**Designation: ~~D8025 – 16~~ D8025 – 23**

# Standard Test Method for Determination of Select Pesticides in Water by Multiple Reaction Monitoring Liquid Chromatography Tandem Mass Spectrometry[1]

This standard is issued under the fixed designation D8025; 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 ~~procedure~~ test method covers a method for analysis of selected pesticides in a water matrix by filtration followed with liquid chromatography/electrospray ionization tandem mass spectrometry analysis. The samples are prepared in ~~20 %~~ 20 % methanol, filtered, and analyzed by liquid chromatography/tandem mass spectrometry. This method was developed for an agricultural run-off study, not for low level analysis of pesticides in drinking water. This method may be modified for lower level analysis. The analytes are qualitatively and quantitatively determined by this method. This method adheres to multiple reaction monitoring (MRM) mass spectrometry.

1.2 A full collaborative study to meet the requirements of Practice D2777 has not been completed. This standard contains single-operator precision and bias based on single-operator data. Publication of standards that have not been fully validated is done to make the current technology accessible to users of standards, and to solicit additional input from the user community.

1.3 A reporting limit check sample (RLCS) is analyzed during every batch to ensure that if an analyte was present in a sample at or near the reporting limit it would be positively identified and accurately quantitated within set quality control limits. A method detection limit (MDL) study was not done for this method, the method detection limits would be much lower than the reporting limits in this method and would be irrelevant. A RLCS was determined to be more applicable for this standard. If this method is adapted to report much lower or near the MDL then a MDL study would be warranted.

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

1.5 The Reporting Range for the target analytes are listed in Table 1.

1.5.1 The reporting limit in this test method is the minimum value below which data are documented as non-detects. The reporting limit is calculated from the concentration of the Level 1 calibration standard as shown in Table 6 after taking into account an 8 mL water sample volume and a final diluted sample volume of 10 mL (80 % water/20 % methanol).

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

[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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**TABLE 1 Reporting Range**

| Analyte | Reporting Ranges, (ng/L) |
|---|---|
| 2,4-D | 250–10 000 |
| Acetochlor | 250–10 000 |
| Alachlor | 250–10 000 |
| Aldicarb | 250–10 000 |
| Atrazine | 62.5–2 500 |
| Desethylatrazine | 62.5–2 500 |
| Desisopropylatrazine | 125–5 000 |
| Azoxystrobin | 31.2–1 250 |
| Bentazon | 250–10 000 |
| Carbaryl | 250–10 000 |
| Chlorpyrifos | 250–10 000 |
| Clopyralid | 25 000–1 000 000 |
| Clothianidin | 62.5–2 500 |
| Diazinon | 62.5–2 500 |
| Dicamba | 12 500–500 000 |
| Fipronil | 250–10 000 |
| Imidacloprid | 62.5–2 500 |
| Malathion | 125–5 000 |
| Methomyl | 250–10 000 |
| Metolachlor | 62.5–2 500 |
| Metribuzin | 125–5 000 |
| Picloram | 6 250–250 000 |
| Propiconazole | 62.5–2 500 |
| Simazine | 62.5–2 500 |
| Tebuconazole | 62.5–2 500 |
| Thiamethoxam | 62.5–2 500 |
| Triclopyr | 1 250–5 000 |

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*



## 2. Referenced Documents

2.1 *ASTM Standards:*[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
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 Document:*

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

## 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 *batch QC, n*—all the quality control samples and standards included in an analytical procedure.

3.2.2 *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.

3.2.2.1 *Discussion*—

The IRM ~~shall~~must be obtained from a different lot of material than is used for calibration.

[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

3.2.3 *reporting limit, RL, n*—the minimum concentration below which data are documented as non-detects.

3.2.4 *reporting limit check sample, RLCS, n*—a sample used to ensure that if the analyte was present at the reporting limit, it would be confidently identified.

