Designation: D8018 – 15

Standard Test Method for Determination of (Tri-n-butyl)-n-tetradecylphosphonium chloride (TTPC) in Soil by Multiple Reaction Monitoring Liquid Chromatography/Mass Spectrometry (LC/MS/MS)[1]

This standard is issued under the fixed designation D8018; 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 (Tri-n-butyl)-n-tetradecylphosphonium chloride (TTPC) in a soil matrix by extraction with acetone, filtration, dilution with water, and analysis by liquid chromatography/tandem mass spectrometry. TTPC is a biocide that strongly adsorbs to soils.[2] The sample extracts are prepared in a solution of 75 % acetone and 25 % water because TTPC has an affinity for surfaces and particles. The reporting range for this method is from 250 to 10 000 ng/kg. This analyte is qualitatively and quantitatively determine by this method. This method adheres to multiple reaction monitoring (MRM) mass spectrometry.

1.2 The Method Detection Limit (Note 1) (MDL) and Reporting Range (Note 2) for the target analyte are listed in Table 1.

NOTE 1—The MDL is determined following the Code of Federal Regulations, 40 CFR Part 136, Appendix B, as a guide utilizing solvent extraction of soil. Two-gram sample of Ottawa Sand was utilized. A detailed process determining the MDL is explained in the reference and is beyond the scope of this standard to be explained here.

NOTE 2—Reporting range concentration is calculated from Table 2 concentrations assuming a 50 µL injection of the Level 1 calibration standard for TTPC, and the highest level calibration standard with a 20 mL final extract volume of a 2 g soil sample. Volume variations will change the reporting limit and ranges.

1.2.1 The reporting limit in this test method is the minimum value below which data are documented as non-detects. Analyte detections between the method detection limit and the reporting limit are estimated concentrations and are not reported following this test method. The reporting limit is calculated from the concentration of the Level 1 calibration standard as shown in Table 2 for TTPC after taking into account a 2 g sample weight and a final extract volume of 20 mL in 75 % acetone/25 % water. The final extract volume is 20 mL because a 15 mL volume of acetone is added to each soil sample and only the liquid layer after extraction is filtered leaving the solid behind followed by the addition of 5 mL of water to the acetone extract.

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

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.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

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

D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis

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

2.2 *Other Documents:*[4]

EPA Publication SW-846 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods

40 CFR Part 136, Appendix B Definition and Procedure for the Determination of the Method Detection Limit

3. Terminology

3.1 For determinations of terms used in this standard, refer to Terminology D5681.

[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.

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[2] More information on TTPC can be found at http://www.buruenergy.com/wp-content/uploads/BE-Environmental-Properties-of-Proposed- Biocide-BE-91.pdf (2014) and http://iaspub.epa.gov/sor_internet/registry/substreg/searchandretrieve/advancedsearch/externalSearch.do?p_type=CASNO&p=81741-28-8 (2014).

[3] 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.

[4] Available from National Technical Information Service (NTIS), 5301 Shawnee Rd., Alexandria, VA 22312, http://www.ntis.gov or at http://www.epa.gov/epawaste/hazard/testmethods/index.htm.

TABLE 1 Method Detection Limit and Reporting Range[A]

Analyte	MDL (ng/kg)	Reporting Range (ng/kg)
TTPC	32.7	250–10 000

[A]Acronyms are defined in 3.3.

3.2 *Definitions of Terms Specific to This Standard:*

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

3.2.2 *reporting limit check sample, RLCS, n*—this sample is to verify that if the analyte was present at the reporting limit, it would be confidently identified.

3.3 *Acronyms:*

3.3.1 *CCC, n*—Continuing Calibration Check

3.3.2 *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*—Precision and Accuracy

3.3.13 *PPT, n*—parts-per-trillion

3.3.14 *QA, adj*—Quality Assurance

3.3.15 *QC, adj*—Quality Control

3.3.16 *RL, n*—Reporting Limit

3.3.17 *RLCS, n*—Reporting Limit Check Sample

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 *TTPC*—n-(Tri-n-butyl)-n-tetradecylphosphonium chloride

3.3.25 *VOA, n*—Volatile Organic Analysis

4. Summary of Test Method

4.1 The operating conditions presented in this test method have been successfully used in the determination of TTPC in soil; however, this test method is intended to be performance based and alternative operating conditions can be used to perform this method provided data quality objectives are attained. Mention of trade names or suppliers is not an endorsement of use, it is provided for informational purposes only. Any apparatus, supply, standard, or reagent may be used provided that it is shown to be acceptable to meet the performance criteria of the method.

