Designation: D5315 – 04 (Reapproved 2011)

Standard Test Method for Determination of N-Methyl-Carbamoyloximes and N-Methylcarbamates in Water by Direct Aqueous Injection HPLC with Post-Column Derivatization[1]

This standard is issued under the fixed designation D5315; 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 is a high-performance liquid chromatographic (HPLC) test method applicable to the determination of certain n-methylcarbamoyloximes and n-methylcarbamates in ground water and finished drinking water (**1**)[2]. This test method is applicable to any carbamate analyte that can be hydrolyzed to a primary amine. The following compounds have been validated using this test method:

Analyte	Chemical Abstract Services Registry Number [A]
Aldicarb	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
Baygon	114-26-1
Carbaryl	63-25-2
Carbofuran	1563-66-2
3-Hydroxycarbofuran	16655-82-6
Methiocarb	2032-65-7
Methomyl	16752-77-5
Oxamyl	23135-22-0

[A] Numbering system of Chemical Abstracts, Inc.

1.2 This test method has been validated in a collaborative round-robin study (**2**) and estimated detection limits (EDLs) have been determined for the analytes listed in 1.1 (Table 1). Observed detection limits may vary between ground waters, depending on the nature of interferences in the sample matrix and the specific instrumentation used.

1.3 This test method is restricted to use by, or under the supervision of, analysts experienced in both the use of liquid chromatography and the interpretation of liquid chromatograms. Each analyst should demonstrate an ability to generate acceptable results with this test method using the procedure described in 12.3.

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

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

1.6 *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 and health practices and determine the applicability of regulatory limitations prior to use.* Additional guidance on laboratory safety is available and suitable references for the information are provided(**3-5**).

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D1129 Terminology Relating to Water

D1192 Guide for Equipment for Sampling Water and Steam in Closed Conduits (Withdrawn 2003)[4]

D1193 Specification for Reagent Water

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

D3370 Practices for Sampling Water from Closed Conduits

D3694 Practices for Preparation of Sample Containers and for Preservation of Organic Constituents

E682 Practice for Liquid Chromatography Terms and Relationships

2.2 *U.S. Environmental Protection Agency Standard:*

EPA Method 531.1, Revision 3.0, USEPA, EMSL-Cincinnati, 1989[5]

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

[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.

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

[5] Published by the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268, 1989.

TABLE 1 Relative Retention Times for the Primary and Confirmation Columns and EDLs for the 10 Carbamate Pesticides

Analyte	Retention Time (minutes)		
	Primary [A]	Confirmation [B]	EDL [C]
Aldicarb	27.0	21.4	1.0
Aldicarb sulfone	15.2	12.2	2.0
Aldicarb sulfoxide	15.0	17.5	2.0
Baygon (Propoxur)	29.6	23.4	1.0
Carbaryl	30.8	25.4	2.0
Carbofuran	29.3	24.4	1.5
3-Hydroxycarbofuran	23.3	19.0	2.0
Methiocarb	34.9	28.6	4.0
Methomyl	18.4	14.8	0.50
Oxamyl	17.4	14.6	2.0

[A] Primary column—250 by 4.6 mm inside diameter Altex Ultrasphere ODS, 5 µm.
[B] Confirmation column—250 by 4.6 mm inside diameter Supelco LC-1, 5 µm.
[C] Estimated method detection limit in micrograms per litre.

EPA Method 531.2, Revision 1.0, USEPA, EMSL-Cincinnati, 2001[6]

3. Terminology

3.1 *Definitions*—For definitions of water terms used in this test method, refer to Terminology D1129. For definitions of other terms used in this test method, refer to Practice E682.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *calibration standard* (CAL)—a solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.2.2 *field duplicates* (FD1 and FD2)—two separate samples collected at the same time, placed under identical circumstances, and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 provide a measure of the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

3.2.3 *field reagent blank* (FRB)—reagent water placed in a sample container in the laboratory and treated in all respects as a sample, including being exposed to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine whether method analytes or other interferences are present in the field environment.

3.2.4 *internal standard*—a pure analyte(s) added to a solution in known amount(s) and used to measure the relative responses of other analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.

3.2.5 *laboratory duplicates* (LD1 and LD2)—two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 provide a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.2.6 *laboratory-fortified blank* (LFB)—an aliquot of reagent water to which known quantities of the test method analytes are added in the laboratory. The LFB is analyzed exactly as a sample is; its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise methods at the required test method detection limit.

3.2.7 *laboratory-fortified sample matrix* (LFM)—an aliquot of an environmental sample to which known quantities of the test method analytes are added in the laboratory. The LFM is analyzed exactly as a sample is; 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.

3.2.8 *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.

3.2.9 *laboratory reagent blank* (LRB)—an aliquot of reagent water treated exactly the same as a sample, including being exposed to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine whether method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.2.10 *primary dilution standard solution*—a solution of several analytes prepared in the laboratory from stock standard solutions and diluted as necessary to prepare calibration solutions and other necessary analyte solutions.

3.2.11 *quality control sample* (QCS)—a sample matrix containing test method analytes or a solution of test method analytes in a water miscible solvent that is used to fortify water or environmental samples. The QCS is obtained from a source external to the laboratory and is used to check the laboratory performance with externally prepared test materials.

3.2.12 *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.

3.2.13 *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. It is measured with the same procedures used to measure other sample components. The purpose of a surrogate analyte is to monitor the method performance with each sample.

4. Summary of Test Method

4.1 The water sample is filtered, and a 200 to 400-µL aliquot is injected onto a reverse phase HPLC column. Separation of the analytes is achieved using gradient elution chromatography. After elution from the HPLC column, the analytes are hydrolyzed with sodium hydroxide (2.0 g/L NaOH) at 95°C. The methylamine formed during hydrolysis is reacted with

[6] Published by the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268, 2001.

o-phthalaldehyde (OPA) and 2-mercaptoethanol to form a highly fluorescent derivative that is detected by a fluorescence detector (**5**).

4.2 This method is applicable to any carbamte analyte that can be hydrolyzed to a primary amine, not necessarily methylamine.

5. Significance and Use

5.1 N-methylcarbamates and n-methylcarbomoyloximes are used in agriculture as insecticides and herbicides. They are sometimes found in both surface and ground waters and can be toxic to animals and plants at moderate to high concentrations. The manufacturing precursors and degradation products may be equally as hazardous to the environment.

6. Interferences

6.1 Test method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatuses that lead to discrete artifacts or elevated baselines in liquid chromatograms. Specific sources of contamination have not been identified. All reagents and apparatus must be routinely demonstrated to be free of interferences under the analysis conditions by running laboratory reagent blanks in accordance with 12.2.

6.1.1 Glassware must be cleaned scrupulously. Clean all glassware as soon as possible after use by rinsing thoroughly with the last solvent used in it.

6.1.2 After drying, store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store the glassware inverted or capped with aluminum foil.

6.1.3 The use of high-purity reagents and solvents helps to minimize interference problems.

6.2 Interfering contamination may occur when a sample containing low concentrations of analytes is analyzed immediately after a sample containing relatively high concentrations of analytes. A preventive technique is between-sample rinsing of the sample syringe and filter holder with two portions of water. Analyze one or more laboratory method blanks after analysis of a sample containing high concentrations of analytes.

6.3 Matrix interference may be caused by contaminants present in the sample. The extent of matrix interference will vary considerably from source to source, depending upon the water sampled. Positive analyte identifications must be confirmed using the alternative conformational columns, or LC/MS.

6.4 The quality of the reagent water used to prepare standards and samples must conform to D1193, especially in TOC content. High reagent water TOC causes a deterioration of column selectivity, baseline stability, and analyte sensitivity.

6.5 Eliminate all sources of airborne primary amines, especially ammonia, which are absorbed into the mobile phases and effect sensitivity.

