TDL.ORG TEXAS DIGITAL LIBRARY

Journal of Regulatory Science

http:\\journalofregulatoryscience.org

Regulatory Science

Journal of Regulatory Science 02 (2016) 9–18

Direct Determination of 2,4-dichlorophenoxyacetic acid in Soybean and Corn by Liquid chromatography/tandem mass spectrometry

Narong Chamkasem[a,*], Cynthia Morris[a]

[a]*Southeast Regional Laboratory, U.S. Food and Drug Administration, 60 Eighth Street, N.E., Atlanta, GA, 30309*

Abstract

A simple high-throughput liquid chromatography/tandem mass spectrometry (LC-MS/MS) method was developed for the determination of 2,4-dichlorophenoxy acetic acid (2,4-D) in soybean and corn using a reversed-phase with weak anion-exchange and cation-exchange mixed-mode Acclaim™ Trinity™ Q1 column. The method involved an alkaline hydrolysis with sodium hydroxide in order to convert all forms of 2,4-D into a salt form prior to extraction with acidified acetonitrile. The acetonitrile was salted out of the extract with sodium chloride and magnesium sulfate. The acetonitrile extract was diluted 1:1 with water and filtered before analysis by LC-MS/MS in a negative mode. Recoveries were evaluated at 10, 100, and 500 ng/g with seven replicates. Mean recovery ranged from 86 to 107% with relative standard deviation of less than 10%. Matrix suppression was not observed in this procedure; therefore, no internal standard was needed. The method was used successfully for the determination of 2,4-D in soybean and corn samples containing incurred residue. The procedure proved to be quick, accurate, precise, sensitive, and selective.

Keywords:
2,4-D, soybean, corn, LC-MS/MS, direct determination

1. Introduction

Herbicide-resistant crop technology has led to a 239 million kilogram increase in herbicide use in the United States between 1996 and 2011 [1]. In September 2014, EPA approved the use of new herbicide, Enlist Duo, a combination of 2,4-D and glyphosate, on genetically engineered corn and soybeans that will tolerate 2,4-D and glyphosate [2]. It was developed by Dow AgroSciences, as the answer to severe weed resistance problems that are limiting crop productions around the country. As a result of this approval, the volume of 2,4-D sprayed could drive herbicide usage upward significantly. According to FDA (40CFR180.142), the tolerance of 2,4-D for soybean and corn are 0.02 and 0.05 μg/g [3]. A quick, accurate, and sensitive method to determine this herbicide in food grains must be developed to support regulatory action.

2,4-D is one of most the popular acid herbicides used to kill broad leaf weeds and the second most used selective herbicide in the world, next to glyphosate [4]. It has proven to be an endocrine disruptor. GC, GC/MS and LC-MS/MS have been used for the determination of acid herbicide residues [5–7]. The analyte needs to be derivatized before GC analysis which will affect the accuracy of the results. Additionally, the reagents used are normally toxic. LC-MS/MS does not require a derivatization step and can provide better sensitivity and selectivity.

The polar nature and high water solubility of this acidic herbicide make its specific extraction very difficult in the case of complicated matrices such as cereals. They contain a large number of co-extracted components that may adversely affect extraction efficiency and quantitative determination. For this reason, the analysis of the residue requires specific sample preparation in order to release the corresponding acids and provide high extraction efficiency. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction method has been used for pesticides extraction in crops successfully over the past 10 years [8]. However, acetonitrile alone in conventional QuEChERS is a poor solvent to extract 2,4-D and other acid herbicides from these samples [9–11]. Acidified acetonitrile with formic acid was used to improve extraction of 2,4-D in crops [7, 12]. Acid was used to suppress the dissociation of 2,4-D and to facilitate the transfer of the undissociated molecular species to the organic phase. Because 2,4-D might exist in different forms (free acid, salt, and ester), direct extraction of sample without proper pretreatment will likely lead to a decrease in recovery.

*Corresponding author:Narong Chamkasem, Tel: (404)-253-2302; FAX: 404-253-1209. E-mail address: narong.chamkasem@fda.hhs.gov, Address:Southeast Regional Laboratory, U.S. Food and Drug Administration, 60 Eighth Street, N.E., Atlanta, GA, 30309

Table 1. Preparation of calibration standards.

acetonitrile:water 1:1 (µL)	990	950	900	975	950	900	850
2,4-D standard 0.1 µg/mL (µL)	10	50	100	0	0	0	0
2,4-D standard 1 µg/mL (µL)	0	0	0	25	50	100	150
final volume (µL)	1000	1000	1000	1000	1000	1000	1000
final concentration (ng/mL)	1	5	10	25	50	100	150

Table 2. Preparation of fortified samples.

fortification level (ng/g)	standard 2,4-D 1 ng/µL (µL)	10 ng/µL (µL)	100 ng/µL (µL)	expected conc. in the final extract (ng/mL)
0	0	0	0	0
10	50	0	0	2.5
100	0	50	0	25
500	0	0	25	125

Alkaline hydrolysis was used prior to acetonitrile extraction to hydrolyze the salt and ester forms of 2,4-D which were later converted to the acid form with pH adjustment to improve the extraction yield of 2,4-D in crops and soil samples [4, 13, 14].

This study describes a single laboratory validation of an LC-MS/MS method under a negative ion-spray ionization mode for the direct determination of 2,4-D in soybean and corn. It also provides a quick and reliable extraction method that requires small sample size, non-toxic solvent, and an effective sample cleanup procedure to ensure a rugged, sensitive, and selective method.

