Designation: D5317 – 20

Standard Test Method for Determination of Chlorinated Organic Acid Compounds in Water by Gas Chromatography with an Electron Capture Detector[1]

This standard is issued under the fixed designation D5317; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers a gas chromatographic procedure for the quantitative determination of selected chlorinated acids and other acidic herbicides in water. Similar chemicals may also be determined by this test method, but it is the user's responsibility to verify the applicability of this test method to any compounds not listed in this scope. The acid form of the following compounds were interlaboratory tested using this test method, and the results were found acceptable:[2]

Analyte	Chemical Abstract Services Registry Number
Bentazon	25057-89-0
2,4-D	94-75-7
2,4-DB	94-82-6
DCPA acid metabolites [2]	
Dicamba	1918-00-9
3,5-Dichlorobenzoic acid	51-36-5
Dichlorprop	120-36-5
5-Hydroxydicamba	7600-50-2
Pentachlorophenol (PCP)	87-86-5
Picloram	1918-02-1
2,4,5-T	93-76-5
2,4,5-TP (Silvex)	93-72-1

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

1.3 This test method has been validated in an interlaboratory test for reagent water and finished tap water. The analyst should recognize that precision and bias reported in Section 18 may not be applicable to other waters.

1.4 This test method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography (GC) and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this test method using the procedure described in 19.3. It is the user's responsibility to ensure the validity of this test method for waters of untested matrices.

1.5 Analytes that are not separated chromatographically, that is, 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 exists (16.6, 16.7, and 16.8).

1.6 When this test method is used to analyze unfamiliar samples for any or all of the analytes given in 1.1, analyte identifications must be confirmed by at least one additional qualitative technique.

1.7 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.8 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* For specific warning statements, see Sections 6, 8, 9, and 10.

1.9 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water

[1] This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

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[2] DCPA monoacid and diacid metabolites are included in the scope of this test method; DCPA diacid metabolite is used for validation studies.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

D3370 Practices for Sampling Water from Flowing Process Streams
D3856 Guide for Management Systems in Laboratories Engaged in Analysis of Water
D4210 Practice for Intralaboratory Quality Control Procedures and a Discussion on Reporting Low-Level Data (Withdrawn 2002)[4]
D5789 Practice for Writing Quality Control Specifications for Standard Test Methods for Organic Constituents (Withdrawn 2002)[4]

2.2 *EPA Standard:*[5]

Method 515.1 Revision 4.0, Methods for the Determination of Organic Compounds in Drinking Water

2.3 *OSHA Standard:*[6]

29 CFR 1910 OSHA Safety and Health Standards, General Industry

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this standard, refer to Terminology D1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *internal standard, n*—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.

3.2.1.1 *Discussion*—The internal standard must be an analyte that is not a sample component.

3.2.2 *surrogate analyte, n*—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.

3.2.2.1 *Discussion*—The purpose of a surrogate analyte is to monitor method performance with each sample.

4. Summary of Test Method

4.1 The compounds listed in 1.1, in water samples, are converted into sodium salts by adjusting the pH to 12 with sodium hydroxide solution (240 g/L) and shaking for 1 h. Extraneous neutral material is removed by extraction with methylene chloride. The sample is acidified, the acids are extracted with ethyl ether and converted to methyl esters using diazomethane. After the excess reagent is removed, the methyl esters are determined by capillary column GC using an electron capture (EC) detector. Other detection systems, such as microcoulometric and electrolytic conductivity, are not as sensitive as EC for measurement of chlorinated acid esters but are more specific and less subject to interferences. A mass spectrometer may also be used as a detector.

4.2 This test method provides a magnesium silicate[7] cleanup procedure to aid in the elimination of interferences that may be present.

5. Significance and Use

5.1 Chlorinated phenoxyacid herbicides, and other organic acids are used extensively for weed control. Esters and salts of 2,4-D and silvex have been used as aquatic herbicides in lakes, streams, and irrigation canals. Phenoxy acid herbicides can be toxic even at low concentrations. For example, the 96 h, TL_m for silvex is 2.4 mg/L for bluegills (**1**).[8] These reasons make apparent the need for a standard test method for such compounds in water.

6. Interferences

6.1 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 19.2.