3.3 *Acronyms:*

3.3.1 *CCC, n*—Continuing Calibration Check

3.3.2 *CRW, n*—Chicago River Water

3.3.3 *IC, n*—Initial Calibration

3.3.4 *LC, n*—Liquid Chromatography

3.3.5 *LCS/LCSD, n*—Laboratory Control Sample/Laboratory Control Sample Duplicate

3.3.6 *MDL, n*—Method Detection Limit

3.3.7 *MeOH, n*—Methanol

3.3.8 *mM, n*—millimolar, $1 \times 10^{-3}$ moles/L



3.3.9 *MRM, n*—Multiple Reaction Monitoring

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

3.3.11 *NA, adj*—Not Available

3.3.12 *ND, n*—non-detect



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

3.3.14 *ppt, n*—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 *RLCS, n*—Reporting Limit Check Sample

3.3.19 *RSD, n*—Relative Standard Deviation

3.3.20 *RT, n*—Retention Time

3.3.21 *SDS, n*—Safety Data Sheets

3.3.22 *SRM, n*—Single Reaction Monitoring

3.3.23 *SS, n*—Surrogate Standard

3.3.24 *TC, n*—Target Compound

3.3.25 *VOA, n*—Volatile Organic Analysis

## 4. Summary of Test Method

4.1 The operating conditions presented in this standard have been successfully used in the determination of the select pesticides in water; however, this standard is intended to be performance based and alternative operating conditions can be used to perform this method provided data quality objectives are attained.

4.2 For pesticide analysis, samples are shipped to the lab on ice and analyzed within 14 days of collection. A sample (8 mL) is transferred to an amber VOA vial, an isotopically labeled pesticide surrogate mix is added to all samples followed by a pesticide spike solution which is added only to the Reporting Limit Check Samples, Laboratory Control and Matrix Spike samples before the addition of methanol. Then 2 mL of methanol is added to each sample and hand shaken or vortexed for ~~1 minute.~~ 1 min. The samples are then filtered through a PTFE membrane syringe driven filter unit and then analyzed by LC/MS/MS. All concentrations reported only to the reporting limit.

4.3 The analysis of the sample requires two separate analysis methods, one using the LC gradient conditions in Table 2 with the Methanol/Water/Ammonium Formate and the second using the LC gradient conditions in Table 3 with the Methanol/Water/Formic acid. Each analysis set is to be analyzed separately in two different complete sample sequences which includes the exact same samples and may even include the same calibration level standards. The only analytes reported from the formic acid run conditions are 2,4-D, Clopyralid, Dicamba, Picloram, Triclopyr, 2,4-D (Ring-D3) and Dicamba-D3, the rest of the analytes are reported from the ammonium formate analysis run.

4.4 The pesticides are identified by comparing the single reaction monitoring (SRM) transition and its confirmatory SRM transitions if correlated to the known standard SRM transition (Tables 4 and 5) and quantitated utilizing an external calibration. The final report issued for each sample lists the concentration of pesticides, if detected, or RL, if not detected, in ng/L and surrogate recovery.



## 5. Significance and Use

5.1 Pesticides may be used in various agricultural and household products. These products may enter waterways at low levels through run-off or misuse near water resources. Hence, there is a need for quick, easy and robust method to determine pesticide concentration in water matrices for understanding the sources and concentration levels in affected areas.

5.2 This method has been single-laboratory validated in reagent water and surface waters (Tables 12-14).

## 6. Interferences

6.1 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.~~ 250 °C for 15 min to 30 min. All glassware is subsequently rinsed or sonicated, or both, with acetone, n-propanol, acetonitrile, or a combination thereof.

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

**TABLE 2 Gradient Conditions for Neutral Liquid Chromatography**

| Time (min) | Flow (mL/min) | Percent 95 % Water: 5 % Methanol | Percent Methanol | Percent 200 mM Ammonium Formate (95 % Water: 5 % Methanol) |
|---|---|---|---|---|
| 0 | 0.3 | 95 | 0 | 5 |
| 1.5 | 0.3 | 95 | 0 | 5 |
| 9 | 0.3 | 0 | 95 | 5 |
| 12 | 0.3 | 0 | 95 | 5 |
| 13 | 0.3 | 95 | 0 | 5 |
| 16 | 0.3 | 95 | 0 | 5 |

