Designation: D7600 – 09$^{\varepsilon 2}$

StandardTest Method for Determination of Aldicarb, Carbofuran, Oxamyl and Methomyl by Liquid Chromatography/Tandem Mass Spectrometry[1]

This standard is issued under the fixed designation D7600; 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—This test method was changed editorially in February 2012.
ε[2] NOTE—Table 1 was editorially corrected in May 2013.

1. Scope

1.1 This procedure covers the determination of aldicarb, carbofuran, oxamyl and methomyl (referred to collectively as carbamates in this test method) in surface 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 This test method has been developed by US EPA Region 5 Chicago Regional Laboratory (CRL).

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

1.4 The Detection Verification Level (DVL) and Reporting Range for the carbamates are listed in Table 1.

1.4.1 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.

1.4.2 The reporting limit is the concentration of the Level 1 calibration standard as shown in Table 2 for the carbamates.

1.5 *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.*

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
D3856 Guide for Management Systems in Laboratories Engaged in Analysis of Water
D3694 Practices for Preparation of Sample Containers and for Preservation of Organic 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:*

EPA publication SW-846 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods[3]

3. Terminology

3.1 *Definitions:*

3.1.1 *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).

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

3.1.3 *carbamates, n*—in this test method, aldicarb, carbofuran, oxamyl and methomyl collectively.

3.2 *Abbreviations:*

3.2.1 *ppt*—parts per trillion, ng/L

[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 Dec. 1, 2009. Published January 2010. DOI: 10.1520/D7600-09E02.

[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.

TABLE 1 Detection Verification Level and Reporting Range

Analyte	DVL (ng/L)†	Reporting Range (µg/L)
Aldicarb	100	1–100
Carbofuran	100	1–100
Oxamyl	100	1–100
Methomyl	100	1–100

† Measurement editorially corrected.

3.2.2 *ND*—non-detect

4. Summary of Test Methods

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 between 0°C and 6°C and analyzed within 7 days of collection. In the lab, the samples are spiked with surrogate, filtered using a syringe driven Millex HV PVDF filter unit and analyzed directly by LC/MS/MS.

4.3 Aldicarb, carbofuran, oxamyl, methomyl and 4-bromo-3,5-dimethylphenyl-*N*-methylcarbamate (BDMC, 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 aldicarb, carbofuran, oxamyl, methomyl and the BDMC surrogate recovery.

5. Significance and Use

5.1 The *N*-methyl carbamate (NMC) pesticides: aldicarb, carbaryl, carbofuran, formetanate hydrochloride, methiocarb, methomyl, oxamyl, pirimicarb, propoxur, and thiodicarb have been identified by EPA as working through a common mechanism. They affect the nervous system by reducing the ability of the enzyme cholinesterase. Cholinesterase 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.[4]

5.2 This method has been investigated for use with reagent and surface water for the selected carbamates: aldicarb, carbofuran, oxamyl and methomyl.

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 a detergent, 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, then 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 that are co-extracted from 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] 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-Waters*—XBridge C18, 150 mm × 2.1 mm, 3.5 µm particle size, or equivalent.

7.1.3 *Tandem Mass Spectrometer (MS/MS) System*—A MS/MS system capable of MRM analysis.[6] A 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 0.45 µm (Millipore Corporation, Catalog # SLHV033NS) 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 0.45 µm (Millipore Corporation, Catalog # SLHV033NS) 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.

[4] Additional information about carbamate pesticides can be found on the Internet at http://www.epa.gov (2009).

[5] A Waters Alliance High Performance Liquid Chromatography (HPLC) System was used to develop this test method. The multi-laboratory study included Agilent, Thermo Electron and Waters LC systems.

[6] A Waters Quattro micro API mass spectrometer was used to develop this test method. The multi-laboratory study included Agilent, Applied Biosystems, Thermo Electron, Varian and Waters mass spectrometers.

