Designation: D7597 – 09ε2

StandardTest Method for Determination of Diisopropyl Methylphosphonate, Ethyl Hydrogen Dimethylamidophosphate, Ethyl Methylphosphonic Acid, Isopropyl Methylphosphonic Acid, Methylphosphonic Acid and Pinacolyl Methylphosphonic Acid in Water by Liquid Chromatography/Tandem Mass Spectrometry[1]

This standard is issued under the fixed designation D7597; 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—Added research report footnote to Section 16 editorially in June 2013.

1. Scope

1.1 This procedure covers the determination of diisopropyl methylphosphonate (DIMP), ethyl hydrogen dimethylamidophosphate (EHDMAP), ethyl methylphosphonic acid (EMPA), isopropyl methylphosphonic acid (IMPA), methylphosphonic acid (MPA) and pinacolyl methylphosphonic acid (PMPA) (referred to collectively as organophosphonates in this test method) in surface water by direct injection using liquid chromatography (LC) and detected with tandem mass spectrometry (MS/MS) using electrospray ionization (ESI). These analytes are qualitatively and quantitatively determined by this method. This method adheres to single reaction monitoring (SRM) 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 organophosphonates are listed in Table 1.

1.4.1 The DVL is required to be at a concentration at least three times below the reporting limit (RL) and have a signal/noise ratio greater than 3:1. Fig. 1 displays the signal/noise ratios at the DVLs for the organophosphonates in the ESI positive mode and Fig. 2 in the ESI negative mode.

1.4.2 The reporting limit is the concentration of the Level 1 calibration standard as shown in Table 2 for the organophosphonates except for MPA in the ESI negative mode which is at Level 2 due to not meeting the DVL criteria at the lower concentration level. The DVL for MPA in the ESI negative mode is at 20 µg/L, which forces a raised reporting limit. However, the multi-laboratory validation required a spike of all target analytes at Level 1 concentrations. The mean recovery for MPA in the ESI negative mode at this level was 98.7 % as shown in Table 3. If your instrument's sensitivity can meet the requirements in this test method, MPA may have a 50 µg/L reporting limit.

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

[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.

TABLE 1 Detection Verification Level and Reporting Range

Analyte	ESI Mode	DVL (µg/L)	Reporting Range (µg/L)
Diisopropyl methylphosphonate	Positive	1	5–150
Ethyl hydrogen dimethylamidophosphate	Negative	0.25	5–150
Ethyl hydrogen dimethylamidophosphate	Positive	0.25	5–150
Ethyl methylphosphonic acid	Negative	5	50–1500
Ethyl methylphosphonic acid	Positive	5	50–1500
Isopropyl methylphosphonic acid	Negative	10	50–1500
Isopropyl Methylphosphonic acid	Positive	5	50–1500
Methylphosphonic acid	Negative	20	100–1500
Methylphosphonic acid	Positive	10	50–1500
Pinacolyl methylphosphonic acid	Negative	5	50–1500
Pinacolyl methylphosphonic acid	Positive	5	50–1500

