Designation: D7597 – 16 (Reapproved 2017)

Standard Test 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.

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 test method. This test method adheres to single reaction monitoring (SRM) mass spectrometry.

1.2 This test method has been developed by U.S. 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.*

1.6 *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
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

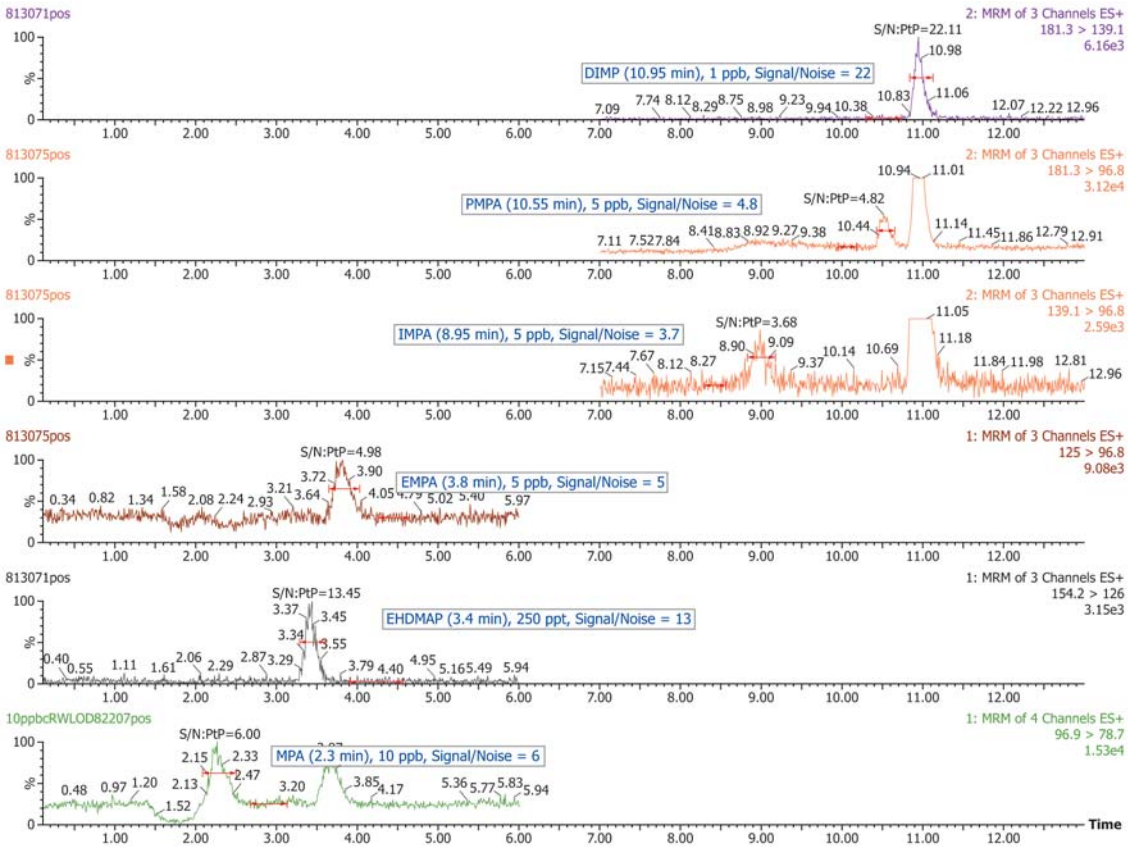

FIG. 1 Example ESI Positive Mode SRM Chromatograms Signal/Noise Ratios

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

2.2 *Other Documents:*[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.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *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).