[7] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. 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 Formulary,* U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

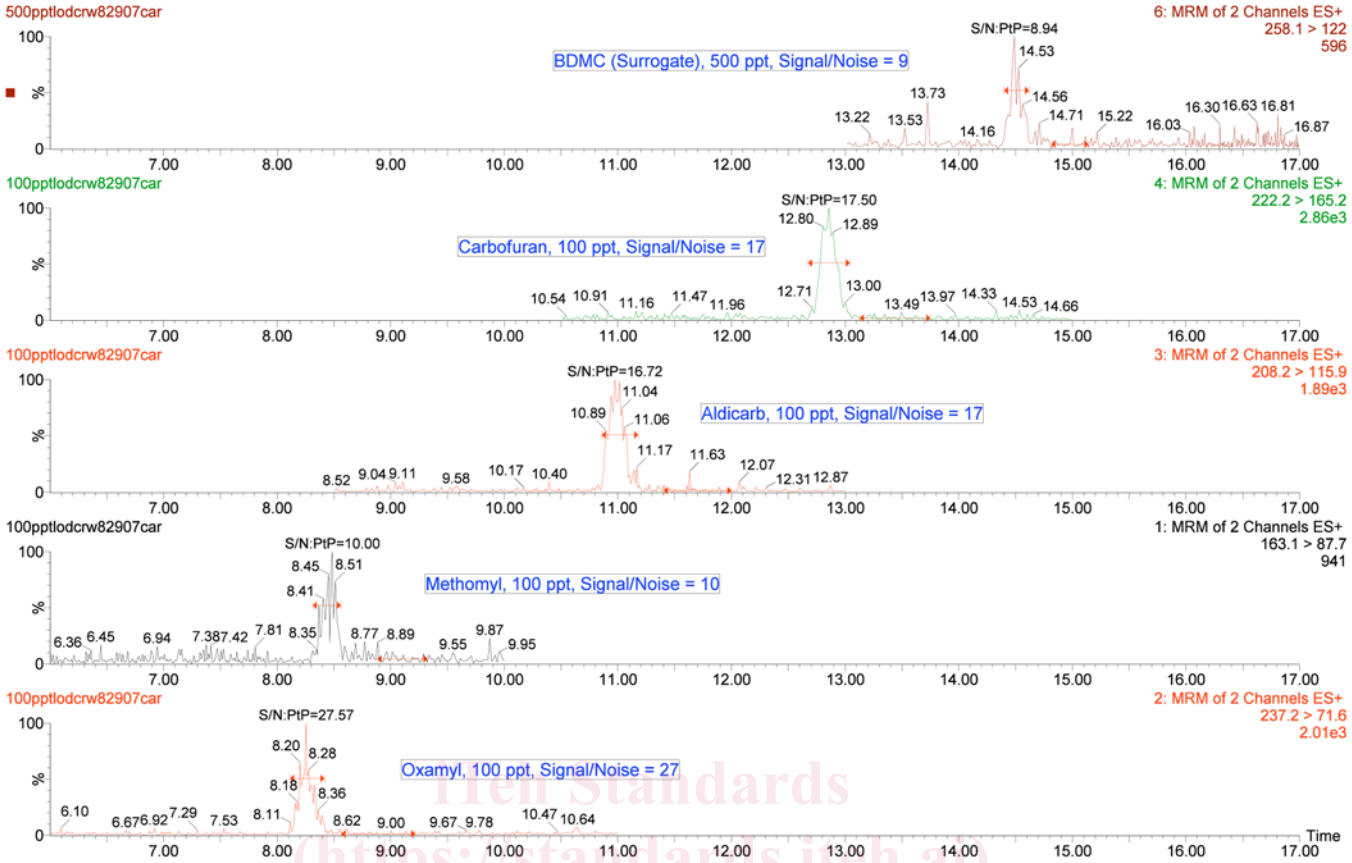

FIG. 1 Example Primary SRM Chromatograms Signal/Noise Ratios

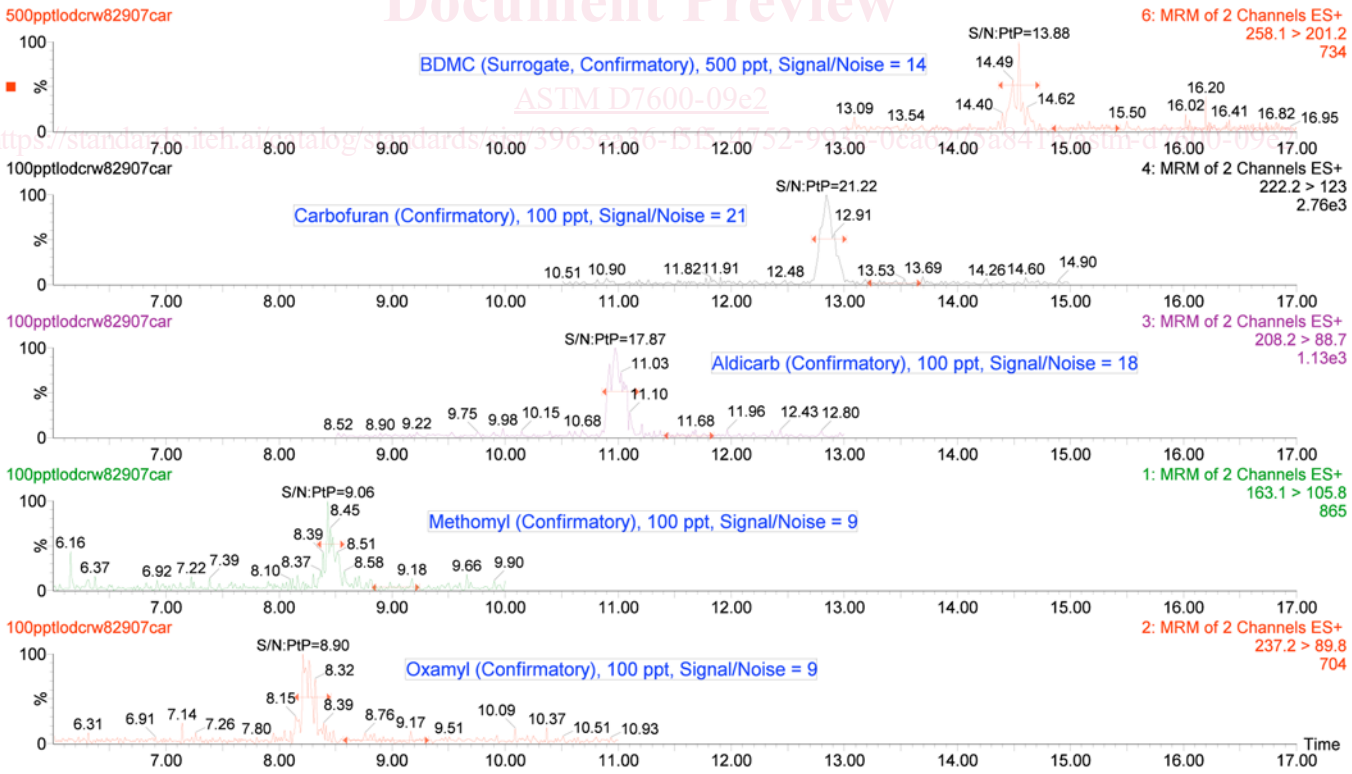

FIG. 2 Example Confirmatory SRM Chromatograms Signal/Noise Ratios

TABLE 2 Concentrations of Calibration Standards (PPB)

Analyte/Surrogate	LV 1	LV 2	LV 3	LV 4	LV 5	LV 6
Aldicarb	1	5	10	25	50	100
Carbofuran	1	5	10	25	50	100
Oxamyl	1	5	10	25	50	100
Methomyl	1	5	10	25	50	100
BDMC (Surrogate)	2	10	20	50	100	200

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 acetate (CAS # 631-61-8).

8.8 Ammonium hydroxide (Concentrated, CAS # 1336-21-6).

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

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

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

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

8.13 4-Bromo-3,5-dimethylphenyl-N-methylcarbamate (BDMC, CAS # 672-99-1).