2. MATERIAL AND METHODS

2.1. Chemicals and Materials

Pesticide standard of 2,4-D ($\geqslant$ 99% purity) was purchased from LGC Standards (Manchester, NH). Acetonitrile and water of HPLC grade were obtained from Fisher Scientific (Pittsburgh, PA). Formic acid was obtained as 98% solution for mass spectrometry from Fluka (Buchs, Switzerland.). Sodium hydroxide and sulfuric acid were purchased from Fisher Scientific (Pittsburgh, PA). Anhydrous magnesium sulfate powder (6 g) and sodium chloride powder (1.5 g) grade were purchased from UCT (Bristol, PA). Extracting solvent (1% formic acid in acetonitrile) was prepared by mixing 10 mL of formic acid in 990 mL of acetonitrile. Mini-UniPrep 0.2 μm RC syringeless filters were obtained from Agilent Technologies (Palo Alto, CA). EDP 3 electronic pipettes at different capacities (0-10 μL, 10-100 μL, and 100-1000 μL) were purchased from Rainin Instrument LLC (Oakland, CA) and were used for standard fortification.

A solution of 500 mM ammonium formate/formic acid (pH 2.9) was prepared as follows: 15.76 g of ammonium formate were dissolved in approximately 300 mL of HPLC water and adjusted with 98% formic acid (approx. 28.3 mL) until the pH reached 2.9 (using pH meter), and the solution was diluted to 500 mL with water. The HPLC mobile phase A was prepared by mixing 100 mL of the 500 mM buffer solution with 900 mL of purified water to obtain a solution of 50 mM ammonium formate. Mobile phase B was acetonitrile.

2.2. Standard Preparation

The stock solution of 2,4-D at 100, 10, 1, and 0.1 μg/mL were prepared by dissolving the stock standard in methanol. The solutions were maintained at 4 °C in amber glass bottles. The calibration standards were prepared at the concentrations from 1 to 150 ng/mL with acetonitrile:water (1:1 v/v) as shown in Table 1.

2.3. Sample Preparation and Extraction Procedure

Organic soybean and corn were obtained from a local market. The samples were ground with a food processor until they had powder-like texture. The samples were weighed at 5 g each in 50-mL polypropylene centrifuge tubes (Fisher Scientific, Pittsburgh, PA) and fortified with standard solutions at 10, 100 and 500 ng/g (7 replicates) as listed in Table 2. The samples were allowed to stand at room temperature for 1 hour along with a sample blank and then stored in a freezer overnight to let the analyte be absorbed by the samples. On the extraction day, the spiked samples were allowed to thaw to room temperature. Purified water (15 mL) and a solution of 5 N sodium hydroxide

Table 3. MRM conditions and retention time of 2,4-D for LC-MS/MS analysis.

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	DP	CE	EP	CXP	Retention Time (min)
2,4-D.1	218.9	160.9	-20	-20	-10	-18	5.8
2,4-D.2	220.9	162.9	-35	-20	-10	-15	5.8

Compound dependent parameters: DP = declustering potential, CE = collision energy, EP = entrance potential, CXP = collision cell exit potential

(300 μL) were added into each tube using an automatic pipette. The tubes were capped tightly and shaken for 30 minutes on a SPEX 2000 Geno grinder (SPEX Sample Prep LLC, Metuchen, NJ) at 2000 stroke/min. A solution of 5 N sulfuric acid (300 μL) and 10 mL of the extracting solvent were added into the tubes. The tubes were capped and shaken on the Geno grinder for 2 min at the same speed. A mixture of 6 g of anhydrous magnesium sulfate powder and 1.5 g of sodium chloride powder were added into the tubes. The tubes were capped and shaken for another 2 minutes. The sample tubes were then centrifuged at 3000 rpm for 5 min. The acetonitrile phase (250 μL) was pipetted into the sample chamber of a Mini-UniPrep 0.2 um RC syringeless filter containing 250 μL of purified water. The filter plunger was partially inserted and the filter assembly was vortexed briefly on a vortex mixer. At this point, some precipitates were formed due to the poor solubility of non-polar co-extractive in 1:1 water:acetonitrile. The dilution-and-filtration step is a faster technique to minimize some of the co-extract lipophilic compounds in the acetonitrile extract than the overnight freezing technique [14]. Finally, the filter plunger was depressed into the sample chamber until it reached the bottom. Clean filtrate was passed through the filter element to fill the sample reservoir. The filter replaced the need of syringe filter, syringe, auto-sampler vials, septa, and cap for a quick sample filtration. A 10 μL volume of sample was injected into the LC-MS/MS system. Validation criteria included method detection limit (MDL), method quantification limit (MQL) accuracy (recovery %), precision,(relative standard deviation, RSD%) and comparison to residue levels in samples reported by other sources. Protocols for validation followed those provided in the FDA Foods Program Guidelines, SRL-QMS.5.4.5 [15] and AOAC Requirement for Single Laboratory Validation of Chemical Methods [16].

2.4. LC-MS/MS Analysis

LC-MS/MS analysis was performed by using a Shimadzu HPLC system. The instrument was equipped with two LC-20AD pumps, a Sil-20AC autosampler, and a CTO-20AC column oven (Shimadzu, Kyoto, Japan), coupled with a 5500 Q-TRAP mass spectrometer from AB SCIEX (Foster City, CA). The Analyst software (version 1.6) was used for instrument control and data acquisition. Nitrogen and air from TriGas Generator (Parker Hannifin Co., Haverhill, MA) were used for nebulizer and collision gas in LC-MS/MS. An Acclaim™ Trinity™ Q1 (3 μm, 100 x 3 mm) from Thermo Scientific (Sunnyvale, CA) and a C18 SecurityGuard guard column (4 x 3 mm) from Phenomenex (Torrance, CA) were used for HPLC separation at 40 °C with sample injection volume of 10 μL. The mobile phase was A:B at 3:7 50 mM ammonium formate (pH 2.9):acetonitrile isocratic at a flow rate of 0.5 mL/min for a total run time of 10 min. A diverter valve was connected between the HPLC column outlet and mass spectrometer to direct the column flow to waste at 0-4.8 min and 6.8-10 min. The MS determination was performed in negative electrospray mode with monitoring of the two most abundant MS/MS (precursor/product) ion transitions using a scheduled MRM program of 100 seconds. These were used so that quantification and confirmation could be performed with a single injection along with retention time to confirm the presence of the 2,4-D in the sample. Analyte-specific MS/MS conditions and HPLC retention times for the analytes were shown in Table 3. The MS source conditions were as follows: curtain gas (CUR) of 30 psi, ion spray voltage (ISV) of -4500 volts, collisionally activated dissociation gas (CAD) is high, nebulizer gas (GS1) of 60 psi, heater gas (GS2) of 60 psi, source temperature (TEM) of 600 °C.

