METHOD 505

ANALYSIS OF ORGANOHALIDE PESTICIDES AND COMMERCIAL POLYCHLORINATED BIPHENYL (PCB) PRODUCTS IN WATER BY MICROEXTRACTION AND GAS CHROMATOGRAPHY

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1.0 SCOPE AND APPLICATION

1.1 This method[1,2,3] is applicable to the determination of the following analytes in finished drinking water, drinking water during intermediate stages of treatment, and the raw source water:

Analyte	Chemical Abstract Services Registry Number
Alachlor	15972-60-8
Aldrin	309-00-2
Atrazine	1912-24-9
Chlordane	57-74-9
alpha-Chlorodane	5103-71-9
gamma-Chlorodane	5103-74-2
Dieldrin	60-57-1
Endrin	72-20-8
Heptachlor	76-44-8
Heptachlor Epoxide	1024-57-3
Hexachlorobenzene	118-74-1
Hexachlorocyclopentadiene	77-74-4
Lindane	58-89-9
Methoxychlor	72-43-5
cis-Nonachlor	5103-73-1
trans-Nonachlor	39765-80-5
Simazine	122-34-9
Toxaphene	8001-35-2
Aroclor 1016	12674-11-2
Aroclor 1221	11104-28-2
Aroclor 1232	11141-16-5
Aroclor 1242	53469-21-9
Aroclor 1248	12672-29-6
Aroclor 1254	11097-69-1
Aroclor 1260	11096-82-5

1.2 The analyst must demonstrate the applicability of the method by collecting precision and accuracy data on fortified samples (i.e., groundwater, tap water)[4] and provide qualitative confirmation of results by Gas Chromatography/Mass Spectrometry (GC/MS)[5], or by GC analysis using dissimilar columns.

1.3 Method detection limits (MDL)[6] for the above organohalides and Aroclors have been experimentally determined (Section 13.2). Actual detection limits are highly dependent upon the characteristics of the gas chromatographic system used (e.g., column type, age, and proper conditioning; detector condition; and injector mode and condition).

1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of GC 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 11.0.

2.0 SUMMARY OF METHOD

2.1 Thirty-five mL of sample are extracted with 2 mL of hexane. One to 2 µL of the extract are then injected into a gas chromatograph equipped with a linearized electron capture detector for separation and analysis. Analytes are quantitated using procedural standard calibration (Section 3.12).

2.2 The extraction and analysis time is 40-70 minutes per sample depending upon the analytes and the analytical conditions chosen. (See Section 6.9).

3.0 DEFINITIONS

3.1 Laboratory Duplicates (LD1 and LD2) -- Two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.2 Field Duplicates (FD1 and FD2) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.3 Laboratory Reagent Blank (LRB) -- An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.4 Field Reagent Blank (FRB) -- Reagent water placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.5 Laboratory Performance Check Solution (LPC) -- A solution of method analytes, surrogate compounds, and internal standards used to evaluate the performance of the instrument system with respect to a defined set of method criteria.

3.6 Laboratory Fortified Blank (LFB) -- An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements at the required method detection limit.

3.7 Laboratory Fortified Sample Matrix (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.8 Stock Standard Solution -- A concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.

3.9 Primary Dilution Standard Solution (PDS) -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.10 Calibration Standard (CAL) -- A solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.11 Quality Control Sample (QCS) -- A sample matrix containing method analytes or a solution of method analytes in a water miscible solvent which is used to fortify reagent water or environmental samples. The QCS is obtained from a source external to the laboratory, and is used to check laboratory performance with externally prepared test materials.

3.12 Procedural Standard Calibration -- A calibration method where aqueous calibration standards are prepared and processed (e.g., purged, extracted, and/or derivatized) in exactly the same manner as a sample. All steps in the process from addition of sampling preservatives through instrumental analyses are included in the calibration. Using procedural standard calibration compensates for any inefficiencies in the processing procedure.

4.0 INTERFERENCES

4.1 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 9.2.

4.1.1 Glassware must be scrupulously cleaned[2]. Clean all glass- ware 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 one hour. Do not heat volumetric ware. Thermally stable materials, such as PCBs, might not be eliminated by this treatment. Thorough rinsing with acetone 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.

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

Warning: When a solvent is purified, stabilizers put into the solvent by the manufacturer are removed thus potentially making the solvent hazardous. Also, when a solvent is purified, preservatives put into the solvent by the manufacturer are removed thus potentially reducing the shelf-life.

4.2 Interfering contamination may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. Between-sample rinsing of the sample syringe and associated equipment with hexane can minimize sample cross contamination. After analysis of a sample containing high concentrations of analytes, one or more injections of hexane should be made to ensure that accurate values are obtained for the next sample.

4.3 Matrix interferences may be caused by contaminants that are coextract- ed from the sample. Also, note that all the analytes listed in the scope and application section are not resolved from each other on any one column, i.e., one analyte of interest may be an interferant for another analyte of interest. The extent of matrix interferences will vary considerably from source to source, depending upon the water sampled. Cleanup of sample extracts may be necessary. Analyte identifications should be confirmed (Section 11.4).

4.4 It is important that samples and working standards be contained in the same solvent. The solvent for working standards must be the same as the final solvent used in sample preparation. If this is not the case, chromatographic comparability of standards to sample may be affected.

4.5 Caution must be taken in the determination of endrin since it has been reported that the splitless injector may cause endrin degradation[7]. The analyst should be alerted to this possible interference resulting in an erratic response for endrin.

4.6 Variable amounts of pesticides and commercial PCB products from aqueous solutions adhere to glass surfaces. It is recommended that sample transfers and glass surface contacts be minimized, and that adequate rinsing of glass surfaces be performed.

4.7 Aldrin, hexachlorocyclopentadiene, and methoxychlor are rapidly oxidized by chlorine. Dechlorination with sodium thiosulfate at time of collection will stop further oxidation of these compounds.

Warning: An interfering, erratic peak has been observed within the retention window of heptachlor during many analyses of reagent, tap, and groundwater. It appears to be related to dibutyl phthalate; however, the specific source has not yet been definitively determined. The observed magnitude and character of this peak randomly varies in numerical value from successive injections made from the same vial.

5.0 SAFETY

5.1 The toxicity and carcinogenicity of chemicals used in this method have not been precisely defined; each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining awareness of OSHA regulations regarding safe handling of chemicals used in this method. Additional references to laboratory safety are available[8-10] for the information of the analyst.

5.2 The following organohalides have been tentatively classified as known or suspected human or mammalian carcinogens: aldrin, commercial PCB products, chlordane, dieldrin, heptachlor, hexachlorobenzene, and toxaphene. Pure standard materials and stock standard solutions of these compounds should be handled in a hood or glovebox.

Warning: When a solvent is purified, stabilizers put into the solvent by the manufacturer are removed thus potentially making the solvent hazardous.

6.0 **EQUIPMENT AND SUPPLIES** (All specifications are suggested. Catalog numbers are included for illustration only.)

6.1 Sample Containers -- 40 mL screw cap vials (Pierce #13075 or equivalent) each equipped with a size 24 cap with a flat, disc-like TFE facing backed with a polyethylene film/foam extrusion (Fisher #02-883-3F or equivalent). Prior to use, wash vials and septa with detergent and rinse with tap and distilled water. Allow the vials and septa to air dry at room temperature, place the vials in a 400°C oven for one hour, then remove and allow to cool in an area known to be free of organics.

6.2 Vials -- auto sampler, screw cap with septa, 1.8 mL, Varian #96-000099-00 or equivalent or any other autosampler vials not requiring more than 1.8 mL sample volumes.

6.3 Auto Sampler -- Hewlett-Packard 7671A, or equivalent.

6.4 Micro Syringes -- 10 µL and 100 µL.

6.5 Micro Syringe -- 25 µL with a 2 inch by 0.006 inch needle - Hamilton 702N or equivalent.

6.6 Pipettes -- 2.0 mL and 5.0 mL transfer.

6.7 Volumetric Flasks -- 10 mL and 100 mL, glass stoppered.

6.8 Standard Solution Storage Containers -- 15 mL bottles with PTFE-lined screw caps.

6.9 Gas Chromatograph -- Analytical system complete with temperature programmable GC and split/splitless injector suitable for use with capillary columns and all required accessories including syringes, analytical columns, gases, a linearized electron capture detector and stripchart recorder. A data system is recommended for measuring peak areas. Table 1 lists retention times observed for method analytes using the columns and analytical conditions described below.

6.9.1 Three gas chromatographic columns are recommended. Column 1 (Section 6.9.2) should be used as the primary analytical column unless routinely occurring analytes are not adequately resolved. Validation data presented in this method were obtained using this column. Columns 2 and 3 are recommended for use as confirmatory columns when GC/MS confirmation is not available. Alternative columns may be used in accordance with the provisions described in Section 9.4.

6.9.2 Column 1 (primary column) - 0.32 mm ID x 30 M long fused silica capillary with chemically bonded methyl polysiloxane phase (DB-1, 1.0 µm film, or equivalent). Helium carrier gas flow is about 25 cm/sec. linear velocity, measured at 180° with 9 psi column head pressure. The oven

temperature is programmed from 180-260°C at 4°C/min. and held at 260°C until all expected compounds have eluted. Injector temperature: 200°C. Splitless Mode: 0.5 minute. Detector temperature: 290°C. Sample chromatograms for selected pesticides are presented in Figures 1 and 2. Chromatograms of the Aroclors, toxaphene, and technical chlordane are presented in Figures 3 through 11.

6.9.3 Column 2 (alternative Column 1) - 0.32mm ID x 30 M long fused silica capillary with a 1:1 mixed phase of dimethyl silicone and polyethylene glycol (Durawax-DX3, 0.25 µm film, or equivalent). Helium carrier gas flow is about 25 cm/sec. linear velocity and oven temperature is programmed from 100-210°C at 8°C/min., and held at 210°C until all expected compounds have eluted. Then the post temperature is programmed to 240°C at 8°C/min. for five minutes.

6.9.4 Column 3 (alternative Column 2) - 0.32mm ID x 25 M long fused silica capillary with chemically bonded 50:50 Methyl-Phenyl silicone (OV-17, 1.5 µm film thickness, or equivalent). Helium carrier gas flow is about 40 cm/sec. linear velocity and oven temperature is programmed from 100-260°C at 4°C/min. and held at 260°C until all expected compounds have eluted.

