Fast Analysis of Chlorinated Phenoxy Herbicides in Environmental Samples by Negative ESI LC-MS/MS – 14337

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ABSTRACT

This paper describes the analysis procedure and results for environmental herbicide samples by a negative ESI LC-MS/MS method. After the preparation of aqueous and soil samples, the extracts are directly injected into a high performance liquid chromatography system coupled to negative eletrospray ionization (ESI-) tandem mass spectrometry. The sample preparation incorporates modified hydrolysis steps from EPA 8151 to ensure herbicides containing a variety of functional groups are captured in the reported concentration. Herbicides in the final extract have been converted to their carboxylic acid form for LC-MS/MS analysis. The liquid chromatograph uses a narrow-bore C-18 column and acidic acetonitrile mobile phase. The triple quadruple mass spectrometer uses a Multiple Reaction Monitoring (MRM) mode for each herbicide compound enabling highly selective identification. The method was certified for herbicide targets from EPA 8151. Parallel blind performance evaluation tests run concurrently between EPA 8151 and TestAmerica's EPA 8321 LC-MS/MS herbicide method indicate that the LC-MS/MS method has superior performance on accuracy, precision, sensitivity, and selectivity in addition to the aforementioned advantages of sample preparation efficiency and TAT for both aqueous and solid samples. The increased reliability of the qualitative identification of the herbicides using the LC-MS/MS method produces more reliable and defensible data.

Keywords: phenoxyacetic herbicide, chlorinated, LC-MS/MS, Multiple Reaction Monitoring.

INTRODUCTION

Herbicides have been widely used to control weeds in agricultural and domestic areas since the 1950s. There is an increasing concern on its contamination of ground water and soil. Some phenoxyacetic acid herbicides are very toxic [1] and banned in the United States. Now phenoxyacetic herbicides are monitored at trace levels in drinking water, ground water, and soil. These compounds are commonly found in a variety of forms including acids, salts, or esters. Traditionally, EPA 8151 is used for analyzing herbicides in environmental samples [2]. EPA 8151 sample preparation converts all forms of herbicides into the methyl ester form which are then analyzed by dual-column GC-ECD. This approach is time consuming, includes excessive solvent use, potentially explosive reagents, is highly technique dependent, and results in relatively high reporting limits for some target compounds.

High performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has become more prevalent in the past ten years in the testing industries because the LC-MS/MS

significantly enhances sensitivity and selectivity [4]. US EPA first introduced 8321A using High performance liquid chromatography coupled with thermospray-mass spectrometry (TS-MS) in 1996 and revised it in 2007 as 8321B [3]. Although LC-MS/MS is different from LC-TS/MS, this method provides general guidance and regulatory framework for this LCMS technology in environmental testing. This application presents an analytical approach for the analysis of phenoxyacetic herbicides in water and soil based on EPA 8321 by direct injection of herbicide acid form into LC-MS/MS. Test America has developed the method which mitigates or eliminates many of the issues inherent in the EPA 8151 methodology. The direct injection approach permits trace level analysis without the traditional high volume enrichment during sample preparation. The preparation for the LC-MS/MS method is safer, solvent use is minimized, no derivatization is required, no confirmation analysis is needed, faster turn around time (TAT) is achievable, and low detection limits can be attained, even in complex matrices.

METHOD DESCRIPTION

Experimental Materials and Instrumentation

Reagent and solvents used for sample preparation and mobile phases were obtained from J.T Baker (Center Valley, PA) and EMD (Germany). The standards were obtained from o2si smart solution (Charleston, SC) and Accustandard (New Haven, CT).

The method was developed using Waters Acquity UPLC instrument coupled with Xevo TQS MS/MS spectrometer system with negative ESI mode. The following describes the experimental conditions for the quantifications of 10 common phenoxyacetic herbicides (listed in Table III) in environmental aqueous and solid samples.

UPLC Conditions

The primary column used in this study was Water Acquity UPLC BEH C18, 1.7um x 2.1mm x 100mm (or equivalent). The injection volume was 50 uL. HPLC mobile phase condition is listed in Table I.