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

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

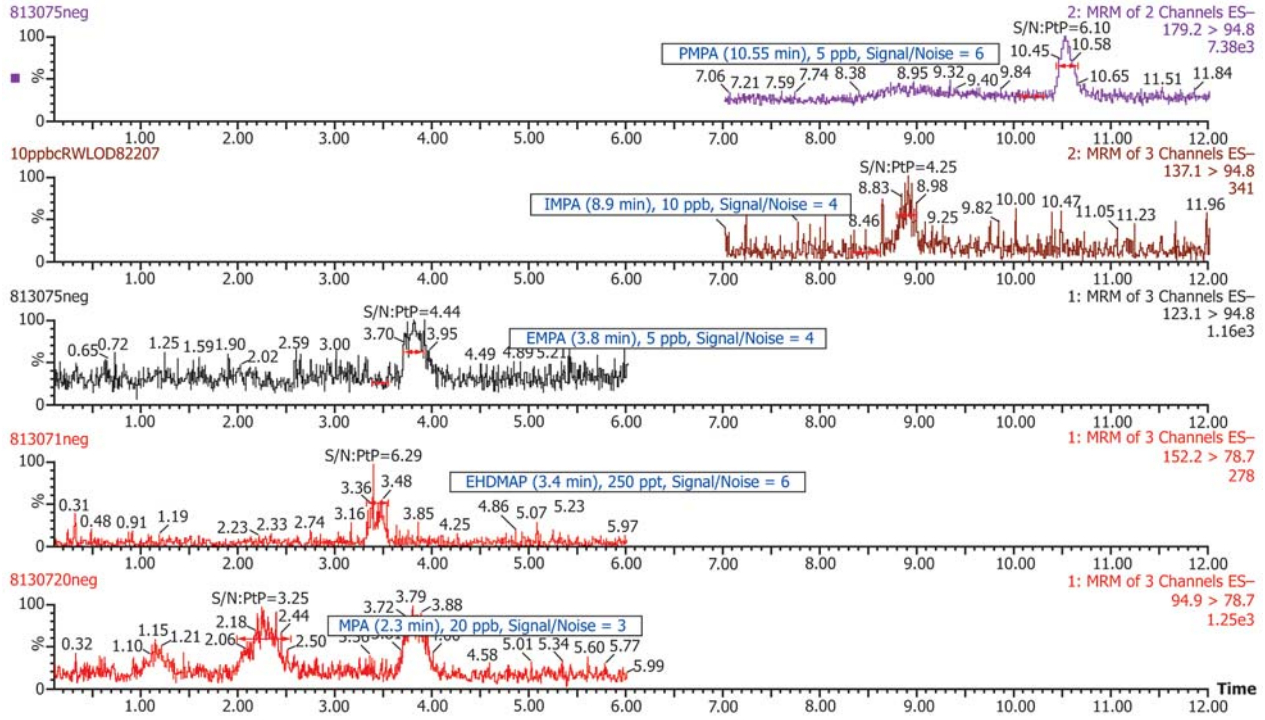

FIG. 2 Example ESI Negative Mode SRM Chromatograms Signal/Noise Ratios

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

3.3 *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 *PPB, n*—parts per billion

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 *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 Method

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

4.2 For organophosphonate analysis, samples are shipped to the lab between 0°C and 6°C and analyzed within 1 day of

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

collection. In the lab, the samples are spiked with surrogate, filtered using a syringe-driven 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 test method has been investigated for use with reagent and surface water.

6. Interferences

6.1 Test 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.[6] 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*[7]—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 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.[8] 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 luer-lock tip glass syringe capable of holding 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.

7.2.2 *Filter Unit*[9]—A 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.[10] 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).

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[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).

[6] A Waters Alliance (a trademark of the Waters Corporation, Milford, MA) High Performance Liquid Chromatography (HPLC) System, or equivalent, was found suitable for use. The multi-laboratory study included Agilent and Waters LC systems.

[7] A Waters Atlantis (a trademark of the Waters Corporation, Milford, MA) dC18, 150 mm × 2.1 mm, 3 µm particle size, or equivalent, has been found suitable for use.

[8] A Waters Quattro (a trademark of the Waters Corporation, Milford, MA) micro API mass spectrometer, or equivalent, was found suitable for use. The multi-laboratory study included applied Biosystems and Waters mass spectrometers.

[9] A Millex (a trademark of Merck KGAA, Darmstadt, Germany) HV Syringe Driven Filter Unit PVDF 0.45 µm (Millipore Corporation, Catalog # SLHV033NS) 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.

[10] *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.

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 test 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 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 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.

