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RESEARCH PAPER

Direct determination of glyphosate, glufosinate, and AMPA in soybean and corn by liquid chromatography/tandem mass spectrometry

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Abstract Glyphosate, glufosinate, and aminomethylphosphonic acid (AMPA) are amphoteric, low mass, high water soluble, and do not have chromophore. They are very difficult to be retained on a reversed phase HPLC and detected by UV or fluorescence detectors. A liquid chromatography/tandem mass spectrometry (LC-MS/MS) method was developed to determine these analytes in soybean and corn using a reversed phase with weak anion-exchange and cation-exchange mixed-mode Acclaim™ Trinity™ Q1 column. The sample was shaken with water containing ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) and acetic acid for 10 min to precipitate protein and extract the analytes into the solution. The supernatant was passed thru an Oasis HLB SPE to retain suspended particulates and non-polar interferences. The sample was directly injected and analyzed in 6 min by LC-MS/MS with no sample concentration or derivatization steps. Three isotopically labeled internal standards corresponding to each analyte were used to counter matrix suppression effect. Linearity of the detector response with a minimum coefficient of determination (R^2) of more than 0.995 was demonstrated in the range of 10 to 1000 ng/mL for each analyte. Accuracy (recovery %) and precision (relative standard deviation or RSD %) were evaluated at the fortification levels of 0.1, 0.5, and 2 μg/g in seven replicates in both soybean and corn samples.

Keywords Glyphosate · Soybean · Corn · LC-MS/MS · Direct determination

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Introduction

Glyphosate (*N*-phosphonomethyl glycine) and glufosinate (2-amino-4-[hydroxy(methyl)phosphoryl]butanoic acid) are non-selective post-emergence herbicides used for the control of a broad spectrum of grasses and broad leaf weed species in agricultural and industrial fields. AMPA is the major metabolite of glyphosate and also classified as a toxicologically significant compound [1]. According to recent reports, there has been a dramatic increase in the usage of these herbicides which are of risk to both human health and the environment [2]. Glyphosate and glufosinate have high efficacy, low toxicity, and an affordable price when compared with other pesticides. These factors lead to its wide utilization on several crops. Farmers also use glyphosate as a desiccant to rapidly kill aboveground growth of crops such as wheat. This allows for rapid dry down for easy harvest. Due to the low toxicity of glyphosate, the maximum residues levels (MRLs) established around the world are generally greater than the limits for other pesticides. In the USA (40CFR180.364 and 40CFR180.473), the tolerance of glyphosate for soybean and corn are 20 and 5 μg/g and the tolerance of glufosinate in soybean and corn are 2 and 0.2 μg/g [3]. However, some crops such as wheat and oats do not have a tolerance for glyphosate. Therefore, any glyphosate detected above the limit of quantification in these two commodities would be violative. A quick, accurate, and sensitive method to determine these herbicides in food grains must be developed to support the regulatory actions.

Glyphosate, glufosinate, and AMPA are very polar compounds and insoluble in organic solvents. These properties make the use of classical organic solvent extraction very difficult. An aqueous extraction method was used to extract glyphosate and AMPA from soil, plant, and animal matrices [4]. This method required the use of lengthy cleanup procedures that involved both anion and cation exchange columns.

Typical silica-based reversed-phase C18 columns experience difficulty with the retention of such polar compounds and may generate non-resolved co-eluting peaks, often with polar analytes eluting in the void volume. The lack of chromophore or fluorophore also necessitates the use of derivatization techniques for the determination of these analyte residues by liquid chromatography and gas chromatography [4–6]. Vreeken and co-workers developed an analytical method to analyze glyphosate, AMPA, and glufosinate in water samples using a reversed-phase liquid chromatography separation after pre-column derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) and detection by LC-MS/MS [7]. Schreiber and Cabrices streamlined the derivatization by using a special autosampler for automation to determine these polar analytes in corn and soybean [8]. The derivatization technique is not highly regarded by analysts as it requires the optimization of a number of parameters (temperature, reaction time, concentration and purity of the reagents, laboratory handling time). An Acclaim® Mix-mode WAX-1 (reversed-phase/weak anion-exchange) was used to directly determined glyphosate in water [9]. This column experienced bad peak shape of glyphosate after 80–100 sample injections and requires long column generation with EDTA solution to eliminate metal ions accumulated during the analysis. Anion exchange (Dionex IonPac AS11) column was used at high pH mobile phase (pH 11) for glyphosate analysis, and the alkaline-compatible HPLC components were required [10]. An Oblisc-N (HILIC column) was used for underivaztized glyphosate with MS detection in rice, maize, and soybean [11]. However, it had poor column robustness and poor retention time reproducibility.

