APPENDIX A TO PART 136
METHODS FOR ORGANIC CHEMICAL ANALYSIS OF MUNICIPAL AND INDUSTRIAL WASTEWATER

METHOD 604—PHENOLS

1.   Scope and Application

1.1   This method covers the determination of phenol and certain substituted phenols. The following parameters may be determined by this method:

Parameter	STORET No.	CAS No.
4-Chloro-3-methylphenol	34452	59-50-7
2—Chlorophenol	34586	95-57-8
2,4-Dichlorophenol	34601	120-83-2
2,4-Dimethylphenol.	34606	105-67-9
2,4-Dinitrophenol	34616	51-28-5
2-Methyl-4,6-dinitrophenol	34657	534-52-1
2-Nitrophenol	34591	88-75-5
4-Nitrophenol	34646	100-02-7
Pentachlorophenol	39032	87-86-5
Phenol	34694	108-95-2
2,4,6-Trichlorophenol	34621	88-06-2

1.2   This is a flame ionization detector gas chromatographic (FIDGC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR Part 136.1. 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 derivatization, cleanup, and electron capture detector gas chromatography (ECDGC) that can be used to confirm measurements made by FIDGC. Method 625 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for all of the parameters listed above, using the extract produced by this method.

1.3   The method detection limit (MDL, defined in Section 14.1)[1] for each parameter is 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. The MDL listed in Table 1 for each parameter was achieved with a flame ionization detector (FID). The MDLs that were achieved when the derivatization cleanup and electron capture detector (ECD) were employed are presented in Table 2.

1.4   Any modification of this method, beyond those expressly permitted, shall be considered as a major modification subject to application and approval of alternate test procedures under 40 CFR Parts 136.4 and 136.5.

1.5   This method is restricted to use by or under the supervision of analysts experienced in the use of a gas chromatograph 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.

2.   Summary of Method

2.1   A measured volume of sample, approximately 1 L, is acidified and extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to 2-propanol during concentration to a volume of 10 mL or less. The extract is separated by gas chromatography and the phenols are then measured with an FID.[2]

2.2   A preliminary sample wash under basic conditions can be employed for samples having high general organic and organic base interferences.

2.3   The method also provides for a derivatization and column chromatography cleanup procedure to aid in the elimination of interferences.[2,3] The derivatives are analyzed by ECDGC.

3.   Interferences

3.1   Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.1.3.

   3.1.1   Glassware must be scrupulously cleaned.[4] Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. Solvent rinsing should be followed by detergent washing with hot water, and rinses with tap water and distilled water. The glassware should then be drained dry, and heated in a muffle furnace at 400°C for 15-30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Thorough rinsing with such solvents usually eliminates PCB interference. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored 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   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 being sampled. The derivatization cleanup procedure in Section 12 can be used to overcome

many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Tables 1 and 2.

3.3   The basic sample wash (Section 10.2) may cause significantly reduced recovery of phenol and 2,4-dimethylphenol. The analyst must recognize that results obtained under these conditions are minimum concentrations.

4.   Safety

4.1   The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should 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 analyst.

4.2   Special care should be taken in handling pentafluorobenzyl bromide, which is a lachrymator, and 18-crown-6-ether, which is highly toxic.

5.   Apparatus and Materials

5.1   Sampling equipment, for discrete or composite sampling.

    5.1.1   Grab sample bottle—1 L or 1 qt, amber glass, fitted with a screw cap lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The bottle 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)—The sampler must incorporate glass sample containers for the collection of a minimum of 250 mL of sample. 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 should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled 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—2 L, with Teflon stopcock.

    5.2.2   Drying column—Chromatographic column, 400 mm long x 19 mm ID, with coarse frit filter disc.

5.2.3 Chromatographic column—100 mm long x 10 mm ID, with Teflon stopcock.

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 Snyder column, Kuderna-Danish—Two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.8 Vials—10-15 mL, amber glass, with Teflon-lined screw cap.

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

5.3 Boiling chips—Approximately 10/40 mesh. Heat to 400°C for 30 minutes or Soxhlet extract 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 weighting 0.0001 g.

5.6 Gas chromatograph—An analytical system complete with a temperature programmable gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column for underivatized phenols—1.8 m long x 2 mm ID glass, packed with 1% SP-1240DA on Supelcoport (80/100 mesh) or equivalent. This column was used to develop the method performance statements in Section 14. Guidelines for the use of alternate column packings are provided in Section 11.1.

5.6.2 Column for derivatized phenols—1.8 m long x 2 mm ID glass, packed with 5% OV-17 on Chromosorb W-AW-DMCS (80/100 mesh) or equivalent. This column has proven effective in the analysis of wastewaters for derivatization products of the parameters listed in the scope (Section 1.1), and was used to develop the method performance statements in Section 14. Guidelines for the use of alternate column packings are provided in Section 11.1.

