Method 617

The Determination of Organohalide Pesticides and PCBs in Municipal and Industrial Wastewater

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1. SCOPE AND APPLICATION

1.1 This method covers the determination of certain organohalide pesticides and PCBs. The following parameters can be determined by this method:

Parameter	*Storet No.*	*CAS No.*
Aldrin	39330	309-00-2
α-BHC	39337	319-84-6
ß-BHC	39338	319-85-7
δ-BHC	39259	319-86-8
γ-BHC	39340	58-89-9
Captan	39640	133-06-2
Carbophenothion	--	786-19-6
Chlordane	39350	57-74-9
4,4′-DDD	39310	72-54-8
4,4′-DDE	39320	72-55-9
4,4′-DDT	39300	50-29-3
Dichloran	--	99-30-9
Dicofol	39780	115-32-2
Dieldrin	39380	60-57-1
Endosulfan I	34356	959-98-8
Endosulfan II	34361	33213-65-9
Endosulfan sulfate	34351	1031-07-8
Endrin	39390	72-20-8
Endrin aldehyde	34366	7421-93-4
Heptachlor	39410	76-44-8
Heptachlor epoxide	39420	1024-57-3
Isodrin	39430	465-73-6
Methoxychlor	39480	72-43-5
Mirex	39755	2385-85-5
PCNB	39029	82-68-8
Perthane	39034	72-56-0
Strobane	--	8001-50-1
Toxaphene	39400	8001-35-2
Trifluralin	39030	1582-09-8
PCB-1016	34671	12674-11-2
PCB-1221	39488	11104-28-2
PCB-1232	39492	11141-16-5
PCB-1242	39496	53469-21-9
PCB-1248	39500	12672-29-6
PCB-1254	39504	11097-69-1
PCB-1260	39508	11096-82-5

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.

1.3 The method detection limit (MDL, defined in Section 15) for many of the parameters are listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 The sample extraction and concentration steps in this method are essentially the same as in Method 614. Thus, a single sample may be extracted to measure the parameters included in the scope of both of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures. Under gas chromatography, the analyst is allowed the latitude to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters (see Section 12).

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. SUMMARY OF METHOD

2.1 A measured volume of sample, approximately 1 L, is extracted with 15% methylene chloride in hexane using a separatory funnel. The extract is dried and concentrated to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by electron capture gas chromatography.

2.2 Method 617 represents an editorial revision of two previously promulgated U.S. EPA methods for pesticides and for PCBs.[1] While complete method validation data is not presented herein, the method has been in widespread use since its promulgation, and represents the state of the art for the analysis of such materials.

2.3 This method provides selected cleanup procedures to aid in the elimination of interferences which may be encountered.

3. INTERFERENCES

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.[2] Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric ware. Thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Interferences by phthalate esters can pose a major problem in pesticide analysis when the EC detector is used. These compounds generally appear in the chromatogram as large late-eluting peaks, especially in the 15% and 50% fractions from the Florisil column cleanup. Common flexible plastics contain varying amounts of phthalates. These phthalates are easily extracted or leached from such materials during laboratory operations. Cross-contamination of clean glassware occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Interferences from phthalates can be minimized by avoiding the use of plastics in the laboratory. Exhaustive cleanup of reagents and glassware may be required to eliminate background phthalate contamination.[3,4] The interferences from phthalate esters can be avoided by using a microcoulometric or electrolytic conductivity detector.

3.3 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. SAFETY

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals

specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified [5-7] for the information of the analyst.

4.2 The following parameters covered by this method have been tentatively classified as known or suspected human or mammalian carcinogens: aldrin, benzene hexachlorides, chlordane, heptachlor, PCNB, PCBs, and toxaphene. Primary standards of these toxic materials should be prepared in a hood.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel: 125-mL, 1000-mL, and 2000-mL, with TFE-fluorocarbon stopcock, ground-glass or TFE stopper.

5.2.2 Drying column: Chromatographic column 400 mm long by 19 mm ID with coarse-fritted disc.

5.2.3 Chromatographic column: 400 mm long by 19 mm ID with coarse-fritted disc at bottom and TFE-fluorocarbon stopcock (Kontes K-420540-0224 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground-glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish: three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Vials: Amber glass, 10- to 15-mL capacity with TFE-fluorocarbon-lined screw-cap.

5.3 Boiling chips: Approximately 10/40 mesh. Heat at 400°C for 30 minutes or perform a Soxhlet extraction with methylene chloride.

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

5.5 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.

5.6 Shaker: Laboratory, reciprocal action.

5.7 Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.7.1 Column 1: 180 cm long by 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 method performance statements in Section 15. Alternative columns may be used in accordance with the provisions described in Section 12.1.

5.6.2 Column 2: 180 cm long by 4 mm ID glass, packed with 3% OV-1 on Supelcoport (100/120 mesh) or equivalent.

5.6.3 Detector: Electron capture. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope and was used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. REAGENTS

6.1 Reagent water: Reagent water is defined as a water in which an interferant is not observed at the method detection limit of each parameter of interest.

6.2 Acetone, hexane, isooctane, methylene chloride: Pesticide-quality or equivalent.

6.3 Ethyl ether: Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Cat. No. P1126-8, and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.

6.4 Acetonitrile, hexane-saturated: Mix pesticide-quality acetonitrile with an excess of hexane until equilibrium is established.

6.5 Sodium sulfate: ACS, granular, anhydrous. Heat in a shallow tray at 400°C for a minimum of four hours to remove phthalates and other interfering organic substances. Alternatively, heat 16 hours at 450 to 500°C in a shallow tray or Soxhlet extract with methylene chloride for 48 hours.

6.6 Sodium chloride solution, saturated: Prepare saturated solution of NaCl in reagent water and extract with hexane to remove impurities.

6.7 Sodium hydroxide solution (10N): Dissolve 40 g ACS grade NaOH in reagent water and dilute to 100 mL.

6.8 Sulfuric acid solution (1+1): Slowly add 50 mL H_2SO_4 (sp. gr. 1.84) to 50 mL of reagent water.

6.9 Mercury: Triple-distill.

6.10 Florisil: PR grade (60/100 mesh). Purchase activated at 675°C and store in dark in glass container with ground-glass stopper or foil-lined screw-cap. Before use, activate each batch at least 16 hours at 130°C in a foil-covered glass container.

6.11 Stock standard solutions (1.00 µg/µL): Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.

6.11.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in pesticide-quality isooctane and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially-prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.11.2 Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw-cap vials. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.11.3 Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure.

