Designation: E2866 – 21

Standard Test Method for Determination of Diisopropyl Methylphosphonate, Ethyl Methylphosphonic Acid, Isopropyl Methylphosphonic Acid, Methylphosphonic Acid, and Pinacolyl Methylphosphonic Acid in Soil by Pressurized Fluid Extraction and Analyzed by Liquid Chromatography/Tandem Mass Spectrometry[1]

This standard is issued under the fixed designation E2866; 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 Methylphosphonic Acid (EMPA), Isopropyl Methylphosphonic Acid (IMPA), Methylphosphonic Acid (MPA), and Pinacolyl Methylphosphonic Acid (PMPA), referred to collectively as organophosphonates (OPs) in this test method, in soil. This method is based upon solvent extraction of a soil by pressurized fluid extraction (PFE). The extract is filtered and analyzed by liquid chromatography/tandem mass spectrometry (LC/MS/MS). OPs are qualitatively and quantitatively determined by this method.

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

1.3 The method detection limit[2] (MDL), electrospray ionization (ESI) mode, and reporting range[3] for the OPs are listed in Table 1.

1.4 *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.*

1.5 *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:*[4]

D1193 Specification for Reagent Water

D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water

D5681 Terminology for Waste and Waste Management

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[5]

40 CFR Part 136, Appendix B The Code of Federal Regulations[6]

3. Terminology

3.1 *Definitions*—For definitions of terms used in this test method, refer to Terminology D5681.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *analytical column, n*—the particles of the solid stationary phase fill the whole inside volume of a tube (column)

[1] This test method is under the jurisdiction of ASTM Committee D34 on Waste Management and is the direct responsibility of Subcommittee D34.01.06 on Analytical Methods.

Current edition approved May 1, 2021. Published May 2021. Originally approved in 2012. Last previous edition approved in 2016 as E2866 – 12 (2016). DOI: 10.1520/E2866-21.

[2] The MDL is determined following the Code of Federal Regulations, 40 CFR Part 136, Appendix B utilizing solvent extraction of soil by PFE. A detailed process determining the MDL is explained in the reference and is beyond the scope of this standard to be explained here.

[3] Reporting range concentrations are calculated from Table 4 concentrations assuming a 100 µL injection of the lowest and highest level calibration standards with a 40 mL final extract volume of a 10 g soil sample. Volume variations will change the reporting limit and ranges. The reporting limit (RL), lowest concentration of the reporting range, is calculated from the concentration of the Level 1 calibration standard as shown in Table 4.

[4] 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.

[5] 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.

[6] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, http://www.access.gpo.gov.

TABLE 1 Method Detection Limit and Reporting Range

Analyte	ESI Mode	MDL (PPB)	Reporting Range (PPB)
Diisopropyl methylphosphonate	Positive	2.7	40–2000
Ethyl methylphosphonic acid	Negative	2.3	40–2000
Ethyl methylphosphonic acid	Positive	1.3	40–2000
Isopropyl methylphosphonic acid	Negative	5.7	40–2000
Isopropyl methylphosphonic acid	Positive	2.8	40–2000
Methylphosphonic acid	Positive	8.7	40–2000
Pinacolyl methylphosphonic acid	Negative	5.3	40–2000

that the mobile phase passes through using the pressure generated by the liquid chromatography system.

3.2.2 *filter unit, n*—in this standard, a filter that is supported with an inert housing to the solvents as described in Section 7 of this standard.

3.2.3 *filtration device, n*—a device used to remove particles from the extract that may clog the liquid chromatography system. Described in 7.3 of this standard.

3.2.4 *glass fiber filter, n*—a porous glass fiber material onto which solid particles present in the extraction fluid, which flows through it, are largely caught and retained, thus removing them from the extract.

3.2.5 *hypodermic syringe, n*—in this standard, a luer-lock-tipped glass syringe capable of holding a syringe-driven filter unit as described in 7.3 of this standard.

3.2.6 *liquid chromatography (LC) system, n*—in this standard, a separation system using liquid as the mobile phase and a stationary phase packed into a column. The use of small particles packed inside a column and a high inlet pressure enables the separation of components in a mixture.