3. RESULTS AND DISCUSSION

3.1. Chromatography Optimization

The Acclaim™ Trinity™ Q1 column is a weak cation, weak anion/reversed phase column that is capable of separating ionized compounds along with neutral ones. Previously, a mobile phase containing 50 mM ammonium formate (pH 2.9) was used with this column to determine glyphosate, AMPA, and glufosinate in soybean and corn by LC-MS/MS (Figure 1) [17]. Since glyphosate and 2,4-D may be applied in the same crops to kill grass and broad leaf weeds in genetically modified crops, it would be practical to analyze these two compounds using the same column on the same LC-MS/MS instrument to save time

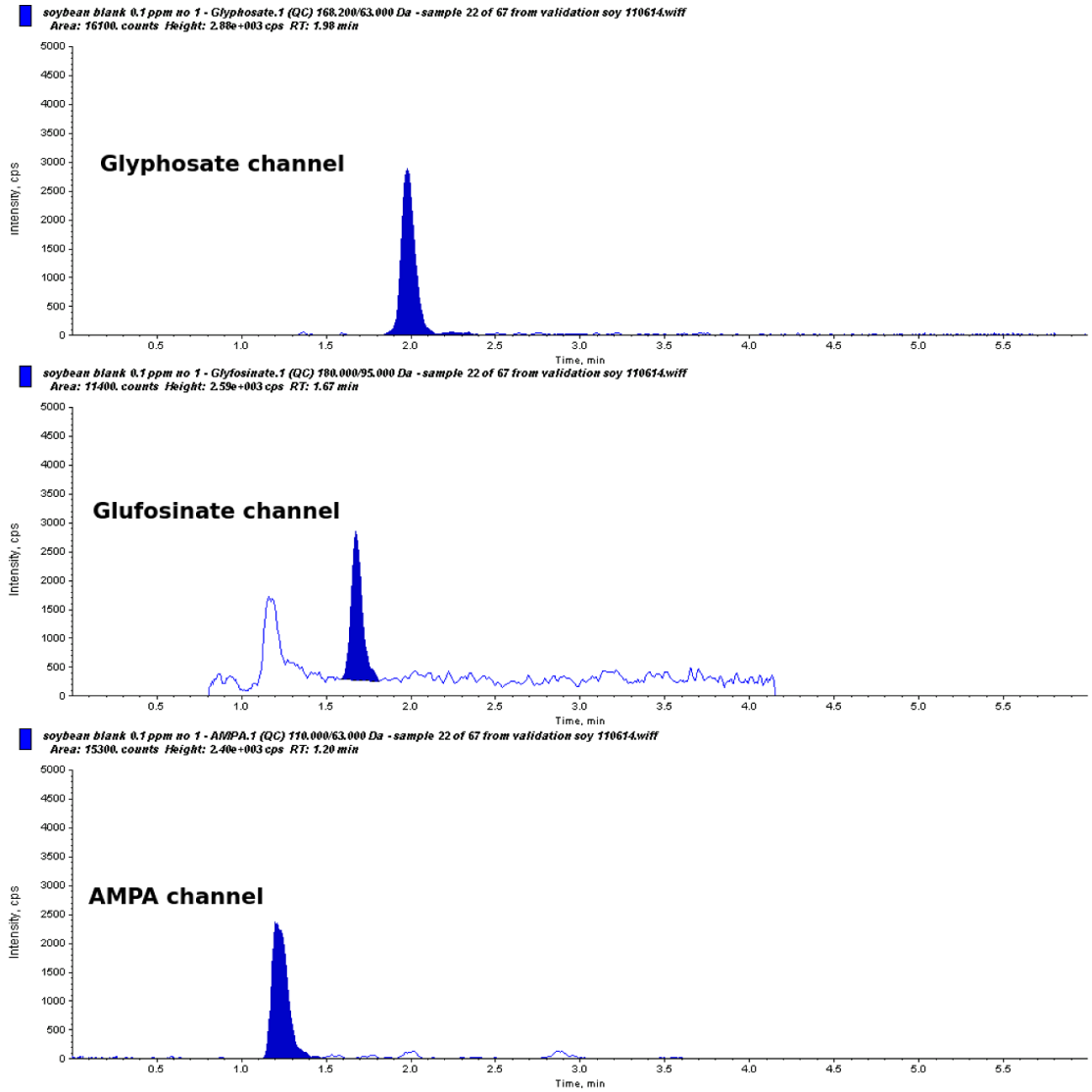

Figure 1: Chromatogram of soybean blank fortified at 0.1 μg/g of glyphosate, glufosinate and AMPA on the Acclaim™ Trinity™ Q1 with 50 mM ammonium formate (pH 2.9) at a flow rate of 0.5 mL/min.