This study describes a single laboratory validation of an LC-MS method under a negative ion-spray ionization mode for the direct determination of glyphosate, glufosinate, and AMPA 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.

Materials and method

Chemical and material

Pesticide standard (≥99 % purity) were purchased from LGC Standards (Manchester, NH) consisting of glyphosate, AMPA, glufosinate, glyphosate $^{13}C2^{15}N$ (100 μg/mL), AMPA ^{13}C ^{15}N (100 μg/mL), and glufosinate D3. Methanol, 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). Acetic acid, ammonium formate, and ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) were purchased from Fisher Scientific (Pittsburgh, PA). Extracting solvent (50 mM acetic acid/10 mM Na_2EDTA) was prepared by mixing 572 μL of acetic acid and 0.74 g of Na_2EDTA in 200 mL of purified water. Oasis HLB (60 mg) solid-phase extraction cartridge was obtained from Waters (Milford, MA). EDP 3 electronic pipettes at different capacities (0–10, 10–100, 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 was 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 was prepared by mixing 100 mL of the 500 mM buffer solution with 900 mL of purified water, so the final concentration was 50 mM.

Standard preparation

The stock solution of glyphosate, glufosinate, and AMPA at 50, 10, and 1 μg/mL were prepared by dissolving the stock standard in 1:1 (*v*/*v*) water/methanol solution. The solutions were maintained at 4 °C in polypropylene tubes to avoid adsorption to glass. The internal standard (IS) solution of glyphosate $^{13}C2^{15}N$, AMPA $^{13}C^{15}N$, and glufosinate D3 at 2 and 10 μg/mL were prepared by dissolving the stock standard in 1:1 (*v*/*v*) water/methanol solution. The calibration standards were prepared in the extracting solvent or blank matrix extract (after SPE cleanup) with IS solutions for the calibration curves as described in Table 1.

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 2 g each in 50-mL centrifuge tubes (Fisher Scientific, Pittsburgh, PA) and fortified with native standard solutions at 0.1, 0.5, and 2 μg/g (7 replicates). The IS solution (100 μL) at the concentration of 10 μg/mL was added into the samples, so the concentration was 0.5 μg/g for all samples. The samples were allowed to stand at room temperature for 1 h and then stored in a freezer overnight to let the analytes to be absorbed by the sample. A set of five non-fortified samples without IS were also prepared and used for matrix-matched standard. On the extraction day, the spiked samples were allowed to thaw to room temperature. The extracting solvent (10 mL) was added to each tube using an automatic pipette. The tubes were capped tightly and shaken for 10 min on a SPEX 2000 Geno grinder (SPEX Sample Prep LLC, Metuchen, NJ) at 2000 stroke/min then centrifuged at 3000 rpm for 5 min using a Q-Sep 3000 centrifuge (Restek, Bellefonte, PA). Three milliliters of the supernatant was

Table 1 Preparation of calibration standard solutions

Sample extract or extracting solvent (μL)	425	425	425	425	425	425	425
Extracting solvent (μL)	45	37.5	25	0	37.5	25	0
Pesticide mix 1 μg/mL (μL)	5	12.5	25	50	0	0	0
Pesticide mix 10 μg/mL (μL)	0	0	0	0	12.5	25	50
IS 2 μg/mL (μL)	25	25	25	25	25	25	25
Total volume (μL)	500	500	500	500	500	500	500
IS concentration (ng/mL)	100	100	100	100	100	100	100
Final concentration (ng/mL)	10	25	50	100	250	500	1000

passed through an Oasis HLB cartridge (60 mg), previously conditioned with 2 mL methanol and 2 mL of the extracting solvent, and the last milliliter of the extract was collected into an autosampler vial. A 10-μL volume of sample was injected into the LC-MS/MS system. The corresponding matrix concentration in the sample is 0.2 g/mL

LC-MS/MS Analysis

A 5500 Q-TRAP mass spectrometer from AB SCIEX (Foster City, CA) coupled with a Shimadzu HPLC system equipped with two LC-20AD Pumps, a Sil-20AC autosampler, and a CTO-20AC column oven (Shimadzu, Kyoto, Japan) was used. An Acclaim™ Trinity™ Q1 (3 μm, 100 × 3 mm) from Thermo Scientific (Sunnyvale, CA) and a C18 SecurityGuard guard column (4 × 3 mm) from Phenomenex (Torrance, CA) were used for HPLC separation at 35 °C with sample injection volume of 10 μL. The mobile phase is 50 mM ammonium formate (pH 2.9) at a flow rate of 0.5 mL/min for a total run time of 6 min. The MS determination was performed in negative electrospray mode using a scheduled SRM program of 60 s for each analyte. Analyte-specific MS/MS conditions and LC retention times for the analytes are shown in Table 2. 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, and source temperature (TEM) of 350 °C. Nitrogen and air from TriGas Generator (Parker Hannifin Co., Haverhill, MA) were used for nebulizer and collision gas in LC-MS/MS.

