METHOD 555

DETERMINATION OF CHLORINATED ACIDS IN WATER BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH A PHOTODIODE ARRAY ULTRAVIOLET DETECTOR

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1.0 SCOPE AND APPLICATION

1.1 This is a high performance liquid chromatographic (HPLC) method for the determination of certain chlorinated acids in ground water and finished drinking water. The following compounds can be determined by this method:

Analyte	Chemical Abstract Services Registry Number
Acifluorfen	50594-66-6
Bentazon	25057-89-0
Chloramben[a]	133-90-4
2,4-D	94-75-7
2,4-DB	94-82-6
Dicamba	1918-00-9
3,5-Dichlorobenzoic acid	51-36-5
Dichlorprop	120-36-5
Dinoseb	88-85-7
5-Hydroxydicamba[a]	7600-50-2
MCPA	
MCPP	
4-Nitrophenol[a]	100-02-7
Pentachlorophenol[b] (PCP)	87-86-5
Picloram[a]	1918-02-1
2,4,5-T	93-76-5
2,4,5-TP	93-72-1

[a]Analytes measurable from 20 mL sample volume only.
[b]Use a 100 mL sample for pentachlorophenol in order to attain a MDL of 0.3 µg/L. The MLC for this compound is 1.0 µg/L.

1.2 This method is applicable to the determination of salts and esters of analyte acids. The form of each analyte is not distinguished by this method. Results are calculated and reported for each listed analyte as the total free acid.

1.3 This method has been validated in a single laboratory and method detection limits (MDLs)[1] have been determined from a 20 mL sample for the analytes above. Observed MDLs may vary among ground waters, depending on the nature of interferences in the sample matrix and the specific instrumentation used.

1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC and in the interpretation of chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 9.3.

1.5 Analytes that are not separated chromatographically cannot be individually identified and measured in the same calibration mixture or water sample unless an alternative technique for identification and quantitation exists (Section 11.3).

1.6 When this method is used to analyze unfamiliar samples, analyte identifications must be confirmed by at least one additional qualitative technique.

2.0 SUMMARY OF METHOD

2.1 A measured sample volume of approximately 100 mL is adjusted to pH 12 with 6 N sodium hydroxide, shaken, and allowed to set for 1 hr to hydrolyze chlorinated esters. The sample is acidified with H_3PO_4, filtered, and the chlorinated acids are extracted from a 20 mL aliquot. The 20 mL aliquot is pumped through an HPLC cartridge (containing C_{18}-silica), trapping the chlorinated acids. The concentrator cartridge is valved in-line with the C_{18} analytical column following extraction. The analytes are separated and measured by photodiode array - ultraviolet detection (PDA-UV).

NOTE: A liquid-solid extraction disk is perfectly acceptable for use in the in-line extraction of the analytes providing all quality control (QC) criteria in Section 9.0 are met or exceeded.

2.2 The method measures the analytes from 20 mL volumes. Volumes of up to 100 mL may be analyzed by this procedure for certain analytes. The analytes which may **not** be determined in a larger volume are indicated in Section 1.1.

3.0 DEFINITIONS

3.1 Laboratory Duplicates (LD1 AND LD2) -- Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate 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 or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents 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) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the labora-tory and treated as a sample in all respects, including shipment to the sampling site, 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 Fortified Blank (LFB) -- An aliquot of reagent water or other blank matrix 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.

3.6 Laboratory Fortified Sample Matrix (LFM) -- An aliquot of an environmental sample to which know 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.

3.7 Stock Standard Solution (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial supplier.

3.8 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.

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

3.10 Quality Control Sample (QCS) -- A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards, It is used to check laboratory performance with externally prepared test materials.

3.11 Method Detection Limit (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.12 External Standard (ES) -- A pure analyte(s) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the external standard(s) is used to calibrate the instrument response for the corresponding analytes(s). The instrument response is used to calculate the concentrations of the analyte(s) in the sample.

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 liquid chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by analyzing laboratory reagent blanks as described in Section 9.2.

4.1.1 Glassware must be scrupulously cleaned[3]. Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with dilute acid, 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 distillation in all-glass systems may be required.

WARNING: When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous. Removal of preservatives by distillation may also reduce the shelf-life of the solvent.

4.2 The acid forms of the analytes are strong organic acids which react readily with alkaline substances and can be lost during sample preparation. Glassware must be acid-rinsed with 1 N hydrochloric acid prior to use to avoid analyte losses due to adsorption.

4.3 Matrix interferences may be caused by contaminants that are coextracted from the sample. Also, note that all method analytes are not resolved from each other on a single column, i.e., one analyte of interest may interfere with another analyte of interest. The extent of matrix interferences will vary

considerably from source to source, depending upon the water sampled. The procedures in Section 11.0 can be used to overcome many of these interferences. Tentative identifications should always be confirmed (Section 11.3).

5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. Accordingly, exposure to these chemicals must be reduced to the lowest possible level. 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 safety data sheets should also be made available to all personnel involved in the chemical analysis.

WARNING: When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous. Therefore, storage of large volumes of purified solvents may be hazardous. Therefore, only small volumes of solvents should be purified just before use.

6.0 EQUIPMENT AND SUPPLIES

6.1 Sample Bottle -- Borosilicate, 125 mL volume, graduated, fitted with Teflon-lined screw cap. Protect samples from light. The container must be washed and dried as described in Section 4.1.1 before use to minimize contamination. Cap liners may be cut to fit from Teflon sheets and extracted with methanol overnight prior to use.