7.0 REAGENTS AND STANDARDS

Warning: When a solvent is purified, stabilizers put into the solvent by the manufacturer are removed thus potentially making the solvent hazardous. Also, when a solvent is purified, preservatives put into the solvent by the manufacturer are removed thus potentially making the shelf-life short.

7.1 Reagents

7.1.1 Hexane Extraction Solvent -- UV Grade, Burdick and Jackson #216 or equivalent.

7.1.2 Methyl Alcohol, ACS Reagent Grade -- Demonstrated to be free of analytes.

7.1.3 Sodium Chloride, NaCl, ACS Reagent Grade -- For pretreatment before use, pulverize a batch of NaCl and place in a muffle furnace at room temperature. Increase the temperature to 400°C and hold for 30 minutes. Store in a glass (not plastic) bottle to avoid phthalate contamination..

7.1.4 Sodium thiosulfate, $Na_2S_2O_3$, ACS Reagent Grade -- For preparation of solution (0.04 g/mL), mix 1 g of $Na_2S_2O_3$ with reagent water and bring to 25 mL volume in a volumetric flask. Verify the stability of this solution and replace as necessary.

7.2 Reagent Water -- Reagent water is defined as water free of interference when employed in the procedure described herein.

7.2.1 A Millipore Super-Q Water System or its equivalent may be used to generate deionized reagent water.

7.2.2 Test reagent water each day it is used by analyzing it according to Section 9.2.

7.3 Stock Standard Solutions (SSS) -- These solutions may be obtained as certified solutions or prepared from pure standard materials using the following procedures:

7.3.1 Prepare stock standard solutions (5000 µg/mL) by accurately weighing about 0.0500 g of pure material. Dissolve the material in methanol and dilute to volume in a 10 mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

7.3.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

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

7.4 Primary Dilution Standard Solutions (PDS) -- Use stock standard solutions to prepare primary dilution standard solutions that contain the analytes in methanol. The primary dilution standards should be prepared at concentrations that can be easily diluted to prepare aqueous calibration standards (Section 10.2.1) that will bracket the working concentra- tion range. Store the primary dilution standard solutions with minimal headspace and check frequently for signs of deterioration or evaporation, especially just before preparing calibration standards. The storage time described for stock standard solutions in Section 7.3.3 also applies to primary dilution standard solutions.

Note: Primary dilution standards for toxaphene, chlordane and each of the Aroclors must be prepared individually.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Sample Collection

8.1.1 Collect all samples in 40 mL bottles into which 3 mg of sodium thiosulfate crystals have been added to the empty bottles just prior to shipping to the sampling site. Alternately, 75 µL of freshly prepared sodium thiosulfate solution (0.04 g/mL) may be added to empty 40 mL bottles just prior to sample collection.

8.1.2 When sampling from a water tap, open the tap and allow the system to flush until the water temperature has stabilized (usually about 10 minutes). Adjust the flow to about 500 mL/min. and collect samples from the flowing stream.

8.1.3 When sampling from a well, fill a wide-mouth bottle or beaker with sample, and carefully fill 40 mL sample bottles.

8.2 Sample Preservation

8.2.1 The samples must be chilled to 4°C at the time of collection and maintained at that temperature until the analyst is prepared for the extraction process. Field samples that will not be received at the laboratory on the day of collection must be packaged for shipment with sufficient ice to insure that they will be maintained at 4°C until arrival at the laboratory.

8.3 Sample Storage

8.3.1 Store samples and extracts at 4°C until extraction and analysis.

8.3.2 Extract all samples as soon as possible after collection. Results of holding time studies suggest that all analytes with the possible exception of heptachlor were adequately stable for 14 days when stored under these conditions. In general, heptachlor showed inconsistent results. If heptachlor is to be determined, samples should be extracted within seven days of collection. The maximum holding time for all other analytes is 14 days. Analyte stability may be affected by the matrix; therefore, the analyst should verify that the preservation technique is applicable to the samples under study.

8.3.3 Because of the potential for extract volume loss due to evaporation, it is recommended that extracts be analyzed immediately after preparation. If this is not possible, extracts may be stored at 4°C or less in tightly capped vials (Section 6.2) for up to 24 hours.

9.0 QUALITY CONTROL

9.1 Minimum quality control (QC) requirements are initial demonstration of laboratory capability, analysis of laboratory reagent blanks (LRB), laboratory fortified blanks (LFB), laboratory fortified sample matrix (LFM), and quality control samples (QCS). A MDL for each analyte must also be determined.

9.2 Laboratory Reagent Blanks (LRB) -- Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is extracted or reagents are changed, an LRB must be analyzed. If within the retention time window of any analyte the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

9.3 Initial Demonstration of Capability

9.3.1 Select a representative fortified concentration (about 10 times MDL or at a concentration in the middle of the calibration range established in Section 10.0) for each analyte. Prepare a standard concentrate containing each analyte at 1000 times the selected concentration. With a syringe, add 35 µL of the concentrate to each of four to seven 35 mL aliquots of reagent water, and analyze each aliquot according to procedures beginning in Section 11.0.

9.3.2 For each analyte the mean recovery value for these samples must fall in the range of ±30% of the fortified amount. The RSD for these measurements must be 20% or less. For those compounds that meet the acceptance criteria, performance is considered acceptable. For those compounds that fail these criteria, this procedure must be repeated using fresh replicate samples until satisfactory performance has been demonstrated.

9.3.3 For each analyte, determine the MDL. Prepare a minimum of seven LFBs at a low concentration. Use calibration data obtained in Section 10.0 to estimate a concentration for each analyte that will produce a peak with a three to five times signal to noise response. Extract and analyze each replicate according to Sections 11.0 and 12.0. It is recommended that these LFBs be prepared and analyzed over a period of several days, so that day to day precision is reflected in the precision measurements. Calculate mean recovery and standard deviation for each analyte. Use the equation given in Section 13.0 to calculate the MDL.

9.3.4 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. It is expected that as laboratory personnel gain experience with this method the quality of data will improve beyond those required here.

9.4 The analyst is permitted to modify GC columns, GC conditions, concentration techniques (i.e., evaporation techniques), internal standards or surrogate compounds. Each time such method modifications are made, the analyst must repeat the procedures in Section 9.3.

9.5 Assessing Laboratory Performance -- Laboratory Fortified Blank (LFB)

9.5.1 The laboratory must analyze at least one LFB per sample set (all samples extracted within a 24-hour period). If the sample set contains more than 20 samples, analyze one LFB for every 20 samples. Ideally the fortifying concentration of each analyte in the LFB sample should be the same as that selected in Section 9.3.1. Calculate accuracy as percent recovery (X_i). If the recovery of any analyte falls outside the control limits (see Section 9.5.2), that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses. Because this LFB is prepared and analyzed in the same way as a calibration verification standard, it can be used to satisfy the calibration requirement in Section 10.2.3.

Note: It is suggested that one multi-component analyte (toxaphene, chlordane or an Aroclor) LFB also be analyzed with each sample set. By selecting a different multi-component analyte for this LFB each work shift, LFB data can be obtained for all of these analytes over the course of several days

9.5.2 Until sufficient data become available from within their own laboratory, usually a minimum of results from 20-30 analyses, the laboratory may assess laboratory performance against the control limits in Section 9.3.2 that are derived from the data in Table 2. When sufficient internal performance data becomes available, develop control limits from the mean percent recovery ($\bar{X}$) and standard deviation (S) of the percent recovery. These data are used to establish upper and lower control limits as follows:

UPPER CONTROL LIMIT = $\bar{X}$ + 3S
LOWER CONTROL LIMIT = $\bar{X}$ - 3S

After each five to 10 new recovery measurements, new control limits should be calculated using only the most recent 20-30 data points. These calculated control limits should not exceed the fixed limits in Section 9.3.2.

9.5.3 It is recommended that the laboratory periodically determine and document its detection limit capabilities for analytes of interest.

Caution: No attempts to establish low detection limits should be made before instrument optimization and adequate conditioning of both the column and the GC system. Conditioning includes the processing of LFB and LFM samples containing moderate concentration levels of these analytes.

9.5.4 At least quarterly the laboratory should analyze quality control samples (QCS). If acceptance criteria are not met, corrective action should be taken and documented.

9.6 Assessing Analyte Recovery -- Laboratory Fortified Sample Matrix (LFM)

9.6.1 The laboratory must add a known concentration to a minimum of 10% of the routine samples or one LFM per set, whichever is greater. The fortified concentration should not be less than the background concentration of the sample selected for fortification. Ideally the LFM concentration should be the same as that used for the LFB (Section 9.3.1). Periodically, samples from all routine sample sources should be fortified.

9.6.2 Calculate the percent recovery (R_i) for each analyte, corrected for background concentrations measured in the unfortified sample.

9.6.3 If the recovery of any such analyte falls outside the range of ±35% of the fortified amount, and the laboratory performance for that analyte is shown to be in control (Section 9.5), the recovery problem encountered with the dosed sample is judged to be matrix related, not system related. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.7 The laboratory may adopt additional quality control 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. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation and storage.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Establish GC operating parameters equivalent to those indicated in Section 6.9.

Warning: Endrin is easily degraded in the injection port if the injection port or front of the column is dirty. This is the result of buildup of high boiling residue from sample injection. Check for degradation problems daily by injecting a mid-level standard containing only endrin. Look for the degradation products of endrin (endrin ketone and endrin aldehyde). If degradation of endrin exceeds

20%, take corrective action before proceeding with calibration. Calculate percent breakdown as follows:

$$\frac{\text{Total endrin degradation peak area (endrin aldehyde + endrin ketone)}}{\text{Total endrin peak area (endrin + endrin aldehyde + endrin ketone)}} \times 100\%$$

10.2 At least three calibration standards are needed; five are recommended. Guidance on the number of standards is as follows: A minimum of three calibration standards are required to calibrate a range of a factor of 20 in concentration. For a factor of 50 use at least four standards, and for a factor of 100 at least five standards. The lowest standard should represent analyte concentrations near, but above, their respective MDLs. The remaining standards should bracket the analyte concentrations expected in the sample extracts, or should define the working range of the detector.

