

Analytical method for fenpyroximate in soil

Reports: ECM: EPA MRID No.: 50013402. Brown, D. 2016. Validation of an analytical method for the determination of residues of fenpyroximate in soil by LC-MS/MS. Study Reference No.: RES-00061. Report prepared by ResChem Analytical Limited, Derby, United Kingdom, and sponsored by Nihon Nohyaku Co., Ltd., Tokyo, Japan, and submitted by Nichino America, Inc., Wilmington, Delaware; 47 pages (including page 1a). Final report issued June 21, 2016.

ILV: EPA MRID No. 50021401. Coleman, H. 2016. Independent Laboratory Validation of 'Validation of an analytical method for the determination of residues of fenpyroximate in soil by LC-MS/MS'. Study No.: XG/16/005. Report prepared by Battelle UK Ltd., Essex, United Kingdom, sponsored by Nihon Nohyaku Co., Ltd., Tokyo, Japan, and submitted by Nichino America, Inc., Wilmington, Delaware; 49 pages. Final report issued September 1, 2016.

Document No.: MRIDs 50013402 & 50021401

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with UK and OECD Good Laboratory Practice (GLP) standards (p. 2; Appendix C, p. 46 of MRID 50013402). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 1a-2, 4; Appendix C, p. 46). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).


ILV: The study was conducted in accordance with OECD and UK GLP standards, which are accepted by Regulatory Authorities throughout the European Community, the United States of America and Japan (p. 3; Appendix 4, p. 37 of MRID 50021401). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4; Appendix 5, p. 49). A statement of the authenticity of the study report was included with the quality assurance and GLP statements (p. 4).

Classification: This analytical method is classified as acceptable. The fenpyroximate dataset doesn't have an endpoint that can be used as a level of concern in soil.

PC Code: 129131

**Final EPA
Reviewer:**


James Lin,
Environmental Engineer

Signature: 
Date: 6/24/19

6/12/17

**CDM/CSS-
Dynamac JV
Reviewers:**

Lisa Muto,
Environmental Scientist

Signature: 
Date: 6/12/17

Kathleen Ferguson, Ph.D.,
Environmental Scientist

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This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.

Executive Summary

This analytical method, Analytical Method RES-00061, is designed for the quantitative determination of fenpyroximate in soil at the LOQ of 0.01 mg/kg using LC/MS/MS. The fenpyroximate dataset doesn't have an endpoint that can be used as a level of concern in soil. The ECM used characterized sandy loam and clay soil matrices; the ILV used characterized sandy loam and clay loam soil matrices. The specific sources of the ECM and ILV soils were not reported. Although the specific number of trials was not reported, the reviewer assumed that the method was validated after one trial with insignificant modifications to the analytical method. All submitted ILV and ECM data pertaining to linearity, repeatability, reproducibility and specificity was acceptable. The LOD was not reported in the ECM.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Fenpyroximate	50013402 ¹	50021401 ²		Soil	21/06/2016	Nichino America, Inc.	LC/MS/MS	0.01 mg/kg

1 In the ECM, loamy sand soil matrix [Lufa Speyer soil type 2.2; 75.8 ± 3.9% sand 16.3 ± 2.5% silt 7.9 ± 1.8% clay; pH 5.5 ± 0.1 (0.1M CaCl₂), 1.61 ± 0.15% organic carbon] and clay soil matrix [Lufa Speyer soil type 6S; 25.6 ± 3.2% sand 34.4 ± 2.6% silt 40.0 ± 2.1% clay; pH 7.1 ± 0.1 (0.1M CaCl₂), 1.73 ± 0.05% organic carbon] were used (USDA soil texture classification; p. 10; Appendix B, p. 45 of MRID 50013402). The specific soil source was not reported. The soil characterization was performed by Bezirks Verband Pfalz.

