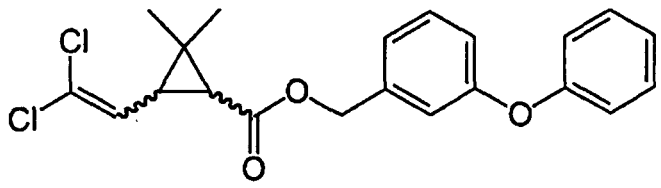
1.0 Introduction

1.1 Background

Permethrin (CAS#: 52645-53-1) is the active ingredient of certain pesticides in the synthetic pyrethroid family developed by FMC. Primera Analytical Solutions Corp. (hereafter referred to as PASC) has been contracted by FMC Corporation to conduct an independent laboratory validation (ILV) to demonstrate that the analytical methods reported in ML10-1602-PWG (for water) and ML06-1286-PWG (for soil) from Morse Laboratories, Inc can be performed with acceptable recoveries for quantitative determination of permethrin in water and soil by LC-MS/MS. This study was conducted as part of data call-in for the EPA registration review.



Permethrin

1.2 Purpose

This report summarizes the validation of quantitative LC/MS/MS methods for determination of permethrin in water and soil. The results demonstrated that the analytical methods are suitable for their intended uses.

1.3 Scope

According to the validation protocol PASC-PRT-0204 Ver01, this report applies to the validation methods for the analysis of permethrin in water and soil, which were developed by Morse Laboratories, Inc and reported in ML10-1602-PWG and ML06-1286-PWG.

2.0 References

2.1 Willoh, J.M., 2010, "Validation of Morse Laboratories, LLC Analytical Method (METH-201): "Determination of Residues of Bifenthrin, Permethrin, Cyfluthrin, Deltamethrin, Esfenvalerate, Fenpropathrin, *Lambda*-cyhalothrin and permethrin in Wastewater (Influent and Effluent)," Dated September 30, 2010. Morse Labs Project No.: ML10-1602-PWG. Date of the Report December 3, 2010. Unpublished study performed by Morse Laboratories, LLC, and submitted by the Pyrethroid Working Group (PWG), 200 pp. (MRID 48638501)

2.2 Reed, R.L., 2006, "Validation of the Residue Analytical Method: "Residue Analytical Method for the Determination of Residues of Bifenthrin, Permethrin, Cyfluthrin, Deltamethrin, Esfenvalerate, Fenpropathrin, *Lambda*-cyhalothrin and permethrin in Sediment,". Morse Labs Project No.: ML06-1286-PWG. Date of the Report November 29, 2006. Unpublished study performed by Morse Laboratories, LLC, and submitted by the Pyrethroid Working Group (PWG), 418 pp. (MRID 47053001 and 47053002)

2.3 EPA EFED Registration Review Preliminary Problem Formulation for Permethrin (06/20/11)

2.4 PASC-PRT-0204 ver01, "Independent Laboratory Validation of the Method for the Analysis of Permethrin in Water and Soil by LC-MS/MS"

2.5 PASC-SOP-0011 Ver04, "Method Validation/Qualification Procedure"

2.6 EPA OPPTS 850.7100, "Ecological Effects Test Guidelines-Data Reporting for Environmental Chemistry Methods"

2.7 Data Package under Project 058-0612C at PASC

3.0 Materials and Equipment

3.1 Materials and Chemicals

3.1.1 Analytes

Permethrin standards were provided by the sponsor.

Common name:	Cis-Permethrin
IUPAC 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:	391.28
Supplier:	FMC Corporation
FMC No.:	035171
FMC Reference No.:	G0101:6
Purity:	99.3%
Specification Date:	Nov-2007
Expiration Date:	Oct-2012
PASC ID:	11179

Common name:	Trans-Permethrin
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:	391.28
Supplier:	FMC Corporation
FMC No.:	030960
FMC Reference No.:	G026:89-2
Purity:	99.0%
Specification Date:	Feb-2011
Expiration Date:	Feb-2016
PASC ID:	11178

3.1.2 Matrix

Water (PASC ID 120036) was obtained from local Delaware River (Yardley, Pennsylvania). Soil (PASC ID 120022) was provided by FMC Corporation. The non-GLP soil characterization data can be found in the Attachment II.

3.1.1 Hexanes (PHARMCO-AAPER, Lot# PB001717HX95)

3.1.2 Methanol (J.T.Baker, Lot# K43E14)

3.1.3 Diethyl ether (Sigma-Aldrich, Lot# 12796KM)

3.1.4 Sodium Chloride (Sigma-Aldrich, Lot# MKAA0670)

3.1.5 Sodium sulfate (Sigma-Aldrich, Lot# MKBF3701V)

3.1.6 SPE Cartridge (Varian, Bond Elut SI, 500 mg 3 mL, Lot# 100411)

3.2 Equipment

3.2.1 SHIMADZU SIL-HTC Autosampler and SHIMADZU LC-10AD*vp* pumps coupled with Applied Biosystems 4000 Triple Quadrupole mass spectrometer (LETS #137)

3.2.1 Analytical Balance capable of weighing to 0.1 mg (LETS #169)

3.2.2 Analyst® 1.4.2 Software

3.2.3 Centrifuge, Model SorVall T6000,Thermal Scientific (LETS# 76)

3.2.4 Refrigerator, Sanyo Labcool, 2-8 °C (LETS#118)

4.0 Instrument Conditions/Parameters

4.1 Chromatographic Conditions

4.1.1 **Method A**: producing a single peak for cis-/trans- isomers

Column: Sunfire C8, 3.5 µm, 20 mm x 2.1 mm (Serial# 01013504210903; Catalog#: 186002697)

Flow rate: 0.4 mL/min.

