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Glyphosate Analysis by LC/MS/MS using FMOC Derivitization or Underivitized Method

Matthew Trass[1], Allen Misa[1] and James Turner[2]
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA[1]
Phenomenex Ltd, Queens Ave, Macclesfield, SK10 2BN, UK[2]

Introduction

Glyphosate is the active ingredient in the broad spectrum herbicide Roundup®. It is a contact herbicide which is believed to disrupt the enzymatic pathway responsible for the synthesis of aromatic amino acids (tyrosine, tryptophan and phenylalanine). Originally manufactured by Monsanto, and now by many agrochemical companies across the world, glyphosate was the most widely used herbicide in 2013. Glyphosate is only effective as a herbicide if it comes into contact with growing foliage. Any glyphosate which comes into contact with soil is rapidly metabolized by microbes in the soil to produce AMPA. AMPA binds to soil particles, making the risk of glyphosate or AMPA contaminating ground water low. Despite this, there is a concern that water sources can become contaminated by run-off of glyphosate and deposition of soil containing AMPA.

In analytical studies for glyphosate contamination, it is commonplace to analyze for both parent glyphosate and AMPA. Glyphosate is a small, polar molecule (log P -3.1) and does not contain a chromophore. Retention and detection are therefore challenging. In this study we have developed two approaches to analyzing glyphosate and AMPA by LC/MS/MS. In the first approach we derivatize the analytes using FMOC (Fluorenylmethyloxycarbonyl chloride) and analyze them using a reversed phase procedure. In the second approach we utilize the HILIC separation mode on an amino column.

Materials and Methods

Reagents and Chemicals
All standards were purchased from Sigma-Aldrich.

Experimental Conditions

Sample Preparation
Derivatization Protocol (Method 1 only):

In a 2 mL vial, spike 50 µL of 10 µg/mL Glyphosate and AMPA standards into 1 mL of 0.4 M borate buffer. Add 200 µL of 2 mg/mL FMOC in acetone (make fresh daily) and vortex.

LC/MS/MS Conditions

LC/MS/MS was performed using a Gemini® NX-C18 3 µm 50 x 2.0 mm HPLC column (p/n 00B-4453-B0) or a Luna® NH_2 3 µm 50 x 2.0 mm HPLC column (p/n 00B-4377-B0) on an Agilent® 1200 LC system (Agilent Technologies, Palo Alto, CA, USA) with an upper pressure limit of 600 bar, equipped with a binary pump, autosampler and interfaced with an API 5000™ triple quadrupole mass spectrometer (AB SCIEX, Framingham, MA, USA). The ionization source is electrospray ionization (ESI-) in negative mode.

Method 1.
FMOC Derivatized analytes on a Gemini NX-C18 HPLC Column

Column: Gemini 3 µm NX-C18
Dimensions: 50 x 2.0 mm
Part No.: 00B-4453-B0
Mobile Phase: A: 5 mM Ammonium bicarbonate pH 9
B: 50:50 Acetonitrile/methanol
Gradient:

Time (min)	% B
0.0	20
4.0	90
4.1	20
7.0	20

Flow Rate: 0.40 mL/min
Inj. Volume: 20 µL
Temperature: Ambient
Detection: API 5000 (AB SCIEX) Tandem Mass Spec (MS/MS)
Electrospray ionization (ESI-) in negative mode.
Sample: 1. Glyphosate
2. AMPA

Table 1.
MRM transitions & retention times for FMOC derivatized Glyphosate and AMPA

Analyte	Q1	Q3	Retention Time (min)	Mode
Glyphosate	390.4	168.0/150.2	1.44	-ve
AMPA	332.2	110.0/135.9	2.32	-ve

Figure 1.
Glyphosate (1) and AMPA (2) standards derivatized with FMOC on a Gemini 3 µm NX-C18

