The Detection and Quantification of Dicamba and Acid Herbicides in Agricultural Samples

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Highly sensitive detection of a suite of historically challenging acid herbicides in complex environmental (soil) and agricultural matrices address increased demand for method performance and instrument sensitivity. Quantitative determination of dicamba, 2,4-D, 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 derivatisation is demonstrated.

Introduction

In the world of farming, weeds are a problem. If left untreated, the overgrowth can reduce yields. Farmers and growers have turned to modern synthetic herbicides such as glyphosate for help. Recent industry news has highlighted that the extensive and consistent use of glyphosate raised health concerns and has even spawned glyphosate-resistant weeds [1].

Because of the glyphosate-resistant weeds, farmers and growers are using alternative acid herbicides (AcHs), such as the well-characterised 2,4-dichlorophenoxyacetic acid (2,4-D), dicamba, triclopyr and others, to help destroy the persistent threat [2]. However, like with glyphosate, health concerns have arisen [3].

The problem with dicamba is that it does not always stay where it is applied. The herbicide can migrate via spray drift from the fields where it is intentionally applied, to nearby fields and farms, damaging crops that have not been engineered with dicamba-tolerant genes. Research by the University of Missouri Extension, USA found that the migration has widely damaged non-resistant crops and other plants [4].

Despite complaints and concerns about drift across plots during spray application, the United States (US) Environmental Protection Agency (EPA) ruled in favor of the continued use of dicamba [5]. The EPA introduced regulations around application patterns to help control migration - things like the time of day you are allowed to apply and the conditions to avoid, such as high winds. Nevertheless, AcHs remain a prevalent concern in environmental monitoring and crop contamination analysis.

One of the most common analytical approaches for AcHs is the EPA Method 8151: Chlorinated Herbicides by GC Using Methylation or Pentafluorobenzylation Derivatization [6]. However, the sample derivatisation process can be time-consuming and costly. This limitation is driving the need for an alternative method to quantify AcHs and metabolites at low levels in relevant environmental matrices, using liquid chromatography tandem mass spectrometry (LC-MS/MS) methods.

Understanding the Metabolites

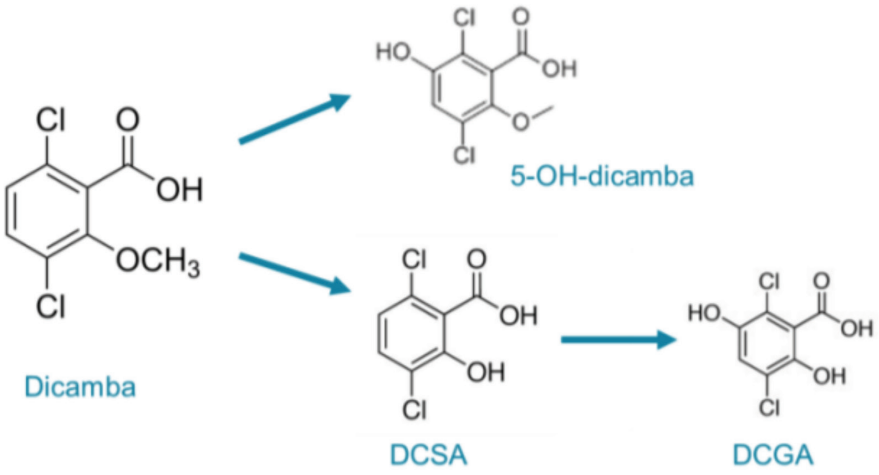

Figure 1: Major dicamba metabolites are important to monitor. Metabolites of concern are 5 OH dicamba and 3,6-dichlorosalicylic acid (DCSA). DCSA is the major degradant in the environment and is more persistent in the environment than the parent dicamba. DCSA can further transform into DCGA.

In this study, we have identified three primary dicamba metabolites that are generated in the environment.

- 5-OH-dicamba
- 3,6-dichlorosalicylic acid (DCSA) which demonstrates a higher persistence in the environment than the original parent dicamba
- A secondary metabolite of DCSA, DCGA (3,6-dichlorogentisic acid)

Experimental

GC-MS/MS has historically enabled multiple pesticide residues to be screened, but LC-MS/MS represents an ideal replacement

technology for analysing AcHs and their metabolites. It eliminates the need for derivatisation and can achieve greater sensitivity. A recent literature review of chlorophenoxy acid herbicide methods, have also shown LC-MS/MS to be a more rugged analytical approach [7-8].