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

3.2.8 *pressurized fluid extraction, n*—the process of transferring the analytes of interest from the solid matrix, a soil, into the extraction solvent using pressure and elevated temperature.

3.2.9 *reporting range, n*—the quantitative concentration range for an analyte in this standard.

3.2.10 *tandem mass spectrometer, n*—an arrangement in which ions are subjected to two sequential stages of analysis according to the quotient mass/charge.

3.3 *Abbreviations:*

3.3.1 *DIMP*—diisopropyl methylphosphonate

3.3.2 *EMPA*—ethyl methylphosphonic acid

3.3.3 *IMPA*—isopropyl methylphosphonic acid

3.3.4 *LC*—liquid chromatography

3.3.5 *LCS/LCSD*—laboratory control spike/laboratory control spike duplicate

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

3.3.7 *MPA*—methylphosphonic acid

3.3.8 *MRM*—multiple reaction monitoring

3.3.9 *MS*—matrix spike

3.3.10 *NA*—not applicable

3.3.11 *ND*—non-detect

3.3.12 *PFE*—pressurized fluid extraction

3.3.13 *PMPA*—pinacolyl methylphosphonic acid

3.3.14 *PPB*—parts per billion

3.3.15 *QC*—quality control

3.3.16 *SD*—standard deviation

3.3.17 *SRM*—single reaction monitoring

3.3.18 *VOA*—volatile organic analysis

4. Summary of Test Method

4.1 For OPs soil analysis, samples are shipped to the lab between 0 °C and 6 °C. The samples are to be extracted, filtered, and analyzed by LC/MS/MS within seven days of collection.

4.2 The OPs 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 This is a performance-based method, and modifications are allowed to improve performance.

5.1.1 Due to the rapid development of newer instrumentation and column chemistries, changes to the analysis described in this standard are allowed as long as better or equivalent performance data result. Any modifications shall be documented and performance data generated. The user of the data generated by this standard shall be made aware of these changes and given the performance data demonstrating better or equivalent performance.

5.2 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 were 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.[7,8]

5.3 This test method is for the analysis of selected organophosphorous based pesticide degradation products.

5.4 This method has been investigated for use with various soils.

[7] Additional information about organophosphate pesticides is available at http://www.epa.gov (2011).

[8] Additional information about chemical weapon agents is available at http://www.opcw.org (2011).

6. Interferences

6.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other apparatus producing discrete artifacts or elevated baselines. All of these materials are demonstrated to be free from interferences by analyzing laboratory reagent blanks under the same conditions as samples.

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

6.3 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*[9]—A complete LC system is required in order to analyze samples. An LC system that is capable of performing at the flows, pressures, controlled temperatures, sample volumes, and requirements of the standard shall be used.

7.1.2 *Analytical Column*[10]—A column that achieves adequate resolution shall be used. The retention times and order of elution may change depending on the column used and need to be monitored. A reverse-phase analytical column that combines the desirable characteristics of a reversed-phase HPLC column with the ability to separate polar compounds was used to develop this test method. MPA elutes early in the chromatograph, at approximately 2 min, which is just beyond the instrument void volume of 1.5 min. A column is required that elutes MPA after the instrument void volume.

7.1.3 *Tandem Mass Spectrometer (MS/MS) System*[11]—An MS/MS system capable of multiple reaction monitoring (MRM) analysis or any system that is capable of performing at the requirements in this standard shall be used.

7.2 *Pressurized Fluid Extraction Device (PFE):*[12]

7.2.1 A PFE system was used for this test method with appropriately sized extraction cells. Cells are available that will accommodate the 10 g sample sizes used in this test method. Cells shall be made of stainless steel or other material capable of withstanding the pressure requirements (≥2000 psi) necessary for this procedure. A pressurized fluid extraction device shall be used that can meet the necessary requirements in this test method.

7.2.2 *Glass Fiber Filters.*[13]

7.2.3 *Amber VOA Vials*—40 mL for sample extracts and 60 mL for PFE.

7.3 *Filtration Device:*

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

7.3.1.1 A 50 mL lock-tip glass syringe size is recommended since a 40 mL sample extract may result.

7.3.2 *Filter Unit*[14]—Filter units of polyvinylidene fluoride (PVDF) were used to filter the PFE extracts.

