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Glyphosate and Aminomethylphosphonic acid (AMPA) analysis in plants using LC-MS/MS

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Novel Aspects

A Glyphosate and AMPA quantitative analysis method was developed on a triple quadrupole mass spectrometer for plant analysis without derivatization.

Introduction

Glyphosate (Gly) is one of the most widely used herbicides in the world. It acts as an enzyme inhibitor to stunt the growth and vitality of plants. The potential health effects on humans exposed to glyphosate have been a concern. Therefore, methods that analyze glyphosate and aminomethylphosphonic acid (AMPA), the main degradant of glyphosate, are highly desired. An LCMS glyphosate and AMPA quantitation method was therefore developed using a Shimadzu triple quadrupole mass spectrometer for research use.

Method – Mass Spectrometry

A LCMS-8060 triple quadrupole mass spectrometer was used to analyze glyphosate and AMPA in ESI negative mode. The MRM transitions (Table 1) were determined and optimized using LabSolutions software. Instrument conditions are listed in Table 2.

Table 1. MRM transitions for Gly and AMPA

Analyte	MRM	Q1 Bias (V)	Collision Energy (V)	Q3 Bias (V)
Glyphosate	168.10>62.90	17	23	15
	168.1>78.95		37	19
AMPA	110.00>79.00	21	27	27
	110.00>63.00		23	13

Table 2. MRM transitions for Gly and AMPA

Heating gas	: 20 L/min
Drying gas	: 3 L/min
Nebulizing gas	: 3 L/min
Interface temperature	: 350 °C
Heat block temperature	: 400 °C
DL temperature	: 250 °C

Method- Reversed Phase Liquid Chromatography

A Bio-Rad Micro-Guard Cation H+ Cartridge (30 x 4.6 mm) was used for the separation. Mobile phase was composed of A: 0.1% FA in H2O, B: ACN, and C: 0.2% phosphoric acid in H2O. A divert valve was used for mobile phase C to wash the column without contacting the mass spectrometer. Chromatography details can be found in Table 3. In effort to minimize glyphosate adsorption, post autosampler injection port tubing was changed to peek.

Table 3. Chromatography method

Time (min)	Command	Value
0	Valve	To Waste
0	B%	20%
0	A+B flow	0.5 mL/min
0.5	Valve	To MS
1	B%	20%
2.5	B%	0%
4	A+B flow	1 mL/min
6.9	Valve	To waste
7	A+B flow	0.5 mL/min
7	C flow	0.5 mL/min
10	B%	0%
10.09	C flow	0.5 mL/min
10.1	B%	20%
10.1	C flow	0 mL/min
12.6	Controller	Stop

Solid standards of glyphosate and AMPA were acquired from Sigma-Aldrich. Dilution and extraction solvent was 0.1% formic acid. Six point calibration curves were created for both AMPA and glyphosate to ensure instrument sensitivity from 0.1 ppb to 10 ppb.
Barley and lemongrass were chosen as sample matrices. They were ground to increase extraction surface area. 1 gram of plant samples were put in to each extraction vial along with 10 mL of 0.1% FA. Glyphosate and AMPA in various concentrations were spiked in the sample to create matrix matched external calibration curve for lemongrass and standard addition for barley. Samples were filtered and directly inject (20 µL) onto the system for analysis.

Results- Standards in neat

Decent chromatographic peaks were obtained in both LLOQ and ULOQ for both Gly and AMPA. Chromatograms of blank, LLOQ (0.1 ppb), and 10 ppb were shown in Figure 1.

