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A Simple Procedure for Determination of Aminocyclopyrachlor and Aminopyralid in Soil, Corn Meal, and Soy Meal using Liquid Chromatography/Tandem Mass Spectrometry

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Abstract

An analytical method has been developed that efficiently extracts aminocyclopyrachlor and aminopyralid from a variety of matrices including soil, soy meal, and corn meal without the need of time-consuming matrix cleanup steps such as solid phase extraction. Following extraction, the samples are analyzed by positive mode LC-ESI-MS/MS. Spiking studies were conducted at low- and intermediate-concentrations to determine the method detection limit as well as to evaluate the accuracy and precision of the method. Generally, aminocyclopyrachlor and aminopyralid recoveries were acceptable following extraction from soil, soy meal, and corn meal.

Keywords: pesticide, aminocyclopyrachlor, aminopyralid, mass spectrometry, FIFRA, EPA

1. Introduction

Herbicides are widely used worldwide for weed control or as plant growth regulators and account for the largest portion of pesticide expenditures in the world [2]. In 2012, U.S. herbicide expenditures accounted for 21% of the total world expenditures for pesticides. The major use for herbicides is for agricultural practices; home and garden use; and industrial, commercial and government applications. The mostly used herbicide active ingredients in 2012 included glyphosate, atrazine, S-metolachlor, 2,4-D and acetochlor. Among those five herbicide active ingredients, glyphosate, atrazine and 2,4-D are broad-spectrum usage herbicides that can be used in a wide range of weed control applications.

Aminocyclopyrachlor (Figure 1), developed by DuPont Crop Protection, represents the first herbicide of the pyrimidine carboxylic acid class and is used as a selective herbicide for control of invasive broadleaf weeds, woody species, and vines on industrial non-crop sites. Aminocyclopyrachlor is also used for the control of noxious broadleaf weeds and brush in rangeland in some areas. It was conditionally registered for use by the EPA under the trade name of Imprelis® and first used in 2010. Aminopyralid (Figure 1), developed by Dow AgroScience LLC, is also a selective herbicide that belongs to the pyridine carboxylic acid class and was conditionally registered for use by the EPA in 2005. Both aminocyclopyrachlor and aminopyralid are classified as synthetic auxins that function as growth regulators [4]. Aminocyclopyrachlor and aminopyralid are both approved for weed and brush control on railroads, roadsides and c right-of-ways. Because both pesticides have similar uses, it is important to be able to distinguish between the two products during non-target injury investigations.

In the United States, the use of pesticides is governed by the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) administrated by the Environmental Protection Agency (EPA). Pesticide registration by EPA requires analytical methods submitted by the registrant that can quantify trace level of the active ingredient in environmental matrices such as soil and water [12]. Under FIFRA section 26, the states have the primary law enforcement responsibilities to investigate pesticide misuse. Investigation of pesticide usage involves collection of investigation samples under sampling protocols and sample analysis to determine the presence or absence of pesticide active ingredients. The presence of the active ingredients serves as evidence that the pesticide has been used on the investigation site. State government laboratories implement rigid quality standards to ensure the validity and defensibility of the analytical data [5]. Even though pesticide registrants are required by the EPA to submit appropriate environmental and crop methods, the peti-

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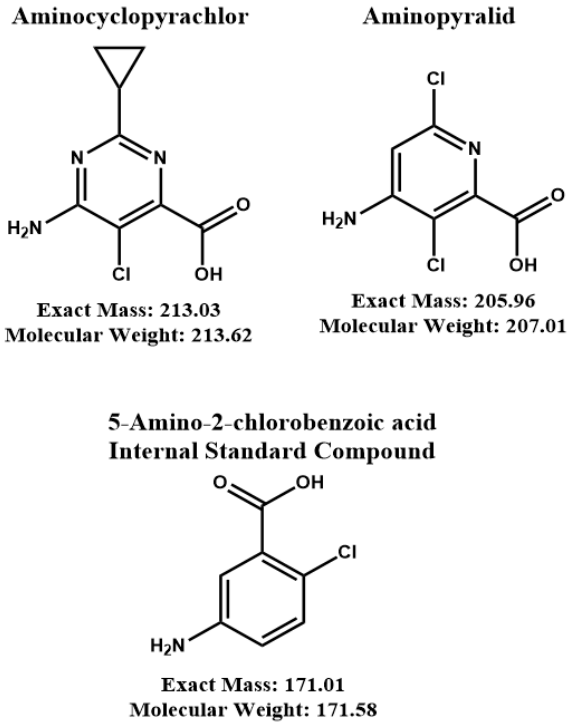


