Test Material:	Fenpropathrin
MRID:	49491402
Title:	Independent Laboratory Validation for "Analytical Method for the Determination of Fenpropathrin Metabolites CONH2-Fenpropathrin, 4'-OH-Fenpropathrin, and TMPA in Drinking Water by LC-MS/MS"
EPA PC Code:	127901
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** 

Date: 4/9/15

Secondary Reviewer: Lynne Binari **Signature:** 

Date: 4/9/15

QC/QA Manager: Joan Gaidos **Signature:** 

Date: 4/9/15

Analytical method for fenpropathrin metabolites $CONH_2$-fenpropathrin, 4'-OH-fenpropathrin, and TMPA in water

Reports:	ECM: EPA MRID No. 49491402 (Appendix 1, Appendix 1, pp. 62-89). Schoenau, E.A. 2014. Analytical Method for the Determination of Fenpropathrin Metabolites CONH2-Fenpropathrin, 4'-OH-Fenpropathrin, and TMPA in Drinking Water by LC-MS/MS. Method No.: GPL-MTH-085. Report prepared by Golden Pacific Laboratories, LLC, Fresno, California; sponsored and submitted by Valent U.S.A. Corporation, Dublin, California (Appendix 1, p. 56); 28 pages. Final report issued July 28, 2014. ILV: EPA MRID No. 49491402. Li, F. 2014. Independent Laboratory Validation for "Analytical Method for the Determination of Fenpropathrin Metabolites CONH2-Fenpropathrin, 4'-OH-Fenpropathrin, and TMPA in Drinking Water by LC-MS/MS". Laboratory Project ID/CPS Study No.: 14-CPS-014. Report prepared by Critical Path Services, LLC (CPS), Garnet Valley, Pennsylvania; sponsored and submitted by Valent U.S.A. Corporation, Dublin, California (Appendix 1, p. 56); 98 pages. Final report issued October 7, 2014.
Document No.:	MRID 49491402 (ILV & ECM)
Guideline:	850.6100
Statements:	ECM: It was not reported if the study was conducted in compliance with any GLP regulations. Statements of No Data Confidentiality, GLP, Quality Assurance and Authenticity Certification were not provided. A signatures page was provided (Appendix 1, Appendix 1, p. 62). ILV: The study was conducted in accordance with the USEPA FIFRA GLP (40 CFR Part 160; p. 3). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 5). A Certification of Authenticity was not provided.
Classification:	This analytical method is classified as supplemental. The LOQ and LOD were not determined using scientifically acceptable procedures. The ILV did not report LODs. The water matrices were not characterized. Calibration curves and calibration raw data were not reported in the ECM.
PC Code:	127901
Reviewer:	Jim Carleton, Ph.D. Senior Scientist, USEPA **Date:** 8/6/15

JAMES CARLETON Digitally signed by JAMES CARLETON DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=JAMES CARLETON, dnQualifier=0000001907 Date: 2015.08.06 14:20:17 -04'00'

All page numbers refer to those listed at the bottom-most center of the MRID pages.

Executive Summary

This analytical method, GPL-MTH-085, is designed for the quantitative determination of fenpropathrin metabolites $CONH_2$-fenpropathrin, 4'-OH-fenpropathrin, and TMPA in water at the stated LOQ of 1.00 µg/L using LC/MS/MS. The ECM validation was conducted using drinking water. The ILV successfully validated the method for all three analytes after one trial using surface water. The water matrices of the ECM and ILV were not characterized. In the ECM and ILV, analytes were identified using two ion transitions; only one transition was used

for quantification for all analytes. The ILV recommended that the amount of phosphate buffer needed to adjust the pH of the test sample be measured prior to analysis and the ECM correct a typographical error in the procedure for preparation of a standard. The LOD was not reported in the ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
$CONH_2$-Fenpropathrin	49491402 (Appendix 1, Appendix 1, pp. 62-89)	49491402		Water	07/28/2014	Valent U.S.A Corporation	LC/MS/MS	1.00 µg/L
4'-OH-Fenpropathrin								
TMPA								

I. Principle of the Method

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

Water (10 mL) was fortified (100 µL of 100/100 ng/mL mixed standard or 1.0/1.0 µg/mL mixed standard) then combined with 10 mL of methanol via manual shaking (*ca*. 5 seconds; Appendix 1, Appendix 1, pp. 64-65, 71, 73-74). An aliquot (*ca*. 1.5 mL) of the sample was then filtered (PTFE 0.45-µm), and analyzed by LC/MS/MS. If necessary, additional dilutions were made using methanol:water (1:1, v:v).

