Overcoming the Challenges of Analysing Anionic Polar Pesticides in Food

Benjamin Wuyts, PhD and Euan Ross
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European Headquarters
Introduction

- Multiresidue analyses determine as many residues as possible in the smallest number of analyses
  - Generic extraction, no/limited cleanup, highly selective determination step (GC- and LC-MS/MS or HRMS)
  - A number of different very successful implementations
    - *e.g.* QuEChERS, mini Luke...

- Polar pesticides in many cases are not amenable to the generic multiresidue approach as they are challenging to analyse.

- The source of these difficulties arise from the physicochemical properties of these compounds, which impact and complicate each stage of the analysis.

- Historically these compounds have been analysed in a series of selective single residue methods (SRM), adding significant costs so were often excluded from surveillance.

- As well as glyphosate, EU screening labs also want to include AMPA (glyphosate metabolite) and a number of other challenging polar pesticides in a single method.
A Selection of Polar/Ionic Pesticides

- Cyromazine
- Amitrole
- Ethylenethiourea
- Propylenethiourea
- Chlormequat
- Mepiquat
- Maleic hydrazide

- Ethephon
- Glufosinate
- Glyphosate
- Aminomethylphosphonic acid (AMPA)
- Perchlorate
- Chlorate
Some Example EU MRLs in Different Commodities

- Where usage is approved, MRLs are often set relatively high
  - Glyphosate in barley: 20 mg/kg
  - Glufosinate on sunflower seeds: 5 mg/kg
  - Ethephon on blueberries: 20 mg/kg

- Where no MRLs have been set the default MRL “at or about the limit of determination” applies
  - For glyphosate and glufosinate: 0.1 mg/kg
  - For ethephon: 0.05 mg/kg

- Exception is for food intended for infants and young children where the MRL for these compounds is 0.01 mg/kg throughout

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Glyphosate EU MRL mg/kg (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange</td>
<td>0.5</td>
</tr>
<tr>
<td>Apples</td>
<td>0.1</td>
</tr>
<tr>
<td>Table Grapes</td>
<td>0.5</td>
</tr>
<tr>
<td>Blueberries</td>
<td>0.1</td>
</tr>
<tr>
<td>Mango</td>
<td>0.1</td>
</tr>
<tr>
<td>Wheat</td>
<td>10</td>
</tr>
<tr>
<td>Milk</td>
<td>0.05</td>
</tr>
<tr>
<td>Soyabean</td>
<td>20</td>
</tr>
<tr>
<td>Honey</td>
<td>0.05</td>
</tr>
</tbody>
</table>
What’s All The Fuss About Glyphosate?

First commercialised by Monsanto under the name ‘Roundup’.

Widely adopted herbicide for agricultural and domestic use across the globe.

Use as a desiccant on cereal crops to aid harvest - results in an increased frequency of residues in cereal-based products such as bread and breakfast cereals and beer.

‘WHO’ Group 2A - limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals.

Glyphosate's EU license has been extended for 18 months during last hours before expiration and will be re-evaluated at the end of 2017.
Why is Glyphosate Such a Challenge to Analyse?

<table>
<thead>
<tr>
<th>Method Step</th>
<th>Challenges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Preparation</td>
<td>Glyphosate is very polar, water soluble and insoluble in organic solvents, and will bind to metal ions, therefore making the extraction possibilities limited. Derivatization can be very time consuming.</td>
</tr>
<tr>
<td>Separation</td>
<td>Insufficient retention on column using reverse phase, requires derivatization, HILIC or IEX stationary phase.</td>
</tr>
<tr>
<td>Analysis</td>
<td>Within the extract, other water soluble matrix compounds can also be found (proteins, sugars, amino acids, salts, etc.) that interfere with the determination of glyphosate. The use of derivatization is not compound specific and can lead to selectivity issues.</td>
</tr>
</tbody>
</table>
Analysis of Polar Pesticides

- Broad range of analytical approaches available
  - LC or IC with MS and/ or MS/MS detection
  - Each with associated benefits and challenges

**Options for extraction and cleanup**

- Extraction with aqueous and aqueous/organic solvents?
- SPE for cleanup using pass through (e.g. Oasis HLB) or trap and elute (SAX, MAX or WAX)
- Partition with organic solvents for cleanup
- Derivatisation with FMOC?

