SUMMARY

A method for the quantitation of prallethrin in surface water was developed and validated. The test/reference substance prallethrin [(S)-2-methyl-4-oxo-3-(2-propynyl)-2-cyclopenten-1-yl(1R)-cis,trans-2,2-dimethyl-3-(2-methyl-1-propenyl) cyclopropanecarboxylate] was analyzed using gas chromatography with mass spectrometry and negative chemical ionization (GC-MS NCI) from natural surface water samples fortified with 0.01 µg/L (LOQ) and 0.1 µg/L (10X LOQ) of prallethrin. The limit of quantitation in the test system was 0.01 µg/L. The limit of detection was defined as 20% of LOQ which represented 0.9 ng/mL of prallethrin in solution using the current methodology.

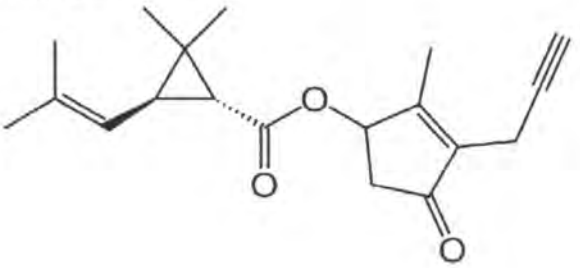
Method validation was conducted with one reagent blank, two untreated controls and five control samples spiked at each fortification level (0.01 µg/L and 0.1 µg/L). The samples were extracted/partitioned in triplicate with hexane. The combined organic solvent phase was dried through anhydrous sodium sulfate, then concentrated by rotary film evaporation followed by a stream of nitrogen gas. The final concentrated extracts were reconstituted with toluene. Lindane was added to calibrants and sample extracts as an internal standard prior to analysis by GC-MS.

Prallethrin was quantified using a 1/x weighted linear curve of the reference substance whose concentration ranged from 0.9 ng/mL to 180 ng/mL. All calibrants and fortified samples contained the internal standard lindane. The quantitation of prallethrin was based on the peak area response (PAR or response ratio) between prallethrin and the internal standard. The amount of prallethrin was determined for the quantitation ion m/z 167 and for the confirmation ions m/z 168 and m/z 132.

No significant matrix effect was found when comparing the response ratio of a solvent based calibrant and with that of a matrix based calibrant.

MATERIAL AND METHODS

Reference/Test Substance

Name:	Prallethrin
CAS Name:	(S)-2-methyl-4-oxo-3-(2-propynyl)-2-cyclopenten- 1-yl (1R)-cis,trans-2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylate
IUPAC Name:	(S)-2-methyl-4-oxo-3-(2-propynyl)cyclopent-2-enyl (1R)-cis-trans-chrysanthemate
Purity:	100%
Supplier:	Sumitomo Chemical Company
Lot no.:	13SC8359513
Molecular formula:	$C_{19}H_{24}O_3$
Molecular weight:	300.4 g/mole
Structure:	

Origin of Reference/Test Substance

The reference/test substance identified as prallethrin (lot no. 13SC8359513) was provided by 12L Research USA, Inc. and received at PTRL West on March 11, 2014. Upon receipt at PTRL West, the reference/test substance was given the PTRL inventory no. 2451W-001. The reference/test substance was maintained refrigerated when not in use. The Certificate of Analysis is provided in Appendix B.

Other Chemicals

The internal standard, lindane (gamma-1,2,3,4,5,6-hexachlorocyclohexane), lot no. SZBB321XV, was provided by Sigma-Aldrich and received at PTRL West on May 14, 2014. The internal standard was given the PTRL inventory no. 100W-0054 and stored at room temperature when not in use.

HPLC grade acetone, toluene, and hexane were obtained from Burdick & Jackson; sodium sulfate was obtained from Fisher Scientific.