7. Apparatus

7.1 *Sampling Equipment:*

7.1.1 *Sample Bottle,* 60-mL screw cap glass vials[7] and caps[8] equipped with a PTFE-faced silicone septa. Prior to use, wash the vials and septa as described in 6.1.1.

7.2 *Filtration Apparatus:*

7.2.1 *Macrofiltration Device,* to filter derivatization solutions and mobile phases used in HPLC. It is recommended that 47-mm, 0.45-µm pore size filters be used.[9]

7.2.2 *Microfiltration Device,* to filter samples prior to HPLC analysis. Use a 13-mm filter holder[10] and 13-mm diameter, 0.2-µm polyester filters.[11]

7.3 *Syringes and Valves:*

7.3.1 *Hypodermic Syringe,* 10 mL, glass, with Luer-Lok[12] tip.

7.3.2 *Syringe Valve,* three-way.[13]

7.3.3 *Syringe Needle,* 7 to 10 cm long, 17-gage, blunt tip.

7.3.4 *Micro Syringes,* various sizes.

7.4 *Miscellaneous:*

7.4.1 *Solution Storage Bottles,* amber glass, 10 to 15-mL capacity with TFE-fluorocarbon-lined screw cap.

7.5 *High-Performance Liquid Chromatograph (HPLC):*

7.5.1 *HPLC System,* [14] capable of injecting 200 to 1000-µL aliquots and performing ternary linear gradients at a constant flow rate. A data system is recommended for measuring peak areas. Table 2 lists the retention times observed for test method analytes using the columns and analytical conditions described below.

7.5.2 *Column 1 (Primary Column),* 250 mm long by 4.6-mm inside diameter, stainless steel, packed with 5-µm C-18 material.[15] Mobile phase is established at 1.0 mL/min as a linear gradient from 15:85 methanol: water to 100 % methanol in 32 min. Data presented in this test method were obtained using this column.[16]

7.5.3 *Column 2 (Alternative Column),* 250 mm long by 4.6-mm inside diameter, stainless steel, packed with 5-µm silica beads coated with trimethylsilyl.[17] Mobile phase is established at 1.0 mL/min as a linear gradient from 15:85 methanol: water to 100 % methanol in 32 min.

7.5.4 *Column 3 (Alternative Column, used for EPA 531.2 validation),* 150 mm long by 3.9 mm inside diameter, stainless

[7] Sample bottle vial, Pierce No. 13075, available from Pierce Chemical Co., 3747 N. Meridian Rd., Rockford, IL 61101, or equivalent.

[8] Sample bottle cap, Pierce No. 12722, available from Pierce Chemical Co., 3747 N. Meridian Rd., Rockford, IL 61101, or equivalent.

[9] Millipore Type HA, 0.45 µm for water, and Millipore Type FH, 0.5µ m for organics, available from Millipore Corp., 80 Ashby Rd., Bedford, MA 01730, or equivalent.

[10] Millipore stainless steel XX300/200, available from Millipore Corp., 80 Ashby Rd., Bedford, MA 01730, or equivalent.

[11] Nucleopore 180406, available from Costar Corp., 1 Alewife Center, Cambridge, MA 02140, or equivalent.

[12] Luer-Lok connectors are available from most laboratory suppliers.

[13] Hamilton HV3-3, available from Hamilton Co., P.O. Box 10030, Reno, NV 89502, or equilivalent.

[14] Consult HPLC manufacturer's operation manuals for specific instructions relating to the equipment.

[15] Beckman Ultrasphere ODS, available from Beckman Instruments, 2500 Harbor Blvd., Fullerton, CA 92634, has been found suitable.

[16] Newer manufactured columns have not been able to resolve aldicarb sulfone from oxamyl.

[17] Supelco LC-1, available from Supelco, Inc., Supelco Park, Bellefonte, PA 16823, has been found suitable.

TABLE 2 Retention Times for Method Analytes Retention Time[A]

Analyte	Primary [B]	Confirmation [C]	Confirmation [D]
	Minutes		
Aldicarb sulfoxide	6.80	17.5	
Aldicarb sulfone	7.77	12.2	
Oxamyl	8.20	14.6	
Methomyl	8.94	14.8	
3-Hydroxycarbofuran	13.65	19	
Aldicarb	16.35	21.4	
Baygon (Propoxur)	18.86	24.4	
Carbofuran	19.17	23.4	
Carbaryl	20.29	25.4	
Methiocarb	24.74	28.6	
BDMC	25.28		...

[A] Columns and analytical conditions are described in 7.5.2, 7.5.3.
[B] Beckman Ultasphere ODS.
[C] Supelco LC-1.
[D] Waters Carbamate Analysis Column using ternary gradient conditions.

steel, packed with 5-mm C_{18}[18]. Mobile phase is a ternary methanol, acetonitrile, water gradient over 24 minutes. See Annex A1.

7.5.5 *Post Column Reactor,* capable of mixing reagents into the mobile phase. The reactor should be constructed using PTFE tubing and should be equipped with pumps to deliver 0.1 to 1.0 mL/min of each reagent; mixing tees; and two 1.0-mL delay coils, with one thermostated at 90°C.[19,18]

7.5.6 *Fluorescence Detector,* capable of excitation at 230 nm and detection of emission energies greater than 418 nm[20], or variable wavelength fluorescence detector capable of 340 nm excitation, 465 nm emission with a 18 nm band width, and 16 mL flow cell[18].

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 of the American Chemical Society where such specifications are available.[21] 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. For trace analysis using organic solvents for liquid-liquid extraction or elution from solid sorbents, solvents specified as distilled-in-glass, nano-grade, or pesticide-grade frequently have lower levels of interfering impurities. In all cases, sufficient reagent blanks must be processed with the samples to ensure that all of the compounds of interest are not present as blanks due to reagents or glassware.

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Type I of Specification D1193. It must be shown that this water does not contain contaminants at concentrations sufficient to interfere with the analysis. The reagent water used to generate the validation data in this test method was distilled water.[22]

8.3 *Buffer Solutions:*

8.3.1 *Monochloroacetic Acid (pH 3) ($ClCH_3CO_2H$) Buffer Solution*—Prepare by mixing 156 mL of monochloroacetic acid ($ClCH_3CO_2H$) solution (236.2 g/L) and 100 mL of potassium acetate (KCH_3CO_2) solution (245.4 g/L).

8.3.2 *Buffered Water,* to prepare 1 L, mix 10 mL of monochloroacetic acid buffer (pH 3) and 990 mL of water.

8.4 *Helium,* for degassing solutions and solvents.

8.5 *HPLC Mobile Phase:*

8.5.1 *Water,* HPLC grade[23], or equivalent Type I Reagent Water.

8.5.2 *Methanol,* HPLC grade. Filter and degas before use.

8.5.3 *Acetonitrile,* HPLC grade. Filter and degass before use.

8.6 *Internal Standard Solution* —Prepare an internal standard solution by weighing approximately 0.0010 g of pure BDMC (4-Bromo-3,5-Dimethylphenyl N-Methylcarbamate, 98 % purity)[24] to two significant figures. Dissolve the BDMC in methanol 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 it at room temperature. The addition of 5 µL of the internal standard solution to 50 mL of sample results in a final internal standard concentration of 10 µg/L. Replace the solution when ongoing quality control indicates a problem.

NOTE 1—BDMC has been shown to be an effective internal standard for

[18] Waters Carbamate Analysis Column, available from Waters Corp., Milford, MA, 01757.

[19] ABI URS 051 and URA 100, available from ABI Analytical, Inc., 170 Williams Drive, Ramsey, NJ 07446, or equivalent.

[20] A Schoffel Model 970 fluorescence detector was used to generate the validation data presented in this test method. Now available from Kratos Division of ABI Analytical, Inc., 170 Williams Drive, Ramsey, NJ 07446.