6.2 Glassware

6.2.1 Volumetric flask, Class A -- 100 mL, with ground glass stoppers.

6.2.2 Graduated cylinder -- 100 mL.

6.2.3 Disposable pipets, transfer -- Borosilicate glass.

6.2.4 Glass syringe -- 50 mL, with Luer-Lok fitting.

6.2.5 Volumetric pipette, Class A -- 20 mL.

6.3 Balance -- Analytical, capable of accurately weighing to the nearest 0.0001 g.

6.4 Liquid Chromatograph -- Analytical system complete with gradient programmable HPLC suitable for use with analytical HPLC columns and all required accessories including an injector, analytical column, semi-prep guard column, and photodiode array UV detector. A data system is necessary for measuring the peak areas and for assessing the confirmation of the peak identification. A personal computer (PC) of at least the AT-class is generally

needed to control and collect data from the photodiode array UV detector. Table 1 lists the retention times observed for the method analytes using the column and analytical conditions described below. Figure 1 is a schematic drawing of the analytical system including the sample concentrator column (semi-prep guard column).

6.4.1 Primary column -- 250 mm x 4.6 mm I.D. ODS-AQ, 5 µm spherical (YMC Ltd.). Any column may be used if equivalent or better performance (better peak shape, better analyte efficiency, or more complete separation of analytes) can be demonstrated. Mobile phase flow rate is established at 1.0 mL/min (linear velocity of 6.0 cm/min). Two mobile phase components are used: A -- 0.025 M H_3PO_4; B -- Acetonitrile. A gradient solvent program is used to separate the analytes: 90:10 A:B to 10:90 A:B in 30 minutes, linear ramp/hold at 10:90 for 10 minutes. Reverse the gradient and establish initial conditions: 10:90 A:B to 90:10 A:B in 10 minutes, linear ramp. Allow column backpressure to restablize for 5-10 minutes before beginning the next analysis. Total restabilization time will be determined by each analyst.

6.4.2 Confirmation column -- 300 mm x 3.9 mm I.D. Nova-Pak C_{18}, 4 µm spherical (Waters Chromatography Division, Millipore). Any column may be used if equivalent or better performance (better peak shape, better analyte efficiency, or more complete separation of analytes) can be demonstrated. Mobile phase and conditions same as primary column.

6.4.3 Sample concentrator column -- 30 mm x 10 mm I.D. ODS-AQ, 5 µm spherical (YMC Ltd). An alternative concentrator column may be used if all QC criteria in Section 9.0 can be equalled or improved. Also, a liquid-solid extraction disk may be used if all QC criteria in Section 9.0 can be equalled or improved.

6.4.4 Six-port switching valve -- Rheodyne Model 7000 (Rheodyne Corp).

6.4.5 Sample delivery pump -- A piston-driven pump capable of delivering aqueous sample at a flow rate of 5.0 mL/min. An analytical HPLC pump may serve as the sample delivery pump. A Waters Model 6000A was used to generate the data presented in this method.

6.4.6 Detector -- Photodiode array. Ultraviolet (PDA-UV), LKB-Bromma Model 2140 Rapid Spectral Detector or equivalent. Detector parameters: Scan Range - 210-310 nm at 1 scan/sec, detector integration - one second.

6.4.7 Data handling system -- DOS-based Personal Computer, AT-class machine or machine of greater capability with 640 K RAM or more, an 80 Mb hard disk or larger, VGA monitor or equivalent.

7.0 REAGENTS AND STANDARDS

7.1 Acetonitrile -- HPLC Grade or equivalent.

7.2 Sodium Sulfite -- Granular, anhydrous, ACS grade.

7.3 Sodium Hydroxide (NaOH) -- Pellets, ACS grade.

7.3.1 NaOH, 6 N -- Dissolve 216 g NaOH in 900 mL reagent water.

7.4 Phosphoric Acid -- 85% AR, ACS grade.

7.4.1 0.025 M -- Mix 2.0 mL of H_3PO_4 in 998 mL of reagent water.

7.5 Stock Standard Solutions (1.00 µg/µL) -- Stock standard solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedure:

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

7.5.2 Transfer the stock standard solutions into Teflon-lined sealed screw cap amber vials. Store at room temperature and protect from light.

7.5.3 Stock standard solutions should be replaced after two months or sooner if comparison with laboratory fortified blanks, or QC samples indicate a problem.

7.6 Hydrochloric Acid -- ACS grade.

7.6.1 HCl, 1 N -- Dilute 50 mL in 600 mL of reagent water.

7.7 Filters -- 0.45 µm, Nylon, 25 mm i.d. (Gelman Sciences).

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Grab samples must be collected in glass containers. Conventional sampling practices[2] should be followed; however, the bottle must not be prerinsed with sample before collection.

8.2 Sample Preservation and Storage

8.2.1 Add hydrochloric acid (1:1) to the sample to produce a pH of 2. The pH may be measured in the field using pH indicator strips.

8.2.2 Residual chlorine should be reduced at the sampling site by the addition of a reducing agent. Add 4-5 mg of sodium sulfite (this may be added as a solid with shaking until dissolved) to each 100 mL of water.

