TABLE 6410:IV. SUGGESTED INTERNAL AND SURROGATE STANDARDS

Base/Neutral Fraction	Acid Fraction
Aniline-d_5	2-Fluorophenol
Anthracene-d_{10}	Pentafluorophenol
Benzo(a)anthracene-d_{12}	Phenol-d_5
4,4′-Dibromobiphenyl	2-Perfluoromethyl phenol
4,4′-Dibromooctafluorobiphenyl	
Decafluorobiphenyl	
2,2′-Difluorobiphenyl	
4-Fluoroaniline	
1-Fluoronaphthylene	
2-Fluoronaphthylene	
Naphthalene-d_8	
Nitrobenzene-d_5	
2,3,4,5,6-Pentafluorobiphenyl	
Phenanthrene-d_{10}	
Pyridine-d_5	

centration near, but above, the MDL and others corresponding to the expected range of sample concentrations or defining the working range of the GC/MS system.

k. Quality control (QC) check sample concentrate: Obtain a check sample concentrate containing each compound at a concentration of 100 μg/mL in acetone. Multiple solutions may be required. PCBs and multicomponent pesticides may be omitted. If such a sample is not available from an external source, prepare using stock standards prepared independently from those used for calibration.

5. Procedure

a. Extraction: Extraction by means of a separatory funnel, ¶ 1), is most common, but if emulsions will prevent acceptable solvent recovery, use continuous extraction, ¶ 2).

1) Separatory funnel extraction—Normally use a sample volume of 1 L. For sample volumes of 2 L, use 250-, 100-, and 100-mL volumes of methylene chloride for the serial extraction of the base/neutrals and 200-, 100-, and 100-mL volumes of methylene chloride for the acids.

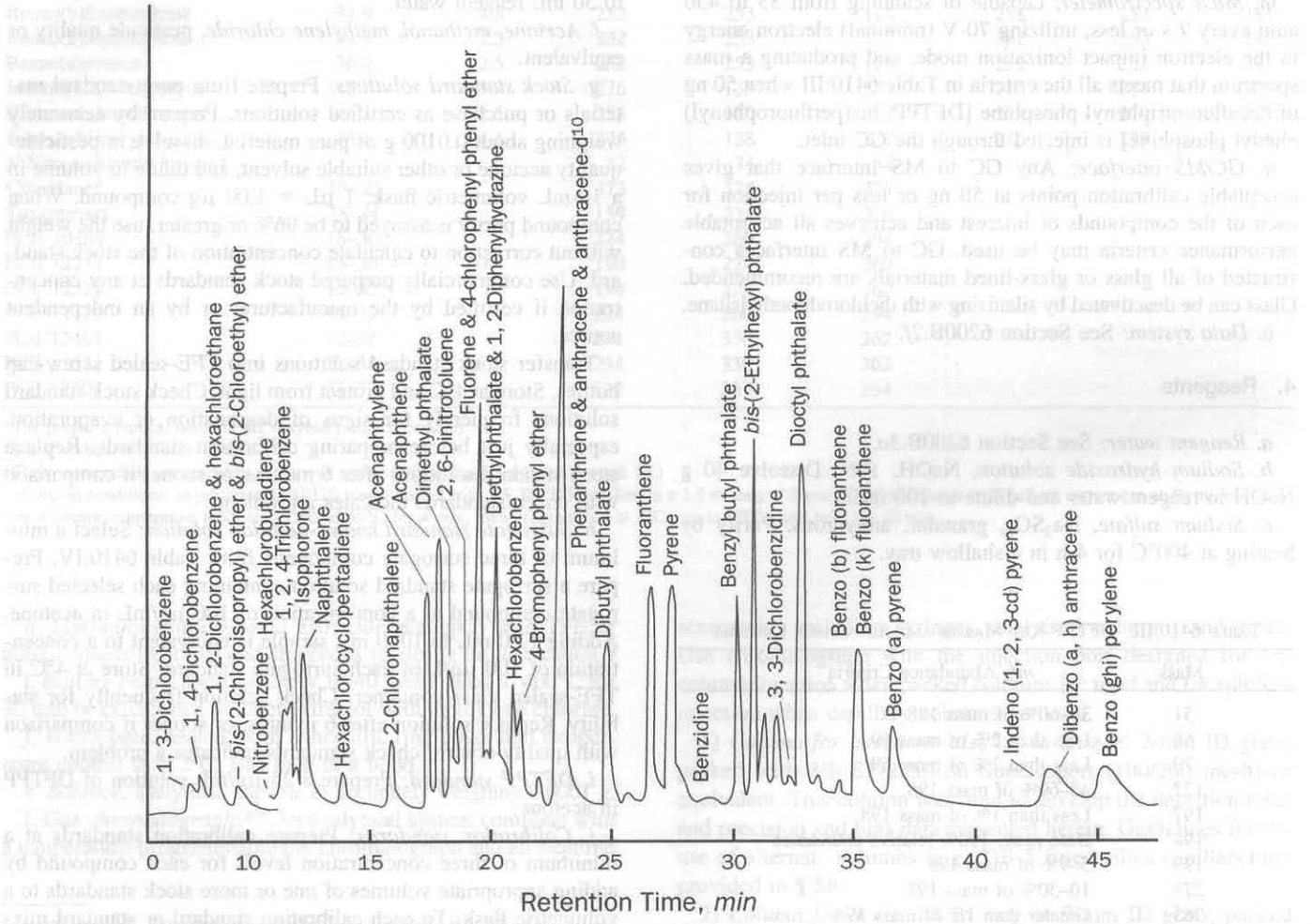

Figure 6410:1. Gas chromatogram of base/neutral fraction. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

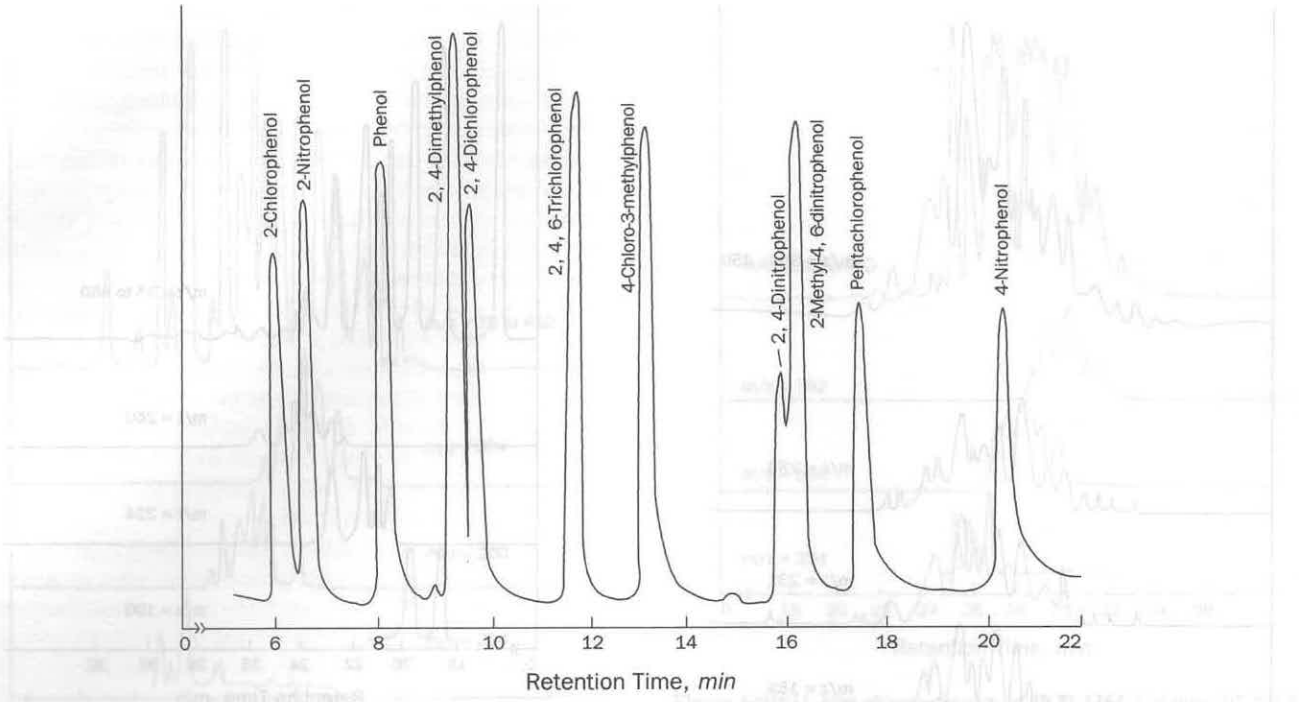

Figure 6410:2. Gas chromatogram of acid fraction. Column: 1% SP-1240DA on Supelcoport; program: 70°C for 2 min, 8°C/min to 200°C; detector: mass spectrometer.

Mark water meniscus on side of sample bottle for later determination of sample volume. Pour entire sample into a 2-L separatory funnel. Pipet 1.00 mL surrogate standard solution into separatory funnel and mix well. Check pH with wide-range pH paper and adjust to pH > 11 with NaOH solution.

Add 60 mL methylene chloride to sample bottle, seal, and shake for 30 s to rinse inner surface. Transfer solvent to separatory funnel and extract sample by shaking for 2 min with periodic venting to release excess pressure. Let organic layer separate from water phase for a minimum of 10 min. If emulsion interface between layers is more than one-third the volume of the

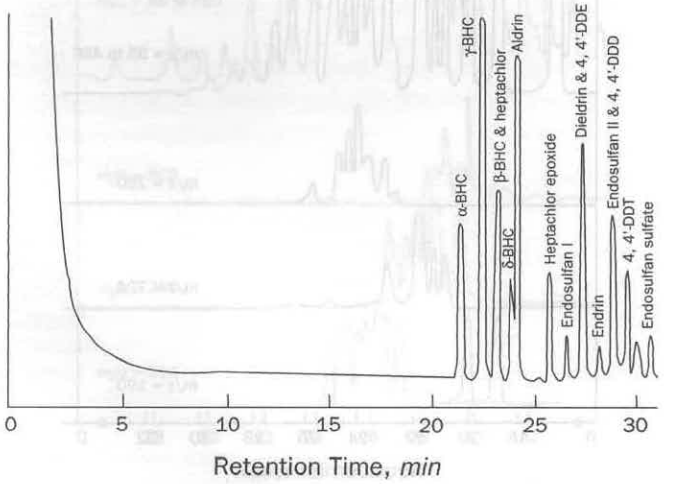

Figure 6410:3. Gas chromatogram of pesticide fraction. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

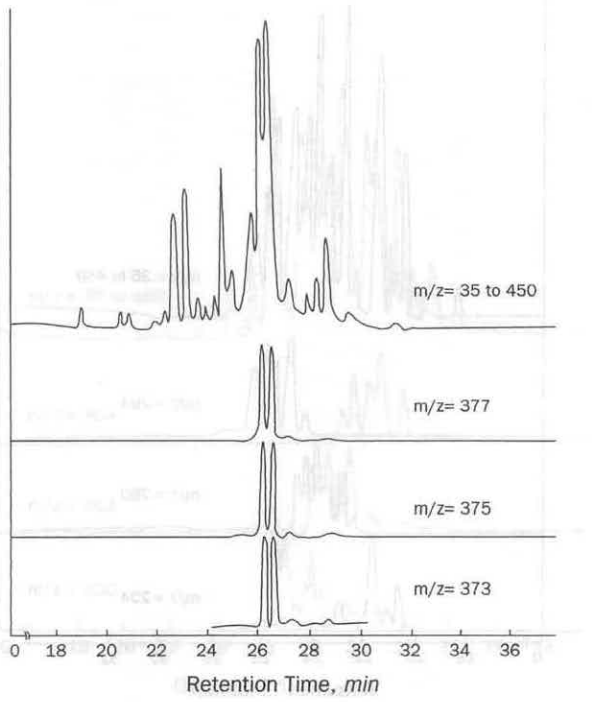

Figure 6410:4. Gas chromatogram of chlordane. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

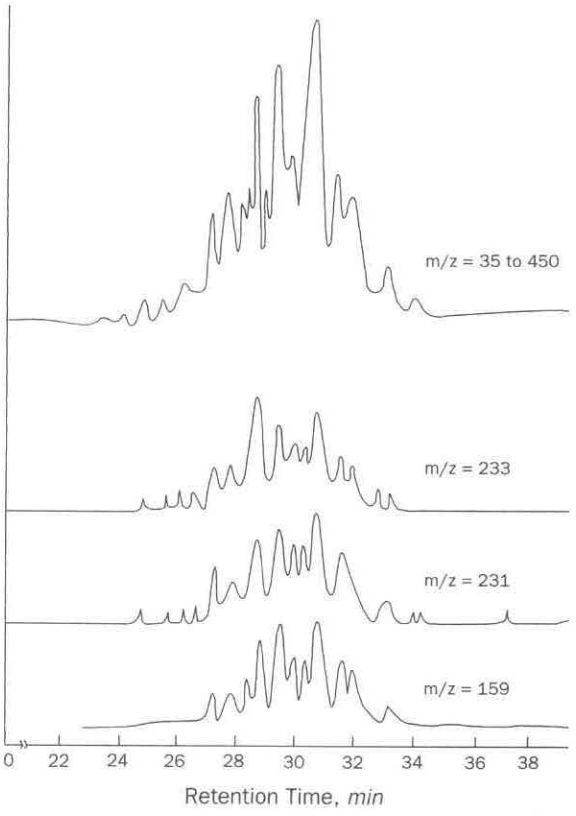

Figure 6410:5. Gas chromatogram of toxaphene. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

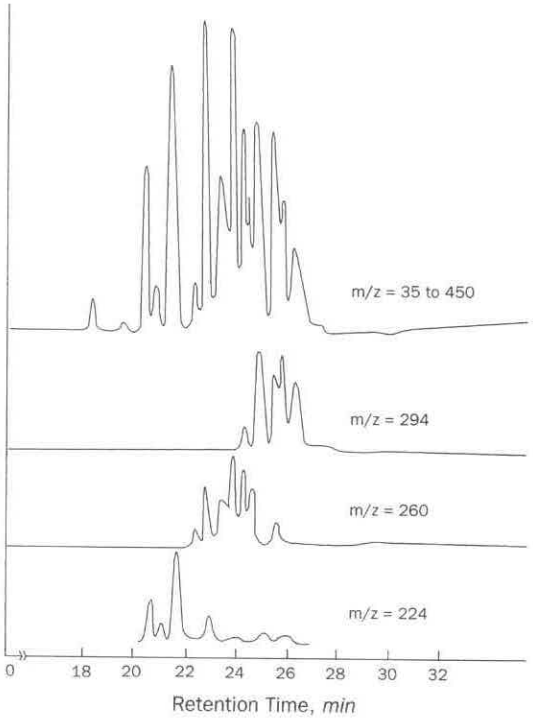

Figure 6410:6. Gas chromatogram of PCB-1016. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

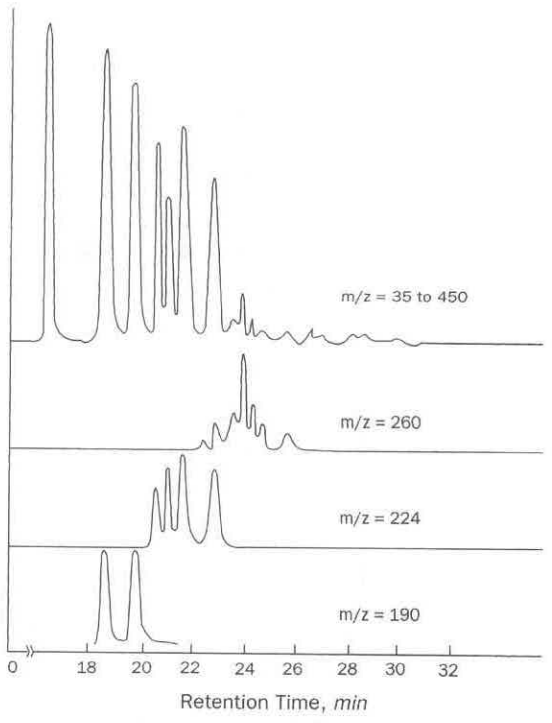

Figure 6410:7. Gas chromatogram of PCB-1221. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

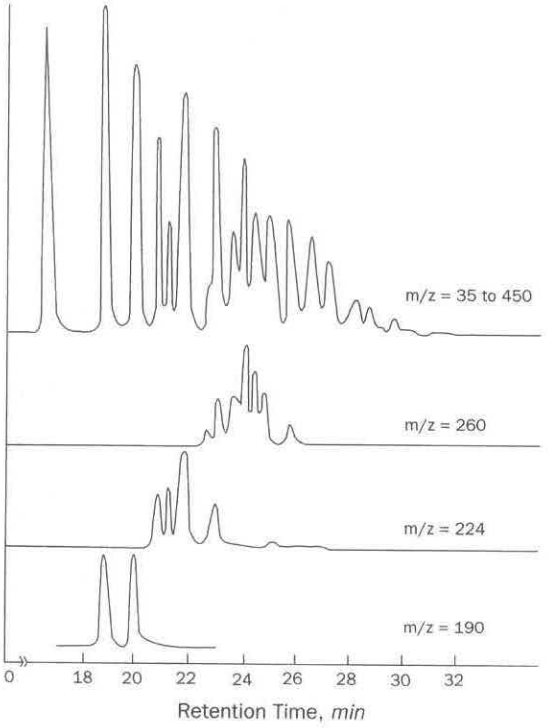

Figure 6410:8. Gas chromatogram of PCB-1232. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

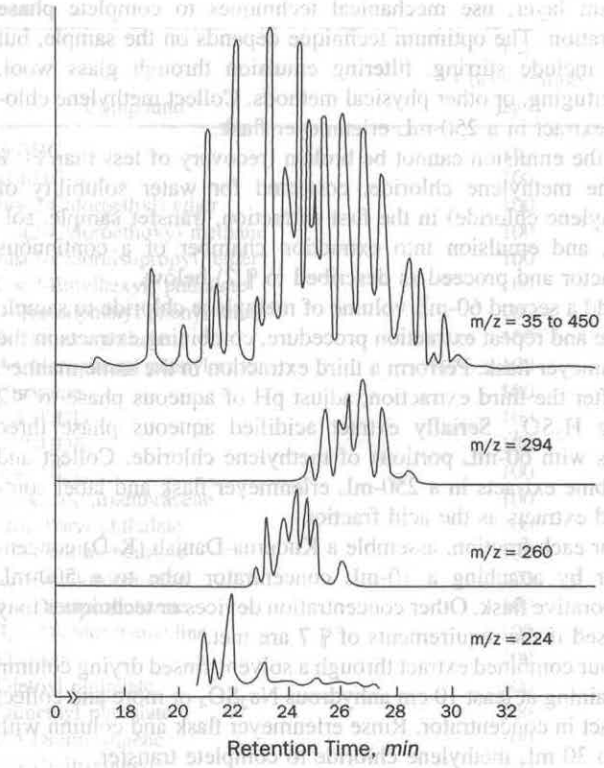

Figure 6410:9. Gas chromatogram of PCB-1242. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

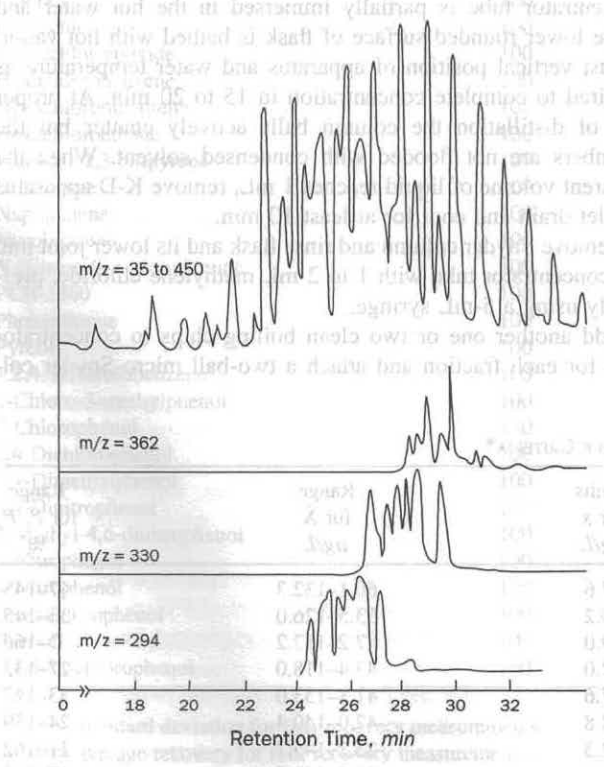

Figure 6410:10. Gas chromatogram of PCB-1248. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

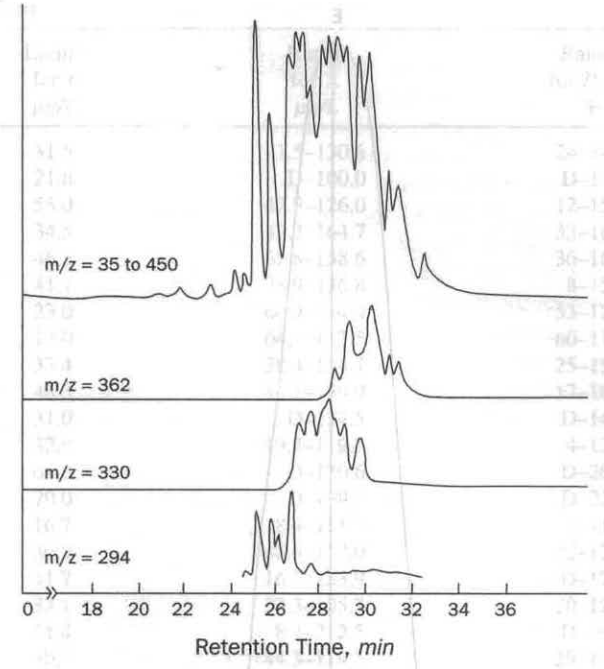

Figure 6410:11. Gas chromatogram of PCB-1254. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

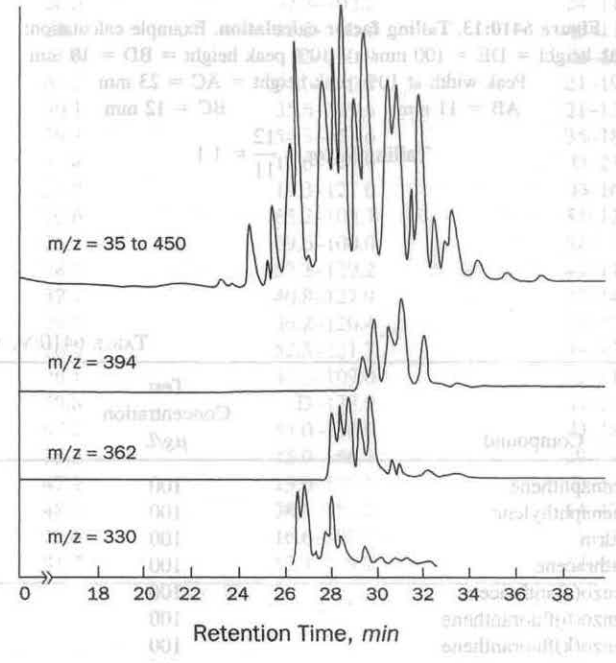

Figure 6410:12. Gas chromatogram of PCB-1260. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

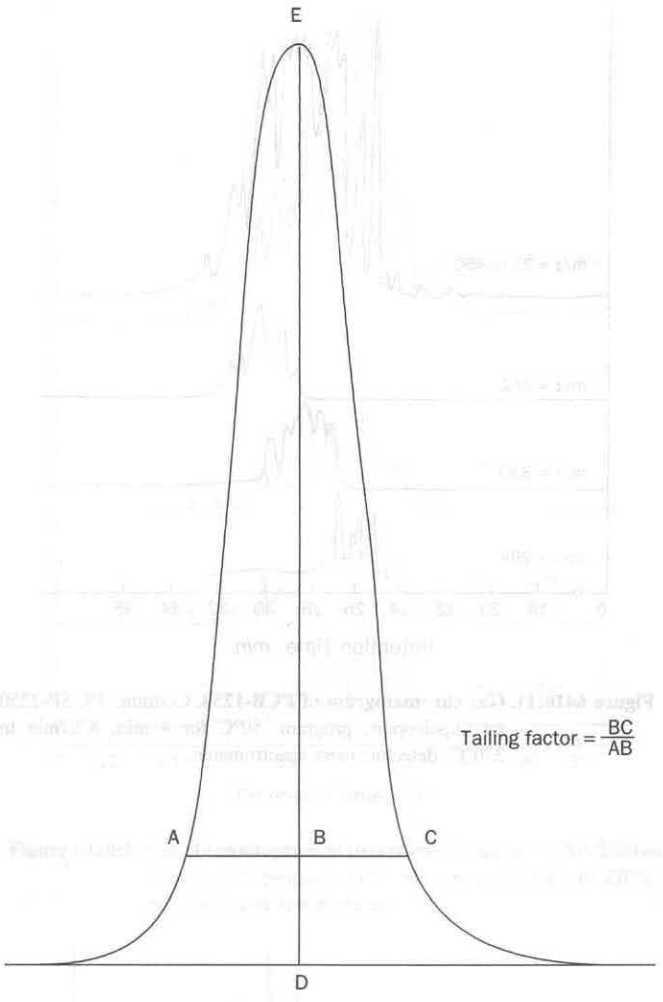

Figure 6410:13. Tailing factor calculation. Example calculation:
Peak height = DE = 100 mm 10% peak height = BD = 10 mm
Peak width at 10% peak height = AC = 23 mm
AB = 11 mm BC = 12 mm

$$\text{Tailing factor} = \frac{12}{11} = 1.1$$

solvent layer, use mechanical techniques to complete phase separation. The optimum technique depends on the sample, but may include stirring, filtering emulsion through glass wool, centrifuging, or other physical methods. Collect methylene chloride extract in a 250-mL erlenmeyer flask.

If the emulsion cannot be broken (recovery of less than 80% of the methylene chloride, corrected for water solubility of methylene chloride) in the first extraction, transfer sample, solvent, and emulsion into extraction chamber of a continuous extractor and proceed as described in ¶ 2) below.

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

After the third extraction, adjust pH of aqueous phase to <2 using H_2SO_4. Serially extract acidified aqueous phase three times with 60-mL portions of methylene chloride. Collect and combine extracts in a 250-mL erlenmeyer flask and label combined extracts as the acid fraction.

For each fraction, assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used if the requirements of ¶ 7 are met.

Pour combined extract through a solvent-rinsed drying column containing at least 10 cm anhydrous Na_2SO_4 or more and collect extract in concentrator. Rinse erlenmeyer flask and column with 20 to 30 mL methylene chloride to complete transfer.

Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet Snyder column by adding about 1 mL methylene chloride to the top. Place K-D apparatus on a hot water bath (60 to 65°C) in a hood so that concentrator tube is partially immersed in the hot water and entire lower rounded surface of flask is bathed with hot vapor. Adjust vertical position of apparatus and water temperature as required to complete concentration in 15 to 20 min. At proper rate of distillation the column balls actively chatter but the chambers are not flooded with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove K-D apparatus and let drain and cool for at least 10 min.

Remove Snyder column and rinse flask and its lower joint into the concentrator tube with 1 to 2 mL methylene chloride, preferably using a 5-mL syringe.

Add another one or two clean boiling chips to concentrator tube for each fraction and attach a two-ball micro-Snyder col-

TABLE 6410:V. QC ACCEPTANCE CRITERIA*

Compound	Test Concentration µg/L	Limits for s µg/L	Range for $\bar{X}$ µg/L	Range for P, P_s %
Acenaphthene	100	27.6	60.1–132.3	47–145
Acenaphthylene	100	40.2	53.5–126.0	33–145
Aldrin	100	39.0	7.2–152.2	D–166
Anthracene	100	32.0	43.4–118.0	27–133
Benzo(a)anthracene	100	27.6	41.8–133.0	33–143
Benzo(b)fluoranthene	100	38.8	42.0–140.4	24–159
Benzo(k)fluoranthene	100	32.3	25.2–145.7	11–162
Benzo(a)pyrene	100	39.0	31.7–148.0	17–163
Benzo(ghi)perylene	100	58.9	D–195.0	D–219
Benzyl butyl phthalate	100	23.4	D–139.9	D–152

TABLE 6410:V. CONT.

Compound	Test Concentration µg/L	Limits for *s* µg/L	Range for $\bar{X}$ µg/L	Range for *P*, P_s %
δ-BHC	100	31.5	41.5–130.6	24–149
β-BHC	100	21.6	D–100.0	D–110
bis(2-Chloroethyl) ether	100	55.0	42.9–126.0	12–158
bis(2-Chloroethoxy) methane	100	34.5	49.2–164.7	33–184
bis(2-Chloroisopropyl) ether†	100	46.3	62.8–138.6	36–166
bis(2-Ethylhexyl) phthalate	100	41.1	28.9–136.8	8–158
4-Bromophenyl phenyl ether	100	23.0	64.9–114.4	53–127
2-Chloronaphthalene	100	13.0	64.5–113.5	60–118
4-Chlorophenyl phenyl ether	100	33.4	38.4–144.7	25–158
Chrysene	100	48.3	44.1–139.9	17–168
4,4′-DDD	100	31.0	D–134.5	D–145
4,4′-DDE	100	32.0	19.2–119.7	4–136
4,4′-DDT	100	61.6	D–170.6	D–203
Dibenzo(a,h)anthracene	100	70.0	D–199.7	D–227
Di-*n*-butyl phthalate	100	16.7	8.4–111.0	1–118
1,2-Dichlorobenzene	100	30.9	48.6–112.0	32–129
1,3-Dichlorobenzene	100	41.7	16.7–153.9	D–172
1,4-Dichlorobenzene	100	32.1	37.3–105.7	20–124
3,3′-Dichlorobenzidine	100	71.4	8.2–212.5	D–262
Dieldrin	100	30.7	44.3–119.3	29–136
Diethyl phthalate	100	26.5	D–100.0	D–114
Dimethyl phthalate	100	23.2	D–100.0	D–112
2,4-Dinitrotoluene	100	21.8	47.5–126.9	39–139
2,6-Dinitrotoluene	100	29.6	68.1–136.7	50–158
Di-*n*-octylphthalate	100	31.4	18.6–131.8	4–146
Endosulfan sulfate	100	16.7	D–103.5	D–107
Endrin aldehyde	100	32.5	D–188.8	D–209
Fluoranthene	100	32.8	42.9–121.3	26–137
Fluorene	100	20.7	71.6–108.4	59–121
Heptachlor	100	37.2	D–172.2	D–192
Heptachlor epoxide	100	54.7	70.9–109.4	26–155
Hexachlorobenzene	100	24.9	7.8–141.5	D–152
Hexachlorobutadiene	100	26.3	37.8–102.2	24–116
Hexachloroethane	100	24.5	55.2–100.0	40–113
Indeno(1,2,3-cd)pyrene	100	44.6	D–150.9	D–171
Isophorone	100	63.3	46.6–180.2	21–196
Naphthalene	100	30.1	35.6–119.6	21–133
Nitrobenzene	100	39.3	54.3–157.6	35–180
N-Nitrosodi-*n*-propylamine	100	55.4	13.6–197.9	D–230
PCB-1260	100	54.2	19.3–121.0	D–164
Phenanthrene	100	20.6	65.2–108.7	54–120
Pyrene	100	25.2	69.6–100.0	52–115
1,2,4-Trichlorobenzene	100	28.1	57.3–129.2	44–142
4-Chloro-3-methylphenol	100	37.2	40.8–127.9	22–147
2-Chlorophenol	100	28.7	36.2–120.4	23–134
2,4-Dichlorophenol	100	26.4	52.5–121.7	39–135
2,4-Dimethylphenol	100	26.1	41.8–109.0	32–119
2,4-Dinitrophenol	100	49.8	D–172.9	D–191
2-Methyl-4,6-dinitrophenol	100	93.2	53.0–100.0	D–181
2-Nitrophenol	100	35.2	45.0–166.7	29–182
4-Nitrophenol	100	47.2	13.0–106.5	D–132
Pentachlorophenol	100	48.9	38.1–151.8	14–176
Phenol	100	22.6	16.6–100.0	5–112
2,4,6-Trichlorophenol	100	31.7	52.4–129.2	37–144

**s* = standard deviation for four recovery measurements,
$\bar{X}$ = average recovery for four recovery measurements,
P, *Ps* = percent recovery measured, and
D = detected; results must be greater than zero.

† The proper chemical name is 2,2′-oxybis(1-chloropropane).

NOTE: These criteria are based directly upon the method performance data in Table 6410:VI. Where necessary, the limits for recovery were broadened to assure applicability of the limits to concentrations below those used to develop Table 6410:VI.

umn. Prewet Snyder column by adding about 0.5 mL of methylene chloride to the top. Place K-D apparatus on a hot-water bath (60 to 65°C) so that concentrator tube is partially immersed in hot water and continue concentrating as directed above without further solvent addition until apparent volume of liquid reaches about 0.5 mL. After cooling, remove Snyder column and rinse flask and its lower joint into the concentrator tube with approximately 0.2 mL acetone or methylene chloride. Adjust final volume to 1.0 mL with solvent. Stopper concentrator tube and store refrigerated if further processing will not be done immediately. If extract is to be stored longer than 2 d, transfer to a TFE-sealed screw-cap vial and label base/neutral or acid fraction as appropriate.

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

2) Continuous extraction—Mark water meniscus on side of sample bottle, and determine sample volume later as described in ¶ 1). Check pH with wide-range pH paper and adjust to pH > 11 with NaOH solution. Transfer sample to continuous extractor and, using a pipet, add 1.00 mL surrogate standard solution and mix well. Add 60 mL methylene chloride to sample bottle, seal, and shake for 30 s to rinse inner surface. Transfer solvent to extractor. Repeat rinse with an additional 50- to 100-mL portion methylene chloride and add rinse to extractor.

Add 200 to 500 mL methylene chloride to distilling flask, add sufficient reagent water to ensure proper operation, and extract for 24 h. Let cool and detach distilling flask. Dry, concentrate, and seal extract as in ¶ 1) above.

Charge a clean distilling flask with 500 mL methylene chloride and attach it to continuous extractor. Carefully, while stirring, adjust pH of aqueous phase to less than 2 with H_2SO_4. Extract for 24 h. Dry, concentrate, and seal extract as in ¶ 1) above.

b. GC/MS operating conditions: Table 6410:I summarizes the recommended gas chromatographic operating conditions for the base/neutral fraction and Table 6410:II for the acid fraction. Included in these tables are retention times and MDLs that can be achieved under these conditions. Examples of the separations obtained with these columns are shown in Figures 6410:1 through 12. Other packed or capillary (open-tubular) columns or chromatographic conditions may be used if the requirements of ¶ 7 are met.

c. GC/MS performance tests: At the beginning of each day on which analyses are to be performed, check GC/MS system to see if acceptable performance criteria are achieved for DFTPP.[8] Each day that benzidine is to be determined, the tailing factor criterion described in ¶ 2) must be achieved. Each day that the acids are to be determined, the tailing factor criterion described in ¶ 3) must be achieved.

These performance tests have the requirements given in Section 6200B.4*b*, but use following conditions:

Electron energy: 70 V (nominal)

Mass range: 35 to 450 amu

Scan time: To give at least 5 scans per peak but not to exceed 7 s per scan.

1) DFTPP performance test—At beginning of each day, inject 2 μL (50 ng) DFTPP standard solution. Obtain a background-corrected mass spectrum of DFTPP and confirm that all the key *m/z* criteria in Table 6410:III are achieved. If not, retune mass spectrometer and repeat test until all criteria are achieved. Meet performance criteria before any samples, blanks, or standards are analyzed. The tailing factor tests in ¶s 2) and 3) may be performed simultaneously with the DFTPP test.

2) Column performance test for base/neutrals—At beginning of each day that base/neutral fraction is to be analyzed for benzidine, calculate benzidine tailing factor. Inject 100 ng benzidine either separately or as a part of a standard mixture that may contain DFTPP, and calculate tailing factor, which must be less than 3.0. Calculation of the tailing factor is illustrated in Figure 6410:13.[9] Replace column packing if tailing factor criterion cannot be met.

3) Column performance test for acids—At beginning of each day that acids are to be determined, inject 50 ng pentachlorophenol either separately or as a part of a standard mix that may contain DFTPP. The tailing factor for pentachlorophenol must be less than 5. Calculation of the tailing factor is illustrated in Figure 6410:13.[9] Replace column packing if tailing factor criterion cannot be met.

d. Calibration of GC/MS system: Calibrate system daily after performance tests.