and cost of analysis. 2,4-D is a relatively strong acid with a pKa of 2.73 and was widely analyzed on a reversed-phase gradient HPLC using acidic mobile phase of water and acetonitrile [4, 11, 13, 14]. The attempt to use this column in a similar manner was not successful due to the slow equilibrium behavior of the stationary phase that produces poor retention time reproducibility. 2,4-D was not eluted from the column using 50 mM ammonium formate (pH 2.9) in a reasonable time (less than 15 min). Several isocratic elution experiments of the 50 mM ammonium formate (pH 2.9) with different proportions of acetonitrile were then evaluated. It was found that when the concentration of acetonitrile was increased from 30% to 70%, the retention time was decreased from 18 to 5.2 minutes (Figure 2). However, by replacing ammonium formate solution with 0.1% formic acid in water as it was normally used in most of the 2,4-D HPLC methods, the 2,4-D peak shape was too broad to be practical. Therefore, the separation mechanism for 2,4-D on this column may be the combination of reversed-phase and ion-exchange modes where a certain amount of salt and acetonitrile are needed to elute it from the column. Higher acetonitrile content (90%) did not give higher response or sharper peak shape. Moreover, the solubility of 50 mM of ammonium formate in 90% or higher acetonitrile was very poor. Therefore, the mobile phase A:B 50 mM ammonium formate pH (2.9):acetonitrile at 3:7 (v/v) was chosen for this work. The major benefit of this setup is that after a set of samples are analyzed for 2,4-D, the same set of samples can be analyzed for glyphosate, glufosinate, and AMPA by switching to 100% mobile phase A with the appropriate acquisition method. However, the HPLC column should be equilibrated with the second mobile phase for at least 1 hour at a flow rate 0.5 mL/min before use.

3.2. Optimization of Sample Extraction Procedure

Acetonitrile alone could not extract 2,4-D and other acidic herbicides from food crops after the salting out step as part of

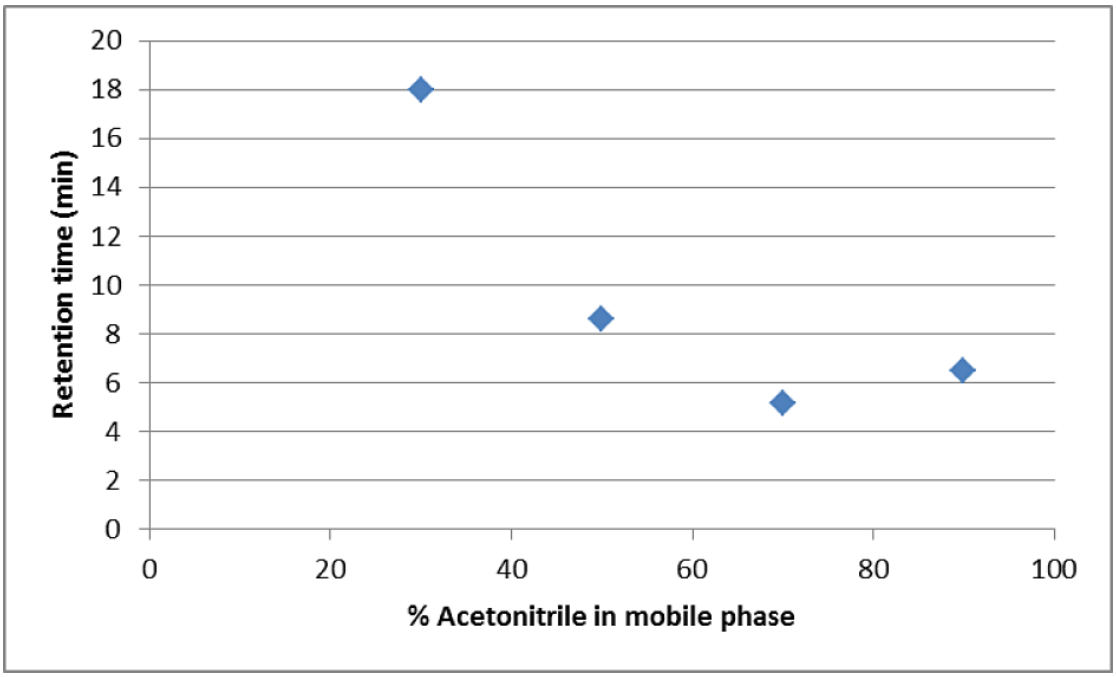

Figure 2: Effect of acetonitrile concentration in the mobile phase on the retention of 2,4-D on the Acclaim™ Trinity™ Q1 column.

the QuEChERS extraction procedure [9, 11]. 2,4-D is a relatively strong acid (pKa = 2.73); therefore, an aqueous solvent with low pH is necessary to suppress 2,4-D into a protonated neutral form before being extracted into acetonitrile [7]. Primary and secondary amine (PSA) sorbent, a weak anionic exchanger which was routinely used in the dispersive cleanup step of the QuEChERS method to absorb polar organic acids, sugars and fatty acid from food matrix would also strongly interact with 2,4-D [11]. Therefore, the dispersive cleanup step with PSA was not used in this work. To improve extraction efficiency of 2,4-D from plant matrix, alkaline hydrolysis with sodium hydroxide was used to free up bound residue before the extraction steps [4, 13, 14]. In order to effectively evaluate the extraction procedure, four different matrices with incurred residue of 2,4-D were used in the experiment. They were corn, soybean, sunflower seeds, and roasted peanuts. Initially, two extraction procedures were evaluated:1) alkaline hydrolysis followed by pH adjustment prior to acetonitrile extraction and 2) 1% formic acid in acetonitrile extraction.

Method 1) Alkaline hydrolysis followed by pH adjustment prior to acetonitrile extraction followed by a partition step.

