenol	34606
4,6-Dinitro-2-methylphenol	34657
2,4-Dinitrophenol	34616
2-Nitrophenol	34591
4-Nitrophenol	34646
Pentachlorophenol	39032
Phenol	34694
2,4,6-Trichlorophenol	34621
8-Methyldecanoic acid	76992
Undecanoic acid	77648

1. Application

This method is suitable for the determination of acid extractable compounds in water and water–suspended-sediment mixtures containing at least 3–5 µg/L of the analyte.

2. Summary

Acidic organic compounds are extracted from water and water–suspended-sediment mixtures with methylene chloride. The extract is concentrated and subjected to analysis by gas chromatography (GC) using a flame-ionization detector (FID) or a mass spectrometric (MS) detector.

3. Interferences

Compounds having chemical and physical properties similar to the compounds of interest may interfere.

4. Apparatus

4.1 *Boiling chip,* micro, carbon chips: Rinse with hexane, air dry, and heat at 300°C overnight. Treat with 5 percent aqueous sulfuric acid for 5 min. Rinse with deionized water until the washwater is neutral to pH paper. Heat at 130°C overnight.

4.2 *Concentrator, Kuderna-Danish (K-D),* 500-mL, all glass, with ground-glass joints, a 10.0-mL receiver, and a one-ball Snyder column.

4.3 *Evaporative concentrator,* Organomation N-Evap, or equivalent: Water bath must be maintained at 50°to 55°C.

4.4 *Gas chromatograph/mass spectrometer/data system,* Hewlett-Packard 5985B GC/MS (gas chromatograph/mass spectrometer), or equivalent: The gas chromatograph is used with one of the following options:

4.4.1 *Column,* fused silica capillary column, 25 m×0.20 mm id (inside diameter), SE–54 bonded column, 0.33-µm film thickness.

Detector, mass spectrometer.

Injection temperature, 260°C.

Carrier gas, 1 mL/min, helium.

Transfer line temperature, 285°C.

Mode, splitless injection.

Program rate, 45° to 300°C, 2.5-min initial hold, 6°C/min, 15-min final hold.

4.4.2 *Columns,* two fused silica capillary columns, 25 m×0.20 mm id, SE–54 bonded column, 0.33-µm film thickness.

Detectors, mass spectrometer and a flame-ionization detector, 285°C.

Injection temperature, 260°C.
Mode, splitless injection.
Carrier gas, 1 mL/min, helium.
Transfer line, 285°C.
Program rate, 45° to 300°C, 2.5-min initial hold, 6°C/min, 15-min final hold.

4.5 *pH paper,* hydrion, pH range 1 to 14.

5. Reagents

5.1 *Acidic organic compound standards,* EPA analytical reference grade or highest purity available: Weigh about 20 mg of the standard to three significant figures, quantitatively transfer it to a 100-mL volumetric flask, and dilute to volume with methylene chloride. Prepare dilutions in methylene chloride to obtain solutions containing 2, 10, and 20 ng/μL.

5.2 *DFTPP (Decafluorotriphenylphosphine) solution,* 50 ng/μL: Dilute 20 μL DFTPP (Supelco, or equivalent, 25 mg/mL solution) to 10 mL in a volumetric flask with methylene chloride.

5.3 *Internal standard,* perdeuterophenanthrene (phenanthrene-d_{10}), Kor Isotopes, Division of Kor, Inc., or equivalent; perdeuteronaphthalene (naphthalene-d_8), Aldrich Chemical Co., or equivalent; and perdeuterochrysene (chrysene-d_{12}), Kor Isotopes, Division of Kor, Inc., or equivalent: Weigh about 20 mg perdeuteronaphthalene, perdeuterophenanthrene, and perdeuterochrysene to three significant figures, quantitatively transfer to a 100-mL volumetric flask, and dilute to volume with methylene chloride.

5.4 *Sodium hydroxide,* pellets, reagent grade.

5.5 *Sodium hydroxide solution,* 37 percent (weight/volume; w/v): Dissolve 185 g sodium hydroxide pellets in 500 mL organic-free water and reflux 8 h. Cool and store at 4°C.

5.6 *Sodium sulfate,* anhydrous, granular, Mallinckrodt 8024, or equivalent: Heat at 300°C overnight, slurry with enough diethyl ether to cover the crystals, and acidify to pH 2 or less by adding a few millileters of concentrated sulfuric acid. Determine the pH by removing a portion of the slurry, evaporating the ether, adding water to the crystals, and testing the aqueous phase with pH paper. Evaporate the ether by allowing the slurry to stand in an open container under a fume hood. Store at 130°C in a glass-stoppered bottle.