[21] "Reagent Chemicals, American Chemical Society Specifications," Am. Chemical Soc., 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."

[22] Available from the Magnetic Springs Water Co., 1801 Lone Eagle St., Columbus, OH 43228.

[23] Available from Burdick and Jackson. Distributed by Scientific Products, 1430 Waukegan Road, McGraw Park, IL 60085-6787.

[24] Available from Aldrich Chemical Co., Inc., 1001 West Saint Paul Ave., Milwaukee, WI 53233.

TABLE 3 Instrument Quality Control Standard

Test	Analyte	Concentration, µ g/L	Requirements
Sensitivity	3-Hydroxycarbofuran	2	Detection of analyte; *S/N* > 3
Chromatographic performance	aldicarb sulfoxide	100	0.90 < PGF [A] < 1.1

[A] PGF = peak Gaussian factor

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

where:

$W(1/2)$ = peak width at half height, and
$W(1/10)$ = peak width at tenth height.

the method analytes (**1**), but other compounds may be used if the quality control requirements in Section 11 are met.

8.7 *Laboratory Performance Check Solution*—Prepare the concentrate by adding 20 µL of the 3-hydroxycarbofuran stock standard solution (8.11), 1.0 mL of the aldicarb sulfoxide stock standard solution (8.11), and 1 mL of the internal standard fortification solution (8.7) to a 10-mL volumetric flask (Table 3). Dilute to volume with methanol. Mix concentrate thoroughly. Prepare a check solution by placing 100 µL of the concentrate solution into a 100-mL volumetric flask. Dilute to volume with buffered water. Transfer to a TFE-fluorocarbon-sealed screw-cap bottle and store it at room temperature. The solution should be replaced when ongoing quality control indicates a problem.

8.8 *Methanol,* distilled-in-glass quality or equivalent.

8.9 *Post Column Derivatization Solutions:*

8.9.1 *Sodium Hydroxide* (2 g/L)—Dissolve 2.0 g of sodium hydroxide (NaOH) in water. Dilute to 1.0 L with water. Filter and degas just before use.

8.9.2 *2-Mercaptoethanol* (1 + 1)—Mix 10.0 mL of 2-mercaptoethanol and 10.0 mL of acetonitrile. Cap and store in hood.

NOTE 2—**Caution:** Work in a hood due to reagent volatility and odor.

8.9.3 *Sodium Borate Solution* (19.1 g/L)—Dissolve 19.1 g of sodium borate ($Na_2B_4O_7 \times 10H_2O$) in water. Dilute to 1.0 L with water. The sodium borate will dissolve completely at room temperature if prepared one day before use.

8.9.4 *OPA Reaction Solution*—Dissolve 100 ± 10 mg of o-phthalaldehyde (melting point range from 55 to 58°C) in 10 mL of methanol. Add to 1.0 L of sodium borate solution (19.1 g/L). Mix, filter, and degas with helium. Add 100 µL of 2-mercaptoethanol (1 + 1) and mix. Make up fresh solutions daily.

8.10 Sodium thiosulfate ($Na_2S_2O_3$).

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

8.11.1 Prepare stock standard solutions by weighing approximately 0.0100 g of pure material. Dissolve the material in methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If the 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 either the manufacturer or an independent source.

8.11.2 Transfer the stock standard solution into TFE-fluorocarbon-sealed screw-cap vials. Store it at room temperature and protect it from light.

8.11.3 Stock standard solutions should be replaced after two months, or sooner, if comparison with laboratory-fortified blanks, or quality-control samples indicate a problem.

9. Sample Collection and Handling

9.1 Collect the samples in accordance with Specification D1192, Practices D3370, or Practices D3694.

9.2 Additionally, grab samples must be collected in glass containers. Follow conventional sampling practices (**6**); however, the bottle must not be prerinsed with sample before collection.

10. Preservation of Samples

10.1 *Sample Preservation/pH Adjustment*—Oxamyl, 3-hydroxycarbofuran, aldicarb sulfoxide, and carbaryl can all degrade rapidly in neutral and basic waters held at room temperature (**7, 8**). This short-term degradation is of concern during the periods of time that samples are being shipped and that processed samples are held at room temperature in autosampler trays. Samples targeted for the analysis of these three analytes must be preserved at a pH of 3, as shown as follows. The pH adjustment also minimizes analyte biodegradation.

10.1.1 Add 1.8 mL of monochloroacetic acid buffer solution (pH 3) to the 60-mL sample bottle. Add buffer to the sample bottle either at the sampling site or in the laboratory before shipping to the sampling site.

10.1.2 If residual chlorine is present, add 80 mg of sodium thiosulfate per litre of sample to the sample bottle prior to collecting the sample.

10.1.3 After the sample is collected in a bottle containing buffer, seal the sample bottle and shake it vigorously for 1 min.

10.1.4 Samples must be iced or refrigerated at 4°C from the time of collection until storage; they must be stored at – 10°C until analyzed. Preservation study results indicate that test method analytes are stable in water samples for at least 28 days when adjusted to pH 3 and stored at – 10°C. However, analyte stability may be affected by the matrix; the analyst should therefore verify that the preservation technique is applicable to the samples under study.

11. Calibration

11.1 Establish HPLC operating parameters equivalent to those indicated in 7.5. Calibrate the HPLC system using either the internal (11.2) or the external (11.3) standard technique.

11.2 *Internal Standard Calibration Procedure*—The analyst must select one or more internal standards similar in analytical behavior to the analytes of interest. In addition, the analyst

must demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. BDMC has been identified as a suitable internal standard.

11.2.1 Prepare calibration standards at a minimum of three (recommended, five) concentration levels for each analyte of interest by adding volumes of one or more of the stock standards to a volumetric flask. Add a known constant amount of one or more internal standards to each calibration standard, and dilute to volume with buffered water. The lowest standard should represent analyte concentration near, but above, their respective estimated detection limit (EDL) (Table 1). The remaining standards should bracket the analyte concentrations expected in the sample extracts, or they should define the working range of the detector.

11.2.2 Analyze each calibration standard in accordance with the procedure in 13.2. Tabulate the peak height or area responses against the concentration for each compound and internal standard.

11.2.3 Calculate response factors (RF) for each analyte, surrogate, and internal standard using Eq 1 as follows:

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

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.

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

11.2.5 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.

11.2.6 Single-point calibration is a viable alternative to a calibration curve. Prepare single-point standards from the secondary dilution standards. Prepare the single-point standards at a concentration deviating from the sample extract response by no more than 20 %.

11.2.7 Verify calibration standards periodically (recommended at least quarterly) by analyzing a standard prepared from reference material obtained from an independent source. The results from these analyses must be within the limits used to check calibration routinely.

11.3 *External Standard Calibration Procedure:*

11.3.1 Prepare calibration standards at a minimum of three (recommended five) concentration levels for each analyte of interest by adding volumes of one or more stock standards to a volumetric flask. Dilute to volume with buffered water. The lowest standard should represent analyte concentrations near, but above, the respective EDLs. The remaining standards should bracket the analyte concentrations expected in the sample extracts, or they should define the working range of the detector.

11.3.2 Beginning with the standard of lowest concentration, analyze each calibration standard in accordance with 13.2, and tabulate the response (peak height or area) versus the concentration in the standard. Use the results 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 % relative standard deviation, assume linearity through the origin and use 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 measuring 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 in order to verify the concentration curve. For extended analysis periods (longer than 8 h), it is strongly recommended that check standards be interspersed with the samples at regular intervals during the course of the analyses. 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 results still do not agree, generate a new calibration curve or use a single-point calibration standard in accordance with 11.3.4.

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

11.3.5 Verify the calibration standards periodically, (recommended, at least quarterly), by analyzing a standard prepared from reference material obtained from an independent source. The results from these analyses must be within the limits used to check calibration routinely.