2 In the ILV, sandy loam soil matrix [Soil 13/006 Lufa 2.2 Residues; 74% sand 13% silt 13% clay; pH 5.4 (1:1 soil:water ratio), 3.17% organic carbon] and clay loam soil matrix [16/011-South Witham; 42% sand 20% silt 38% clay; pH 7.6 (1:1 soil:water), 2.9% organic carbon] were used (USDA soil texture classification; p. 12; Appendices 3-4, pp. 47-48 of MRID 50021401). Both soils were obtained from Battelle UK; the specific soil source was not reported. The soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

I. Principle of the Method

Soil samples (25 g) were fortified with fenpyroximate in a 25-mL polypropylene tube (pp. 14, 17 of MRID 50013402). The samples were sequentially extracted twice with methanol (3 x 50 mL), once with acetone (50 mL), once with methanol:water (1:1, v:v; 50 mL) and once with methanol:0.1 M hydrochloride acid (1:1, v:v; 50 mL) via shaking on an orbital shaker (150 orbits/min., 20 minutes). After centrifugation (1500 rpm for 2 minutes), the extract was filtered through cotton wool and transferred to a clean container. The volume of the combined extract was adjusted to 500 mL using de-ionized water. An aliquot of the sample was transferred to an autosampler vial and analyzed by HPLC/MS/MS.

Samples were analyzed for fenpyroximate using an Agilent 1100 Binary HPLC coupled to an AB Sciex API 4000 MS equipped with an Ascentis Express C18 column (2.1 mm x 50 mm, 2.7 μ m) using a mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [percent A:B at 0-3.5 min. 40:60] with MS/MS-ESI (electrospray ionization) detection in positive ion mode and multiple reaction monitoring (MRM; p. 15 of MRID 50013402). Injection volume was 10 μ L. Fenpyroximate was identified using two ion transitions (quantitation and confirmation, respectively): m/z 422 \rightarrow 366 and m/z 422 \rightarrow 135. Expected retention time was *ca.* 2.6 minutes.

In the ILV, the ECM was performed as written with insignificant modifications to the analytical instrumentation (pp. 15-16; Figure 1, p. 24; Appendix 2, pp. 45-46 of MRID 50021401). An Agilent 1290 Series HPLC coupled to a MDS Sciex API 5500 Triple Quadrupole MS or MDS Sciex API 6500 Triple Quadrupole MS was used for analyte identification. Fenpyroximate was identified using the same two ion transitions; expected retention time was *ca.* 2.5 minutes. No other modifications of the ECM were reported.

The Limit of Quantification (LOQ) for soil was 0.01 mg/kg in the ECM and ILV (pp. 8, 12, 19, 23, 25 of MRID 50013402; pp. 10, 13, 18 of MRID 50021401). The Limit of Detection (LOD) was reported as 0.0002 mg/kg for clay loam and 0.0004 mg/kg for sandy loam soil in the ILV. In the ECM, a value for the LOD was not specified; however, the LOD was confirmed to be less than 30% of the LOQ, as demonstrated by the lowest calibration standard (0.00015 μ g/mL; equivalent to 30% of the LOQ, 0.003 mg/kg).

II. Recovery Findings

ECM (MRID 50013402): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of fenpyroximate at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 \times LOQ) in soil matrices (Tables 5-8, pp. 20-23). Fenpyroximate was identified using two ion transitions; performance data (recovery results) from quantitation and confirmation analyses were comparable. Loamy sand soil matrix [Lufa Speyer soil type 2.2; 75.8 \pm 3.9% sand 16.3 \pm 2.5% silt 7.9 \pm 1.8% clay; pH 5.5 \pm 0.1 (0.1M CaCl₂), 1.61 \pm 0.15% organic carbon] and clay soil matrix [Lufa Speyer soil type 6S; 25.6 \pm 3.2% sand 34.4 \pm 2.6% silt 40.0 \pm 2.1% clay; pH 7.1 \pm 0.1 (0.1M CaCl₂), 1.73 \pm 0.05% organic

carbon] were used (USDA soil texture classification; p. 10; Appendix B, p. 45). The specific soil source was not reported. The soil characterization was performed by Bezirks Verband Pfalz.

