

Analytical method for fenpyroximate in water

Reports: ECM: MRID No.: 50013401. Brown, D. 2016. Validation of an analytical method for the determination of residues of fenpyroximate in surface water by LC-MS/MS. Study Reference No.: RES-00059. 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; 36 pages (including page 1a). Final report issued June 15, 2016.

ILV: MRID No. 50013403. Coleman, H. 2016. Independent Laboratory Validation of 'Validation of an analytical method for the determination of residues of fenpyroximate in surface water by LC-MS/MS'. Study No.: XG/16/004. 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; 37 pages. Final report issued August 16, 2016.

Document No.: MRIDs 50013401 & 50013403

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. 35 of MRID 50013401). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 1a-2, 4; Appendix C, p. 35). 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 50013403). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4; Appendix 3, p. 37). 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 LOQ of 0.01 µg/L is less than the lowest toxicological level of concern in water of 0.016 µg/L.

PC Code: 129131

**Final EPA
Reviewer:**

James Lin,
Environmental Engineer

Signature:



Date:

6/24/19

**CDM/CSS-
Dynamac JV**

Lisa Muto,
Environmental Scientist

Signature:



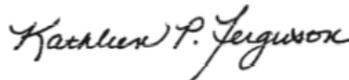
Date:

6/12/17

Reviewers:

Kathleen Ferguson, Ph.D.,
Environmental Scientist

Signature:



Date:

6/12/17

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-00059, is designed for the quantitative determination of fenpyroximate in water at the LOQ of 0.01 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water. The ECM used characterized surface (river) water matrix; the ILV used surface water matrix. The specific source of the ILV water was 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 and sample preparation. 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	50013401 ¹	50013403 ²		Water	15/06/2016	Nichino America, Inc.	LC/MS/MS	0.01 µg/L

1 In the ECM, the surface (river) water matrix (pH 7.6, 201 mg/L total hardness as CaCO₃, 4.74 mg/L dissolved organic carbon), obtained from River Derwent in Derby, United Kingdom, was used (p. 10; Appendix B, p. 34 of MRID 50013401).

2 In the ILV, the surface water matrix (pH 6.0, 118 mg/L total hardness as CaCO₃, 3.5 ppm total organic carbon, 3.1 ppm dissolved organic carbon), obtained from Battelle UK, was used (characterization by Agvise Laboratories, Northwood, North Dakota; p. 10; Appendix 2, pp. 33-34 of MRID 50013403). The specific water source was not reported.

I. Principle of the Method

Water samples (20 mL) were fortified with fenpyroximate in a 50-mL polypropylene tube (pp. 13, 17 of MRID 50013401). After shaking, the samples were extracted twice with 2.5 mL of hexane (2 x 2.5 mL) via vortex mixing for 30 seconds. After centrifugation (3000 rpm for 5 minutes), the hexane phase was transferred to a clean 15-mL polypropylene centrifuge tube. The combined extract was evaporated to dryness using a Dri-block heater at 50°C and a gentle stream of air. The residue was reconstituted in 1.0 mL of acetonitrile:water (1:1, v:v) using an ultrasonic bath and vortex mixer. 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 µm) 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. 14 of MRID 50013401). Injection volume was 5 µL. Fenpyroximate was identified using two ion transitions (quantitation and confirmation, respectively): m/z 422→366 and m/z 422→135. Expected retention time was *ca.* 2.4 minutes.

In the ILV, the ECM was performed as written with insignificant modifications to the analytical instrumentation (pp. 12-14; Figure 1, p. 20; Appendix 3, p. 36 of MRID 50013403). An Agilent 1290 Series HPLC coupled to a MDS Sciex API 5500 Triple Quadrupole MS was used for analyte identification. Fenpyroximate was identified using the same two ion transitions; expected retention time was *ca.* 3.5 minutes. Additionally, the 100×LOQ sample extract (0.1 mL) was further diluted with 0.9 mL of acetonitrile:water (1:1, v:v; p. 17). No other modifications of the ECM were reported.

