SUMMARY

A method for quantitation of *cis*- Permethrin and *trans*- Permethrin in water, soil, and sediment test systems was validated.

The test substance containing a mixture of *cis*-Permethrin and *trans*-Permethrin (1:1) was analyzed using external standardization by Liquid Chromatography with Tandem Mass Spectrometry Detection (LC-MS/MS). Water samples were fortified with 5.0 ppt (LOQ) and 50 ppt (10X LOQ) of total Permethrin (sum of cis and trans isomers). Soil/sediment samples were fortified with 1.0 ppb (LOQ) and 10 ppb (10X LOQ) of total Permethrin. The limit of detection was defined as approximately 20% LOQ using the current methodology.

The experiment for each matrix (test system) was conducted with one reagent blank, two untreated controls and five control samples spiked for each fortification level: one at LOQ level and another at 10X LOQ level. Permethrin was extracted from samples, followed by a clean-up step with solid-phase extraction (SPE). The final concentrated extract was reconstituted with methanol and analyzed by LC-MS/MS.

cis-Permethrin and *trans*-Permethrin contents were quantitated against separate 1/x weighted linear curves of the reference substances *cis*-Permethrin and *trans*-Permethrin whose concentrations ranged from 0.5 ng/mL to 50 ng/mL for each isomer or from 1 ng/mL to 100 ng/mL for total Permethrin in soil or sediment test system. In water test system *cis*-Permethrin and *trans*-Permethrin contents were quantitated against separate 1/x weighted linear curves of the reference substances *cis*-Permethrin and *trans*-Permethrin whose concentrations ranged from 0.25 ng/mL to 37.5 ng/mL for each isomer or from 0.5 ng/mL to 75 ng/mL for total Permethrin. The calibration for each isomer in all matrices yielded acceptable linearity (correlation coefficient $r > 0.992$) over the range examined. The quantitation of Permethrin was based on the peak area response and concentration of the calibration standards. The amount of Permethrin of each isomer was determined with the quantitation MS/MS ion transition from m/z 391 to m/z 183 or the alternative quantitation MS/MS ion transition from m/z 393 to m/z 183. Recoveries from fortified samples were determined by calculating the found concentration of individual isomer and averaging by the relevant fortification level.

The LOD in soil or sediment matrix is estimated to be 0.2 ppb and the LOD in water matrix is estimated to be 1 ppt of total Permethrin using either MS/MS transition.

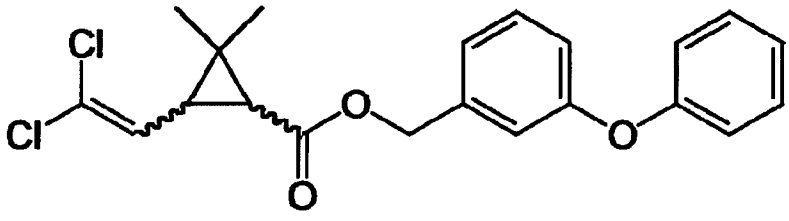
MATERIAL AND METHODS

Test and Reference Substance

Name:	*cis*-Permethrin
Supplier:	FMC Corporation
Lot No.:	G0101:6
Chemical name:	3-Phenoxybenzyl (1RS)-cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
CAS No.:	61949-76-6
Molecular formula:	$C_{21}H_{20}Cl_2O_3$
Molecular weight:	391.3 grams/mole
Purity:	99.3%
Expiration Date:	November 2017

Name:	*trans*-Permethrin
Supplier:	FMC Corporation
Lot No.:	G0101:8
IUPAC name:	3-Phenoxybenzyl (1RS)- trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
CAS No.:	61949-77-7
Molecular formula:	$C_{21}H_{20}Cl_2O_3$
Molecular weight:	391.3 grams/mole
Purity:	99.0%
Expiration Date:	April 2018

Structures:



Origin of Samples

The test and reference substances identified as *cis*-Permethrin (Lot No. G0101:6) and *trans*-Permethrin (Lot No. G0101:8) were provided by FMC Corporation. Upon receipt at PTRL West, the reference substances were given PTRL inventory No. shown below:

Reference Substance	PTRL Inventory no.	Receipt Date
cis-Permethrin	2007W-001	February 24, 2010
trans-Permethrin	2007W-002	February 24, 2010

The reference and test substances were stored in freezer when not in use for long-term. Certificates of Analysis for the reference and test substances are provided in Appendix B.

Other Chemicals

HPLC grade water, methanol, Hexanes and Diethyl ether were obtained from Burdick & Jackson; formic acid, sodium sulfate and sodium chloride was obtained from Fisher Scientific.