6.1.1 Glassware must be scrupulously cleaned (**2**). Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thoroughly rinsing with dilute acid, tap, and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 1 h. Do not heat volumetric ware. 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. Thermally stable materials such as PCBs may not be eliminated by this treatment.

6.1.2 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.)

6.2 The acid forms of the analytes are strong organic acids that react readily with alkaline substances and can be lost during sample preparation. Glassware and glass wool must be acid-rinsed with hydrochloric acid (1 + 9) and the sodium sulfate must be acidified with sulfuric acid prior to use to avoid analyte loses due to adsorption.

6.3 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] The last approved version of this historical standard is referenced on www.astm.org.

[5] Available from United States Environmental Protection Agency (EPA), William Jefferson Clinton Bldg., 1200 Pennsylvania Ave., NW, Washington, DC 20460, http://www.epa.gov.

[6] Available from U.S. Government Printing Office, Superintendent of Documents, 732 N. Capitol St., NW, Washington, DC 20401-0001, http://www.access.gpo.gov.

[7] Florisil, a trademark of, and available from, Floridin Co., 2 Gateway Center, Pittsburgh, PA 15222, or its equivalent, has been found satisfactory for this purpose.

[8] The boldface numbers in parentheses refer to the list of references at the end of this test method.

6.4 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, which 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 **(3)**.

6.5 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.

6.6 Matrix interferences may be caused by contaminants that are coextracted from the sample. Also, note that all analytes listed in Table 1 are not resolved from each other on any one column, that is, one analyte of interest may be an interferent 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 Section 16 can be used to overcome many of these interferences. Positive identifications should be confirmed. See 16.6, 16.7, and 16.8.

6.7 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.

7. Apparatus and Equipment

7.1 *Sample Bottle*—Borosilicate amber, 1-L volume with graduations, fitted with screw caps lined with polytetrafluoroethylene (PTFE). Protect samples from light. The container must be washed and dried as described in 6.1.1 before use to minimize contamination. Cap liners are cut to fit from sheets and extracted with methanol overnight prior to use.

7.2 *Glassware.*

7.2.1 *Separatory Funnel,* 2000-mL, with PTFE stopcocks, ground glass or PTFE stoppers.

7.2.2 *Tumbler Bottle,* 1.7-L with PTFE-lined screw cap. Cap liners are cut to fit from sheets and extracted with methanol overnight prior to use.

7.2.3 *Concentrator Tube,* Kuderna-Danish (K-D), 10 or 25-mL, graduated. Calibration must be checked at the volumes employed in the procedure. Ground-glass stoppers are used to prevent evaporation of extracts.

7.2.4 *Evaporative Flask,* K-D, 500-mL. Attach to concentrator tube with springs.

7.2.5 *Snyder Column,* K-D, three ball macro.

7.2.6 *Snyder Column,* K-D, two ball micro.

7.2.7 *Flask,* round bottom, 500-mL with 24/40 ground glass joint.

7.2.8 *Vials,* glass, 5 to 10-mL capacity with PTFE-lined screw cap.

7.3 *Boiling Stone,* PTFE.

7.4 *Water Bath,* heated, capable of temperature control (±2°C). The bath should be used in a hood.

7.5 *Diazomethane Generator*—Assemble from two 20- by 155-mm test tubes, two neoprene rubber stoppers, and a source of nitrogen as shown in Fig. 1.

7.6 *Glass Wool,* acid washed and heated at 450°C.

TABLE 1 Retention Times and Estimated Method Detection Limits for Method Analytes

Analyte	CAS No.	Retention Time[A] (min)		EDL[B]
		Primary	Confirmation	
3,5-Dichlorobenzoic Acid	51-36-5	18.6	17.7	0.061
DCAA (surrogate)	19719-28-9	22.0	14.9	...
Dicamba	1918-00-9	22.1	22.6	0.081
Dichlorprop	120-36-5	25.0	25.6	0.26
2,4-D	94-75-7	25.5	27.0	0.2
DBOB (int. std.)	10386-84-2	27.5	27.6	...
Pentachlorophenol	87-86-5	28.3	27.0	0.076
2,4,5-TP	93-72-1	29.7	29.5	0.075
5-Hydroxydicamba	7600-50-2	30.0	30.7	0.04
2,4,5-T	93-76-5	30.5	30.9	0.08
2,4-DB	94-82-6	32.2	32.2	0.8
Bentazon	25057-89-0	33.3	34.6	0.2
Picloram	1918-02-1	34.4	37.5	0.14
DCPA Acid Metabolites[C]	...	35.8	37.8	0.02