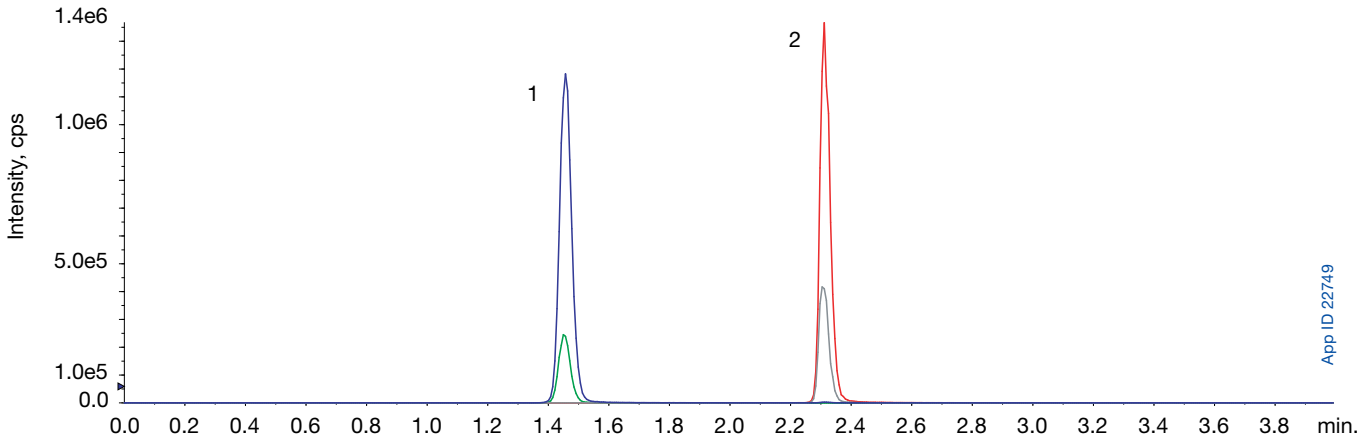

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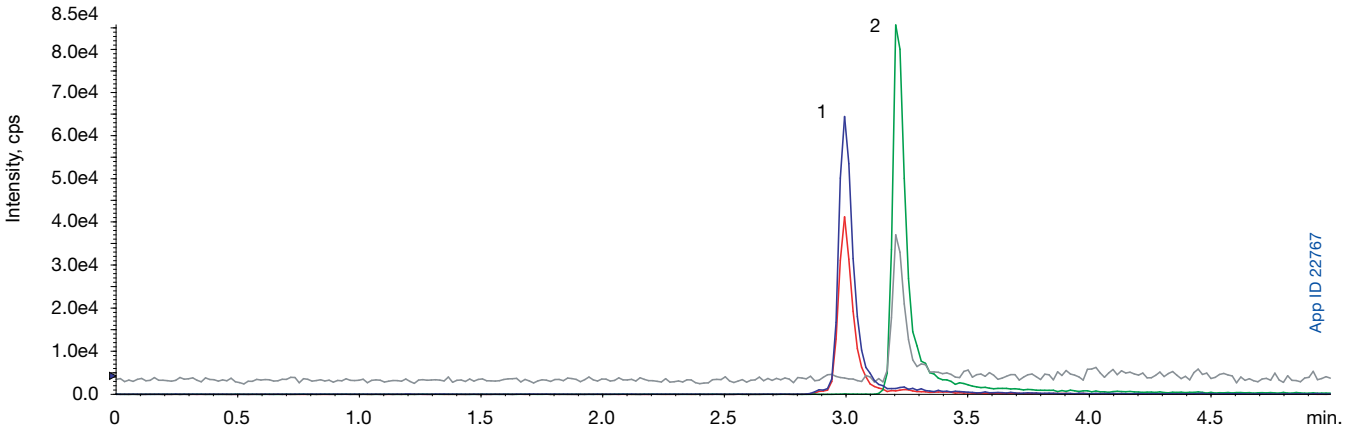
Figure 2.
Glyphosate (1) and AMPA (2) underivatized – Method 2 (Luna® 3 µm NH_2)



Method 2.
Underivatized analytes on Luna NH_2 Amino HPLC Column

Column: Luna 3 µm NH_2
Dimensions: 50 x 2.0 mm
Part No.: 00B-4377-B0
Mobile Phase: A: 10 mM Ammonium Bicarbonate pH 10
B: Acetonitrile