Select three or more internal standards similar in analytical behavior to the compounds of interest. Demonstrate that the measurement of the internal standards is not affected by method or matrix interferences. Some recommended internal standards are listed in Table 6410:IV. Use base peak *m/z* as the primary *m/z* for quantification. If interferences are noted, use one of the next two most intense *m/z* quantities for quantification. Using injections of 2 to 5 μL, analyze each calibration standard according to ¶ *e* below and tabulate area of primary characteristic *m/z* (Tables 6410:I and II) against concentration for each compound and internal standard. Calculate response factors (RF) for each compound by the equation given in Section 6200B.4*c*2). If the RF value over the working range is a constant (<35% RSD), it can be assumed to be invariant; use the average RF for calculations. Alternatively, use the results to plot a calibration curve of response ratios, A_s/A_{is} vs. *RF*.

Verify working calibration curve or RF on each working day by measuring one or more calibration standards. If the response for any compound varies from the predicted response by more than 20%, repeat test using a fresh calibration standard. Alternatively, prepare a new calibration curve for that compound.

e. Sample analysis: Add internal standard to sample extract, mix thoroughly, and immediately inject 2 to 5 μL of sample extract or standard into GC/MS system using solvent-flush technique[10] to minimize losses due to adsorption, chemical reaction, or evaporation. Smaller (1.0-μL) volumes may be injected if automatic devices are used. Record volume injected to nearest 0.05 μL. If response for any *m/z* exceeds the working range of the GC/MS system, dilute extract and reanalyze. Make all qualitative and quantitative measurements as described below and in ¶ 6. When extract is not being used, store at 4°C, protected from light, in screw-cap vial equipped with unpierced TFE-lined septum.

Obtain EICPs for the primary *m/z* and the two other masses listed in Tables 6410:I and II. See ¶ *d* for masses to be used with internal and surrogate standards. Use the following criteria to make a qualitative identification:

- The characteristic masses of each compound maximize in the same or within one scan of each other.

TABLE 6410:VI. METHOD BIAS AND PRECISION AS FUNCTIONS OF CONCENTRATION*

Compound	Bias as Recovery, X' µg/L	Single-Analyst Precision, s_r' µg/L	Overall Precision, S' µg/L
Acenaphthene	$0.96C + 0.19$	$0.15\bar{X} - 0.12$	$0.21\bar{X} - 0.67$
Acenaphthylene	$0.89C + 0.74$	$0.24\bar{X} - 1.06$	$0.26\bar{X} - 0.54$
Aldrin	$0.78C + 1.66$	$0.27\bar{X} - 1.28$	$0.43\bar{X} + 1.13$
Anthracene	$0.80C + 0.68$	$0.21\bar{X} - 0.32$	$0.27\bar{X} - 0.64$
Benzo(a)anthracene	$0.88C - 0.60$	$0.15\bar{X} + 0.93$	$0.26\bar{X} - 0.28$
Benzo(b)fluoranthene	$0.93C - 1.80$	$0.22\bar{X} + 0.43$	$0.29\bar{X} + 0.96$
Benzo(k)fluoranthene	$0.87C - 1.56$	$0.19\bar{X} + 1.03$	$0.35\bar{X} + 0.40$
Benzo(a)pyrene	$0.90C - 0.13$	$0.22\bar{X} + 0.48$	$0.32\bar{X} + 1.35$
Benzo(ghi)perylene	$0.98C - 0.86$	$0.29\bar{X} + 2.40$	$0.51\bar{X} - 0.44$
Benzyl butyl phthalate	$0.66C - 1.68$	$0.18\bar{X} + 0.94$	$0.53\bar{X} + 0.92$
β-BHC	$0.87C - 0.94$	$0.20\bar{X} - 0.58$	$0.30\bar{X} - 1.94$
δ-BHC	$0.29C - 1.09$	$0.34\bar{X} + 0.86$	$0.93\bar{X} - 0.17$
bis(2-Chloroethyl) ether	$0.86C - 1.54$	$0.35\bar{X} - 0.99$	$0.35\bar{X} + 0.10$
bis(2-Chloroethoxy) methane	$1.12C - 5.04$	$0.16\bar{X} + 1.34$	$0.26\bar{X} + 2.01$
bis(2-Chloroisopropyl) ether†	$1.03C - 2.31$	$0.24\bar{X} + 0.28$	$0.25\bar{X} + 1.04$
bis(2-Ethylhexyl) phthalate	$0.84C - 1.18$	$0.26\bar{X} + 0.73$	$0.36\bar{X} + 0.67$
4-Bromophenyl phenyl ether	$0.91C - 1.34$	$0.13\bar{X} + 0.66$	$0.16\bar{X} + 0.66$
2-Chloronaphthalene	$0.89C + 0.01$	$0.07\bar{X} + 0.52$	$0.13\bar{X} + 0.34$
4-Chlorophenyl phenyl ether	$0.91C + 0.53$	$0.20\bar{X} - 0.94$	$0.30\bar{X} - 0.46$
Chrysene	$0.93C - 1.00$	$0.28\bar{X} + 0.13$	$0.33\bar{X} - 0.09$
4,4′-DDD	$0.56C - 0.40$	$0.29\bar{X} - 0.32$	$0.66\bar{X} - 0.96$
4,4′-DDE	$0.70C - 0.54$	$0.26\bar{X} - 1.17$	$0.39\bar{X} - 1.04$
4,4′-DDT	$0.79C - 3.28$	$0.42\bar{X} + 0.19$	$0.65\bar{X} - 0.58$
Dibenzo(a,h)anthracene	$0.88C + 4.72$	$0.30\bar{X} + 8.51$	$0.59\bar{X} + 0.25$
Di-*n*-butyl phthalate	$0.59C + 0.71$	$0.13\bar{X} + 1.16$	$0.39\bar{X} + 0.60$
1,2-Dichlorobenzene	$0.80C + 0.28$	$0.20\bar{X} + 0.47$	$0.24\bar{X} + 0.39$
1,3-Dichlorobenzene	$0.86C - 0.70$	$0.25\bar{X} + 0.68$	$0.41\bar{X} + 0.11$
1,4-Dichlorobenzene	$0.73C - 1.47$	$0.24\bar{X} + 0.23$	$0.29\bar{X} + 0.36$
3,3′-Dichlorobenzidine	$1.23C - 12.65$	$0.28\bar{X} + 7.33$	$0.47\bar{X} + 3.45$
Dieldrin	$0.82C - 0.16$	$0.20\bar{X} - 0.16$	$0.26\bar{X} - 0.07$
Diethyl phthalate	$0.43C + 1.00$	$0.28\bar{X} + 1.44$	$0.52\bar{X} + 0.22$
Dimethyl phthalate	$0.20C + 1.03$	$0.54\bar{X} + 0.19$	$1.05\bar{X} - 0.92$
2,4-Dinitrotoluene	$0.92C - 4.81$	$0.12\bar{X} + 1.06$	$0.21\bar{X} + 1.50$
2,6-Dinitrotoluene	$1.06C - 3.60$	$0.14\bar{X} + 1.26$	$0.19\bar{X} + 0.35$
Di-*n*-octylphthalate	$0.76C - 0.79$	$0.21\bar{X} + 1.19$	$0.37\bar{X} + 1.19$
Endosulfan sulfate	$0.39C + 0.41$	$0.12\bar{X} + 2.47$	$0.63\bar{X} - 1.03$
Endrin aldehyde	$0.76C - 3.86$	$0.18\bar{X} + 3.91$	$0.73\bar{X} - 0.62$
Fluoranthene	$0.81C + 1.10$	$0.22\bar{X} - 0.73$	$0.28\bar{X} - 0.60$
Fluorene	$0.90C - 0.00$	$0.12\bar{X} + 0.26$	$0.13\bar{X} + 0.61$
Heptachlor	$0.87C - 2.97$	$0.24\bar{X} - 0.56$	$0.50\bar{X} - 0.23$
Heptachlor epoxide	$0.92C - 1.87$	$0.33\bar{X} - 0.46$	$0.28\bar{X} + 0.64$
Hexachlorobenzene	$0.74C + 0.66$	$0.18\bar{X} - 0.10$	$0.43\bar{X} - 0.52$
Hexachlorobutadiene	$0.71C - 1.01$	$0.19\bar{X} + 0.92$	$0.26\bar{X} + 0.49$
Hexachloroethane	$0.73C - 0.83$	$0.17\bar{X} + 0.67$	$0.17\bar{X} + 0.80$
Indeno(1,2,3-cd)pyrene	$0.78C - 3.10$	$0.29\bar{X} + 1.46$	$0.50\bar{X} + 0.44$
Isophorone	$1.12C + 1.41$	$0.27\bar{X} + 0.77$	$0.33\bar{X} + 0.26$
Naphthalene	$0.76C + 1.58$	$0.21\bar{X} - 0.41$	$0.30\bar{X} - 0.68$
Nitrobenzene	$1.09C - 3.05$	$0.19\bar{X} + 0.92$	$0.27\bar{X} + 0.21$
N-Nitrosodi-*n*-propylamine	$1.12C - 6.22$	$0.27\bar{X} + 0.68$	$0.44\bar{X} + 0.47$
PCB-1260	$0.81C - 10.86$	$0.35\bar{X} + 3.61$	$0.43\bar{X} + 1.82$
Phenanthrene	$0.87C - 0.06$	$0.12\bar{X} + 0.57$	$0.15\bar{X} + 0.25$
Pyrene	$0.84C - 0.16$	$0.16\bar{X} + 0.06$	$0.15\bar{X} + 0.31$
1,2,4-Trichlorobenzene	$0.94C - 0.79$	$0.15\bar{X} + 0.85$	$0.21\bar{X} + 0.39$
4-Chloro-3-methylphenol	$0.84C + 0.35$	$0.23\bar{X} + 0.75$	$0.29\bar{X} + 1.31$
2-Chlorophenol	$0.78C + 0.29$	$0.18\bar{X} + 1.46$	$0.28\bar{X} + 0.97$
2,4-Dichlorophenol	$0.87C + 0.13$	$0.15\bar{X} + 1.25$	$0.21\bar{X} + 1.28$
2,4-Dimethylphenol	$0.71C + 4.41$	$0.16\bar{X} + 1.21$	$0.22\bar{X} + 1.31$
2,4-Dinitrophenol	$0.81C - 18.04$	$0.38\bar{X} + 2.36$	$0.42\bar{X} + 26.29$
2-Methyl-4,6-dinitrophenol	$1.04C - 28.04$	$0.10\bar{X} + 42.29$	$0.26\bar{X} + 23.10$

TABLE 6410:VI. CONT.

Compound	Bias as Recovery, X' $\mu g/L$	Single-Analyst Precision, s_r' $\mu g/L$	Overall Precision, S' $\mu g/L$
2-Nitrophenol	$1.07C - 1.15$	$0.16\bar{X} + 1.94$	$0.27\bar{X} + 2.60$
4-Nitrophenol	$0.61C - 1.22$	$0.38\bar{X} + 2.57$	$0.44\bar{X} + 3.24$
Pentachlorophenol	$0.93C + 1.99$	$0.24\bar{X} + 3.03$	$0.30\bar{X} + 4.33$
Phenol	$0.43C + 1.26$	$0.26\bar{X} + 0.73$	$0.35\bar{X} + 0.58$
2,4,6-Trichlorophenol	$0.91C - 0.18$	$0.16\bar{X} + 2.22$	$0.22\bar{X} + 1.81$

*X' = expected recovery for one or more measurements of a sample containing a concentration of C,
s_r' = expected single-analyst standard deviation of measurements at an average concentration found of $\bar{X}$,
S' = expected interlaboratory standard deviation of measurements at an average concentration found of $\bar{X}$,
C = true value for the concentration, and
$\bar{X}$ = average recovery found for measurements of samples containing a concentration of C.
† The proper chemical name is 2,2′-oxybis(1-chloropropane).

• The retention time falls within ±30 s of the retention time of the authentic compound.

• The relative peak heights of the three characteristic masses in the EICPs fall within ±20% of the relative intensities of these masses in a reference mass spectrum obtained from a standard analyzed in the GC/MS system or from a reference library.

Structural isomers that have very similar mass spectra and less than 30 s difference in retention time can be identified explicitly only if the resolution between authentic isomers in a standard mix is acceptable. Acceptable resolution is achieved if the baseline to valley height between the isomers is less than 25% of the sum of the two peak heights. Otherwise, structural isomers are identified as isomeric pairs.

*f. Screening procedure for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD):* CAUTION: *In screening a sample for 2,3,7,8-TCDD, do not handle reference material without taking extensive safety precautions.* It is sufficient to analyze the base/neutral extract by selected ion monitoring (SIM) GC/MS techniques, as follows:

Concentrate base/neutral extract to a final volume of 0.2 mL. Adjust temperature of base/neutral column to 220°C. Operate mass spectrometer to acquire data in the SIM mode using the ions at *m/z* 257, 320, and 322 and a dwell time no greater than 333 ms/mass. Inject 5 to 7 µL of base/neutral extract. Collect SIM data for a total of 10 min. The possible presence of 2,3,7,8-TCDD is indicated if all three masses exhibit simultaneous peaks at any point in the selected ion current profiles. For each occurrence where the possible presence of 2,3,7,8-TCDD is indicated, calculate and retain the relative abundances of each of the three masses. False positives may be caused by the presence of single or coeluting combinations of compounds whose mass spectra contain all of these masses. Conclusive results of the presence and concentration level of 2,3,7,8-TCDD can be obtained only from a properly equipped laboratory using a specialized test method.[11]

6. Calculation

When a compound has been identified, base quantitation on the integrated abundance from the EICP of the primary characteristic *m/z* given in Tables 6410:I and II. Use base peak *m/z* for internal and surrogate standards. If sample produces an interference for the primary *m/z*, use a secondary characteristic *m/z* to quantitate.

Calculate sample concentration using the response factor (RF) determined in ¶ 5*d* and the equation:

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

where:

A_s = area of characteristic *m/z* for compound or surrogate standard to be measured,
A_{is} = area of characteristic *m/z* for internal standard,
I_s = amount of internal standard added to each extract, µg, and
V_e = volume of water extracted, L.

Report results in µg/L without correction for recovery. Report all QC data with sample results.

7. Quality Control

a. Quality control program: See Section 6200A.5.

b. Initial quality control: Proceed according to Section 6200A.5*a*1) and 2). Use Table 6410:V for acceptance criteria.

c. Analyses of samples with known additions: Use quality acceptance criteria given in Table 6410:V.

d. Quality-control check standard analysis: Proceed as in Section 6200A.5*a*3); prepare QC check standard with 1.0 mL QC check standard concentrate and 1 L reagent water.

e. Bias assessment and records: Assess method bias and maintain records. For example, after the analysis of five wastewater samples, calculate the average percent recovery ($\bar{P}$) and the standard deviation of the percent recovery (s_p). Express bias assessment as a percent recovery interval from $\bar{P} - 2s_p$ to $\bar{P} + 2s_p$. If $\bar{P} = 90\%$ and $s_p = 10\%$, the recovery interval is expressed as 70–110%. Update bias assessment for each compound regularly, (e.g., after each five to ten new accuracy measurements).

f. Use of surrogate compounds: As a quality control check, make known additions to all samples of surrogate standard solution as described in ¶ 5*a*1), and calculate percent recovery of each surrogate compound.

g. Additional quality-assurance practices: Other desirable practices depend on the needs of the laboratory and the nature of

the samples. Analyze field duplicates to assess precision of environmental measurements. Whenever possible, analyze standard reference materials and participate in relevant performance evaluation studies. Certain compounds, such as phthalates, are common laboratory contaminants. When these are measured above the detection limits in sample blanks, locate their source and repeat the analysis after taking corrective action.

8. Precision and Bias

This method was tested by 15 laboratories using reagent water, drinking water, surface water, and industrial wastewaters with additions at six concentrations over the range 5 to 1300 μg/L.[3] Single-operator precision, overall precision, and method bias were found to be related directly to the compound concentration and essentially independent of the sample matrix. Linear equations describing these relationships are presented in Table 6410:VI.

9. References

1. U.S. Environmental Protection Agency. 1984. Method 625—Base/neutrals and acids. 40 CFR Part 136, 43385; *Federal Register* 49, No. 209.
2. U.S. Environmental Protection Agency. 1977. Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants. Environmental Monitoring and Support Lab., Cincinnati, Ohio.
3. U.S. Environmental Protection Agency. 1984. EPA Method Study 30, Method 625—Base/Neutrals, Acids, and Pesticides. EPA-600/4-84-053, National Technical Information Serv., PB84-206572, Springfield, Va.
4. American Society for Testing and Materials. 1978. Standard practices for preparation of sample containers and for preservation of organic constituents. ASTM Annual Book of Standards, Part 31, D3694-78. Philadelphia, Pa.
5. U.S. Environmental Protection Agency. 1984. Definition and procedure for the determination of the method detection limit. 40 CFR Part 136, Appendix B. *Federal Register* 49, No. 209.
6. Olynyk, P., W.L. Budde & J.W. Eichelberger. 1980. Method Detection Limit for Methods 624 and 625. Unpublished report.
7. American Society for Testing and Materials. 1976. Standard practices for sampling water. ASTM Annual Book of Standards, Part 31, D3370-76. Philadelphia, Pa.
8. Eichelberger, J.W., L.E. Harris & W.L. Budde. 1975. Reference compound to calibrate ion abundance measurement in gas chromatography-mass spectrometry. *Anal. Chem.* 47:995.
9. McNair, N.M. & E.J. Bonelli. 1969. Basic Chromatography. Consolidated Printing, Berkeley, Calif.
10. Burke, J.A. 1965. Gas chromatography for pesticide residue analysis; some practical aspects. *J. Assoc. Offic. Anal. Chem.* 48:1037.
11. U.S. Environmental Protection Agency. 1984. Method 613—2,3,7,8-Tetrachlorodibenzo-*p*-dioxin. 40 CFR Part 136, 43368; *Federal Register* 49, No. 209.

6420 PHENOLS*

6420 A. Introduction

1. Sources and Significance

Phenols are found in many wastewaters and some raw source waters in the United States. They generally are traceable to industrial effluents or landfills. These compounds have a low taste threshold in potable waters and also may have a detrimental effect on human health at higher levels.

2. Selection of Method

For methods of determining total phenols in water and wastewater, see Section 5530.

The methods presented in this section are intended for the determination of individual phenolic compounds. For specific compounds covered, see each method. Method 6420B is a gas chromatographic (GC) method using liquid-liquid extraction and either flame ionization detection (FID) or derivatization and electron capture detection (ECD) to determine a wide variety of phenols at relatively low concentrations. In addition, Method 6420C, a liquid-liquid extraction gas chromatographic/mass spectrometric (GC/MS) method, can be used to determine the phenols at slightly higher concentrations.

* Approved by Standard Methods Committee, 2000.

6420 B. Liquid-Liquid Extraction Gas Chromatographic Method

This method[1] is applicable to the determination of phenol and certain substituted phenols* in municipal and industrial discharges. When analyzing unfamiliar samples for any or all of these compounds, support the identifications by at least one additional qualitative technique. Alternatively, use the derivatization, cleanup, and electron capture detector gas chromatography (ECD/GC) procedure to confirm measurements made by the flame ionization detector gas chromatographic (FID/GC) proce-

* 4-Chloro-3-methylphenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4-dimethylphenol, 2,4-dinitrophenol, 2-methyl-4,6-dinitrophenol, 2-nitrophenol, 4-nitrophenol, pentachlorophenol, phenol, 2,4,6-trichlorophenol.

TABLE 6420:I. CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LEVELS

Compound	Retention Time *min*	Method Detection Level *µg/L*
2-Chlorophenol	1.70	0.31
2-Nitrophenol	2.00	0.45
Phenol	3.01	0.14
2,4-Dimethylphenol	4.03	0.32
2,4-Dichlorophenol	4.30	0.39
2,4,6-Trichlorophenol	6.05	0.64
4-Chloro-3-methylphenol	7.50	0.36
2,4-Dinitrophenol	10.00	13.0
2-Methyl-4,6-dinitrophenol	10.24	16.0
Pentachlorophenol	12.42	7.4
4-Nitrophenol	24.25	2.8

Column conditions: Supelcoport (80/100 mesh) coated with 1% SP-1240DA packed in a 1.8-m long × 2-mm-ID glass column with nitrogen carrier gas at 30 mL/min flow rate. Column temperature was 80°C at injection, programmed immediately at 8°C/min to 150°C final temperature. MDLs determined with an FID.

TABLE 6420:II. SILICA GEL FRACTIONATION AND ELECTRON CAPTURE GAS CHROMATOGRAPHY OF PFBB DERIVATIVES

Parent Compound	Percent Recovery by Fraction* 1	2	3	4	Retention Time *min*	Method Detection Level *µg/L*
2-Chlorophenol	—	90	1	—	3.3	0.58
2-Nitrophenol	—	—	9	90	9.1	0.77
Phenol	—	90	10	—	1.8	2.2
2,4-Dimethylphenol	—	95	7	—	2.9	0.63
2,4-Dichlorophenol	—	95	1	—	5.8	0.68
2,4,6-Trichlorophenol	50	50	—	—	7.0	0.58
4-Chloro-3-methylphenol	—	84	14	—	4.8	1.8
Pentachlorophenol	75	20	—	—	28.8	0.59
4-Nitrophenol	—	—	1	90	14.0	0.70

Column conditions: Chromosorb W-AW-DMCS (80/100 mesh) coated with 5% OV-17 packed in a 1.8-m long × 2.0-mm-ID glass column with 5% methane/95% argon carrier gas at 30 mL/min flow rate. Column temperature held isothermal at 200°C. MDLs determined with an ECD.

* Eluent composition:
Fraction 1 - 15% toluene in hexane.
Fraction 2 - 40% toluene in hexane.
Fraction 3 - 75% toluene in hexane.
Fraction 4 - 15% 2-propanol in toluene.

dure. The method for base/neutrals and acids (Section 6410B) provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for qualitative and quantitative confirmation of results using the extract produced.

1. General Discussion

a. Principle: See Section 6010C for discussion of gas chromatographic principles. A measured volume of sample is acidified and extracted with methylene chloride. The extract is dried and exchanged to 2-propanol during concentration. The extract is separated by gas chromatography and phenols are measured with a flame ionization detector.[2]

The method provides for a derivatization and column chromatography cleanup procedure to aid in the elimination of interferences.[2,3] Derivatives are analyzed by an electron capture detector.

b. Interferences:

1) General precautions—See Section 6410B.1*b*.

2) Other countermeasures—The cleanup procedure in ¶ 5*c* can be used to overcome many of these inteferences, but unique samples may require additional cleanup to achieve the method detection levels.

The basic sample wash (¶ 5*a*) may cause low recovery of phenol and 2,4-dimethylphenol. Results obtained under these conditions are minimum concentrations.

c. Detection levels: The method detection level (MDL) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[4] The MDL concentrations listed in Tables 6420:I and II were obtained by using reagent water.[5] Similar results were achieved with representative wastewaters. The MDL actually obtained in a given analysis will vary, depending on instrument sensitivity and matrix effects.

d. Safety: The toxicity or carcinogenicity of each reagent used in this method has not been defined precisely. Take special care in handling pentafluorobenzyl bromide, which is a lachrymator, and 18-crown-6-ether, which is highly toxic.

2. Sampling and Storage

See Section 6410B.2.

3. Apparatus

Use all the apparatus specified in Section 6410B.3*a–g* and *i–k*, and in addition:

a. Chromatographic column, 100 mm long × 10 mm ID, with TFE stopcock.

b. Reaction flask, 15- to 25-mL round-bottom, with standard tapered joint, fitted with a water-cooled condenser and U-shaped drying tube containing granular calcium chloride.

c. Gas chromatograph:† An analytical system complete with a temperature-programmable gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. Preferably use a data system for measuring peak areas.

1) *Column for underivatized phenols,* 1.8 m long × 2-mm ID glass, packed with 1% SP1240DA on Supelcoport (80/100 mesh) or equivalent. The detection level and precision and bias data presented herein were developed with this column. For guide-

† Gas chromatographic methods are extremely sensitive to the materials used. Mention of trade names by *Standard Methods* does not preclude the use of other existing or as-yet-undeveloped products that give *demonstrably* equivalent results.

lines for the use of alternate columns (e.g., capillary or megabore) see ¶ 5*b*1).

2) *Column for derivatized phenols*, 1.8 m long × 2-mm ID, glass, packed with 5% OV-17 on Chromosorb W-AW-DMCS (80/100 mesh) or equivalent. This column was used to develop the detection limit and precision and bias data presented herein. For guidelines for the use of alternate columns (e.g., capillary or megabore) see ¶ 5*b*1).

3) *Detectors*, flame ionization (FID) and electron capture (ECD). Use the FID to determine parent phenols. Use the ECD when determining derivatized phenols. For guidelines for use of alternative detectors see ¶ 5*b*1).

4. Reagents

Use reagents listed in Section 6410B.4*a–f*, and in addition:

a. Sodium hydroxide solution, NaOH, 1*N:* Dissolve 4 g NaOH in reagent water and dilute to 100 mL.

b. Sulfuric acid, H_2SO_4, 1*N:* Slowly add 58 mL conc H_2SO_4 to 500 mL reagent water and dilute to 1 L.

c. Potassium carbonate, K_2CO_3, powdered.

d. Pentafluorobenzyl bromide (α-bromopentafluorotoluene), 97% minimum purity. (CAUTION: *This chemical is a lachrymator.*)

e. 18-Crown-6-ether (1,4,7,10,13,16-hexaoxacyclooctadecane), 98% minimum purity. (CAUTION: *This chemical is highly toxic.*)

f. Derivatization reagent: Add 1 mL pentafluorobenzyl bromide and 1 g 18-crown-6-ether to a 50-mL volumetric flask and dilute to volume with 2-propanol. Prepare fresh weekly. Prepare in a hood. Store at 4°C and protect from light.

g. Acetone, hexane, methanol, methylene chloride, 2-propanol, toluene, pesticide quality or equivalent.

h. Silica gel, 100/200 mesh.‡ Activate at 130°C overnight and store in a desiccator.

i. Stock standard solutions: Prepare from pure standard materials or purchase as certified solutions. Prepare as directed in Section 6410B.4*g*, but dissolve material in 2-propanol.

j. Calibration standards: Prepare standards appropriate to chosen means of calibration.

1) *External standards:* Prepare at a minimum of three concentration levels for each compound by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with 2-propanol. Prepare one standard at a concentration near, but above, the MDL (see Table 6420:I or II) and the others to correspond to the expected range of sample concentrations or to define the working range of the detector.

2) *Internal standards:* Prepare at a minimum of three concentration levels for each compound by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with 2-propanol. Prepare one standard at a concentration near, but above, the MDL and the others to correspond to the expected range of sample concentrations or to define the working range of the detector.

‡ Davison grade 923 or equivalent.

k. Quality control (QC) check sample concentrate: Obtain a check sample concentrate containing each compound at a concentration of 100 µg/mL in 2-propanol. If such a sample is not available from an external source, prepare using stock standards prepared independently from those used for calibration.

5. Procedure

a. Extraction: Mark water meniscus on side of sample bottle for later determination of volume. Pour entire sample into a 2-L separatory funnel. For samples high in organic content, solvent wash sample at basic pH as prescribed in next paragraph, to remove potential interferences. During wash, avoid prolonged or exhaustive contact with solvent, which may result in low recovery of some phenols, notably phenol and 2,4-dimethylphenol. For relatively clean samples, omit wash and extract directly.

To wash, adjust pH to 12.0 or greater with NaOH solution. Add 60 mL methylene chloride and shake the funnel for 1 min with periodic venting to release excess pressure. Discard solvent layer. Repeat wash up to two additional times if significant color is being removed.

Before extraction, adjust to pH of 1 to 2 with H_2SO_4. Extract three times with methylene chloride as directed in Section 6410B.5*a*1). Assemble Kuderna-Danish apparatus, concentrate extract to 1 mL, and remove, drain, and cool K-D apparatus as directed in Section 6410B.5*a*1).

Increase temperature of hot water bath to 100°C. Remove Snyder column and rinse flask and its lower joint into concentrator tube with 1 to 2 mL 2-propanol. Preferably use a 5-mL syringe for this operation. Attach a two-ball micro-Snyder column to concentrator tube and prewet column by adding about 0.5 mL 2-propanol to the top. Place micro-K-D apparatus on water bath so that concentrator tube is partially immersed in hot water. Adjust vertical position of apparatus and water temperature so as to complete concentration in 5 to 10 min. (CAUTION: If temperature is raised too quickly the sample may be blown out of the K-D apparatus). At proper rate of distillation the column balls actively chatter but the chambers are not flooded. When the apparent volume of liquid reaches 2.5 mL, remove K-D apparatus and let drain and cool for at least 10 min. Add 2 mL 2-propanol through top of micro-Snyder column and resume concentrating as before. When the apparent volume of liquid reaches 0.5 mL, remove K-D apparatus and let drain and cool for at least 10 min.

Remove micro-Snyder column and rinse lower joint into concentrator tube with a minimum amount of 2-propanol. Adjust extract volume to 1.0 mL. Stopper concentrator tube and store at 4°C if further processing will not be done immediately. If extract is to be stored longer than 2 d, transfer to a TFE-sealed screw-cap vial. If sample extract requires no further cleanup, proceed with chromatographic analysis (¶ *b*). If sample requires further cleanup, proceed to ¶ *c*.

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

b. Flame ionization detector gas chromatography (FID/GC):

1) Operating conditions—Table 6420:I summarizes the recommended operating conditions for the gas chromatograph and gives retention times and MDLs that can be achieved under these conditions. An example of the separations obtained with this

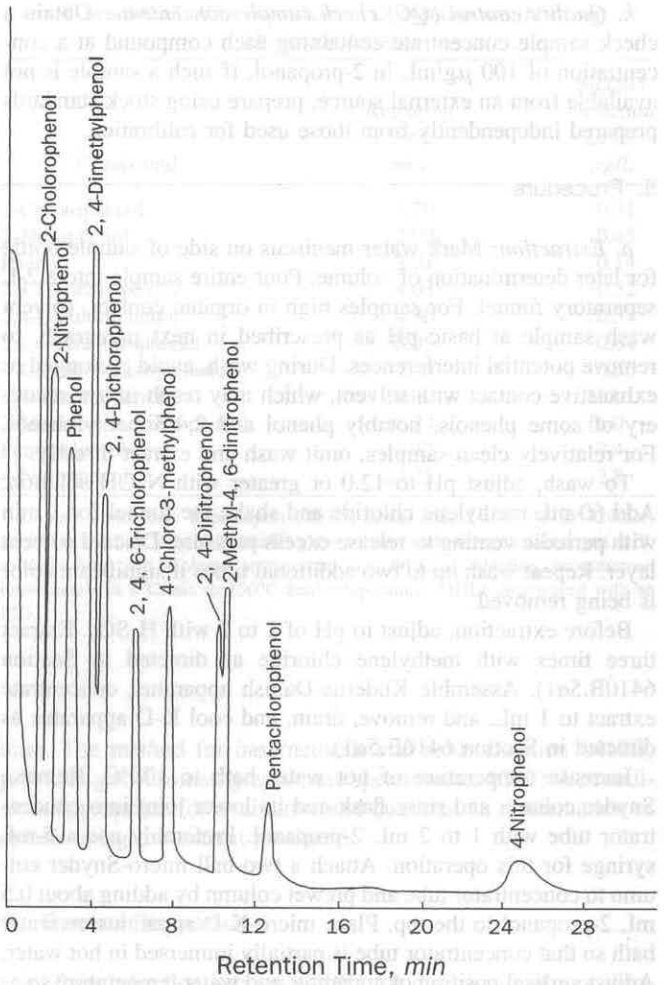

Figure 6420:1. Gas chromatogram of phenols. Column: 1% SP-1240DA on Supelcoport; program: 80°C at injection, immediate 8°C/min to 150°C; detector: flame ionization.

column is shown in Figure 6420:1. Other packed or capillary (open-tubular) columns, chromatographic conditions, or detectors may be used if the requirements of ¶ 7 are met.

2) Calibration—To calibrate the system for underivatized phenols, establish gas chromatographic operating conditions equivalent to those given in Table 6420:I. Calibrate using the external or the internal standard technique as follows:

a) External standard calibration procedure—Prepare standards as directed in ¶ 4*j*1) and follow the procedure of ¶ 3) below. Tabulate data and obtain calibration curve or calibration factor as directed in Section 6200B.4*c*3).

b) Internal standard calibration procedure—Prepare samples as directed in ¶ 4*j*2) and follow the procedure of ¶ 3) below. Tabulate data and calculate response factors as directed in Section 6200B.4*c*2).

Verify working calibration curve, calibration factor, or RF on each working day by measuring one or more calibration standards. If the response for any compound varies from the predicted response by more than ±15%, prepare a new calibration curve for that compound.

3) Sample analysis—If the internal standard calibration procedure is used, add internal standard to sample extract and mix thoroughly immediately before injecting 2 to 5 µL sample extract or standard into gas chromatograph using the solvent-flush technique.[6] Smaller (1.0-µL) volumes may be injected if automatic devices are used. Record volume injected to nearest 0.05 µL and resulting peak size in area or peak height units.

Identify compounds in sample by comparing peak retention times with peaks of standard chromatograms. Base width of retention time window used to make identifications on measurements of actual retention time variations of standards over the course of a day. To calculate a suggested window size, use three times the standard deviation of a retention time for a compound. Analyst's experience is important in interpreting chromatograms.

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

If peak response cannot be measured because of interferences, use the alternative gas chromatographic procedure (¶ *c* below).

c. Derivatization and electron capture detector gas chromatography (ECD/GC):

1) Derivatization—Pipet 1.0 mL of the 2-propanol solution of standard or sample extract into a glass reaction vial. Add 1.0 mL derivatizing reagent (¶ 4*f*); this is sufficient to derivatize a solution having a total phenolic content not exceeding 0.3 mg/mL. Add about 3 mg K_2CO_3 and shake gently. Cap mixture and heat for 4 h at 80°C in a hot water bath. Remove from hot water bath and let cool. Add 10 mL hexane and shake vigorously for 1 min. Add 3.0 mL distilled, deionized water and shake for 2 min. Decant a portion of the organic layer into a concentrator tube and cap with a glass stopper.

2) Cleanup—Place 4.0 g silica gel in a chromatographic column. Tap column to settle silica gel and add about 2 g anhydrous Na_2SO_4 to the top. Pre-elute column with 6 mL hexane. Discard eluate and just before exposing Na_2SO_4 layer to air, pipet onto the column 2.0 mL hexane solution, ¶ 1) above, that contains the derivatized sample or standard. Elute column with 10.0 mL hexane and discard eluate. Elute column, in order, with 10.0 mL 15% toluene in hexane (Fraction 1), 10.0 mL 40% toluene in hexane (Fraction 2), 10.0 mL 75% toluene in hexane (Fraction 3), and 10.0 mL 15% 2-propanol in toluene (Fraction 4). Prepare all elution mixtures on a volume:volume basis. Elution patterns for the phenolic derivatives are shown in Table 6420:II. Fractions may be combined as desired, depending on the specific phenols of interest or level of interferences.

3) Operating conditions—Table 6420:II summarizes the recommended operating conditions for the gas chromatograph and gives retention times and MDLs that can be achieved under these conditions. An example of the separations obtained with this column is shown in Figure 6420:2.

4) Calibration—Calibrate system daily by preparing a minimum of three 1-mL portions of calibration standards, ¶ 4*j*1), containing each of the phenols of interest and derivatized as above. Analyze 2 to 5 µL of each column eluate collected as in ¶ 5) below and tabulate peak height or area responses against calculated equivalent mass of underivatized phenol injected. Prepare a calibration curve for each compound.