4.2 For TTPC analysis, samples are shipped to the lab on ice and analyzed within 14 days of collection. A sample (~2 g) is transferred to a VOA vial, a TTPC spike solution is added to Laboratory Control and Matrix Spike samples before the addition of acetone. An isotopically labeled TTPC surrogate could be added at this point, presently requires a custom synthesis and should be incorporated into this method by the user if requested by the customer.[5] Then add 15 mL of acetone and hand shake or vortex for one minute. The samples are allowed to settle, and are then filtered through a Nylon membrane syringe driven filter unit[6] leaving the solids behind, 5 mL of ASTM Type 1 water is added to the filtered extract and then analyzed by LC/MS/MS. All concentrations reported, only to the reporting limit, using this method are based upon a dry weight basis.

4.3 TTPC is identified by comparing the single reaction monitoring (SRM) transition and its confirmatory SRM transitions if correlated to the known standard SRM transition (Table 3) and quantitated utilizing an external calibration. The final report issued for each sample lists the concentration of TTPC, if detected, or RL, if not detected, in ng/kg (Dry Weight Basis) and surrogate recovery, if available.

5. Significance and Use

5.1 This test method has been developed by the US EPA Region 5 Chicago Regional Laboratory (CRL).

5.2 TTPC may be used in various industrial and commercial products for use as a biocide. Products containing TTPC have been approved for controlling algal, bacterial, and fungal slimes in industrial water systems.[2] TTPC should not be persistent in water but may be deposited in sediments at concentrations of concern. Hence, there is a need for quick, easy, and robust method to determine TTPC concentration at trace levels in various soil matrices for understanding the sources and concentration levels in affected soils and sediments.

5.3 This method has been used to determine TTPC in sand, a commercial top soil and four ASTM reference soils (Table 4).

6. Interferences

6.1 All glassware is washed in hot water with detergent and rinsed in hot water followed by distilled water. The glassware is then dried and heated in an oven at 250°C for 15 to 30 minutes. All glassware is subsequently rinsed or sonicated, or both, with acetone, n-propanol, or acetonitrile, or combinations thereof.

6.2 TTPC should not be a common contaminant found in a laboratory, unless involved in the analysis or matrices that

[5] A custom synthesized surrogate, TTPC (D29), may be an inexpensive viable surrogate.

[6] A Whatman Puradisc™ 25 NYL Disposable Filter unit (Diameter 25 mm, 0.2 µm Nylon membrane syringe driven filter unit has been found suitable for use for this method, any filter unit may be used that meets the performance of this method may be used. The use of PTFE, PVDF, and polypropylene filter units resulted in poor performance.

TABLE 2 Concentrations of Calibration Standards (ng/L)

Concentrations (ng/L)	LV1	LV2	LV3	LV4	LV5	LV6	LV7	LV8
TTPC	25	50	100	200	400	600	800	1000

TABLE 3 Retention Times, SRM Ions, and Analyte-Specific Mass Spectrometer Parameters

Chemical	Primary/ Confirmatory	SRM Transition	Cone (V)	Collision (eV)	Retention Time (minutes)	Primary/ Confirmatory SRM Area Ratio
TTPC	Primary (Quantitation)	399.5 → 229.3	40	45	8.1	NA
	First Confirmatory	399.5 → 75.9	40	46		0.92
	Second Confirmatory	399.5 → 343.5	40	40		3.02