Gradient:	**Time (min)**	**% B**
	0.0	80
	5.0	10
	5.1	80
	8.0	80

Flow Rate: 0.40 mL/min
Inj. Volume: 5 µL
Temperature: Ambient
Detection: API 5000™ (AB SCIEX) Tandem Mass Spec (MS/MS)
Electrospray ionization (ESI-) in negative mode.
Sample: 1. Glyphosate
2. AMPA
Note: 100 ng/mL in initial mobile phase

Table 2.
MRM transitions & retention times for underivatized Glyphosate and AMPA

Analyte	Q1	Q3	Retention Time (min)	Mode
Glyphosate	168.1	63.1/80.9	3.00	-ve
AMPA	110.1	62.9/78.9	3.25	-ve

Results and Discussion

Two different analytical approaches have been developed for the analysis of Glyphosate and its primary metabolite AMPA. The first method requires derivatization and utilizes a conventional reversed phase HPLC method performed on a highly robust TWIN-NX™ Technology column. This provides an extremely stable chromatographic platform allowing for the analysis of relatively dirty samples at high pH 9 while maintaining excellent performance for an extended period of time. The second method does not require derivatization and uses an amino-type stationary phase to retain the highly-polar analytes.

Figure 1. displays the chromatography for the FMOC derivatized procedure – method 1. The analyte peaks are well resolved and exhibit excellent peak symmetry and efficiency. This results in a robust and sensitive method. The HPLC conditions used are well within the operating parameters of Gemini® NX-C18 allowing for stable chromatography over an extended number of injections.

Figure 2. displays the chromatography for the underivatized procedure – method 2. The peak-shape and retention is also very good considering the polarity of the analytes, although resolution is greatly reduced relative to the derivatized approach. An advantage to the underivatized approach is the time and expense saved during sample preparation. In order to obtain acceptable chromatography without derivatization, a high-pH mobile phase is used. The drawback is that the method conditions can cause column life to be reduced. As always, column life is sample dependent, however with high pH HPLC conditions, it is recommended that the chromatography is closely monitored particularly as the column exceeds >150 injections.

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Conclusions

The methodology we have described allows the determination of two very polar molecules, glyphosate and its metabolite AMPA. We have presented two very different approaches to overcome the analytical challenges associated with small polar-molecules. The derivatized method displays excellent peak-shape and resolution, provides a rugged separation over an extended period, but involves an extra sample preparation step. The underivatized method also displays good peak-shape with adequate resolution, removes a sample preparation step, but can result in reduced column-lifetime. Depending on the priorities of your analytical laboratory, one of these analytical approaches can be successfully implemented to analyze glyphosate and its primary metabolite AMPA.

Gemini Ordering Information

3 µm Microbore, Minibore and Narrow Bore Columns (mm)									SecurityGuard™ Cartridges (mm)
Phases	20 x 2.0	30 x 2.0	50 x 2.0	100 x 2.0	150 x 2.0	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0*
									/10pk
NX-C18	00M-4453-B0	00A-4453-B0	00B-4453-B0	00D-4453-B0	00F-4453-B0	00B-4453-Y0	00D-4453-Y0	00F-4453-Y0	AJ0-8367

for ID: 2.0-3.0 mm

3 µm Analytical Columns (mm)					SecurityGuard Cartridges (mm)
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*
					/10pk
NX-C18	00B-4453-E0	00D-4453-E0	00F-4453-E0	00G-4453-E0	AJ0-8368

for ID: 3.2-8.0 mm

* SecurityGuard™ Analytical Cartridges require holder, Part No.: KJ0-4282

5 µm Minibore and Narrow Bore Columns (mm)								SecurityGuard Cartridges (mm)
Phases	30 x 2.0	50 x 2.0	150 x 2.0	50 x 3.0	100 x 3.0	150 x 3.0	250 x 3.0	4 x 2.0*
								/10pk
NX-C18	00A-4454-B0	00B-4454-B0	00F-4454-B0	00B-4454-Y0	00D-4454-Y0	00F-4454-Y0	00G-4454-Y0	AJ0-8367

for ID: 2.0-3.0 mm

5 µm Analytical Columns (mm)					SecurityGuard Cartridges (mm)
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*
					/10pk
NX-C18	00B-4454-E0	00D-4454-E0	00F-4454-E0	00G-4454-E0	AJ0-8368

for ID: 3.2-8.0 mm

guarantee

If Gemini analytical columns do not provide at least an equivalent separation as compared to a competing column of the same particle size, similar phase and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

