APPLICATION BRIEF

Environmental

UHPLC/SQ MS Analysis of Glyphosate After Derivatization with FMOC

Glyphosate is a widely used herbicide for both agricultural and urban landscape management applications. It has been shown to have an endocrine disrupting capability that led the U.S. Environmental Protection Agency (EPA) to require testing for glyphosate in drinking and surface water.

We present a method for the analysis of glyphosate and its major degradation product, aminomethylphosphonic acid (AMPA), using UHPLC/SQ MS. Both of these molecules were derivatized using 9-fluorenylmethoxycarbonyl chloride (FMOC) to increase the sensitivity of response by mass spectrometry. Figure 1 (see next page) shows the derivatization reactions of FMOC with glyphosate and AMPA. A post-column diverter valve was also used to redirect the salts eluting in the void volume (0-2 min) and the excess derivatizing agent and its byproducts (11-13 min) through the waste line, maintaining a cleaner LC/MS system.

Experimental Conditions

Target Analytes: Glyphosate (N-(phosphonomethyl) glycine), AMPA (aminomethylphosphonic acid)

Sample Preparation Conditions

A borate buffer (50 µL of 5% tetraborate solution) was added to 100 µL of a series of standard solutions (containing glyphosate, AMPA and ^{13}C, ^{15}N glyphosate at various concentrations). The derivatizing agent FMOC (50 µL of 10 mg/mL of fluorenylmethyloxycarbonyl chloride in acetonitrile) was added to these solutions.

The standard solutions were incubated overnight at room temperature in the dark. The following day, the reaction in each of the standards solutions was stopped by the addition of phosphoric acid (500 µL of 2% solution). The excess FMOC that had precipitated in the solutions upon adding the acid was re-dissolved back into the solution by the addition of methanol (300 µL). The final standard solutions contained concentrations of glyphosate and AMPA ranging from 5 ng/mL to 500 ng/mL for each analyte and internal standard ^{13}C, ^{15}N glyphosate (at a concentration of 100 ng/mL).

Liquid Chromatography Conditions

Pump Type:	PerkinElmer® Flexar™ FX-15		
Column:	Phenomenex®, Prodigy C18 (3.2 mm x 150 mm, 5 µm)		
Mobile Phase:	A: 5 mM ammonium formate in water B: acetonitrile		
Flow Rate:	0.4 mL/min		
Injection Volume:	4 µL in partial fill mode		
Gradient:	**Time (min)**	**%A**	**%B**
	2	80	20
	7	60	40
	1	10	90
	5	10	90

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Mass Spectrometer Conditions

Ionization: Ultraspray™ ESI – Negative mode

The $[M-H]^-$ ions for each of the analytes were monitored in two different time periods:

Time Period 1: (0-8.0 min) SIM ions 390.0 and 392.0 for glyphosate and ^{13}C, ^{15}N respectively; dwell time of 200 ms each

Time Period 2: (8.0-11.0 min) SIM ion 332.0 for AMPA; dwell time of 150 ms

Capillary Exit Voltage: -70 V

Results

The derivatized FMOC products of glyphosate and its degradation product, AMPA, were well separated from each other by UHPLC (Figure 2). The calibration curves for glyphosate and AMPA are plotted as a ratio to the internal standard and show excellent linearity for the concentration range of 5-500 ng/mL (r^2 = 0.998 for both), Figures 3 and 4 respectively. We were easily able to detect 5 ppb concentrations of both glyphosate and AMPA using UHPLC in tandem with the Flexar SQ 300 MS detector.

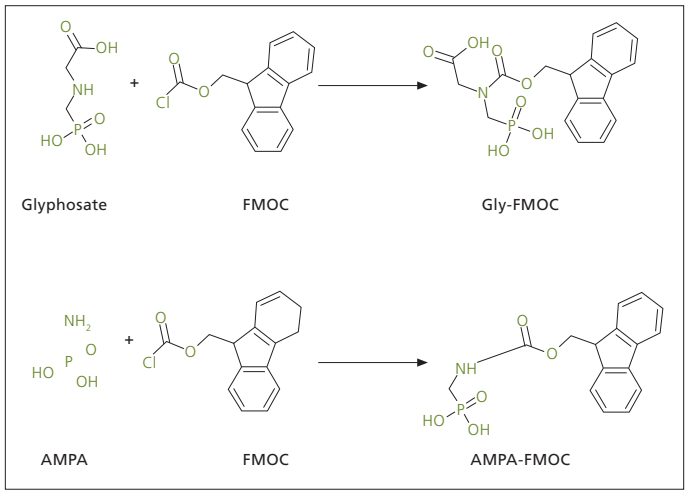

Figure 1. Derivatization reactions of glyphosate and AMPA with FMOC.

