Quantitation of Acid Herbicides in Agricultural Field Samples

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Acid Herbicides (AcHs)

- Widespread global use as weed control agents and plant growth regulators for agricultural crops, lawns, and gardens
- Active ingredients in AcH products account for more use than all the other types of pesticides combined
  - 2,4-dichlorophenoxyacetic acid (2,4-D), dicamba, triclopyr, and other AcHs
- EPA recently ruled in favor of continued use of dicamba
  - Despite complaints and concern about drift across plots during spray application
  - Adjustments in regulation around application patterns
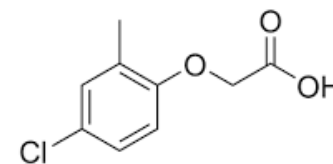
FOOD FOR THOUGHT

With OK From EPA, Use Of Controversial Weedkiller Is Expected To Double

October 13, 2017 · 5:14 PM ET

DAN CHARLES

Dicamba

MCPA

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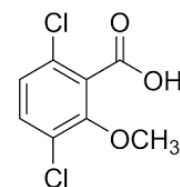
Acid Herbicides Analysis

- Historically determined by gas chromatography with element selective detectors such as electron capture

- EPA Method 8151: CHLORINATED HERBICIDES BY GC USING METHYLATION OR PENTAFLUOROBENZYLATION DERIVATIZATION
  - Derivatization to form volatile species required
  - Derivatization + GC widely being considered more inefficient and unreliable

- LC-MS/MS as a GC replacement technology, eliminating the need for the derivatization step
  - In a literature review of chlorophenoxy acid herbicide methods, LC-MS/MS was the prevalent technology cited

- LC-MS/MS methods use predominately negative mode electrospray ionization (ESI−)
  - Acidic functional groups easiest to ionize as their conjugate base

Metabolites

- Major dicamba metabolites also relevant
  - 5OH-dicamba, DCSA (DCGA)
  - Environmental persistence

Dicamba

5-OH-dicamba

DCSA

DCGA
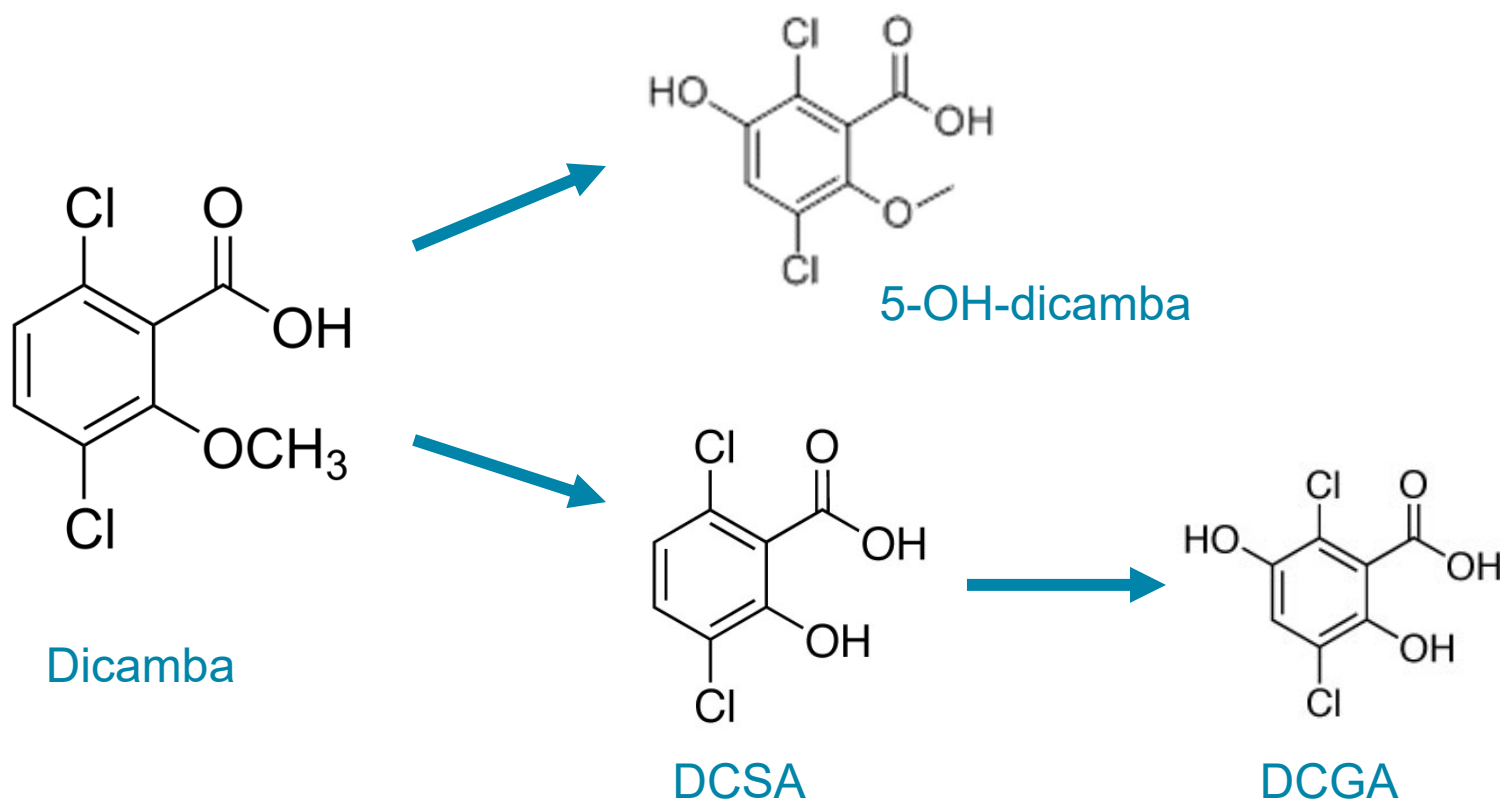
Overview

Key Points Demonstrated

- Methods and data are presented which demonstrate LC-MS/MS for accurate and sensitive quantitation of dicamba, acid herbicides, and some metabolites in water, soil, and soy foliage samples.

