

**METHOD 515.1. DETERMINATION OF CHLORINATED ACIDS IN WATER BY GAS
CHROMATOGRAPHY WITH AN ELECTRON CAPTURE DETECTOR**

Revision 4.1

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R.C. Dressman and J.J. Lichtenberg - EPA 600/4-81-053, Revision 1.0 (1981)

J.W. Hodgeson - Method 515, Revision 2.0 (1986)

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R.L. Graves - Method 515.1, Revision 4.0 (1989)

**NATIONAL EXPOSURE RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268**

METHOD 515.1

DETERMINATION OF CHLORINATED ACIDS IN WATER BY GAS CHROMATOGRAPHY WITH AN ELECTRON CAPTURE DETECTOR

1. SCOPE AND APPLICATION

- 1.1 This is a gas chromatographic (GC) method applicable to the determination of certain chlorinated acids in ground water and finished drinking water. The following compounds can be determined by this method:

<u>Analyte</u>	Chemical Abstract Services <u>Registry Number</u>
Acifluorfen*	50594-66-6
Bentazon	25057-89-0
Chloramben*	133-90-4
2,4-D	94-75-7
Dalapon*	75-99-0
2,4-DB	94-82-6
DCPA acid metabolites (a)	
Dicamba	1918-00-9
3,5-Dichlorobenzoic acid	51-36-5
Dichlorprop	120-36-5
Dinoseb	88-85-7
5-Hydroxydicamba	7600-50-2
4-Nitrophenol*	100-02-7
Pentachlorophenol (PCP)	87-86-5
Picloram	1918-02-1
2,4,5-T	93-76-5
2,4,5-TP	93-72-1

(a)DCPA monoacid and diacid metabolites included in method scope; DCPA diacid metabolite used for validation studies.

*These compounds are only qualitatively identified. These compounds are not quantitated because control over precision has not been accomplished.

- 1.2 This method is also applicable to the determination of salts and esters of analyte acids. The form of each acid 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 estimated detection limits (EDLs) and method detection limits (MDLs) have been determined for the analytes above (Sect.13). Observed detection limits may vary between ground waters, depending upon 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 GC and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Sect. 9.3.
- 1.5 Analytes that are not separated chromatographically i.e., which have very similar retention times, cannot be individually identified and measured in the same calibration mixture or water sample unless an alternate technique for identification and quantitation exist (Sect. 11.9).
- 1.6 When this method is used to analyze unfamiliar samples for any or all of the analytes above, analyte identifications must be confirmed by at least one additional qualitative technique.

2. SUMMARY OF METHOD

- 2.1 A measured volume of sample of approximately 1 L is adjusted to pH 12 with 6 N sodium hydroxide and shaken for 1 hr to hydrolyze derivatives. (Note: Since many of the herbicides contained in this method are applied as a variety of esters and salts, it is vital to hydrolyze them to the parent acid prior to extraction.) Extraneous organic material is removed by a solvent wash. The sample is acidified, and the chlorinated acids are extracted with ethyl ether by shaking in a separatory funnel or mechanical tumbling in a bottle. The acids are converted to their methyl esters using diazo-methane as the derivatizing agent or alternatively, trimethylsilyldiazomethane (TMSD). Excess derivatizing reagent is removed, and the esters are determined by capillary column/GC using an electron capture detector (ECD).
- 2.2 The method provides aa optional Florisil separation procedure to aid in the elimination of interferences that may be encountered.

3. DEFINITIONS

- 3.1 INTERNAL STANDARD -- A pure analyte(s) added to a solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.
- 3.2 SURROGATE ANALYTE -- A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction and is measured with the same procedures used to measure other sample components. The purpose of a surrogate analyte is to monitor method performance with each sample.
- 3.3 LABORATORY DUPLICATES (LD1 and LD2) -- Two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

- 3.4 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.
- 3.5 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.
- 3.6 FIELD REAGENT BLANK (FRB) -- Reagent water placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.
- 3.7 LABORATORY PERFORMANCE CHECK SOLUTION (LPC) -- A solution of method analytes, surrogate compounds, and internal standards used to evaluate the performance of the instrument system with respect to a defined set of method criteria.
- 3.8 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements at the required method detection limit.
- 3.9 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.
- 3.10 STOCK STANDARD SOLUTION -- A concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.
- 3.11 PRIMARY DILUTION STANDARD SOLUTION -- 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.
- 3.12 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

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

4. INTERFERENCES

- 4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Sect. 9.2.
- 4.1.1 Glassware must be scrupulously cleaned.(1) 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 1 hr. Do not heat volumetric glassware. 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. Also, when a solvent is purified, preservatives added by the manufacturer are removed, thus potentially reducing the shelf-life.
- 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 and glass wool must be acid-rinsed with 1N hydrochloric acid and the sodium sulfate must be acidified with sulfuric acid prior to use to avoid analyte losses due to adsorption.
- 4.3 Organic acids and phenols, especially chlorinated compounds, cause the most direct interference with the determination. Alkaline hydrolysis and subsequent extraction of the basic sample removes many chlorinated hydrocarbons and phthalate esters that might otherwise interfere with the electron capture analysis.
- 4.4 Interferences by phthalate esters can pose a major problem in pesticide analysis when using the ECD. These compounds generally appear in the chromatogram as large peaks. Common flexible plastics contain varying amounts of phthalates, that are easily extracted or leached during laboratory operations. Cross contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Interferences from phthalates can best be

minimized by avoiding the use of plastics in the laboratory. Exhaustive purification of reagents and glassware may be required to eliminate background phthalate contamination.(1)

- 4.5 Interfering contamination may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. Between-sample rinsing of the sample syringe and associated equipment with methyl-t-butyl-ether (MTBE) can minimize sample cross contamination. After analysis of a sample containing high concentrations of analytes, one or more injections of MTBE should be made to ensure that accurate values are obtained for the next sample.
- 4.6 Matrix interferences may be caused by contaminants that are coextracted from the sample. Also, note that all analytes listed in the Scope and Application Section are not resolved from each other on any one column, i.e., one analyte of interest may be an interferant for another analyte of interest. The extent of matrix interferences will vary considerably from source to source, depending upon the water sampled. The procedures in Sect. 11 can be used to overcome many of these interferences. Positive identifications should be confirmed (Sect. 11.9).
- 4.7 It is important that samples and working standards be contained in the same solvent. The solvent for working standards must be the same as the final solvent used in sample preparation. If this is not the case, chromatographic comparability of standards to sample may be affected.

5. **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. Additional references to laboratory safety are available and have been identified (2-4) for the information of the analyst.
- 5.2 DIAZOMETHANE -- A toxic carcinogen which can explode under certain conditions. The following precautions must be followed:
 - 5.2.1 Use only a well ventilated hood -- do not breath vapors.
 - 5.2.2 Use a safety screen.
 - 5.2.3 Use mechanical pipetting aides.
 - 5.2.4 Do not heat above 90°C -- **EXPLOSION** may result.

