Determination of Chlorinated Acid Herbicides in Soil by LC/MS/MS

Application Note

Environmental

Author

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Abstract

Chlorinated acid herbicides were analyzed at the picogram level on column without any derivatization using liquid chromatography and tandem mass spectrometry (LC/MS/MS). Good linearity was observed for all the selected analytes, from low pg to low ng levels on column.

Introduction

Chlorinated acid herbicides are a popular class of broad-leaf weed killer in lawn and grain crops. Due to their widespread use, environmental contamination in water and soil from run-off is a serious concern.

Traditional analytical methods based on gas chromatography (GC) and/or mass spectrometry (MS) require derivatization of the analytes. Combining LC and electrospray ionization (ESI) in negative ion mode, these herbicides can be analyzed without derivatization. The multiple reaction monitoring (MRM) mode in MS/MS operation provides low pg detection limits.

This application note is based on standards and sample preparation procedures from the Montana Department of Agriculture in Bozeman, Montana.

Experimental

Standard and Sample Preparation

A stock solution of each analyte at 200 ppm is prepared in methanol. Intermediate mixed solutions for fortifying soil samples and making calibration standards are made by accurately mixing aliquots of each standard stock solution. The concentrations of each analyte in the intermediate solution used in this study are listed in Table 1.

Table 1. Acid Herbicide Mixed Intermediate Standards in Methanol

Clopyralid	5930 (pg/µL)	3,6-dichloro-2-pyridinecarboxylic acid
Picloram	1800	4-amino-3,5,6-trichloropicolinic acid
Dicamba	8200	3,6-dichloro-2-methoxybenzoic acid
2,4-D	1740	2,4-dichlorophenoxyacetic acid
MCPA	5480	2-methyl-4-chlorophenoxyacetic acid
Triclopyr	1240	[(3,5,6-trichloro-2-pyridinyl)oxy] acetic acid
2,4-DP	1410	2,4-dichlorophenoxypropionic acid or dichloroprop
MCPP	2710	2-(2-methyl-4-chlorophenoxy) propionic acid
2,4-DB	6900	2,4-dichlorophenoxybutyric acid

Sample extraction and cleanup procedures are shown below.

Sample Extraction Procedure

1. Weigh 20 ± 0.1 g of soil.
2. Add 50 mL of 0.5N KOH in 10% KCl extracting solution to each sample. Mix thoroughly by shaking.
3. Place samples in boiling water bath for 15 minutes.
4. Place samples on horizontal shaker for 15 minutes.
5. Centrifuge samples at 1200 to 1500 rpm for 15 minutes.
6. Transfer a 3.0-mL aliquot into a 13-mL conical centrifuge tube and add 150 µL of 12 N sulfuric aicd.
7. Vortex and confirm the pH is <1.5. If not, add additional acid solution.

Sample Cleanup Procedure

1. Add 2 mL of chloroform to the acidified extract.
2. Vortex 30 seconds and centrifuge at 3000 rpm for 2 minutes.
3. Remove the lower chloroform layer into another centrifuge tube. Repeat these three steps two more times.
4. Evaporate the chloroform extract to just dryness.
5. Immediately add 4.0 mL HPLC-grade water, vortex briefly, sonicate 5 minutes, and briefly vortex again. Fill autosampler vial.

See Reference 1 for more detailed information on sample preparation.

Instrumentation

LC:	1200 LC
Column:	ZORBAX Extend-C18, RRHT, 2.1 mm × 100 mm, 1.8 µm
Column temperature:	60 °C
Mobile phases:	A: 0.04% Glacial acetic acid in water
	B: Acetonitrile (ACN)
Flow rate:	0.3 mL/min
Injection volume:	1.0 µL

Gradient:

Time, min.	%B
0	0
1	40
2	52
3	60
4	100
8	100
9	0

MS:	G6410A QQQ
Ionization:	ESI (–)
Mass range:	120 to 400 amu
Capillary:	3500 V
Nebulizer pressure:	40 psi
Drying gas flow:	9 L/min
Gas temperature:	200 °C
Skimmer:	35 V

MRM parameters are listed in Table 2.

