



Designation: D7645 – 23

# Standard Test Method for Determination of Aldicarb, Aldicarb Sulfone, Aldicarb Sulfoxide, Carbofuran, Methomyl, Oxamyl, and Thiofanox in Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)<sup>1</sup>

This standard is issued under the fixed designation D7645; 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 reappraisal. A superscript epsilon ( $\epsilon$ ) indicates an editorial change since the last revision or reappraisal.

## 1. Scope

1.1 This test method covers the determination of aldicarb, aldicarb sulfone, aldicarb sulfoxide, carbofuran, methomyl, oxamyl, and thiofanox (referred to collectively as carbamates in this test method) in water by direct injection using liquid chromatography (LC) and detected with tandem mass spectrometry (MS/MS). These analytes are qualitatively and quantitatively determined by this test method. This test method adheres to multiple reaction monitoring (MRM) mass spectrometry.

1.2 The Detection Verification Level (DVL) and Reporting Range for the carbamates are listed in [Table 1](#).

1.2.1 The DVL is required to be at a concentration at least 3 times below the Reporting Limit (RL) and have a signal/noise ratio greater than 3:1. [Fig. 1](#) displays the signal/noise ratios of the primary single reaction monitoring (SRM) transitions, and [Fig. 2](#) displays the confirmatory SRM transitions at the DVLs for the carbamates.

1.2.2 The reporting limit is the concentration of the Level 1 calibration standard as shown in [Table 2](#) for the carbamates.

1.3 *Units*—The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *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.*

1.5 *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 Recom-*

*mendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

## 2. Referenced Documents

### 2.1 ASTM Standards:<sup>2</sup>

- [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](#)
- [D3694 Practices for Preparation of Sample Containers and for Preservation of Organic Constituents](#)
- [D3856 Guide for Management Systems in Laboratories Engaged in Analysis of Water](#)
- [D4841 Practice for Estimation of Holding Time for Water Samples Containing Organic and Inorganic Constituents](#)
- [D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis](#)
- [E2554 Practice for Estimating and Monitoring the Uncertainty of Test Results of a Test Method Using Control Chart Techniques](#)

### 2.2 Other Documents:<sup>3</sup>

- [EPA Publication SW-846 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods](#)
- [EPA Method 531 Measurement of \*N\*-Methyl Carbamoyloximes and \*N\*-Methyl Carbamates in Drinking Water by Direct Aqueous Injection HPLC with Post Column Derivatization](#)
- [EPA Method 531.2 Measurement of \*N\*-Methyl Carbamoyloximes and \*N\*-Methyl Carbamates in Water by Direct Aqueous Injection HPLC with Post Column Derivatization](#)

<sup>1</sup> 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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<sup>2</sup> For referenced ASTM standards, visit the ASTM website, [www.astm.org](http://www.astm.org), or contact ASTM Customer Service at [service@astm.org](mailto:service@astm.org). For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

<sup>3</sup> Available from National Technical Information Service (NTIS), U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA, 22161 or at <http://www.epa.gov/epawaste/hazard/testmethods/index.htm>.

**TABLE 1 Detection Verification Level and Reporting Range**

Analyte	DVL (ng/L)	Reporting Range (µg/L)
Aldicarb	250	1-100
Aldicarb Sulfone	250	1-100
Aldicarb Sulfoxide	250	1-100
Carbofuran	250	1-100
Methomyl	250	1-100
Oxamyl	250	1-100
Thiofanox	250	1-100

**EPA Method 538 Determination of Selected Organic Contaminants in Drinking Water by Direct Aqueous Injection-Liquid Chromatography/Tandem Mass Spectrometry (DAI-LC/MS/MS)**

### 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 *carbamates, n*—in this test method, aldicarb, aldicarb sulfone, aldicarb sulfoxide, carbofuran, methomyl, oxamyl, and thiofanox collectively.

3.2.2 *detection verification level, DVL, n*—a concentration that has a signal/noise ratio greater than 3:1 and is at least 3 times below the Reporting Limit (RL).

3.2.3 *independent reference material, IRM, n*—a material of known purity and concentration obtained either from the National Institute of Standards and Technology (NIST) or other reputable supplier; the IRM must be obtained from a different lot of material than is used for calibration.