TABLE I. UPLC conditions

Parameters	**Description**			
Mobile Phase	A = 0.1% Formic Acid in 40% ACN in Water (v/v) B = Acetonitrile			
Column Temp	30 ± 0.1ºC			
Gradient	Time (min)	Flow (mL/min)	A (%)	B (%)
	0.0	0.4	100	0
	4.5	0.4	100	0
	5.0	0.4	0	100
	7.0	0.4	0	100
	7.5	0.4	100	0
	12.0	0.4	100	0

MS/MS Parameters

All compounds were detected using negative ionization ESI in Multiple Reaction Monitoring (MRM) mode using selected MRM transitions for each target compound, surrogate, and internal standard (Table III). The following mass spectrometer conditions were used (Table II):

TABLE II. Mass spectrometer operating conditions

Parameter	Setting
Scan Mode	ESI Negative Ion
Corona Current	20.0 µA
Multiplier Voltage	550 Volts
Desolvation Temperature	300 °C
Source Temperature	120 °C
Desolvation Gas Flow	800 L/hr
Cone Gas Flow	50 L/hr

TABLE III. Selected characteristic ions and scan conditions

Analyte	MRM (m/z)	Cone Volt (V)	Collision Energy (eV)
Dalapon	141 > 97	15	10
2,4-Dichlorophenyl acetic acid (DCAA), surrogate	249 > 159	15	12
2,4-D	219 > 161	25	13
Dicamba	219 > 175	10	7
MCPA	199 > 141	18	15
Dichlorprop	233 > 161	25	14
MCPP	213 > 141	20	14
2,4,5-T	253 > 195	20	12
2,4-DB	247 > 161	15	12
2,4,5-TP (Silvex)	267 > 195	17	15
Dinoseb	239 > 193	35	25
2,4-Dichlorophenoxyacetic acid (13C6), internal standard	225 > 167	25	13
2,4,5-Trichlorophenoxyacetic acid (13C6), internal standard	259 > 201	20	12

Sample Preparation Procedure

10mL of aqueous sample and 2 grams of solid sample were used for the process. For solid samples, 10 mL of HPLC water was added before pH adjustment. All samples were first spiked with appropriate amount of surrogate. QC samples were then spiked with appropriate amount of herbicide standard. Then the sample is adjusted to pH 12 to hydrolyze esterified herbicides with 1M KOH, left to sit for two hours at room temperature, and then it is acidified to pH 2 to bring all herbicides to their acid form for LC-MS/MS analysis with formic acid. The internal standard was then added to the final extract prior to injection into LC column.

For complex matrices, such as samples containing high level of dissolved salts or total organic carbon (TOC), sample clean up steps using appropriate cartridges or filters are necessary prior to or after pH adjustment, because the interference can cause ion enhancement and/or ion suppression on the target MS/MS signals.

RESULTS AND DISCUSSION

Method development was performed using Waters Masslynx and TA Chrom software. The MDL and spiked matrix samples were performed using HPLC water and sand. The method was also tested using PT aqueous and solid samples. Results showed consistent performance for both standards and PT samples over several months of work.

The LC method uses a fast gradient program for a complete analysis in less than ten minutes and an analysis cycle time of 12 minutes. Due to the structural similarity of the acidic herbicides, incomplete peak resolution (RT overlap) is observed under faster separation conditions as shown in Figure 1. However, the increased selectivity of MS/MS is readily apparent. The MRM chromatograms in Figure 1 are interference free within the acquire window. In addition to the MRM peak and its retention time, the greater signal/noise ratio provides high confidence of target identification and quantification.

Calibration and Sensitivity

The result of LOD and LOQ study and the associated initial calibration is listed in Table IV below. The LOD is at sub-ppb level and the LOQ is currently set at lower ppb level. The linearity of the associated calibration is shown in the second column of Table IV. The calibration %RSD ≤ 20 or r2 ≥ 0.995 indicates good linearity for all the herbicides in this study. The calibration concentration range was from 0.5 to 40 ppb for the targets. The method has a good linear range for low level herbicide samples.