Results and discussion

Chromatography optimization

Glyphosate, glufosinate, and AMPA are zwitterionic in aqueous solution that makes them difficult to be retained by a reversed-phase column. Several mixed phase mode columns including (1) Obelisc R (SIELC Technologies, Wheeling, IL), (2) zwitterionic-type mixed mode, Scherzo SM-C18 (Imtakt USA, Philadelphia, PA), (3) mixed beads of cation and anion exchange particles, and Nanopolymer Silica Hybrid, Acclaim™ (Thermo Scientific, Sunnyvale, CA) were evaluated for the use of this study. Different mobile phase parameters were evaluated which included pH (2.9 to 5), acetonitrile concentration (0–100 %), and salt concentration (0–100 mM). It was found that the proposed mobile phase containing 50 mM ammonium formate (pH 2.9) at a flow rate of 0.5 mL/min for the Acclaim™ Trinity™ Q1 (3 μm, 100 × 3 mm) produced the optimum condition for peak shape, retention time, and sensitivity for these three analytes. The

Table 2 Retention time and SRM conditions for LC/MS analysis

Analyte	Precursor ion (*m/z*)	Product ion (*m/z*)	DP	CE	EP	CXP	Retention time (min)
AMPA.1	110	63	−60	−24	−10	−10	1.1
AMPA.2	110	79	−60	−26	−10	−10	1.1
AMPA $^{13}C^{15}N$ (IS)	112	63	−60	−24	−10	−10	1.1
Glufosinate.1	180	95	−46	−23	−10	−10	1.65
Glufosinate.2	180	85	−46	−26	−10	−10	1.65
Glufosinate D3 (IS)	183	63	−46	−26	−10	−10	1.65
Glyphosate.1	168.2	63	−110	−30	−10	−10	2.05
Glyphosate.2	168.2	79	−110	−55	−10	−10	2.05
Glyphosate $^{13}C2^{15}N$ (IS)	171	63	−110	−30	−10	−10	2.05

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

most important parameter for good analyte retention on column was the pH of the mobile phase. At low pH (2.9), glyphosate was retained well in a reasonable time with good peak shape. At higher pH (4.5), glyphosate was retained at a much longer retention time with a wide and tailing peak shape. Acetonitrile in the mobile phase increased ion-spray efficiency and increased the retention, but it resulted in very broad glyphosate peak at pH 2.9. Therefore, acetonitrile was not added in the mobile phase. High salt concentration shortened the retention time of the analytes, but decreased analyte response due to ion suppression. The reversed-phase guard column was used to retained non-polar compounds that may be irreversibly absorbed on the analytical column. After each set of the samples was analyzed, the column was flushed with acetonitrile approximately 20 column volumes to wash out these non-polar compounds.

Optimization of sample extraction procedure

For high protein sample such as soybean, protein precipitation is a common protocol for rapid sample cleanup and extraction [12]. An organic solvent and acid have been used for effecting protein precipitation by exerting specific interactive effects on the protein structure. An organic solvent lowers the dielectric constant of the protein solution and also displaces the ordered water molecules around the hydrophobic regions on the protein surface, the former enhancing electrostatic attractions among charged protein molecules and the latter minimizing hydrophobic interactions among the proteins. Acidic reagents form insoluble salts with the positively charged amino groups of the proteins at pH values below their isoelectric points. EDTA was used to improve extracting efficiency of tetracycline in milk [13–15]. It was found during the method development stage that EDTA significantly improved extraction yield of glyphosate in the sample. A direct determination of these analytes in milk was validated with excellent recovery using 50 mM acetic acid/10 mM Na_2EDTA as the extracting solvent [16]. Acetic acid lowered the pH of the sample to precipitate the protein, and Na_2EDTA may prevent chelation complex between polyvalent metal ions in the sample and the analytes. Na_2EDTA does not dissolve well in either acetonitrile or methanol. The solvent crash method with solvent plus Na_2EDTA is not an appropriate option. Therefore, the proposed extracting solution containing 50 mM acetic acid/10 mM Na_2EDTA was used in the method.