Equipment/Materials List

Laboratory balances
0.2 mm sieve
Silanized glass wool
Weighing boats
Pasteur pipettes
Pipetmen with plastic disposable tips (adjustable volume pipetors)
Vortex mixer
Thermometers
Büchi rotavapor with water bath
Turbovap® LV nitrogen evaporator with water bath
GC vials
Glass inserts with flat bottom (0.4 mL capacity)

Glassware:

- Beakers
- Glass funnels (6 cm diameter)
- Graduated glass cylinders
- Separatory funnels (500 mL capacity)
- Pear shaped flasks (250 mL capacity)
- Glass rods
- Glass conical tubes (15 mL capacity)
- Volumetric glassware (flasks, glass precision syringes)
- Amber bottles and vials with Teflon® lined caps

Agilent 7000 Series Triple Quad Mass Spectrometer (GC-QQQ) with Agilent 7890A Series gas chromatograph (note, the tandem mass spectrometry capability of this instrument was not utilized)
Mass Hunter Data System Software

Test System

Source of Test System

Natural surface water collected from Wildcat Creek in Richmond, California was collected by PTRL West under a related study (P2578W) (Reference no. 1). The test system was collected on March 3, 2014 and upon arrival at PTRL West was assigned the inventory no. 2578W-004. The water sample was stored refrigerated (typically < 4°C) in the dark when not in use.

Characterization of the Test System

The natural water used in the study was characterized by Agvise Laboratories, Inc. (604 Highway 15 West, Northwood, North Dakota) under a related study (P2578W) (Reference no. 1). The water was characterized for pH, calcium, magnesium, sodium, hardness, sodium adsorption ratio, dissolved organic carbon content and conductivity. Water was sieved through a 200 micron sieve prior to fortification and analysis. The water characterization report, methods of characterization as well as collection documentation are presented in Appendix C.

Preparation of Internal Standard Solutions

Lindane was added to calibrants and samples as an internal standard just prior to analysis to mitigate detector signal variability.

Preparation of Internal Standard Stock Solution

An internal standard stock solution was prepared by weighing an aliquot (5.18 mg) g of Lindane into a 5 mL volumetric flask. The solution was diluted to the mark with toluene. An additional volume of toluene (0.17 mL) was added into the flask to yield a nominal concentration of 1,000 µg/mL. The concentration of the internal standard solution was corrected for the purity of the internal standard (99.8%). The internal solution was vortexed to mix, transferred into a bottle and stored frozen (typically < -4°C) when not in use.

Preparation of Internal Standard Working Solutions

Internal standard working solutions were prepared by measuring aliquots of the stock solution (1,000 µg/mL) and/or of the previously prepared diluted solutions via adjustable volume pipetors with plastic disposable tips and transferring into separate volumetric flasks containing some toluene. Final solutions were diluted to the mark with same solvent. Actual concentrations are shown below:

Solution used	Aliquot soln (mL)	Final volume (mL)	int std working soln (µg/mL)[1]
1,000 µg/mL	1.0	10	100
100 µg/mL	0.1	10	1
100 µg/mL	1.0	10	10

[1] Theoretical conc. (µg/mL) = [theoretical conc. x aliquot (mL)] ÷ final volume (mL)

Internal standard working solutions were vortexed to mix, transferred into amber bottles and stored frozen (typically < -4°C) when not in use.

Preparation of Prallethrin Stock Solution

A stock solution containing prallethrin was prepared by weighing an aliquot of the reference substance (10.10 mg) in a glass weighing boat and transferring into a 10 mL volumetric flask. The stock solution was dissolved and diluted to the mark with toluene. An additional volume of toluene (0.1 mL) was added into the flask to yield a nominal concentration of 1,000 µg/mL. A purity correction factor was not required as the purity of the reference substance was 100%. The stock solution was transferred into an amber bottle and stored frozen (typically < -4°C) when not in use.

Preparation of Prallethrin Intermediate Solutions

Intermediate solutions were prepared by measuring aliquots of prallethrin stock solution (1,000 µg/mL) and/or of the previously prepared diluted solutions and transferring into separate volumetric flasks. Adjustable volume pipetors with plastic disposable tips were used for measuring aliquots. Final solutions were diluted to the mark with toluene. Actual concentrations are shown below:

Solution used	Aliquot soln (mL)	Final volume (mL)	Theoretical conc. (µg/mL) [2]	Sample ID
1,000 µg/mL	1.0	10	100	100 µg/mL
1,000 µg/mL	0.1	10	10	10 µg/mL
100 µg/mL	0.1	10	1	1 µg/mL
10 µg/mL	0.1	10	0.1	100 ng/mL
1 µg/mL	0.2	10	0.02	20 ng/mL

[2] Theoretical conc. (µg/mL) = [theoretical conc. soln used x aliquot (mL)] ÷ final volume (mL)

Intermediate solutions were vortexed to mix, transferred into amber bottles and stored frozen (typically < -4°C) when not in use.