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

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

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:*[8]

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 test 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. 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

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

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 methylphosphonate-D_{14}, pinacolyl methylphosphonic acid-$^{13}C_6$ and methylphosphonic acid-D_3 is prepared at Level 7 concentration and aliquots of that solution are diluted to prepare Levels 1 through 6. 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 7) by adding to a 100-mL volumetric flask individual methanol solutions of the following: 750 µL of 0.1g/L solutions of MPA-D3 and PMPA-13C6, 15 µL of 1 g/L solutions of DIMP, DIMP-D14 and EHDMAP and 150 µL of 1 g/L solutions of EMPA, IMPA, MPA and PMPA then dilute to 100 mL with water. The preparation of the Level 7 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 water to prepare the desired calibration levels in 2 mL amber glass LC vials. The calibration vials must be used within 24 hours to ensure optimum results. Stock calibration standard solutions are routinely replaced every 3 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 is used monitoring the SRM transitions of each analyte. Calibration software is utilized to conduct the quantitation of the target analytes and surrogates. The SRM transition of each analyte is used for quantitation and confirmation. This gives confirmation by isolating the parent ion, fragmenting it the product ion fragment, and also 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 ppb or ppm 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 (or both) 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, other than the high or low, causes the curve to be <0.99 this point must be re-injected or a new calibration curve must be regenerated. If the low or high point is excluded, a six point curve is acceptable using a quadratic fit. An initial seven point curve over the calibration range is suggested 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. Each calibration point used to generate the curve must have a calculated percent deviation less than 25 % from the generated curve.

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 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 the target compounds and surrogates at a concentration in the calibration range of Levels 3 to 5. 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 6.

12.4.3 This study should be repeated until the single operator precision and mean recovery are within the limits in Table 6. If a concentration other than the recommended concentration is used, refer to Practice D5847 for information on applying the F test and t test in evaluating the acceptability of the mean and standard deviation.

12.4.3.1 The QC acceptance criteria for the Initial Demonstration of Performance in Table 6 were generated from a multi-laboratory method validation involving six laboratories. The descriptive statistics from this validation are shown in Section 16, Precision and Bias. 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 Practices D2777, D5847, E2554 or Method 8000B in EPA Publication SW-846 may be helpful.

12.5 *Surrogate Spiking Solution:*

12.5.1 A surrogate standard solution containing MPA-D3, PMPA-13C6 and DIMP-D14 is added to all samples. A stock surrogate spiking solution is prepared in methanol at 50 ppm for MPA-D3 and PMPA-13C6 and 5 ppm for DIMP-D14. Spiking 125 µL of this spiking solution into a 25 mL water sample results in a concentration of 250 ppb for MPA-D3 and PMPA-13C6, and 25 ppb for DIMP-D14 of the surrogates in the sample. The result obtained for the surrogate recovery must fall within the limits of Table 6. If the limits are not met, the

TABLE 6 QC Acceptance Criteria

Analyte	ESI Mode	Test Conc. (µg/L)	Initial Demonstration of Performance			Lab Control Sample	
			Recovery (%)		Precision	Recovery (%)	
			Lower Limit	Upper Limit	Maximum % RSD	Lower Limit	Upper Limit
Diisopropyl methylphosphonate	Positive	25	67	139	10	67	139
Ethyl hydrogen dimethylamidophosphate	Negative	25	16	152	15	17	152
Ethyl hydrogen dimethylamidophosphate	Positive	25	25	154	11	25	154
Ethyl methylphosphonic acid	Negative	250	76	134	11	75	134
Ethyl methylphosphonic acid	Positive	250	51	149	11	51	149
Isopropyl methylphosphonic acid	Negative	250	59	137	24	55	142
Isopropyl methylphosphonic acid	Positive	250	75	119	14	72	122
Methylphosphonic acid	Negative	250	66	133	14	65	134
Methylphosphonic acid	Positive	250	4	179	16	4	179
Pinacolyl methylphosphonic acid	Negative	250	69	133	9	69	133
Pinacolyl methylphosphonic acid	Positive	250	60	147	8	61	147
DIMP-D14 (Surrogate)	Positive	25	86	119	16	79	126
PMPA-13C6 (Surrogate)	Negative	250	82	117	16	76	123
MPA-D3 (Surrogate)	Negative	250	44	179	25	42	181
MPA-D3 (Surrogate)	Positive	250	72	137	23	66	143

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 organophosphonates found in the blank must be below the DVL. If the concentrations of the organophosphonates 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 the organophosphonates at a concentration containing the organophosphonates and surrogates 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 EMPA, MPA, IMPA and PMPA each at 250 ppm and EHDMAP and DIMP each at 25 ppm. Spiking 25 µL of this stock solution into 25 mL of water to yield a concentration of 250 ppb for EMPA, MPA, IMPA and PMPA and 25 ppb for EHDMAP and DIMP in the sample. The result obtained for the LCS must fall within the limits in Table 6.