5.6.3 Detectors—Flame ionization and electron capture detectors. The FID is used when determining the parent phenols. The ECD is used when determining the derivatized phenols. Guidelines for the use of alternative detectors are provided in Section 11.1.

6.   Reagents

6.1   Reagent water—Reagent water is defined as a water in which an interferent is not observed at the MDL of the parameters of interest.

6.2   Sodium hydroxide solution (10 N)—Dissolve 40 g of NaOH (ACS) in reagent water and dilute to 100 mL.

6.3   Sodium hydroxide solution (1 N)—Dissolve 4 g of NaOH (ACS) in reagent water and dilute to 100 mL.

6.4   Sodium sulfate—(ACS) Granular, anhydrous. Purify by heating at 400°C for four hours in a shallow tray.

6.5   Sodium thiosulfate—(ACS) Granular.

6.6   Sulfuric acid (1+1)—Slowly, add 50 mL of H_2SO_4 (ACS, sp. gr. 1.84) to 50 mL of reagent water.

6.7   Sulfuric acid (1 N)—Slowly, add 58 mL of H_2SO_4 (ACS, sp. gr. 1.84) to reagent water and dilute to 1 L.

6.8   Potassium carbonate—(ACS) Powdered.

6.9   Pentafluorobenzyl bromide (α-Bromopentafluorotoluene)—97% minimum purity.

     NOTE: This chemical is a lachrymator (See Section 4.2.).

6.10   18-crown-6-ether (1,4,7,10,13,16-Hexaoxacyclooctadecane)—98% minimum purity.

     NOTE: This chemical is highly toxic.

6.11   Derivatization reagent—Add 1 mL of pentafluorobenzyl bromide and 1 g of 18-crown-6-ether to a 50 mL volumetric flask and dilute to volume with 2-propanol. Prepare fresh weekly. This operation should be carried out in a hood. Store at 4°C and protect from light.

6.12   Acetone, hexane, methanol, methylene chloride, 2-propanol, toluene—Pesticide quality or equivalent.

6.13   Silica gel—100/200 mesh, Davison, grade-923 or equivalent. Activate at 130°C overnight and store in a desiccator.

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

     6.14.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in 2-propanol 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.

6.14.2 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.

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

6.15 Quality control check sample concentrate. See Section 8.2.1.

7. Calibration

7.1 To calibrate the FIDGC for the anaylsis of underivatized phenols, establish gas chromatographic operating conditions equivalent to those given in Table 1. The gas chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure for FIDGC

7.2.1 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 and diluting to volume with 2-propanol. One of the external standards should be at a concentration near, but above, the MDL (Table 1) and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.2.2 Using injections of 2-5 μL, analyze each calibration standard according to Section 11 and tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (<10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.3 Internal standard calibration procedure for FIDGC—To use this approach, the analyst must select one or more internal standards that are 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. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

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 2-propanol. One of the standards should be at a concentration near, but above, the MDL and the

other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.3.2   Using injections of 2-5 µL, analyze each calibration standard according to Section 11 and tabulate peak height or area responses against concentration for each compound and internal standard. Calculate response factors (RF) for each compound using Equation 1.

Equation 1

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

where:
A$_s$ = Response for the parameter to be measured.
A$_{is}$ = Response for the internal standard.
C$_{is}$ = Concentration of the internal standard (µg/L).
C$_s$=Concentration of the parameter to be measured (µg/L).

If the RF value over the working range is a constant (<10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A$_s$/A$_{is}$, vs. concentration ratios C$_s$/C$_{is}$[*].

7.4   The working calibration curve, calibration factor, or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±15%, a new calibration curve must be prepared for that compound.

7.5   To calibrate the ECDGC for the analysis of phenol derivatives, establish gas chromatographic operating conditions equivalent to those given in Table 2.

7.5.1   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 and diluting to volume with 2-propanol. One of the external standards should be at a concentration near, but above, the MDL (Table 2) and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.5.2   Each time samples are to be derivatized, simultaneously treat a 1 mL aliquot of each calibration standard as described in Section 12.

7.5.3   After derivatization, analyze 2-5 µL of each column eluate collected according to the method beginning in Section 12.8 and tabulate peak height or area responses

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[*]This equation corrects an error made in the original method publication (49 FR 43234, October 26, 1984). This correction will be formalized through a rulemaking in FY97.

against the calculated equivalent mass of underivatized phenol injected. The results can be used to prepare a calibration curve for each compound.

7.6   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 interferences from the reagents.

8.   Quality Control

8.1   Each laboratory that uses 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 an ongoing analysis of spiked samples to evaluate and document data quality. The laboratory must maintain records to document the quality of data that is generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method. When results of sample spikes indicate atypical method performance, a quality control check standard must be analyzed to confirm that the measurements were performed in an in-control mode of operation.