7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with isooctane. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.2.2 Using injections of 1 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.3 Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest 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 isooctane. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

7.3.2 Using injections of 1-5 µL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

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

where

A_s = *Response for the parameter to be measured*

A_{si} = *Response for the internal standard*

C_{is} = *Concentration of the internal standard, in ug/L*

C_s =*Concentration of the parameter to be measured, in ug/L*

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s / A_{is} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 The cleanup procedure in Section 11 utilizes Florisil chromatography. Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil which is used, the use of the lauric acid value is suggested. This procedure[8] determines the adsorption from hexane solution of lauric acid, in milligrams, per gram of Florisil. The amount of Florisil to be used for each column is calculated by dividing this factor into 110 and multiplying by 20 g.

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

7.6 The multipeak materials included in this method present a special calibration problem. Recommended procedures for calibration, separation and measurement of PCBs is discussed in detail in the previous edition of this method.[1] Illustrated methods for the calibration and measurement of chlordane and strobane/toxaphene are available elsewhere.[9]

8. QUALITY CONTROL

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on

performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone, 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Table 2 provides single-operator recovery and precision for many of the organohalide pesticides. Similar results should be expected from reagent water for all parameters listed in this method. Compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

Upper Control Limit (UCL) = R + 3s
Lower Control Limit (LCL) = R − 3s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts[10] that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.[10]

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[11] should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction. Chemical preservatives should not be used in the field unless more than 24 hours will elapse before delivery to the laboratory. If the samples will not be extracted within 48 hours of collection, the sample should be adjusted to a pH range of 6.0 to 8.0 with sodium hydroxide or sulfuric acid. Record the volume of acid or base used.

9.3 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

10. SAMPLE EXTRACTION

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel.

10.2 Add 60 mL 15% (v/v) methylene chloride in hexane to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Drain the aqueous phase into a 1000-mL Erlenmeyer flask and collect the extract in a 250-mL Erlenmeyer flask. Return the aqueous phase to the separatory funnel.

10.3 Add a second 60-mL volume of 15% methylene chloride in hexane to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the 250-mL Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500- mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of hexane to complete the quantitative transfer.

10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 80 to 85°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

10.7 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than 2 days, they should be transferred to PTFE-sealed screw-cap bottles. If the sample extract requires no further cleanup, proceed with gas chromatographic analysis. If the sample requires cleanup, proceed to Section 11.

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

11. CLEANUP AND SEPARATION

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various industrial and municipal effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

11.2 Acetonitrile partition: The following acetonitrile partitioning procedure may be used to isolate fats and oils from the sample extracts. This procedure is applicable to all of the parameters in this method except mirex.

11.2.1 Quantitatively transfer the previously concentrated extract to a 125-mL separatory funnel with enough hexane to bring the final volume to 15 mL. Extract the sample four times by shaking vigorously for 1 minute with 30-mL portions of hexane-saturated acetonitrile.

11.2.2 Combine and transfer the acetonitrile phases to a 1-L separatory funnel and add 650 mL of reagent water and 40 mL of saturated sodium chloride solution. Mix thoroughly for 30 to 45 seconds. Extract with two 100-mL portions of hexane by vigorously shaking for 15 seconds.

11.2.3 Combine the hexane extracts in a 1-L separatory funnel and wash with two 100-mL portions of reagent water. Discard the water layer and pour the hexane layer through a drying column containing 7 to 10 cm of anhydrous sodium sulfate into a 500-mL K-D flask equipped with a 10-mL concentrator tube. Rinse the separatory funnel and column with three 10-mL portions of hexane.

11.2.4 Concentrate the extracts to 6 to 10 mL in the K-D as directed in Section 10.6. Adjust the extract volume to 10 mL with hexane.

11.2.5 Analyze by gas chromatography unless a need for further cleanup is indicated.

11.3 Florisil column cleanup: The following Florisil column cleanup procedure has been demonstrated to be applicable to most of the organochlorine pesticides and PCBs listed in Table 3. It should also be applicable to the cleanup of extracts for PCNB, strobane, and trifluralin.

11.3.1 Add a weight of Florisil (nominally 20 g), predetermined by calibration (Sections 7.4 and 7.5), to a chromatographic column. Settle the Florisil by tapping the column. Add anhydrous sodium sulfate to the top of the Florisil to form a layer 1 to 2 cm deep. Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the elution of the hexane by closing the stopcock on the chromatography column. Discard the eluate.

11.3.2 Adjust the sample extract volume to 10 mL with hexane and transfer it from the K-D concentrator tube to the Florisil column. Rinse the tube twice with 1 to 2 mL hexane, adding each rinse to the column.

11.3.3 Place a 500-mL K-D flask and clean concentrator tube under the chromatography column. Drain the column into the flask until the sodium sulfate layer is nearly exposed. Elute the column with 200 mL of 6% (v/v) ethyl ether in hexane (Fraction 1) using a drip rate of about 5 mL/min. Remove the K-D flask and set aside for later concentration. Elute the column again, using 200 mL of 15% (v/v) ethyl ether in hexane (Fraction 2), into a second K-D flask. Perform a third elution using 200 mL of 50% (v/v) ethyl ether in hexane (Fraction 3) into a separate K-D flask. The elution patterns for the pesticides and PCBs are shown in Table 3.

11.3.4 Concentrate the eluates by standard K-D techniques (Section 10.6), using the water bath at about 85°C. Adjust final volume to 10 mL with hexane. Analyze by gas chromatography.

11.4 Removal of sulfur: Elemental sulfur will elute in Fraction 1 of the Florisil cleanup procedure. If a large amount of sulfur is present in the extract, it may elute in all fractions. If so, each fraction must be further treated to remove the sulfur. This procedure cannot be used with heptachlor, endosulfans, or endrin aldehyde.

11.4.1 Pipette 1.00 mL of the concentrated extract into a clean concentrator tube or a vial with a TFE-fluorocarbon seal. Add 1 to 3 drops of mercury and seal.

11.4.2 Agitate the contents of the vial for 15 to 30 seconds.

11.4.3 Place the vial in an upright position on a reciprocal laboratory shaker and shake for up to 2 hours.

11.4.4 If the mercury appears shiny after this treatment, analyze the extract by gas chromatography. If the mercury is black, decant the extract into a clean vial and repeat the cleanup with fresh mercury.

12. GAS CHROMATOGRAPHY

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.2 Calibrate the system daily as described in Section 7.

12.3 If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 1 to 5 µL of the sample extract using the solvent-flush technique.[12] Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used. Multipeak materials present a special analytical problem beyond the scope of this discussion. Illustrated procedures for calibration and measurement are available for PCBs[1] and pesticides.[9]

12.5 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

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

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

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

where

A = *Amount of material injected, in ng*

V_i = *Volume of extract injected, in µL*

V_t = *Volume of total extract, in µL*

V_s = *Volume of water extracted, in mL*

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

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

where

A_s = *Response for parameter to be measured*

A_{is} = *Response for the internal standard*

I_s = *Amount of internal standard added to each extract, in µg*

V_o = *Volume of water extracted, in L*

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. GC/MS CONFIRMATION

14.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative compound identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 per scan

utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC-to-MS interface constructed of all glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.