- Quantitation was achieved to ng/g levels (in sample) for many analytes, and 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

Analytical Conditions

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
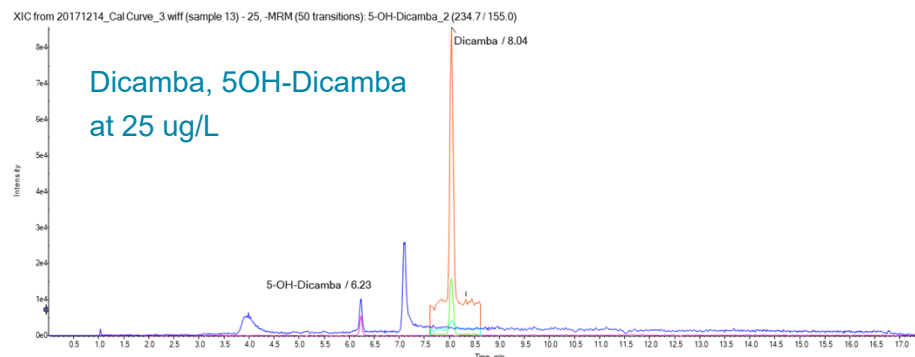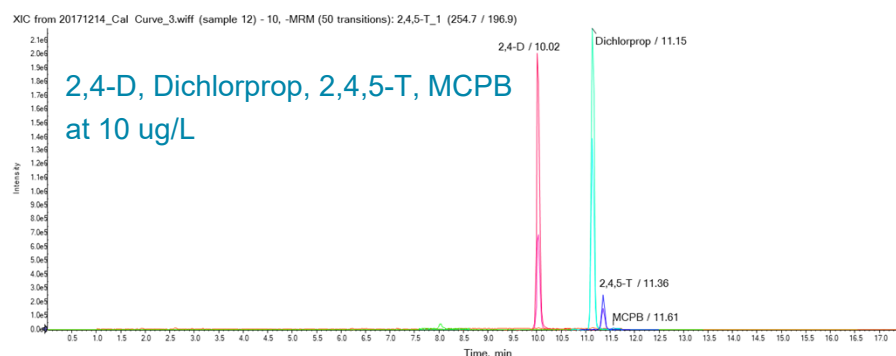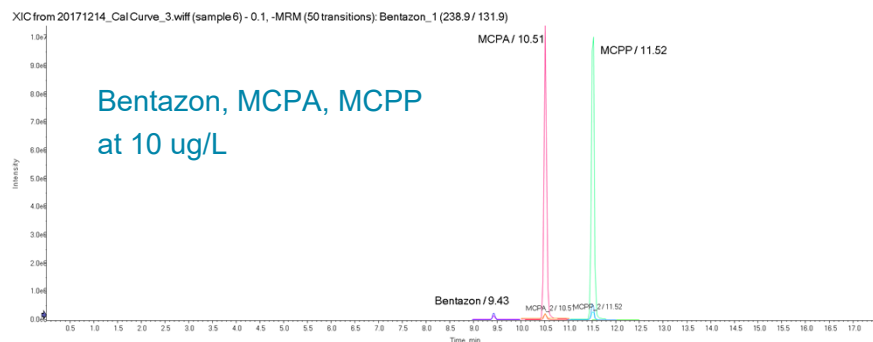- Run time was 17 minutes
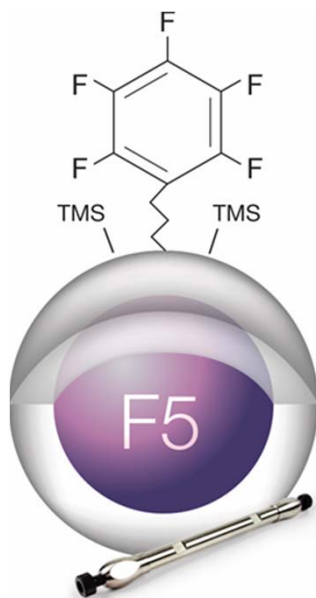
MS Conditions

- 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

Chromatography

Bentazon, MCPA, MCPP
at 10 ug/L

2,4-D, Dichlorprop, 2,4,5-T, MCPB
at 10 ug/L

Dicamba, 5OH-Dicamba
at 25 ug/L

- F5 stationary phase demonstrated excellent retention and resolution for these small, polar species

- 17 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

- Mobile phases are water and methanol with 0.1% formic acid

- Gradient program:

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

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Data Processing

- Acid herbicide LODs mostly <1 ng/mL range, with some exceptions including 5OH-dicamba
  - d3-Dicamba ISTD
  - Occurrence data collected for soils collected from various spatial targets
  - Occurrence data collected for soy plant tissue

- Data processing performed using MultiQuant software

- d3-Dicamba used as ISTD

- Signal to noise evaluated Peak-to-Peak with 1 point smooth

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Data Analysis and Method Performance Evaluation

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

Calibration regression and linear response

- Calibration curves over concentration levels from 0.025 - 50 µg/L

- Calibrators made as mixtures in solvent

- Linear regression models with 1/x weighting to fit all calibration curves

- LOD was defined as the lowest calibrator S/N ≥3

- LOQ was defined as the lowest calibrator with S/N ≥10

Calibration for 2,4,5-T_1: y = 0.86289 x + 0.03389 (r = 0.99768) (weighting: 1 / x)
Calibration for 2,4,5-T_2: y = 0.51113 x + 0.02908 (r = 0.99655) (weighting: 1 / x)