Figure 1: Structures of aminocyclopyrachlor and aminopyralid.

tioner's methods tend to be validated only for the crop/animal matrices for which the petitioner is seeking a usage label. Due to the complicated nature of pesticide misuse investigations, analyzing pesticides in various environmental and agricultural matrices remains a challenge to both the regulatory and scientific community.

QuEChERS was developed by the United State Department of Agriculture (USDA) laboratory from 2001 and 2002 and soon gained wide popularity in the pesticide analysis community for its "Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS)" feature [1]. For aminocyclopyrachlor and aminopyralid, both contain anionic and cationic functional groups, contributing to the properties that have made these herbicides historically difficult to extract from a variety of matrices [7, 8]. Using a QuEChERS method to extract a variety of acid herbicides from rice, Sack et al. observed an 18% recovery for aminocyclopyrachlor when fortified at 5 ng/g [9]. Furthermore, Nanita et al. demonstrated the limited utility of older extraction techniques and detection methods listed in the FDA-PAM pesticide analysis manual [6]. However, at the time this manuscript was prepared, there were very limited publications detailing co-extraction of aminocyclopyrachlor and aminopyralid from differing matrices. The current study presents a simple and straightforward process to co-extract and analyze aminocyclopyrachlor and aminopyralid from environmental matrices (i.e. soil and water) and agricultural materials.

2. Experimental Chemicals and Reagents

Aminocyclopyrachlor and aminopyralid were obtained from the United States Environmental Protection Agency Standard Repository (Fort Meade, MD 20755-5350). As labeled versions of the analytes are not readily available, 5-Amino-2-chlorobenzoic acid (Sigma Aldrich, St. Louis, MO) was used as a chemically-similar internal standard (Figure 1). Acetonitrile, methanol, formic acid, 0.1 v/v % formic acid in water, and ammonium acetate (Fisher Scientific, Hampton, NH) were used without further purification. Ultrapure water was provided by a Millipore Synergy UV purification system (Burlington, MA). A mixed intermediate spiking solution containing aminocyclopyrachlor and aminopyralid was prepared at 2 μg/mL in methanol. The 2 μg/mL spiking solution was then used to prepare calibration standards at concentrations from 0.2 - 200 ng/mL. Calibration standards and samples were prepared in 5% methanol in water, 10 mM total in ammonium acetate. The extraction solution was prepared by mixing 800 mL of acetonitrile, 200 mL of purified water, 1 mL of formic acid, and 3.1 grams of ammonium acetate. Additionally, the extraction solution was prepared with 5-Amino-2-chlorobenzoic acid at a final concentration of 100 ng/mL. 5-Amino-2-chlorobenzoic acid was utilized as the internal standard for both aminocyclopyrachlor and aminopyralid to monitor for and normalize response data for potential matrix effects. Acceptance criteria for internal standard recovery was 50 - 150% relative to the average recovery observed for the calibration standards.

Time, min	Channel A %	Channel B %
0.00	95	5
5.00	41	59
8.00	1	99
10.0	1	99
10.1	95	5
15.0	95	5

Flow Rate = 0.3 mL/min.
Channel A: 0.1% formic acid in HPLC grade water
Channel B: Methanol

Table 1: HLPC Gradient Used for Separation of Aminocyclopyrachlor and Aminopyralid.