**Liquid Chromatography options include**

- Reversed-phase (RP) LC
- Ion chromatography (IC)
- Hydrophilic interaction liquid chromatography (HILIC)
- Porous graphitised/graphitic carbon (PGC)
- Mixed mode
Reversed-phase LC (e.g. C$_{18}$)

- Need retention greater than that equivalent to two column volumes
- Void volume for 4.6 x 150 mm column is 1.74 ml
- Flow rate is 0.75 ml/min so $T_0$ is 2.33 minutes
- **Peak is un-retained!**
  - Along with lots of other co-extractives...
Derivatization Method
Sample Extraction and Derivatization

Sample Pre-Treatment
• Chopping, Grinding and homogenization

Sample Extraction
• Samples are spiked with labelled internal standards
• Samples are extracted by an aqueous extraction

Sample Cleanup
• Some matrix effects may be removed using D.SPE or Pass Through SPE sorbents such as MCX, HLB or C18

Derivatization
• Commonly carried out using FMOC-Cl
• Samples furthered clean to remove excess reagent

Analysis
• LC-MS/MS using C18 based reversed phase chemistries such as BEH C18 or HSS T3
Using FMOC:
Glyphosate, AMPA and Glufosinate

0.02 mg/kg in barley

ESI -ve

3.39
8628

3.39
74520

ESI +ve

2.99
2193

3.78
17046

Glufosinate

ESI +ve

3.41
6255

3.39
17630

ESI -ve

2.99
5192

3.78
5543

Glyphosate

ESI -ve

2.99
2193

3.78
17046

AMPA

ESI +ve

3.41
6255

3.39
17630

0.02 mg/kg in tea

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Derivatization Method
The Pros and Cons

**Pros To Derivatization**

- Allows for retention by reverse phase (C18)
- Molecular mass is increased facilitating better MS/MS detection.
- Can be automated, to reduce handling and increase efficiency.
- Established approach

**Cons To Derivatization**

- Multiple steps need to be optimised, such as reagent concentration and reaction time
- Specificity - FMOC reacts with other compounds in the sample
- Sample cleanup is typically required before and after derivatization
- High cost per sample
Avoiding Derivatization
What Are the Options?

- Reversed-phase (RP) LC
- Porous graphitised/graphitic carbon (PGC)
- Ion chromatography (IC)
- Hydrophilic interaction liquid chromatography (HILIC)
- Mixed mode
# QuPPe Method\(^{(2)}\)  
**Anionic Pesticides**

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<tr>
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<td>Separation principle</td>
<td>Anion Exchange</td>
<td>Anion Exchange</td>
<td>Carbon</td>
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## NEGATIVE MODE

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<th>M 2</th>
<th>M 3</th>
<th>M 4.1</th>
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</tbody>
</table>

## POSITIVE MODE

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### QuPPe Method

#### Sample Extraction

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample Pre-Treatment</strong></td>
<td>• Chopping, Grinding and homogenization</td>
</tr>
</tbody>
</table>
| **Sample Extraction**                                               | • Weigh 10g* of sample into a 50ml tube  
• Add labelled internal standards  
• Add 10ml of methanol with 1% formic acid                         |
| **Shake and Centrifuge**                                            | • Shake for 15 minutes  
• Centrifuge for 5 minutes                                         |
| **Sample Clean-up (Optional)**                                     | • Dispersive SPE or pass-through SPE with a hydrophobic sorbent i.e. C18                             |
| **Filter extract and transfer to vial**                             | • Filter extract and transfer to polypropylene vials                                                  |

* Adjusted depending on water content as per QuPPe method

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Avoiding Derivatization
What Are the Options?