5.7 *Solvents,* diethyl ether, unpreserved, distilled in glass, pesticide analysis quality, Burdick and Jackson, or equivalent; methylene chloride, isopropanol, glass distilled pesticide quality, Burdick and Jackson, or equivalent.

5.8 *Sulfuric acid, concentrated,* (sp. gr. 1.84), reagent grade, Mallinckrodt, or equivalent.

5.9 *Sulfuric acid,* (1 + 3): Prepare by adding one part concentrated sulfuric acid to three parts organic-free water. Store in a refrigerator at 4°C.

5.10 *Surrogate standards,* perdeuterophenol (phenol-d_6), Aldrich Chemical Co., or equivalent; dibromobenzene, FDA, or equivalent; 2,4,6-tribromophenol, FDA, or equivalent: Weigh 4 mg of each of the three compounds to three significant figures, quantitatively transfer to a 100-mL volumetric flask, and dilute to volume with isopropanol.

5.11 *Water,* organic-free.

6. Procedure

All glassware must be washed in warm detergent solution, rinsed with organic-free water, and heated at 300°C overnight. All glassware must be rinsed with (1 + 3) aqueous sulfuric acid, then rinsed with organic-free water until the washwater is neutral to pH paper, and then heated at 130°C overnight. Immediately before use, the glassware is rinsed with methylene chloride. Stopcock grease should not be used on ground-glass joints.

6.1 Immediately upon receipt of the sample in the laboratory, acidify to pH 2 or lower (as indicated by pH paper) with (1 + 3) sulfuric acid (approximately 3 mL), and store at 4°C. Extraction must begin within 48 h after receipt of the sample.

NOTE: Extraction may be carried out on the aqueous phase following extraction of the extractable base/neutral compounds (method O–3118).

6.2 A blank must accompany each set of samples. For each sample and blank, rinse a 2-L separatory funnel and a 500-mL Erlenmeyer flask with methylene chloride.

6.3 Weigh the capped sample bottle to three significant figures and record the weight for subsequent calculations.

6.4 Pour the sample into the separatory funnel and allow the sample bottle to drain completely.

6.5 Weigh the capped empty sample bottle to three significant figures and calculate and record the net sample weight.

6.6 Add 1.0 mL of the surrogate standard (5.10) to the sample in the separatory funnel.

6.7 Add 100 mL methylene chloride to the empty sample bottle and gently swirl to wash the

sides of the container with the solvent. The Teflon liner inside the cap is not rinsed because of the potential for contamination from solvent that has contacted the cap threads and the surface beneath the liner. Pour the contents of the bottle into the separatory funnel.

6.8 Stopper the separatory funnel and shake vigorously for at least 1 min, venting often to release the pressure. Allow the layers to separate.

6.9 Drain the organic layer into a 500-mL Erlenmeyer flask.

6.10 Add 50 mL methylene chloride to the separatory funnel. Stopper the separatory funnel and shake for at least 1 min, venting often to release the pressure. Allow the layers to separate.

6.11 Drain the organic layer into the Erlenmeyer flask.

6.12 Extract the sample one more time by repeating steps 6.9 and 6.10.

6.13 Add approximately 30 g sodium sulfate to the Erlenmeyer flask. Cover the Erlenmeyer flask with aluminum foil and set aside for no longer than 2 h. (Significant loss of analytes can occur from sample adsorption onto the sodium sulfate if the extract is allowed to remain in contact with the sodium sulfate for longer than 2 h.)

6.14 Quantitatively transfer the dried extract to a K-D apparatus, add a boiling chip, and attach a Snyder column.

6.15 Concentrate to about 5 mL by heating the apparatus on a 90°C water bath in a fume hood.

6.16 Allow the K-D flask to cool. Dry the apparatus with a towel, especially around the ground-glass joint.

6.17 Separate the Snyder column from the K-D flask and rinse the walls of the K-D flask with approximately 2 mL methylene chloride. Dry the joints with a towel. Separate the receiver from the K-D flask and rinse the ground-glass joint of the K-D flask into the receiver with methylene chloride.

6.18 Reduce the volume of the extract in the receiver to less than 0.9 mL on the evaporative concentrator. During the concentration procedure, rinse the receiver walls two or three times with small portions of methylene chloride.

6.19 Stopper the receiver with a ground-glass stopper and store the extract in a freezer until analysis can proceed.

NOTE: The extract obtained from the base/neutral extraction (method O-3118) and the extract from this acidic extraction can be combined for GC/MS analysis immediately before injection.

6.20 Immediately before analysis, add 0.05 mL of internal standard. Adjust the final volume of the sample extract in the receiver to 1.0 mL.