Analyte	R.T. Min.	Q1 m/z	Q3 m/z	Dwell (msec)	CUR (psi)	GS1 (psi)	GS2 (psi)	TEM (°C)	CAD (psi)	DP (volts)	CE (volts)
Aminocyclopyrachlor 1	6.8	214.1	68.0	150	40	60	60	400	5.00	105.0	105
Aminocyclopyrachlor 2	6.8	216.1	68.0	150	40	60	60	400	5.00	105.0	105
Aminopyralid 1	8.8	209.2	136.1	150	40	60	60	400	5.00	55.0	42.0
Aminopyralid 2	8.8	207.2	160.9	150	40	60	60	400	5.00	55.0	42.0
5-Amino-2-chlorobenzoic acid (Internal Standard)	7.5	172.1	93.10	150	40	60	60	400	5.00	70.0	25.0

R.T. = retention time in minutes
Min. = minutes

Table 2: Mass Spectrometer Parameters for Aminocyclopyrachlor and Aminopyralid on AB Sciex 4000 QT.

3. Sample Preparation and Analysis

Test matrices of dried soy beans, corn, pinto beans, and oranges were purchased from local retail grocers. Prior to extraction, the soy beans, and corn, were ground to a fine powder using a consumer coffee grinder (KitchenAid model # BCG1110B). Five grams of soil or ground test matrix was added to 50-mL centrifuge tubes and spiked with the appropriate amount of the mixed spiking solution. After 10 minutes, 10 mL of extraction solution was added to the samples. Samples were shaken for 3 minutes at 2500 rpm on an orbital shaker (VWR DVX-250, Radnor, PA). Following shaking, the samples were centrifuged at 3000 rpm for 3 minutes (Heraeus Instruments Biofuge Statos, Hanau, Germany) and the supernatant decanted. The extraction process was repeated once more by addition of 10 mL of extraction buffer. Samples were taken through the shaking and centrifugation steps and the extracts from the two extraction cycles were pooled. A 2-mL aliquot was then blown to dryness using a TurboVap solvent removal system (Caliper Life Sciences, Charlotte, NC). Once dry, the sample was reconstituted in 2 mL of the calibration standard/sample diluent with 100 ng/mL of 5-Amino-2-chlorobenzoic acid added as internal standard. The contents were mixed on a vortex mixer and then sonicated for 5 minutes. Prior to analysis, the extract was filtered through a 13-mm nylon syringe filter with 0.45 μm diameter pores (Fisher Scientific) and transferred to an amber glass autosampler vial for analysis.

The HPLC system consisted of an Agilent 1200 series (Santa Clara, CA) equipped with degasser, binary pump, refrigerated autosampler tray and a temperature controlled column compartment. The HPLC system is interfaced with an AB Sciex ABI 4000 QT mass spectrometer (Framingham, MA). The chromatographic separation was conducted using a Phenomenex (Torrance, CA) Luna Phenyl-Hexyl 4.6 mm x 150 mm with 3 μm diameter particle size column. The column eluent was introduced through an ESI source with the mass spectrometer operated in the positive ion mode. Data was collected using multiple reaction monitoring for the quantitation of aminopyralid and aminocyclopyrachlor. System control, acquisition, and data analysis was provided by Analyst 1.6.2 software. Samples were analyzed by LC-MS/MS injecting 50 μL of sample using an acetonitrile/water/formic acid gradient. Details can be found in Table 1. See Table 2 for operating and data-collection details for the AB Sciex 4000 QT MS/MS system. See Figures 2, 3, and 4 for examples of the chromatographic data typically observed from extracted samples containing aminocyclopyrachlor, aminopyralid, and the internal standard 5-Amino-2-chlorobenzoic acid.

3.1. Limit of Detection (LOD)

For the purpose of this study to test pesticides under EPA regulation, the limit of detection calculation was based on the