**TABLE 3 Gradient Conditions for Acidic Liquid Chromatography**

| Time (min) | Flow (mL/min) | Percent 95 % Water: 5 % Methanol | Percent Methanol | Percent 4 % Formic Acid (95 % Water: 5 % Methanol) |
|---|---|---|---|---|
| 0 | 0.3 | 95 | 0 | 5 |
| 1.5 | 0.3 | 95 | 0 | 5 |
| 6 | 0.3 | 0 | 95 | 5 |
| 9 | 0.3 | 0 | 95 | 5 |
| 10 | 0.3 | 95 | 0 | 5 |
| 13 | 0.3 | 95 | 0 | 5 |

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

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

| Chemical | Primary/ Confirmatory | MRM Transition | ESI Mode Neg/Pos | Cone (V) | Collision (eV) |
|---|---|---|---|---|---|
| 2,4-D[A] | Primary | 218.9→160.9 | Neg | 20 | 14 |
| | Confirmatory | 220.9→162.9 | | | 14 |
| Acetochlor | Primary | 270.1→224.1 | Pos | 20 | 10 |
| | Confirmatory | 270.1→148 | | | 18 |
| Alachlor | Primary | 270.1→162 | Pos | 30 | 12 |
| | Confirmatory | 270.1→238.1 | | | 18 |
| Aldicarb | Primary | 213→88.9 | Pos | 20 | 16 |
| | Confirmatory | 213→116 | | | 12 |
| Atrazine | Primary | 216.1→174 | Pos | 20 | 18 |
| | Confirmatory | 216.1→95.9 | | | 24 |
| Desethylatrazine | Primary | 188→146 | Pos | 30 | 16 |
| | Confirmatory | 188→78.8 | | | 22 |
| Desisopropylatrazine | Primary | 174→96 | Pos | 30 | 16 |
| | Confirmatory | 174→78.8 | | | 16 |
| Azoxystrobin | Primary | 404.2→372.2 | Pos | 20 | 14 |
| | Confirmatory | 404.2→344.2 | | | 24 |
| Bentazon | Primary | 239→197 | Pos | 20 | 20 |
| | Confirmatory | 239→175 | | | 20 |
| Carbaryl | Primary | 202.1→145 | Pos | 10 | 10 |
| | Confirmatory | 202.1→127 | | | 25 |
| Chlorpyrifos | Primary | 350→197.9 | Pos | 30 | 20 |
| | Confirmatory | 350→322 | | | 11 |
| Clopyralid[A] | Primary | 189.9→145.9 | Neg | 5 | 10 |
| | Confirmatory | 191.9→147.9 | | | 10 |
| Clothianidin | Primary | 250→169 | Pos | 20 | 15 |
| | Confirmatory | 250→131.9 | | | 11 |
| Diazinon | Primary | 305.1→169 | Pos | 30 | 20 |
| | Confirmatory | 305.1→153 | | | 20 |
| Dicamba[A] | Primary | 218.9→174.9 | Neg | 10 | 8 |
| | Confirmatory | 220.9→176.9 | | | 8 |