7.3.2.1 A filter unit that meets the requirements of the test method shall 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.[15] 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 mean reagent water conforming to ASTM Type I 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*—Nitrogen (purity ≥97 %) and argon (purity ≥99.999 %).

8.4 Acetonitrile (CH_3CN, CAS No. 75-05-8).

8.5 2-Propanol (C_3H_8O, CAS No. 67-63-0).

8.6 Methanol (CH_3OH, CAS No. 67-56-1).

8.7 Formic acid (HCO_2H, ≥95 %, CAS No. 64-18-6).

8.8 Diisopropyl methylphosphonate ($C_7H_{17}O_3P$, DIMP, CAS No. 1445-75-6).

8.9 Ethyl methylphosphonic acid ($C_3H_9O_3P$, EMPA, CAS No. 1832-53-7).

8.10 Isopropyl methylphosphonic acid ($C_4H_{11}O_3P$, IMPA, CAS No. 1832-54-8).

[9] A Waters Acquity UPLC H-Class System was used to develop this test method and generate the precision and bias data presented in Section 16. Any HPLC system that produces results that meet or exceed the performance criteria of this test method may be used.

[10] A Waters-Atlantis dC18, 150 mm by 2.1 mm, 3 µm particle size, was used to develop this test method and generate the precision and bias data presented in Section 16. Any column that produces results that meet or exceed the performance criteria of this test method may be used.

[11] A Waters Quattro micro API mass spectrometer was used to develop this test method and generate the precision and bias data presented in Section 16. Any mass spectrometer that produces results that meet or exceed the performance criteria of this test method may be used.

[12] A Dionex Accelerated Solvent Extraction (ASE 200) system with appropriately sized extraction cells was used to develop this test method and generate the precision and bias data presented in Section 16. Any extraction system that produces results that meet or exceed the performance criteria of this test method may be used.

[13] Whatman Glass Fiber Filters 19.8 mm, Part No. 047017, specially designed for the PFE system,[12] were used to develop this test method and generate the precision and bias data presented in Section 16. Any filter that produces results that meet or exceed the performance criteria of this test method may be used.

[14] Millex-GV Syringe-Driven Filter Units PVDF 0.22 µm (Catalog No. SLGV033NS) were used to develop this test method and generate the precision and bias data presented in Section 16. Any filter that produces results that meet or exceed the performance criteria of this test method may be used.

[15] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, D.C. 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 Methylphosphonic acid (CH_5O_3P, MPA, CAS No. 993-13-5).

8.12 Pinacolyl methylphosphonic acid ($C_7H_{17}O_3P$, PMPA, CAS No. 616-52-4).

8.13 Diisopropyl methylphosphonate-D_{14} ($C_7H_3D_{14}O_3P$, DIMP-D_{14}, unlabeled CAS No. 1445-75-6).

8.13.1 DIMP-D_{14} represents deuterium labeled diisopropyl methylphosphonate where the two isopropyl moieties contain all ^2H.

8.14 Methylphosphonic acid-D_3 ($CH_2D_3O_3P$, MPA-D_3, unlabeled CAS No. 993-13-5).

8.14.1 MPA-D_3 represents deuterium labeled methylphosphonic acid where the methyl moiety contains all ^2H.

8.15 Pinacolyl methylphosphonic acid-$^{13}C_6$ ($C_7H_{17}O_3P$, PMPA-$^{13}C_6$, unlabeled CAS No. 616-52-4).

8.15.1 PMPA-$^{13}C_6$ represents ^{13}C labeled pinacolyl methylphosphonic acid where all the trimethylpropyl carbon atoms are uniformly labeled ^{13}C.

8.16 Ottawa sand (CAS No. 14808-60-7) or equivalent.

8.17 Drying agent.[16]

9. Hazards

9.1 Normal laboratory safety applies to this method. Analysts shall wear safety glasses, gloves, and lab coats when working in the lab. Analysts shall review the Safety Data Sheets (SDS) for all reagents used in this test method and shall be fully trained to perform this test method.