- Reversed-phase (RP) LC
- Porous graphitised/graphitic carbon (PGC)
- Ion chromatography (IC)
- Hydrophilic interaction liquid chromatography (HILIC)
- Mixed mode
Using Porous Graphitised Carbon: Selected Anionic Compounds in Mango

- QuPPe Extraction: 1% formic acid in water/MeOH (1:1 v/v)

- Hypercarb using 1% acetic acid (aq) and methanol

- Xevo TQ-XS, electrospray in negative ion mode

- Column needs considerable conditioning* with extracts to cover certain active sites on the surface

- Significant variation in retention times mitigated by the use of stable isotope analogues and use of relative retention time for identification purposes

* QuPPe(2) page 24, section 5.7.3.1
Avoiding Derivatization
What Are the Options?

Reversed-phase (RP) LC
Porous graphitised/graphitic carbon (PGC)
Ion chromatography (IC)
Hydrophilic interaction liquid chromatography (HILIC)
Mixed mode
Using Anion Exchange: Waters IC-Pak Anion HR

- Anion exchange column, non-suppressed
- IC column with volatile buffers
  - 1 mM citric acid (aq) at pH 11 (DMA)
- Xevo TQ-XS, electrospray in negative ion mode
- Uses low amounts of buffers not recommended for LC-MS/MS (1 mM citric acid)
- Poor efficiency of desolvation with high aqueous mobile phase
Avoiding Derivatization
What Are the Options?

Current Application Work

- Ion chromatography (IC)
- Hydrophilic interaction liquid chromatography (HILIC)
- Mixed mode

Benjamin Wuyts, PhD
European Field Applications Chemist
Waters Corporation
Xevo TQ-XS

- Most sensitive MS we have ever produced with market-leading performance
  - **Ultimate sensitivity** from StepWave XS
  - **Easy method transfer**/accessible sensitivity with XDR detector
  - **Tool-free probes** for improved customer experience
Xevo TQ-XS

- Redesigned ion guide to optimise performance for challenging compounds

RF voltage constant improving robustness while mass switching

Segmented quadrupole second stage – focussed ion beam

Wider profile first stage, more space for ion cloud

Horizontal plates provide more controlled extraction of ions from gas flow – less energetic collisions

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COLUMN 1

Acclaim Trinity Q1

Mixed Mode Column
Column 1
Background

- **Acclaim Trinity Q1**
  - Mixed mode: 
    - WCX, WAX, RP
    - Silica-based
  - Rationale:
    - Referenced FDA\(^3\) paper (N. Chamkasem et al, 2015)
# Column 1

## LC-MS Settings

### Acclaim Trinity Q1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ion mode</th>
<th>Transitions</th>
<th>Cone voltage (V)</th>
<th>Collision energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate</td>
<td>ESI-</td>
<td>168 &gt; 62.9, 168 &gt; 80.9, 168 &gt; 150</td>
<td>30</td>
<td>16, 16, 8</td>
</tr>
<tr>
<td>AMPA</td>
<td>ESI-</td>
<td>110 &gt; 62.9, 110 &gt; 78.9, 110 &gt; 80.9</td>
<td>30</td>
<td>15, 15, 11</td>
</tr>
<tr>
<td>Glufosinate</td>
<td>ESI-</td>
<td>180 &gt; 62.9, 180 &gt; 84.95, 180 &gt; 134</td>
<td>30</td>
<td>16, 16, 16</td>
</tr>
</tbody>
</table>

### Column

- **Acclaim Trinity Q1 column (3 µm, 3 x 100 mm)**
- **Solvent A**: 50mM Ammonium Formate (pH 2.9)
- **Solvent B**: ACN
- **Column Temperature**: 35°C
- **Injection Volume**: 5 – 30 µL
- **Flow Rate**: 0.5 mL/min
- **Mobile Phase**: 95% A
Column 1
Example Chromatography

Acclaim Trinity Q1

- Chromatography (50 ng/mL)

- Glufosinate

- Glyphosate

- AMPA
Column 1
Linearity

Acclaim Trinity Q1

- Linearity
  - Glyphosate
  - Glufosinate
  - AMPA

- Linearity: 1 – 1000 ng/mL
- Residuals below 15%
- Correlation coefficient > 0.999
Column 1
Precision and Injection Volume

