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Development and Independent Laboratory Validation of an Analytical Method for the Direct Determination of Glyphosate, Glufosinate, and Aminomethylphosphonic Acid in Honey by Liquid Chromatography/Tandem Mass Spectrometry

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Abstract

A simple high-throughput liquid chromatography/tandem mass spectrometry (LC-MS/MS) method was developed for the determination of glyphosate, glufosinate and aminomethylphosphonic acid (AMPA) in honey using a reversed-phase column with weak anion-exchange and cation-exchange mixed-mode. One gram of sample was shaken with water containing ethylenediaminetetraacetic acid disodium salt and acetic acid for five minutes. After centrifugation, the supernatant was mixed with internal standard and directly injected and analyzed in ten minutes by LC-MS/MS with no sample concentration or derivatization steps. Two precursor/product ion transitions were monitored in the method for each target compound to achieve true positive identification. Three isotopically labelled internal standards corresponding to each analyte were used to correct for matrix suppression effects and/or instrument signal drift. The average recovery for all analytes at 25, 50, 100, and 500 ng/g (n=11) ranged from 87 to 111% with a relative standard deviation of less than 12%.

Keywords: glyphosate, honey, LC-MS/MS, direct determination

1. Introduction

Glyphosate (N-phosphonomethyl glycine) and glufosinate [ammonium(S)-2-amino-4-[hydroxyl (methyl) phosphinoyl] butyrate] are non-selective post emergence herbicides used for the control of a broad spectrum of grasses and broadleaf weed species in agricultural and industrial fields. AMPA is the major metabolite of glyphosate and is classified as a toxicologically significant compound [11]. 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 [15]. Glyphosate and glufosinate have high efficacy, low toxicity and an affordable price, when compared with other herbicides. These factors lead to its wide utilization on several crops. Farmers also use glyphosate as a desiccant to rapidly kill above ground growth of crops such as wheat. This allows for rapid dry down for easy harvest. The use of glyphosate in agriculture has increased significantly with the introduction of transgenic crops such as Roundup-Ready®soybeans and corn, which enable farmers to directly apply low cost broad spectrum herbicide products to their fields without harming crops. In the United States, glyphosate is currently the most widely used herbicide, with 180 to 185 million pounds applied in the agricultural sector during 2007, 5 to 8 million pounds used in the home and garden markets, and 13-15 million pounds used in industrial, commercial and governmental weed control applications [15]. This high level of use has led to concerns about its effects on humans and the environment. A recent study by researchers from Boston University and Abraxis LLC found significant amounts of glyphosate in honey (41 out of 69 samples collected in the Philadelphia, US metropolitan area) with a concentration range between 17 and 163 ng/g using enzyme linked immune sorbent assay (ELISA) [13]. This method is quick, inexpensive, and sensitive; however, it does not have confirmation method to prevent false positives. A quick, accurate, and sensitive method with a positive confirmation method to determine these herbicides in honey must be developed to support regulatory actions.

Glyphosate, glufosinate, and AMPA are very polar compounds and insoluble in organic solvents. Therefore, the use of classical organic solvent extraction is very difficult and ineffec-

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tive. As a result, the isolation and quantification of these herbicides have posed a challenge to the analytical chemist. Alferness and Iwata used an aqueous extraction method to extract glyphosate and AMPA from soil, plant and animal matrices [1]. 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 chromophophore or fluorophore also necessitates the use of derivatization techniques for the determination of these analyte residues by liquid chromatography and gas chromatography [12, 9, 7]. 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 [16]. Schreiber and Cabrices streamlined the derivatization by using a special autosampler for automation to determine these polar analytes in corn and soybean [14]. The derivatization technique is problematic as it requires the optimization of a number of parameters (temperature, reaction time, concentration and purity of the reagents, laboratory handling time). Anion exchange, Hydrophilic Interaction Liquid Chromatography (HILIC), and mixed-mode columns have been used with LC-MS/MS to determine underivatized glyphosate and other polar pesticides in food matrices with limited success [6, 2, 3]. An LC/MS method was developed using a mixed-mode HPLC column (Acclaim Trinity Q1) to directly determine these three analytes in milk and soybean [4, 5]. This method should be applicable for honey as well.

This paper describes an inter-lab validation of an LC-MS/MS method using a negative ion-spray ionization mode for the direct determination of glyphosate, glufosinate, and AMPA in honey. A quick and reliable extraction method that requires small sample size, non-toxic solvent, and an effective sample cleanup procedure to ensure method ruggedness, sensitivity, and selectivity is provided.