- 5.2.5 Avoid grinding surfaces, ground glass joints, sleeve bearings, glass stirrers -- **EXPLOSION** may result.
 - 5.2.6 Store away from alkali metals -- **EXPLOSION** may result.
 - 5.2.7 Solutions of diazomethane decompose rapidly in the presence of solid materials such as copper powder, calcium chloride, and boiling chips.
 - 5.2.8 The diazomethane generation apparatus used in the esterification procedures (Sect. 11.4 and 11.5) produces micromolar amounts of diazomethane to minimize safety hazards.
- 5.3 ETHYL ETHER -- Nanograde, redistilled in glass, if necessary.
- 5.3.1 Ethyl ether is an extremely flammable solvent. If a mechanical device is used for sample extraction, the device should be equipped with an explosion-proof motor and placed in a hood to avoid possible damage and injury due to an explosion.
 - 5.3.2 Ethyl ether must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Cat. No. PI126-8, and other suppliers).
- 5.4 WARNING: When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous.

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

- 6.1 SAMPLE BOTTLE -- Borosilicate, 1-L volume with graduations (Wheaton Media/Lab bottle 219820 or equivalent), fitted with screw caps lined with TFE-fluorocarbon. Protect samples from light. The container must be washed and dried as described in Sect. 4.1.1 before use to minimize contamination. Cap liners are cut to fit from sheets (Pierce Catalog No. 012736) and extracted with methanol overnight prior to use.
- 6.2 GLASSWARE
- 6.2.1 Separatory funnel -- 2000-mL, with TFE-fluorocarbon stopcocks, ground glass or TFE-fluorocarbon stoppers.
 - 6.2.2 Tumbler bottle -- 1.7-L (Wheaton Roller Culture Vessel or equivalent), with TFE-fluorocarbon lined screw cap. Cap liners are cut to fit from sheets (Pierce Catalog No. 012736) and extracted with methanol overnight prior to use.
 - 6.2.3 Concentrator tube, Kuderna-Danish (K-D) -- 10- or 25-mL, graduated (Kontes K-570050-2525 or Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stoppers are used to prevent evaporation of extracts.

- 6.2.4 Evaporative flask, K-D -- 500-mL (Kontes K-57000I-0500 or equivalent). Attach to concentrator tube with springs.
- 6.2.5 Snyder column, K-D -- three-ball macro (Kontes K-503000-012I or equivalent).
- 6.2.6 Snyder column, K-D -- two-ball micro (Kontes K-56900I-0219 or equivalent).
- 6.2.7 Flask, round-bottom -- 500-mL with 24/40 ground glass joint.
- 6.2.8 Vials -- glass, 5- to 10-mL capacity with TFE-fluorocarbon lined screw cap.
- 6.2.9 Disposable pipets -- sterile plugged borosilicate glass, 5-mL capacity (Corning 7078-5N or equivalent).
- 6.3 SEPARATORY FUNNEL SHAKER -- Capable of holding 2-L separatory funnels and shaking them with rocking motion to achieve thorough mixing of separatory funnel contents (available from Eberbach Co. in Ann Arbor, MI or other suppliers).
- 6.4 TUMBLER -- Capable of holding tumbler bottles and tumbling them end-over-end at 30 turns/min (Associated Design and Mfg. Co., Alexandria, VA and other suppliers).
- 6.5 BOILING STONES -- Teflon, Chemware (Norton Performance Plastics No. 015021 and other suppliers).
- 6.6 WATER BATH -- Heated, capable of temperature control ($\pm 2^{\circ}\text{C}$). The bath should be used in a hood.
- 6.7 BALANCE -- Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 6.8 DIAZOMETHANE GENERATOR -- Assemble from two 20 x 150 mm test tubes, two Neoprene rubber stoppers, and a source of nitrogen as shown in Figure 1 (available from Aldrich Chemical Co.). When esterification is performed using diazomethane solution, the diazomethane collector is cooled in an approximately 2-L thermos for ice bath or a cryogenically cooled vessel (Thermoelectrics Unlimited Model SK-12 or equivalent).
- 6.9 GLASS WOOL -- Acid washed (Supelco 2-0383 or equivalent) and heated at 450°C for 4 hr.
- 6.10 GAS CHROMATOGRAPH -- Analytical system complete with temperature programmable GC suitable for use with capillary columns and all required accessories including syringes, analytical columns, gases, detector and stripchart recorder or computerized data system. A data system is recommended for measuring peak areas. Table 1 lists retention times observed for method analytes using the columns and analytical conditions described below.
 - 6.10.1 Column 1 (Primary column) -- 30 m long x 0.25 mm I.D. DB-5 bonded fused silica column, 0.25 μm film thickness (J&W Scientific). Helium carrier gas

flow is established at 30 cm/sec linear velocity and oven temperature is programmed from 60°C to 300°C at 4°C/min. Data presented in this method were obtained using this column. The injection volume was 2 µL splitless mode with 45 second delay. The injector temperature was 250°C and the detector was 320°C. Alternative columns may be used in accordance with the provisions described in Sect. 9.4.

6.10.2 Column 2 (Confirmation column) -- 30 m long x 0.25 mm I.D. DB-1701 bonded fused silica column, 0.25 µm film thickness (J&W Scientific). Helium carrier gas flow is established at 30 cm/sec linear velocity and oven temperature is programmed from 60°C to 300°C at 4°C/min.

6.10.3 Detector -- Electron capture. This detector has proven effective in the analysis of method analytes in fortified reagent and artificial ground waters.

7. REAGENTS AND STANDARDS - WARNING: When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous. Also, when a solvent is purified, preservatives added by the manufacturer are removed, thus potentially reducing the shelf-life.

7.1 ACETONE, METHANOL, METHYLENE CHLORIDE, MTBE -- Pesticide quality or equivalent.

7.2 ETHYL ETHER, UNPRESERVED -- Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Cat. No. PI126-8, and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips.

7.3 SODIUM SULFATE, GRANULAR, ANHYDROUS, ACS GRADE -- Heat treat in a shallow tray at 450°C for a minimum of 4 hr to remove interfering organic substances. Acidify by slurring 100 g sodium sulfate with enough ethyl ether to just cover the solid. Add 0.1 mL concentrated sulfuric acid and mix thoroughly. Remove the ether under vacuum. Mix 1 g of the resulting solid with 5 mL of reagent water and measure the pH of the mixture. The pH must be below pH 4. Store at 130°C.