Table 2. Method Parameters for MRM

Name	RT	MW	Quant	Qual	Frag V	Col cell	Dwell	Segment
Clopyralid	3.47	191	190 > 146	192 > 148	80	5	70	1
Picloram	3.69	240	239 > 195	241 > 197	80	5	70	1
Dicamba	4.31	220	219 > 175	219 > 145	60	0	150	2
2,4-D	5.02	220	219 > 161	221 > 163	80	15	25	3
MCPA	5.09	200	199 > 141	201 > 143	100	10	25	3
Triclopyr	5.26	255	254 > 196	256 > 198	80	10	25	3
2,4-DP	5.42	234	233 > 161	235 > 163	80	5	25	3
MCPP	5.46	214	213 > 141	215 > 143	100	10	25	3
2,4-DB	5.66	248	247 > 161	249 > 163	80	10	25	3

Due to the concentration differences for these analytes in the intermediate solution (Table 1), Dicamba's concentration was used as the "concentration level" shown in Table 3.

Table 3. Concentration Levels (8000 to 10 pg/µL) Used in This Study

Solution concentration level	8000	800	400	200	80	40	20	10
Clopyralid	5930	593	296.5	148.2	59.3	29.7	14.8	7.4
Picloram	1800	180	90	45	18	9.0	4.5	2.3
Dicamba	8200	820	410	205	82	41.0	20.5	10.3
2,4-D	1740	174	87	43.5	17.4	8.7	4.4	2.2
MCPA	5480	548	274	137	54.8	27.4	13.7	6.9
Triclopyr	1240	124	62	31	12.4	6.2	3.1	1.6
2,4-DP	1410	141	70.5	35.2	14.1	7.1	3.5	1.8
MCPP	2710	271	135.5	67.7	27.1	13.6	6.8	3.4
2,4-DB	6900	690	345	172.5	69	34.5	17.3	8.6

For example, concentration level 8000 is the intermediate solution, and concentration level 20 is a 1:400 dilution of the intermediate solution.

Results and Discussion

Figure 1 shows the overlaid chromatograms of all nine herbicides from the MRM analysis. The run time was less than 6 minutes. Using a 1.8-µm particle size column, the peak widths of these analytes are in the range of 0.1 to 0.2 min. The narrower peak width helps to achieve a higher signal-to-noise ratio.

The MRM results of all nine herbicides at 1.6 to 10.3 pg on column are shown in Figure 2.

As shown in Table 3, the linearity range, incorporating 10, 20, 40, 80, 200, 400, 800, and 8000 pg on column, mimics the concentration of Dicamba.

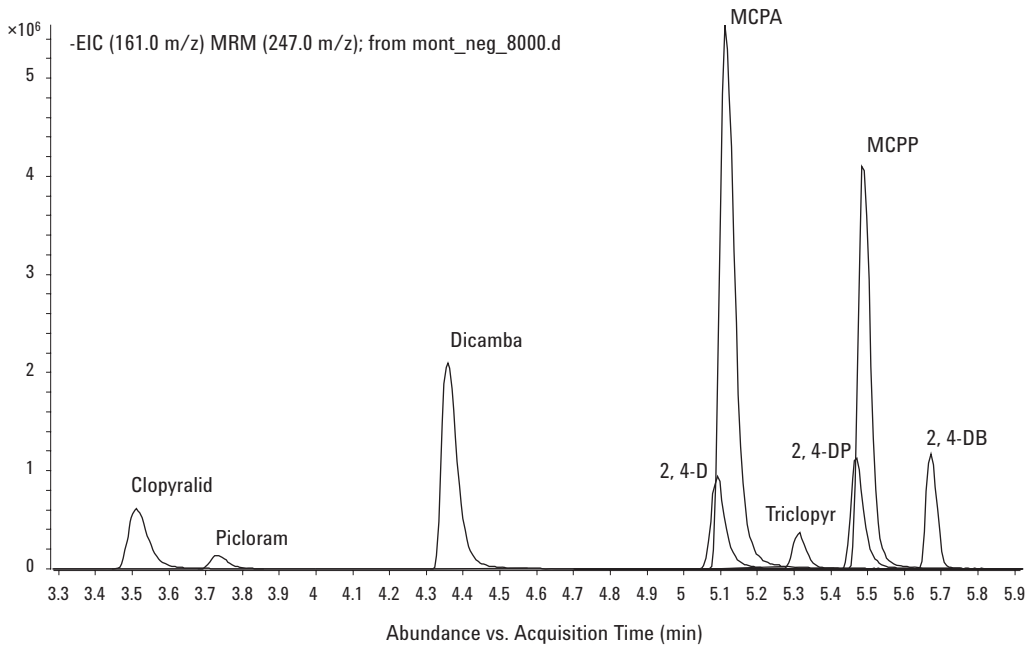

Figure 1. Overlaid MRM results from the nine selected herbicides.