#### 3.3 Acronyms:

- 3.3.1 *CCC, n*—Continuing Calibration Check
- 3.3.2 *IC, n*—Initial Calibration
- 3.3.3 *LC, n*—Liquid Chromatography
- 3.3.4 *LCS/LCSD, n*—Laboratory Control Sample/  
Laboratory Control Sample Duplicate
- 3.3.5 *MeOH, n*—Methanol
- 3.3.6 *mM, n*—millimolar,  $1 \times 10^{-3}$  moles/L
- 3.3.7 *MRM, n*—Multiple Reaction Monitoring
- 3.3.8 *MS/MSD, n*—Matrix Spike/Matrix Spike Duplicate
- 3.3.9 *NA, adj*—Not Available
- 3.3.10 *ND, n*—non-detect
- 3.3.11 *P&A, n*—Precision and Accuracy
- 3.3.12 *PPB, n*—parts per billion
- 3.3.13 *PPT, n*—parts per trillion
- 3.3.14 *QA, adj*—Quality Assurance
- 3.3.15 *QC, adj*—Quality Control
- 3.3.16 *RL, n*—Reporting Limit
- 3.3.17 *RSD, n*—Relative Standard Deviation
- 3.3.18 *RT, n*—Retention Time
- 3.3.19 *SDS, n*—Safety Data Sheets

3.3.20 *SRM, n*—Single Reaction Monitoring

3.3.21 *SS, n*—Surrogate Standard

3.3.22 *TC, n*—Target Compound

3.3.23 *µM, n*—micromolar,  $1 \times 10^{-6}$  moles/L

3.3.24 *VOA, n*—Volatile Organic Analysis

### 4. Summary of Test Method

4.1 This is a performance-based method, and modifications are allowed to improve performance.

4.2 For carbamate analysis, samples are shipped to the lab acidified between above freezing and 6 °C and analyzed within 14 days of collection. In the lab, the samples are spiked with surrogates, filtered using a syringe driven filter unit, and analyzed directly by LC/MS/MS.

4.3 The carbamates, methomyl-<sup>13</sup>C<sub>2</sub>, <sup>15</sup>N (surrogate) and carbofuran-<sup>13</sup>C<sub>6</sub> (surrogate) are identified by retention time and two SRM transitions. The target analytes and surrogate are quantitated using the primary SRM transitions utilizing an external calibration. The final report issued for each sample lists the concentration of carbamates and the surrogate recoveries.

### 5. Significance and Use

5.1 This test method has been developed by U.S. EPA Region 5 Chicago Regional Laboratory (CRL).

5.2 The *N*-methyl carbamate (NMC) pesticides: aldicarb, carbofuran, methomyl, oxamyl, and thiofanox have been identified by EPA as working through a common mechanism. These affect the nervous system by reducing the ability of enzymes. Enzyme inhibition was the primary toxicological effect of regulatory concern to EPA in assessing the NMC's food, drinking water, and residential risks. In most of the country, NMC residues in drinking water sources are at levels that are not likely to contribute substantially to the multi-pathway cumulative exposure. Shallow private wells extending through highly permeable soils into shallow, acidic ground water represent what the EPA believes to be the most vulnerable drinking water. Aldicarb sulfone and aldicarb sulfoxide are breakdown products of aldicarb and should also be monitored due to their toxicological effects.<sup>4</sup>

5.3 This test method has been investigated for use with reagent, surface, and drinking water for the selected carbamates: aldicarb, aldicarb sulfone, aldicarb sulfoxide, carbofuran, methomyl, oxamyl, and thiofanox.

### 6. Interferences

6.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other apparatus producing discrete artifacts or elevated baselines. All of these materials are demonstrated to be free from interferences by analyzing laboratory reagent blanks under the same conditions as samples.

<sup>4</sup> Additional information about Carbamate pesticides area available from United States Environmental Protection Agency (EPA), <http://www.epa.gov>.