8.1.1   The analyst must make an initial, one-time, demonstration of 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 advances that are occurring in chromatography, the analyst is permitted certain options (detailed in Sections 10.6 and 11.1) to improve the separations or lower the cost of measurements. Each time such a modification is made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3   Before processing any samples the analyst must analyze a reagent water blank to demonstrate that interferences from the analytical system and glassware are under control. Each time a set of samples is extracted or reagents are changed a reagent water blank must be processed as a safeguard against laboratory contamination.

8.1.4   The laboratory must, on an ongoing basis, spike and analyze a minimum of 10% of all samples to monitor and evaluate laboratory data quality. This procedure is described in Section 8.3.

8.1.5   The laboratory must, on an ongoing basis, demonstrate through the analyses of quality control check standards that the operation of the measurement system is in control. This procedure is described in Section 8.4. The frequency of the check standard analyses is equivalent to 10% of all samples analyzed but may be reduced if spike recoveries from samples (Section 8.3) meet all specified quality control criteria.

8.1.6   The laboratory must maintain performance records to document the quality of data that is generated. This procedure is described in Section 8.5.

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

8.2.1   A quality control (QC) check sample concentrate is required containing each parameter of interest at a concentration of 100 μg/mL in 2-propanol. The QC check sample concentrate must be obtained from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory in Cincinnati, Ohio, if available. If not available from that source, the QC check sample concentrate must be obtained from another external source. If not available from either source above, the QC check sample concentrate must be prepared by the laboratory using stock standards prepared independently from those used for calibration.

8.2.2   Using a pipet, prepare QC check samples at a concentration of 100 μg/L by adding 1.0 mL of QC check sample concentrate to each of four 1-L aliquots of reagent water.

8.2.3   Analyze the well-mixed QC check samples according to the method beginning in Section 10.

8.2.4   Calculate the average recovery $\overline{X}$ in μg/L, and the standard deviation of the recovery (s) in μg/L, for each parameter using the four results.

8.2.5   For each parameter compare s and $\overline{X}$ with the corresponding acceptance criteria for precision and accuracy, respectively, found in Table 3. If s and $\overline{X}$ for all parameters of interest meet the acceptance criteria, the system performance is acceptable and analysis of actual samples can begin. If any individual s exceeds the precision limit or any individual $\overline{X}$ falls outside the range for accuracy, the system performance is unacceptable for that parameter.

   NOTE: The large number of parameters in Table 3 present a substantial probability that one or more will fail at least one of the acceptance criteria when all parameters are analyzed.

8.2.6   When one or more of the parameters tested fail at least one of the acceptance criteria, the analyst must proceed according to Section 8.2.6.1 or 8.2.6.2.

   8.2.6.1 Locate and correct the source of the problem and repeat the test for all parameters of interest beginning with Section 8.2.2.

   8.2.6.2 Beginning with Section 8.2.2, repeat the test only for those parameters that failed to meet criteria. Repeated failure, however, will confirm a general problem with the measurement system. If this occurs, locate and correct the source of the problem and repeat the test for all compounds of interest beginning with Section 8.2.2.

8.3   The laboratory must, on an ongoing basis, spike at least 10% of the samples from each sample site being monitored to assess accuracy. For laboratories analyzing one to ten samples per month, at least one spiked sample per month is required.

   8.3.1   The concentration of the spike in the sample should be determined as follows:

8.3.1.1 If, as in compliance monitoring, the concentration of a specific parameter in the sample is being checked against a regulatory concentration limit, the spike should be at that limit or one to five times higher than the background concentration determined in Section 8.3.2, whichever concentration would be larger.

8.3.1.2 If the concentration of a specific parameter in the sample is not being checked against a limit specific to that parameter, the spike should be at 100 µg/L or one to five times higher than the background concentration determined in Section 8.3.2, whichever concentration would be larger.

8.3.1.3 If it is impractical to determine background levels before spiking (e.g., maximum holding times will be exceeded), the spike concentration should be (1) the regulatory concentration limit, if any, or, if none, (2) the larger of either five times higher than the expected background concentration or 100 µg/L.

8.3.2   Analyze one sample aliquot to determine the background concentration (B) of each parameter. If necessary, prepare a new QC check sample concentrate (Section 8.2.1) appropriate for the background concentrations in the sample. Spike a second sample aliquot with 1.0 mL of the QC check sample concentrate and analyze it to determine the concentration after spiking (A) of each parameter. Calculate each percent recovery (P) as 100(A-B)%/T, where T is the known true value of the spike.