7.4 SODIUM THIOSULFATE, GRANULAR, ANHYDROUS -- ACS grade.

7.5 SODIUM HYDROXIDE (NAOH), PELLETS -- ACS grade.

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

7.6 SULFURIC ACID, CONCENTRATED -- ACS grade, sp. gr. 1.84.

7.6.1 Sulfuric acid, 12 N -- Slowly add 335 mL concentrated sulfuric acid to 665 mL of reagent water.

7.7 POTASSIUM HYDROXIDE (KOH), PELLETS -- ACS grade.

- 7.7.1 KOH, 37% (w/v) -- Dissolve 37 g KOH pellets in reagent water and dilute to 100 mL.
- 7.8 CARBITOL (DIETHYLENE GLYCOL MONOETHYL ETHER) -- ACS grade. Available from Aldrich Chemical Co.
- 7.9 DIAZALD, ACS grade -- Available from Aldrich Chemical Co.
- 7.10 DIAZALD SOLUTION -- Prepare a solution containing 10 g Diazald in 100 mL of a 50:50 by volume mixture of ethyl ether and carbitol. This solution is stable for one month or longer when stored at 4°C in an amber bottle with a Teflon-lined screw cap.
- 7.11 TRIMETHYLSILYLDIAZOMETHANE (TMSD) -- Available from Aldrich Chemical Co. as a 2 molar solution in hexane. TMSD is stable during storage in this solution.
- 7.12 SODIUM CHLORIDE (NaCl), CRYSTAL, ACS GRADE -- Heat treat in a shallow tray at 450°C for a minimum of 4 hr to remove interfering organic substances.
- 7.13 4,4'-DIBROMOOCTAFLUOROBIPHENYL (DBOB) -- 99% purity, for use as internal standard (available from Aldrich Chemical Co).
- 7.14 2,4-DICHLOROPHENYLACETIC ACID (DCAA) -- 99% purity, for use as surrogate standard (available from Aldrich Chemical Co).
- 7.15 MERCURIC CHLORIDE -- ACS grade (Aldrich Chemical Co.) - for use as a bactericide (optional- see Section 8).
- 7.16 REAGENT WATER -- Reagent water is defined as water that is reasonably free of contamination that would prevent the determination of any analyte of interest. Reagent water used to generate the validation data in this method was distilled water obtained from the Magnetic Springs Water Co., Columbus, Ohio.
- 7.17 SILICIC ACID, ACS GRADE.
- 7.18 FLORISIL -- 60-100/PR mesh (Sigma No. F-9127). Activate by heating in a shallow container at 150°C for at least 24 and not more than 48 hr.
- 7.19 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:
- 7.19.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in MTBE 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.

- 7.19.2 Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw cap amber vials. Store at room temperature and protect from light.
- 7.19.3 Stock standard solutions should be replaced after two months or sooner if comparison with laboratory fortified blanks, or QC samples indicate a problem.
- 7.20 INTERNAL STANDARD SOLUTION -- Prepare an internal standard solution by accurately weighing approximately 0.0010 g of pure DBOB. Dissolve the DBOB in MTBE and dilute to volume in a 10-mL volumetric flask. Transfer the internal standard solution to a TFE-fluorocarbon-sealed screw cap bottle and store at room temperature. Addition of 25 μL of the internal standard solution to 10 mL of sample extract, or 12.5 μL to 5 mL of sample extract, results in a final internal standard concentration of 0.25 $\mu\text{g/mL}$. Solution should be replaced when ongoing QC (Sect. 9) indicates a problem. Note that DBOB has been shown to be an effective internal standard for the method analytes, but other compounds may be used if the quality control requirements in Sect. 9 are met.
- 7.21 SURROGATE STANDARD SOLUTION -- Prepare a surrogate standard solution by accurately weighing approximately 0.0010 g of pure DCAA. Dissolve the DCAA in MTBE and dilute to volume in a 10-mL volumetric flask. Transfer the surrogate standard solution to a TFE-fluorocarbon-sealed screw cap bottle and store at room temperature. Addition of 50 μL of the surrogate standard solution to a 1-L sample prior to extraction results in a surrogate standard concentration in the sample of 5 $\mu\text{g/L}$ and, assuming quantitative recovery of DCAA, a surrogate standard concentration in the final extract of 0.5 $\mu\text{g/mL}$. Solution should be replaced when ongoing QC (Sect. 9) indicates a problem. Note DCAA has been shown to be an effective surrogate standard for the method analytes, but other compounds may be used if the quality control requirements in Sect. 9 are met.
- 7.22 LABORATORY PERFORMANCE CHECK SOLUTIONS -- Prepare a diluted dinoseb solution by adding 10 μL of the 1.0 $\mu\text{g}/\mu\text{L}$ dinoseb stock solution to the MTBE and diluting to volume in a 10-mL volumetric flask. To prepare the check solution, add 40 μL of the diluted dinoseb solution, 16 μL of the 4-nitrophenol stock solution, 6 μL of the 3,5-dichlorobenzoic acid stock solution, 50 μL of the surrogate standard solution, 25 μL of the internal standard solution, and 250 μL of methanol to a 5-mL volumetric flask and dilute to volume with MTBE. Methylate sample as described in Sects. 11.4 or 11.5. Dilute the sample to 10 mL in MTBE. Transfer to a TFE-fluorocarbon-sealed screw cap bottle and store at room temperature. Solution should be replaced when ongoing QC (Sect. 9) indicates a problem.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

- 8.1 Grab samples must be collected in glass containers. Conventional sampling practices (5) should be followed; however, the bottle must not be prerinsed with sample before collection.
- 8.2 SAMPLE PRESERVATION AND STORAGE

- 8.2.1 If residual chlorine is present, add 80 mg of sodium thiosulfate (or 50 mg sodium sulfite) per liter of sample to the sample bottle prior to collecting the sample.
- 8.2.2 After the sample is collected in a bottle containing the dechlorinating agent, seal the bottle and shake until dissolved.
- 8.2.3 The samples must be iced or refrigerated at 4°C away from light from the time of collection until extraction. Preservation study results indicated that most method analytes present in samples were stable for 14 days when stored under these conditions. Analyte stability may be affected by the matrix; therefore, the analyst should verify that the preservation technique is applicable to the samples under study.
- 8.2.4 All performance data presented in this method are from samples preserved with mercuric chloride. No suitable preservation agent (biocide) has been found other than mercuric chloride. However the use of mercuric chloride is not required due to its toxicity and potential harm to the environment.
- 8.2.5 In some circumstances where biological degradation of target pesticides might be expected, use of mercuric chloride may be appropriate to minimize the possibility of false-negative results. If mercuric chloride is to be used, add it (Sect. 7.8) to the sample bottle in amounts to produce a concentration of 10 mg/L. Add 1 mL of a solution containing 10 mg/mL of mercuric chloride in reagent water to the sample bottle at the sampling site or in the laboratory before shipping to the sampling site. A major disadvantage of mercuric chloride is that it is a highly toxic chemical; mercuric chloride must be handled with caution, and samples containing mercuric chloride must be disposed of properly.

8.3 EXTRACT STORAGE

- 8.3.1 Extracts should be stored at 4°C away from light. Preservation study results indicate that most analytes are stable for 28 days; however, the analyst should verify appropriate extract holding times applicable to the samples under study.

9. QUALITY CONTROL

- 9.1 Minimum quality control (QC) requirements are initial demonstration of laboratory capability, determination of surrogate compound recoveries in each sample and blank, monitoring internal standard peak area or height in each sample and blank (when internal standard calibration procedures are being employed), analysis of laboratory reagent blanks, laboratory fortified samples, laboratory fortified blanks, and QC samples. A MDL for each analyte must also be determined.
- 9.2 LABORATORY REAGENT BLANKS (LRB). Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is extracted or reagents are changed, 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. Suggested concentrations are 10 times the EDL or a concentration that represents a mid-point in the calibration range. Prepare a primary dilution standard (in methanol) containing each analyte at 1000 times selected concentration. With a syringe, add 1 mL of the concentrate to each of four to seven 1-L aliquots of reagent water, and analyze each aliquot according to procedures beginning in Sect. 11.
- 9.3.2 For each analyte the recovery value for all of these samples must fall in the range of $\pm 30\%$ of the fortified amount, with the RSD of the measurements 30% or less. For those compounds that meet the acceptable criteria, performance is considered acceptable and sample analysis may begin. For those compounds that fail these criteria, this procedure must be reported using fresh samples until satisfactory performance has been demonstrated.
- 9.3.3 For each analyte, determine the MDL. Prepare a minimum of 7 LFBs at a low concentration. Fortification concentrations in Table 3 may be used as a guide, or use calibration data obtained in Section 10 to estimate a concentration for each analyte that will produce a peak with a 3-5 times signal to noise response. Extract and analyze each replicate according to Sections 11 and 12. It is recommended that these LFBs be prepared and analyzed over a period of several days, so that day to day variations are reflected in precision measurements. Calculate mean recovery and standard deviation for each analyte. Use the standard deviation and the equation given in Table 3 to calculate the MDL.
- 9.3.4 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. It is expected that as laboratory personnel gain experience with this method the quality of data will improve beyond those required here.