TABLE 4 Single-Laboratory Recovery Data in Six Soil Types

Sample	**Ottawa Sand (2500 ng/kg spike)**	**ASTM Frederick Sand (2500 ng/kg spike)**	**ASTM Silt (2500 ng/kg spike)**
MB 1	<RL	<RL	<RL
MB 2	<RL	<RL	<RL
P&A 1	2074.5	2121.4	1477.8
P&A 2	2244.6	2145.9	1482.3
P&A 3	2286.4	2171.3	1364.2
P&A 4	2077.8	2215.4	1543.9
P&A 5	2192.1	2038.5	1545.7
P&A 6	1953.1	2079.2	1462.1
Average Recovery (ng/kg)	2138.1	2128.6	1479.3
% Average Recovery	85.5	85.1	59.2
Standard Deviation	125.0	63.7	66.5
RSD (%)	5.8	3.0	4.5
Sample	**ASTM Lean Clay (2500 ng/kg spike)**	**ASTM Fat Clay (2500 ng/kg spike)**	**Top Soil (2500 ng/kg spike)**
MB 1	<RL	<RL	<RL
MB 2	<RL	<RL	<RL
P&A 1	394.6	790.2	1764.4
P&A 2	986.4	783.2	1750.1
P&A 3	386.4	772.4	1758.9
P&A 4	392.4	774.9	1771.6
P&A 5	435.3	791.7	1659.6
P&A 6	375.5	751.7	1778.3
Average Recover (ng/kg)	395.1	777.4	1747.2
% Average Recovery	15.8	31.1	69.9
Standard Deviation	20.8	14.8	44.0
RSD (%)	5.3	1.9	2.5

contain TTPC. TTPC has been found to continue to adhere to glassware and syringes after routine glassware washing. Rinsing glassware with acetone, n-propanol, or acetonitrile, or both, or even sonication, may be required to remove TTPC. All of the materials and supplies are routinely demonstrated to be free from interferences and TTPC by analyzing laboratory blanks under the same conditions as the samples. If found, measures should be taken to remove the contamination or data should be qualified, background subtraction of blank contamination is not allowed.

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 in the sample. The extent of matrix interferences can vary considerably depending on variations in the sample matrices.

6.5 Automatic pipettes with polypropylene tips are used with this method. The use of glass syringes for standards preparation, spiking and calibrations generated erratic results and should be avoided. A thoroughly cleaned 20 mL hypodermic glass syringe with a nylon filter is used to filter the 20 mL sample extracts and has been shown to perform well when filtering these large volumes. Preparing small volumes of samples and standards, like 1 mL calibration standards, may be affected by adhesion of TTPC to the syringe barrel or plunger. The use of PTFE, PVDF, and polypropylene filter units resulted in poor performance and low recoveries.

NOTE 3—The use of polypropylene disposable syringes to filter samples and polypropylene LC vials with polyethylene caps have been shown to perform in the performance criteria of the method and may be used.

7. Apparatus

7.1 *LC/MS/MS System:*

7.1.1 *Liquid Chromatography System*[7]—A complete LC system is required in order to analyze samples, this should include a sample injection system, a solvent pumping system

[7] A Waters Acquity UPLC H-Class System, or equivalent, has been found suitable for use.

capable of mixing solvents, a sample compartment capable of maintaining required temperature and a temperature controlled column compartment. A LC system that is capable of performing at the flows, pressures, controlled temperatures, sample volumes, and requirements of the standard shall be used.

7.1.2 *Analytical Column*[8]—A reverse phase C18 particle column was used to develop this test method. Any column that achieves adequate resolution may be used. The retention times and order of elution may change depending on the column used and need to be monitored.

7.2 *Tandem Mass Spectrometer System*[9]—A MS/MS system capable of multiple reaction monitoring (MRM) analysis or any system that is capable of meeting the requirements in this standard shall be used.

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

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

7.4 *Class A Volumetric Glassware.*

7.5 *Filtration Device:*

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

7.5.2 A 20 mL Lock Tip Glass Syringe size is recommended since a 20 mL sample size is used in this test method.

7.5.3 *Filter Unit*[10]—Nylon filter units were used to filter the samples.

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

7.7 *VOA Vials*—40 mL.

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.[11] 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 be understood to mean reagent water conforming to Type 1 of Specification D1193. It shall 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 Acetone (CAS # 67-64-1).

8.5 Acetonitrile (CAS # 75-05-8).

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

8.7 Ammonium Acetate (CAS # 631-61-8).

8.8 2-Propanol (isopropyl alcohol, CAS # 67-63-0).

8.9 Ottawa Sand (CAS # 14808-60-7).

8.10 (Tri-n-butyl)-n-tetradecylphosphonium chloride (CAS # 81741-28-8).