10. Glassware Washing, Sampling, and Preservation

10.1 *Glassware Washing*—All glassware is washed in hot tap water with a detergent and rinsed in hot water conforming to ASTM Type I of Specification D1193. The glassware is then dried and heated in an oven at 250 °C for 15 to 30 min. All glassware is subsequently cleaned with acetone and methanol, respectively.

10.2 *Sampling*—Grab samples must be collected in precleaned glass jars with polytetrafluoroethylene (PTFE) lined caps demonstrated to be free of interferences. This test method requires at least a 10 g sample size per analysis. A 100 g sample amount should be collected to allow for quality control samples and re-analysis. Field blanks are needed to follow conventional sampling practices.

10.3 *Preservation*—Store samples between 0 °C and 6 °C from the time of collection until analysis. Analyze the samples within seven days of collection. If the samples are above 6 °C when received or during storage or not analyzed within seven days of collection, the data are qualified, estimated, and noted in the case narrative that accompanies the data.

[16] Varian – Chem Tube – Hydromatrix, 1 kg (Part No. 198003) was used to develop this test method and generate the precision and bias data presented in Section 16 by recommendation of the PFE manufacturer. (Note: Some drying agents have been shown to clog PFE transfer lines.) Any drying agent that produces results that meet or exceed the performance criteria of this test method may be used.

TABLE 2 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. Preparation of LC/MS/MS

11.1 *LC Operating Conditions Used to Develop This Test Method:*[9]

11.1.1 Injection volumes of all calibration standards and samples are 100 µL and are composed of primarily water. 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 2.

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

11.1.3 *Wash and Purge Solvent*—60 % Acetonitrile/40 % 2-Propanol; Pre- and post-inject wash solvent: 6 s.

11.1.4 Specific instrument manufacturer wash and purge specifications shall be followed in order to eliminate sample carry-over in the analysis.

11.2 *Mass Spectrometer Parameters:*[11]

11.2.1 To acquire the maximum number of data points per SRM channel while maintaining adequate sensitivity, the tune parameters shall be optimized according to the instrument. Each peak requires at least ten scans per peak for adequate quantitation. This test method contains five target compounds and three surrogates which are in different SRM experiment windows in order to optimize the number of scans and sensitivity. Variable parameters regarding retention times, SRM transitions, and cone and collision energies are shown in Table 3. Mass spectrometer parameters used in the development of this method are listed below:

The instrument is set in the Electrospray source setting.
Capillary Voltage: 3.5 kV
Cone: Variable depending on analyte (Table 3)
Extractor: 2 V
RF Lens: 0.2 V
Source Temperature: 120 °C
Desolvation Temperature: 300 °C
Desolvation Gas Flow: 700 L/h
Cone Gas Flow: 25 L/h
Low Mass Resolution 1: 14.0
High Mass Resolution 1: 14.0
Ion Energy 1: 0.8 V
Entrance Energy: –1 V
Collision Energy: Variable depending on analyte (Table 3)
Exit Energy: 2 V
Low Mass Resolution 2: 14
High Mass resolution 2: 14
Ion Energy 2: 1.0 V
Multiplier: 650 V
Gas Cell Pirani Gauge: 0.60 Pa
Inter-Channel Delay: 0.02 s
Inter-Scan Delay: 0.1 s if acquiring in one ESI mode, 0.4 s if acquiring in both.
Repeats: 1

TABLE 3 Retention Times, SRM Transitions, 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	8.8	181.2 > 139.1	25	6
Ethyl methylphosphonic acid	Negative	3.6	123.0 > 94.9	30	12
Ethyl methylphosphonic acid	Positive	3.6	125.0 > 96.9	25	10
Isopropyl methylphosphonic acid	Negative	7.5	137.0 > 94.9	28	13
Isopropyl methylphosphonic acid	Positive	7.5	139.1 > 96.9	25	7
Methylphosphonic acid	Positive	2.0	96.9 > 78.8	45	15
Pinacolyl methylphosphonic acid	Negative	8.6	179.1 > 94.9	35	18
DIMP-D_{14} (Surrogate)	Positive	8.8	195.2 > 147.1	23	7
PMPA-$^{13}C_6$ (Surrogate)	Negative	8.6	185.1 > 94.9	35	18
MPA-D_3 (Surrogate)	Positive	2.0	99.9 > 81.8	40	15

Span: 0 Daltons
Dwell: 0.1 s

12. Calibration and Standardization

12.1 The mass spectrometer shall be calibrated per manufacturer specifications before analysis. In order to obtain valid and accurate analytical values within the confidence limits, the following procedures shall be followed when performing the test method.