Lecithin is a phospholipid found in soybeans that could be extracted along with the analytes in aqueous solution. It may accumulate at the head of the analytical column under high aqueous mobile phase condition and degrade column performance. Therefore, the Oasis HLB cartridge was added to the method to filter the aliquot and trap the phospholipids and other non-polar compounds in the final extract. Special clean-up cartridges specifically designed for phospholipids such as Captiva (Agilent Technology, Santa Clara, CA) and HybridSPE-plus (SupelCo, Bellefonte, PA) were also evaluated, but gavepoor recovery, presumably because glyphosate and glufosinate have phosphate functional groups similar to those in phospholipids.

To evaluate the optimal extraction time, a soybean sample (2 g) containing incurred residue of glyphosate (~10 μg/g) was put in five 50-mL plastic centrifuge tubes and 10 mL of the extracting solvent was added into each tube. The tubes were shaken on the SPEX 2000 Geno grinder at 2000 stroke/min at 2, 5, 10, 30, and 60 min and then centrifuged at 3000 rpm for 5 min using the Q-Sep 3000 centrifuge. The supernatant was passed through an Oasis HLB cartridge (60 mg), previously conditioned with 2 mL methanol and 2 mL of the extracting solvent, and the last milliliter of the extract was collected into an autosampler vial. Ten microliters of the sample extract was injected into the LC-MS/MS system. The results showed that there was no significant difference in glyphosate concentration in sample extract after the samples were shaken at 5, 10, 30, and 60 min. At 2 min of shaking, the concentration of glyphosate was approximately 70 % of the sample shaken at 5 min. This suggests that 5 min is sufficient for the extraction of glyphosate. However, to ensure efficient extraction even with some variability among samples, an extraction time of 10 min was chosen for this method.

Evaluation of matrix effects

Matrix effect (%ME) in the sample extract was calculated as the slope of calibration curve of analyte in sample matrix divided by the slope of calibration curve of analyte in solvent and 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. Table 3 shows the %ME of all three analytes in both matrices. Glyphosate had minimum degree of suppression (95–101 %) in both matrices, while AMPA had severe suppression (17–

Table 3 Matrix effect evaluation in soybean and corn samples (using calibration curve with linear fit)

	Slope of cal. curve in solvent	Slope of cal. curve matrix	Matrix effect (%ME)
Soybean			
Glyphosate	772	731	95
Glufosinate	755	562	74
AMPA	1499	258	17
Corn			
Glyphosate	812	823	101
Glufosinate	779	718	92
AMPA	1516	455	30

30 %). Glufosinate has less % ME in soybean (74 %) than in corn (92 %). Based on this data, IS may not be needed for glyphosate and glufosinate analysis in soybean and corn (reduces the cost of analysis). However, it is necessary to use IS for AMPA analysis to correct for matrix suppression.

Method validation

The calibration standard solutions at concentrations from 10 to 1000 ng/mL were prepared in both sample matrices (soybean and corn) and extracting solvent with the addition of IS

Table 4 Recovery (%) and RSD (%) data obtained in the validation experiments ($n = 7$)

Matrix	Fortification level (μg/g)	Analyte	Calibration method			
			Matrix with IS	Matrix no IS	Solvent with IS	Solvent no IS
		Glyphosate				
Soybean	0.1	Recovery (%)	103	101	102	97
		RSD (%)	4.3	4.7	3.3	4.7
Corn	0.1	Recovery (%)	100	89	104	105
		RSD (%)	4.8	6.3	3.6	5.4
Soybean	0.5	Recovery (%)	102	100	101	96
		RSD (%)	3.9	2.9	3.5 1	3.0
Corn	0.5	Recovery (%)	104	96	104	99.4
		RSD (%)	4.2	4.0	4.18	3.9
Soybean	2	Recovery (%)	102	103	100	98
		RSD (%)	2.4	3.07	2.36	3.0
Corn	2	Recovery (%)	107	97	106	98
		RSD (%)	3.8	2.7	3.77	2.8
		Glufosinate				
Soybean	0.1	Recovery (%)	102	95	101	76
		RSD (%)	4.3	5.2	4.13	4.9
Corn	0.1	Recovery (%)	92	96	99	97
		RSD (%)	8.64	9.9	4.8	9.1
Soybean	0.5	Recovery (%)	102	100	98	75
		RSD (%)	3.9	1.6	3.83	1.7
Corn	0.5	Recovery (%)	103	99	104	94
		RSD (%)	3.9	3.7	3.7	3.6
Soybean	2	Recovery (%)	98	104	94	76
		RSD (%)	3.0	3.8	3.07	3.8
Corn	2	Recovery (%)	103	99	101	92
		RSD (%)	5.3	3.4	5.3	3.3
		AMPA				
Soybean	0.1	Recovery (%)	101	57	106	NA
		RSD (%)	6.3	28.8	4.5	NA
Corn	0.1	Recovery (%)	96	NA	113	NA
		RSD (%)	11.9	NA	6.5	NA
Soybean	0.5	Recovery (%)	108	78	107	NA
		RSD (%)	6.3	5.76	4.4	NA
Corn	0.5	Recovery (%)	103	8.2	111	NA
		RSD (%)	8.3	48.6	7.8	NA
Soybean	2	Recovery (%)	105	80	108	2
		RSD (%)	7.6	11.2	5.8	63.5
Corn	2	Recovery (%)	105	52	110	10.4
		RSD (%)	6.9	5.8	6.9	9.3