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 and Preservation*—Grab samples are collected in glass containers with polytetrafluoroethylene lined caps. As part of the overall quality assurance program for this test method, field blanks exposed to the same field conditions as samples are collected and analyzed according to this test method to assess the potential for field contamination. This test method is based on a 2 g sample size per analysis. If different sample sizes are used, spiking solution amounts may need to be modified. EPA publication SW-846 may be used as a sampling guide. Samples shall be shipped on ice with a trip blank. Once received the sample temperature is taken and should be less than 6°C. If the receiving temperature is greater than 6°C, the sample temperature is noted in the case narrative accompanying the data. Samples should be stored refrigerated between 0 and 6°C from the time of collection until analysis. The sample should be analyzed within 14 days of collection. No holding time study has been done on the various soil matrices tested in this test method. Holding time may vary depending on the matrix and individual laboratories should determine the holding time in their matrix.[12]

11. Preparation of LC/MS/MS

11.1 *LC Chromatograph Operating Conditions:*

11.1.1 Injections of all standards and samples are made at a 50 µL volume. Other injection volumes may be used to optimize conditions. Standards and sample extracts shall be in a 75:25 acetone:water solution. In the case of extreme concentration differences amongst samples, it is wise to analyze a blank after a concentrated sample and before a dilute sample to minimize carry-over of analytes from injection to injection. However, there should not be carry-over between samples. The LC utilized to develop this test method has a flow through LC

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

[9] A Waters Xevo TQ-S triple quadrupole mass spectrometer, or equivalent, has been found suitable for use.

[10] A Whatman Puradisc™ 25 NYL Disposable Filter unit (Diameter 25 mm, 0.2 µm Nylon membrane syringe driven filter unit has been found suitable for use for this method, any filter unit may be used that meets the performance of this method may be used. The use of PTFE, PVDF, and polypropylene filter units resulted in poor performance.

[11] 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.

[12] A guide to help and determine sample holding times can be found at http://www.epa.vom/esd/cmb/resaerch/bs_033cmb06.pdf (2014).

needle design. The gradient conditions for liquid chromatography are shown in Table 5.

11.2 *LC Sample Manager Conditions:*

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

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

11.2.3 *Seal Wash*—Solvent: 50 % methanol/50 % water; Time: 5 minutes.

11.3 *Mass Spectrometer Parameters:*

11.3.1 To acquire the maximum number of data points per SRM channel while maintaining adequate sensitivity, the tune parameters may be optimized according to the instrument used. Each peak requires at least ten scans per peak for adequate quantitation. 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 test method are listed below:

The instrument is set in the Electrospray positive source setting	
Capillary Voltage:	1 kV
Cone:	Variable depending on analyte
Extractor:	2 Volts
Source Temperature:	150°C
Desolvation Gas Temperature:	500°C
Desolvation Gas Flow:	900 L/hr
Cone Gas Flow:	150 L/hr
Collision Gas Flow:	0.15 mL/min
Low Mass Resolution 1:	3
High Mass Resolution 1:	14
Ion Energy 1:	1
Entrance Energy:	1
Collision Energy:	Variable depending on analyte
Exit Energy:	1
Low Mass Resolution 2:	2.8
High Mass Resolution 2:	14
Ion Energy 2:	1
Gain:	1.0
Multiplier:	512.82
Inter-Scan Delay:	0.003 seconds

12. Calibration and Standardization

12.1 The mass spectrometer shall be calibrated as per manufacturer's specifications before analysis. Analytical values satisfying test method criteria have been achieved using the following procedures. Prepare all solutions in the lab using Class A volumetric glassware.

12.2 *Calibration and Standardization*—To calibrate the instrument, analyze eight calibration standards of the TTPC compound prior to sample analysis as shown in Table 2. Calibration stock standard solution is prepared from the target spike solution directly to ensure consistency. Stock standard Solution A containing the TTPC 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 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. At a minimum, five calibration levels are required when using a linear calibration curve and six calibration levels are required when using a quadratic calibration curve. An initial eight point curve may be used to allow for the dropping of the lower level calibration points if the individual laboratory's instrument cannot achieve low detection limits. This should allow for at least a five or six point calibration curve to be obtained. No problems were encountered while using the eight point calibration curve in developing this test method.

12.2.1 Calibration stock standard Solution A (Level 8, Table 2) is prepared from the target spike solution directly to ensure consistency. 500 µL of TTPC Target Spike Solution (100 µg/L, 12.7) is added to a 50 mL volumetric flask and diluted to 50 mL with 75:25 acetone:water. The preparation of the Level 8 standard can be accomplished using appropriate volumes and concentrations of stock solutions as per a particular laboratory's standard procedure.