Acclaim Trinity Q1

- Repeatability

<table>
<thead>
<tr>
<th>Conc (ng/mL)</th>
<th>RSD (% n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7.2</td>
</tr>
<tr>
<td>50</td>
<td>4.2</td>
</tr>
<tr>
<td>AMPA</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>14.9</td>
</tr>
<tr>
<td>50</td>
<td>4.8</td>
</tr>
<tr>
<td>Glufosinate</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>12.0</td>
</tr>
<tr>
<td>50</td>
<td>8.4</td>
</tr>
</tbody>
</table>

- Injection volume linearity

- Inj vol linearity: 0.2 – 30 µL
- Residuals below 15%
- Correlation coefficient > 0.999
**Column 1 Sensitivity**

**Acclaim Trinity Q1**

- **Sensitivity**
  - LLOQ: $S/N = 10$
  - LLOQ < 1 ng/mL

<table>
<thead>
<tr>
<th></th>
<th>LLOQ (ng/mL)</th>
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<tbody>
<tr>
<td>Glyphosate</td>
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</tr>
<tr>
<td>AMPA</td>
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</tbody>
</table>
Column 1
Linearity in Matrix

Acclaim Trinity Q1

- Linearity in food matrices
  - Matrix: honey

- Calibration curves (n=3)
- Linearity: 1 – 100 ng/mL
- Residuals below 15%
- Correlation coefficient > 0.995
Column 1
Matrix Robustness

Acclaim Trinity Q1

- Sample prep (QuPPe extraction)

<table>
<thead>
<tr>
<th></th>
<th>Wheat</th>
<th>Lentils</th>
<th>Barley</th>
<th>Beer</th>
<th>Honey</th>
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</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>15</td>
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<td><strong>AMPA</strong></td>
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<td>11.9</td>
<td>13.5</td>
<td>12.9</td>
<td>14.0</td>
</tr>
</tbody>
</table>

- EU directive
- Fast (< 30 min)
- Easy

Example chromatogram of 25 ng/mL pesticides in lentils matrix

Gly, Gluf: RSD < 15%
AMPA: RSD < 20%
Column 1
Maintenance

Acclaim Trinity Q1

- Maintenance
  - Column wash

Peak tailing glyphosate ↔ metal ion contamination?
Column 1
LC Maintenance

Acclaim Trinity Q1

- Maintenance
  - LC system

Before cleanup

[Graph showing Glufosinate and Glyphosate before cleanup]

After cleanup

[Graph showing Glufosinate and Glyphosate after cleanup]

Glufosinate

Glyphosate

AMPA
Highly Sensitive Analysis of Polar Pesticides in Food Matrices on the Xevo TQ-XS

Benjamin Wuyts,¹ Dimple Shah,² Euan Ross,¹ Jonathan Fox,¹ Eimear McCall¹
¹Waters Corporation, Brussels, Belgium; ²Waters Corporation, Milford, MA, USA

- Remaining issue
  - %RSD AMPA
  - Too close to void volume (retention factor (K) = 0.22 min)

Search for different column chemistry which increases AMPA’s retention time
COLUMNS 2

IC-Pak Anion HR

Anion Exchange
Column 2
Background

IC-Pak Anion HR

- **IC**
  - Polymethacrylate resin with quaternary ammonium sites
  - (QuPPe\(^{2}\): IonPac AS11 method)

- **HR** (high resolution, 4.6x75mm, 6µm)

- Quantifies anions at ng/mL level: F\(^-\), Cl\(^-\), NO\(_2\)\(^-\), Br\(^-\), NO\(_3\)\(^-\), HPO\(_4\)\(^{2-}\), SO\(_4\)\(^{2-}\)

- **LC settings:**

<table>
<thead>
<tr>
<th>Column</th>
<th>Waters IC-Pak A HR (6 µm, 4.6 x 75 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent A</td>
<td>Water</td>
</tr>
<tr>
<td>Solvent B</td>
<td>1 mM citric acid + DMA (pH 11)</td>
</tr>
<tr>
<td>Column Temperature</td>
<td>35°C</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>2 µL</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>0.4 mL/min</td>
</tr>
</tbody>
</table>
Column 2
Example Chromatography