2. Material and Methods

2.1. Chemicals and Materials

Pesticide standards (≥ 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 (1 mg). 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. 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 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 A was HPLC grade water and mobile phase B was prepared by mixing 100 mL of the 500 mM buffer solution with 900 mL of purified water (final concentration was 50 mM).

2.2. Standard Preparation

The individual stock solutions of glyphosate, glufosinate, and AMPA at 1 mg/mL were prepared in water. These stock solutions were used to prepare standard mix solutions at 50, 10, 2, 1 and 0.5 ng/uL. 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 ng/μL was prepared by dissolving the stock standard in water and stored in a plastic tube. The mixed standard solutions were further diluted with the extracting solvent to obtain standard mixes from 2.5 to 250 ng/mL. The calibration standards were mixed with IS solution at 100 ng/mL for the calibration curves.

2.3. Sample Preparation and Extraction Procedure

Two organic honey samples were obtained from a local market. The samples were weighed at 1 ± 0.1 g each in 50-mL plastic centrifuge tubes (Fisher Scientific, Pittsburgh, PA) and fortified with native standard solutions at 25, 50, 100, and 500 ng/g. The samples were allowed to stand at room temperature for 1 h. Extracting solvent (4.3 mL) was added to each tube using an automatic pipette. The tubes were capped tightly and shaken for 5 min on a SPEX 2000 Geno grinder (SPEX Sample Prep LLC, Metuchen, NJ) at 2,000 stroke/min, then centrifuged at 3,000 x rpm for 5 min using a Q-Sep 3000 centrifuge (Restek, Bellefonte, PA). The clear liquid (180 μL) was pipetted into a plastic 300- μL autosampler vial (Wheaton, Millville, NJ) containing 20 μL of 2 ng/μL IS solution. The vial was capped and mixed on a vortex mixer for 30 sec. A 20 μL of sample was injected into the LC-MS/MS system.

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 6500 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) analytical column 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 35 °C with sample injection volume of 20 μL. The mobile phase was 100% A (water) for 30 sec

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	DP [a]	CE [b]	EP [c]	CXP [d]	Retention
AMPA.1	110	63	-60	-24	-10	-10	2.4
AMPA.2	110	79	-60	-26	-10	-10	2.4
AMPA $^{13}C^{15}N$ (IS)	112	63	-60	-24	-10	-10	2.4
Glufosinate.1	180	95	-46	-23	-10	-10	2.6
Glufosinate.2	180	85	-46	-26	-10	-10	2.6
Glufosinate D3 (IS)	183	63	-46	-26	-10	-10	2.6
Glyphosate.1	168.2	63	-110	-30	-10	-10	2.8
Glyphosate.2	168.2	79	-110	-55	-10	-10	2.8
Glyphosate $^{13}C2^{15}N$ (IS)	171	63	-110	-30	-10	-10	2.8

[a]DP = declustering potential
[b]CE = collision energy
[c]EP = entrance potential
[d]CXP = collision exit potential

Table 1: Retention time and MRM conditions for LC/MS analysis.

at a flow rate of 0.5 mL/min then stepped up to 100% B (ammonium formate/formic acid buffer) immediately for 4 min to elute the analytes. The column was equilibrated with 100% A at a flow rate of 0.7 mL/min for 6 min for a total run time of approximately 10 min. A diverter valve connected between the HPLC column and the MS interface was used to direct the LC eluent to waste just before the AMPA peak (2 min) and after the glyphosate peak (3.7 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 60 sec for each analyte. Analyte-specific MS/MS conditions and LC retention times for the analytes are shown in Table 1. 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 350 °C.