12.2.2 Aliquots of Solution A are then diluted with 75:25 acetone:water to prepare the desired calibration levels in 2 mL amber glass LC vials (Table 6). The calibration vials shall be used within 24 hours to ensure optimum results. Calibration standards are not filtered.

12.2.3 Inject each standard and obtain its chromatogram. An external calibration technique is used to monitor the primary and confirmatory SRM transitions of TTPC. Calibration software is utilized to conduct the quantitation of the target analyte using the primary SRM transition. The ratios of the primary/confirmatory SRM transitions area counts are given in Table 3 and will vary depending on the individual tuning conditions. The primary/confirmatory SRM transitions area ratio shall be within 35 % of the individual labs' accepted primary/confirmatory SRM transitions area ratio. The primary SRM transition of TTPC is used for quantitation and the confirmatory SRM transitions for confirmation. This gives added confirmation by isolating the parent ion, forming three product ions via fragmentation, and relating it to the retention time in the calibration standard.

12.2.4 Depending on sensitivity and matrix interference issues dependent on sample type, a confirmatory SRM transition may be substituted as the primary SRM transition for

TABLE 5 Gradient Conditions for Liquid Chromatography

Time (min)	Flow (mL/min)	Percent 95 % Water: 5 % Acetonitrile	Percent Acetonitrile	Percent 400 mm Ammonium Acetate (95 % Water: 5 % Acetonitrile)
0	0.3	95	0	5
1	0.3	95	0	5
4	0.4	0	95	5
11	0.4	0	95	5
12	0.4	95	0	5
15	0.4	95	0	5

TABLE 6 Preparation of Calibration Standards

Solution	LV1	LV2	LV3	LV4	LV5	LV6	LV7	LV8
A[A]	25 µL	50 µL	100 µL	200 µL	400 µL	600 µL	800 µL	1000 µL
B[B]	975 µL	950 µL	900 µL	800 µL	600 µL	400 µL	200 µL	0 µL

[A]Solution A: Level 8 stock solution prepared according to 12.2 and at Table 2 concentrations.
[B]Solution B: 75 % Acetone : 25 % Water.

quantitation during analysis. This shall be explained in a narrative accompanying the data. New primary/confirmatory ion ratios will then be determined if switching the SRM transitions used to quantitate and confirm. The new primary/confirmatory SRM transitions area ratio is required to be within 35 % of the individual labs' new primary/confirmatory SRM transitions area ratio.

12.2.5 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 ppt 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 (X = 0, Y = 0) is not recommended.

12.2.6 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. Each calibration point used to generate the curve shall have a calculated percent deviation less than 30 % from the generated curve. If the low or high point(s), or both, are excluded, minimally a five point curve is acceptable but the reporting range shall be modified to reflect this change.

12.2.7 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 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(s), or both, are excluded, minimally a six point curve is acceptable but the reporting range shall be modified to reflect this change. Each calibration point used to generate the curve shall have a calculated percent deviation less than 30 % from the generated curve.

12.2.8 The retention time window of the SRM transitions shall 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.

12.2.9 A midpoint calibration check standard shall be analyzed at the end of each batch of 30 samples within 24 hours after the initial calibration curve was generated, the criteria in the individual labs' quality system may be more restrictive pertaining to the number of samples. This end calibration check should come from the same calibration standard solution that was used to generate the initial curve. The results from the end calibration check standard shall have a percent deviation less than 30 % from the calculated concentration for the target analyte. If the results are not within these criteria, corrective action including reoccurrence minimization is performed and either all samples in the batch are re-analyzed against a new calibration curve or the affected results are 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 or other anomaly, 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 analyte, the results may be reported unqualified.

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, new instrument, etc., an instrument qualification study including method detection limit (MDL), calibration range determination and precision and bias determination shall be performed to demonstrate laboratory capability.

12.3.1 Analyze at least four replicates of a spiked sand sample containing TTPC at an extract concentration in the calibration range of Levels 3-6. A 250 ng/L extract concentration, or 2500 ng/kg (based upon a 2 g sand sample), was used to set the QC acceptance criteria in this method. The matrix and chemistry should be similar to the matrix used in this test method. Each replicate shall be taken through the complete analytical test method including any sample manipulation and extraction steps.