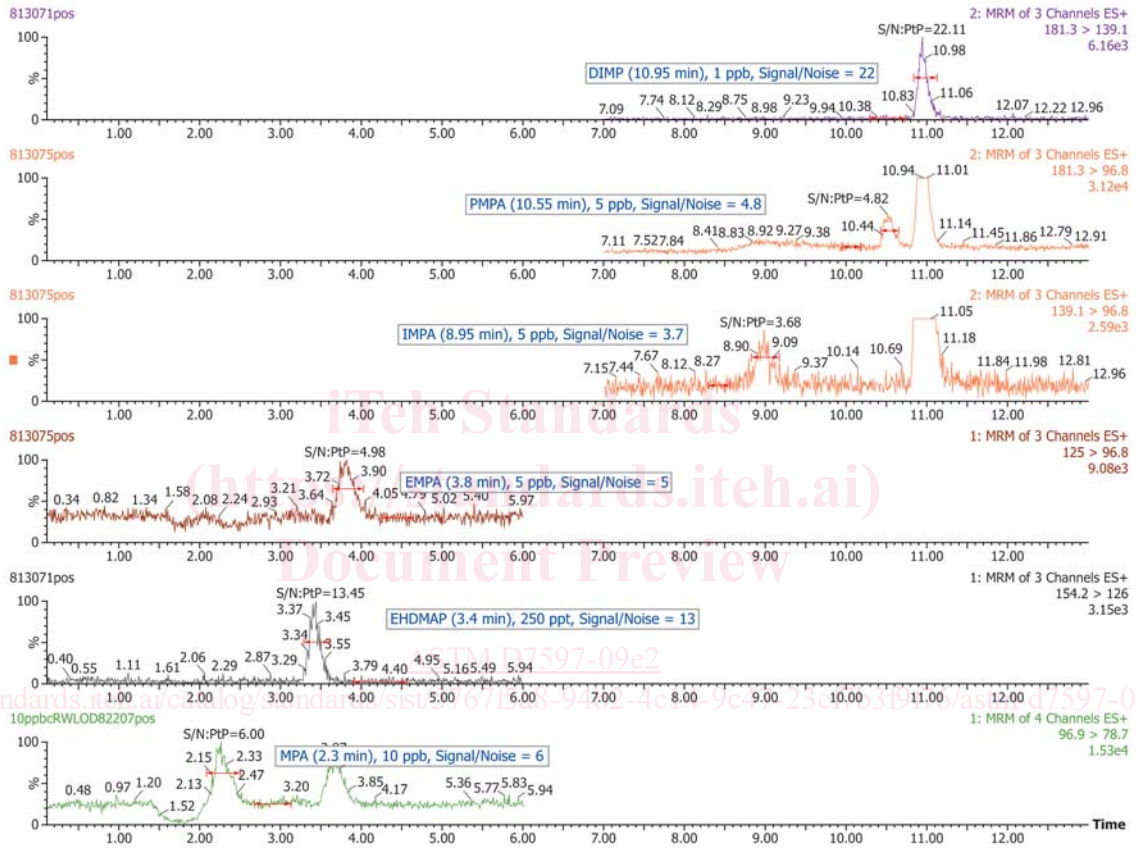

FIG. 1 Example ESI Positive Mode SRM Chromatograms Signal/Noise Ratios

E2554 Practice for Estimating and Monitoring the Uncertainty of Test Results of a Test Method Using Control Chart Techniques

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 *organophosphonates, n*—in this test method, diisopropyl methylphosphonate (DIMP), ethyl hydrogen dimethylamidophosphate (EHDMAP), ethyl methylphosphonic acid (EMPA), isopropyl methylphosphonic acid (IMPA), methylphosphonic acid (MPA) and pinacolyl methylphosphonic acid (PMPA).

3.2 *Abbreviations:*

3.2.1 *ND*—non-detect

4. Summary of Test Methods

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

[3] Available from United States Environmental Protection Agency (EPA), Ariel Rios Bldg., 1200 Pennsylvania Ave., NW, Washington, DC 20460, http://www.epa.gov.