2,4,5-T calibration
0.1 – 50 ug/L
r = 0.998
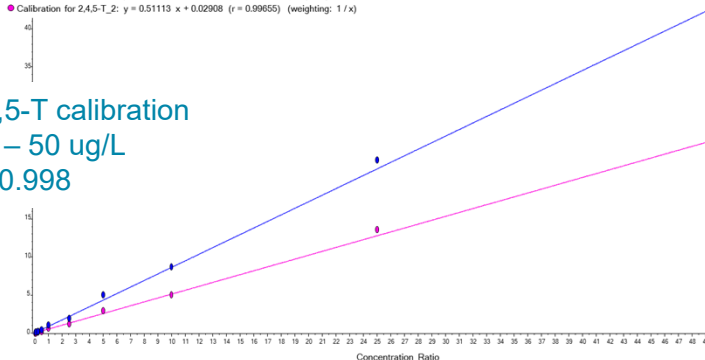
Calibration for 5-OH-Dicamba_2: y = 0.04246 x + 0.01901 (r = 0.90673) (weighting: 1 / x)
Calibration for 5-OH-Dicamba_3: y = 0.02226 x + 0.00231 (r = 0.98814) (weighting: 1 / x)

5OH-Dicamba calibration
0.25 – 50 ug/L
r = 0.907
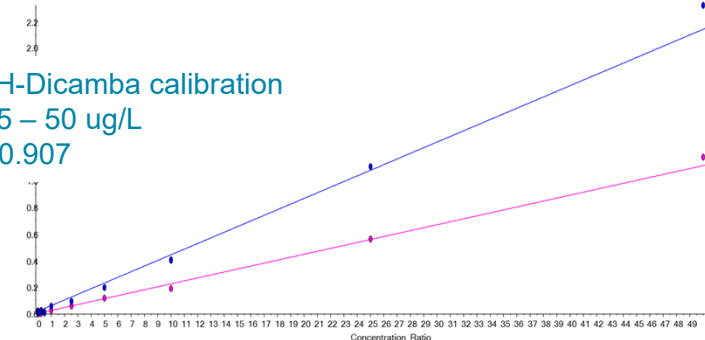
Calibration for Dicamba_1: y = 0.19566 x + -0.08603 (r = 0.99562) (weighting: 1 / x)
Calibration for Dicamba_2: y = 0.04204 x + 0.00810 (r = 0.99059) (weighting: 1 / x)

Dicamba calibration
0.25 – 50 ug/L
r = 0.996

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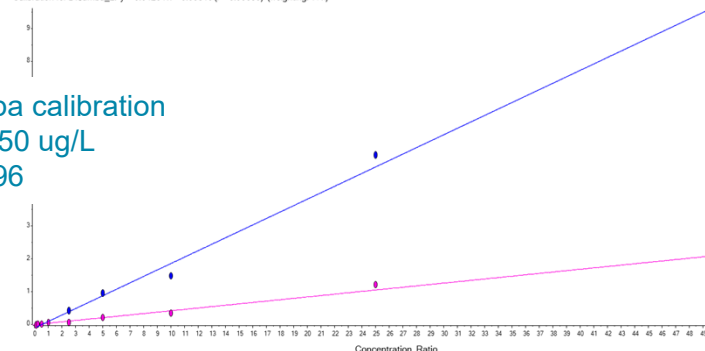
Agricultural Samples

- Soil samples and soy foliage collected from impacted fields in the US Midwest

- Sampling locations varied about the field site

- "Foliage" vs. a human consumed food product

- 7 soy foliage samples
  - Target field, planted rows, and increasing distances from field

- 5 soil samples
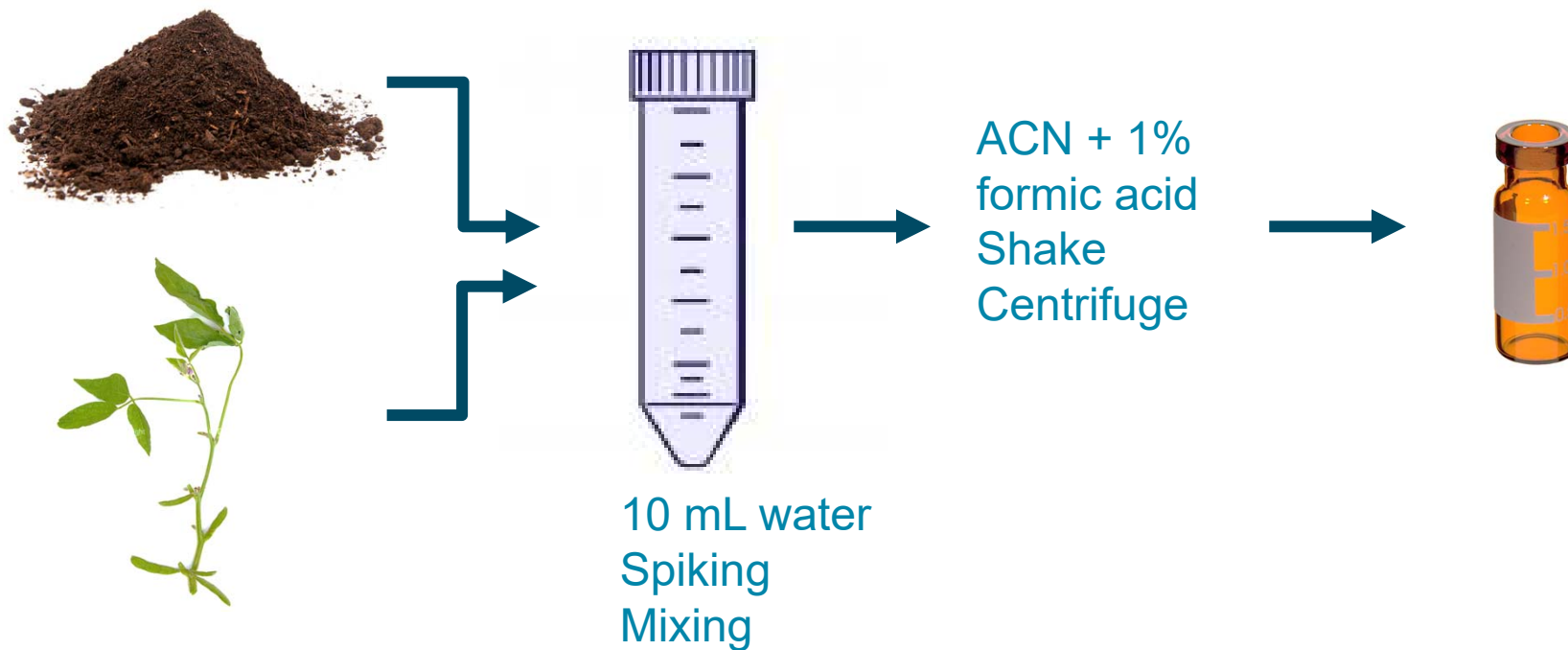 - 3 from target field, and 2 from increasing distances

