

Analytical method for clopyralid in compost

Reports: ECM: EPA MRID No.: 51120701 (Appendix D, pp. 48-106). Beato, B.D. 2020. Method Validation Study for the Determination of Residues of Clopyralid in Compost by Liquid Chromatography with Tandem Mass Spectrometry. Dow AgroSciences Study ID: 191812. Report prepared, sponsored, and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 59 pages. Final report issued February 25, 2020.

ILV: EPA MRID No. 51120701. Skaggs, C. 2020. Independent Laboratory Validation of Clopyralid in Compost. Sponsor Study ID: 170234. Performing Laboratory Study No.: SGS-19-01-15. Report prepared by SGS North America, Inc., Brookings, South Dakota, and sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 106 pages. Final report issued April 13, 2020.

Document No.: MRID 51120701

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160), except that the electronic signature device used for solvent preparation sheets was not validated according to internal SOPs (Appendix D, p. 50). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (Appendix D, pp. 49-51). A statement of the authenticity of the study report was included with the quality assurance statement (Appendix D, p. 51).

ILV: The study was conducted in accordance with USEPA FIFRA GLP (40 CFR Part 160), which are compatible with OECD GLP standards (as revised 1997), ENV/MC/CHEM(98)17, and OECD, Paris (1998; p. 3; Appendix G, p. 66). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).


Classification: This analytical method is classified as supplemental. The reported method LOQ of the ILV (0.67 ng/g) differed from that of the ECM (0.70 ng/g); since the ILV LOQ was less than the ECM LOQ, the reviewer considered the ECM LOQ to be validated by the ILV performance data. The ILV used one unspecified compost matrix. ECM representative 10×LOQ chromatograms were not provided.

PC Code: 117403

EFED Final


Reviewer:

James Lin
Environmental Engineer

Signature: 
Date: 08-12-2020

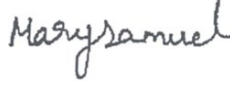
**CDM/CSS-
Dynamac JV**

Lisa Muto, M.S.
Environmental Scientist

Signature: 

Reviewers:

Mary Samuel, M.S.,
Environmental Scientist

Date: 05/29/2020
Signature: 
Date: 05/29/2020

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, Dow AgroSciences Study No. 191812, is designed for the quantitative determination of clopyralid in compost at an LOQ of 0.70 ng/g using LC/MS/MS. The ILV validated the method for clopyralid at an LOQ of 0.67 ng/g using one unspecified compost in the first trial with insignificant modifications to the analytical instrumentation, equipment, and parameters. Since the ILV LOQ was less than the ECM LOQ, the reviewer considered the ECM LOQ to be validated by the ILV performance data. The ILV did not include the difference of the LOQ as a method deviation. All ECM and ILV precision, accuracy, linearity, and specificity data was acceptable, but ECM and ILV representative 10×LOQ chromatograms were not provided.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide ¹	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Clopyralid	51120701 (Appendix D) ¹	51120701 ²		Compost	25/02/2020	Dow AgroSciences LLC	LC/MS/MS	0.70 ng/g ³

1 In the ECM, pasture and manure compost were obtained from Dow Agrosciences LLC Samples Management Group (Appendix D, p. 59). No further information regarding test matrices was provided; it was reported that complete source documentation was included in the study file.

2 In the ILV, compost used in this study was provided by Dow Agrosciences; the compost source was not further specified (p. 10). No further information regarding test matrix was provided.

3 The reported method LOQ of the ILV (0.67 ng/g) differed from that of the ECM (0.70 ng/g). The reviewer believed that this difference was due to the ILV reporting the actual versus nominal concentration (p. 13; Appendix D, p. 58). Since the ILV LOQ was less than the ECM LOQ, the reviewer considered the ECM LOQ to be validated by the ILV performance data. The ILV did not include the difference of the LOQ as a method deviation (p. 15).