Preparation of Prallethrin Solvent Calibrants

Eight calibrants were prepared by transferring an appropriate volume of the prallethrin intermediate solutions via adjustable volume pipetors with plastic disposable tips into separate 10 mL volumetric flasks and diluting to the mark with toluene. Calibrants were mixed and transferred in amber bottles.

intermediate soln used	Aliquot soln (mL)	Volume (mL)	Theoretical conc. (ng/mL)[3] Prallethrin
100 ng/mL	0.1	10	1
20 ng/mL	1	10	2
1 µg/mL	0.05	10	5
1 µg/mL	0.1	10	10
1 µg/mL	0.2	10	20
10 µg/mL	0.05	10	50
10 µg/mL	0.1	10	100
10 µg/mL	0.2	10	200

[3]Theoretical conc stds (ng/mL) = [theoretical conc sol used (ng/mL) x aliquot (mL)] ÷ final volume (mL)

For GC/MS analysis, aliquots (0.09 mL) of prallethrin calibrants were combined with an aliquot (0.01 mL) of lindane internal standard solution (10 µg/mL) in GC vials containing 0.4 mL glass inserts with flat bottom. Final mixed calibrants were capped and vortexed to mix. Adjustable volume pipetors with plastic disposable tips were used to measure all calibrants whereas a 25 µL glass precison syringe was used to measure and distribute the internal standard solution (10 µg/mL).

The concentration of mixed prallethrin calibrants ranged from 0.9 ng/mL to 180 ng/mL as shown below:

Calibrant soln used	Aliquot soln (mL)	Final volume (mL)	Resulting Calibrant Concentration (ng/mL)[4] prallethrin
1 ng/mL	0.09	0.1	0.9
2 ng/mL	0.09	0.1	1.8
5 ng/mL	0.09	0.1	4.5
10ng/mL	0.09	0.1	9
20 ng/mL	0.09	0.1	18
50 ng/mL	0.09	0.1	45
100 ng/mL	0.09	0.1	90
200 ng/mL	0.09	0.1	180

[4]Resulting Calibrant Conc. (ng/mL) = [calibrant sol used x aliquot (mL)] ÷ final volume (mL)

The final concentration for the internal standard solution in all calibrants was 1,000 ng/mL. Calibrants (no internal standard solution added) were stored frozen (typically < -4°C) when not in use whereas mixed calibrants (internal standard solution added) were stored refrigerated (typically < 10°C).

The internal standard was incorporated into the method after reconstituting final water extracts in toluene. Therefore, equivalent quantities of lindane were added to sample extracts and calibrant solutions prior to analysis. An alternate method would be to reconstitute the final water sample extracts in toluene that already contains the internal standard and preparing calibrant solutions with internal standard added ahead of time; however, this approach was not taken in this study.

Preparation of Matrix Based Calibrant (Spiked Control)

A matrix based calibrant was prepared at one concentration level (40 ng/mL prallethrin) as follows:

An aliquot (0.07 mL) of control sample (untreated water) was spiked with 0.02 mL of 200 ng/mL prallethrin solvent calibrant and 0.01 mL of 10 µg/mL Lindane internal standard solution in a GC vial containing a 0.4 mL glass insert with flat bottom. The vial was capped and vortexed to mix and analyzed by GC-MS. Final concentration of internal standard solution in the spiked control: 1,000 ng/mL. Glass precision syringes were used for volume measurements.

Preparation of Prallethrin Fortification Solutions

Fortification solutions were prepared by measuring an aliquot of prallethrin intermediate solution (10 µg/mL) and/or of the previously prepared diluted solution and transferring into separate volumetric flasks containing some acetone. Final solutions were diluted to the mark with same solvent. Actual concentrations are shown below:

Solution used	Aliquot soln (mL)	Final volume (mL)	Theoretical conc. (ng/mL)	Fort Solution
10 µg/mL	0.25	10	250	10X LOQ
250 ng/mL	1.0	10	25	LOQ

Fortification solutions were vortexed to mix, transferred into amber bottles and stored frozen (typically < -4°C) when not in use.

Fortification Procedure

Fortification of untreated water samples was conducted at two fortification levels as shown below:

Fortification Level (µg/L)	Prallethrin
0.01	0.1 mL of 25 ng/mL in 250 mL water
0.1	0.1 mL of 250 ng/mL in 250 mL water

Fortification was conducted to determine the percent recovery within the method validation. This procedure was performed in quintuplicate during method validation at each fortification level.