Equipment List

Laboratory Balances
0.2 mm sieve
Thermometers
Silanized glass wool
Pasteur pipettes
Beakers
Glass funnels (6 cm diameter)
Graduated glass cylinders
Hamilton glass precision syringes
Volumetric flasks
Pipetmen with plastic disposable tips
Separatory funnels (500 mL capacity)
Pear shaped flasks (100 mL capacity)
Glass conical tubes (15 mL capacity)
Amber bottles and vials with Teflon® lined caps
Vortex mixer
Büchi rotavapor with water bath
Turbovap® LV nitrogen evaporator

AB Sciex API 4000 Series Triple Quad Mass Spectrometer with Agilent 1100 Series Liquid Chromatograph (LC-MS/MS)

Test System

Source of Test System

Water is a natural surface water collected in Denton, Maryland. Soil is a sandy loam collected in Vacaville, California. Sediment is a river sediment characterized as a clay loam, collected from the Goose River, Grand Forks County, North Dakota. The water, soil and sediment samples were stored refrigerated (typically < 4°C) in the dark when not in use.

Characterization of the Test System

The water, soil and sediment used in the study were characterized by Agvise Laboratories, Inc. (604 Highway 15 West, Northwood, North Dakota). The characterization reports and methods of characterization are presented in Appendix C.

Test Method

The analytical method for the analysis of Permethrin validated at PTRL West by Liquid Chromatography with Tandem Mass Spectrometry Detection (LC-MS/MS) was based on the analytical method (Reference 1) provided by the sponsor.

The water, soil and sediment samples were spiked with known concentrations of Permethrin (racemic ratio of *cis*-Permethrin and *trans*-Permethrin (1:1)). Permethrin was extracted from samples, followed by a clean-up step with solid-phase extraction (SPE). The final concentrated extract was reconstituted with methanol and analyzed by LC-MS/MS. The percent recovery was determined using external standardization where linear curves for Permethrin calibration standards were analyzed along with the samples.

Preparation of cis-Permethrin Stock Solution

A stock solution of the *cis*-Permethrin reference substance (white powder) was prepared by weighing an aliquot of the reference substance (12.92 mg) onto glass boat and then

into a 10 mL volumetric flask. The stock solution was dissolved and diluted to the mark with methanol to yield a nominal concentration of 1.28 mg/mL. The concentration of stock solution was corrected for the purity of the reference substance (99.3%). The stock solution was transferred into an amber bottle and stored refrigerated (typically < 10°C) when not in use.

Preparation of trans-Permethrin Stock Solution

A stock solution of the *trans*-Permethrin reference substance (white powder) was prepared by weighing an aliquot of the reference substance (13.03 mg) onto glass boat and then into a 10 mL volumetric flask. The stock solution was dissolved and diluted to the mark with methanol to yield a nominal concentration of 1.29 mg/mL. The concentration of stock solution was corrected for the purity of the reference substance (99.0%). The stock solution was transferred into an amber bottle and stored refrigerated (typically < 10°C) when not in use.

Preparation of cis-Permethrin and trans-Permethrin Mixed Stock Solution

The 100 µg/mL Permethrin mixed stock solution was prepared by measuring 0.391 mL of *cis*-Permethrin stock solution and 0.388 mL of trans-Permethrin stock solution prepared above via pipetman with disposable plastic tips and transferring into 10 mL volumetric flask containing some methanol. Final solution was diluted to the mark with methanol. This mixed stock solution contains 50 µg/mL of each isomer or 100 µg/mL of total Permethrin.

The mixed stock solution was vortexed to mix, transferred into amber bottle and stored refrigerated (typically < 10°C) when not in use.

Preparation of cis-Permethrin and trans-Permethrin Mixed Standard Solutions

The 1000 ng/mL Permethrin mixed standard solution was prepared by transferring 0.10 mL of the Permethrin mixed stock solution via pipetman with disposable plastic tips into a 10 mL volumetric flask and diluted to the mark with methanol. This mixed standard solution contains 500 ng/mL of each isomer or 1000 ng/mL of total Permethrin. The 1000 ng/mL mixed standard solution was vortexed to mix, transferred into amber bottle and stored refrigerated (typically < 10°C) when not in use.