3. Results and Discussion

3.1. Optimization of Sample Extraction Procedure

A honey sample (1 g) was spiked with the analytes at 100 ng/g and shaken with 5 mL of water for 5 min on a Geno grinder at 2,000 stroke/min. After the centrifugation, the supernatant was injected along with standard solution in water at the same concentration using the isocratic elution with 50 mM ammonium formate buffer solution previously used in the milk and soybean method [4, 5]. The result was disappointing due to the poor peak shape and poor response of AMPA. Honey contains mostly sugars which are polar compounds which tend to coelute with AMPA near the solvent front. A few sample cleanup procedures were evaluated to eliminate sugar in the sample. Due to their phosphonate structures, glyphosate, glufosinate, and AMPA could be retained and efficiently purified on an anion-exchange SPE cartridge while the sugars, nonionic compounds, would pass through. A strong anion solid phase cartridge was previously used as a cleanup step for the determination of these analytes in beer and tea [10]. Three anion exchange cartridges (NH2, WAX, and SAX 500 mg/6 mL) were evaluated. One gram of honey spiked with 100 ng of the analytes was shaken with 5 mL of water. One milliliter was loaded on these cartridges (previously conditioned with water and methanol). The cartridges were washed with 3 mL of water and eluted with 6 mL of 1:9 1N HCl:methanol. The eluent was analyzed by LC/MS. Due to the high concentration of methanol in the sample, only 1 μL of the extract was injected to maintain a good peak shape of AMPA. The result was mixed. Strong anion exchange SPE (SAX) did not effectively retain AMPA, while amino propyl (NH2) phase lost glufosinate during the loading step. Weak anion exchange (WAX) SPE gave good overall recovery for all analytes. However, in order to decrease the detection limit, the sample had to be evaporated to decrease the sample volume and to evaporate the methanol to improve the peak shape. The evaporation step was time-consuming and the high concentration of hydrochloric acid left in the sample may affect the peak shape. Honey solution made of a higher honey concentration (1 g in 3 mL of water) was loaded on the WAX SPE to increase the concentration of the analytes in the sample. High sugar content reduced effectiveness of the WAX SPE to retain AMPA and resulted in poor recovery. Because of the poor loading efficiency on the WAX SPE (at high honey content in the solution) and the long evaporation time (to decrease sample volume), the cleanup procedure was not further evaluated. The alternative solution to minimize matrix effect was to modify the chromatographic condition to move analytes peaks further away from the solvent front by using a gradient elution.