Extraction Method

1. Sieve natural surface water through a 200 micron sieve.
2. Measure 250 mL aliquots of water in 500 mL glass separatory funnels. All glassware: separatory funnels, 250 mL pear shaped flasks, 6 cm diameter funnels, 15 mL conical tubes were previously rinsed with hexane and acetone.
3. Fortify as necessary using a 100 µL glass precision syringe.
4. Extract samples with 25 mL x 2 hexane for 40 seconds by manual shaking.
5. Extract samples with 15 mL x 1 hexane for 40 seconds by manual shaking. After third extraction, break emulsion with glass rods.
6. Combine all hexane extracts in 250 mL pear shaped flasks.
7. Dry combined hexane extracts through a funnel plugged with silanized glass wool, topped with approximate 10 g ± 0.1 g sodium sulphate anhydrous in the 250 mL pear shaped flasks.
8. Rinse separatory funnels and wash sodium sulphate with 10 mL x 2 hexane collecting rinsate into the pear shaped flasks.
9. Concentrate extracts by rotary evaporation (approximately 2 mL) over a water bath at approximately 40°C.
10. Transfer concentrated extracts into 15 mL conical glass tubes by Pasteur pipette.
11. Rinse pear shaped flasks with 3 mL x 3 hexane and combined rinsates into the 15 mL conical tubes.
12. Concentrate concentrated extracts to dryness using Turbovap® LV evaporator with nitrogen at 40°C.
13. Reconstitute samples in 0.5 mL toluene via a 1 mL pipetman. Vortex to mix.
14. Combine 0.09 mL aliquot of reconstituted samples with 0.01 mL of lindane internal standard solution (10 µg/mL) in GC vials containing 0.4 mL glass inserts.

An additional small aliquot of one of the controls was not spiked with internal standard solution. Final samples were capped and vortexed to mix.

15. Analyze samples by GC-MS.

16. Remaining extracts are transferred in GC vials with glass inserts and stored refrigerated (typically < 10°C) when not in use.

A schematic diagram of the extraction method is presented in Figure 1.

Gas Chromatography with Mass Spectrometry Analytical Method (GC-MS)

GC conditions

Column: J&W DB-5 ms, 30 m x 250 µm x 0.25 µm
Injection volume: 1 µL
Injector temp: 250°C
Splitless mode with 25 psi pressure pulse for 0.5 min
Restek Single Goose Neck liner, deactivated
Temp program:

- Initial conditions: 120°C for 2 minutes
- Ramp: 15°C/minute to 255°C
- Final temp: 255°C hold for 3 minutes

Flow rate (He): 1 mL/min
Pressure: 11.654 psi
Run time: 14 minutes
Retention times:

- Lindane int std: 7.2 min
- Prallethrin: 9.5 min

MS conditions

Negative Chemical Ionization mode
Transfer line temp: 260°C
MS source temp: 150°C
Solvent delay: 4 minutes

Time segments

Time segment	Start Time (min)	Scan Type	gain
1	4	MS1 SIM	5
2	8	MS1 SIM	5

Time Events

Time segment 1

Compound name	Mass	MS1 resolution	Dwell (ms)
Int std	255*	unit	150
Int std	253	unit	150

*Only 255 (most abundant ion) was used

Time segment 2

Compound name	Mass	MS1 resolution	Dwell (ms)
Prallethrin	168	unit	100
Prallethrin	167	unit	100
Prallethrin	132	unit	100

Note: the full scan (50 – 400 amu) for prallethrin and lindane are provided in Figures 2 and 3, respectively. Based on prallethrin full scan, m/z ion 167 was considered as the quantitation ion (most abundant ion); m/z 168 and 132 were considered as the confirmation (qualifier) ions.

GC-MS Analysis

Samples were analyzed interspersed between the calibrants. Calibrants and samples were analyzed in single injections. Toluene was analyzed as the solvent blank.

Two standard solutions were reanalyzed at the end of the sequence as check standards (quality control standards) to ensure good chromatography and consistent instrument performance. The stability of the signal was monitored by comparing the response of a quality control standard injection (peak area prallethrin/ peak area internal standard) at the end of the sequence to the response of a comparable quantitation standard within the sequence.