Ten additional fortification and/or calibration standard solutions were prepared by transferring an appropriate volume of the Permethrin mixed fortification or calibration standard solutions via pipetman with disposable plastic tips into separate 5 mL or 10 mL volumetric flasks and diluted to the mark with methanol. Final calibrants were vortexed to mix, transferred into amber bottles and stored refrigerated (typically < 10°C) when not in use. The concentration of Permethrin standard solutions ranged from 0.5 ng/mL to 200 ng/mL as shown below:

Theoretical Conc. (ng/mL)[1] Total Permethrin	Volume of standard Solution (mL)	Standard Solution Used (ng/mL)	Final Volume (mL)
200	1.0	1000	5
100	1.0	1000	10
75	0.75	1000	10
50	0.50	1000	10
25	0.25	1000	10
10	1.0	100	10
5	1.0	50	10
2.5	1.0	25	10
1	1.0	10	10
0.5	1.0	5	10

[1] Theoretical conc stds (ng/mL) = [theoretical conc soln used (µg/mL) x 1,000 ng/µg x aliquot (mL)] ÷ final volume (mL)

Fortification Procedure

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

Fortification Level (ppt)	Permethrin Solution
5.00	0.100 mL of 25 ng/mL in 500 mL of water
50.0	0.125 mL of 200 ng/mL in 500 mL of water

Fortification of untreated soil and sediment samples was conducted at two fortification levels as shown below:

Fortification Level (ppb)	Permethrin Solution
1.0	0.250 mL of 200 ng/mL in 50 grams of soil or sediment
10	0.500 mL of 1 µg/mL in 50 grams of soil or sediment

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 for each matrix.

Extraction Method for Permethrin in Water

1. Place 500 mL of water sample into 1 L separatory funnel.
2. Fortify if necessary.
3. Add 50 mL of hexane, 10 g of sodium chloride and 50 mL of MeOH to the funnel.
4. Shake funnel vigorously for 1 min.
5. Let phases to separate for about 10 min.
6. Drain low water layer to a suitable container.
7. Drain the upper hexane layer through a glass funnel with glass wool plug and 20 g of sodium sulfate into 250 mL flask.
8. Return the aqueous/methanol phase to the separatory funnel. Rinse container with 50 mL of hexane, add the rinse to the sep. funnel.
9. Vigorously shake for 1 min. Let phases to separate for 10 min. Drain and discard the lower aqueous phase.
10. Drain the hexane phase through the sodium sulfate into the same 250 mL flask.
11. Rinse sodium sulfate with 10 mL of hexane.
12. Transfer combined hexane extract portion by portion into 15 mL disposable glass tube and turboevaporate at 40°C. Rinse the flask with about 5 mL of hexane, add to the tube to evaporate.
13. Turboevaporate to small volume about 0.2 mL at 40°C. Manually evaporate to dryness with nitrogen.
14. Reconstitute in 2.0 mL of hexane. Sonicate, vortex to dissolve completely.

Clean-up.

15. Place Bond Elut Silica SPE 500 mg, 3 mL onto vacuum manifold. Add 3 mL of hexane and draw through under vacuum to the level of frit at 2 mL/min. rate. Discard the elute.
16. Transfer the sample from step 14 (2 mL) onto the cartridge and allow to percolate through under gravity or low vacuum. Discard the eluate.

17. Rinse the tube with 1 mL of hexane. Vortex. Add the rinse to the cartridge. Discard the eluate.
18. Elute the analytes from the cartridge with 6 mL of hexane:diethyl ether, 9:1, v:v under gravity or low vacuum, collecting into 15 mL glass tube.
19. Turboevaporate to dryness under a stream of nitrogen at 40°C.
20. Reconstitute in 1.0 mL of MeOH.
21. Aliquot in autosampler vials for HPLC-MS/MS analysis.

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

Extraction Method for Permethrin in Soil and Sediment

1. Weigh 50g of sample in 250 mL plastic new disposable centrifuge bottle. Do not reuse bottles!
2. Fortify if necessary.
3. Add 75 mL MeOH: water, 1:1, v:v and 50 mL of hexane.
4. Place on shaker for 60 minutes.
5. Centrifuge at 4000 rpm for 5 min. All emulsion should be dispersed.
6. Remove upper hexane layer into clean bottle (should be about 50 mL).
7. Transfer 10 mL of hexane extract into 15 mL disposable glass tube.
8. Turboevaporate to dryness under a stream of nitrogen at 40°C.
9. Re-dissolve in 2 mL of hexane. Sonicate to dissolve completely.

Clean-up.

10. Place Bond Elut Silica SPE 500 mg, 3 mL onto vacuum manifold. Add 3 mL of hexane and draw through under vacuum to the level of frit at 2 mL/min. rate. Dicard the eluate.
11. Transfer the sample from step 8 (2 mL) onto the cartridge and allow to percolate through under gravity or low vacuum. Discard the eluate.
12. Wash cartridge with 1 mL of hexane. Discard the eluate.
13. Elute the analytes from the cartridge with 6 mL of hexane:diethyl ether, 9:1, v:v under gravity or low vacuum, collecting into 15 mL glass tube.
14. Turboevaporate to dryness under a stream of nitrogen at 40°C.
15. Reconstitute in 1.0 mL of MeOH.
16. Aliquot in autosampler vials for HPLC-MS/MS analysis.