| Fipronil | Primary | 435→330 | Pos | 30 | 15 |
| | Confirmatory | 435→318 | | | 23 |
| Imidacloprid | Primary | 256.1→209.1 | Pos | 30 | 17 |
| | Confirmatory | 256.1→175 | | | 23 |
| Malathion | Primary | 331.1→127 | Pos | 20 | 12 |
| | Confirmatory | 331.1→285 | | | 6 |
| Methomyl | Primary | 163→87.9 | Pos | 10 | 10 |
| | Confirmatory | 163→105.9 | | | 11 |
| Metolachlor | Primary | 284.1→252.1 | Pos | 30 | 16 |
| | Confirmatory | 284.1→176.1 | | | 25 |
| Metribuzin | Primary | 215.1→187.1 | Pos | 30 | 16 |
| Picloram[A] | Primary | 240.9→196.9 | Neg | 10 | 11 |
| | Confirmatory | 238.9→194.9 | | | 11 |
| Propiconazole | Primary | 342.1→158.9 | Pos | 30 | 22 |
| | Confirmatory | 342.1→205 | | | 17 |
| Simazine | Primary | 202→132 | Pos | 40 | 17 |
| | Confirmatory | 202→124 | | | 17 |
| Tebuconazole | Primary | 308.2→70 | Pos | 40 | 20 |
| | Confirmatory | 308.2→125 | | | 28 |
| Thiamethoxam | Primary | 292.1→211.1 | Pos | 20 | 12 |
| | Confirmatory | 292.1→131.9 | | | 20 |
| Triclopyr[A] | Primary | 253.9→195.9 | Neg | 10 | 12 |
| | Confirmatory | 253.9→217.9 | Neg | 10 | 6 |
| | | Surrogates | | | |
| 2,4-D (Ring-D3)[A] | Primary | 221.9→163.8 | Neg | 20 | 14 |
| Atrazine (ethyl-D5) | Primary | 221.1→179 | Pos | 20 | 17 |
| Desethylatrazine (iso-propyl-D7) | Primary | 195→146.9 | Pos | 20 | 18 |
| Desisopropylatrazine (ethyl-D5) | Primary | 179→100.9 | Pos | 30 | 18 |
| Bentazon -D7 | Primary | 246.1→182 | Pos | 20 | 20 |
| Carbofuran (Ring-13C6) | Primary | 228.1→171 | Pos | 20 | 12 |
| Clothianidin -D3 | Primary | 253→131.9 | Pos | 20 | 15 |
| Diazinon (Diethyl-D10) | Primary | 315.2→170 | Pos | 30 | 20 |
| Dicamba -D3[A] | Primary | 223.9→179.9 | Neg | 10 | 8 |
| Imidacloprid -D4 | Primary | 260.1→213.1 | Pos | 30 | 15 |
| Methomyl (Acetohydroxamate-13C2 15N) | Primary | 166→90.8 | Pos | 10 | 8 |
| Simazine (Diethyl-D10) | Primary | 212.1→134 | Pos | 40 | 18 |
| Tebuconazole (tert-Butyl-D9) | Primary | 317.2→69.9 | Pos | 40 | 20 |
| Thiamethoxam -D3 | Primary | 295→214.1 | Pos | 30 | 12 |