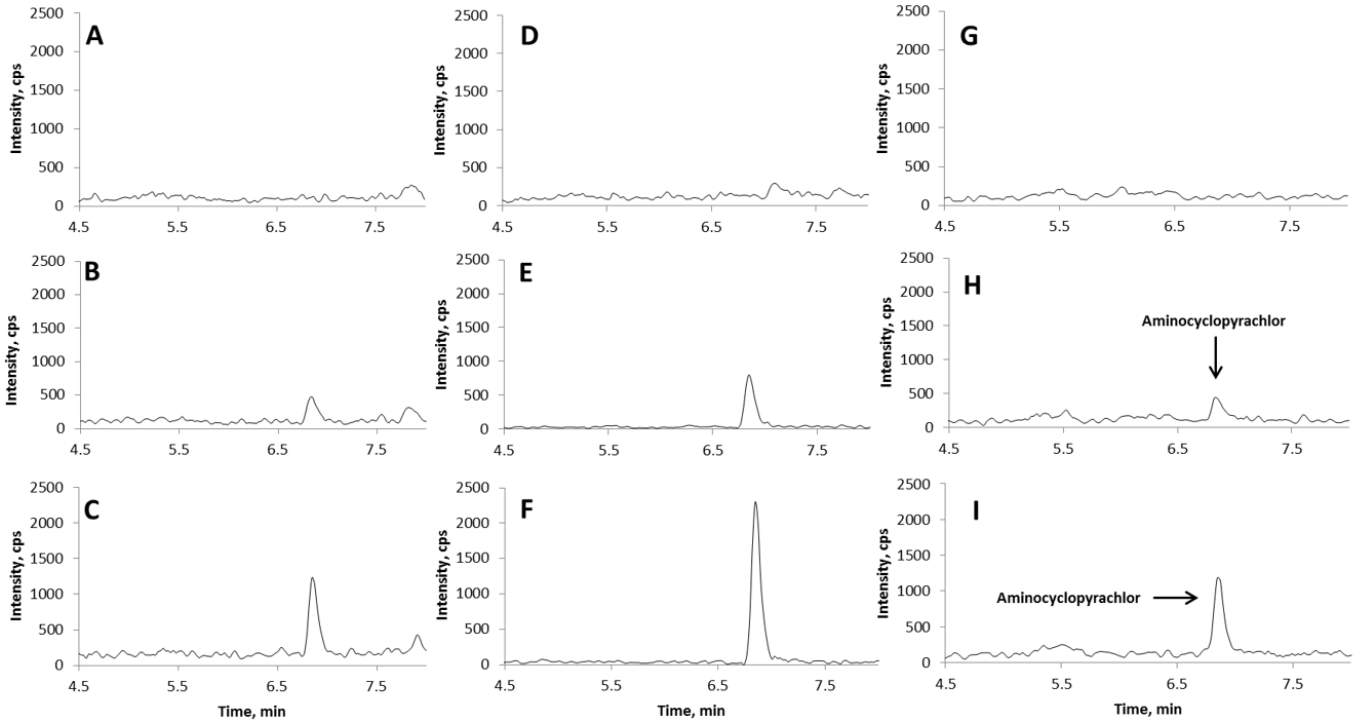


Figure 2: Chromatographs of Aminocyclopyrachlor Extracted from corn meal, soil and soy meal.