8.2.3 The samples must be iced or refrigerated at 4°C away from light from the time of collection until extraction. The samples must be analyzed within 14 days of collection. However, analyte stability may be affected by the matrix. Therefore, the analyst should verify that the preservation technique is applicable to the samples under study. If the 14-day holding time is exceeded, the data should be flagged so that the data user is aware of possible analyte degradation.

8.2.4 Field reagent blanks (FRB) -- Processing a FRB is recommended along with each set, which is composed of the samples collected from the same general sample site at approximately the same time. At the laboratory, fill a sample container with reagent water, seal, and ship to the sampling site along with the empty sample containers. During sample collection, open the FRB and add H6 (Section 8.2.1) and sodium sulfite (Section 8.2.2) Return the FRB to the laboratory with filled sample bottles.

9.0 QUALITY CONTROL

9.1 Minimum QC requirements are initial demonstration of laboratory capability, analysis of laboratory reagent blanks, laboratory fortified samples, laboratory fortified blanks, and QC samples.

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, a 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 for each analyte. Prepare a sample concentrate (in acetonitrile) containing each analyte at 1000 times the selected concentration. With a syringe, add 100 µL of the concentrate to each of at least four 100 mL aliquots of reagent water, and analyze each aliquot according to procedures beginning in Section 11.0.

9.3.2 Calculate the recoveries, the relative standard deviation, and the MDLs[5]. For each analyte the recovery value for all four of these samples must fall in the range of R ±30%, using the value for R for reagent water in Table 2. As the calibration procedure employs a fortified reagent water blank for the determination of the calibration curves or factors, the recovery values for the analytes should, by definition, be within this range. If the mean recovery of any analyte fails this demonstration, repeat the measurement of that analyte to demonstrate acceptable performance.

9.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples using a new, unfamiliar method prior to obtaining some experience with it. As laboratory personnel gain experience with this method the quality of data should improve beyond what is required here.

9.4 The analyst is permitted to modify LC columns, LC conditions, and detectors. Each time such method modifications are made, the analyst must repeat the procedures in Section 9.3.

NOTE: The LC column and guard cartridge used to generate the data in this method were found to be unique C_{18}-silica columns. Before substituting other C_{18} columns, a careful review of the literature is recommended.

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

9.5.1 The laboratory must analyze at least one LFB sample with every 20 samples or one per sample set (all samples analyzed within a 24-hour period) whichever is greater. The concentration of each analyte in the LFB should be 10 times the MDL or the MCL, whichever is less. 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.

9.5.2 Until sufficient data become available from within the laboratory, usually a minimum of results from 20-30 analyses, the laboratory should 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 become 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 5-10 new recovery measurements, new control limits should be calculated using only the most recent 20-30 data points. These calculated control limits should never exceed those established in Section 9.3.2.

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

9.5.4 At least quarterly, analyze a QC sample from an outside source.

9.5.5 Laboratories are encouraged to participate in external performance evaluation studies such as the laboratory certification programs offered by many states or the studies conducted by USEPA. Performance evaluation studies serve as independent checks on the analyst's performance.

9.6 Assessing Analyte Recovery -- Laboratory Fortified Sample Matrix

9.6.1 The laboratory should add a known concentration to a minimum of 10% of the routine samples or one sample per set, whichever is greater. The concentration should not be less than the background concentration of the sample selected for fortification. Ideally, the concentration should be the same as that used for the laboratory fortified blank (Section 9.5). Over time, samples from all routine sample sources should be fortified.

9.6.2 Calculate the percent recovery, P of the concentration for each analyte, after correcting the analytical result, X, from the fortified sample for the background concentration, b, measured in the unfortified sample,

$$P = 100\ (X - b)\ /\ \text{fortifying concentration},$$

and compare these values to control limits appropriate for reagent water data collected in the same fashion. If the analyzed unfortified sample is found to contain **NO** background concentrations, and the added concentrations are those specified in Section 9.5, the appropriate control limits would be the acceptance limits in Section 9.5. If, on the other hand, the analyzed unfortified sample is found to contain background concentration, b, estimate the standard deviation at the background data, s_b, using regressions or comparable background data and similarly, estimate the mean, $\bar{X}_a$, and standard deviation, s_a, of analytical results or the total concentration after fortifying. Then the appropriate percentage control limits would be $P \pm 3s_p$, where:

$$\bar{P} = 100\bar{X}\ /\ (b + \text{fortifying concentration})$$

and $s_P = 100\ (s_a^2 + s_b^2)^{1/2}$ / fortifying concentration

For example, if the background concentration for Analyte A was found to be 1 µg/L and the added amount was also 1 µg/L, and upon analysis the laboratory fortified sample measured 1.6 µg/L, then the calculated P for this sample would (1.6 µg/L minus 1.0 µg/L)/1.0 µg/L or 60%. This calculated P is compared to control limits derived from prior reagent water data. Assume it is known that analysis of an interference free sample at 1.0 µg/L yields an s of 0.12 µg/L and similar analysis at 2.0 µg/L yields $\bar{X}$ and S of 2.01 µg/L and 0.20 µg/L, respectively. The appropriate limits to judge the reasonableness of the percent recovery, 60%, obtained on the fortified matrix sample is computed as follows:

$$[100\ (2.01\ \mu g/L)\ /\ 2.0\ \mu g/L]$$

$$\pm 3\ (100)\ [(0.12\ \mu g/L)^2 + (0.20\ \mu g/L)^2]^{1/2}/1.0\ \mu g/L =$$

$$100.5\% \pm 300\ (0.233) =$$

$$100.5\% \pm 70\% \text{ or } 30\% \text{ to } 170 \text{ recovery of the added analyte.}$$

9.6.3 If the recovery of any such analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Section 9.5), the recovery problem encountered with the fortified 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 QC 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. The 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 HPLC operating parameters equivalent to those indicated in Section 6.4.1. The HPLC system should be calibrated using the external standard technique (Section 10.2).

