Designation: D5315 – 04 (Reapproved 2017)ε1

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.

ε[1] NOTE—Warning notes were editorially updated throughout in December 2017.

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, health, and environmental 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**).

1.7 *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

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

[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.

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.

E682 Practice for Liquid Chromatography Terms and Relationships

2.2 *U.S. Environmental Protection Agency Standard:*[5]

EPA Method 531.1 Revision 3.0, USEPA, EMSL-Cincinnati, 1989

EPA Method 531.2 Revision 1.0, USEPA, EMSL-Cincinnati, 2001

3. Terminology

3.1 *Definitions:*

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

3.1.2 For definitions of other terms used in this standard, refer to Practice E682.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *calibration standard* (CAL), *n*—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), *n*—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), *n*—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, n*—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), *n*—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), *n*—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), *n*—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), *n*—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), *n*—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, n*—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), *n*—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, n*—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, n*—a pure analyte(s), which is extremely unlikely to be found in any sample, and which is

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

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

4.2 This test 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 Specification 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[6] and caps[7] 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.[8]

7.2.2 *Microfiltration Device,* to filter samples prior to HPLC analysis. Use a 13-mm filter holder[9] and 13-mm diameter, 0.2-µm polyester filters.[10]

7.3 *Syringes and Valves:*

7.3.1 *Hypodermic Syringe,* 10 mL, glass, with Luer-Lok[11] tip.

7.3.2 *Syringe Valve,* three-way.[12]

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,*[13]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.[14] Mobile phase is established at 1.0 mL/min as a

[6] Sample bottle vial, Pierce No. 13075, available from Pierce Chemical Co., 3747 N. Meridian Rd., Rockford, IL 61101, or equivalent, has been found suitable for use.

[7] Sample bottle cap, Pierce No. 12722, available from Pierce Chemical Co., 3747 N. Meridian Rd., Rockford, IL 61101, or equivalent, has been found suitable for use.

[8] 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, has been found suitable for use.

[9] Millipore stainless steel XX300/200, available from Millipore Corp., 80 Ashby Rd., Bedford, MA 01730, or equivalent, has been found suitable for use.

[10] Nucleopore 180406, available from Costar Corp., 1 Alewife Center, Cambridge, MA 02140, or equivalent, has been found suitable for use.

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

[12] Hamilton HV3-3, available from Hamilton Co., P.O. Box 10030, Reno, NV 89502, or equivalent, has been found suitable for use.

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

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