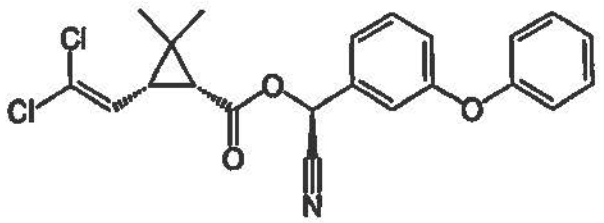
1.0 Introduction

1.1 Background

Cypermethrin (CAS#: 52315-07-8) 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 FMC analytical methods reported in ML10-1602-PWG (for water) and ML06-1288-PWG (for soil) from Morse Laboratories, Inc can be performed with acceptable recoveries for quantitative determination of cypermethrin in water and soil by LC-MS/MS. This study was conducted as part of data call-in for the EPA registration review.



Cypermethrin

1.2 Purpose

This report summarizes the validation of a quantitative LC/MS/MS method for determination of cypermethrin in water and soil. The results demonstrated that the analytical method is suitable for its intended use.

1.3 Scope

This report applies to the validation methods for the analysis of cypermethrin in water and soil, which were developed by and reported in ML10-1602-PWG and ML06-1286-PWG from Morse Laboratories, Inc, according to validation protocol PASC-PRT-0203 Ver01.

2.0 References

2.1 Willoh, J.M., 2010, "Validation of Morse Laboratories, LLC Analytical Method (METH-201): "Determination of Residues of Bifenthrin, Cypermethrin, 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 Unpublished study performed by Morse Laboratories, LLC, and submitted by the Pyrethroid Working Group (PWG), 200 pp.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, Cypermethrin, 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 Unpublished study performed by Morse Laboratories, LLC, and submitted by the Pyrethroid Working Group (PWG), 418 pp.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 Registration Eligibility Decision for Cypermethrin (revised 01/14/08)

2.4 PASC-PRT-0203, "Independent Laboratory Validation of the Method for the Analysis of Cypermethrin 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 058-0612B at PASC

3.0 Materials and Equipment

3.1 Materials and Chemicals

3.1.1 Analytes

Cypermethrin standard was provided by the sponsor.

Common name:	Cypermethrin
IUPAC Name:	α-Cyano-3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
CAS No.:	52315-07-8
Molecular formula:	$C_{22}H_{19}Cl_2NO_3$

Molecular weight:	416.30
Supplier:	FMC Corporation
FMC No.:	30980
FMC Reference No.:	CY-61
Purity:	95.2%
Specification Date:	Dec-2010
Expiration Date:	Dec-2012
PASC ID:	11040

3.1.2 Matrix

Water (PASC ID 110616) was obtained from local Delaware River (Yardley, Pennsylvania). Soil (PASC ID 110053) 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 (PHARMCO-AAPER, Lot# KKG20G)

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# 10041)

3.2 Equipment

3.2.1 SHIMADZU SIL-HTC Autosampler and SHIMADZU LC-10ADvp 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 #128)

3.2.2 Analyst® 1.4.2 Software

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

4.0 Instrument Conditions/Parameters

4.1 Chromatographic Conditions

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

4.2 Autosampler Properties

Syringe Size (µl)	100
Injection Volume (µl)	20
Sampling Speed (µl/Sec)	5
Needle Stroke (mm)	52

4.3 Mass Spectrometer Method Properties

Acquisition Duration	7 minutes
Ionization Mode	ESI
Scan Type	MRM
Polarity	Positive
Resolution Q1	Low
Resolution Q3	Low
Gas 1 (GS1)	70
Gas 2 (GS2)	60
Collision Gas (CAD)	6
Curtain Gas (CUR)	15
Ion Spray Voltage (IS)	5500
Temperature (TEM)	100

4.4 Mass Transitions and Voltages

Analyte	Q1	Q3	Time(msec.)	DP*	EP*	CE*	CXP*
Cypermethrin	416.0	190.9	200	46	10	17	12

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

5.0 Validation Procedures

The method validation was conducted on Nov. 29 - Dec. 09, 2011 at Primera labs in Princeton, New Jersey.

