

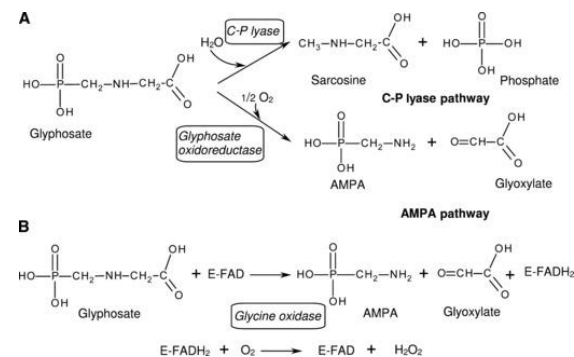
Direct LC/MS/MS Analysis of Glyphosate, AMPA and other Polar Pesticides

What is the best way to do
this analysis?

Jerry Zweigenbaum
Agilent User's meeting



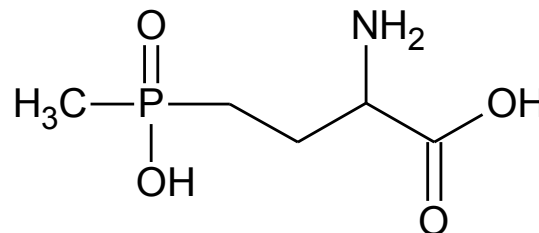
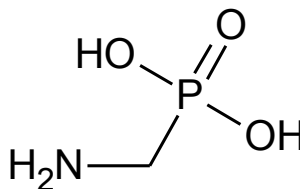
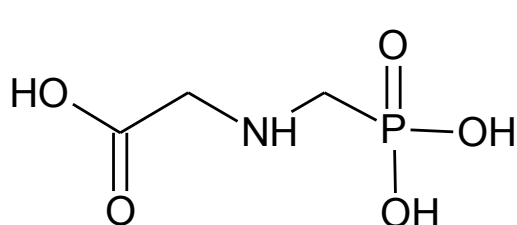
Target Analytes Herbicides



Compounds	Compounds
Glyphosate	Ethephon
AMPA	HEPA
Glufosinate	MPPA
N-acetyl Glufosinate	Fosetyl
Maleic hydrazide	

Issues with Analysis of Glyphosate

Strong Acid/Weak Acid/Zwitterion?



- Chelate with metal ions
 - New SS
 - Old SS
 - Erratic behavior
 - Some systems ok
- Some systems show loss of analyte
 - Use of EDTA
 - Passivation with phosphoric acid

Chromatographic Methods all LC/MS/MS

At this meeting alone:

1. HILIC silica based
2. iHILIC polymer based
3. Reversed-phase chromatography
4. Anion exchange chromatography with suppresser column
5. Anion exchange without suppressor column
6. Cation exchange chromatography
7. Mixed mode chromatography
8. Ion pair chromatography (with reversed-phase column)
9. Capillary Electrophoresis (not chromatography but we will count it)

??Thoughts on the chromatography (or electrophoresis)??



Chromatographic Conditions

Parameter																									
Liquid Chromatograph	1260 Infinity Quaternary Bio-inert LC with 100 µL injection loop																								
Analytical Column	Metrohm Asupp 4, 4.0x200 mm																								
Flowrate	0.7 mL/min																								
Temperature	45 °C																								
Mobile Phase	A: Water B: 50 mM Ammonium acetate C: 50 mM Ammonium Hydroxide																								
Gradient	<table><tr><th>Time (min)</th><th>A %</th><th>B %</th><th>C %</th></tr><tr><td>0.0</td><td>25</td><td>45</td><td>30</td></tr><tr><td>3.0</td><td>25</td><td>45</td><td>30</td></tr><tr><td>7.0</td><td>0</td><td>90</td><td>10</td></tr><tr><td>16.5</td><td>0</td><td>90</td><td>10</td></tr><tr><td>17.0</td><td>25</td><td>45</td><td>30</td></tr></table>	Time (min)	A %	B %	C %	0.0	25	45	30	3.0	25	45	30	7.0	0	90	10	16.5	0	90	10	17.0	25	45	30
Time (min)	A %	B %	C %																						
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17.0	25	45	30																						
Runtime	20.0 min																								