Sample Preparation

- Acidified acetonitrile extraction without cleanup

- Addition of water

10 mL water
Spiking
Mixing

ACN + 1%
formic acid
Shake
Centrifuge

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Measurements in Matrix

Concentrations detected in:

Soil

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

Soy foliage

Sample	Compound	Concentration (ng/g, in sample)
TARGET FIELD 80 N	2,4,5-T	4.06
TARGET FIELD 80 N	Dichlorprop	2.1
Between trout rose beans	2,4,5-T	7.28
Between trout rose beans	Dicamba	4.34
Horral Beans berts	2,4,5-T	5.6
Horral Beans berts	2,4,5-TP	0.14
Horral Beans berts	2,4-D	0.7
Horral Beans berts	Dichlorprop	1.26
CENTER OF FIELD	2,4-D	1.54
CENTER OF FIELD	Dichlorprop	1.12

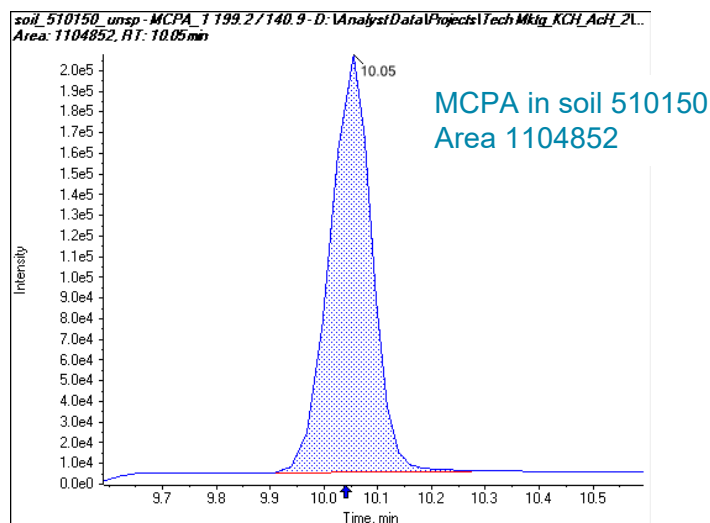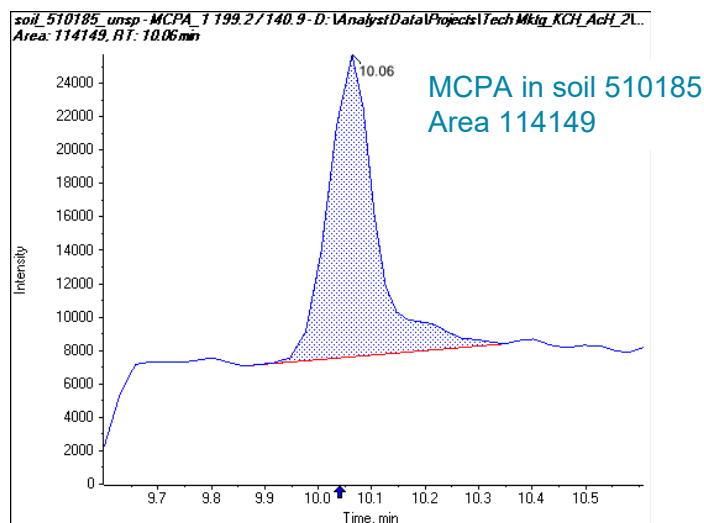| Sample	Compound	Concentration (ng/g, in sample)
GRASS LANE	2,4,5-T	7.42
GRASS LANE	2,4,5-TP	0.42
GRASS LANE	2,4-D	0.14
GRASS LANE	Dichlorprop	0.56
40' from target	2,4-D	3.36
40' from target	Acifluorfen	6.16
40' from target	Dicamba	2.66
TARGET FIELD	2,4,5-TP	0.98
TARGET FIELD	2,4-D	3.08
TARGET FIELD	Dicamba	2.8
TARGET FIELD	Dichlorprop	2.52
TARGET FIELD	MCPP	2.8