- 9.4 The analyst is permitted to modify GC columns, GC conditions, concentration techniques (i.e., evaporation techniques), internal standard or surrogate compounds. Each time such method modifications are made, the analyst must repeat the procedures in Sect. 9.3
- 9.5 ASSESSING SURROGATE RECOVERY.
- 9.5.1 When surrogate recovery from a sample or method blank is <70% or >130%, check (1) calculations to locate possible errors, (2) standard solutions for degradation, (3) contamination, and (4) instrument performance. If those steps do not reveal the cause of the problem, reanalyze the extract.
- 9.5.2 If a LRB extract reanalysis fails the 70-130% recovery criterion, the problem must be identified and corrected before continuing.
- 9.5.3 If sample extract reanalysis meets the surrogate recovery criterion, report only data for the reanalyzed extract. If sample extract continues to fail the recovery criterion, report all data for that sample as suspect.
- 9.6 ASSESSING THE INTERNAL STANDARD
- 9.6.1 When using the internal standard calibration procedure, the analyst must monitor the IS response (peak area or peak height) of all samples during each analysis day. The IS response for any sample chromatogram should not deviate from the daily calibration check standard's IS response by more than 30%.
- 9.6.2 If >30% deviation occurs with an individual extract, optimize instrument performance and inject a second aliquot of that extract.
- 9.6.2.1 If the reinjected aliquot produces an acceptable internal standard response, report results for that aliquot.
- 9.6.2.2 If a deviation of greater than 30% is obtained for the reinjected extract, analysis of the samples should be repeated beginning with Sect. 11, provided the sample is still available. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.
- 9.6.3 If consecutive samples fail the IS response acceptance criterion, immediately analyze a calibration check standard.
- 9.6.3.1 If the check standard provides a response within 20% of the predicted value, then follow procedures itemized in Sect. 9.6.2 for each sample failing the IS response criterion.
- 9.6.3.2 If the check standard provides a response which deviates more than 20% of the predicted value, then the analyst must recalibrate, as specified in Sect. 10.

9.7 ASSESSING LABORATORY PERFORMANCE - LABORATORY FORTIFIED BLANK

- 9.7.1 The laboratory must analyze at least one laboratory fortified blank (LFB) sample with every 20 samples or one per sample set (all samples extracted within a 24-hr period) whichever is greater. The concentration of each analyte in the LFB should be 10 times EDL or a concentration which represents a mid-point in the calibration. Calculate accuracy as percent recovery (X_i). If the recovery of any analyte falls outside the control limits (see Sect. 9.7.2), that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.
- 9.7.2 Until sufficient data become available from within their own laboratory, usually a minimum of results from 20 to 30 analyses, the laboratory should assess laboratory performance against the control limits in Sect. 9.3.2 that are derived from the data in Table 2. When sufficient internal performance data becomes available, develop control limits from the mean percent recovery (\bar{X}) and standard deviation (S) of the percent recovery. These data are used to establish upper and lower control limits as follows:

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

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

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

- 9.7.3 It is recommended that the laboratory periodically determine and document its detection limit capabilities for the analytes of interest.
- 9.7.4 At least quarterly, analyze a QC sample from an outside source.

9.8 ASSESSING ANALYTE RECOVERY - LABORATORY FORTIFIED SAMPLE MATRIX

- 9.8.1 The laboratory must 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 (Sect. 9.7). Over time, samples from all routine sample sources should be fortified.

- 9.8.2 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, i.e.,:

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

and compare these values to control limits appropriate for reagent water data collected in the same fashion. The value for P must fall between 65%-135% of the fortified concentration.

- 9.8.3 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 (Sect. 9.7), 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.

9.9 ASSESSING INSTRUMENT SYSTEM - LABORATORY PERFORMANCE CHECK SAMPLE

-Instrument performance should be monitored on a daily basis by analysis of the LPC sample. The LPC sample contains compounds designed to monitor instrument sensitivity, column performance (primary column) and chromatographic performance. LPC sample components and performance criteria are listed in Table 4. Inability to demonstrate acceptable instrument performance indicates the need for reevaluation of the instrument system. The sensitivity requirements are set based on the EDLs published in this method. If laboratory EDLs differ from those listed in this method, concentrations of the LPC compounds must be adjusted to be compatible with the laboratory EDLs.

- 9.10 The laboratory may adopt additional quality control practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation and storage.

10. **CALIBRATION AND STANDARDIZATION**

- 10.1 Establish GC operating parameters equivalent to those indicated in Sect. 6.10. The GC system may be calibrated using either the internal standard technique (Sect. 10.2) or the external standard technique (Sect. 10.3). NOTE: Calibration standard solutions must be prepared such that no unresolved analytes are mixed together.

- 10.2 INTERNAL STANDARD CALIBRATION PROCEDURE -- To use this approach, the analyst must select one or more internal standards compatible 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. DBOB has been identified as a suitable internal standard.

- 10.2.1 Prepare calibration standards at a minimum of three (recommend five) concentration levels for each analyte 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 of the internal standards and 250 μ L methanol, and dilute to volume with MTBE. Esterify acids with diazomethane as described in Sect. 11.4 or 11.5.

Guidance on the number of standards is as follows: A minimum of three calibration standards are required to calibrate a range of a factor of 20 in concentration. For a factor of 50 use at least four standards, and for a factor of 100 at least five standards. One calibration standard should contain each analyte of concern at a concentration 2 to 10 times greater than the method detection limit for that compound. The other calibration standards should contain each analyte of concern at concentrations that define the range of the sample analyte concentrations or should define the working range of the detector.

- 10.2.2 Analyze each calibration standard according to the procedure (Sect. 11.9). Tabulate response (peak height or area) against concentration for each compound and internal standard. Calculate the response factor (RF) for each analyte and surrogate using Equation 1.

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)} \quad \text{Equation 1}$$

where:

A_s = Response for the analyte to be measured.

A_{is} = Response for the internal standard.

C_{is} = Concentration of the internal standard (μ g/L).

C_s = Concentration of the analyte to be measured (μ g/L).

- 10.2.3 If the RF value over the working range is constant (20% RSD or less) 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. C_s .
- 10.2.4 The working calibration curve or RF must be verified on each working day by the measurement of a minimum of two calibration check standards, one at the beginning and one at the end of the analysis day. These check standards should be at two different concentration levels to verify the calibration curve. For extended periods of analysis (greater than 8 hr), it is strongly recommended that check standards be interspersed with samples at regular intervals during the course of the analyses. If the response for any analyte varies from the predicted response by more than $\pm 20\%$, the test must be repeated using a fresh calibration standard. If the results still do not agree, generate a

new calibration curve. For those analytes that failed the calibration verification, results from field samples analyzed since the last passing calibration should be considered suspect. Reanalyze sample extracts for these analytes after acceptable calibration is restored.