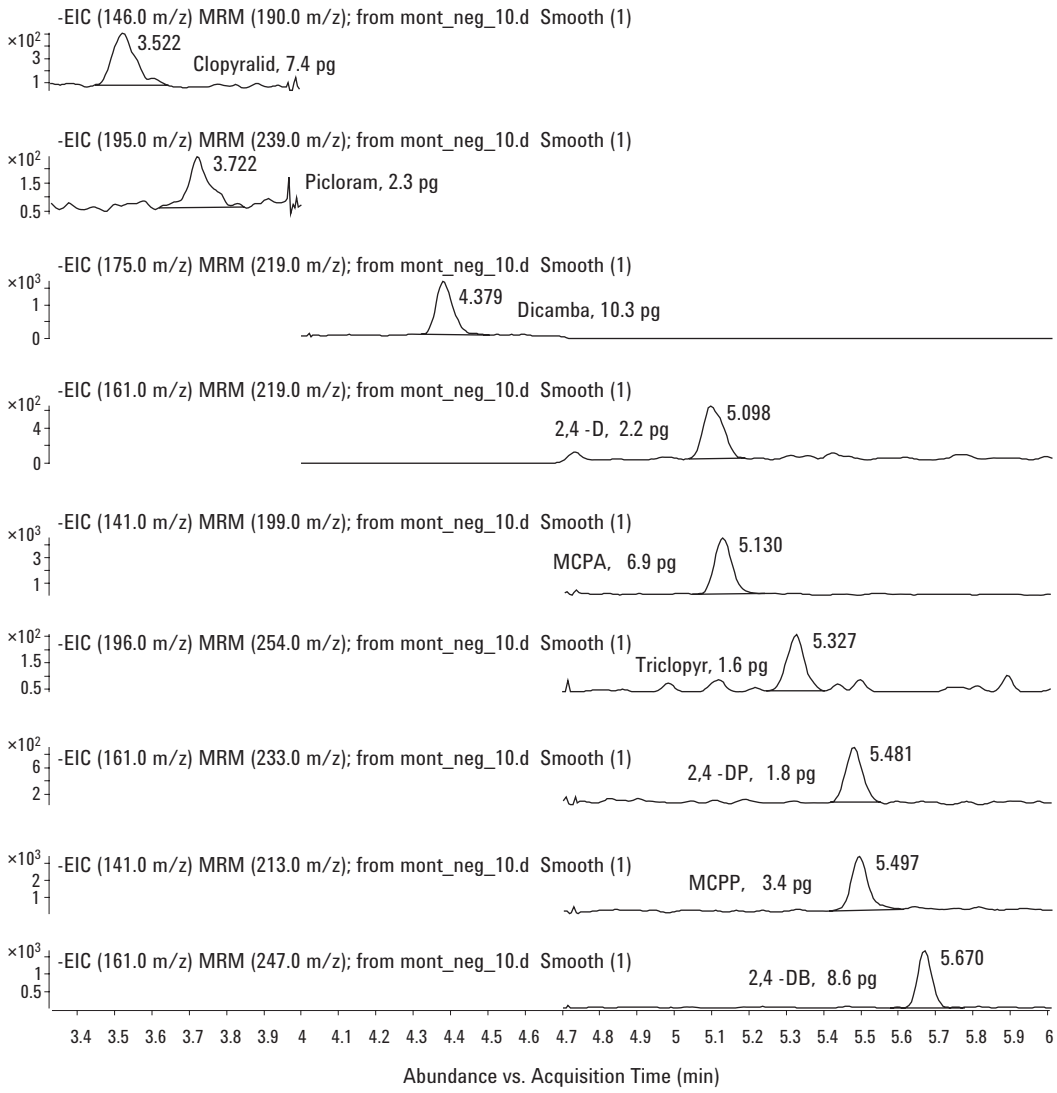

Figure 2. MRM results.