12.7.2 If the result is not within these limits, analysis of samples is halted until the problem is corrected, and either all samples in the batch must be re-analyzed, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.8 *Matrix Spike (MS):*

12.8.1 To check for interferences in the specific matrix being tested, perform a MS on at least one sample from each batch of 10 or fewer samples by spiking the sample with a known concentration of organophosphonates and following the analytical method. Prepare a stock matrix spiking solution in methanol containing EMPA, MPA, IMPA and PMPA each at 250 ppm and EHDMAP and DIMP each at 25 ppm. Spiking 25 µL of this stock solution into 25 mL of water to yield a concentration of 250 ppb for EMPA, MPA, IMPA and PMPA and 25 ppb for EHDMAP and DIMP in the sample.

12.8.2 The matrix spike analysis is important in the analysis of the organophosphonates. Every different matrix sampled must have an associated matrix spike sample analyzed. The chemistry of the various surface waters may impact the recoveries and must be monitored on a case by case basis. Both the ESI positive and negative modes of analysis are required for this test method due to the possible ion enhancement or suppression of signal in certain matrices. An example of this effect was found in the analysis of Chicago River water for methylphosphonic acid. The recovery of methylphosphonic acid in the ESI positive mode was 90 % in reagent water and 141 % in Chicago River water. The recovery of methylphosphonic acid in the ESI negative mode was 93 % in reagent water and below the reporting limit in Chicago River water. Due to these effects, the matrix spike QC acceptance criteria is wide and the laboratory should generate their own in-house QC acceptance criteria after the analysis of 15–20 matrix spike samples of a particular surface water matrix. All results must be reported to the project manager so informed decisions to be made.

12.8.3 If the spiked concentration plus the background concentration exceeds that of the Level 7 calibration standard, the sample must be diluted to a level near the midpoint of the calibration curve.

12.8.4 Calculate the percent recovery of the spike (P) using Eq 1:

$$P = 100\,\frac{\left|\,A(V_s+V) - BVs\,\right|}{CV} \tag{1}$$

where:

A = concentration found in spiked sample,
B = concentration found in unspiked sample,
C = concentration of analyte in spiking solution,
V_s = volume of sample used,
V = volume of spiking solution added, and
P = percent recovery.

12.8.5 The percent recovery of the spike shall fall within the limits in Table 7. If the percent recovery is not within these limits, a matrix interference may be present in the selected

TABLE 7 MS/MSD QC Acceptance Criteria

Analyte	ESI Mode	Test Conc. (µg/L)	MS/MSD		
			Recovery (%)		Precision
			Lower Limit	Upper Limit	Maximum RPD (%)
Diisopropyl methylphosphonate	Positive	25	60	135	30
Ethyl hydrogen dimethylamidophosphate	Negative	25	3	158	51
Ethyl hydrogen dimethylamidophosphate	Positive	25	Detect	215	19
Ethyl methylphosphonic acid	Negative	250	Detect	176	42
Ethyl methylphosphonic acid	Positive	250	Detect	179	39
Isopropyl methylphosphonic acid	Negative	250	Detect	148	32
Isopropyl methylphosphonic acid	Positive	250	Detect	183	24
Methylphosphonic acid	Negative	250	Detect	120	64
Methylphosphonic acid	Positive	250	Detect	362	14
Pinacolyl methylphosphonic acid	Negative	250	65	131	28
Pinacolyl methylphosphonic acid	Positive	250	72	134	37
DIMP-D14 (Surrogate)	Positive	25	63	130	45
PMPA-13C6 (Surrogate)	Negative	250	74	138	34
MPA-D3 (Surrogate)	Negative	250	Detect	149	35
MPA-D3 (Surrogate)	Positive	250	Detect	305	26

sample. Under these circumstances, one of the following remedies must be employed: the matrix interference must be removed, all samples in the batch must be analyzed by a test method not affected by the matrix interference, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.8.6 The matrix spike/matrix spike duplicate (MS/MSD) limits in Table 7 were generated by five laboratories across the country using surface waters collected near their facilities. The matrix variation between the different surface waters may have a tendency to generate significantly wider control limits than those generated by a single laboratory in one surface water matrix. It is recommended that the laboratory generate their own in-house QC acceptance criteria which meets or exceeds the criteria in this standard.