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

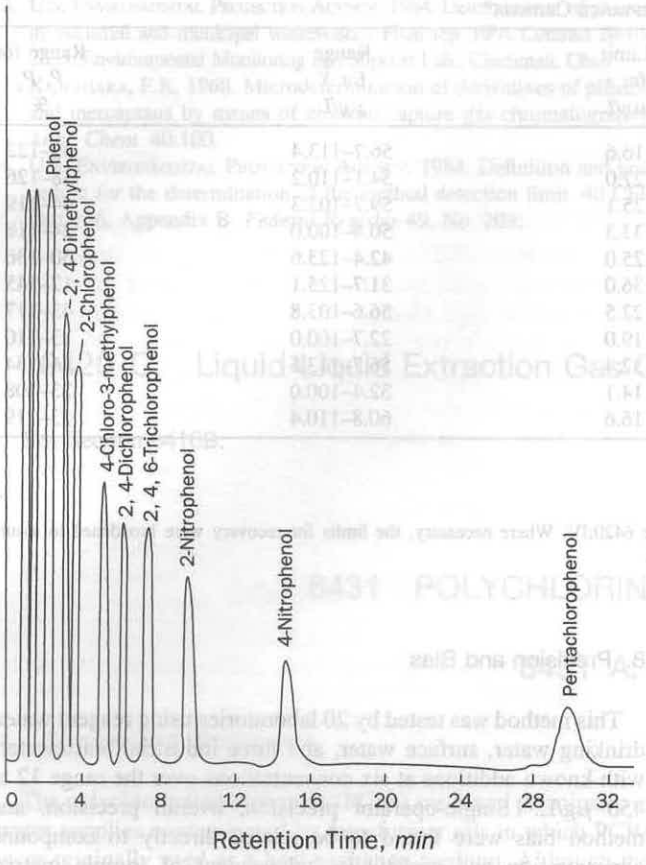

Figure 6420:2. Gas chromatogram of PFB derivatives of phenols. Column: 5% OV-17 on Chromosorb W-AW-DMCS; temperature: 200°C; detector: electron capture.

5) Sample analysis—Inject 2 to 5 μL column fractions into the gas chromatograph using the solvent-flush technique. Smaller (1.0-μL) volumes can be injected if automatic devices are used. Record volume injected to nearest 0.05 μL and resulting peak size in area or peak height units. If peak response exceeds linear range of system, dilute extract and reanalyze.

6. Calculation

a. FID/GC analysis: Determine concentration of individual compounds. If the external standard calibration procedure is used, calculate amount of material injected from peak response using calibration curve or calibration factor determined previously. Calculate sample concentration from the equation:

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

where:

A = amount of material injected, ng,
V_i = volume of extract injected, μL,
V_t = volume of total extract, μL, and
V_s = volume of water extracted, mL.

If the internal standard calibration procedure is used, calculate concentration in sample using the response factor (RF) determined above and the equation:

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

where:

A_s = response for compound to be measured,
A_{is} = response for internal standard,
I_s = amount of internal standard added to each extract, μg, and
V_o = volume of water extracted, L.

b. Derivatization and ECD/GC analysis: To determine concentration of individual compounds in the sample, use the equation:

$$\text{Concentration, } \mu g/L = \frac{(A)\,(V_t)\,(B)\,(D)}{(V_i)\,(V_s)\,(C)\,(E)}$$

where:

A = mass of underivatized phenol represented by area of peak in sample chromatogram, determined from calibration curve in ¶ 5*c*4), ng,
V_i = volume of eluate injected, μL,
V_t = total volume of column eluate or combined fractions from which V_i was taken, μL,
V_s = volume of water extracted in ¶ 5*a*, mL,
B = total volume of hexane added in ¶ 5*c*1), mL,
C = volume of hexane sample solution added to cleanup column in ¶ 5*c*2), mL,
D = total volume of 2-propanol extract before derivatization, mL, and
E = volume of 2-propanol extract carried through derivatization in ¶ 5*c*1), mL.

Report results in μg/L without correction for recovery. Report QC data with sample results.

7. Quality Control

a. Quality control program: See Section 6200A.5.

b. Initial quality control: To establish the ability to generate data with acceptable bias and precision, perform the following operations:

Using a pipet, prepare QC check samples at a concentration of 100 μg/L by adding 1.00 mL of 100 μg/mL QC check sample concentrate to each of four 1-L portions reagent water. Analyze check samples according to the method of ¶ 5 and proceed with the check described in Section 6200A.5*a*1) and 2). Use acceptance criteria given in Table 6420:III.

c. Analyses of laboratory-fortified samples: On an ongoing basis, make known additions to at least 10% of the samples from each sample site being monitored. For laboratories analyzing one to ten samples per month, analyze at least one such sample with a known addition per month. Use the procedure detailed in Section 6200A.5*c*7) and 8), but use an addition of 100 μg/L rather than 20 μg/L and compare percent recovery for each compound with the corresponding QC acceptance criteria found in Table 6420:III. If the known addition was at a concentration lower than 100 μg/L, use either the QC acceptance criteria in

TABLE 6420:III. QC ACCEPTANCE CRITERIA*

Compound	Test Conc. µg/L	Limit for s µg/L	Range for $\bar{X}$ µg/L	Range for P, P_s %
4-Chloro-3-methylphenol	100	16.6	56.7–113.4	49–122
2-Chlorophenol	100	27.0	54.1–110.2	38–126
2,4-Dichlorophenol	100	25.1	59.7–103.3	44–119
2,4-Dimethylphenol	100	33.3	50.4–100.0	24–118
2-Methyl-4,6-dinitrophenol	100	25.0	42.4–123.6	30–136
2,4-Dinitrophenol	100	36.0	31.7–125.1	12–145
2-Nitrophenol	100	22.5	56.6–103.8	43–117
4-Nitrophenol	100	19.0	22.7–100.0	13–110
Pentachlorophenol	100	32.4	56.7–113.5	36–134
Phenol	100	14.1	32.4–100.0	23–108
2,4,6-Trichlorophenol	100	16.6	60.8–110.4	53–119

* s = standard deviation for four recovery measurements,
$\bar{X}$ = average recovery for four recovery measurements, and
P, P_s = percent recovery measured.

NOTE: These criteria are based directly upon the method performance data in Table 6420:IV. Where necessary, the limits for recovery were broadened to assure applicability of the limits to concentrations below those used to develop Table 6420:IV.

Table 6420:III or optional QC acceptance criteria calculated for the specific addition concentration based on the equations in Table 6420:IV.

d. Quality-control check standard analysis: If analysis of any compound fails to meet the acceptance criteria for recovery, prepare and analyze a QC check standard containing each compound that failed. NOTE: The frequency for the required analysis of a QC check standard will depend on the number of compounds being tested for simultaneously, the complexity of the sample matrix, and the performance of the laboratory.

Prepare the QC check standard by adding 1.0 mL of QC check sample concentrate to 1 L reagent water and proceed as in Section 6200A.5*a*3) using Table 6420:III.

e. Bias assessment and records: Assess method bias and maintain records as directed in Section 6410B.7*e*.

8. Precision and Bias

This method was tested by 20 laboratories using reagent water, drinking water, surface water, and three industrial wastewaters with known additions at six concentrations over the range 12 to 450 µg/L.[7] Single-operator precision, overall precision, and method bias were found to be related directly to compound concentration and essentially independent of sample matrix. Linear equations describing these relationships for a flame ionization detector are presented in Table 6420:IV.

9. References

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Method 604—Phenols. 40 CFR Part 136, 43290; *Federal Register* 49, No. 209.

TABLE 6420:IV. METHOD BIAS AND PRECISION AS FUNCTIONS OF CONCENTRATION*

Compound	Bias, as Recovery, X' µg/L	Single-Analyst Precision, s_r' µg/L	Overall Precision, S' µg/L
4-Chloro-3-methylphenol	$0.87C - 1.97$	$0.11\bar{X} - 0.21$	$0.16\bar{X} + 1.41$
2-Chlorophenol	$0.83C - 0.84$	$0.18\bar{X} + 0.20$	$0.21\bar{X} + 0.75$
2,4-Dichlorophenol	$0.81C + 0.48$	$0.17\bar{X} - 0.02$	$0.18\bar{X} + 0.62$
2,4-Dimethylphenol	$0.62C - 1.64$	$0.30\bar{X} - 0.89$	$0.25\bar{X} + 0.48$
2-Methyl-4,6-dinitrophenol	$0.84C - 1.01$	$0.15\bar{X} + 1.25$	$0.19\bar{X} + 5.85$
2,4-Dinitrophenol	$0.80C - 1.58$	$0.27\bar{X} - 1.15$	$0.29\bar{X} + 4.51$
2-Nitrophenol	$0.81C - 0.76$	$0.15\bar{X} + 0.44$	$0.14\bar{X} + 3.84$
4-Nitrophenol	$0.46C + 0.18$	$0.17\bar{X} + 2.43$	$0.19\bar{X} + 4.79$
Pentachlorophenol	$0.83C + 2.07$	$0.22\bar{X} - 0.58$	$0.23\bar{X} + 0.57$
Phenol	$0.43C + 0.11$	$0.20\bar{X} - 0.88$	$0.17\bar{X} + 0.77$
2,4,6-Trichlorophenol	$0.86C - 0.40$	$0.10\bar{X} + 0.53$	$0.13\bar{X} + 2.40$

* X' = expected recovery for one or more measurements of a sample containing a concentration of C,
s_r' = expected single-analyst standard deviation of measurements at an average concentration found of $\bar{X}$,
S' = expected interlaboratory standard deviation of measurements at an average concentration found of $\bar{X}$,
C = true value for the concentration, and,
$\bar{X}$ = average recovery found for measurements of samples containing a concentration of C.

2. U.S. Environmental Protection Agency. 1984. Determination of phenols in industrial and municipal wastewaters. Final rep. EPA Contract 68-03-2625, Environmental Monitoring and Support Lab., Cincinnati, Ohio.
3. Kawahara, F.K. 1968. Microdetermination of derivatives of phenols and mercaptans by means of electron capture gas chromatography. *Anal. Chem.* 40:100.
4. U.S. Environmental Protection Agency. 1984. Definition and procedure for the determination of the method detection limit. 40 CFR Part 136, Appendix B. *Federal Register* 49, No. 209.
5. U.S. Environmental Protection Agency. 1984. Development of detection limits, EPA Method 604, Phenols. Special letter report for EPA Contract 68-03-2625, Environmental Monitoring and Support Lab., Cincinnati, Ohio.
6. Burke, J.A. 1965. Gas chromatography for pesticide residue analysis; some practical aspects. *J. Assoc. Offic. Anal. Chem.* 48:1037.
7. U.S. Environmental Protection Agency. 1984. EPA Method Study 14, Method 604—Phenols. EPA-600/4-84-044, National Technical Information Serv., PB84-196211, Springfield, Va.

6420 C. Liquid-Liquid Extraction Gas Chromatographic/Mass Spectrometric Method

See Section 6410B.

6431 POLYCHLORINATED BIPHENYLS (PCBs)*

6431 A. Introduction

1. Sources and Significance

The polychlorinated biphenyls (PCBs) are found principally in water supplies contaminated by transformer oils in which PCBs were originally used as a heat-exchange medium. Although the use of these compounds has been banned, there are still numerous transformers in existence that contain PCBs, which results in their occasional discharge into potable water or wastewater. These compounds are toxic, bioaccumulative, and extremely stable, and thus there is a need to monitor them in wastewaters.

2. Selection of Method

The liquid-liquid extraction (LLE) gas chromatographic (GC) method is used to monitor both the PCBs and the organochlorine pesticides simultaneously. This method has excellent sensitivity. The LLE gas chromatographic/mass spectrometric (GC/MS) method also can be used to detect PCBs, but with substantially less sensitivity.

PCBs usually are measured as commercial mixtures of isomers rather than as individual isomers (congeners).

* Approved by Standard Methods Committee, 2000.

6431 B. Liquid-Liquid Extraction Gas Chromatographic Method

See Sections 6630B and C.

6431 C. Liquid-Liquid Extraction Gas Chromatographic/Mass Spectrometric Method

See Section 6410B.

6440 POLYNUCLEAR AROMATIC HYDROCARBONS*

6440 A. Introduction

1. Sources and Significance

The polynuclear aromatic hydrocarbons (PAHs) often are by-products of petroleum processing or combustion. Many of these compounds are highly carcinogenic at relatively low levels. Although they are relatively insoluble in water, their highly hazardous nature merits their monitoring in potable waters and wastewaters.

* Approved by Standard Methods Committee, 2000.

2. Selection of Method

Method 6440B encompasses both a high-performance liquid chromatographic (HPLC) method with UV and fluorescence detection and a gas chromatographic (GC) method using flame ionization detection. Method 6440C is a gas chromatographic/mass spectrometric (GC/MS) method that also can detect these compounds at somewhat higher concentrations. Certain of these compounds may also be measured by closed-loop stripping analysis (see Section 6040).

6440 B. Liquid-Liquid Extraction Chromatographic Method

This method[1] is applicable to the determination of certain polynuclear aromatic hydrocarbons (PAHs)* in municipal and industrial discharges. When analyzing unfamiliar samples for any or all of these compounds, support the identifications by at least one additional qualitative technique. The method for base/neutrals and acids (Section 6410B) provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for qualitative and quantitative confirmation of results using the extract produced.

1. General Discussion

a. Principle: A measured volume of sample is extracted with methylene chloride. The extract is dried, concentrated, and separated by the high-performance liquid chromatographic (HPLC) or gas chromatographic (GC) method. If other analyses having essentially the same extraction and concentration steps are to be performed, extraction of a single sample will be sufficient for all the determinations. Ultraviolet (UV) and fluorescence detectors are used with HPLC to identify and measure the PAHs. A flame ionization detector is used with GC.[2]

The method provides a silica gel column cleanup to aid in eliminating interferences. When cleanup is required, sample concentration levels must be high enough to permit separate treatment of subsamples before the solvent-exchange steps.

Chromatographic conditions (¶ 5*d*) appropriate for the simultaneous measurement of combinations of these compounds may be selected but they do not adequately resolve the following four pairs of compounds: anthracene and phenanthrene; chrysene and benzo(a)anthracene; benzo(b)fluoranthene and benzo(k)fluoranthene; and dibenzo(a,h)anthracene and indeno(1,2,3-cd)pyrene. Unless reporting the sum of an unresolved pair is acceptable, use the liquid chromatographic method, which does resolve all 16 listed PAHs.

b. Interferences: See Section 6410B.1*b* for precautions concerning glassware, reagent purity, and matrix interferences. Interferences in liquid chromatographic techniques have not been assessed fully. Although HPLC conditions described allow for unique resolution of specific PAHs, other PAH compounds may interfere. PAHs in water samples containing particulate matter may actually be absorbed onto the particulate matter. This may result in hidden coeluting peaks and consequently false fingerprint or erroneous quantitation. The use of capillary GC or MS detection can remedy this situation.

c. Detection levels: The method detection level (MDL) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[3] The MDL concentrations listed in Table 6440:I were obtained with reagent water.[4] Similar results were achieved with representative wastewaters. MDLs for the GC method were not determined. The MDL actually obtained in a given analysis will vary, depending on instrument sensitivity and matrix effects. This method has been tested for linearity of known-addition recovery from reagent water and has been demonstrated to be applicable over the concentration range from 8 × MDL to 800 × MDL,[4] with the following exception: benzo(ghi)perylene recovery at 80 × and 800 × MDL were low (35% and 45%, respectively).

d. Safety: The toxicity or carcinogenicity of each reagent has not been defined precisely. The following compounds have been classified tentatively as known or suspected, human or mammalian carcinogens: benzo(a)anthracene, benzo(a)pyrene, and dibenzo(a,h)anthracene. Prepare primary standards of these compounds in a hood and wear NIOSH/MESA-approved toxic gas respirator when handling high concentrations.

2. Sampling and Storage

For collection and general storage requirements, see Section 6410B.2. Because PAHs are light-sensitive, store samples, ex-

* Acenaphthene, acenaphthylene, anthracene, benzo-(a)anthracene, benzo-(a)pyrene, benzo(b)fluoranthene, benzo(ghi)perylene, benzo(k)fluoranthene, chrysene, dibenzo(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene, and pyrene.

TABLE 6440:I. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY CONDITIONS AND METHOD DETECTION LEVELS

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

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

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

tracts, and standards in amber or foil-wrapped bottles to minimize photolytic decomposition.

3. Apparatus

Use all the apparatus specified in Section 6410B.3*a–g* and *i–k*, and in addition:

a. Chromatographic column, 250 mm long × 10-mm ID with coarse frit filter disk at bottom and TFE stopcock.

b. High-performance liquid chromatograph (HPLC): An analytical system complete with column supplies, high-pressure syringes, detectors, and compatible strip-chart recorder. Preferably use a data system for measuring peak areas and retention times.

1) *Gradient pumping system*, constant flow.

2) *Reverse phase column*, HC-ODS Sil-X, 5-μm particle diam, in a 25-cm × 2.6-mm ID stainless steel column.† This column was used to develop MDL and precision and bias data presented herein. For guidelines for the use of alternate column packings see ¶ 5*d*1).

3) *Detectors*, fluorescence and/or UV. Use the fluorescence detector for excitation at 280 nm and emission greater than 389 nm cutoff.‡ Use fluorometers with dispersive optics for excitation utilizing either filter or dispersive optics at the emission detector. Operate the UV detector at 254 nm and couple it to the fluorescence detector. These detectors were used to develop MDL and precision and bias data presented herein. For guidelines for the use of alternate detectors see ¶ 5*d*1).

† Perkin Elmer No. 089-0716 or equivalent.

‡ Corning 3-75 or equivalent.

TABLE 6440:II. GAS CHROMATOGRAPHIC CONDITIONS AND RETENTION TIMES

Compound	Retention Time *min*
Naphthalene	4.5
Acenaphthylene	10.4
Acenaphthene	10.8
Fluorene	12.6
Phenanthrene	15.9
Anthracene	15.9
Fluoranthene	19.8
Pyrene	20.6
Benzo(a)anthracene	24.7
Chrysene	24.7
Benzo(b)fluoranthene	28.0
Benzo(k)fluoranthene	28.0
Benzo(a)pyrene	29.4
Dibenzo(a,h)anthracene	36.2
Indeno(1,2,3-cd)pyrene	36.2
Benzo(ghi)perylene	38.6

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

c. Gas chromatograph:§ An analytical system complete with temperature-programmable gas chromatograph suitable for on-column or splitless injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. Preferably use a data system for measuring peak areas.

1) *Column*, 1.8 m long × 2-mm ID glass, packed with 3% OV-17 on Chromosorb W-AW-DCMS (100/120 mesh) or equivalent. This column was used to develop the retention time data in Table 6440:II. For guidelines for the use of alternate columns (e.g. capillary or megabore) see ¶ 5*d*2).

2) *Detector*, flame ionization. This detector is effective except for resolving the four pairs of compounds listed in ¶ 1*a*. With the use of capillary columns, these pairs may be resolved with GC. For guidelines for the use of alternate detectors see ¶ 5*d*2).

4. Reagents

a. Reagent water: See Section 6200B.3*a*.

b. Sodium thiosulfate, $Na_2S_2O_3 \cdot 5H_2O$, granular.

c. Cyclohexane, methanol, acetone, methylene chloride, pentane, pesticide quality or equivalent.

d. Acetonitrile, HPLC quality, distilled in glass.

e. Sodium sulfate, Na_2SO_4, granular, anhydrous. Purify by heating at 400°C for 4 h in a shallow tray.

f. Silica gel, 100/200 mesh, desiccant.‖ Before use, activate for at least 16 h at 130°C in a shallow glass tray, loosely covered with foil.

§ Gas chromatographic methods are extremely sensitive to the materials used. Mention of trade names by *Standard Methods* does not preclude the use of other existing or as-yet-undeveloped products that give *demonstrably* equivalent results.

‖ Davison, grade 923 or equivalent.

g. Stock standard solutions: Prepare as directed in Section 6410B.4*g*, using acetonitrile as the solvent.

h. Calibration standards: Prepare standards appropriate to chosen means of calibration following directions in Section 6420B.4*j*, except that acetonitrile is the diluent instead of 2-propanol. See Table 6440:I for MDLs.

i. Quality control (QC) check sample concentrate: Obtain a check sample concentrate containing each compound at the following concentrations in acetonitrile: 100 μg/mL of any of the six early-eluting PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, and anthracene); 5 μg/mL of benzo(k)fluoranthene; and 10 μg/mL of any other PAH. If such a sample is not available from an external source, prepare using stock standards prepared independently from those used for calibration.

5. Procedure

a. Extraction: Mark water meniscus on side of sample bottle for later determination of volume. Pour entire sample into a 2-L separatory funnel and extract as directed in Section 6410B.5*a*1) without any pH adjustment.

After extraction, concentrate by adding one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet Snyder column by adding about 1 mL methylene chloride to the top. Place K-D apparatus on a hot water bath (60 to 65°C) in a hood so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of flask is bathed with hot vapor. Adjust vertical position of apparatus and water temperature as required to complete the concentration in 15 to 20 min. At proper rate of distillation the column balls actively chatter but the chambers are not flooded with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove K-D apparatus and let drain and cool for at least 10 min.

Remove Snyder column and rinse flask and its lower joint into concentrator tube with 1 to 2 mL methylene chloride. Preferably use a 5-mL syringe for this operation. Stopper concentrator tube and store refrigerated if further processing will not be done immediately. If extract is to be stored longer than 2 d, transfer to a TFE-sealed screw-cap vial and protect from light. If sample extract requires no further cleanup, proceed with gas or liquid chromatographic analysis (¶s *c* through *f* below). If sample requires further cleanup, first follow procedure of ¶ *b* before chromatographic analysis.

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

b. Cleanup and separation: Use procedure below or any other appropriate procedure; however, first demonstrate that the requirements of ¶ 7 can be met.

Before using silica-gel cleanup technique, exchange extract solvent to cyclohexane. Add 1 to 10 mL sample extract (in methylene chloride) and a boiling chip to a clean K-D concentrator tube. Add 4 mL cyclohexane and attach a two-ball micro-Snyder column. Prewet column by adding 0.5 mL methylene chloride to the top. Place micro-K-D apparatus on a boiling (100°C) water bath so that concentrator tube is partially immersed in hot water. Adjust vertical position of apparatus and water temperature so as to complete concentration in 5 to 10 min. At proper rate of distillation the column balls actively chatter but the chambers are not flooded. When apparent volume of liquid reaches 0.5 mL, remove K-D apparatus and let drain and cool for at least 10 min. Remove micro-Snyder column and rinse its lower joint into concentrator tube with a minimum amount of cyclohexane. Adjust extract volume to about 2 mL.

To perform silica-gel column cleanup, make a slurry of 10 g activated silica gel in methylene chloride and place in a 10-mm-ID chromatographic column. Tap column to settle silica gel and elute with methylene chloride. Add 1 to 2 cm anhydrous Na_2SO_4 to top of silica gel. Pre-elute with 40 mL pentane. Elute at rate of about 2 mL/min. Discard eluate and just before exposure of Na_2SO_4 layer to the air, transfer all the cyclohexane sample extract onto column using an additional 2 mL cyclohexane. Just before exposure of Na_2SO_4 layer to air, add 25 mL pentane and continue elution. Discard this pentane eluate. Next, elute column with 25 mL methylene chloride/pentane (4 + 6) (v/v) into a 500-mL K-D flask equipped with a 10-mL concentrator tube. Concentrate collected fraction to less than 10 mL as in ¶ 5*a*. After cooling, remove Snyder column and rinse flask and its lower joint with pentane.

c. Reconcentration: Concentrate further as follows:

1) For high-performance liquid chromatography—To extract in a concentrator tube, add 4 mL acetonitrile and a new boiling chip. Attach a two-ball micro-Snyder column and concentrate solvent as in ¶ 5*a* (but set water bath at 95 to 100°C.) After cooling, remove micro-Snyder column and rinse its lower joint into the concentrator tube with about 0.2 mL acetonitrile. Adjust extract volume to 1.0 mL.

2) For gas chromatography—To achieve maximum sensitivity with this method, concentrate extract to 1.0 mL. Add a clean boiling chip to methylene chloride extract in concentrator tube. Attach a two-ball micro-Snyder column. Prewet column by adding about 0.5 mL methylene chloride to the top. Place micro-K-D apparatus on a hot water bath (60 to 65°C) and continue concentration as in ¶ 5*b*. Remove micro-Snyder column and rinse its lower joint into concentrator tube with a minimum amount of methylene chloride. Adjust final volume to 1.0 mL and stopper concentrator tube.

d. Operating conditions:

1) High-performance liquid chromatography—Table 6440:I summarizes the recommended operating conditions for HPLC and gives retention times, capacity factors, and MDLs that can be achieved under these conditions. Preferably use the UV detector for determining naphthalene, acenaphthylene, acenapthene, and fluorene and the fluorescence detector for the remaining PAHs. Examples of separations obtained with this HPLC column are shown in Figures 6440:1 and 2. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of ¶ 7 are met.

2) Gas chromatography—Table 6440:II summarizes the recommended operating conditions for the gas chromatograph and gives retention times that were obtained under these conditions. An example of the separations is shown in Figure 6440:3. Other packed or capillary (open-tubular) columns, chromatographic conditions, or detectors may be used if the requirements of ¶ 7 are met.

e. Calibration: Calibrate system daily using either external or internal standard procedure.

1) External standard calibration procedure—Prepare standards as directed in ¶ 4*h* and follow either procedure of ¶ *f* below.

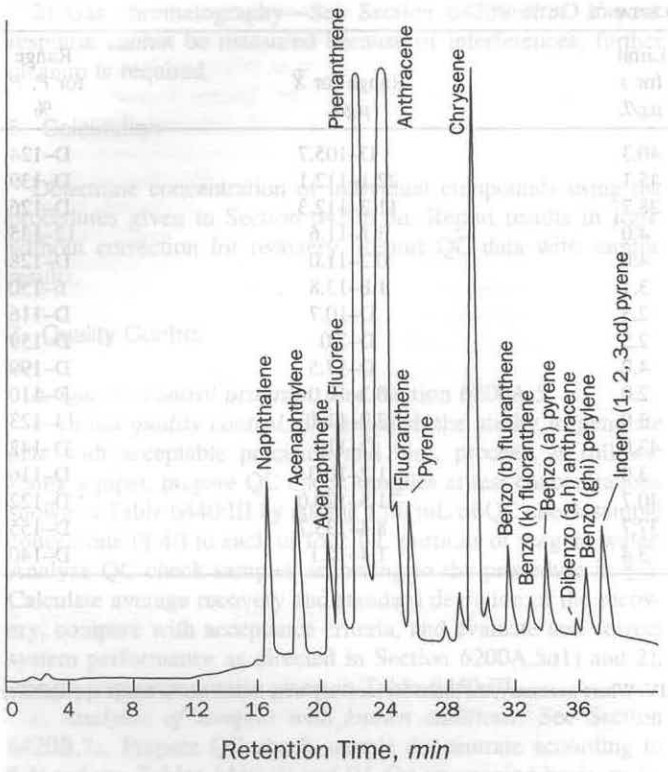

Figure 6440:1. Liquid chromatogram of polynuclear aromatic hydrocarbons. Column: HC—ODS SIL-X; mobile phase: 40% to 100% acetonitrile in water; detector: ultraviolet at 254 nm.

Tabulate data and obtain calibration curve or calibration factor as directed in Section 6200B.4*c*3).

2) Internal standard calibration procedure—Prepare standards as directed in ¶ 4*h* and follow either procedure of ¶ *f* below.

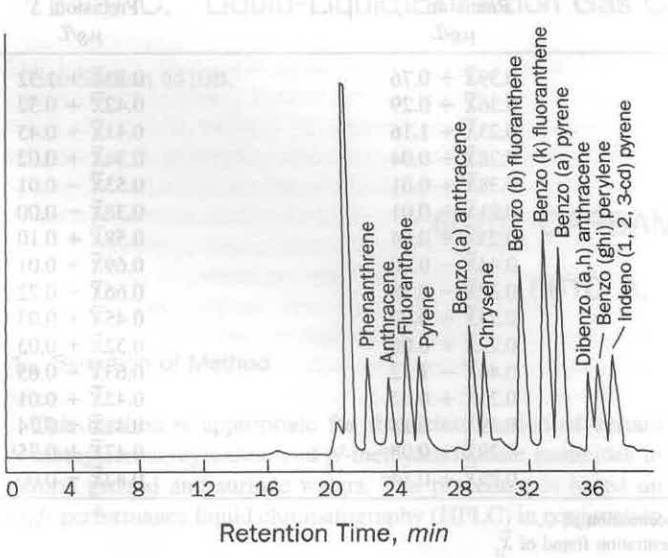

Figure 6440:2. Liquid chromatogram of polynuclear aromatic hydrocarbons. Column: HC—ODS SIL-X; mobile phase: 40% to 100% acetonitrile in water; detector: fluorescence.

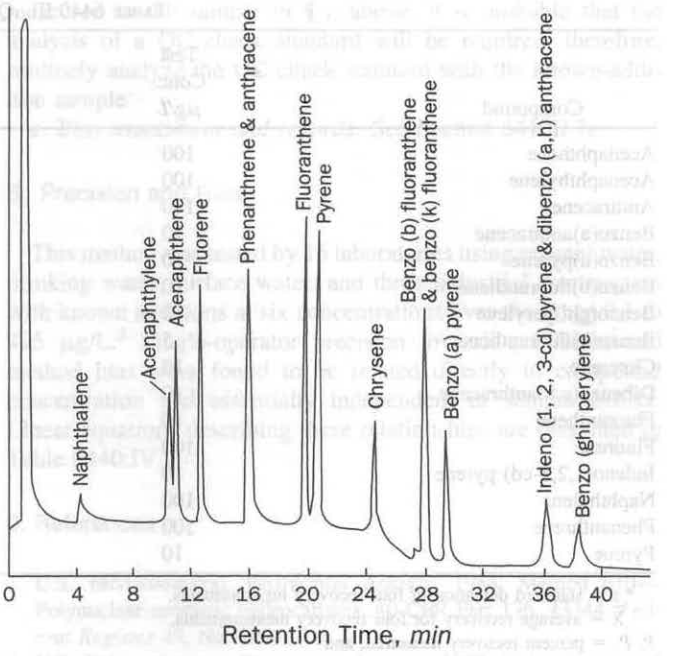

Figure 6440:3. Gas chromatogram of polynuclear aromatic hydrocarbons. Column: 3% OV-17 on Chromosorb W-AW-DCMS; program: 100°C for 4 min, 8°C/min to 280°C; detector: flame ionization.

Tabulate data and calculate response factors as directed in Section 6200B.4*c*2).

Verify working calibration curve, calibration factor, or RF on each working shift by measuring one or more calibration standards. If the response for any compound varies from the predicted response by more than ± 15%, repeat test using a fresh calibration standard. Alternatively, prepare a new calibration curve for that compound.

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

f. Sample analysis:

1) High-performance liquid chromatography—If the internal standard calibration procedure is being used, add internal standard to sample extract and mix thoroughly. Immediately inject 5 to 25 μL sample extract or standard into HPLC using a high-pressure syringe or a constant-volume sample injection loop. Record volume injected to nearest 0.1 μL and resulting peak size in area or peak height units. Re-equilibrate HPLC column at initial gradient conditions for at least 10 min between injections.

Identify compounds in sample by comparing peak retention times with peaks of standard chromatograms. Base width of retention time window used to make identifications on measurements of actual retention time variations of standards over the course of a day. To calculate a suggested window size use three times the standard deviation of a retention time for a compound. Analyst's experience is important in interpreting chromatograms.

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

If peak response cannot be measured because of interferences, further cleanup is required.

TABLE 6440:III. QC ACCEPTANCE CRITERIA*

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

* s = standard deviation of four recovery measurements,
$\bar{X}$ = average recovery for four recovery measurements,
P, P_s = percent recovery measured, and
D = detected; result must be greater than zero.

NOTE: These criteria are based directly upon the method performance data in Table 6440:IV. Where necessary, the limits for recovery were broadened to assure applicability of the limits to concentrations below those used to develop Table 6440:IV.

TABLE 6440:IV. METHOD BIAS AND PRECISION AS FUNCTIONS OF CONCENTRATION*

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

* X' = expected recovery for one or more measurements of a sample containing a concentration of C,
s_r = expected single-analyst standard deviation of measurements at an average concentration found of $\bar{X}$,
S' = expected interlaboratory standard deviation of measurements at an average concentration found of $\bar{X}$,
C = true value for concentration, and
$\bar{X}$ = average recovery found for measurements of samples containing a concentration of C.

2) Gas chromatography—See Section 6420B.5*b*3). If peak response cannot be measured because of interferences, further cleanup is required.

6. Calculation

Determine concentration of individual compounds using the procedures given in Section 6420B.6*a*. Report results in μg/L without correction for recovery. Report QC data with sample results.

7. Quality Control

a. Quality-control program: See Section 6200A.5.

b. Initial quality control: To establish the ability to generate data with acceptable precision and bias, proceed as follows: Using a pipet, prepare QC check samples at test concentrations shown in Table 6440:III by adding 1.00 mL of QC check sample concentrate (¶ 4*i*) to each of four 1-L portions of reagent water. Analyze QC check samples according to the procedure in ¶ 5. Calculate average recovery and standard deviation of the recovery, compare with acceptance criteria, and evaluate and correct system performance as directed in Section 6200A.5*a*1) and 2), using acceptance criteria given in Table 6440:III.

c. Analyses of samples with known additions: See Section 6420B.7*c*. Prepare QC check sample concentrate according to ¶ 4*i* and use Tables 6440:III and IV. On an ongoing basis, make known additions to at least 10% of the samples from each sample site being monitored. For laboratories analyzing one to ten samples per month, analyze at least one such sample with a known addition per month. Use the procedure described in Section 6200A.5*c*7) and 8).

d. Quality-control check standard analysis: See Section 6420B.7*d*. Prepare QC check standard according to ¶ 4*i* and use Table 6440:III. If all compounds in Table 6440:III are to be measured in the sample in ¶ *c* above, it is probable that the analysis of a QC check standard will be required; therefore, routinely analyze the QC check standard with the known-addition sample.

e. Bias assessment and records: See Section 6410B.7*e*.

8. Precision and Bias

This method was tested by 16 laboratories using reagent water, drinking water, surface water, and three industrial wastewaters with known additions at six concentrations over the range 0.1 to 425 μg/L.[5] Single-operator precision, overall precision, and method bias were found to be related directly to compound concentration and essentially independent of sample matrix. Linear equations describing these relationships are presented in Table 6440:IV.

9. References

1. U.S. Environmental Protection Agency. 1984. Method 610—Polynuclear aromatic hydrocarbons. 40 CFR Part 136, 43344; *Federal Register* 49, No. 209.
2. U.S. Environmental Protection Agency. 1982. Determination of polynuclear aromatic hydrocarbons in industrial and municipal wastewaters. EPA-600/4-82-025, National Technical Information Serv., PB82-258799, Springfield, Va.
3. U.S. Environmental Protection Agency. 1984. Definition and procedure for the determination of the method detection limit. 40 CFR Part 136, Appendix B. *Federal Register* 49, No. 209.
4. Cole, T., R. Riggin & J. Glaser. 1980. Evaluation of method detection limits and analytical curve for EPA Method 610 PNA's. International Symp. Polynuclear Aromatic Hydrocarbons, 5th, Battelle's Columbus Lab., Columbus, Ohio.
5. U.S. Environmental Protection Agency. 1984. EPA Method Study 20, Method 610—PNA's. EPA-600/4-84-063, National Technical Information Serv., PB84-211614, Springfield, Va.

6440 C. Liquid-Liquid Extraction Gas Chromatographic/Mass Spectrometric Method

See Section 6410B.

6610 CARBAMATE PESTICIDES*

6610 A. Introduction

1. Selection of Method

This method is appropriate for the determination of certain *N*-methylcarbamoyloxime and *N*-methylcarbamate pesticides in natural ground and surface waters. The procedure is based on high-performance liquid chromatography (HPLC) in conjunction with a post-column derivatization system and a fluorescence detector.

Carbamate pesticides are heat-sensitive and labile, and hence not amenable to analysis by gas chromatography. HPLC is the method of choice, but without preconcentration the usual ultraviolet detector is not adequate because of low sensitivity. A procedure to separate seven carbamate pesticides by HPLC followed by post-column alkaline hydrolysis has been reported.[1] A fluorescent adduct[2–4] is produced and is measured with a fluorescence detector.[5–8]

* Approved by Standard Methods Committee, 1999.
Joint Task Group: Andrew D. Eaton (chair), David Munch, Richard Reding.