[A] Indicates analyzed under acidic LC conditions.

**TABLE 5 SRM Ions, Retention times and SRM Ion Ratios**

| Chemical | Primary/ Confirmatory | MRM Transition | Retention Time Minutes | Primary/Confirmatory SRM Area Ratio |
|---|---|---|---|---|
| 2,4-D[A] | Primary | 218.9→160.9 | 7.6 | 1.5 |
| | Confirmatory | 220.9→162.9 | | |
| Acetochlor | Primary | 270.1→224.1 | 10.3 | 2.5 |
| | Confirmatory | 270.1→148 | | |
| Alachlor | Primary | 270.1→162 | 10.3 | 0.4 |
| | Confirmatory | 270.1→238.1 | | |
| Aldicarb | Primary | 213→88.9 | 8 | 2.1 |
| | Confirmatory | 213→116 | | |
| Atrazine | Primary | 216.1→174 | 9.2 | 3.6 |
| | Confirmatory | 216.1→95.9 | | |
| Desethylatrazine | Primary | 188→146 | 7.6 | 5.4 |
| | Confirmatory | 188→78.8 | | |
| Desisopropylatrazine | Primary | 174→96 | 6.6 | 1.3 |
| | Confirmatory | 174→78.8 | | |
| Azoxystrobin | Primary | 404.2→372.2 | 9.6 | 3.7 |
| | Confirmatory | 404.2→344.2 | | |
| Bentazon | Primary | 239→197 | 6.4 | 1.1 |
| | Confirmatory | 239→175 | | |
| Carbaryl | Primary | 202.1→145 | 8.8 | 3.6 |
| | Confirmatory | 202.1→127 | | |
| Chlorpyrifos | Primary | 350→197.9 | 11.4 | 1.8 |
| | Confirmatory | 350→322 | | |
| Clopyralid[A] | Primary | 189.9→145.9 | 5.2 | 1.5 |
| | Confirmatory | 191.9→147.9 | | |
| Clothianidin | Primary | 250→169 | 6.9 | 1.6 |
| | Confirmatory | 250→131.9 | | |
| Diazinon | Primary | 305.1→169 | 10.6 | 1.6 |
| | Confirmatory | 305.1→153 | | |
| Dicamba[A] | Primary | 218.9→174.9 | 7.1 | 1.5 |
| | Confirmatory | 220.9→176.9 | | |
| Fipronil | Primary | 435→330 | 10.3 | 5.7 |
| | Confirmatory | 435→318 | | |
| Imidacloprid | Primary | 256.1→209.1 | 6.9 | 1.1 |
| | Confirmatory | 256.1→175 | | |
| Malathion | Primary | 331.1→127 | 9.9 | 1.2 |
| | Confirmatory | 331.1→285 | | |
| Methomyl | Primary | 163→87.9 | 6.1 | 1.8 |
| | Confirmatory | 163→105.9 | | |
| Metolachlor | Primary | 284.1→252.1 | 10.3 | 2.4 |
| | Confirmatory | 284.1→176.1 | | |
| Metribuzin | Primary | 215.1→187.1 | 8.5 | NA |
| Picloram[A] | Primary | 240.9→196.9 | 5.9 | 1 |
| | Confirmatory | 238.9→194.9 | | |
| Propiconazole | Primary | 342.1→158.9 | 10.6 | 7.2 |
| | Confirmatory | 342.1→205 | | |
| Simazine | Primary | 202→132 | 7.6 | 5.4 |
| | Confirmatory | 202→124 | | |
| Tebuconazole | Primary | 308.2→70 | 10.5 | 11.2 |
| | Confirmatory | 308.2→125 | | |
| Thiamethoxam | Primary | 292.1→211.1 | 6.3 | 4 |
| | Confirmatory | 292.1→131.9 | | |
| Triclopyr[A] | Primary | 253.9→195.9 | 7.8 | 2.9 |
| | Confirmatory | 253.9→217.9 | | |
| Surrogates | | | | |
| 2,4-D (Ring-D3)[A] | Primary | 221.9→163.8 | 7.6 | NA |
| Atrazine (ethyl-D5) | Primary | 221.1→179 | 9.2 | NA |
| Desethylatrazine (iso-propyl-D7) | Primary | 195→146.9 | 7.6 | NA |
| Desisopropylatrazine (ethyl-D5) | Primary | 179→100.9 | 6.6 | NA |
| Bentazon -D7 | Primary | 246.1→182 | 6.4 | NA |
| Carbofuran (Ring-13C6) | Primary | 228.1→171 | 8.6 | NA |
| Clothianidin -D3 | Primary | 253→131.9 | 6.9 | NA |
| Diazinon (Diethyl-D10) | Primary | 315.2→170 | 10.6 | NA |
| Dicamba -D3[A] | Primary | 223.9→179.9 | 7.1 | NA |
| Imidacloprid -D4 | Primary | 260.1→213.1 | 6.9 | NA |
| Methomyl (Acetohydroxamate-13C2, 15N) | Primary | 166→90.8 | 6.1 | NA |
| Simazine (Diethyl-D10) | Primary | 212.1→134 | 7.6 | NA |
| Tebuconazole (tert-Butyl-D9) | Primary | 317.2→69.9 | 10.5 | NA |
| Thiamethoxam -D3 | Primary | 295→214.1 | 6.3 | NA |

[A] Indicates analyzed under acidic LC conditions.

## 7. Apparatus

7.1 *LC/MS/MS System:*

7.1.1 *Liquid Chromatography ~~System~~System*—~~—~~A complete LC system is required in order to analyze samples, this should include a sample injection system, an autosampler, a solvent pumping system capable of mixing solvents, a sample compartment capable of maintaining required temperature and a temperature controlled column compartment. A LC system that is capable of performing at the flows, pressures, controlled temperatures, sample volumes, and requirements of the standard ~~shall~~must be used.

7.1.2 *Analytical Column*[4]—A reverse phase C18 particle 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 used and need to be monitored.

7.2 *Tandem Mass Spectrometer ~~System~~System*—~~—~~A MS/MS system capable of multiple reaction monitoring (MRM) analysis or any system that is capable of meeting the requirements in this standard ~~shall~~must be used. Electrospray ionization is utilized for this standard.