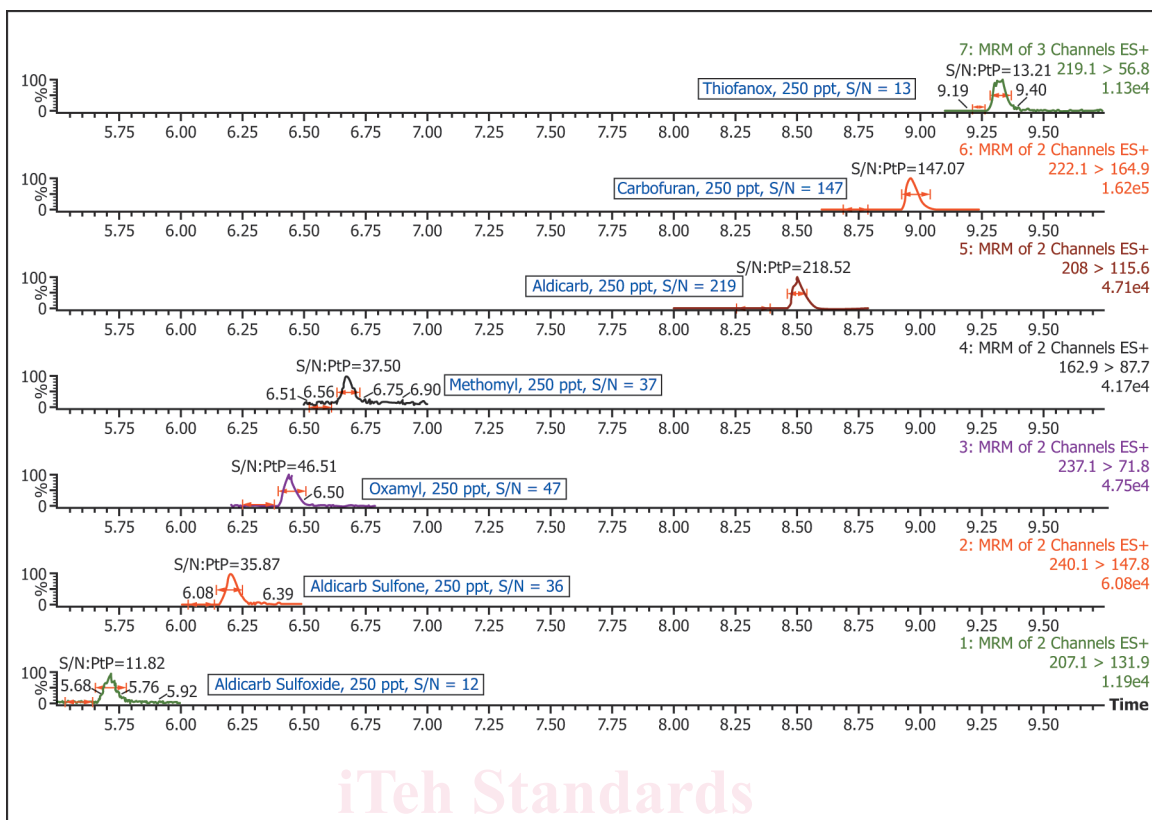


FIG. 1 Example Primary SRM Chromatograms Signal/Noise Ratios

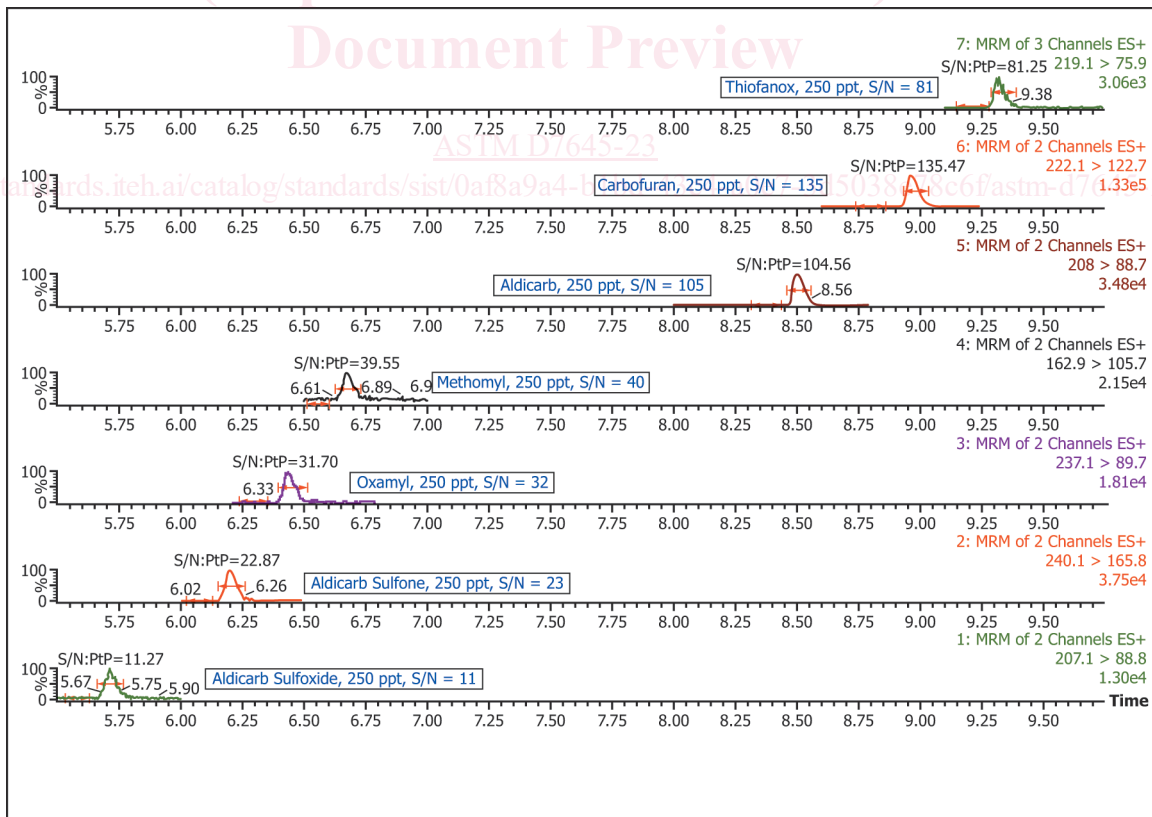


FIG. 2 Example Confirmatory SRM Chromatograms Signal/Noise Ratios

**TABLE 2 Concentrations of Calibration Standards (PPB)**

Analyte/Surrogate	LV 1	LV 2	LV 3	LV 4	LV 5	LV 6	LV 7	LV 8
Aldicarb	1	5	10	25	35	50	75	100
Aldicarb Sulfone	1	5	10	25	35	50	75	100
Aldicarb Sulfoxide	1	5	10	25	35	50	75	100
Carbofuran	1	5	10	25	35	50	75	100
Methomyl	1	5	10	25	35	50	75	100
Oxamyl	1	5	10	25	35	50	75	100
Thiofanox	1	5	10	25	35	50	75	100
Carbofuran- <sup>13</sup> C <sub>6</sub> (Surrogate)	1	5	10	25	35	50	75	100
Methomyl- <sup>13</sup> C <sub>2</sub> , <sup>15</sup> N (Surrogate)	1	5	10	25	35	50	75	100

6.2 All glassware is washed in hot water with detergent and rinsed in hot water followed by distilled water. The glassware is then dried and heated in an oven at 250 °C for 15 min to 30 min. All glassware is subsequently cleaned with acetone followed by methanol.

6.3 All reagents and solvents should be pesticide residue purity or higher to minimize interference problems.

6.4 Matrix interferences may be caused by contaminants in the sample. The extent of matrix interferences can vary considerably from sample source depending on variations of the sample matrix.

## 7. Apparatus

### 7.1 LC/MS/MS System:

7.1.1 *Liquid Chromatography (LC) System*—A complete LC system is needed to analyze samples. This should include a sample injection system, a solvent pumping system capable of mixing solvents, a sample