8.3.3   Compare the percent recovery (P) for each parameter with the corresponding QC acceptance criteria found in Table 3. These acceptance criteria were calculated to include an allowance for error in measurement of both the background and spike concentrations, assuming a spike to background ratio of 5:1. This error will be accounted for to the extent that the analyst's spike to background ratio approaches 5:1.[8] If spiking was performed at a concentration lower than 100 µg/L, the analyst must use either the QC acceptance criteria in Table 3, or optional QC acceptance criteria calculated for the specific spike concentration. To calculate optional acceptance criteria for the recovery of a parameter: (1) Calculate accuracy (X') using the equation in Table 4, substituting the spike concentration (T) for C; (2) calculate overall precision (S') using the equation in Table 4, substituting X' for $\overline{X}$; (3) calculate the range for recovery at the spike concentration as $(100\ X'/T) \pm 2.44(100\ S'/T)\%$.[8]

8.3.4   If any individual P falls outside the designated range for recovery, that parameter has failed the acceptance criteria. A check standard containing each parameter that failed the criteria must be analyzed as described in Section 8.4.

8.4   If any parameter fails the acceptance criteria for recovery in Section 8.3, a QC check standard containing each parameter that failed must be prepared and analyzed.

NOTE: The frequency for the required analysis of a QC check standard will depend upon the number of parameters being simultaneously tested, the complexity of the sample matrix, and the performance of the laboratory.

8.4.1   Prepare the QC check standard by adding 1.0 mL of QC check sample concentrate (Section 8.2.1 or 8.3.2) to 1 L of reagent water. The QC check standard needs only to contain the parameters that failed criteria in the test in Section 8.3.

8.4.2   Analyze the QC check standard to determine the concentration measured (A) of each parameter. Calculate each percent recovery (P_s) as 100 (A/T)%, where T is the true value of the standard concentration.

8.4.3   Compare the percent recovery (P_s) for each parameter with the corresponding QC acceptance criteria found in Table 3. Only parameters that failed the test in Section 8.3 need to be compared with these criteria. If the recovery of any such parameter falls outside the designated range, the laboratory performance for that parameter is judged to be out of control, and the problem must be immediately identified and corrected. The analytical result for that parameter in the unspiked sample is suspect and may not be reported for regulatory compliance purposes.

8.5   As part of the QC program for the laboratory, method accuracy for wastewater samples must be assessed and records must be maintained. After the analysis of five spiked wastewater samples as in Section 8.3, calculate the average percent recovery ($\overline{P}$) and the standard deviation of the percent recovery (s_p). Express the accuracy assessment as a percent recovery interval from $\overline{P}$-2s_p to $\overline{P}$+2s_p. If $\overline{P}$=90% and s_p =10%, for example, the accuracy interval is expressed as 70-110%. Update the accuracy assessment for each parameter on a regular basis (e.g., after each 5-10 new accuracy measurements).

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 assess the precision of the environmental measurements. 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 analyze standard reference 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[9] should be followed, except that 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 Tygon tubing and other potential sources of contamination.

9.2   All samples must be iced or refrigerated at 4°C from the time of collection until extraction. Fill the sample bottles and, if residual chlorine is present, add 80 mg of sodium thiosulfate per liter of sample and mix well. EPA Methods 330.4 and 330.5 may be used for measurement of residual chlorine.[10] Field test kits are available for this purpose.

9.3   All samples must be extracted within seven days of collection and completely analyzed within 40 days of extraction.[2]

10.   Sample Extraction

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

10.2   For samples high in organic content, the analyst may solvent wash the sample at basic pH as prescribed in Sections 10.2.1 and 10.2.2 to remove potential method interferences. Prolonged or exhaustive contact with solvent during the wash may result in low recovery of some of the phenols, notably phenol and 2,4-dimethylphenol. For relatively clean samples, the wash should be omitted and the extraction, beginning with Section 10.3, should be followed.

   10.2.1   Adjust the pH of the sample to 12.0 or greater with sodium hydroxide solution.

   10.2.2   Add 60 mL of methylene chloride to the sample by shaking the funnel for one minute with periodic venting to release excess pressure. Discard the solvent layer. The wash can be repeated up to two additional times if significant color is being removed.

10.3   Adjust the sample to a pH of 1-2 with sulfuric acid.

10.4   Add 60 mL of methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two 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. Collect the methylene chloride extract in a 250 mL Erlenmeyer flask.

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

10.6   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 concentrator if the requirements of Section 8.2 are met.

10.7   Pour the combined extract through a solvent-rinsed 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 20-30 mL of methylene chloride to complete the quantitative transfer.

10.8   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 of methylene chloride to the top.   Place the K–D apparatus on a hot water bath (60-65°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

15-20 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.

