Designation: D7645 – 10

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)[1]

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 reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

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 method. This 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 and health practices and determine the applicability of regulatory limitations prior to use.*

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

2. Referenced Documents

2.1 *ASTM Standards:*[2]

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 Good Laboratory Practices in Laboratories Engaged in Sampling and 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 in a Single Laboratory Using a Control Sample Program

2.2 *Other Documents:*[3]

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*-Methylcarbamoyloximes and *N*-Methylcarbamates in Water by Direct Aqueous Injection HPLC with Postcolumn Derivatization

[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.

Current edition approved June 1, 2010. Published July 2010. DOI: 10.1520/D7645-10.

[2] 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.

[3] 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.

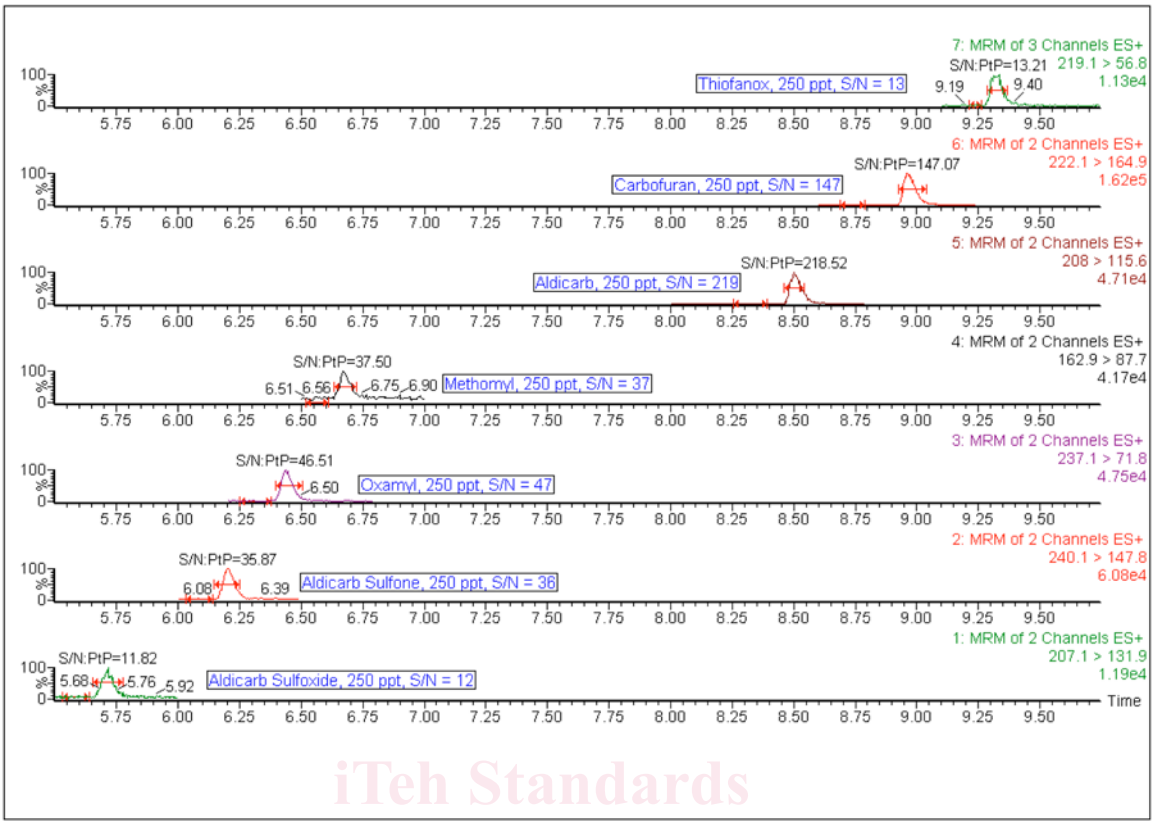

FIG. 1 Example Primary SRM Chromatograms Signal/Noise Ratios

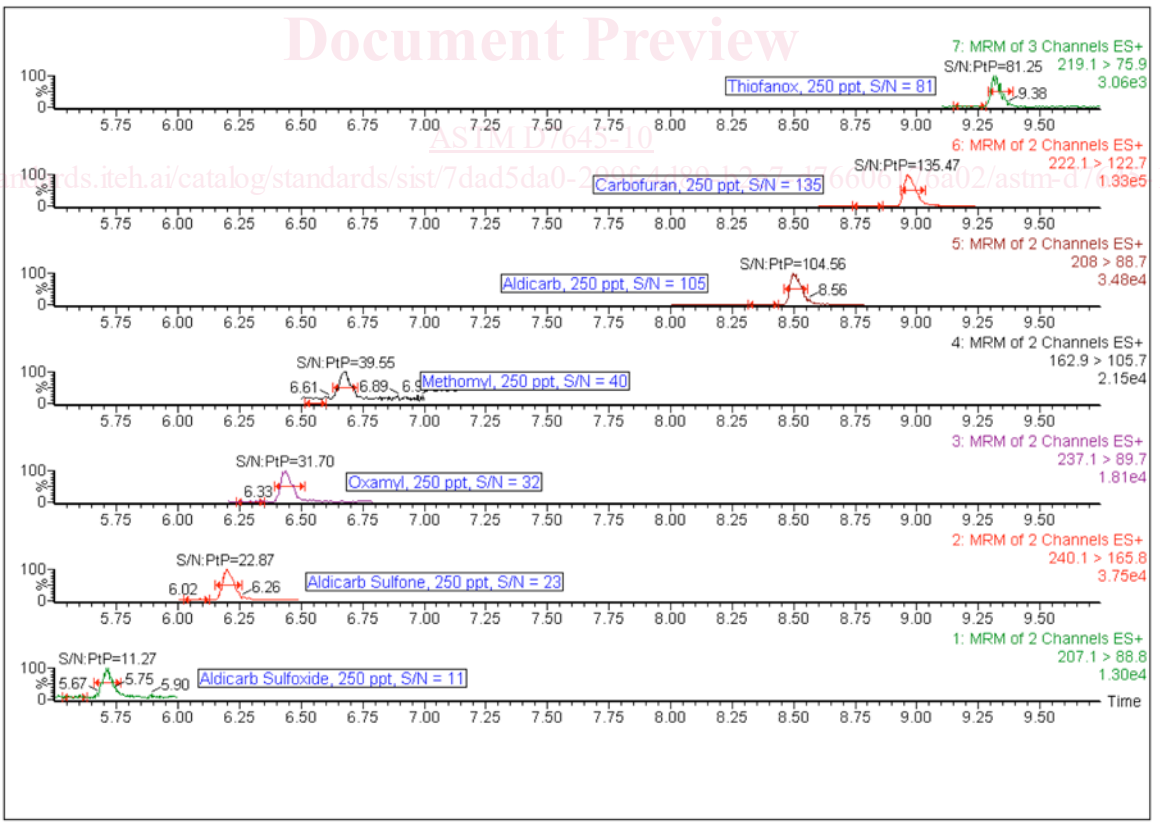

FIG. 2 Example Confirmatory SRM Chromatograms Signal/Noise Ratios

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-$^{13}C_6$ (Surrogate)	1	5	10	25	35	50	75	100
Methomyl-$^{13}C_2$,^{15}N (Surrogate)	1	5	10	25	35	50	75	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 *carbamates*, *n*—in this test method, aldicarb, aldicarb sulfone, aldicarb sulfoxide, carbofuran, methomyl, oxamyl, and thiofanox collectively.

3.1.2 *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).

3.1.3 *reporting limit, RL*, *n*—the concentration of the lowest-level calibration standard used for quantification.

3.2 *Abbreviations:*

3.2.1 *mM*—millimolar, 1×10^{-3} moles/L

3.2.2 *ND*—non-detect

3.2.3 *ppt*—parts per trillion, ng/L