12.2 *Calibration and Standardization*—To calibrate the instrument, analyze eight calibration standards containing the eight concentration levels of the organophosphonates and surrogates prior to analysis as shown in Table 4. A calibration stock standard solution is prepared from standard materials or purchased as certified solutions. Stock standard Solution A (Level 8) containing the organophosphonates diisopropyl methylphosphonate-D_{14}, pinacolyl methylphosphonic acid-$^{13}C_6$, and methylphosphonic acid-D_3 is prepared at Level 8 concentration and aliquots of that solution are diluted to prepare Levels 1 through 7. The following steps will produce standards with the concentration values shown in Table 4. The analyst is responsible for recording initial component weights carefully when working with pure materials and correctly carrying the weights through the dilution calculations. Calibration standards are not filtered.

12.2.1 Prepare stock standard Solution A (Level 8) by adding to a 50 mL volumetric flask individual methanol solutions of the following: 250 µL of 100 µg/mL solutions of DIMP, EMPA, IMPA, MPA, PMPA, MPA-D_3, and PMPA-$^{13}C_6$, and 25 µL of 1000 µg/mL of DIMP-D_{14} and then dilute to 50 mL with water. The preparation of the Level 8 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 shall 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 at concentrations shown in Table 4; calibration standards are not filtered. The calibration standard vials shall be used within 24 h to ensure optimum results. Stock calibration standard solutions are replaced every 14 days if not previously discarded for quality control failure.

12.2.3 Inject each calibration standard and obtain its chromatogram. External calibration curves are generated from the calibration standards monitoring the SRM transition 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. The use of SRM transitions gives additional confirmation than by the selective ion monitoring technique because the parent ion is isolated and fragmented to the product ion.

12.2.4 The calibration software manual shall be consulted to use the software correctly. The quantitation method is set as an external calibration using the peak areas in ppb units. Concentrations may be calculated using the data system software to generate linear regression or quadratic calibration curves. Forcing the calibration curve through the origin is not recommended. Each calibration point used to generate the curve shall have a calculated percent deviation less than 30 % from the generated curve. Refer to 12.2.4.1 and 12.2.4.2 to determine if linear or quadratic calibration curves may be used.

12.2.4.1 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 shall be re-injected or a new calibration curve shall be regenerated. If the low or high point is excluded, or both, minimally a five-point curve is acceptable; the reporting range shall be modified to reflect this change.

12.2.4.2 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 shall be re-injected or a new calibration curve shall be regenerated. If the low or high point is excluded, or both, a six-point curve is acceptable using a quadratic fit. An initial eight-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 shall be modified to reflect this change.

12.2.5 The retention time window of the SRM transitions shall be within 5 % of the retention time of the analyte in a midpoint calibration standard. A midpoint calibration standard is defined at or between Levels 4 and 6 in Table 4 in this test method. 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 re-inject the sample. If the retention time is still incorrect in the sample, refer to the analyte as an unknown.

TABLE 4 Concentrations of Calibration Standards (PPB)

Analyte/Surrogate	LV 1	LV 2	LV 3	LV 4	LV 5	LV 6	LV 7	LV 8
Diisopropyl methylphosphonate	10	25	50	100	200	300	400	500
Ethyl methylphosphonic acid	10	25	50	100	200	300	400	500
Isopropyl methylphosphonic acid	10	25	50	100	200	300	400	500
Methylphosphonic acid	10	25	50	100	200	300	400	500
Pinacolyl methylphosphonic acid	10	25	50	100	200	300	400	500
DIMP-D_{14} (Surrogate)	10	25	50	100	200	300	400	500
PMPA-$^{13}C_6$ (Surrogate)	10	25	50	100	200	300	400	500
MPA-D_3 (Surrogate)	10	25	50	100	200	300	400	500

