Designation: ~~E2866 – 12~~ E2866 – 12 (Reapproved 2016)

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 and health practices and determine the applicability of regulatory limitations prior to use.*

[1] This test method is under the jurisdiction of ASTM Committee E54 on Homeland Security Applications and is the direct responsibility of Subcommittee E54.03 on Decontamination.

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[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 gram 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.

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

2. Referenced Documents

2.1 *ASTM Standards:*[4]

D653 Terminology Relating to Soil, Rock, and Contained Fluids

D1193 Specification for Reagent Water

D3694 Practices for Preparation of Sample Containers and for Preservation of Organic Constituents

D3740 Practice for Minimum Requirements for Agencies Engaged in Testing and/or Inspection of Soil and Rock as Used in Engineering Design and Construction

D3856 Guide for Management Systems in Laboratories Engaged in Analysis of Water

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 The Code of Federal Regulations, Appendix B[6]

3. Terminology

3.1 *Definitions:*

3.1.1 *analytical column, n*—the particles of the solid stationary phase fill the whole inside volume of a tube (column) that the mobile phase passes through using the pressure generated by the liquid chromatography system.

3.1.2 *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.

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

3.1.4 *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 ,~~ retained, thus removing them from the extract.

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

3.1.6 *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.

3.1.7 *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.

3.1.8 *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.

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

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

3.2 *Abbreviations:*

3.2.1 *DIMP*—diisopropyl methylphosphonate

3.2.2 *EMPA*—ethyl methylphosphonic acid

3.2.3 *IMPA*—isopropyl methylphosphonic acid

3.2.4 *LC*—liquid chromatography

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

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

3.2.7 *MPA*—methylphosphonic acid

3.2.8 *MRM*—multiple reaction monitoring

3.2.9 *MS*—matrix spike

3.2.10 *NA*—not applicable

[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.

3.2.11 *ND*—non-detect

3.2.12 *PFE*—pressurized fluid extraction

3.2.13 *PMPA*—pinacolyl methylphosphonic acid

3.2.14 *PPB*—parts per billion

3.2.15 *QC*—quality control

3.2.16 *SD*—standard deviation

3.2.17 *SRM*—single reaction monitoring

3.2.18 *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 7 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.

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*[9,10]—A complete LC system is required in order to analyze samples. A 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*[11,10]—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

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

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

[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. The sole source of supply known to the committee at this time is Waters Corporation, Milford, MA 01757.

[10] If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

[11] A Waters-Atlantis® dC18, 150 mm × 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. The sole source of supply known to the committee at this time is Waters Corporation, Milford, MA 01757.

this test method. MPA elutes early in the chromatograph, at approximately 2 minutes, which is just beyond the instrument void volume of 1.5 minutes. A column is required that elutes MPA after the instrument void volume.

7.1.3 *Tandem Mass Spectrometer (MS/MS) System*[12,10]—A 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)*[13,10]:

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.*[14,10]

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*[15,10]—Filter units of polyvinylidene fluoride (PVDF) were used to filter the PFE extracts.

7.3.2.1 *Discussion*—A filter unit shall be used that meets the requirements of the test method.

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.[16] 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 # 75-05-8).

8.5 2-Propanol (C_3H_8O, CAS # 67-63-0).

8.6 Methanol (CH_3OH, CAS # 67-56-1).

8.7 Formic Acid (HCO_2H, ≥95%, CAS # 64-18-6).

8.8 Diisopropyl Methylphosphonate ($C_7H_{17}O_3P$, DIMP, CAS # 1445-75-6).

8.9 Ethyl Methylphosphonic Acid ($C_3H_9O_3P$, EMPA, CAS # 1832-53-7).

8.10 Isopropyl Methylphosphonic Acid ($C_4H_{11}O_3P$, IMPA, CAS # 1832-54-8).

8.11 Methylphosphonic Acid (CH_5O_3P, MPA, CAS # 993-13-5).

8.12 Pinacolyl Methylphosphonic Acid ($C_7H_{17}O_3P$, PMPA, CAS # 616-52-4).

8.13 Diisopropyl Methylphosphonate-D_{14} ($C_7H_3D_{14}O_3P$, DIMP-D_{14}, Unlabeled CAS # 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 # 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 # 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.

[12] 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. The sole source of supply known to the committee at this time is Waters Corporation, Milford, MA 01757.

[13] 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. The sole source of supply known to the committee at this time is Dionex Corporation, Sunnyvale, CA 94088.

[14] Whatman Glass Fiber Filters 19.8 mm, Part # 047017, specially designed for the PFE system,[13] were used to develop this test method and generate the precision and bias data presented in Section 16. The sole source of supply known to the committee at this time is Dionex Corporation, Sunnyvale, CA 94088.

[15] Millex®-GV Syringe Driven Filter Units PVDF 0.22 µm (Catalog # SLGV033NS) were used to develop this test method and generate the precision and bias data presented in Section 16. The sole source of supply known to the committee at this time is Millipore Corporation.

[16] *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 *Annual Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulators*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

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

8.16 Ottawa Sand (CAS # 14808-60-7) or equivalent.

8.17 Drying Agent.[17,10]

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 Material Safety Data Sheets (MSDS) 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 minutes. All glassware is subsequently cleaned with acetone and methanol, respectively.

10.2 *Sampling*—Grab samples must be collected in pre-cleaned 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 7 days of collection. If the samples are above 6°C when received or during storage or not analyzed within 7 days of collection, the data are qualified estimated and noted in the case narrative that accompanies the data.

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 Seconds.

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*[12]:

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 10 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

[17] Varian – Chem Tube – Hydromatrix®, 1kg (Part # 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. The sole source of supply known to the committee at this time is Agilent Technologies, Inc., 5301 Stevens Creek Blvd., Santa Clara, CA 95051. (Note: Some drying agents have been shown to clog PFE transfer lines.)

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

Desolvation Gas Flow: 700 L/hr
Cone Gas Flow: 25 L/hr
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
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 hours 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