Sample (5 g) was shaken with 15 mL of water and 300 μL of 5 N sodium hydroxide solution in a 50-mL centrifuge tube on a Geno grinder at 2000 stroke/min for 30 min. Sulfuric acid solution (5N) was added (300 μL) into the tube along with 10 mL of acetonitrile. The tube was shaken for another 2 min at the same speed. A mixture of 6 g of magnesium sulfate and 1.5 g of sodium chloride were added into the tube. The tube was capped and shaken for another 2 minutes. The sample tube was then centrifuged at 3000 rpm for 5 min. The acetonitrile extract (1 μL) was injected to LC-MS/MS.

Method 2) Acidic aqueous acetonitrile extraction followed by a partition step.

Sample (5 g) was shaken with 15 mL of water and 10 mL of 1% formic acid in acetonitrile in a 50-mL centrifuge tube on a Geno grinder at 2000 stroke/min for 30 minutes. A mixture of 6 g of magnesium sulfate and 1.5 g of sodium chloride were added into the tube. The tube was capped and shaken for another 2 minutes. The sample tubes were then centrifuged at 3000 rpm for 5 min. The acetonitrile extract (1 μL) was injected to LC-MS/MS.

Table 4 demonstrates that the extraction method 1 with alkaline hydrolysis yielded more 2,4-D in corn, soybean and sunflower seed than extraction method 2. The sodium hydroxide solution was needed to convert all forms of 2,4-D (acid, salt, and ester) into a salt form, and then the pH of the solution was lowered with sulfuric acid. As a result, 2,4-D was converted into its acid form and could be extracted with acetonitrile. Surprisingly, for the roasted peanuts, the method 1 yielded much less 2,4-D than method 2. Roasted peanuts may raise pH of the sample extract so that after adding the sulfuric acid solution, the pH may not be low enough to fully suppress the ionization of 2,4-D. To improve the extraction yield, method 1 was modified by replacing acetonitrile with 1% formic in acetonitrile to lower the pH of the sample extract (Method 3).

As seen in Table 4, method 3 improved the amount of 2,4-D found in the roasted peanuts. Results were in line with the amount of 2,4-D found in method 2. This experiment had shown that alkaline hydrolysis was needed to extract 2,4-D from sample matrix and pH of the sample extract must be low enough to suppress 2,4-D into the acid form. Therefore, the extraction method 3 was chosen for this work.

Since the alkaline hydrolysis was one of the important fac-

Table 4. Incurred residue of 2,4-D found in the samples using three different extraction methods.

Matrix	Extraction method	residue found (ng/g)	CV (%) n = 2
corn	1	2.11	9.34
corn	2	1.49	9.18
soybean	1	13.51	4.45
soybean	2	10.59	2.94
sunflower seed	1	13.51	4.45
sunflower seed	2	10.59	2.94
Roasted peanut	1	6.21	2.58
Roasted peanut	2	11.97	0.24
Roasted peanut	3	11.82	3.47

Table 5. Alkaline hydrolysis experiments on 2,4-D extraction yield from soybean.

Sample treatment	2,4-D found (ng/g)	% improvement
without hydrolysis 30 min shake	4.78	
with hydrolysis 5 min shake	5.18	8.4
with hydrolysis 15 min shake	5.14	7.5
with hydrolysis 30 min shake	5.9	23.4
with hydrolysis 60 min shake	4.74	-0.8

% improvement = [(2,4-D found in the treatment) - 4.78) /4.78] x 100

tors for the 2,4-D extraction efficiency, the duration of hydrolysis was also evaluated. Five 5-g of soybean samples with incurred residue of 2,4-D were put into five 50-mL centrifuge tubes. Tube no 1 was shaken with 15 mL of water for 30 minutes (no alkaline hydrolysis as the baseline sample). Tube no 2, 3, 4, and 5 were shaken for 5, 15, 30, and 60 min on the Geno grinder at 2000 stroke/min after the addition of 15 mL of purified water and 300 μL of 5 N sodium hydroxide. The samples were acidified immediately with 300 μL of 5 N sulfuric acid and extracted with 10 mL of 1% formic acid in acetonitrile for 2 min. After salting out with magnesium sulfate and sodium chloride per method 3, the acetonitrile layer (1 μL) was injected to LC-MS/MS. Table 5 showed that 30-minutes was the optimum time to extract 2,4-D from the soybean samples. It improved the extraction yield up to 23 % over the non-hydrolysis method. Longer hydrolysis time up to 60 minutes may have had a detrimental effect on the extraction yield. Therefore, the 30-minutes alkaline hydrolysis was chosen in this work.

3.3. Evaluation of Matrix Effects

Many components extracted with the analyte during the extraction process can affect the quality of the analytical results. This is known as matrix effect and is normally unavoidable. The matrix effect on the detector response was investigated by comparison of the response differences between matrix-matched standard solutions and pure solvent standard solutions. Matrix effect (%ME) in the sample extract was calculated as the ratio of analyte response in sample matrix and analyte response in water multiplied by 100. Therefore, a value of 100% means that no matrix effect is present. If the value is less than 100%, it means that there is matrix suppression. If the value is more than 100%, it means that there is matrix enhancement. The acetonitrile extract of soybean and corn samples (no incurred residue) were diluted with water at the ratios of 1:1, 1:2, and 1:3, respectively. These samples were filtered to eliminate small particulates and then fortified with 2,4-D at 50 ng/mL and injected along with a 2,4-D solution in water at the same concentration. The injection volume was set at 1, 5, and 10 μL to evaluate the degree of matrix effects. Table 6 demonstrated that there was