12.8.6.1 The laboratory should generate their own in-house QC acceptance criteria after the analysis of 15–20 matrix spike samples of a particular surface water matrix. References on how to generate QC acceptance criteria are ASTM standards Practices D5847, D2777, E2554 or Method 8000B in EPA Publication SW-846 may be helpful.

12.9 *Duplicate:*

12.9.1 To check the precision of sample analyses, analyze a sample in duplicate with each batch of 20 or fewer samples. If the sample contains the analyte at a level greater than 5 times the detection limit of the test method, the sample and duplicate may be analyzed unspiked; otherwise, an MSD should be used.

12.9.2 Calculate the relative percent difference (RPD) between the duplicate values (or MS/MSD values) as shown in Eq 2. Compare to the RPD limit in Table 7. Relative percent difference:

$$RPD = \frac{|\ MSR - MSDR\ |}{(MSR + MSDR)/2} \times 100 \qquad (2)$$

where:

RPD = relative percent difference,
MSR = matrix spike recovery, and
MSDR = matrix spike duplicate recovery.

The vertical bars in Eq 2 indicate the absolute value of the difference, hence RPD is always expressed as a positive value.

12.9.3 If the result exceeds the precision limit, the batch must be re-analyzed or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

13. Procedure

13.1 The water samples are shipped chilled between 0°C and 6°C in ≥25 mL pre-cleaned amber glass bottles with Teflon-lined caps and stored in the laboratory between 0°C and 6°C. The samples must be analyzed within 1 day of collection. If the samples are above 6°C when received or during storage, or not analyzed within 1 day of collection the data is qualified estimated and noted in the case narrative that accompanies the data.

13.2 In the laboratory, a 25-mL Class A glass volumetric flask is used to measure a 25-mL aliquot of the sample. Every sample is then spiked with the surrogate as described in 12.5. The laboratory control and matrix spike samples are then spiked with the target compounds as described in 12.7 and 12.8. The samples are then shaken in order to mix the spike solutions throughout the water sample. The sample, the entire 25-mL volume, is filtered through the filtration device described in 7.2. An aliquot of that filtered sample is placed into 2-mL amber glass LC vials for analysis.

13.3 Once a passing calibration curve is generated the analysis of samples may begin. An order of analysis may be; method blank(s), laboratory control sample(s), sample(s), duplicate(s), matrix spike sample(s) followed by an end calibration check standard.

14. Calculation or Interpretation of Results

14.1 For quantitative analysis of the organophosphonates and surrogates, the SRM transitions are identified by comparison of retention times in the sample to those of the standards. External calibration curves are used to calculate the amounts of DIMP, EHDMAP, EMPA, IMPA, MPA and PMPA, and MPA-D3, PMPA-13C6 and DIMP-D14 surrogates. Calculate the concentration in µg/L (ppb) for each analyte. The individual organophosphonates may be reported if present at or above the reporting limit. If the concentration of the analyte is determined to be above the calibration range, the sample is diluted with reagent water to obtain a concentration near the mid-point of the calibration range and re-analyzed.

14.2 DIMP and PMPA are separated by the LC conditions in this test method. If there is overlap encountered due to poor chromatography which may be caused by a variation in elution solvent concentrations or column degradation this is not a concern if the analyte specific SRM transitions are employed. DIMP and PMPA have a same molecular weight parent ion which produces the same product fragment under certain conditions. DIMP does not produce a 179.2 m/z parent ion in the ESI negative mode. Therefore, the optimum transition for PMPA analysis is using the ESI negative mode using 179.2 > 94.8. DIMP in the ESI positive mode does produce a 181.3 m/z parent ion which is the same m/z as the PMPA parent ion. However, the DIMP parent ion produces a 139.1 m/z product ion by loss of one isopropyl moiety whereas PMPA is not able to lose that fragment. Therefore the optimum SRM transition for DIMP is in the ESI positive mode using 181.3 > 139.1.

