

Analysis of Clopyralid in Manure Compost and Sludge Fertilizer Using a Triple Quadrupole Mass Spectrometer

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User Benefits

- ◆ High-sensitivity analysis of clopyralid in manure compost and sludge fertilizer is possible using an improved method from Testing Methods for Fertilizers (FAMIC)
- ◆ Filter blockages during the pretreatment step can be avoided by using an improved purification procedure.

Introduction

Clopyralid (Fig. 1) is a herbicide in common use in North America and Australia, which is found in residual quantities in imported animal feed and thereby in the manure of livestock raised on this feed. Even at low concentrations, clopyralid in manure-based fertilizer can have an adverse effect on the growth of vegetables and flowers such as tomatoes, soybeans and sunflowers, incurring demand for a high sensitivity detection method.

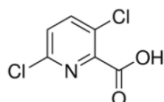


Fig. 1 Structure of clopyralid

In this Application News, we demonstrate the analysis of clopyralid in manure compost and sludge fertilizer samples using the LCMS-8050 triple quadrupole mass spectrometer (Fig. 2). The method employed is an improved version¹⁾ of a method originally listed in Testing Methods for Fertilizers (2020)²⁾ published by the Food and Agricultural Materials Inspection Center (FAMIC).

Note: Shimadzu Corporation contributed to the report *Analysis of clopyralid in manure compost using liquid chromatography-mass spectrometry: Validation through multilaboratory collaborative testing*³⁾, also published by FAMIC.

Table 1: Analysis Conditions

[HPLC conditions] (Nexera™ X3)	
Column	: Shim-pack™ Scepter C18-120, 1.9 μm (100 mm × 2.1 mm I.D., 1.9 μm) P/N:227-31012-05
Mobile phase	: A: 0.1% formic acid in water B: Methanol
Time program	: B.Conc. 5% (0 min) → 60% (5 min) → 95% (6 min) → 5% (7-10 min) 3% (17.01-20 min) Introduced into the MS at 1.4-5.5 min only using a flow switching valve.
Flow rate	: 0.4 mL/min
Injection volume	: 5 μL
Column temp.	: 40 °C
[MS conditions] (LCMS-8050)	
Ionization	: ESI (Positive mode)
Mode	: MRM
Interface voltage	: +0.5 kV
Nebulizing gas flow	: 3 L/min
Drying gas flow	: 10 L/min
Heating gas flow	: 10 L/min
DL temp.	: 150 °C
Block heater temp.	: 150 °C
Interface temp.	: 400 °C
Probe position	: +1 mm
MRM transition	: m/z 191.90>146.10 CE -21 V m/z(Ref1) 191.90>110.10 CE -34 V



Fig. 2 The LCMS-8050

Analysis Conditions

The analysis conditions of the improved method are shown in Table 1, and the MRM chromatogram for a standard solution of 0.5 ng/mL clopyralid is shown in Fig. 3. Under these analysis conditions, clopyralid was eluted at approx. 3.5 min.

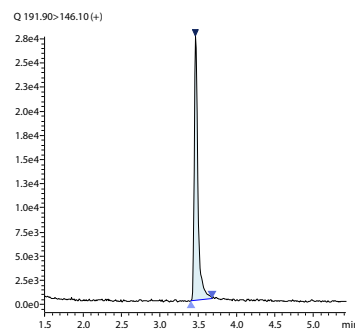


Fig. 3 MRM chromatogram for clopyralid (0.5 ng/mL)

Calibration Curve

Fig. 4 shows the calibration curve obtained for clopyralid in the concentration range 0.5-100 ng/mL. Excellent linearity was achieved with $r^2 > 0.999$.

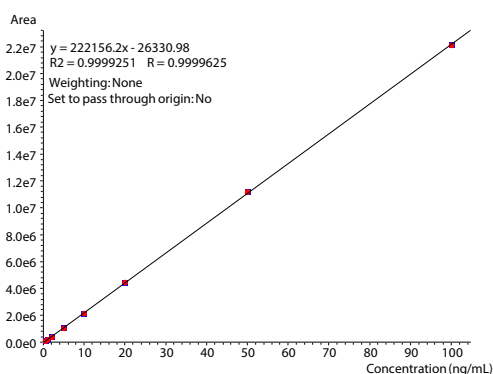


Fig. 4 Calibration curve for clopyralid

■ Pretreatment of Manure Compost and Sludge Fertilizer

6 manure compost samples (2 each of cattle, pig, and horse manure compost samples) and 2 sludge fertilizer samples were prepared. After drying at 40°C for approx. 60-70 hours, the samples were crushed with a pulverizer until they passed through a 500 µm screen. Then pretreatment was carried out according to the improved method¹⁾. In the original method²⁾, it was found that impurities in the extracted liquid tended to block the filter of the copolymer cartridge column, causing problems in purification. Therefore, we employed an improved procedure for the clean up (1) step described in section 8.2.c (4.2) of Testing Methods for Fertilizers (2020). Fig. 5 shows the original pretreatment workflow. In the clean up (1) step, before introducing the sample liquids into the copolymer column, we added a centrifugal separation step. For details, see references 1) and 3).