I. Principle of the Method

Samples (1.00 ± 0.05 g) of compost were weighed into 50-mL centrifuged tubes and fortified, as necessary, at the LOD, LOQ, $10 \times \text{LOQ}$, and $57 \times \text{LOQ}$ (p. 10; Appendix D, p. 58; Appendix D, Appendix I, pp. 96, 102-104). The samples were extracted with 20 mL of methanol:10N sodium hydroxide (100:1, v:v) via shaking for at least 1 hour on reciprocating shaker (*ca.* 240 excursions/minute). The samples were sonicated for 10 minutes at 70-80% amplitude using pulse on for 20 seconds and off for 5 seconds. After standing overnight (at least 12 hours), the samples were then centrifuged (5 minutes at 3000 rpm). An aliquot (10.0 mL) of the supernatant was pipetted into 45-mL glass tubes then evaporated to near dryness using a Turbovap set at 40°C and a gentle stream of nitrogen. The samples were reconstituted with 4.00 mL of 1N HCl and mixed well. The sample was purified via Oasis MAX SPE cartridge (150 mg, 6 mL) pre-conditioned with 4 mL each of methanol then water. The sample was applied with two vial rinsings of 3 mL of water. The cartridge was washed with 2 x 4 mL of methanol:water:acetic acid (50:49:1, v:v:v). After the cartridge was dried for at least 30 seconds, the analyte was eluted with 2 x 4.00 mL aliquots of ethyl acetate:trifluoroacetic acid (98:2, v:v) into culture tubes (16 x 100 mm) containing 20 μL of the 1-butanol:glycerol (90:10, v:w) solution. The sample was evaporated (*ca.* 1 hour) to dryness using a Turbovap set at 40°C and a gentle stream of nitrogen. The samples were mixed with 100 μL of the 50.0 ng/mL internal standard solution then evaporated to dryness using a Turbovap set at 40°C and a gentle stream of nitrogen (the method noted that it was critical that all methanol was removed from the sample via evaporation prior to derivatization). The residue was reconstituted in 200 μL of acetonitrile:pyridine:1-butanol (22:2:1, v:v:v) then derivatized by pipetting 100 μL of acetonitrile:butyl chloroformate (9:1, v:v). After vortexing for a few seconds, the mixture was allowed to react at room temperature for *ca.* 15 minutes. The reaction was quenched with sonication for *ca.* 30 seconds with 250 μL of 0.1% formic acid in water. The samples were filtered (13 mm, 0.2 μm PTFE), transferred to low volume autosampler vials or autosampler vials with low volume glass inserts, then analyzed by LC/MS/MS. The method noted that some compost matrices, such as pasture grass compost, may require additional filtering.

Samples were analyzed for analytes by Agilent 1290 Series HPLC (Waters HSS T3 column, 2.1 mm x 100 mm, 1.8 μm , and KrudKatcher Ultra pre-column filter, 0.5 μm x 0.004 in.; column temperature 40°C) using a mobile phase of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid [percent A:B at 0.0-2.0 min. 45:55, 3.5 min. 37:63, 4.0-5.0 min. 5:95, 5.5-6.5 min. 45:55] with AB SCIEX QTrap 5500 MS using MS/MS-ESI (electrospray ionization; temperature 600°C) detection in positive polarity and multiple reaction monitoring (MRM; Appendix D, p. 60; Appendix D, Appendix I, pp. 105-106). Injection volume was 20 μL . Clopyralid was identified using two ion transitions (quantitative and confirmatory, respectively): m/z 248 \rightarrow 110 and m/z 250 \rightarrow 112 (m/z 253 \rightarrow 115 for clopyralid IS). Expected retention time was *ca.* 3.1 minutes for clopyralid (Appendix D, Figure 8, p. 83).

In the ILV, the ECM was performed as written, except for insignificant modifications to the analytical instrumentation, equipment, and parameters (pp. 8, 11, 15; Table 4, p. 25). A Shimadzu Nexera XR HPLC (Acquity UPLC HSS T3 column, 2.1 mm x 100 mm, 1.8 μm ; column temperature 40°C) coupled with AB Biosystems/MDS Sciex API6500+ MS using MS/MS-ESI was used for all analyses. Significant parameters were the same as the ECM.

Clopyralid was identified using two ion transitions (quantitative and confirmatory, respectively): m/z 247.9→110.0 and m/z 250.0→112.0 (m/z 253.1→150.9 for clopyralid IS). The monitored ion transition for the internal standard differed from that of the ECM. Expected retention time was *ca.* 3.6 minutes for clopyralid. No other modifications to the ECM were reported.

The Limit of Quantification (LOQ) for clopyralid was reported as 0.70 ng/g in the ECM and 0.67 ng/g in the ILV (p. 8; Appendix D, pp. 58, 62, 66; Appendix D, Table 11, p. 72; Appendix D, Appendix I, p. 102). The Limit of Detection (LOD) for clopyralid was reported as 0.210 ng/g in the ECM and 0.201 ng/g in the ILV. In the ECM, the LOQ and LOD were calculated as 0.171-0.468 ng/g and 0.0514-0.140 ng/g, respectively, for pasture compost and 0.698-1.01 ng/g and 0.209-0.303 ng/g, respectively, for manure compost.

