APPLICATION NOTE

Liquid Chromatography / Mass Spectrometry

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Direct Analysis of Glyphosate and Similar Polar Pesticides in Oatmeal by UHPLC-MS/MS

Introduction

Glyphosate (N-(phosphonomethyl) glycine), an organophosphorus compound, is used to kill weeds (e.g. annual broadleaf weeds and grasses) that compete with crops. Since its introduction to market approximately 40 years ago, glyphosate has become one of the world's most widely used herbicides due to its relatively low toxicity in comparison with other herbicides towards mammals. The adoption of glyphosate by farmers intensified after the introduction of genetically engineered "glyphosate tolerant" crops, such as corn and soybeans, that can withstand glyphosate treatment unlike the weeds the herbicide is meant to destroy. Like other pesticides, glyphosate is directly administered to food products and can come in contact with both food workers and the environment, resulting in the bio burden of exposure in uncontrolled regional populations. As a registered herbicide product under a number of regulatory organizations, glyphosate has been considered nontoxic with minimal risk to human health with persistent exposure at trace levels. However, recent toxicity evaluations by different organizations have put glyphosate at the center of a dispute. The World Health Organization's (WHO) International Agency for Research on Cancer classified it as "probably carcinogenic to humans" in March of 2015[1]. However, in November of 2015, the European Food Safety Authority (EFSA) published a report claiming that there was no scientific evidence linking glyphosate to cancer[2].

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Independent of the dispute in the scientific community, federal regulations have been established by food authorities in several countries. The typical maximum residual level for glyphosate is between 0.05 to 500 mg/kg, but may vary depending on the food commodity.

Glyphosate is a very polar compound with high solubility in water and low solubility in most organic solvents. These properties mean that these compounds do not retain well on conventional C18 LC columns and non-polar GC columns. Therefore, the derivatization with fluorenylmethyloxycarbonyl chloride (FMOC-Cl) is a common procedure to improve extraction and separation of glyphosate and other related compounds with LC and GC based methods. These methods based on derivatization are labor-intensive, time-consuming and less reproducible.

There is a growing need to develop a method for analysis of glyphosate and other related polar compounds without derivatization. Recently, the EU Reference Laboratories (EURL) published two methods that can directly analyze glyphosate (GLY), its metabolite, aminomethylphosphonic acid (AMPA), and glufosinate (GLU) without derivatization. One method used an ion exchange column with a long run time (23 min), while the second method utilized a Hypercarb column, which requires a special priming/reconditioning procedure and showed significant chromatographic peak tailing[3]. Our study reports a 12 minute LC/MS/MS method with an amino-based column to analyze glyphosate and other related polar compounds in underivatized states, with exceptional selectivity and sensitivity.

Experimental

A PerkinElmer Altus® A-30 UPLC® system was used with a PerkinElmer QSight™ 210 triple quadrupole mass spectrometer. Instrument control, data acquisition and processing was performed using the PerkinElmer Simplicity 3Q™ software.

The LC method conditions are provided in Table 1.

Table 1. LC method.

Column	Shodex NH2P-50 2D column, 2.0 x 150 mm, 5 µm
Mobile Phase	A: 5 mM ammonium acetate (pH11.0) in water; B: acetonitrile
Flow Rate	0.25 mL/min.
Oven Temp.	35 °C
Injection Volume	10 µL
Gradient Conditions	

Time(min)	Mobile Phase	
	A (%)	B (%)
0.00	20	80
2.00	20	80
2.01	80	20
8.00	80	20
8.01	20	80
12.00	20	80

The mass spectrometer was equipped with an electrospray ionization source operating in negative ion mode. The mass spectrometer source conditions are shown in Table 2:

Table 2. Mass spectrometer source conditions.

Parameter	Setting
Dry Gas	150
Nebulizer Gas	220
Heating Gas Temp	500 °C
Electrospray Voltage	-4500 V

MRM settings for each analyte were optimized by infusing neat standard solutions. The parameters for each analyte's MRM transition are listed in Table 3. The dwell time for each MRM was set at 30 ms.

Table 3. Optimized MRM settings.