10.2.1 To prepare a calibration standard (CAL), add an appropriate volume of a secondary dilution standard to a 35 mL aliquot of reagent water in a 40 mL bottle. Do not add less than 20 µL of an alcoholic standard to the reagent water. Use a 25 µL micro syringe and rapidly inject the methanolic standard into the middle point of the water volume. Remove the needle as quickly as possible after injection. Mix by inverting and shaking the capped bottle several times. Aqueous standards must be prepared fresh daily.

10.2.2 Starting with the standard of lowest concentration, prepare, extract, and analyze each calibration standard beginning with Section 11.2 and tabulate peak height or area response versus the concentration in the standard. The results are to be used to prepare a calibration curve for each compound by plotting the peak height or area response versus the concentration. Alternatively, if the ratio of concentration to response (calibration factor) is a constant over the working range (20% RSD or less), linearity to the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

Note: Toxaphene, chlordane, and Aroclor standards must be injected separately. See Section 12.2 for information on quantitation of multi-component analytes.

10.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of a minimum of two calibration check standards, one at the beginning and one at the end of the analysis day. These check standards should be at two different concentration levels to verify the calibration curve. For extended periods of analysis (greater than eight hours), it is strongly recommended that check standards be interspersed with samples at regular intervals during the course of the analyses. If the response for any analyte varies from the

predicted response by more than the criteria in Section 9.3.2, the test must be repeated using a fresh calibration standard. If the results still do not agree, generate a new calibration curve. For those analytes that failed the calibration verification, results from field samples analyzed since the last passing calibration should be considered suspect. Reanalyze sample extracts for these analytes after acceptable calibration is restored.

Note: It is suggested that a calibration verification standard of one multi-component analyte, either chlordane, toxaphene or an Aroclor also be analyzed each work shift. By selecting a different multi-component analyte for this calibration verification each work shift, continuing calibration data can be obtained for all of these analytes over the course of several days.

10.3 Instrument Performance -- Check the performance of the entire analytical system daily using data gathered from analyses of laboratory reagent blanks (LRB), (CAL), and laboratory duplicate samples (LD1 and LD2).

10.3.1 Significant peak tailing in excess of that shown for the target compounds in the method chromatograms (Figures 1-11) must be corrected. Tailing problems are generally traceable to active sites on the GC column, improper column installation, or operation of the detector.

10.3.2 Check the precision between replicate analyses. Poor precision is generally traceable to pneumatic leaks, especially at the injection port. If the GC system is apparently performing acceptably but with decreased sensitivity, it may be necessary to generate a new curve or set of calibration factors to verify the decreased responses before searching for the source of the problem.

10.3.3 Observed relative area responses of endrin (Section 10.1) must meet the following general criteria:

10.3.3.1 The breakdown of endrin into its aldo and keto forms must be adequately consistent during a period in which a series of analyses is made. Equivalent relative amounts of breakdown should be demonstrated in the LRB, LFB, CAL, and QCS. Consistent break- down resulting in these analyses would suggest that the breakdown occurred in the instrument system and that the methodology is in control.

10.3.3.2 Analyses of laboratory fortified matrix (LFM) samples must also be adequately consistent after corrections for potential background concentrations are made.

11.0 PROCEDURE

11.1 Sample Preparation

11.1.1 Remove samples from storage and allow them to equilibrate to room temperature.

11.1.2 Remove the container caps. Withdraw and discard a 5 mL volume using a 10 mL graduated cylinder. Replace the container caps and weigh the containers with contents to the nearest 0.1 g and record these weights for subsequent sample volume determinations (Section 11.3).

11.2 Extraction and Analysis

11.2.1 Remove the container cap of each sample, and add 6 g NaCl (Section 7.1.3) to the sample bottle. Using a class A, TD transfer or automatic dispensing pipet, add 2.0 mL of hexane. Recap and shake vigorously by hand for one minute. Invert the bottle and allow the water and hexane phases to separate.

11.2.2 Remove the cap and carefully transfer approximately 0.5 mL of hexane layer into an autosampler vial using a disposable glass pipet.

11.2.3 Transfer the remaining hexane phase, being careful not to include any of the water phase, into a second autosampler vial. Reserve this second vial at 4°C for reanalysis if necessary.

11.2.4 Transfer the first sample vial to an autosampler set up to inject 1-2 µL portions into the gas chromatograph for analysis (See Section 6.9 for GC conditions). Alternately, 1-2 µL portions of samples, blanks, and standards may be manually injected, although an autosampler is strongly recommended.

11.3 Determination of Sample Volume in Bottles Not Calibrated

11.3.1 Discard the remaining sample/hexane mixture from the sample bottle. Shake off the remaining few drops using short, brisk wrist movements.

11.3.2 Reweigh the empty container with original cap and calculate the net weight of sample by difference to the nearest 0.1 g (Section 11.1.2 through Section 11.3.2). This net weight (in grams) is equivalent to the volume (in mL) of water extracted (Section 12.4). Alternatively, by using 40 mL bottles precalibrated at 35 mL levels, the gravimetric steps can be omitted, thus increasing the speed and ease of this extraction process.

11.4 Identification of Analytes

11.4.1 Identify a sample component by comparison of its retention time to the retention time of a reference chromatogram. If the retention time of an unknown compound corresponds, within limits, to the retention time of a standard compound, then identification is considered positive.

11.4.2 The width of the retention time window used to make identifi- cations 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.

11.4.3 Identification requires expert judgement when sample compo- nents are not resolved chromatographically. When peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima), or any time doubt exists over the identification of a peak on a chromatogram, appropriate alternative techniques to help confirm peak identification need be employed. For example, more positive identification may be made by the use of an alternative detector which operates on a chemical/physical principle different from that originally used, e.g., mass spectrometry, or the use of a second chromatography column. Suggested alternative columns are described in Section 6.9.

Note: Identify multi-component analytes by comparison of the sample chromatogram to the corresponding calibration standard chromatograms of chlordane, toxaphene and the Aroclors. Identification of multi-component analytes is made by pattern recognition, in which the experience of the analyst is an important factor.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Identify the organohalides in the sample chromatogram by comparing the retention time of the suspect peak to retention times generated by the calibration standards and the laboratory fortified blanks. Identify the multicomponent compounds using all peaks that are characteristic of the specific compound from chromatograms generated with individual standards.

12.2 To quantitate multi-component analytes, one of the following methods should be used.

Option 1 - Calculate an average response factor or linear regression equation for each multi-component analyte using the combined area of all the component peaks in each of the calibration standard chromatograms.

Option 2 - Calculate an average response factor or linear regression equation for each multi-component analyte using the combined areas of three to six of the most intense and reproducible peaks in each of the calibration standard chromatograms.

When quantifying multi-component analytes in samples, the analyst should use caution to include only those peaks from the sample that are attributable to the multi-component analyte. Option 1 should not be used if there are significant interference peaks within the chlordane, Aroclor or toxaphene pattern.

12.3 Use the multi-point calibration curve or calibration factor (Section 10.2.3) to directly calculate the uncorrected concentration (Ci) of each analyte in the sample (e.g., calibration factor x response). Do not use the daily calibration standard to quantitate method analytes in samples. If any analyte response exceeds the calibration range, dilute the extract and reanalyze.

12.4 Calculate the sample volume (V_s) as equal to the net sample weight:

V_s = gross weight (Section 11.1.2) - bottle tare (Section 11.3.2).

12.5 Calculate the corrected sample concentration as:

$$\text{Concentration, } \mu g/L = \frac{35\ (C_i)}{(V_s)}$$

12.6 Results should be reported with an appropriate number of significant figures. Experience indicates that three significant figures may be used for concentrations above 99 µg/L, two significant figures for concentrations between 1-99 µg/L, and one significant figure for lower concentrations.

13.0 METHOD PERFORMANCE

13.1 Single laboratory (NERL-Cincinnati) accuracy and precision at several concentrations in reagent, ground, and tap water matrices are presented in Table 2. These results were obtained from data generated with a DB-1 column, and with quantitation Option 2 as described in Section 12.2.

13.2 With these data, the method detection limits (MDL) in Table 2 were calculated using the formula:

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

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

S = standard deviation of replicate analyses.

13.3 This method has been tested by 10 laboratories using reagent water and groundwater fortified at three concentration levels. Single operator precision, overall precision, and method accuracy were found to be directly related to the concentration of the analyte and virtually independent of the sample matrix. Linear equations to describe the relationships are presented in Table 3.

14.0 POLLUTION PREVENTION

14.1 This method utilizes a microextraction procedure that requires the use of very small volumes of hexane, thus making this method safe for use by the laboratory analyst and harmless to the environment. For information concerning pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations, and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

15.0 WASTE MANAGEMENT

15.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing the waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel," also available from the American Chemical Society at the address in Section 14.1.

16.0 REFERENCES

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4. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories", EPA-600/4-79-019, U. S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.

5. Munch, J. W. "Method 525.2-Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Chromatography/Mass Spectrometry" in Methods for the Determination of Organic Compounds in Drinking Water; Supplement 3 (1995). USEPA, National Exposure Research Laboratory, Cincinnati, Ohio 45268.

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7. Bellar, T.A., Stemmer, P., Lichtenberg, J.J. "Evaluation of Capillary Systems for the Analysis of Environmental Extracts", EPA-600/S4-84-004, March 1984.

8. "Carcinogens-Working with Carcinogens", Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute of Occupational Safety and Health, Publication No. 77-206, August 1977.

9. "OSHA Safety and Health Standards, General Industry", (29CFR1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).