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 0°C 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-$^{13}C_2$,^{15}N (surrogate) and carbofuran-$^{13}C_6$ (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 in support of the National Homeland Security Research Center, US EPA by 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.[4]

5.3 This 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.

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 to 30 minutes. 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 in order to analyze samples.[5] Any 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–Waters*—ACQUITY UPLC® BEH C18, 2.1 × 100 mm, 1.7 µm particle size was used to develop

[4] Additional information about Carbamate pesticides can be found on the Internet at http://www.epa.gov (2010).

[5] A Waters ACQUITY UltraPerformance Liquid Chromatography (UPLC®) System was used to develop this test method. All parameters in this test method are based on this system and may vary depending on your instrument.

this test method. Any column that achieves adequate resolution may be used. The retention times and order of elution may change depending on the column used and need to be monitored.

NOTE 1—Any column that can achieve baseline resolution of these analytes may be used. Baseline resolution simplifies data analysis and can reduce the chance of ion suppression, leading to higher limits of detection.

7.1.3 *Tandem Mass Spectrometer (MS/MS) System*—A MS/MS system capable of MRM analysis.[6] Any system that is capable of performing at the requirements in this standard may be used.

7.2 *Filtration Device*:

7.2.1 *Hypodermic Syringe*—A Lock Tip Glass Syringe capable of holding a Millex® HV Syringe Driven Filter Unit PVDF 0.22 µm or similar may be used.

7.2.1.1 A 25 mL Lock Tip Glass Syringe size is recommended since a 25 mL sample size is used in this test method.

7.2.2 *Filter*—Millex® HV Syringe Driven Filter Unit PVDF 0.22 µm (Millipore Corporation, Catalog # SLGV033NS) or similar may be used.