NA not applicable

(Table 1). These standard solutions were injected along with the fortified samples and sample blank as previously described. For comparison purposes, four different quantification methods were used to determine the accuracy and precision of the recovery results. They were (a) standard in matrix with internal standard calibration method, (b) standard in matrix with external calibration method, (c) standard in solvent with internal standard calibration method, and (d)

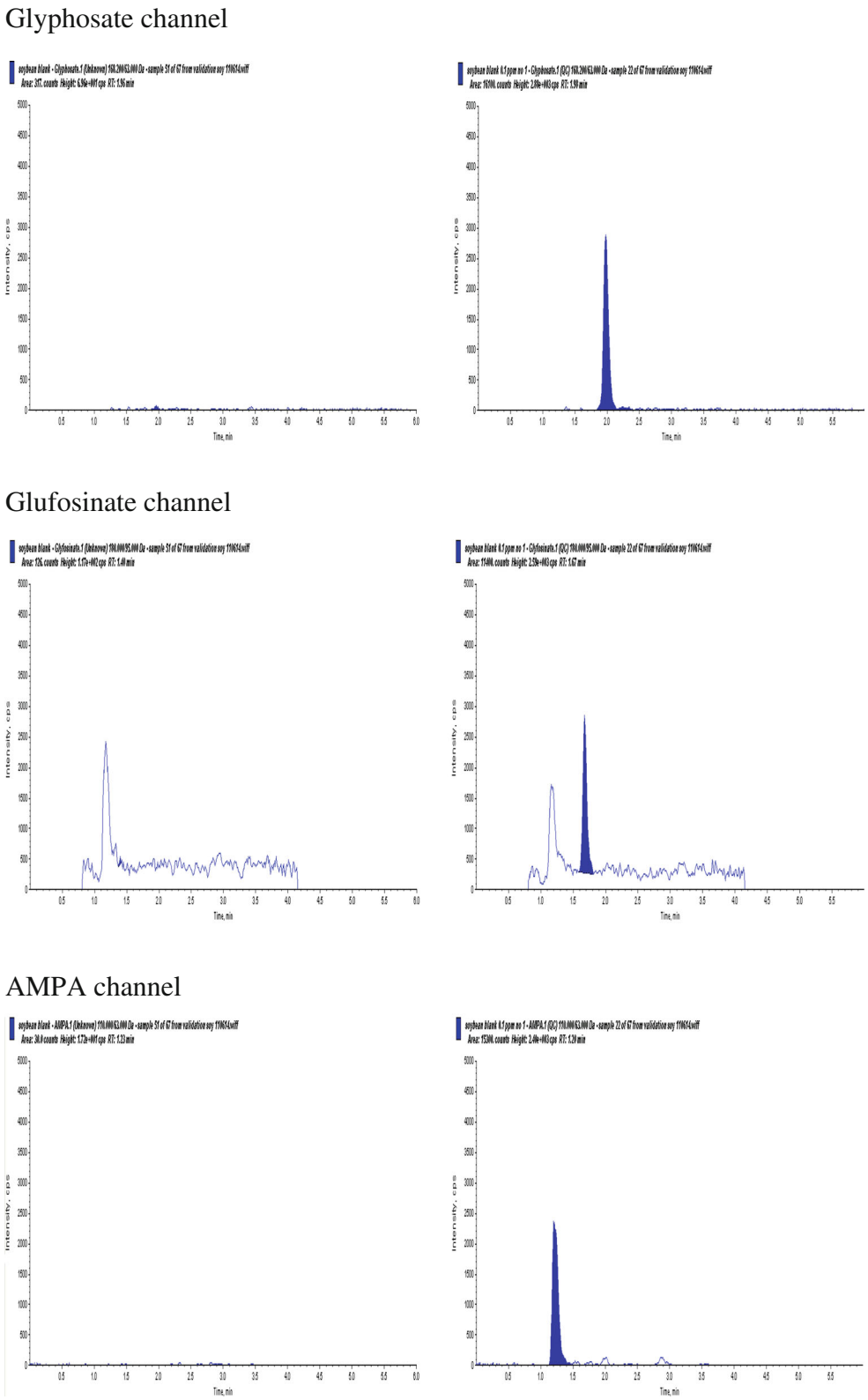

Fig. 1 Chromatogram of soybean blank (*left*) and soybean blank fortified at 0.1 μg/g of glyphosate, glufosinate, and AMPA (*right*)

