Quantitation of dicamba and acid herbicides in agricultural field samples on SCIEX QTRAP® 6500+ System

Herbicides and their metabolites in soy and soil

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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, the analysis of AcHs has been performed using a complex sample preparation procedure to derivatize the analytes followed by detection with gas chromatography and an electron capture detector. The US EPA Method 8151: CHLORINATED HERBICIDES BY GC USING METHYLATION OR PENTAFLUOROBENZYLATION DERIVATIZATION has been the most common analytical approach for these analytes. This method, however, is extremely difficult to perform correctly, is not

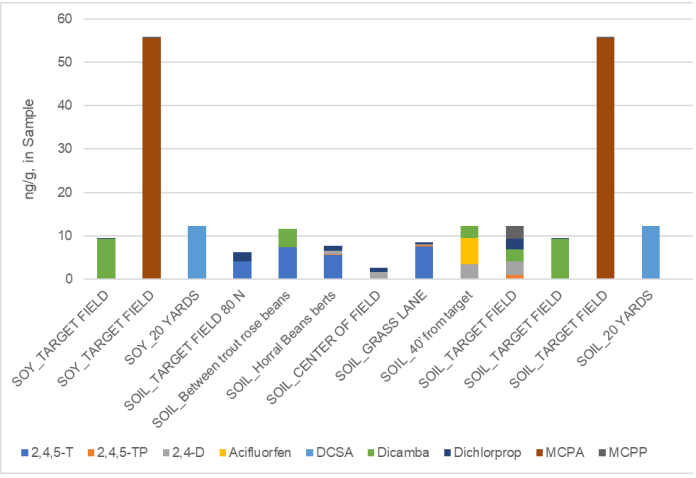

Figure 1. Quantitation of acid herbicides and metabolites in real-world samples. Measured concentrations of some detected AcHs in the field-collected soy foliage and soil samples. LOQs for the described method range from 0.1 – 140 ng/g in matrix. Samples were collected not only from targeted fields, but also from increasing distances from the field center.

rugged and is time consuming. LC-MS/MS as a replacement technology would eliminate the need for the derivatization step thus making this a more rugged analytical approach. A recent literature review of chlorophenoxy acid herbicide methods demonstrated that LC-MS/MS was the prevalent technology cited. Acidic functional groups are easiest to ionize as their conjugate base, and LC-MS/MS methods can utilize negative mode electrospray ionization (ESI−) with great sensitivity.

Key points

- Quantitation was achieved to ng/L concentrations for many analytes in neat calibration solutions, corresponding to ng/g levels in field samples.
- An isotopic internal standard, d3-Dicamba, was employed to assess recovery, precision, and robustness of the method. The ISTD peak area %CV was 21% across both soy foliage and soil matrices.
- Recoveries were generally between 70-150% and replicate precision were within %CV of 20%.
- Important dicamba metabolites 5OH-Dicamba, DCSA, and DCGA were included in the analytical method.

Experimental

Sample preparation:

5 g of soil sample or soybean foliage were collected from impacted and non-impacted agricultural field sites. Internal standard was added to the sample pre-extraction. Sample was homogenized and extracted with formic acid fortified 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, and a 17-minute gradient program (Table 2) provided chromatographic resolution for performance in complex extract matrices.

MS conditions:

A SCIEX QTRAP® 6500+ System was employed for its sensitivity and robustness. Optimized MRM transitions were selected and utilized for maximum sensitivity. Isotopically labelled target analytes were utilized as internal standards for achieving the highest quality quantitation data in complex soil and plant extracts. Table 1 details the instrument conditions utilized in this method.

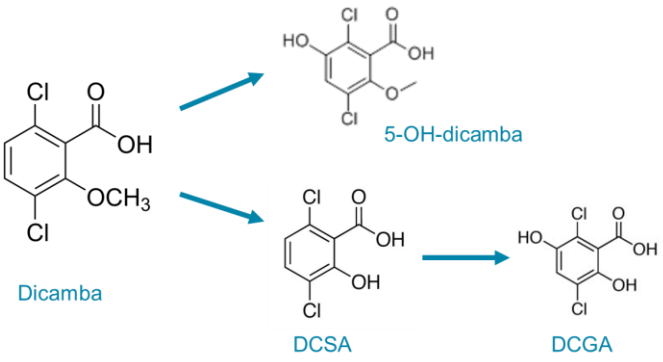

Figure 2. Major dicamba metabolites are also relevant. Metabolites of concern are 5-OH Dicamba and 3,6-dichlorosalicylic acid (DCSA). DCSA is the major degradate in the environment and is more persistent in the environment than the parent Dicamba. DCSA can further transform into DCGA.