Measurements in Matrix
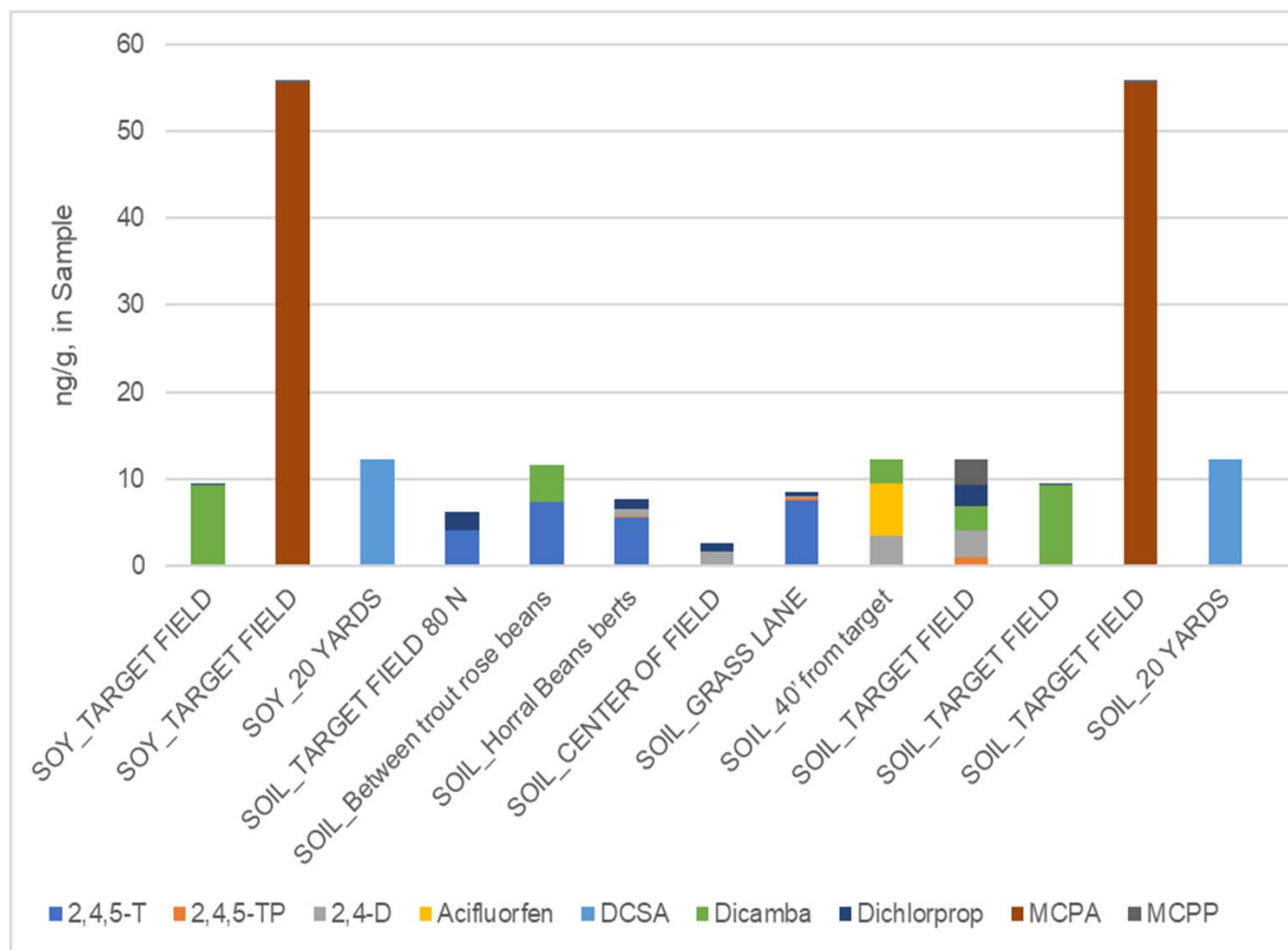
Measurements in Matrix

"Real – world" agricultural samples acquired for analysis

- 2,4,5-T and Dichloprop detected most frequently
  – In most vegetation samples and one soil sample

- 2,4-D detected in most vegetation samples but not in any soil samples

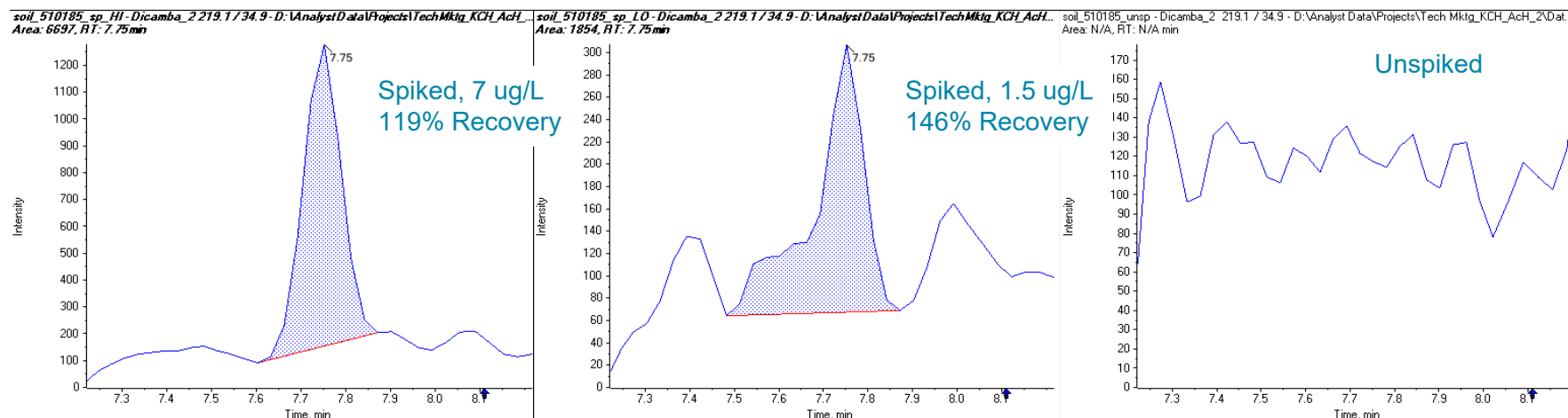- One soil sample (from a target field) showed a very high level of MCPA (>50ng/g in sample)
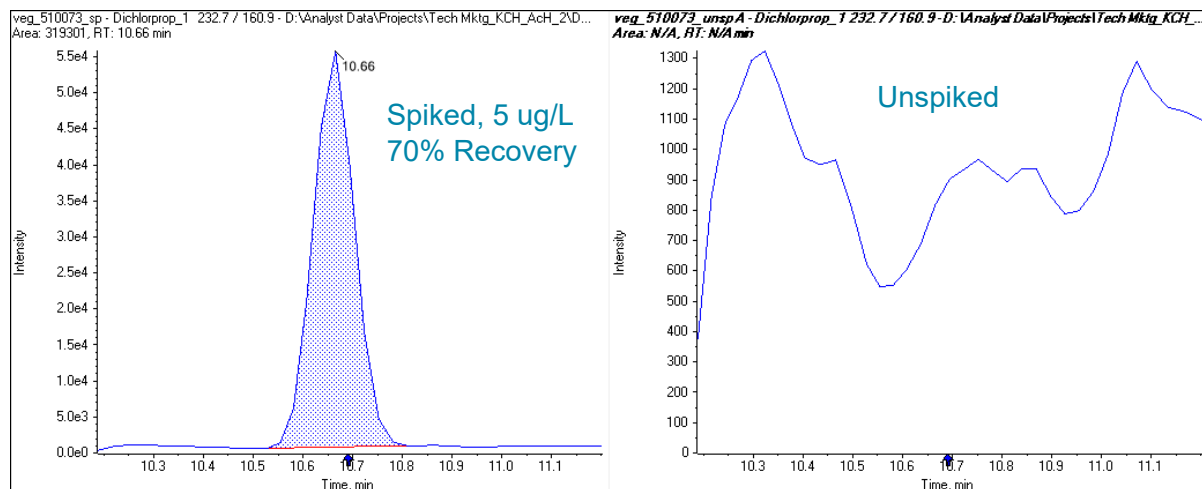