10. "Safety in Academic Chemistry Laboratories", American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. RETENTION TIMES FOR METHOD ANALYTES

Analyte	Retention Time[a], Min		
	Primary	Confirm. 1	Confirm. 2
Hexachlorocyclopentadiene	5.5	6.8	5.2
Simazine	10.9	25.7	19.9
Atrazine	11.2	22.6	19.6
Hexachlorobenzene	11.9	13.4	15.6
Lindane	12.3	18.4	18.7
Alachlor	15.1	19.7	21.1
Heptachlor	15.9	17.5	20.0
Aldrin	17.6	18.4	21.4
Heptachlor Epoxide	19.0	24.6	24.6
gamma-Chlordane	19.9	25.9	26.0
alpha-Chlordane	20.9	26.6	26.6
trans-Nonachlor	21.3	24.8	26.3
Dieldrin	22.1	45.1	27.8
Endrin	23.2	33.3	29.2
cis-Nonachlor	24.3	39.0	30.4
Methoxychlor	30.0	58.5	36.4

	Primary[b]
Aroclor 1016	13.6, 14.8, 15.2, 16.2, 17.7
Aroclor 1221	7.7, 9.0, 15.9, 19.1, 24.7
Aroclor 1232	11.2, 14.7, 13.6, 15.2, 17.7
Aroclor 1242	11.2, 13.6, 14.7, 15.2, 17.7, 19.8
Aroclor 1248	14.8, 16.2, 17.1, 17.7, 19.8, 22.0
Aroclor 1254	19.1, 21.9, 23.4, 24.9, 26.7
Aroclor 1260	23.4, 24.9, 26.7, 28.2, 29.9, 21.3
Chlordane	15.1, 15.9, 20.1, 20.9, 21.3
Toxaphene	21.7, 22.5, 26.7, 27.2

[a]Columns and analytical conditions are described in Sections 6.9.2 through 6.9.4.
[b]Column and conditions described in Section 6.9.2. More than one peak listed does not indicate the total number of peaks characteristic of the multi-component analyte. Listed peaks indicate only the ones chosen for summation in the quantification.

TABLE 2. SINGLE LABORATORY ACCURACY, PRECISION, AND METHOD DETECTION LIMITS (MDLs) FOR ANALYTES FROM REAGENT WATER, GROUND WATER, AND TAP WATER[a]

			Accuracy and Standard Deviation Data					
			Reagent Water		Ground Water		Tap Water	
Analyte	MDL µg/L	Concentration* µg/L	R[c]	S_R[d]	R	S_R	R	S_R
Alachlor	0.225	0.50	102	13.4	–	–	–	–
Aldrin	0.007	0.05	106	20.0	86	16.3	–	–
Atrazine	2.4	5.0	85	16.2	95	7.3	108	10.9
		20.0	95	5.2	86	9.1	91	3.1
alpha-Chlordane	0.006	0.06	95	3.5	83	4.4	85	7.1
		0.35	86	17.0	94	10.2	91	2.4
gamma-Chlordane	0.012	0.06	95	0.4	86	5.3	83	14.7
		0.35	86	18.5	95	14.5	91	6.0
Chlordane	0.14	0.17	–	8.0	–	–	105	12.4
		3.4	–	3.6	–	–	95	9.6
Dieldrin	0.012	0.10	87	17.1	67	10.1	92	15.7
		3.6	114	9.1	94	8.6	81	14.0
Endrin	0.063	0.10	119	29.8	94	20.2	106	14.0
		3.6	99	6.5	100	11.3	85	12.4
Heptachlor	0.003	0.032	77	10.2	37	6.8	200	22.6
		1.2	80	7.4	71	9.8	106	16.8
Heptachlor Epoxide	0.004	0.04	100	15.6	90	14.2	112	7.5
		1.4	115	6.6	103	6.9	81	5.9
Hexachlorobenzene	0.002	0.003	104	13.5	91	10.9	100	15.6
		0.09	103	6.6	101	4.4	88	13.4
Hexachlorocyclopentadiene	0.13	0.15	73	5.1	87	5.1	191	18.5
		0.35	73	11.7	69	4.8	109	14.3
Lindane	0.003	0.03	91	6.5	88	7.7	103	8.1
		1.2	111	5.0	109	3.4	93	18.4
Methoxychlor	0.96	2.10	100	21.0	–	–	–	–
		7.03	98	10.9	–	–	–	–

TABLE 2. SINGLE LABORATORY ACCURACY, PRECISION, AND METHOD DETECTION LIMITS (MDLs) FOR ANALYTES FROM REAGENT WATER, GROUND WATER, AND TAP WATER[a]

			Accuracy and Standard Deviation Data					
			Reagent Water		Ground Water		Tap Water	
Analyte	MDL µg/L	Concentration* µg/L	R[c]	S_R[d]	R	S	R	S
cis-Nonachlor	0.027	0.06	110	15.2	101	7.2	93	14.3
		0.45	82	21.3	93	18.3	87	5.4
trans-Nonachlor	0.011	0.06	95	9.6	83	7.1	73	4.1
		0.35	86	21.8	94	17.2	86	5.1
Simazine	6.8	25	99	8.3	97	9.2	102	13.4
		60	65	3.6	59	18.0	67	6.2
Toxaphene	1.0	10	–	–	–	–	110	9.5
		80	–	–	–	–	114	13.5
Aroclor 1016	0.08	1.0	–	–	–	–	97	7.5
Aroclor 1221	15.0	180	–	–	–	–	92	9.6
Aroclor 1232	0.48	3.9	–	–	–	–	86	7.3
Aroclor 1242	0.31	4.7	–	–	–	–	96	7.4
Aroclor 1248	0.102	3.6	–	–	–	–	–	–
		3.4	–	–	–	–	84	9.9
Aroclor 1254	0.102	1.8	–	–	–	–	–	–
		1.7	–	–	–	–	85	11.8
Aroclor 1260	0.189	2.0	–	–	–	–	–	–
		1.8	–	–	–	–	88	19.8

[a]Data corrected for amount detected in blank and represents the mean of five to eight samples.
[b]MDL = method detection limit in sample in µg/L; calculated by multiplying standard deviation (S) times the students' t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom.
[c]R = average percent recovery.
[d]S_R = Standard deviation about percent recovery.
*Refers to concentration levels used to generate R and SR data for the three types of water matrices, not for MDL determinations.
–No analyses conducted.

TABLE 3. METHOD ACCURACY AND PRECISION AS FUNCTIONS OF CONCENTRATION-METHOD 505 REAGENT WATER

Parameter	Applicable Conc. Range (µg/L)	Accuracy as Recovery X (µg/L)	Single Analyst Precision S_r (µg/L)	Overall Precision S (µg/L)
Atrazine	(3.06-45.90)	1.122C+0.97	$0.000\bar{x}+1.21$	$0.045\bar{x}+2.23$
Simazine	(12.55-50.20)	0.892C+1.446	$-0.049\bar{x}+3.52$	$0.209\bar{x}+1.23$
Hexachlorobenzene	(0.01-0.37)	1.028C-0.00	$0.108\bar{x}+0.00$	$0.227\bar{x}+0.00$
Lindane	(0.04-1.39)	1.009C-0.00	$0.057\bar{x}+0.01$	$0.142\bar{x}+0.00$
Alachlor	(0.50-37.50)	1.004C-0.08	$0.077\bar{x}+0.10$	$0.105\bar{x}+0.16$
Heptachlor	(0.04-1.41)	1.002C+0.02	$0.107\bar{x}+0.01$	$0.211\bar{x}+0.02$
Aldrin	(0.04-1.42)	1.066C+0.00	$0.031\bar{x}+0.02$	$0.264\bar{x}-0.00$
Heptachlor Epoxide	(0.04-1.42)	0.952C+0.00	$0.032\bar{x}+0.02$	$0.129\bar{x}+0.02$
Dieldrin	(0.10-7.53)	1.027C+0.00	$0.091\bar{x}+0.01$	$0.198\bar{x}+0.02$
Endrin	(0.10-7.50)	0.958C+0.01	$0.116\bar{x}+0.01$	$0.136\bar{x}+0.02$
Methoxychlor	(0.20-15.00)	0.950C+0.15	$0.115\bar{x}+0.12$	$0.125\bar{x}+0.20$
Chlordane	(0.51-50.90)	1.037C+0.06	$0.084\bar{x}+0.06$	$0.125\bar{x}+0.19$
Toxaphene	(5.63-70.40)	1.087C+0.24	$0.131\bar{x}-0.31$	$0.269\bar{x}+0.69$
PCB-1016	(0.50-49.80)	0.856C+0.31	$0.106\bar{x}+0.31$	$0.147\bar{x}+0.45$
PCB-1254	(0.50-50.40)	0.872C-0.01	$0.122\bar{x}+0.11$	$0.281\bar{x}+0.05$

*The concentration range applicable to the multi-laboratory study from which the data was generated.