8.13.1 BDMC is used as a surrogate in this standard.

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*—Grab samples must be collected in ≥25-mL pre-cleaned amber glass bottles with Teflon-lined caps demonstrated to be free of interferences. This test method requires a 25-mL sample size per analysis. Conventional sampling practices should be followed. Refer to Guide D3856 and Practices D3694.

10.2 *Preservation*—Store samples between 0°C and 6°C from the time of collection until analysis. Analyze the sample within 7 days of collection.

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 100 µL. 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.

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

TABLE 3 Gradient Conditions for Liquid Chromatography

Time (min)	Flow (µL/min)	Percent CH_3CN	Percent 95 % Water/ 5 % CH_3CN	Percent 50 mmolar NH_4OAc/NH_4OH in 95 % Water/5 % CH_3CN
0	300	0	95	5
2	300	0	95	5
4	300	30	65	5
6	300	35	60	5
8	300	35	60	5
10	300	75	20	5
11.5	300	75	20	5
12	300	95	0	5
18	300	95	0	5
20	300	0	95	5
23	300	0	95	5

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

11.1.4 *Needle Wash*—Solvent: 50 % Acetonitrile/50 % Water; Normal wash, approximately 13 second wash time.

11.1.5 *Autosampler Purge*—Three loop volumes.

11.1.6 Specific instrument manufacturer wash/purge specifications should be followed in order to eliminate sample carry-over in the analysis of carbamates.

11.2 *Mass Spectrometer Parameters*[6]:

11.2.1 In order 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 only one surrogate and four target compounds. The MRM experiment windows were set to acquire methomyl and oxamyl in one experiment window while aldicarb, carbofuran and BDMC are in their individual MRM experiment windows. This is required because the chromatographic resolution separating oxamyl and methomyl was not achieved. Variable parameters regarding retention times, SRM Transitions and cone and collision energies are shown in Table 4.

The instrument is set in the Electrospray (+) positive setting.
Capillary Voltage: 3.5 kV
Cone: Variable depending on analyte (Table 4)
Extractor: 2 Volts
RF Lens: 0.2 Volts
Source Temperature: 120°C
Desolvation Temperature: 300°C
Desolvation Gas Flow: 500 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: 2
Low Mass Resolution 2: 15
High Mass resolution 2: 15
Ion Energy 2: 0.5
Multiplier: 650
Gas Cell Pirani Gauge: 3.3×10^{-3} Torr
Inter-Channel Delay: 0.02 seconds
Inter-Scan Delay: 0.1 seconds
Repeats: 1
Span: 0 Daltons
Dwell: 0.1 Seconds

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)	Collision Energy (eV)
Aldicarb	Primary	11.00	10	7	208.2 > 115.9	2.12
	Confirmatory		10	15	208.2 > 88.7	
Carbofuran	Primary	12.85	27	12	222.2 > 165.2	1.20
	Confirmatory		27	20	222.2 > 123	
Oxamyl	Primary	8.25	15	8	237.2 > 71.6	2.38
	Confirmatory		15	8	237.2 > 89.8	
Methomyl	Primary	8.45	17	8	163.1 > 87.7	1.58
	Confirmatory		17	8	163.1 > 105.8	
BDMC (Surrogate)	Primary	14.50	25	24	258.1 > 122	1.31
	Confirmatory		25	9	258.1 > 201.2	

12. Calibration and Standardization

12.1 The mass spectrometer must be calibrated per manufacturer specifications before analysis. In order that analytical values obtained using this test method are valid and accurate within the confidence limits of the test method, the following procedures must be followed when performing the test method.