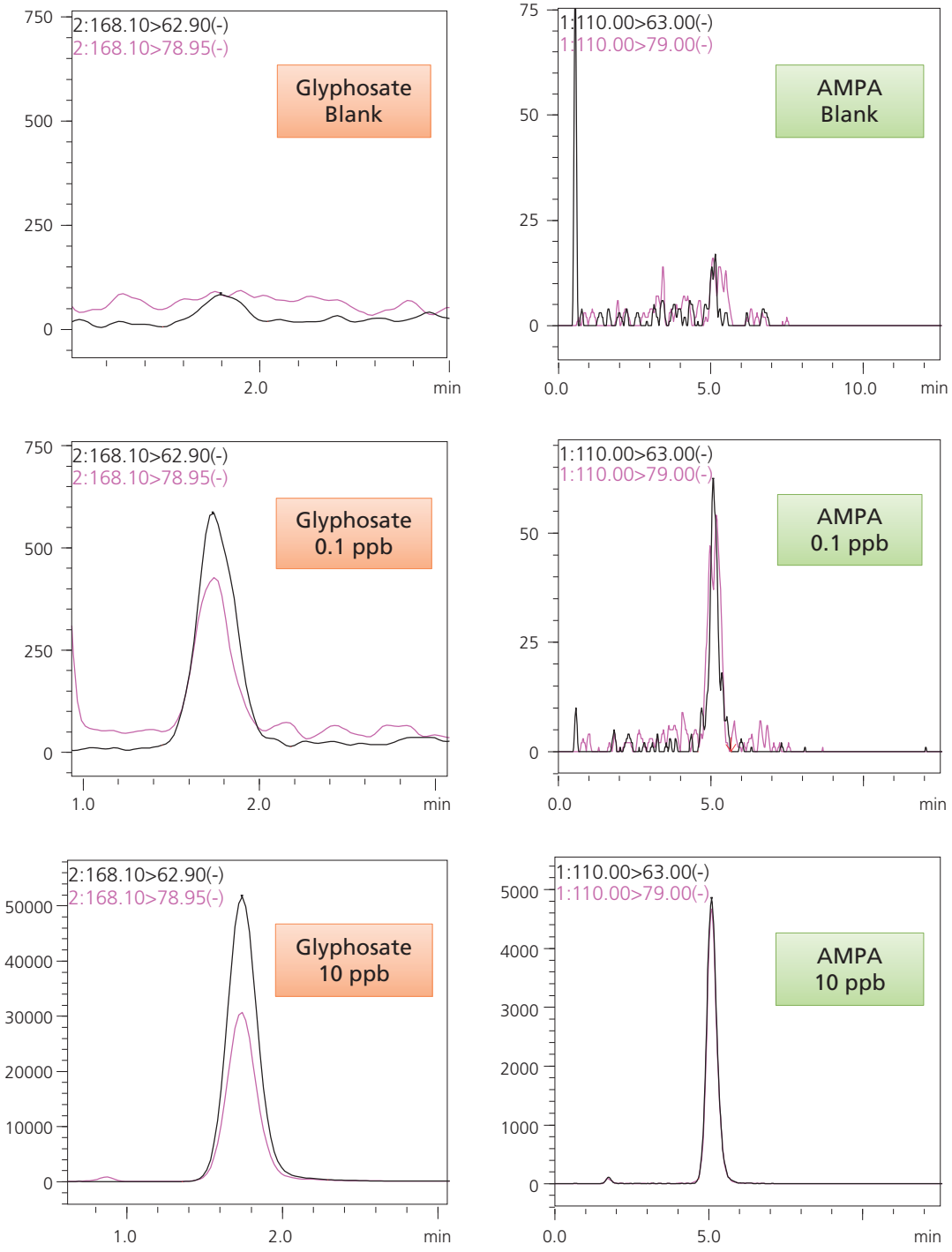

Figure 1. Chromatograms of Gly and AMPA in blank, at 0.1 ppb, and 10 ppb

Calibration curves of Gly and AMPA were obtained (triplicates) in neat standard from 0.1 ppb to 10 ppb. (Figure 2). Acceptable linearity was obtained with R^2=0.9999 for Gly and R^2=0.9997 for AMPA.

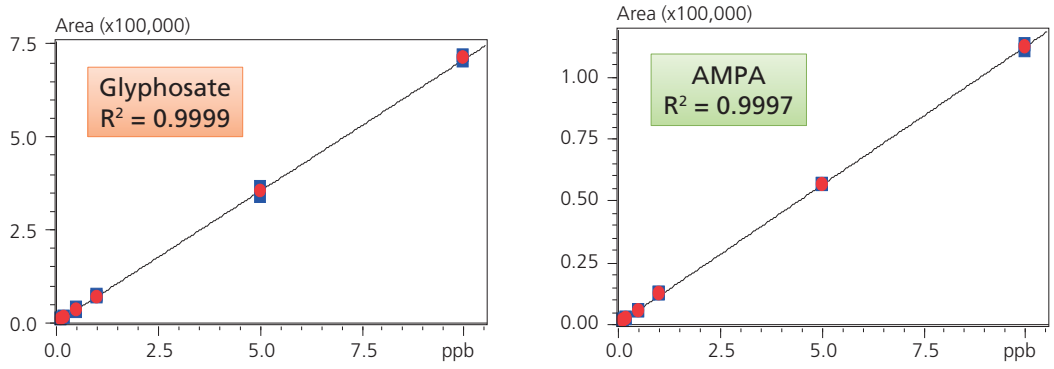

Figure 2. Calibration curves of Gly and AMPA in neat standards

Results- Glyphosate and AMPA in plant matrix

USDA approved organic lemongrass was used as a low glyphosate matrix to generate matrix matched calibration curve. Due to the complexity of matrix and the lack of sample cleaning steps for this initial study, source saturation was observed at 10 ppb. A linear curve was still maintained from 0.1 ppb to 5 ppb. Linearity of R^2= 0.993 for glyphosate and R^2=0.994 for AMPA were obtained. This result shows the LCMS-8060 has the ability to perform matrix matched calibration even at low level (0.1 ppb).