6.21 Mass spectrometer tuning:

6.21.1 Use perfluorotributylamine to tune the mass spectrometer in a manner that results in a satisfactory calibration of mass assignments as well as agreement with the criteria listed in step 6.21.2.

6.21.2 Set the MS to scan the mass range 40–450 amu (atomic mass units). Temperature program the GC from 45° to 275°C at 20°C per min with an initial hold of 1.5 min. Inject 50 ng (1 μL of solution, step 5.2). Obtain a background corrected mass spectrum of DFTPP and verify that all of the following criteria are met:

Mass	*Ion abundance criteria*
51	30–60 percent of mass 198
68	<2 percent of mass 69
70	<2 percent of mass 69
127	40–60 percent of mass 198
197	<1 percent of mass 198
198	Base peak, 100 percent relative abundance
199	5–9 percent of mass 198
275	10–30 percent of mass 198
365	>1 percent of mass 198
441	<mass 443
422	>40 percent of mass 198
443	17–23 percent of mass 442

6.22 Analyze the extract by injection of an aliquot into the GC/MS system optimized as follows:

6.22.1 For systems configured with option A, analyze the mass range 40 to 450 amu at a scan rate sufficient to obtain a minimum of 5 scans per chromatographic peak. Record the total ion current chromatogram and the mass spectrum of each peak.

6.22.2 For systems configured with option B, analyze the mass range 40 to 450 amu at a scan rate sufficient to obtain a minimum of 5 scans per chromatographic peak. Record the total ion current chromatogram and the mass spectrum of each peak as well as the data (retention time and integrated area of each peak) from the FID.

6.23 Process the data to determine the identity of the extractable acidic organic compounds in the following manner:

6.23.1 Identification of the target compound is accomplished by a computerized reverse search procedure employing a 25-scan retention time window.

6.23.2 Identification of the extractable acid compounds that are not target compounds is accomplished by a computerized library search versus the National Bureau of Standards library reference spectra on each peak. The best computer matches of mass spectra are reviewed manually.

6.24 Determine the largest characteristic ion and quantitate the area on this ion for any identified peak, including the internal standard peak and the surrogate standards peaks. Alternatively, if the system is configured with option B, the quantitation can be carried out on the FID trace rather than on the mass spectrum. The integrated area of an identified peak is recorded for subsequent calculations. The better chromatogram (FID or MS) is used for quantitation.

6.25 Confirm the surrogate compounds found in the sample by injecting an aliquot of the corresponding surrogate standards (step 5.10) into the gas chromatograph and analyze according to steps 6.22 through 6.24. Record the integrated area obtained.

6.26 Confirm any identified extractable acid compounds found in the sample by injecting an aliquot of the corresponding acidic organic compound standard (step 5.1) of about the same concentration into the gas chromatograph and analyze according to steps 6.22 through 6.24. If the concentration of the compound of interest exceeds the highest standard, dilute the extract and reanalyze it. Record the integrated area obtained. If an acid standard is not available, quantitate relative to the internal standard (see step 7.6).

7. Calculations

7.1 Calculations of response factors and relative response factors:

7.1.1 Compute the response factor of the surrogate standard (step 6.25) and each compound in the acid standard (step 6.26) using the following equation:

$$RF_i = \frac{A_{si}}{C_{si}},$$

where

RF_i = response factor of compound i in standard, in area/ng

C_{si} = amount of compound i injected, in ng/μL, and,

A_{si} = area of compound i peak.

7.1.2 Calculate the response factor of th internal standard using the following equation:

$$RFI = \frac{AI_1}{CI_1},$$

where

RFI = response factor of internal standard, in area/ng,

CI_1 = amount of internal standard in acid standard (step 5.1) injected, in ng, and

AI_1 = area of internal standard peak.

7.1.3 Calculate a relative response factor by the following equation:

$$RRF_i = \frac{RF_i}{RFI},$$

where

RRF_i = relative response factor,

RF_i = response factor of compound i, and

RFI = response factor of internal standard.

7.2 Calculations of recoveries of surrogates: Calculate the percent recovery of each surrogate standard recovered from the original water–suspended-sediment mixture using the following equation:

$$\text{Percent recovery} = \frac{A \times CI_2 \times V_3 \times 100}{RRF_i \times AI_2 \times C_s \times V_4},$$

where

A = area of identified surrogate peak in sample extract,

CI_2 = amount of internal standard injected, in ng,

RRF_i = relative response factor of surrogate i,

AI_2 = area of internal standard peak in sample extract,

C_s = concentration of standard, in ng/mL,

V_3 = final volume of extract, in mL, and

V_4 = volume of sample injected, in μL.