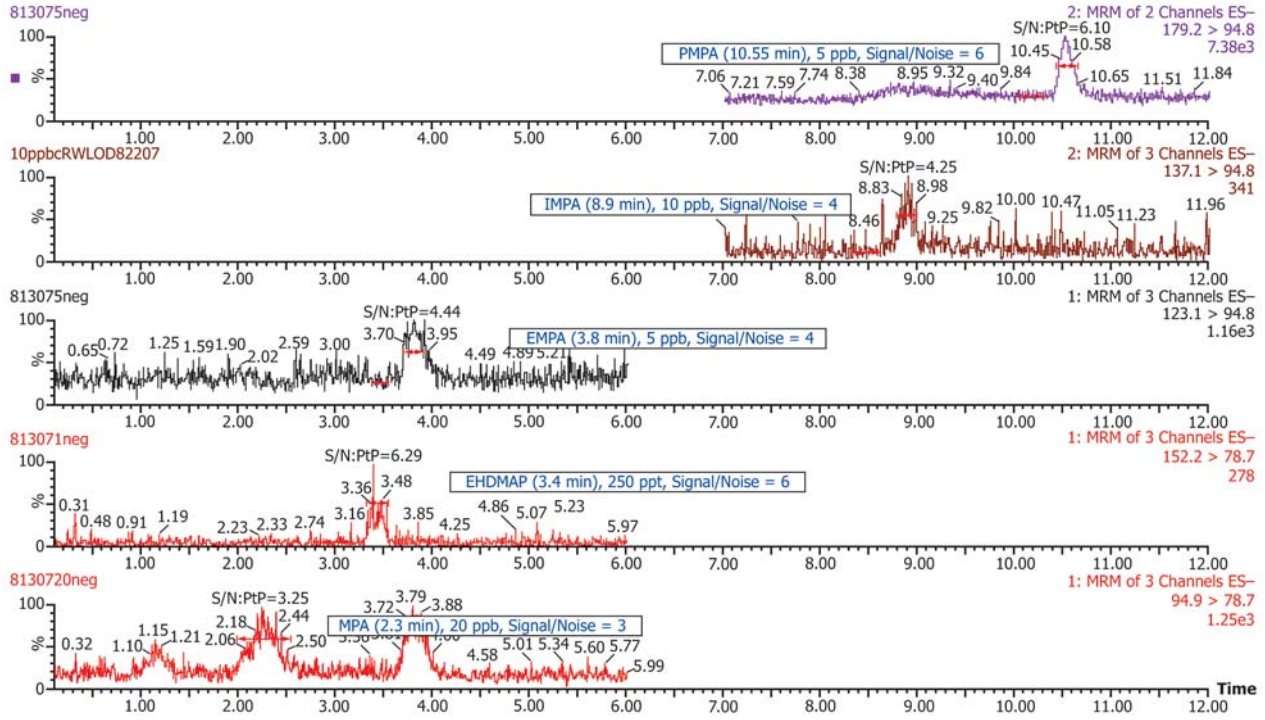

FIG. 2 Example ESI Negative Mode SRM Chromatograms Signal/Noise Ratios

TABLE 2 Concentrations of Calibration Standards (PPB)

Analyte/Surrogate	LV 1	LV 2	LV 3	LV 4	LV 5	LV 6	LV 7
Diisopropyl methylphosphonate	5	10	20	35	50	100	150
Ethyl hydrogen dimethylamidophosphate	5	10	20	35	50	100	150
Ethyl methylphosphonic acid	50	100	200	350	500	1000	1500
Isopropyl methylphosphonic acid	50	100	200	350	500	1000	1500
Methylphosphonic acid	50	100	200	350	500	1000	1500
Pinacolyl methylphosphonic acid	50	100	200	350	500	1000	1500
DIMP-D14 (Surrogate)	5	10	20	35	50	100	150
PMPA-13C4 (Surrogate)	25	50	100	175	250	500	750
MPA-D3 (Surrogate)	25	50	100	175	250	500	750

4.2 For organophosphonate analysis, samples are shipped to the lab between 0°C and 6°C and analyzed within 1 day 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 The organophosphonates and the surrogates; diisopropyl methylphosphonate-D_{14}, pinacolyl methylphosphonic acid-$^{13}C_6$ and methylphosphonic acid-D_3 are identified by retention time and one SRM transition. The target analytes and surrogates are quantitated using the SRM transitions utilizing an external calibration. The final report issued for each sample lists the concentration of each organophosphonate target compound and each surrogate recovery.

5. Significance and Use

5.1 Organophosphate pesticides affect the nervous system by disrupting the enzyme that regulates acetylcholine, a neurotransmitter. They were developed during the early 19th century, but their effects on insects, which are similar to their effects on humans, were discovered in 1932. Some are poisonous and were used as chemical weapon agents. Organophosphate pesticides are usually not persistent in the environment.[4,5]

5.2 This test method is for the analysis of selected organophosphorous-based chemical weapon agent degradation products from Sarin (GB), Soman (GD), Tabun (GA) and VX. This method has been investigated for use with reagent and surface water.