NOTE 1—The chromatographic peak shape for EMPA in the Nebraska soil was poor compared to the other soils tested. A blank soil, "unspiked soil," and matrix spike soils were analyzed for each soil type for quality control purposes. The EMPA peak shape in the calibration curve and Nebraska soil is shown in Fig. X1.1 in both the ESI positive and negative modes. Monitoring the SRM transition for EMPA in both the positive and negative electrospray modes resulted in similar chromatographic peak shape in the matrix spike sample. A comparison to an unspiked soil shall be made by the analyst to determine presence or absence of the target analyte in soils where chromatographic peak shape may be an issue. Data for EMPA and IMPA are collected in both the electrospray positive and negative modes providing more information for an analyst to make such a decision in those cases. The PMPA, DIMP, IMPA, and MPA chromatographic peak shapes were shown to be less affected by the various matrices tested.

12.2.6 A midpoint calibration check standard shall be analyzed at the end of each batch of 20 samples or within 24 h after the initial calibration curve was generated. This end calibration check shall be the same calibration standard that was used to generate the initial curve. The results from the end calibration check standard shall have a percent deviation less than 35 % from the calculated concentration for the target analytes and surrogates. If the results are not within these criteria, the problem shall be corrected and either all samples in the batch shall be re-analyzed against a new calibration curve or the affected results shall 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 shall be made and analyzed. If this new end calibration check standard has a percent deviation less than 35 % from the calculated concentration for the target analytes and surrogates, the results shall be reported unqualified if all other quality control parameters are acceptable.

12.3 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 or new instrument, perform a precision and bias study to demonstrate laboratory capability and verify that all technicians are adequately trained and follow relevant safety procedures. Refer to Practice D2777 as a guide.

12.3.1 Analyze at least four replicates of a sample containing the target compounds and surrogates at a concentration between 200 and 800 ppb in Ottawa sand. This test method was tested at 400 ppb. Each replicate shall be taken through the complete analytical test method including any sample preservation and pretreatment steps.

12.3.2 Calculate the mean (average) percent recovery and relative standard deviation (RSD) of the four values and compare to the acceptable ranges of the quality control (QC) acceptance criteria for the initial demonstration of performance in Table 5.

12.3.3 This study shall be repeated until the single-operator precision and mean recovery are within the limits in Table 5.

12.3.4 The QC acceptance criteria for the initial demonstration of performance in Table 5 are preliminary until a collaborative study is conducted. Single-laboratory data is shown in Section 16. The analyst shall be aware that the performance data generated from single-laboratory data tend to be significantly tighter than those generated from multi-laboratory data. The laboratory shall generate its own in-house QC acceptance criteria which meet or exceed the criteria in this test method. References on how to generate QC acceptance criteria are found in Practice E2554 or Method 8000B in EPA publication SW-846.

12.4 *Surrogate Spiking Solution:*

12.4.1 A surrogate standard solution containing MPA-D_3, PMPA-$^{13}C_6$, and DIMP-D_{14} is added to each 10 g soil sample. A stock surrogate spiking solution is prepared in methanol at 40 ppm for MPA-D_3, PMPA-$^{13}C_6$, and DIMP-D_{14}. The surrogates are added to each sample to achieve a concentration of 400 ppb (that is, 100 µL of a 40 ppm methanol solution containing MPA-D_3, PMPA-$^{13}C_6$, and DIMP-D_{14} is added to a 10 g soil sample). The result obtained for the surrogate recovery shall fall within the limits of Table 5. If the limits are not met, the affected results shall be qualified with an indication that they do not fall within the performance criteria of the test method.

12.5 *Method Blank:*

12.5.1 Analyze a sample blank, Ottawa sand, with each batch of 20 or fewer samples. The concentration of target analytes found in the blank must be three times below the reporting limit. If the concentration of target analytes is 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 shall be qualified with an indication that there is a blank contamination and report the concentration found in the blank sample.

