Designation: ~~D7600 – 09ε3~~ D7600 – 16

Standard Test 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.~~
~~ε[3] NOTE—Added research report footnote to Section 16 editorially in June 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 test method. This test 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 Using Control Chart Techniques

2.2 *Other Documents:*[3]
EPA publication SW-846 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods

3. Terminology

3.1 *Definitions:*

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

[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] 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)~~
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 ~~*Definitions:*~~*Definitions of Terms Specific to This Standard:*

3.2.1 *detection verification* ~~*level (DVL),*~~*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.~~

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

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 shall be obtained from a different lot of material than is used for calibration

3.3 ~~*Abbreviations:*~~*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 *MDL, n*—Method Detection Limit

3.3.6 *MeOH, n*—Methanol

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

3.3.8 *MRM, n*—Multiple Reaction Monitoring

3.3.9 *MS/MSD, n*—Matrix Spike/Matrix Spike Duplicate

3.3.10 *NA, adj*—Not Available

3.3.11 *ND, n*—non-detect

3.3.12 *P&A, n*—Precision and Accuracy

3.3.13 ~~*ppt*—~~*PPB, n*—parts per ~~trillion, ng/L~~billion

3.3.14 ~~*ND*—~~*PPT, n*—~~non-detect~~parts per trillion