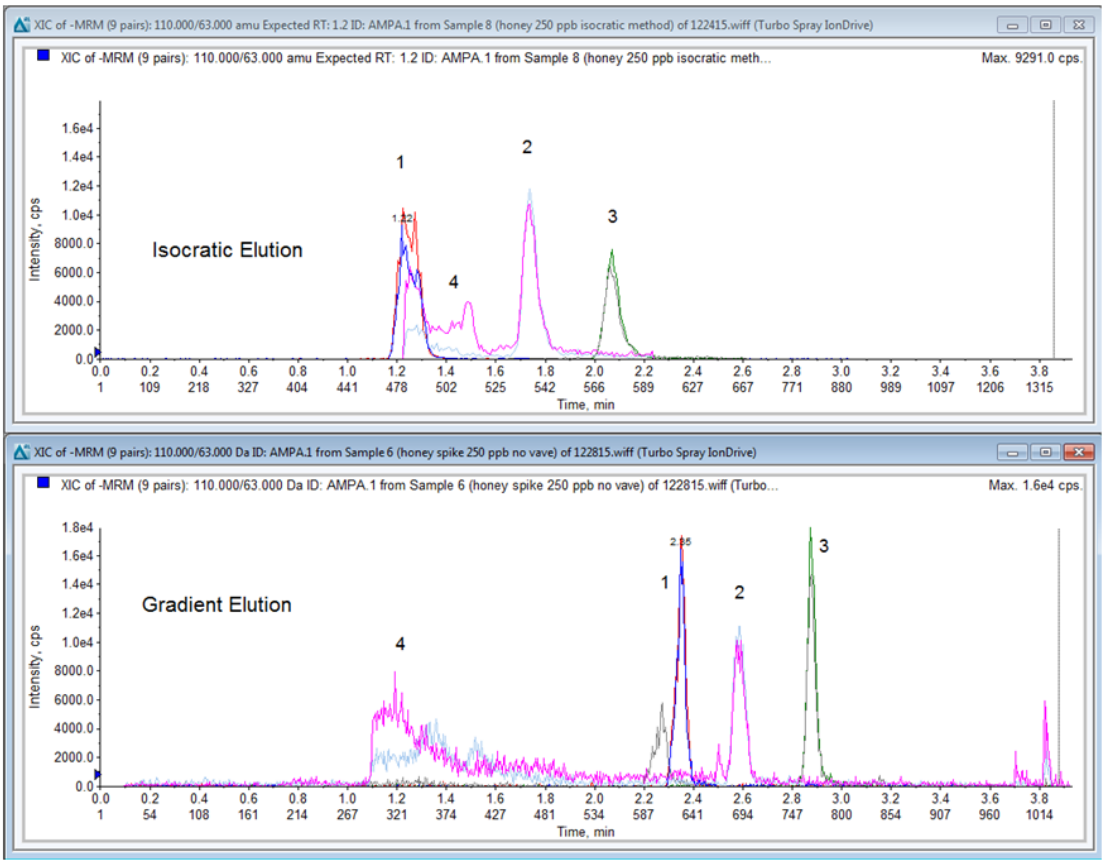

Figure 1: Chromatograms of honey samples (spiked at 250 ng/g) analyzed by isocratic and step gradient elution (peak 1 = AMPA, peak 2 = glufosinate, peak 3 = glyphosate, peak 4 = honey matrix).

3.2. Chromatrography Optimization

The isocratic elution of the Acclaim Q1 column with 50 mM ammonium formate was previously developed for glyphosate analysis in milk and soybean [4, 5]. This condition worked well with these matrices which did not contain a high concentration of sugar. In order to increase analyte retention on this column, a much lower salt concentration mobile phase at the initial condition must be used. Different gradient conditions were evaluated. Finally, the mobile phase condition was optimized by using a step gradient of 100% water for 30 sec at a flow rate of 0.5 mL/min to sufficiently retain AMPA (2.4 min), immediately followed by 100% 50 mM ammonium formate (pH 2.9) immediately for another 4 min to elute glufosinate and glyphosate. The column was then quickly equilibrated with 100% water at a flow rate of 0.7 mL/min for 6 min for a total run time of 10 min. Honey solution in water (1 g/5 mL) containing the analytes at 250 ng/g was injected into the LC/MS using the isocratic mode and the step gradient mode for comparison (Figure 1). The step gradient produced a better peak shape of AMPA, and the analytes eluted further away from the sample matrix (mostly sugars). This HPLC condition allowed the diluted honey solution to be directly injected without concentration and achieved good sensitivity and good peak shape for all analytes. The extracting solvent containing diluted acetic acid and EDTA provided good extraction of glyphosate in milk by precipitating the protein and preventing glyphosate from binding with metal ions. It was observed that a sharper peak shape of glyphosate was obtained when the standard solution contained EDTA instead of water alone. An aqueous solution of EDTA (50 mM) was previously used to restore the column performance for glyphosate after a few sets of samples were analyzed on the Acclaim WAX-1. It was believed that trace metals in the column may broaden the glyphosate peak. EDTA was used to eliminate the metal ions in the system [6].

The moisture content in honey reported in the literature is approximately 17-20% [8]. Therefore, 1 g of honey should contain approximately 0.2 mL of water. However, when it was shaken with 4.8 mL of the extracting solvent, the final volume was 5.5 mL. At least ten different honey samples showed the same results. This indicated that the extra 0.5 mL may have come from honey. It was found that in order to adjust the final volume of the solution to 5 mL, only 4.3 mL of the extracting solvent was needed to mix with 1 g of honey. The final volume of 5 mL of the honey extract was then used for the analyte concentration calculation.

Since there was no sample cleanup step in this method, it was necessary to use a diverter valve to bypass the HPLC eluate away from the LC/MS interface to waste at the beginning of the run until just before the AMPA retention time. This step prevented sugar in honey from entering the heated ion source

Analyte	Fortification level (ng/g)	Recovery (%)	RSD (%)	LOD ng/g	LOQ ng/g	Linearity R square
Honey A, honey from Ivory Coast (n = 4)						
Glyphosate	25	92	7.0	5	16	0.9997
	50	102	2.1			
	100	92	2.6			
	500	96	2.8			
Glufosinate	25	107	6.3	5	17	0.9991
	50	107	5.3			
	100	91	3.0			
	500	98	2.7			
AMPA	25	106	1.6	1	4	0.9998
	50	104	3.0			
	100	90	3.4			
	500	103	3.1			
Honey B [a], organic honey (n =7)						
Glyphosate	25	90	12.1	8	26	0.9981
	50	93	4.6			
	100	87	8.0			
	500	102	8.1			
Glufosinate	25	107	6.8	5	18	0.9990
	50	94	5.9			
	100	90	8.2			
	500	101	7.3			
AMPA	25	111	5.6	5	16	0.9994
	50	103	3.5			
	100	93	6.7			
	500	103	3.5			

[a] Honey B contains approximately 10 ng/g of glyphosate residue.