Ordering Information continued on next page >

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Luna Ordering Information

3 µm Minibore Columns (mm)					SecurityGuard™ Cartridges (mm)
Phases	30 x 2.0	50 x 2.0	100 x 2.0	150 x 2.0	4 x 2.0
					/10pk
NH_2	00A-4377-B0	00B-4377-B0	00D-4377-B0	00F-4377-B0	AJ0-4301
					for ID: 2.0-3.0 mm

3 µm Narrow Bore and Analytical Columns (mm)						SecurityGuard Cartridges (mm)	
Phases	50 x 3.0	150 x 3.0	50 x 4.6	100 x 4.6	150 x 4.6	4 x 2.0	4 x 3.0
						/10pk	/10pk
NH_2	00B-4377-Y0	00F-4377-Y0	00B-4377-E0	00D-4377-E0	00F-4377-E0	AJ0-4301	AJ0-4302
						for ID: 2.0-3.0 mm	3.2-8.0 mm

5 µm Minibore Columns (mm)			SecurityGuard Cartridges (mm)
Phases	150 x 2.0	250 x 2.0	4 x 2.0*
			/10pk
NH_2	00F-4378-B0	00G-4378-B0	AJ0-4301
			for ID: 2.0-3.0 mm

* SecurityGuard™ Analytical Cartridges require holder, Part No.: KJ0-4282

5 µm Narrow Bore and Analytical Columns (mm)							SecurityGuard Cartridges (mm)	
Phases	50 x 3.0	150 x 3.0	250 x 3.0	30 x 4.6	50 x 4.6	75 x 4.6	4 x 2.0*	4 x 3.0*
							/10pk	/10pk
NH_2	00B-4378-Y0	00F-4378-Y0	00G-4378-Y0	00A-4378-E0	00B-4378-E0	00C-4378-E0	AJ0-4301	AJ0-4302
							for ID: 2.0-3.0 mm	3.2-8.0 mm

5 µm Analytical Columns (mm)				SecurityGuard Cartridges (mm)
Phases	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*
				/10pk
NH_2	00D-4378-E0	00F-4378-E0	00G-4378-E0	AJ0-4302
				for ID: 3.2-8.0 mm

Australia
t: 02-9428-6444
f: 02-9428-6445
auinfo@phenomenex.com

Austria
t: 01-319-1301
f: 01-319-1300
anfrage@phenomenex.com

Belgium
t: 02 503 4015 (French)
t: 02 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada
t: (800) 543-3681
f: (310) 328-7768
info@phenomenex.com

Denmark
t: 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland
t: 09 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France
t: 01 30 09 21 10
f: 01 30 09 21 11
franceinfo@phenomenex.com

Germany
t: 06021-58830-0
f: 06021-58830-11
anfrage@phenomenex.com

India
t: 040-3012 2400
f: 040-3012 2411
indiainfo@phenomenex.com

Ireland
t: 01 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy
t: 051 6327511
f: 051 6327555
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico
t: 001-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands
t: 030-2418700
f: 030-2383749
nlinfo@phenomenex.com

New Zealand
t: 09-4780951
f: 09-4780952
nzinfo@phenomenex.com

Norway
t: 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico
t: (800) 541-HPLC
f: (310) 328-7768
info@phenomenex.com

Sweden
t: 08 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom
t: 01625-501367
f: 01625-501796
ukinfo@phenomenex.com

United States
t: (310) 212-0555
f: (310) 328-7768
info@phenomenex.com

All other countries:
Corporate Office USA
t: (310) 212-0555
f: (310) 328-7768
info@phenomenex.com

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guarantee

If Luna analytical columns do not provide at least an equivalent separation as compared to a competing column of the same particle size, similar phase and dimensions, send in your comparative data within 45 days and keep the Luna column for FREE.

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