Analytical method for tolfenpyrad in water

Reports: ECM: EPA MRID No.: 50142026. Wright, D.R., J. Gilbert. 2011. Tolfenpyrad: Validation of an Analytical Method for the Determination of Residues in Surface and Distilled water. Covance Study No.: 8245058. Clint ID No.: 1002870. Report prepared by Covance Laboratories Limited, North Yorkshire, United Kingdom, and sponsored by Nihon Nohyaku Co., Ltd., Tokyo, Japan, and submitted by Nichino America, Inc., Wilmington, Delaware; 55 pages. Final report issued June 9, 2011.

ILV: EPA MRID No. 50142028. Cremin, P. 2013. Independent Laboratory Validation of the Analytical Method for Tolfenpyrad in Surface and Distilled Water. PTRL Study No.: 2304W. Report prepared by PTRL West, Hercules, California, sponsored by Nihon Nohyaku Co., Ltd., Tokyo, Japan, and submitted by Nichino America, Inc., Wilmington, Delaware; 81 pages. Final report issued January 10, 2013.

Document No.: MRIDs 50142026 & 50142028

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with UK and OECD Good Laboratory Practice (GLP) standards (p. 3 of MRID 50142026). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-5).

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 50142028). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-5).

Classification: This analytical method is classified as supplemental. In the ILV, performance data for tolfenpyrad in distilled water did not meet OCSPP guidelines requirements for precision at 10×LOQ. The ILV water matrices were not characterized or fully described. The LOD values of the ECM differed from those of the ILV.

PC Code: 090111

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Executive Summary

This analytical method, Analytical Method CLE 8245058-01V, is designed for the quantitative determination of tolfenpyrad in water at the LOQ of 0.01 µg/L using LC/MS/MS. The ECM used characterized surface and distilled water matrices; the ILV used uncharacterized surface and distilled water matrices. The specific source of the ILV water was not reported. The repeatability and reproducibility of the method was validated for both matrices at the LOQ; however, the reproducibility of the method at 10×LOQ was validated for only surface water, since the ILV did not provide acceptable performance data in distilled water at 10×LOQ. 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 and specificity was acceptable. The LOD values of the ECM differed from those of the ILV.

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						
Tolfenpyrad	50142026[1]	50142028[2]		Water	09/06/2011	Nichino America, Inc.	LC/MS/MS	0.01 µg/L

1 In the ECM, surface (stream) water matrix (pH 8.1, 112 mg/L total hardness as $CaCO_3$, 8.0 ppm dissolved organic carbon), obtained from Oak Beck, Harrogate, United Kingdom, and distilled water matix (<2 ppm non-volatile residues, <2 ppb heavy metals) was obtained from Rathburns, United Kingdom, were used (pp. 9, 12-13; Appendix 2, pp. 25-26 of MRID 50142026).

2 In the ILV, surface water matrix (Sample ID 2304W-001), obtained from Alvarado park, Richmond, California, and distilled water matrix (Sample ID 2304W-002) was purchased from Lucky supermarket, Hercules, California, were used (p. 10 of MRID 50142028). The water matrices were not characterized, and the specific surface water source was not reported.

I. Principle of the Method

Water samples (10 ± 0.05 mL) were fortified with tolfenpyrad in a 15-mL centrifuge tube (p. 13; Appendix 3, pp. 34-35, 40 of MRID 50142026). The sample was transferred to a clean 20-mL volumetric flask. The centrifuge tube was rinsed twice with 4 mL of methanol, and the rinses were combined with the sample in the volumetric flask. The volume of the sample was brought to 20 mL using methanol. An aliquot (0.5 mL) of the sample extract was diluted with 0.5 mL of methanol:water (1:1, v:v) in an autosampler vial and analyzed by HPLC/MS/MS. The method noted that a larger dilution could be performed for higher concentration samples.

Samples were analyzed for tolfenpyrad using a Waters Acquity HPLC coupled to an Applied Biosystems API 5000 MS equipped with an Phenomenex Luna C18 (2)-HST column (2.0 mm x 100 mm, 2.5 µm; column temperature 40°C nominally) and Phenomenex C18 guard column (2 mm x 4 mm) using a gradient mobile phase of (A) 0.1% formic acid in water and (B) acetonitrile [percent A:B at 0-0.5 min. 75:25, 10.25-16.26 min. 0:100, 20.25 min. 75:25] with MS/MS-ESI (electrospray ionization) detection in positive ion mode and multiple reaction monitoring (MRM; Appendix 3, pp. 34-37 of MRID 50142026). Injection volume was 50 µL. Tolfenpyrad was identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 384.2→197.1 and *m/z* 384.2→153.1. Observed retention time was *ca*. 10.35 minutes (Appendix 5, pp. 48-54).