The Limit of Quantification (LOQ) for water was 0.01 µg/L in the ECM and ILV (pp. 8, 20-21 of MRID 50013401; pp. 9, 11 of MRID 50013403). The Limit of Detection (LOD) was reported as 0.002 µg/L 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 (0.00005 µg/mL; equivalent to 25% of the LOQ, 0.0025 µg/L).

II. Recovery Findings

ECM (MRID 50013401): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of fenpyroximate at fortification levels of 0.01 µg/L (LOQ), 0.10 µg/L (10×LOQ) and 1.0 µg/L (100×LOQ) in the surface water matrix (Tables 3-4, pp. 18-19). Fenpyroximate was identified using two ion transitions; performance data (recovery results) from quantitation and confirmation analyses were comparable. The surface (river) water matrix (pH 7.6, 201 mg/L total hardness as CaCO₃, 4.74 mg/L dissolved organic carbon) was obtained from River Derwent in Derby, United Kingdom (p. 10; Appendix B, p. 34).

ILV (MRID 50013403): Mean recoveries and RSDs were within guideline requirements for analysis of fenpyroximate at fortification levels of 0.01 µg/L (LOQ), 0.10 µg/L (10×LOQ) and 1.0 µg/L (100×LOQ) in the surface water matrix (Table 2, p. 19). Fenpyroximate was identified using two ion transitions; performance data (recovery results) from quantitation and confirmation analyses were comparable. The surface water matrix (pH 6.0, 118 mg/L total hardness as CaCO₃, 3.5 ppm total organic carbon, 3.1 ppm dissolved organic carbon) was obtained from Battelle UK (characterization by Agvise Laboratories, Northwood, North Dakota; p. 10; Appendix 2, pp. 33-34). The specific water source was 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 and sample preparation (pp. 9, 12-14, 16-17).

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

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
Surface (River) Water						
Quantitation Ion Transition						
Fenpyroximate	0.01	5	93.9-109.5	102.6	5.8	5.65
	0.1	5	96.5-109.2	102.7	5.2	5.10
	1.0	5	91.5-97.1	95.8	2.4	2.51
Confirmation Ion Transition						
Fenpyroximate	0.01	5	85.1-110.7	97.6	9.8	10.04
	0.1	5	92.3-105.8	98.9	5.2	5.22
	1.0	5	93.4-98.5	95.9	1.9	1.99

Data (uncorrected recovery results, p. 16) were obtained from Tables 3-4, pp. 18-19 of MRID 50013401 and DER Attachment 2.

1 The surface (river) water matrix (pH 7.6, 201 mg/L total hardness as CaCO₃, 4.74 mg/L dissolved organic carbon) was obtained from River Derwent in Derby, United Kingdom (p. 10; Appendix B, p. 34).

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 Water^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface () Water						
Quantitation Ion Transition						
Fenpyroximate	0.01	5	67.0-82.5	73.3	5.9	8.1
	0.1	5	69.5-76.0	72.4	2.5	3.4
	1.0	5	63.5-76.5	71.6	5.1	7.1
Confirmation Ion Transition						
Fenpyroximate	0.01	5	67.5-77.5	72.4	3.7	5.1
	0.1	5	69.0-76.0	71.6	2.7	3.8
	1.0	5	62.5-76.0	70.3	5.5	7.8

Data (uncorrected recovery results, p. 15) were obtained from Table 2, p. 19 of MRID 50013403.

1 The surface water matrix (pH 6.0, 118 mg/L total hardness as CaCO₃, 3.5 ppm total organic carbon, 3.1 ppm dissolved organic carbon) was obtained from Battelle UK (characterization by Agvise Laboratories, Northwood, North Dakota; p. 10; Appendix 2, pp. 33-34). The specific water source was not reported.