15. Report

15.1 Determine the results in units of µg/L (ppb) in a water sample. Calculate the concentration in the sample using the linear or quadratic calibration curve generated. All data that does not meet the specifications in the test method must be appropriately qualified.

16. Precision and Bias[11]

16.1 The determination of precision and bias was conducted through EPA and generated applicable data to determine the precision and bias as described in Practice D2777.

[11] Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR:D19-1187. Contact ASTM Customer Service at service@astm.org.

16.2 This test method was tested by US EPA Region 5 Chicago Regional Laboratory (CRL) on reagent water. The samples were spiked with target compounds and surrogates as described in Section 12. Tables 8 and 9 contain the recoveries and standard deviation (SD) for the surrogates and target compounds.

16.3 This test method was tested by US EPA Region 5 Chicago Regional Laboratory (CRL) on Chicago River water. The samples were spiked with target compounds and surrogates as described in Section 12. Tables 10 and 11 contain the recoveries and standard deviation (SD) for the surrogates and target compounds.

16.4 *Multi-Laboratory Validation*—This test method has been tested by six laboratories using reagent water and up to five local surface waters. The incorporation of the testing laboratory's individual local surface water was chosen to validate the test method using various surface water matrices. The surface waters were from California, Mississippi, Illinois, Massachusetts and Virginia. The reagent and local surface waters were spiked across the reporting range in quadruplicate for reagent water and duplicate for surface water. The multi-laboratory data for reagent water is shown in Table 3 and for surface waters in Table 12. Results of this collaborative study may not be typical of the results for matrices other than those studied. Grubbs' outliers were removed.

17. Quality Control

17.1 A crucial part of a test method is quality control. A laboratory should follow their in-house QA/QC procedures and should meet or exceed the criteria given in this test method. The quality-control criteria are given in the various test method sections. Section 10 contains the sampling and preservation requirements and Section 12 contains the majority of quality control requirements when following this test method. Section 12 includes requirements for calibration, precision and bias study to demonstrate laboratory capability, initial demonstration of performance, surrogate, method blank, reporting limit check, laboratory control, matrix spike and duplicate sample requirements. An IRM should be incorporated into the analysis periodically to verify that standard concentrations are comparable between sources. The IRM criteria should be based upon the laboratories QA/QC policies and the individual data quality objectives.

18. Keywords

18.1 liquid chromatography; mass spectrometry; organophosphonates

TABLE 8 Single-Laboratory Recovery Data in Reagent Water ESI Positive Mode

Precision and Accuracy Samples (ESI+)	Measured ppb from 250 ppb Spikes					Measured ppb from 25 ppb Spikes		
	MPA	MPA-D3	EMPA	IMPA	PMPA	EHDMAP	DIMP	DIMP-D14
Method Blank	ND	234.9	ND	ND	ND	ND	ND	26.5
1	223.5	241.9	236.8	244.4	254.9	21.5	26.0	26.0
2	235.5	241.8	251.0	253.7	268.5	22.0	25.7	25.7
3	221.2	238.9	247.1	252.5	268.2	22.0	26.8	26.2
4	219.2	233.8	252.8	260.6	270.1	22.0	26.7	25.1
Average Recovery	224.9	238.3	246.9	252.8	265.4	21.9	26.3	25.9
Average Percent Recovery	89 %	95.3 %	98.8 %	101.1 %	106.2 %	87.5 %	105.2 %	103.6 %
Standard Deviation (SD)	7.31	3.79	7.16	6.64	7.07	0.25	0.54	0.53
% Relative SD	3.3 %	1.6 %	2.9 %	2.6 %	2.7 %	1.1 %	2.0 %	2.1 %