12. Quality Control

12.1 Minimum quality control (QC) requirements are as follows: an initial demonstration of laboratory capability; monitoring of the internal standard peak area or height in each sample and blank when internal standard calibration procedures are being used; and an analysis of laboratory reagent blanks, laboratory-fortified samples, laboratory-fortified blanks, and quality control samples.

12.2 *Laboratory Reagent Blanks*—Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. A laboratory reagent blank (LRB) must be analyzed each time a set of samples is extracted or reagents are changed. If, within the retention time window of any analyte of interest, the LRB produces a peak that would prevent the determination of that analyte, locate the source of contamination and eliminate the interference before processing the samples.

12.3 *Initial Demonstration of Capability:*

12.3.1 Select a representative concentration (approximately 10 times EDL) for each analyte. Prepare a sample concentrate (in methanol) containing each analyte at 1000 times the selected concentration. With a syringe, add 50 µL of the

TABLE 4 Acceptance Limits for the Analysis of a Laboratory Quality Control Sample as Percent of Mean Recovery

Analyte	Concentration Level [A]	Mean Recovery [B]	Overall Standard Deviation [B]	Acceptance Limits, [C] %
Aldicarb	10.0	9.46	0.58	81.6–118
Aldicarb sulfone	20.0	19.3	1.33	79.3–121
Aldicarb sulfoxide	20.0	19.6	1.35	79.3–121
Baygon (Propoxur)	10.0	9.52	0.78	75.4–124
Carbaryl	20.0	19.5	1.35	79.2–121
Carbofuran	20.0	19.1	0.68	89.3–111
3-Hydroxycarbofuran	20.0	19.2	1.31	79.5–120
Methiocarb	50.0	47.0	3.93	74.9–125
Methomyl	5.00	4.92	0.37	77.4–122
Oxamyl	20.0	19.4	1.44	77.7–122

[A] Concentration level ca 10 times the estimated method detection limit.
[B] Calculated from the mean recovery and overall standard deviation regression equations from the collaborative study.
[C] Acceptance limits are defined as the mean recovery ± 3 standard deviations as percent.

concentrate to each of at least four 50-mL aliquots of water, and analyze each aliquot according to the procedures beginning in Section 13.

12.3.2 For each analyte, the recovery value for all four of these samples must fall in the recovery range shown in Table 4. For those compounds meeting the acceptance criteria, the performance is judged as acceptable and sample analysis may begin. For those compounds failing these criteria, this procedure must be repeated, using four fresh samples, until satisfactory performance has been demonstrated.

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

12.4 The analyst is permitted to modify HPLC columns, HPLC conditions, internal standards, or detectors to improve separations or lower analytical costs. The analyst must repeat the procedures described in 12.3 each time such test method modifications are made.

12.5 *Assessing the Internal Standards:*

12.5.1 When using the internal standard calibration procedure, the analyst is expected to monitor the internal standard response (the peak area or peak height) of all samples during each analysis day. The internal standard response for any sample chromatogram should not deviate from the internal standard response of the daily calibration check standard by more than 30 %.

12.5.2 If greater than 30 % deviation occurs with an individual sample, optimize instrument performance and inject a second aliquot.

12.5.2.1 If the reinjected aliquot produces an acceptable internal standard response, report the results for that aliquot.

12.5.2.2 If a deviation of greater than 30 % is obtained for the reinjected sample, repeat the analysis of the sample, beginning with Section 13, provided that the samples are still available. Otherwise, report the results obtained from the reinjected sample, but annotate them as suspect.

12.5.3 If consecutive samples fail the internal standard response acceptance criterion, analyze a calibration check standard immediately.

12.5.3.1 If the check standard provides a response factor within 20 % of the predicted value, follow the procedures outlined in 12.5.2 for each sample failing the internal standard response criterion.

12.5.3.2 If the check standard provides a response factor that deviates by more than 20 % of the predicted value, the analyst must then recalibrate, as specified in Section 11.

12.6 *Assessing Laboratory Performance Laboratory-Fortified Blanks:*

12.6.1 The laboratory must analyze at least one laboratory-fortified blank (LFB) sample with every 20 samples, or one per sample set (all samples being analyzed within a 24-h period), whichever is greater. The fortification concentration of each analyte in the LFB should be ten times the EDL or the MCL, whichever is less. Calculate the accuracy as percent recovery (X_j). If the recovery of any analyte falls outside the control limits (see 12.7.2), that analyte is judged to be out of control, and the source of the problem must be identified and resolved before continuing the analyses.

12.6.2 Until sufficient data become available from within-their own laboratory, usually after obtaining the results from a minimum of 20 to 30 analyses, analysts should assess laboratory performance against the control limits in 12.3.2 that are derived from the data given in Table 5. When sufficient internal performance data become available, develop control limits from the mean percent recovery, X, and standard deviation, S, of the percent recovery. These data are used to establish upper and lower control limits as follows:

$$\text{upper control limit} = X + 3S$$

$$\text{lower control limit} = X - 3S$$

After each five to ten new recovery measurements, calculate new control limits using only the most recent 20 to 30 data points. These calculated control limits should never exceed those established in 12.3.2.

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

12.6.4 Analyze a quality control sample from an outside source at least on a quarterly basis.

12.6.5 Laboratories are encouraged to participate in external performance evaluation studies such as the laboratory certification programs offered by many states or the studies conducted by the U.S. Environmental Protection Agency (EPA). Performance evaluation studies serve as independent checks on the performance of the analyst.

12.7 *Assessing Analyte Recovery/Laboratory Fortified Sample Matrix:*

12.7.1 The laboratory must add a known concentration to a minimum of 5 % of the routine samples or one sample concentration per set, whichever is greater. The concentration should not be less than the background concentration of the sample selected for fortification. The concentration should ideally be the same as that used for the laboratory fortified

TABLE 5 Summary Statistics and Regression Equation for EPA Method 531.1 Collaborative Study Data Sets