The injection sequence for the method validation sample set was: toluene (solvent blank), 0.9 ng/mL calibrant, reagent blank, control sample 1 for 10XLOQ set (no internal standard), control sample 1 for 10XLOQ set (internal standard added), 1.8 ng/mL calibrant, control sample 1 for LOQ set (internal standard added), control sample 2 for

LOQ set (internal standard added), 4.5 ng/mL calibrant, fortified sample A1, fortified sample A2, 9 ng/mL calibrant, fortified sample A3, fortified sample A4, 18 ng/mL calibrant, fortified sample A5, fortified sample B1, 45 ng/mL calibrant, fortified sample B2, fortified sample B3, 90 ng/mL calibrant, fortified sample B4, fortified sample B5, 180 ng/mL calibant, spiked control sample (matrix based calibrant), 18 ng/mLQC standard, 45 ng/mLQC standard.

Methods of Calculation

Preparation of Stock Standard Solutions

$$\text{Volume of solvent (mL)} = \frac{(W) \times 1000\,\mu g/mg \times (P)}{(FC)}$$

where W = Milligrams of neat standard
P = Chemical purity of neat standard
FC = Final Concentration (µg/mL)

Quantitation

Separation of prallethrin was achieved by GC. The detection was by MS (NCI) in SIM mode. The target analyte was identified by the coincidence of its retention time with its reference substance and MS characteristics. The quantitation of prallethrin was conducted by peak area response factor (PAR or response ratio) of prallethrin relative to the internal standard. The peak area of prallethrin divided by the peak area for the internal standard, lindane, yields a PAR for the samples and the calibrants. The content of prallethrin in samples was quantitated against a 1/x weighted linear curve ($y = mx + b$) from prallethrin calibrants where:

y = peak area response (PAR)
x = ng/mL analyte
m = slope
b = intercept

The calculation of weighted curve equation (linear regression) and concentration (ng/mL) present in samples and calibrants was conducted using Mass Hunter software. The

amount of prallethrin was determined for the quantitation ion at m/z 167 and for the confirmation ions at m/z 168 and m/z 132.

Recoveries from fortified samples were determined by calculating the found concentration and dividing by the relevant fortification level.

Residue in water

Found conc (µg/L prallethrin) =

$$\frac{\text{calculated conc (ng/mL) of Prallethrin x dilution factor x Final vol. (mL)}}{\text{1000 ng/µg x sample volume (L)}}$$

Example: Fortified sample FA1 (m/z 167)

$$\text{µg/L prallethrin} = \frac{4.727 \text{ ng/mL} \times 1.1 \times 0.5 \text{ mL}}{1000 \text{ ng/µg} \times 0.25 \text{ L}} = 0.0104$$

Percent recovery of prallethrin in water

$$\% \text{ Recovery} = \frac{\text{µg/L detected - µg/L Control}}{\text{Fortification Level (µg/L)}} \times 100$$

Example: Fortified sample FA1 (m/z 167)

$$\% \text{ prallethrin} = \frac{0.0104 \text{ µg/L}}{0.01 \text{ µg/L}} \times 100 = 104\%$$

*No prallethrin residue was detected in the control for the LOQ sample set (m/z 167 ion).

Transcriptions (spreadsheets) of the raw data to support calculations for this study are presented in Appendix D.

Limit of Detection

The limit of detection was defined as 20% LOQ as defined by the lowest calibrant (0.9 ng/mL). This is equivalent to 0.002 µg/L in water.

Limit of Quantitation

The limit of quantitation was assigned as the lowest fortification level of prallethrin validated by the analytical method. The LOQ for prallethrin in water was 0.01 µg/L.

Time Required for Completion of a Sample Set

A sample set consists of a minimum of five fortified water samples (at one level i.e. LOQ), two controls (untreated water sample), and one reagent blank. Time required per one sample set from initiation of extraction until the completion of instrumental analysis and data evaluation is as follows:

- Sample preparation, including sieving of natural water through a 0.2 micron sieve, liquid-liquid partition and extract concentration take approximately 8 hours
- GC-MS analysis and data processing (three fragment ions) take approximately 4 hours

TOTAL = approximately 12 hours for one analyst to complete a sample set (approximately one and a half calendar days). This does not include preparation of calibrants, fortification and internal standard solutions.

Statistical Methods

Means, standard deviation, relative standard deviation, and 1/x linear regression were the only statistical methods employed in this study.

Figure 1. Schematic Diagram of the Analytical Method.