7.3 Calculations of concentrations of analytes: Calculate the concentration of each identified acid extractable compound in the original water or water–suspended-sediment mixture using the following equation:

$$\text{Concentration (μg/L)} = \frac{A \times CI_2 \times V_3 \times 1{,}000}{RRF \times AI_2 \times W \times V_4},$$

where

A = area of identified peak in sample,

CI_2 = amount of internal standard injected, in ng,

RRF = relative response factor,
AI_2 = area of internal standard peak in sample extract,
W = weight of sample extracted, expressed in mL (1.000g = 1.000 mL),
V_3 = final volume of extract, in mL, and
V_4 = volume of sample injected.

7.4 Calculations of concentrations of other compounds: The concentrations of all other identified extractable acid compounds in the original water sample, for which there are no standards, are calculated relative to the concentration of the internal standard and are semiquantitative for the purpose of a general organic scan. The response factor of the compound is assumed to be exactly equal to that of the internal standard. Calculate the concentration using the following equation:

$$\text{Concentration } (\mu g/L) = \frac{A \times V_3 \times 1{,}000}{RFI \times W \times V_4},$$

where
RFI = response factor of internal standard, in area/ng,
A = area of identified peak in sample,
W = weight of sample extracted, expressed in mL (1.000 g = 1.000 mL),
V_3 = final volume of extract, in mL, and
V_4 = volume of sample injected.

8. Report

8.1 Report concentrations of extractable acidic organic compounds (except 4-chloro-3-methyl phenol, 2,4,6-trichlorophenol, 2,4-dinitrophenol, 4-nitrophenol, 4,6-dinitro-2-methylphenol, and pentachlorophenol) in water and water–suspended-sediment mixtures as follows: less than 6.0 µg/L, as "less than 6.0 µg/L"; 6.0 µg/L and above, two significant figures.

8.2 Report concentrations of 4-chloro-3-methylphenol, 4-nitrophenol, 4, 6-dinitro-2-methylphenol, and pentachlorophenol as follows: less than 30 µg/L, as "less than 30 µg/L"; 30 µg/L and above, two significant figures.

8.3 Report concentrations of 2,4,6-trichlorophenol and 2,4-dinitrophenol as follows: less than 20 µg/L, as "less than 20 µg/L"; 20 µg/L and above, two significant figures.

9. Precision

9.1 Surrogate recoveries must be between 30 percent and 130 percent unless a matrix effect can be demonstrated. Water and water–suspended-sediment samples were spiked with surrogate standards and recoveries were determined by two operators in a single laboratory over an 8-mo period. Results are as follows:

Compound	Ion used for quantitation	Concentration range (ng/µL)	Number of samples analyzed	Average recovery (percent)	Relative standard deviation (percent)
Phenol-d_6	99	42–81	45	50	30
2,4-Dibromophenol	252	57–104	46	68	39
2,4,6-Tribromophenol	332	61–75	44	83	34

9.2 Deionized water samples were spiked with acid extractable compounds and recoveries were determined by two operators in a single laboratory over a 1-yr period. Results are as follows:

Compound	Ion used for quantitation	Concentration range (ng/µL)
4-Chloro-3-methyphenol	142	50–292
2-Chlorophenol	128	50-355
2,4-Dichlorophenol	162	50-316
2,4-Dimethylphenol	122	43-319
2,4-Dinitrophenol	184	58-436
2-Methyl-4,6-dinitrophenol	198	50-571
2-Nitrophenol	139	50-271
4-Nitrophenol	139	50-326
Pentachlorophenol	266	144-349
Phenol	94	50-331
2,4,6-Trichlorophenol	196	50-236

Compound	Number of samples analyzed	Average recovery (percent)	Relative standard deviation (percent)
4-Chloro-3-methyphenol	18	80	27
2-Chlorophenol	20	73	25
2,4-Dichlorophenol	18	84	21
2,4-Dimethylphenol	19	74	23
2,4-Dinitrophenol	15	67	26
2-Methyl-4,6-dinitrophenol	5	66	33
2-Nitrophenol	17	78	32
4-Nitrophenol	17	61	44
Pentachlorophenol	15	77	31
Phenol	19	53	44
2,4,6-Trichlorophenol	17	83	31

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U.S. Environmental Protection Agency, 1979, Base/neutrals, acids, and pesticides—Method 625: Federal Register v. 44, no. 233, p. 69540.