Table 6. Matrix effect evaluation of soybean and corn extract

Matrix	Dilution ratio of acetonitrile extract:water	Injection volume (µL)	Matrix effect (%ME)
Soybean	1 to 1	1	107
	1 to 2	1	105
	1 to 3	1	102
	1 to 1	5	109
	1 to 2	5	103
	1 to 3	5	101
	1 to 1	10	112
	1 to 2	10	104
	1 to 3	10	104
Corn	1 to 1	1	98
	1 to 2	1	102
	1 to 3	1	103
	1 to 1	5	107
	1 to 2	5	102
	1 to 3	5	99
	1 to 1	10	112
	1 to 2	10	104
	1 to 3	10	104

Table 7. Recovery (%) and RSD (%) data obtained in the validation experiments (n = 7)

Matrix	Fortification level (ng/g)	Recovery (%)	RSD (%)
Soybean	10	85.6	5.45
	100	90.5	2.93
	500	95.4	3.19
Corn	10	100.2	2.32
	100	98.3	2.7
	500	107.5	9.66

no significant matrix effect on both matrices at three different dilution ratios and three different injection volumes. Therefore, the dilution ratio of 1:1 and inject volume of 10 μL were used in this method in order to lower the limit of quantification.

3.4. Method Validation

The calibration standard solutions at concentrations from 1 to 150 ng/mL were prepared in acetonitrile:water(1:1 v/v) (Table 1). These standard solutions were injected along with the fortified samples and sample blank as described in the Table 2. The linearity was evaluated and they showed satisfactory linearity with coefficient of determination (R^2) of more the 0.998 (1/x weighing). The specificity of the method was evaluated by analyzing blank samples and blank samples spiked at the lowest fortification level of 10 ng/g. No relevant signal (above 20%) was observed at any of the transitions selected in the blank sample. A reagent blank was injected immediately after the 150 ng/mL standard and no analyte signals were detected above 10% of the 1 ng/mL standard at the retention time of 2,4-D.

The method detection limit (MDL) for 2,4-D was calculated

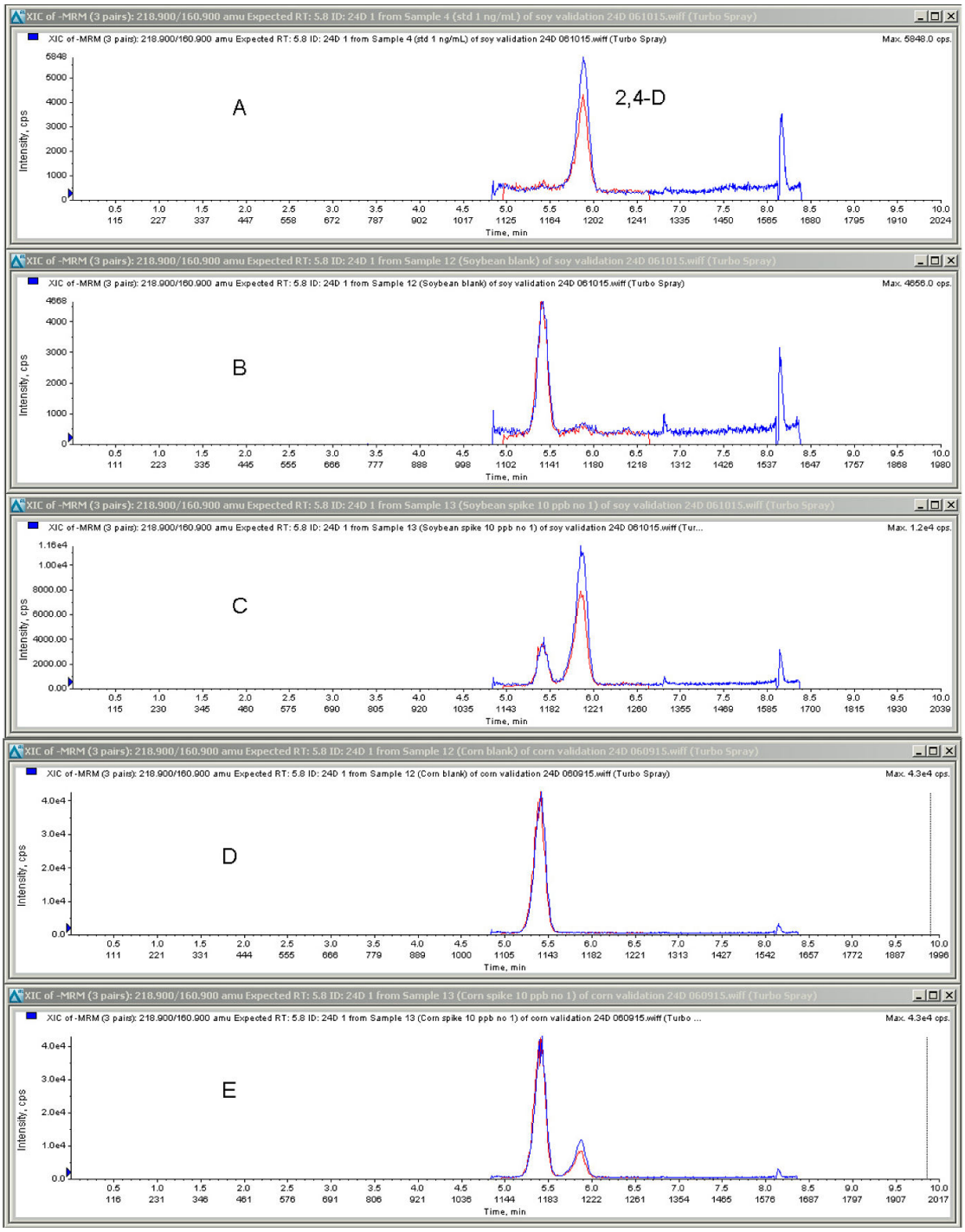

Figure 3: Chromatograms of 2,4-D standard 1 ng/mL (A), soybean blank (B), soybean blank fortified with 2,4-D at 10 ng/g (C), corn blank (D), and corn blank fortified with 2,4-D at 10 ng/g

according to FDA guidelines with 7 replicates of the lowest fortification level (10 ng/g). The MDL was calculated by multiplying standard deviation of 2,4-D found in 7 fortified samples by the t value at a degree of freedom of 6 (3.14). The MDL for 2,4-D for soybean and corn samples were 1.46 and 0.72 ng/g, respectively. The method quantification limit (MQL) was three times the MDL which were 4.38 and 2.16 ng/g for soybean and corn.