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.

[4] Additional information about organophosphate pesticides is available on the Internet at http://www.epa.gov (2009).

[5] Additional information about chemical weapon agents is available on the Internet at http://www.opcw.org (2009).

TABLE 3 Multi-Laboratory Recovery Data in Reagent Water

Analyte	ESI Mode	Spike Conc. (ppb)	# Results	# Labs	Bias			Precision			
					Mean Recovery (%)	Min Recovery (%)	Max Recovery (%)	Overall SD (%)	Pooled within-lab SD (%)	Overall RSD (%)	Pooled within-lab RSD (%)
DIMP	Pos	5	16	4	95.1	65.8	136.0	17.6	19.4	21.3	22.4
DIMP	Pos	10	19	5	98.2	80.0	121.0	6.1	5.5	13.6	13.8
DIMP	Pos	25	26	6	102.9	74.4	128.0	5.6	5.7	14.5	14.1
DIMP	Pos	125	22	5	96.6	80.4	120.0	4.4	4.5	11.0	11.4
DIMP-D14	Pos	25	86	6	102.6	54.8	127.6	9.8	9.5	11.2	10.9
EHDMAP	Neg	5	12	3	57.5	0.0	220.0	31.4	22.3	71.1	123.6
EHDMAP	Neg	10	16	4	47.1	0.0	178.0	13.3	10.8	66.8	142.0
EHDMAP	Neg	25	22	5	84.1	54.0	141.2	6.9	6.6	22.9	27.3
EHDMAP	Neg	125	18	4	87.4	64.2	141.6	5.0	5.1	25.4	29.1
EHDMAP	Pos	5	16	4	77.0	0.0	134.2	7.4	8.0	51.4	66.8
EHDMAP	Pos	10	20	5	70.0	0.0	143.0	7.7	10.0	53.6	76.5
EHDMAP	Pos	25	26	6	89.6	60.0	128.8	5.2	6.2	23.5	26.2
EHDMAP	Pos	125	22	5	87.5	59.0	123.2	5.2	6.0	23.9	27.3
EMPA	Neg	50	16	4	110.1	74.8	170.6	22.4	17.2	25.6	23.3
EMPA	Neg	100	20	5	108.3	87.7	175.0	11.1	8.7	24.8	22.9
EMPA	Neg	250	26	6	104.8	82.0	122.6	6.1	5.5	11.8	11.3
EMPA	Neg	1250	22	5	101.5	87.2	126.4	8.4	8.1	11.2	11.0
EMPA	Pos	50	16	4	95.4	77.6	122.8	12.9	13.3	13.1	13.7
EMPA	Pos	100	20	5	96.0	61.4	132.5	9.5	9.5	15.9	16.6
EMPA	Pos	250	26	6	99.7	70.0	133.2	5.9	5.4	18.2	18.2
EMPA	Pos	1250	21	5	93.9	84.0	108.4	2.7	3.0	7.7	8.2
IMPA	Neg	50	16	4	88.0	56.6	140.4	23.7	26.0	23.5	26.7
IMPA	Neg	100	20	5	88.0	68.5	118.0	12.9	14.3	13.4	15.2
IMPA	Neg	250	26	6	98.1	72.8	144.0	13.1	11.9	19.2	19.6
IMPA	Neg	1250	21	5	90.7	73.1	103.0	5.5	6.1	8.7	9.6
IMPA	Pos	50	16	4	98.3	47.8	139.6	19.2	20.5	27.2	27.7
IMPA	Pos	100	19	5	95.4	72.3	120.5	9.8	10.3	12.4	13.0
IMPA	Pos	250	26	6	97.0	79.2	188.4	7.4	7.6	10.9	11.2
IMPA	Pos	1250	21	5	91.3	70.4	115.5	5.1	5.2	11.4	12.5
MPA	Neg	50	16	4	98.7	3.3	175.0	14.2	25.3	60.5	61.3
MPA	Neg	100	20	5	100.0	41.9	142.0	8.9	9.2	30.8	30.8
MPA	Neg	250	26	6	99.5	66.0	124.5	7.6	7.6	14.5	14.5
MPA	Neg	1250	22	5	102.7	81.8	130.5	10.5	9.9	12.4	12.1
MPA	Pos	50	16	4	68.3	9.8	139.6	13.4	20.3	36.6	53.6
MPA	Pos	100	20	5	80.5	48.4	149.7	14.0	12.6	26.8	33.3
MPA	Pos	250	26	6	91.7	33.9	153.7	8.0	7.8	31.8	34.7
MPA	Pos	1250	22	5	95.8	31.8	208.2	12.6	8.3	43.4	45.3
MPA-D3	Neg	250	84	6	111.2	57.2	190.8	16.2	12.5	30.0	26.9
MPA-D3	Pos	250	68	5	104.4	58.4	151.8	14.3	14.0	18.0	17.3
PMPA	Neg	50	15	4	87.8	77.6	124.4	8.4	8.2	13.8	15.7
PMPA	Neg	100	19	5	91.6	83.6	98.2	2.8	3.0	4.4	4.8
PMPA	Neg	250	26	6	101.0	77.2	123.8	5.0	4.8	12.1	12.0
PMPA	Neg	1250	22	5	99.2	84.8	126.5	4.9	4.8	12.3	12.4
PMPA	Pos	50	16	4	90.8	60.8	148.8	15.8	16.1	25.6	28.2
PMPA	Pos	100	15	4	95.2	86.8	114.0	3.8	4.0	7.9	8.3
PMPA	Pos	250	20	5	103.8	85.2	136.1	4.5	3.8	15.3	14.7
PMPA	Pos	1250	12	3	99.8	88.8	117.5	5.2	5.0	7.5	7.5
PMPA-13C6	Neg	250	83	6	99.5	74.8	128.3	9.4	9.2	11.1	11.1