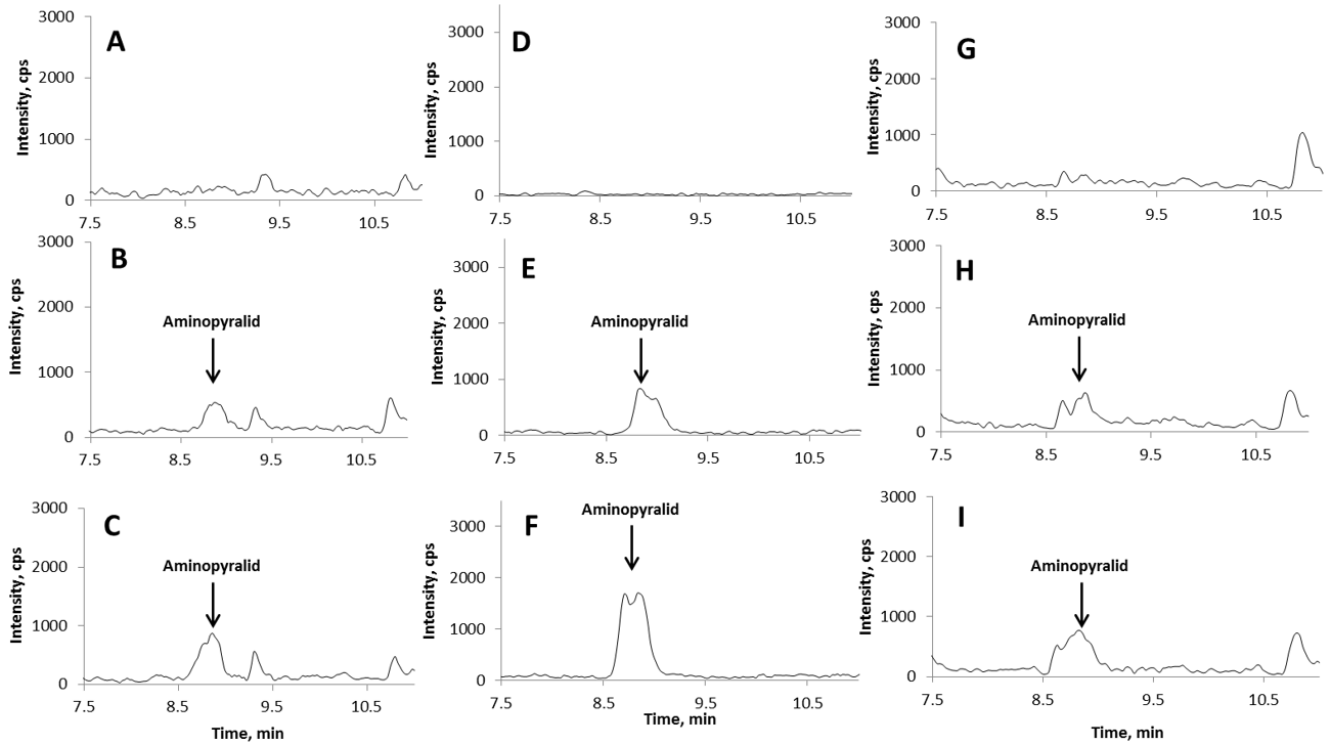


Figure 3: Chromatographs of Aminopyralid Extracted from corn meal, soil and soy meal.

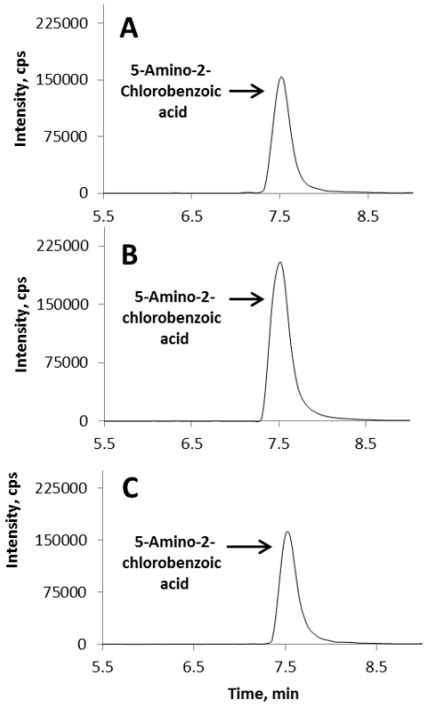
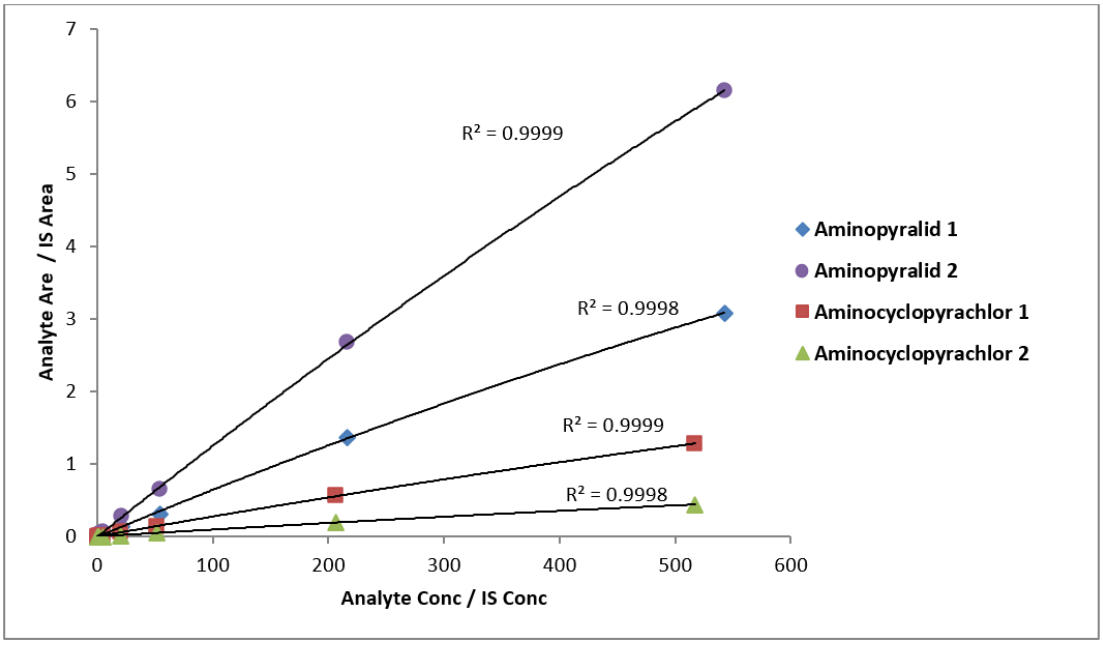