MCPA in soil 510185
Area 114149

MCPA in soil 510150
Area 1104852

Recovery in Matrix

Dicamba in soil: Spiked (high), spiked (low), unspiked

soil_510185_sp_HI - Dicamba_2 219.1 / 34.9 - D:\Analyst Data\Projects\Tech Mktg_KCH_AcH...
Area: 6697, RT: 7.75 min

Spiked, 7 ug/L
119% Recovery

soil_510185_sp_LO - Dicamba_2 219.1 / 34.9 - D:\Analyst Data\Projects\Tech Mktg_KCH_AcH...
Area: 1854, RT: 7.75 min

Spiked, 1.5 ug/L
146% Recovery

soil_510185_unsp - Dicamba_2 219.1 / 34.9 - D:\Analyst Data\Projects\Tech Mktg_KCH_AcH_2\Dat...
Area: N/A, RT: N/A min

Unspiked

Dichloprop in soy foliage: Spiked, unspiked

veg_510073_sp - Dichlorprop_1 232.7 / 160.9 - D:\Analyst Data\Projects\Tech Mktg_KCH_AcH_2\D...
Area: 319301, RT: 10.66 min

Spiked, 5 ug/L
70% Recovery

veg_510073_unsp A - Dichlorprop_1 232.7 / 160.9 - D:\Analyst Data\Projects\Tech Mktg_KCH_...
Area: N/A, RT: N/A min

Unspiked

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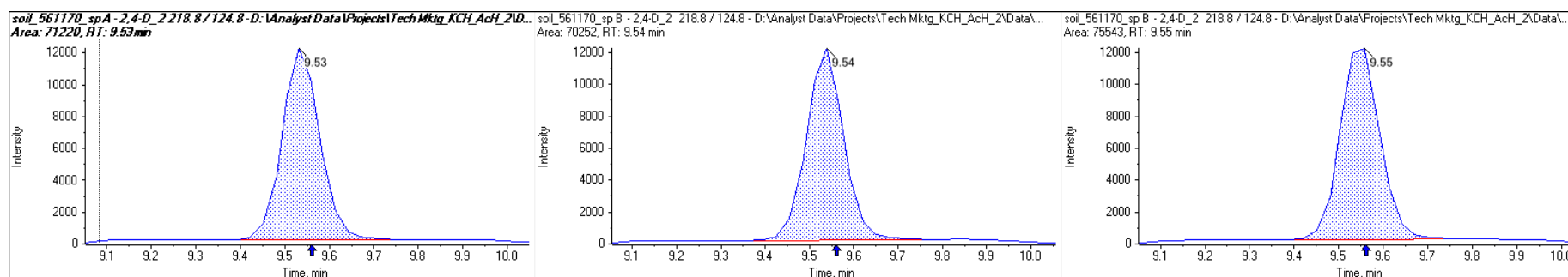
Precision in Matrix

Acifluorfen, endogenous detection in soy foliage: 7% CV for triplicate injections
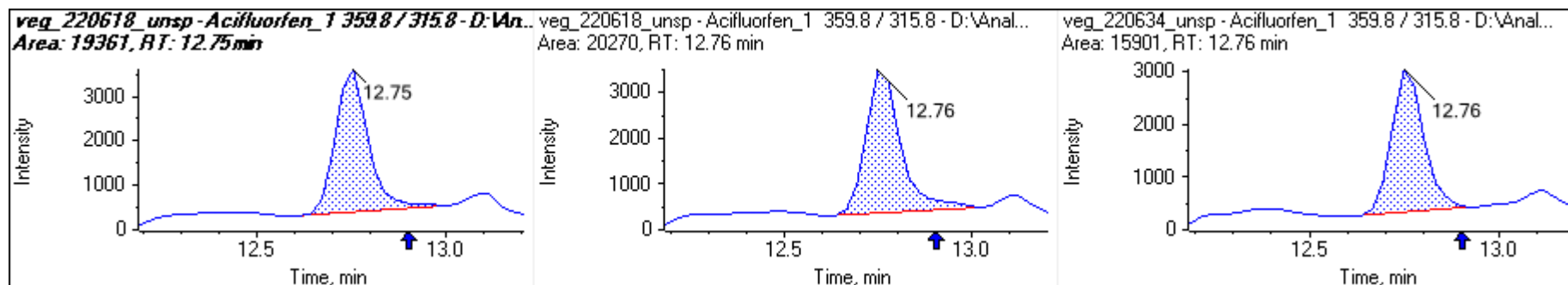
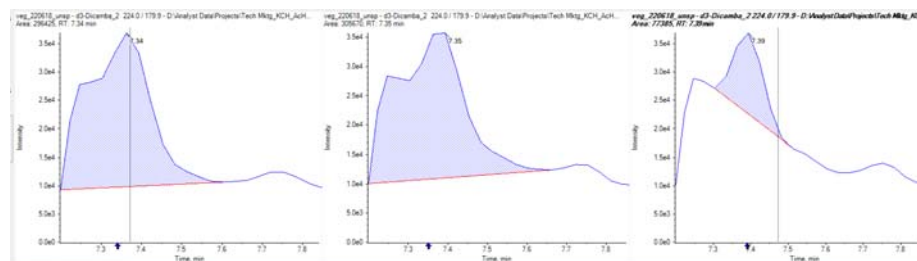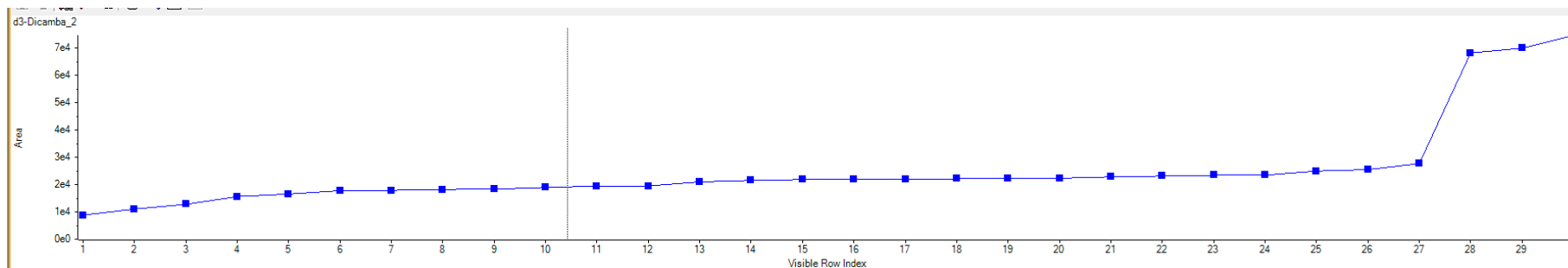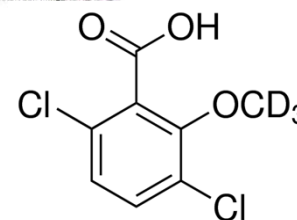