Analyte	C [A]	Reagent Water				Finished Drinking Water			
		X [B]	S_R [C]	S_r [D]	Regr. Equations	X [B]	S_R [C]	S_r [D]	Regr. Equations
Aldicarb	3.24	3.24	0.33	0.37	X = 0.926C + 0.202	3.27	0.24	0.22	X = 1.032C + 0.031
	4.84	4.56	0.69		S_R = 0.022X + 0.370 [E]	5.09	0.40		S_R = 0.101X – 0.042 [E]
	9.70	9.26	0.91	0.15	S_r = 0.32 [F]	10.87	2.10	0.51	S_r = 0.040X + 0.046
	12.90	12.37	0.57			13.84	1.73		
	19.40	18.12	0.12	0.44		19.08	0.43	1.04	
	27.40	25.30	0.72			26.77	1.90		
Aldicarb sulfone	6.44	6.71	0.63	0.58	X = 0.942C + 0.446	6.18	0.30	0.35	X = 0.968C – 0.097
	9.68	9.11	0.37		S_R = 0.062X + 0.132	9.24	0.53		S_R = 0.039X + 0.119 [E]
	19.30	18.26	2.24	0.98	S_r = 0.025X + 0.382	18.42	1.34	0.38	S_r = 0.008X + 0.276
	25.80	25.07	1.11			24.55	1.14		
	38.60	37.10	2.04	1.43		36.75	1.70	0.79	
	54.80	52.83	3.56			54.60	1.57		
Aldicarb sulfoxide	6.40	6.58	0.66	0.39	X = 0.941C + 0.876 [G]	5.65	0.77	0.22	X = 0.952C + 0.460 [G]
	8.00	8.53	0.51		S_R = 0.058X + 0.211	8.18	0.60		S_R = 0.021X + 0.440
	19.20	17.99	2.42	1.18	S_r = 0.040X + 0.103	18.30	0.84	0.40	S_r = 0.024X + 0.050
	24.00	23.34	0.92			22.72	0.97		
	40.00	39.31	1.53	1.59		38.68	1.32	1.46	
	56.00	54.66	3.71			55.55	1.38		
Baygon (Propoxur)	3.16	3.35	0.33	0.26	X = 0.916C + 0.360	3.20	0.16	0.20	X = 0.994C + 0.101
	4.76	4.47	0.74		S_R = 0.058X + 0.230	4.92	0.35		S_R = 0.086X – 0.114
	9.50	9.13	0.68	0.41	S_r = 0.040X + 0.092	9.55	0.68	0.24	S_r = 0.046X – 0.005
	12.70	12.02	0.95			13.06	0.23		
	19.00	17.62	0.99	1.12		18.93	2.80	1.48	
	27.00	25.65	1.62			26.26	1.79		
Carbaryl	6.38	6.66	0.58	0.62	X = 0.949C + 0.542	6.49	0.63	0.52	X = 0.958C + 0.439
	9.58	9.48	0.83		S_R = 0.058X + 0.219	9.82	0.28		S_R = 0.068X + 0.015
	19.20	18.73	1.32	0.63	S_r = 0.016X + 0.480	18.62	1.11	0.57	S_r = 0.039X + 0.167
	25.60	24.11	1.61			24.87	1.79		
	38.20	36.39	2.26	1.51		36.67	3.42	2.66	
	54.20	52.41	3.34			52.99	3.56		
Carbofuran	4.76	5.18	0.74	0.48	X = 0.923C + 0.636	4.87	0.49	0.37	X = 0.970C + 0.220
	7.16	6.90	0.29		S_R = 0.006X + 0.564 [E]	7.03	0.31		S_R = 0.042X + 0.178
	14.30	13.58	0.33	0.41	S_r = 0.022X + 0.322	14.23	0.41	0.31	S_r = 0.008X + 0.316
	19.10	18.67	0.87			19.27	0.57		
	28.00	26.99	1.22	1.46		27.47	2.19	0.80	
	40.60	38.71	1.15			39.33	2.59		
3-Hydroxycarbofuran	6.36	6.59	0.79	0.84	X = 0.940C + 0.438	6.39	0.51	0.45	X = 0.979C + 0.153
	9.56	9.01	0.99		S_R = 0.038X + 0.578	9.51	0.91		S_R = 0.085X + 0.045 [E]
	19.10	18.41	1.47	0.47	S_r = 0.013X + 0.697 [E]	18.58	1.29	1.25	S_r = 0.044X + 0.114
	25.40	24.41	1.29			25.26	4.76		
	38.20	36.60	1.96	2.09		37.30	1.65	1.80	
	54.20	51.82	2.34			53.67	2.67		
Methiocarb	12.80	12.96	3.02	1.93	X = 0.923C + 0.887	13.00	0.76	0.61	X = 0.958C + 0.474
	19.20	17.94	2.35		S_R = 0.035X + 2.286	18.12	1.91		S_R = 0.057X + 0.322
	38.40	36.56	2.71	1.86	S_r = 0.005X + 1.839	38.08	3.21	1.29	S_r = 0.034X + 0.046
	51.40	48.35	4.50			49.53	3.97		
	77.00	72.44	4.92	2.58		74.25	2.70	3.52	
	109.0	101.9	7.70			105.2	4.56		
Methomyl	1.60	1.61	0.21	0.17	X = 0.976C + 0.043	1.66	0.22	0.24	X = 0.988C + 0.000
	2.40	2.40	0.22		S_R = 0.048X + 0.133	2.67	0.34		S_R = 0.040X + 0.000
	4.80	4.53	0.60	0.41	S_r = 0.053X + 0.069	4.79	0.13	0.09	S_r = 14 [F]
	6.40	6.42	0.40			6.59	1.03		
	9.60	9.41	0.17	0.61		9.31	0.45	0.09	
	13.60	13.49	0.93			13.63	0.22		
Oxamyl	6.40	6.84	0.88	1.02	X = 0.936C + 0.659	6.43	0.81	0.24	X = 0.998C + 0.045
	9.60	9.25	1.25		S_R = 0.038X + 0.699	9.65	0.80		S_R = 0.023X + 0.672 [E]
	19.20	18.16	1.48	0.52	S_r = 1.04 [F]	19.00	1.67	0.68	S_r = 0.025X + 0.048
	25.60	24.82	1.75			25.75	1.48		
	38.40	37.09	1.16	1.58		37.94	1.35	1.08	
	54.40	52.49	3.27			54.99	0.79		

[A] Spike concentration, µg/L.
[B] Mean recovery, µg/L.
[C] Overall standard deviation, µg/L.
[D] Single-analyst standard deviation, µg/L.
[E] Coefficient of determination of weighted equation was weak (COD < 0.5).
[F] Weighted linear regression equation had negative slope; average precision is reported.
[G] Lowest spike recovery (6.40 µg/L) not used for this regression (see text).

blank (see 12.6). Samples from all routine sample sources should be fortified over time.

12.7.2 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 using Eq 2:

$$P = 100\,(X - b)/\textit{fortifying concentration} \quad (2)$$

Compare these values to the control limits appropriate for water data collected in the same fashion. If the analyzed unfortified sample is found to contain *NO* background concentrations, and the added concentrations are those specified in 12.7, the appropriate control limits would then be the acceptance limits given in 12.7. If, on the other hand, the analyzed unfortified sample is found to contain background concentration, *b*, estimate the standard deviation at the background concentration, *sb*, using regressions or comparable background data and, similarly, estimate the mean, X_a, and standard deviation, *sa*, of analytical results at the total concentration after fortifying. The appropriate percent control limits would be $P \pm 3sp$, where:

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

$$sp = 100\,(sa^2 + sb^2)^{1/2}/\text{fortifying concentration}$$

NOTE 3—For example, if the background concentration for Analyte A was found to be 1 µg/L and the added amount was also 1 µg/L, and upon analysis the laboratory fortified sample measured 1.6 µg/L, then the calculated *P* for this sample would be (1.6 µg/L – 1.0 µg/L)/1 µg/L or 60 %. This calculated *P* is compared to control limits derived from prior water data. Assume that it is known that analysis of an interference free sample at 1 µg/L yields an s of 0.12 µg/Ls and similar analysis at 2.0 µg/L yields *X* and *s* of 2.01 µg/L and 0.20 µg/L, respectively. The appropriate limits by which to judge the reasonableness of the percent recovery, 60 %, obtained on the fortified matrix sample are computed as follows:

$$[100\,(2.01\ \mu g/L)/2.0\ \mu g/L] \pm 3\,(100)\,[(0.12\ \mu g/L)^2 + (0.20\ \mu g/L)^2]^{1/2}/1.0\ \mu g/L = 100.5\,\% \pm 300\,(0.233)$$
$$= 100.5\,\% \pm 70\,\% \text{ or } 30\,\% \text{ to } 170\,\% \text{ recovery of the added analyte}$$

12.7.3 If the recovery of any such analyte falls outside the designated range and the laboratory performance for the analyte is shown to be in control (12.6), the recovery problem encountered with the dosed sample is judged to be matrix related rather than system related. The result for that analyte in the unfortified sample is labeled suspect/matrix in order to inform the data user that the results are suspect due to matrix effects.

12.8 *Assessing Instrument System/Laboratory Performance Check Sample*—Monitor instrument performance daily by analysis of the LPC sample. The LPC sample contains compounds designed to indicate appropriate instrument sensitivity, column performance (primary column), and chromatographic performance. LPC sample components and performance criteria are given in Table 3. An 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 test method. If laboratory EDLs (Table 1) differ from those listed in this test method, concentrations of the instrument quality-control standard compounds must be adjusted to be compatible with the laboratory EDLs.

