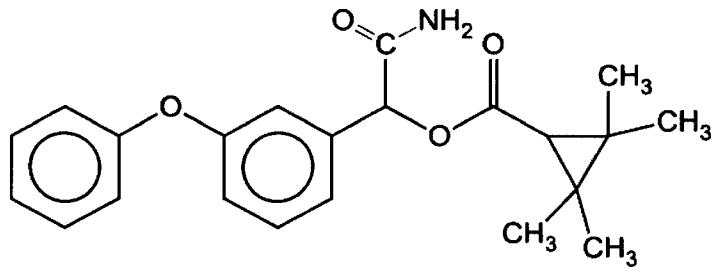
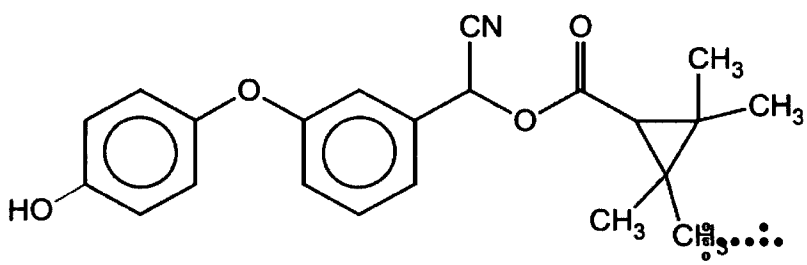
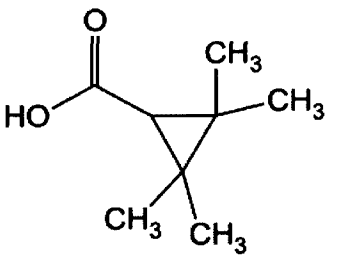
Analytical Reference Standards

Standard Name:	$CONH_2$-Fenpropathrin
Lot Number:	AS 2129b
CPS ID:	14-CPS-Aug28-01
Valent Sample Archive No.:	V-Arc-2312
Source:	Valent USA
Purity:	99.6%
Molecular Formula:	$C_{22}H_{25}NO_4$
Average Mass:	367.2
Molecular Structure:	

Standard Name:	4′-OH-Fenpropathrin
Lot Number:	AS 2354a
CPS ID:	14-CPS-Aug28-02
Valent Sample Archive No.:	V-Arc-2314
Source:	Valent USA
Purity:	96.4%
Molecular Formula:	$C_{23}H_{22}NO_4$
Average Mass:	365.2
Molecular Structure:	

Standard Name:	TMPA
Lot Number:	AS 2357a
CPS ID:	14-CPS-Aug28-03
Valent Sample Archive No.:	V-Arc-2317
Source:	Valent USA
Purity:	99.7%
Molecular Formula:	$C_8H_{14}O_2$
Average Mass:	142.1
Molecular Structure:	

Other

Upon completion of the study, a copy of the protocol and the final report will be archived at Critical Path Services, LLC (CPS). The original protocol, final report, raw data, correspondence, and other documentation will be transferred to the Valent Archives, Valent U.S.A. Corporation, 6560 Trinity Court, Dublin, California, 94568.

2.0 INTRODUCTION

The objective of this study was to validate Valent method (Golden Pacific Laboratories method GPL-MTH-085) "Analytical Method for the Determination of Fenpropathrin Metabolites $CONH_2$-Fenpropathrin, 4'-OH-Fenpropathrin, and TMPA in Drinking Water by LC-MS/MS" [1]. This method passed the ILV for all three analytes in surface water on the first attempt with no major modifications.

This study was designed to fulfill the requirements of the US EPA Ecological Effects Test Guidelines OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation [2]. In addition, this study was conducted in compliance with US EPA FIFRA (40 CFR Part 160) GLP standards [3].

3.0 MATERIALS AND METHODS

3.1 Test Substances

Standard name:	$CONH_2$-Fenpropathrin
Lot number:	AS 2129b
CPS ID:	14-CPS-Aug28-01
Sample archive no.:	V-Arc-2312
Manufacturer's ID:	Sumitomo Lot 08SC8046993
Purity:	99.6%
Date of analysis:	April 07, 2014
Expiration date:	April 07, 2015
Storage conditions:	Frozen

Standard name:	4'-OH-Fenpropathrin
Lot number:	AS 2354a
CPS ID:	14-CPS-Aug28-02
Sample archive no.:	V-Arc-2257
Manufacturer's ID:	Sumitomo Lot S-3206 No.36
Purity:	96.4%
Date of analysis:	April 22, 2014
Expiration date:	April 22, 2016
Storage conditions:	Frozen

Standard name:	TMPA
Lot number:	AS 2357a
CPS ID:	14-CPS-Aug28-03
Sample archive no.:	V-Arc-2317
Manufacturer's ID:	Sumitomo Lot F-16
Purity:	99.7%
Date of analysis:	April 22, 2014
Expiration date:	April 22, 2016
Storage conditions:	Frozen

3.2 Test System

The test system used for the validation was surface water collected at Upper Merion Township Park (175 West Valley Road, King of Prussia, PA 19406). The samples were stored in a refrigerator when not in use.