Therefore, the corresponding on column amounts for Triclopyr are: 1.6, 3.1, 6.2, 12.4, 31, 62, 124, and 1240 pg. The calibration model used was a linear model that included origin with no weighting. All analytes showed excellent linearity.

In the repeatability study, seven 1-µL injections of the level 40 solution (Table 4) were analyzed to calculate the RSD. At this low concentration, all RSDs were <15%, with the majority in the single digits.

The matrix effect from three different matrices was evaluated. Water and soil extracts were spiked with the herbicide standards (50 µL of the level 400 standard were added to 950 µL of water, silt, clay, or sandy extracts). The resulting concentration of the analytes is equivalent to the level 20 solution (Table 3).

Table 5 shows the RSD of eight 1-µL injections of the level 20 solutions, that is, <20 pg of each ana-

Table 4. Linearity (10 to 8000 pg/µL) and Repeatability

Compound	R^2 (linear fit, include origin)	Repeatability, %RSD (n=7)	Amount on column
Clopyralid	0.9995	3.5	29.7 pg
Picloram	0.9991	13.1	9.0
Dicamba	0.9999	2.4	41.0
2,4-D	0.9998	6.5	8.7
MCPA	0.9999	2.2	27.4
Triclopyr	0.9993	11.9	6.2
2,4-DP	0.9998	4.5	7.1
MCPP	0.9999	5.0	13.6
2,4-DB	0.9973	8.4	34.5

lyte on column, in three different matrices. As expected, analytes with lower absolute responses showed higher RSD value.

Table 5. RSDs from Eight Injections of <20 pg of Each Analyte in Three Matrices

	Clay	Sandy	Silt	On column
Triclopyr	27	22	22	3.1 pg
MCPP	6	5	7	6.8
MCPA	2	6	5	13.7
Clopyralid	14	15	13	14.8
2,4-DP	9	8	13	3.5
2,4-DB	9	3	9	17.3
2,4-D	11	14	13	4.4

The repeatabilities of responses in three matrices for all analytes were <15% except for Triclopyr, which was >20%.

The responses of ≤20 pg analytes among water and matrices are compared in Figure 3. The listed response for each matrix is the average of responses from eight injections. The RSDs for water and the three matrices are shown in Figure 3 and are comparable. In general, the variation of the responses among water and different matrices is less than 5% for all analytes except for 2,4-D, which has an RSD close to 10% due to the higher responses from the silt matrix. This shows that the method described in this application note is free from matrix interferences from clay, sand, and silt.

Conclusion

Using LC/MS/MS, chlorinated acid herbicides were analyzed at pg levels on column without any derivatization. At <40 pg on column, the repeatability of seven 1-µL injections showed RSD <15%. Good linearity was observed for all analytes from low pg to low ng on column.

The RSDs of <20 pg analytes in the selected matrices were comparable to that in water (RSDs ~5%), except for 2,4-D, due to the higher response from the silt.

Acknowledgement

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Reference

1. "Determination of Chlorinated Acid Herbicides in Soils by Liquid Chromatography-Electrospray/Mass Spectrometry/Mass Spectrometry," by Angela Schaner and Laura Luckey, Revision 2, April 2, 2004. Montana Department of Agriculture, Laboratory Bureau, McCall Hall, Montana State University, Bozeman, MT 59717.

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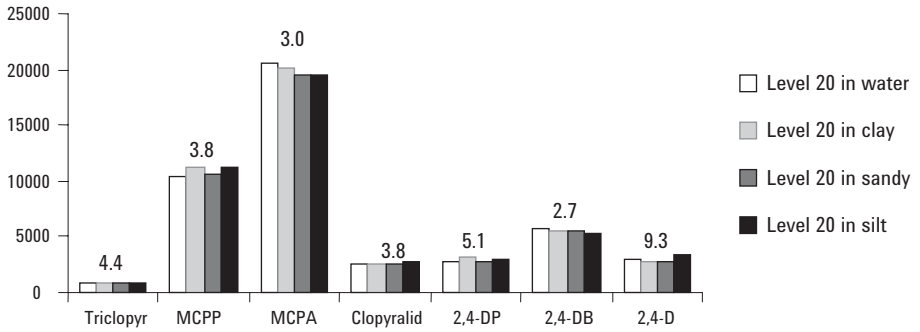

Figure 3. Analyte RSDs from water and soil extracts are comparable.

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