compartment capable of maintaining required temperature and a temperature controlled column compartment. A system that is capable of performing at the flows, pressures, controlled temperatures, sample volumes, and requirements of the standard may be used.

7.1.2 *Analytical Column*<sup>5</sup>—A C18 column was used to develop this test method.

7.2 *Tandem Mass Spectrometer (MS/MS) System*—A MS/MS system capable of MRM analysis. Any system that is capable of performing at the requirements in this standard may be used.

### 7.3 Filtration Device:

7.3.1 *Hypodermic Syringe*—A lock tip glass syringe capable of holding a syringe-driven filter unit or similar may be used.

7.3.1.1 A 25-mL lock tip glass syringe size is recommended since a 25-mL sample size is used in this test method.

7.3.2 *Filter Unit*<sup>6</sup>—PVDF filter units were used to filter the samples.

<sup>5</sup> A Waters ACQUITY UPLC (a trademark of Waters Technologies Corporation in Wilmington, DE) BEH C18, 2.1 mm × 100 mm, 1.7 μm particle size was used, if you are aware of an alternative column that meets the performance of the standard, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,<sup>1</sup> which you may attend.

<sup>6</sup> A Millex HV Syringe Driven Filter Unit PVDF 0.22 μm (Millipore Corporation, Catalog #SLGV033NS; Millex is a trademark of Merck KGAA, Darmstadt, Germany) was used, if you are aware of an alternative filter that meets the performance of the standard, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,<sup>1</sup> which you may attend.

