Test Material: Fenpropathrin

MRID: 49491403

Title: Independent Laboratory Validation for "Analytical Method for the Determination of Fenpropathrin Metabolites CONH2-Fenpropathrin and TMPA in Soil by LC-MS/MS"

EPA PC Code: 127901

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lisa Muto   **Signature:**

Date: 4/9/15

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Date: 4/9/15

Analytical method for fenpropathrin metabolites CONH₂-fenpropathrin and TMPA in soil

Reports:	ECM: EPA MRID No. 49491403 (Appendix 1, Appendix 1, pp. 50-72). Schoenau, E.A. 2014. Analytical Method for the Determination of Fenpropathrin Metabolites CONH2-Fenpropathrin and TMPA in Soil by LC-MS/MS. Method No.: GPL-MTH-084. Report prepared by Golden Pacific Laboratories, LLC, Fresno, California; sponsored and submitted by Valent U.S.A. Corporation, Dublin, California (Appendix 1, p. 44); 23 pages. Final report issued July 28, 2014.
	ILV: EPA MRID No. 49491403. Li, F. 2014. Independent Laboratory Validation for "Analytical Method for the Determination of Fenpropathrin Metabolites CONH2-Fenpropathrin and TMPA in Soil by LC-MS/MS". Laboratory Project ID/CPS Study No.: 14-CPS-015. 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. 44); 78 pages. Final report issued October 7, 2014.
Document No.:	MRID 49491403 (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. 50). 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 determinations of the LOQ and LOD were not based on scientifically acceptable procedures. The ILV did not report LODs. The soil 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

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

Executive Summary

This analytical method, GPL-MTH-084, is designed for the quantitative determination of fenpropathrin metabolites CONH₂-fenpropathrin and TMPA in soil at the stated LOQ of 0.01 µg/g using LC/MS/MS. The soil matrices of the ECM and ILV were not characterized or classified. The ILV successfully validated the method for both analytes after one trial. In the ECM and ILV, analytes were identified using two ion transitions; only one transition was used for quantification for all analytes. 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	49491403 (Appendix 1, Appendix 1, pp. 50-72)	49491403		Soil*	07/28/2014	Valent U.S.A Corporation	LC/MS/MS	0.01 µg/g
TMPA								

* The soils were not characterized in the ECM or ILV.

I. Principle of the Method

Soil (10.0 ± 0.1 g) in a 125 or 250 mL HDPE Nalgene bottle was fortified (100 µL of 1.0 µg/mL standard or 10 µg/mL standard) then extracted with 50 mL of methanol:water (9:1, v:v) via mechanical shaking (*ca*. 200 rpm for 45 minutes; Appendix 1, Appendix 1, pp. 51, 56, 58-59, 61). After centrifugation (3 minutes at 3000 rpm), an aliquot (*ca*. 20 mL) of the soil extract was then filtered (PTFE 0.45-µm). A 10-mL aliquot of the filtered soil extract was evaporated to remove the methanol under a gentle stream of nitrogen with a water bath set at 40°C. The remaining aqueous sample was reconstituted with 5 mL of 100 mM phosphate buffer (pH = 7.2) via manual shaking (*ca*. 5 seconds). 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:water (1:1, v:v; 3 mL each). The eluate was discarded before analytes were eluted. CONH$_2$-Fenpropathrin was eluted with 5 mL of methanol. The volume of the eluate was adjusted to 10 mL with water, then further diluted 1+3 (DF = 4) using methanol:water (1:1, v:v) and analyzed by LC/MS/MS. An alternative dilution of 0.2+0.8 (or equivalent dilution) can be made directly into an HPLC vial. If necessary, additional dilutions were made using methanol:water:formic acid (50:50:1, v:v:v). 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 for CONH$_2$-fenpropathrin using an AB Sciex API 4000 LC/MS/MS with electrospray ionization (ESI; Appendix 1, Appendix 1, pp. 56-57). 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 and multiple reaction monitoring (MRM). CONH$_2$-Fenpropathrin was 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). Expected retention time was *ca*. 2.4 minutes.

Samples are analyzed for <u>TMPA</u> using an AB Sciex API 5000 LC/MS/MS with electrospray ionization (ESI; Appendix 1, Appendix 1, pp. 57-58). 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). 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.