10.9 Increase the temperature of the hot water bath to 95-100°C. Remove the Synder column and rinse the flask and its lower joint into the concentrator tube with 1-2 mL of 2-propanol. A 5-mL syringe is recommended for this operation. Attach a two-ball micro-Snyder column to the concentrator tube and prewet the column by adding about 0.5 mL of 2-propanol to the top. Place the micro-K–D apparatus on the water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete concentration in 5-10 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 2.5 mL, remove the K–D apparatus and allow it to drain and cool for at least 10 minutes. Add an additional 2 mL of 2-propanol through the top of the micro-Snyder column and resume concentrating as before. When the apparent volume of liquid reaches 0.5 mL, remove the K–D apparatus and allow it to drain and cool for at least 10 minutes.

10.10 Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a minimum amount of 2-propanol. Adjust the extract volume to 1.0 mL. Stopper the concentrator tube and store refrigerated at 4 °C if further processing will not be performed immediately. If the extract will be stored longer than two days, it should be transferred to a Teflon-sealed screw-cap vial. If the sample extract requires no further cleanup, proceed with FIDGC analysis (Section 11). If the sample requires further cleanup, proceed to Section 12.

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

11. Flame Ionization Detector Gas Chromatography

11.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are retention times and MDL that can be achieved under these conditions. An example of the separations achieved by this column is shown in Figure 1. Other packed or capillary (open-tubular) columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.

11.2 Calibrate the system daily as described in Section 7.

11.3 If the internal standard calibration procedure is used, the internal standard must be added to the sample extract and mixed thoroughly immediately before injection into the gas chromatograph.

11.4 Inject 2-5 μL of the sample extract or standard into the gas chromatograph using the solvent-flush technique.[11] Smaller (1.0 μL) volumes may be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 μL, and the resulting peak size in area or peak height units.

11.5 Identify the parameters in the sample by comparing the retention times of the peaks in the sample chromatogram with those of the peaks in standard chromatograms. 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 for a compound may be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

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

11.7 If the measurement of the peak response is prevented by the presence of interferences, an alternative gas chromatographic procedure is required. Section 12 describes a derivatization and column chromatographic procedure which has been tested and found to be a practical means of analyzing phenols in complex extracts.

12. Derivatization and Electron Capture Detector Gas Chromatography

12.1 Pipet a 1.0 mL aliquot of the 2-propanol solution of standard or sample extract into a glass reaction vial. Add 1.0 mL of derivatizing reagent (Section 6.11). This amount of reagent is sufficient to derivatize a solution whose total phenolic content does not exceed 0.3 mg/mL.

12.2 Add about 3 mg of potassium carbonate to the solution and shake gently.

12.3 Cap the mixture and heat it for four hours at 80°C in a hot water bath.

12.4 Remove the solution from the hot water bath and allow it to cool.

12.5 Add 10 mL of hexane to the reaction flask and shake vigorously for one minute. Add 3.0 mL of distilled, deionized water to the reaction flask and shake for two minutes. Decant a portion of the organic layer into a concentrator tube and cap with a glass stopper.

12.6 Place 4.0 g of silica gel into a chromatographic column. Tap the column to settle the silica gel and add about 2 g of anhydrous sodium sulfate to the top.

12.7 Preelute the column with 6 mL of hexane. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, pipet onto the column 2.0 mL of the hexane solution (Section 12.5) that contains the derivatized sample or standard. Elute the column with 10.0 mL of hexane and discard the eluate. Elute the column, in order, with: 10.0 mL of 15% toluene in hexane (Fraction 1); 10.0 mL of 40% toluene in hexane (Fraction 2); 10.0 mL of 75% toluene in hexane (Fraction 3); and 10.0 mL of 15% 2-propanol in toluene (Fraction 4). All elution mixtures are prepared on a volume: volume basis. Elution patterns for the phenolic derivatives are shown in Table 2. Fractions may be combined as desired, depending upon the specific phenols of interest or level of interferences.

12.8 Analyze the fractions by ECDGC. Table 2 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are retention times and

MDL that can be achieved under these conditions. An example of the separations achieved by this column is shown in Figure 2.

12.9   Calibrate the system daily with a minimum of three aliquots of calibration standards, containing each of the phenols of interest that are derivatized according to Section 7.5.

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

13.   Calculations

13.1   Determine the concentration of individual compounds in the sample analyzed by FIDGC (without derivatization) as indicated below.

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 determined in Section 7.2.2. The concentration in the sample can be calculated from Equation 2.

Equation 2

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

where:

A = Amount of material injected (ng).
V_i = Volume of extract injected (µL).
V_t = Volume of total extract (µL).
V_s = Volume of water extracted (mL).

13.1.2 If the internal standard calibration procedure is used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 and Equation 3.

Equation 3

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

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
I_s = Amount of internal standard added to each extract (µg).
V_o = Volume of water extracted (L).

13.2   Determine the concentration of individual compounds in the sample analyzed by derivatization and ECDGC according to Equation 4.