3.3.15 *QA, adj*—Quality Assurance

3.3.16 *QC, adj*—Quality Control

3.3.17 *RL, n*—Reporting Limit

3.3.18 *RSD, n*—Relative Standard Deviation

3.3.19 *RT, n*—Retention Time

3.3.20 *SDS, n*—Safety Data Sheets

3.3.21 *SRM, n*—Single Reaction Monitoring

3.3.22 *SS, n*—Surrogate Standard

3.3.23 *TC, n*—Target Compound

3.3.24 *µM, n*—micromolar, 1×10^{-6} moles/L

3.3.25 *VOA, n*—Volatile Organic Analysis

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~~ syringe-driven filter unit and analyzed directly by LC/MS/MS.

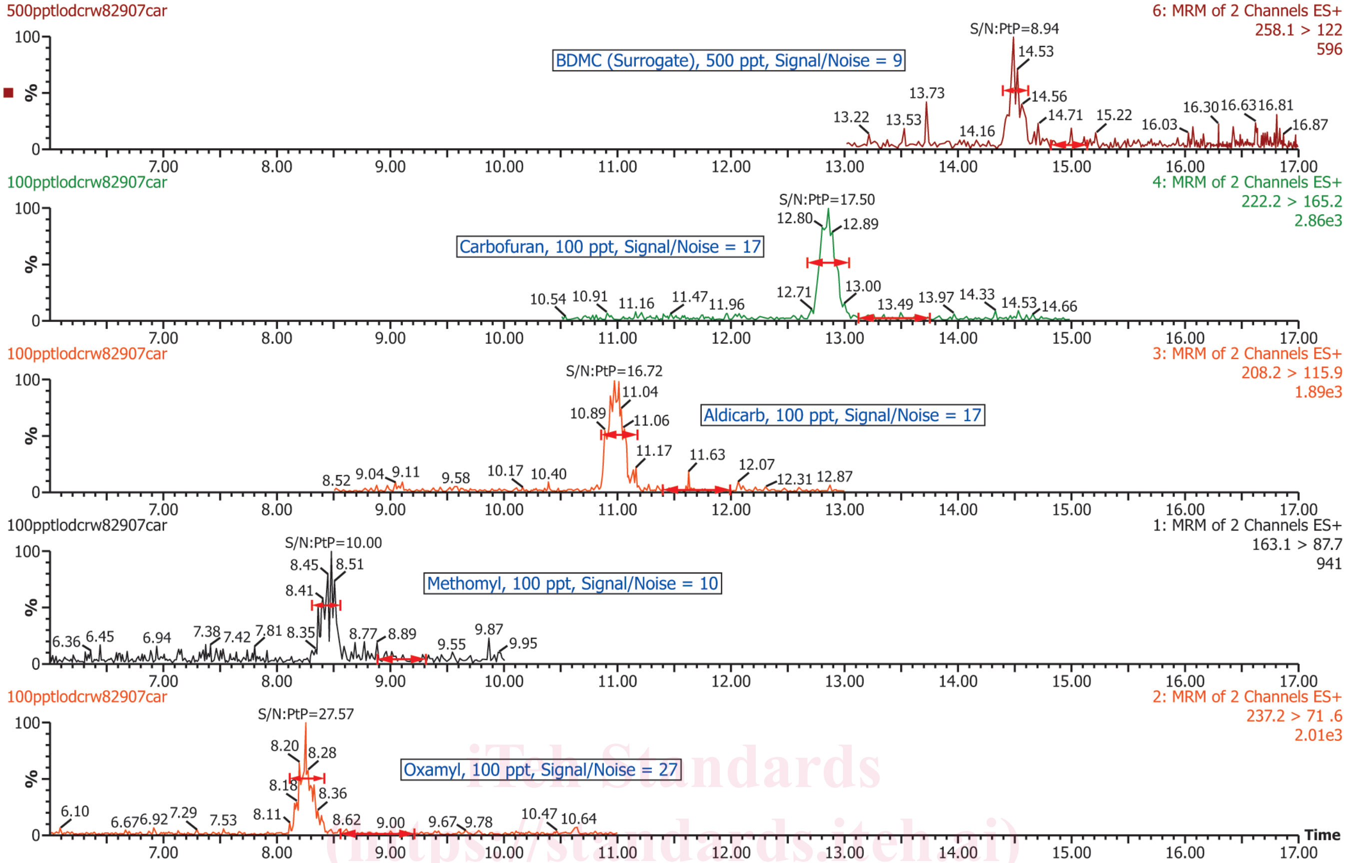
500pptlodcrw82907car
6: MRM of 2 Channels ES+
258.1 > 122
596
S/N:PtP=8.94
BDMC (Surrogate), 500 ppt, Signal/Noise = 9
14.53 13.73 14.56 13.22 13.53 14.16 14.71 15.22 16.03 16.30 16.63 16.81 16.87
100pptlodcrw82907car
4: MRM of 2 Channels ES+
222.2 > 165.2
2.86e3
S/N:PtP=17.50
Carbofuran, 100 ppt, Signal/Noise = 17
12.80 12.89 10.54 10.91 11.16 11.47 11.96 12.71 13.00 13.49 13.97 14.33 14.53 14.66
100pptlodcrw82907car
3: MRM of 2 Channels ES+
208.2 > 115.9
1.89e3
S/N:PtP=16.72
Aldicarb, 100 ppt, Signal/Noise = 17
11.04 10.89 11.06 11.17 11.63 12.07 8.52 9.04 9.11 9.58 10.17 10.40 12.31 12.87
100pptlodcrw82907car
1: MRM of 2 Channels ES+
163.1 > 87.7
941
S/N:PtP=10.00
Methomyl, 100 ppt, Signal/Noise = 10
8.45 8.51 8.41 6.36 6.45 6.94 7.38 7.42 7.81 8.35 8.77 8.89 9.55 9.87 9.95
100pptlodcrw82907car
2: MRM of 2 Channels ES+
237.2 > 71 .6
2.01e3
S/N:PtP=27.57
Oxamyl, 100 ppt, Signal/Noise = 27
8.20 8.28 8.18 8.36 6.10 6.67 6.92 7.29 7.53 8.11 8.62 9.00 9.67 9.78 10.47 10.64
100 % 0
7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00
Time