14.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.[13]

14.3 At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all decafluorotriphenyl phosphine (DFTPP) performance criteria are achieved.[14]

14.4 To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.

14.4.1 All ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to ±10%. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20 to 40%.

14.4.2 The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.

14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.

14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.

14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. METHOD PERFORMANCE

15.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[15] The MDL concentrations listed in Table 1 were obtained using reagent water.[16]

15.2 In a single laboratory, Susquehanna University, using spiked tap water samples, the average recoveries presented in Table 2 were obtained. The standard deviation of the percent recovery is also included in Table 2.[16]

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Table 1. Gas Chromotagraphy of Pesticides and PCBs

Parameter	***Retention Time (min)***		***Method Detection Limit (µg/L)***
	Column 1	***Column 2***	
Aldrin	2.40	4.10	0.009
α-BHC	1.35	1.82	0.004
β-BHC	1.90	1.97	ND
δ-BHC	2.15	2.20	ND
γ-BHC	1.70	2.13	0.002
Captan	6.22	5.00	ND
Carbophenothion	10.9	10.90	ND
4,4′-DDD	7.83	9.08	0.012
4,4′-DDE	5.13	7.15	0.004
4,4′-DDT	9.40	11.75	0.032
Dichloran	1.85	2.01	ND
Dicofol	2.86	4.59	ND
Dieldrin	5.45	7.23	0.011
Endosulfan I	4.50	6.20	0.11
Endosulfan II	8.00	8.28	0.17
Endosulfan sulfate	14.22	10.70	ND
Endrin	6.55	8.19	ND
Endrin aldehyde	11.82	9.30	ND
Heptachlor	2.00	3.35	0.004
Heptachlor epoxide	3.50	5.00	0.003
Isodrin	3.00	4.83	ND
Methoxychlor	18.20	26.60	0.176
Mirex	14.60	15.50	0.015
PCNB	1.63	2.01	0.002
Trifluralin	0.94	1.35	0.013

*For multipeak materials, see Figures 2 through 10 for chromatographic conditions and retention patterns.

ND = Not Determined

Column 1 conditions: Supelcoport (100/120 mesh) coated with 1.5% SP-2250/1.95% SP-2401 in a glass column 1.8 m long by 4 mm ID with 95% argon/5% methane carrier gas at a flow rate of 60 mL/min. Column temperature: isothermal at 200°C. An electron capture detector was used with this column to determine the MDL.

Column 2 conditions: Supelcoport (100/120 mesh) coated with 3% OV-1 packed in a glass column 1.8 m long by 4 mm ID with 95% argon/5% methane carrier gas at a flow rate of 60 mL/min. Column temperature: isothermal at 200°C.

Table 2. Single-Operator Accuracy and Precision for Tap Water

Parameter	***Average Percent Recovery***	***Standard Deviation (%)***	***Spike Range (µg/L)***	***Number of Analyses***
Aldrin	78.1	5.4	0.03–3.0	21
δ-BHC	95.3	8.9	0.01–1.0	21
γ-BHC	95.1	7.2	0.01–1.0	21
4,4′-DDD	94.4	5.0	0.08–8.0	21
4,4′-DDE	89.8	3.7	0.05–5.0	21
4,4′-DDT	91.0	4.5	0.2–20	21
Dieldrin	98.2	4.9	0.06–6.0	21
Endosulfan I	101.0	7.6	0.05–5.0	21
Endosulfan II	92.9	4.8	0.09–9.0	21
Heptachlor	84.4	6.4	0.02–2.0	21
Heptachlor epoxide	93.7	3.9	0.03–3.0	21
Methoxychlor	96.6	6.7	0.6–60	21
Mirex	89.1	4.8	0.2–20	21
PCNB	82.6	6.2	0.01–1.0	21
Trifluralin	94.3	10.5	0.03–3.0	21

Table 3. Distribution and Recovery of Chlorinated Pesticides and PCBs Using Florisil Column Chromatography

	Percent Recovery by Fraction		
Parameter	***No. 1***	***No. 2***	***No. 3***
Aldrin	100		
α-BHC	100		
β-BHC	97		
δ-BHC	98		
γ-BHC	100		
Captan	+	+	
Carbofenthion	100		
Chlordane	100		
4,4′-DDD	99		
4,4′-DDE	98		
4,4′-DDT	100		
Dicofol	+	+	
Dieldrin	0	100	
Endosulfan I	37	64	91
Endosulfan II	0	7	106
Endosulfan sulfate	0	0	
Endrin	4	96	
Endrin aldehyde	0	68	26
Heptachlor	100		
Heptachlor epoxide	100		
Isodrin	100		
Methoxychlor	100		
Mirex	100		
Perthane	100		
Toxaphene	96		
PCB-1016	97		
PCB-1221	97		
PCB-1232	95		
PCB-1242	97		
PCB-1248	103		
PCB-1254	90		
PCB-1260	95		

+ = Compound occurs in both 6% and 15% fractions.

Florisil eluate composition by fraction:
Fraction 1 = 200 mL of 65% ethyl ether in hexane
Fraction 2 = 200 mL of 15% ethyl ether in hexane
Fraction 3 = 200 mL of 50% ethyl ether in hexane