Figure 1 (page 6) shows the MRM spectra for all the targets at 0.5 ppb from the associated initial calibration of the LOD study. In Figure 1, MRM transition is denoted by A > B. The retention time and signal/noise ratio of a peak are marked at the left side of the peak. Positive peak identification criteria used in this study were peak shown within 0.5 minute retention time window and signal/noise ratio ≥ 5. The center of the retention time window is from the middle level of ICAL. From the signal/noise ratio shown in Figure 1, under the developed MRM method and instrument conditions, the LC-MS/MS system is very sensitive for all the targets compared to GC or HPLC systems.

TABLE IV. The limit of detection and quantification

Analyte	Calibration %RSD / r2	Water		Solid	
		LOD (ug/L)	LOQ (ug/L)	LOD (ug/Kg)	LOQ (ug/Kg)
Dalapon	16	0.82	2	1.9	10
2,4-D	14	0.42	2	1.5	5
Dicamba	10	0.57	2	1.4	5
MCPA	10	0.69	5	2.3	20
Dichlorprop	0.996	0.46	2	2.3	5
MCPP	16	0.75	5	2.9	20
2,4,5-T	7.7	0.57	2	2.9	5
2,4-DB	9.9	0.17	1	2.5	5
2,4,5-TP (Silvex)	10	0.5	2	1.6	5
Dinoseb	20	0.15	0.5	0.8	2

pH Effect on Recovery

pH value is critical to get good QC recovery and consistent results over time. It has been proven that pH variation from sample preparation to sample injection into the mass spectrometer is the main factor for failing to get reliable consistent results. Consistent pH must be maintained in the following steps.

First of all, sample preparation is a two-step process, basic hydrolysis followed by an acidification step. Our experiments suggest to maintain pH ≥12 for the hydrolysis and pH = 2-3 for the acidification for the targets in Table IV. The pH = 2-3 in the final extract must be maintained from the sample extracts to the sample injection into the mass spectrometer to ensure all herbicides are in their acid form when analyzed.

Second, to maintain the acid form of herbicides during the course of LC separation and MS/MS analysis, the mobile phase must maintain the same pH (2-3) as the pH of the extracts. Volatile organic acids such as formic acid or acetic acid are recommended for mass spectrometer.