TABLE 9 Single-Laboratory Recovery Data in Reagent Water ESI Negative Mode

Precision and Accuracy Samples (ESI–)	Measured ppb from 250 ppb Spikes						Measured ppb from 25 ppb Spikes
	MPA	MPA-D3	EMPA	IMPA	PMPA	PMPA-13C6	EHDMAP
Method Blank	ND	230.6	ND	ND	ND	252.6	ND
1	227.0	246.2	246.7	246.0	249.3	253.5	22.8
2	223.0	234.8	252.9	243.9	253.1	244.6	21.7
3	234.2	240.2	258.2	249.0	259.4	246.3	21.2
4	244.8	242.4	264.2	253.1	268.0	254.4	23.6
Average Recovery	232.3	238.8	255.5	248.0	257.5	250.3	22.3
Average Percent Recovery	92.9 %	95.5 %	102.2 %	99.2 %	103.0 %	100.1 %	89.3 %
Standard Deviation (SD)	9.56	6.18	7.47	3.99	8.17	4.50	1.08
% Relative SD	4.1 %	2.6 %	2.9 %	1.6 %	3.2 %	1.8 %	4.8 %

TABLE 10 Single-Laboratory Recovery Data in Chicago River Water ESI Positive Mode

Precision and Accuracy Samples (ESI+)	Measured ppb from 250 ppb Spikes					Measured ppb from 25 ppb Spikes		
	MPA	MPA-D3	EMPA	IMPA	PMPA	EHDMAP	DIMP	DIMP-D14
Blank Chicago River Water	ND	353.2	ND	ND	ND	ND	ND	26.3
1	353.2	300.3	253.8	244.7	275.0	21.2	26.3	26.1
2	344.1	293.6	250.1	246.2	268.5	20.9	26.5	25.5
3	365.0	305.0	244.8	215.6	266.8	21.0	25.4	25.0
4	348.5	291.7	248.0	239.7	271.1	20.6	26.0	25.9
Average Recovery	352.7	308.8	249.2	236.6	270.4	20.9	26.1	25.8
Average Percent Recovery	141.1 %	123.5 %	99.7 %	94.6 %	108.1 %	83.7 %	104.2 %	103.0 %
Standard Deviation (SD)	9.00	25.40	3.78	14.24	3.57	0.25	0.48	0.52
% Relative SD	2.6 %	8.2 %	1.5 %	6.0 %	1.3 %	1.2 %	1.8 %	2.0 %

TABLE 11 Single-Laboratory Recovery Data in Chicago River Water ESI Negative Mode

Precision and Accuracy Samples (ESI–)	Measured ppb from 250 ppb Spikes						Measured ppb from 25 ppb Spikes
	MPA	MPA-D3	EMPA	IMPA	PMPA	PMPA-13C6	EHDMAP
Blank Chicago River Water	ND	41.5	ND	ND	ND	260.8	ND
1	ND	42.7	265.0	266.6	274.5	265.4	21.0
2	ND	44.1	268.8	268.9	274.8	265.9	19.9
3	ND	43.4	271.1	253.6	277.4	263.7	21.8
4	ND	44.3	273.7	254.2	276.2	268.9	22.0
Average Recovery	—	43.2	269.7	260.8	275.7	264.9	21.2
Average Percent Recovery	—	17.3 %	107.9 %	104.3 %	110.3 %	106.0 %	84.7 %
Standard Deviation (SD)	—	1.14	3.69	8.05	1.34	2.98	0.95
% Relative SD	—	2.6 %	1.4 %	3.1 %	0.5 %	1.1 %	4.5 %