2. References

1. MOYE, H.A., S.J. SCHERRER & P.A. ST. JOHN. 1977. Dynamic labeling of pesticides for high performance liquid chromatography: Detection of *N*-methylcarbamates and *o*-phthalaldehyde. *Anal. Lett.* 10:1049.
2. SIMONS, S.S., JR. & D.D. JOHNSON. 1977. Communication to the editor. *J. Amer. Chem. Soc.* 98:7098.
3. KRAUSE, R.T. 1978. Further characterization and refinement of an HPLC post-column fluorometric labeling technique for the determination of carbamate insecticides. *J. Chromatogr. Sci.* 16:261.
4. KRAUSE, R.T. 1979. Resolution, sensitivity and selectivity of a high-performance liquid chromatographic post-column fluorometric labeling technique for determination of carbamate insecticides. *J. Chromatogr.* 185:615.
5. HILL, K.M., R.H. HOLLOWELL & L.A. DALCORTIVO. 1984. Determination of *N*-methylcarbamate pesticides in well water by liquid chromatography and post-column fluorescence derivatization. *Anal. Chem.* 56:2465.
6. FOERST, D.C. & H.A. MOYE. 1985. Aldicarb and related compounds in drinking water via direct aqueous injection HPLC with post-column derivatization. *In* Advances in Water Analysis and Treatment, Proc. 12th Annu. AWWA Water Quality Technology Conf., Dec. 2–5, 1984, Denver, Colo., p. 189. American Water Works Assoc., Denver, Colo.
7. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1991. Method 531.1—Measurement of *N*-methylcarbamoyloximes and *N*-methylcarbamates in water by direct aqueous injection HPLC with post-column derivatization. *In* Methods for the Determination of Organic Compounds in Drinking Water. EPA-600/4-88-039, rev. July 1991, U.S. Environmental Protection Agency, Cincinnati, Ohio.
8. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1988. National Pesticide Survey Analytical Methods. Method 5. Measure *N*-methyl-carbamoyloximes and *N*-methylcarbamates in groundwater by direct aqueous injection HPLC with post-column derivatization. U.S. Environmental Protection Agency, Cincinnati, Ohio.

6610 B. High-Performance Liquid Chromatographic Method

1. General Discussion

a. Principle: This HPLC method is applicable to the determination of certain carbamate pesticides in water.* The sample is filtered and injected onto a reverse-phase HPLC column. The constituents are separated by gradient elution chromatography. After elution the *N*-methyl compounds are hydrolyzed with sodium hydroxide. The resulting methylamine reacts with *o*-phthalaldehyde (OPA) and 2-mercaptoethanol (MERC) to form a highly fluorescent isoindole product that is detected with a fluorescence detector.

b. Interferences: The post-column reaction generally is sensitive to primary amines and secondary ammonium cations and will form fluorescent adducts that may cause interference depending on their elution time and fluorescence intensity. Interferences also may be caused by contaminants in solvents, reagents, glassware, and sample-processing equipment. Specific sources of contamination have not been identified. Demonstrate that reagents and apparatus are free from interferences under the conditions of the analysis by analyzing laboratory method blanks. Use only high-purity reagents and solvents.

High chlorine concentrations, which may be encountered near a chlorine injection point, cause interference and loss of some constituents. Collect samples before chlorination or as far as practical from an injection point. If a raw water source is to be evaluated, collect sample before chlorination.

Matrix interferences may be caused by contaminants in the sample. They vary considerably from source to source. Severe interferences are not expected from most ground and surface waters, but the method may not be suitable for waste streams, landfill leachate, or wastewater effluents. Confirmatory analysis may be used to increase the confidence of identification and quantitation for compounds determined by the primary analysis.

Interfering contamination in the analysis may occur when a low-level sample is analyzed immediately after a sample containing relatively high concentrations of test compounds. To minimize contamination use disposable syringes, filters, and sample vials. Analyze a reagent water blank after analyzing a sample containing high concentrations of carbamate pesticides, to demonstrate that no carryover contamination is present.

c. Detection levels: This method has been validated in a multiple-laboratory test. Estimated detection levels (EDLs) are listed in Table 6610:I. Observed detection levels may vary between laboratories and samples, depending on interferences and specific instrumentation.

2. Sampling and Storage

If residual chlorine is present in the water to be sampled, add 8 mg sodium thiosulfate/100 mL sample to bottle before collecting the sample. Fill bottle only to shoulder with water to be sampled, leaving space for expansion on freezing. Keep samples cold (4°C) from time of collection until receipt in the laboratory. Oxamyl, 3-hydrocarbofuran, and carbaryl can degrade quickly in water at room temperature. This short-term degradation is of concern during sample processing and holding at room temperature in autosampler trays. Preserve samples targeted for the analysis of these three compounds by adjusting to pH 3 with monochloroacetic acid buffer solution (1.5 mL buffer/50 mL sample) or HCl (approximately 2.0 mL 0.1*N* HCl/50 mL) or potassium hydrogen tartrate (50 mg/50 mL). If HCl is used for finished water, dechlorinate sample before adding acid or transfer dechlorinated sample to separate bottle with acid. For maximum protection, add buffer to sample bottle before taking sample. In the laboratory, store samples at 4°C until analysis. Samples are stable for at least 14 d when adjusted to pH 3 and stored at 4°C (see Tables 6610:II, 6610:III, and 6610:IV). The current regulatory limit is 28 d, but 14 d is recommended.

* Aldicarb sulfoxide, aldicarb sulfone, oxamyl, methomyl, 3-hydroxycarbofuran, aldicarb, baygon (propoxur), carbofuran, carbaryl, and methiocarb.

TABLE 6610:I. RECOVERY OF KNOWN ADDITIONS OF SELECTED CARBAMATES FROM WATER AND ESTIMATED DETECTION LEVELS (EDL)

Constituent	Addition µg/L	No. Data Points	Recovery Average %	Standard Deviation µg/L	Relative Standard Deviation %	EDL* µg/L
Aldicarb	1.0	8	107	0.07	7	1.0
Aldicarb sulfoxide	2.0	8	47	0.20	21	2.0
Aldicarb sulfone	2.0	8	83	0.34	20	2.0
Baygon (Propoxur)	1.0	7	101	0.32	32	1.0
Carbaryl	2.0	8	97	0.44	23	2.0
Carbofuran	1.5	7	90	0.17	12	1.5
3-Hydroxycarbofuran	2.0	8	108	0.63	29	2.0
Methiocarb	4.0	8	82	0.64	19	4.0
Methomyl	0.50	7	102	0.09	18	0.50
Oxamyl	2.0	8	82	0.29	7	2.0

* EDL = estimated detection level in sample: calculate by multiplying standard deviation times the Student's t value appropriate for a 99% confidence level and a standard deviation estimate with $n-1$ degrees of freedom, or a level of compound in sample yielding a peak with a signal-to-noise ratio of approximately 5, whichever value is higher. There were no detectable apparent residues of any constituent in the blank water.

3. Apparatus

a. High-performance liquid chromatograph (HPLC): An analytical system complete with column supplies, high-pressure syringes, detectors, and compatible strip-chart recorder. Preferably use a data system for measuring peak areas and retention times. Use system capable of injecting 200- to 500-µL portions and of performing binary linear gradients at a constant flow rate. See Figure 6610:1.

1) *Primary column:* 150 mm long × 3.9 mm ID stainless steel packed with 4 µm Novapak C18.† If other columns, conditions, or detectors are used, demonstrate that acceptable results are obtained.

2) *Alternate column:* 250 mm long × 4.6 mm ID stainless steel packed with 5 µm Beckman Ultrasphere ODS.† Data presented herein were obtained with this column. However, newer manufactured columns have not been able to resolve aldicarb sulfone from oxamyl.

3) *Confirmatory column:* 250 mm long × 4.6 mm ID stainless steel packed with 5 µm Supelco LC-1.† This is a trimethylsilyl bonded silica column.

4) *Post-column reactor:* Use a post-column reactor capable of mixing reagents into the mobile phase and equipped with pumps to deliver 0.1 to 1.0 mL/min of each reagent. Use a delivery rate of 0.5 mL/min for both sodium hydroxide and OPA solution. Use PTFE tubing‡ for coils and other post-column lines. Stainless steel, polyetheretherketone (PEEK), and/or nickel lines have been used successfully.

5) *Detector:* Use fluorescence detector capable of excitation at approximately 330 nm and detection of emission energies greater than 418 nm.

b. Filters: For macrofiltration of derivatization solutions and mobile phases, use 47-mm filters.§ For microfiltration of samples before HPLC analysis, use 13-mm filter holder‖ and 13-mm-diam 0.2-µm polyester filters.# If disposable filters** and syringes are to be used, test and verify that comparable results are obtained.

c. Analytical balance, capable of weighing to the nearest 0.0001 g.

d. Sample bottles, 120-mL (or other convenient size) screw-cap polypropylene or glass bottles. Less preferably, use polyethylene bottles.

4. Reagents

Use reagent-grade chemicals of high purity and HPLC-grade (tested on HPLC and verified to give no impurity peaks) solvents or equivalent.

a. Methanol, CH_3OH, HPLC-grade, or equivalent.

b. Reagent water: Generate water with less than 0.1 mg TOC/L by using a water purification system.†† Alternatively, purchase HPLC-grade water commercially (verify absence of interferences if water is purchased). For additional alternatives, see Section 1080.

c. Sodium hydroxide, NaOH, 0.05*N:* Dissolve 2.0 g NaOH in 1.0 L reagent water. Filter and degas with helium before use. Alternatively, dilute 50% NaOH solution to minimize CO_2 absorption (6.7 mL/L).

d. 2-Mercaptoethanol solution: Mix 10.0 mL 2-mercaptoethanol and 10.0 mL acetonitrile. Cap. Store in refrigerator. (CAUTION: *Stench.*)

e. Sodium borate solution: Dissolve 19.1 g $Na_2B_4O_7 \cdot 10H_2O$ in reagent water. Dilute to 1 L. Prepare a day before use to insure complete dissolution.

f. OPA reaction solution: Dissolve 0.100 g *o*-phthalaldehyde in 10 mL methanol. Add to 1.0 L sodium borate solution. Mix, filter, and degas with helium. Add 100 µL 2-mercaptoethanol

† HPLC methods are extremely sensitive to the materials used. Mention of trade names by *Standard Methods* does not preclude the use of other existing or as-yet-undeveloped products that give *demonstrably* equivalent results.

‡ Kratos URS 051 and URA 100, or equivalent.

§ Millipore Type HA, 0.45 µm, for water, and Millipore Type FH, 0.5 µm, for organics, or equivalent.

‖ Millipore stainless steel, XX300/200 or equivalent.

#Nuclepore 180406, 7035 Commerce Circle, Pleasanton, CA 94566-3294, or equivalent.

** Gelman Sciences Acro LC 13, 0.45-µm disposable filter assembly, or equivalent, for aqueous samples.

†† Millipore, Super-Q, or equivalent.

TABLE 6610:II. STORAGE STABILITY OF SAMPLES WITH KNOWN ADDITIONS

Constituent	Added Concentration µg/L	Day	Storage Temperature °C	Average Recovery %	Relative Standard Deviation %
Aldicarb	5.0	0	—	100	9
		14	−10	100	4
			+4	110	2
		28	−10	100	6
			+4	83	1
Aldicarb sulfone	10	0	—	99	9
		14	−10	93	3
			+4	99	3
		28	−10	97	0
			+4	86	8
Aldicarb sulfoxide	10	0	—	100	9
		14	−10	91	6
			+4	100	2
		28	−10	98	2
			+4	91	5
Baygon (Propoxur)	5.0	0	—	98	10
		14	−10	91	2
			+4	100	4
		28	−10	88	2
			+4	93	9
Carbaryl	10	0	—	100	6
		14	−10	92	4
			+4	95	3
		28	−10	99	18
			+4	89	5
Carbofuran	7.5	0	—	100	9
		14	−10	95	3
			+4	110	7
		28	−10	95	2
			+4	93	3
3-Hydroxycarbofuran	10	0	—	95	9
		14	−10	89	6
			+4	100	3
		28	−10	100	8
			+4	95	11
Methiocarb	20	0	—	110	4
		14	−10	100	1
			+4	98	1
		28	−10	99	0
			+4	94	6
Methomyl	2.5	0	—	110	12
		14	−10	90	6
			+4	96	5
		28	−10	93	2
			+4	96	5
Oxamyl	10	0	—	98	6
		14	−10	85	4
			+4	95	5
		28	−10	100	11
			+4	94	9

Conditions: Samples preserved by adjustment to pH 3 with monochloroacetic acid buffer, and protected from light.

solution and mix. If protected from oxygen, solution is stable for at least 3 d; otherwise, prepare daily.

g. Monochloroacetic acid, 2.5M: Dissolve 236 g monochloroacetic acid in 1 L reagent water.

h. Potassium acetate solution, 2.5M: Dissolve 245 g potassium acetate in 1 L reagent water.

i. Monochloroacetic acid buffer: Mix 156 mL 2.5*M* monochloroacetic acid with 100 mL 2.5*M* potassium acetate solution.

TABLE 6610:III. STABILITY OF 10 μG/L CARBAMATES WITH DIFFERENT PRESERVATIVES

Analyte and Preservative	Concentration μg/L									
	Day 0		Day 3		Day 8		Day 28		Average, Day 0–8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Recovery %	SD
Aldicarb sulfoxide:										
HCl	9.88	0.23	10.15	0.34	9.42	0.31	8.76	0.09	98	0.41
MCAA	10.20	0.57	10.45	0.12	10.02	0.79	9.32	0.11	102	0.53
Tartrate	10.38	0.27	10.30	0.27	9.86	0.18	9.64	0.17	102	0.32
Aldicarb sulfone:										
HCl	10.33	0.06	10.18	0.38	10.06	0.27	9.45	0.26	102	0.26
MCAA	10.41	0.39	10.48	0.41	10.42	0.52	9.62	0.14	104	0.39
Tartrate	10.36	0.18	10.31	0.08	10.46	0.14	9.70	0.18	104	0.14
Oxamyl:										
HCl	10.47	0.28	10.04	0.23	10.49	0.46	9.78	0.37	103	0.37
MCAA	10.26	0.14	10.16	0.63	10.55	0.28	9.62	0.21	103	0.40
Tartrate	10.54	0.73	10.26	0.48	10.42	0.24	9.60	0.31	104	0.47
Methomyl:										
HCl	9.94	0.35	9.18	0.11	9.70	0.17	8.40	0.26	96	0.39
MCAA	9.93	0.17	9.66	0.52	10.24	0.34	9.27	0.13	99	0.41
Tartrate	10.23	0.77	9.91	0.27	10.23	0.19	9.38	0.32	101	0.45
3-Hydroxycarbofuran:										
HCl	9.94	0.24	9.84	0.07	10.49	0.01	9.81	0.03	101	0.33
MCAA	10.22	0.26	9.89	0.09	10.27	0.12	9.75	0.01	101	0.23
Tartrate	10.36	0.20	9.91	0.15	10.25	0.02	9.92	0.10	102	0.24
Aldicarb:										
HCl	9.78	0.28	9.26	0.10	9.15	0.16	7.49	0.04	94	0.34
MCAA	9.69	0.07	9.46	0.09	9.21	0.06	8.04	0.11	95	0.22
Tartrate	10.12	0.30	9.76	0.15	9.98	0.21	9.38	0.08	100	0.25
Baygon (Propoxur):										
HCl	10.38	0.13	9.93	0.11	10.36	0.14	9.80	0.03	102	0.24
MCAA	10.22	0.13	9.79	0.14	10.16	0.04	9.79	0.20	101	0.23
Tartrate	10.41	0.29	10.01	0.14	10.23	0.15	9.79	0.09	102	0.25
Carbofuran:										
HCl	10.18	0.42	10.13	0.29	9.95	0.28	9.71	0.11	101	0.31
MCAA	9.96	0.23	9.41	0.40	10.16	0.08	9.90	0.77	98	0.41
Tartrate	9.95	0.09	10.00	0.42	10.37	0.23	9.58	0.11	101	0.31
Carbaryl:										
HCl	7.99	0.42	7.70	0.71	8.23	0.85	5.70	0.82	80	0.63
MCAA	7.59	0.85	6.29	0.96	6.82	0.39	5.68	1.08	69	0.88
Tartrate	7.74	2.15	7.10	1.50	8.28	0.59	7.56	1.20	77	1.44
Methiocarb:										
HCl	5.27	0.62	5.15	0.91	6.65	1.20	3.19	0.75	57	1.09
MCAA	4.90	1.17	4.05	1.19	4.40	0.66	3.61	1.45	45	0.97
Tartrate	5.41	2.41	4.50	1.60	6.30	1.42	5.63	2.40	54	1.79

NOTES: Southern California tap water dechlorinated with sodium thiosulfate was acidified as shown below and analyzed in triplicate on each day in a single laboratory.
HCl — preserved samples were adjusted to pH 3 with HCl.
MCAA — preserved samples were adjusted to pH 3 with MCAA buffer solution.
Tartrate — preserved samples had 50 mg potassium hydrogen tartrate added/50 mL.
Methiocarb standard may have degraded before use, accounting for the low recoveries.

CAUTION: *Handle with care; long-term health effects from exposure have not been determined.*

j. Hydrochloric acid, HCl, 0.1*N:* Dilute 8.3 mL conc HCl to 1 L with reagent water.

k. Potassium hydrogen tartrate, reagent-grade.

l. Stock pesticide solutions: Prepare stock standard solutions (1.00 μg/μL) by accurately weighing approximately 0.0100 g of each compound into separate 10-mL volumetric flasks. Dissolve in about 5 mL methanol and dilute to mark with methanol. Larger volumes may be used at the convenience of the analyst. Alternatively, purchase certified mixtures. These solutions are stable for several months when stored in a freezer at −10°C.

m. Calibration standards:

TABLE 6610:IV. STABILITY OF 2 µG/L CARBAMATES WITH DIFFERENT PRESERVATIVES

Analyte and Preservative	Sample Source*	Concentration µg/L Day 0	Day 6	Day 7	Day 14	Day 21	Day 28	Avg. Recovery %	RSD %
Aldicarb sulfoxide:									
HCl	CA	1.78	—	1.63	1.64	—	1.67	84	4
HCl	VA	—	1.87	—	—	—	1.44	83	18
MCAA	CA	1.83	—	1.67	1.69	—	1.49	84	8
MCAA	VA	—	2.04	—	—	—	1.76	95	10
Tartrate	VA	2.08	—	—	—	1.95	1.73	96	9
Aldicarb sulfone:									
HCl	CA	1.61	—	1.75	1.96	—	1.50	85	12
HCl	VA	—	1.91	—	—	—	1.77	92	5
MCAA	CA	1.74	—	1.76	1.79	—	1.72	88	2
MCAA	VA	—	1.90	—	—	—	1.84	94	2
Tartrate	VA	1.96	—	—	—	1.90	1.76	94	5
Oxamyl:									
HCl	CA	1.81	—	1.73	1.79	—	1.52	86	8
HCl	VA	—	1.83	—	—	—	1.85	92	1
MCAA	CA	1.64	—	1.82	1.84	—	1.58	86	8
MCAA	VA	—	1.80	—	—	—	1.91	93	4
Tartrate	VA	1.95	—	—	—	1.74	1.80	92	6
Methomyl:									
HCl	CA	1.83	—	1.60	1.60	—	1.52	82	8
HCl	VA	—	1.65	—	—	—	1.50	79	7
MCAA	CA	1.89	—	1.65	1.70	—	1.60	86	7
MCAA	VA	—	1.73	—	—	—	1.72	86	0
Tartrate	VA	1.95	—	—	—	1.77	1.75	91	6
3-Hydroxycarbofuran:									
HCl	CA	2.03	—	1.93	1.83	—	1.92	96	4
HCl	VA	—	1.99	—	—	—	1.94	98	2
MCAA	CA	1.90	—	1.99	1.91	—	1.81	95	4
MCAA	VA	—	1.99	—	—	—	2.02	100	1
Tartrate	VA	1.99	—	—	—	1.97	1.93	98	2
Aldicarb:									
HCl	CA	1.88	—	1.83	1.75	—	1.61	88	7
HCl	VA	—	1.72	—	—	—	1.17	72	27
MCAA	CA	1.90	—	1.89	1.84	—	1.73	92	4
MCAA	VA	—	1.88	—	—	—	1.64	88	10
Tartrate	VA	1.94	—	—	—	1.86	1.95	96	3
Baygon (Propoxur):									
HCl	CA	1.97	—	1.99	2.10	—	1.69	97	9
HCl	VA	—	2.11	—	—	—	1.89	100	8
MCAA	CA	1.89	—	1.97	2.08	—	1.56	94	12
MCAA	VA	—	2.01	—	—	—	2.01	101	0
Tartrate	VA	2.12	—	—	—	1.94	2.04	102	4
Carbofuran:									
HCl	CA	1.87	—	1.82	1.83	—	1.40	87	13
HCl	VA	—	2.07	—	—	—	1.96	101	4
MCAA	CA	1.94	—	1.83	1.68	—	1.51	87	11
MCAA	VA	—	1.83	—	—	—	1.86	92	1
Tartrate	VA	1.98	—	—	—	1.84	1.93	96	4
Carbaryl:									
HCl	CA	2.02	—	1.40	1.65	—	1.69	85	15
HCl	VA	—	1.37	—	—	—	1.47	71	5
MCAA	CA	1.75	—	1.29	1.76	—	1.91	84	16
MCAA	VA	—	1.62	—	—	—	1.34	74	13
Tartrate	VA	1.50	—	—	—	1.39	1.21	68	11
Methiocarb:									
HCl	CA	1.72	—	0.84	1.04	—	1.90	69	37
HCl	VA	—	0.85	—	—	—	0.88	43	2
MCAA	CA	1.78	—	0.73	1.15	—	2.04	71	42
MCAA	VA	—	0.89	—	—	—	0.86	44	12
Tartrate	VA	0.80	—	—	—	0.87	0.66	39	14

* Tap water sample from two sites, California groundwater (CA) and Virginia surface water (VA), preserved as in Table 6610:III but analyzed only once each day.
NOTE: Some compounds fortified below recommended minimum reporting level, because fortifying mix contained all compounds at the same concentration level.

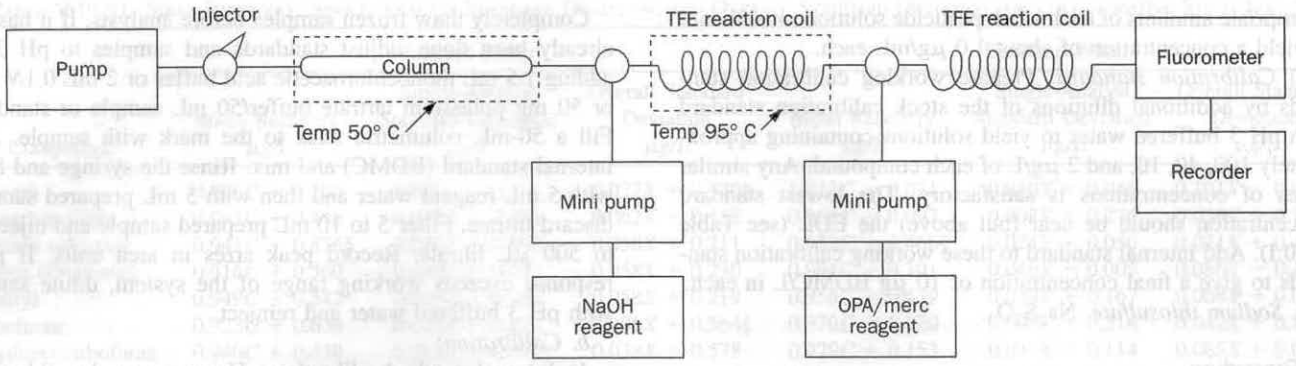

Figure 6610:1 Schematic of post-column reaction HPLC system.

1) *Internal standard:* Prepare an internal standard solution by accurately weighing approximately 0.0010 g 4-bromo-3,5-dimethylphenyl *N*-methylcarbamate (BDMC), dissolving in methanol, and diluting to volume in a 10-mL volumetric flask. Add 5 μL of internal standard solution to 50 mL sample to give an internal standard concentration of 10 μg/L.

2) *Stock calibration standard:* Prepare a mixed concentrated standard of the compounds listed in Table 6610:I by adding

TABLE 6610:V. PRIMARY AND CONFIRMATORY CHROMATOGRAPHIC CONDITIONS AND RETENTION TIMES FOR SELECTED CARBAMATE PESTICIDES

Constituent	Retention Time, *min*: Primary System	Confirmatory System	Alternate System
Aldicarb sulfoxide	6.80	8.5	14.94
Aldicarb sulfone	7.77	8.57	15.23
Oxamyl	8.20	10.03	17.36
Methomyl	8.94	10.39	18.39
3-Hydroxycarbofuran	13.65	12.51	23.32
Aldicarb	16.35	14.11	27.01
Baygon (Propoxur)	18.86	16.02	29.25
Carbofuran	19.17	16.33	29.61
Carbaryl	20.29	17.17	30.78
Methiocarb	24.74	20.45	34.93
BDMC (internal standard)	25.28	20.58	35.50

Primary conditions:	
Column	150-mm-long × 3.9-mm-ID Waters NovaPak C18 (4 μm)
Mobile phase	Linear gradient from 10:90 methanol:water, hold 2 min, then linear gradient to 80:20 methanol:water in 25 min
Flow rate	1.0 mL/min
Injection volume	500 μL
Detector	Fluorescence; excitation 330 nm; emission >418 nm
Confirmatory conditions:	
Column	250-mm-long × 4.6-mm-ID Supelco LC-1 (5 μm)
Mobile phase	Linear gradient from 15:85 methanol:water to 100% methanol in 32 min
Flow rate	1.0 mL/min
Injection volume	500 μL
Detector	Fluorescence; excitation 330 nm; emission >418 nm
Alternate conditions:	
Column	250-mm-long × 4.6-mm-ID Beckman Ultrasphere ODS (5 μm)
Mobile phase	Linear gradient from 15:85 methanol:water to 100% methanol in 32 min
Flow rate	1.0 mL/min
Injection volume	500 μL
Detector	Fluorescence; excitation 330 nm; emission >418 nm
Post-column reactor condition for all detectors:	
Hydrolysis	NaOH (0.05*N*), flow rate 0.5 mL/min, 1.0-mL reaction coil at 95°C
Derivatization	OPA solution, flow rate 0.5 mL/min, 1.0-mL reaction coil at ambient temperature

appropriate amounts of each stock pesticide solution to methanol to yield a concentration of about 1.0 μg/mL each.

3) *Calibration standard:* Prepare working calibration standards by additional dilutions of the stock calibration standard with pH 3 buffered water to yield solutions containing approximately 100, 40, 10, and 2 μg/L of each compound. Any similar series of concentrations is satisfactory. The lowest standard concentration should be near (but above) the EDL (see Table 6610:I). Add internal standard to these working calibration standards to give a final concentration of 10 μg BDMC/L in each.

n. Sodium thiosulfate, $Na_2S_2O_3$.

5. Procedure

a. Liquid chromatography: Table 6610:V summarizes the recommended operating conditions for the liquid chromatograph, including retention times. An example of the separations achieved under these conditions is shown in Figure 6610:2.

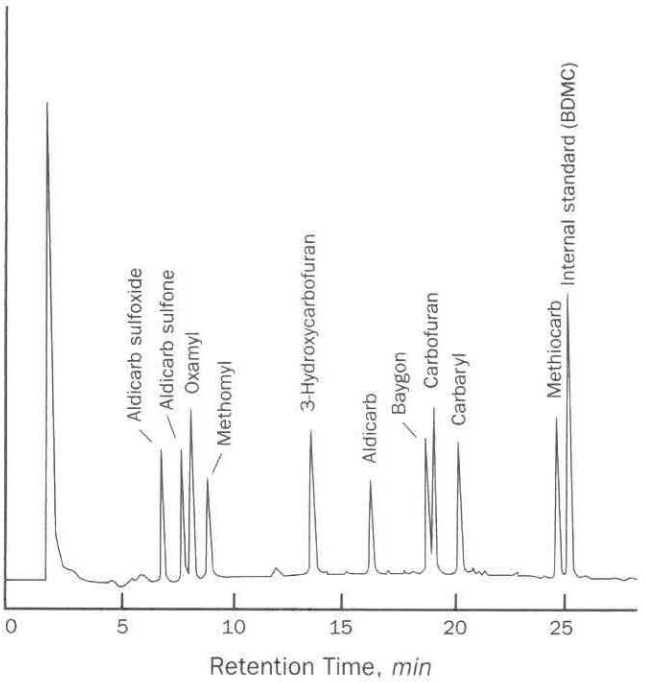

Figure 6610:2. Schematic HPLC-PCD chromatogram of carbamate mix indicating relative response, separations, and retention. Concentrations are shown below.

Compound	Concentration μg/L
Aldicarb sulfoxide	8.6
Aldicarb sulfone	7.6
Oxamyl	12.6
Methomyl	4.7
3-Hydroxycarbofuran	13.8
Aldicarb	6.5
Baygon (Propoxur)	11.6
Carbofuran	11.3
Carbaryl	6.1
Methiocarb	20.4

Completely thaw frozen samples before analysis. If it has not already been done, adjust standards and samples to pH 3 by adding 1.5 mL monochloroacetic acid buffer or 2 mL 0.1*N* HCl or 50 mg potassium tartrate buffer/50 mL sample or standard. Fill a 50-mL volumetric flask to the mark with sample. Add internal standard (BDMC) and mix. Rinse the syringe and filter with 5 mL reagent water and then with 5 mL prepared sample; discard filtrate. Filter 5 to 10 mL prepared sample and inject up to 500 μL filtrate. Record peak sizes in area units. If peak response exceeds working range of the system, dilute sample with pH 3 buffered water and reinject.

b. Calibration:

1) Internal standard calibration—Using prepared working calibration standards, inject 500 μL of each standard. The lowest calibration standard should represent compound concentrations near, but above, their respective EDLs. The remaining calibration standards should bracket expected concentration range of samples or should define the working range of the detector. Tabulate peak area responses against concentration for each compound and the internal standard. Calculate response factors (*RF*) for each compound, using the equation:

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

where:

A_s = peak area for compound in working standard,
A_{is} = peak area of internal standard,
C_{is} = concentration of internal standard, μg/L, and
C_s = concentration of compound in working standard, μg/L.

If the *RF* value over the working range is constant (≤ 20% RSD), use average *RF* for calculations.

Alternatively, plot a calibration curve using (A_s/A_{is}) vs. C_s.

Verify working calibration curve or *RF* for each working shift by measuring one or more calibration standards. Preferably verify at different levels throughout a working day. If the response for any compound varies from the predicted response by more than ±20%, repeat the test using a fresh calibration standard and, if necessary, prepare a new calibration curve.

Verify calibration standards at least quarterly by analyzing a standard prepared using reference material obtained from an independent source.

2) External standard calibration—Use working calibration standards, ¶ 4*m*3). Starting with the lowest concentration, analyze each working calibration standard, using 500-μL injections. Tabulate responses, peak height or area versus compound concentration in the standard. Prepare a calibration curve for each compound. Alternatively, if the ratio of response to concentration (calibration factor) is constant (≤20% RSD) over the working range of the curve, use a calibration factor in place of the calibration curve.

Verify calibration curve each working shift by analysis of a working calibration standard at the beginning of the shift and a different concentration at the end.

6. Calculations

Determine the concentrations of individual compounds using the following equations:

TABLE 6610:VI. MEAN RECOVERY, SINGLE-ANALYST STANDARD DEVIATION, AND OVERALL STANDARD DEVIATION FOR COLLABORATIVE STUDY DATA*

	Reagent Water			Drinking Water		
Compound	Mean Recovery μg/L	Single-Analyst Standard Deviation μg/L	Overall Standard Deviation μg/L	Mean Recovery μg/L	Single-Analyst Standard Deviation μg/L	Overall Standard Deviation μg/L
Aldicarb	$0.926C + 0.202$	0.32†	$0.022X + 0.370$‡	$1.032C + 0.031$	$0.040X + 0.046$	$0.101X - 0.042$‡
Aldicarb sulfone	$0.942C + 0.446$	$0.025X + 0.382$	$0.062X + 0.132$	$0.968C - 0.097$	$0.008X + 0.276$	$0.039X + 0.119$‡
Aldicarb sulfoxide	$0.941C + 0.876$§	$0.040X + 0.103$	$0.058X + 0.211$	$0.952C + 0.460$§	$0.024X + 0.050$	$0.021X + 0.440$
Baygon (Propoxur)	$0.916C + 0.360$	$0.040X + 0.092$	$0.058X + 0.230$	$0.994C + 0.101$	$0.046X - 0.005$	$0.086X - 0.114$
Carbaryl	$0.949C + 0.542$	$0.016X + 0.480$	$0.058X + 0.219$	$0.958C + 0.439$	$0.039X + 0.167$	$0.068X + 0.015$
Carbofuran	$0.923C + 0.636$	$0.022X + 0.322$	$0.006X + 0.564$‡	$0.970C + 0.220$	$0.008X + 0.316$	$0.042X + 0.178$
3-Hydroxycarbofuran	$0.940C + 0.438$	$0.013X + 0.697$‡	$0.038X + 0.578$	$0.979C + 0.153$	$0.044X + 0.114$	$0.085X + 0.045$‡
Methiocarb	$0.923C + 0.887$	$0.005X + 1.839$	$0.035X + 2.286$	$0.958C + 0.474$	$0.034X + 0.046$	$0.057X + 0.322$
Methomyl	$0.976C + 0.043$	$0.053X + 0.069$	$0.048X + 0.133$	$0.988C + 0.000$	0.14†	$0.040X + 0.000$
Oxamyl	$0.936C + 0.659$	1.04†	$0.038X + 0.699$	$0.998C + 0.045$	$0.025X + 0.048$	$0.023X + 0.672$‡

* C = true concentration, μg/L; X = mean recovery, μg/L.

† Weighted linear regression equation had negative slope; average precision is reported.

‡ Coefficient of determination of weighted equation was weak (<0.5).

§ Lowest addition recovery not used for this regression.

Source: EDGELL, K.W., L.A. BIEDERMAN & J.E. LONGBOTTOM. 1991. Measurement of *N*-methylcarbamoyloximes and *N*-methylcarbamates in water by direct aqueous injection HPLC with post column derivatization: Collaborative study. *J. Assoc. Offic. Anal. Chem.* 74:309.

For internal standard quantitation,

$$C_x = \frac{A_x \times C_{is}}{A_{is} \times RF}$$

and for external standard quantitation,

$$C_x = \frac{A_x \times C_s}{A_s}$$

where:

C_x = compound concentration, μg/L, and
A_x = response of sample compound (peak area), and other terms are as defined previously.

7. Quality Control

A minimum quality control program should include the following quality assurance elements: initial demonstration of laboratory capability, laboratory control standards, monitoring of systems performance, blank samples, quality control check samples, duplicate analyses, demonstration of adequate recoveries, assessment of matrix effects, and demonstration of storage stability. Follow QC procedures in 6020. Also see Part 1000 for general quality control requirements.

8. Precision and Bias

Single-laboratory recovery data are summarized in Tables 6610:II, 6610:III, and 6610:IV. Comparable results should be obtained in other laboratories. Results are comparable if the calculated percent relative standard deviation (RSD) does not exceed 3 times the single-laboratory RSD or 30%, whichever is greater, and the mean recovery lies within the interval R ± 3SD or R ± 30%, whichever is greater, where R is the average recovery in Table 6610:II.

Results of an eight-laboratory collaborative test are shown in Table 6610:VI. Test involved six concentrations for each compound (overall range 1 to 109 μg/L).

Alternate columns and conditions may give improved separation and sensitivity.

6630 ORGANOCHLORINE PESTICIDES*

6630 A. Introduction

1. Sources and Significance

The organochlorine pesticides commonly occur in waters that have been affected by agricultural discharges. Some of the listed compounds are degradation products of other pesticides detected by this method. Several of the pesticides are bioaccumulative and relatively stable, as well as toxic or carcinogenic; thus they require close monitoring.

2. Selection of Method

Methods 6630B and C consist of gas chromatographic (GC) procedures following liquid-liquid extraction of water samples. They are relatively sensitive methods that can be used to detect numerous pesticides. Differences between the methods are minimal after extraction. Method 6630D is a gas chromatographic/mass spectrometric (GC/MS) method that can detect all of the target compounds, but at much higher concentrations. The use of extracted ion traces or time scheduled selective ion monitoring (SIM) can reduce the concentration level needed for detection.[1,2] All these methods also are useful for determination of polychlorinated biphenyls (PCBs) (also see Section 6431A).

* Approved by Standard Methods Committee, 2000.