NOTE: Calibration standard solutions must be prepared such that no unresolved analytes are mixed together.

The method analytes have been separated into two calibration solutions (See Table 1 for Groups A and B). The analytes in these solutions have been found to be resolved under the LC conditions listed. Mixtures of these analytes at concentration levels of 100 µg/mL (in acetonitrile) are suggested as a possible

secondary dilution standard. Figures 2 and 3 are typical chromatograms of Groups A and B as separated on the primary HPLC column.

10.2 External Standard Calibration Procedure

10.2.1 Prepare calibration standards (CAL) at a minimum of three (five are recommended) concentration levels for each analyte of interest by adding volumes of one or more stock standards to volumetric flasks. Alternatively, add various volumes of a primary dilution standard solution of Group A or B (Section 10.1) to a volumetric flask. Dilute to volume with the aqueous mobile phase (0.025 M H_3PO_4). The lowest standard should contain analyte concentrations near, but above, the respective MDL. The remaining standards should bracket the analyte concentrations expected in the sample extracts, or should define the working range of the detector.

10.2.2 Starting with the standard of the lowest concentration, process each calibration standard according to Section 11.1 and tabulate response (peak area) versus injected quantity in the standard. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to concentration (response factor) is a constant over the working range (20% RSD or less), linearity through the origin can be assumed and the average ratio or response factor can be used in place of a calibration curve.

10.2.3 The working calibration curve or response factor must be verified on each working day by the measurement of a CAL, analyzed at the beginning of the analysis day. It is highly recommended that an additional check standard be analyzed at the end of the analysis day. For extended periods of analysis (greater than eight hours), it is strongly recommended that check standards be interspersed with samples at regular intervals during analyses. If the response for any analyte varies from the predicted response by more than ±25%, the test must be repeated using a fresh calibration standard. If the results still do not agree, generate a new calibration curve.

10.2.4 Verify calibration standards periodically, recommend at least quarterly, by analyzing a standard prepared from reference material obtained from an independent source. Results from these analyses must be within the limits used to routinely check calibration.

11.0 PROCEDURE

11.1 Hydrolysis, Preparation, and Extraction.

11.1.1 Add preservative to blanks and QC check standards. Mark the water meniscus on the side of the sample bottle for later determination of sample volume (Section 11.1.5).

11.1.2 Add 1.7 mL of 6 N NaOH to the sample, seal, and shake. Check the pH of the sample with pH paper; if the sample does not have a pH greater than or equal to 12, adjust the pH by adding more 6 N NaOH. Let the sample sit at room temperature for one hour, shaking the sample bottle and contents periodically.

11.1.3 Add 2 mL of concentrated H_3PO_4 to the sample, seal, and shake to mix. Check the pH of the sample with pH paper; if the sample does not have a pH less than or equal to two, adjust the pH by adding more H_3PO_4.

11.1.4 From the homogeneous sample, remove a 20 mL aliquot for analysis. Filter the aliquot through a 0.45 µm filter into a graduated cylinder or other convenient graduated container. Using an HPLC pump (or HPLC reagent delivery pump), pump the 20 mL aliquot through the on-line concentrator column at a flowrate of 5.0 mL/min (See Figure 1). The use of a liquid-solid extraction disk is perfectly acceptable providing all QC criteria in Section 9.0 are met or exceeded. After passing the sample through the concentrator column, follow with an additional 10 mL of the aqueous mobile phase (0.025 M H_3PO_4).

11.1.5 After analysis is completed, determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 100 mL graduated cylinder. Record the sample volume to the nearest 1 mL.

11.2 High Performance Liquid Chromatography

11.2.1 Section 6.4.1 summarizes the recommended operating conditions for the HPLC. Included in Table 1 are retention times observed using this method. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of Section 9.3 are met.

11.2.2 Calibrate the system daily as described in Section 10.0.

11.2.3 After loading the sample (or calibration standard) onto the concentrator column, valve the sample into the analytical stream, backflushing the concentrator column. The photodiode array detector (PDA-UV) is set to scan and record from 210-310 nm, one scan per second during the entire chromatographic run (40 minutes). Extract the 230 nm trace from the stored data and record the resulting peak size in area units for all analytically significant peaks.

11.2.4 If the responses for the peaks exceed the working range of the system, dilute an additional 20 mL aliquot of the sample with reagent water, adjust the pH to 12 with NaOH, and reanalyze according to Section 11.1.2.

11.3 Identification of Analytes

11.3.1 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.