3.3 Equipment and Reagents

The equipment and reagents used for the method validation were as outlined on pages 5 through 7 in the Valent method (Golden Pacific Laboratories method GPL-MTH-085; included in the protocol in Appendix 1), and precisely documented in the study records (Notebook pages 4–8). Identical or equivalent equipment and materials were used.

3.3.1 Equipment and Apparatuses

Analytical balance (Mettler Toledo)
Manual pipettor of multiple volumes
Electronic pipettor of multiple volumes
Refrigerator/freezer (Nor-Lake® Scientific)
Top-loading balance (Mettler Toledo)
Ultrasonic cleaner 5210 (Branson)
Polypropylene conical tubes (50-mL)
5-mL plastic syringes
PTFE syringe filter, 0.45 µm, 25 cm (Pall Life Sciences)
Oasis® Max cartridges, 60 mg/3cc (Waters)
SPE manifold (Supelco)
Centrifuge tubes, 50-mL and 15-mL
Various sizes of volumetric flasks
Various size of glass graduated cylinders
LC-MS/MS—Agilent 1200 binary pump HPLC system and autosampler, coupled to an Applied Biosystems® API 4000™ mass spectrometer with an electrospray ionization interface

3.3.2 Reagents

Acetone (Pharmco-AAPER)
Acetonitrile (EMD)
Methanol (EMD)
Formic acid (Sigma-Aldrich®)
Milli-Q water
Ammonium hydroxide (Sigma-Aldrich)
Monobasic sodium phosphate, monohydrate (Sigma-Aldrich®)
Dibasic sodium phosphate, anhydrate (EMD)
Milli-Q water

3.4 Experimental Design

3.4.1 Establishment of the Method

Prior to performing the ILV, the analyte retention times, instrument detection limits, and linearity of instrument responses to a range of analyte concentrations were determined. The SPE cartridge recovery was also checked before the validation.

3.4.2 Standard Solutions Preparation

The primary stock solution for each reference standard was prepared by weighing approximately 50.0 mg of compound onto a tared piece of weigh paper and transferring to a 50-mL glass volumetric flask. Acetone was added up to volume and the solution was sonicated appropriately.

Fortification solution FS1B, containing 1.00 µg/mL of $CONH_2$-fenpropathrin and 4′-OH-fenpropathrin, was prepared by adding an appropriate amount of each primary stock solution into a 100-mL volumetric flask and diluting up to volume with methanol. A second fortification solution, FS2B, was prepared at a concentration of 0.100 µg/mL (of each analyte) by measuring 10.0 mL of the FS1B into a 100-mL volumetric flask and diluting to volume with methanol.

TMPA fortification standard FS3B (4.00 µg/mL) was prepared by pipetting 40 µL of TMPA stock standard (1.00 mg/mL) into a 10-mL volumetric flask and bringing to volume with methanol. The TMPA fortification standard FS4B (0.400 µg/mL) was prepared by measuring 1.00 of FS3B into a 10-mL volumetric flask and bringing to volume with methanol.

The calibration standard solutions containing $CONH_2$-fenpropathrin and 4′-OH-fenpropathrin were prepared from an intermediate standard, IM1B (100 µg/L), which was prepared from FS1B in methanol/water (50:50, v/v) solution. The calibration standards were prepared by adding an appropriate amount of IM1B standard to 10-mL volumetric flasks and diluting to the total volume with methanol/water (50:50, v/v) solution. Calibration standards for $CONH_2$-fenpropathrin and 4′-OH-fenpropathrin ranged from 0.250 µg/L to 10.0 µg/L.

TMPA calibration standard solutions were prepared from an intermediate standard, IM2B, containing TMPA at 2.00 µg/mL, which was prepared from FS3B in methanol/water/formic acid (50:50:1, v/v/v) solution. The TMPA calibration standards were prepared by adding an appropriate amount of IM3B standard to 10-mL volumetric flasks and diluting to the total volume with methanol/water/formic acid (50:50:1, v/v/v) solution. Calibration standards for TMPA ranged from 2.50 µg/L to 100 µg/L.

All standard solutions were refrigerated (~4°C) when not in use.