12.9 *Optional Additional Quality Control Practices*—The laboratory may adopt additional quality-control practices for use with this test method. The most productive specific practices depend on 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 the contamination of samples under site conditions, transportation, and storage.

13. Procedure

13.1 *pH Adjustment and Filtration :*

13.1.1 Add preservative to any samples not previously preserved (Section 10). Adjust the pH of the sample to pH 3 ± 0.2 by adding 1.5 mL of 2.5 *M*-monochloroacetic acid buffer solution (8.3.1) to each 50 mL of sample. This step should not be necessary if the sample pH was adjusted during sample collection as a preservation precaution. Fill a 50-mL volumetric flask to the mark with the sample. Add 5 µL of the internal standard solution if the internal standard calibration procedure is being used and mix by inverting the flask several times.

13.1.2 Affix the three-way valve to a 10-mL syringe. Place a clean filter in the filter holder, and affix the filter holder and the 7 to 10-cm syringe needle to the syringe valve. Rinse the needle and syringe with water. Prewet the filter by passing 5 mL of water through the filter. Draw another 10 mL of sample into the syringe, expel it through the filter, and collect the last 5 mL for analysis. Rinse the syringe with water. Discard the filter.

13.2 *Liquid Chromatography:*

13.2.1 Recommended operating conditions for the liquid chromatograph are summarized in 7.5. Table 1 lists the retention times observed using this test method. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of 12.4 are met.

13.2.2 Calibrate the system daily, as described in Section 11. The standards and sample must be in buffered water having a pH of 3.

13.2.3 Inject 200 to 400 µL of the sample. Record the volume injected and the resulting peak size in area units.

13.2.4 If the response for the peak exceeds the working range of the system, dilute the sample with buffered water (pH of 3) and reanalyze.

13.3 *Identification of Analytes :*

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

13.3.2 Base the width of the retention time window used to make identifications on measurements of actual retention time variations of standards over the course of one day. Use three times the standard deviation of a retention time to calculate a suggested window size for a compound.

13.3.3 Identification requires expert judgment when sample components are not resolved chromatographically. When peaks obviously represent more than one sample component (that is, a broadened peak with shoulder(s) or a valley between two or more maxima), or whenever doubt exists over identification of a peak on a chromatogram, use appropriate alternative techniques to help confirm peak identification. For example, a more positive identification may be made by using an alternative

detector that operates on a chemical/physical principle different from that originally used, for example, mass spectrometry or the use of a second chromatography column. A suggested alternative column is described in 7.5.3 and 7.5.5.

14. Calculation

14.1 Determine the concentration of individual compounds in the sample using the following equation:

$$C_z = \frac{A_x \times Q_s}{A_s \times RF} \quad (3)$$

where:

C_x = analyte concentration, µg/L,
A_x = response of the sample analyte,
A_s = response of the standard (either internal or external), in units consistent with those used for the analyte response,
RF = response factor (with an external standard, RF = 1, because the standard is the same compound as the measured analyte), and
Q_s = concentration of the internal standard present, or concentration of the external standard that produced As, µg/L.

15. Report

15.1 Report compounds that clearly meet the criteria given in 13.3 to two significant figures.

15.2 When peaks obviously represent more than one sample component (that is, a broadened peak with shoulders or a valley between two or more maxima) or whenever doubt exists over identification of a peak on a chromatogram, appropriate alternative techniques need to be used to help confirm peak identification. For example, a more positive identification may be made by the use of an alternative detector that operates on a chemical/physical principle different from that originally used, for example, mass spectrometry or the use of a second chromatography column.

15.3 If the recovery of any analyte in the laboratory-fortified sample matrix falls outside the designated range and the laboratory performance of the analyte is shown to be in control (12.6), the recovery problem encountered with the dosed sample is judged to be matrix related. The result for that analyte in the unfortified sample is labeled suspect/matrix in order to inform the data user that the results are suspect due to matrix effects.

15.4 If the internal standard response for any sample deviates any more than 30 % of the daily calibration check standard, the sample should be reanalyzed. If the deviation is still greater than 30 % and the original sample is unavailable, report the data but annotate it as suspect.

16. Precision and Bias[25]

16.1 The collaborative study for performance evaluation of this test method was conducted in accordance with Practice D2777 – 86.

16.2 Eight laboratories participated in the study. The study design was based on Youden's nonreplicate plan for collaborative tests of analytical methods. Reagent and finished drinking water were spiked with the 12 analytes, each at six concentration levels, prepared as three Youden pairs. Analyses of the spiked reagent water evaluated the proficiency of this test method on a sample free from interferences. Analyses of the spiked finished drinking water allowed an analysis of variance test. Only Aldicarb sulfoxide was affected by sample matrix. The comparison of results between reagent water and finished tap water are shown in Table 6.

16.3 The overall standard deviation (S_R) shows precision associated with measurements generated by the eight laboratories (Table 5). Single analyst standard deviation (S_r) is the precision associated with performance in an individual laboratory (Table 5). Both precision estimates were made using a concentration that was about 10 times the EDL. The pooled, overall precisions in reagent water for the 10 analytes at approximately 10 times the EDL, expressed as RSD_R, was 6.9 %. The precision ranged from 3.6 % for carbofuran to 8.4 % for methiocarb. The pooled, overall precision in drinking water for the 10 analytes at approximately 10 times the EDL, expressed as RSD_R was 6.3 %. The precision ranged from 4.0 % for methomyl to 9.7 % for aldicarb. There is no significant difference between the reagent water matrix and the various finished drinking water matrices.

16.4 This method has evolved significantly since first approved. Method validation data for this updated method originated during EPA Method 531.2 validation, and is shown in Annex A1.

17. Keywords

17.1 carbamates; direct aqueous injection; drinking water; HPLC; *N*-methylcarbamates; *N*-methylcarbamoyloximes.

[25] Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR:D19-1150.

TABLE 6 Precision Statistics Calculated from Regression Equations by Compound and By Water Type [A]

Analyte	Concentration, µg/L [B]	Reagent Water		Finished Drinking Water		Reagent Water		Finished Drinking Water	
		S_r	S_R	S_r	S_R	RSD_r,%	RSD_R, %	RSD_r,%	RSD_R, %
Aldicarb	10.0	0.32	0.58	0.46	1.00	3.4	6.1	4.4	9.7
Aldicarb sulfone	20.0	0.86	1.33	0.43	0.87	4.5	6.9	2.2	4.7
Aldicarb sulfoxide	20.0	0.89	1.35	0.52	0.85	4.5	6.9	2.7	4.4
Baygon (Propoxur)	10.0	0.47	0.78	0.46	0.75	5.0	8.2	4.6	7.5
Carbaryl	20.0	0.79	1.35	0.93	1.35	4.1	6.9	4.8	6.9
Carbofuran	20.0	0.74	0.68	0.47	1.00	3.9	3.6	2.4	5.1
3-Hydroxycarbofuran	20.0	0.95	1.31	0.98	1.72	4.9	6.8	5.0	8.7
Methiocarb	50.0	2.07	3.93	1.69	3.08	4.4	8.4	3.5	6.4
Methomyl	5.0	0.33	0.37	0.14	0.20	6.7	7.5	2.8	4.0
Oxamyl	20.0	1.04	1.44	0.55	1.13	5.4	7.4	2.7	5.7
Average						4.7	6.9	3.5	6.3
Standard deviation						0.91	1.34	1.07	1.92

[A] S_r and S_R = standard deviations for repeatability and reproducibility, respectively. RSD_r and RSD_R = corresponding relative standard deviations.
[B] Concentration value is 10 to 15 times estimated MDL.

ANNEX

(Mandatory Information)

A1. SUMMARY OF CHANGES IN EPA METHOD 531.2

A1.1 EPA Method 531.1, the basis for Test Method D5315, was updated in 2001 to EPA Method 531.2. This update reflects the changes. See Fig. A1.1.