In the ILV, the ECM was performed as written with insignificant modifications to the analytical instrumentation, except that the sample was not diluted prior to LC/MS/MS analysis (pp. 10, 13-16, 21 of MRID 50142028). A Dionex Ultimate 3000 LC coupled to an Applied Biosystems MDS/SCIEX API 4000 MS was used for analyte identification. LC parameters which differed from the ECM were the use of a Phenomenex Fusion RP 100A column (2.0 mm x 100 mm; column temperature 40°C) and Phenomenex Fusion guard column (2 mm x 4 mm). Tolfenpyrad was identified using two ion transitions which were similar to those used in the ECM (quantitation and confirmation, respectively): *m/z* 384.2→197.3 and *m/z* 384.2→153.2; expected retention time was *ca*. 11.5 minutes. 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. 9-10, 13-14, 16 of MRID 50142026; pp. 18, 21 of MRID 50142028). In the ECM, the Limit of Detection (LOD) was calculated as 0.0008 µg/L in surface water and 0.0011 µg/L in distilled water. In the ILV, the LOD was reported as 0.0025 µg/L for both matrices.

II. Recovery Findings

ECM (MRID 50142026): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of tolfenpyrad at fortification levels of 0.01 µg/L (LOQ) and 0.10 µg/L (10×LOQ) in the surface and distilled water matrices (Tables 1-4, pp. 19-20). Tolfenpyrad was identified using two ion transitions; performance data (recovery results) from quantitation and confirmation analyses were comparable. Surface (stream) water matrix (pH 8.1, 112 mg/L total hardness as $CaCO_3$, 8.0 ppm dissolved organic carbon), obtained from Oak Beck, Harrogate, United Kingdom, and distilled water matix (<2 ppm non-volatile residues, <2 ppb heavy metals) was obtained from Rathburns, United Kingdom, were used (pp. 9, 12-13; Appendix 2, pp. 25-26).

ILV (MRID 50142028): Mean recoveries and RSDs were within guideline requirements for analysis of tolfenpyrad at fortification levels of 0.01 µg/L (LOQ) and 0.10 µg/L (10×LOQ) in the surface and distilled water matrices, except for the 10×LOQ analysis in distilled water [RSDs 31% (Q) and 37% (C); Tables 2-3, pp. 24-25; DER Attachment 2]. For the 10×LOQ analysis in distilled water, means, s.d.s and RSDs were reviewer-calculated based on n = 5 since the study author excluded one recovery value from each sample set after assuming that sample was prepared with a double fortification (p. 19; Table 2, p. 24). The means and RSDs which were calculated by the study author based on n = 4 were 102% and 3%, respectively, for the quantitation ion transition and 99% and 4%, respectively, for the confirmation ion transition. Tolfenpyrad was identified using two ion transitions; performance data (recovery results) from quantitation and confirmation analyses were comparable. Surface water matrix (Sample ID 2304W-001), obtained from Alvarado park, Richmond, California, and distilled water matrix (Sample ID 2304W-002) was purchased from Lucky supermarket, Hercules, California, were used (p. 10). The water matrices were not characterized, and the specific surface 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. 10, 13-16, 21).

Table 2. Initial Validation Method Recoveries for Tolfenpyrad in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Surface (Stream) Water					
	Quantitation Ion Transition					
Tolfenpyrad	0.0100 (LOQ)	5	93-104	99	4.3	4.4
	0.1000	5	101-105	102	1.7	1.6
	Confirmation Ion Transition					
Tolfenpyrad	0.0100 (LOQ)	5	93-99	96	2.8	2.9
	0.1000	5	98-106	101	3.0	3.0
	Distilled Water					
	Quantitation Ion Transition					
Tolfenpyrad	0.0100 (LOQ)	5	96-102	98	2.5	2.6
	0.1000	5	93-100	97	3.0	3.2
	Confirmation Ion Transition					
Tolfenpyrad	0.0100 (LOQ)	5	79-100	94	8.7	9.3
	0.1000	5	90-98	94	3.0	3.3

Data (uncorrected recovery results, Appendix 3, p. 38) were obtained from Tables 1-4, pp. 19-20 of MRID 50142026.

1 The surface (stream) water matrix (pH 8.1, 112 mg/L total hardness as $CaCO_3$, 8.0 ppm dissolved organic carbon) was obtained from Oak Beck, Harrogate, United Kingdom (pp. 9, 12-13; Appendix 2, pp. 25-26). Distilled water matix (<2 ppm non-volatile residues, <2 ppb heavy metals) was obtained from Rathburns, United Kingdom.