12.3.2 Calculate the mean (average) percent recovery and relative standard deviation (RSD) of the four values and

TABLE 7 QC Acceptance Criteria

NOTE 1—Table 7 data is preliminary until a multi-lab validation study is completed.

Analyte	Spike Conc. ng/kg	Initial Demonstration of Performance			Laboratory Control Sample	
		Recovery (%)		Precision	Recovery (%)	
		Lower Limit	Upper Limit	Maximum % RSD	Lower Control Limit (LCL) %	Upper Control Limit (UCL) %
TTPC	2500	70	130	30	70	130

compare to the acceptable ranges of the QC acceptance criteria for the Initial Demonstration of Performance in Table 7.

12.3.3 This study should be repeated until the single operator precision and mean recovery are within the limits in Table 7. If a concentration other than the recommended concentration is used, refer to Test Method D5847 for information on applying the F test and t test in evaluating the acceptability of the mean and standard deviation.

12.3.3.1 The QC acceptance criteria for the Initial Demonstration of Performance in Table 7 were generated from the single-laboratory data shown in the Precision and Bias Section 16. It is recommended that each laboratory determine in-house QC acceptance criteria which meet or exceed the criteria in this test method. References generating QC acceptance criteria are Practices D2777, D5847, E2554, or Method 8000 in EPA Publication SW-846.

12.4 *Surrogate Spiking Solution:*

12.4.1 A surrogate spiking solution containing TTPC (D29), or similar isotopically labeled standard should be used and added to all samples. The surrogate concentration should be at a similar concentration as the target spike. At the time of standard development a labelled TTPC surrogate was not available.[13] QA/QC criteria will have to be established by the laboratory and should be similar to the unlabeled TTPC target analyte it is the isotopically labeled counterpart.

12.5 *Method Blank:*

12.5.1 A method blank for every 30 samples is prepared in 2 g of Ottawa Sand to investigate for contamination during sample preparation and extraction. The concentration of target analytes in the blank shall be at less than half the reporting limit or the data shall be qualified as having a blank issue and the reporting limit shall be raised to at least three times above the blank contamination concentration.

12.6 *Reporting Limit Check Sample (RLCS):*

12.6.1 Each batch or within the 24 hour analysis window a reporting limit check sample shall be analyzed. The reporting limit check sample is processed like a Laboratory Control Sample just spiked at or near (one to two times) the reporting limit. The concentration of the RLCS may be reported below the reporting limit since the spike is at or near the reporting limit. This sample is to check if the analytes were present at the reporting limit, they would be identified. The recovery limits for the RLCS are 35 to 150 %, if any analytes are outside of these limits the QC failure is explained in a narrative accompanying the data.

12.6.2 Two grams of Ottawa Sand is added to a 40 mL VOA vial. The sample is spiked with 50 µL of a 10 µg/L Reporting Limit Check solution in 75 % acetone/25 % water (prepared by dilution from the 100 µg/L target spike solution) is added to the reporting limit check sample to prepare 250 ng/kg (25 ng/L in 20 mL extract) of TTPC in a ~2 g sand sample. The sample is then extracted and prepared as a sample as described in Section 13.

12.7 *Laboratory Control Sample (LCS):*

12.7.1 Analyze at least one LCS with the TTPC at a mid-level extract concentration. The concentration of TTPC at an extract concentration in the calibration range of Levels 3-6 should be used. A 250 ng/L extract concentration, or 2500 ng/kg (based upon a 2 g sand sample), was used to set the QC acceptance criteria in this method. The LCS is prepared following the analytical method and analyzed with each batch of 30 samples or less. Prepare a stock matrix spiking solution. The target spike solution is prepared by preparing a 100 µg/L solution in 75 % acetone/25 % water from a concentrated stock standard. The concentrated stock standard concentration can vary when preparing from neat material, usually between 50 to 100 mg/L TTPC in 75 % acetone/25 % water. A ~2 g amount of Ottawa sand is added to a 40 mL VOA vial. The sample is spiked with 50 µL of a 100 µg/L target spike solution and then taken through the sample preparation step in Section 13.

12.7.2 The result obtained for the LCS shall fall within the limits in Table 7. Spiking solutions are routinely replaced every year if not previously discarded for quality control failure.