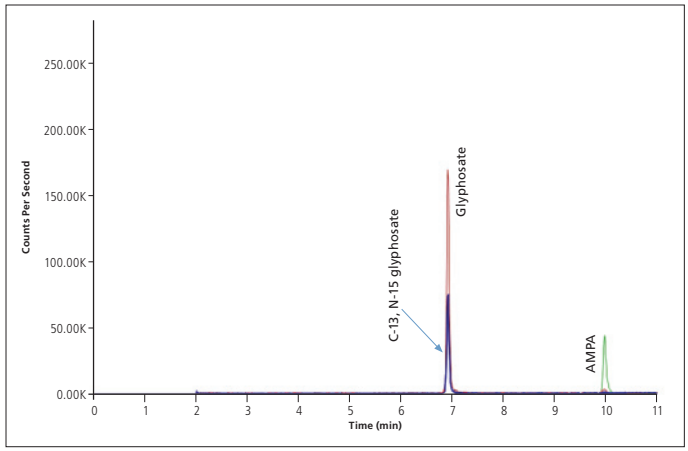

Figure 2. UHPLC/SQ MS elution profile of glyphosate (red trace), ^{13}C, ^{15}N glyphosate (blue trace) and AMPA (green trace).

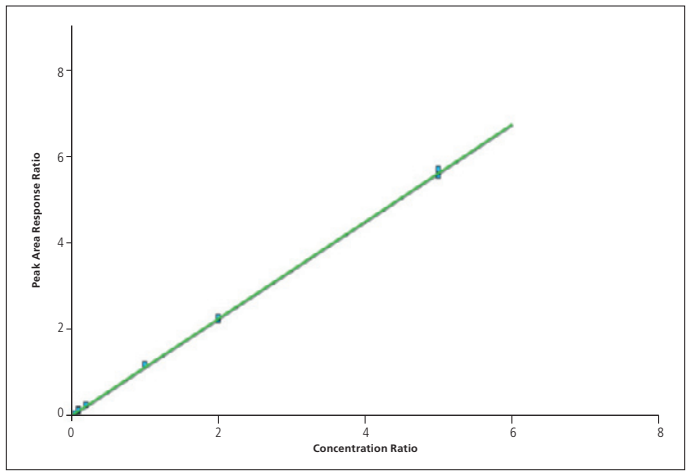

Figure 3. Calibration curve of glyphosate (5-500 ng/mL, n=3) plotted as a ratio to the internal standard, ^{13}C, ^{15}N glyphosate (r^2 = 0.998).

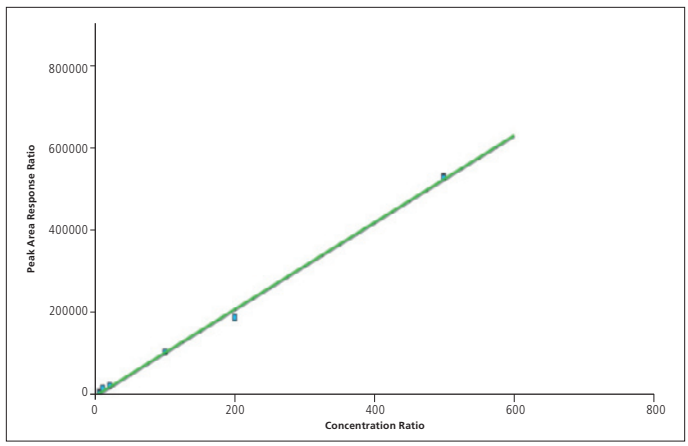

Figure 4. Calibration curve of AMPA (5-500 ng/mL, n=3, r^2 = 0.998) plotted as a ratio to the internal standard, ^{13}C, ^{15}N glyphosate.

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