FIG. 1 Example Primary SRM Chromatograms Signal/Noise Ratios

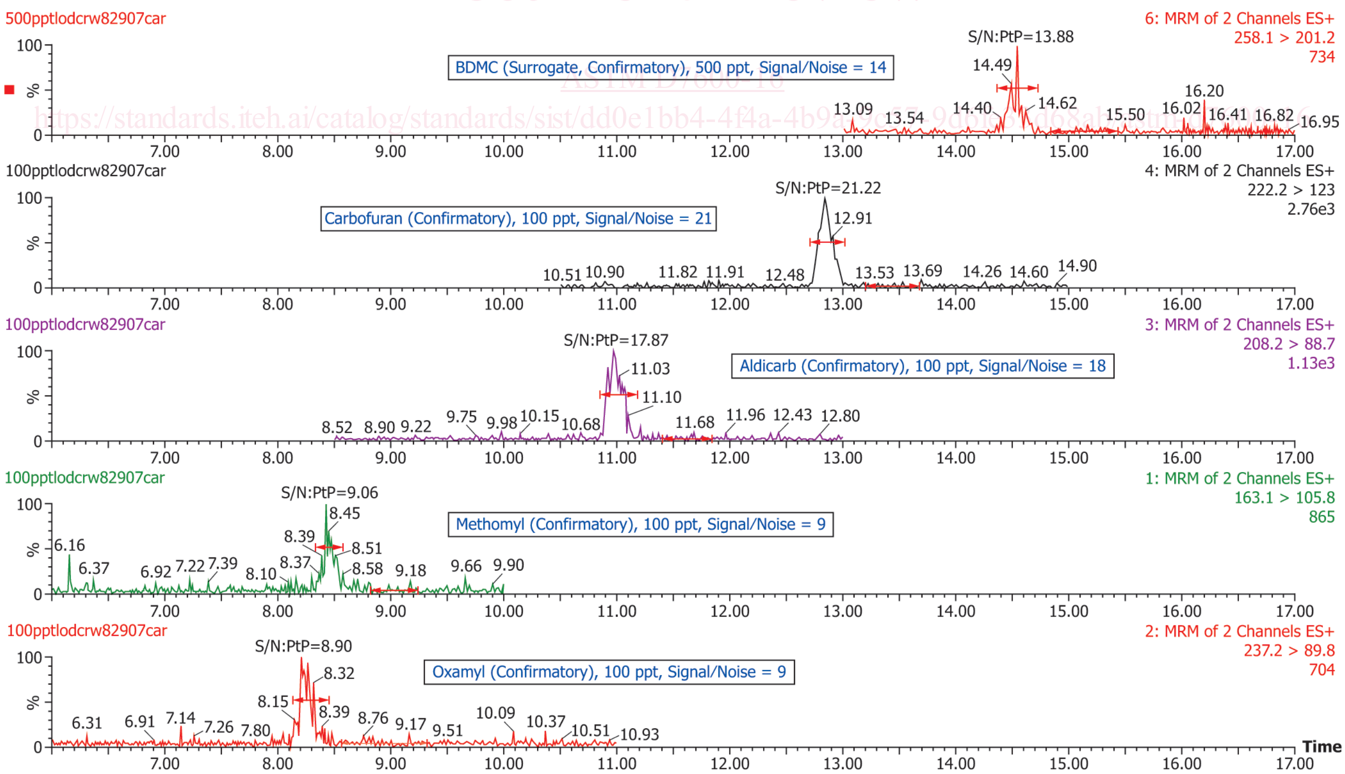
500pptlodcrw82907car
6: MRM of 2 Channels ES+
258.1 > 201.2
734
S/N:PtP=13.88
BDMC (Surrogate, Confirmatory), 500 ppt, Signal/Noise = 14
14.49 14.62 13.09 13.54 14.40 15.50 16.02 16.20 16.41 16.82 16.95
100pptlodcrw82907car
4: MRM of 2 Channels ES+
222.2 > 123
2.76e3
S/N:PtP=21.22
Carbofuran (Confirmatory), 100 ppt, Signal/Noise = 21
12.91 10.51 10.90 11.82 11.91 12.48 13.53 13.69 14.26 14.60 14.90
100pptlodcrw82907car
3: MRM of 2 Channels ES+
208.2 > 88.7
1.13e3
S/N:PtP=17.87
Aldicarb (Confirmatory), 100 ppt, Signal/Noise = 18
11.03 11.10 8.52 8.90 9.22 9.75 9.98 10.15 10.68 11.68 11.96 12.43 12.80
100pptlodcrw82907car
1: MRM of 2 Channels ES+
163.1 > 105.8
865
S/N:PtP=9.06
Methomyl (Confirmatory), 100 ppt, Signal/Noise = 9
8.45 8.39 8.51 6.16 6.37 6.92 7.22 7.39 8.10 8.37 8.58 9.18 9.66 9.90
100pptlodcrw82907car
2: MRM of 2 Channels ES+
237.2 > 89.8
704
S/N:PtP=8.90
Oxamyl (Confirmatory), 100 ppt, Signal/Noise = 9
8.32 8.15 8.39 6.31 6.91 7.14 7.26 7.80 8.76 9.17 9.51 10.09 10.37 10.51 10.93
100 % 0
7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00
Time

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

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 test 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~~System:*

7.1.1 *Liquid Chromatography (LC) System*—A complete LC system is needed in order to analyze samples.[5] 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-Waters—~~Column*[6] ~~—XBridge C18, 150 mm × 2.1 mm, 3.5 µm particle size, or equivalent.~~A C18 column was used to develop 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 that is used and need to be monitored.

7.1.3 *Tandem Mass Spectrometer (MS/MS) System*—A MS/MS system capable of MRM analysis.[7] A system that is capable of performing at the requirements in this standard may be used.

7.2 *Filtration ~~Device~~Device:*

7.2.1 *Hypodermic syringe*—A ~~lock~~luer-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.~~syringe-driven filter unit.

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.

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

[5] A Waters Alliance High Performance Liquid Chromatography (HPLC) System ~~was used to develop this test method.~~ (a trademark of the Waters Corporation, Milford, MA), or equivalent, was found suitable for use. The multi-laboratory study included ~~Agilent, Thermo Electron~~ Agilent and Waters LC systems.

[6] A Waters (a trademark of the Waters Corporation, Milford, MA) XBridge C18, 150 mm × 2.1 mm, 3.5 µm particle size, or equivalent, has been found suitable for use.

[7] A Waters Quattro micro API mass spectrometer ~~was used to develop this test method.~~ (a trademark of the Waters Corporation, Milford, MA), or equivalent, was found suitable for use. The multi-laboratory study included ~~Agilent, Applied Biosystems, Thermo Electron, Varian~~ Applied Biosystems and Waters mass spectrometers.

7.2.2 ~~*Filter—*~~*Filter unit*[8] ~~—Millex HV Syringe Driven Filter Unit PVDF 0.45 µm (Millipore Corporation, Catalog # SLHV033NS) or similar may be used.~~PVDF filter units were used to filter the samples.

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.[9] 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 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)~~(SDS) for all reagents used in this test 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~~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.

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~~Parameters:*[7]~~:~~

[8] A Millex HV Syringe Driven Filter Unit PVDF 0.45 µm (Millipore Corporation, Catalog # SLHV033NS; a trademark of the Waters Corporation, Milford, MA) has been found suitable for use for this test method, any filter unit may be used that meets the performance of this test method may be used.

[9] *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.

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

11.2.1 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	