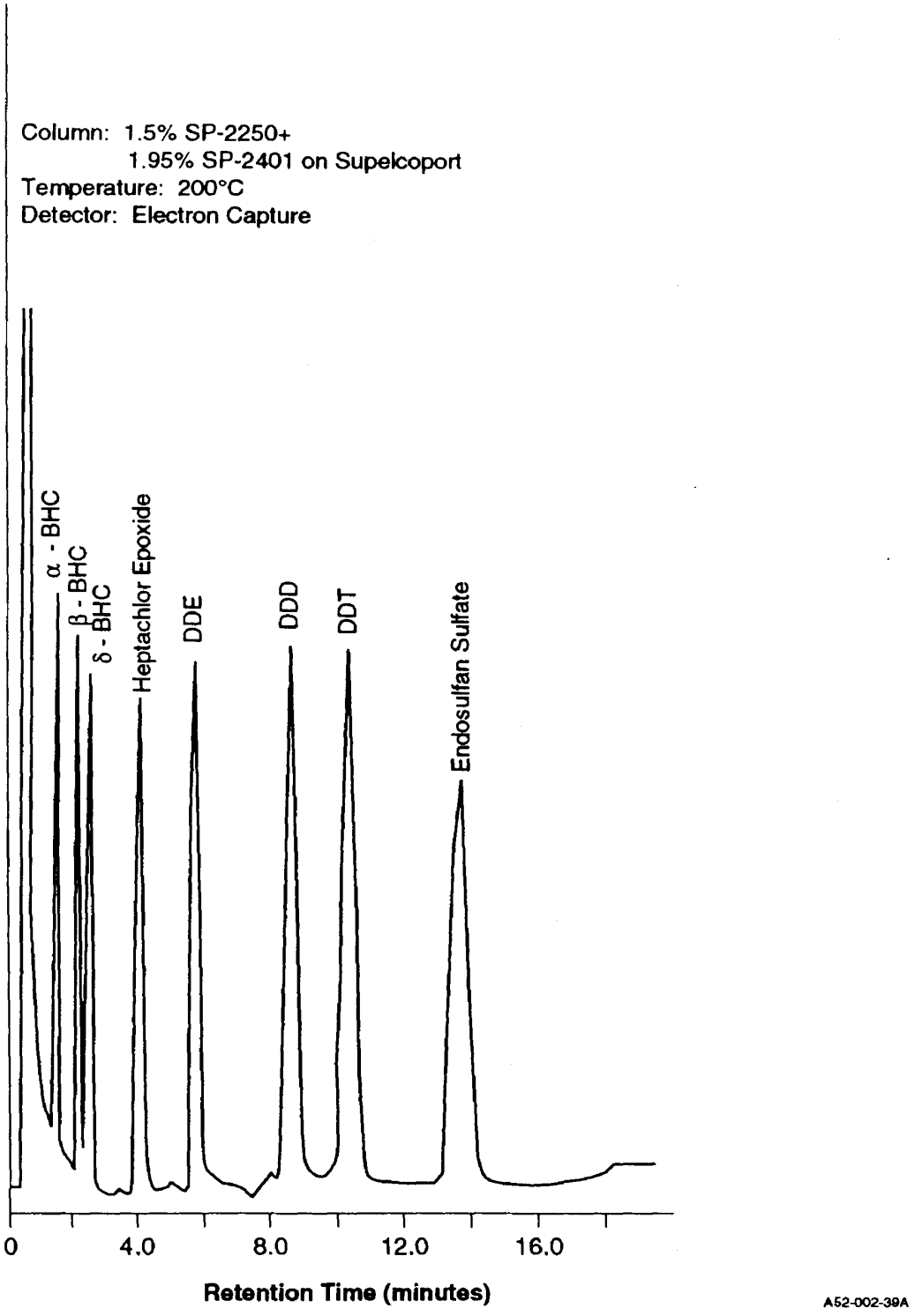

Figure 1. Gas Chromatogram of Pesticides

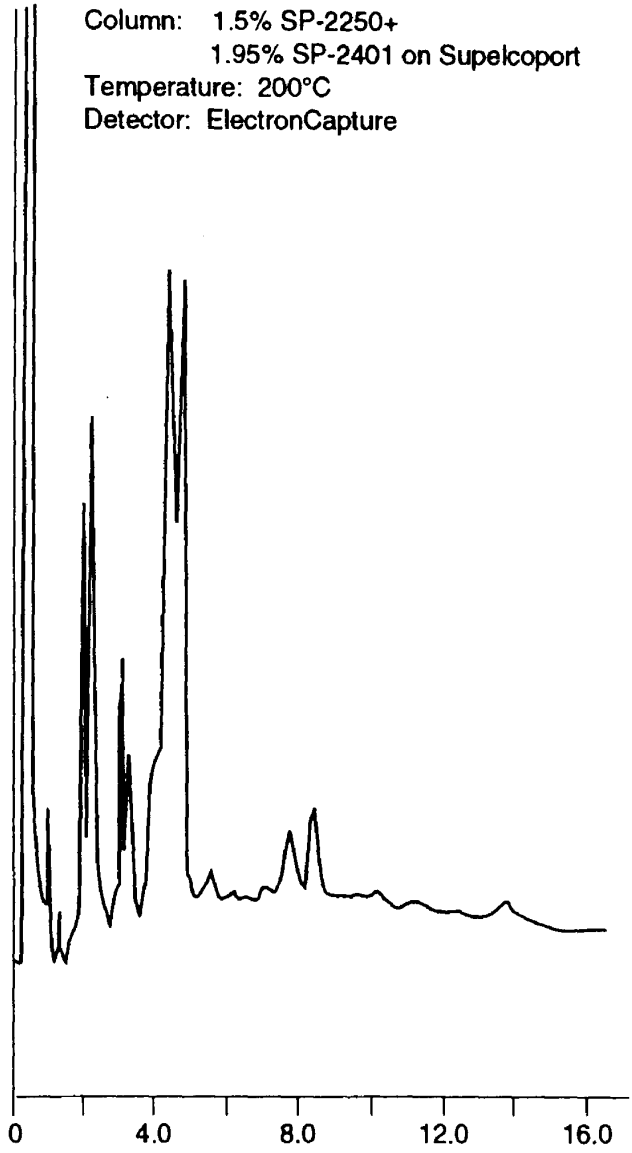

Figure 2. Gas Chromatogram of Chlordane