Acidic functional groups are easy to ionise as their conjugate base. While there are several new AcHs that are more easily ionised by ESI positive modes, this method mainly focuses on the legacy AcHs that require negative mode electrospray ionisation (ESI−) for high sensitivity.

Recent work [9] presented an LC-MS/MS analysis to quantify dicamba, related AcHs and the metabolite compounds mentioned in real-world agricultural samples - water, soil, and soy plant tissue (foliage) samples. These samples were collected from fields either targeted by or close to AcH application in the US Midwest. Samples evaluated included:

- 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

The goal was to demonstrate the sensitivity and recovery of the compounds in complex matrices. The application note presents quantification method performance data showing ng/g levels in samples for many of these analytes. The dicamba isotopic internal standard, d3-dicamba, was employed to assess and optimise recovery of method performance for both linearity and accuracy.

A Phenomenex Kinetex® F5 column (2.6 µm, 100 x 3 mm) was employed for these highly polar, low molecular weight species. This is a unique stationary phase that is novel for food and environmental analysis. The electronegativity of the fluorines means that it works well for inherently charged negative mode compounds. Strong dipole-dipole and ion-dipole interactions are the suspected interaction mechanisms. Some of these carry an acidic charge, are small and thus poorly retained on a more traditional stationary phase like a C18. The F5 column does a good job of retaining very low molecular weight, negatively charged species. A flow rate of 0.500 mL/min and a 17-minute gradient program (Table 1) provided chromatographic resolution for performance in complex extract matrices.

Table 1: LC gradient time program.

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

We defined the known retention time values in the acquisition method, in order to optimise the cycle time for best peak shape for quantification performance. Figure 2 shows example elution profiles. Mobile phases are water and methanol with 0.1% formic acid.

For the MS, we used the SCIEX QTRAP® 6500+ LC-MS/MS System for its sensitivity and robustness. The Turbo V™ Ion Source operated in negative mode electrospray ionisation (ESI) was used for optimal ionisation of acidic species. The multiple reaction monitoring (MRM) experiment monitored two transitions for each analyte and we optimised compound-specific voltages designated for maximum sensitivity.

Results

We found that AcH limits of detection (LODs) were largely less than <1 ng/mL, with some exceptions, including 5-OH-dicamba, which had poor sensitivity in comparison to some other AcH compounds. To optimise the method performance for linearity and accuracy, we utilised the isotopically labeled d3-dicamba as the internal standard (ISTD) for all analytes. Data was processed using the SCIEX MultiQuant™ Software.

Table 2 shows LOD and LOQ values for the compound IDs we analysed. You can see 2,4-D, dicamba, dichlorprop and new metabolites DCGA, DCSA, MCPA, MCPB and MCPP. Also note the low limits of quantification (LOQ) for these historically challenging compounds. The highest LOQs are for DCGA and 2-4-DB, which are at 10 ng/mL.

The signal-to-noise ratio at 1 ppb, demonstrates excellent sensitivity. 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 (Figure 3).