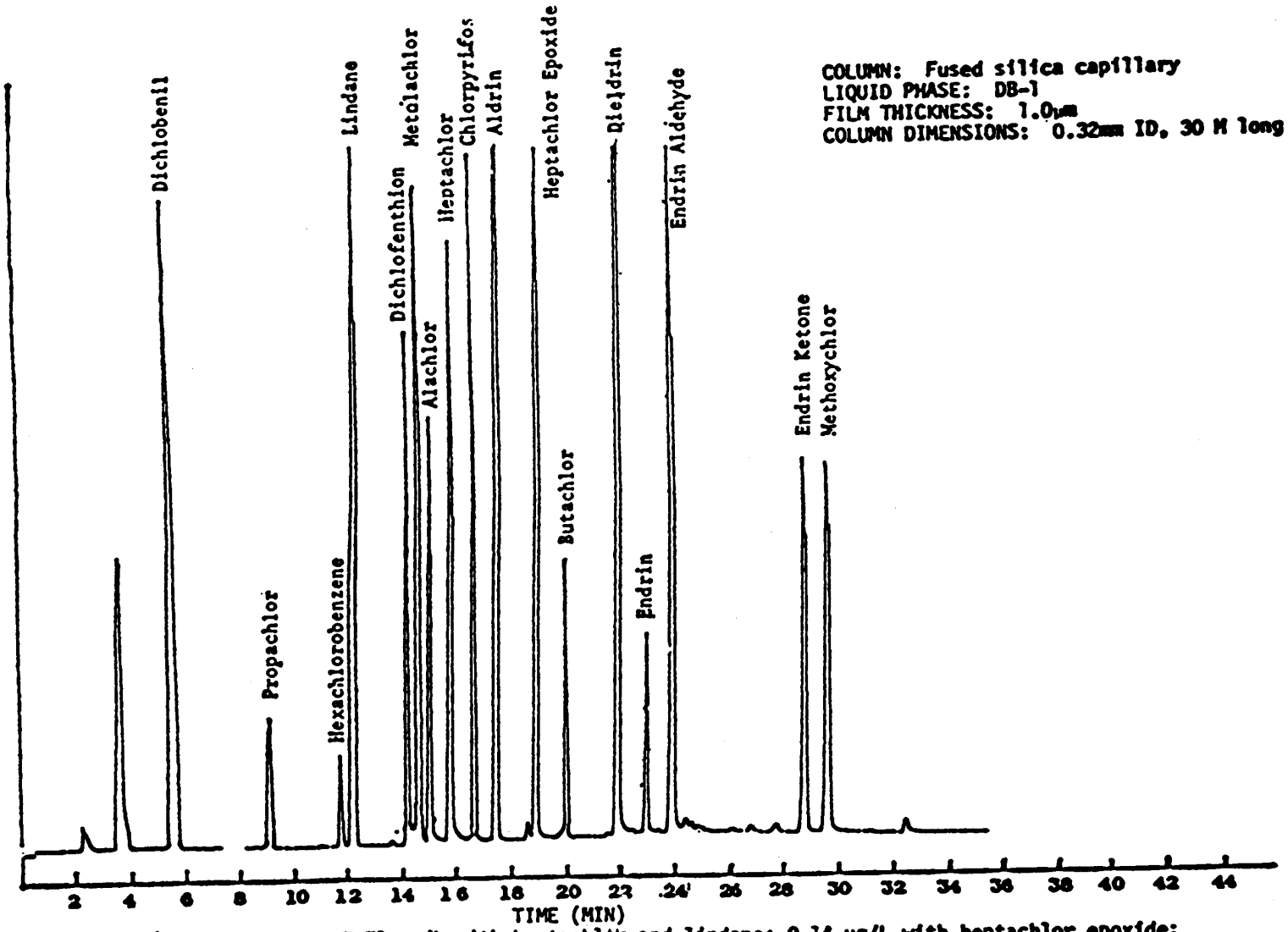

Figure 1. Hexane spiked at 7.71 ug/L with heptachlor and lindane; 9.14 ug/L with heptachlor epoxide; 11.4 ug/L with aldrin and hexachlorobenzene; 23 ug/L with butachlor, chlorpyrifos, chlorpyrifos-methyl, diclobenil, dieldrin, endrin, metolochlor, and propachlor; and 44.9 ug/L with methoxychlor.

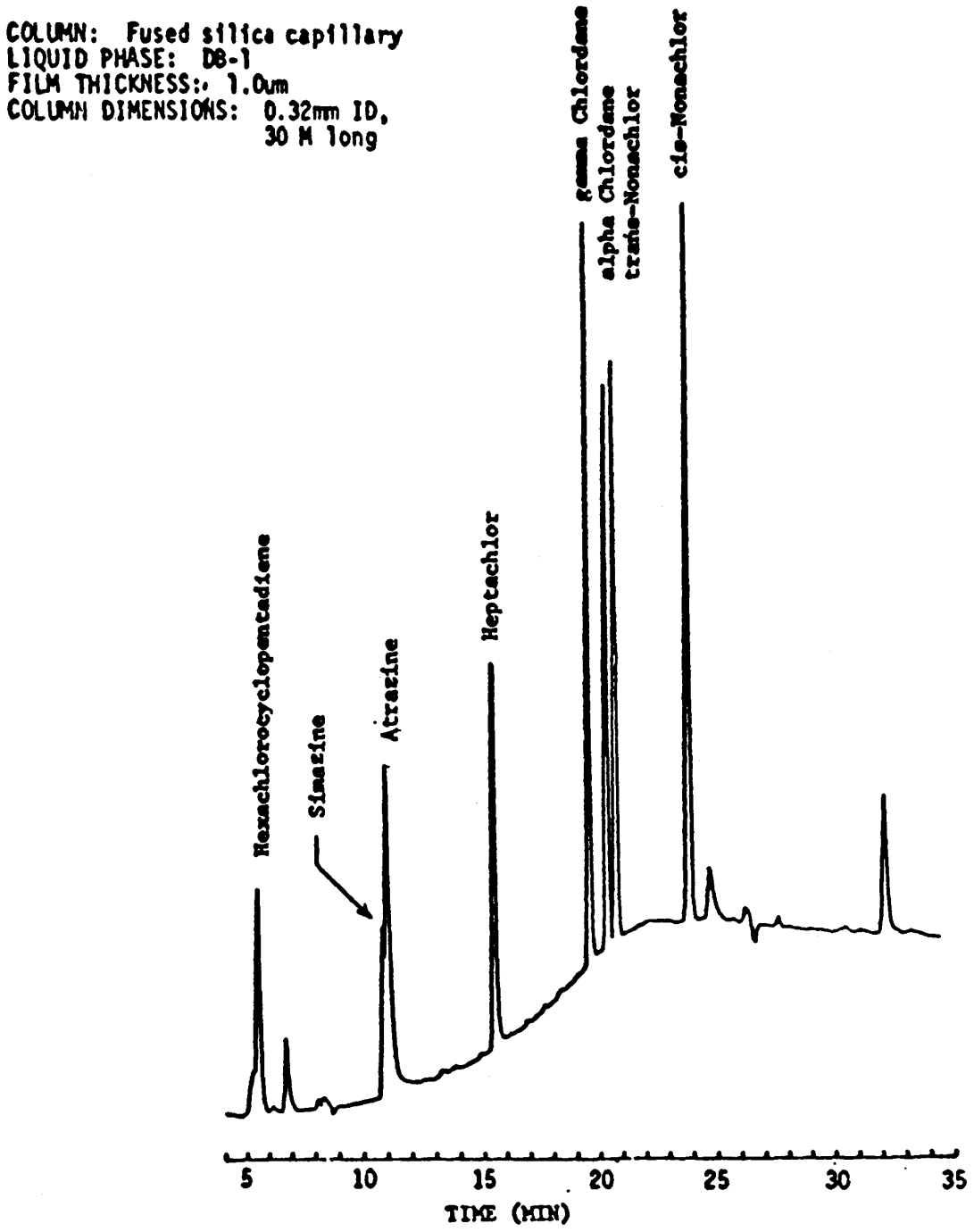

Figure 2. Extract of reagent water spiked at 20 ug/L with atrazine, 60 ug/L with simazine, 0.45 ug/L with cis-nonachlor, and 0.35 ug/L with hexachlorocyclopentadiene, heptachlor, alpha chlordane, gamma chlordane, and trans-nonachlor.

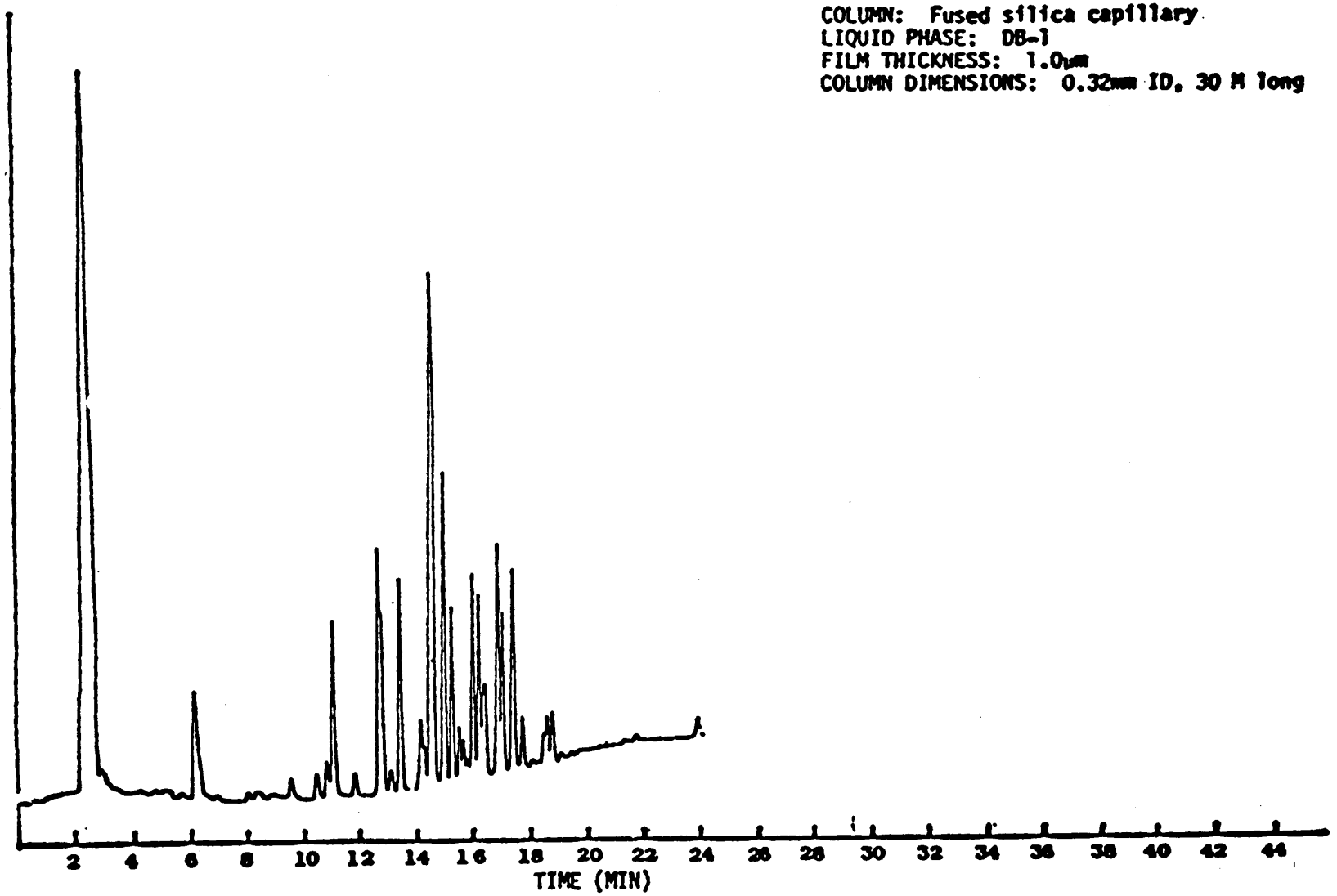

Figure 3. Hexane spiked at 11.4 ug/L with Aroclor 1016.