## 8. Reagents and Materials

8.1 *Purity of Reagents*—High Performance Liquid Chromatography (HPLC) pesticide residue analysis and spectrophotometry grade chemicals must be used in all tests. Unless indicated otherwise, it is intended that all reagents must conform to the Committee on Analytical Reagents of the American Chemical Society.<sup>7</sup> Other reagent grades may be used provided they are first determined to be of sufficiently high purity to permit their use without affecting the accuracy of the measurements.

8.2 *Purity of Water*—Unless otherwise indicated, references to water must be understood to mean reagent water conforming to Type 1 of Specification D1193. It must be demonstrated that this water does not contain contaminants at concentrations sufficient to interfere with the analysis.

8.3 *Gases*—Ultrapure nitrogen and argon.

8.4 Acetonitrile (CAS # 75-05-8).

8.5 Methanol (CAS # 67-56-1).

8.6 Acetone (CAS # 67-64-1).

8.7 Ammonium Formate (CAS # 540-69-2).

8.8 Acetic Acid (Glacial, CAS # 64-19-7).

8.9 Aldicarb (CAS # 116-06-3).

8.10 Aldicarb Sulfone (CAS # 1646-88-4).

8.11 Aldicarb Sulfoxide (CAS # 1646-87-3).

8.12 Carbofuran (CAS # 1563-66-2).

8.13 Oxamyl (CAS # 23135-22-0).

8.14 Methomyl (CAS # 16752-77-5).

8.15 Thiofanox (CAS # 39196-18-4).

8.16 Methomyl-<sup>13</sup>C<sub>2</sub>, <sup>15</sup>N (acetohydroxamate-<sup>13</sup>C<sub>2</sub>, <sup>15</sup>N, CAS # (unlabeled) 16752-77-5).

8.17 Carbofuran-<sup>13</sup>C<sub>6</sub> (Ring-<sup>13</sup>C<sub>6</sub>, CAS # (unlabeled) 1563-66-2).

<sup>7</sup> *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.



## 9. Hazards

9.1 Normal laboratory safety applies to this test method. Analysts should wear safety glasses, gloves, and lab coats when working in the lab. Analysts should review the Safety Data Sheets (SDS) for all reagents used in this test method.

## 10. Sampling

10.1 *Sampling and Preservation*—Grab samples should be collected in  $\geq 25$  mL pre-cleaned amber glass bottles with inert-lined caps demonstrated to be free of interferences. All samples are acidified with glacial acetic acid to  $\text{pH} \leq 3.8$  upon collection. A few drops or less of glacial acetic acid is required per 40 mL water sample collected. Chlorinated drinking water samples are also dechlorinated with ascorbic acid; 10 mg of ascorbic acid is added to each 40 mL volume of water prior to collection. Drinking water samples must be dechlorinated upon collection. Aldicarb oxidizes when residual chlorine is present in the sample. This test method is based on a 25 mL sample size per analysis. If different sample sizes are used, spiking solution amounts and preservatives will need to be modified. Conventional sampling practices should be followed. Refer to Guide [D3856](#) and Practices [D3694](#). Store samples between above freezing and 6 °C from the time of collection until analysis. Analyze the sample within 14 days of collection.

NOTE 1—Less sample volume is acceptable, but the spike amounts and sample preservatives must be adjusted accordingly.

10.1.1 EPA Method 531.2 demonstrated that carbamates are more stable under acidic conditions. Potassium dihydrogen citrate buffer is used in Method 531.2 to bring the pH to  $\sim 3.8$ , but this buffer is incompatible with LC/MS/MS. Therefore, the pH adjustment is accomplished with acetic acid in this test method. EPA Method 531.2 demonstrated that carbamates under acidic conditions are stable for at least 28 days. EPA Method 531 demonstrated that oxamyl and methomyl are stable for at least 70 days at  $\text{pH } 3 \pm 0.2$ . Holding time is dependent upon an individual matrix and will vary. Practice [D4841](#) may be used to conduct a holding time study on an individual matrix.

## 11. Preparation of LC/MS/MS

### 11.1 LC Chromatograph Operating Conditions:

11.1.1 Injection volumes of all calibration standards and samples are made at 50  $\mu\text{L}$  volume using a full loop injection. If a 50  $\mu\text{L}$  volume loop is installed in the LC, a “full loop” mode is the preferred technique when performing fast, qualitative analyses. This mode should be used whenever accuracy and precision are the primary concerns. The first sample analyzed after the calibration curve is a blank to ensure there is no carry-over. The gradient conditions for the liquid chromatograph are shown in [Table 3](#).