ILV (MRID 50021401): Mean recoveries and RSDs were within guideline requirements for analysis of fenpyroximate at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10×LOQ) in soil matrices (Tables 2-3, pp. 22-23). Fenpyroximate was identified using two ion transitions; performance data (recovery results) from quantitation and confirmation analyses were comparable. Sandy loam soil matrix [Soil 13/006 Lufa 2.2 Residues; 74% sand 13% silt 13% clay; pH 5.4 (1:1 soil:water ratio), 3.17% organic carbon] and clay loam soil matrix [16/011-South Witham; 42% sand 20% silt 38% clay; pH 7.6 (1:1 soil:water), 2.9% organic carbon] were used (USDA soil texture classification; p. 12; Appendices 3-4, pp. 47-48). Both soils were obtained from Battelle UK; the specific soil source was not reported. The soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. Although the specific number of trials was not reported, the reviewer assumed that the method was validated after one trial with insignificant modifications to the analytical method (pp. 10, 15-16, 19-20).

Table 2. Initial Validation Method Recoveries for Fenpyroximate in Soil^{1,2}

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
Clay Soil						
Quantitation Ion Transition						
Fenpyroximate	0.01	5	97.7-101.2	99.4	1.3	1.29
	0.1	5	100.6-103.3	102.0	1.0	0.99
Confirmation Ion Transition						
Fenpyroximate	0.01	5	95.2-102.9	98.4	2.8	2.88
	0.1	5	100.7-104.4	102.5	1.8	1.72
Loamy Sand Soil						
Quantitation Ion Transition						
Fenpyroximate	0.01	5	96.0-97.4	96.7	0.5	0.56
	0.1	5	97.6-100.1	99.0	1.0	0.97
Confirmation Ion Transition						
Fenpyroximate	0.01	5	92.3-99.3	96.3	2.9	3.02
	0.1	5	97.4-100.6	98.7	1.2	1.26

Data (uncorrected recovery results, p. 16) were obtained from Tables 5-8, pp. 20-23 of MRID 50013402 and DER Attachment 2.

1 Loamy sand soil matrix [Lufa Speyer soil type 2.2; 75.8 ± 3.9% sand 16.3 ± 2.5% silt 7.9 ± 1.8% clay; pH 5.5 ± 0.1 (0.1M CaCl₂), 1.61 ± 0.15% organic carbon] and clay soil matrix [Lufa Speyer soil type 6S; 25.6 ± 3.2% sand 34.4 ± 2.6% silt 40.0 ± 2.1% clay; pH 7.1 ± 0.1 (0.1M CaCl₂), 1.73 ± 0.05% organic carbon] were used (USDA soil texture classification; p. 10; Appendix B, p. 45). The specific soil source was not reported. The soil characterization was performed by Bezirks Verband Pfalz.

2 Fenpyroximate was identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 422→366 and *m/z* 422→135.

3 Standard deviations were reviewer-calculated since these values were not calculated in the study report (see DER Attachment 2). Rules of significant figures was followed when reporting results.

Table 3. Independent Validation Method Recoveries for Fenpyroximate in Soil^{1,2}

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Loam Soil						
Quantitation Ion Transition						
Fenpyroximate	0.01	5	94.5-101	96.9	2.6	2.7
	0.1	5	90.3-93.5	92.0	1.1	1.2
Confirmation Ion Transition						
Fenpyroximate	0.01	5	89.7-104	96.1	6.1	6.3
	0.1	5	91.1-95.3	92.9	1.5	1.6
Clay Loam Soil						
Quantitation Ion Transition						
Fenpyroximate	0.01	5	93.1-94.7	94.3	0.7	0.7
	0.1	5	93.1-98.9	96.0	2.6	2.7
Confirmation Ion Transition						
Fenpyroximate	0.01	5	85.7-99.9	94.3	6.0	6.4
	0.1	5	94.5-99.7	97.7	2.0	2.1

Data (uncorrected recovery results, p. 17) were obtained from Tables 2-3, pp. 22-23 of MRID 50021401.