II. Recovery Findings

ECM (Appendix D of MRID 51120701): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of clopyralid in two compost matrices at the fortification levels of 0.70 ng/g (LOQ), 7.00 ng/g (10×LOQ), and 40 ng/g (57×LOQ; $n = 6$ for all analyses; Appendix D, Tables 3-10, pp. 68-72). All analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. Pasture and manure compost were obtained from Dow Agrosiences LLC Samples Management Group (Appendix D, p. 59). No further information regarding test matrices was provided; it was reported that complete source documentation was included in the study file.

ILV (MRID 51120701): Mean recoveries and RSDs were within guideline requirements for analysis of clopyralid in two compost matrices at the fortification levels of 0.67 ng/g (LOQ), 6.7 ng/g (10×LOQ), and 38 ng/g (57×LOQ; $n = 5$ for all analyses pp. 9, 12-14). All analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were not comparable for the LOQ analyses but were comparable for 10×LOQ and 57×LOQ. The compost used in this study was provided by Dow Agrosiences; the compost source was not further specified (p. 10). No further information regarding test matrix was provided. The method for clopyralid in compost was validated in the first trial with insignificant modifications to the analytical instrumentation, equipment, and parameters (pp. 9, 11, 15, 17; Table 4, p. 25).

Table 2. Initial Validation Method Recoveries for Clopyralid in Compost^{1,2}

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Pasture Compost						
Quantitation Ion Transition						
Clopyralid	0.700 (LOQ)	6	99-106	102	2.50	2.45
	7.00	6	95-100	97	1.75	1.81
	40.0	6	93-97	95	1.63	1.71
Confirmatory Ion Transition						
Clopyralid	0.700 (LOQ)	6	94-112	103	6.52	6.34
	7.00	6	96-100	98	1.37	1.40
	40.0	6	94-98	96	1.47	1.54
Manure Compost						
Quantitation Ion Transition						
Clopyralid	0.700 (LOQ)	6	95-119	106	10.0	9.36
	7.00	6	82-104	96	8.11	8.43
	40.0	6	68-99	87	10.8	12.4
Confirmatory Ion Transition						
Clopyralid	0.700 (LOQ)	6	86-124	109	14.4	13.2
	7.00	6	83-103	96	7.73	8.07
	40.0	6	69-99	87	10.3	11.8

Data (uncorrected recovery results; Appendix D, Figure 5, p. 80) were obtained from Appendix D, Tables 3-10, pp. 68-72 of the study report.

1 Clopyralid was identified using two ion transitions (quantitative and confirmatory, respectively): m/z 248→110 and m/z 250→112.

2 Pasture and manure compost were obtained from Dow Agrosiences LLC Samples Management Group and homogenized prior to use (Appendix D, p. 59). No further information regarding test matrices was provided; it was reported that complete source documentation was included in the study file.

Table 3. Independent Validation Method Recoveries for Clopyralid in Compost^{1,2}

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Compost						
Quantitation ion						
Clopyralid	0.67 (LOQ)	5	79-97	85	8.0	9.4
	6.7	5	75-81	79	2.3	2.9
	38	5	72-76	75	1.7	2.3
Confirmatory ion						
Clopyralid	0.67 (LOQ)	5	91-116	104	9.4	9.1
	6.7	5	78-83	80	2.3	2.9
	38	5	74-79	77	2.3	3.0

Data (uncorrected recovery results, p. 15) were obtained from pp. 9, 12-14 of the study report.

1 Clopyralid was identified using two ion transitions (quantitative and confirmatory, respectively): m/z 247.9→110.0 and m/z 250.0→112.0.

2 The compost used in this study was provided by Dow Agrosiences; the compost source was not further specified (p. 10). No further information regarding test matrix was provided.