Separation on Metrohm Column

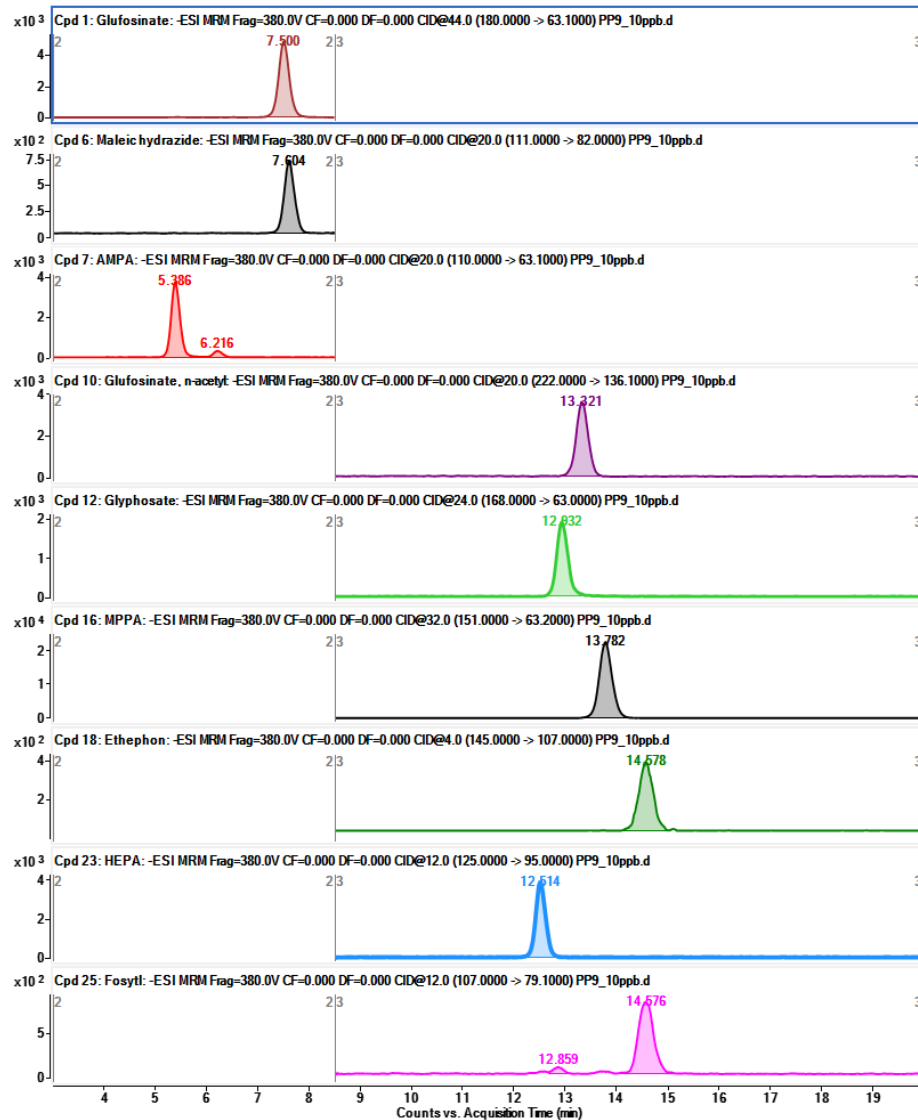
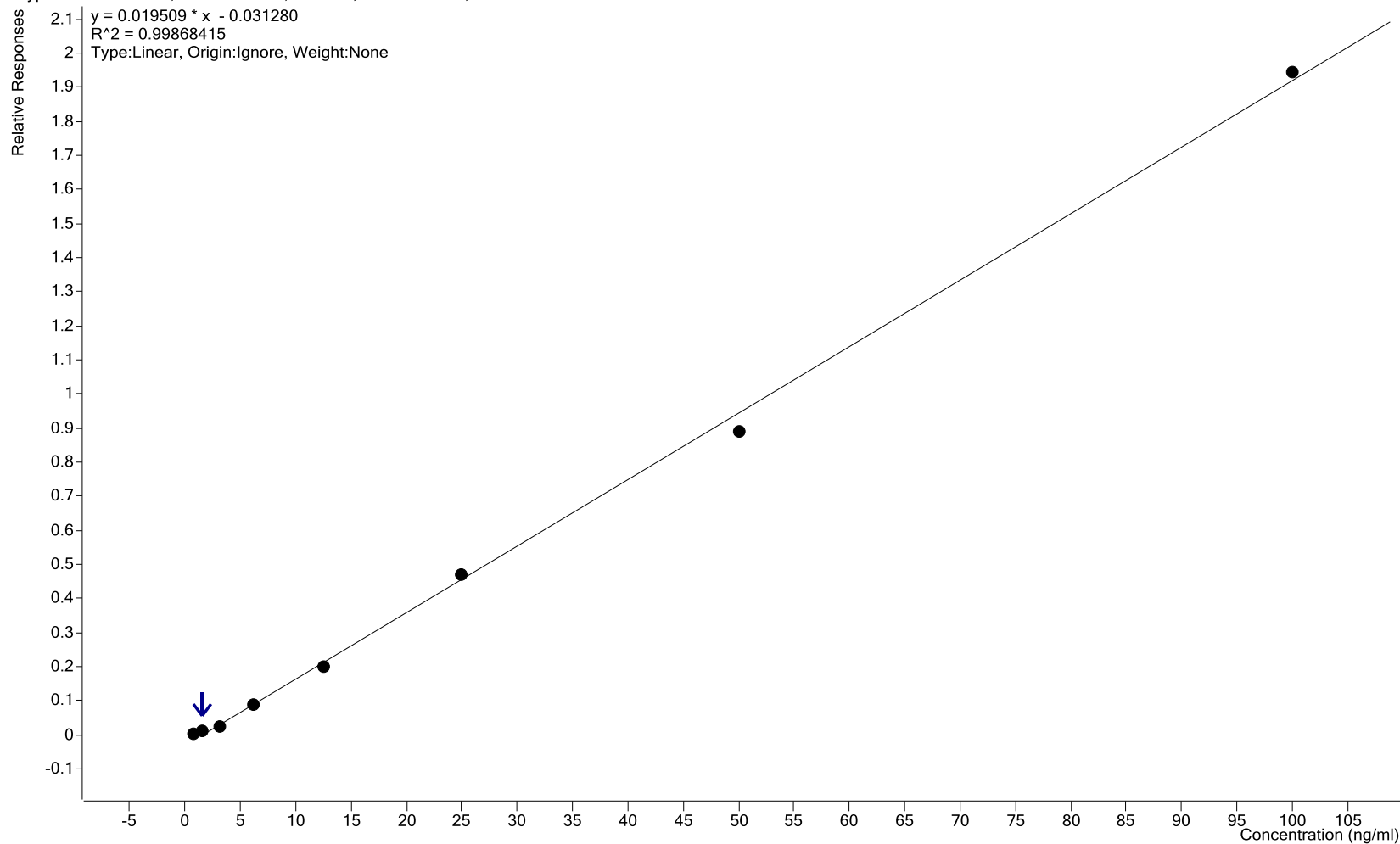