3.4.3 Sample Validation Sets, Fortification, and Extraction Procedure

Sample Validation Sets

An analytical set was prepared for $CONH_2$-fenpropathrin and 4'-OH-fenpropathrin; a separate set was also prepared for TMPA. Each analytical set consisted of 13 samples: one reagent blank, two untreated controls, five untreated controls fortified at the LOQ (1.00 µg/L), and five untreated controls fortified at 10× LOQ (10.0 µg/L).

Sample Preparation for $CONH_2$-Fenpropathrin and 4'-OH-Fenpropathrin Analysis

1. Pipetted 10.0-mL sample into a 20-mL scintillation vial.
2. Added the appropriate amount of fortification solution to the sample.
   a. For the reagent blank and control samples, added nothing.
   b. For the LOQ samples, added 100 µL of FS2B (0.100 µg/mL $CONH_2$-fenpropathrin and 4'-OH-fenpropathrin) fortification solution.
   c. For the 10× LOQ samples, added 100 µL of FS1B (1.00 µg/mL $CONH_2$-fenpropathrin and 4'-OH-fenpropathrin) fortification solution.
3. Added 10.0 mL of methanol to each sample, capped the sample vials, and shook for ~5 seconds.
4. Filtered approximately 1.5 mL sample through a 0.45 µm syringe filter into an HPLC vial for LC-MS/MS analysis.

Sample Preparation for TMPA Analysis

1. Measured 40-mL sample into a 50-mL centrifuge tube.
2. Added the appropriate amount of fortification solution to the sample.
   a. For the reagent blank and control samples, added nothing.
   b. For the LOQ samples, added 100 µL of FS4B (0.400 µg/mL TMPA) fortification solution.
   c. For the 10× LOQ samples, added 100 µL of FS3B (4.00 µg/mL TMPA) fortification solution.
3. Added 2.50 mL of 100 mM phosphate buffer to each sample and mixed.
4. Placed Waters Oasis® Max cartridge (60mg/3cc) on an SPE manifold. Conditioned the cartridge with 3.00 mL methanol followed by 3.00 mL water.
5. Loaded the sample to the cartridge.
6. Washed the cartridge with 3.00 mL water, 3.00 mL of 0.15 mM ammonium hydroxide solution, and 3.00 mL of methanol. Discarded all washes.
7. Placed a 15-mL centrifuge tube under the cartridge, eluted TMPA with 2.00 mL of 2% formic acid in methanol solution, and collected into a 15-mL tube tube (eluent 1). Vacuum was applied to pull the liquid completely.
8. Placed a clean 15-mL centrifuge tube under the cartridge, eluted with 2.00 mL of methanol/water/formic acid (50:50:1, v/v/v) solution (eluent 2).
9. Brought the total volume of eluent 1 to 4.00 mL with water and transferred a ~1.5-mL aliquot of eluent 1 into an autosampler vial for LC-MS/MS analysis. Eluent 2 was stored in a refrigerator.

3.4.4 Sample Processing and Analysis

The samples were analyzed as described by the Valent method (Golden Pacific Laboratories method GPL-MTH-085). The samples were analyzed with six calibration standards interspersed with the samples in a sequence. The continuing calibration standards (2.00 µg/L for $CONH_2$-fenpropathrin and 4'-OH-fenpropathrin and 20.0 µg/L for TMPA) were injected at the beginning, middle, and end of the sequence. The coefficient of determination of the continuing calibration standards was acceptable (<15%) for the $CONH_2$-fenpropathrin, 4'-OH-fenpropathrin, and TMPA analyses.

3.5 LC-MS/MS Instrumentation

Instrumentation
Agilent 1200 HPLC System (Agilent Technologies)
API 4000™ Tandem Mass Spectrometer, MS/MS (Applied Biosystems®)
HPLC Column: Phenomenex Luna® C18, 30 × 2 mm, 3 µm
Software: Applied Biosystems®, Analyst® 1.6.2
Refer to Table 2 for the details of the instrument conditions.

3.6 Data Acquisition and Reporting

Peak integration was performed by Analyst® software version 1.6.2. The MS detector responses (peak area) for various injected standard concentrations were used to generate an external calibration curve for the analytes of interest. The overall purpose of the external calibration curve was to display acceptable linearity (r^2 ≥0.99) of the assigned calibration range. The recoveries of the analytes from the fortified samples were calculated by multi-point calibration.

Recovery results of each analyte were computed for each sample. The equations used for quantification are presented in Appendix 2. A statistical treatment of the data includes the calculation of means, standard deviations (SD), RSDs as percentages (%), and the 95% confidence intervals. All statistics were calculated using Microsoft® Office Excel 2003.