Base/neutral extractable compounds, total recoverable, gas chromatographic/mass spectrometric (O-3118-83)

Parameter	*Code*
Acenaphthene	34205
Acenaphthylene	34200
Anthracene	34220
Benzidine	39120
Benzo(a)anthracene	34526
Benzo(b)fluoranthene	34230
Benzo(k)fluoranthene	34242
Benzo(g,h,i)perylene	34521
Benzo(a)pyrene	34247
4-Bromophenyl phenyl ether	34636
Butyl benzyl phthalate	34292
bis(2-Chloroethoxy)methane	34278
bis(2-Chlororethyl) ether	34273
bis(2-Chloroisopropyl) ether	34283
2-Chloronaphthalene	34581
4-Chlorophenyl phenyl ether	34641
Chrysene	34320
Dibenz(a,h)anthracene	34556
1,2-Dichlorobenzene	34536
1,3-Dichlorobenzene	34566
1,4-Dichlorobenzene	34571
3,3'-Dichlorobenzidine	34631
Diethyl phthalate	34336
Dimethyl phthalate	34341
Di-n-butyl phthalate	39110
2,4-Dinitrotoluene	34611
2,6-Dinitrotoluene	34626
Di-n-octyl phthalate	34596
bis(2-Ethylhexyl) phthalate	39100
Fluoranthene	34376
Fluorene	34381
Hexachlorobenzene	39700
Hexachlorobutadiene	39702
Hexachlorocyclopentadiene	34386
Hexachloroethane	34396
Indeno(1,2,3-cd)pyrene	34403
Isophorone	34408
Naphthalene	34696
Nitrobenzene	34447
N-Nitrosodimethylamine	34438
N-Nitrosodiphenylamine	34433
N-Nitrosodi-n-propylamine	34428
Phenanthrene	34461
Pyrene	34469
2,3,7,8-Tetrachlorodibenzo-p-dioxin	34675
1,2,4-Trichlorobenzene	34551

1. Application

This method is suitable for the determination of methylene chloride extractable base/neutral compounds in water and water–suspended-sediment mixtures containing at least 5 µg/L of the analyte.

2. Summary of method

Organic base/neutral compounds are extracted from water and water-suspended-sediment mixtures with methylene chloride. The extract is concentrated and analyzed by gas chromatography (GC) using a flame-ionization detector (FID) or a mass spectrometric (MS) detector.

3. Interferences

Any compound having chemical and physical properties similar to an analyte of interest may interfere.

4. Apparatus

4.1 *Boiling chips,* micro, carbon chips: Rinse with hexane, air dry, and heat at 300°C overnight.

4.2 *Concentrator, Kuderna-Danish (K-D),* 500 mL, all glass, with ground-glass joints, a 10.0-mL receiver, and a one-ball Snyder column.

4.3 *Evaporative concentrator,* Organomation N-Evap, or equivalent: Water bath must be maintained at 50° to 55°C.

4.4 *Gas chromatograph/mass spectrometer/data system,* (GC/MS) Hewlett-Packard 5985 B GC/MS, or equivalent: The gas chromatograph is used with one of the following options:

4.4.1 *Columns,* fused silica capillary column, 25 m×0.20 mm id (inside diameter), SE-54 bonded column, 0.33-µm film thickness.

Detector, mass spectrometer (MS).
Injection temperature, 260°C.
Carrier gas, 1mL/min, helium.
Transfer line temperature, 285°C.
Mode, splitless injection.
Program rate, 45° to 300°C, 2.5-min initial hold, 6°C/min, 15-min final hold.

4.4.2 *Columns,* two fused silica capillary columns, 25 m×0.20 mm id, SE-54 bonded column, 0.33-µm film thickness.

Detectors, mass spectrometer, FID 285°C.
Injection temperature, 260°C.
Mode, splitless injection.
Carrier gas, 1 mL/min, helium.
Transfer line, 285°C.
Program rate, 45° to 300°C, 2.5-min initial hold, 6°C/min, 15-min final hold.

5. Reagents

5.1 *Base/neutral standards,* EPA analytical reference grade or highest purity available: Weigh 20 mg of the compound to three significant figures, quantitatively transfer to a 100-mL volumetric flask, and dilute to volume with methylene chloride. Prepare dilutions in methylene chloride to obtain solutions containing 2, 10, and 20 ng/µL.

5.2 *DFTPP (Decafluorotriphenylphosphine) solution,* 50 ng/μL: Dilute 20 μL DFTPP (Supelco, or equivalent, 25-mg/mL solution) to 10 mL in a volumetric flask with methylene chloride.

5.3 *Internal standards,* perdeuteronaphthalene (naphthalene-d_8), Aldrich Chemical Co., or equivalent; perdeuterophenanthrene (phenanthrene-d_{10}), Kor Isotopes, Division of Kor, Inc., or equivalent; and perdeuterochrysene (chrysene-d_{12}), Kor Isotopes, Division of Kor, Inc., or equivalent. Weigh about 20 mg of perdeuteronaphthalene, perdeuterophenanthrene, and perdeuterochrysene to three significant figures, quantitatively transfer to a 100-mL volumetric flask, and dilute to volume with methylene chloride.