A1.2 The use of the binary water / methanol gradient has been changed to incorporate the use of the non-fluorescent quenching acetonitrile after the resolution of the 4 early eluting analytes, Aldicard Sulfone, Aldicarb Sulfoxide, Oxamyl, and Methomyl.

A1.3 Aldicarb and its 2 degradation products, aldicarb sulfone and aldicarb sulfoxide, may be regulated in the future. This HPLC gradient modification addresses this separation of the analytes given in A1.2. See Fig. A1.2.

A1.4 Since initial method approval in 1986, HPLC Fluorescence Detectors has evolved significant capability not available when this initial method was validated. N-Methyl Carbamte detection has been lowed to sub-ppb concentrations.

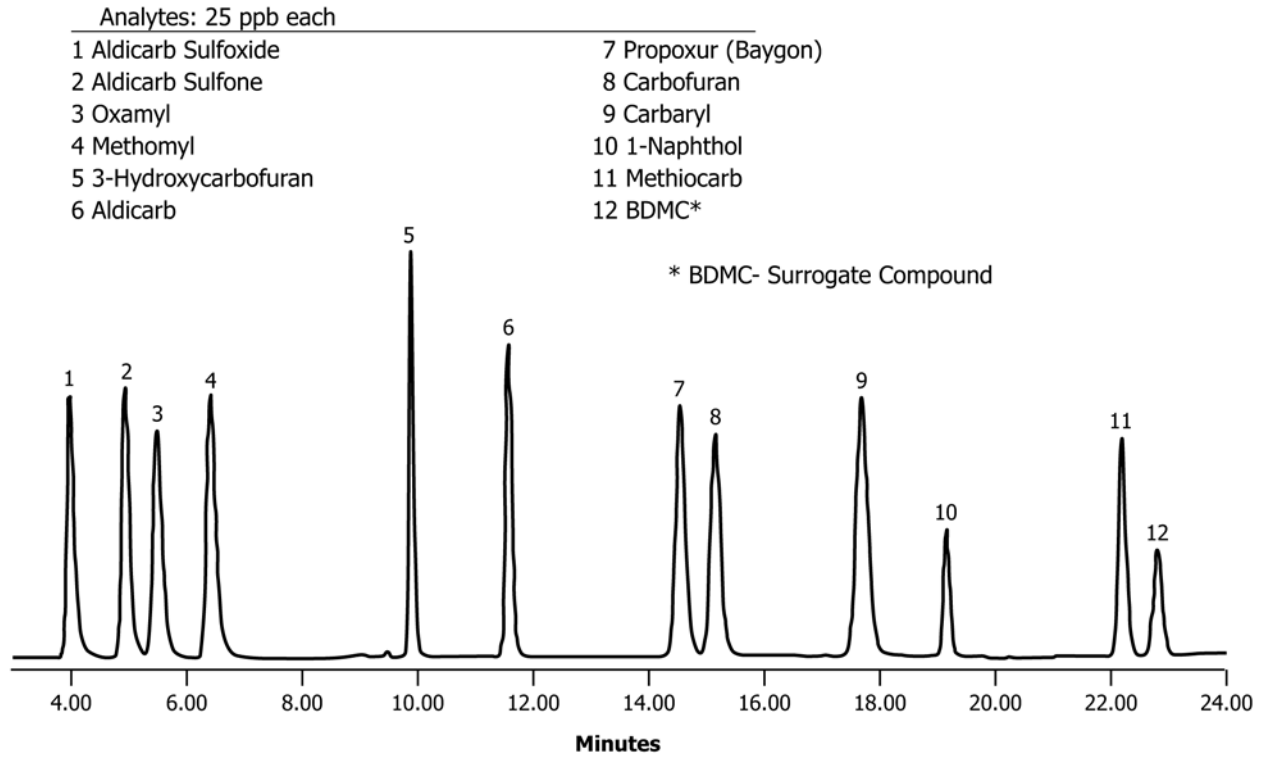

FIG. A1.1 Carbamate chromatogram using the Waters carbamate column and ternary gradient.

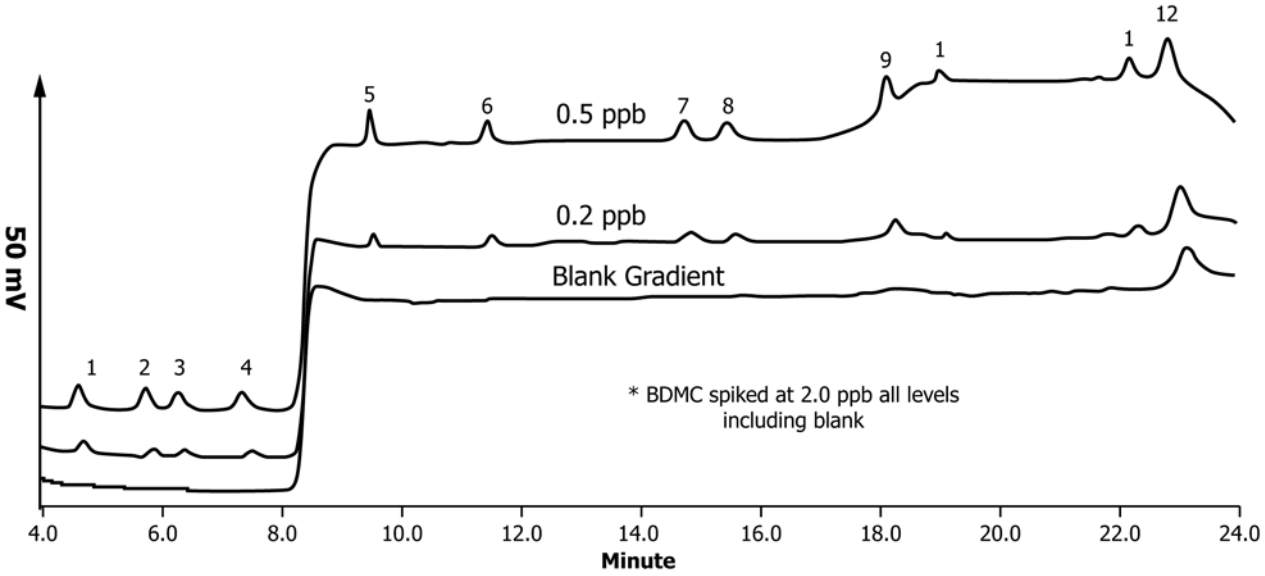

FIG. A1.2 Carbamate chromatogram using the Waters carbamate analysis column and ternary gradient.

TABLE A1.1 Instrument Method Conditions

Time (min)	% Water	% Methanol	% Acetronile
initial	88.0	12.0	0.0
5.30	88.0	12.0	0.0
5.40	68.0	16.0	16.0
14.00	68.0	16.0	16.0
16.10	50.0	25.0	25.0
20.00	50.0	25.0	25.0
22.00	88.0	12.0	0.0
30.00	88.0	12.0	0.0

Column: Waters carbamate 3.9 × 150 mm packed with 4.0 µm C_{18} stationary phase.

Postcolumn Reactor: Reaction coil set at 80°C, flow rate for Postcolumn Reagent #1 and #2 = 0.5 mL/min (each) for Waters unit, 0.3 mL/min for the Pickering unit.

Fluorescence Detector: 340 nm excitation, 465 nm emission with an 18 nm band width; Gain = 100; Attn. = 16; Response = Standard; 16µL flow cell.

HPLC: A ternary gradient comprised of water, methanol, and acetonitrile with a flow of 1.5 mL/min as shown in the table.