Table 2 LC-MS/MS System Operating Parameters

HPLC System:	Agilent Model 1200
Software:	Applied Biosystems®, Analyst® 1.6.2
HPLC Column:	Phenomenex Luna® C18, 30 × 2 mm, 3 µm
Needle Wash:	(50:50, v/v) water/methanol
Wash Time:	Flush port, 15 seconds

$CONH_2$-Fenpropathrin and 4'-OH-Fenpropathrin Analysis

Mobile Phase:	(A - Aqueous):	0.2% formic acid in HPLC-grade water
	(B - Organic):	0.2% formic acid in acetonitrile
Injection Volume:	10.0 µL	
Run Time:	6.5 minutes	

Gradient:

Time (min)	A (%)	B (%)	Flow
0.0	60.0	40.0	500
2.0	30.0	70.0	500
3.5	30.0	70.0	500
3.6	10.0	90.0	500
4.6	10.0	90.0	500
4.7	60.0	40.0	500
6.5	60.0	40.0	500

Mass Spectrometer Conditions:

Parameter	Setting			
Ion Source:	TurboSpray			
Scan Type:	MRM			
Curtain Gas (CUR):	25			
Temperature (TEM):	100			
Ion Source Gas 1 (GS1):	60			
Ion Source Gas 2 (GS2):	50			
Interface Heater (ihe):	ON			
Analyte	$CONH_2$-Fenpropathrin		4'-OH-Fenpropathrin	
Polarity	Positive (0-3.75 min)		Negative (3.76-6.50 min)	
Declustering Potential (DP):	66.00		-100	
Entrance Potential (EP):	10.00		-10	
Dwell Time (msec)	150		150	
Collision Gas (CAD):	6.00		6.00	
Ion Spray Voltage (IS):	5500		-4500	
Transitions Monitored:	Quant.	Conf.	Quant.	Conf.
	368.2→125.2	368.2→97.1	364.0→140.9	364.0→212.9
Collision Cell Exit Potential (CXP):	8.00	6.00	-1.00	-3.00
Collision Energy (CE):	15.0	43.0	-28.0	-22.0

TMPA Analysis

Mobile Phase: (A - Aqueous): Water
(B - Organic): Acetonitrile
Injection Volume: 50.0 µL
Run Time: 6.5 minutes
Gradient:

Time (min)	A (%)	B (%)	Flow
0.0	90.0	10.0	500
3.0	40.0	60.0	500
4.0	40.0	60.0	500
4.1	10.0	90.0	500
4.5	10.0	90.0	500
4.6	90.0	10.0	500
6.5	90.0	10.0	500

Mass Spectrometer Conditions:

Parameter	Setting	
Ion Source:	TurboSpray	
Scan Type:	MRM	
Curtain Gas (CUR):	30	
Temperature (TEM):	500	
Ion Source Gas 1 (GS1):	70	
Ion Source Gas 2 (GS2):	50	
Interface Heater (ihe):	ON	
Polarity	Negative	
Declustering Potential (DP):	-65.00	
Entrance Potential (EP):	-5.00	
Dwell Time (msec)	150	
Collision Gas (CAD):	6.00	
Ion Spray Voltage (IS):	-4500	
Transitions Monitored:	Quant.	Conf.
	141.0→106.9	141.0→97.0
Collision Cell Exit Potential(CXP):	-5.00	-10
Collision Energy (CE):	-26	-18

APPENDIX 2 CALCULATIONS

For calculation of the concentrations, calibration curves were used. These curves were calculated automatically after each sequence run with the Applied Biosystems® Analyst® software version 1.6.2 using a linear regression with 1/concentration weighting. Further calculations were performed using Microsoft® Office Excel 2003.

The linear equation is expressed as:

$$y = mx + b$$

where

y = peak area
x = concentration (ng/mL)

The concentration of analyte in the final sample solution can be calculated as follows:

$$\text{Final Sample Concentration C (ng/mL)} = (y-b)/m$$

The residue of analytes in test samples is calculated as follows:

$$\text{Residue (ppb)} = \frac{C \times (1/1000) \times FV \times DF}{V}$$

where

C = Concentration in final sample (ng/mL)
FV = Final sample volume (20.0 mL for $CONH_2$-fenpropathrin and 4′-OH-fenpropathrin; 4.00 mL for TMPA)
V = Sample volume (10.0 mL for $CONH_2$-fenpropathrin and 4′-OH-fenpropathrin; and 40.0 mL for TMPA)
DF = Additional dilution factor

Recoveries are calculated using the following equation:

$$\text{Recovery (\%)} = \frac{\text{Analyzed Final Sample Concentration}}{\text{Theoretical Final Sample Concentration}} \times 100$$