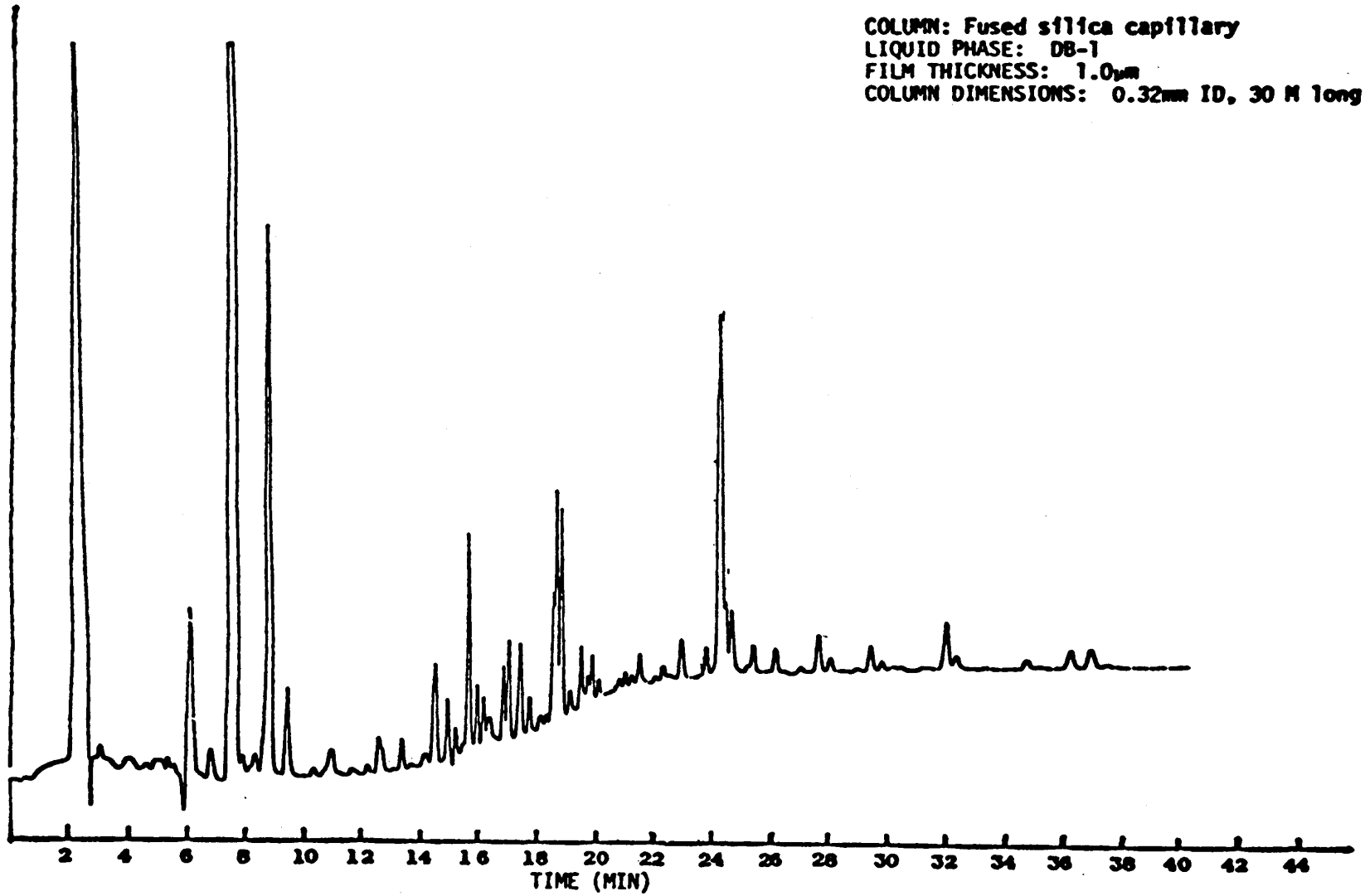

Figure 4. Hexane spiked at 171.4 ug/L with Aroclor 1221.

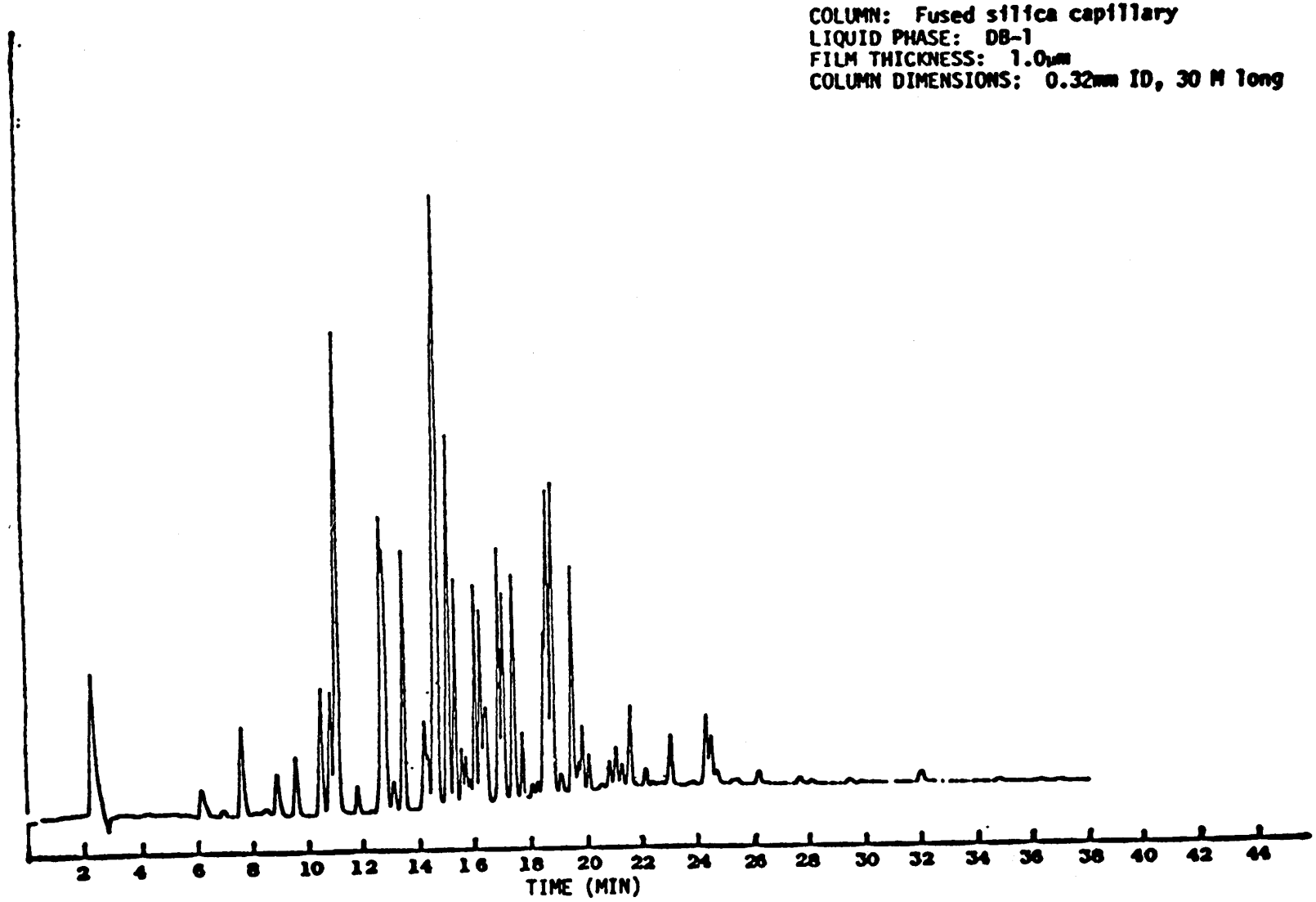

Figure 5. Hexane spiked at 57.1 ug/L with Aroclor 1232.

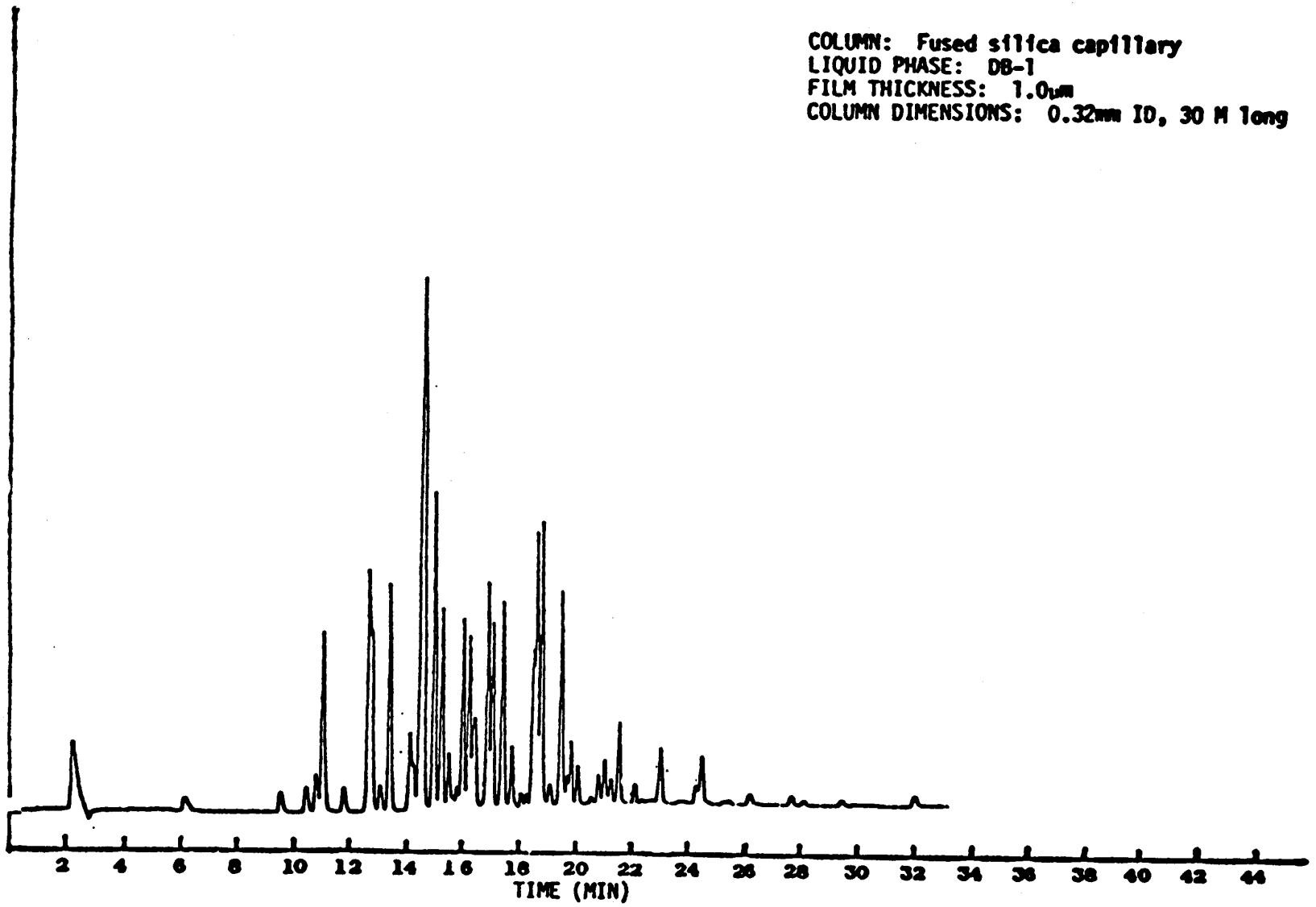

Figure 6. Hexane spiked at 57.1 ug/L with Aroclor 1242.