### 11.2 LC Sample Manager Conditions:

11.2.1 *Wash Solvents*—Weak wash is 2.4 mL of 95 % water/5 % methanol. Strong wash is 1.2 mL of methanol. The strong wash solvent is needed to eliminate carry-over between injections of carbamate samples. The weak wash is used to remove the strong wash solvent. Instrument manufacturer specifications should be followed in order to eliminate sample carry-over.

**TABLE 3 Gradient Conditions for Liquid Chromatography**

Time (min)	Flow ( $\mu\text{L}/\text{min}$ )	Percent	
		95 % Water/ 5 % Methanol, 5 mM $\text{NH}_4\text{CO}_2\text{H}$	95 % Methanol/ 5 % Water, 5 mM $\text{NH}_4\text{CO}_2\text{H}$
0.0	300	100	0
2.0	300	100	0
3.0	300	95	5
5.0	300	85	15
10.0	300	0	100
11.5	300	0	100
12.0	300	100	0
14.0	300	100	0

11.2.2 *Temperatures*—Column, 30 °C; Sample compartment, 15 °C.

11.2.3 *Seal Wash*—Solvent: 50 Acetonitrile/50 Water; Time: 5 min.

### 11.3 Mass Spectrometer Parameters:

11.3.1 To acquire the maximum number of data points per SRM channel while maintaining adequate sensitivity, the tune parameters may be optimized according to the instrument. Each peak requires at least 10 scans per peak for adequate quantitation. This test method contains two surrogates, which are isotopically labeled methomyl and carbofuran, and seven carbamates, which are split up into seven MRM acquisition functions to optimize sensitivity. Variable parameters regarding retention times, SRM transitions, and cone and collision energies are shown in [Table 4](#). Mass spectrometer parameters used in the development of this test method are listed below:

The instrument is set in the Electrospray positive source setting.  
 Capillary Voltage: 3.5 kV  
 Cone: Variable depending on analyte ([Table 4](#))  
 Extractor: 2 Volts  
 RF Lens: 0.1 Volts  
 Source Temperature: 120 °C  
 Desolvation Temperature: 375 °C  
 Desolvation Gas Flow: 800 L/h  
 Cone Gas Flow: 25 L/h  
 Low Mass Resolution 1: 14.5  
 High Mass Resolution 1: 14.5  
 Ion Energy 1: 0.5  
 Entrance Energy:  $-1$   
 Collision Energy: Variable depending on analyte ([Table 4](#))  
 Exit Energy: 0  
 Low Mass Resolution 2: 14.5  
 High Mass resolution 2: 14.5  
 Ion Energy 2: 0.7  
 Multiplier: 650  
 Gas Cell Pirani Gauge:  $7.0 \times 10^{-3}$  Torr  
 Inter-Channel Delay: 0.005 s  
 Inter-Scan Delay: 0.005 s  
 Dwell: 0.075 s

## 12. Calibration and Standardization

12.1 The mass spectrometer must be calibrated in accordance with manufacturer specifications before analysis. In order to obtain accurate analytical values through using this test method within the confidence limits, the following procedures must be followed when performing the test method. Prepare all solutions in the lab using Class A volumetric glassware.

12.2 *Calibration and Standardization*—To calibrate the instrument, analyze eight calibration standards containing the eight concentration levels of the carbamates, methomyl- $^{13}\text{C}_2$ ,

**TABLE 4 Retention Times, SRM Ions, and Analyte-Specific Mass Spectrometer Parameters**

Analyte	Primary/ Confirmatory	Retention Time (min)	Cone Voltage (Volts)	Collision Energy (eV)	SRM Mass Transition (Parent > Product)	Primary/ Confirmatory SRM Area Ratio
Aldicarb	Primary	8.50	10	7	208.0 > 115.6	1.4
	Confirmatory		10	16	208.0 > 88.7	
Aldicarb Sulfone	Primary	6.20	13	13	240.1 > 147.8	1.6
	Confirmatory		13	11	240.1 > 165.8	
Aldicarb Sulfoxide	Primary	5.72	16	6	207.1 > 131.9	1.1
	Confirmatory		16	14	207.1 > 88.8	
Carbofuran	Primary	8.96	22	12	222.1 > 164.9	1.2
	Confirmatory		22	20	222.1 > 122.7	
Methomyl	Primary	6.68	15	10	162.9 > 87.7	1.8
	Confirmatory		15	10	162.9 > 105.7	
Oxamyl	Primary	6.44	11	11	237.1 > 71.8	2.6
	Confirmatory		11	7	237.1 > 89.7	
Thiofanox	Primary	9.32	12	8	219.1 > 56.8	4.8
	Confirmatory		12	5	219.1 > 75.9	
Carbofuran- <sup>13</sup> C <sub>6</sub> (Surrogate)	Primary	8.96	22	11	228.1 > 170.9	1.3
	Confirmatory		22	21	228.1 > 128.8	
Methomyl- <sup>13</sup> C <sub>2</sub> , <sup>15</sup> N (Surrogate)	Primary	6.68	18	8	165.8 > 90.7	1.7
	Confirmatory		18	9	165.8 > 108.7	