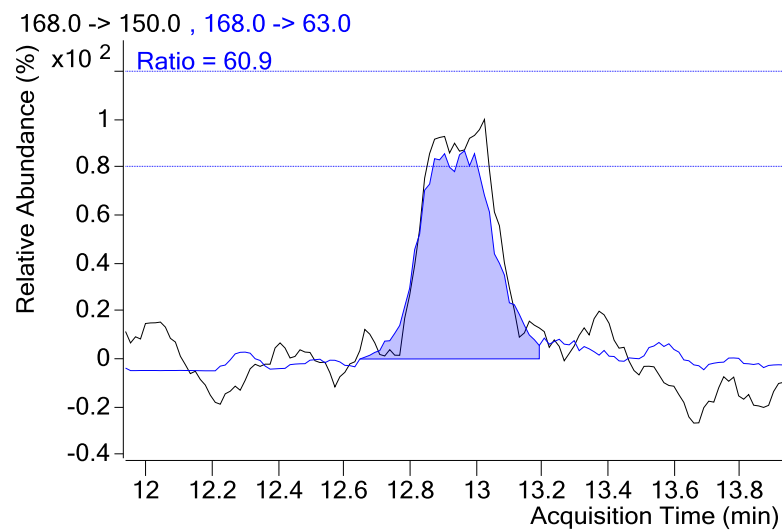
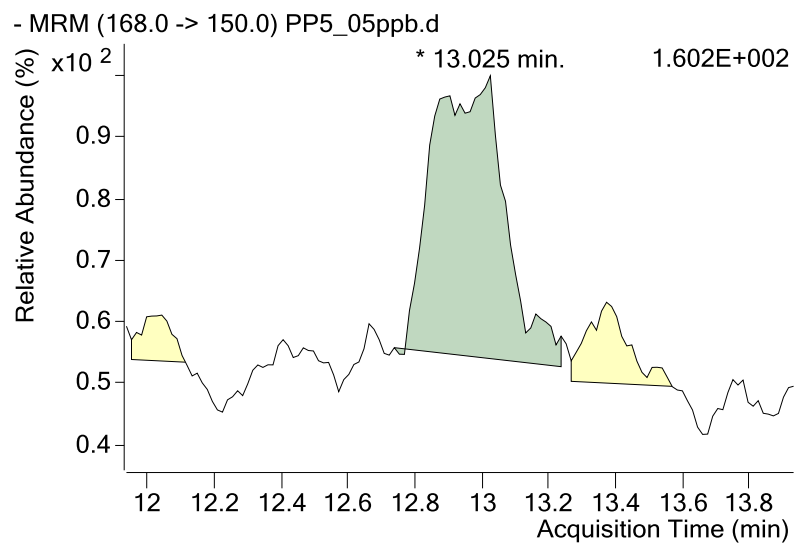
Figure 1. MRM chromatogram of 9 polar pesticides at 10 ppb each.