Samples are analyzed using an AB Sciex API 4000 LC/MS/MS with electrospray ionization (ESI; Appendix 1, Appendix 1, pp. 68-69). The following LC conditions were used: Phenomenex Luna C18 column (30 mm x 2 mm, 3 µm, column temperature ambient) using a mobile phase of (A) 0.2% formic acid in acetonitrile and (B) 0.2% formic acid in water [percent A:B (v:v) at 0.0 min. 40:60, 2.0-3.5 min. 70:30, 3.6-4.6 min. 90:10, 4.7-6.5 min 40:60]. Injection volume was 10 µL. The following MS/MS conditions were used: ESI in positive ion mode detection for $CONH_2$-fenpropathrin and negative ion mode detection for 4'-OH-fenpropathrin and multiple reaction monitoring (MRM). Analytes were identified using two ion transitions; one for quantitation (Q, "primary") and one for confirmation (C). Ion transitions monitored were as follows: *m/z* 368.0→125.0 (Q) and *m/z* 368.0→97.0 (C) for $CONH_2$-fenpropathrin and *m/z* 364.2→141.0 (Q) and *m/z* 364.2→213.0 (C) for 4'-OH-fenpropathrin. Expected retention times were *ca*. 2.5 and 2.7 minutes for $CONH_2$-fenpropathrin and 4'-OH-fenpropathrin, respectively.

For TMPA

Water (20 mL) was fortified (100 µL of 200 ng/mL standard in methanol or 2.00 µg/mL standard in methanol) then combined with 1 mL of 100 mM phosphate buffer (pH = 7.2) via manual shaking (*ca*. 5 seconds; Appendix 1, Appendix 1, pp. 68, 71). A 60 mg, 3 cc MAX Oasis solid phase extraction (SPE) column was pre-conditioned with methanol then water (3 mL each; the study author noted that this SPE column should not be substituted). After the sample was loaded

onto the column, the column was washed sequentially with water, 0.15 M aqueous ammonium hydroxide solution and methanol (3 mL each). The eluate was discarded before TMPA was eluted with 2 mL of 2% formic acid in methanol under vacuum. The volume of the eluate was adjusted to 4 mL using water and analyzed by LC/MS/MS. If necessary, additional dilutions were made using methanol:water:formic acid (50:50:1, v:v:v).

Samples are analyzed using an AB Sciex API 5000 LC/MS/MS with electrospray ionization (ESI; Appendix 1, Appendix 1, pp. 69-70). The study author noted that concentration of TMPA in the sample should be increased if the signal cannot be differentiated from the background noise levels. The following LC conditions were used: Phenomenex Luna C18 column (30 mm x 2 mm, 3 µm, column temperature ambient) using a mobile phase of (A) acetonitrile and (B) water [percent A:B (v:v) at 0.0 min. 10:90, 3.0-4.0 min. 60:40, 4.1-4.5 min. 90:10, 4.6-6.5 min 10:90]. Injection volume was 50 µL. The following MS/MS conditions were used: ESI in negative ion mode detection and multiple reaction monitoring (MRM). Analytes were identified using two ion transitions; one for quantitation (Q, "primary") and one for confirmation (C). Ion transitions monitored were as follows: *m/z* 141.0→106.9 (Q) and *m/z* 141.0→97.0 (C) for TMPA. Expected retention time was *ca*. 2.8 minutes. The method defines that the TMPA confirmation ion pair (*m/z* 141.0→97.0) cannot be used for quantitation for <10×LOQ levels.

ILV

The samples were processed using the same procedure as that of the ECM, except that the volume of water sample and phosphate buffer solution were increased (20 mL to 40 mL and 1.00 mL to 2.50 mL, respectively) for the TMPA analysis due to the sensitivity of the instrument used (pp. 15-18; Table 2, pp. 22-23). Both analytes were analyzed using an AB API 4000 LC/MS/MS with ESI interface. Fortifications of TMPA were performed using 100 µL of 0.400 µg/mL standard in methanol or 4.00 µg/mL standard in methanol.

In the ECM and ILV, the LOQ was 1.00 µg/L for all three analytes (p. 12; Appendix 1, Appendix 1, pp. 63, 73). In the ECM, the LOD was reported as 0.5 µg/L for all analytes; the LOD was not reported in the ILV.