8. Reagents and Materials

8.1 *Purity of Reagents*—High Performance Liquid Chromatography (HPLC) pesticide residue analysis and spectrophotometry grade chemicals shall be used in all tests. Unless indicated otherwise, it is intended that all reagents shall conform to the Committee on Analytical Reagents of the American Chemical Society.[7] 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 shall 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-$^{13}C_2$,^{15}N (acetohydroxamate-$^{13}C_2$,^{15}N, CAS # (unlabeled) 16752-77-5).[8]

8.17 Carbofuran-$^{13}C_6$ (Ring-$^{13}C_6$, CAS # (unlabeled) 1563-66-2).[8]

9. Hazards

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

10. Sampling

10.1 *Sampling and Preservation*—Grab samples should be collected in ≥25 mL pre-cleaned amber glass bottles with Teflon® lined caps demonstrated to be free of interferences. All samples are acidified with glacial acetic acid to pH ≤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 0°C and 6°C from the time of collection until analysis. Analyze the sample within 14 days of collection.

NOTE 2—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 ~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 pH 3 ±0.2. Holding time is dependent upon your individual matrix and will vary. Practice D4841 may be used to conduct a holding time study on your individual matrix.

11. Preparation of LC/MS/MS

11.1 *LC Chromatograph Operating Conditions:*[5]

11.1.1 Injection volumes of all calibration standards and samples are made at 50 µL volume using a full loop injection. If a 50 µL 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.

[6] A Waters Quattro Premier™ XE tandem quadrupole mass spectrometer was used to develop this test method. All parameters in this test method are based on this system and may vary depending on your instrument.

[7] Reagent Chemicals, American Chemical Society Specifications, American Chemical Society, Washington, D.C. For Suggestions on the testing of reagents not listed by the American Chemical Society, see Annual Standards for Laboratory Chemicals, BDH Ltd., Poole, Dorset, U.K., and the United States Pharmacopeia and National Formulators, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

[8] A source for the labeled carbamates is Cambridge Isotope Laboratories, 50 Frontage Road, Andover, MA 01810-5413.

TABLE 3 Gradient Conditions for Liquid Chromatography

Time (min)	Flow (µL/min)	Percent 95% Water/ 5% Methanol, 5 mM NH_4CO_2H	Percent 95% Methanol/ 5% Water, 5 mM NH_4CO_2H
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

NOTE 3—If your instrument does not have a 50 µL injection capability a different volume may be used. This is a performance-based method and modifications are allowed as long as minimum performance criteria are met.

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.

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

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

11.3 *Mass Spectrometer Parameters:*[6]

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 your instrument. Each peak requires at least 10 scans per peak for adequate quantitation. This standard 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 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/hr
Cone Gas Flow: 25 L/hr
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: 7.0×10^{-3} Torr
Inter-Channel Delay: 0.005 seconds
Inter-Scan Delay: 0.005 seconds
Dwell: 0.075 seconds

12. Calibration and Standardization

12.1 The mass spectrometer must be calibrated per 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-$^{13}C_2,^{15}N$ and carbofuran-$^{13}C_6$ 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-$^{13}C_2,^{15}N$ in methanol and carbofuran-$^{13}C_6$ in 1,4-dioxane[9] 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 hours 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.

[9] Carbofuran-$^{13}C_6$ is purchased as a solution in 1,4-dioxane from Cambridge Isotope Laboratories.

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-$^{13}C_6$ (Surrogate)	Primary	8.96	22	11	228.1 > 170.9	1.3
	Confirmatory		22	21	228.1 > 128.8	
Methomyl-$^{13}C_2$,^{15}N (Surrogate)	Primary	6.68	18	8	165.8 > 90.7	1.7
	Confirmatory		18	9	165.8 > 108.7	

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.

12.2.5 Linear calibration may be used if the coefficient of determination, r^2, is >0.98 for the analyte. 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. If one of the calibration standards other than the high or low point causes the r^2 of the curve to be <0.98, this point must be re-injected or a new calibration curve must be regenerated. If the low and/or high point is excluded, minimally a six point curve is acceptable but the reporting range must be modified to reflect this change.

12.2.6 Quadratic calibration may be used if the coefficient of determination, r^2, is >0.99 for the analyte. 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. If one of the calibration standards causes the curve to be <0.99, this point must be re-injected or a new calibration curve must be regenerated. Minimally a six point curve is acceptable using a quadratic fit. Each calibration point used to generate the curve must have a calculated percent deviation less than 25% from the generated curve.

12.2.6.1 An initial eight point curve over the calibration range is an option in the event that the low and/or high point must be excluded to obtain a coefficient of determination >0.99. In this event, the reporting range must be modified to reflect this change.

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 hours after the initial calibration curve was generated. This end 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 8 point calibration curve was used to set the QC acceptance criteria in this 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