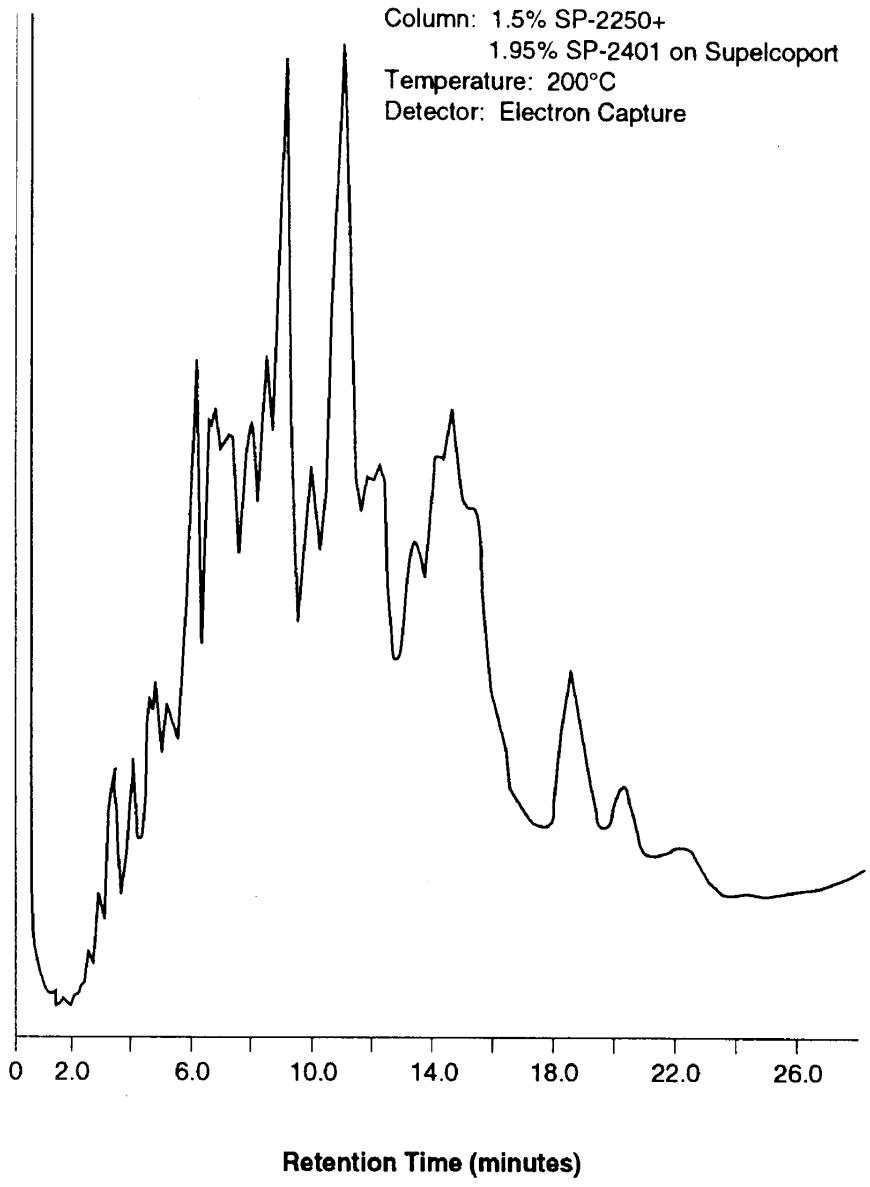

Figure 3. Gas Chromatogram of Toxaphene

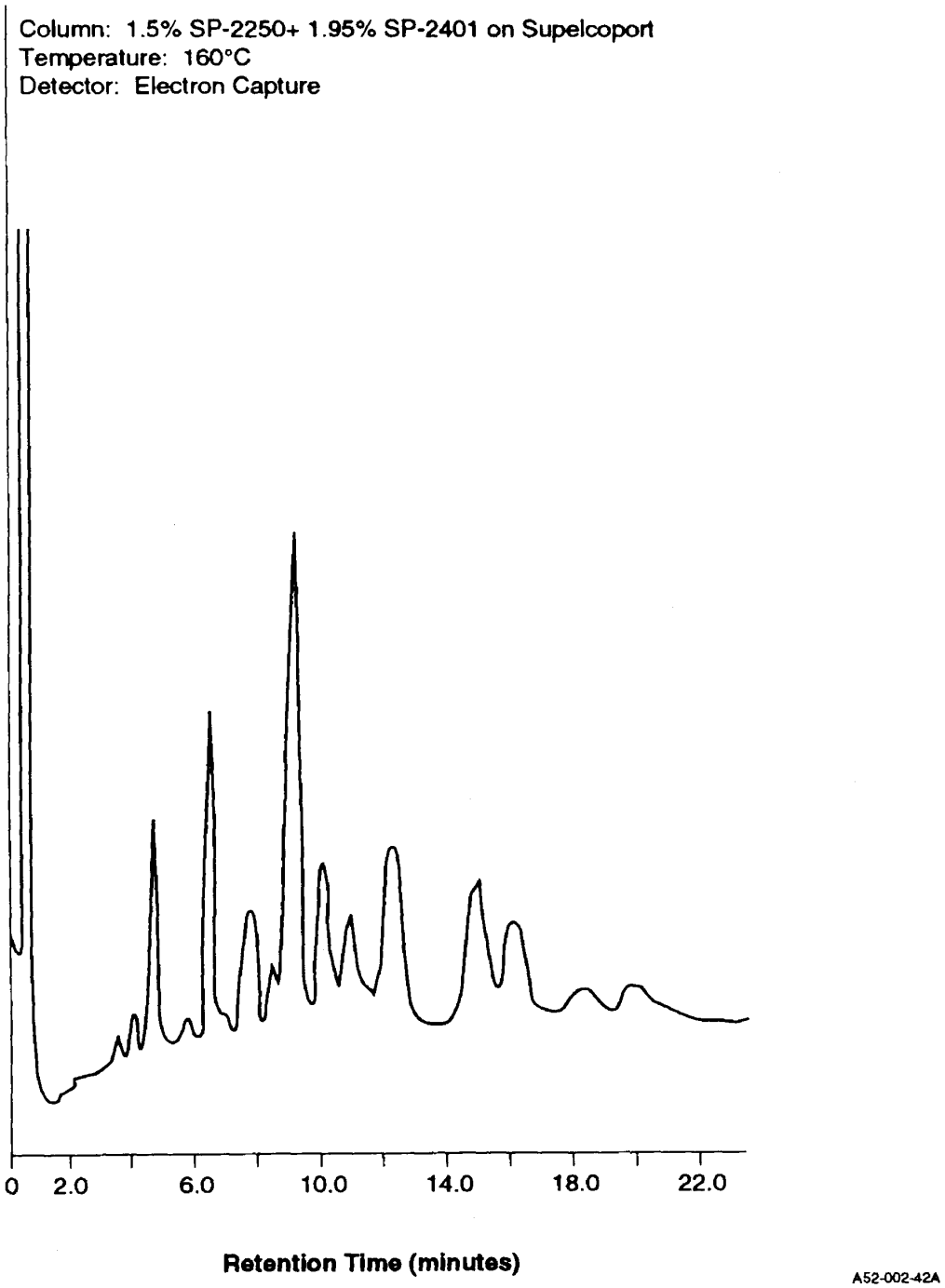

Figure 4. Gas Chromatogram of PCB-1016