Equation 4

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

where:
- A = Mass of underivatized phenol represented by area of peak in sample chromatogram, determined from calibration curve in Section 7.5.3 (ng).
- V_i = Volume of eluate injected (µL).
- V_t = Total volume of column eluate or combined fractions from which V_i was taken (µL).
- V_s = Volume of water extracted in Section 10.11 (mL).
- B = Total volume of hexane added in Section 12.5 (mL).
- C = Volume of hexane sample solution added to cleanup column in Section 12.7 (mL).
- D = Total volume of 2-propanol extract prior to derivatization (mL).
- E = Volume of 2-propanol extract carried through derivatization in Section 12.1 (mL).

13.3   Report results in µg/L without correction for recovery data. All QC data obtained should be reported with the sample results.

14.   Method Performance

14.1   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.[1] The MDL concentrations listed in Tables 1 and 2 were obtained using reagent water.[12] Similar results were achieved using representative wastewaters. The MDL actually achieved in a given analysis will vary depending on instrument sensitivity and matrix effects.

14.2   This method was tested by 20 laboratories using reagent water, drinking water, surface water, and three industrial wastewaters spiked as six concentrations over the range 12-450 µg/L.[13] Single operator precision, overall precision, and method accuracy were found to be directly related to the concentration of the parameter and essentially independent of the sample matrix. Linear equations to describe these relationships for a flame ionization detector are presented in Table 4.

References

1.   40 CFR Part 136, Appendix B.

2.   "Determination of Phenols in Industrial and Municipal Wastewaters," EPA 600/4-84-ABC, National Technical Information Service, PBXYZ, Springfield, Virginia 22161, November 1984.

3.   Kawahara, F. K. "Microdetermination of Derivatives of Phenols and Mercaptans by Means of Electron Capture Gas Chromatography," *Analytical Chemistry, 40,* 1009 (1968).

4.   ASTM Annual Book of Standards, Part 31, D3694-78. "Standard Practices for Preparation of Sample Containers and for Preservation of Organic Constituents," American Society for Testing and Materials, Philadelphia.

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

6.   "OSHA Safety and Health Standards, General Industry," (29 CFR part 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976).

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

8.   Provost, L. P. and Elder, R. S. "Interpretation of Percent Recovery Data," *American Laboratory, 15,* 58-63 (1983). (The value 2.44 used in the equation in Section 8.3.3 is two times the value 1.22 derived in this report.)

9.   ASTM Annual Book of Standards, Part 31, D3370-76. "Standard Practices for Sampling Water," American Society for Testing and Materials, Philadelphia.

10.   "Methods 330.4 (Titrimetric, DPD-FAS) and 330.5 (Spectrophotometric, DPD) for Chlorine, Total Residual," Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.

11.   Burke, J. A. "Gas Chromatography for Pesticide Residue Analysis; Some Practical Aspects," *Journal of the Association of Official Analytical Chemists, 48,* 1037 (1965).

12.   "Development of Detection Limits, EPA Method 604, Phenols," Special letter report for EPA Contract 68-03-2625, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

13.   "EPA Method Study 14 Method 604-Phenols," EPA 600/4-84-044, National Technical Information Service, PB84-196211, Springfield, Virginia 22161, May 1984.

Table 1—Chromatographic Conditions and Method Detection Limits

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

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

Table 2—Silica Gel Fractionation and Electron Capture Gas Chromatography of PFBB Derivatives

Parent compound	Percent recovery by fraction[a]				Retention time (min)	Method Detection limit (µg/L)
	1	**2**	**3**	**4**		
2-Chlorophenol	.....	90	1	.....	3.3	0.58
2-Nitrophenol	.....	.....	9	90	9.1	0.77
Phenol	.....	90	10	.....	1.8	2.2
2,4-Dimethylphenol	.....	95	7	.....	2.9	0.63
2,4-Dichlorophenol	.....	95	1	.....	5.8	0.68
2,4,6-Trichlorophenol	50	50	.....	.....	7.0	0.58
4-Chloro-3-methylphenol	.....	84	14	.....	4.8	1.8
Pentachlorophenol	75	20	.....	.....	28.8	0.59
4-Nitrophenol	.....	.....	1	90	14.0	0.70

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

[a]Eluant composition:
Fraction 1–15% toluene in hexane.
Fraction 2–40% toluene in hexane.
Fraction 3–75% toluene in hexane.
Fraction 4–15% 2-propanol in toluene.

Table 3—QC Acceptance Criteria—Method 604

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

s = Standard deviation of four recovery measurements, in µg/L (Section 8.2.4).
$\overline{X}$ = Average recovery for four recovery measurements, in µg/L (Section 8.2.4).
P, P_s = Percent recovery measured (Section 8.3.2, Section 8.4.2).

