Quantitative Analysis of Dicamba and Related Acid Herbicides and Metabolites

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ABSTRACT

With the EPA ruling to continue to allow the application of Dicamba to important agricultural crops, interest in this and related herbicide compounds and their degradation products has increased in the US. Dicamba, 2,4-D, and other herbicides comprise a large portion of the widely applied chemical herbicide compounds. Quantitative determination of these and other related acid herbicides and metabolites to low levels in relevant environmental matrices represents a crucial analytical need in the environmental and agricultural testing spaces. The ability to effectively and reliably perform quantitative analysis in complex extracts of soil and plant tissues by LC-MS/MS without the need for chemical derivatization is demonstrated. Limits of detection are reported for a suite of acid herbicides and select degradation products.

INTRODUCTION

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Widespread global use as weed control agents and plant growth regulators for agricultural crops, lawns, and gardens makes the active ingredients in Acid Herbicide (AcH) products account for more use than all the other types of pesticides combined. These predominant herbicide chemicals include the well- characterized 2,4-dichlorophenoxyacetic acid (2,4-D), Dicamba, triclopyr, and other AcHs. The US EPA recently ruled in favor of continued use of Dicamba despite complaints and concern about drift across plots during spray application. While adjustments in regulation around application patterns were made, these AcHs remain a prevalent concern in environmental monitoring and crop contamination analysis.

Historically, AcHs detection and concentration measurements were determined by analysis with gas chromatography with element selective detectors such as electron capture. The US EPA Method 8151: CHLORINATED HERBICIDES BY GC USING METHYLATION OR PENTAFLUOROBENZYLATION DERIVATIZATION has historically represented a dominant analytical approach in the testing industry. This method, however, requires derivatization to form volatile species. Derivatization plus GC analysis is now widely being considered more inefficient and unreliable than alternative approaches.

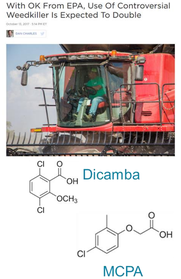

Figure 1. Recent EPA focus on Dicamba brings AcHs to the forefront of testing concerns.

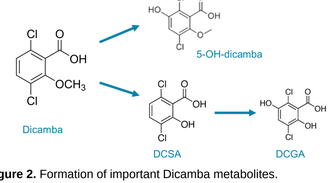

Figure 2. Formation of important Dicamba metabolites.

Major Dicamba metabolites are also relevant.
Metabolites of concern are 5-OH Dicamba and 3,6-dichlorosalicylic acid (DCSA). DCSA is the major degradate in the environment, and is more persistent in the environment than the parent Dicamba.

LC-MS/MS as a GC replacement technology would eliminate the need for the derivatization step.
In a literature review of chlorophenoxy acid herbicide methods, LC-MS/MS was the prevalent technology cited. Acidic functional groups are easiest to ionize as their conjugate base, and LC-MS/MS methods have the ability to utilize negative mode electrospray ionization (ESI−).

MATERIALS AND METHODS

Sample Preparation:
5 g of soil sample or soybean foliage were collected from impacted and non-impacted agricultural field sites. Sample was homogenized and extracted with acidified acetonitrile. Sample was shaken for 15 minutes then centrifuged at 4000 rpm. The supernatant was diluted with aqueous mobile phase into 2 mL amber autosampler vials for LC-MS/MS analysis.

HPLC Conditions:
Chromatographic separation of these highly polar, low molecular species was achieved using a Phenomenex Kinetex F5 column. Excellent analyte retention and peak quality is demonstrated using this relatively novel stationary phase. Reproducibility and robustness over multiple injections is reported.

HPLC Conditions
- SCIEX ExionLC™ AD
- Chromatographic gradient conditions using a Phenomenex Kinetex® F5 column (2.6 µm, 100 x 3 mm)
- Flow rate of 0.500 mL/min
- Column oven temperature 25° C and a 50 µL injection was used
- Run time was 17 minutes

MS Conditions
- SCIEX 6500+ QTRAP system
- Turbo V™ source operated in positive mode electrospray ionization (ESI)
- MRM experiment monitored 2 transitions for each analyte
  - Optimized compound-specific voltages were designated for maximum sensitivity and specificity

MS/MS Conditions:
A SCIEX QTRAP® 6500+ system is employed for its sensitivity and robustness. Optimized MRM transitions are selected and utilized for maximum sensitivity. Isotopically labelled version of a subset of the target analytes are employed as internal standards for achieving the highest quality quantitation data in complex soil and plant extracts. Quantitative method performance and recovery values were investigated and reported.

RESULTS

Chromatography
The F5 stationary phase demonstrated excellent retention and resolution for these small, polar species. The17 minute gradient maximizes separation from matrix interferences. RT values were specified for each MRM transition to optimize cycle time for best peak shape and quantitation.

Table 1. LC Gradient Program

Time	%B
1	40
4	52
12	85
13.5	90
14.5	90
14.6	2
17.5	2

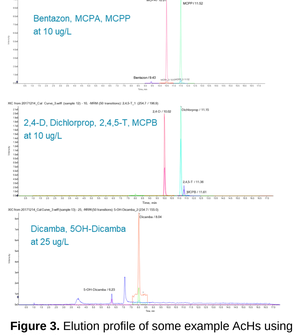

Figure 3. Elution profile of some example AcHs using the Kinetex F5 stationary phase.

Quantitation
Acid herbicide LODs were found to be mostly <1 ng/mL range, with some exceptions including 5OH-Dicamba. The isotopically labelled d3-Dicamba was utilized as an internal standard for all analytes. Calibration curves made over concentration levels from 0.025 - 50 µg/L (Figure 4).