3. References

1. Zaugg, S.D., M.W. Sandstrom, S.G. Smith & K.M. Fehlberg. 1995. Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Pesticides in Water by C-18 Solid Phase Extraction and Capillary Column Gas Chromatography/Mass Spectrometry with Selected-Ion Monitoring. USGS Open File Rep. 95-181, U.S. Geological Survey, Earth Science Information Center, Denver, Colo.
2. Stan H.-J & T. Heberer. 1995. Identification and confirming analysis based on gas chromatography-mass spectrometry. *In* H.J. Stan. Analysis of Pesticides in Ground and Surface Water 1. Chemistry of Plant Protection, Vol. 11, p. 141. Springer, Berlin, Germany.

6630 B. Liquid-Liquid Extraction Gas Chromatographic Method I

1. General Discussion

a. Application: This gas chromatographic procedure is suitable for quantitative determination of the following specific compounds: BHC, lindane (γ-BHC), heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, captan, DDE, DDD, DDT, methoxychlor, endosulfan, dichloran, mirex, and pentachloronitrobenzene. Under favorable circumstances, strobane, toxaphene, chlordane (tech.), and others also may be determined when relatively high concentrations of these complex mixtures are present and the chromatographic fingerprint is recognizable in packed or capillary column analysis. Trifluralin and certain organophosphorus pesticides, such as parathion, methylparathion, and malathion, which respond to the electron-capture detector, also may be measured. However, the usefulness of the method for organophosphorus or other specific pesticides must be demonstrated before it is applied to sample analysis.

b. Principle: In this procedure, the pesticides are extracted with a mixed solvent, diethyl ether/hexane or methylene chloride/hexane by either liquid-liquid extraction using a separatory funnel or by continuous liquid-liquid extraction. The extract is concentrated by evaporation and, if necessary, is cleaned up by column adsorption-chromatography. The individual pesticides then are determined by gas chromatography. Although procedures detailed below refer primarily to packed columns, capillary column chromatography also may be used. See Section 6010C.2*a*1) for discussion of gas chromatographic principles and 6010C.2*b*2) for discussion of electron-capture detector.

As each component passes through the detector a quantitatively proportional change in electrical signal is measured on a strip-chart recorder. Each component is observed as a peak on the recorder chart. The retention time is indicative of the particular pesticide and peak height/peak area is proportional to its concentration.

Variables may be manipulated to obtain important confirmatory data. For example, the detector system may be selected on the basis of the specificity and sensitivity needed. The detector used in this method is an electron-capture detector that is very sensitive to chlorinated compounds. Additional confirmatory identification can be made from retention data on two or more columns where the stationary phases are of different polarities. A two-column procedure that has been found particularly useful is specified. If sufficient pesticide is available for detection and measurement, confirmation by a more definitive technique, such as mass spectrometry, is desirable.

c. Interference: See Sections 6010C.2*a*2) and 6010C.2*b*2). Some compounds other than chlorinated compounds respond to the electron-capture detector. Among these are oxygenated and unsaturated compounds. Sometimes plant or animal extractives obscure pesticide peaks. These interfering substances often can be removed by auxiliary cleanup techniques. A magnesia-silica gel column cleanup and separation procedure is used for this purpose. Such cleanup usually is not required for potable waters.

1) Polychlorinated biphenyls (PCBs)—Industrial plasticizers, hydraulic fluids, and old transformer fluids that contain PCBs are a potential source of interference in pesticide analysis. The presence of PCBs is suggested by a large number of partially resolved or unresolved peaks that may occur throughout the entire chromatogram. Particularly severe PCB interference will require special separation procedures.

2) Phthalate esters—These compounds, widely used as plasticizers, cause electron-capture detector response and are a source of interferences. Water leaches these esters from plastics, such as polyethylene bottles and plastic tubing. Phthalate esters can be separated from many important pesticides by the magnesia-silica gel column cleanup. They do not cause response to halogen-specific detectors such as microcoulometric or electrolytic conductivity detectors.

d. Detection limits: The ultimate detection limit of a substance is affected by many factors, for example, detector sensitivity, extraction and cleanup efficiency, concentrations, and detector signal-to-noise level. Lindane (γ-BHC) usually can be determined at 10 ng/L in a sample of relatively unpolluted water; the DDT detection limit is somewhat higher, 20 to 25 ng/L. Increased sensitivity is likely to increase interference with all pesticides.

e. Sample preservation: Some pesticides are unstable. Transport under iced conditions, store at 4°C until extraction, and do not hold more than 7 d. When possible, extract upon receipt in the laboratory and store extracts at 4°C until analyzed. Analyze extracts within 40 d.

2. Apparatus

Clean thoroughly all glassware used in sample collection and pesticide residue analyses. Clean glassware as soon as possible after use. Rinse with water or the solvent that was last used in it, wash with soapy water, rinse with tap water, distilled water, redistilled acetone, and finally with pesticide-quality hexane. As a precaution, glassware may be rinsed with the extracting solvent just before use. Heat heavily contaminated glassware in a muffle furnace at 400°C for 15 to 30 min. High-boiling-point materials, such as PCBs, may require overnight heating at 500°C, but no borosilicate glassware can exceed this temperature without risk.

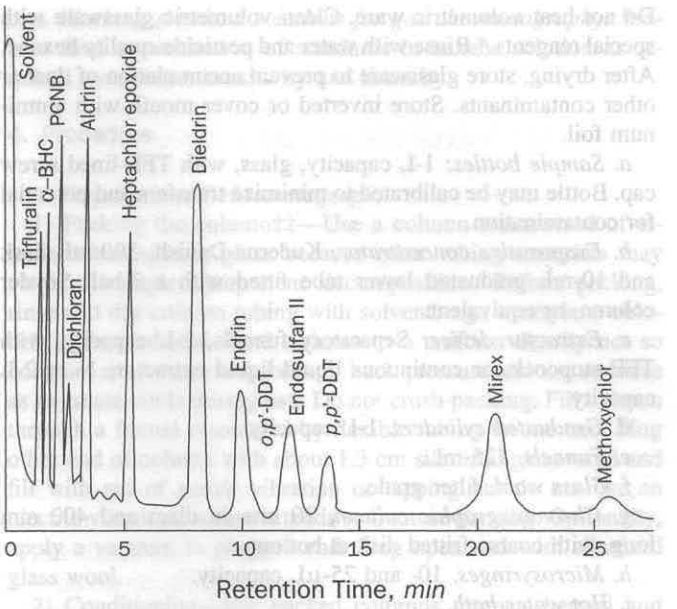

Figure 6630:1. Results of gas chromatographic procedure for organochlorine pesticides. Column packing: 1.5% OV-17 + 1.95% QF-1; carrier gas: argon/methane at 60 mL /min; column temperature: 200°C; detector: electron capture in pulse mode.

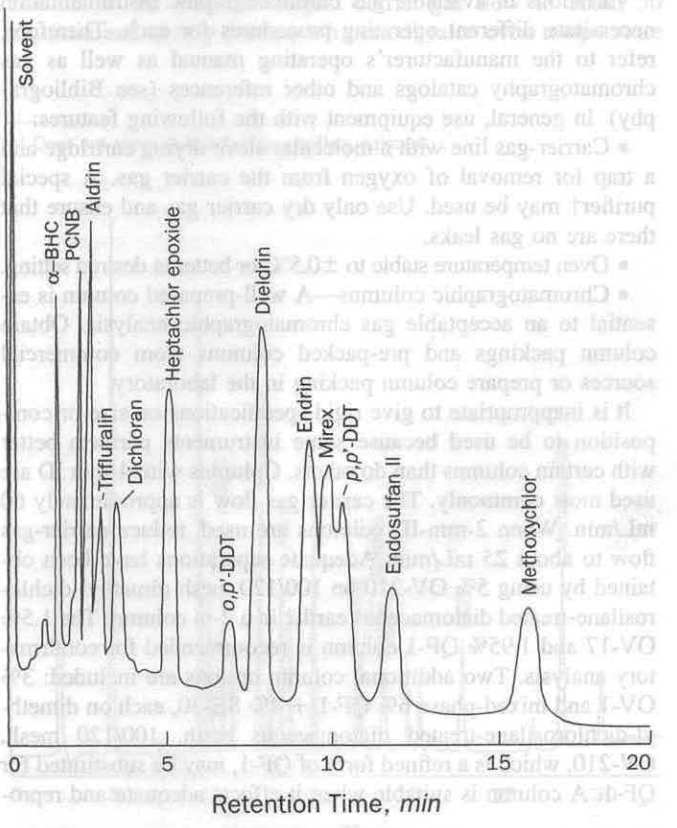

Figure 6630:2. Results of gas chromatographic procedure for organochlorine pesticides. Column packing: 5% OV-210; carrier gas: argon/methane at 70 mL /min; column temperature: 180°C; detector: electron capture.

Do not heat volumetric ware. Clean volumetric glassware with special reagents.* Rinse with water and pesticide-quality hexane. After drying, store glassware to prevent accumulation of dust or other contaminants. Store inverted or cover mouth with aluminum foil.

a. Sample bottles: 1-L capacity, glass, with TFE-lined screw cap. Bottle may be calibrated to minimize transfers and potential for contamination.

b. Evaporative concentrator, Kuderna-Danish, 500-mL flask and 10-mL graduated lower tube fitted with a 3-ball Snyder column, or equivalent.

c. Extractor device: Separatory funnels, 2-L capacity, with TFE stopcock, or continuous liquid-liquid extractor, 1- or 2-L capacity.

d. Graduated cylinders, 1-L capacity.

e. Funnels, 125-mL.

f. Glass wool, filter grade.

g. Chromatographic column, 20 mm in diam and 400 mm long, with coarse fritted disk at bottom.

h. Microsyringes, 10- and 25-μL capacity.

i. Hot water bath.

j. Gas chromatograph, equipped with:

1) *Glass-lined injection port.*

2) *Electron-capture detector.*

3) *Recorder:* Potentiometric strip chart, 25-cm, compatible with detector and associated electronics.

4) *Borosilicate glass column,* 1.8 m × 4-mm ID or 2-mm ID.

Variations in available gas chromatographic instrumentation necessitate different operating procedures for each. Therefore, refer to the manufacturer's operating manual as well as gas chromatography catalogs and other references (see Bibliography). In general, use equipment with the following features:

- Carrier-gas line with a molecular sieve drying cartridge and a trap for removal of oxygen from the carrier gas. A special purifier† may be used. Use only dry carrier gas and ensure that there are no gas leaks.
- Oven temperature stable to ±0.5°C or better at desired setting.
- Chromatographic columns—A well-prepared column is essential to an acceptable gas chromatographic analysis. Obtain column packings and pre-packed columns from commercial sources or prepare column packing in the laboratory.

It is inappropriate to give rigid specifications on size or composition to be used because some instruments perform better with certain columns than do others. Columns with 4-mm ID are used most commonly. The carrier-gas flow is approximately 60 mL/min. When 2-mm-ID columns are used, reduce carrier-gas flow to about 25 mL/min. Adequate separations have been obtained by using 5% OV-210 on 100/120 mesh dimethyl-dichlorosilane-treated diatomaceous earth‡ in a 2-m column. The 1.5% OV-17 and 1.95% QF-1 column is recommended for confirmatory analysis. Two additional column options are included: 3% OV-1 and mixed-phase 6% QF-1 + 4% SE-30, each on dimethyl-dichlorosilane-treated diatomaceous earth, 100/120 mesh. OV-210, which is a refined form of QF-1, may be substituted for QF-1. A column is suitable when it effects adequate and reproducible resolution. Sample chromatograms are shown in Figures 6630:1 through 6630:4.

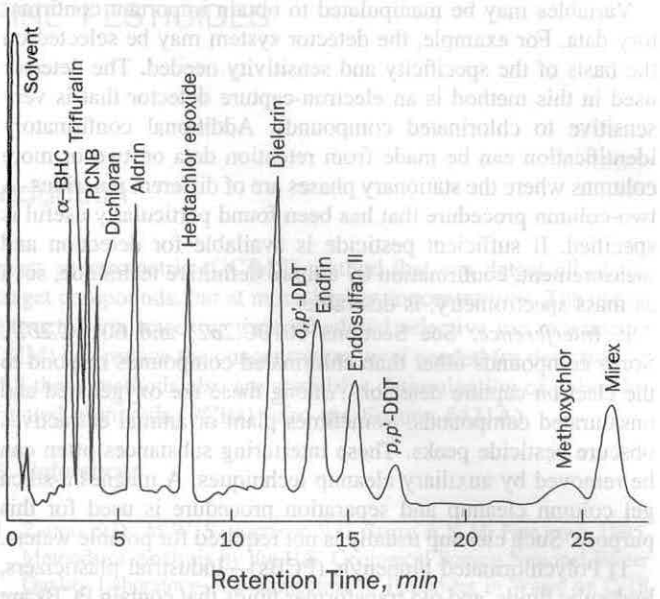

Figure 6630:3. Chromatogram of pesticide mixture. Column packing: 6% QF-1 + 4% SE-30; carrier gas; argon/methane at 60 mL/min; column temperature: 200°C; detector: electron capture.

Alternately, used fused silica capillary columns§ 30 m long with 0.32-mm ID or 0.25-mm ID and 0.25-μm film thickness,

§ Primary column DB-1701, J&W Scientific, or equivalent; confirmatory column DB-5 or equivalent.

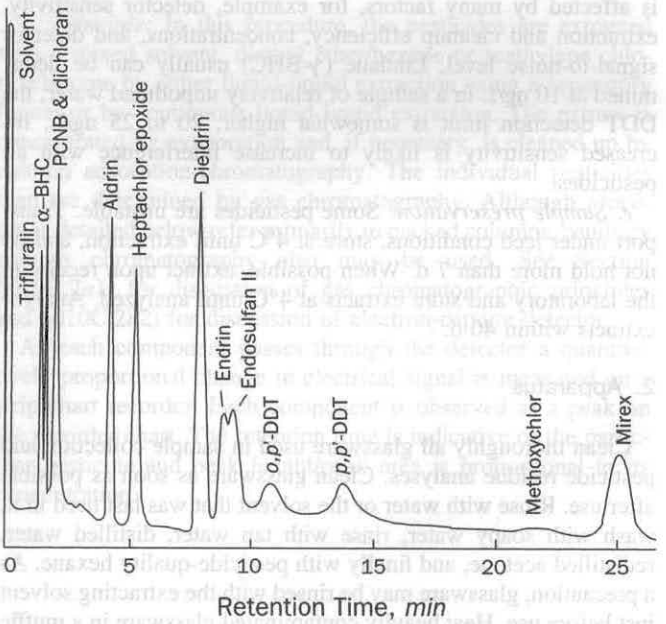

Figure 6630:4. Chromatogram of pesticide mixture. Column packing: 3% OV-1; carrier gas: argon/methane at 70 mL/min; column temperature: 180°C; detector: electron capture.

* No Chromix, Godax, 6 Varick Place, New York, NY, or equivalent.
† Hydrox, Matheson Gas Products, P. O. Box E, Lyndhurst, NJ, or equivalent.
‡ Gas Chrom Q, Applied Science Labs., Inc., P. O. Box 440, State College, PA, or equivalent.

for primary and confirmatory analyses (see Figure 6630:5). Co-elution of Alpha-chlordane and Endosulfan I on the recommended confirmatory column makes it less desirable for primary analysis. Another type of detector, such as a mass spectrometer, also can be used for confirmatory analysis. Use carrier gas flows recommended by manufacturer according to the ID of the column, along with nitrogen make-up gas for the proper operation of the electron capture detector.

3. Reagents‖

Use solvents, reagents, and other materials for pesticide analysis that are free from interferences under the condition of the analysis. Specific selection of reagents and distillation of solvents in an all-glass system may be required. "Pesticide quality" solvents usually do not require redistillation; however, always determine a blank before use.

a. Hexane.

b. Petroleum ether, boiling range 30 to 60°C.

c. Diethyl ether: CAUTION: *Explosive peroxides tend to form.* Test for presence of peroxides# and, if present, reflux over granulated sodium-lead alloy for 8 h, distill in a glass apparatus, and add 2% methanol. Use immediately or, if stored, test for peroxides before use.

d. Ethyl acetate.

e. Methylene chloride.

*f. Magnesia-silica gel,*** PR grade, 60 to 100 mesh. Purchase activated at 676°C and store in the dark in glass container with glass stopper or foil-lined screw cap; do not accept in plastic container. Before use, activate each batch overnight at 130°C in foil-covered glass container.

g. Sodium sulfate, Na_2SO_4, anhydrous, granular: Do not accept in plastic container. If necessary, bake in a muffle furnace to eliminate interferences.

h. Silanized glass wool.

i. Column packing:

1) Solid support—Dimethyl dichlorosilane-treated diatomaceous earth,†† 100 to 120 mesh.

2) Liquid phases—OV-1, OV-210, 1.5% OV-17 (SP 2250) + 1.95% QF-1 (SP 2401), and 6% QF-1 + 4% SE-30, or equivalent.

j. Carrier gas: One of the following is required:

1) *Nitrogen gas,* purified grade, moisture- and oxygen-free.

2) *Argon-methane* (95 + 5%) for use in pulse mode.

k. Pesticide reference standards: Obtain purest standards available (95 to 98%) from gas chromatographic and chemical supply houses.

l. Stock pesticide solutions: Dissolve 100 mg of each pesticide in ethyl acetate and dilute to 100 mL in a volumetric flask; 1.00 mL = 1.00 mg.

m. Intermediate pesticide solutions: Dilute 1.0 mL stock solution to 100 mL with ethyl acetate; 1.0 mL = 10 μg.

n. Working standard solutions for gas chromatography: Prepare final concentration of standards in hexane solution as required by detector sensitivity and linearity.

4. Procedure

a. Preparation of chromatograph:

1) Packing the column‡‡—Use a column constructed of silanized borosilicate glass because other tubing materials may catalyze sample component decomposition. Before packing, rinse and dry column tubing with solvent, e.g., methylene chloride, then methanol. Pack column to a uniform density not so compact as to cause unnecessary back pressure and not so loose as to create voids during use. Do not crush packing. Fill column through a funnel connected by flexible tubing to one end. Plug other end of column with about 1.3 cm silanized glass wool and fill with aid of *gentle* vibration or tapping but do not use an electric vibrator because it tends to fracture packing. Optionally, apply a vacuum to plugged end. Plug open end with silanized glass wool.

2) Conditioning—For packed columns, proper thermal and pesticide conditioning are essential to eliminate column bleed and to provide acceptable gas chromatographic analysis. The following procedure provides excellent results: Connect packed column to the injection port. *Do not* connect column to detector; however, maintain gas flow through detector by using the purge-gas line, or in dual-column ovens, by connecting an unpacked column to the detector. Adjust carrier-gas flow to about 50 mL /min and slowly (over a 1-h period) raise oven temperature

‡‡ Does not apply to fused silica capillary columns.

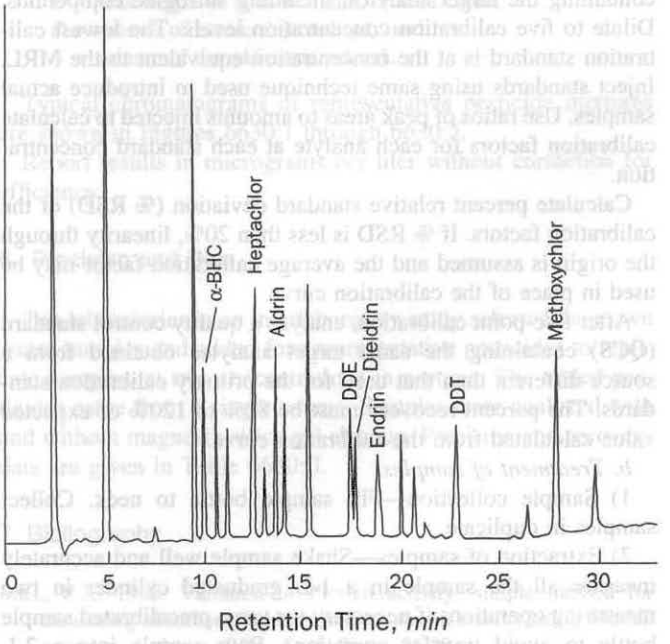

Figure 6630:5. Chromatogram of pesticide mixture. Column DB-5, 30 m long, multilevel program temperature, electron-capture detector.

‖ Gas chromatographic methods are extremely sensitive to the materials used. Mention of trade names by *Standard Methods* does not preclude the use of other existing or as-yet-undeveloped products that give *demonstrably* equal results.

Use E. M. Quant™, MCB Manufacturing Chemists, Inc., 2909 Highland Ave., Cincinnati, OH, or equivalent.

** Florisil™ or equivalent.

†† Gas-Chrom Q™, Supelcoport, or equivalent.

to 230°C. After 24 to 48 h at this temperature the column is ready for pesticide conditioning.

Adjust oven temperature and carrier-gas flow rate to approximate operating levels. Make six consecutive 10-μL injections of a concentrated pesticide mixture through column at about 15-min intervals. Prepare this injection mixture from lindane (γ-BHC), heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, and *p,p'*-DDT, each compound at a concentration of 200 ng/μL. After pesticide conditioning, connect column to detector and let equilibrate for at least 1 h, preferably overnight. Column is then ready for use.

For capillary columns, conditioning is minimal. After installation, bake column for 1 h at 280 to 300°C. Do not exceed the maximum temperature rating specified by the manufacturer for the column.

3) Injection technique

a) Develop an injection technique with constant rhythm and timing. The "solvent flush" technique described below has been used successfully and is recommended to prevent sample blowback or distillation within the syringe needle. Flush syringe with solvent, then draw a small volume of clean solvent into syringe barrel (e.g., 1 μL in a 10-μL syringe). Remove needle from solvent and draw 1 μL of air into barrel. For packed columns, draw 3 to 4 μL of sample extract into barrel; for capillary columns, use 1 μL. Remove needle from sample extract and draw approximately 1 μL air into barrel. Record volume of sample extract between air pockets. Rapidly insert needle through inlet septum, depress plunger, withdraw syringe. After each injection thoroughly clean syringe by rinsing several times with solvent.

b) Inject standard solutions of such concentration that the injection volume and peak height of the standard are approximately the same as those of the sample.

4) Calibration procedure—Prepare a stock calibration mix containing the target analytes, including surrogate compounds. Dilute to five calibration concentration levels. The lowest calibration standard is at the concentration equivalent to the MRL. Inject standards using same technique used to introduce actual samples. Use ratios of peak areas to amounts injected to calculate calibration factors for each analyte at each standard concentration.

Calculate percent relative standard deviation (% RSD) of the calibration factors. If % RSD is less than 20%, linearity through the origin is assumed and the average calibration factor may be used in place of the calibration curve.

After five-point calibration, analyze a quality control standard (QCS) containing the same target analytes obtained from a source different than that used for the primary calibration standards. The percent recovery must be 80% to 120% of expected value calculated from the calibration curve.

b. Treatment of samples:

1) Sample collection—Fill sample bottle to neck. Collect samples in duplicate.

2) Extraction of samples—Shake sample well and accurately measure all the sample in a 1-L graduated cylinder in two measuring operations if necessary (or use a precalibrated sample bottle to avoid transfer operation). Pour sample into a 2-L separatory funnel or 1- to 2-L continuous liquid-liquid extractor. Rinse sample bottle and cylinder with 60 mL 15% diethyl ether or methylene chloride in hexane, pour this solvent into extraction device. For separatory funnel, shake vigorously for 2 min. Let phases separate for at least 10 min. For continuous liquid-liquid extractors, see 6410B.5*a*2), but do not adjust pH.

Drain water phase from separatory funnel into sample bottle and carefully pour organic phase through a 2-cm-OD column containing 8 to 10 cm of Na_2SO_4 into a Kuderna-Danish apparatus fitted with a 10-mL concentrator tube. Pour sample back into separatory funnel.

Rinse sample bottle with 60 mL mixed solvent, use solvent to repeat sample extraction, and pass organic phase through Na_2SO_4. Complete a third extraction with 60 mL of mixed solvent that was used to rinse sample bottle again, and pass organic phase through Na_2SO_4. Wash Na_2SO_4 with several portions of hexane and drain well. Fit Kuderna-Danish apparatus with a three-ball Snyder column and reduce volume to about 7 mL in a hot water bath (90 to 95°C). At this point all methylene chloride present in the initial extracting solvent has been distilled off. Cool, remove concentrator tube from Kuderna-Danish apparatus, rinse ground-glass joint, and dilute to 10 mL with hexane. Make initial gas chromatographic analysis at this dilution.

3) Gas chromatography—Inject 3 to 4 μL of extract solution into a packed column or 1 μL on a fused silica capillary column (split or splitless). Always inject the same volume. Inspect resulting chromatogram for peaks corresponding to pesticides of concern and for presence of interferences.

a) If there are presumptive pesticide peaks and no significant interference, rechromatograph the extract solution on an alternate column.

b) Inject standards frequently to insure optimum operating conditions. If necessary, concentrate or dilute (*do not use methylene chloride*) the extract so that peak size of pesticide is very close to that of corresponding peaks in standard. (See dilution factor, ¶ 5*a*).

c) If significant interference is present, separate interfering substances from pesticide materials by using the cleanup procedure described in the following paragraph.

4) Magnesia-silica gel cleanup—Adjust sample extract volume to 10 mL with hexane. Place a charge of activated magnesia-silica gel§§ (weight determined by lauric-acid value, see Appendix) in a chromatographic column. After settling gel by tapping column, add about 1.3 cm anhydrous granular Na_2SO_4 to the top. Pre-elute column, after cooling, with 50 to 60 mL petroleum ether. Discard eluate and just before exposing sulfate layer to air, quantitatively transfer sample extract into column by careful decantation and with subsequent petroleum ether washings (5 mL maximum). Adjust elution rate to about 5 mL/min and, separately, collect the eluates in 500-mL Kuderna-Danish flasks equipped with 10-mL receivers.

Make first elution with 200 mL 6% ethyl ether in petroleum ether, and the second with 200 mL 15% ethyl ether in petroleum ether. Make third elution with 200 mL 50% ethyl ether-petroleum ether and the fourth with 200 mL 100% ethyl ether. Follow with 50 to 100 mL petroleum ether to insure removal of all ethyl ether from the column. Alternatively, to separate PCBs elute initially with 0% ethyl ether in petroleum ether and proceed as above to yield four fractions.

§§ Florisil™ or equivalent.

TABLE 6630:I. RETENTION RATIOS OF VARIOUS ORGANOCHLORINE PESTICIDES RELATIVE TO ALDRIN

Liquid phase*	1.5% OV-17 + 1.95% QF-1	5% OV-210	3% OV-1	6% QF-1 + 4% SE-30
Column temperature	200°C	180°C	180°C	200°C
Argon/methane carrier flow	60 mL/min	70 mL/min	70 mL/min	60 mL/min
Pesticide	RR	RR	RR	RR
∝-BHC	0.54	0.64	0.35	0.49
PCNB	0.68	0.85	0.49	0.63
Lindane (γ-BHC)	0.69	0.81	0.44	0.60
Dichloran	0.77	1.29	0.49	0.70
Heptachlor	0.82	0.87	0.78	0.83
Aldrin	1.00	1.00	1.00	1.00
Heptachlor epoxide	1.54	1.93	1.28	1.43
Endosulfan I	1.95	2.48	1.62	1.79
p,p'-DDE	2.23	2.10	2.00	1.82
Dieldrin	2.40	3.00	1.93	2.12
Captan	2.59	4.09	1.22	1.94
Endrin	2.93	3.56	2.18	2.42
o,p'-DDT	3.16	2.70	2.69	2.39
p,p'-DDD	3.48	3.75	2.61	2.55
Endosulfan II	3.59	4.59	2.25	2.72
p,p'-DDT	4.18	4.07	3.50	3.12
Mirex	6.1	3.78	6.6	4.79
Methoxychlor	7.6	6.5	5.7	4.60
Aldrin (Min absolute)	3.5	2.6	4.0	5.6

* All columns glass, 180 cm × 4 mm ID, solid support Gas-Chrom Q (100/200 mesh).

Concentrate eluates in Kuderna-Danish evaporator in a hot water bath as in ¶ 4*b*2) preceding, dilute to appropriate volume, and analyze by gas chromatography.

Eluate composition—By use of an equivalent quantity of any batch of magnesia-silica gel as determined by its lauric acid value (see Appendix) the pesticides will be separated into the eluates indicated below:

6% Ethyl Ether Eluate

Aldrin	Heptachlor	Pentachloro-nitrobenzene
BHC	Heptachlor epoxide	Strobane
Chlordane	Lindane (γ-BHC)	Toxaphene
DDD	Methoxychlor	Trifluralin
DDE	Mirex	PCBs
DDT		

15% Ethyl Ether Eluate
Endosulfan I
Endrin
Dieldrin
Dichloran
Phthalate esters

50% Ethyl Ether Eluate
Endosulfan II
Captan

If present, certain thiophosphate pesticides will occur in each of the above fractions as well as in the 100% ether fraction. For additional information regarding eluate composition and the procedure for determining the lauric acid value, refer to the FDA Pesticide Analytical Manual (see Bibliography). For elution pattern test procedure see Appendix, Section 4.

5) Determination of extraction efficiency—Add known amounts (at concentrations similar to those expected in samples) of pesticides in ethyl acetate solution to 1 L water sample and carry through the same procedure as for samples. Dilute an equal amount of intermediate pesticide solution (¶ 3*m* above) to the same final volume. Call peak height from standard "*a*" and peak height from sample to which pesticide was added "*b*," whereupon the extraction efficiency equals *b/a*. Periodically determine extraction efficiency and a control blank to test the procedure. Also analyze one set of duplicates with each series of samples as a quality-control check.

5. Calculation

a. Dilution factor: If a portion of the extract solution was concentrated, the dilution factor, *D*, is a decimal; if it was diluted, the dilution factor exceeds 1.

b. Determine pesticide concentrations by direct comparison to a single standard when the injection volume and response are within 10% of those of the sample pesticide of interest (Table 6630:I). Calculate concentration of pesticide:

$$\mu g/L = \frac{A \times B \times C \times D}{E \times F \times G}$$

where:

A = ng standard pesticide,
B = peak height of sample, mm, or area count,
C = extract volume, μL,
D = dilution factor,
E = peak height of standard, mm, or area count,
F = volume of extract injected, μL, and
G = volume of sample extracted, mL.

Typical chromatograms of representative pesticide mixtures are shown in Figures 6630:1 through 6630:5.

Report results in micrograms per liter without correction for efficiency.

6. Precision and Bias

Ten laboratories in an interlaboratory study selected their own water samples and added four representative pesticides to replicate samples, at two concentrations in acetone. The added pesticides came from a single source. Samples were analyzed with and without magnesia-silica gel cleanup. Precision and recovery data are given in Table 6630:II.

7. Bibliography

MILL, P.A. 1968. Variation of Florisil activity: simple method for measuring absorbent capacity and its use in standardizing Florisil columns. *J. Assoc. Offic. Anal. Chem.* 51:29.

FOOD AND DRUG ADMINISTRATION. 1968 (revised 1978). Pesticide Analytical Manual, 2nd ed. U.S. Dep. Health, Education & Welfare, Washington, D.C.

TABLE 6630:II. PRECISION AND BIAS DATA FOR SELECTED ORGANOCHLORINE PESTICIDES

Pesticide	Level Added *ng/L*	Pretreatment	Mean Recovery *ng/L*	Recovery *%*	Precision* *ng/L* S_T	Precision* *ng/L* S_o
Aldrin	15	No cleanup	10.42	69	4.86	2.59
	110		79.00	72	32.06	20.19
	25	Cleanup†	17.00	68	9.13	3.48‡
	100		64.54	65	27.16	8.02‡
Lindane (γ-BHC)	10	No cleanup	9.67	97	5.28	3.47
	100		72.91	73	26.23	11.49‡
	15	Cleanup†	14.04	94	8.73	5.20
	85		59.08	70	27.49	7.75‡
Dieldrin	20	No cleanup	21.54	108	18.16	17.92
	125		105.83	85	30.41	21.84
	25	Cleanup	17.52	70	10.44	5.10‡
	130		84.29	65	34.45	16.79‡
DDT	40	No cleanup	40.30	101	15.96	13.42
	200		154.87	77	38.80	24.02
	30	Cleanup†	35.54	118	22.62	22.50
	185		132.08	71	49.83	25.31

* S_T = overall precision and S_o = single-operator precision.
† Use of magnesia-silica gel column cleanup before analysis.
‡ $S_o < S_T/2$.

MONSANTO CHEMICAL COMPANY. 1970. Monsanto Methodology for Arochlors—Analysis of Environmental Materials for Biphenyls, Analytical Chemistry Method 71-35. St. Louis, Mo.

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1971. Method for Organic Pesticides in Water and Wastewater. National Environmental Research Center, Cincinnati, Ohio.

STEERE, N.V., ed. 1971. Handbook of Laboratory Safety. Chemical Rubber Company, Cleveland, Ohio.

GOERLITZ, D.F. & E. BROWN. 1972. Methods for analysis of organic substances in water. *In* Techniques of Water Resources Investigations of the United States Geological Survey, Book 5, Chapter A3, p. 24. U.S. Dep. Interior, Geological Survey, Washington, D.C.

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1978. Method for Organochlorine Pesticides in Industrial Effluents. National Environmental Research Center, Cincinnati, Ohio.

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1978. Method for Polychlorinated Biphenyls in Industrial Effluents. National Environmental Research Center, Cincinnati, Ohio.

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Handbook for Analytical Quality Control in Water and Wastewater Laboratories. National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio.

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1980. Analysis of Pesticide Residues in Human and Environmental Samples. Environmental Toxicology Div., Health Effects Laboratory, Research Triangle Park, N.C.

Appendix—Standardization of Magnesia-Silica Gel* Column by Weight Adjustment Based on Adsorption of Lauric Acid

A rapid method for determining adsorptive capacity of magnesia-silica gel is based on adsorption of lauric acid from hexane solution. An excess of lauric acid is used and the amount not adsorbed is measured by alkali titration. The weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of gel for batches having different adsorptive capacities.

1. Reagents

a. Ethyl alcohol, USP or absolute, neutralized to phenolphthalein.

b. Hexane, distilled from all-glass apparatus.

c. Lauric acid solution: Transfer 10.000 g lauric acid to a 500-mL volumetric flask, dissolve in hexane, and dilute to 500 mL; 1.00 mL = 20 mg.

d. Phenolphthalein indicator: Dissolve 1 g in alcohol and dilute to 100 mL.

e. Sodium hydroxide, 0.05*N*: Dilute 25 mL 1*N* NaOH to 500 mL with distilled water. Standardize as follows: Weigh 100 to 200 mg lauric acid into 125-mL erlenmeyer flask; add 50 mL neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point; and calculate milligrams lauric acid per milliliter NaOH (about 10 mg/mL).

2. Procedure

Transfer 2.000 g magnesia-silica gel to a 25-mL glass-stoppered erlenmeyer flask. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 mL lauric acid solution (400 mg), stopper, and shake occasionally during 15 min. Let adsorbent settle and pipet 10.0 mL supernatant into a 125-mL erlenmeyer flask. Avoid including any gel. Add 50 mL neutral alcohol and 3 drops phenolphthalein indicator solution; titrate with 0.05*N* NaOH to a permanent end point.

3. Calculation of Lauric Acid Value and Adjustment of Column Weight

Calculate amount of lauric acid adsorbed on gel as follows:

$$\text{Lauric acid value} = \text{mg lauric acid/g gel} \quad 200\ (\text{mL required for titration} \times \text{mg lauric acid/mL } 0.05N\ \text{NaOH})$$

To obtain an equivalent quantity of any batch of gel, divide 110 by lauric acid value for that batch and multiply by 20 g. Verify proper elution of pesticides by the procedure given below.

4. Test for Proper Elution Pattern and Recovery of Pesticides

Prepare a test mixture containing aldrin, heptachlor epoxide, *p,p'*-DDE, dieldrin, parathion, and malathion. Dieldrin and parathion should elute in the 15% eluate; all but a trace of malathion in the 50% eluate, and the others in the 6% eluate.

* Florisil™ or equivalent.

6630 C. Liquid-Liquid Extraction Gas Chromatographic Method II

This method[1] is applicable to the determination of organochlorine pesticides and PCBs*† in municipal and industrial discharges. When analyzing unfamiliar samples for any or all of these compounds, support the identifications by at least one additional qualitative technique. This method includes analytical conditions for a second, confirmatory gas chromatographic column. Alternatively, analyze by a gas chromatographic/mass spectrometric (GC/MS) method for base/neutrals and acids (Section 6410B) using the extract produced by this method.

Additional PCB congeners can be determined if standards are included.