Figure 4: Chromatographs of 5-Amino-2-chlorobenzoic acid following addition to blanks extracted from corn meal, soil, and soy meal.



Note: Quadratic curve fit with 1/x weighting.

Figure 5: Calibration Curve Plots for Aminocyclopyrachlor and Aminopyralid.

EPA's method detection limit (MDL) determination outlined in Appendix B of Title 40 CFR 136 [3]. By definition, the MDL is the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte. A

	Aminocyclopyrachlor			Aminopyralid		
Matrix	Spiking Level (ng/g)	% Recovery ± RSD[a]	LOD[b] (ng/g)	Spiking Level (ng/g)	% Recovery ± RSD	LOD (ng/g)
Soil	2.1	115 ± 7.5	0.52	2.2	102 ± 8.6	0.66
	4.1	109 ± 18		4.3	103 ± 11	
	20.7	107 ± 13		21.7	96 ± 10	
Corn	2.1	101 ± 31	1.9	2.2	101 ± 31	0.84
	4.1	98 ± 15		4.3	80 ± 11	
	20.7	100 ± 9		21.7	70 ± 5	
Soy	2.1	76 ± 33	1.5	2.2	76 ± 33	2.8
	4.1	78 ± 26		4.3	93 ± 28	
	20.7	100 ± 27		21.7	77 ± 19	

[a] The recovery and relative standard deviation calculation is based on duplicate spiking experiments performed on three different days.

[b] LOD is calculated based on 9 spikes (triplicate experiments performed on three different days) at the lowest spiking level using EPA MDL definition.

Table 3: Aminocyclopyrachlor and Aminopyralid method performance parameters.

minimum of seven aliquots of the sample is processed through the entire analytical procedure. The MDL is calculated as follows:

LOD = t(n-1, 1-μ = 0.99)(S), where

LOD = the method detection limit (MDL)

t(n-1, μ-1 = 0.99) = the students' t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom. For nine replicates, t = 2.896

S = standard deviation of the replicate analyses

4. Results and Discussion

4.1. Limit of Detection and Calibration Linear Dynamic Range

Studies were undertaken to determine LOD in soil, soybean meal, and corn meal. These test matrices were spiked with known amounts of mixed aminocyclopyrachlor and aminopyralid solution and subjected to the extraction procedure.

Following filtration, the sample was subjected to LC-ESI-MS/MS analysis and analyte recoveries were determined by external calibration with standards prepared with a dynamic range covering 3 orders of magnitude from 0.2 - 200 ng/mL. Calibration curves were generated using a quadratic regression with 1/x weighting which routinely generated curves with r values of > 0.999 (Figure 5). The internal standard response from 5-Amino-2-chlorobenzoic acid was used for data normalization and matrix interference monitoring (Figure 4). Method development study results are presented in Table 3 detailing fortification levels and percent recoveries for aminocyclopyrachlor and aminopyralid from the test matrices. The LOD studies revealed that soil had the lowest LOD (minimum detection level), approximately 0.50 and 0.66 ng/g for aminocyclopyrachlor and aminopyralid, respectively. Extractions from soy meal produced an LOD of 1.5 and 2.8 ng/g for aminocyclopyrachlor and aminopyralid, respectively. Corn meal extractions produced an LOD for aminocyclopyrachlor and aminopyralid of 1.9 and 0.84 ng/g, respectively.