Table 1. Ion source parameters. Electrospray Ionization (ESI) conducted in positive ion mode.

Parameter	Setting
Curtain Gas (CUR)	*20*
Collision Gas	*10*
Ion Spray voltage (IS)	*5500*
Temperature (TEM)	*650*
Nebulizer Gas (GS1)	*50*
Heater Gas (GS2)	*50*

Results

Chromatography:

The F5 stationary phase demonstrated excellent retention and resolution for these small, polar species. The LC gradient (Table 2) was utilized to maximize separation from matrix interferences. RT values were specified for each MRM transition to optimize cycle time for best peak shape and quantitation. Figure 3 shows example elution profiles.

Table 2. LC Gradient program.

Time (min)	%B
1	*40*
4	*52*
12	*85*
13.5	*90*
15.5	*90*
15.6	*2*

Quantification:

Acid herbicide LODs (Limits of Detection) were found to be mostly <1 ng/mL, with some exceptions including 5OH-dicamba, which had an LOD of 1 ng/mL. Isotopically labelled d3-Dicamba was utilized as an internal standard for all analytes. The calibration range was 0.025 - 50 µg/L (Figure 4).

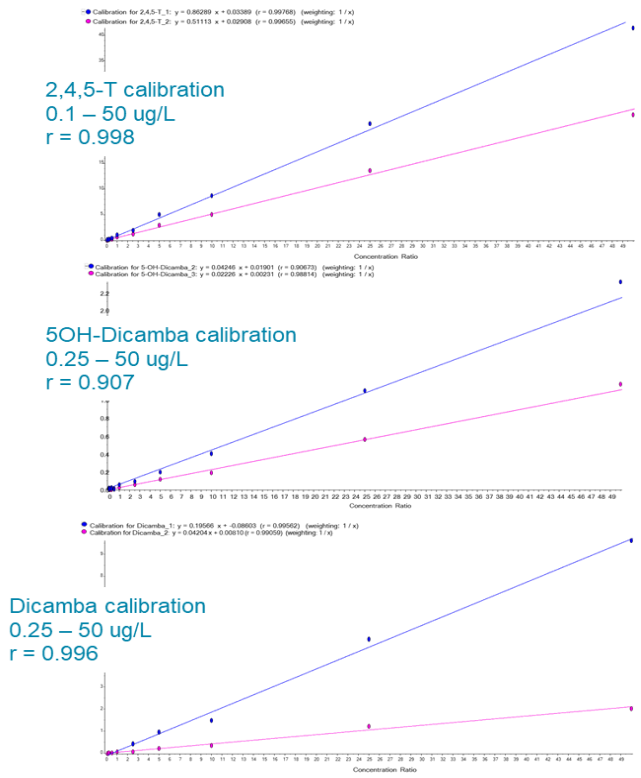

Figure 4. Quantitative calibration curves. Calibration curves of some example AcHs demonstrating sensitivity, linear response, and dynamic range.

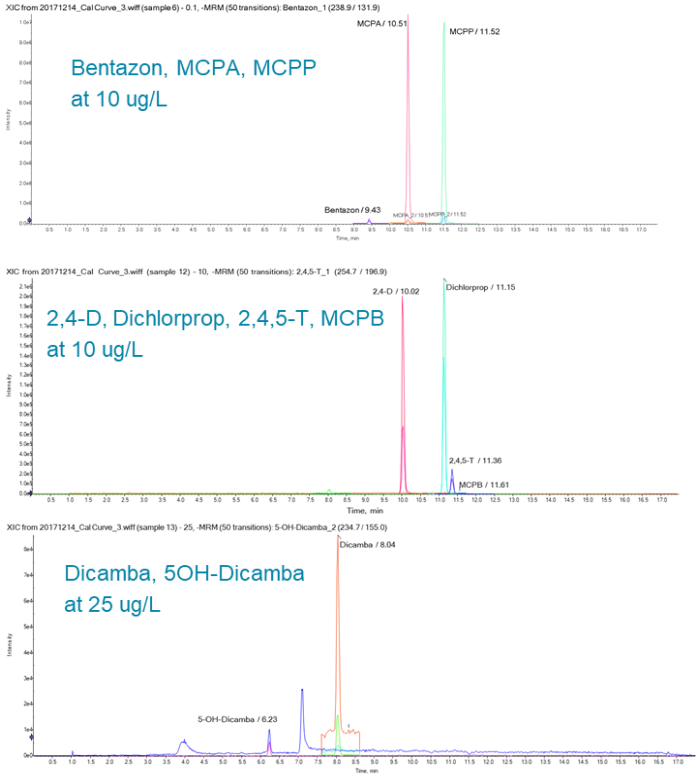

Figure 3. Chromatography. Elution profile of some example AcHs using the Kinetex F5 stationary phase.