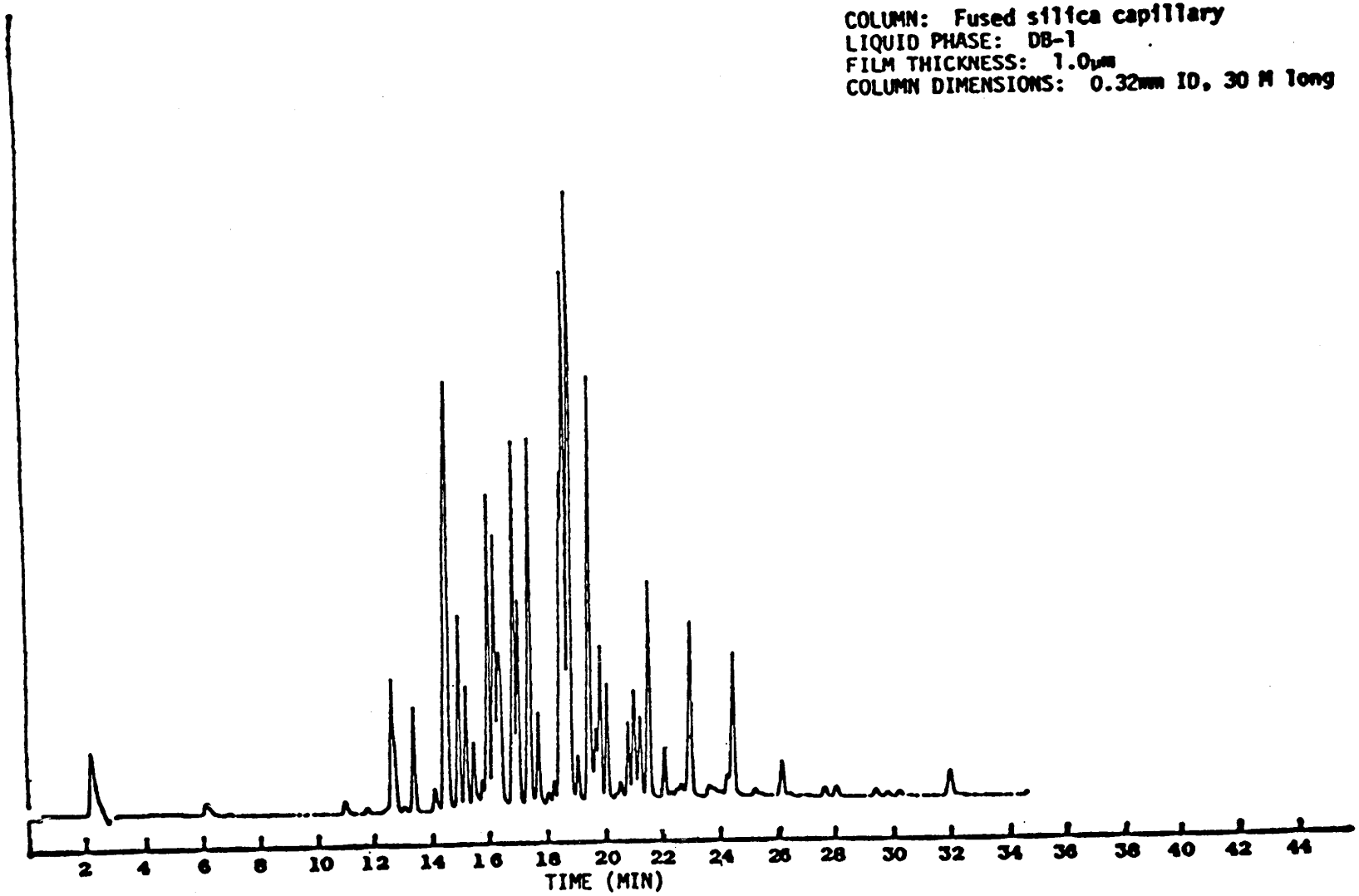

Figure7. Hexane spiked at 57.1 ug/L with Aroclor 1248.

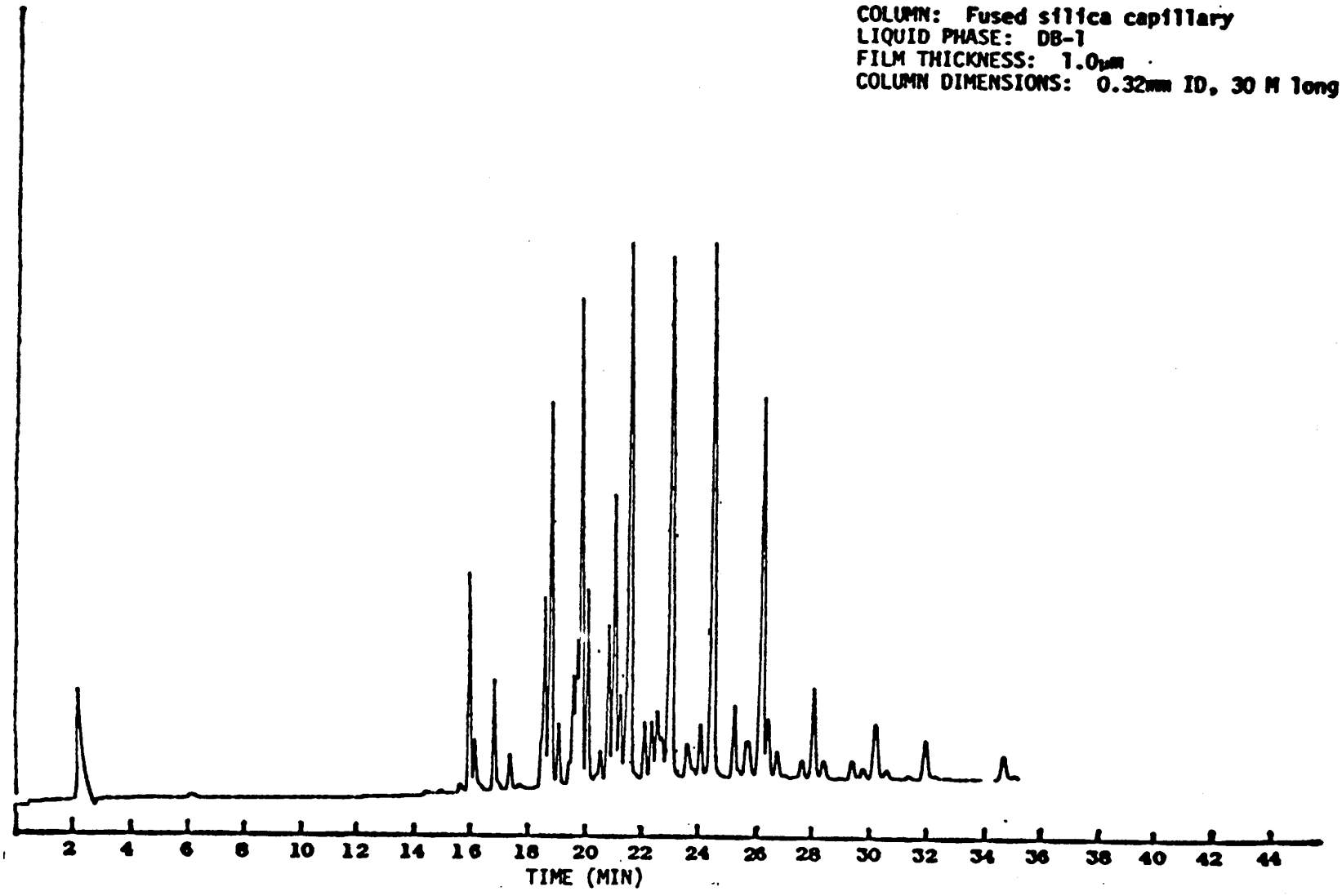

Figure 8. Hexane spiked at 42.9 ug/L with Aroclor 1254.