II. Recovery Findings

ECM (MRID 49491402; Appendix 1, Appendix 1, pp. 62-89): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of $CONH_2$-fenpropathrin, 4'-OH-fenpropathrin and TMPA in drinking water at the LOQ (1 ppb) and 10x LOQ (10 ppb; Appendix 1, Appendix 1, p. 73; Appendix 1, Appendix 1, Tables 1-3, pp. 87-89). The method defines that the TMPA confirmation ion pair (*m/z* 141.0→97.0) cannot be used for quantitation for <10×LOQ levels due to limited sensitivity (Appendix 1, Appendix 1, p. 70). Analytes were identified using two ion transitions; only one transition was used for quantification for all analytes (Appendix 1, Appendix 1, Figures 1-12, pp. 75-86; Appendix 1, Appendix 1, Tables 1-3, pp. 87-89). Therefore, quantitation ion and confirmation ion recovery results could not be compared. The water matrix was not characterized.

ILV (MRID 49491402): Mean recoveries and RSDs were within guideline requirements for analysis of $CONH_2$-fenpropathrin, 4'-OH-fenpropathrin and TMPA in surface water at the LOQ and 10x LOQ (pp. 12, 18; Table 1, p. 21). Analytes were identified using two ion transitions; only one transition was used for quantification for all analytes (Table 1, p. 21; Figures 4-30, pp. 28-54). Therefore, quantitation ion and confirmation ion recovery results could not be compared. The water matrix was collected from Upper Merion Township Park, King of Prussia, Pennsylvania; it was not characterized or further described (p. 14). The method was validated with the first trial for all analytes (p. 18).

Table 2. Initial Validation Method Recoveries for $CONH_2$-Fenpropathrin, 4'-OH-Fenpropathrin and TMPA in Drinking Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)[1]
	Quantitation Ion					
$CONH_2$-Fenpropathrin *m/z* 368.0→125.0	1.00 (LOQ)	5	104-110	107	2.17	2.03
	10.0	5	105-107	106	0.707	0.667
4'-OH-Fenpropathrin *m/z* 364.2→141.0	1.00 (LOQ)	5	102-106	105	1.64	1.56
	10.0	5	103-106	104	1.22	1.17
TMPA *m/z* 141.0→106.9	1.00 (LOQ)	5	85.4-91.4	88.1	2.26	2.57
	10.0	5	93.8-102	97.6	3.42	3.50
	Confirmation Ion					
$CONH_2$-Fenpropathrin *m/z* 368.0→97.0	1.00 (LOQ)	Data not reported.				
	10.0					
4'-OH-Fenpropathrin *m/z* 364.2→213.0	1.00 (LOQ)					
	10.0					
TMPA *m/z* 141.0→97.0	1.00 (LOQ)					
	10.0					

Data (uncorrected recovery results, Appendix 1, Appendix 1, pp. 72-73) were obtained from Appendix 1, Appendix 1, Tables 1-3, pp. 87-89 of the study report.
1 Coefficient of Variance in study tables (Appendix 1, Appendix 1, Tables 1-3, pp. 87-89).

Table 3. Independent Validation Method Recoveries for $CONH_2$-Fenpropathrin, 4'-OH-Fenpropathrin and TMPA in Surface Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)[1]
	Quantitation Ion					
$CONH_2$-Fenpropathrin *m/z* 368.0→125.0	1.00 (LOQ)	5	101-105	103	1.42	1.42
	10.0	5	106-109	107	1.14	1.06
4'-OH-Fenpropathrin *m/z* 364.2→141.0	1.00 (LOQ)	5	97.6-102	100	2.24	2.25
	10.0	5	104-110	107	2.41	2.26
TMPA *m/z* 141.0→106.9	1.00 (LOQ)	5	71.0-88.1	78.3	7.18	9.17
	10.0	5	70.6-83.2	76.5	4.80	6.27
	Confirmation Ion					
$CONH_2$-Fenpropathrin *m/z* 368.0→97.0	1.00 (LOQ)	5	Data not reported.			
	10.0	5				
4'-OH-Fenpropathrin *m/z* 364.2→213.0	1.00 (LOQ)	5				
	10.0	5				
TMPA *m/z* 141.0→97.0	1.00 (LOQ)	5				
	10.0	5				

Data (uncorrected recovery results, Appendix 2, p. 90) were obtained from Table 1, p. 21 of the study report.