<u>ILV</u>

The samples were processed using the same procedure as that of the ECM, except that the volume of filtered soil extract sample was increased (10 mL to 20 mL) for the TMPA analysis due to the sensitivity of the instrument used (pp. 13-15; Table 2, pp. 20-22). Both analytes were analyzed using an AB API 4000 LC/MS/MS with ESI interface.

In the ECM and ILV, the LOQ was 0.01 µg/g (0.0100 mg/kg; 0.01 ppm) for both analytes (p. 10; Appendix 1, Appendix 1, pp. 51, 61). In the ECM, the LOD was reported as 0.005 µg/g for both analytes; the LOD was not reported in the ILV.

II. Recovery Findings

<u>ECM (MRID 49491403; Appendix 1, Appendix 1, pp. 50-72)</u>: Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of $CONH_2$-fenpropathrin and TMPA in soil at the LOQ (0.01 ppm) and 10x LOQ (0.1 ppm; Appendix 1, Appendix 1, p. 51; Appendix 1, Appendix 1, Tables 1-2, pp. 71-72). 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. 58). Analytes were identified using two ion transitions; only one transition was used for quantification for both analytes (Appendix 1, Appendix 1, Figures 1-8, pp. 63-70; Appendix 1, Appendix 1, Tables 1-2, pp. 71-72). Therefore, quantitation ion and confirmation ion recovery results could not be compared. The soil matrix was not characterized.

<u>ILV (MRID 49491403)</u>: Mean recoveries and RSDs were within guideline requirements for analysis of $CONH_2$-fenpropathrin and TMPA in soil at the LOQ and 10x LOQ (p. 10; Table 1, p. 19). Analytes were identified using two ion transitions; only one transition was used for quantification for both analytes (Table 1, p. 19; Figures 3-20, pp. 25-42). Therefore, quantitation ion and confirmation ion recovery results could not be compared. The soil matrix was collected at Tift County, Georgia; it was not characterized or further described (p. 11). The method was validated with the first trial for both analytes (p. 16).

Table 2. Initial Validation Method Recoveries for CONH₂-Fenpropathrin and TMPA in Soil*

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)[1]
	Quantitation Ion					
CONH₂-Fenpropathrin m/z 368.0→125.0	0.01 (LOQ)	5	79.1-84.5	82.7	2.49	3.01
	0.1	5	85.6-87.8	86.7	0.858	0.990
TMPA m/z 141.0→106.9	0.01 (LOQ)	5	72.5-82.9	79.3	3.99	5.03
	0.1	5	77.2-85.9	82.3	3.54	4.30
	Confirmation Ion					
CONH₂-Fenpropathrin m/z 368.0→97.0	0.01 (LOQ)					
	0.1		Data not reported.			
TMPA m/z 141.0→97.0	0.01 (LOQ)					
	0.1					

Data (uncorrected recovery results, Appendix 1, Appendix 1, pp. 60-61) were obtained from Appendix 1, Appendix 1, Tables 1-2, pp. 71-72 of the study report.
* The soil was not characterized or described.
1 Coefficient of Variance in study tables (Appendix 1, Appendix 1, Tables 1-2, pp. 71-72).

Table 3. Independent Validation Method Recoveries for CONH₂-Fenpropathrin and TMPA in Soil*

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)[1]
	Quantitation Ion					
CONH₂-Fenpropathrin m/z 368.0→125.0	0.01 (LOQ)	5	74.3-86.0	80.4	5.2	6.5
	0.1	5	72.5-85.0	79.1	5.7	7.2
TMPA m/z 141.0→106.9	0.01 (LOQ)	5	72.2-116	95.4	15.8	16.6
	0.1	5	84.4-104	94.8	7.1	7.5
	Confirmation Ion					
CONH₂-Fenpropathrin m/z 368.0→97.0	0.01 (LOQ)	5				
	0.1	5	Data not reported.			
TMPA m/z 141.0→97.0	0.01 (LOQ)	5				
	0.1	5				

Data (uncorrected recovery results, Appendix 2, p. 73) were obtained from Table 1, p. 19 of the study report.
* The soil was not characterized or described, other than source (p. 11).