12.7.3 If the result is not within these limits, sample analysis is halted until corrective action resolving the problem has been performed. Impacted samples in the batch are either re-analyzed, or the results are flagged with a qualifier stating 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 samples by spiking the sample with a known concentration of TTPC and following the analytical method. A batch of samples for this test method is defined as 30 or fewer samples, for each additional set of 30 samples another complete set of quality control samples is required. Prepare a stock matrix spiking solution. The target spike solution is prepared by preparing a 100 µg/L solution in 75 % acetone/25 % water from a concentrated stock standard. Spike 50 µL of this stock solution into 2 g of the site sample to yield a concentration of 2500 ng/kg TTPC in the sample.

12.8.2 If the spiked concentration plus the background concentration exceeds that of the Level 8 calibration standard, the sample shall be diluted using 75 % acetone/25 % water to a level near the midpoint of the calibration curve.

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

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

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.

12.8.4 The percent recovery of the spike shall fall within the limits in Table 8. If the percent recovery is not within these limits, a matrix interference may be present. Under these circumstances either all samples in the batch may be analyzed

[13] A custom synthesis of TTPC (D29) was undertaken by Cambridge Isotope Laboratories and may be commercially available soon.

TABLE 8 MS/MSD QC Acceptance Criteria

NOTE 1—Table 8 data is preliminary until a multi-lab validation study is completed.

Analyte	Spike Conc. ng/kg	MS/MSD Recovery (%)		Precision RPD (%)
		Lower Limit	Upper Limit	
TTPC	2500	70	130	30

by a test method not affected by the matrix interference, or the results shall be qualified indicating that they do not fall within the performance criteria of the test method. It has been found that in some cases the matrix spike concentration may be minimal compared to the concentration in the native sample. If this is the case, the sample may be spiked at a higher level of the generated data may be reported explaining in the narrative accompanying the data that the spike was negligible compared to the native concentration found in the sample.

12.8.5 The matrix spike/matrix spike duplicate (MS/MSD) limits in Table 8 were generated by a single-laboratory study using the data in the Precision and Bias Section 16. The limits in Table 8 are preliminary until a multi-lab validation study is completed. The matrix variation between different soils may have a tendency to generate significantly wider control limits than those generated for this test method. It is recommended that each laboratory determine in-house QC acceptance criteria meeting or exceeding the criteria stated in this test method.

12.8.5.1 Each laboratory should 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 generating QC acceptance criteria are Practices D5847, D2777, E2554, or Method 8000 in EPA publication SW-846.

12.9 *Duplicate:*

12.9.1 To check the precision of sample analyses, analyze a sample in duplicate with each batch of samples. A batch of samples for this test method is defined as 30 or fewer samples, for each additional set of 30 samples another complete set of quality control samples is required. If the sample contains the analyte at a level greater than five times the reporting limit of the method, the sample and duplicate may be analyzed unspiked; otherwise, a matrix spike/matrix spike duplicate 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 8.

$$RPD = \frac{|[M\ S] - [M\ S\ D]|}{([M\ S]+[M\ S\ D])/2} \times 100 \quad (2)$$

where:

RPD = relative percent difference,
MS = measured concentration in the matrix spike QC sample (to calculate duplicate RSD use Sample Concentration), and
MSD = measured concentration in the matrix spike duplicate QC sample (to calculate duplicate RPD use Sample Duplicate Concentration).

12.9.3 If the result exceeds the precision limit (Table 8), the batch shall be reanalyzed or the results are flagged with a qualifier stating that they do not fall within the performance criteria of the test method.

13. Procedure

13.1 This test method is based upon a 2 g sample size per analysis. The samples shall be analyzed within 14 days of collection. If samples are received or stored above 6°C, or are not analyzed within 14 days of collection, it is noted in the case narrative that accompanies the data.

13.2 Each batch of samples (30 or less) shall contain at least a method blank, a laboratory control sample, matrix spike, duplicate and a reporting limit check sample at a minimum.

13.3 In the laboratory, 2 g of sample (measured to the hundredth of a gram) is placed in a 40 mL VOA vial. The laboratory control, reporting limit check and matrix spike samples are then spiked with the target compounds as described in Section 12. The samples are then shaken, as thoroughly as possible depending on the soil, in order to mix the spike solutions throughout the sample.