IC-Pak A HR

- Chromatography
  - 100 ng/mL

- Sensitivity
  - LLOQ: S/N = 10
  - LLOQ < 2 ng/mL

<table>
<thead>
<tr>
<th></th>
<th>LLOQ (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate</td>
<td>1.9</td>
</tr>
<tr>
<td>Glufosinate</td>
<td>0.5</td>
</tr>
<tr>
<td>AMPA</td>
<td>0.8</td>
</tr>
</tbody>
</table>

- Issues
  - Suboptimal flow rate
  - pH 11
  - Citric acid

Search for different column chemistry with acceptable LC conditions
COLUMN 3

Metrosep A Supp 5

HILIC
Column 3
Background

Metrosep A Supp 5

- HILIC
  - Polyvinyl alcohol with quarternary ammonium groups

- Dimensions: **5 µm, 2 x 150 mm**

- These dimensions offer the optimum combination of selectivity and capacity

- With its low eluent flow, this column is particularly suitable for IC-MS coupling
Column 3
LC-MS Conditions

Metrosep A Supp 5

- LC-MS settings
  - 5 polar pesticides added (Gly, Gluf, AMPA: same as previous)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ion mode</th>
<th>Transitions</th>
<th>Cone voltage (V)</th>
<th>Collision energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorate</td>
<td>ESI-</td>
<td>82.8 &gt; 66.8</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Ethephon</td>
<td>ESI-</td>
<td>142.85 &gt; 78.8</td>
<td>20</td>
<td>17 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>142.85 &gt; 106.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fosethyl Aluminium</td>
<td>ESI-</td>
<td>108.85 &gt; 62.85</td>
<td>20</td>
<td>18 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>108.85 &gt; 80.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphonic acid</td>
<td>ESI-</td>
<td>80.8 &gt; 62.8</td>
<td>20</td>
<td>14 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80.8 &gt; 78.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maleic hydrazide</td>
<td>ESI-</td>
<td>110.85 &gt; 54.9</td>
<td>20</td>
<td>15 20 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>110.85 &gt; 81.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>110.85 &gt; 82.85</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Column</th>
<th>Metrosep A Supp 5 (5 µm, 2.0 x 150 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent A</td>
<td>80:20 water:ACN</td>
</tr>
<tr>
<td>Solvent B</td>
<td>80:20 45 mM Ammonium Bicarbonate: ACN</td>
</tr>
<tr>
<td>Column Temperature</td>
<td>Room temperature <em>(doesn’t fit in column oven)</em></td>
</tr>
<tr>
<td>Injection Volume</td>
<td>10 µL</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>0.21 mL/min</td>
</tr>
</tbody>
</table>
Column 3
Example Chromatography

Metrosep A Supp 5

- Chromatography

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>Transitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMPA</td>
<td>109.85 &gt; 62.85</td>
</tr>
<tr>
<td></td>
<td>109.85 &gt; 78.85</td>
</tr>
<tr>
<td></td>
<td>109.85 &gt; 80.85</td>
</tr>
<tr>
<td>Fos Al</td>
<td>108.85 &gt; 62.85</td>
</tr>
<tr>
<td></td>
<td>108.85 &gt; 80.8</td>
</tr>
<tr>
<td>Phosp acid</td>
<td>80.8 &gt; 62.8</td>
</tr>
<tr>
<td></td>
<td>80.8 &gt; 78.8</td>
</tr>
</tbody>
</table>
Column 3 Optimisation

Metrosep A Supp 5

- Chromatography

Optimizing gradient conditions

AMP A

Fosethyl AL

Co-elution

Separation

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Column 3
Final Chromatography

Metrosep A Supp 5
- Chromatography
- Sensitivity
  - LLOQ: S/N = 10
  - LLOQ ≤ 3.1 ng/mL