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.[6] A system that is capable of performing at the flows, pressures, controlled temperatures, sample volumes and requirements of the standard may be used.

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

7.1.2 *Analytical Column-Waters*—Atlantis dC18, 150 mm × 2.1 mm, 3 µm particle size, or equivalent.

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*

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.[8] Other reagent grades may be used provided they are first determined they are 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 Formic acid (≥95%, CAS # 64-18-6).

8.8 Diisopropyl methylphosphonate (DIMP, CAS # 1445-75-6).

8.9 Ethyl hydrogen dimethylamidophosphate (EHDMAP, CAS # 2632-86-2).

8.10 Ethyl methylphosphonic acid (EMPA, CAS # 1832-53-7).

8.11 Isopropyl methylphosphonic acid (IMPA, CAS # 1832-54-8).

8.12 Methylphosphonic acid (MPA, CAS # 993-13-5).

8.13 Pinacolyl methylphosphonic acid (PMPA, CAS # 616-52-4).

8.14 Diisopropyl methylphosphonate-D_{14} (DIMP-D14, Unlabeled CAS # 1445-75-6).

8.14.1 DIMP-D14 represents deuterium labeled diisopropyl methylphosphonate where the two isopropyl moieties contain all ^{2}H.

8.15 Methylphosphonic acid-D_3 (MPA-D3, Unlabeled CAS # 993-13-5).

8.15.1 MPA-D3 represents deuterium labeled methylphosphonic acid where the methyl moiety contains all ^{2}H.

8.16 Pinacolyl methylphosphonic acid-$^{13}C_6$ (PMPA-13C6, Unlabeled CAS # 616-52-4).

8.16.1 PMPA-13C6 represents ^{13}C labeled pinacolyl methylphosphonic where all the trimethylpropyl carbon atoms are uniformly labeled ^{13}C.

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 1 day of collection.