Compound	Transitions m/z	EV /V	CE /eV
(GLY)	167.6/62.9*	-19	35
	167.6/149.6		14
(AMPA)	109.7/63.0*	-27	-30
	109.7/78.9		37
(GLU)	179.6/63.0*	-20	53
	179.6/84.9		27

** Quantifier ion*

Sample Preparation

1.0 g of oatmeal sample was weighed into a centrifuge tube, 10 mL of water/ acetonitrile (V/V, 2/1) was added to the tube and the mixture was then shaken/vortexed for one minute, ultra-sonicated for 15 minutes and centrifuged for five minutes at 6000 rpm. The recovered supernatant was filtered through a 0.22 µm nylon membrane filter for LC/MS/MS analysis. To avoid possible interaction between analytes and glass surfaces, plastic sample vials were used during the analysis and samples were analyzed immediately after preparation.

Standards Calibration Solutions

Matrix matched calibration standards were prepared by adding different levels of analytes (5.0, 10.0, 100.0, 200.0 and 500.0 ng/mL, respectively) in oatmeal matrix extract.

Results and Discussion

Figure 1 shows typical MRM chromatograms for the three analytes spiked to 10 ng/mL (0.1 mg/kg) in oatmeal extract. All three analytes were well retained on the column and showed good peak shape and signal to noise. GLY and AMPA were eluted at very similar retention times due to their similar chemical structure. GLU was baseline separated from the other analytes. No matrix interferences, which can affect peak integration, were observed.

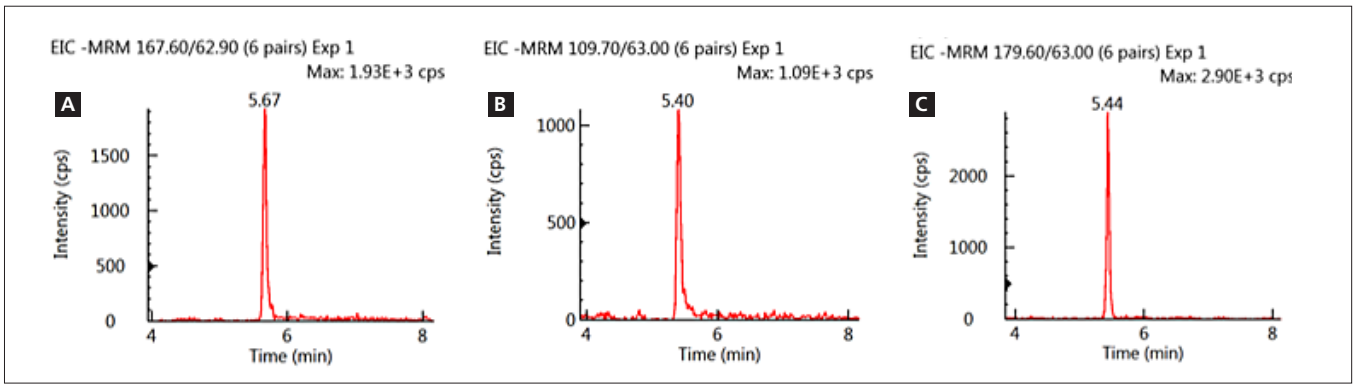

Figure 1. MRM Chromatograms of GLY (A), AMPA (B), and GLU (C) spiked at 10 ng/ml in oatmeal extract.

It is commonly known that LC/MS/MS, especially when working in ESI mode, is susceptible to matrix effects, affecting quantitational accuracy. In this study, signal intensities of standards in neat solution were compared with those of standards in matrix-matched solution at different concentration levels to calculate matrix effects (ME). An ME value of less than 100% indicates matrix suppression, whereas an ME value larger than 100% indicates matrix enhancement. As seen in Table 4, both GLY and AMPA show matrix suppression, while GLU shows matrix enhancement. Using matrix-matched standards, one can often compensate for matrix effects, which may allow for good quantitational accuracy without the use of internal standards. Therefore, calibration curves were generated by running matrix-matched calibration standards as described in the experimental section.