1 Sandy loam soil matrix [Soil 13/006 Lufa 2.2 Residues; 74% sand 13% silt 13% clay; pH 5.4 (1:1 soil:water ratio), 3.17% organic carbon] and clay loam soil matrix [16/011-South Witham; 42% sand 20% silt 38% clay; pH 7.6 (1:1 soil:water), 2.9% organic carbon] were used (USDA soil texture classification; p. 12; Appendices 3-4, pp. 47-48). Both soils were obtained from Battelle UK; the specific soil source was not reported. The soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

2 Fenpyroximate was identified using two ion transitions (quantitation and confirmation, respectively): m/z 422→366 and m/z 422→135.

III. Method Characteristics

The LOQ for soil was 0.01 mg/kg in the ECM and ILV (pp. 8, 12, 19, 23, 25 of MRID 50013402; pp. 10, 13, 18 of MRID 50021401). The LOD was reported as 0.0002 mg/kg for clay loam and 0.0004 mg/kg for sandy loam soil in the ILV, reportedly based on 3xs the chromatographic noise. In the ECM, a value for the LOD was not specified; however, the LOD was confirmed to be less than 30% of the LOQ, as demonstrated by the lowest calibration standard (0.00015 µg/mL; equivalent to 30% of the LOQ, 0.003 mg/kg). The response of the lowest calibration standard was visually confirmed by the ECM study author to be greater than three times the signal to noise for each mass transition. No calculations were provided for the LOQ or LOD in the ECM or ILV.

Table 4. Method Characteristics

Analyte		Fenpyroximate	
Limit of Quantitation (LOQ)		0.01 mg/kg	
Limit of Detection (LOD)	ECM	Not specified; less than 30% of the LOQ.	
	ILV	0.0002 mg/kg (clay loam) 0.0004 mg/kg (sandy loam)	
Linearity (calibration curve r^2 and concentration range) ¹	ECM	Clay	$r^2 = 0.9990$ (Q) $r^2 = 0.9988$ (C)
		Loamy Sand	$r^2 = 0.9998$ (Q) $r^2 = 1.0000$ (C)
	ILV	Sandy Loam	$r^2 = 0.9978$ (Q & C)
		Clay Loam	$r^2 = 0.9994$ (Q) $r^2 = 0.9996$ (C)
	Concentration range	0.05-5.0 ng/mL	
Repeatable	ECM ²	Yes at LOQ and 10×LOQ.	
	ILV ^{3,4}		
Reproducible		Yes at LOQ and 10×LOQ.	
Specific	ECM	Yes, no matrix interferences were observed.	
	ILV		

Data were obtained from pp. 8, 12, 19, 23, 25; Tables 5-8, pp. 20-23 (recovery data); Figures 2-3, pp. 28-29 and Figures 10-11, pp. 36-37 (calibration curve); Figures 6-9, pp. 32-35 and Figures 14-17, pp. 40-43 (chromatograms) of MRID 50013402; pp. 10, 13, 18; Tables 2-3, pp. 22-23 (recovery data); Figures 3-4, pp. 26-27 (calibration curve); Figures 8-11, pp. 31-34 and Figures 15-18, pp. 38-41 (chromatograms) of MRID 50021401; DER Attachment 2. Q = Quantitation ion transition; C = Confirmatory ion transition.

1 Reported correlation coefficients were reviewer-calculated from r values reported in the study report (Figures 2-3, pp. 28-29 and Figures 10-11, pp. 36-37 of MRID 50013402; Figures 3-4, pp. 26-27 of MRID 50021401; DER Attachment 2). Matrix-matched standards were used in the ECM, even though matrix effects were not significant (p. 18 of MRID 50013402). Matrix-matched standards were used in the ILV since matrix effects were significant at the LOQ (not at 10×LOQ; pp. 10, 18 of MRID 50021401).

2 In the ECM, loamy sand soil matrix [Lufa Speyer soil type 2.2; 75.8 ± 3.9% sand 16.3 ± 2.5% silt 7.9 ± 1.8% clay; pH 5.5 ± 0.1 (0.1M CaCl₂), 1.61 ± 0.15% organic carbon] and clay soil matrix [Lufa Speyer soil type 6S; 25.6 ± 3.2% sand 34.4 ± 2.6% silt 40.0 ± 2.1% clay; pH 7.1 ± 0.1 (0.1M CaCl₂), 1.73 ± 0.05% organic carbon] were used (USDA soil texture classification; p. 10; Appendix B, p. 45 of MRID 50013402). The specific soil source was not reported. The soil characterization was performed by Bezirks Verband Pfalz.