Acid herbicide LODs in the ng/L range, with metabolite LODs in the ug/L range

Compound ID	LOD (ng/mL, in vial)	LOQ (ng/mL, in vial)	LOQ (ng/g, in sample)	S/N at 1ppb	%CV at 1ppb	%CV at 25ppb	Cal Range
2,4,-T	0.1	0.25	3.5	132	12%	11%	0.1 - 50
2,4,5-TP	0.025	0.05	0.7	72	18%	6%	0.025 - 50
2,4,-D	0.025	0.05	0.7	226	6%	7%	0.05 - 50
2,4-DB	5	10	140	--	--	3%	5 - 50
5OH-Dicamba	1	2.5	35	49	26%	3%	0.5 - 50
Acifluorfen	<0.1	0.1	1.4	17	10%	11%	0.1 - 50
Bentazon	<0.01	<0.01	<0.14	1883	5%	3%	0.1 - 25
DCGA	5	10	140	--	--	7%	--
DCSA	1	2.5	1.4	7	7%	8%	0.05 - 50
Dicamba	0.25	1	14	25	14%	11%	0.25 - 50
Dichlorprop	0.025	0.05	0.7	586	2%	5%	0.025 - 50
MCPA	1	2.5	<0.14	4	1%	3%	0.01 - 100
MCPB	0.5	1	14	384	6%	2%	0.5 - 50
MCPP	<0.01	<0.01	<0.14	560	3%	3%	0.01 - 100

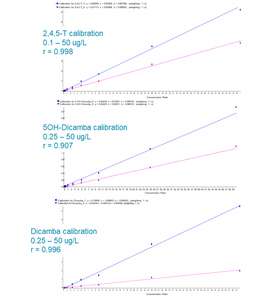

Figure 4. Calibration curves of some example AcHs demonstrating sensitivity, linear response, and dynamic range.

Data Collected from Field Samples
Occurrence data collected for soils collected from various spatial targets within impacted and non-impacted fields in the US Midwest. Occurrence data was collected for soy plant tissue (foliage) and soil.
7 soy foliage samples
Target field, planted rows, and increasing distances from field
5 soil samples
3 from target field, and 2 from increasing distances

Concentrations detected in:

Soil

Sample	Compound	Concentration (ng/g, in sample)
TARGET FIELD	Dicamba	9.38
	Dichlorprop	0.14
TARGET FIELD	MCPA	55.58
	MCPP	0.28
20 YARDS	DCSA	12.32

Soy foliage

Sample	Compound	Concentration (ng/g, in sample)
TARGET FIELD IN N	2,4,5-T	4.06
	Dichlorprop	2.1
Between trout rose beans	2,4,5-T	7.28
	Dicamba	4.34
Hornet Beans beds	2,4,5-T	5.6
	2,4,5-TP	0.14
	2,4-D	0.7
	Dichlorprop	1.26
CENTER OF FIELD	2,4-D	1.54
	Dichlorprop	1.12
GRASS LANE	2,4,5-T	7.42
	2,4,5-TP	0.42
	2,4-D	0.14
	Dichlorprop	0.56
40' from target	2,4-D	3.36
	Acifluorfen	6.16
	Dicamba	2.66
TARGET FIELD	2,4,5-TP	0.98
	2,4-D	3.08
	Dicamba	2.8
	Dichlorprop	2.52
	MCPP	2.8

Figure 5. Measured concentrations of some detected AcHs in the field-collected samples. 2,4,5-T and Dichloprop detected most frequently; 2,4-D detected in most vegetation samples but not in any soil samples.

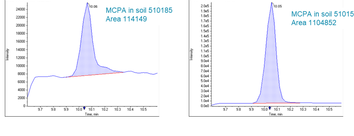

Figure 6. One soil sample (from a target field) showed a very high level of MCPA (>50ng/g in sample).

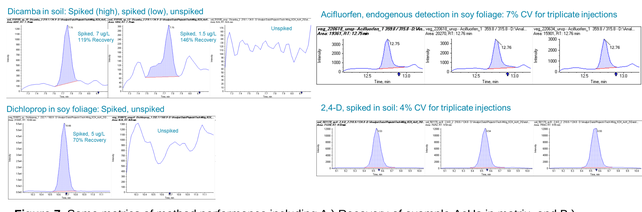

Figure 7. Some metrics of method performance including A.) Recovery of example AcHs in matrix, and B.) precision in matrix as represented by %CV of peak area, shown as both spiked replicates and background detected replicates for example AcHs.

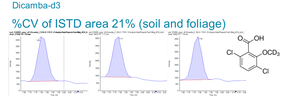

Figure 8. The reproducibility of the isotopic ISTD was demonstrated by %CV of peak area of 21%. Includes peaks measured in both soy foliage and soil matrices.

CONCLUSIONS

Quantitation was achieved to ng/L levels for many analytes in neat calibration solutions, corresponding to ng/g levels in the field samples.Isotopic internal standard of Dicamba was employed to assess recovery and maximize method performance for linearity and accuracy. Agricultural samples were analyzed to demonstrate sensitivity, recovery, and precision in complex matrices. Target field samples demonstrated highest frequency of analyte detection compared to samples collected further from fields.

REFERENCES

1 Guo *et al*., Talanta, 2015.
2 Sack *et al*., J. Ag. Food Chem. 2015.

TRADEMARKS/LICENSING

AB Sciex is doing business as SCIEX.

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