1. General Discussion

a. Principle: A measured volume of sample is extracted with methylene chloride either by liquid-liquid extraction using separatory funnels or by continuous liquid-liquid extraction. The extract is dried and exchanged to hexane during concentration. If other determinations having essentially the same extraction and concentration steps are to be performed, a single sample extraction is sufficient. The extract is separated by gas chromatography and the compounds are measured with an electron capture detector.[2] See Section 6010C for discussion of gas chromatographic principles.

The method provides procedures for magnesia-silica gel column cleanup and elemental sulfur removal to aid in the elimination of interferences. When cleanup is required, sample concentration levels must be high enough to permit separate treatment of subsamples. Chromatographic conditions appropriate for the simultaneous measurement of combinations of compounds may be selected.

b. Interferences: See Section 6410B.1*b*1) for precautions concerning glassware, reagent purity, and matrix interferences.

Phthalate esters may interfere in pesticide analysis with an electron capture detector. These compounds generally appear in the chromatogram as large, late-eluting peaks, especially in the 15 and 50% fractions from magnesia-silica gel. Common flexible plastics contain phthalates that are easily extracted during laboratory operations. Cross-contamination of clean glassware can occur when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Minimize interferences from phthalates by avoiding use of plastics. Exhaustive cleanup of reagents and glassware may be required to eliminate phthalate contamination.[3,4] Phthalate ester interference can be avoided by using a microcoulometric or electrolytic conductivity detector.

c. Detection levels: The method detection level (MDL) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[5] The MDL concentrations listed in Table 6630:III were obtained with reagent water.[6] Similar results were achieved with representative wastewaters. The MDL actually obtained in a given analysis will vary, depending on instrument sensitivity and matrix effects. This method has been tested for linearity of known-addition recovery from reagent water and is applicable over the concentration range from 4 × MDL to 1000 × MDL with the following exceptions: Chlordane recovery at 4 × MDL was low (60%); toxaphene recovery was linear over the range of 10 × MDL to 1000 × MDL.[6] It is difficult to determine MDLs for mixtures such as these. To calculate the MDLs given, a few of the GC peaks in each mixture were used. Depending on the particular peaks selected, these results may or may not be reproducible in other laboratories.

d. Safety: The toxicity or carcinogenicity of each reagent has not been defined precisely. The following compounds have been classified tentatively as known or suspected, human or mamma-

TABLE 6630:III. CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LEVELS*

Compound	Retention Time *min*		Method Detection Level *μg/L*
	Column 1	Column 2	
α-BHC	1.35	1.82	0.003
γ-BHC	1.70	2.13	nd
β-BHC	1.90	1.97	nd
Heptachlor	2.00	3.35	0.003
δ-BHC	2.15	2.20	0.009
Aldrin	2.40	4.10	0.004
Heptachlor epoxide	3.50	5.00	0.083
Endosulfan I	4.50	6.20	0.014
4,4′-DDE	5.13	7.15	0.004
Dieldrin	5.45	7.23	0.002
Endrin	6.55	8.10	0.006
4,4′-DDD	7.83	9.08	0.011
Endosulfan II	8.00	8.28	0.004
4,4′-DDT	9.40	11.75	0.012
Endrin aldehyde	11.82	9.30	0.023
Endosulfan sulfate	14.22	10.70	0.066
Chlordane	mr	mr	0.014
Toxaphene	mr	mr	0.24
PCB-1016	mr	mr	nd
PCB-1221	mr	mr	nd
PCB-1232	mr	mr	nd
PCB-1242	mr	mr	0.065
PCB-1248	mr	mr	nd
PCB-1254	mr	mr	nd
PCB-1260	mr	mr	nd

Column 1 conditions: Supelcoport (100/120 mesh) coated with 1.5% SP-2250/1.95% SP-2401 packed in a 1.8 m long × 4 mm ID glass column with 5% methane/95% argon carrier gas at 60 mL/min flow rate. Column temperature held isothermal at 200°C, except for PCB-1016 through PCB-1248 at 160°C.

Column 2 conditions: Supelcoport (100/120 mesh) coated with 3% OV-1 packed in a 1.8 m long × 4 mm ID glass column with 5% methane/95% argon carrier gas at 60 mL/min flow rate. Column temperature held isothermal at 200°C for the pesticides; at 140°C for PCB-1221 and 1232; and at 170°C for PCB-1016 and 1242 to 1268.

*mr = multiple peak response. See Figures 6630:2 through 10.
nd = not determined.

* Aldrin, α-BHC, β-BHC, δ-BHC, γ-BHC, chlordane, 4,4′-DDD, 4,4′-DDE, 4,4′-DDT, dieldrin, endosulfan I, endosulfan II, endosulfan sulfate, endrin, endrin aldehyde, heptachlor, heptachlor epoxide, toxaphene, PCB-1016, PCB-1221, PCB-1232, PCB-1242, PCB-1248, PCB-1254, PCB-1260.

† The PCBs constitute a class of 209 compounds. This procedure is designed to determine nine commercial formulations known as the Aroclors, each of which is a mixture of PCBs.

lian carcinogens: 4,4′-DDT, 4,4′-DDD, the BHCs, and the PCBs. Prepare primary standards of these compounds in a hood and wear a NIOSH/MESA-approved toxic gas respirator when handling high concentrations. Treat and dispose of Hg used for sulfur removal as a hazardous waste.

2. Sampling and Storage

For collection and storage requirements, see Section 6410B.2. If samples will not be extracted within 72 h of collection, adjust pH to the range 5.0 to 9.0 with NaOH or H_2SO_4. Record volume of acid or base used. If aldrin is to be determined, add sodium thiosulfate when residual chlorine is present.

3. Apparatus

Use apparatus specified in Section 6410B.3*a–e*, *g*, and *i–k*.

In addition:

a. Chromatographic column, 400 mm long × 22 mm ID, with TFE stopcock and coarse frit filter disk.‡

b. Gas chromatograph:§ An analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, and strip-chart recorder. Preferably use a data system for measuring peak areas. See column specifications in 6630B.2*j* for both packed columns and fused silica capillary columns.

1) *Column 1*, 1.8 m long × 4 mm ID, glass, packed with 1.5% SP-2250/1.95% SP-2401 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the detection level and precision and bias data presented herein. For guidelines for the use of alternate column packings see ¶ 5*c*.

Although procedures detailed below refer primarily to packed columns, capillary columns may be used if equivalent results can be demonstrated.

2) *Column 2*, 1.8 m long × 4 mm ID, glass, packed with 3% OV-1 on Supelcoport (100/120 mesh) or equivalent.

3) *Detector*, electron-capture. This detector was used to develop the detection level and precision and bias data presented herein. For use of alternate detectors see ¶ 5*c*.

4. Reagents

This method requires reagents described in Section 6410B.4*a–e*, and in addition:

a. Acetone, hexane, isooctane, methylene chloride, pesticide quality or equivalent.

b. Ethyl ether, nanograde redistilled in glass if necessary. Demonstrate before use freedom from peroxides by means of test strips.‖ Remove peroxides by procedures provided with the test strips. After cleanup, add 20 mL ethyl alcohol preservative per liter of ether.

c. Magnesia-silica gel,# 60/100 mesh. Purchase activated at 1250°F and store in the dark in glass containers with ground-glass stoppers or foil-lined screw caps. Before use, activate each batch for at least 16 h at 130°C in a foil-covered glass container; let cool.

d. Mercury, triple-distilled.

e. Copper powder, activated.

f. Stock standard solutions: Prepare as directed in Section 6410B.4*g*, using isooctane as the solvent.

g. Calibration standards: See Section 6420B.4*j*. Dilute with isooctane and use MDL values from Table 6630:III.

h. Quality control (QC) check sample concentrate: Obtain a check sample concentrate containing each compound at the following concentrations in acetone: 4,4′-DDD, 10 μg/mL; 4,4′-DDT, 10 μg/mL; endosulfan II, 10 μg/mL; endosulfan sulfate, 10 μg/mL; endrin, 10 μg/mL; any other single-component pesticide, 2 μg/mL. If this method will be used only to analyze for PCBs, chlordane, or toxaphene, the QC check sample concentrate should contain the most representative multicomponent compound at a concentration of 50 μg/mL in acetone. If such a sample is not available from an external source, prepare using stock standards prepared independently from those used for calibration.

5. Procedure

a. Extraction: Mark water meniscus on side of sample bottle for later determination of volume. Pour entire sample into a 2-L separatory funnel or a 1- or 2-L continuous liquid-liquid extractor for a period of 18 h. Extract with methylene chloride as directed in Section 6410B.5*a*1), without any pH adjustment or solvent wash.

After extracting and concentrating with a three-ball Snyder column, increase temperature of hot water bath to about 80°C. Momentarily remove Snyder column, add 50 mL hexane and a new boiling chip, and reattach Snyder column. Concentrate extract as before but use hexane to prewet column. Complete concentration in 5 to 10 min.

Remove Snyder column and rinse flask and its lower joint into the concentrator tube with 1 to 2 mL hexane. Preferably use a 5-mL syringe for this operation. Stopper concentrator tube and store refrigerated if further processing will not be done immediately. If extract is to be stored longer than 2 d, transfer to a TFE-sealed screw-cap vial. If extract requires no further cleanup, proceed with gas chromatographic analysis. If further cleanup is required, follow procedure of ¶ *b* before chromatographic analysis.

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

b. Cleanup and separation: Use either procedure below or any other appropriate procedure; however, first demonstrate that the requirements of ¶ 7 can be met. The magnesia-silica gel column allows for a select fractionation of compounds and eliminates polar interferences. Elemental sulfur, which interferes with the electron-capture gas chromatography of certain pesticides, can be removed by the technique described below.

1) Magnesia-silica gel column cleanup—Place a weight of magnesia-silica gel (nominally 20 g) predetermined by calibration, ¶ *d*3), into a chromatographic column. Tap column to settle

‡ Kontes K-42054 or equivalent.

§ Gas chromatographic methods are extremely sensitive to the materials used. Mention of trade names by *Standard Methods* does not preclude the use of other existing or as-yet-undeveloped products that give *demonstrably* equivalent results.

‖ E. Merck, EM Science Quant or equivalent.

#Florisil or equivalent.

gel and add 1 to 2 cm anhydrous Na_2SO_4 to the top. Add 60 mL hexane to wet and rinse. Just before exposure of the Na_2SO_4 layer to air, stop elution of hexane by closing stopcock on column. Discard eluate. Adjust sample extract volume to 10 mL with hexane and transfer it from K-D concentrator tube onto column. Rinse tube twice with 1 to 2 mL hexane, adding each rinse to the column. Place a 500-mL K-D flask and clean concentrator tube under chromatographic column. Drain column into flask until Na_2SO_4 layer is nearly exposed. Elute column with 200 mL 6% ethyl ether in hexane (v/v) (Fraction 1) at a rate of about 5 mL/min. Remove K-D flask and set aside. Elute column again, using 200 mL 15% ethyl ether in hexane (v/v) (Fraction 2), into a second K-D flask. Elute a third time using 200 mL 50% ethyl ether in hexane (v/v) (Fraction 3). The elution patterns for the pesticides and PCBs are shown in Table 6630:IV. Concentrate fractions for 15 to 20 min as in ¶ *a*, using hexane to prewet the column, and set water bath temperature at about 85°C. After cooling, remove Snyder column and rinse flask and its lower joint into concentrator tube with hexane. Adjust volume of each fraction to 10 mL with hexane and analyze by gas chromatography, ¶s *c* through *e* below.

2) Sulfur interference removal—Elemental sulfur usually will elute entirely in Fraction 1 of the magnesia-silica gel column cleanup. To remove sulfur interference from this fraction or the original extract, pipet 1.00 mL concentrated extract into a clean

TABLE 6630:IV. DISTRIBUTION OF CHLORINATED PESTICIDES AND PCBS INTO MAGNESIA-SILICA GEL COLUMN FRACTIONS[5]

Compound	Recovery by Fraction* %		
	1	2	3
Aldrin	100	—	—
α-BHC	100	—	—
β-BHC	97	—	—
δ-BHC	98	—	—
γ-BHC	100	—	—
Chlordane	100	—	—
4,4′-DDD	99	—	—
4,4′-DDE	98	—	—
4,4′-DDT	100	—	—
Dieldrin	0	100	—
Endosulfan I	37	64	—
Endosulfan II	0	7	91
Endosulfan sulfate	0	0	106
Endrin	4	96	—
Endrin aldehyde	0	68	26
Heptachlor	100	—	—
Heptachlor epoxide	100	—	—
Toxaphene	96	—	—
PCB-1016	97	—	—
PCB-1221	97	—	—
PCB-1232	95	4	—
PCB-1242	97	—	—
PCB-1248	103	—	—
PCB-1254	90	—	—
PCB-1260	95	—	—

* Eluent composition:
Fraction 1–6% ethyl ether in hexane
Fraction 2–15% ethyl ether in hexane
Fraction 3–50% ethyl ether in hexane

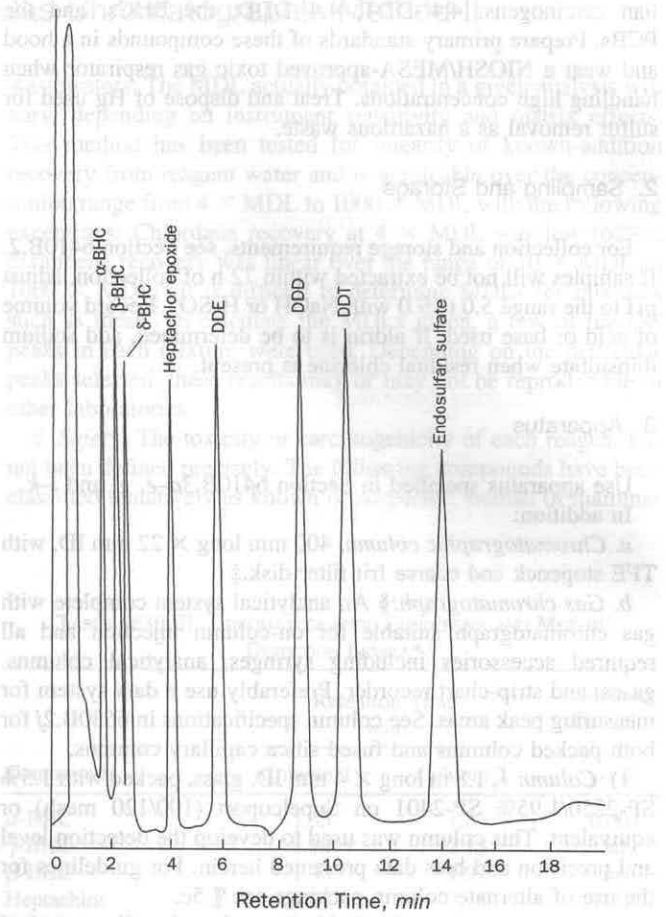

Figure 6630:6. Gas chromatogram of pesticides. Column: 1.5% SP-2250/1.95% SP-2401 on Supelcoport; temperature: 200°C; detector: electron capture.

concentrator tube or TFE-sealed vial. Add 1 to 3 drops of mercury and seal.[7] Mix for 15 to 30 s. If prolonged shaking (2 h) is required, use a reciprocal shaker. Alternatively, use activated copper powder for sulfur removal.[8] Analyze by gas chromatography.

c. Gas chromatography operating conditions: Table 6630:III summarizes the recommended operating conditions for the gas chromatograph and gives retention times and MDLs that can be achieved under these conditions. Examples of separations obtained with Column 1 are shown in Figures 6630:6 to 15. Other packed or capillary (open-tubular) columns,[9] chromatographic conditions, or detectors may be used if the requirements of ¶ 7 are met.

d. Calibration: Calibrate system daily by either external or internal procedure. NOTE: For quantification and identification of mixtures such as PCBs, chlordane, and toxaphene, take extra precautions.[9–11]

1) External standard calibration procedure—Prepare standards as directed in ¶ 4*g* and follow procedure of Section 6420B.5*b*3). Tabulate data and obtain calibration curve or calibration factor as directed in Section 6200B.4*c*3).

2) Internal standard calibration procedure—Prepare standards as directed in ¶ 4*g* and follow procedure of Section 6420B.5*b*3).

Tabulate data and calculate response factors (RF) as directed in Section 6200B.4*c*2).

Verify working calibration curve, calibration factor, or RF on each working shift by measuring one or more calibration standards. If the response for any compound varies from the predicted response by more than ±15%, repeat test using a fresh calibration standard. Alternatively, prepare a new calibration curve for that compound.

3) Magnesia-silica gel standardization—Gel from different batches or sources may vary in adsorptive capacity. To standardize the amount used, use the lauric acid value[12], which measures the adsorption from a hexane solution of lauric acid (mg/g gel). Determine the amount to be used for each column by dividing 110 by this ratio and multiplying the quotient by 20 g.

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

e. Sample analysis: See Section 6420B.5*b*3). If peak response cannot be measured because of interferences, further cleanup is required.

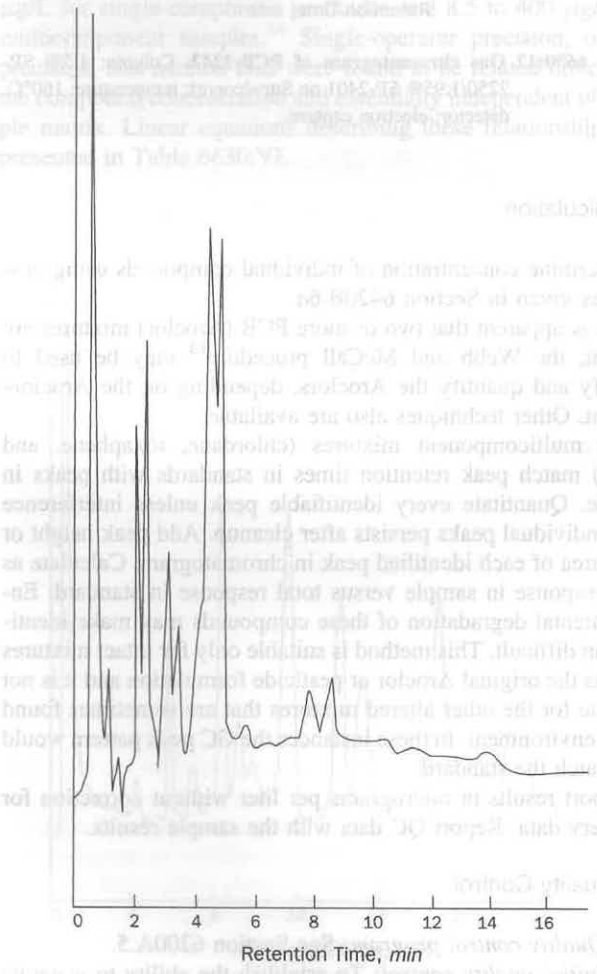

Figure 6630:7. Gas chromatogram of chlordane. Column: 1.5% SP-2250/1.95% SP-2401 on Supelcoport; temperature: 200°C; detector: electron capture.

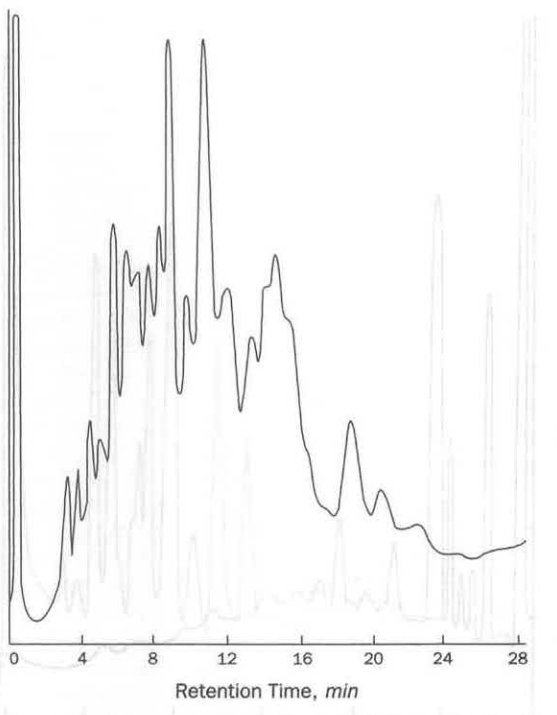

Figure 6630:8. Gas chromatogram of toxaphene. Column: 1.5% SP-2250/1.95% SP-2401 on Supelcoport; temperature: 200°C; detector: electron capture.

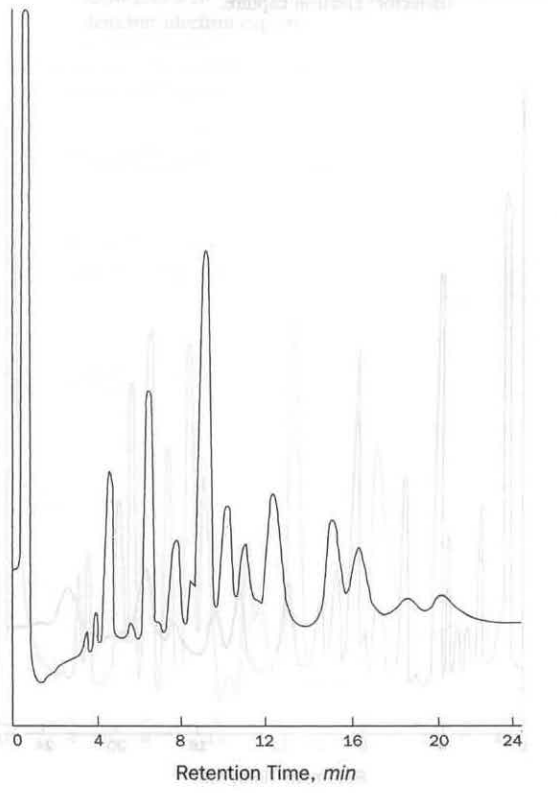

Figure 6630:9. Gas chromatogram of PCB-1016. Column: 1.5% SP-2250/1.95% SP-2401 on Supelcoport; temperature: 160°C; detector: electron capture.

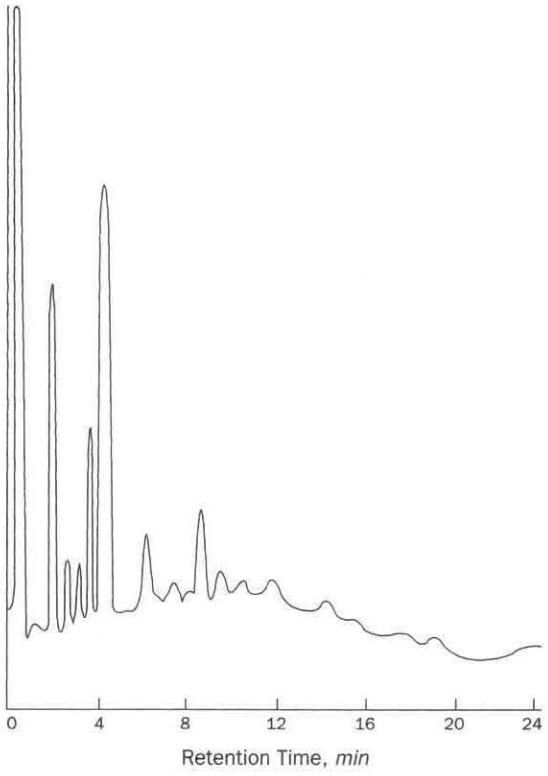

Figure 6630:10. Gas chromatogram of PCB-1221. Column: 1.5% SP-2250/1.95% SP-2401 on Supelcoport; temperature: 160°C; detector: electron capture.

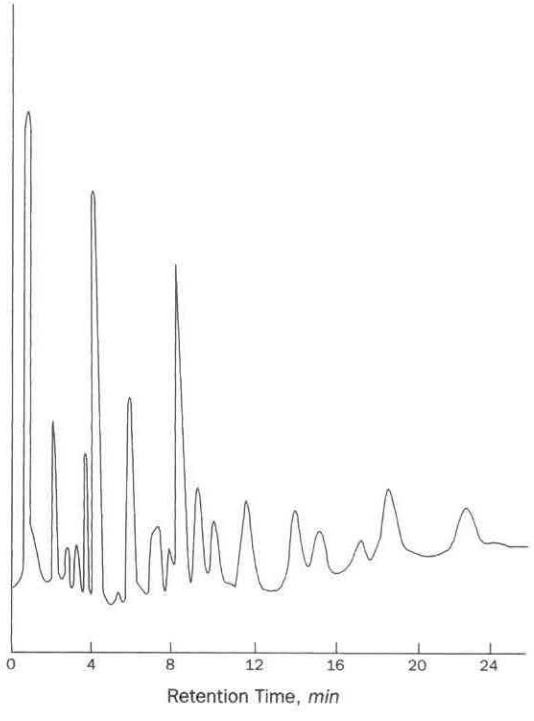

Figure 6630:11. Gas chromatogram of PCB-1232. Column: 1.5% SP-2250/1.95% SP-2401 on Supelcoport; temperature: 160°C; detector: electron capture.

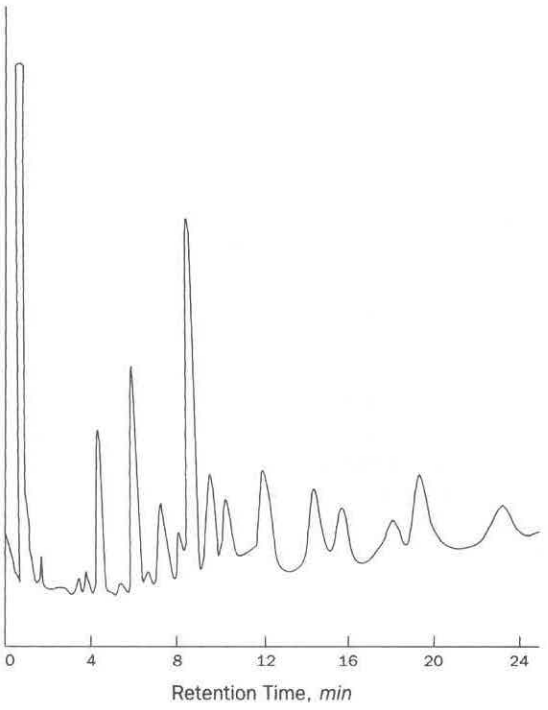

Figure 6630:12. Gas chromatogram of PCB-1242. Column: 1.5% SP-2250/1.95% SP-2401 on Supelcoport; temperature: 160°C; detector: electron capture.

6. Calculation

Determine concentration of individual compounds using procedures given in Section 6420B.6*a*.

If it is apparent that two or more PCB (Aroclor) mixtures are present, the Webb and McCall procedure[13] may be used to identify and quantify the Aroclors, depending on the Aroclors present. Other techniques also are available.

For multicomponent mixtures (chlordane, toxaphene, and PCBs) match peak retention times in standards with peaks in sample. Quantitate every identifiable peak unless interference with individual peaks persists after cleanup. Add peak height or peak area of each identified peak in chromatogram. Calculate as total response in sample versus total response in standard. Environmental degradation of these compounds may make identification difficult. This method is suitable only for intact mixtures such as the original Aroclor or pesticide formulation and it is not suitable for the other altered mixtures that are sometimes found in the environment. In these instances the GC peak pattern would not match the standard.

Report results in micrograms per liter without correction for recovery data. Report QC data with the sample results.

7. Quality Control

a. Quality control program: See Section 6200A.5.

b. Initial quality control: To establish the ability to generate data with acceptable precision and bias, proceed as follows: Using a pipet, prepare QC check samples at test concentrations shown in Table 6630:V by adding 1.00 mL of QC check sample

concentrate (¶ 4*h*) to each of four 1-L portions of reagent water. Analyze QC check samples according to the method beginning in ¶ 5*a*. Calculate average recovery and standard deviation of the recovery, compare with acceptance criteria and evaluate and correct system performance as directed in Section 6200A.5*a*1) and 2).

c. Analyses of samples with known additions: See Section 6420B.7*c*. Prepare QC check sample concentrates according to ¶ 4*h* and use Tables 6630:III and IV.

d. Quality-control check standard analysis: See Section 6420B.7*d*. Prepare QC check standard according to ¶ 4*h* and use Table 6630:V. If all compounds in Table 6630:V are to be measured in the sample in ¶ *c* above, it is probable that the analysis of a QC check will be required; therefore, routinely analyze the QC check standard with the known-addition sample.

e. Bias assessment and records: See Section 6410B.7*e*.

8. Precision and Bias

This method was tested by 20 laboratories using reagent water, drinking water, surface water, and industrial wastewaters with known additions at six concentrations over the range 0.5 to 30 μg/L for single-component pesticides and 8.5 to 400 μg/L for multicomponent samples.[14] Single-operator precision, overall precision, and method bias were found to be related directly to the compound concentration and essentially independent of sample matrix. Linear equations describing these relationships are presented in Table 6630:VI.

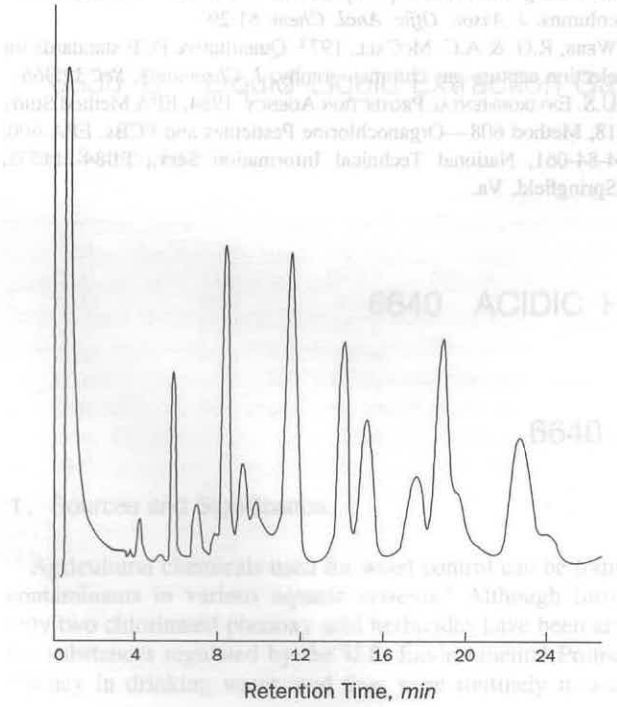

Figure 6630:13. Gas chromatogram of PCB-1248. Column: 1.5% SP-2250/1.95% SP-2401 on Supelcoport; temperature: 160°C; detector: electron capture.

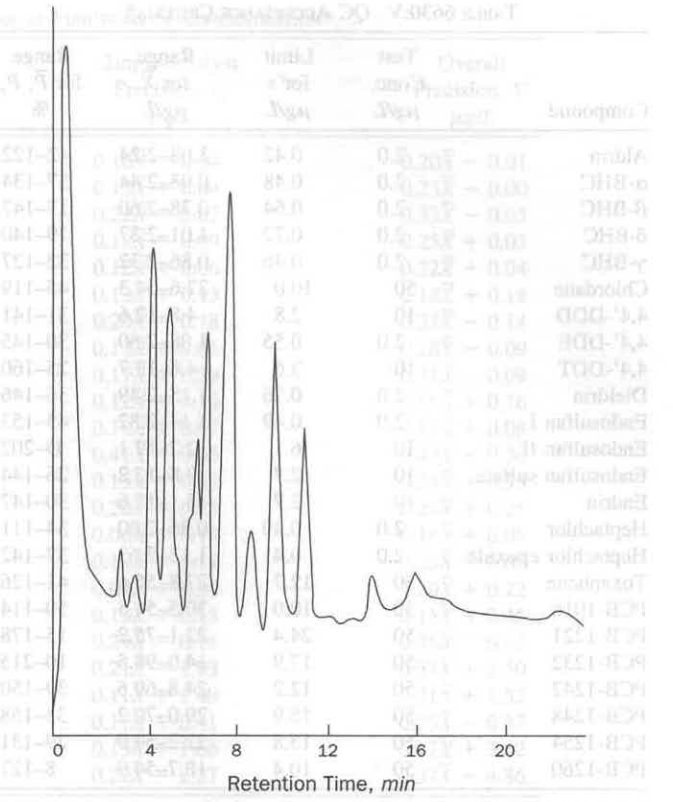

Figure 6630:14. Gas chromatogram of PCB-1254. Column: 1.5% SP-2550/1.95% SP-2401 on Supelcoport; temperature: 200°C; detector: electron capture.

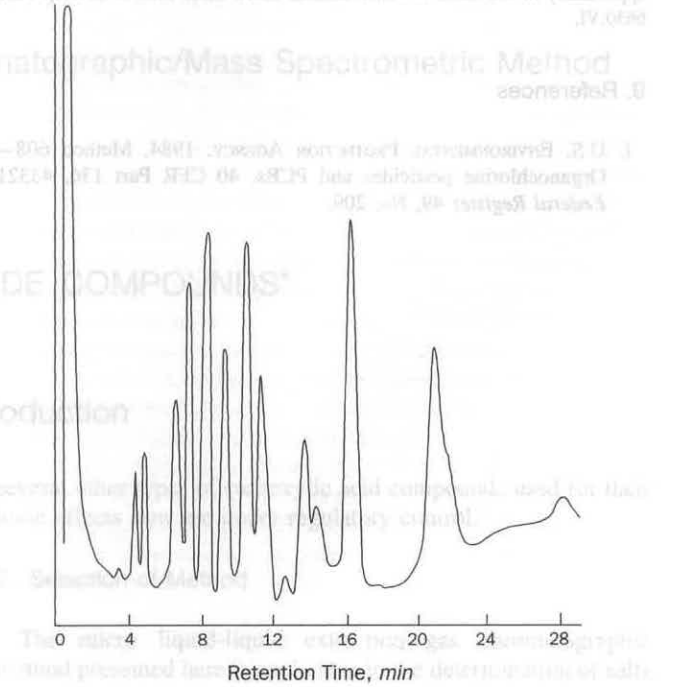

Figure 6630:15. Gas chromatogram of PCB-1260. Column: 1.5% SP-2250/1.95% SP-2401 on Supelcoport; temperature: 200°C; detector: electron capture.

TABLE 6630:V. QC ACCEPTANCE CRITERIA*

Compound	Test Conc. µg/L	Limit for s µg/L	Range for $\bar{X}$ µg/L	Range for $\bar{P}$, P_s %
Aldrin	2.0	0.42	1.08–2.24	42–122
α-BHC	2.0	0.48	0.98–2.44	37–134
β-BHC	2.0	0.64	0.78–2.60	17–147
δ-BHC	2.0	0.72	1.01–2.37	19–140
γ-BHC	2.0	0.46	0.86–2.32	32–127
Chlordane	50	10.0	27.6–54.3	45–119
4,4′-DDD	10	2.8	4.8–12.6	31–141
4,4′-DDE	2.0	0.55	1.08–2.60	30–145
4,4′-DDT	10	3.6	4.6–13.7	25–160
Dieldrin	2.0	0.76	1.15–2.49	36–146
Endosulfan I	2.0	0.49	1.14–2.82	45–153
Endosulfan II	10	6.1	2.2–17.1	D–202
Endosulfan sulfate	10	2.7	3.8–13.2	26–144
Endrin	10	3.7	5.1–12.6	30–147
Heptachlor	2.0	0.40	0.86–2.00	34–111
Heptachlor epoxide	2.0	0.41	1.13–2.63	37–142
Toxaphene	50	12.7	27.8–55.6	41–126
PCB-1016	50	10.0	30.5–51.5	50–114
PCB-1221	50	24.4	22.1–75.2	15–178
PCB-1232	50	17.9	14.0–98.5	10–215
PCB-1242	50	12.2	24.8–69.6	39–150
PCB-1248	50	15.9	29.0–70.2	38–158
PCB-1254	50	13.8	22.2–57.9	29–131
PCB-1260	50	10.4	18.7–54.9	8–127

*s = standard deviation of four recovery measurements,
$\bar{X}$ = average recovery for four recovery measurements,
$\bar{P}$, P_s = percent recovery measured, and
D = detected; result must be greater than zero.

NOTE: These criteria are based directly on the method performance data in Table 6630:VI. Where necessary, the limits for recovery were broadened to assure applicability of the limits to concentrations below those used to develop Table 6630:VI.