5.4 *Sodium chloride,* reagent grade: Heat at 300°C overnight and store in a closed glass container.

5.5 *Sodium hydroxide,* 37 percent (weight/volume): Dissolve 185 g sodium hydroxide pellets in 500 mL organic-free water and reflux 8 hr. Cool and store at 4°C.

5.6 *Sodium sulfate,* granular, anhydrous, reagent grade: Heat at 300°C overnight and store in a glass-stoppered Erlenmeyer flask at 130°C.

5.7 *Solvents,* hexane, isopropanol, and methylene chloride, glass distilled, pesticide analysis quality, Burdick and Jackson, or equivalent.

5.8 *Surrogate standards,* 1-fluoronaphthalene, Aldrich Chemical Co., or equivalent; 2,2′-difluorobiphenyl, Pfaltz and Bauer Co., or equivalent; and p-dibromobenzene, FDA, or equivalent. Weigh 4 mg of each of the three compounds to three significant figures, quantitatively transfer to a 100-mL volumetric flask, and dilute to volume with isopropanol.

5.9 *Water,* organic-free.

6. Procedure

All glassware must be washed in a warm detergent solution, rinsed with organic-free water, and heated at 300°C overnight. Immediately before use, it must be rinsed with methylene chloride. Stopcock grease should not be used on ground-glass joints.

6.1 Immediately upon receipt of the sample, store at 4°C. Extraction must begin within 48 h following receipt of the sample.

6.2 A blank must accompany each group of samples. For each sample and blank, rinse a 2-L separatory funnel and a 500-mL Erlenmeyer flask with methylene chloride.

6.3 Weigh the capped sample bottle to three significant figures and record the weight for subsequent calculations.

6.4 Adjust the sample to pH 11, as indicated by pH paper, by the addition of sodium hydroxide solution.

6.5 Pour the sample into a separatory funnel containing 100 g sodium chloride. Allow the sample bottle to drain into the separatory funnel for several minutes. Stopper and shake until the salt is dissolved.

6.6 Weigh the empty, capped sample bottle to three significant figures, calculate and record the net sample weight.

6.7 Add 1 mL of the surrogate standard (step 5.8) to the sample in the separatory funnel.

6.8 Add 100 mL methylene chloride to the empty sample bottle and swirl to wash the sides of the container with the solvent. The Teflon liner is not rinsed because of the potential of contamination from solvent that has contacted the cap threads and the surface beneath the liner. Pour the contents of the bottle into the separatory funnel.

6.9 Stopper the separatory funnel and shake for at least 1 min, venting often to relieve pressure.

6.10 Drain the organic layer into a 500-mL Erlenmeyer flask containing approximately 30 g sodium sulfate.

6.11 Extract the sample two more times with 50 mL methylene chloride by repeating steps 6.8 through 6.10, collecting the two organic extracts in the Erlenmeyer flask containing the sodium sulfate.

NOTE: The aqueous phase may be retained and extracted for the acidic extractable organic compounds after adjusting the pH to 2 (method O–3117).

6.12 Cover the Erlenmeyer flask with aluminum foil and allow to stand at room temperature for approximately 4 h.

6.13 Quantitatively transfer the dried extract into a K-D apparatus, add a boiling chip, and attach a Snyder column.

6.14 Concentrate the extract to about 5 mL by heating the apparatus on a 80°C water bath in a fume hood.

6.15 Allow the K-D apparatus to cool. Dry the apparatus with a towel, especially around the ground-glass joint of the receiver.

6.16 Separate the Snyder column from the K-D flask and rinse the walls of the K-D flask with approximately 2 mL methylene chloride. Separate the receiver from the K-D flask and rinse the

ground-glass joint of the K-D flask into the receiver with methylene chloride.

6.17 Reduce the volume of the methylene chloride in the receiver to less than 0.9 mL on the evaporative concentrator. During the concentration, rinse the receiver walls two or three times with small portions of methylene chloride.

6.18 Stopper the receiver with a ground-glass stopper and store the extract at 4°C until analysis can proceed.

NOTE: The extract can be combined with the extract from the acidic extractable organic compounds (method O–3117) immediately before injection into the GC/MS.

6.19 Immediately before analysis, add 0.05 mL of internal standard. Adjust the final volume of the sample extract in the receiver to 1.0 mL.

6.20 Mass spectrometer tuning:

6.20.1 Use perfluorotributylamine to tune the mass spectrometer in a manner that results in satisfactory calibration of mass assignments as well as agreement with the criteria listed in step 6.20.2.