Run time: 7 minutes

Mobile phase A: 0.5% Formic acid in DI water

Mobile phase B: Methanol

Elution gradient table:

Time (minutes)	A%	B%	Flow rate (mL/min)
0	80	20	0.4
2	10	90	0.4
4	10	90	0.4
4.5	80	20	0.4
7	80	20	0.4

4.1.2 **Method B**: producing separated peaks for cis- and trans- isomers

Column: Supelco Express C18, 2.7 µm, 50 mm x 2.1 mm (Serial # USMD003163; Catalog #: 53822-U)

Flow rate: 0.4 mL/min

Run time: 8 minutes

Mobile phase A: 0.5% Formic acid in DI water

Mobile phase B: Methanol

Elution gradient table:

Time (minutes)	A%	B%	Flow rate (mL/min)
0	70	30	0.4
3	10	90	0.4
5	10	90	0.4
5.5	70	30	0.4
8	70	30	0.4

4.2 Autosampler Properties (Method A & B)

Injection Volume (µl)	20
Sampling Speed (µl/Sec)	5
Needle Stroke (mm)	50

4.3 Mass Spectrometer Method Properties (Method A & B)

Acquisition Duration	Follow HPLC duration
Ionization Mode	ESI
Scan Type	MRM
Polarity	Positive
Resolution Q1	Unit
Resolution Q3	Unit
Gas 1 (GS1)	55
Gas 2 (GS2)	80
Collision Gas (CAD)	6
Curtain Gas (CUR)	15
Ion Spray Voltage (IS)	5500
Temperature (TEM)	300

4.4 Mass Transitions and Voltages (Method A & B)

Analyte	Q1	Q3	Time (msec)	DP*	EP*	CE*	CXP*
Permethrin	391.07	183.20	400	36	4	19	16

*Note: DP: Declustering Potential; EP: Exit Potential; CE: Collision Energy; CXP: Cell Exit Potential

5.0 Validation Procedures

The method validation was conducted on Jan. 31 – Feb. 27, 2012 at Primera labs in Princeton, New Jersey.

5.1 Standard Solution Preparation

5.1.1 *Stock Standard Solution preparation*: 5.100 mg of cis-permethrin standard (99.3% purity) and 5.110 mg of trans-permethrin standard (99.0% purity) were weighed into a 10-mL volumetric flask, and filled to the mark with methanol. The flasks were shaken to dissolve the analytes completely to obtain a solution with 1.01 mg/mL of total permethrin (cis/trans 1:1). 988 µL of this stock solution was transferred to a 10-mL volumetric flask, and filled to the mark with methanol to obtain a stock solution with 100 µg/mL concentration. 100 µL of this solution was further transferred to a 10-mL volumetric flask, and filled to the mark with methanol to obtain a stock solution with 1000 ng/mL concentration. All the above stock solutions were stored at 2-8°C.

5.1.2 *Calibration Standard Solution preparation:* A series of calibration standard solutions were prepared according to **Table 1**, starting from the stock solution with 1000 ng/mL concentration of permethrin. A separate set of standard solutions were prepared freshly before use for water and soil sample fortification and analyses.

Table 1. Calibration Standard Solution Preparation

Standard Solution ID	Source solution ID	Source solution concentration (ng/mL)	Aliquot Taken (mL)	Total Vol. (mL)	Concentration (ng/mL)
Std-1	Stock	1000	1.00	5.00	200
Std-2	Std-1	200	0.750	1.00	150
Std-3	Std-1	200	2.50	5.00	100
Std-4	Std-3	100	0.750	1.00	75.0
Std-5	Std-3	100	1.00	2.00	50.0
Std-6	Std-5	50.0	1.00	2.00	25.0
Std-7	Std-6	25.0	0.800	2.00	10.0
Std-8	Std-7	10.0	1.00	2.00	5.00

5.2 Residue Sample Preparation

5.2.1 Fortification

Fortification of water samples: 500 mL of river water was transferred into each of twelve 1-L separation funnels. The funnels were labeled as Control 1 to 2, LOQ 1 to 5 and 10xLOQ 1 to 5. The water samples were fortified with standard permethrin solutions as outlined in **Table 2A** below. The samples were then shaken and mixed. An empty separation funnel without water was used as reagent blank and was not fortified.