7.3 *Adjustable Volume Pipettes*—~~10, 20, 100 and 1000 µL and 5 and 10 mL.~~10 µL, 20 µL, 100 µL, 1000 µL, 5 mL, and 10 mL.

7.3.1 *Discussion*—Any pipette may be used providing the data generated meets the performance of the standard.

7.3.2 *Pipette Tips*—Polypropylene pipette tips free of release agents or low retention coating of various sizes.

7.4 *Class A Volumetric Glassware.*



[4] A Waters Acquity (a trademark of the Waters Corporation, Milford, MA) UPLC ~~H-Class System, or equivalent, has been found suitable for use.~~BEH C18, 2.1 mm × 100 mm and 1.7 µm particle size column 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,[1] which you may attend.

[5] ~~A Waters Acquity (a trademark of the Waters Corporation, Milford, MA) UPLC BEH C18, 2.1×100 mm and 1.7 µm particle size column, or equivalent, has been found suitable for use. It was used to develop this test method and generate the precision and bias data presented in Section 16.~~

[6] ~~A Waters Xevo (a trademark of the Waters Corporation, Milford, MA) TQ-S triple quadrupole mass spectrometer, or equivalent, has been found suitable for use.~~

7.5 *Filtration Device:*

7.5.1 *Hypodermic Syringe*—A luer-lock tip glass syringe capable of holding a syringe driven filter unit.

7.5.2 A 10 mL Lock Tip Glass Syringe size is recommended since a 10 mL prepared sample size is used in this test method. If a smaller volume syringe is used, do not wash out the syringe or change filters while filtering the same sample if multiple refills of the syringe are required in order to filter the 10 mL prepared sample.

7.5.3 *Filter Unit*[5]—PTFE filter units were used to filter the samples.

7.6 *Vials*—2-mL autosampler vials (LC vials) with pre-slit PTFE/silicone septa or equivalent.

7.7 *Sonicator.*

7.8 *Oven*—Capable to achieve ~~250°C.~~250 °C.

7.9 *VOA Vials*—Amber, ~~40 mL.~~40 mL.

## 8. Reagents and Materials

8.1 *Purity of Reagents*—High Performance Liquid Chromatography (HPLC) pesticide residue analysis and spectrophotometry grade chemicals ~~shall~~must be used in all tests. Unless indicated otherwise, it is intended that all reagents ~~shall~~must conform to the Committee on Analytical Reagents of the American Chemical Society.[6] 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~~must be understood to mean reagent water conforming to Type 1 of Specification D1193. It ~~shall~~must be demonstrated that this water does not contain contaminants at concentrations sufficient to interfere with the analysis.

8.3 All prepared solutions are routinely replaced every year if not previously discarded for quality control failure.

8.4 *Gases*—Ultrapure nitrogen and argon.

8.5 Formic Acid (CAS # ~~64-18-6)~~64-18-6).

8.6 Acetonitrile (CAS # ~~75-05-8)~~75-05-8).

8.7 Methanol (CAS # ~~67-56-1)~~67-56-1).

8.8 Ammonium Formate (CAS # ~~540-69-2)~~540-69-2).

8.9 2-Propanol (isopropyl alcohol, CAS # ~~67-63-0)~~67-63-0).

8.10 2,4-Dichlorophenoxyacetic acid (2,4-D, CAS # ~~94-75-7)~~94-75-7).

[5] A Millipore IC Millex-LG PTFE/0.2µm membrane syringe driven membrane filter unit (Millex is a trademark of Merck KGAA, Darmstadt, Germany) ~~has been found suitable for use for this method, any filter unit may be used~~ was used, if you are aware of an alternative filter that meets the performance of ~~this method~~ the standard, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] ~~may be used.~~ which you may attend.

[6] ~~*Reagent Chemicals, American Chemical Society Specifications.*~~*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.