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 water was 0.01 µg/L in the ECM and ILV (pp. 8, 20-21 of MRID 50013401; pp. 9, 11, 16 of MRID 50013403). The LOD was reported as 0.002 µg/L 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 (0.00005 µg/mL; equivalent to 25% of the LOQ, 0.0025 µg/L). 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 µg/L
Limit of Detection (LOD)	ECM	Not specified; less than 30% of the LOQ.
	ILV	0.002 µg/L
Linearity (calibration curve r^2 and concentration range) ¹	ECM	$r^2 = 0.9978$ (Q) $r^2 = 0.9996$ (C)
	ILV	$r^2 = 0.9990$ (Q) $r^2 = 0.9984$ (C)
	Concentration range	0.05-5.0 ng/mL
Repeatable	ECM ²	Yes at LOQ, 10×LOQ and 100×LOQ.
	ILV ^{3,4}	
Reproducible		Yes at LOQ, 10×LOQ and 100×LOQ.
Specific	ECM	Yes, no matrix interferences were observed.
	ILV	Yes, matrix interferences were observed at <i>ca.</i> 12-13% of the LOQ (based on peak areas).

Data were obtained from pp. 8, 12, 20-21; Tables 3-4, pp. 18-19 (recovery data); Figures 2-3, pp. 24-25 (calibration curve); Figures 6-10, pp. 28-32 (chromatograms) of MRID 50013401; pp. 9, 11-12; Table 2, p. 19 (recovery data); Figure 3, p. 22 (calibration curve); Figures 7-11, pp. 26-30 (chromatograms) of MRID 50013403; 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. 24-25 of MRID 50013401; Figure 3, p. 22 of MRID 50013403; DER Attachment 2). Matrix-matched standards were used in the ECM, even though matrix effects were not significant (p. 17 of MRID 50013401).

Solvent standards were used in the ILV (pp. 12, 16 of MRID 50013403).

2 In the ECM, the surface (river) water matrix (pH 7.6, 201 mg/L total hardness as CaCO₃, 4.74 mg/L dissolved organic carbon), obtained from River Derwent in Derby, United Kingdom, was used (p. 10; Appendix B, p. 34 of MRID 50013401).

3 In the ILV, the surface water matrix (pH 6.0, 118 mg/L total hardness as CaCO₃, 3.5 ppm total organic carbon, 3.1 ppm dissolved organic carbon), obtained from Battelle UK, was used (characterization by Agvise Laboratories, Northwood, North Dakota; p. 10; Appendix 2, pp. 33-34 of MRID 50013403). The specific water source was not reported.

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 and sample preparation (pp. 9, 12-14, 16-17 of MRID 50013403).

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, 20-21 of MRID 50013401; pp. 9, 11, 16 of MRID 50013403). 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 (25% 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. In the ILV, representative chromatograms showed that matrix interferences were *ca.* 12-13% of the LOQ (based on peak areas; Figures 7-11, pp. 26-30 of MRID 50013403). The study author reported that matrix interferences were acceptable since they were observed at less than 30% of the LOQ in the control and blank samples (p. 9).
3. In the ILV and ECM, the matrix effects were evaluated and found to be insignificant (<20%; p. 17; Tables 1-2, p. 17 of MRID 50013401; p. 16; Table 1, p. 18 of MRID 50013403). Solvent-based standards were used in the ILV, but matrix-matched standards were still used in the ECM.
4. The extract and calibration standard stability was evaluated in the ECM (p. 20; Tables 5-6, p. 20 of MRID 50013401). When refrigerated (2°C to 8°C), the extracts were found to be stable for up to 7 days while calibration standards were found to be stable for up to 12 days.
5. No communication between the ILV testing facility and the method developer occurred during the conduct of the study (p. 17 of MRID 50013403). The ILV contacted with Sponsor prior to the start of the dilution of the 100×LOQ samples to validate this step.
6. 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-yl)methyleneaminoxy)-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