[A] Columns and analytical conditions are described in 7.7.1 and 7.7.2

[B] Estimated method detection limit, µg/L, determined from 7 replicate analyses of a reagent water fortified with analyte at a concentration level yielding signal-to-noise of 5:1. EDL is defined as the standard deviation × student's *t* (99 % CI, *n* – 1 degrees of freedom).

[C] DCPA monoacid and diacid metabolites are included in the scope of this test method; DCPA diacid metabolite is used for validation studies.

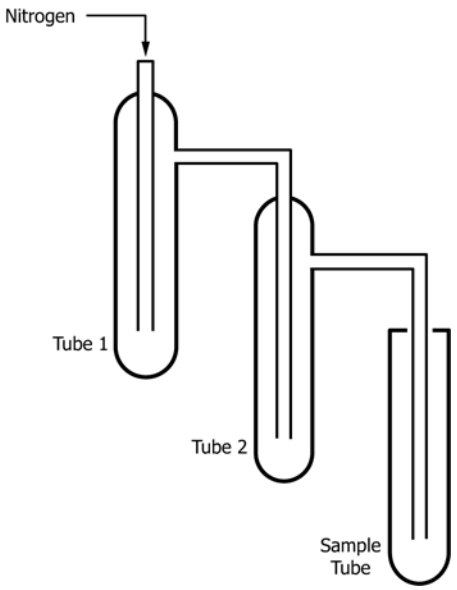

FIG. 1 Gaseous Diazomethane Generator

7.7 *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. A data system is recommended for measuring peak areas. Table 1lists retention times observed for test method analytes using the columns and analytical conditions described below.

7.7.1 *Column 1 (Primary Column),* 30-m long by 0.25-mm inside diameter (ID) DB-5 bonded fused silica column, 0.25-µm film thickness. Establish helium carrier gas flow at 30 cm/s linear velocity and program oven temperature from 60°C to 300°C at 4°C/m. Data presented in this test method were obtained using this column (Table 1). The injection volume is 2-µL splitless mode with 45 s delay. The injector temperature is 250°C and the detector is 320°C. Alternative columns may be used in accordance with the provisions described in 19.3.

7.7.2 *Column 2 (Confirmation Column),* 30-m long by 0.25-mm I.D. DB-1701 bonded fused silica column, 0.25-µm film thickness. Establish helium carrier gas flow at 30 cm/s linear velocity and program oven temperature from 60°C to 300°C at 4°C/m.

7.7.3 *Detector,* electron capture (ECD). This detector has proven effective in the analysis of fortified reagent and artificial ground waters. An ECD was used to generate the validation data presented in this test method. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in 19.3.

8. Reagents and Materials

8.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents to the American Chemical Society, where such specifications are available[9]. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

8.2 *Purity of Water*—Except as otherwise indicated, references to water shall be understood to mean water conforming to Specification D1193, Type II. Additionally, the water shall be free of the interferences described in Section 6.

8.3 *Acetone,* pesticide quality.

8.4 *Diazomethane Esterification Reagents.*

8.4.1 *Diethylene Glycol Monoethyl Ether,* reagent grade.[10]

8.4.2 *N-methyl-N-nitroso-paratoluenesulfonamide,* ACS grade.[11]

8.4.3 *N-methyl-N-nitroso-paratoluenesulfonamide Solution*—Prepare a solution containing 10 g N-methylN-nitroso-paratoluenesulfonamide in 100 mL of 50:50 by volume mixture of ethyl ether and diethylene glycol monoethyl ether. This solution is stable for one month or longer when stored at 4°C in an amber bottle with a PTFE-lined screw cap.

8.4.4 *Diethyl Ether,* reagent grade, redistilled in glass after refluxing over granulated sodium-lead alloy for 4 h. (**Warning—**Use immediately, or if stored, test for ether peroxides by test paper,[12] or other suitable means. If present, repeat reflux and distillation.)

