

## Independent Laboratory Validation of Clopyralid in Compost

### INTRODUCTION

The purpose of this study was to demonstrate that analytical method “Enforcement Method for Determination of Residues of Clopyralid in Compost Using Liquid Chromatography with Tandem Mass Spectrometry” could be performed successfully at an outside facility with no prior experience with the method (Reference 1).

**Principle of the method.** The analyte is extracted from compost with methanol containing 0.1N NaOH. Samples sit overnight, prior to an aliquot being taken and evaporated. Samples are reconstituted with 1N HCl and subjected to SPE. After further reconstitution, including derivatization, samples are analyzed via LC-MS/MS.

**Test conditions.** For validation, the analytical set consisted of one reagent blank, two matrix controls, one control fortified at LOD (limit of detection), five replicates fortified at LOQ (limit of quantitation), five replicates fortified at 10X LOQ, and five replicated fortified at 57X LOQ. The mass transitions used for analysis are listed below.

	<b>Quantitation (<i>m/z</i>)</b>
Clopyralid	<i>m/z</i> 247.9 → 110.0
Clopyralid <sup>1</sup>	<i>m/z</i> 250.0 → 112.0
Clopyralid IS	<i>m/z</i> 253.1 → 150.9

<sup>1</sup>Denotes confirmatory ion.

**Limit of Quantification (LOQ) and Limit of Detection (LOD).** During the independent laboratory validation of the method, the limit of quantitation (LOQ) of clopyralid was confirmed to be 0.67 ng/g (ppb) for compost. The LOD for clopyralid was set at 30% of the defined LOQ.

**Selectivity.** At the retention time of clopyralid and clopyralid IS, no interfering peaks were found. It was also found that matrix effect was insignificant during method adaptation, therefore, solvent calibration standards were used for the validation.

**Linearity.** For both transitions of clopyralid, the *r* value was over 0.995, indicating that the linearity was acceptable. The linear range was from 0.2 ng/ml to 48 ng/ml for clopyralid.

**Standard Stability.** Analytical standards and fortification solutions were stored under refrigerated conditions when not in use. Stock standard and spiking standard stabilities were assessed in a separate study, and solutions of clopyralid were found to be stable in acetonitrile (Reference 2).

**Extract Stability.** Extract stability was established during validation study “Enforcement Method for Determination of Residues of Clopyralid in Compost Using Liquid Chromatography with Tandem Mass Spectrometry” (Reference 1).

## EXPERIMENTAL

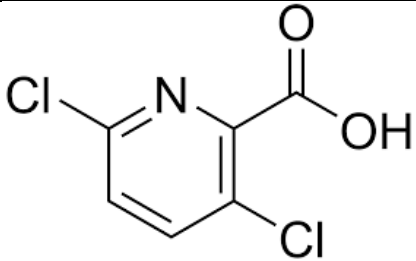
### Test Systems

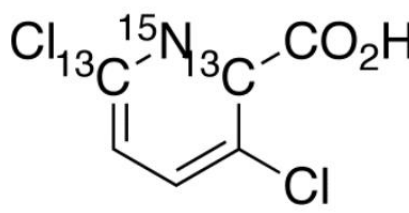
The test system considered in this study was compost. The control sample was provided by Dow AgroSciences and sent to SGS via overnight shipping on dry ice. The compost sample was received by SGS on November 12, 2019. The test system was received frozen and stored under frozen conditions at all times, unless necessary for laboratory analysis.

### Test and Reference Substances

Clopyralid and clopyralid internal standard reference substances were stored at room temperature. Sponsor has retained a reserve sample of these chemicals, and has documentation specifying the location of the synthesis and characterization information available at Dow AgroSciences in Indianapolis, Indiana.

The clopyralid and clopyralid internal standard reference substances were provided by the sponsor and received on October 8 and November 25, 2019, respectively. Upon receipt, the reference substances were stored in the ambient lockbox with the temperature ranging from 20 to 23 °C. The certificates of analysis are presented in Appendix A. A detailed summary of the reference substances is presented below.

Common Name	Clopyralid
Chemical Formula	C <sub>6</sub> H <sub>3</sub> Cl <sub>2</sub> NO <sub>2</sub>
Test Substance Structure	
CAS Number	1702-17-6
Supplier	Dow AgroSciences
Lot / Batch #	910905-5P
Purity	95.9%
Expiration	July 28, 2020

Common Name	<sup>13</sup> C <sub>2</sub> <sup>15</sup> N-Clopyralid
Chemical Formula	C <sub>4</sub> <sup>13</sup> C <sub>2</sub> H <sub>3</sub> Cl <sub>2</sub> <sup>15</sup> NO <sub>2</sub>
Test Substance Structure	
CAS Number	N/A
Supplier	Dow AgroSciences
Lot / Batch #	YM3-160205-050
Purity	N/A
Expiration	July 18, 2022

### Analytical Method

Analytical method “Enforcement Method for Determination of Residues of Clopyralid in Compost Using Liquid Chromatography with Tandem Mass Spectrometry”, was used for the analysis of the samples (Reference 1).