Accuracy (recovery %) and precision (relative standard deviation or RSD %) were evaluated at the fortification levels of 10, 100, and 500 ng/g with seven replicates in both soybean and corn samples (Table 7). The average recovery ranged from 85−107% with the RSD of less than 10% for both matrices. Since the matrix suppression was not an issue as demonstrated previously, a standard solution in solvent can be used for quantification and neither internal standard nor matrix-matched

Table 8. Results of 2,4-D found in samples received from other agencies with reported values

Sample	Tolerance (ppm) 40CFR180.142	Found (µg/g) by this method	Reported value (µg/g)	Source
Soybean (14A)`	0.02	0.008	0.01	USDA
Soybean (14B)	0.02	0.008	0.01	USDA
Corn Forage	6	7.4	9.4	Dow AgroSciences
Corn Stover	50	7.5	8.1	Dow AgroSciences
Soybean forage	0.02	10.5	11.2	Dow AgroSciences
Soybean hay	2	16.3	23.1	Dow AgroSciences

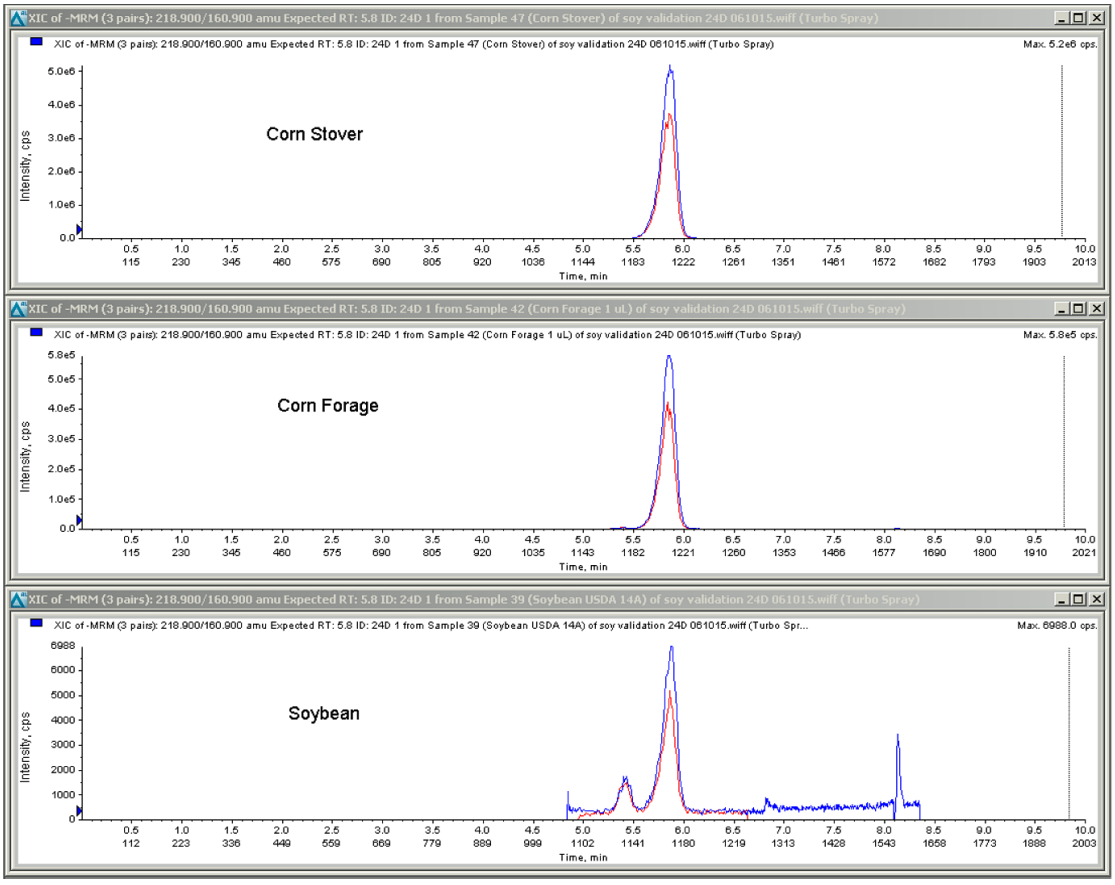

Figure 4: Chromatograms of corn stover, corn forage, and soybean containing incurred residue of 2,4-D at 7.5, 7.4, and 0.008 (μg/g). (sample extract was diluted to the appropriate dilution before analysis

standard is required. This is a significant improvement over other methods [4, 10, 11, 13, 14]. Chromatograms of 2,4-D in soybean blank, soybean blank fortified at 10 ng/g, corn blank and corn blank fortified at 10 ng/g are shown in Figure 3. No significant inferences were observed in the blank samples where the analyte was eluted.

Finally, an additional verification of the method performance was made by analyzing samples with reported residue from other sources. Two soybean samples from USDA and four samples (corn stover, corn forage, soybean forage and soybean hay) from Dow AgroSciences were analyzed by the proposed method. The results were comparable to the results obtained from the validated methods used by the two agencies (Table 8). Chromatograms of corn stover, corn forage, and soybean containing incurred residue of 2,4-D at 7.5, 7.4, and 0.008 μg/g were shown in Figure 4.