<table>
<thead>
<tr>
<th>Compound</th>
<th>LLOQ (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate</td>
<td>0.9</td>
</tr>
<tr>
<td>AMPA</td>
<td>1.5</td>
</tr>
<tr>
<td>Glufosinate</td>
<td>2.8</td>
</tr>
<tr>
<td>Chlorate</td>
<td>1.6</td>
</tr>
<tr>
<td>Ethephon</td>
<td>0.5</td>
</tr>
<tr>
<td>Fosethyl Al</td>
<td>0.8</td>
</tr>
<tr>
<td>Phosphonic acid</td>
<td>2.4</td>
</tr>
<tr>
<td>Maleic hydrazide</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Search for same column chemistry which fits in column oven.
COLUMN 4

HILICpak VT-50 2D

HILIC
Column 4

Background

HILICpak VT-50 2D

- HILIC (same as Metrosep)
  - Polyvinyl alcohol with quaternary ammonium groups
- Dimensions: 5 µm, 2 x 150 mm (same as Metrosep)
- Polymer-based packing material provides excellent chemical stability and minimum deterioration over extended time period

LC settings:

<table>
<thead>
<tr>
<th>Column</th>
<th>HILICpak VT-50 2D (5 µm, 2.0 x 150 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent A</td>
<td>68:20:12 water:ACN:45 mM Ammonium Bicarbonate</td>
</tr>
<tr>
<td>Solvent B</td>
<td>50 mM Ammonium Bicarbonate</td>
</tr>
<tr>
<td>Column Temp</td>
<td>40°C</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>10 µL</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>0.2 mL/min</td>
</tr>
</tbody>
</table>
Column 4
Example Chromatography

HILICpak VT-50 2D

- Chromatography

Mix all 50 ng/mL (A:ACN H2O 45mM / B: 50mM) meth 105

1: MRM of 2 Channels ES-TIC (Phosphonic acid) 1.19e5
2: MRM of 2 Channels ES-TIC (Chlorate) 1.63e4
3: MRM of 2 Channels ES-TIC (Fosethyl Al) 1.91e6
4: MRM of 3 Channels ES-TIC (AMPA) 7.26e4
5: MRM of 3 Channels ES-TIC (Maleic hydrazide) 2.93e4
6: MRM of 2 Channels ES-TIC (Ethephon) 1.11e5
7: MRM of 3 Channels ES-TIC (GLY) 1.39e5
8: MRM of 4 Channels ES-TIC (GLU) 2.53e5
HILICpak VT-50 2D

- Linearity
  - Linearity: 1 – 250 ng/mL
  - Residuals below 20%
  - Correlation coefficient > 0.995

- Glyphosate
- Glufosinate
- Fosetyl Al
- Maleic hydrazide (4 – 250 ng/mL)
- Ethephon
- Phosphonic acid
- AMPA
- Chlorate
Column 4
Precision

HILICpak VT-50 2D

- Repeatability
  - Conc: 2, 10, 50 ng/mL
  - n = 6
  - RSD < 10%

<table>
<thead>
<tr>
<th></th>
<th>Conc (ng/mL)</th>
<th>Mean</th>
<th>RSD (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glyphosate</strong></td>
<td>2</td>
<td>2.1</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.9</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>48.2</td>
<td>1.9</td>
</tr>
<tr>
<td><strong>AMPA</strong></td>
<td>2</td>
<td>1.6</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10.3</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>50.6</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>Glufosinate</strong></td>
<td>2</td>
<td>2.2</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10.0</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>49.1</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>Chlorate</strong></td>
<td>2</td>
<td>1.8</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>11.4</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>52.2</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>Ethephon</strong></td>
<td>2</td>
<td>2.0</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10.0</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>49.9</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>Fosethyl Aluminium</strong></td>
<td>2</td>
<td>2.0</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10.0</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>50.8</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Phosphonic acid</strong></td>
<td>2</td>
<td>1.9</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10.2</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>49.4</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>Maleic hydrazide</strong></td>
<td>2</td>
<td>/</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10.7</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>49.4</td>
<td>1.7</td>
</tr>
</tbody>
</table>
### Column 4
#### Sensitivity

**HILICpak VT-50 2D**

- **Sensitivity**
  - LLOQ: $S/N = 10$
  - LLOQ $\leq 1$ ng/mL
    (except for maleic hydrazide)