Table 2: Recovery (%) and RSD (%) data obtained in the single laboratory validation experiments (FDA laboratory in Georgia).

and forming deposits at the LC/MS interface. The valve also diverted the HPLC eluate after the glyphosate peak to waste at higher mobile phase flow rate, to keep the LC/MS interface clean from the sample matrix.

3.3. Evaluation of Matrix Effects

Matrix effect (%ME) in the sample extract is calculated as the ratio of the analyte response in the sample matrix divided by the response of the analyte in the extracting solvent 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%, matrix enhancement exists. Standard solutions at 50 ng/mL in the extracting solvent and in honey extract (1 g/5 mL) were prepared and injected to evaluate the matrix effect. The %ME of all analytes in honey extract. AMPA and glyphosate demonstrated matrix enhancement approximately 130 and 150%, respectively, while glufosinate had severe suppression (50%). Based on this data, internal standards are needed for accurate quantification of these analytes.

3.4. Method Validation

A single laboratory validation at the FDA laboratory in Atlanta, GA was performed using two organic honey samples collected from a local market. The calibration standard solutions at concentrations from 2.5 to 250 ng/mL were prepared in the extracting solvent with the addition of IS. These standard solutions were injected along with the fortified samples and sample blank. The accuracy and precision of the method was evaluated via recovery experiment on two blank honey samples (A and B) spiked at 25, 50, 100, and 500 ng/g. The specificity of the