Figure 2 shows the calibration curves for GLY, AMPA and GLU. Good linear correlation coefficients ($R^2 \geq 0.997$) were obtained between concentrations of 5 to 500 ng/mL (0.05-5 mg/kg in real sample). For the 5 ng/mL calibrant, the signal-to-noise ratios (S/N) for GLY, AMPA and GLU were 432, 165, and 325, respectively. From these values, the limits of quantitation (LOQs; S/N ≥ 10) were calculated to be 0.12, 0.30 and 0.15 ng/mL. As the EU has set the maximum residue limit (MRL) for glyphosate in oatmeal at 20 mg/kg, the method developed in this study easily meets this requirement.

Table 4. Matrix effect result in oatmeal matrix.

Compound	GLY	AMPA	GLU
Matrix effect (%)	67.3	87.3	107.6

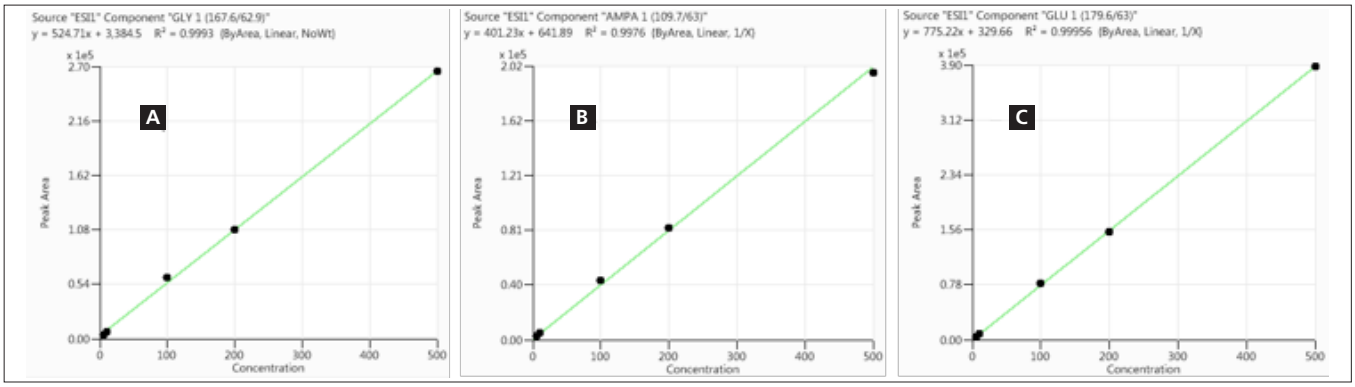

Figure 2. Calibration curves for GLY (A), AMPA (B) and GLU (C) in oatmeal extract, respectively

Table 5. Linear dynamic range, regression coefficients, LOQ and S/N at LOQ level for analytes.

Compound	Range (ng/mL)	R^2	S/N at 5 ng/mL	LOQ in matrix (S/N ≥ 10) Ng/mL
GLY	5-500	0.999	432	0.12
AMPA	5-500	0.997	165	0.30
GLU	5-500	0.999	325	0.15

Recovery of the analytes was evaluated at concentrations of 0.05 and 1 mg/kg. All recoveries were satisfactory, with mean values ranging from 85% to 130%, and relative standard deviations less than 13% for all three analytes (Table 6).

Table 6. Recovery of the analytes from oatmeal sample at different concentration levels.

Compound	Spiked Level (50 µg/kg)		Spiked Level (1 mg/kg)	
	Recovery /%	Recovery /%	Recovery /%	RSD/%
GLY	118	8.74	85	6.91
AMPA	130	11.8	94	8.72
GLU	122	12.9	96	1.93

Conclusion

In this study, we reported a rapid, sensitive and reliable 12 min LC/MS/MS method that allowed direct analysis of GLY, AMPA, and GLU in oatmeal without derivatization. The sample preparation method was a simple water/acetonitrile extraction, which showed good recoveries and minimal matrix effects for all three compounds. The calibration curves for three analytes exhibited good linearity over three orders of magnitude with calibration fit of R^2 greater than 0.997. The LOQs for glyphosate and other related polar compounds were much lower than the EU's MRL of 20 mg/kg in oatmeal.

References

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