<table>
<thead>
<tr>
<th>Compound</th>
<th>LLOQ (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate</td>
<td>0.4</td>
</tr>
<tr>
<td>AMPA</td>
<td>0.4</td>
</tr>
<tr>
<td>Glufosinate</td>
<td>1.0</td>
</tr>
<tr>
<td>Chlorate</td>
<td>0.7</td>
</tr>
<tr>
<td>Ethephon</td>
<td>0.04</td>
</tr>
<tr>
<td>Fosetyl Al</td>
<td>0.02</td>
</tr>
<tr>
<td>Phosphonic acid</td>
<td>0.2</td>
</tr>
<tr>
<td>Maleic hydrazide</td>
<td>2.5</td>
</tr>
</tbody>
</table>
## Summary

<table>
<thead>
<tr>
<th>Column</th>
<th>Acclaim Trinity Q1</th>
<th>IC-Pak Anion HR</th>
<th>Metrosep A Supp 5</th>
<th>HILICpak VT-50 2D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mode</strong></td>
<td>Mixed</td>
<td>Anion</td>
<td>HILIC</td>
<td>HILIC</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td></td>
<td>LLOQ (ng/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glyphosate</td>
<td>0.1</td>
<td>1.9</td>
<td>0.9</td>
<td>0.4</td>
</tr>
<tr>
<td>AMPA</td>
<td>0.2</td>
<td>0.8</td>
<td>1.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Glufosinate</td>
<td>0.9</td>
<td>0.5</td>
<td>2.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Chlorate</td>
<td>/</td>
<td>/</td>
<td>1.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Ethephon</td>
<td>/</td>
<td>/</td>
<td>0.5</td>
<td>0.04</td>
</tr>
<tr>
<td>Fosethyl Al</td>
<td>/</td>
<td>/</td>
<td>0.8</td>
<td>0.02</td>
</tr>
<tr>
<td>Phosphonic acid</td>
<td>/</td>
<td>/</td>
<td>2.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Maleic hydrazide</td>
<td>/</td>
<td>/</td>
<td>3.1</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Linearity (ng/mL)</strong></td>
<td>1 - 1000</td>
<td>/</td>
<td>/</td>
<td>1 - 250</td>
</tr>
<tr>
<td><strong>Repeatability (%RSD)</strong></td>
<td>&lt; 15</td>
<td>/</td>
<td>/</td>
<td>&lt; 10</td>
</tr>
<tr>
<td><strong>Matrix robustness (% RSD)</strong></td>
<td>AMPA&lt;20</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td><strong>Drawbacks</strong></td>
<td>Peak shape AMPA</td>
<td>Dimensions, pH, citric acid</td>
<td>Doesn’t fit in column oven</td>
<td>Matrix analysis needed</td>
</tr>
</tbody>
</table>
Conclusions

- The results in this webcast indicate the ongoing pursuit of an optimal LC-MS method to analyze polar pesticides

- The Xevo TQ-XS showed to be highly sensitive for the polar pesticides, indicated by a sensitivity below 5 ng/mL for all pesticides in combination with 4 different column chemistries

- Three columns were evaluated to date.
  - The Acclaim Trinity column was unable to generate stable peak shape for AMPA (using the QuPPe extraction),
  - The IC-Pak Anion HR uses less optimal LC conditions
  - The Metrosep A Supp 5 column doesn’t fit in standard Waters column ovens.

- The Shodex HILICpak VT-50 2D demonstrated promising results in terms of solvent standard sensitivity (< 1 ng/mL for 7 out of 8 pesticides) and repeatability (% RSD < 10)
Next step

- **Optimizing** the established method
  - **Shodex HILICpak VT-50 2D**
    - Analysis of pesticides in different food matrices
      - Linearity, sensitivity, recovery, repeatability
    - Include more polar pesticides

- **Feedback** from customers
  - Which samples (matrices, pesticides)
  - What concentration
  - Application specific focus
  - Collaborations
Acknowledgements & References

Thank You For Your Attention

- External
  - Primoris Belgium CVBA
  - NofaLab Laboratories, Netherlands

- References
Live Q&A Session