standard in solvent with external standard calibration method. The linearity was evaluated, and they showed satisfactory linearity with coefficient of determination (R^2) of more the 0.995. The specificity of the method was evaluated by analyzing reagent blank, blank sample, and blank sample spiked at the lowest fortification level (0.1 μg/g). No relevant signal (above 20 %) was observed at any of the transitions selected in the blank sample. A reagent blank was injected

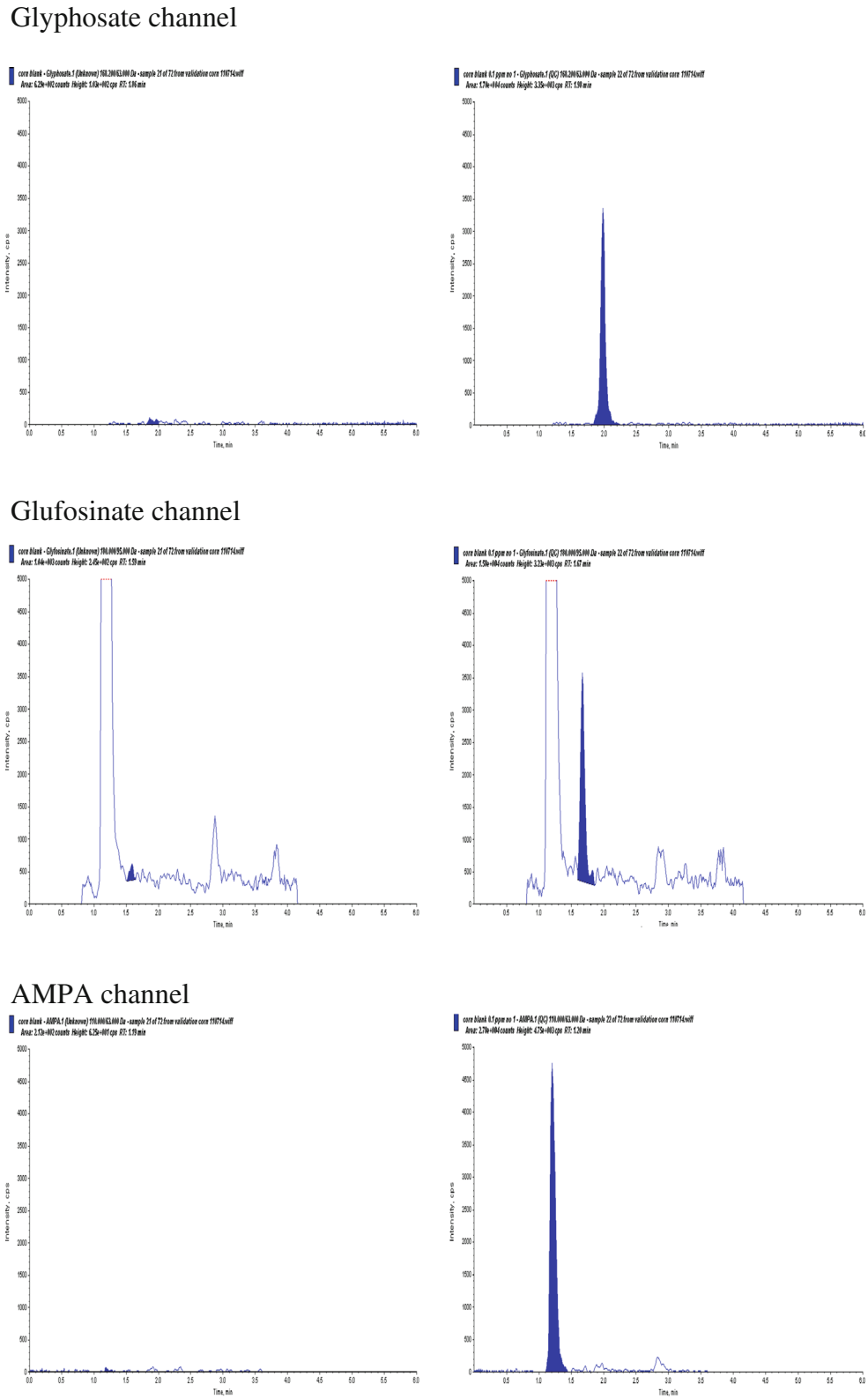

Fig. 2 Chromatogram of corn blank (*left*) and corn blank fortified at 0.1 μg/g of glyphosate, glufosinate, and AMPA (*right*)

immediately after the 1000 ng/mL standard, and no analyte signals were detected above 10 % of the 10 ng/mL standard.