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

11.3.3 Identification requires expert judgment when sample components are not resolved chromatographically. When peaks obviously represent more that one sample component (i.e., broadened peak with shoulder(s) or vallies between two or more maxima, or any time doubt exists over the identification or a peak on a chromatogram, appropriate alternative techniques, to help confirm peak identification, should be used. For this method, the use of the PDA-UV detector affords the analyst the option of using a secondary wavelength for the analysis of the questionable identification. The response ratio for a compound of interest at two wavelengths may be determined from standards of known purity. If the wavelength response ratio and the retention time matches a given unknown to a method analyte, more certainty may be assigned to the identification of the unknown. If this method of compound confirmation is employed, each analyst will need to determine the wavelength response ratio for each analyte. Table 3 lists suggested alternative wavelengths for each analyte in the scope of the method. An alternative LC column may be used to separate and confirm the identification of unknown peaks. A suggested alternative column is described in Section 6.4.2.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Calculate analyte concentrations in the sample from the response for the analyte using the calibration procedure described in Section 10.0.

12.2 Calculate the amount of sample analyte injected from the peak response using the calibration curve or calibration response factor determined in Section 10.2. The concentration (C) in the sample can be calculated from Equation 1.

Equation 1

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

where: A = Amount of standard injected (ng).
V_i = Volume of standard injected (mL).
V_t = Volume of sample injected (mL).
V_s = Volume of water sample (mL).

13.0 METHOD PERFORMANCE

13.1 In a single laboratory, analyte recoveries from reagent water were determined at two concentration levels. Results were used to determine analyte MDLs[5] and demonstrated method range. Analyte MDLs and analyte recoveries and standard deviations about the percent recoveries at one concentration are given in Table 2.

13.2 In a single laboratory, analyte recoveries from dechlorinated tap water and ground waters were determined at one concentration level, 10 ug/L. Results were used to demonstrate applicability of the method to different tap and ground water matrices. Analyte recoveries from tap water and ground water are given in Table 4. MDLs calculated from results of analyses of six 100 mL reagent water samples at 0.5 µg/L concentrations for each analyte are listed in Table 5.

14.0 POLLUTION PREVENTION

14.1 This method utilizes the new in-line liquid-solid extraction technology which requires the use of very small quantities of organic solvents. This feature eliminates the hazards involved with the use of large volumes of potentially harmful organic solvents needed for conventional liquid-liquid extractions. Also, this method uses no derivatizing reagents, which are toxic or explosive, to form gas chromatographable derivatives. These features make this method much safer for use by the analyst in the laboratory and a great deal less harmful to the environment.

14.2 For information about 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 Due to the nature of this method, there is little need for waste management. No large volumes of solvents or hazardous chemicals are used. The matrices of concern are finished drinking water or source water. However, the Agency requires that laboratory waste management practices be consistent with all applicable rules and regulations, and that laboratories 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, particularly the hazardous waste identification rules and land disposal restrictions. 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 14.2.

16.0 REFERENCES

1. Glazer, J.A., Foerst, D.L., McKee, G.D., Quave, S.A., and Budde, W.L. Environ. Sci. Technol. 15, 1981, pp. 1426-1435.

2. "Pesticide Methods Evaluation," Letter Report #33 for EPA Contract No. 68-03-2697. Available from U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

3. ASTM Annual Book of Standards, Part 11, Volume 11.02, D3694-82, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 86, 1986.

4. Giam, C.S., Chan, H.S., and Nef., G.S. "Sensitive Method for Determination of Phthalate Ester Plasticizers in Open-Ocean Biota Samples," Analytical Chemistry, 47, 2225 (1975).

5. 40 CFR, Part 136, Appendix B.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. RETENTION TIMES FOR METHOD ANALYTES

Analyte	Group	Retention Times[a] (minutes) Primary Column	Confirmation Column
Picloram	(A)	19.0	12.8
5-Hydroxydicamba	(A)	19.7	13.5
Chloramben	(A)	21.1	14.8
4-Nitrophenol	(B)	21.6	5.0
Dicamba	(A)	24.0	18.2
Bentazon	(A)	25.2	19.5
MCPA	(B)	25.5	20.1
2,4-D	(A)	25.6	20.1
3,5-Dichlorobenzoic acid	(B)	26.7	21.3
MCPP	(B)	27.2	21.8
Dichloroprop	(A)	27.3	21.8
2,4,5-T	(B)	27.5	22.4
2,4-DB	(B)	28.0	22.8
2,4,5-TP	(A)	29.2	23.9
Acifluorfen	(A)	30.7	25.5
Dinoseb	(B)	32.8	27.7
Pentachlorophenol	(B)	33.4	28.3

[a]Columns and analytical conditions are described in Sections 6.4.1 and 6.4.2.

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

Analyte	MDL (µg/L)[b]	Concentration (µg/L)	Reagent Water	
			R[c]	S_R[d]
Acifluorfen	1.7	10.0	104	1.7
Bentazon	4.6	10.0	126	14.6
Chloramben	3.1	10.0	83	10.0
2,4-D	1.3	10.0	112	4.2
2,4-DB	1.9	10.0	92	5.9
Dicamba	2.1	10.0	104	6.6
3,5-Dichlorobenzoic acid	2.1	10.0	94	6.7
Dichlorprop	1.7	10.0	108	5.4
Dinoseb	1.5	10.0	97	4.8
5-Hydroxydicamba	2.2	10.0	132	7.0
MCPA	0.8	10.0	93	2.5
MCPP	1.7	10.0	95	5.5
4-Nitrophenol	1.2	10.0	95	4.0
Pentachlorophenol (PCP)	1.6	10.0	99	5.2
Picloram	0.5	10.0	104	1.7
2,4,5-T	1.3	10.0	93	4.1
2,4,5-TP	1.8	10.0	90	5.8

[a]Data represent the average of six to seven samples. Sample volume = 20 mL.
[b]MDL = method detection limit; defined in Appendix B to 40 CFR Part 136 - Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11.
[c]R = average percent recovery.
[d]S_r = standard deviation of the percent recovery.