Fortification of soil samples: 10.0 g of soil were measured into each of twelve 50-mL centrifuge tubes. The tubes were labeled as Control 1 to 2, LOQ 1 to 5, and 10xLOQ 1 to 5. The soil samples were fortified with standard permethrin solutions as outlined in **Table 2B** below. The samples were then shaken and mixed. An empty centrifuge tube without soil was used as reagent blank and was not fortified.

Table 2A. Water Sample Fortifications

Sample ID	Water (ml)	Fortification Solution ID	Solution Concentration (ng/mL)	Fortification Volume (µL)	Fortification Level (ppt)
Reagent Blank	0	N/A	N/A	0	0
Control 1 and 2	500	N/A	N/A	0	0
LOQ 1 to 5	500	Std-6	25.0	100	5.00
10xLOQ 1 to 5	500	Std-3	100	250	50.0

Table 2B. Soil Sample Fortifications

Sample ID	Soil (g)	Fortification Solution ID	Solution concentration (ng/mL)	Fortification Volume (µL)	Fortification Level (ppb)
Reagent Blank	0	N/A	N/A	0	0
Control 1 and 2	10.0	N/A	N/A	0	0
LOQ 1 to 5	10.0	Std-3	100	100	1.00
10xLOQ 1 to 5	10.0	Stock	1000	100	10.0

5.2.2 Extraction and clean-up

Extraction and sample clean-up procedures were performed according to methods described in Morse Laboratories Reports (References 2.1 and 2.2) with minor modifications.

For water samples, 10 g of NaCl was added to each water sample, followed by extraction with hexane. The hexane extract was filtered through Na_2SO_4 and evaporated to dryness and re-dissolved in hexane, and cleaned up with a silica SPE cartridge. The eluate was evaporated to dryness and reconstituted in methanol for LC-MS/MS injection.

For soil samples, methanol/water (1:1, v/v) was added as described in the method and the sample was extracted with hexane. The extraction solution was evaporated to dryness and re-dissolved in hexane, and then cleaned up with a silica SPE cartridge. The eluate was evaporated to dryness and reconstituted in methanol for LC-MS/MS injection.

The extraction/clean-up procedures were summarized in **Tables 3A** and **3B**.

Table 3A. Extraction/Clean-up Summary for Water Samples

Sample ID	Fortification Level (ppt)	Extraction Volume (mL)	Reconstitution Volume (mL)	Nominal Injection Concentration (ng/mL)
Reagent Blank	0	100	0.500	0
Control 1 and 2	0	100	0.500	0
LOQ 1 to 5	5.00	100	0.500	5.00
10xLOQ 1 to 5	50.0	100	0.500	50.0

Table 3B. Extraction/Clean-up Summary for Soil Samples

Sample ID	Fortification Level (ppb)	Extraction Volume (mL)	Reconstitution Volume (mL)	Nominal Injection Concentration (ng/mL)
Reagent Blank	0	10.0	2.00	0
Control 1 and 2	0	10.0	2.00	0
LOQ 1 to 5	1.00	10.0	2.00	5.00
10xLOQ 1 to 5	10.0	10.0	2.00	50.0

5.2.3 Modification to the original methods

The following modifications were applied to the methods:

1) The quantification method was changed from GC to LC/MS/MS.
2) The final reconstitution solvent was changed from acetone to methanol to accommodate the LC/MS/MS quantification method.
3) 10 g of NaCl was added to each water sample before the hexane extraction step. The addition of NaCl facilitated layer separation and improved extraction efficiency.

5.3 Chromatographic Conditions

Two chromatographic methods were used during the validation. Using Method A, cis- and trans-permethrin isomers were eluted as a single peak with retention time (RT) at ~3.1 min. Using Method B, the two isomers were eluted as separated peaks with RT of trans-permethrin at 4.6 min and RT of cis-permethrin at 4.9 min. The detailed HPLC method parameters are listed in Section 4.1.

5.4 Injection Sequence

Five replicate samples at two fortification levels were used to evaluate the method efficiency. Calibration standards were injected within the analysis set to ensure detector linearity and stable response.

The validation set contained at least one reagent blank, two unfortified matrix controls, five matrix control samples fortified at 5.00 ppt for water or 1.00 ppb for soil as LOQ level and five matrix control samples fortified at 50.0 ppt for water or 10.0 ppb for soil as 10x LOQ level. The injection sequence is outlined in **Table 4**:

Table 4. Injection Sequence

Injection Sequence	Sample Type
1-2	Solvent Blank
3-4	Matrix Control Blank 1 to 2
5	Reagent Blank
6	Standard Solution 2.50 ng/mL
7	Standard solution 5.00 ng/mL
8-12	Fortified sample extracts LOQ 1-5
13	Standard solution 10.0 ng/mL
14	Standard solution 25.0 ng/mL
15	Standard solution 50.0 ng/mL
16-20	Fortified sample extracts 10xLOQ 1-5
21	Standard solution 100 ng/mL
22	Standard solution 150 ng/mL
23	Standard solution 200 ng/mL
24	Solvent Blank