12.6 *Laboratory Control Sample (LCS):*

12.6.1 To ensure that the test method is in control, analyze an LCS prepared with the OPs and surrogates at a concentration between 200 and 800 ppb. This test method was tested at 400 ppb. 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,

TABLE 5 Quality Control Acceptance Criteria (Test Concentration at 400 ppb)

Analyte	ESI Mode	Initial Demonstration of Performance			Lab Control Sample	
		Recovery (%)		Precision	Recovery (%)	
		Lower Limit	Upper Limit	Maximum % RSD	Lower Limit	Upper Limit
Diisopropyl methylphosphonate	+	70	130	30	70	130
Ethyl methylphosphonic acid	+	70	130	30	70	130
Ethyl methylphosphonic acid	-	70	130	30	70	130
Isopropyl methylphosphonic acid	+	70	130	30	70	130
Isopropyl methylphosphonic acid	-	70	130	30	70	130
Methylphosphonic acid	+	50	150	40	50	150
Pinacolyl methylphosphonic acid	-	70	130	30	70	130
DIMP-D_{14} (Surrogate)	+	70	130	30	70	130
PMPA-$^{13}C_6$ (Surrogate)	-	70	130	30	70	130
MPA-D_3 (Surrogate)	+	50	150	40	50	150

MPA, IMPA, DIMP, and PMPA each at 40 ppm. An Ottawa sand sample is spiked with the matrix spiking solution to achieve a concentration of 400 ppb (that is, 100 µL of a 40 ppm methanol solution containing of DIMP, EMPA, IMPA, MPA, and PMPA is added to a 10 g soil sample). The results obtained for the LCS shall fall within the limits in Table 5.

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

12.7 *Matrix Spike (MS):*

12.7.1 To check for interferences in the specific matrix being tested, perform an MS on at least one sample from each batch of ten or fewer samples. This is accomplished by spiking the sample with a known concentration of OPs and following the analytical method. Prepare a stock matrix spiking solution in methanol containing EMPA, MPA, IMPA, DIMP, and PMPA each at 40 ppm. Spiking 100 µL of this stock solution into 10 g of soil to yield a concentration of 400 ppb for EMPA, MPA, IMPA, PMPA, and DIMP in the soil.

12.7.2 If the spiked concentration plus the background concentration exceeds that of the Level 8 calibration standard, the sample shall be diluted to a level near the midpoint of the calibration curve.

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

$$P = 100\,\frac{|A(V_s+V) - BV_s|}{CV} \quad (1)$$

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.

12.7.4 The percent recovery of the spike shall fall within the limits in Table 6. If the percent recovery is not within these limits, a matrix interference may be present in the selected sample, a matrix suppression or enhancement of the response or extraction efficiency of the analyte, or both, may be poor in the soil matrix. The results shall be qualified with an indication that they do not fall within the performance criteria of the test method. The recoveries of OPs in the matrix spike samples are required for all data generated and shall accompany the analytical results due to the variation in recoveries in the various soil matrices as shown in Section 16. It has been demonstrated that in certain soil types, primarily clay, recoveries are low or, for MPA, less than the reporting limit (see Section 16).

12.7.4.1 Various extraction solvents and procedures were studied. The extraction procedures included PFE, sonication, and tumbling. The solvents included water, methanol, and acetonitrile in various combinations. The adjustment of pH was also investigated and included the use of ammonium hydroxide, acetic acid, and sodium hydroxide. Water was shown to produce the overall best results in these studies.

12.7.5 The matrix spike/matrix spike duplicate (MS/MSD) limits in Table 6 are from a single-lab study. Matrix spike recovery data for six different soils is included in Section 16. The matrix spike recovery data is variable amongst the soils tested. The matrix variation between different soils may tend to generate significantly wider control limits than those generated by a single laboratory in one soil matrix. It is recommended that the laboratory generate its own in-house QC acceptance criteria which meet or exceed the criteria shown in Table 6 in this test method.

12.7.5.1 The laboratory shall generate its own in-house QC acceptance criteria after the analysis of 15 to 20 matrix spike samples of a particular soil matrix. References on how to generate QC acceptance criteria are found in Practice E2554 or Method 8000B in EPA publication SW-846.

12.8 *Duplicate:*

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

12.8.2 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 6.

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

where:

RPD = relative percent difference,