A schematic diagram of the soil and sediment extraction method is presented in Figure 2.

Liquid Chromatography with Tandem Mass Spectrometry Analytical Method (LC-MS/MS)

LC conditions

Column: Supelco Express Ascentis® C18, 2.7µm, 50mm X 2.1mm (S/N USMD005652)
Injection volume: 20 µL
Flow rate : 0.4 mL/min
Run time: 9 minutes
Mobile Phase:

- *A: 0.5% Formic acid in HPLC grade water*
- *B: Methanol*

Gradient Program:

Time (minutes)	%A	%B	Flow rate (mL/min)
0.0	70	30	0.4
3.0	10	90	0.4
6.0	10	90	0.4
6.5	70	30	0.4
9.0	70	30	0.4

MS conditions

ESI Positive mode

ESI Parameters

Collision Gas (CAD)	6
Curtain Gas (CUR)	15
Gas 1 (GS1)	55
Gas 2 (GS2)	80
Ion Spray Voltage (IS)	5500
Temperature (TEM)	300
Declustering Potention (DP)	39
Exit Potential (EP)	9
Collision Energy (CE)	30

Cell Exit Potential (CXP)	16

MRM Parameters

Compound name	Precursor ion	MS1 resolution	Product ion	MS2 resolution	Dwell (msec)
Permethrin	391	unit	183	low	400
Permethrin	393	unit	183	low	400

Note: Based on the analytical method (Reference 1) provided by the sponsor, MS/MS transition 391→183 m/z is used for quantitation. In this study the alternative MS/MS transition 393→183 is used for quantitation only when 391→183 transition fails to resolve existing interference.

LC-MS/MS Analysis

Samples were analyzed interspersed between the calibrants so as to assess the response of the calibrants if they had been affected by matrix samples (signal suppression or enhancement). Since separate linear curves were prepared for each isomer, samples were interspersed between Permethrin calibration standards. Calibrants and samples were analyzed in single injection.

Methods of Calculation

Quantitation

Separation of *cis*-Permethrin and *trans*-Permethrin was achieved by LC-MS/MS. The isomers were identified by the coincidence of their retention times with their respective reference standards and MS characteristics. The quantitation of *cis*-Permethrin and *trans*-Permethrin was conducted by peak area of each isomer relative to the theoretical concentration of the calibrants. The content of *cis*-Permethrin and *trans*-Permethrin in samples was quantitated against separate 1/x weighted linear curves ($y = mx + b$) of *cis*-Permethrin and *trans*-Permethrin calibrants where:

y = peak area
x = ng/mL isomer injected

m = slope
b = intercept

Weighting of the calibration curve of each isomer was applied so as to provide better curve fit at the lower concentration levels of each isomer. The calculation of weighted curve equations (linear regression) and concentration (ng/mL) present in samples and calibrants was conducted using Analyst® software. The amount of Permethrin of each isomer was determined for the quantitation MRM of 391→183 or 393→183.

Recoveries from fortified samples were determined by averaging the found concentration of both isomers and dividing by the relevant fortification level.

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

Calibration Range

The calibration curve was generated by Analyst® software range from 1 ng/mL to 100 ng/mL of total Permethrin (sum of cis and trans isomers) for soil and sediment validation while range from 0.5 ng/mL to 75 ng/mL of total Permethrin for water validation.

Limit of Quantitation

The limit of quantitation (LOQ) was set at 5.0 ppt for total Permethrin (sum of cis and trans isomers) in water which represented 2.5 ng/mL of total Permethrin (1.25 ng/mL of each isomer) in calibration standard solution as validated in this study.

The limit of quantitation (LOQ) was set at 1.0 ppb in soil and sediment for total Permethrin which represented 10 ng/mL of total Permethrin (5 ng/mL of each isomer) in calibration standard solution as validated in this study.

Limit of Detection

The limit of detection (LOD) was defined as approximately 20% of LOQ which represented 0.5 ng/mL of total Permethrin (sum of cis and trans isomers) in calibration standard solution. So the LOD for soil and sediment is estimated to be 0.2 ppb and the LOD for water is estimated to be 1 ppt of total Permethrin.