8.5 *4,4" Dibromooctafluorobiphenyl* (DBOB), 99 % purity, for use as internal standard.

8.6 *2,4 Dichlorophenylacetic Acid* (DCAA), 99 % purity, for use as surrogate standard.

8.7 *Ethyl Acetate,* pesticide quality.

8.8 *Magnesium Silicate,* PR grade (60 to 100 mesh) purchased activated at 1250°F (650°C) and continuously stored at 130°C.

8.9 *Glass Wool,* acid washed.

8.10 *Herbicide Standards*—Acids and methyl esters, analytical reference grade.

8.11 *Hexane,* pesticide quality.

8.12 *Mercuric Chloride.*

8.13 *Methyl-t-butyl Ether,* pesticide quality.

8.14 *Methylene Chloride,* pesticide quality.

8.15 *Potassium Hydroxide Solution (37 g/100 mL)*—Dissolve 37 g of potassium hydroxide (KOH) in water, mix and dilute to 100 mL.

8.16 *Silicic Acid.*

8.17 *Sodium Chloride* (NaCl), heat-treated in a shallow tray at 450°C for a minimum of 4 h to remove any potential interfering organic substances.

8.18 *Sodium Hydroxide Solution (240 g/L)*—Dissolve 240 g of sodium hydroxide (NaOH) in water, mix and dilute to 1 L.

8.19 *Sodium Sulfate, Acidified*—Slurry 100 g of the sodium sulfate that has been heat treated in a shallow tray at 450°C for a minimum of 4 h with sufficient diethyl ether to just cover the solid. Add 0.1 mL of concentrated sulfuric acid (sp gr 1.84) and mix thoroughly. Remove the ether with vacuum. Ensure that a pH below 4 can be obtained from mixing 1 g of the solid with 5 mL of water. Store continuously at 130°C.

8.20 *Sodium Thiosulfate,* anhydrous ($Na_2S_2O_3$), reagent grade.

8.21 *Standard Solution, Stock (1.00 µg/µL)*—Stock standard solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedure:

8.21.1 Prepare stock standard solutions by weighing approximately 0.0100 g of pure material to three significant

[9] *ACS Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

[10] Carbitol, a registered trademark of and available from Sigma Chemical Co., P.O. Box 14508, St Louis, MO 63178-9916, or its equivalent, has been found suitable for this purpose.

[11] Diazald, a registered trademark, is available from Aldrich Chemical Company, Inc., 1001 West Saint Paul Avenue, Milwaukee, WI 53233, and has been found satisfactory for this purpose.

[12] EM Quant, a trademark of, and available from, EM Laboratories, Inc., 500 Executive Blvd., Elmsford, NY 10523, or its equivalent, has been found satisfactory for this purpose.

figures. Dissolve the material in MTBE and dilute to volume in a 10-mL volumetric flask. Larger volumes may be prepared 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.

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

8.21.3 Replace stock standard solutions after two months or sooner if comparison with laboratory fortified blanks, or quality control sample indicates a problem.

8.22 *Standard Solution, Internal*—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 PTFE sealed screw cap bottle and store at room temperature. Addition of 25 µL of the internal standard solution to 10 mL of sample extract results in a final internal standard concentration of 0.25 µg/mL. Solution should be replaced when ongoing quality control in Section 19 indicates a problem. Note that DBOB has been shown to be an effective internal standard for the test method analytes (**4**), but other compounds may be used if the quality control requirements in Section 19 are met.

8.23 *Surrogate Standard Solution*—Prepare a surrogate standard solution by weighing approximately 0.0010 g of pure DCAA to three significant figures. Dissolve the DCAA in MTBE and dilute to volume in a 10-mL volumetric flask. Transfer the surrogate standard solution to a PTFE 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 µg/L and, assuming quantitative recovery of DCAA, a surrogate standard concentration in the final extract of 0.5 µg/mL. Solution should be replaced when ongoing quality control described in Section 19 indicates a problem.

NOTE 1—DCAA has been shown to be an effective surrogate standard for the method analytes (**4**), but other compounds may be used if the quality control requirements in 19.4 are met.