10.3 EXTERNAL STANDARD CALIBRATION PROCEDURE

- 10.3.1 Prepare calibration standards as described in Sect 10.2.1, omitting the use of an internal standard.
 - 10.3.2 Starting with the standard of lowest concentration, analyze each calibration standard according to Sect. 11.9 and tabulate response (peak height or area) versus the concentration in the standard. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to concentration (calibration factor) is a constant over the working range (20% RSD or less), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.
 - 10.3.3 The working calibration curve or calibration factor must be verified on each working day as described in Section 10.2.4.
- 10.4 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. PROCEDURE

11.1 MANUAL HYDROLYSIS, PREPARATION, AND EXTRACTION.

- 11.1.1 Add preservative(s) (Sect.8) to LRBs and LFBs. Mark the water meniscus on the side of the sample bottle for later determination of sample volume (Sect. 11.1.9). Pour the entire sample into a 2-L separatory funnel. Fortify sample with 50 µL of the surrogate standard solution.
- 11.1.2 Add 250 g NaCl to the sample, seal, and shake to dissolve salt.
- 11.1.3 Add 17 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 1 hr, shaking the separatory funnel and contents periodically. Note: Since many of the analytes contained in this method are applied as a variety of esters and salts, it is vital to hydrolyze them to the parent acid prior to extraction. This step must be included in the analysis of all extracted field samples, LRBs, LFBs, LFMs, and QCS.
- 11.1.4 Add 60 mL methylene chloride to the sample bottle to rinse the bottle, transfer the methylene chloride to the separatory funnel and extract the sample by vigorously shaking the funnel for 2 min with periodic venting to

release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. 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 through glass wool, centrifugation, or other physical methods. Discard the methylene chloride phase (Sect. 14,15).

- 11.1.5 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, discarding the methylene chloride layer. Perform a third extraction in the same manner.
- 11.1.6 Add 17 mL of 12 N H_2SO_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 2, adjust the pH by adding more 12 N H_2SO_4 .
- 11.1.7 Add 120 mL ethyl ether to the sample, seal, and extract the sample by vigorously shaking the funnel for 2 min with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. 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 through glass wool, centrifugation, or other physical methods. Remove the aqueous phase to a 2-L Erlenmeyer flask and collect the ethyl ether phase in a 500-mL round-bottom flask containing approximately 10 g of acidified anhydrous sodium sulfate. Periodically, vigorously shake the sample and drying agent. Allow the extract to remain in contact with the sodium sulfate for approximately 2 hours.
- 11.1.8 Return the aqueous phase to the separatory funnel, add a 60-mL volume of ethyl ether to the sample, and repeat the extraction procedure a second time, combining the extracts in the 500-mL erlenmeyer flask. Perform a third extraction with 60 mL of ethyl ether in the same manner.
- 11.1.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11.2 AUTOMATED HYDROLYSIS, PREPARATION, AND EXTRACTION. -- Data presented in this method were generated using the automated extraction procedure with the mechanical separatory funnel shaker.

- 11.2.1 Add preservative (Sect. 8.2) to any samples not previously preserved, e.g., LRBs and LFBs. Mark the water meniscus on the side of the sample bottle for later determination of sample volume (Sect. 11.2.9). Fortify sample with 50 μL of the surrogate standard solution. If the mechanical separatory funnel shaker is used, pour the entire sample into a 2-L separatory funnel. If the mechanical tumbler is used, pour the entire sample into a tumbler bottle.

- 11.2.2 Add 250 g NaCl to the sample, seal, and shake to dissolve salt.
- 11.2.3 Add 17 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. Shake sample for 1 hr using the appropriate mechanical mixing device. Note: Since many of the analytes contained in this method are applied as a variety of esters and salts, it is vital to hydrolyze them to the parent acid prior to extraction. This step must be included in the analysis of all extracted field samples, LRBs, LFBs, LFMs, and QCS.
- 11.2.4 Add 300 mL methylene chloride to the sample bottle to rinse the bottle, transfer the methylene chloride to the separatory funnel or tumbler bottle, seal, and shake for 10 s, venting periodically. Repeat shaking and venting until pressure release is not observed during venting. Reseal and place sample container in appropriate mechanical mixing device. Shake or tumble the sample for 1 hr. Complete and thorough mixing of the organic and aqueous phases should be observed at least 2 min after starting the mixing device.
- 11.2.5 Remove the sample container from the mixing device. If the tumbler is used, pour contents of tumbler bottle into a 2-L separatory funnel. Allow the organic layer to separate from the water phase for a minimum of 10 min. 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 through glass wool, centrifugation, or other physical methods. Drain and discard the organic phase. If the tumbler is used, return the aqueous phase to the tumbler bottle.
- 11.2.6 Add 17 mL of 12 N H₂SO₄ 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 2, adjust the pH by adding more 12 N H₂SO₄.
- 11.2.7 Add 300 mL ethyl ether to the sample, seal, and shake for 10 s, venting periodically. Repeat shaking and venting until pressure release is not observed during venting. Reseal and place sample container in appropriate mechanical mixing device. Shake or tumble sample for 1 hr. Complete and thorough mixing of the organic and aqueous phases should be observed at least 2 min after starting the mixing device.
- 11.2.8 Remove the sample container from the mixing device. If the tumbler is used, pour contents of tumbler bottle into a 2-L separatory funnel. Allow the organic layer to separate from the water phase for a minimum of 10 min. 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 through glass wool, centrifugation, or other physical methods. Drain and discard the aqueous phase. Collect the extract

in a 500-mL round-bottom flask containing about 10 g of acidified anhydrous sodium sulfate. Periodically vigorously shake the sample and drying agent. Allow the extract to remain in contact with the sodium sulfate for approximately 2 hr.

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

11.3 EXTRACT CONCENTRATION

- 11.3.1 Assemble a K-D concentrator by attaching a concentrator tube to a 500-mL evaporative flask.
- 11.3.2 Pour the dried extract through a funnel plugged with acid washed glass wool, and collect the extract in the K-D concentrator. Use a glass rod to crush any caked sodium sulfate during the transfer. Rinse the round-bottom flask and funnel with 20 to 30 mL of ethyl ether to complete the quantitative transfer.
- 11.3.3 Add 1 to 2 clean boiling stones to the evaporative flask and attach a macro Snyder column. Prewet the Snyder column by adding about 1 mL ethyl ether to the top. Place the K-D apparatus on a hot water bath, 60 to 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. 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 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.
- 11.3.4 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of ethyl ether. Add 2 mL of MTBE and a fresh boiling stone. Attach a micro-Snyder column to the concentrator tube and prewet the column by adding about 0.5 mL of ethyl ether 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 to 10 min. When the apparent volume of liquid reaches 0.5 mL, remove the micro K-D from the bath and allow it to drain and cool. Remove the micro Snyder column and add 250 μ L of methanol. If the gaseous diazomethane procedure (Sect. 11.4) or trimethylsilyldiazomethane procedure (11.6) is used for esterification of pesticides, rinse the walls of the concentrator tube while adjusting the volume to 5.0 mL with MTBE. If the pesticides will be esterified using the diazomethane solution (Sect. 11.5), rinse the walls of the concentrator tube while adjusting the volume to 4.5 mL with MTBE.