Time Required for Completion of a Sample Set

A sample set can be divided into two subsample sets for efficient handling. A subsample set consisted of a reagent blank, two controls (untreated water samples) and five fortified water samples (at one level i.e. LOQ). Time required for one subsample set from initiation of extraction until the completion of instrumental analysis and data evaluation is as follows:

- Sample preparation takes approximately 6 hours
- LC-MS/MS analysis and data processing (one or two MS/MS transitions for each isomer) take approximately 6 hours

TOTAL = approximately 12 hours for one analyst to complete a subsample set (approximately one a half calendar days) or 24 hours (3 calendar days) to complete two subsample sets to satisfy the validation requirements.

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-Water.

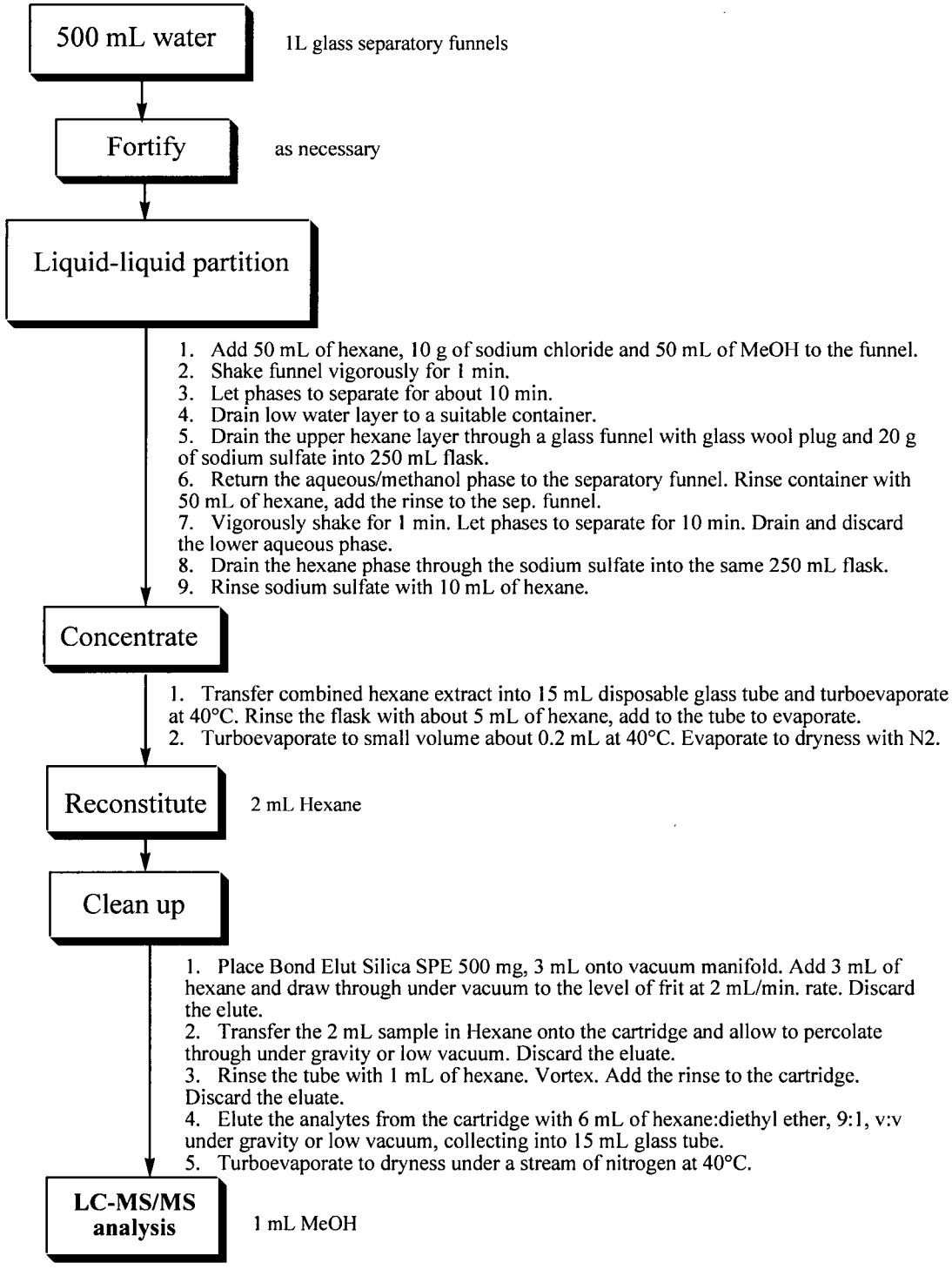


Figure 2. Schematic Diagram of the Analytical Method-Soil and Sediment.