<sup>15</sup>N and carbofuran-<sup>13</sup>C<sub>6</sub> prior to analysis as shown in [Table 2](#). A calibration stock standard solution is prepared from standard materials or they are purchased as certified solutions. Stock Standard Solution A containing the carbamates and surrogates is prepared at Level 8 concentration and aliquots of that solution are diluted to prepare Levels 1 through 7. The following steps will produce standards with the concentration values shown in [Table 2](#). The analyst is responsible for recording initial component weights carefully when working with pure materials and correctly carrying the weights through the dilution calculations.

12.2.1 Prepare Stock Standard Solution A (Level 8) by adding to a 50 mL volumetric flask individual solutions of the following: 100 µL of aldicarb, aldicarb sulfone, aldicarb sulfoxide, carbofuran, methomyl, oxamyl, and thiofanox, each at 50 ppm in methanol and 50 µL of methomyl-<sup>13</sup>C<sub>2</sub>, <sup>15</sup>N in methanol and carbofuran-<sup>13</sup>C<sub>6</sub> in 1,4-dioxane each at 100 ppm, dilute to 50 mL with water. The preparation of the Level 8 standard can be accomplished using different volumes and concentrations of stock solutions as is accustomed in the individual laboratory. Depending on the prepared stock concentrations, the solubility at that concentration will have to be ensured.

12.2.2 Aliquots of Solution A are then diluted with water to prepare the desired calibration levels in 2-mL amber glass LC vials. The calibration vials must be used within 24 h to ensure optimum results. Stock calibration standards are routinely replaced every 7 days if not previously discarded for quality control failure. Calibration standards are not filtered.

12.2.3 Inject each standard and obtain its chromatogram. An external calibration technique is used to monitor the primary and confirmatory SRM transitions of each analyte. Calibration software is utilized to conduct the quantitation of the target analytes and surrogates using the primary SRM transition. The ratios of the primary/confirmatory SRM transition area counts are given in [Table 4](#) and will vary depending on the individual tuning conditions. The primary/confirmatory SRM transition area ratio must be within 35 % of the individual labs' accepted primary/confirmatory SRM transition area ratio. The primary

SRM transition of each analyte is used for quantitation and the confirmatory SRM transition for confirmation. This gives added confirmation by isolating the parent ion, forming two product ions via fragmentation, and relating it to the retention time in the calibration standard.

12.2.4 The calibration software manual should be consulted to use the software correctly. The quantitation method is set as an external calibration using the peak areas in ppt or ppb units, as long as the analyst is consistent. Concentrations may be calculated using the data system software to generate linear regression or quadratic calibration curves. Forcing the calibration curve through the origin is not recommended. Curves should be evaluated using relative error or relative standard error.<sup>8</sup>

12.2.5 Linear calibration may be used if the point of origin is excluded and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. Each calibration point used to generate the curve must have a calculated percent deviation less than 25 % from the generated curve.

12.2.6 Quadratic calibration may be used if the point of origin is excluded, and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. Each calibration point used to generate the curve must have a calculated percent deviation less than 25 % from the generated curve.

12.2.7 The retention time window of the SRM transitions must be within 5 % of the retention time of the analyte in a midpoint calibration standard. If this is not the case, re-analyze the calibration curve to determine if there was a shift in retention time during the analysis and the sample needs to be re-injected. If the retention time is still incorrect in the sample, refer to the analyte as an unknown.