4.2. Accuracy and Precision

To test the ruggedness of the extraction procedure, two more spiking levels were tested for all of the matrices in this study. Additional spiking levels of 4.1 and 20.7 ng/g were used for aminocyclopyrachlor in soil, corn, and soy meal. For aminopyralid, soil, corn, and soy meal were additionally spiked at 4.3 and 21.7 ng/g. The recoveries for aminocyclopyrachlor in soil, soy, and corn at the 4.1 ng/g level were 109% ± 18%, 78% ± 26%, and 98% ± 15%, respectively. Aminopyralid recoveries were 103% ± 11%, 93% ± 28%, and 80% ± 11% in soil, soy, and corn at the 4.3 ng/g level. Recoveries at the 20.7 ng/g level for soil, soy, and corn for aminocyclopyrachlor were 107% ± 13%, 100% ± 27%, and 100% ± 9%, respectively. For aminopyralid at the 21.7 ng/g level, recoveries of 96% ± 10%, 77% ± 19%, 70% ± 5% in soil, soy, and corn, respectively. This information is also presented in tabular form in Table 3. The EPA residue chemistry test guidelines uses 70 - 120% as the acceptable recoveries for spiked samples and evaluation of relative standard deviations as a function of residue level [11]. The EPA ecological effects test guidelines suggests mean recovery between 70 and 120% and relative standard deviation of replicate measurements less than 20% as quality objectives and recognizes that not all methods can meet the precision objectives [12]. In the study presented here, most of our recoveries are between 70 to 120% and the relative standard deviations are less than 20%.

4.3. Matrix Effect

During development, we also investigated aminocyclopyrachlor and aminopyralid extractions from pinto bean meal and orange puree as an example of matrices with high protein content or are highly acidic. Unfortunately, significant matrix effects were observed with pinto meal and orange puree during the analysis, generally observed as signal enhancement (data not shown).

To decrease the effects of matrix enhancement observed in difficult matrices, one could employ matrix-matched calibration standards that could theoretically normalize the enhancement phenomenon. Dilution of the final sample extract is another means of decreasing matrix effects, but at the expense of decreasing the sensitivity of the method. Additionally, matrix clean up steps could be introduced to eliminate the matrix elements responsible for signal enhancement. Nanita et al. incorporated a filtration approach that retained matrix and allowed nearly quantitative filtration of aminocyclopyrachlor through the filter, which significantly minimized matrix effects [6]. Tian et al. employed dispersive SPE with GBC (graphitized black carbon) to minimize matrix effects and achieve acceptable recoveries of aminopyralid in addition to two other pyridine carboxylic acids reproducibly in a variety of vegetable- and fruit-based matrices [10]. Unfortunately, Sack et al. concluded that the QuEChERS approach to extraction and minimization of matrix effect does not provide adequate recoveries of aminocyclopyrachlor but provides acceptable recoveries of aminopyralid from matrices identified in the FDA total diet study [9]. Taken together and with regard to orange fruit matrix, it is conceivable that one of the methods could be employed to minimize matrix interference to reliably quantitate aminocyclopyrachlor in orange puree or other highly acidic matrices.

5. Conclusions

Straightforward extraction method without extensive matrix cleanup steps was developed for aminocyclopyrachlor and aminopyralid and successfully demonstrated with soil, soy bean meal, corn meal matrices. Overall, the recoveries were acceptable and the method provides a reliable and simplified approach to the extraction, detection, and quantification of two auxin mimic herbicides containing both anionic and cationic functional groups.

6. Declaration of Conflicting Interest

The authors declare no conflict of interest to the presented work.

7. Article Information

This article was received April 26, 2018, in revised form July 2, 2018, and made available online November 5, 2018.

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