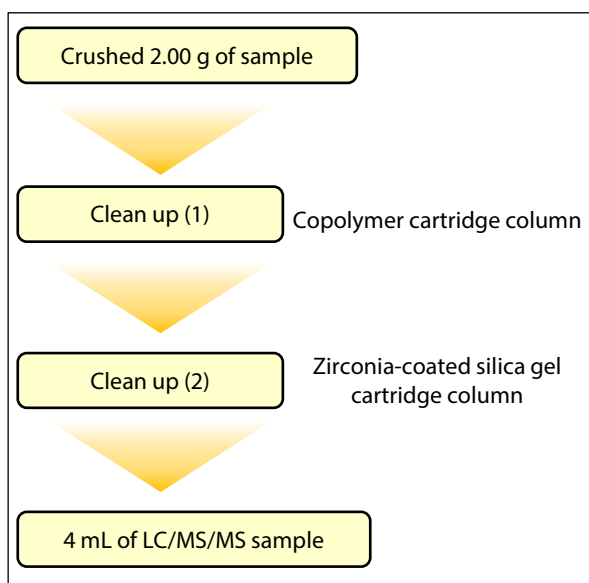


Fig. 5 Sample pretreatment

■ Analysis of Manure Compost and Sludge Fertilizer

The quantitative results for each sample are shown in Table 2, and MRM chromatograms (one for each sample type) are shown in Fig. 6. The amount of clopyralid detected varied between 18.5-143 µg/kg.

Table 2 Quantitative results for each sample

Sample	Cattle manure compost		Pig manure compost	
Concentration in sample [µg/kg]	125	143	63.0	64.8
Sample	Horse manure compost		Composted sludge fertilizer	
Concentration in sample [µg/kg]	18.5	27.4	18.8	19.9

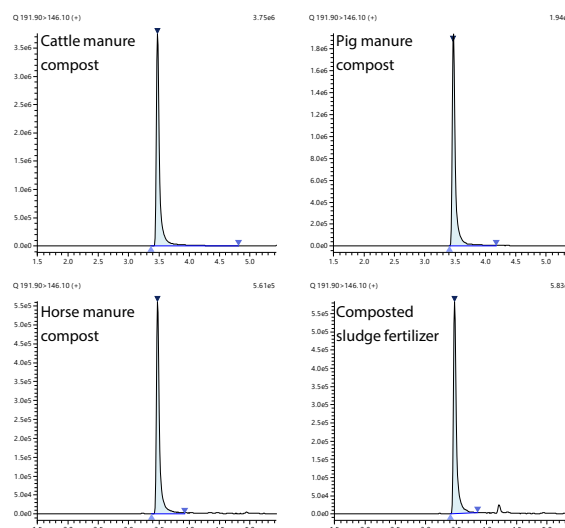


Fig. 6 MRM chromatogram for each sample (only one of each type of sample shown)

■ Conclusion

We carried out an analysis of clopyralid according to an improved version of the Testing Methods for Fertilizers (2020) method using the LCMS-8050 and a Shim-pack Scepter C18-120 column. We demonstrated that clopyralid could be detected with high sensitivity in samples of manure compost and sludge fertilizer.

References

- 1) *Analysis of clopyralid in manure compost using liquid chromatography-mass spectrometry: An improved purification method*, Research Report of Fertilizer Vol.14, 2021
<http://www.famic.go.jp/ffis/fert/rf/obj/rf14-09.pdf>
[Pretreatment steps and analytical conditions given in English]
- 2) Testing Methods for Fertilizers (2020), Food and Agricultural Materials Inspection Center (FAMIC)
- 3) *Analysis of clopyralid in manure compost using liquid chromatography-mass spectrometry: Validation through multilaboratory collaborative testing*, Research Report of Fertilizer Vol.14, 2021
<http://www.famic.go.jp/ffis/fert/rf/obj/rf14-10.pdf>
[Pretreatment steps and analytical conditions given in English]

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