13.4 To all samples, 15 mL of acetone is added and hand shaken/vortexed for ~1 minute. After mixing, the solids in the sample vials are allowed to settle. The supernatant of the sample is filtered through a Nylon filter syringe driven filter unit (refer to 13.5 before use) to remove particulates in the samples, and leave solids behind. Five millilitres of water is then added to the acetone extract. An aliquot of the solution is transferred to a LC vial and a cap is applied. The final volume of the solution is estimated to be 20 mL for quantitation purposes.

13.5 All the samples are filtered through a nylon filter unit using the cleaned glass syringe. **Note: It is important that this syringe is cleaned with water and acetone, 2-propanol or acetonitrile, or both, before use.** The filter does not require rinsing prior to use. TTPC should not be found in facilities that manufacture filter units.

13.6 The syringe shall be cleaned between each filtration. It is the analyst's responsibility to ensure that the syringe is clean. A suggested method for cleaning the syringe between filtrations is to first rinse with at least five syringe volumes of water, followed by at least three volumes of acetone, three volumes of 2-propanol, three volumes of acetonitrile and a final rinse with water.

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

14. Calculation or Interpretation of Results

14.1 For quantitative analysis of the TTPC, the SRM transitions are identified by comparison of retention times in the sample to those of the standards. TTPC is identified by comparing the sample primary SRM transition and its confirmatory SRM transitions if correlated to the known standard SRM transitions. Two confirmatory transitions are available for TTPC (Table 3). The primary/confirmatory SRM ion ratios

shall meet the criteria set in the quantitation method by ±35 %. The primary/confirmatory SRM ion ratios is the average of the individual levels primary/confirmatory SRM ion ratios in the calibration curve on the day of analysis. These ratios will vary depending on the instrument acquisition parameters and must be checked for every sample batch. External calibration curves are used to calculate the amounts of TTPC. Calculate the concentration in ng/kg (Dry Weight Basis, ppt) for TTPC. TTPC 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 a solution of 75 % acetone/25 % water to obtain a concentration near the mid-point of the calibration range and reanalyzed.

14.1.1 If there is no confirmatory transition for the analyte (refer to Table 3), or the ion ratios do not meet the criteria the compound is determined to be an unknown.

14.2 *Example Calculation of Sample Concentration Reported:*

14.2.1 The concentration of sample is calculated using the Eq 3.

$$C_s(n\ g/k\ g) = \frac{[C_i\ (n\ g/L)] \times [V_s\ (L)]}{[W_d\ (k\ g)]} \quad (3)$$

where:

C_s = concentration of target analyte in sample,
C_i = concentration of target analyte in sample from instrument,
V_s = volume of sample, and
W_d = dry weight of sample.

14.2.2 The analysis of TTPC may require dilution per sample. Example calculation is given in Eq 4.

$$\frac{V_f}{V_i}(C_u) = C_f \quad (4)$$

where:

V_f = final volume,
V_i = initial volume,
C_u = uncorrected concentration, and
C_f = final concentration (corrected for dilution).

15. Report

15.1 Determine the results in units of ng/kg (ppt) in a soil sample on a dry weight basis. Calculate the concentration in the sample using the linear or quadratic calibration curve generated. All data that do not meet the specifications in the test method shall be appropriately qualified.

16. Precision and Bias

16.1 The determination of precision and bias was conducted by US EPA Region 5 Chicago Regional Laboratory (CRL) and generated applicable data to determine the precision and bias as described in Practice D2777 for a single laboratory validation study.

16.2 This test method was tested by CRL on Ottawa Sand and four ASTM reference soils (CH-1, ML-1, CL-1, and SP-1).[14] ASTM reference soil CH-1 is Fat Clay (CH)- Vicksburg Buckshot Clay, ASTM reference soil ML-1 is Silt (ML)-Vicksburg Silt, ASTM reference soil CL-1 is Lean Clay (CL)-Annapolis Clay and ASTM reference soil SP-1 is Sand (SP)-Frederick Sand. The samples were spiked with the TTPC to obtain a 2500 ng/kg concentration as described in Section 12. Table 4 contains the recoveries for TTPC in the Ottawa sand, ASTM soils, and a commercial top soil.

17. Keywords

17.1 liquid chromatography; mass spectrometry; soil; (tri-n-butyl)-n-tetradecylphosphonium chloride (TTPC)

[14] Reference to the ASTM soils and soil reports can be found at http://www.durhamgeo.com/downloads/ASTM%20Soil%20Reports.html (2014).