11.4 ESTERIFICATION OF ACIDS USING GASEOUS DIAZOMETHANE -- Results presented in this method were generated using the gaseous diazomethane derivatization procedure. See Section 11.5 and 11.6 for alternative procedures.

- 11.4.1 Assemble the diazomethane generator (Figure 1) in a hood.

- 11.4.2 Add 5 mL of ethyl ether to Tube 1. Add 1 mL of ethyl ether, 1 mL of carbitol, 1.5 mL of 37% aqueous KOH, and 0.2 grams Diazald to Tube 2. Immediately place the exit tube into the concentrator tube containing the sample extract. Apply nitrogen flow (10 mL/min) to bubble diazomethane through the extract for 1 min. Remove first sample. Rinse the tip of the diazomethane generator with ethyl ether after methylation of each sample. Bubble diazomethane through the second sample extract for 1 min. Diazomethane reaction mixture should be used to esterify only two samples; prepare new reaction mixture in Tube 2 to esterify each two additional samples. Samples should turn yellow after addition of diazomethane and remain yellow for at least 2 min. Repeat methylation procedure if necessary.
- 11.4.3 Seal concentrator tubes with stoppers. Store at room temperature in a hood for 30 min.
- 11.4.4 Destroy any unreacted diazomethane by adding 0.1 to 0.2 grams silicic acid to the concentrator tubes. Allow to stand until the evolution of nitrogen gas has stopped (approximately 20 min). Adjust the sample volume to 5.0 mL with MTBE.
- 11.5 ESTERIFICATION OF ACIDS USING DIAZOMETHANE SOLUTION -- Alternative procedure.
- 11.5.1 Assemble the diazomethane generator (Figure 2) in a hood. The collection vessel is a 10- or 15-mL vial, equipped with a Teflon-lined screw cap and maintained at 0-5°C.
- 11.5.2 Add a sufficient amount of ethyl ether to tube 1 to cover the first impinger. Add 5 mL of MTBE to the collection vial. Set the nitrogen flow at 5-10 mL/min. Add 2 mL Diazald solution (Sect. 7.10) and 1.5 mL of 37% KOH solution to the second impinger. Connect the tubing as shown and allow the nitrogen flow to purge the diazomethane from the reaction vessel into the collection vial for 30 min. Cap the vial when collection is complete and maintain at 0-5°C. When stored at 0-5°C this diazomethane solution may be used over a period of 48 hr.
- 11.5.3 To each concentrator tube containing sample or standard, add 0.5 mL diazomethane solution. Samples should turn yellow after addition of the diazomethane solution and remain yellow for at least 2 min. Repeat methylation procedure if necessary.
- 11.5.4 Seal concentrator tubes with stoppers. Store at room temperature in a hood for 30 min.
- 11.5.5 Destroy any unreacted diazomethane by adding 0.1 to 0.2 grams silicic acid to the concentrator tubes. Allow to stand until the evolution of nitrogen gas has stopped (approximately 20 min). Adjust the sample volume to 5.0 mL with MTBE.

11.6 ESTERIFICATION OF ACIDS USING TRIMETHYLSILYLDIAZOMETHANE (TMSD) -- Alternative procedure. It should be noted that the gas chromatographic background is increased when TMSD is used as the derivatizing reagent instead of the generated diazomethane. Although no method analyte is affected by this increased background, the recommended surrogate, 2,4-dichloro-phenylacetic acid, is masked by an interfering peak. This renders the surrogate useless at 1 µg/L or lower. Any compound found suitable when TMSD is used is acceptable as a surrogate.

11.6.1 Carry out the hydrolysis, clean-up, and extraction of the method analytes as described up to Sect. 11.4.

11.6.2 Add 50 µL of the 2 M TMSD solution to each 5 mL sample extract.

11.6.3 Place the tube containing the extract into a heating block at 50°C and heat the extract for 1 hour.

11.6.4 Allow the extract to cool to room temperature, then add 100 µL of 2 M acetic acid in methanol to react any excess TMSD.

11.6.5 Proceed with the identification and measurement of the analytes using GC/ECD according to the procedures described in the method.

11.7 FLORISIL SEPARATION (optional)

11.7.1 Place a small plug of glass wool into a 5-mL disposable glass pipet. Tare the pipet, and measure 1 g of activated Florisil into the pipet.

11.7.2 Apply 5 mL of 5 percent methanol in MTBE to the Florisil. Allow the liquid to just reach the top of the Florisil. In this and subsequent steps, allow the liquid level to just reach the top of the Florisil before applying the next rinse, however, do not allow the Florisil to go dry. Discard eluate.

11.7.3 Apply 5 mL methylated sample to the Florisil leaving silicic acid in the tube. Collect eluate in K-D tube.

11.7.4 Add 1 mL of 5 percent methanol in MTBE to the sample container, rinsing walls. Transfer the rinse to the Florisil column leaving silicic acid in the tube. Collect eluate in a K-D tube. Repeat with 1-mL and 3-mL aliquots of 5 percent methanol in MTBE, collecting eluates in K-D tube.

11.7.5 If necessary, dilute eluate to 10 mL with 5 percent methanol in MTBE.

11.7.6 Seal the vial and store in a refrigerator if further processing will not be performed immediately. Analyze by GC-ECD.

11.8 GAS CHROMATOGRAPHY

11.8.1 Sect. 6.10 summarizes the recommended operating conditions for the GC. Included in Table 1 are retention times observed using this method. Other GC

columns or chromatographic conditions may be used if the requirements of Sect. 9.3 are met.

- 11.8.2 Calibrate or verify the calibration of the system daily as described in Sect. 10. The standards and extracts must be in MTBE.
- 11.8.3 If the internal standard calibration procedure is used, fortify the extract with 25 μL of internal standard solution. Thoroughly mix sample and place aliquot in a GC vial for subsequent analysis.
- 11.8.4 Inject 2 μL of the sample extract. Record the resulting peak size in area units.
- 11.8.5 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze. If internal standard calibration is used, add an additional amount of the IS, so that the amount in the diluted extract will match the calibration standards.

11.9 IDENTIFICATION OF ANALYTES

- 11.9.1 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.
- 11.9.2 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.
- 11.9.3 Identification requires expert judgement when sample components are not resolved chromatographically. When GC peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima, or any time doubt exists over the identification of a peak on a chromatogram, appropriate alternative techniques, to help confirm peak identification, need to be employed. For example, more positive identification may be made by the use of an alternative detector which operates on a chemical/physical principle different from that originally used, e.g., mass spectrometry, or the use of a second chromatography column. A suggested alternative column is described in Sect. 6.10.

12. DATA ANALYSIS AND CALCULATIONS

- 12.1 Calculate analyte concentrations in the sample from the response for the analyte using the calibration procedure described in Sect. 10. Use the multi-point calibration to make all calculations. Do not use the daily calibration verification data to quantitate analytes in samples.