The method detection limit (MDL) for each compound was calculated by multiplying standard deviation of 7 replicate recovery of the lowest fortification samples (0.1 μg/g) with t value (3.14). By using matrix-matched standard with IS, the MDL for glyphosate, glufosinate, and AMPA were 14, 14, and 18 ng/g for soybean sample and 15, 25, and 18 ng/g for corn

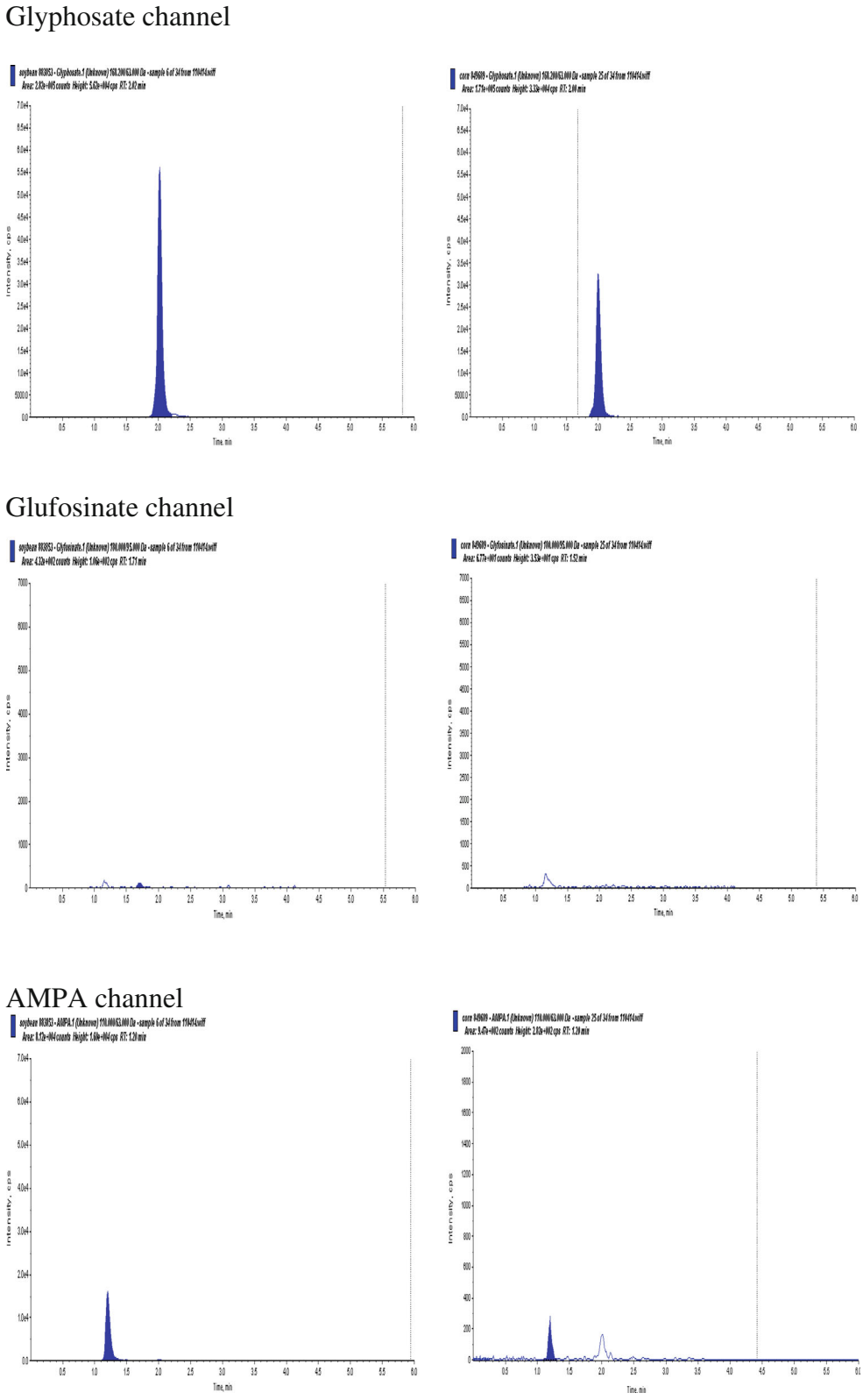

Fig. 3 Chromatogram of soybean containing 11.0 μg/g of glyphosate and 4.9 μg/g of AMPA (*left*) and corn containing 6.5 μg/g of glyphosate and 0.065 μg/g of AMPA (*right*)

sample, respectively. The method quantification limit (MQL) was three times the MDL which were 42, 42, and 54 ng/g for soybean and 45, 75, and 54 ng/g for corn, respectively.