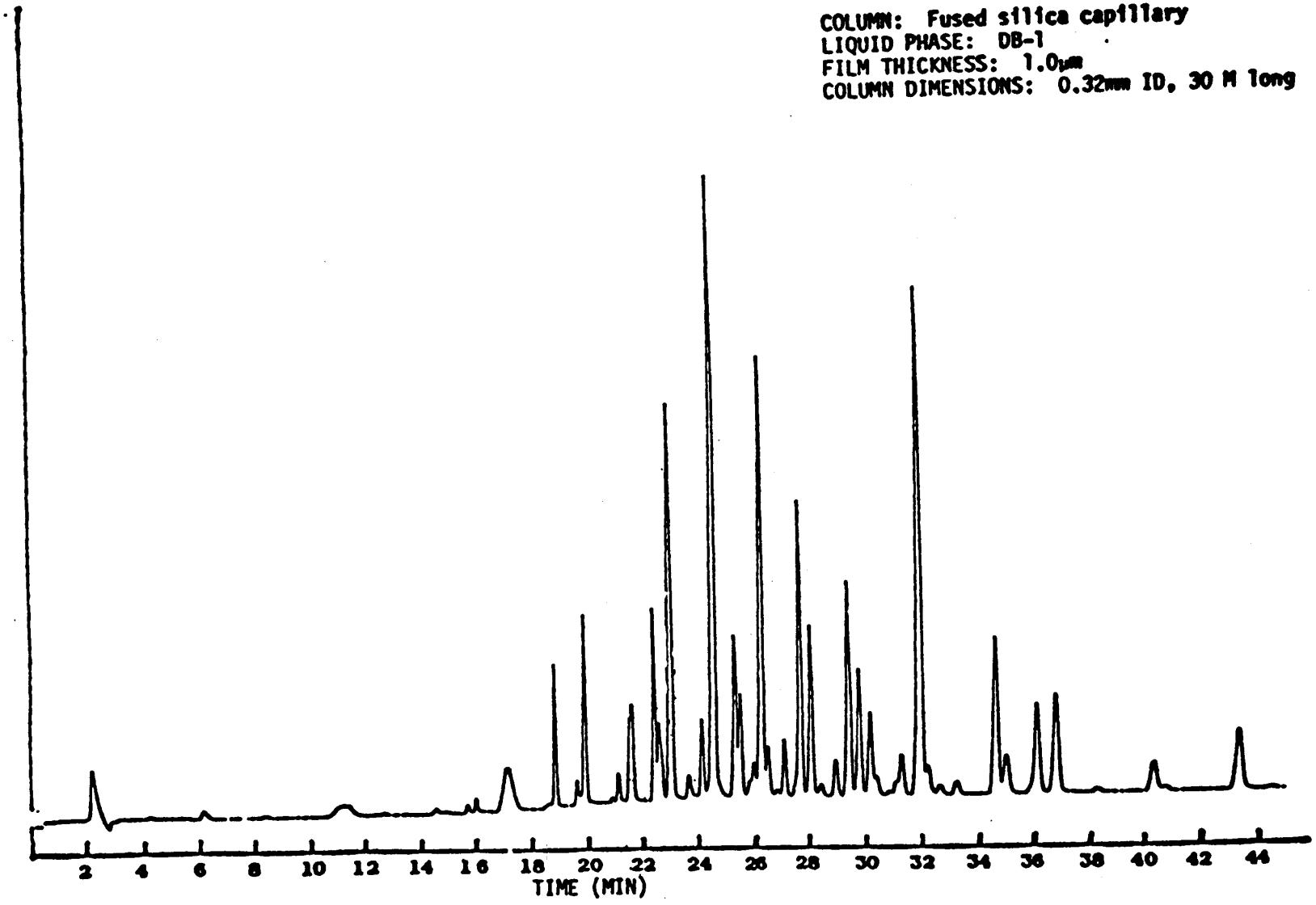

Figure 9. Hexane spiked at 34.3 ug/L with Aroclor 1260.

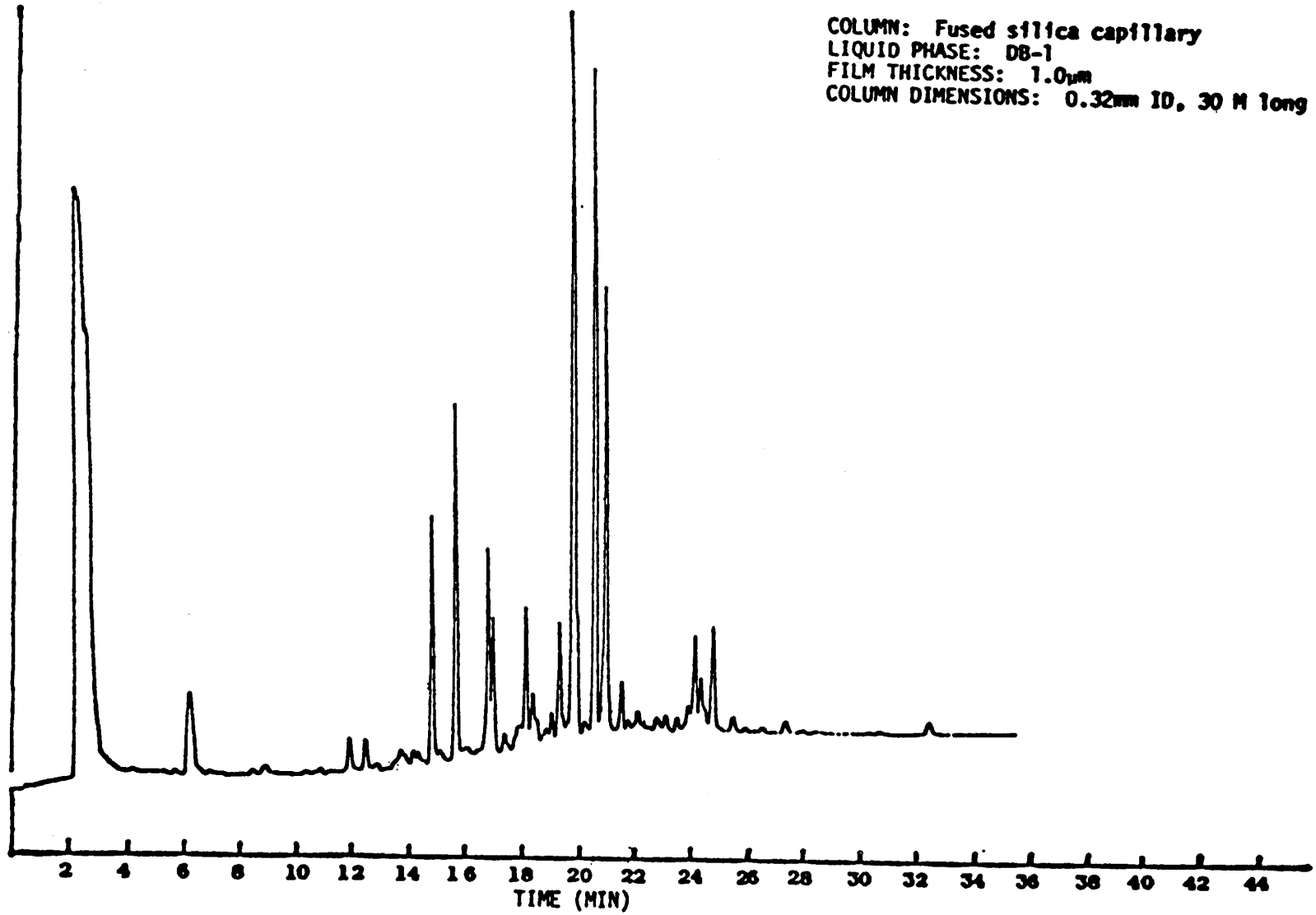

Figure 10. Hexane spiked at 28.6 ug/L with chlordane.

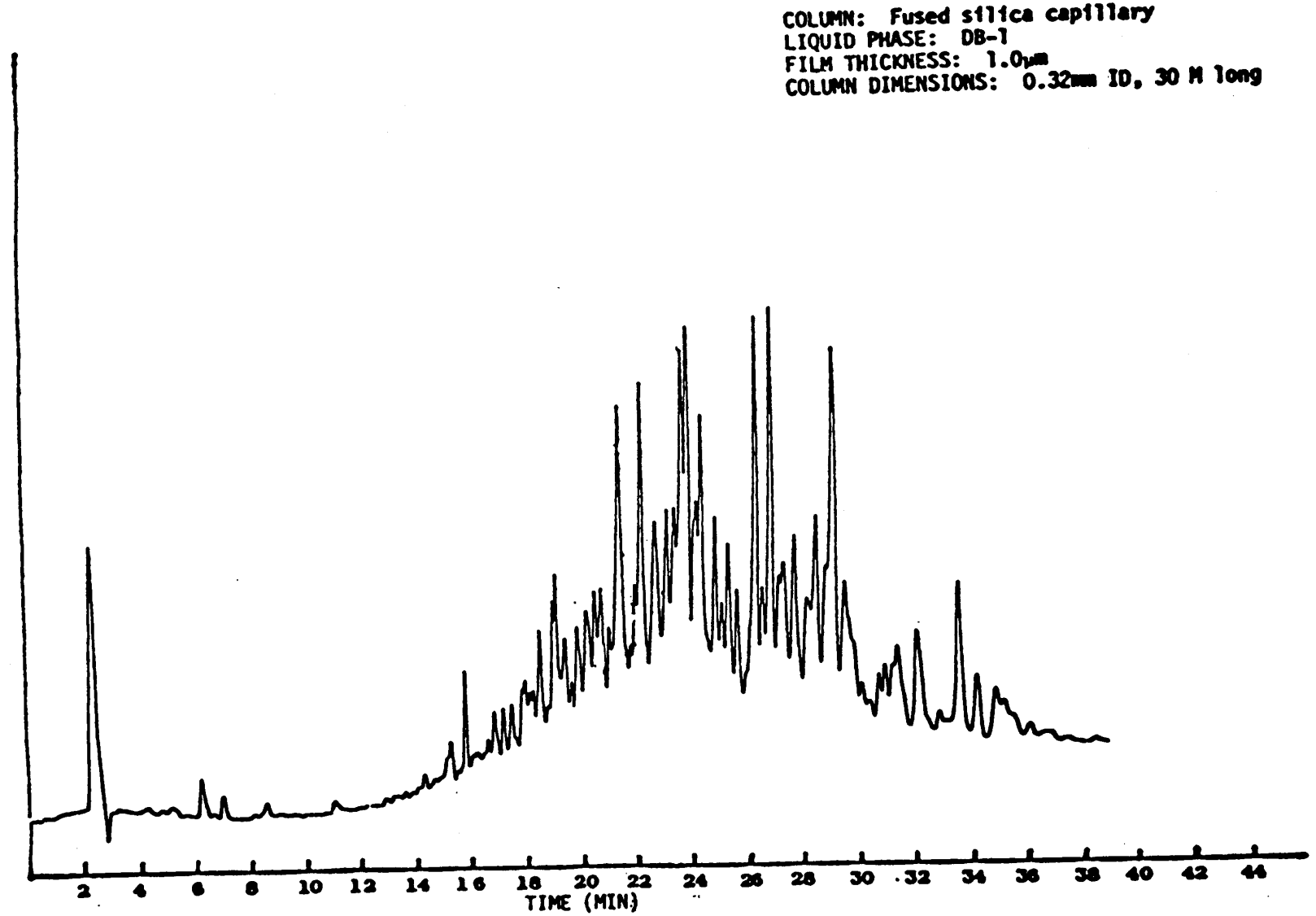

Figure 11. Hexane spiked at 57.1 ug/L with toxaphene.

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