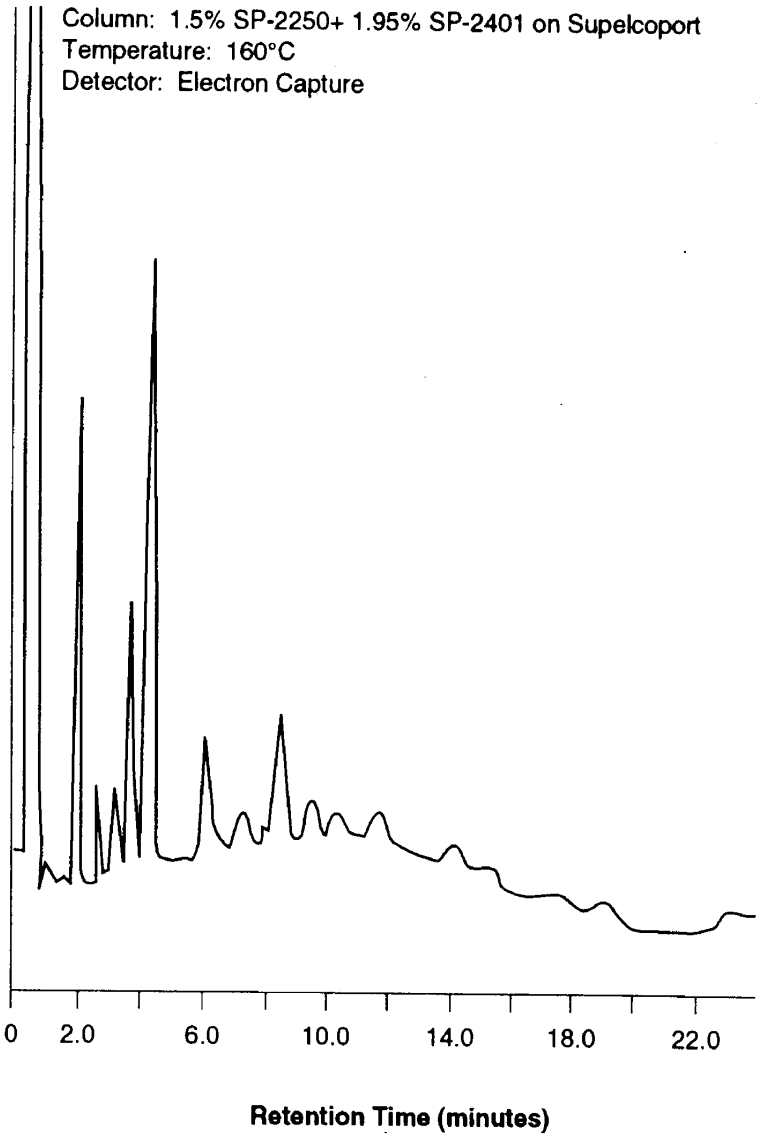

Figure 5. Gas Chromatogram of PCB-1221

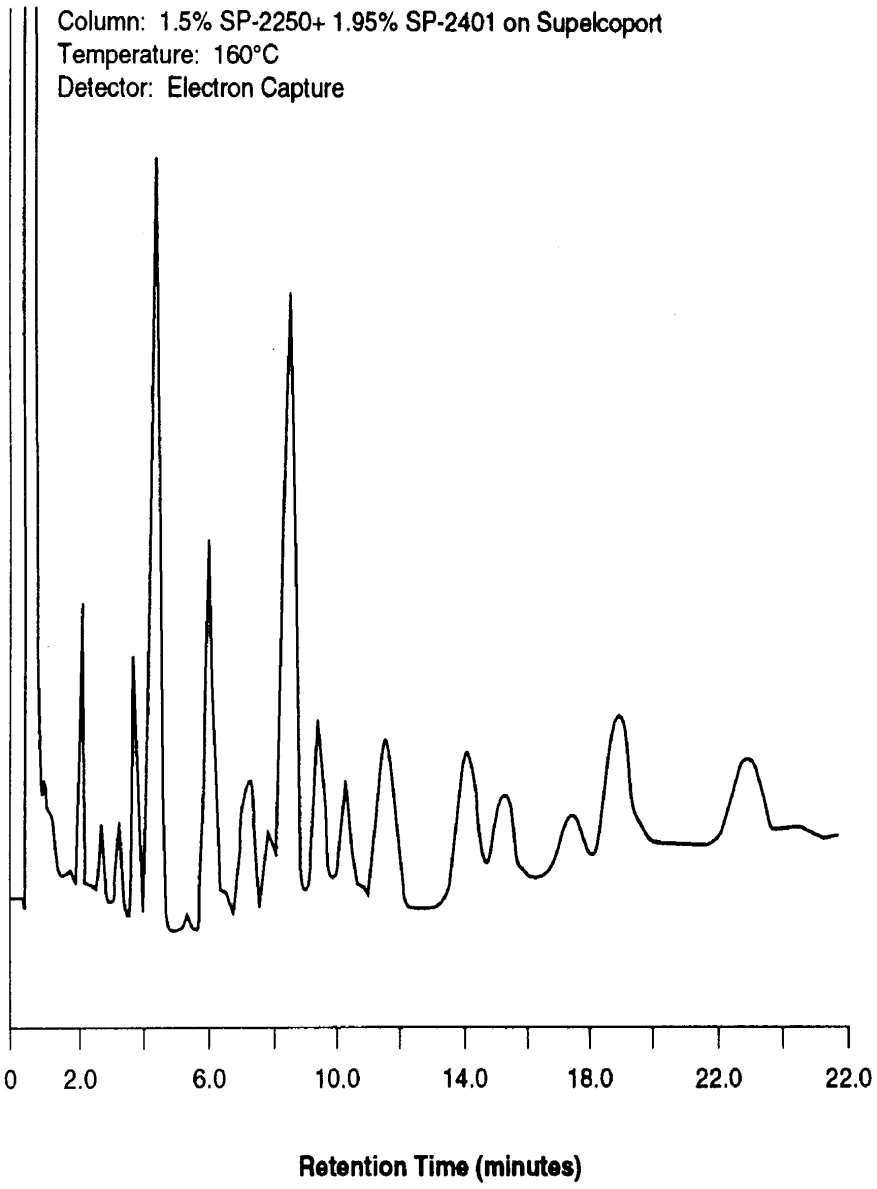

Figure 6. Gas Chromatogram of PCB-1232

