Analysis of Glyphosate & AMPA in Water by Fully Automated Online SPE - LC/MS/MS

Tarun Anumol
Agilent Technologies Inc.

Shane Snyder
University of Arizona

August 3, 2015
Glyphosate Application and Properties

• Thought to be the most widely used pesticide in the world
  - A non-selective herbicide, absorbed rapidly by plants
  - Sold as ‘Roundup’ by Monsanto in US

• Expected to have low toxicity and high efficiency

• Highly water soluble and involatile
  - Very polar and ionic

• Fairly Rapid degradation in soil and is biodegradable
Aminomethylphosphonic acid (AMPA)
General properties

- Chief *degradation product* of glyphosate
- Similarly low acute toxicity like glyphosate
- Chronic toxicity to aquatic animals has not been evaluated
- Can be degraded by bacteria in soil to phosphoric acid
Glyphosate & AMPA
Presence in water

• Presence detected in several US streams and rivers\(^1\)

• Detected in European groundwater sources\(^2\)

• Maximum allowable concentration in drinking water set by the European Community for several polar pesticides of 0.1 µg/L

\(^1\) Battaglin et al., Glyphosate, Other Herbicides, and Transformation Products in Midwestern Streams. JAWRA Journal of the American Water Resources Association 2005, 41, (2), 323-332.

New Toxicological Characterization

World Health OrganizationLabels Glyphosate Probable Carcinogen

Health Agency Says Widely Used Herbicide Likely Carcinogenic
Herbicide, glyphosate, is sold by Monsanto under Roundup brand

Glyphosate is a 'probably carcinogenic' pesticide. Why do cities still use it?

Cities use glyphosate to control weeds in parks and along verges. Now that the WHO says the pesticide is 'probably carcinogenic to humans', is it time to stop?

Netherlands Bans Monsanto’s Roundup to Protect Citizens from Carcinogenic Glyphosate

By Jennifer Lalley
Global Research, May 26, 2015
Natural News 24 May 2015

In the Netherlands, people who have been spraying their lawns and gardens with Roundup will have to find another way to protect their land from pests. Late last year, the Dutch parliament voted to ban the sale of glyphosate-based herbicides to private parties. The ban, under which agricultural use is excluded, was initially proposed several years ago. However, it is thought that Monsanto influence prevented it from taking place at the time.
Agilent Infinity Flexcube OSPE system

Schematic system set-up
1260 Infinity LC – 6460 MS(MS)

- Solvents required for SPE - rinsing, conditioning
- Triple quad MS for quantification

- Solvents for LC
- Column Compartment
- Flexible Cube
- Autosampler
- Thermostat
- 6460 MS
1200 Infinity Series Online SPE product and concept

Solvent selection valve for up to three solvents

Reciprocating single-piston pump for flows up to 4 ml/min (60 bar)

up to two Quick-Change valves, according to application
Online SPE valve setup

LOAD position
Online SPE valve setup

ELUTE position
Derivatization reaction with FMOC

Workflow

1. Collect 25 mL sample

   ![Glyphosate](image)

2. Derivatization

   - 25 ml sample
   - 250 uL of 10 ug/L surrogate
   - 1 ml EDTA
   - 1 ml Borate buffer
   - 2.5 ml ACN
   - 0.5 ml of 20 mM FMOC
   - Heat at 100 C for 30 min
   - 50 uL Phosphoric Acid

3. Flexcube 900 uL

4. 6460 LC/MS/MS
Separation Parameters
Agilent 1290 LC & Flexcube

LC Column: Poroshell EC 120 C-18, 3x50 mm, 2.7 µm
Flowrate: 0.35 mL/min
Injection Volume: 900 µL
Column Compartment Temperature: 30°C

LC Mobile Phase:
A – Water + 5mM Ammonium Acetate
B – Acetonitrile

Flexcube Solvents:
A - [95/5(v/v): HPLC Water/Acetonitrile] + 0.1% Acetic Acid
B - 1/1/1 (v/v/v): Acetonitrile/Methanol/Isopropanol
## Gradient Profiles

### Flexcube

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Function</th>
<th>Volume</th>
<th>Flowrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Pump Solvent A</td>
<td>4 mL</td>
<td>1 mL/min</td>
</tr>
<tr>
<td>4</td>
<td>Valve Change Position to Elution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Pump Solvent B</td>
<td>4 mL</td>
<td>1 mL/min</td>
</tr>
<tr>
<td>10</td>
<td>Pump solvent A</td>
<td>2 mL</td>
<td>1 mL/min</td>
</tr>
</tbody>
</table>