3 In the ILV, sandy loam soil matrix [Soil 13/006 Lufa 2.2 Residues; 74% sand 13% silt 13% clay; pH 5.4 (1:1 soil:water ratio), 3.17% organic carbon] and clay loam soil matrix [16/011-South Witham; 42% sand 20% silt 38% clay; pH 7.6 (1:1 soil:water), 2.9% organic carbon] were used (USDA soil texture classification; p. 12; Appendices 3-4, pp. 47-48 of MRID 50021401). Both soils were obtained from Battelle UK; the specific soil source was not reported. The soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

4 Although the specific number of trials was not reported, the reviewer assumed that the method was validated after one trial with insignificant modifications to the analytical method (pp. 10, 15-16, 19-20 of MRID 50021401).

IV. Method Deficiencies and Reviewer's Comments

1. The estimation of LOQ and LOD in ECM and ILV was not based on scientifically acceptable procedures as defined in 40 CFR Part 136 ILV (pp. 8, 12, 19, 23, 25 of MRID 50013402; pp. 10, 13, 18 of MRID 50021401). No calculations were provided for the LOQ or LOD in the ECM or ILV. The LOD was not specifically reported in the ECM (reported as less than 30% of the LOQ), but it was compared to the lowest calibration standard (30% of the LOQ) which was visually confirmed to be greater than three times the signal to noise for each mass transition. In the ILV, the LOD was based on 3xs the chromatographic noise.
2. The definition of the LOD in the ECM was based on the lowest calibration standard, 0.00015 µg/mL (pp. 8, 12, 19, 23, 25; Figure 5, p. 31 of MRID 50013402). In most of the study report, the lowest calibration standard was reported as equivalent to 30% of the LOQ; however, the reviewer noted that, in one instance, the lowest calibration standard was reported as equivalent to 25% of the LOQ (p. 23). The reviewer assumed that this was a typographical error.
3. The ECM matrices of clay and loamy sand were referred to as clayey loam and loamy sand, respectively, in the study report based on the German DIN classification (p. 10; Appendix B, p. 45 of MRID 50013402). The ILV matrices of sandy loam and clay loam were referred to as sandy loam and clayey loam, respectively, in the study report based on the ADAS or other classification (p. 12; Appendices 3-4, pp. 47-48 of MRID 50021401). The reviewer referred to the soils based on their USDA soil texture classification in the DER and DER Attachments.
4. In the ECM, the matrix effects were evaluated and found to be insignificant, but matrix-matched standards were still used (pp. 18-19; Tables 1-4, pp. 18-19 of MRID 50013402). Matrix-matched standards were used in the ILV since matrix effects were significant at the LOQ (not at 10×LOQ; pp. 10, 18; Table 1, p. 21 of MRID 50021401).
5. The extract and calibration standard stability was evaluated in the ECM (pp. 23-25; Tables 9-11, pp. 24-25 of MRID 50013402). When refrigerated (2°C to 8°C), the extracts were found to be stable for up to 12 days while calibration standards were found to be stable for up to 13 days.
6. No communication between the ILV testing facility and the method developer occurred (p. 19 of MRID 50021401).
7. The timeframe required to complete the method validation for one set was not reported in the ILV or ECM.

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**Fenpyroximate****IUPAC Name:** tert-Butyl (E)- α -(1,3-dimethyl-5-phenoxy-pyrazol-4-ylmethyleneaminoxy)-p-toluate**CAS Name:** 1,1-Dimethylethyl 4-[[[(E)-[(1,3-dimethyl-5-phenoxy-1H-pyrazol-4-yl)methylene]amino]oxy]methyl]benzoate**CAS Number:** 134098-61-6**SMILES String:** O=C(OC(C)(C)C)c1ccc(CON=Cc2c(Oc3ccccc3)n(C)nc2C)cc1