8.11 Acetochlor (CAS # ~~34256-82-1)~~34256-82-1).

8.12 Alachlor (CAS # ~~15972-60-8)~~15972-60-8).

8.13 Aldicarb (CAS # ~~116-06-3)~~116-06-3).

8.14 Atrazine (CAS # ~~1912-24-9)~~1912-24-9).

8.15 Desethylatrazine (CAS # ~~6190-65-4)~~6190-65-4).

8.16 Desisopropylatrazine (CAS # ~~1007-28-9)~~1007-28-9).

8.17 Azoxystrobin (CAS # ~~131860-33-8)~~131860-33-8).

8.18 Bentazon (CAS # ~~25057-89-0)~~25057-89-0).

8.19 Carbaryl (CAS # ~~63-25-2)~~63-25-2).

8.20 Chlorpyrifos (CAS # ~~2921-88-2)~~2921-88-2).

8.21 Clopyralid (CAS # ~~1702-17-6)~~1702-17-6).

8.22 Clothianidin (CAS # ~~210880-92-5)~~210880-92-5).

8.23 Diazinon (CAS # ~~333-41-5)~~333-41-5).

8.24 Dicamba (CAS # ~~1918-00-9)~~1918-00-9).

8.25 Fipronil (CAS # ~~120068-37-3)~~120068-37-3).

8.26 Imidacloprid (CAS # ~~138261-41-3)~~138261-41-3).

8.27 Malathion (CAS # ~~121-75-5)~~121-75-5).

8.28 Methomyl (CAS # ~~16752-77-5)~~16752-77-5).

8.29 Metolachlor (CAS # ~~51218-45-2)~~51218-45-2).

8.30 Metribuzin (CAS # ~~21087-64-9)~~21087-64-9).

8.31 Picloram (CAS # ~~1918-02-1)~~1918-02-1).

8.32 Propiconazole (CAS # ~~60207-90-1)~~60207-90-1).

8.33 Simazine (CAS # ~~122-34-9)~~122-34-9).

8.34 Tebuconazole (CAS # ~~107534-96-3)~~107534-96-3).

8.35 Thiamethoxam (CAS # ~~153719-23-4)~~153719-23-4).

8.36 Triclopyr (CAS # ~~55335-06-3)~~55335-06-3).

8.37 *Isotopically Labeled Pesticide Standards (Surrogates)*—There are not isotopically labeled surrogates for every target analyte. The labeled surrogate only mimics its unlabeled target analyte. The isotopically labeled carbofuran was chosen to mimic carbaryl. (Note—P&A data show that the labeled carbofuran is not a good surrogate for carbaryl even though they are structurally similar.) Surrogates may be added or deleted from the below list if new ones become available or if the existing ones are not readily available. The surrogate list is long and expensive to maintain. If surrogates are not available at the time of analysis it will be mentioned in the case narrative that accompanies the data, if extra surrogates are added this will also be mentioned in the case ~~narrative.~~narrative (CAS #'s are for the unlabeled native analyte).

8.37.1 2,4-Dichlorophenoxyacetic acid (2,4-D (Ring-D3), CAS # ~~94-75-7)~~94-75-7).