### Analytical

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile Phase</th>
<th>Flow rate (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5% B</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>5% B</td>
<td>0.4</td>
</tr>
<tr>
<td>8</td>
<td>55% B</td>
<td>0.4</td>
</tr>
<tr>
<td>9</td>
<td>95% B</td>
<td>0.4</td>
</tr>
<tr>
<td>10</td>
<td>95% B</td>
<td>0.4</td>
</tr>
<tr>
<td>10.1</td>
<td>5% B</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Stop time: 12 min  
Post time: 1 min
## Analyte transitions for LC-MS/MS analysis

<table>
<thead>
<tr>
<th>Compound</th>
<th>Precursor ion</th>
<th>Product ion</th>
<th>Fragmentor voltage</th>
<th>Collision Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate</td>
<td>392</td>
<td>179</td>
<td>100</td>
<td>24</td>
</tr>
<tr>
<td>glyphosate $^{13}\text{C}_2,^{15}\text{N}_1$</td>
<td>394</td>
<td>90.1</td>
<td>100</td>
<td>16</td>
</tr>
<tr>
<td>AMPA</td>
<td>334</td>
<td>112</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>AMmPA $^{13}\text{C}_1,^{15}\text{N}_1,\text{D}_2$</td>
<td>338</td>
<td>116</td>
<td>100</td>
<td>10</td>
</tr>
</tbody>
</table>

**Q1: Most Abundant Transition; Q2: Second-most Abundant Transition**

**Analysis:**

- ESI Positive
- Mode: MRM
- Dwell time: 20 ms
- Cell Accelerator Voltage: 2 eV
Source Optimization for Agilent 6460 MS/MS
Linearity: Calibration Curves

Glyphosate: 0.2-500 ng/L
10 cal stds.
$R^2 > 0.995$

AMPA: 0.2-500 ng/L
10 cal stds.
$R^2 > 0.999$
Sample Chromatogram

- Glyphosate 392 -> 179
- Glyphosate 392 -> 88
- AMPA 334 -> 179
- AMPA 334 -> 112

10 ng/L in Mili-Q water
Limit of detection and quantification (LOD/LOQ)

Limit of Detection (LOD): \( S/N > 3 \) for most abundant transition
Limit of Quantification (LOQ): \( S/N > 9 \) for both transitions

<table>
<thead>
<tr>
<th>Analyte</th>
<th>S/N for 0.2 ng/L standard</th>
<th>Expected LOQ (ng/L)</th>
<th>Expected LOD (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate (Q1)</td>
<td>25.8</td>
<td>0.1</td>
<td>0.025</td>
</tr>
<tr>
<td>Glyphosate (Q2)</td>
<td>18.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMPA (Q1)</td>
<td>13.1</td>
<td>0.2</td>
<td>0.045</td>
</tr>
<tr>
<td>AMPA (Q2)</td>
<td>10.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Surface & Ground Water analysis

- Samples analyzed from a ground water source in AZ and a surface water (Colorado river).
- Surface Water (n=4): Glyphosate – 1.5 ng/L ; AMPA – 0.5 ng/L
- Ground Water (n=4): Glyphosate – ND ; AMPA - ND

- Samples were spiked at two levels (20 ng/L and 100 ng/L) to determine method recoveries.
## Method Performance

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Ground Water</th>
<th>Surface Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 ng/L spike (n=5)</td>
<td>100 ng/L spike (n=5)</td>
</tr>
<tr>
<td></td>
<td>Recovery (%)</td>
<td>Recovery (%)</td>
</tr>
<tr>
<td></td>
<td>RSD (%)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>88.6</td>
<td>93.4</td>
</tr>
<tr>
<td></td>
<td>2.7</td>
<td>2.4</td>
</tr>
<tr>
<td>AMPA</td>
<td>98.9</td>
<td>94.1</td>
</tr>
<tr>
<td></td>
<td>7.7</td>
<td>4.5</td>
</tr>
</tbody>
</table>

- Recoveries for AMPA and glyphosate in both waters was 75-100%
- RSD (%) was <10% in all spikes
Conclusions

• A sensitive and robust method for trace level analysis of Glyphosate & AMPA has been developed using the Agilent 1290 UHPLC coupled to the 6460 MS/MS

• The Flexcube allows automated online sample enrichment thus reducing labor time while drastically reducing required sample volume

• A second transition is added for additional validation and increased specificity

• Very low ng/L levels of quantitation are possible

• Recoveries in both surface and ground waters are good.
Acknowledgements

Agilent Technologies
- Sheher Mohsin
- Craig Marvin
- Joe Weitzel

University of Arizona
- Colin Richards
QUESTIONS ???

tarun.anumol@agilent.com
Ph (O): 302-636-1517