6.20.2 Set the MS to scan the mass range 40–450 amu (atomic mass units). Temperature program the GC from 45° to 275°C at 20°C/min with an initial hold of 1.5 min. Inject 50 ng (1 μL of solution, step 5.2). Obtain a background corrected mass spectrum of DFTPP and verify that all of the following criteria are met:

Mass	*Ion abundance criteria*
51	30–60 percent of mass 198
68	<2 percent of mass 69
70	<2 percent of mass 69
127	40–60 percent of mass 198
197	<1 percent of mass 198
198	Base peak, 100 percent relative abundance
199	5–9 percent of mass 198
275	10–30 percent of mass 198
365	>1 percent of mass 198
441	<mass 443
442	>40 percent of mass 198
443	17–23 percent of mass 442

6.21 Analyze the extract by injection of an aliquot into the GC/MS system optimized as follows:

6.21.1 For systems configured with option A, analyze the mass range 40 to 450 amu at a scan rate sufficient to obtain a minimum of 5 scans per chromatographic peak. Record the total ion current chromatogram and the mass spectrum of each peak.

6.21.2 For systems configured with option B, analyze the mass range 40 to 450 amu at a scan rate sufficient to obtain a minimum of 5 scans per chromatographic peak. Record the total ion current chromatogram and the mass spectrum of each peak as well as the retention time and integrated area of each peak from the FID.

6.22 Process the data to determine the identity of the extractable base/neutral compounds including the priority pollutants in the following manner:

6.22.1 Identification of the target compounds is accomplished by a computerized reverse search procedure employing a 25-scan retention time window.

6.22.2 Identification of the extractable base/neutral compounds that are not target compounds is accomplished by a computerized library search versus the National Bureau of Standards library reference spectra on each peak.

6.23 Determine the largest characteristic ion and quantitate the area on this ion for any identified peak, including the internal standard peak and the surrogate standards peaks. Alternatively, if the system is configured with option B, the quantitation can be carried out on the FID response rather than on the mass spectrometer response. The integrated area of an identified peak from the FID is recorded for subsequent calculations. The better chro matogram (FID or MS) is used for quantitation.

6.24 Confirm the surrogate compounds found in the sample by injecting an aliquot of the corresponding surrogate standards (step 5.8) into the gas chromatograph and analyze according to steps 6.21 through 6.23. Record the integrated area obtained.

6.25 Confirm any identified extractable base/neutral compounds found in the sample by injecting an aliquot of the corresponding base/neutral standard into the gas chromatograph and analyze according to steps 6.21 through 6.23. Record the integrated area obtained. If a base/neutral standard is not available, quantitate relative to the internal standard (see step 7.6).

7. Calculations

7.1 Calculations of response factors and relative response factors:

7.1.1 Calculate the response factor for each compound in the base/neutral standard or surrogate standard (step 6.23) using the following equation:

$$RF_i = \frac{A_{si}}{C_{si}},$$

where

RF_i = response factor of compound i in standard, in area/ng,

C_{si} = amount of compound i injected, in ng/μL, and

A_{si} = area of compound i peak in the standard.

7.1.2 Calculate the response factor of the internal standard using the following equation:

$$RFI = \frac{AI_1}{CI_1},$$

where

RFI = response factor of internal standard, in area/ng,

CI_1 = amount of internal standard in base/neutral standard, in ng injected, and

AI_1 = area of internal standard peak.

7.1.3 Calculate a relative response factor by the following equation:

$$RRF_i = \frac{RF_i}{RFI},$$

where

RRF_i = relative response factor, compound i,

RF_i = response factor of compound i, and

RFI = response factor of internal standard.

7.2 Calculations of recoveries of surrogates: Calculate the percent recovery of each surrogate standard recovered from the original Water- suspended-sediment mixture using the following equation:

$$\text{Percent recovery} = \frac{A \times CI_2 \times V_3 \times 100}{RRF_i \times AI_2 \times C_s \times V_4},$$

where

A = area of identified surrogate peak in sample extract,

CI_2 = amount of internal standard injected, in ng,

RRF_i = relative response factor of compound i,

AI_2 = area of internal standard peak in sample extract,

C_s = concentration of standard, in ng/μL,

V_3 = final volume of extract, in mL, and

V_4 = volume injected, in μL.

7.3 Calculation of concentrations of analytes: Calculate the concentration of each identified extractable base/neutral priority pollutant in the original water–suspended-sediment mixture using the following equation:

$$\text{Concentration (μg/L)} = \frac{A \times CI_2 \times V_3 \times 1{,}000}{RRF_i \times AI_2 \times W \times V_4},$$

where

A = area of identified peak in sample extract,

CI_2 = amount of internal standard injected, in ng,

RRF_i = relative response factor of compound i,

AI_2 = area of internal standard peak in sample extract,

W = weight of sample extracted, expressed in mL (1.000 g = 1.000 mL),

V_3 = final volume of extract, in mL, and

V_4 = volume injected, in μL.