5.1 Standard Solution Preparation

5.1.1 *Stock Standard Solution preparation*: 26.33 mg of cypermethrin standard (95.2% purity) were weighed into a 25-mL volumetric flask, and filled to the mark with methanol. The flasks were shaken to dissolve the analytes completely to obtain a solution with 1.00 mg/mL concentration. 100 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 10 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 100 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 100 ng/mL concentration of cypermethrin. A separate set of standard solutions were prepared freshly before use for water matrix and soil matrix.

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	100	2.50	5.00	50.0
Std-2	Std-1	50.0	2.50	5.00	25.0
Std-3	Std-2	25.0	2.00	5.00	10.0
Std-4	Std-3	10.0	2.50	5.00	5.00
Std-5	Std-4	5.00	2.50	5.00	2.50
Std-6	Std-5	2.50	2.00	5.00	1.00
Std-7	Std-6	1.00	2.50	5.00	0.500
Std-8	Std-7	0.500	2.50	5.00	0.250

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 cypermethrin solutions as outlined in **Table 2A** below. The samples were then shaken and mixed. An empty separation funnel without water was used for 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 cypermethrin solutions as outlined in **Table 2B** below. The samples were then shaken and mixed. An empty centrifuge tube without soil was used for reagent blank and was not fortified.

Table 2A. Water Sample Fortifications

Sample ID	Water (ml)	Fortification Solution ID	Fortification solution concentration (ng/mL)	Fortification Volume (µL)	Fortification Level (ppt)
Reagent Blenk	0	N/A	N/A	0	0
Control 1 and 2	500	N/A	N/A	0	0
LOQ 1 to 5	500	Std-5	2.50	100	0.500
10xLOQ 1 to 5	500	Std-2	25.0	100	5.00

Table 2B. Soil Sample Fortifications

Sample ID	Soil (g)	Fortification Solution ID	Fortification 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	10.0	100	0.100
10xLOQ 1 to 5	10.0	Stock	100	100	1.00

5.2.2 Extraction and clean-up

Extraction and sample clean-up procedures were performed according to methods described in Morse Laboratories Reports (Reference 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	0.500	100	0.500	0.500
10xLOQ 1 to 5	5.00	100	0.500	5.00

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	0.100	10.0	2.00	0.500
10xLOQ 1 to 5	1.00	10.0	2.00	5.00

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 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 0.500 ppt for water or 0.100 ppb for soil as LOQ level and five matrix control samples fortified at 5.00 ppt for water or 1.00 ppb for soil as 10x LOQ level. The injection sequences are outlined in **Tables 4A** and **4B** below:

Table 4A. Injection Sequence for Water Samples

Injection Sequence	Sample Type
1-4	Solvent Blank
5-6	Matrix Control Blank 1 to 2
7	Reagent Blank
8	Standard Solution 0.25 ng/mL
9	Standard solution 0.5 ng/mL
10	Standard solution 1 ng/mL
11-15	Fortified sample extracts LOQ 1-5
16	Standard solution 2.5 ng/mL
17	Standard solution 5 ng/mL
18	Standard solution 10 ng/mL
19-23	Fortified sample extracts 10xLOQ 1-5
24	Standard solution 25 ng/mL
25	Standard solution 50 ng/mL

Table 4B. Injection Sequence for Soil Samples

Injection Sequence	Sample Type
1-2	Solvent Blank
3-4	Matrix Control Blank 1 to 2
5	Reagent Blank
6	Standard Solution 0.25 ng/mL
7	Standard solution 0.5 ng/mL
8-12	Fortified sample extracts LOQ 1-5
13	Standard solution 1 ng/mL
14	Standard solution 2.5 ng/mL
15	Standard solution 5 ng/mL
16-20	Fortified sample extracts 10xLOQ 1-5
21	Standard solution 10 ng/mL
22	Standard solution 25 ng/mL
23	Standard solution 50 ng/mL