NOTE:　　These criteria are based directly upon the method performance data in Table 4. Where necessary, the limits for recovery have been broadened to assure applicability of the limits to concentrations below those used to develop Table 4.

Table 4—Method Accuracy and Precision as Functions of Concentration—Method 604

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

X′ = Expected recovery for one or more measurements of a sample containing a concentration of C, in μg/L.

s_r' = Expected single analyst standard deviation of measurements at an average concentration found of $\overline{X}$, in μg/L.

S′ = Expected interlaboratory standard deviation of measurements at an average concentration found of $\overline{X}$, in μg/L.

C = True value for the concentration, in μg/L.

$\overline{X}$ = Average recovery found for measurements of samples containing a concentration of C, in μg/L.

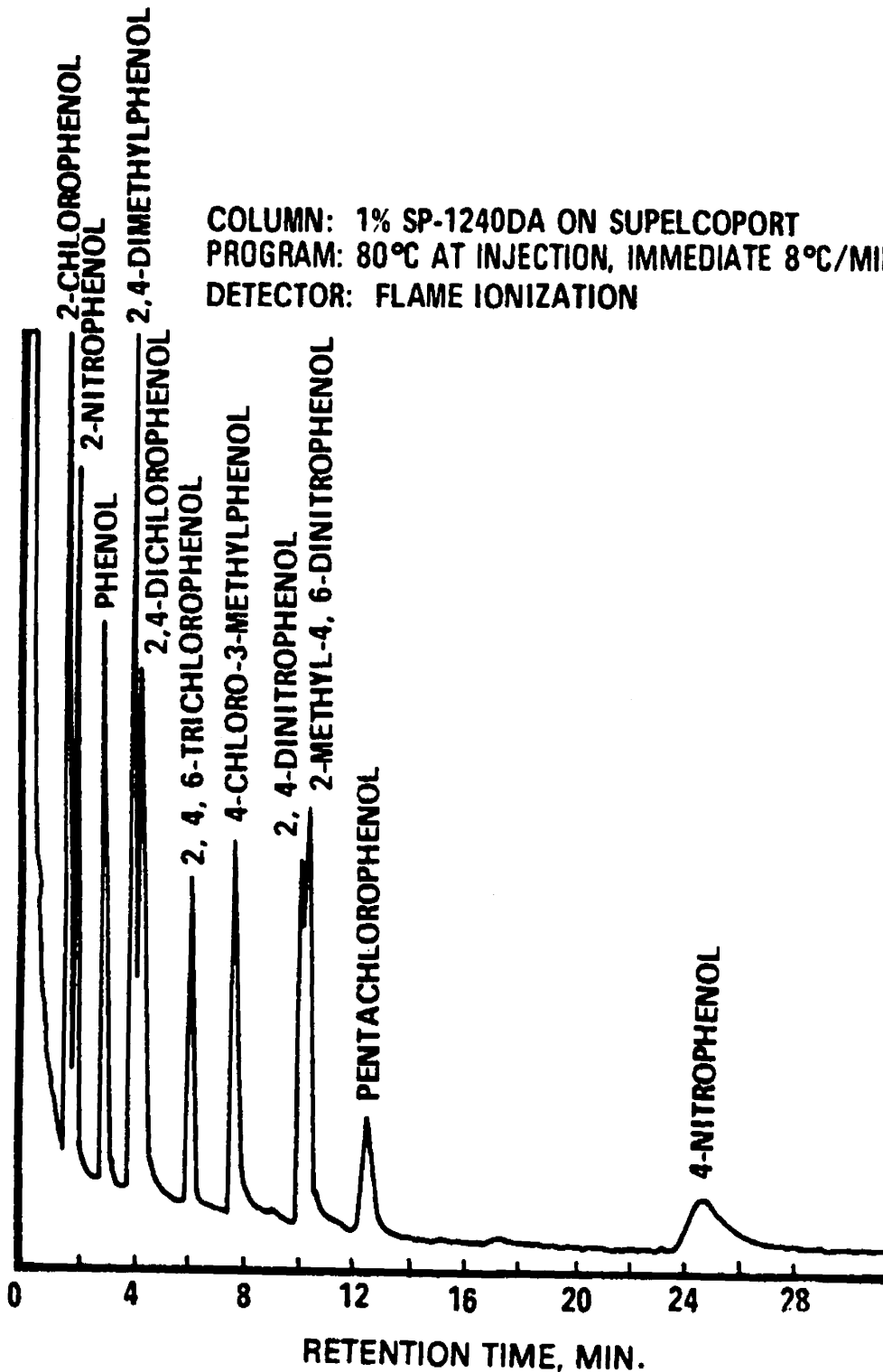COLUMN: 1% SP-1240DA ON SUPELCOPORT
PROGRAM: 80°C AT INJECTION, IMMEDIATE 8°C/MIN TO 150°C
DETECTOR: FLAME IONIZATION

2-CHLOROPHENOL
2-NITROPHENOL
PHENOL
2,4-DIMETHYLPHENOL
2,4-DICHLOROPHENOL
2, 4, 6-TRICHLOROPHENOL
4-CHLORO-3-METHYLPHENOL
2, 4-DINITROPHENOL
2-METHYL-4, 6-DINITROPHENOL
PENTACHLOROPHENOL
4-NITROPHENOL

RETENTION TIME, MIN.