TABLE 3. CONFIRMATION WAVELENGTHS AND AREA RESPONSE RATIOS FOR METHOD ANALYTES

Analyte	Confirmation Wavelength (nm)	Area Response Ratio[a]
Acifluorfen	293	1.72
Bentazon	240	1.08
Chloramben	214	0.61
2,4-D	285	4.02
2,4-DB	285	5.93
Dicamba	220	0.66
3,5-Dichlorobenzoic acid	285	5.15
Dichlorprop	285	4.07
Dinoseb	268	0.48
5-Hydroxydicamba	293	1.89
MCPA	285	6.66
MCPP	285	6.49
4-Nitrophenol	310	0.56
Pentachlorophenol (PCP)	290	5.65
Picloram	223	0.82
2,4,5-T	290	4.00
2,4,5-TP	293	3.84

[a]Area Response Ratio = Peak Area for 230 nm / Peak Area for Conf. Wavelength

TABLE 4. SINGLE LABORATORY PRECISION AND ACCURACY DATA FROM TAP WATER AND GROUND WATER[a]

	Dechlorinated			
	Tap Water		Ground Water	
Analyte	R[b]	S_R[c]	R[b]	S_R[c]
Acifluorfen	65.7	±27.	87.3	±17.
Bentazon	86.1	±6.0	90.1	±9.0
Chloramben	100	±5.5	88.2	±5.9
2,4-D	117	±9.6	105	±8.1
2,4-DB	91.2	±7.0	97.2	±6.1
Dicamba	94.3	±6.1	86.0	±7.7
3,5-Dichlorobenzoic acid	90.2	±7.2	92.1	±5.5
Dichlorprop	92.9	±6.1	98.3	±10.
Dinoseb	94.1	±6.2	91.2	±4.1
5-Hydroxydicamba	110	±5.5	108	±7.0
MCPA	92.7	±5.0	85.2	±5.7
MCPP	91.4	±7.7	84.3	±5.9
4-Nitrophenol	89.2	±10.	103	±3.4
Pentachlorophenol (PCP)	102	±4.2	92.6	±9.1
Picloram	99.0	±4.9	84.3	±5.8
2,4,5-T	88.2	±7.8	90.0	±6.2
2,4,5-TP	90.3	±5.9	77.8	±8.9

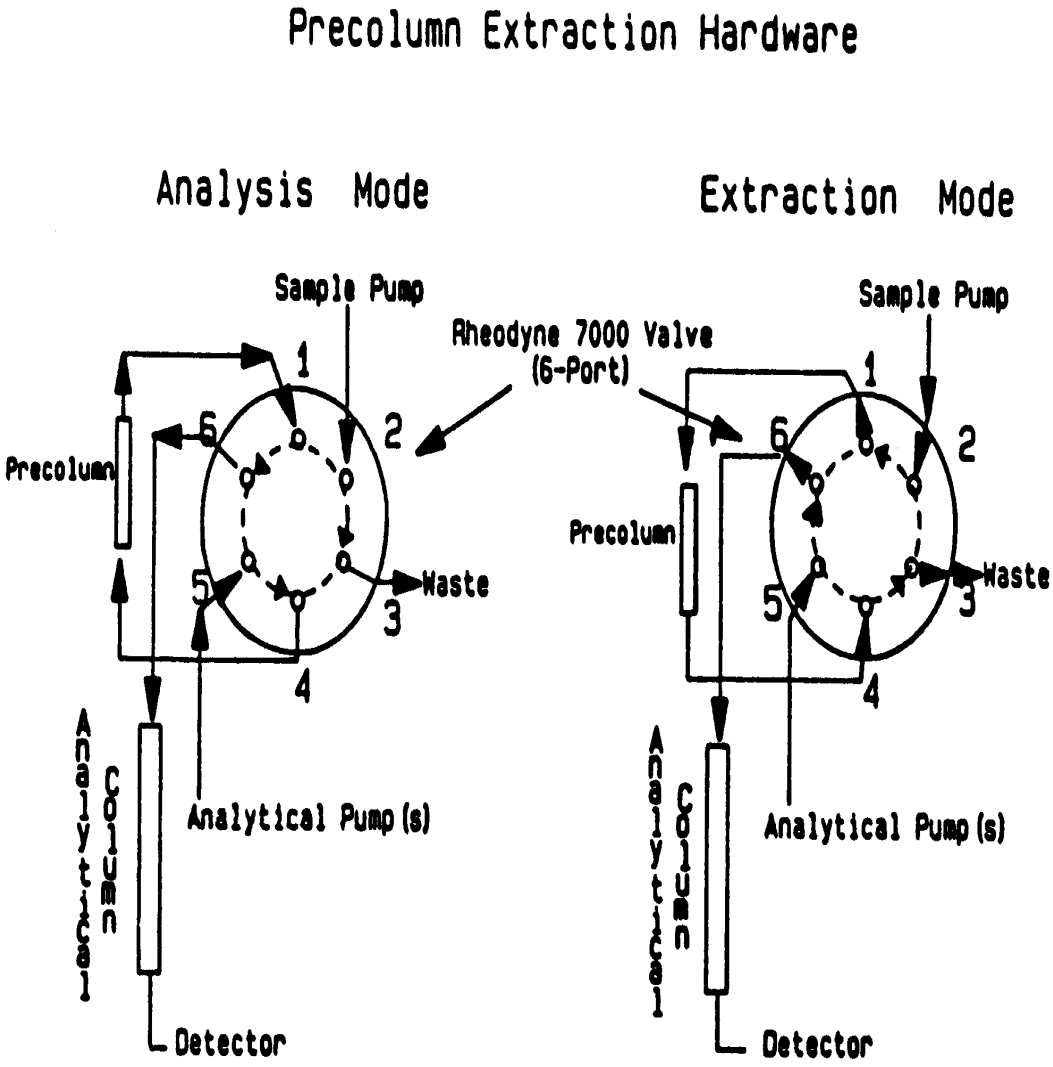
[a]Average of six samples fortified at 10 µg/L.
[b]Mean percent recovery, corrected for background levels.
[c]Standard deviation of the mean percent recovery.