Residues of clopyralid were extracted from compost with methanol containing 0.1N NaOH. Samples sat overnight, prior to an aliquot being taken and evaporated. Samples are reconstituted with 1N HCl and subjected to SPE. After further reconstitution, including derivatization, samples are analyzed via LC-MS/MS.

Analyte	Transition (m/z)		Ionization Mode	Retention Time (min)
	Primary	Secondary		
Clopyralid	247.9 → 110.0	250.0 → 112.0	Positive	~3.6
Clopyralid IS	253.1 → 150.9	N/A		~3.6

### Percent Recovery Calculation

To determine the recovery in fortified samples, the following equation is used:

$$\%Recovery = \frac{\text{Calculated Residue found } (\frac{ng}{g})}{\text{Nominal amount fortified } (\frac{ng}{g})} \times 100$$

Compost LOQ 5 for clopyralid:

$$\% \text{ Recovery: } (0.66384 \text{ ng/g}) / (0.67239 \text{ ng/g}) \times 100 = 96.50\%$$

### Changes to the Method

The original method used a mass transition of 253.1 → 115.0 for the clopyralid internal standard; however, during method suitability testing, it was determined that this transition was very noisy and consistently produced split peaks. Therefore, a different and less noisy mass transition (253.1 → 150.9) was used. The mass spec used in the original validation was an API 5500 QTrap; therefore, since a 6500+ MS/MS was used in this ILV, it stands to reason that a different mass transition could be used

## REFERENCES

1. "Enforcement Method for Determination of Residues of Clopyralid in Compost Using Liquid Chromatography with Tandem Mass Spectrometry," Study ID: 191812.
2. OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation; United States Environmental Protection Agency, U.S. Government Printing Office: Washington, DC, 2012; EPA-712-C-001.
3. SANCO/825/00 rev. 8.1 (16 November 2010), Guidance Document on Pesticide Residue Analytical Methods; Directorate General Health and Consumer Protection, European Commission.

Intermediate Standard Solutions

Target Concentration (ng/mL)	Initial Solution (ng/mL)	Conc. of Initial Solution (µg/mL) <sup>1</sup>	Aliquot (mL)	Final Volume (mL) <sup>2</sup>	Final Concentration (µg/mL)
10000	Clopyralid stock solution	96.1	1.0	10	9.6
1000	10000	9.6	1.0	10	0.96
100	1000	0.96	1.0	10	0.096
5	100	0.01	0.5	10	0.005
500	Clopyralid IS stock solution	100	0.05	10	0.5
50	500	0.5	1.0	10	0.05

<sup>1</sup> Corrected for purity.

<sup>2</sup> Prepared in methanol.

Calibration Standard Solutions

Standard ID	Target Conc. (ng/mL)	Initial solution (ng/mL)	Conc. of Initial Solution (ng/mL)	Aliquot (mL)	Final volume (mL) <sup>1</sup>	Final Concentration (ng/g)
A	1.05	100 Int. Std.	96.1	0.105	10	0.202
B	2.00	100 Int. Std.	96.1	0.200	10	0.384
C	3.50	100 Int. Std.	96.1	0.350	10	0.672
D	5.00	100 Int. Std.	96.1	0.500	10	0.961
E	10.0	100 Int. Std.	96.1	1.000	10	1.92
F	25.0	1000 Int. Std.	961	0.250	10	4.80
G	50.0	1000 Int. Std.	961	0.500	10	9.61
H	100	1000 Int. Std.	961	1.000	10	19.2
I	150	10000 Int. Std.	9606	0.150	10	28.8
J	250	10000 Int. Std.	9606	0.250	10	48.0

**Table 4 Instrument Conditions and Parameters**

<b>HPLC Conditions</b>			
Chromatographic System:	Shimadzu Nexera XR		
Column:	Acquity UPLC HSS T3; 1.8 $\mu$ m, 2.1 $\times$ 100 mm S/N: 02283930915105		
Temperature:	40 $^{\circ}$ C		
Flow rate ( $\mu$ L/min)	600		
Gradient:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.0	45	55
	2.0	45	55
	3.5	37	63
	4.0	5	95
	5.0	5	95
	5.5	45	55
	6.5	45	55
Mobile Phase A:	0.1% formic acid in water		
Mobile Phase B:	0.1% formic acid in acetonitrile		
Injection Volume:	20 $\mu$ L		

<b>MS/MS Conditions</b>						
Detection System:	AB BioSystems/MDS Sciex API 6500+ LC/MS/MS					
Ionization:	Turbo Ion Spray					
Polarity:	Positive					
Curtain gas (CUR):	20.00					
Temperature (TEM):	600 $^{\circ}$ C					
Collision gas setting (CAD):	10.00					
GS1:	45.00					
GS2:	55.00					
IS:	5500					
Entrance potential (EP):	10.00					
Scan type:	MRM					
<b>MRM Conditions</b>	Transition (m/z)	Dwell (msec)	DP	CE	CXP	Retention Time (min)
Clopyralid	247.9 $\rightarrow$ 110.0	200	76	63	12	3.6
	250.0 $\rightarrow$ 112.0		100	60	14	
Clopyralid IS	253.1 $\rightarrow$ 150.9	200	51	37	22	3.6