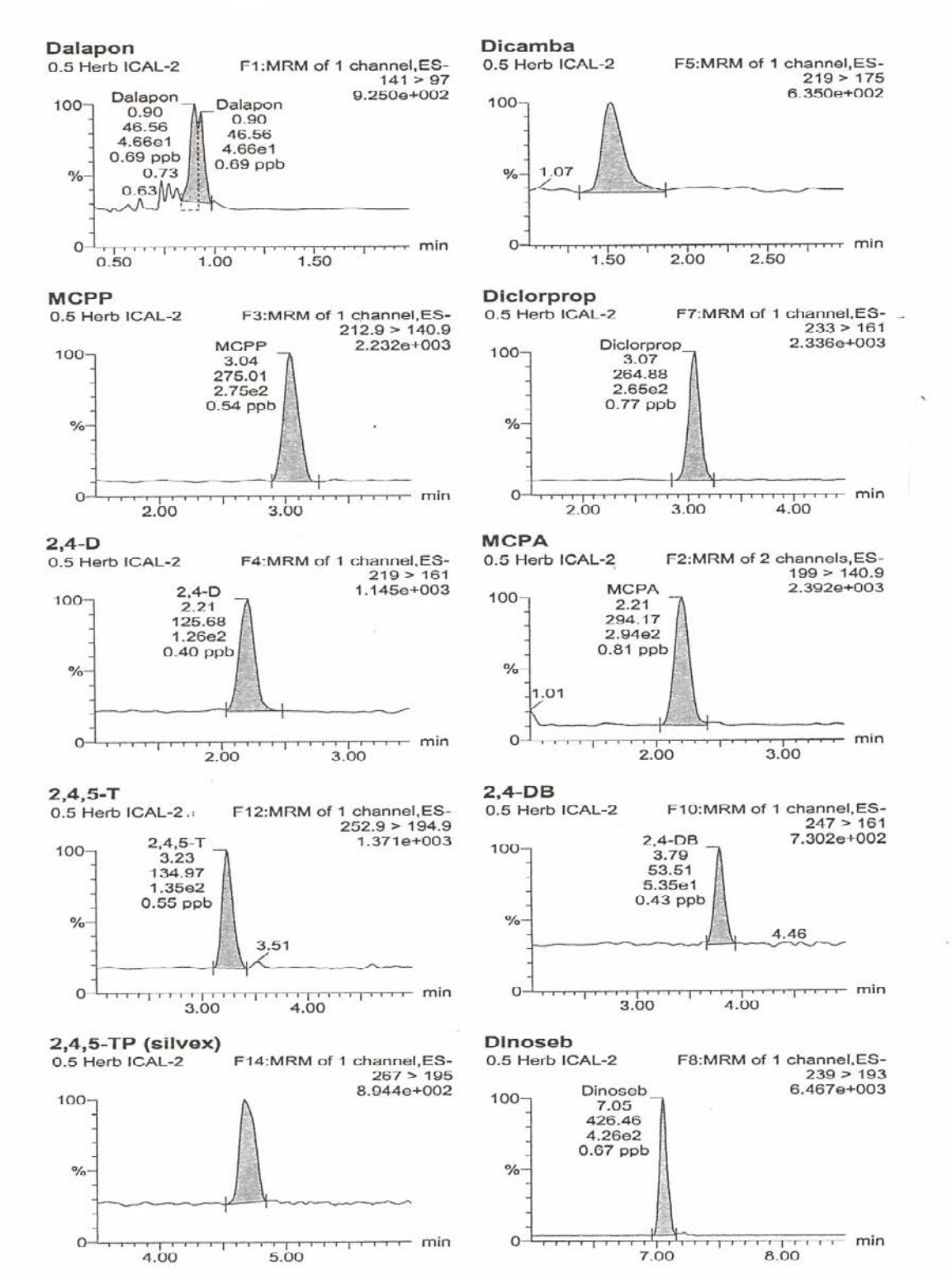

Figure 1: The chromatogram of low level of calibration (0.5 ug/L).

Method Stability

Besides sensitivity and selectivity, method stability is a key factor for commercial testing laboratories. The method performance has to be as consistent as possible during daily analysis. Two series of tests were performed on the stability of this method. One test was on clean water and sand matrices and the other was on real aqueous and soil sample matrices. Each test simulated a standard 20 sample analytical batch each day for three continuous days. Continuing calibration verification (CCV) and internal standard recovery in each injection provide valuable insight into overall method stability. The goal during development was to consistently achieve CCV recoveries within ±30% of expected value for all compounds (Table V). The percent recovery of each internal standard was calculated by comparing the internal standard response factor of each injection to the average response factor of the internal standard from the ICAL. Percent recovery was also used to assess consistency of the internal standard, with a goal of ±50% (Table VI).

TABLE V. Continuing calibration verification %Recovery results

Analyte	CCV (%Recovery)					CCV S. Dev
	1	2	3	4	5	
Dalapon	98.5	94.8	97.6	100.8	113.5	7.3
2,4-D	76.5	101.8	81	93.1	107.7	13.3
Dicamba	86.1	99.4	91	107.7	102.3	8.7
MCPA	88.6	105.2	96.2	102.7	114.7	9.8
Dichlorprop	86.5	98.4	105.7	97.4	110.3	9.1
MCPP	91.3	105.1	97.9	95.2	82.6	8.3
2,4,5-T	95	109.9	83.7	71.3	108.2	16.4
2,4-DB	115.7	122.4	107.1	106.4	113.4	6.6
2,4,5-TP (Silvex)	88.1	106.9	93.4	90.2	97.1	7.4
Dinoseb	103.3	97.5	87.6	82.1	89.9	8.4
2,4-DCAA (surr)	122.9	103.7	108.4	94.9	112.4	10.4