9. References

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Method 608—Organochlorine pesticides and PCBs. 40 CFR Part 136, 43321; *Federal Register* 49, No. 209.
2. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1982. Determination of pesticides and PCBs in industrial and municipal wastewaters. EPA-600/4-82-023, National Technical Information Serv., PB82-214222, Springfield, Va.
3. GIAM, C.S., H.S. CHAN & G.S. NEF. 1975. Sensitive method for determination of phthalate ester plasticizers in open-ocean biota samples. *Anal. Chem.* 47:2225.
4. GIAM, C.S. & H.S. CHAN. 1976. Control of Blanks in the Analysis of Phthalates in Air and Ocean Biota Samples. U.S. National Bur. Standards, Spec. Publ. 442.
5. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1995. Definition and procedure for the determination of the method detection limit. 40 CFR Part 136, Appendix B. *Federal Register* 49, No. 209.
6. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1980. Method Detection Limit and Analytical Curve Studies, EPA Methods 606, 607, and 608. Special letter rep. for EPA Contract 68-03-2606, Environmental Monitoring and Support Lab., Cincinnati, Ohio.
7. GOERLITZ, D.F. & L.M. LAW. 1971. Note on removal of sulfur interferences from sediment extracts for pesticide analysis. *Bull. Environ. Contam. Toxicol.* 6:9.
8. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1980. Manual of Analytical Methods for the Analysis of Pesticides in Human and Environmental Samples. EPA-600/8-80-038, Health Effects Research Lab., Research Triangle Park, N.C.
9. ALFORD-STEVENS, A, et al. 1986. Characterization of commercial Aroclors by automated mass spectrometric determination of polychlorinated biphenyls by level of chlorination. *Anal. Chem.* 58: 2014.
10. ALFORD-STEVENS, A. 1986. Analyzing PCB's. *Environ. Sci. Technol.* 20:1194.
11. ALFORD-STEVENS, A. 1987. Mixture analytes. *Environ. Sci. Technol.* 21: 137.
12. MILLS, P.A. 1968. Variation of florisil activity: Simple method for measuring absorbent capacity and its use in standardizing florisil columns. *J. Assoc. Offic. Anal. Chem.* 51:29.
13. WEBB, R.G. & A.C. MCCALL. 1973. Quantitative PCB standards for election capture gas chromatography. *J. Chromatog. Sci.* 11:366.
14. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. EPA Method Study 18, Method 608—Organochlorine Pesticides and PCBs. EPA-600/4-84-061, National Technical Information Serv., PB84-211358, Springfield, Va.

TABLE 6630:VI. METHOD PRECISION AND BIAS AS FUNCTIONS OF CONCENTRATION*

Compound	Bias, as Recovery, X' $\mu g/L$	Single-Analyst Precision, s_r $\mu g/L$	Overall Precision, S' $\mu g/L$
Aldrin	$0.81C + 0.04$	$0.16\bar{X} - 0.04$	$0.20\bar{X} - 0.01$
α-BHC	$0.84C + 0.03$	$0.13\bar{X} + 0.04$	$0.23\bar{X} - 0.00$
β-BHC	$0.81C + 0.07$	$0.22\bar{X} - 0.02$	$0.33\bar{X} - 0.05$
δ-BHC	$0.81C + 0.07$	$0.18\bar{X} + 0.09$	$0.25\bar{X} + 0.03$
γ-BHC	$0.82C - 0.05$	$0.12\bar{X} + 0.06$	$0.22\bar{X} + 0.04$
Chlordane	$0.82C - 0.04$	$0.13\bar{X} + 0.13$	$0.18\bar{X} + 0.18$
4,4′-DDD	$0.84C + 0.30$	$0.20\bar{X} - 0.18$	$0.27\bar{X} - 0.14$
4,4′-DDE	$0.85C + 0.14$	$0.13\bar{X} + 0.06$	$0.28\bar{X} - 0.09$
4,4′-DDT	$0.93C - 0.13$	$0.17\bar{X} + 0.39$	$0.31\bar{X} - 0.09$
Dieldrin	$0.90C + 0.02$	$0.12\bar{X} + 0.19$	$0.16\bar{X} + 0.16$
Endosulfan I	$0.97C + 0.04$	$0.10\bar{X} + 0.07$	$0.18\bar{X} + 0.08$
Endosulfan II	$0.93C + 0.34$	$0.41\bar{X} - 0.65$	$0.47\bar{X} - 0.20$
Endosulfan sulfate	$0.89C - 0.37$	$0.13\bar{X} + 0.33$	$0.24\bar{X} + 0.35$
Endrin	$0.89C - 0.04$	$0.20\bar{X} + 0.25$	$0.24\bar{X} + 0.25$
Heptachlor	$0.69C + 0.04$	$0.06\bar{X} + 0.13$	$0.16\bar{X} + 0.08$
Heptachlor epoxide	$0.89C + 0.10$	$0.18\bar{X} - 0.11$	$0.25\bar{X} - 0.08$
Toxaphene	$0.80C + 1.74$	$0.09\bar{X} + 3.20$	$0.20\bar{X} + 0.22$
PCB-1016	$0.81C + 0.50$	$0.13\bar{X} + 0.15$	$0.15\bar{X} + 0.45$
PCB-1221	$0.96C + 0.65$	$0.29\bar{X} - 0.76$	$0.35\bar{X} - 0.62$
PCB-1232	$0.91C + 10.79$	$0.21\bar{X} - 1.93$	$0.31\bar{X} + 3.50$
PCB-1242	$0.93C + 0.70$	$0.11\bar{X} + 1.40$	$0.21\bar{X} + 1.52$
PCB-1248	$0.97C + 1.06$	$0.17\bar{X} + 0.41$	$0.25\bar{X} - 0.37$
PCB-1254	$0.76C + 2.07$	$0.15\bar{X} + 1.66$	$0.17\bar{X} + 3.62$
PCB-1260	$0.66C + 3.76$	$0.22\bar{X} - 2.37$	$0.37\bar{X} - 4.86$

* X' = expected recovery for one or more measurements of a sample containing a concentration of C,
s_r = expected single-analyst standard deviation of measurements at an average concentration found of $\bar{X}$,
S' = expected interlaboratory standard deviation of measurements at an average concentration found of $\bar{X}$,
C = true value for the concentration, and
$\bar{X}$ = average recovery found for measurements of samples containing a concentration of C.

6630 D. Liquid-Liquid Extraction Gas Chromatographic/Mass Spectrometric Method

See Section 6410B.

6640 ACIDIC HERBICIDE COMPOUNDS*

6640 A. Introduction

1. Sources and Significance

Agricultural chemicals used for weed control can be found as contaminants in various aquatic systems.[1] Although formerly only two chlorinated phenoxy acid herbicides have been among the substances regulated by the U.S. Environmental Protection Agency in drinking water, and thus were routinely measured, several other types of carboxylic acid compounds used for their toxic effects now are under regulatory control.[2]

2. Selection of Method

The micro liquid-liquid extraction gas chromatographic method presented here is applicable to the determination of salts and esters of analyte acids. The form of each acid is not routinely distinguished by this method. Results are calculated and reported for each listed compound as the total free acid. Since only the free acid is quantified, the herbicide Lactofen will be quantified

* Approved by Standard Methods Committee, 2001.
Joint Task Group: Russell Chinn (chair), Wenta Liao, David J. Munch, Theresa A. Prato.

as Acifluorfen because their structures represent different esters of the same acid herbicide. Additionally, this method is unable to quantify the degradation products of Dacthal separately from the parent compound unless the optional wash step is performed. If this wash step is not performed, all forms of Dacthal (the parent compound and the mono- and di-acid degradation products) will be quantified as Dacthal.

3. Sampling and Storage

Collect grab samples in accordance with conventional sampling practices (see Section 1060B), using amber glass containers with polytetrafluoroethylene (PFTE) lined screw caps and capacities of at least 40 mL. Add sodium sulfite crystals (approximately 2 mg/40 mL sample) if residual chlorine is present in the sample. Fill sample bottles but take care not to flush out the sodium sulfite. Because the target analytes of this method are not volatile, it is not necessary to ensure that the sample bottles are completely headspace-free. Ice samples during shipment so that the temperature does not exceed 10°C during the first 48 h after collection. Confirm that samples are at or below 10°C when they are received at the laboratory. Store samples in the laboratory at or below 6°C and protect from light until extraction. Do not freeze samples.

Because of the several pH adjustments made to the samples in the course of this method, the addition of organic or inorganic biocides, including hydrochloric acid, has been omitted. The analyst should be aware of the potential for the biological degradation of the analytes. Extract samples as soon as possible and within no more than 14 d.

4. References

1. U.S. Environmental Protection Agency. 1990. National Pesticide Survey: Summary Results of EPA's National Survey of Pesticides in Drinking Water Wells. EPA 57019-90-015, U.S. Environmental Protection Agency, Cincinnati, Ohio.
2. U.S. Environmental Protection Agency. 1992. National Primary Drinking Water Regulations; Synthetic Organic Chemicals and Inorganic Chemicals; Final Rule. 40 CFR Parts 141 and 142, Part 111; *Federal Register* 57, No.138.

6640 B. Micro Liquid-Liquid Extraction Gas Chromatographic Method

1. General Discussion

a. Application: This is a gas chromatography (GC) method for the determination of chlorinated acids in drinking water. It can be used to measure various acidic organic compounds and their corresponding acid salts, although the form of each acid is not differentiated and the calculated amount of each is expressed as free acid. Accuracy, precision, and detection level data have been generated in reagent water and finished ground and surface waters for compounds listed in Table 6640:I. Also see 6640A.2.

b. Principle: A 40-mL sample is adjusted to pH $\geq$ 12 with 4*N* sodium hydroxide and is kept for 1 h at room temperature to hydrolyze derivatives. Note: Because the chlorphenoxy acid herbicides are formulated as a variety of esters and salts, the hydrolysis step is required and may not be skipped. After hydrolysis, an optional wash step using a 90:10 hexane:methyl *tert*-butyl ether (MtBE) mixture may be performed as a sample cleanup and to remove Dacthal. It is only necessary to perform this wash if the quantitation of the Dacthal degradation products is desired. The aqueous sample then is acidified with sulfuric acid to pH $\leq$ 1 and extracted with 4 mL of MtBE that contains the internal standard. The chlorinated acids, which have been partitioned into the MtBE, then are converted to methyl esters by derivatization with diazomethane. The target esters are separated and detected by capillary column gas chromatography using an electron capture detector (GC/ECD). Analytes are quantified using an internal-standard-based calibration curve.

c. Interferences: Clean all glassware meticulously by washing with detergent and tap water, rinsing with tap water, and rinsing with reagent water. A final rinse with solvents may be needed. In place of a solvent rinse, heat nonvolumetric glassware in a muffle furnace at 400°C for 2 h. Do not heat volumetric glassware in an oven above 120°C. Store glassware inverted or capped with aluminum foil. Method interferences may be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other sample-processing hardware that lead to discrete artifacts and/or elevated base lines in the chromatograms. Interferences by phthalate esters can pose a

Table 6640:I. Single-Laboratory Method Detection Levels in Reagent Water

Compound*	Fortification Level µg/L	Primary Column MDL µg/L	Secondary Column MDL µg/L
Dalapon	0.100	0.05	0.07
3,5-Dichlorobenzoic acid	0.050	0.21	0.05
Dicamba	0.050	0.03	0.04
Dichlorprop	0.100	0.43	0.12
2,4-D	0.100	0.06	0.07
Pentachlorophenol	0.010	0.01	0.08
Silvex	0.025	0.03	0.02
2,4,5-T	0.025	0.02	0.03
2,4-DB	0.100	0.25	0.18
Chloramben	0.050	0.06	0.08
Dinoseb	0.100	0.17	0.08
Bentazon	0.100	0.07	0.19
Dacthal	0.050	0.11	0.11
Quinclorac	0.050	0.08	0.11
Picloram	0.050	0.08	0.05
Acifluorfen	0.250	0.31	0.09

* To assure exact identification of analytes, CAS numbers are listed as follows: Acifluorfen, 50594-66-6; Bentazon, 25057-89-0; Chloramben, 133-90-4; 2,4-D, 94-75-7; Dacthal, 1861-32-1; Dalapon, 75-99-0; 2,4-DB, 94-82-6; Dicamba, 1918-00-9; 3,5-Dichlorobenzoic acid, 51-36-5; Dichloroprop, 120-36-5; Dinoseb, 88-85-7; pentachlorophenol 87-86-5; Picloram, 1918-02-1; Quinclorac, 84087-01-4; Silvex, 93-72-1; 2,4,5-T, 93-76-5.

major problem in pesticide analysis when an electron capture detector (ECD) is used. These compounds generally appear in the chromatogram as large peaks. Common flexible plastics contain varying amounts of phthalates that are easily extracted or leached during laboratory operations. Cross-contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are present. Interferences from phthalates can best be minimized by avoiding the use of plastics in the laboratory. Exhaustive purification of reagents and glassware may be required to eliminate background phthalate contamination.[1,2] Routinely demonstrate that all sources of reagents are free from interferences, i.e., less than one-third the method reporting limit (MRL) for each target analyte, under the conditions of the analysis, by analyzing laboratory reagent blanks. Do not subtract blank values from sample results.

Use a grade of sodium sulfate suitable for pesticide residue analysis. If the suitability of the available sodium sulfate is in question, extract and analyze a laboratory reagent blank to test for interferences before sample processing. Matrix interferences may be caused by contaminants that are extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the water sampled. Also, contaminants in local ground water and surface water may interfere with the analysis of Dalapon on the primary column. A slow initial temperature program can ensure separation of the interferent from Dalapon. Confirm analyte identifications with the confirmation column specified, another column that is dissimilar to the primary column, or by GC/MS if the concentrations are high enough.

d. Method detection level (MDL): Method detection levels are compound-, instrument-, and matrix-dependent. The MDL is defined as the statistically calculated minimum amount that can be measured with 99% confidence that the reported value is greater than zero.[3] Experimentally determined MDLs for the analytes are provided in Table 6640:I. The MDL differs from, and is usually lower than (but never above), the minimum reporting level (MRL). The concentration range for target analytes in this method was evaluated between 0.5 μg/L and 20 μg/L for a 40-mL sample.

e. Safety: See Section 1090. The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. Regard each chemical compound as a potential health hazard and minimize exposure to these chemicals. Maintain in the laboratory a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of MSDSs also should be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available.[4–6]

The toxicity of the extraction solvent methyl *t*-butyl ether (MtBE) has not been well defined. Susceptible individuals may experience adverse affects upon skin contact or inhalation of vapors. Therefore, use protective clothing and gloves, and use MtBE only in a chemical fume hood or glove box. The same precaution applies to pure standard materials.

Diazomethane is a toxic carcinogen that can explode under certain conditions. Use the diazomethane generator behind a safety shield in a well-ventilated fume hood. Never allow the generator to be heated above 90°C and avoid all grinding surfaces such as ground-glass joints, sleeve bearings, and glass stirrers. To minimize safety hazards, the diazomethane generator apparatus used in the esterification procedure produces micromolar amounts of diazomethane in solution. If the procedure is followed carefully, no possibility for explosion exists.

Although hydrogen can be safely used as a carrier gas, the potential for fire or explosion does exist if the gas system is mishandled. If unsure of the safety guidelines for using hydrogen as a carrier gas, seek advice from the instrument manufacturer.

2. Apparatus

a. Sample containers, amber glass bottles with a minimum volume of 40 mL, fitted with PTFE-lined screw caps.

b. Extraction vials, 60-mL clear glass vials with PTFE-lined screw caps.

c. Autosampler vials, 2.0-mL vials with screw or crimp cap and a PTFE-faced seal.

d. Standard solution storage containers, 10- to 20-mL amber glass vials with PTFE-lined screw caps.

e. Clear vials, 7-mL glass, disposable, with PTFE-lined screw caps for extract drying and derivatization.

f. Pasteur pipets, glass, disposable.

g. Pipets, Class A, 2.0-mL and 4.0-mL glass, or adjustable volume dispensers.

h. Volumetric flasks, Class A, suggested sizes 5 mL, 10 mL, and 100 mL.

i. Microsyringes, various sizes.

j. Balance, analytical, capable of weighing to the nearest 0.0001 g.

k. Diazomethane generator: See Figure 6251:3 for a diagram of an all-glass system custom-made for these validation studies. Micromolar generators also are available from commercial sources.* If a generator from a commercial source is used, follow manufacturer's instructions.

l. Gas chromatograph, capillary GC.† Modifications should include a high-pressure (50 psi) split/splitless injector, fast temperature ramp oven (50°C/min) and a low-volume (150-μL) micro ECD detector. A data system capable of fast sampling (20 points/peak) also is required.

m. Primary GC column,‡ 180-μm ID, 40 m long, 0.2-μm film thickness, fused silica capillary with chemically bonded (14% cyanopropylphenymethylpolysiloxane), or equivalent bonded, fused silica column.

n. Confirmation GC column,§ 180-μm ID, 40 m long, 0.2-μm film thickness, fused silica capillary with chemically bonded (5% phenylmethylpolysiloxane), or equivalent bonded, fused silica column. Columns of other dimensions may be used as long as the QC criteria of the method are met.

3. Reagents

Use reagent-grade or better chemicals in all analyses. For reagents *b* through *f*, use high-purity HPLC-grade materials

* Aldrich Catalog, No. Z10, 889-8, or equivalent.
† Hewlett Packard Model 6890 or equivalent.
‡ DB-1701 or equivalent.
§ DB-5 or equivalent.

demonstrated to be free from analytes and interferences. Unless otherwise indicated, use reagents conforming to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first determined that the reagent is of sufficiently high purity to permit its use without lessening the quality of the determination.

Observe safety precautions; see ¶ 1*e*.

a. Reagent water: Use purified water that does not contain any measurable quantities of any target analytes or interfering compounds greater than 1/3 the MRL for each compound of interest.

b. Methyl tert*-butyl ether (MtBE).*

c. Acetone.

d. Carbitol (diethylene glycol monoethyl ether).

e. Hexane:MtBE wash solvent, 90:10 (v/v), unpreserved.

f. Hexane.

g. Sodium sulfate, Na_2SO_4, pesticide-grade, granular, anhydrous. If interferences are observed (see ¶ 1*c* above), it may be necessary to heat the sodium sulfate in a shallow tray at 400°C for up to 4 h to remove phthalates and other interfering organic substances. Alternatively, extract it with methylene chloride in a Soxhlet apparatus for 48 h. Store in a capped glass bottle rather than a plastic container.

h. Acidified sodium sulfate: Acidify by slurrying 100 g of muffled sodium sulfate with enough ethyl ether to just cover the solid. Add 0.5 mL conc sulfuric acid dropwise while mixing thoroughly. Remove ether under vacuum. Mix 1 g of resulting solid with 5 mL reagent water and measure pH of the mixture. The pH must be below 4. Store in a desiccator or at 100°C to keep reagent dry.

i. Copper II sulfate pentahydrate, $CuSO_4 \cdot 5H_20$.

j. Sodium hydroxide solution, NaOH, 4*N*: Dissolve 16 g NaOH pellets in reagent water and dilute to 100 mL.

k. Potassium hydroxide solution, KOH, 37% (w/v): Dissolve 37 g KOH pellets in reagent water and dilute to 100 mL.

l. Sodium sulfite, Na_2SO_3, for use as a dechlorinating agent.

m. N*-methyl-*N*-nitroso-*p*-toluenesulfonamide*‖ *(MNTS) solution:* Prepare a solution containing 5 g MNTS in 50 mL of a 50:50 (v/v) mixture of MtBE and carbitol. This solution is stable for 1 month or longer when stored at 4°C in an amber bottle with a PTFE-lined screw cap.

n. Sulfuric acid, H_2SO_4, conc.

o. Silica gel, 35 to 60 mesh.

p. Hydrogen, 99.999% pure or better, GC carrier gas.

q. Nitrogen, 99.999% pure or better, ECD make-up gas.

r. Internal standard solutions: 4,4′-Dibromooctafluorobiphenyl (99+%) is used as an internal standard for the method. This compound has been shown to be an effective internal standard for the method analytes, but other compounds may be used if the QC requirements are met. The solutions listed in 1) and 2) below are stable for at least 30 d. Replace solution if ongoing QC indicates a problem.

1) *Internal standard stock solution,* 2.0 mg/mL: Prepare an internal standard stock solution by accurately weighing approximately 0.0200 g of neat 4,4′- dibromooctafluorobiphenyl. Dissolve the neat material in MtBE and dilute to volume in a 10-mL volumetric flask. Transfer solution to an amber glass vial with a PTFE-lined screw cap and store at ≤ 0 °C. The resulting concentration of the stock internal standard solution will be approximately 2.0 mg/mL.

2) *Internal standard primary dilution standard,* 2.5 μg/mL: Prepare an internal standard fortification solution at approximately 2.5 μg/mL by adding 12.5 μL of the stock standard to 10 mL MtBE. Transfer primary dilution to an amber glass vial with a PTFE-lined screw cap and store at ≤ 0 °C.

3) *MtBE extraction solvent with internal standard,* 2.5 μg/mL: The internal standard 4,4′-dibromooctafluorobiphenyl is added to the extraction solvent before analyte extraction to compensate for any volumetric differences encountered during sample processing. Make this solution just before extraction. The addition of 1 mL of primary dilution standard (2.5 μg/mL) to 99 mL MtBE results in a final internal standard concentration of 25 ng/mL.

s. Surrogate (SUR) analyte standard solution: 2,4-dichlorophenylacetic acid (99+%) is used as a surrogate compound to evaluate extraction and derivatization procedures. This compound is an effective surrogate for the method analytes, but other compounds may be used if QC requirements are met. The solutions listed in 1) and 2) below are stable for at least 30 d. Replace solution if ongoing QC indicates a problem.

1) *Surrogate stock solution,* 1.0 mg/mL: Prepare a surrogate stock standard solution of 2,4-dichlorophenylacetic acid by weighing approximately 0.0100 g of neat material. Dissolve the neat material in acetone and dilute to volume in a 10-mL volumetric flask. Transfer solution to an amber glass vial with a PTFE-lined screw cap and store at ≤ 0°C. The resulting concentration will be 1.0 mg/mL.

2) *Surrogate primary dilution standard/sample fortification solution,* 100 μg/mL: Prepare a primary dilution standard at approximately 100 μg/mL by the addition of 1 mL stock standard to 10 mL acetone. Transfer primary dilution to an amber glass vial with a PTFE-lined screw cap and store at 0°C. The addition of 10 μL of primary dilution standard to the 40-mL aqueous sample results in a surrogate concentration of 25 ng/mL.

t. Analyte standard solutions: Obtain analytes as neat or solid free acid standards or as commercially prepared ampulized solutions from a reputable standard manufacturer. Do not use pre-methylated standards for preparing analyte standards. Prepare analyte stock and primary dilution standards as described below. The solutions listed in 1) and 2) below are stable for at least 30 d. Replace solution if ongoing QC indicates a problem.

1) *Analyte stock standard solution:* Prepare separate stock standard solutions for each analyte of interest at a concentration of 1 to 5 mg/mL in acetone. Method analytes may be obtained as neat materials or ampulized solutions (> 99% purity) from a number of commercial suppliers. Store stock standard solutions at ≤ 0°C.

For analytes that are solids in their pure form, prepare stock standard solutions by accurately weighing approximately 0.01 to 0.05 g pure material in a 10-mL volumetric flask. Dilute to volume with acetone.

For analytes that are liquid in their pure form at room temperature, place approximately 9.8 mL acetone in a 10-mL volu-

‖ Diazald ®, Aldrich Chemical Co., or equivalent.

metric flask. Let flask stand, unstoppered, for about 10 min to allow solvent film to evaporate from the inner walls of flask, and weigh to the nearest 0.1 mg. Use a 10-μL syringe and immediately add 10.0 μL standard material to the flask by keeping syringe needle just above the surface of the acetone. Be sure that the standard material falls dropwise directly into the acetone without contacting inner wall of flask. Calculate concentration in milligrams per milliliter from net gain in weight. Dilute to volume, stopper, then mix by inverting flask several times.

2) *Primary dilution standard (PDS):* Prepare primary dilution standard solution by combining and diluting stock standard solutions with acetone. Store primary dilution standard solution at ≤ 0°C. As a guideline to the analyst, the analyte concentrations, in micrograms per milliliter, used in the primary dilution standard solution during method development are listed as follows.

Acifluorfen	5.0
Bentazon	10
Chloramben	5.0
2,4-D	10
Dalapon	10
2,4-DB	10
Dacthal acid degradation	5.0
Dicamba	5.0
3,5-Dichlorobenzoic acid	5.0
Dichlorprop	10
Dinoseb	10
Pentachlorophenol	1.0
Picloram	5.0
2,4,5-T	2.5
2,4,5-TP (Silvex)	2.5
Quinclorac	5.0

u. Calibration standards: Prepare a five-point calibration curve by fortifying reagent water with the primary dilution standard. Let the standard reach room temperature, then add a designated amount of each calibration standard in acetone into separate, 40-mL portions of reagent water to produce a calibration curve ranging from below or at the MRL to approximately 10 to 20 times the lowest calibration level. Treat these aqueous calibration standards like samples and therefore add all preservatives and other reagents. Extract by the procedure described below. Store calibration standard solutions in acetone at ≤0°C.

4. Procedure

a. Sample preparation: Remove samples from storage and let them equilibrate to room temperature. Place 40 mL water sample in a pre-cleaned, 60-mL glass vial with a PTFE-lined screw cap, using a graduated cylinder. Add 10 μL room temperature surrogate standard (100 μg/mL 2,4-dichlorophenylacetic acid in acetone) to the aqueous sample.

b. Hydrolysis: Because many of the herbicides included in this method are applied as a variety of esters and salts, hydrolyze them to the parent acid before extraction. Include this step in analysis of all extracted field samples, laboratory reagent blanks (LRBs), laboratory-fortified matrices (LFMs) and calibration standards. Failure to perform this step may result in data that are biased low for some targets in field samples.[7] Add 1 mL 4*N* NaOH solution to each glass vial, then cap and shake for several seconds. Check sample pH with pH paper or a pH meter. If sample does not have a pH ≥ 12, adjust pH by adding more 4*N* NaOH solution. Let sample remain at room temperature for 1 h, shaking contents periodically.

c. Hexane:MtBE wash (optional): Use this wash step to quantify the Dacthal degradation products from the parent Dacthal. This wash aids in sample cleanup and removes any Dacthal from the sample that would interfere with the quantitation of the Dacthal degradation products. After hydrolysis, add 5 mL 90:10 (v:v) hexane:MtBE and shake vigorously for 3 min. Let phases separate for approximately 5 min, then remove and discard the top hexane/MtBE layer. If differentiation between Dacthal and its degradation products is not required, this step may be omitted.

d. Microextraction: Adjust pH to 1 by adding approximately 1mL conc sulfuric acid. Cap, shake, and then check pH with a pH meter or narrow-range pH paper. Add more sulfuric acid as needed to properly adjust pH.

Quickly add approximately 2 g copper II sulfate pentahydrate and shake until dissolved. This colors the aqueous phase blue and allows the analyst to better distinguish between the aqueous phase and the organic phase.

Quickly add approximately 16 g muffled sodium sulfate and shake until almost all is dissolved. Sodium sulfate addition increases the ionic strength of the aqueous phase, further driving the chlorophenoxy acids into the organic phase, and also decreases MtBE solubility in the aqueous phase, allowing greater volumetric recovery. Add this salt and the copper II sulfate pentahydrate quickly so that the heat generated from addition of the acid will help dissolve the salts.

Add exactly 4.0 mL MtBE extraction solvent with internal standard to each vial and shake vigorously for 3 min. Let phases separate for approximately 5 min.

e. Preparation of diazomethane: Assemble diazomethane generator (Figure 6251:3) in a hood. See ¶ 2*k* above. The collection vessel is a 10- or 15-mL glass vial equipped with a PTFE-lined screw cap and maintained at 0 to 5°C.

Add a sufficient amount of MtBE (approximately 7 mL) to first tube to cover the impinger. Add 10 mL MtBE to collection vial. Set nitrogen flow at 5 to 10 mL/min. Add 4 mL MNTS solution (¶ 3*m*) and 3 mL 37% KOH solution to the second impinger. Connect tubing as shown in Figure 6251:3 and let nitrogen flow purge diazomethane from reaction vessel into the collection vial for 30 min. Cap vial when collection is complete and maintain at 0 to 5°C. When stored at 0 to 5°C, this diazomethane solution may be used over a period of 72 h.

Using a Pasteur pipet, transfer sample extract (upper MtBE layer) to a 7-mL glass vial. Add 0.6 g acidified sodium sulfate and shake. This step dries the MtBE extract.

Using a Pasteur pipet, transfer extract to a second, 7-mL glass vial. Add 250 μL diazomethane solution to each vial. The contents of the vial should remain slightly yellow, indicating an excess of diazomethane. More diazomethane may be added if necessary. Let the esterification reaction proceed for 30 min.

Remove any unreacted diazomethane by adding 0.1 g silica gel. Effervescence (evolution of nitrogen) is an indication that excess diazomethane was present. Let extracts stand for 0.5 h.

Transfer extract to an autosampler vial. A duplicate vial may be filled with excess extract.

Analyze sample extracts as soon as possible. The sample extract may be stored up to 21 d if kept at 0°C or less. Keep extracts away from light in amber glass vials with PTFE-lined caps.

f. Gas chromatography: Use apparatus specified in ¶s 2*l, m, n,* and hydrogen carrier gas, ¶ 3 *p.* CAUTION: *Observe safety guidelines when using hydrogen as a carrier gas.* If further safety information is required, seek advice from the instrument manufacturer regarding hydrogen use.

Pay strict attention to established column installation guidelines for proper cutting and placement of capillary columns within the instrument. If a loss of response is noted for an analyte, trimming approximately 1 m from the head of the column may restore response. A guard column is recommended if conditions in the laboratory necessitate frequent column trimming.

Chromatographic conditions and analyte retention times for the primary column are given in Table 6640:II A chromatogram from this column is provided in Figure 6640:1. Chromatographic conditions and analyte retention times for the secondary column are given in Table 6640:III. A chromatogram from this column is provided in Figure 6640:2.

g. Calibration: Establish GC operating parameters equivalent to the suggested specifications in Table 6640:II. Calibrate GC system using the internal standard (IS) technique. Other columns or conditions may be used if equivalent or better performance can be demonstrated. Prepare a set of at least five calibration standards as described in ¶ 3*u*. The lowest concentration of calibration standard must be at or below the MRL; preferably use a minimum of four calibration standards between the MRL and the upper quantitative range.

Use GC data system software to generate a linear regression or quadratic calibration curve using the internal standard. This curve may be generated by plotting A_x/A_{is} vs. Q_x/Q_{is}, where A_x and A_{is} are integrated peak areas of the analyte and internal standard, respectively, and Q_x and Q_{is} are quantity of analyte and internal standard injected in concentration units, respectively.

5. Quality Control

Quality control (QC) requirements include the initial demonstration of capability (IDC), the determination of the method detection level (MDL), and subsequent analysis in each analysis batch of a laboratory reagent blank (LRB), continuing calibration check (CCC) standards, a laboratory-fortified sample matrix (LFM), and either a laboratory-fortified sample matrix duplicate (LFMD) or a field duplicate (FD) sample. This section details the specific requirements for each QC parameter. The criteria discussed below are considered the minimum acceptable QC criteria. Laboratories are encouraged to institute additional QC practices to meet their specific needs.

Process all QC samples through all steps of the procedure (¶s 4*a* through *f*), including hydrolysis and methylation. Add sample preservatives as described in 6640A.3 before extracting and analyzing the QC samples.

a. Initial demonstration of capability IDC: Requirements for this demonstration are described below.

1) Initial demonstration of low system background—Before any samples are analyzed, demonstrate that a LRB is reasonably free of contamination and that the criteria in ¶ 5*c* below are met. Process all QC samples through all steps of the method.

2) Initial demonstration of precision—Prepare, extract, and analyze four to seven replicate LFBs fortified at 5 µg/L, or near the mid-range of the initial calibration curve. Process all QC samples through all steps of the analysis procedure. The relative standard deviation (RSD) of the results of the replicate analyses should be less than 20%.

3) Initial demonstration of accuracy—Using the same set of replicate data generated for ¶ 2) above, calculate average recovery. The average recovery of the replicate values should be within ± 20% of the true value.

4) Method detection level (MDL)—Prepare, extract, and analyze at least seven replicate LFBs at a concentration estimated to be near the MDL, over a period of at least 3 d (both extraction and analysis should be conducted over at least 3 d) using the analysis procedure of ¶s 4*a* through *f*. Process all QC samples through all steps of the method. Select a fortification level with a signal of two to five times the noise level and at or below the laboratory's MRL. The appropriate concentration will depend on the sensitivity of the GC/ECD system. Add sample preservatives as described in 6640A.3 to these samples. Calculate the MDL as follows:

$$MDL = S t_{(n-1,\,1-\alpha=0.99)}$$

where:

$t_{(n-1,\,1-\alpha=0.99)}$ = Student's t value for the 99% confidence level with $n-1$ degrees of freedom,
n = number of replicates, and
S = standard deviation of replicate analyses.

NOTE: Calculated MDLs need only be less than 1/3 of the laboratory's MRL to be considered acceptable. Do not subtract blank values when performing MDL calculations. The MDL is a statistical determination of precision only.[1] If the MDL replicates are fortified at a low enough concentration, it is likely that they will not meet precision and accuracy criteria, and may result in a calculated MDL that is higher than the fortified concentration.

b. Minimum reporting level (MRL): The MRL is the threshold concentration of an analyte that a laboratory can expect to quantitate accurately in an unknown sample. The MRL should not be established at an analyte concentration that is less than either three times the MDL or a concentration yielding a signal-to-noise (S/N) ratio of less than five. Depending upon the study's data quality objectives, it may be set at a higher concentration. Although the lowest calibration standard should be at or below the MRL, the MRL cannot be established at a concentration lower than the lowest calibration standard.

c. Laboratory reagent blank (LRB): An LRB is required with each extraction batch of samples to determine the background system contamination. If the LRB produces a peak within the retention-time window of any analyte that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

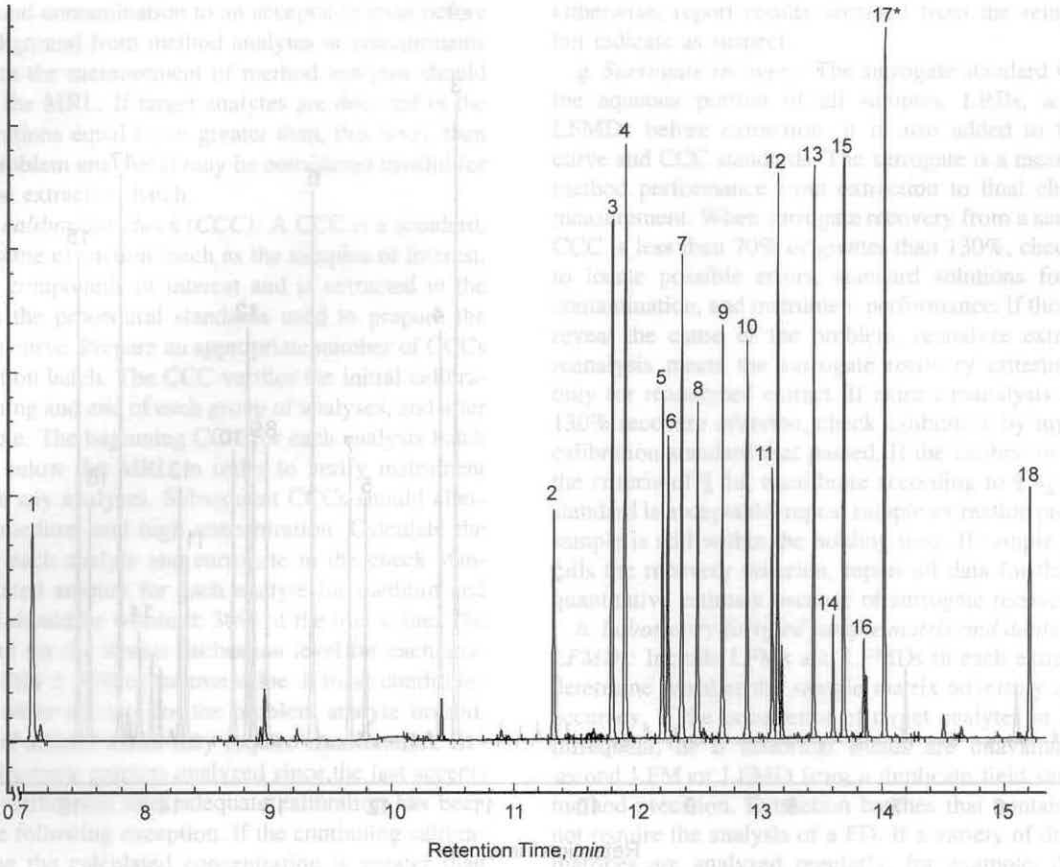

Figure 6640:1. Chromatogram of chlorphenoxy herbicides on a primary column. Peak identifications and chromatographic conditions are given in Table 6640:II. Peak has been attenuated for illustration.