8.24 *Sulfuric Acid Solution (335 + 665)*—Carefully add, with constant mixing, 335 mL of concentrated sulfuric acid to 665 mL of water.

8.25 *Toluene,* pesticide quality.

8.26 *Hydrochloric Acid (HCl) (1 + 9)*—Carefully add, with constant mixing, 100 mL of concentrated HCl to 900 mL of water.

9. Hazards

9.1 The toxicity or carcinogenicity of each reagent used in this test 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 test 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 (29 CFR 1910) (**5, 6**) for the information of the analyst.

9.2 *Diazomethane*—A toxic carcinogen that can explode under certain conditions. The following precautions must be followed:

9.2.1 Use only a well ventilated hood—do not breathe vapors.

9.2.2 Use a safety screen.

9.2.3 Use mechanical pipetting aids.

9.2.4 Do not heat above 90°C—Explosion may result.

9.2.5 Avoid grinding surfaces, ground glass joints, sleeve bearings, glass stirrers—Explosion may result.

9.2.6 Store away from alkali metals—Explosion may result.

9.2.7 Solutions of diazomethane decompose rapidly in the presence of solid materials such as copper powder, calcium chloride, and boiling chips.

9.2.8 The diazomethane generation apparatus used in the esterification procedures 13.2 produces micromolar amounts of diazomethane to minimize safety hazards.

9.3 *Ethyl Ether,* pesticide quality, redistilled in glass, if necessary.

9.3.1 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.

9.3.2 Must be free of peroxides as indicated by test strips.[12] (**Warning—**When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous.)

10. Sample Collection, Preservation, and Storage

10.1 Collect the sample in accordance with Practices D3370 in an amber glass bottle. Do not prerinse the bottle with sample before collection.

10.2 Add mercuric chloride to the sample bottle in an amount to produce a concentration of 10 mg HgCl by adding 1 mL of a 10 mg HgCl/mL solution to the sample bottle at the sampling site, or in the laboratory before shipping to the sampling site. (**Warning—**Mercuric chloride is highly toxic. If the use of another bacteriacide can be shown to be equivalent to $HgCl_2$, it can be used provided all quality control criteria in Sections 18 and 19 are met.)

10.3 Test for the presence of chlorine with potassium iodide-starch test paper previously moistened with dilute acid. Darkening of the test paper indicates the presence of chlorine (and a few other oxidizing materials). Add 80 mg $Na_2S_2O_3$ to the bottle before adding the sample.

10.4 After the sample is collected in the bottle containing preservative, seal the bottle and shake vigorously for 1 min.

10.5 Immediately store the sample at 4°C away from light until extraction. Preservation study results indicate that the

analytes (measured as total acid) present in samples are stable for 14 days when stored under these conditions (**4**). 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.

11. Calibration

11.1 Establish GC operating parameters equivalent to those indicated in 7.7. The GC system may be calibrated using either the internal standard technique (11.2) or the external standard technique (11.3).

NOTE 2—Calibration standard solutions must be prepared such that no unresolved analytes are mixed together.

11.2 *Internal Standard Calibration Procedure*—Select one or more internal standards compatible in analytical behavior to the compounds of interest. Demonstrate that the measurement of the internal standard is not affected by test method or matrix interferences. DBOB has been identified as a suitable internal standard.

11.2.1 Prepare calibration standards 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 a volumetric flask. To each calibration standard, add a known constant amount of one or more of the internal standards and 250-µL methanol, and dilute to volume with MTBE. Esterify acids with diazomethane as described in 13.2 or 13.3. The lowest standard should represent analyte concentrations near, but above, the respective estimated detection levels (EDLs). The remaining standards should bracket the analyte concentrations expected in the sample extracts, or should define the working range of the detector (Table 1).

11.2.2 Analyze each calibration standard according to the procedure (Section 16). 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 the following equation:

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

where:

A_s = response for the analyte to be measured,
A_{is} = response for the internal standard,
C_{is} = concentration of the internal standard, µg/L, and
C_s = concentration of the analyte to be measured, µg/L.

11.2.3 If the *RF* value over the working range is constant (20 % RSD or less) use the average *RF* for calculations. Alternatively, use the results to plot a calibration curve of response ratios (A_s/A_{is}) versus C_s.