Figure 1. Gas chromatogram of phenols.

COLUMN: 5% OV-17 ON CHROMOSORB W-AW-DMCS
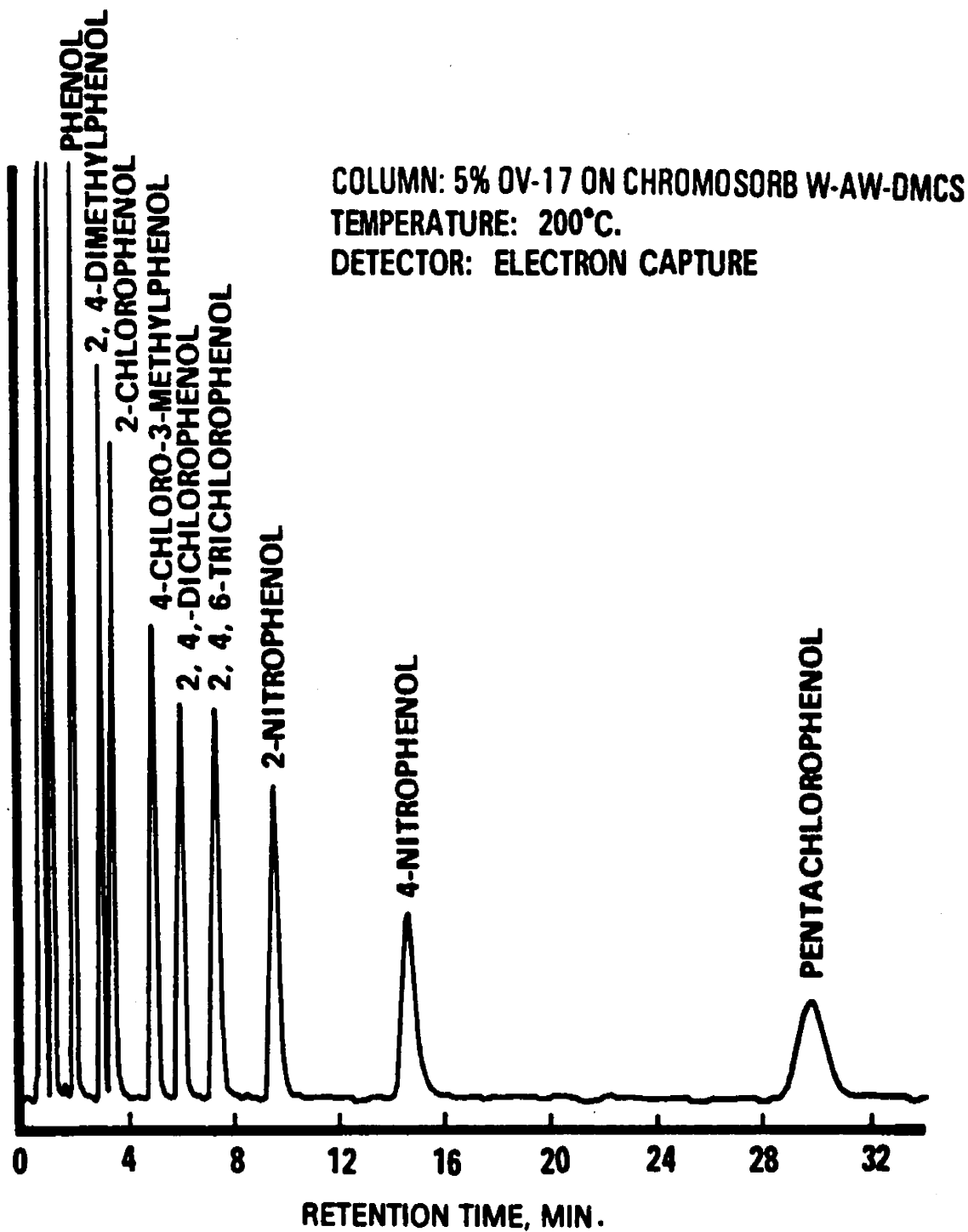TEMPERATURE: 200°C.
DETECTOR: ELECTRON CAPTURE

Figure 2. Gas chromatogram of PFB derivatives of phenols.