2 Tolfenpyrad was identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 384.2→197.1 and *m/z* 384.2→153.1.

Table 3. Independent Validation Method Recoveries for Tolfenpyrad 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					
Tolfenpyrad	0.01	5	90-120	112	13	12
	0.1	5	83-105	93	10	10
	Confirmation Ion Transition					
Tolfenpyrad	0.01	5	100-120	110	10	9
	0.1	5	82-103	93	9	10
	Distilled Water					
	Quantitation Ion Transition					
Tolfenpyrad	0.01	5	84-113	102	11	11
	0.1	5[3]	98-184	118	37	**31**
	Confirmation Ion Transition					
Tolfenpyrad	0.01	5	99-105	102	2	2
	0.1	5[3]	93-196	118	44	**37**

Data (uncorrected recovery results, pp. 16-18) were obtained from Tables 2-3, pp. 24-25 of MRID 50142028 and DER Attachment 2.

1 The surface water matrix (Sample ID 2304W-001) was obtained from Alvarado park, Richmond, California (p. 10). The specific surface water source was not reported. The distilled water matrix (Sample ID 2304W-002) was purchased from Lucky supermarket, Hercules, California.

2 Tolfenpyrad was identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 384.2→197.3 and *m/z* 384.2→153.2.

3 Reported means, s.d.s and RSDs were reviewer-calculated based on n = 5 since the study author excluded one recovery value from each sample set after assuming that sample was prepared with a double fortification (p. 19; Table 2, p. 24). The means and RSDs which were calculated by the study author based on n = 4 were 102% and 3%, respectively, for the quantitation ion transition and 99% and 4%, respectively, for the confirmation ion transition.

III. Method Characteristics

In the ECM and ILV, the LOQ for water was 0.01 µg/L (pp. 9-10, 13-14, 16 of MRID 50142026; pp. 18, 21 of MRID 50142028). In the ECM and ILV, the LOQ was defined as the fortification level for which acceptable mean recoveries and RSDs (mean 70-120%; RSD ≤20%) were obtained. In the ECM, the LOD was calculated as 0.0008 µg/L in surface water and 0.0011 µg/L in distilled water. The LOD calculations were derived by estimation of the equivalent concentration of tolfenpyrad for a peak response equal to three times baseline noise (height) in control matrix extracts compared to the lowest calibration standard (height of the peal equivalent to 0.001 µg/L). The ECM LODs were less than 30% of the LOQ, and its signal-to-noise ratio should be greater than three. In the ILV, the LOD was reported as 0.0025 µg/L for both matrices. In the ILV, the LOD was defined as the concentration of the lowest linearity calibrant injected, 1 ng/L of tolfenpyrad.

Table 4. Method Characteristics

		Tolfenpyrad	
		Surface Water	**Distilled Water**
Limit of Quantitation (LOQ)		0.01 µg/L	
Limit of Detection (LOD)	ECM	0.0008 µg/L	0.0011 µg/L
	ILV	0.0025 µg/L	
Linearity (calibration curve r^2 and concentration range)	ECM	r^2 = 0.9980 (Q) r^2 = 0.9988 (C)	
	ILV[1]	r^2 = 0.9988 (Q) r^2 = 0.9986 (C)	
	Concentration range	0.001-0.1 µg/L	
Repeatable	ECM[2]	Yes at LOQ and 10×LOQ.	
	ILV[3,4]	Yes at LOQ and 10×LOQ.	Yes at LOQ. **No** at 10×LOQ [RSD **31**% (Q) and **37**% (C)].[5]
Reproducible		Yes at LOQ and 10×LOQ.	Yes at LOQ. **No** at 10×LOQ.
Specific	ECM	Yes, no matrix interferences were observed. Confirmation ion 10×LOQ chromatograms were not provided.[6]	
	ILV	Yes, no matrix interferences were observed. Some non-uniform peak integration was noted at the LOQ.	

Data were obtained from pp. 9-10, 13-14, 16; Tables 1-4, pp. 19-20 (recovery data); Appendix 4, pp. 45-46 (calibration curve); Appendix 5, pp. 48-54 (chromatograms) of MRID 50142026; pp. 18, 21; Tables 2-3, pp. 24-25 (recovery data); Figures 1-2, pp. 27-28 (calibration curve); Appendix C, pp. 66-69 and Appendix D, pp. 75-81 (chromatograms) of MRID 50142028; 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 (pp. 12-13; Figures 1-2, pp. 27-28 of MRID 50142028; DER Attachment 2). Solvent standards were used in the ILV.

2 In the ECM, surface (stream) water matrix (pH 8.1, 112 mg/L total hardness as CaCO$_3$, 8.0 ppm dissolved organic carbon), obtained from Oak Beck, Harrogate, United Kingdom, and distilled water matix (<2 ppm non-volatile residues, <2 ppb heavy metals) was obtained from Rathburns, United Kingdom, were used (pp. 9, 12-13; Appendix 2, pp. 25-26 of MRID 50142026).