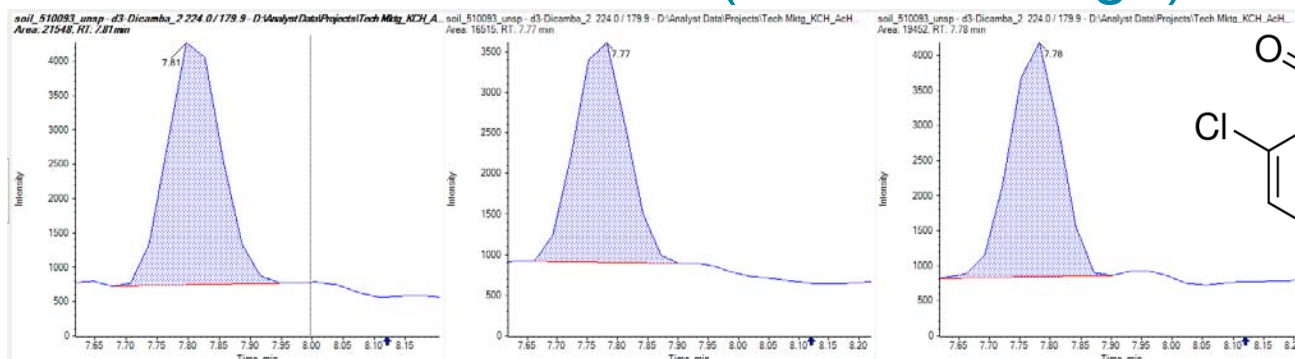2,4-D, spiked in soil: 4% CV for triplicate injections

ISTD Robustness

Dicamba-d3

%CV of ISTD area 21% (soil and foliage)

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Summary

- 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

Quantitative analysis of dicamba residues in raw agricultural commodities with the use of ion-pairing reagents in LC–ESI–MS/MS

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[b] University of Texas at Arlington, Arlington, TX, United States

ARTICLE INFO

ABSTRACT

A sensitive and selective HPLC–MS/MS method was developed for the quantitative analysis of dicamba residues in raw agricultural commodities (RACs). Instead of analysis in the traditionally used negative electrospray ionization (ESI) mode, these anionic compounds were detected in positive ESI with the use of ion-pairing reagents. In this approach, only a small amount (60 μM) of a commercially available dicationic ion-pairing reagent was introduced into the post-column sample stream. This method has been validated in six different types of RACs including corn grain, corn stover, cotton seed, soybean, soy forage and orange with satisfactory quantitative accuracy and precision. The limits of quantitation (LOQ) values for these analytes were 1.0 to 3.0 μg/kg. The standard curves were linear over the range of the tested concentrations (3.0 to 500 μg/kg), with correlation coefficient (r) values ≥ 0.999. Evaluation of ionization effects in RAC matrix extracts using diluent blanks for comparison showed no significant matrix effects were present.

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1. Introduction

Dicamba (3,6-dichloro-2methoxybenzoic acid), a systematic broad-spectrum auxin-type herbicide, has been used for efficient control of most broadleaf weeds in a variety of crops for more than 40 years [1]. Due to the presence of heterogeneous crop matrix components (i.e., sugar, carbohydrate, starch, macromolecule, pigment, fat and structurally similar compounds), analysis of dicamba residues in RACs can be an extremely challenging task [2]. The polar nature and high water solubility of dicamba residues make their selective extraction and chromatographic resolution from these potentially interfering components very difficult. The diversity of various RAC types and composition further complicates the extraction as each matrix can have unique properties and interfering compounds. Established methods for dicamba residue analysis are based on gas chromatography coupled with electron capture detection (GC-ECD) as adopted by Environmental Protection Agency (EPA) in 1993 [3]. These methods often require an additional sample derivatization step, which at the low concentrations normally has several limitations and often results in irreproducible yields, multiple impurities and an increased analysis time [4]. A variety of other analytical methods also have been developed for the analysis of dicamba residues, including GC–MS [5], enzyme-linked immunosorbent assay [6], micellar electrokinetic capillary chromatography (MEKC) [7], capillary liquid chromatography with UV detection [8], and HPLC coupled with UV [9] or MS detection [2, 10–12]. These methods generally suffered

from low sensitivity, which limits their utility for trace residue analysis.