Analytical precision, determined using triplicate injections at varying concentration levels, is shown in Table 3 and Figure 5. The reproducibility of the isotopic ISTD was 21% CV in matrix samples. This value includes peaks measured in both soy foliage and soil matrices representing excellent method reproducibility in matrix.

Table 3. Method performance. Quantitative method performance for acid herbicides and metabolites, including sensitivity and reproducibility data.

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

Data collected from field samples:

Occurrence data were collected from various spatial targets within impacted and non-impacted fields in the US Midwest. Occurrence data were 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

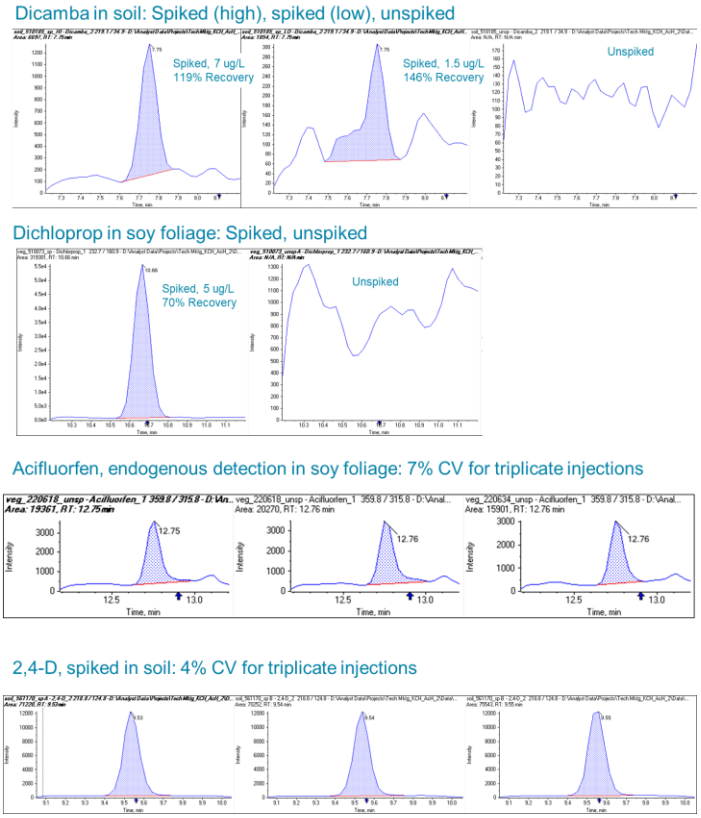

Figure 5. Spike recovery experiments. Spike recovery for dicamba and other acid herbicides in soil and soy matrices, as well as analytical precision for triplicate injections.

2,4,5-T and Dichloprop were detected most frequently; 2,4-D was detected in most vegetation samples but not in any soil samples**.** MCPA showed high concentration detected in one of each type of sample. Samples were collected not only from targeted fields, but also from increasing distances from the field center. One finding of note was the presence of the metabolite DCSA in samples 20 yards from the center, but not at the center (Figure 1).

Summary

The SCIEX QTRAP® 6500+ System was coupled with the ExionLC™ AD System and Phenomenex Kinetex F5 analytical column to attain sensitive quantitation of acid herbicides including dicamba and dicamba metabolites. Quantitation was achieved to ng/L levels for many analytes in neat calibration solutions, corresponding to ng/g levels in the field samples. Spiked and unspiked 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. Endogenous occurrence of several analytes was reported.

References

1. EPA Method 8151A. 1996.
2. Sack *et al.,* (2015) Determination of Acid Herbicides Using Modified QuEChERS with Fast Switching ESI+/ESI– LC-MS/MS. *J. Ag. Food Chem.* **63 (43**), 9657–9665.

Acknowledgments

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