TABLE 12 Multi-Laboratory Recovery Data in Surface 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	8	4	80.0	31.6	130.0	8.3	18.4	34.5	43.1
DIMP	Pos	10	8	4	95.4	81.8	131.0	8.3	7.3	16.8	17.6
DIMP	Pos	25	10	5	97.5	73.2	125.2	7.9	7.1	15.3	15.7
DIMP	Pos	125	8	4	93.6	70.2	115.8	4.9	4.8	15.6	16.7
DIMP-D14	Pos	25	26	4	92.7	58.8	118.0	14.2	15.6	13.8	14.9
DIMP-D14	Pos	50	8	1	108.8	80.2	126.8	16.9	15.5	16.9	15.5
EHDMAP	Neg	5	6	3	67.8	0.0	140.4	21.9	31.4	62.7	92.4
EHDMAP	Neg	10	6	3	68.0	0.0	133.2	8.3	6.8	56.3	82.9
EHDMAP	Neg	25	8	4	80.9	35.6	123.0	10.5	19.4	27.0	33.4
EHDMAP	Neg	125	6	3	79.9	48.6	103.2	8.0	12.5	19.9	24.9
EHDMAP	Pos	5	8	4	112.5	74.0	154.6	3.9	4.6	37.7	33.5
EHDMAP	Pos	10	8	4	116.9	59.0	163.0	8.0	10.4	40.2	34.3
EHDMAP	Pos	25	10	5	105.7	47.2	153.6	5.5	9.2	38.5	36.4
EHDMAP	Pos	125	8	4	97.0	54.3	123.2	3.6	5.8	25.7	26.5
EMPA	Neg	50	8	4	60.0	18.2	92.8	4.1	7.4	33.1	55.2
EMPA	Neg	100	8	4	64.3	24.4	95.3	2.6	4.1	29.6	46.0
EMPA	Neg	250	10	5	84.5	25.5	120.4	9.7	10.8	33.6	39.7
EMPA	Neg	1250	8	4	66.1	21.8	104.6	8.1	24.0	37.1	56.1
EMPA	Pos	50	8	4	83.0	0.0	137.6	3.4	3.2	54.2	65.3
EMPA	Pos	100	8	4	86.2	16.8	140.2	7.0	5.5	45.4	52.7
EMPA	Pos	250	10	5	87.4	25.3	127.0	9.3	8.5	33.5	38.4
EMPA	Pos	1250	8	4	89.3	31.4	125.0	11.8	10.9	37.2	41.7
IMPA	Neg	50	8	4	44.9	8.3	88.2	6.6	10.6	27.3	60.8
IMPA	Neg	100	8	4	47.6	18.1	69.7	6.3	15.1	19.7	41.3
IMPA	Neg	250	10	5	61.1	17.8	107.6	5.3	15.6	30.6	50.1
IMPA	Neg	1250	8	4	49.3	26.2	73.1	6.6	13.3	18.1	36.6
IMPA	Pos	50	8	4	68.7	0.0	124.6	3.9	4.0	49.3	71.7
IMPA	Pos	100	8	4	67.2	14.3	122.0	3.5	10.0	43.1	64.2
IMPA	Pos	250	10	5	74.4	20.3	124.4	4.8	9.3	37.9	51.0
IMPA	Pos	1250	8	4	74.7	24.8	124.8	6.1	10.0	39.9	53.4
MPA	Neg	50	8	4	24.2	0.0	98.0	6.9	7.8	40.1	165.6
MPA	Neg	100	8	4	16.1	0.0	66.9	7.9	16.2	25.5	158.1
MPA	Neg	250	10	5	10.8	0.0	44.8	1.9	11.6	17.4	162.3
MPA	Neg	1250	8	4	28.7	9.4	51.7	1.3	6.9	18.9	65.8
MPA	Pos	50	8	4	113.1	0.0	246.0	19.1	8.6	121.8	107.7
MPA	Pos	100	8	4	123.3	0.0	227.0	7.1	7.3	102.5	83.1
MPA	Pos	250	10	5	121.3	6.3	221.2	4.6	13.8	83.6	68.9
MPA	Pos	1250	8	4	111.9	25.2	253.6	16.5	7.5	86.6	77.3
MPA-D3	Neg	250	34	5	43.8	5.5	106.0	5.3	9.6	36.6	83.4
MPA-D3	Pos	250	26	4	90.8	18.9	193.2	7.9	11.1	66.8	73.6
PMPA	Neg	50	8	4	76.3	48.1	110.6	2.8	4.6	23.2	30.3
PMPA	Neg	100	8	4	91.6	77.7	121.5	7.6	6.9	14.7	16.0
PMPA	Neg	250	10	5	97.9	76.8	120.4	7.4	6.9	13.4	13.7
PMPA	Neg	1250	8	4	105.3	89.6	128.9	8.3	7.1	14.7	13.9
PMPA	Pos	50	6	3	81.8	38.4	138.8	2.0	4.5	45.2	55.2
PMPA	Pos	100	6	3	99.3	79.6	144.0	12.2	10.1	25.5	25.7
PMPA	Pos	250	8	4	103.0	88.0	128.7	9.7	8.5	12.6	12.2
PMPA	Pos	1250	5	2	104.9	88.0	119.8	4.6	4.2	14.6	13.9
PMPA-13C6	Neg	250	34	5	106.1	77.2	134.5	12.3	10.9	15.1	14.2