11. Preparation of LC/MS/MS

11.1 *LC Chromatograph Operating Conditions*[6]:

11.1.1 Injection volumes of all calibration standards and samples are 50 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 4.

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

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

TABLE 4 Gradient Conditions for Liquid Chromatography

Time (min)	Flow (µL/min)	Percent CH_3CN	Percent Water	Percent 2 % Formic Acid in Water
0	300	0	95	5
4	300	0	95	5
5	300	45	50	5
9	300	45	50	5
10	300	95	0	5
13	300	95	0	5
14	300	0	95	5
20	300	0	95	5

[7] A Waters Quattro micro API mass spectrometer was used to develop this test method. The multi-laboratory study included Applied Biosystems and Waters mass spectrometers.

[8] *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.

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 organophosphonates.

11.2 *Mass Spectrometer Parameters*[7]:

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 three surrogates and six target compounds which are located in multiple reaction monitoring (MRM) experiment windows. This method does however require the analysis of the organophosphonates and surrogates, in most cases, be analyzed in both the ESI positive and negatives modes in order to obtain the optimum results from the individual matrices tested. Depending on your instrument, this may be accomplished in one analysis run if the instrument can switch between positive and negative mode fast enough without losing sensitivity and maintaining at least 10 scans per peak. If your instrument is not able to switch between modes fast enough it will require two analyses, one in ESI positive and one in ESI negative. For example, the newer instruments may be capable of switching between positive and negative modes in 50 milliseconds which would require one analysis run for this test method. The older instruments may be capable of switching between positive and negative modes in 300 milliseconds which will require two analysis runs to obtain maximum sensitivity and the adequate number of scans per peak. The single laboratory data in this test method was generated using two analysis runs. Variable parameters regarding retention times, SRM transitions and cone and collision energies are shown in Table 5.

The instrument is set in the Electrospray (+) positive and/or (-) negative source setting.
Capillary Voltage: 3.5 kV
Cone: Variable depending on analyte (Table 3)
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 5)
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 in one ESI mode (0.3 seconds if acquiring in ESI positive and negative mode in same analysis run on a Quattro micro API mass spectrometer)
Repeats: 1
Span: 0 Daltons
Dwell: 0.1 Seconds

12. Preparation of Apparatus

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 seven calibration standards containing the seven concentration levels of the organophosphonates and surrogates 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 7) containing the organophosphonates, diisopropyl

TABLE 5 Retention Times, SRM Ions, and Analyte-Specific Mass Spectrometer Parameters

Analyte	ESI Mode	Retention Time (min)	SRM Mass Transition (Parent > Product)	Cone Voltage (Volts)	Collision Energy (eV)
Diisopropyl methylphosphonate	Positive	10.9	181.3 > 139.1	25	6
Ethyl hydrogen dimethylamidophosphate	Negative	3.4	152.7 > 78.7	30	15
Ethyl hydrogen dimethylamidophosphate	Positive	3.4	154.2 > 126	20	12
Ethyl methylphosphonic acid	Negative	3.8	123.1 > 94.8	30	12
Ethyl methylphosphonic acid	Positive	3.8	125 > 96.8	25	10
Isopropyl methylphosphonic acid	Negative	8.9	137.1 > 94.8	32	13
Isopropyl methylphosphonic acid	Positive	8.9	139.1 > 96.8	18	9
Methylphosphonic acid	Negative	2.3	94.9 > 78.7	35	15
Methylphosphonic acid	Positive	2.3	96.9 > 78.7	45	15
Pinacolyl methylphosphonic acid	Negative	10.5	179.2 > 94.8	35	18
Pinacolyl methylphosphonic acid	Positive	10.5	181.3 > 96.8	15	7
DIMP-D14 (Surrogate)	Positive	10.9	195.3 > 147.2	25	7
PMPA-13C6 (Surrogate)	Negative	10.5	185.3 > 94.8	35	18
MPA-D3 (Surrogate)	Negative	2.3	97.9 > 78.7	35	15
MPA-D3 (Surrogate)	Positive	2.3	99.8 > 81.8	45	15