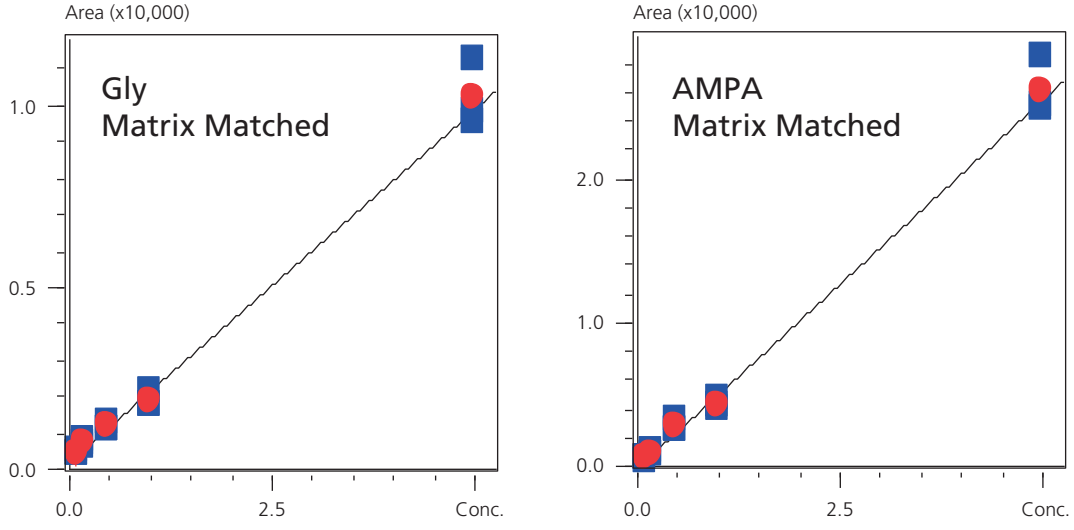

Figure 3. Lemongrass matrix matched calibration for Gly and AMPA

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Barley was chosen to represents matrices already containing glyphosate. For sample matrices containing Gly and AMPA, standard addition can be used to evaluate the levels. Increments of 2 ppb were added to the barley samples. Linear calibration curves were observed with R^2=0.997 for Gly and R^2=0.999 for AMPA. Gly in Barley was found to be 1.084 ppb and AMPA as 0.235 ppb.

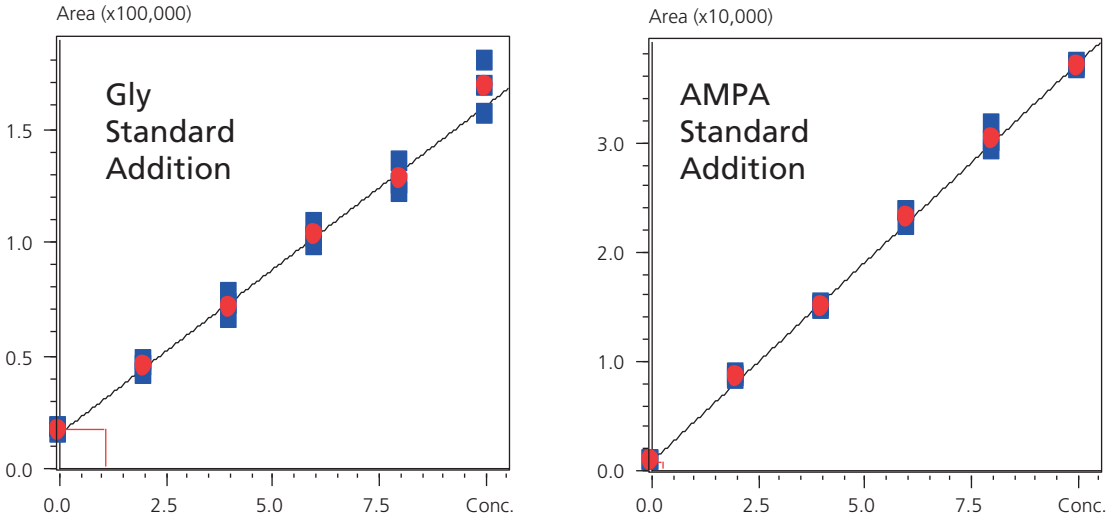

Figure 4. Barley standard addition analysis for Gly and AMPA

Conclusion

MRM transitions for glyphosate and AMPA were identified and optimized using the LCMS-8060 in ESI negative mode. A Bio-Rad Micro-Guard Cation H+ Cartridge was used to obtain separation. Good linearities were obtained from 0.1 ppb to 10 ppb for both glyphosate and AMPA in neat standard. Glyphosate and AMPA were spiked into plant matrices to show feasibility of future research and quality control applications. This analysis demonstrated the capability of LCMS-8060 in quantifying glyphosate and AMPA in various matrices.

Matrix matched calibration curves can be used to quantify Gly and AMPA for samples that do not contain (or with trace amount of) glyphosate. For matrices that contain glyphosate at low levels, standard addition can be used to assess the amount of Gly and AMPA present. Furthermore, Shimadzu LCMS-8060 has a high scanning speed that allows additional herbicides or other compounds of interested to be added to this method for simultaneous analysis.

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Reference

Niemann, Lars, et al. "A critical review of glyphosate findings in human urine samples and comparison with the exposure of operators and consumers." Journal für Verbraucherschutz und Lebensmittelsicherheit 10.1 (2015): 3-12.

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