TABLE A1.2 Retention Time Data[A]

Analyte	Retention Time (min)	Standard Deviation	% RSD
Aldicarb sulfoxide	4.36	0.0092	0.21
Aldicarb sulfone	5.07	0.0089	0.17
Oxamyl	5.74	0.0095	.017
Methomyl	6.53	0.0077	0.12
3-Hydroxycarbofuran	9.82	0.013	0.13
Aldicarb	11.5	0.013	0.11
Propoxur	14.3	0.020	0.14
Carbofuran	14.8	0.024	0.16
Carbaryl	17.0	0.026	0.16
1-Naphthol	18.6	0.019	0.10
Methiocarb	21.8	0.015	0.07
BDMC (SUR)	22.3	0.015	0.07

[A] Retention time data is calculated from precision and accuracy data results presented in Table A1.6 and the calibration curve used to quantitate the data. Retention times may differ depending on the chromatographic conditions and columns used.

TABLE A1.3 Detection Limits in Reagent Water Using the Waters Postcolumn Carbamate System and the Waters Model 474 Detector

Analyte	Fortification Level (µg/L)	Detection Limit[A] (µg/L)	Signal to Noise Ratio
Aldicarb sulfoxide	0.20	0.059	8 : 1
Aldicarb sulfone	0.10	0.051	3 : 1
Oxamyl	0.20	0.065	10 : 1
Methomyl	0.20	0.050	10 : 1
3-Hydroxycarbofuran	0.20	0.029	18 : 1
Aldicarb	0.20	0.026	9 : 1
Propoxur	0.20	0.037	6 : 1
Carbofuran	0.20	0.043	9 : 1
Carbaryl	0.20	0.045	13 : 1
1-Naphthol	0.20	0.063	10 : 1
Methiocarb	0.20	0.061	11 : 1

[A] Detection limits were determined by analyzing seven replicates over three days using the conditions outlined in Table A1.1 with a 1000-µL injection.

TABLE A1.4 Detection Limits in Reagent Water Using the Pickering Model PCX5200 Postcolumn System and the Waters Model 474 Detector

Analyte	Fortification Level (µg/L)	Detection Limit[A] (µg/L)	Signal to Noise Ratio
Aldicarb sulfoxide	0.20	0.056	13 : 1
Aldicarb sulfone	0.20	0.026	15 : 1
Oxamyl	0.20	0.045	9 : 1
Methomyl	0.20	0.045	11 : 1
3-Hydroxycarbofuran	0.20	0.041	11 : 1
Aldicarb	0.20	0.042	7 : 1
Propoxur	0.20	0.040	11 : 1
Carbofuran	0.20	0.058	7 : 1
Carbaryl	0.20	0.065	22 : 1
1-Naphthol	0.20	0.034	9 : 1
Methiocarb	0.20	0.036	5 : 1

[A] Detection limits were determined by analyzing seven replicates over three days using the conditions outlined in Table A1.1 with a 250-µL injection.

TABLE A1.5 Detection Limits in Reagent Water Using the Waters Postcolumn Carbamate Analysis System and the Waters Model 2475 Detector

Analyte	Fortification Level (µg/L)	Detection Limit[A,B] (µg/L)	Signal to Noise Ratio
Aldicarb sulfoxide	0.20	0.038	14 : 1
Aldicarb sulfone	0.20	0.033	9 : 1
Oxamyl	0.20	0.044	4 : 1
Methomyl	0.20	0.054	24 : 1
3-Hydroxycarbofuran	0.20	0.038	7 : 1
Aldicarb	0.20	0.049	12 : 1
Propoxur	0.20	0.061	10 : 1
Carbofuran	0.20	0.050	12 : 1
Carbaryl	0.20	0.043	9 : 1
1-Naphthol	0.20	0.115	3 : 1
Methiocarb	0.20	0.055	5 : 1

[A] Detection limits were determined by analyzing seven replicates over three days using the conditions outlined in Table A1.1 with a 250-µL injection.
[B] These data were collected at American Water Works Service Company.

TABLE A1.6 Precision and Accuracy of Low and High Level Fortified Reagent Water[A]

Analyte	Concentration = 0.20 µg/L (n = 7)		Concentration = 10 µg/L (n = 7)	
	Mean % Recovery	Relative Standard Deviation (%)	Mean % Recovery	Relative Standard Deviation (%)
Aldicarb sulfoxide	112	6.2	106	1.8
Aldicarb sulfone	92	9.5	106	2.6
Oxamyl	101	8.6	106	2.2
Methomyl	101	6.5	106	2.9
3-Hydroxycarbofuran	105	6.8	108	1.2
Aldicarb	95	7.4	106	1.3
Propoxur	109	5.9	109	2.0
Carbofuran	112	6.7	110	2.2
Carbaryl	112	7.0	107	2.1
1-Naphthol	113	12.6	108	3.1
Methiocarb	105	5.9	107	1.5
BDMC (SUR)[B]	108	4.3	101	2.3

[A] Data obtained using conditions in Table A1.1 using a 1000-µl injection.
[B] Surrogate concentration in all samples was 2.0 mg/L.

TABLE A1.7 Precision and Accuracy of Low and High Level Chlorinated Surface Water[A]

Analyte	Concentration = 0.20 µg/L (n = 7)		Concentration = 10 µg/L (n = 7)	
	Mean % Recovery	Relative Standard Deviation (%)	Mean % Recovery	Relative Standard Deviation (%)
Aldicarb sulfoxide	113	7.0	104	2.8
Aldicarb sulfone	104	5.5	106	1.4
Oxamyl	107	6.4	104	2.2
Methomyl	110	9.8	104	1.6
3-Hydroxycarbofuran	128	3.9	107	1.1
Aldicarb	123	2.7	105	1.5
Propoxur	128	6.0	106	2.1
Carbofuran	140	5.6	105	2.5
Carbaryl	112	9.7	106	0.9
1-Naphthol	113	12.1	101	1.3
Methiocarb	104	13.3	107	1.1
BDMC (SUR)[B]	108	2.1	96	3.9

[A] Data obtained using conditions in Table A1.1 using a 1000-µl injection.
[B] Surrogate concentration in all samples was 2.0 mg/L.

TABLE A1.8 Precision and Accuracy of Low and High Level Fortified Chlorinated Ground Water[A]

Analyte	Concentration = 0.20 µg/L (n = 7)		Concentration = 10 µg/L (n = 7)	
	Mean % Recovery	Relative Standard Deviation (%)	Mean % Recovery	Relative Standard Deviation (%)
Aldicarb sulfoxide	111	7.3	106	1.1
Aldicarb sulfone	98	9.2	106	1.4
Oxamyl	99	8.4	105	2.2
Methomyl	99	10.2	105	1.6
3-Hydroxycarbofuran	107	3.0	108	1.1
Aldicarb	100	6.3	105	1.5
Propoxur	112	6.1	107	2.1
Carbofuran	112	4.1	107	2.5
Carbaryl	119	5.1	108	0.9
1-Naphthol	109	8.2	109	1.3
Methiocarb	105	3.9	107	1.1
BDMC (SUR)[B]	109	2.0	97	3.9

[A] Data obtained using conditions in Table A1.1 using a 1000-µl injection.
[B] Surrogate concentration in all samples was 2.0 mg/L.

TABLE A1.9 Sample Holding Time Data for Chlorinated Ground Water Samples Fortified with Method Analytes at 2.0 ug/L

Analyte	% Recovery for Samples Fortified at 2.0 µg/L.				
	Day 0	Day 2	Day 8	Day 15	Day 28
Aldicarb sulfoxide	94	97	93	93	96
Aldicarb sulfone	93	99	97	98	98
Oxamyl	97	97	101	103	101
Methomyl	96	95	99	98	97
3-Hydroxycarbofuran	96	99	96	95	98
Aldicarb	95	100	96	92	93
Propoxur	95	98	97	98	99
Carbofuran	96	99	97	97	100
Carbaryl	96	97	98	94	100
1-Naphthol	96	97	99	95	98
Methiocarb	95	98	96	94	97
BDMC (SUR)	99	105	100	99	99

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