12.2 *Calibration and Standardization*—To calibrate the instrument, analyze six calibration standards containing the six concentration levels of the carbamates and BDMC surrogate prior to analysis as shown in Table 2. A calibration stock standard solution is prepared from standard materials or purchased as certified solutions. Stock standard solution A (Level 6) containing aldicarb, carbofuran, oxamyl, methomyl and BDMC is prepared at Level 6 concentration and aliquots of that solution are diluted to prepare Levels 1 through 5. 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 6) by adding to a 100-mL volumetric flask individual methanol solutions of the following: 50 µL of aldicarb, carbofuran, oxamyl and methomyl each at 0.2 g/L and 50 µL of BDMC at 0.4 g/L, dilute to 100 mL with 90% water/10% methanol. The preparation of the Level 6 standard can be accomplished using different volumes and concentrations of stock solutions as is accustomed in the individual laboratory. Depending on stock concentrations prepared, the solubility at that concentration will have to be ensured.

12.2.2 Aliquots of Solution A are then diluted with 90 % water/10 % methanol 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 a chromatogram for each one. An external calibration technique is used monitoring the primary and confirmatory SRM transition of each analyte. Calibration software is utilized to conduct the quantitation of the target analytes and surrogate using the primary SRM transition. The ratios of the primary/confirmatory SRM transition area counts are given in Table 4. These are given as informative and will vary depending on the individual tuning conditions. The primary/confirmatory SRM transition area ratio must be within 30 % 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, fragmenting it into two product ions, 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 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 or high point is excluded, minimally a five-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 seven-point curve over the calibration range is an option in the event that the low 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 surrogate. 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 surrogate the results may be reported unqualified.

12.3 All samples are prepared using Class A glass volumetric glassware. The sample volume used throughout this test method is 25 mL. Every sample, the entire 25 mL volume, is filtered through the filtration device described in Section 7.2 only after all required spiking solutions are added and mixed throughout the sample.

12.3.1 A new filter unit is used for each sample. The syringe must be cleaned between each filtration. It is the analyst's responsibility to ensure that the syringe is clean. A possible way of cleaning the syringe between filtrations is first by rinsing with at least 5 syringe volumes of water, followed by at least 3 volumes of acetone, then 3 volumes of methanol and finally rinsed with water to remove any residual solvent.

12.4 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., perform a precision and bias study to demonstrate laboratory capability.

12.4.1 Analyze at least four replicates of a sample solution containing aldicarb, carbofuran, oxamyl, methomyl and BDMC at a concentration in the calibration range of Levels 3 to 5. The Level 4 concentration of the 6 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.

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

12.4.3 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 Test Method D5847 for information on applying the F test and t test in evaluating the acceptability of the mean and standard deviation.

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	25	65	135	14	63	136
Carbofuran	25	66	132	15	64	134
Oxamyl	25	76	114	29	61	128
Methomyl	25	82	125	16	77	129
BDMC (Surrogate)	50	59	139	41	42	156

12.4.3.1 The QC acceptance criteria for the initial demonstration of performance in Table 5 were generated from a multi-laboratory method validation involving eight laboratories. The descriptive statistics from this validation are shown in the Precision and Bias Section. The analyst must be aware that the performance data generated from multiple-laboratory data tend to be significantly wider than those generated from single-laboratory data. 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 ASTM Standards D2777, D5847, E2554 or Method 8000B in EPA publication SW-846, which may be helpful.

12.5 *Surrogate Spiking Solution :*

12.5.1 A surrogate standard solution containing BDMC is added to all samples. A stock surrogate spiking solution is prepared in methanol 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.

12.6 *Method Blank:*

12.6.1 Analyze a reagent water blank with each batch of 20 or fewer samples. The concentration of the carbamates found in the blank must be below the DVL. If the concentrations of the carbamates are found above this level, analysis of samples is halted until the contamination is eliminated and a blank shows no contamination at or above this level, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.7 *Laboratory Control Sample (LCS):*

12.7.1 To ensure that the test method is in control, analyze a LCS prepared with aldicarb, carbofuran, oxamyl and methomyl at a concentration in the calibration range of Levels 3 to 5. The LCS is prepared following the analytical method and analyzed with each batch of 20 samples or less. Prepare a stock matrix spiking solution in methanol containing aldicarb, carbofuran, oxamyl and methomyl each at 25 ppm. Spike 25 µL of this stock solution into 25 mL of water to yield a