7.4 Calculations of concentrations of other compounds: The concentrations of all other identified extractable base/neutral compounds in the original water sample for which there are no standards, are calculated relative to the concentration of the internal standard, and are semiquantitative for the purposes of a general organic scan. The response factor of the compound is assumed to be exactly equal to that of the internal standard. Calculate the concentration using the following equation:

$$\text{Concentration (μg/L)} = \frac{A \times V_3 \times 1{,}000}{RFI \times W \times V_4},$$

where

RFI = response factor of internal standard, in area/ng,

A = area of identified peak in sample,

W = weight of sample extracted, expressed in mL (1.000 g = 1.000 mL),

V_3 = final volume of extract, in mL, and

V_4 = volume injected, in μL.

8. Report

8.1 Report concentrations of extractable base/neutral organic compounds (except chrysene, benzo(a) anthracene, dioctyl phthalate, benzo(b) fluoranthene, benzo(k) fluoranthene, benzo(a) pyrene, indeno(1,2,3-cd) pyrene, dibenz(a,h) anthracene, and benzo(g,h,i) perylene) in water and water–suspended-sediment mixtures as follows: less than 5.0 μg/L, as "less than 5.0 μg/L"; 5.0 μg/L and above, two significant figures.

8.2 Report concentrations of chrysene, benzo(a) anthracene, dioctyl phthalate, benzo(b) fluoranthene, benzo(k) fluoranthene, benzo(a) pyrene, indeno(1,2,3-cd) pyrene, dibenz(a,h) anthracene, and benzo(g,h,i) perylene as follows: less than

10 µg/L, as "less than 10 µg/L"; 10µg/L and above, two significant figures.

9. Precision

9.1 Surrogate recoveries must be from 40 percent to 130 percent unless a matrix effect can be demonstrated. Water and water–suspended-sediment samples were spiked with surrogate standards and recoveries were determined by two operators in a single laboratory over an 8-mo period. Results are as follows:

Compound	*Ion used for quantitation*	*Concentration range (ng/µL)*	*Number of samples analyzed*	*Average recovery (percent)*	*Relative standard deviation (percent)*
1-Fluoronaphthalene	146	48–104	45	65	29
p-Dibromobenzene	236	51–107	46	67	26
2,2′-Difluorobiphenyl	190	43–79	46	68	20

9.2 Deionized water samples were spiked with base/neutral extractable compounds and recoveries were determined by two operators in a single laboratory over a 1-yr period. Results are as follows:

Compound	*Ion used for quantitation*	*Concentration range (ng/µL)*	*Number of samples analyzed*	*Average recovery (percent)*	*Relative standard deviation (percent)*
Acenaphthylene	152	190	2	105	---
Anthracene	178	100–177	5	66	45
Benzo(a)anthracene	228	69	5	79	23
Benzo(k)fluoranthene	252	50–71	2	98	---
Benzo(a)pyrene	252	50–100	5	115	22
bis(2-chloroethoxy)methane	93	50–100	3	64	---
4-Bromophenyl phenyl ether	248	100	5	43	6
4-Chlorophenyl phenyl ether	204	50	1	111	---
Chrysene	228	50–113	6	42	46
Dibenz(a,h)anthracene	278	69	1	71	---
Di-n-butyl phthalate	149	100	5	53	19
1,2-Dichlorobenzene	146	50–100	5	56	43
1,3-Dichlorobenzene	146	100–201	6	97	30
1,4-Dicholorobenzene	146	102	1	51	---
Diethyl phthalate	149	50–100	14	69	37
Dimethyl phthalate	163	199	1	19	---
2,4-Dinitrotoluene	165	50–106	6	63	19
Dimethyl phthalate	149	100	15	69	42
bis(2-ethylhexyl) phthalate	149	100	2	42	---
Fluorene	166	50–197	5	99	11
Fluoranthene	202	224	1	98	---
Hexachlorobenzene	284	50–76	3	91	---
Hexachlorobutadine	225	50–74	3	94	---
Indeno(1,2,3-cd)pyrene	276	50	1	104	---
Naphthalene	128	50–130	6	81	17
Nitrobenzene	77	219	1	50	---
N-Nitrosodimethylamine	74	50	1	68	---
N-Nitrosodiphenylamine	169	50	1	48	---
Phenanthrene	178	218	1	94	---
Pyrene	202	50–334	16	94	16
1,2,4-Trichlorobenzene	180	100	5	78	24

Selected references

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