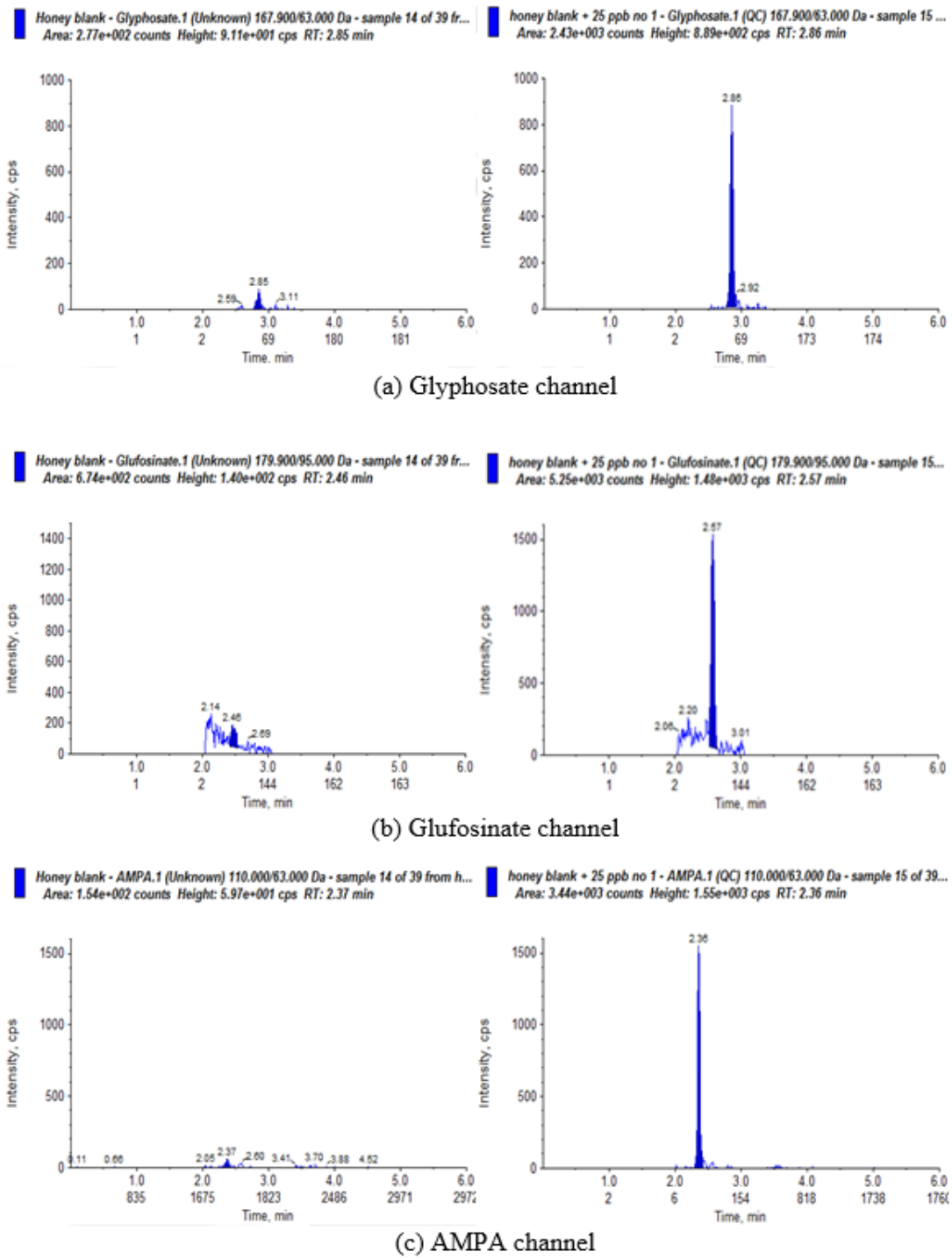

Figure 2: Chromatograms of honey blank (left) and honey blank fortified at 25 ng/g of glyphosate, glufosinate, and AMPA (right).

method was evaluated by analyzing reagent blank, blank sample, and blank sample spiked at the lowest fortification level (25 ng/g). No relevant signal (above 30% of the 25 ng/g sample) was observed at any of the transitions selected in the blank honey sample A. Honey sample B had approximately 10 ng/g of incurred residue of glyphosate. Glyphosate, a herbicide, is not used to treat bee hives; however, bees may carry glyphosate from an agricultural area treated with glyphosate. A reagent blank was injected immediately after the 250 ng/mL standard and only glyphosate approximately 0.5 to 0.8% carry-over was observed. The second injection of the reagent blank showed no trace of glyphosate above 20% of the lowest standard solution of 2.5 ng/mL. It is advisable to inject a reagent blank after injecting a sample containing high concentrations of the analytes to minimize false positive for the next sample.

The sensitivity, expressed in terms of limit of detection (LOD), was estimated as 3 times the standard deviation of the quantitative MRM response from the replicates fortification of honey sample at 25 ng/g. The limit of quantification (LOQ) was estimated as 10 times the standard deviation in a similar manner. Method linearly was determined for each target compounds using a linear regression curve fit (1/x weighing). The average percent recovery, relative standard deviation, method LOD/LOQ, and coefficient of determination (R2) are reported for all compounds in Table 2. Due to the matrix effect (suppression/enhancement), IS must be used for accurate quantification. Quantification was based on calibration standard in the extracting solvent with corresponding IS. No matrix-matched calibration standard was required. A quadratic curve fit (with or without 1/x weighting) is permitted if this provides a better curve fit for the data.

Accuracy (recovery %) and precision (relative standard deviation or RSD %) for the two honey samples (A and B) are also shown in Table 2. The average recoveries were 87-102% with an RSD of 12% for glyphosate, 90-107% with an RSD of 7.3% for glufosinate, and 90-111% with an RSD of 6.7% for AMPA. For honey A, the LOD of glyphosate, glufosinate, and AMPA were 5, 5, and 1 ng/g, respectively. The LOQ of glyphosate, glufosinate, and AMPA were 16, 17, and 4 ng/g, respectively. Since honey B sample had approximately 10 ng/g of incurred residue of glyphosate, the standard deviation of recovery at 25 ng/g was a bit higher (26 ng/g) than the standard deviation of recovery found in honey A (16 ng/g). Since the honey was very viscous, the concentration of the incurred residue may not have been constant throughout the sample. The LOQ of glufosinate in honey B (18 ng/g) was comparable to the LOQ found in honey A. The LOQ of AMPA in honey B was 16 ng/g. Chromatograms of glyphosate, glufosinate, and AMPA in honey A blank and honey A blank fortified at 25 ng/g are shown in Figure 2. Nineteen honey samples were collected from the local market and a private honey farm and analyzed by the proposed method (Table 3). Nine samples (47%) contained glyphosate higher than 16 ng/g (estimated LOQ). Glufosinate and AMPA were not detected in any of the samples.