11.2.4 Verify the working calibration curve or *RF* on each working shift by the measurement of one or more calibration standards. If the response for any analyte varies from the predicted response by more than ±20 %, repeat the test using a fresh calibration standard. If the repetition also fails, generate a new calibration curve for that analyte using freshly prepared standards.

11.2.5 Single-point calibration is a viable alternative to a calibration curve. Prepare single point standards from the secondary dilution standards in MTBE. Prepare the single point standards at a concentration that produces a response that deviates from the sample extract response by no more than 20 %.

11.2.6 Verify calibration standards periodically, at least quarterly is recommended, 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.

11.3 *External Standard Calibration Procedure:*

11.3.1 Prepare calibration standards at a minimum of three (five are recommended) concentration levels for each analyte of interest and surrogate compound by adding volumes of one or more stock standards and 250-µL methanol to a volumetric flask. Dilute to volume with MTBE. Esterify acids with diazomethane (13.2 or 13.3). The lowest standard should represent analyte concentrations near, but above, the respective EDL (Table 1). The remaining standards should bracket the analyte concentrations expected in the sample extracts, or should define the working range of the detector.

11.3.2 Starting with the standard of lowest concentration, analyze each calibration standard according to Section 16 and tabulate response (peak height or area) versus the concentration 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), assume linearity through the origin and put the average ratio or calibration factor in place of a calibration curve.

11.3.3 Verify the working calibration curve or calibration factor 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 h), it is strongly recommended that check standards be interspersed with samples at regular intervals during the course of the analyses. If the response is by more than ±20 %, repeat the test using a fresh calibration standard. If the results still do not agree, generate a new calibration curve or use a single-point calibration standard as described in 11.3.4.

11.3.4 Single-point calibration is a viable alternative to calibration curve. Prepare single-point standards from the secondary dilution standards in MTBE. Prepare the single-point standards at a concentration that produces a response that deviates from the sample extract response by no more than 20 %.

11.3.5 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.

12. Procedure

12.1 *Manual Hydrolysis, Preparation, and Extraction:*

12.1.1 Add preservative to every blank sample and quality-control check the standard. Mark the water meniscus on the side of the sample bottle for later determination of sample

volume (12.1.9). Pour the entire sample into a 2-L separatory funnel. Fortify sample with 50 µL of the surrogate standard solution.

12.1.2 Add 250 g NaCl to the sample, seal, and shake to dissolve salt.

12.1.3 Add 17 mL of NaOH solution (240 g/L) 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 NaOH (240 g/L). Let the sample sit at room temperature for 1 h, and shake the separatory funnel and contents periodically.

12.1.4 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.

12.1.5 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.

12.1.6 Add 17 mL of H_2SO_4 solution (335 + 665) 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 H_2SO_4 solution (335 + 665).

12.1.7 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 either a 500-mL round-bottom flask or a 500-mL Erlenmeyer flask containing approximately 10 g of acidified anydrous sodium sulfate. Periodically, vigorously shake the sample and drying agent. Allow the extract to remain in contact with the sodium sulfate for approximately 2 h.

12.1.8 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 round-bottom or Erlenmeyer flask. Perform a third extraction with 60 mL of ethyl ether in the same manner.

12.1.9 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.

12.2 *Automated Hydrolysis, Preparation, and Extraction:*

12.2.1 Follow the fortification and preservation procedures given in 12.1.1. 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.

12.2.2 Add 250 g of NaCl to the sample, seal, and shake to dissolve salt.

12.2.3 Add 17 mL of NaOH solution (240 g/L) 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 NaOH (240 g/L). Shake sample for 1 h using the appropriate mechanical mixing device.

12.2.4 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 h. Complete and thorough mixing of the organic and aqueous phases should be observed at least 2 min after starting the mixing device.

12.2.5 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.

12.2.6 Add 17 mL of H_2SO_4 solution (335 + 665) 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 H_2SO_4 solution (335 + 665).

12.2.7 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 h. Complete and thorough mixing of the organic and aqueous phases should be observed at least 2 min after starting the mixing device.

12.2.8 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 Erlenmeyer or round-bottom flask containing about 10 g of acidified anhydrous sodium sulfate. Periodically, vigorously shake the sample and drying agent.