3 In the ILV, surface water matrix (Sample ID 2304W-001), obtained from Alvarado park, Richmond, California, and distilled water matrix (Sample ID 2304W-002) was purchased from Lucky supermarket, Hercules, California, were used (p. 10 of MRID 50142028). The water matrices were not characterized, and the specific surface 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. 10, 13-16, 21 of MRID 50142028).

5 The means, s.d.s and RSDs at 10×LOQ were reviewer-calculated based on n = 5 since the study author excluded one recovery value from each sample set after assuming that sample was prepared with a double fortification (p. 19; Table 2, p. 24). The means and RSDs which were calculated by the study author based on n = 4 were 102% and 3%, respectively, for the quantitation ion transition and 99% and 4%, respectively, for the confirmation ion transition.

6 A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

IV. Method Deficiencies and Reviewer's Comments

1. In the ILV, performance data for tolfenpyrad in distilled water did not meet OCSPP guidelines requirements for precision at 10×LOQ [RSDs 31% (Q) and 37% (C); Tables 2-3, pp. 24-25 of MRID 50142028; DER Attachment 2]. OCSPP Guideline 850.6100 criteria for precision states that RSDs for replicates at each spiking level are ≤20%. For the 10×LOQ analysis in distilled water, means, s.d.s and RSDs were reviewer-calculated based on n = 5 since the study author excluded one recovery value from each sample set after assuming that sample was prepared with a double fortification (p. 19; Table 2, p. 24). The means and RSDs which were calculated by the study author based on n = 4 were 102% and 3%, respectively, for the quantitation ion transition and 99% and 4%, respectively, for the confirmation ion transition. OCSPP Guideline 850.6100 criteria states that a minimum of five spiked replicates were analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte. Since the study author only assumed that the sample was double fortified, the reviewer did not exclude this sample from the statistics.

   The reproducibility of the method was validated for both matrices at the LOQ; however, the reproducibility of the method at 10×LOQ was validated for only surface water, since the ILV did not provide acceptable performance data in distilled water at 10×LOQ.

2. The ILV water matrices were not characterized, and the specific surface water source was not reported (p. 10 of MRID 50142028).

3. 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. 9-10, 13-14, 16 of MRID 50142026; pp. 18, 21 of MRID 50142028). In the ECM and ILV, the LOQ was defined as the fortification level for which acceptable mean recoveries and RSDs (mean 70-120%; RSD ≤20%) were obtained. In the ECM, the LOD was calculated by estimation of the equivalent concentration of tolfenpyrad for a peak response equal to three times baseline noise (height) in control matrix extracts compared to the lowest calibration standard (height of the peal equivalent to 0.001 µg/L). The ECM LODs were less than 30% of the LOQ, and its signal-to-noise ratio should be greater than three. In the ILV, the LOD was defined as the concentration of the lowest linearity calibrant injected, 1 ng/L of tolfenpyrad. No calculations were provided for the LOQ or LOD in the ECM or ILV. The LOD values of the ECM differed from those of the ILV.

4. In the ILV, representative chromatograms showed some non-uniform peak integration at the LOQ (Appendix C, pp. 66-69; Appendix D, pp. 75-81 of MRID 50142028).

   In the ECM, Confirmation ion 10×LOQ chromatograms were not provided; however, the reviewer noted that a confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

5. In the ECM, protocol deviations were reported in Appendix 6 (Appendix 6, p. 55 of MRID 50142026).

6. No communication between the ILV testing facility and the method developer occurred during the conduct of the study (p. 21 of MRID 50142028). The ILV study author contacted the Sponsor Representative with emails of routine study updates or routine project management purposes.

7. The analytical method was included in the ECM as Appendix 3 (Appendix 3, pp. 27-43 of MRID 50142026) and in the ILV as Appendix 1 of the Study Protocol (Appendix A, Appendix 1, pp. 45-61 of MRID 50142028).

8. In the ILV, the timeframe required to complete the method validation for one set of 12 samples and one reagent blank was reported as 4 hours for sample preparation and 13 hours for analysis (including 4 hours for instrument tuning; p. 18 of MRID 50142028).

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. https://www.ecfr.gov/cgi-bin/text-idx?SID=f759988d96abd89e79a251b5e5c38df1&mc=true&node=ap40.25.136_17.b&rgn=div9

Attachment 1: Chemical Names and Structures

Tolfenpyrad (OMI-88)

IUPAC Name:	4-Chloro-3-ethyl-1-methyl-N-[4-(p-tolyloxy)benzyl]pyrazole-5-carboxamide
CAS Name:	4-Chloro-3-ethyl-1-methyl-N-[[4-(4-methylphenoxy)phenyl]methyl]-1H-pyrazole-5-carboxamide
CAS Number:	129558-76-5
SMILES String:	CCc1c(c(n(n1)C)C(=O)NCc2ccc(cc2)Oc3ccc(cc3)C)Cl