12.2.8 A midpoint calibration check standard must be analyzed at the end of each batch of 20 samples or within 24 h after the initial calibration curve was generated. This end

<sup>8</sup> Management and Technical Requirements for Laboratories Performing Environmental Analysis; Module 4: Quality Systems for Chemical Testing; The NELAC Institute, 2017.

calibration check should be the same calibration standard that was used to generate the initial curve. The results from the end calibration check standard must have a percent deviation less than 30 % from the calculated concentration for the target analytes and surrogates. If the results are not within these criteria, the problem must be corrected and either all samples in the batch must be re-analyzed against a new calibration curve or the affected results must be qualified with an indication that they do not fall within the performance criteria of the test method. If the analyst inspects the vial containing the end calibration check standard and notices that the sample evaporated affecting the concentration, a new end calibration check standard may be made and analyzed. If this new end calibration check standard has a percent deviation less than 30 % from the calculated concentration for the target analytes and surrogates, the results may be reported unqualified.

12.3 If a laboratory has not performed the test before or if there has been a major change in the measurement system, for example, new analyst, new instrument, etc., a precision and bias study must be performed to demonstrate laboratory capability.

12.3.1 Analyze at least four replicates of a sample solution containing the carbamates and surrogates at a concentration in the calibration range of Levels 5 to 7. The Level 6 concentration of the eight-point calibration curve was used to set the QC acceptance criteria in this test method. The matrix and chemistry should be similar to the solution used in this test method. Each replicate must be taken through the complete analytical test method including any sample preservation and pretreatment steps.

12.3.2 Calculate the mean (average) percent recovery and relative standard deviation (RSD) of the four values and compare to the acceptable ranges of the QC acceptance criteria for the Initial Demonstration of Performance in [Table 5](#).

12.3.3 This study should be repeated until the single operator precision and mean recovery are within the limits in [Table 5](#). If a concentration other than the recommended concentration is used, refer to Practice [D5847](#) for information on applying the F test and t test in evaluating the acceptability of the mean and standard deviation.

12.3.3.1 The QC acceptance criteria for the Initial Demonstration of Performance in [Table 5](#) were generated from a single-laboratory. Data from reagent, surface, and drinking water matrices are shown in Section 16, Precision and Bias. It is recommended that the laboratory generate their own in-house QC acceptance criteria which meets or exceeds the criteria in this standard. References on how to generate QC acceptance criteria are Practices [D2777](#), [D5847](#), [E2554](#), or Method 8000B in EPA Publication SW-846.

#### 12.4 Surrogate Spiking Solution:

12.4.1 A surrogate spiking 50 % methanol/50 % 1,4-dioxane solution containing methomyl-<sup>13</sup>C<sub>2</sub>, <sup>15</sup>N and carbofuran-<sup>13</sup>C<sub>6</sub> is added to all samples. A stock surrogate spiking solution is prepared at 50 ppm. Spiking 25 µL of this spiking solution into a 25 mL water sample results in a concentration of 50 ppb of the surrogate in the sample. The result obtained for the surrogate recovery must fall within the limits of [Table 5](#). If the limits are not met, the affected results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.4.1.1 The surrogate spiking solution was prepared by adding 1.0 mL of a 100 ppm solution of methomyl-<sup>13</sup>C<sub>2</sub>, <sup>15</sup>N in methanol to 1.0 mL of a 100 ppm solution of carbofuran-<sup>13</sup>C<sub>6</sub> in 1,4-dioxane and stored in a 4 mL amber glass vial with an inert-lined cap. Surrogate spiking solutions are routinely replaced every 14 days if not previously discarded for quality control failure.

<https://standards.iteh.ai/catalog/standards/sist/0a18a9a4-b1bd-43c4-a0e7-3d5038e78c6f/astm-d7645-23>

**TABLE 5 QC Acceptance Criteria**

Analyte	Test Conc. (µg/L)	Initial Demonstration of Performance			Lab Control Sample	
		Recovery (%)		Precision	Recovery (%)	
		Lower Limit	Upper Limit	Maximum % RSD	Lower Limit	Upper Limit
Aldicarb	50	65	128	30	65	128
Aldicarb Sulfone	50	68	127	30	68	127
Aldicarb Sulfoxide	50	75	118	30	75	118
Carbofuran	50	39	142	30	39	142
Methomyl	50	67	127	30	67	127
Oxamyl	50	59	143	30	59	143
Thiofanox	50	71	129	30	67	133
Carbofuran- <sup>13</sup> C <sub>6</sub> (Surrogate)	50	40	164	30	40	164
Methomyl- <sup>13</sup> C <sub>2</sub> , <sup>15</sup> N (Surrogate)	50	90	129	30	84	135