Accuracy (recovery %) and precision (relative standard deviation or RSD %) were evaluated at the fortification levels of 0.1, 0.5, and 2 μg/g in seven replicates in both soybean and corn samples (Table 4) using all 4 calibration methods. For glyphosate and glufosinate, the average recovery using (a) standard in matrix with internal standard calibration method, (b) standard in matrix with external calibration method, and (c) standard in solvent with internal standard calibration method was in the range of 92–104 % with the RSD of less than 6 %. The calibration of standard in solvent without the IS had average recovery ranged from 96 to 98 % with the RSD of less than 5 % for glyphosate. However, it had average recovery range from 75 to 76 % with the RSD of less than 5 % for glufosinate. This demonstrates that glyphosate can be effectively extracted from the sample and does not have significant matrix suppression. External standard calibration without the IS can be used to accurately quantify glyphosate in these samples. On the other hand, IS may be used to accurately quantify glufosinate to compensate for the matrix suppression

The recovery of AMPA using calibration curve without IS in both matrices was very low due to matrix suppression as shown in Table 3. The calibration curve from matrix match standard (without IS) improves the recovery of AMPA somewhat, but it is still less than 70 %. AMPA was eluted near the solvent front where polar interferences in the matrix were present. The concentration of these interferences was not predictable depending upon the type of matrix. Therefore, the IS (AMPA ^{13}C ^{15}N) should be used to accurately quantify AMPA in these samples. The recovery of AMPA using IS in sample matrix and in solvent was in the range of 96–113 % with the RSD of less than 12 % in both matrices. Therefore, standard in solvent with IS may be used for the quantification of AMPA to save time and cost of analysis.

Chromatograms of glyphosate, glufosinate, and AMPA in soybean blank and soybean blank fortified at 0.1 μg/g are shown in Fig. 1. Chromatograms of glyphosate, glufosinate, and AMPA in corn blank and corn blank fortified at 0.1 μg/g are shown in Fig. 2. No significant inferences were observed the blank sample where the analytes were eluted. The Acclaim™ Trinity Q1 combined reverse-phase, weak anion, and weak cation exchange properties in one column. This column retains glyphosate, glufosinate, and AMPA by the ion-exchange mechanism similar to the previous work done by Hao et al. on the Acclaim™ WAX-1 column [9]. However, a lower concentration of salt in the mobile phase (50 mM ammonium formate) at a much lower pH significantly improved peak shape and sensitivity with simple isocratic elution. The column was rugged and gave good peak shape and retention time reproducibility over 100 injections of sample matrix without the need for column reconditioning as previously recommended by Hao and coworkers [9].

A soybean sample and a corn sample collected from the market were analyzed by this method (Fig. 3). The soybean sample contained 11 ppm of glyphosate and 4.9 ppm of AMPA. The corn sample contained 6.5 ppm of glyphosate and 0.065 ppm of AMPA. There was no glufosinate detected above 0.03 ppm in either sample.

Conclusion

This work describes a 10-min extraction with aqueous solution of acetic acid and Na_2EDTA which allows a rapid and direct determination of glyphosate, glufosinate, and AMPA residue in soybean and corn samples. Acetic acid precipitates soluble protein (major interference) from the sample extract while Na_2EDTA prevents the analytes from forming a chelation complex with polyvalent metal. Oasis HLB SPE is used to filter the sample extract and trap the phospholipids and other non-polar compounds. The SPE cleanup step is used to maintain HPLC column performance and minimize matrix concentration in the final extract. The mixed-mode Acclaim™ Trinity™ Q1 HPLC column allows the analytes to be retained on the column and separated from each other without a derivatization step. These analytes were commonly derivatized before HPLC analysis to improve their chromatographic retention in reversed-phase LC. Negative mode ion-spray with MS/MS measurement gives excellent sensitivity and selectivity that produce distinct chromatographic peaks with minimal interference. Severe matrix effect on AMPA was clearly observed because it co-eluted with other polar interferences near the solvent front. The use of isotope-labeled AMPA eliminates the matrix suppression problem and provides accurate quantification.

The proposed method is quick, rugged, selective, and sensitive enough to determine glyphosate, glufosinate, and AMPA in soybean, corn, and other food grains. It can be used as an alternate method to the traditional FMOC-based method which requires tedious and time-consuming derivatization and concentration steps.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest. Research was funded by U.S. Food and Drug Administration.

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