3.5. Inter-Laboratory Validation (Spiked and Incurred Samples)

The method was transferred to the second lab for a small scale independent laboratory (State Hygienic Laboratory at the University of Iowa, Coralville, IA) validation under similar instrument conditions using a 5500 Q-TRAP from AB-Sciex. It involved the analysis of spiked samples and samples containing field-incurred residues. This is to prove if the method performance could be duplicated at a different laboratory. Accuracy and reproducibility data were obtained by replicate analysis of spiked sample (blank honey spiked at 25, 50, and 100 ng/g, n = 7,4, and 4). The results are presented in Table 4. The mean recovery for all analytes ranged from 84 to 108% with the RSD ranging from 2.3 to 12.8%. The extraction procedure and HPLC conditions used at the state lab were the same as the FDA lab with no modification. Three honey samples (honey from FL, LA, and IA) containing incurred residue of glyphosate were analyzed by both laboratories and the results were comparable, indicating that the method was reliable for use.

4. Conclusion

This work describes a five-minute extraction with aqueous solution of acetic acid and Na_2EDTA which provides for rapid and direct determination of glyphosate, glufosinate, and AMPA residue in honey samples. After the centrifugation to obtain a clear extract, the sample was injected directly to the LC-MS/MS system. The mixed-mode Acclaim^{TM} Trinity Q1 HPLC column allows the analytes to be retained on the column and separated from each other without a derivatization step. The step gradient elution developed in this method improved the peak shape and retention of the analytes over isocratic elution. Sugar, the major component in the honey sample, was eluted much earlier and diverted to waste to prevent severe matrix suppression of AMPA and keep the ion-source clean. Negative mode ion-spray with MS/MS measurement gives excellent sensitivity and selectivity that produce distinct chromatographic peaks with minimal interference. The use of internal standard for each analyte minimized the matrix effect and provides accurate quantification. The in-house and the inter-laboratory validation studies, using spiked blank honey and honey with incurred residue of glyphosate, demonstrated that the method is quick, rugged, selective, and sensitive enough to determine glyphosate, glufosinate and AMPA in honey at or above the 25 ng/g level. It can be used as an alternative method to the ELISA technique as well as to the traditional FMOC derivatization methods which are tedious and time-consuming.

5. Declaration of Conflicting Interest

The authors declare that there is no conflict of interest.

6. Disclaimer

The views expressed are those of the authors and should not be construed to represent the views or policies of the U.S. Food

Sample	FDA lab [a] glyphosate (ng/g)	State Lab IA [b] glyphosate (ng/g)	Source
wild flower honey	< 16		GA, USA
organic honey	17		Brazil
orange blossom honey	< 16		FL, USA
clover honey	26		GA, USA
orange blossom honey	21		NC, USA
clover honey	40		GA, USA
clover honey	< 16		Canada
wild flower honey/miel	46		Canada
Gourmet honey	< 16		unknown
Manuka honey	< 16		New Zealand
honey	< 16		France
honey from Canada	19		Canada
unknown honey	< 16		Ivory Coast
honey blend with fructose/flavor	< 16		Taiwan
local honey	< 16		GA, USA
organic honey	< 16		TX, USA
honey from FL	24	24	FL, USA
honey from LA	121	123	LA, USA
honey from IA	35	42	IA, USA
353293		653	IA, USA
353294		34	IA, USA
353295		85	MN, USA
353296		103	IA, USA
353297		23	IA, USA
353298		40	IA, USA
IA-1		23	IA, USA
IA-2		< 10	IA, USA
IA-3		44	IA, USA

[a]LOQ = 16 ng/g
[b]LOQ = 10 ng/g

Table 3: Glyphosate found in honey samples collected from the local market.

	Spike Level (ng/g)	n	Recovery (%) Range	Average Recovery (%)	RSD (%)
Glyphosate	25	7	73 - 105	84	12.8
	50	4	79 - 89	86	4.9
	100	4	90 - 97	95	3.2
Glufosinate	25	7	88 – 106	97	6.6
	50	4	91 – 107	98	8.1
	100	4	91 – 112	103	9.0
AMPA	25	7	104 – 112	108	3.9
	50	4	101 – 107	104	2.8
	100	4	103 – 108	105	2.3

Table 4: Recovery (%) and RSD (%) data obtained in the single laboratory validation experiments (State Hygienic Laboratory at the University of Iowa).

and Drug Administration. Any reference to a specific commercial product, manufacturer, or otherwise, is for the information and convenience of the public and does not constitute an endorsement, recommendation or favoring by the U.S. Food and Drug Administration.

7. Article Information

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