III. Method Characteristics

In the ECM and ILV, the LOQ was 1.00 µg/L for all three analytes (pp. 12, 18; Appendix 1, Appendix 1, pp. 63, 73). No justification or calculation was provided for the LOQ. No comparison was made to chromatogram background levels. In the ECM, the LOD was reported as 0.5 µg/L for all analytes; the LOD was not reported in the ILV. The LOD was calculated based on the lowest calibration standard, 0.25 ng/mL ($CONH_2$-fenpropathrin, 4'-OH-fenpropathrin) or 2.5 ng/mL (TMPA), sample size and dilution factor.

Table 4. Method Characteristics in Water

		$CONH_2$-Fenpropathrin	4'-OH-Fenpropathrin	TMPA
Limit of Quantitation (LOQ)		1.00 µg/L		
Limit of Detection (LOD)		0.5 µg/L		
Linearity (calibration curve r^2 and concentration range)	ECM:	No linearity data was reported.		
	ILV[1]:	r^2 = 0.9998	r^2 = 1.0000	r^2 = 0.9990
	Range:	0.250-10.0 µg/L		2.50-100 µg/L
Repeatable		Yes at LOQ and 10x LOQ (quantitative ion only, drinking water).[2]		
Reproducible		Yes at LOQ and 10x LOQ (quantitative ion only, surface water). [2]		
Specific	ECM:	Yes; interferences at the analyte retention times were ≤20% (based on peak height) of the LOQ.		Yes; interferences at the analyte retention times were ≤20% (based on peak height) of the LOQ, except for the confirmation ion analyses. [3]
	ILV:			

Data were obtained from pp. 12, 15, 18; Table 1, p. 21; Figures 1-30, pp. 25-54; Appendix 1, Appendix 1, Figures 1-12, pp. 75-86; Appendix 1, Appendix 1, Tables 1-3, pp. 87-89 of the study report; DER Attachment 2.

1 The reviewer calculated ILV coefficient of determination (r^2) values from the provided r values (DER Attachment 2).

2 Analytes were identified using two ion transitions; only one transition was used for quantification for all analytes (Table 1, p. 21; Figures 4-30, pp. 28-54; Appendix 1, Appendix 1, Figures 1-12, pp. 75-86; Appendix 1, Appendix 1, Tables 1-3, pp. 87-89).

3 The method defines that the TMPA confirmation ion pair (*m/z* 141.0→97.0) can only be used for peak identity confirmation below 10x LOQ (10 µg/L) due to limited sensitivity (Appendix 1, Appendix 1, p. 70). In confirmation ion spectra of the ECM and ILV, baseline noise was greater than the LOQ peak height in some areas and caused difficulty in differentiating the peak from the baseline (Figures 26-28, pp. 50-52; Appendix 1, Appendix 1, Figures 10-11, pp. 84-85).

Typically, a confirmatory method is not required where GC/MS and LC/MS methods are used as the primary method(s) to generate study data.

IV. Method Deficiencies and Reviewer's Comments

1. The determination of the LOQ and LOD were not based on scientifically acceptable procedures as defined in 40 CFR Part 136, Appendix B. No justification or calculation was provided for the LOQ. No comparison was made to chromatogram background levels. The LOD was reported in the ECM based on the lowest concentration standard. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in water was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification. The LOD was not reported in the ILV.

2. The water matrices were not characterized in the ECM or ILV (p. 14; Appendix 1, p. 63).

3. Calibration curves and calibration raw data were not reported in the ECM.

4. Analytes were identified using two ion transitions; only one transition was used for quantification for all analytes (Table 1, p. 21; Figures 4-30, pp. 28-54; Appendix 1, Appendix 1, Figures 1-12, pp. 75-86; Appendix 1, Appendix 1, Tables 1-3, pp. 87-89). Therefore, the reviewer could not compare quantitative and confirmatory ion results. Typically, a confirmatory method is not required where GC/MS and LC/MS methods are used as the primary method(s) to generate study data.

   The method defines that the TMPA confirmation ion pair (*m/z* 141.0→97.0) can only be used for peak identity confirmation below 10x LOQ (10 µg/L) due to limited sensitivity (Appendix 1, Appendix 1, p. 70). In confirmation ion spectra of the ECM and ILV, baseline noise was greater than the LOQ peak height in some areas and caused difficulty in differentiating the peak from the baseline (Figures 26-28, pp. 50-52; Appendix 1, Appendix 1, Figures 10-11, pp. 84-85).