In recent years, HPLC–MS equipped with electrospray ionization (ESI) interface has become the preferred platform for the simultaneous analysis of pesticide residues without derivatization, due to advantages of improved throughput, selectivity, and sensitivity [13]. Generally, applying tandem MS instrumentation (MS/MS) adds further selectivity to the MS detection of compounds in complex RACs. However, analytes with low molecular masses and relatively high polarities pose a general problem to LC–MS/MS sensitivity and selectivity when monitored in the conventionally used negative ESI [14]. These analytes often possess poor ionization efficiency. Impacts on MS sensitivity from often abundant background noise in the low-mass range present additional challenges for low mass dicamba residues. To minimize these factors, we proposed a novel approach with the use of ion-pairing reagent for the sensitive and selective analysis of dicamba residues in RACs. Briefly, it involves the use of specially designed and structurally optimized ion-pairing reagents to pair post-column with the negatively charged analyte [15,16]. The subsequently formed positively charged complexes can be detected and quantified in positive ion mode (see Fig. 1). This technique has several advantages over the routinely used HPLC–MS/MS with negative ESI methods. It moves the detection of analyte from a low m/z region, where the background noise is high, to a higher and more selective m/z region where the background noise is low. Further, the ionization efficiency of the paired analyte is enhanced as shown in

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Determination of Acid Herbicides Using Modified QuEChERS with Fast Switching ESI+/ESI− LC-MS/MS

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ABSTRACT: A method for the determination of 35 acid herbicides in food matrices was developed, validated, and implemented. It utilizes a modified QuEChERS extraction procedure coupled with quantitation by liquid chromatography tandem mass spectrometry (LC-MS/MS). The acid herbicides analyzed are all organic carboxylic acids, including the older chlorophenoxy acid herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D), dicamba, 4-chlorophenoxyacetic acid (4-CPA), quinclorac, and many of the newer imidazolinone herbicides such as imazethapyr and imazaquin. In the procedure, 10 mL of water is added to 5 g of sample and then extracted with 1% formic acid in acetonitrile for 1 min. The acetonitrile is salted out of the extract by adding sodium chloride and magnesium sulfate, followed by centrifugation. The acetonitrile is diluted 1:1 with water to enable quantitation by LC-MS/MS using fast switching between positive and negative electrospray ionization modes. The average recoveries for all the compounds except aminocyclopyrachlor were 95% with a precision of 8%. The method detection limits for all residues were less than 10 ng/g, and the correlation coefficients for the calibration curves was greater than 0.99 for all but two compounds tested. The method was used successfully for the quantitation of acid herbicides in the FDA's total diet study. The procedure proved to be accurate, precise, linear, sensitive, and rugged.

KEYWORDS: acid herbicides, imidazolinone herbicides, chlorophenoxy acid herbicides, QuEChERS, LC-MS/MS

■ INTRODUCTION

Herbicides are widely used in the USA and around the world for weed control and as plant growth regulators for agricultural crops, lawns, and gardens. Active ingredients in herbicides account for more than all the other types of pesticides combined, being over 60% of the U.S. sales in 2007.[1] Fourteen of the top 25 most commonly used pesticides in the US in 2007 are herbicides, and included among them is the acid herbicide (AcH) 2,4-dichlorophenoxyacetic acid (2,4-D). Seven of the top 10 active ingredients used in the home and garden sector are herbicides; five are AcHs including mecoprop, dicamba, triclopyr, pelargonic acid, and 2,4-D, the most commonly used pesticide in the nonagricultural sectors.

Historically, the AcHs were determined by gas chromatography with element selective detectors such as electron capture. Because they are not volatile, they were derivatized to form volatile esters.[2–4] Although sensitive, these methods were inefficient and unreliable. Quantitation by liquid chromatography tandem mass spectrometry (LC-MS/MS) is quickly replacing the GC technology, eliminating the need for the derivitization step. In a recent review of chlorophenoxy acid herbicide methods, LC-MS/MS was the predominant technology cited.[5]

LC-MS/MS methods for the analysis of the older and smaller phenoxy acid herbicides such as 2,4-D, 2,4,5-trichlorophenoxy-acetic acid (2,4,5-T), and dicamba used predominately negative electrospray ionization (ESI−) because the acid herbicides are most easily ionized as their conjugate base.[6–16] However, the newer AcHs such as quinclorac, quizalofop, fluoroxypyr, and the imidazolinones, although easily ionized using ESI−, actually produce transitional ions that are detectable by LC-MS/MS at

the newer AcHs use ESI+ or incorporate both ESI− and ESI+ in separate analytical segments.[17–25]

Recent advances in technology have significantly increased the speed at which LC-MS/MS instruments can switch between ESI+ and ESI−. This enables AcHs and other pesticides to be determined by both ionization modes in a single analytical determination.[26–28]

Several variations of the QuEChERS method[29] have been investigated and evaluated for the extraction and cleanup of various AcHs from complex matrices such as foods, feeds, and waste.[10,12–14,16,17,19,22,27,30–36] Attempts to extract the acid herbicides with nonacidic acetonitrile used in the original QuEChERS method and CEN 15662[29,37] have generally resulted in lower recoveries due to the ionic nature of the acids at pH above 5.[10,12,17,22,30–32,34] QuEChERS extraction using acidified acetonitrile[10,16,17,22,35,38] performed better, although the buffered approach described in AOAC 2007.01 has generally resulted in lower, but erratic recoveries.[38]

Investigators have explored SPE and dispersive SPE (dSPE) cleanup procedures of nonbuffered acidified acetonitrile QuEChERS extracts with mixed success.[10,13,14,27,36] Sorbents investigated include graphitized carbon black (GCB), primary secondary amine (PSA), C18, and alumina. In each case, the cleanup resulted in acceptable recoveries for some acid herbicides and/or erratic recoveries for others. One investigator that studied matrix effects of extracts after multiple cleanup procedures found that cleanup procedures generally did little to reduce matrix effects.[34] Others have described a method for

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