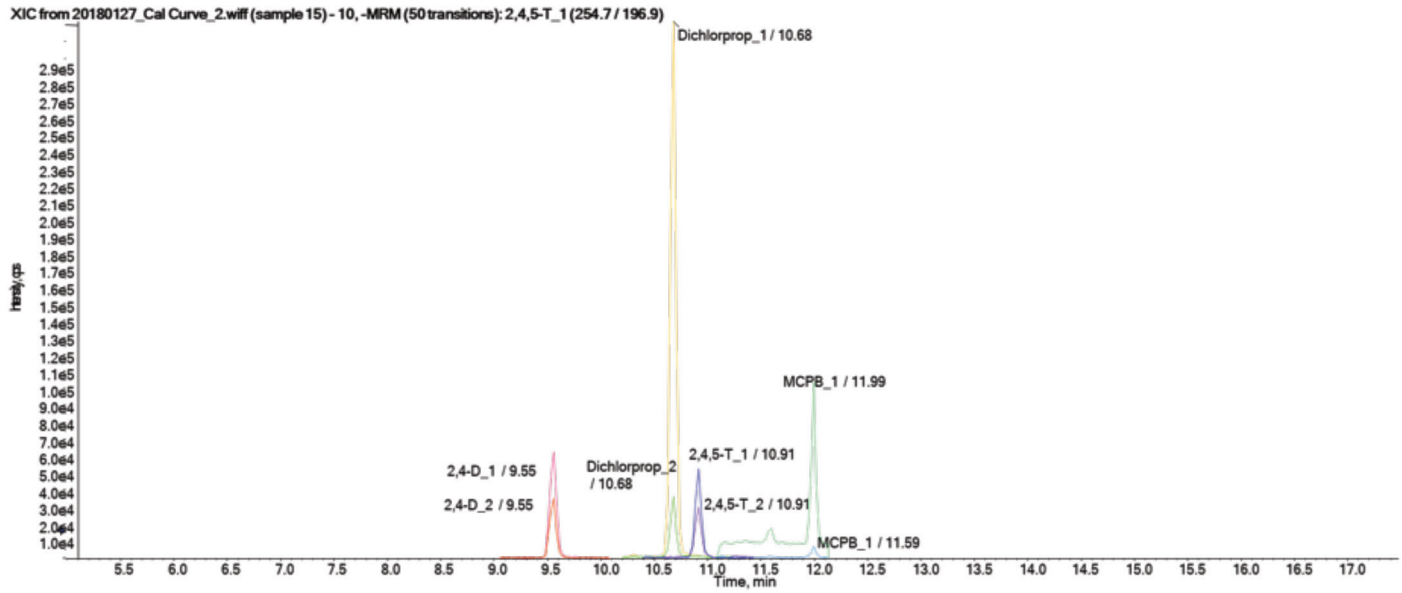

Figure 2: Elution profile of some example AcHs using the Kinetex F5 stationary phase.

Table 2. Data analysis and quantification method performance evaluation for AcHs 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

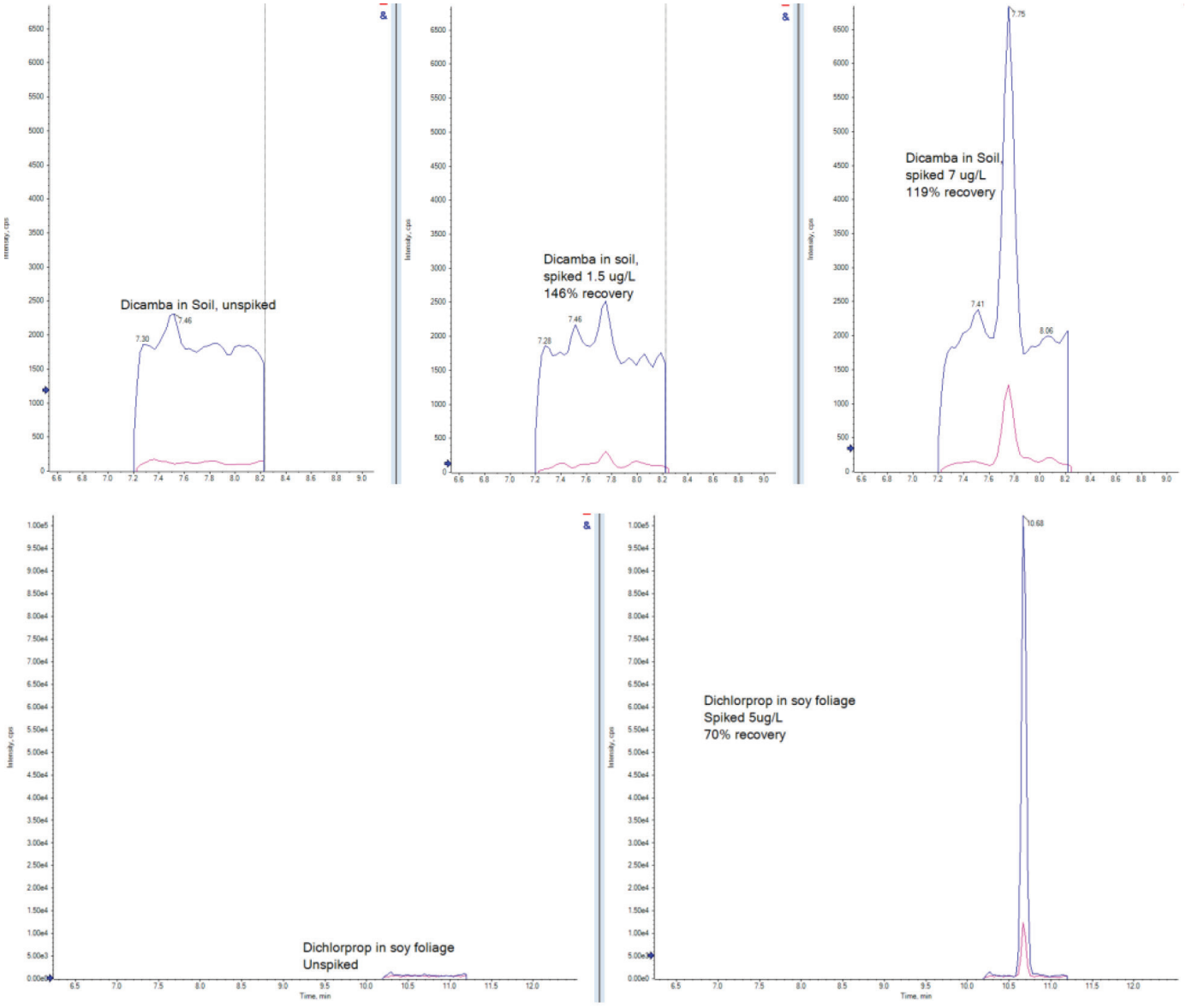

Figure 3:

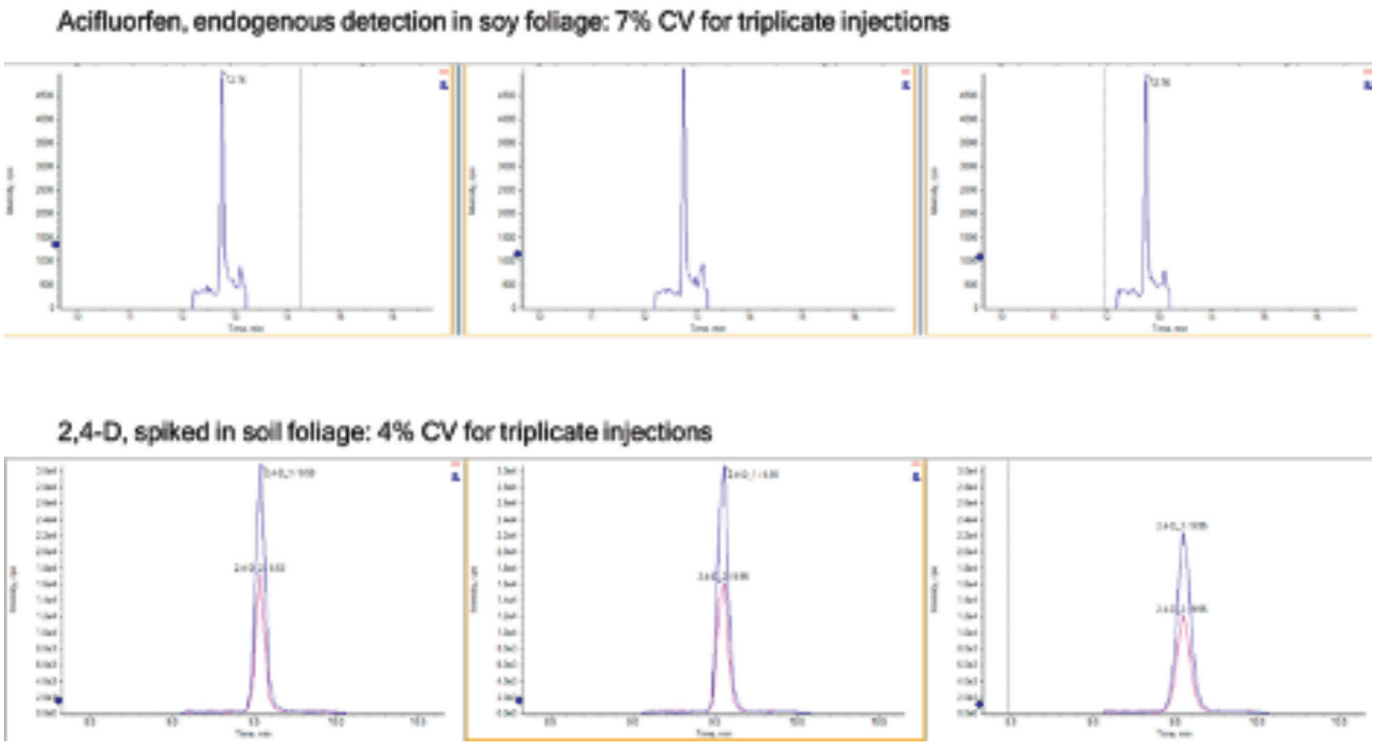

Figure 3: Spike recovery for dicamba and other AcHs in soil and soy matrices, as well as analytical precision for triplicate injections.

Conclusion

Environmental monitoring and crop contamination are becoming more critical, especially as AcH usage becomes more widespread in the agriculture industry. This application demonstrates a sensitive and efficient quantification method for AcHs, including dicamba and its metabolites, in a unique set of field samples analysed on the SCIEX QTRAP 6500+ System with the ExionLC™ AD System and Phenomenex Kinetex F5 analytical column.

References

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