8.37.2 Atrazine (ethyl-D5, CAS # ~~1912-24-9)~~1912-24-9).

8.37.3 Desethylatrazine (iso-propyl-D7, CAS # ~~6190-65-4)~~6190-65-4).

8.37.4 Desisopropylatrazine (ethyl-D5, CAS # ~~1007-28-9)~~1007-28-9).

8.37.5 Bentazon (D7, CAS # ~~25057-89-0)~~25057-89-0).

8.37.6 Carbofuran (Ring-13C6, CAS # ~~1563-66-2)~~1563-66-2).

8.37.7 Clothianidin (D3, CAS # ~~210880-92-5)~~210880-92-5).

8.37.8 Diazinon (diethyl-D10, CAS # ~~333-41-5)~~333-41-5).

8.37.9 Dicamba (D3, CAS # ~~1918-00-9)~~1918-00-9).

8.37.10 Imidacloprid (D4, CAS # ~~138261-41-3)~~138261-41-3).

8.37.11 Methomyl (Acetohydroxamate-13C2, 15N, CAS # ~~16752-77-5)~~16752-77-5).

8.37.12 Simazine (diethyl-D10, CAS # ~~122-34-9)~~122-34-9).

8.37.13 Tebuconazole (tert-Butyl-D9, CAS # ~~107534-96-3)~~107534-96-3).

8.37.14 Thiamethoxam (D3, CAS # ~~153719-23-4)~~153719-23-4).

## 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 Safety Data Sheets (SDS) for all reagents used in this method.

## 10. Sampling and Preservation

10.1 Grab samples are collected in amber glass containers with ~~Teflon™ lined~~ inert-lined caps, such as, 40 mL amber VOA vials. As part of the overall quality assurance program for this test method, field blanks exposed to the same field conditions as samples are collected and analyzed according to this standard to assess the potential for field contamination, refer to Guide D3856 as a guide for sampling. This test method is based upon an 8 mL sample size per analysis. If different sample sizes are used, spiking solution amounts may need to be modified. EPA publication SW-846 may be used as a sampling guide. Samples ~~shall~~must be shipped with a trip blank and ~~at less than 6ºC.~~between freezing and 6 °C.

10.2 Once received the sample temperature is taken and should be less than ~~6ºC.~~6 °C. If the receiving temperature is greater than ~~6ºC,~~6 °C, the sample temperature is noted in the case narrative accompanying the data. Samples should be stored refrigerated between ~~0ºC and 6ºC~~0 °C and 6 °C from the time of collection until analysis.

10.3 The samples should be analyzed within 14 days of collection. No holding time study has been done on water matrices tested in this test method. Holding time may vary depending on the matrix and individual laboratories should determine the holding time in their matrix, refer to Practice D4841.

## 11. Preparation of LC/MS/MS

11.1 *LC Chromatograph Operating Conditions:*

11.1.1 Injections of all standards and samples are made at a ~~25 or 50 µL~~ 25 µL or 50 µL volume. Other injection volumes may be used to optimize conditions. Standards and sample extracts ~~shall~~must be in a 80:20 water:methanol solution. In the case of extreme concentration differences amongst samples, it is wise to analyze a blank after a concentrated sample and before a dilute sample to minimize carry-over of analytes from injection to injection. However, there should not be carry-over between samples. The LC utilized to develop this test method has a flow through LC needle design. The gradient conditions for the two liquid chromatography analysis runs are shown in Tables 2 and 3. The primary SRM transition chromatograms at the lowest calibration level are shown in the Appendix, Figs. X1.1-X1.5.

11.2 *LC Auto Sampler Conditions:*

11.2.1 *Needle Wash Solvent*—~~60 %~~60 % acetonitrile ~~acetonitrile/40 % 2-propanol. 8 second /~~40 % 2-propanol. 8 s wash time before and after injection. Instrument manufacturer's specifications should be followed in order to eliminate sample carry-over.

11.2.2 *Temperatures*—Column, ~~35°C;~~35 °C; Sample compartment, ~~15°C.~~15 °C.

11.2.3 *Seal Wash*—Solvent: ~~50 %~~50 % methanol ~~methanol/50 % /~~50 % water; Time: ~~5 minutes.~~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 used. Each peak requires at least 10 scans per peak for adequate quantitation. Variable parameters regarding 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.

11.3.2 The instrument is set in the Electrospray source setting. The values for the following parameters are shown here for information only. These conditions should be checked and optimized when required.

Methanol/Water/Ammonium Formate Analysis Run Conditions

Capillary Voltage: 1 kV in both ESI modes
Cone: Variable depending on analyte
Source Offset (V) 10
~~Source Temperature: 150°C~~
Source Temperature: 150 °C
~~Desolvation Gas Temperature: 500°C~~
Desolvation Gas Temperature: 500 °C
~~Desolvation Gas Flow: 900 L/hr~~
Desolvation Gas Flow: 900 L/h
~~Cone Gas Flow: 150 L/hr~~
Cone Gas Flow: 150 L/h
Collision Gas Flow: 0.15 mL/min
Low Mass Resolution 1: 2.7
High Mass Resolution 1: 14.7
Ion Energy 1: 0.5
Entrance Energy: 1
Collision Energy: Variable depending on analyte
Exit Energy: 1
Low Mass Resolution 2: 2.8
High Mass resolution 2: 14.7
Ion Energy 2: 1.5
Gain: 1.0
Multiplier: 535
~~Inter-Scan Delay: 0.003 seconds~~
Inter-Scan Delay: 0.003 s
~~Polarity Switching Inter-scan Delay: 0.020 seconds~~