TABLE 5. SINGLE LABORATORY RECOVERY AND PRECISION DATA AND METHOD DETECTION LIMITS (MDLS) FOR ANALYTES FROM REAGENT WATER[a]

Analyte	MDL (µg/L)[b]	Concentration (µg/L)	Reagent Water	
			R[c]	S_R[d]
Acifluorfen	0.40	0.5	114	23.7
Bentazon	0.12	0.5	91	7.3
Chloramben	N.R.	0.5	N.R.	N.R.
2,4-D	0.34	0.5	121	20.2
2,4-DB	0.31	0.5	99	18.5
Dicamba	0.24	0.5	80	14.1
3,5-Dichlorobenzoic acid	0.38	0.5	105	22.5
Dichlorprop	0.33	0.5	110	19.4
Dinoseb	0.26	0.5	99	15.5
5-Hydroxdicamba	N.R.	0.5	N.R.	N.R.
MCPA	0.35	0.5	124	21.0
MCPP	0.19	0.5	125	11.1
4-Nitrophenol	N.R.	0.5	N.R.	N.R.
Pentachlorophenol (PCP)	0.15	0.5	93	8.6
Picloram	N.R.	0.5	N.R.	N.R.
2,4,5-T	0.21	0.5	80	12.7
2,4,5-TP	0.37	0.5	77	21.7

[a]Data represent the average of six samples. Sample Volume = 100 mL
[b]MDL = Method detection limit; defined in Appendix B to 40 CFR 136 - Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11.
[c]R = Average percent recovery.
[d]S_r = Standard deviation of the percent recovery.
N.R. = Not Recovered.