- 12.2 If the internal standard calibration procedure is used, calculate the concentration (C) in the sample using the response factor (RF) determined in Sect. 10.2 and Equation 2, or determine sample concentration from the calibration curve.

$$C (\mu\text{g/L}) = \frac{(A_s)(I_s)}{(A_{is})(\text{RF})(V_o)} \quad \text{Equation 2.}$$

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).

- 12.3 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 Sect. 10.3. The concentration (C) in the sample can be calculated from Equation 3.

$$C (\mu\text{g/L}) = \frac{(A)(V_t)}{(V_i)(V_s)} \quad \text{Equation 3.}$$

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. METHOD PERFORMANCE

- 13.1 In a single laboratory, analyte recoveries from reagent water were used to determine analyte MDLs, EDLs (Table 3) and demonstrate method range. Analyte recoveries and standard deviation about the percent recoveries at one concentration are given in Table 3. All data in Tables 1-3 were obtained using diazomethane for esterification.
- 13.2 In a single laboratory, analyte recoveries from one standard synthetic ground waters were determined at one concentration level. Results were used to demonstrate applicability of the method to different ground water matrices. Analyte recoveries from the one synthetic matrix are given in Table 2.
- 13.3 The performance of dalapon using this method has been variable. Different users have had varying success in the accuracy and precision of dalapon measurements. Because the dalapon methyl ester is much more volatile than the rest of the method analytes, it is suspected that extract concentration technique may be involved with poor recoveries of this analyte. Therefore it is recommended that the analyst use caution to avoid losses due to volatilization.

14. POLLUTION PREVENTION

- 14.1 This method uses significant volumes of organic solvents. It is highly recommended that laboratories use solvent recovery systems to recover used solvent as sample extracts are being concentrated. Recovered solvents should be recycled or properly disposed of.
- 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. WASTE MANAGEMENT

- 15.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions. The laboratory using this method has the responsibility to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance is also required with any sewage discharge permits and regulations. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel," also available from the American Chemical Society at the address in Sect. 14.2.

16. REFERENCES

1. 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.
2. "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, Aug. 1977.
3. "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).
4. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
5. ASTM Annual Book of Standards, Part 11, Volume 11.01, D3370-82, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 130, 1986.

TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA**TABLE 1. RETENTION TIMES FOR METHOD ANALYTES**

Analyte	Retention Time ^a (minutes)	
	Primary	Confirmation
Dalapon	3.4	4.7
3,5-Dichlorobenzoic Acid	18.6	17.7
4-Nitrophenol	18.6	20.5
DCAA (surrogate)	22.0	14.9
Dicamba	22.1	22.6
Dichlorprop	25.0	25.6
2,4-D	25.5	27.0
DBOB (internal standard)	27.5	27.6
Pentachlorophenol (PCP)	28.3	27.0
Chloramben	29.7	32.8
2,4,5-TP	29.7	29.5
5-Hydroxydicamba	30.0	30.7
2,4,5-T	30.5	30.9
2,4-DB	32.2	32.2
Dinoseb	32.4	34.1
Bentazon	33.3	34.6
Picloram	34.4	37.5
DCPA Acid Metabolites	35.8	37.8
Acifluorfen	41.5	42.8

^a

Columns and analytical conditions are described in Sections 6.10.1 and 6.10.2.

**TABLE 2. SINGLE LABORATORY ACCURACY AND PRECISION FOR
ANALYTES FROM REAGENT WATER AND
SYNTHETIC GROUNDWATERS^a**

Analyte	Conc µg/L	Reagent Water		Synthetic Water 1 ^d	
		R ^b	S _r ^c	R	S _R
Acifluorfen	0.2	121	15.7	103	20.6
Bentazon	1	120	16.8	82	37.7
Chloramben	0.4	111	14.4	112	10.1
2,4-D	1	131	27.5	110	5.5
Dalapon	10	100	20.0	128	30.7
2,4-DB	4	87	13.1	0	0
DCPA Acid Metabolites	0.2	74	9.7	81	21.9
Dicamba	0.4	135	32.4	92	17.5
3,5-Dichlorobenzoic Acid	0.6	102	16.3	82	7.4
Dichlorprop	2	107	20.3	106	5.3
Dinoseb	0.4	42	14.3	89	13.4
5-Hydroxydicamba	0.2	103	16.5	88	5.3
4-Nitrophenol	1	131	23.6	127	34.3
Pentachlorophenol (PCP)	0.04	130	31.2	84	9.2
Picloram	0.6	91	15.5	97	23.3
2,4,5-T	0.4	117	16.4	96	3.8
2,4,5-TP	0.2	134	30.8	105	6.3

^a Data corrected for amount detected in blank and represent the mean of 7-8 samples.

^b R = average percent recovery.

^c S_R = standard deviation of the percent recovery.

^d Corrected for amount found in blank; Absopure Nature Artesian Spring Water Obtained from the Absopure Water Company in Plymouth, Michigan.

**TABLE 3. SINGLE LABORATORY ACCURACY, PRECISION, METHOD
DETECTION LIMITS (MDLs) AND ESTIMATED DETECTION LIMITS
(EDLs) FOR ANALYTES FROM REAGENT WATER**

Analyte	Fortified Conc. µg/L	N ^a	Recovery µg/L	RSD %	MDL ^b µg/L	EDL ^c µg/L
Acifluorfen	0.040	7	88	23	0.026	0.04
Bentazon	0.20	7	92	19	0.11	0.20
Chloramben	0.080	7	118	33	0.097	0.097
2,4-D	0.20	7	90	14	0.078	0.20
Dalapon	1.0	6	90	45	1.3	1.3
2,4-DB	4.0	6	87	15	1.7	4.0
DCPA Acid Metabolites	0.20	6	74	13	0.067	0.20
Dicamba	0.080	7	155	22	0.085	0.085
3,5-Dichlorobenzoic Acid	0.060	6	69	38	0.065	0.065
Dichlorprop	0.40	7	110	16	0.22	0.40
Dinoseb	0.40	6	42	34	0.33	0.40
5-Hydroxydicamba	0.040	7	49	28	0.017	0.040
4-Nitrophenol	0.20	7	148	15	0.14	0.20
Pentachlorophenol (PCP)	0.10	6	82	9	0.032	0.10
Picloram	0.12	7	166	24	0.15	0.15
2,4,5-T	0.080	7	87	21	0.045	0.80
2,4,5-TP	0.20	6	134	23	0.21	0.21

^a N = Number of replicates

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

where:

$t_{(n-1, 1-\alpha = 0.99)}$ = Student's t value for the 99% confidence level
with n-1 degrees of freedom

n = number of replicates

S = standard deviation of replicate analyses.

^c EDL = estimated detection limit; defined as either MDL (Appendix B to 40 CFR Part 136 - Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11) or a level of compound in a sample yielding a peak in the final extract with signal-to-noise ratio of approximately 5, whichever value is higher.

TABLE 4. LABORATORY PERFORMANCE CHECK SOLUTION

Test	Analyte	Conc. µg/mL	Requirements
Sensitivity	Dinoseb	0.004	Detection of analyte; S/N >3
Chromatographic performance	4-Nitrophenol	1.6	0.70 <PGF <1.05 ^a
Column performance	3,5-Dichlorobenzoic Acid	0.6	Resolution >0.40 ^b
	4-Nitrophenol	1.6	

^a PGF -- peak Gaussian factor. Calculated using the equation:

$$PGF = \frac{1.83 \times W(1/2)}{W(1/10)}$$

where: W(1/2) is the peak width at half height in seconds and W(1/10) is the peak width in seconds at 10th height.