5. The ILV recommended that the pH of the test sample be measured to determine the amount of phosphate buffer needed to bring the sample pH to *ca*. 7.0 prior to analysis (p. 18). During method establishment, the ILV determined that 2.50 mL of 100 mM phosphate buffer (rather than 1.00 mL of 100 mL phosphate buffer) was required in sample processing to bring the pH of the sample to 7.06 (pp. 17-18). An ECM implementing this ILV recommendation was not submitted.

6. The ECM study author noted that the pH of the sample extract was important for proper SPE clean-up and separation of the analytes (Appendix 1, Appendix 1, p. 62). High pH will cause $CONH_2$-fenpropathrin and 4'-OH-fenpropathrin to degrade to TMPA, and improper pH can also affect the retention of TMPA on the SPE column.

7. The ILV noted the following typographical error in the ECM: the TMPA 2.0 µg/mL solution (in methanol/water/formic acid) was prepared by adding10 mL of the 10 µg/mL TMPA solution into a 100 mL volumetric flask (p. 18; Appendix 1, Appendix 1, p. 65). In the ILV, the TMPA 2.0 µg/mL solution (in methanol/water/formic acid) was prepared by adding 200 µL of the 1.0 mg/mL TMPA stock solution into a 100 mL volumetric flask. The ILV recommended to correct the error in the ECM. A corrected ECM was not submitted.

8. The reviewer noted the following typographical error in the ILV: the LOQ recovery range for $CONH_2$-fenpropathrin was reported as "1.38", instead of the correct range of "101-105" (Table 1, p. 21).

9. In the ILV, chromatograms were provided for three of the calibration standards, reagent blank, matrix blank, LOQ and 10×LOQ for each analyte (Figures 4-30, pp. 28-54). In the ECM, chromatograms were provided for one calibration standard, matrix blank, LOQ and 10×LOQ for each analyte; reagent blanks were not included (Appendix 1, Appendix 1, Figures 1-12, pp. 75-86).

10. The ILV reported that no communication with the study monitor was required (p. 18).

11. It was reported for the ILV that a single analyst completed a sample set consisting of 13 samples in *ca*. 1.5 days (*ca*. 6 hours for extraction and *ca*. 6 hours for analysis; p. 19).

V. References

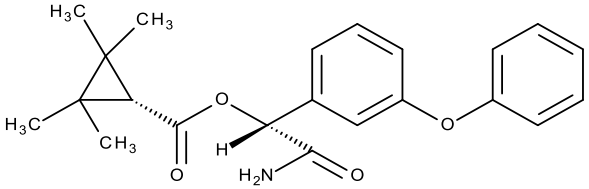
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

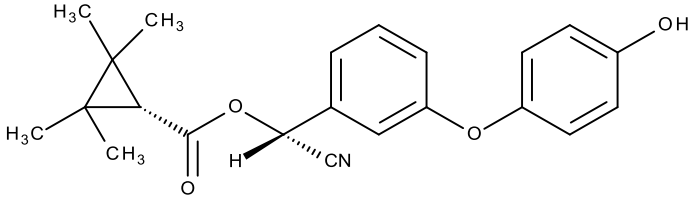
$CONH_2$-Fenpropathrin

IUPAC Name: (RS)-α-carbamoyl-3-phenoxybenzyl 2,2,3.3-tetramethylcyclopropanecarboxylate
CAS Name: Not reported
CAS Number: Not reported
SMILES String: [H][C@](OC(=O)[C@H]1C(C1(C)C)(C)C)(C(=O)N)c2cc(ccc2)Oc3ccccc3



4'-OH-Fenpropathrin

IUPAC Name: (RS)-α-cyano-3-(4-hydroxyphenoxy)benzyl 2,2,3.3-tetramethylcyclopropanecarboxylate
CAS Name: Not reported
CAS Number: Not reported
SMILES String: [H][C@@](OC(=O)[C@H]1C(C1(C)C)(C)C)(c2cc(ccc2)Oc3ccc(cc3)O)C#N



TMPA

IUPAC Name: 2,2,3.3-Tetramethylcyclopropanecarboxylic acid
CAS Name: Not reported
CAS Number: Not reported
SMILES String: CC1(C([C@H]1C(=O)O)(C)C)C