III. Method Characteristics

In the ECM and ILV, the LOQ was 0.01 µg/g (0.0100 mg/kg; 0.01 ppm) for both analytes (p. 10; Appendix 1, Appendix 1, pp. 51, 61). 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 0.005 µg/g for both 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) or 2.5 ng/mL (TMPA), sample size and dilution factor.

Table 4. Method Characteristics in Soil

		$CONH_2$-Fenpropathrin	TMPA
Limit of Quantitation (LOQ)		0.01 µg/g	
Limit of Detection (LOD)		0.005 µg/g	
Linearity (calibration curve r^2 and concentration range)	ECM:	No linearity data were reported.	
	ILV[1]:	r^2 = 0.9976	r^2 = 0.9890
	Range:	0.250-10.0 ng/mL	2.50-100 ng/mL
Repeatable		Yes at LOQ and 10x LOQ (quantitative ion only, soil).[2,3]	
Reproducible		Yes at LOQ and 10x LOQ (quantitative ion only, soil). [2,3]	
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. [4]
	ILV:		

Data were obtained from pp. 10, 13, 16; Table 1, p. 19; Figures 1-20, pp. 23-42; Appendix 1, Appendix 1, Figures 1-8, pp. 63-70; Appendix 1, Appendix 1, Tables 1-2, pp. 71-72 of the study report; DER Attachment 2.

Linearity is satisfactory when r$^2 \geq$ 0.995.

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 both analytes (Table 1, p. 19; Figures 3-20, pp. 25-42; Appendix 1, Appendix 1, Figures 1-8, pp. 63-70; Appendix 1, Appendix 1, Tables 1-2, pp. 71-72).

3 The soil matrices were not characterized in the ECM or ILV (p. 11; Appendix 1, p. 51).

4 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. 58). 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 16-18, pp. 38-40; Appendix 1, Appendix 1, Figures 6-7, pp. 68-69).

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 soil 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 soil matrices were not characterized or classified by soil texture in the ECM or ILV (p. 11; Appendix 1, p. 51).

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. 19; Figures 3-20, pp. 25-42; Appendix 1, Appendix 1, Figures 1-8, pp. 63-70; Appendix 1, Appendix 1, Tables 1-2, pp. 71-72). 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. 58). 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 16-18, pp. 38-40; Appendix 1, Appendix 1, Figures 6-7, pp. 68-69).

5. 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 to degrade to TMPA, and improper pH can also affect the retention of TMPA on the SPE column.

6. In the ECM, the reviewer noted that the concentrations in the final sample set varied slightly from the nominal LOQ and 10×LOQ for both analytes (0.00997 and 0.0997 ppm versus 0.01 and 0.1 ppm; Appendix 1, Appendix 1, Tables 1-2, pp. 71-72).

7. The reviewer noted the following typographical errors in the ILV: the recovery columns were incorrectly titled as "Calculated **ppb (ng/mL)** in Final Sample Solution" (Table 1, p. 19) and the final statement in Section 5.0 Conclusions was "…suitable for determining the residues of $CONH_2$-fenpropathrin and TMPA in soil down to a level of **1.00 ppb**" (p. 16).

8. In the ILV, chromatograms were provided for three of the calibration standards, reagent blank, matrix blank, LOQ and 10×LOQ for each analyte (Figures 3-20, pp. 25-42). 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, Figures 1-8, pp. 63-70).

9. The ILV reported that communication with the study monitor was only required during method development regarding the LC/MS/MS sensitivity of TMPA (p. 16).

10. It was reported for the ILV that a single analyst completed a sample set consisting of 13 samples in *ca*. 1.5 days (*ca*. 7 hours for extraction and *ca*. 6 hours for analysis; p. 16).

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
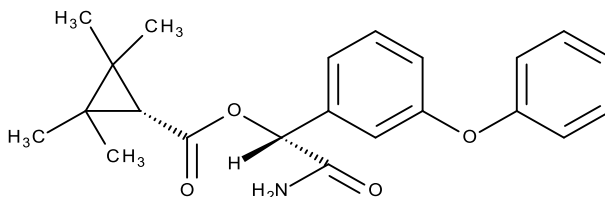   Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures.

CONH₂-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

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