FIGURE 1. SCHEMATIC DIAGRAM OF SAMPLE CONCENTRATION AND ANALYTICAL HPLC HARDWARE



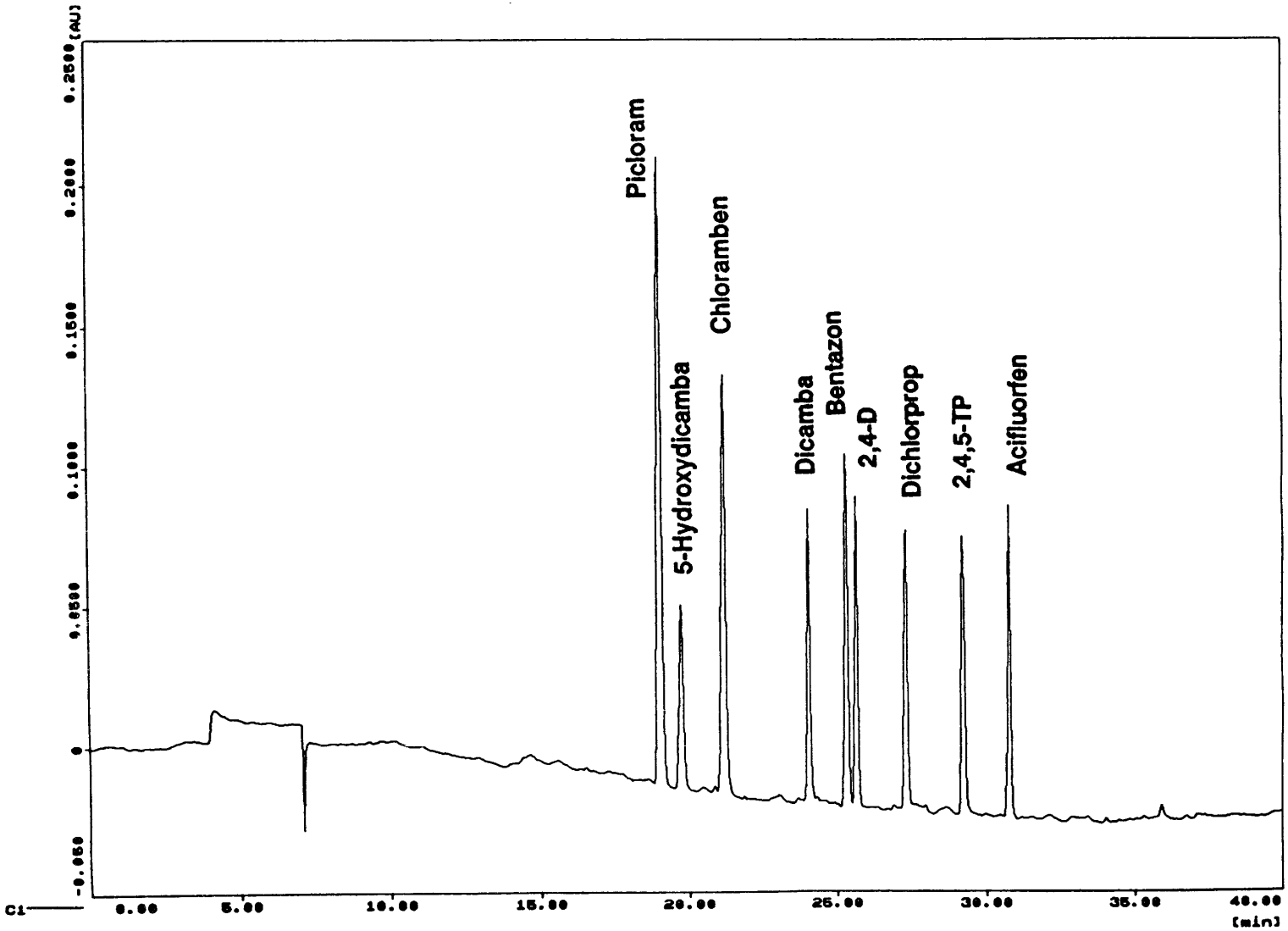


FIGURE 2. TYPICAL HPLC CHROMATOGRAM OF GROUP A ANALYTES

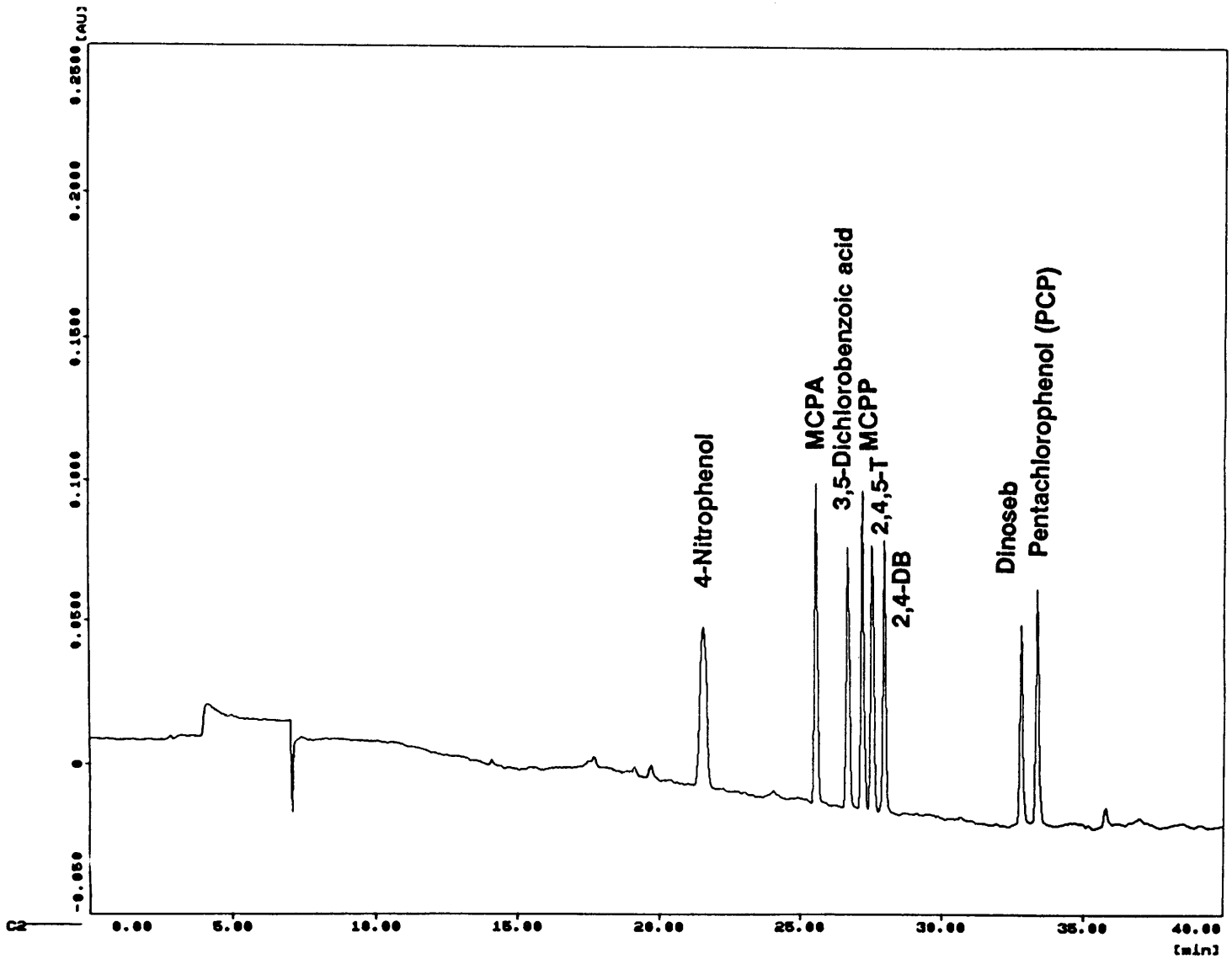


FIGURE 3. TYPICAL HPLC CHROMATOGRAM OF GROUP B ANALYTES