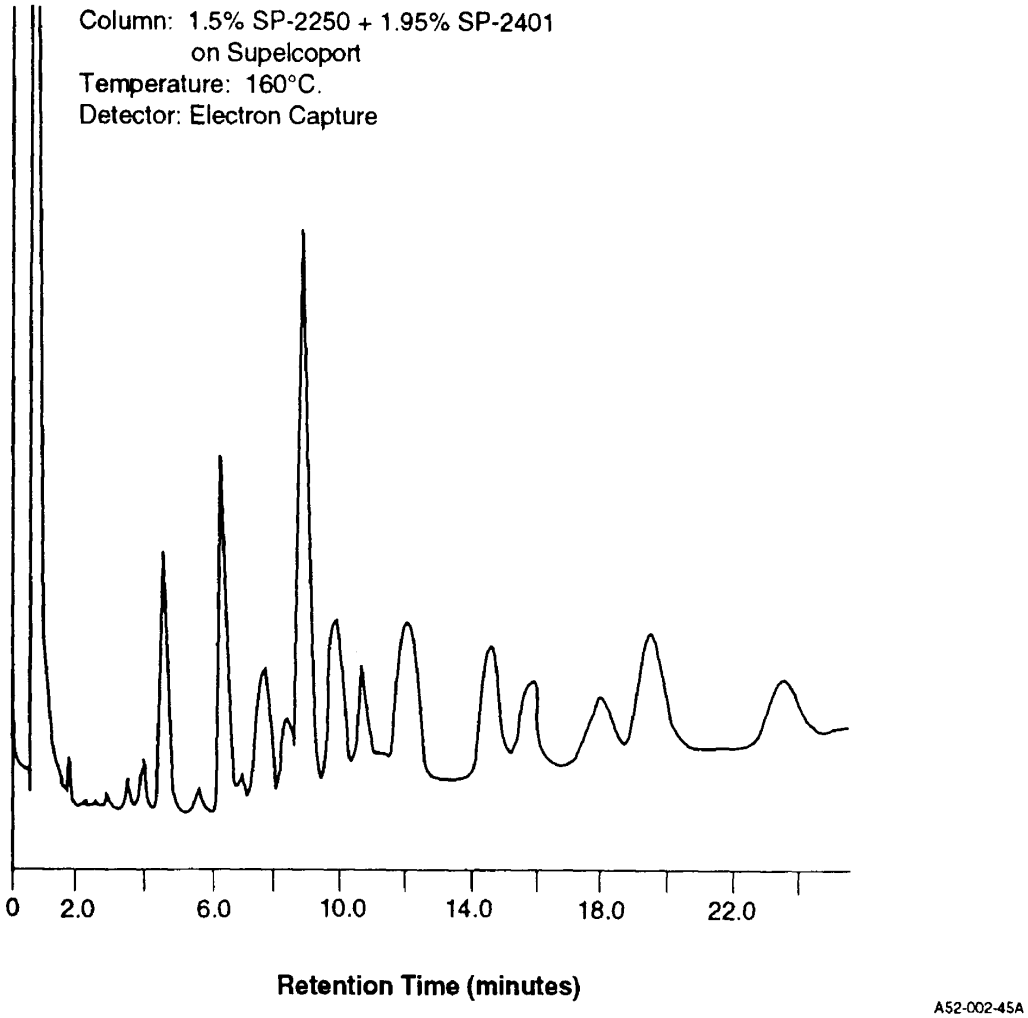

Figure 7. Gas Chromatogram of PCB-1242

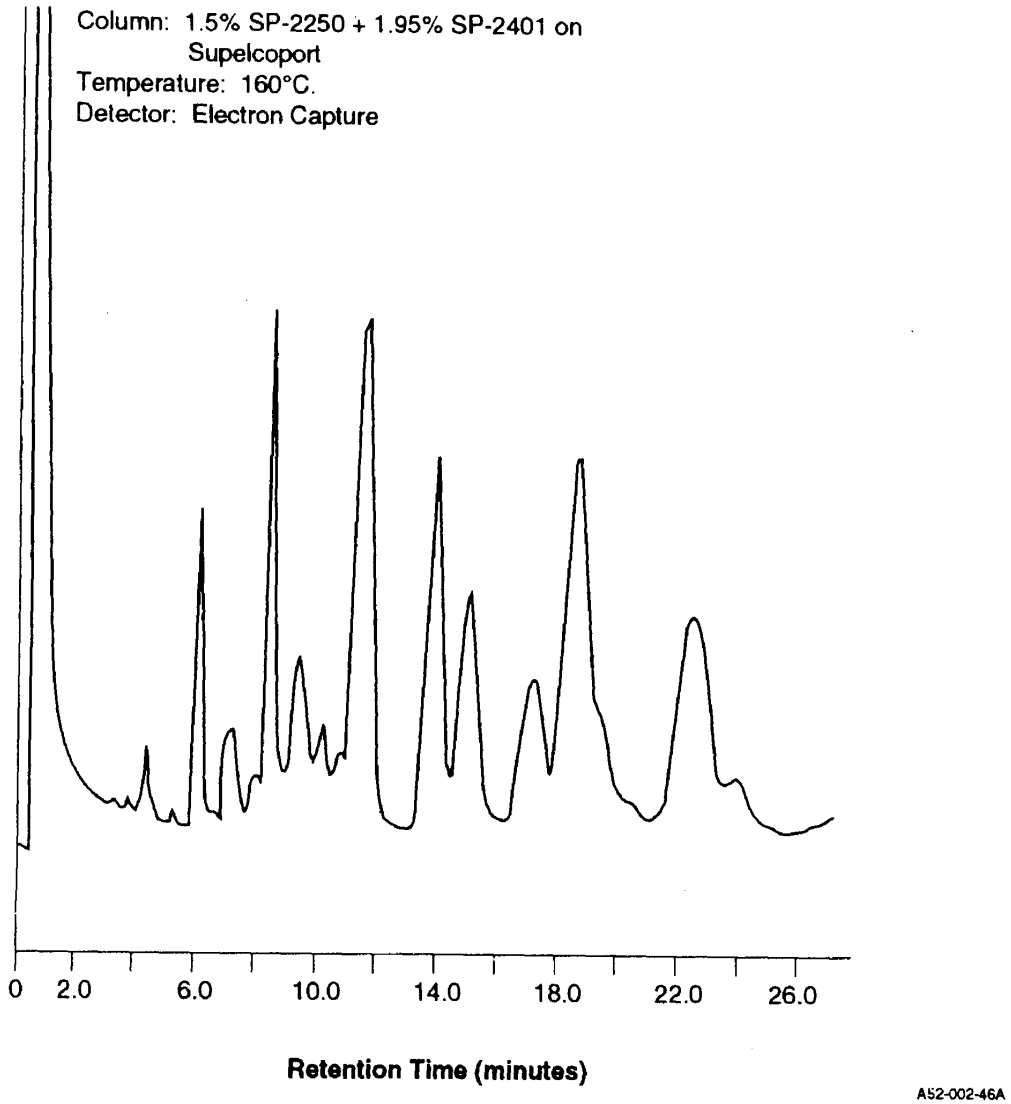

Figure 8. Gas Chromatogram of PCB-1248