TABLE VI. Internal standard %Recovery from 10 continuing injections

Internal Standard[a]	Internal standard %Recovery									
	1	2	3	4	5	6	7	8	9	10
#1	121.3	116.5	114.4	108.5	118.3	125.7	117.6	107.7	124.3	107
#2	115.5	111.7	112	105.1	123.4	118.8	113.6	107.2	100.8	107.8

a Internal Standard #1: 2,4-Dichlorophenoxyacetic acid (13C6)
internal Standard #2: 2,4,5-Trichlorophenoxyacetic acid (13C6)

From the Standard Deviation (S. Dev) column in Table V, we can see that 2,4-D and 2,4,5-T respond more erratically than other targets. But method stability overall remains adequate and stays stable for days in between initial calibrations.

Method Validation Results

As this method was developed based on EPA 8321 (the only LC/MS/MS referenced EPA method) and 8151 (Herbicide target list GC method), it is designed to closely replicate the results obtained from employing GC method 8151 with the benefit of enhanced sensitivity, selectivity, and reduced sample preparation complexity and turn-around-time. A direct comparison of numerical results between the GC method and the LC/MS/MS method is appropriate in this case and can serve as a criterion for validation. This method has passed two set of PT tests on both aqueous and solid matrices. Table VII shows a parallel testing result between this method and 8151 GC method on soil PT (Phenova, Lot#7052-18). A PT result closer to the assigned value was obtained by this method compared to the result obtained from GC, especially for Dinoseb.

TABLE VII. PT validation results (chlorinated acid herbicides in soil)

NELAC Analyte Code	Analyte	Units	8321A Report Value	8151A Report Value	Assigned Value	Acceptance Limits	Performance Evaluation	8321 Analysis Date
8545	2,4-D	ug/Kg	599	380	546	54.6 - 828	Acceptable	20130523
8560	2,4-DB	ug/Kg	356	155	330	33.0 - 686	Acceptable	20130523
8555	Dalapon	ug/Kg	<20	<67	<100	0.00 - 100	Acceptable	20130523
8595	Dicamba	ug/Kg	183	143	215	21.5 - 304	Acceptable	20130523
8605	Dichloprop	ug/Kg	<10	<133	<100	0.00 - 100	Acceptable	20130523
8620	Dinoseb	ug/Kg	410	88.5	642	64.2 - 988	Acceptable	20130523
7775	MCPA	ug/Kg	<50	NA	<1000	0.00 - 1000	Acceptable	20130523
7780	MCPP	ug/Kg	<50	NA	<1000	0.00 - 1000	Acceptable	20130523
8655	2,4,5-T	ug/Kg	296	205	302	99.2 - 401	Acceptable	20130523
8650	2,4,5-TP	ug/Kg	164	85	165	16.5 - 266	Acceptable	20130523

CONCLUSIONS

In the last decade, liquid chromatography coupled with tandem mass spectrometry has been proven to be a powerful technique crossing many disciplines of science due to its sensitivity, selectivity, and adaptability to various matrices. This method development proves its advantages and potential in the environmental industry.

A convenient, high throughput, sensitive, and selective LC-MS/MS method has been developed based on EPA 8321 for detecting phenoxyacetic herbicide in aqueous and solid samples. The analysis provides highly superior specificity and sensitivity when compared to the traditional GC detection methodology. It also provides simple and fast sample preparation with the elimination of excessive organic solvent use. This method makes a rapid 1-2 day turn-around-time possible compared to minimum 4-5 days for the traditional GC method.

The continuing calibration verification and internal standard recovery test results show that this method is stable and maintains the sensitivity and accuracy over the runs. Finally, this method shows acceptable performance for accuracy and precision under conditions normally encountered by production laboratories.

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