III. Method Characteristics

The LOQ for clopyralid was reported as 0.70 ng/g in the ECM and 0.67 ng/g in the ILV (p. 8; Appendix D, pp. 58, 62, 66; Appendix D, Table 11, p. 72; Appendix D, Appendix I, p. 102). The LOD for clopyralid was reported as 0.210 ng/g in the ECM and 0.201 ng/g in the ILV, which was equivalent to 30% of the LOQ. Following the method of Keith, L. H., *et al.* (see section V. References below), the LOD and LOQ for determination of clopyralid in compost were calculated in the ECM using the standard deviation from the LOQ recovery results, 0.0171-0.101 ng/g (Q/C). The LOD was calculated as three times the standard deviation ($3s$), and the LOQ was calculated as ten times the standard deviation ($10s$) of the recovery results. In the ECM, the LOQ and LOD were calculated as 0.171-0.468 ng/g and 0.0514-0.140 ng/g, respectively, for pasture compost and 0.698-1.01 ng/g and 0.209-0.303 ng/g, respectively, for manure compost. The calculated LOQ and LOD values supported the Method LOQ and LOD values, except in the confirmation ion transition analysis in manure compost. No ILV calculations were reported to support the method LOQ or LOD.

Since the ILV LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported ILVLOQ is the lowest level of method validation (LLMV) rather than an LOQ.

Table 4. Method Characteristics

Analyte		Clopyralid	
Limit of Quantitation (LOQ)	ECM (method)	0.70 ng/g	
	ECM (calc)	0.171 ng/g (Q, pasture) 0.468 ng/g (C, pasture)	0.698 ng/g (Q, manure) 1.01 ng/g (C, manure) ¹
	ILV (method)	0.67 ng/g ^{2,3}	
Limit of Detection (LOD)	ECM (method)	0.210 ng/g (30% of the LOQ)	
	ECM (calc)	0.0514 ng/g (Q, pasture) 0.140 ng/g (C, pasture)	0.209 ng/g (Q, manure) 0.303 ng/g (C, manure) ¹
	ILV (method)	0.201 ng/g (30% of the LOQ)	
Linearity (calibration curve r and concentration range)	ECM	r = 0.9999 (Q) r = 0.9998 (C) 0.210-50.0 ng/g	
	ILV	r = 0.99977 (Q) r = 0.99971 (C) 0.202-48 ng/g	
Repeatable	ECM ⁴	Yes at the LOQ, 10×LOQ, and 57×LOQ in pasture and manure compost matrices.	
	ILV ^{5,6}	Yes at the LOQ, 10×LOQ, and 57×LOQ in unspecified compost matrices.	
Reproducible		Yes at the LOQ, 10×LOQ, and 57×LOQ.	
Specific	ECM	Yes, matrix interferences were <i>ca.</i> 11-13% (Q) and <i>ca.</i> 44-55% (C) ^{7,8} of the LOQ (based on peak area). Representative 10×LOQ chromatograms were not presented.	
	ILV	Yes, matrix interferences were either not observed (Q) or <i>ca.</i> 30-46% (C) ^{7,9} of the LOQ (based on peak area). Some minor baseline noise interfered with Q LOQ peak.	

Data were obtained from p. 8 (ILV LOD/LOQ); pp. 9, 12-14 (ILV recovery data); p. 8; Table 3, p. 24; Appendix C, pp. 26, 37 (ILV calibration data and figures); Appendix C, pp. 27-47 (ILV chromatograms); Appendix D, pp. 58, 62, 66; Appendix D, Table 11, p. 72; Appendix D, Appendix I, p. 102 (ECM LOD/LOQ); Appendix D, Tables 3-10, pp. 68-72 (ECM recovery data); Appendix D, p. 62 and Figures 3-4, pp. 78-79 (ECM calibration data and figures); Appendix D, Figures 8-19, pp. 83-94 (ECM chromatograms); of the study report. Q = Quantitation ion transition; C = Confirmation ion transition.

- 1 The ECM calculated LOQ and LOD values for the confirmation ion transition analysis in manure compost did not support the ECM Method LOQ and LOD values.
- 2 The reported method LOQ of the ILV differed from that of the ECM. The reviewer believed that this difference was due to the ILV reporting the actual versus nominal concentration (p. 13). Since the ILV LOQ was less than the ECM LOQ, the reviewer considered the ECM LOQ to be validated by the ILV performance data.
- 3 Since the ILV LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported ILVLOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.
- 4 In the ECM, pasture and manure compost were obtained from Dow Agrosiences LLC Samples Management Group (Appendix D, p. 59). No further information regarding test matrices was provided; it was reported that complete source documentation was included in the study file.
- 5 In the ILV, compost used in this study was provided by Dow Agrosiences; the compost source was not further specified (p. 10). No further information regarding test matrix was provided.
- 6 The ILV validated the method for clopyralid in compost in the first trial with insignificant modifications to the analytical instrumentation, equipment, and parameters (pp. 9, 11, 15, 17; Table 4, p. 25).
- 7 Deviations in the confirmation ion analyses do not affect the specificity of the method since a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.
- 8 Based on Appendix D, Figures 12-17, pp. 87-92, of the study report.
- 9 Based on Figures 18-20, pp. 43-45, of the study report.