4. CONCLUSION

In this work, an acidic acetonitrile extraction of 2,4-D herbicide residue in soybean and corn after alkaline hydrolysis was described. The optimum sample extraction parameters were

evaluated to maximize the extraction yield. The water dilution of the acetonitrile extract with sample filtration effectively reduces co-extractive lipophilic interference in the sample extract. No significant matrix suppression was observed during the matrix evaluation. A calibration curve constructed from standard solutions prepared in solvent was used for the quantification of 2,4-D in the samples with good accuracy and precision at the fortification levels of 10, 100, and 500 ng/g. This is a significant improvement over other methods that require internal standard or matrix matched standard to correct for matrix effect when LC-MS/MS was used. The mixed-mode Acclaim™ Trinity™ Q1 HPLC column was able to retain 2,4-D on the column and elute in less than 10 min using isocratic mobile phase with good peak shape. The proposed method was developed so that the same HPLC column and LC-MS/MS instrument could be used to determined 2,4-D and glyphosate by just changing the mobile phase compositions (from 30:70 mobile phase A:B to 100% mobile phase A) and use of appropriate acquisition methods. This would allow FDA to effectively screen these widely used herbicides in the same samples with good sensitivity, selectivity, accuracy, and precision in the most cost and time effective manner.

5. Article Information

The article was received January the 25th, 2016, in revised form February the 26th, 2016 and available on-line May the 5th, 2016.

References

[1] C. Benbrook, Impacts of genetically engineered crops on pesticides use in the U.S.-the first sixteen years, Environmental Sciences Europe 24.

[2] Keim, B (25 September 2014). "New Generation of GM Crops Puts Agriculture in a 'Crisis Situation'". Wired (Cond Nast.).http://www.wired.com/2014/09/new-gm-crops/ (last access June 25, 2015).

[3] [link].
URL http://www.ecfr.gov/cgi-bin/text-idx?c=ecfr&sid=f41eea8cfec706a8f961b47685450107&tpl=/ecfrbrowse/Title40/40cfr180_main_02.tpl

[4] S. Liu, Z. Bian, F. Yang, Z. Li, Z. Fan, H. Zhang, Y. Wang, Z. Yang, G. Yang, Sampling and Sample Processing in Pesticide Residue Analysis, J. Agric. Food Chem. 63 (2015) 4395.

[5] H. Kuang, X. Chu, C. C. Xu, Simultaneous determination of 13 phenoxy acid herbicide residues in soybean by GC-ECD, Anal. Lett. 39 (2006) 2617.

[6] E. Crespo-Corral, M. Santos-Delgado, L. Polo-Diez, A. Soria, Determination of carbamates, phenylurea, and phenoxy acid herbicide resdues by gas chromatography after potassium ter-butoxide/dimethyl suphoxide/ethyl iodide derivatization reaction, J. Chromatogr. A 1209 (2008) 22.

[7] U. Koesukwiwat, K. Sanguankaew, N. Leepipatpiboon, Rapid determination of phenoxy acid residues in rice by modified QuEChERS extraction and liquid chromatography-tandem mass spectrometry, Anal. Chim. Act. 626 (2008) 10.

[8] M. Anastassiades, S. J. Lehotay, D. Stajnbaher, F. J. Schenck, Fast and easy multiresidue method employing MeCN extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce, J. AOAC Int. 86 (2003) 412.

[9] O. Lacina, M. Zachariasova, J. Urbanova, M. Vaclavikova, T. Cajka, J. Hajslova, Critical assessment of extraction methods for the simultaneous determination of pesticide residues and mycotoxins in fruits, cereals, spices and oil seeds employing ultra-high performance liquid chromatographytandem mass spectrometry, J. Chromatogr. A 1262 (2012) 8.

[10] C. Diez, W. Traag, P. zommer, P. Marinero, J. Atienza, Comparison of an acetonitrile extraction/partitioning and dispersive solid-phase extraction method with classical multi-residue methods for the extraction of herbicide residues in barley samples, J. Chromatogr. A 1131 (2006) 11.

[11] L. Pareja, V. Cesio, H. Heinzen, A. R. Fenandez-Alba, Evaluation of various QuEChERS based methods for the analysis of herbicides and other commonly used pesticides in polished rice by LC-MS/MS, Talanta 83 (2011) 1613.

[12] W. Xiong, X. Tao, S. Pang, X. Yang, G. Tang, Z. Bian, Separation and quantitation of three acidic herbicide residues in tobacco and soil by dispersive solid-phase extraction and UPLCMS/MS, J. Chromatogr. Sci. 52 (2013) 1326.

[13] A. Schaner, J. Konecny, L. Luckey, H. Kickes, Determination of chlorinated herbicides in vegetation and soil by liquid chromatography/electrospray-tandem mass spectrometry, J. AOAC 90 (2007) 1402.

[14] A. Santilio, P. Stefanelli, S. Girolimetti, R. Dommarco, Determination of acidic herbicides in cereals by QuEChERS extraction and LC/MS/MS, J. of Environmental Science and Health Part B 46 (2011) 535.

[15] SRL-QMS.5.4.5.
URL https://qmis.fda.gov/MC/index.cfm?infocard_id=A4E920BB0C06D04EFB

[16] AOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals.
URL http://www.aoac.org/imis15_prod/AOAC_Docs/StandardsDevelopment/SLV_Guidelines_Dietary_Supplements.pdf

[17] N. Chamkasem, T. Harmon, Direct Determination of Glyphosate, Glufosinate, and AMPA in Soybean and Corn by Liquid chromatography/tandem mass spectrometry, Analytical and Bioanalytical Chemistry, in press.