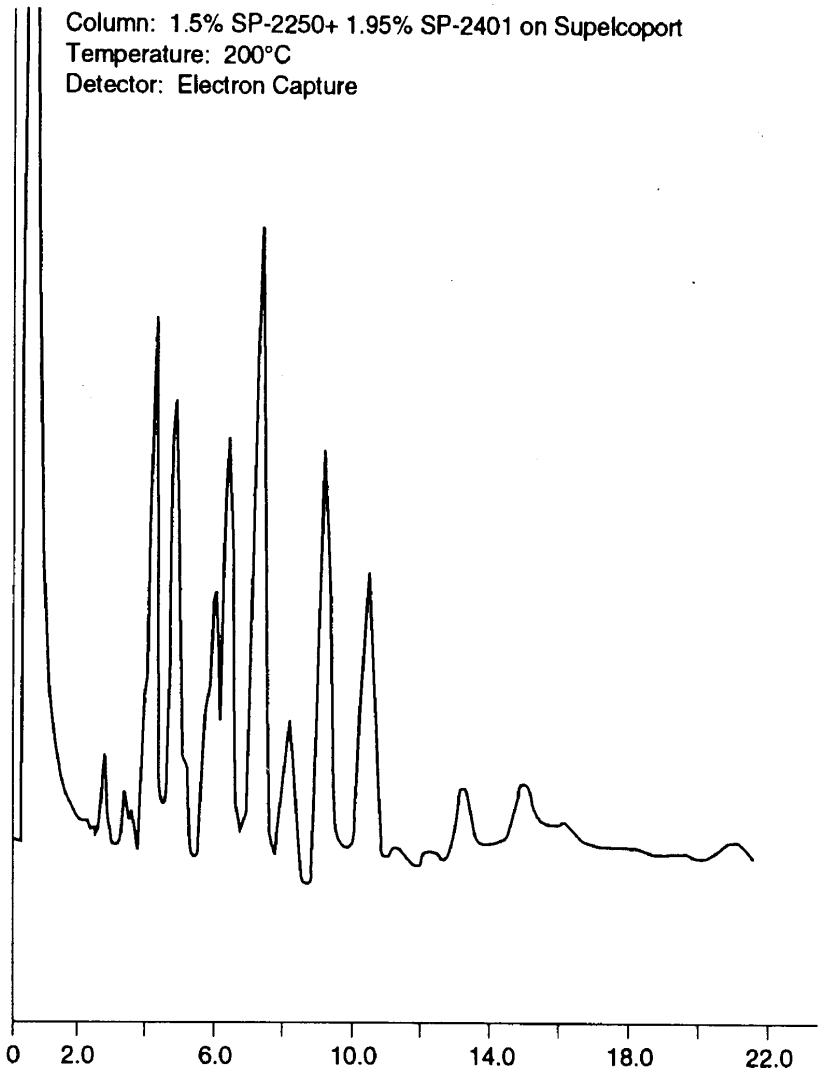

Figure 9. Gas Chromatogram of PCB-1254

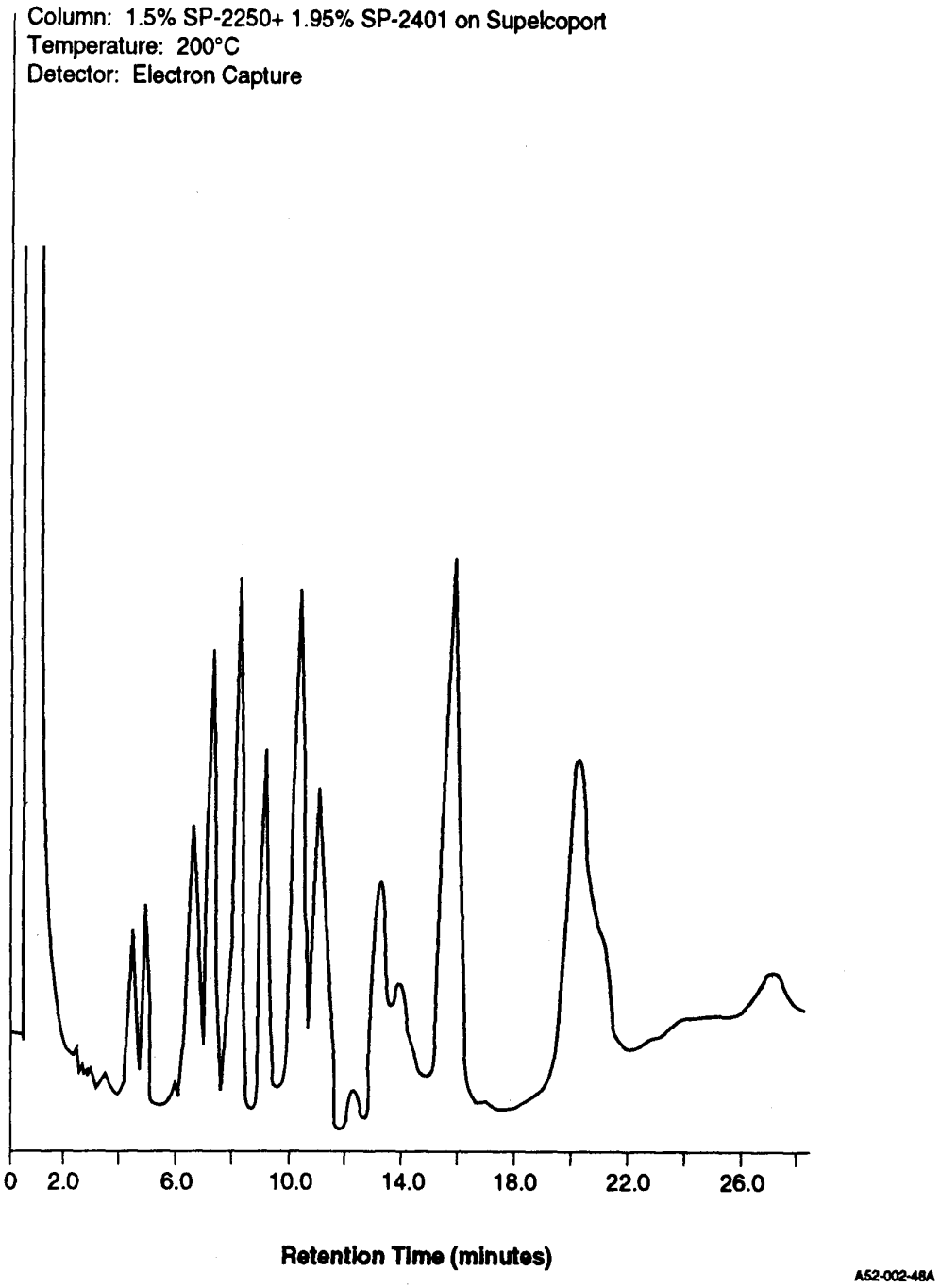

Figure 10. Gas Chromatogram of PCB-1260