IV. Method Deficiencies and Reviewer's Comments

1. The reported method LOQ of the ILV (0.67 ng/g) differed from that of the ECM (0.70 ng/g). The reviewer believed that this difference was due to the ILV reporting the actual versus nominal concentration (p. 13; Appendix D, p. 58). Since the ILV LOQ was less than the ECM LOQ, the reviewer considered the ECM LOQ to be validated by the ILV performance data. The ILV did not include the difference of the LOQ as a method deviation (p. 15).
2. Only one compost which was unspecified was used in the ILV; pasture and manure compost were used in the ECM (p. 10; Appendix D, p. 59).
3. In the ECM, representative 10×LOQ chromatograms were not provided. Representative chromatograms from all matrices and fortifications should be provided for review.
4. The ECM calculated LOQ and LOD values for the confirmation ion transition analysis (1.01 ng/g and 0.303 ng/g, respectively) in manure compost did not support the ECM Method LOQ and LOD values (0.70 ng/g and 0.210 ng/g, respectively; p. 13; Appendix D, pp. 58; Appendix D, Table 11, p. 72). The calculated LOQ and LOD were based on the standard deviation at the LOQ (14.4%, C manure compost; Appendix D, Table 10, p. 72). The reviewer noted that matrix interferences were more evident in pasture compost (*ca.* 55%) than in manure compost (*ca.* 44%; Appendix D, Figures 12-17, pp. 87-92). The reviewer also noted that deficiencies in the confirmation ion analyses do not affect the validity of the method since a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.
5. Communication details were not provided. The ILV reported that communications occurred between the ILV Study Director (C. Skaggs) and Dow AgroSciences Study Representative (Leandro Ap. G. Deziderio) were documented but not provided (pp. 1, 6, 17). The only communication which was reported was the communication of the successful completion of the first ILV trial. No one from Dow AgroSciences was allowed to visit the ILV testing facility. Leandro Ap. G. Deziderio was not listed in the ECM personnel (Appendix D, pp. 52-53).
6. The matrix interferences were significant in the confirmation ion transition for ECM and ILV analyses in all test matrices (ECM, *ca.* 44-55% ; ILV *ca.* 30-46%; Figures 18-20, pp. 43-45; Appendix D, Figures 12-17, pp. 87-92). Deviations in the confirmation ion analyses do not affect the specificity of the method since a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.
7. Carryover was assessed in the ECM (Appendix D, p. 63). No carryover was observed.
8. The matrix effects were found to be insignificant ($\leq 20\%$) for the analytes in the test matrices in the ECM (quantitation and confirmatory transitions) with the use of an

- internal standard (Appendix D, pp. 63-64, and Tables 14-15, p. 74). Solvent-based standards were used in the ECM and ILV.
9. Since stable-isotope labeled internal standards were used, isotopic cross-over was evaluated in the ECM (Appendix D, pp. 59-60, and Table 2, p. 68). The concentration range of calibration curve and concentration of internal standard were chosen to minimize cross-over. No significant mass spectral isotopic crossover was observed.
 10. In the ECM, the calibration solutions and stock solutions were found to be stable in methanol up to 270 days of refrigerated storage (Appendix D, p. 64, and Table 16, p. 75). The final sample extracts were found to be stable up to 5 (pasture) or 7 (manure) days at *ca.* 10°C (Appendix D, p. 63, and Tables 12-13, p. 73).
 11. In the ECM, it was reported that the extraction efficiency of the method was not studied in the study, but a plant metabolism study was referenced (Appendix D, pp. 61, 66).
 12. It was reported for the ILV that one validation sample set required *ca.* 24 hours, which included the samples sitting overnight at room temperature for a minimum of 24 hours (p. 12).

V. References

- Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.* 1983, 55, 2210-2218 (Appendix D, p. 66).
- 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**Clopyralid**

IUPAC Name:	3,6-Dichloropyridine-2-carboxylic acid 3,6-Dichloropicolinic acid
CAS Name:	3,6-Dichloro-2-pyridinecarboxylic acid
CAS Number:	1702-17-6
SMILES String:	Not found