TABLE 6640:II. CHROMATOGRAPHIC CONDITIONS AND AVERAGE RETENTION TIME DATA FOR PRIMARY COLUMN

Peak Number (Figure 6640:1)	Compound	Average RT* *min*	RSD %
1	Dalapon	7.06	0.017
2	3,5 Dichlorobenzoic acid	11.32	0.002
3	2,4-Dichlorophenylacetic acid (SUR)	11.80	0.002
4	Dicamba	11.91	0.002
5	Dichlorprop	12.21	0.002
6	4,4′-Dibromooctafluorobiphenyl (IS)	12.25	0.002
7	2,4-D	12.37	0.002
8	Pentachlorophenol	12.49	0.002
9	Silvex	12.70	0.002
10	2,4,5-T	12.90	0.002
11	2,4-DB	13.10	0.003
12	Chloramben	13.15	0.003
13	Dinoseb	13.44	0.003
14	Bentazon	13.60	0.003
15	Dacthal	13.68	0.002
16	Quinclorac	13.85	0.004
17	Picloram	14.02	0.003
18	Acifluorfen	15.20	0.004

* Average of seven runs.

Primary column: DB-1701, 40 m × 0.180 mm ID, 0.20-mm film thickness, injector temperature 200°C, liner 2 mm straight Siltek® deactivated, injection volume 1 μL of 200 ng/mL (highest level component) standard, splitless injection. Hold to 1 min then purge @ 50 mL/min, detector temperature 360°C, detector make-up gas nitrogen at 20 mL/min. Temperature program: 45°C initial, program at 5°C/min to 80°C, then 50°C/min to 220°C, then 20°C/min to 280°C and hold for 3 min. Data collection via HP GC Chemstation at a rate of 20 Hz.

Carrier gas: Hydrogen (UHP).

Detector make-up gas: Nitrogen (UHP).

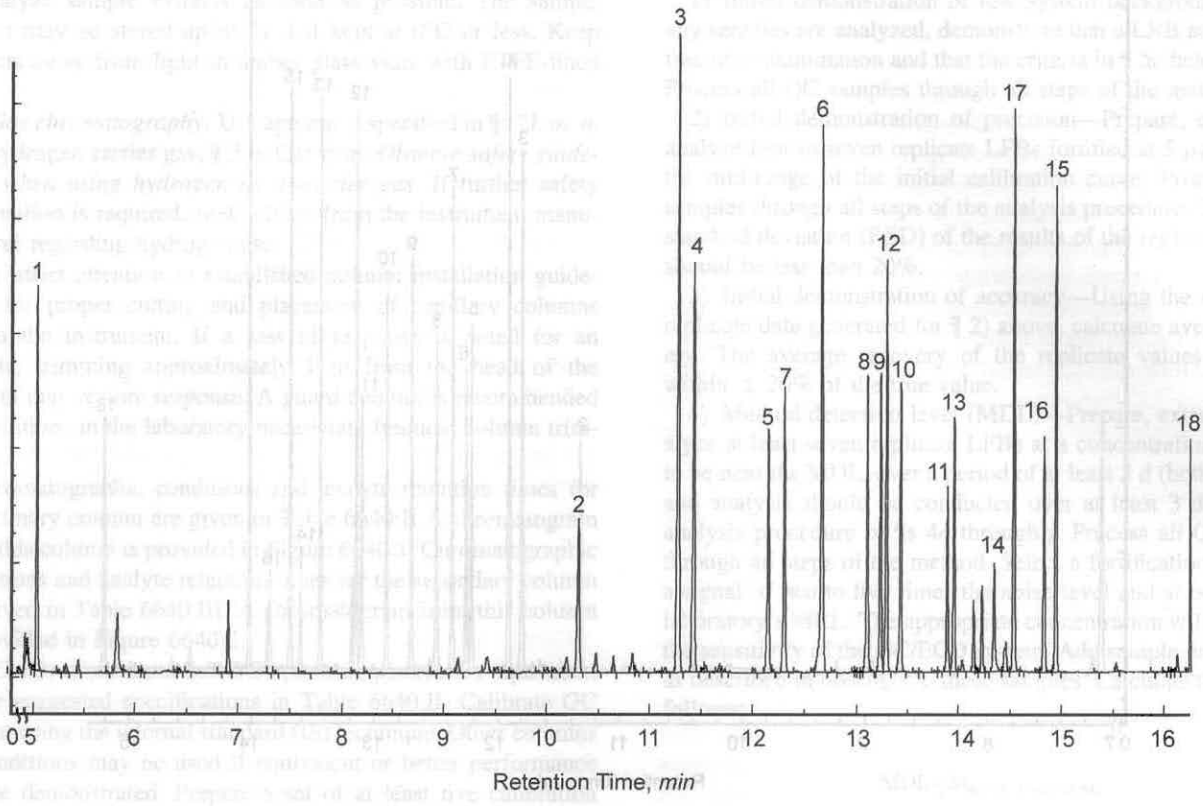

Figure 6640:2. Chromatogram of the chlorphenoxy herbicides on confirmation column. Peak identifications and chromatographic conditions are given in Table 6640:III.

TABLE 6640:III. CHROMATOGRAPHIC CONDITIONS AND AVERAGE RETENTION TIME DATA FOR CONFIRMATION COLUMN

Peak Number (Figure 6640:2)	Compound	Average RT* *min*	RSD %
1	Dalapon	5.04	0.057
2	3,5-Dichlorobenzoic acid	10.33	0.007
3	2,4-Dichlorophenylacetic acid (SUR)	11.31	0.004
4	Dicamba	11.46	0.006
5	Dichlorprop	12.19	0.006
6	4,4′-Dibromooctafluorobiphenyl (IS)	12.71	0.004
7	2,4-D	12.34	0.005
8	Pentachlorophenol	13.16	0.006
9	Silvex	13.29	0.004
10	2,4,5-T	13.48	0.005
11	2,4-DB	13.94	0.005
12	Chloramben	13.34	0.004
13	Dinoseb	14.01	0.004
14	Bentazon	14.28	0.006
15	Dacthal	15.00	0.004
16	Quinclorac	14.88	0.004
17	Picloram	14.59	0.004
18	Acifluorfen	16.38	0.003

* Average of seven runs.

Confirmation column: DB-5, 40 m × 0.180 mm ID, 0.20-mm film thickness, injector temperature 200°C, liner 2 mm straight Siltek® deactivated, injection volume 1 µL of 100 ng/mL (highest level component) standard, splitless injection. Hold to 1 min then purge @ 50 mL/min, detector temperature 360°C, detector make-up gas nitrogen at 20 mL/min. Temperature program: 45°C initial, program at 4°C/min to 80°C, then program at 30°C/min to 280°C and hold for 2 min. Data collection via HP GC Chemstation at a rate of 20 Hz.

Carrier gas: Hydrogen (UHP).

Detector make-up gas: Nitrogen (UHP).

Reduce background contamination to an acceptable level before proceeding. Background from method analytes or contaminants that interfere with the measurement of method analytes should be below 1/3 of the MRL. If target analytes are detected in the LRB at concentrations equal to, or greater than, this level, then all data for the problem analyte(s) may be considered invalid for all samples in the extraction batch.

d. Continuing calibration check (CCC): A CCC is a standard, prepared in the same extraction batch as the samples of interest, that contains all compounds of interest and is extracted in the same manner as the procedural standards used to prepare the initial calibration curve. Prepare an appropriate number of CCCs with each extraction batch. The CCC verifies the initial calibration at the beginning and end of each group of analyses, and after every tenth sample. The beginning CCC for each analysis batch should be at or below the MRL in order to verify instrument sensitivity before any analyses. Subsequent CCCs should alternate between a medium and high concentration. Calculate the concentration of each analyte and surrogate in the check standard. The calculated amount for each analyte for medium and high level CCCs should be within ± 30% of the true value. The calculated amount for the lowest calibration level for each analyte should be within ± 50% of the true value. If these conditions do not exist, consider all data for the problem analyte invalid, and take remedial action, which may require recalibration. Reanalyze any field sample extracts analyzed since the last acceptable calibration verification after adequate calibration has been restored, with the following exception. If the continuing calibration fails because the calculated concentration is greater than 130% (150% for the low-level CCC) for a particular target compound, and field sample extracts show no detection for that target compound, non-detects may be reported without re-analysis. Prepare calibration checks, with the samples being analyzed, at the beginning of each day that samples are analyzed, after every ten samples, and at the end of any group of sample analyses.

e. Laboratory fortified blank (LFB): Because this method utilizes procedural calibration standards, which are fortified reagent waters extracted with each analytical batch, there is no difference between the LFB and the continuing calibration check standard. Consequently, the analysis of an LFB is not required; however the acronym LFB is used for clarity in the IDC.

f. Internal standard (IS): Monitor the peak area of each internal standard in all injections during each analysis day. The IS response (as indicated by peak area) for any chromatographic run should not deviate by more than ± 50% from the average area measured during the initial calibration for that IS. A poor injection could cause the IS area to exceed these criteria. Inject a second portion of the suspect extract to determine whether the failure is due to poor injection or instrument response drift. If the reinjected portion produces an acceptable internal standard response, report the results. If the internal standard area for the reinjected extract deviates more than 50% from the initial calibration average, check the CCC standards that ran before and after the sample. If the CCC fails the criteria of ¶ 5*d*, recalibrate according to ¶ 4*g*. If the CCC is acceptable, repeat sample extraction provided that the sample is still within holding time. Otherwise, report results obtained from the reinjected extract, but indicate as suspect.

g. Surrogate recovery: The surrogate standard is fortified into the aqueous portion of all samples, LRBs, and LFMs and LFMDs before extraction. It is also added to the calibration curve and CCC standards. The surrogate is a means of assessing method performance from extraction to final chromatographic measurement. When surrogate recovery from a sample, blank, or CCC is less than 70% or greater than 130%, check calculations to locate possible errors, standard solutions for degradation, contamination, and instrument performance. If those steps do not reveal the cause of the problem, reanalyze extract. If extract reanalysis meets the surrogate recovery criterion, report data only for reanalyzed extract. If extract reanalysis fails the 70 to 130% recovery criterion, check calibration by injecting the last calibration standard that passed. If the calibration standard fails the criteria of ¶ 5*d*, recalibrate according to ¶ 4*g*. If calibration standard is acceptable, repeat sample extraction provided that the sample is still within the holding time. If sample re-extract also fails the recovery criterion, report all data for that sample as a quantitative estimate because of surrogate recovery.

h. Laboratory-fortified sample matrix and duplicate (LFM and LFMD): Include LFMs and LFMDs in each extraction batch to determine whether the sample matrix adversely affects method accuracy. If the occurrence of target analytes in the samples is infrequent, or if historical trends are unavailable, include a second LFM or LFMD from a duplicate field sample to assess method precision. Extraction batches that contain LFMDs will not require the analysis of a FD. If a variety of different sample matrices are analyzed regularly, for example, drinking water from groundwater and surface water sources, establish method performance for each. Over time, document LFM data for all routine sample sources for the laboratory. Within each extraction batch, fortify a minimum of one field sample as an LFM for every 20 samples extracted. Prepare the LFM by adding to a sample an appropriate amount of analyte as given in ¶ 3*t*2). Select a fortifying concentration that is at least twice the matrix background concentration, if known. Use historical data and rotate through the designated concentrations when selecting a fortifying concentration.

Calculate the percent recovery *(R)* for each analyte by using the equation:

$$R = \frac{(A - B)}{C} \times 100$$

where:

A = measured concentration in fortified sample,
B = measured concentration in unfortified sample, and
C = fortification concentration.

Analyte recoveries may exhibit matrix bias. For samples fortified at or above their native concentration, recoveries should range between 70 and 130%, except for low-level fortification near or at the MRL, where 50 to 150% recoveries may be acceptable. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the LFB, the recovery may be judged to be matrix-biased. Label result for that analyte in the unfortified sample as suspect because of matrix effects.

TABLE 6640:IV. METHOD PRECISION AND BIAS IN SELECTED MATRICES

Matrix	Compound	Low-Level Fortification				Mid-Level Fortification		
		Fortification Concentration µg/L	Mean Recovery %	RSD* %	S/N†	Fortification Concentration µg/L	Mean Recovery %	RSD* %
Reagent water	Dalapon	1.0	108	2.2	578	10	107	2.3
	3,5-Dichlorobenzoic acid	0.50	117	16	9	5.0	96	2.3
	Dicamba	0.50	97	3.7	35	5.0	102	1.5
	Dichlorprop	1.0	95	5.7	19	10	107	0.6
	2,4-D	1.0	98	5.2	23	10	106	1.4
	Pentachlorophenol	0.10	76	7.4	21	1.0	103	1.4
	Silvex	0.25	84	20	9	2.5	107	4.4
	2,4,5-T	0.25	96	7.3	16	2.5	105	1.3
	2,4-DB	1.0	97	8.4	6	10	93	1.6
	Chloramben	0.50	90	9.0	113	5.0	105	2.9
	Dinoseb	1.0	103	6.7	8	10	119	4.6
	Bentazon	1.0	100	2.1	41	10	98	0.7
	Dacthal	0.50	92	6.5	100	5.0	100	1.9
	Quinclorac	0.10	106	9.3	20	1.0	101	2.9
	Picloram	1.0	92	2.6	23	10	99	3.1
	Acifluorfen	0.50	77	5.9	80	5.0	107	4.5
	2,4-Dichlorophenyl-acetic acid (SUR)	25	107	8.0	0	25	112	1.3
Chlorinated surface water	Dalapon	1.0	104	2.1	—	10	100	2.5
	3,5-Dichlorobenzoic acid	0.50	110	3.0	—	5.0	104	2.6
	Dicamba	0.50	93	4.4	—	5.0	102	2.9
	Dichlorprop	1.0	93	11	—	10	101	1.2
	2,4-D	1.0	97	5.2	—	10	101	1.2
	Pentachlorophenol	0.10	86	3.2	—	1.0	100	1.0
	Silvex	0.25	103	4.3	—	2.5	101	1.0
	2,4,5-T	0.25	100	3.5	—	2.5	100	1.6
	2,4-DB	1.0	104	5.9	—	10	101	2.3
	Chloramben	0.50	93	2.0	—	5.0	102	2.6
	Dinoseb	1.0	99	1.2	—	10	101	1.6
	Bentazon	1.0	91	2.2	—	10	98	1.8
	Dacthal	0.50	92	4.6	—	5.0	99	2.0
	Quinclorac	0.10	96	15	—	1.0	105	2.5
	Picloram	1.0	102	2.4	—	10	107	3.2
	Acifluorfen	0.50	98	4.1	—	5.0	92	2.4
	2,4-Dichlorophenyl-acetic acid (SUR)	25	107	1.5	—	25	106	1.4
Chlorinated ground water	Dalapon	1.0	105	1.5	—	10	97	5.5
	3,5-Dichlorobenzoic acid	0.50	104	6.0	—	5.0	95	2.1
	Dicamba	0.50	100	2.2	—	5.0	98	1.6
	Dichlorprop	1.0	109	3.5	—	10	79	0.7
	2,4-D	1.0	108	10	—	10	99	1.6
	Pentachlorophenol	0.10	103	17	—	1.0	100	4.2
	Silvex	0.25	96	3.0	—	2.5	97	1.6
	2,4,5-T	0.25	91	3.4	—	2.5	97	1.5
	2,4-DB	1.0	94	3.1	—	10	92	1.1
	Chloramben	0.50	88	2.7	—	5.0	102	2.6
	Dinoseb	1.0	100	4.0	—	10	105	2.6
	Bentazon	1.0	101	2.8	—	10	101	1.8
	Dacthal	0.50	62‡	9.9	—	5.0	99	1.2
	Quinclorac	0.10	107	5.7	—	1.0	94	1.5
	Picloram	1.0	97	2.8	—	10	103	2.5
	Acifluorfen	0.50	92	4.2	—	5.0	84	2.5
	2,4-Dichlorophenyl acetic acid (SUR)	25	95	3.1	—	25	106	1.1

* $n = 7$

† Signal-to-noise ratios were calculated for each target compound peak by dividing peak height for each compound by peak-to-peak noise, which was determined for each component from method blank over a period of time equal to full peak width in target analyte's retention time window.

‡ Dacthal was present in the chlorinated surface water at about 1/5th fortification level. This was taken into account in the determination of the mean recovery.

If an LFMD is analyzed instead of a FD, calculate relative percent difference (RPD) for values in duplicate LFMs (LFM and LFMD) by using the equation:

$$RPD = \frac{LFM - LFMD}{(LFM + LFMD)/2} \times 100$$

RPDs for duplicate LFMs should fall in the range of ± 30% for samples fortified at or above their native concentration. Greater variability may be observed when LFMs are spiked near the MRL. At the MRL, RPDs should fall in the range of ± 50% for samples fortified at or above their native concentration. If the accuracy of any analyte falls outside the designated range and the laboratory performance for that analyte is shown to be in control in the LFB, the recovery may be judged to be matrix-biased. Label result for that analyte in the unfortified sample as suspect because of matrix effects.

i. Field duplicates (FD1 and FD2): Within each extraction batch, include a minimum of one field duplicate (FD) or LFMD (¶ 5*h*). FDs check the precision associated with sample collection, preservation, storage, and laboratory procedures. If target analytes are not routinely observed in field samples, analyze a LFMD to substitute for this requirement. Extraction batches that contain LFMDs may not require the analysis of a FD. Calculate the relative percent difference (RPD) for duplicate measurements (FD1 and FD2) using the equation:

$$RPD = \frac{FD1 - FD2}{(FD1 + FD2)/2} \times 100$$

RPDs for duplicates should be in the range of ± 30%. Greater variability may be observed when analyte concentrations are near the MRL. At the MRL, RPDs should fall in the range of ± 50%. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the LFB, the recovery may be judged to be matrix-biased. Label result for that analyte in the unfortified sample as suspect because of matrix effects.

j. Quality control (QC) sample: Analyze a QC sample each time that new primary dilution standards, ¶ 3*t*2), are prepared. Ideally, obtain QC sample from second vendor. If a second vendor is not available, use a different lot of the standard. Although use of pre-methylated standards is prohibited for preparing analyte standard solutions, pre-methylated standards may be used to prepare the QC sample. The QC sample may be injected as a calibration standard or fortified into reagent water and analyzed as a LFB. If the QC sample is analyzed as a continuing calibration, acceptance criteria are the same as for the CCC. If the QC sample is analyzed as a LFB, acceptance criteria are the same as for a LFB. If measured analyte concentrations are not of acceptable accuracy, check entire analytical procedure to locate and correct problem source.

6. Precision and Bias

Precision and bias data for this method are presented in Table 6640:IV. The effect of sample holding time on recovery from a chlorinated surface water is shown in Table 6640:V and the effect of extract holding time in Table 6640:VI.

TABLE 6640:V. EFFECT OF SAMPLE HOLDING TIME ON RECOVERY FOR SAMPLES FROM A CHLORINATED SURFACE WATER FORTIFIED WITH METHOD ANALYTES*

Compound	Recovery %			
	Day 0	Day 2	Day 7	Day 14
Dalapon	91	90	91	108
3,5-Dichlorobenzoic acid	98	97	85	91
Dicamba	92	92	96	99
Dichlorprop	89	88	91	95
2,4-D	88	85	87	92
Pentachlorophenol	92	85	86	93
Silvex	91	88	86	94
2,4,5-T	89	89	85	91
2,4-DB	87	84	86	93
Chloramben	86	86	88	100
Dinoseb	105	95	99	111
Bentazon	91	87	95	99
Dacthal	92	91	86	94
Quinclorac	90	80	84	91
Picloram	94	85	85	99
Acifluorfen	109	96	103	123

* Sample storage stability is expressed as a percent recovery value calculated as described in 6640B.5*h*.

TABLE 6640:VI. EFFECT OF EXTRACT HOLDING TIME ON RECOVERY FOR SAMPLES FROM A CHLORINATED SURFACE WATER FORTIFIED WITH METHOD ANALYTES*†

Compound	Recovery %	
	Initial Injection	Day 21 Reinjection
Dalapon	100	89
3,5-Dichlorobenzoic acid	104	113
Dicamba	102	104
Dichlorprop	100	101
2,4-D	101	102
Pentachlorophenol	100	102
Silvex	101	103
2,4,5-T	99	102
2,4-DB	101	102
Chloramben	102	105
Dinoseb	99	81
Bentazon	98	100
Dacthal	99	103
Quinclorac	105	127
Picloram	107	98
Acifluorfen	92	104
2,4-Dichlorophenylacetic acid	106	105

* Sample storage stability is expressed as a percent recovery value calculated as described in 6640B.5*h*.

† All samples fortified at the same level used to collect the mid-level precision and bias.

7. References

1. Giam, C.S., H.S. Chan & G.S. Nef. 1975. Sensitive method for determination of phthalate ester plasticizers in open ocean biota samples. *Anal. Chem.* 47:2225.
2. Giam, C.S. & H.S. Chan. 1976. Control of blanks in the analysis of phthalates in air and ocean biota samples. U.S. National Bur. Standards, Special Publ. 442, p. 701.
3. Glaser, J.A., D.L. Foerst, G.D. McKee, S.A. Quave & W.L. Budde. 1981. Trace analyses for wastewaters. *Environ. Sci. Technol.* 15: 1426.
4. Occupational Safety and Health Administration. 1976. OSHA Safety and Health Standards, General Industry. 29 CRF 1910; OSHA 2206 (rev.), Occupational Safety & Health Admin., Washington, D.C.
5. U.S. Public Health Service. 1977. Carcinogens—Working with Carcinogens. Publ. No. 77-206, U.S. Dep. Health, Education, and Welfare, Public Health Serv., Center for Disease Control, National Inst. Occupational Safety & Health, Atlanta, Ga.
6. Committee on Chemical Safety, American Chemical Society. 1979. Safety in Academic Chemistry Laboratories, 3rd ed. American Chemical Soc., Washington, D.C.
7. Bassett, M.V., B.V. Pepich & D.M. Munch. 2000. The role played by basic hydrolysis in the validity of acid herbicide data — Recommendations for future preparation of herbicide performance evaluation standards. *Environ. Sci. Technol.* 34:1117.

6651 GLYPHOSATE HERBICIDE*

6651 A. Introduction

1. Sources and Significance

Glyphosate [*N*-(phosphonomethyl)glycine] is a broad-spectrum, nonselective, postemergence herbicide that has found widespread agricultural and domestic use. It is sold as a terrestrial and aquatic herbicide under the trade names Roundup® and Rodeo®. Because of low mammalian toxicity (LD50 = 1568 mg/kg rats; oral) there is less concern about water and food contamination than with other pesticides, but the nonselectivity of the herbicide can make nontarget phytotoxicity a problem. Glyphosate's (GLYPH) major metabolite is aminomethylphosphonic acid (AMPA). Contamination of water can occur through runoff and spray drift.

2. Selection of Method

Several methods for determination of GLYPH and AMPA in environmental samples have been developed; those using liquid chromatography are the most precise and accurate. GLYPH and AMPA are not good chromophores or fluorophores and their electrochemical or conductometric detection have not been demonstrated. Sensitive and selective detection has been achieved with the post-column reaction/fluorometric method.[1–3] The absence of a sensitive liquid chromatography technique for confirmation necessitates the use of two different stationary phases.

The liquid chromatographic method presented in 6651B is accurate and precise[3] and includes confirmation by using two columns. Gas chromatography/mass spectrometry confirmation[4] has been used when structural confirmation is required, but the method has not been tested on residues in natural waters.

3. References

1. Moye, H.A., C.J. Miles & S.J. Scherer. 1983. A simplified high-performance liquid chromatographic procedure for the determination of glyphosate herbicide and (aminomethyl)phosphonic acid in fruits and vegetables employing postcolumn fluorogenic labeling. *J. Agric. Food Chem.* 31:69.
2. Cowell, J.E., J.L. Kunstman, P.J. Nord, J.R. Steinmetz & G.R. Wilson. 1986. Validation of an analytical residue method for analysis of glyphosate and metabolite: An interlaboratory study. *J. Agric. Food Chem.* 34:955.
3. Oppenhuizen, M.E. & J.E. Cowell. 1991. Liquid chromatographic determination of glyphosate and (aminomethyl)phosphonic acid in environmental water. *J. Assoc. Offic. Anal. Chem.* 74:317.
4. Deyrup, C.L., S.M. Chang, R.A. Weintraub & H.A. Moye. 1985. Simultaneous esterification and acylation of pesticides for analysis by gas chromatography. 1. Derivatization of glyphosphate and (aminomethyl) phosphonic acid with fluorinated alcohol-perfluorinated anhydrides. *J. Agric. Food Chem.* 33:944.

4. Bibliography

Bardalaye, P.C., W.B. Wheeler & H.A. Moye. 1985. Analytical techniques of glyphosate residue analysis. *In* E. Grossbard & D. Atkinson, eds. The Herbicide Glyphosate. Butterworths, Woburn, Mass.

* Approved by Standard Methods Committee, 2000.

6651 B. Liquid Chromatographic Post-Column Fluorescence Method

1. General Discussion

a. Principle: GLYPH and AMPA are separated by anion- or cation-exchange chromatography and measured by post-column fluorescence derivatization. The post-column reactions consist of oxidation of GLYPH (a secondary amine) to glycine (a primary amine) by hypochlorite solution. Glycine then reacts with an *o*-phthalaldehyde (OPA) and mercaptoethanol (MERC) mixed reagent to form an isoindole that is measured fluorometrically. AMPA (a primary amine) reacts directly with the OPA/MERC reagent and is detected (with decreased sensitivity) in the presence of hypochlorite.

b. Interferences: No matrix interferences in water are known. GLYPH degrades in chlorinated water. GLYPH also is known to sorb strongly to minerals and glass surfaces.

c. Minimum detectable concentration: Minimum detection using this method is 25 μg/L for GLYPH and AMPA by direct injection and 0.5 μg/L with the concentration step.

2. Sampling and Storage

Collect a 500-mL representative sample in a polypropylene or amber glass container. Treatment of sample to remove residual chlorine will prevent glyphosate losses during storage. Destroy chlorine by adding 100 mg/L sodium thiosulfate. Store samples at 4°C away from light and analyze within 2 weeks.

3. Apparatus

a. High-performance liquid chromatograph (HPLC): An analytical system with pump, injector, detectors, and compatible strip chart recorder. Preferably use a data system for measuring peak areas and retention times. Use system capable of injecting 200-μL portions. See Figure 6651:1.

1) *Analytical columns:* Use either a cation exchange resin[1] or an anion-exchange resin[2] packed in a 4.6-mm × 25- to 30-cm column.* Heat columns to between 50 and 60°C to obtain maximum efficiency.

2) *Post-column reactor:* Use system consisting of two separate pumps capable of delivering reaction solutions at 0.1 to 0.5 mL/min and able to withstand pressures of up to 2000 kPa. Include two woven 1-mL TFE reaction coils[3] (0.5-mm ID × 1.4-mm OD × 5 m) with one maintained at 40°C. Turnkey post-column reactor systems are available commercially.

3) *Fluorescence detector:* Use filter or grating fluorimeter capable of sensitively and selectively measuring the isoindole derivative, with excitation wavelength 230 nm (deuterium), 340 nm (quartz halogen or xenon), and emission wavelength 420 to 455 nm.

4. Reagents

a. Reagent water: See Section 1080.

b. Phosphoric acid, H_3PO_4, conc.

c. Sulfuric acid, H_2SO_4, conc. Prepare anion-exchange mobile phase by adding 26 mL conc H_3PO_4 and 2.7 mL conc H_2SO_4 to 5 L water.

d. Hydrochloric acid, HCl, conc.

e. Methanol, CH_3OH, tested on HPLC and verified to give no impurity peaks.

f. Potassium dihydrogen phosphate, KH_2PO_4. Prepare cation-exchange mobile phase by dissolving 0.68 g KH_2PO_4 in 1 L methanol-water (4:96). Adjust to pH 2.1 with conc H_3PO_4. Filter through a 0.22- or 0.45-μm membrane filter and degas.

g. Disodium ethylenediamine tetraacetate dihydrate, EDTA sodium salt solutions: Prepare a 0.001*M* solution by dissolving 0.37 g EDTA dihydrate in 1.0 L water and filter through a 0.22- or 0.45-μm filter. Prepare a 0.03*M* solution by dissolving 11.2 g EDTA dihydrate in 1.0 L water and filtering through a 0.33- or 0.45-μm filter.

h. Sodium chloride, NaCl.

i. Sodium hydroxide, NaOH.

* Aminex, BioRad Labs, A-9 cation exchange and A-27 anion exchange resins, or equivalent.

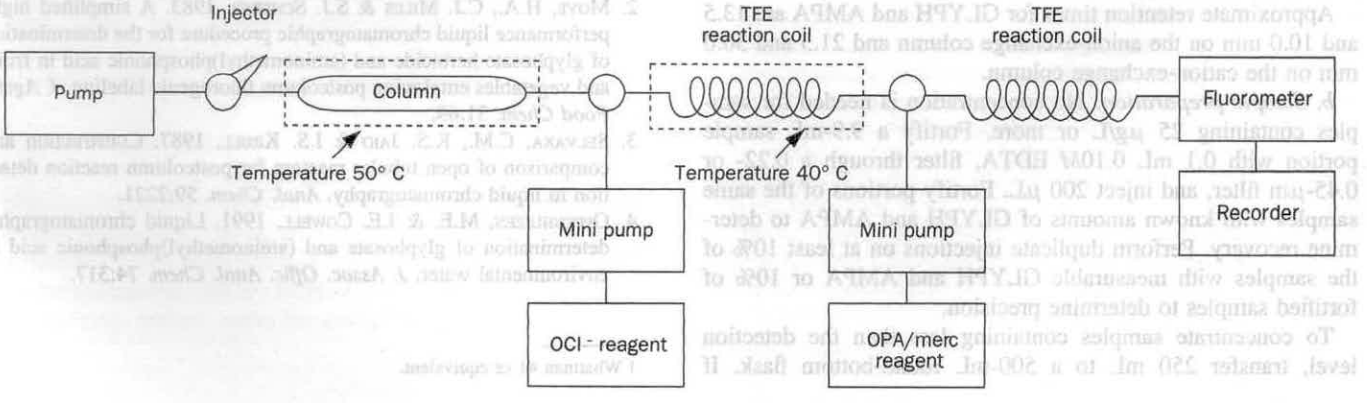

Figure 6651:1. Schematic of post-column reaction HPLC system.

j. Calcium hypochlorite, $Ca(OCl)_2$, 70.9% available chlorine.

k. Oxidation reagent: Dissolve 0.5 g $Ca(OCl)_2$ in 500 mL water with rapid magnetic stirring for 45 min. In a 1.0-L volumetric flask, dissolve 1.74 g KH_2PO_4, 11.6 g NaCl, 0.4 g NaOH, and 10 mL stock $Ca(OCl)_2$ solution. Dilute to volume, mix well, and filter through a 0.22- or 0.45-μm filter.

l. o-Phthalaldehyde, $C_6H_4(CHO)_2$, OPA.

m. 2-mercaptoethanol, $HSCH_2CH_2OH$, MERC.

n. Boric acid powder, H_3BO_3.

o. Potassium hydroxide, KOH.

p. Fluorogenic labeling reagent: Dissolve 100 g boric acid and 72 g KOH in about 700 mL water in a 1.0-L flask. This takes 1 to 2 h. Add 0.8 g OPA dissolved in 5 mL methanol. Add 2.0 mL MERC. Mix well.

q. Glyphosate analytical standard, N-(phosphonomethyl) glycine, 99% or greater.

r. Aminomethylphosphonic acid analytical standard, 99% or greater.

s. Glyphosate and AMPA fortification standards: Prepare a solution containing both 0.1 mg GLYPH/mL and 0.1 mg AMPA/mL in water. Make working solutions of 10.0 and 1.0 μg/mL by serial dilution of this stock solution. Store in a refrigerator, in a polypropylene or amber glass container. Prepare fresh monthly.

t. Glyphosate and AMPA HPLC calibration standards: Prepare a solution containing both 0.1 mg GLYPH/mL and 0.1 mg AMPA/mL in 0.001*M* disodium EDTA solution. Make working solutions of 1.00, 0.50, 0.10, 0.05, and 0.025 μg/mL by serial dilution. Store in a refrigerator, in a polypropylene or amber glass container. Prepare fresh monthly.

5. Procedure

a. HPLC operation: Equilibrate column at 50°C with mobile-phase flow rate of 0.5 mL/min (see Figure 6651:1). Use an approximate flow rate of 0.5 mL/min for the oxidant and 0.3 mL/min for the OPA-MERC reagent but adjust rates to obtain maximum response. While GLYPH reaches a maximum response at some flow rate of oxidative solution, the AMPA response decreases with any addition of this reagent. Thus, an oxidative reagent flow rate that gives an equal response for both GLYPH and AMPA simultaneously is considered optimum for simultaneous measurements. Reagent flow rates will differ for different mobile phases. Establish a standard curve by injecting calibration standards.

Approximate retention times for GLYPH and AMPA are 13.5 and 10.0 min on the anion-exchange column and 21.5 and 30.0 min on the cation-exchange column.

b. Sample preparation: No concentration is needed for samples containing 25 μg/L or more. Fortify a 9.9-mL sample portion with 0.1 mL 0.10*M* EDTA, filter through a 0.22- or 0.45-μm filter, and inject 200 μL. Fortify portions of the same samples with known amounts of GLYPH and AMPA to determine recovery. Perform duplicate injections on at least 10% of the samples with measurable GLYPH and AMPA or 10% of fortified samples to determine precision.

To concentrate samples containing less than the detection level, transfer 250 mL to a 500-mL round-bottom flask. If suspended matter is present, filter sample through coarse filter paper.† For samples used to assess recovery, make known additions. Add 5 mL conc HCl to flask and 5 mL to sample remaining in original container. Concentrate on a rotary evaporator by slowly increasing temperature from 20 to 60°C. Before the first portion is completely evaporated, add remaining sample and two 5-mL rinses of the sample bottle. Evaporate to dryness, and if necessary, remove final traces of water with a stream of dry nitrogen. Dissolve residue in 2.9 mL of mobile phase (adjust pH to 2 if necessary) and 0.10 mL 0.03*M* EDTA solution. Filter through 0.45-μm filter to a test tube and inject into the HPLC system.

6. Calculations

Determine concentration of GLYPH and AMPA by regression analysis of the standard curve. Multiply results for samples that were concentrated by the concentration factor, 166.7 (500 mL original sample/3.0 mL), to determine the original water concentration. Report results in milligrams per liter. Report percent recovery but do not correct for recovery.

7. Quality Control

See Section 6020.

8. Precision and Bias

For six single-operator analyses, the relative standard deviation of duplicate samples (with additions from 0.5 to 5000 μg/L) ranged from 12.1 to 20% with an average of 14.9% for glyphosate. The relative standard deviation for identical AMPA concentrations ranged from 6.5 to 28.8% with an average of 14.5%.[4]

For six single-operator analyses, recoveries of glyphosate (with additions from 0.5 to 5000 μg/L) ranged from 94.6 to 120% with an average of 104.0%. Recoveries of AMPA ranged from 86.0 to 100% with an average of 93.1%.[4]

9. References

1. Cowell, J.E., J.L. Kunstman, P.J. Nord, J.R. Steinmetz & G.R. Wilson. 1986. Validation of an analytical residue method for analysis of glyphosate and metabolite: An interlaboratory study. *J. Agric. Food Chem.* 34:955.
2. Moye, H.A., C.J. Miles & S.J. Scherer. 1983. A simplified high-performance liquid chromatographic procedure for the determination of glyphosate herbicide and (aminomethyl)phosphonic acid in fruits and vegetables employing postcolumn fluorogenic labeling. *J. Agric. Food Chem.* 31:69.
3. Selvaka, C.M., K.S. Jaio & I.S. Krull. 1987. Construction and comparison of open tubular reactors for postcolumn reaction detection in liquid chromatography. *Anal. Chem.* 59:2221.
4. Oppenhuizen, M.E. & J.E. Cowell. 1991. Liquid chromatographic determination of glyphosate and (aminomethyl)phosphonic acid in environmental water. *J. Assoc. Offic. Anal. Chem.* 74:317.

† Whatman #1 or equivalent.