^b Resolution between the two peaks as defined by the equation:

$$R = t / W$$

where: t is the difference in elution times between the two peaks and W is the average peak width, at the baseline, of the two peaks.

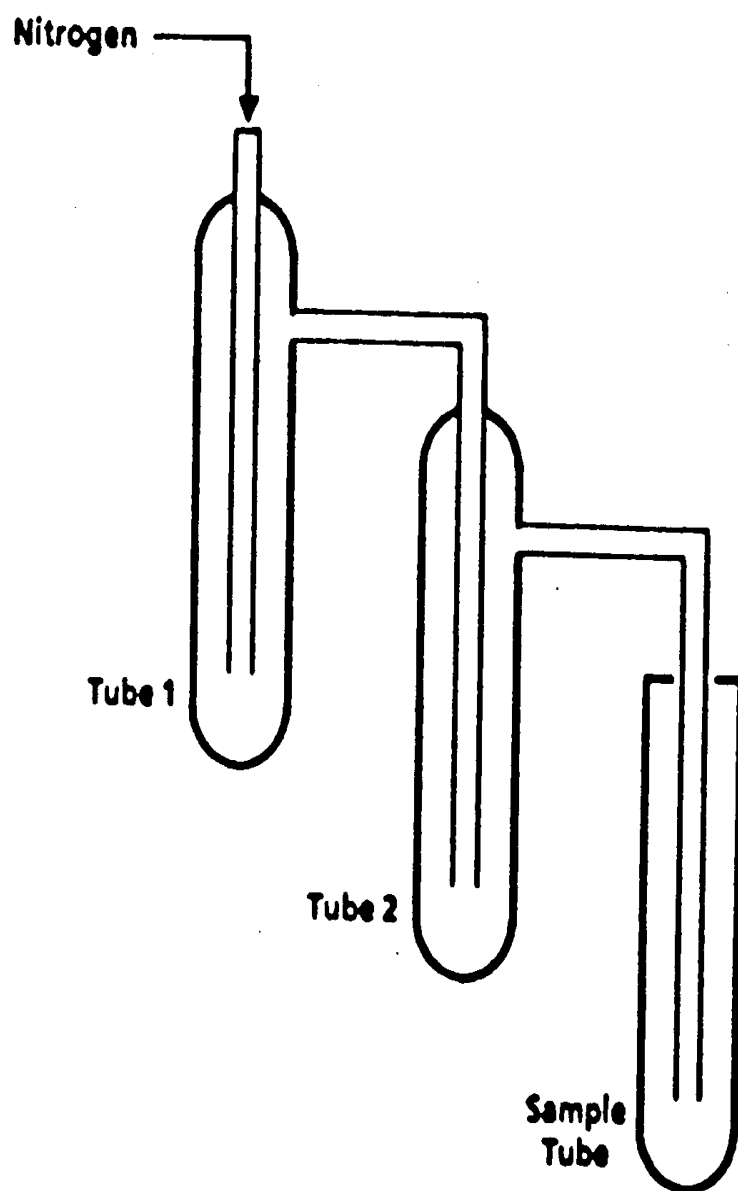


FIGURE 1. GASEOUS DIAZOMETHANE GENERATOR

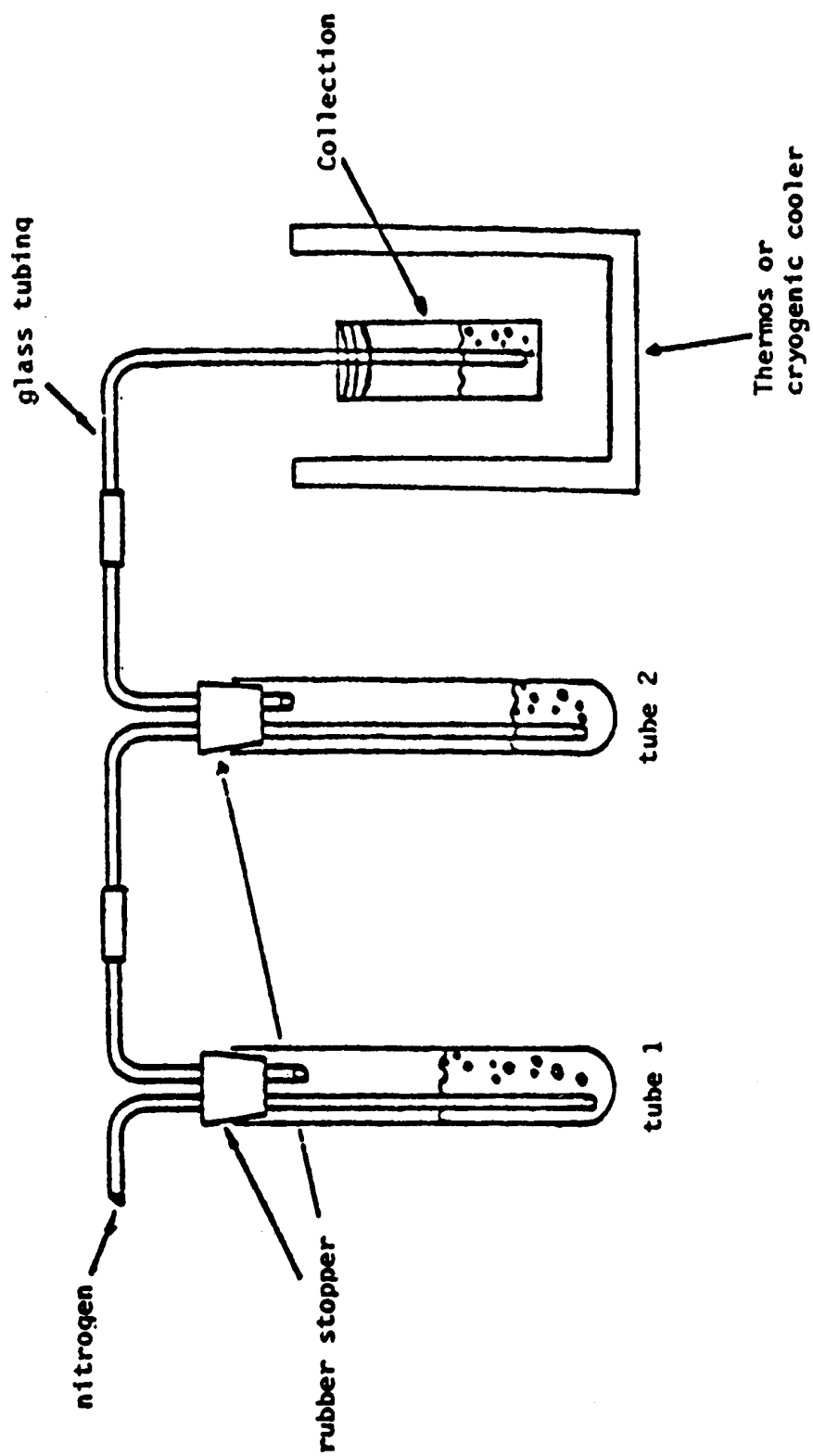


FIGURE 2. DIAZOMETHANE SOLUTION GENERATOR

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