Glyphosate 0.5 ppb to 100 ppb

Glyphosate - 8 Levels, 8 Levels Used, 8 Points, 8 Points Used, 0 QCs

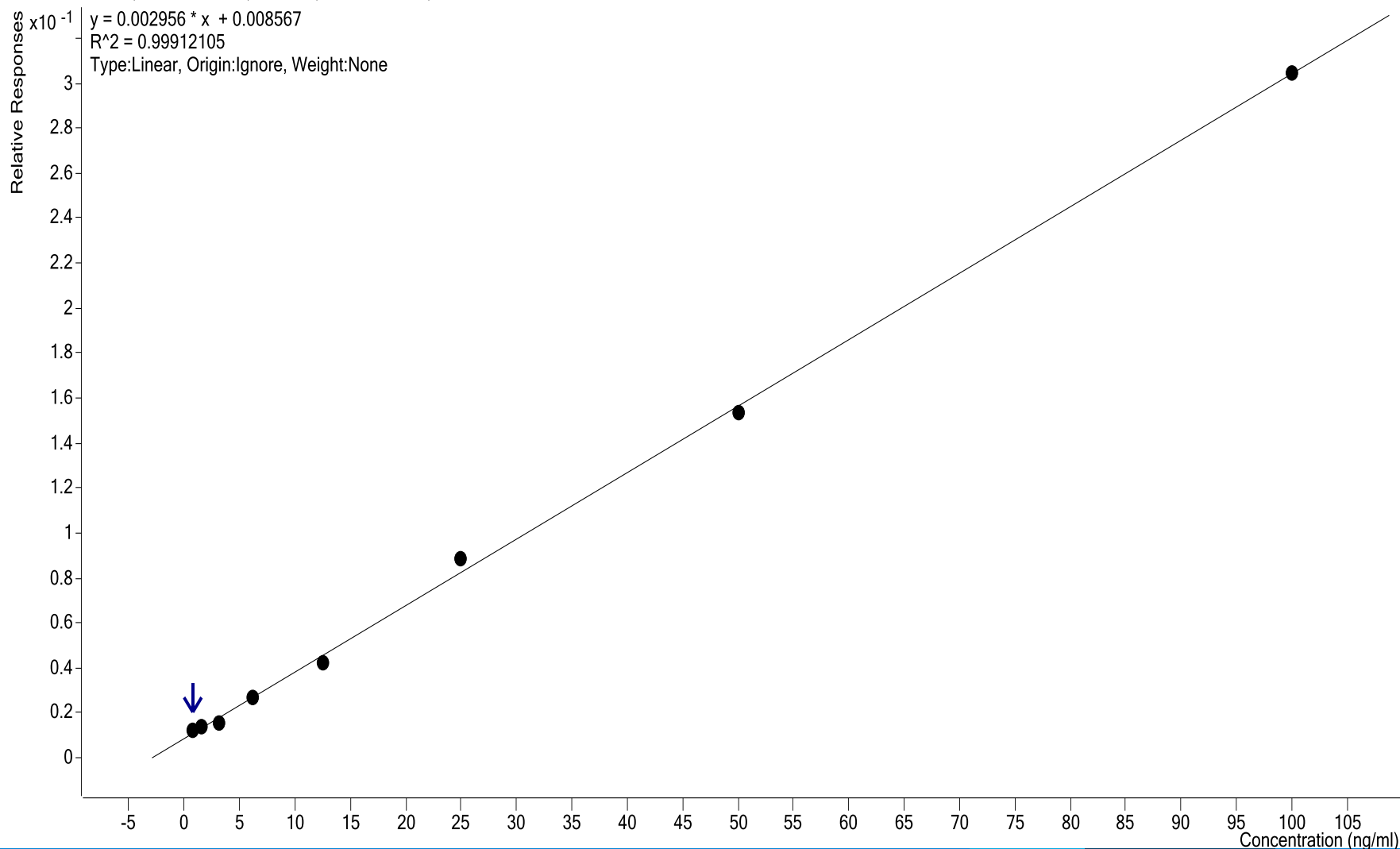


Glyphosate at 0.5 ppb

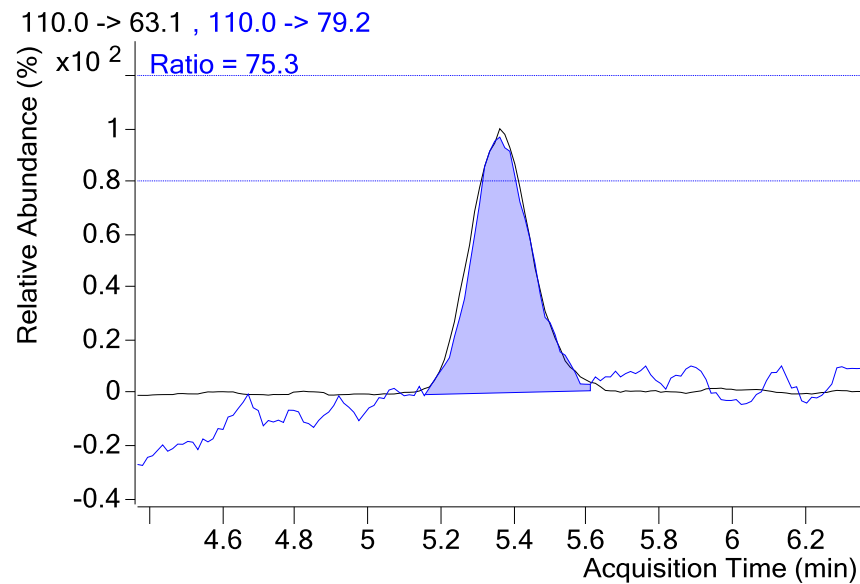
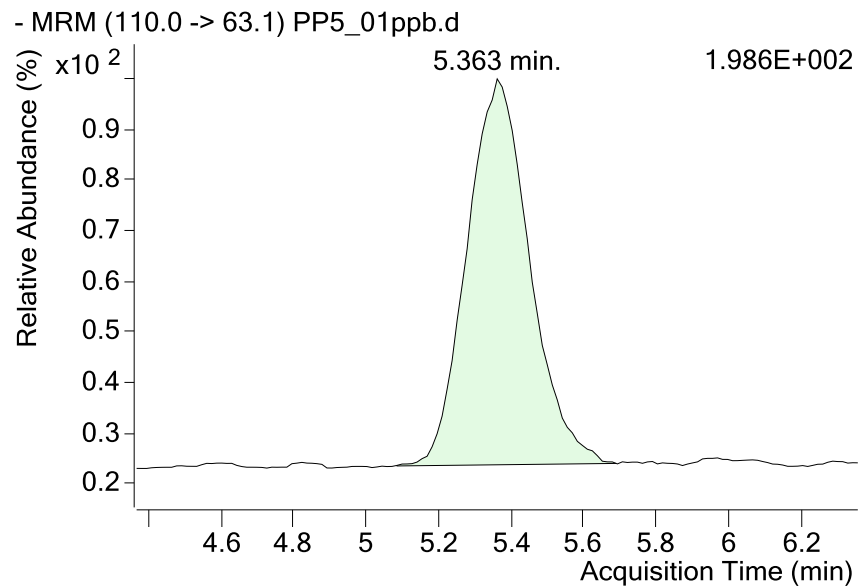


AMPA 0.1 to 100 ppb

AMPA - 8 Levels, 8 Levels Used, 8 Points, 8 Points Used, 0 QCs



AMPA at 0.1 ppb



Sample Prep

At this meeting alone

1. Liquid-liquid extraction MeCl:water, derivatization with FMOC-Cl
2. Extract with water, cleanup on SAX, elute with 1 N HCl, rotovap to dryness, derivatize with TMOA in glacial acetic acid, dried again and taken up in 9:1 water:methanol
3. Extract with water, pass through Plexa SPE and inject
4. QuPPE
5. Buffered extraction with PAX, elution with 1% formic acid
6. Extract with 50mM acetic acid and 10 mM EDTA, pass through Oasis HLB, inject
7. 50 mM acetic acid 10 mM EDTA, pass through an SEC cartridge

What are your thoughts now??



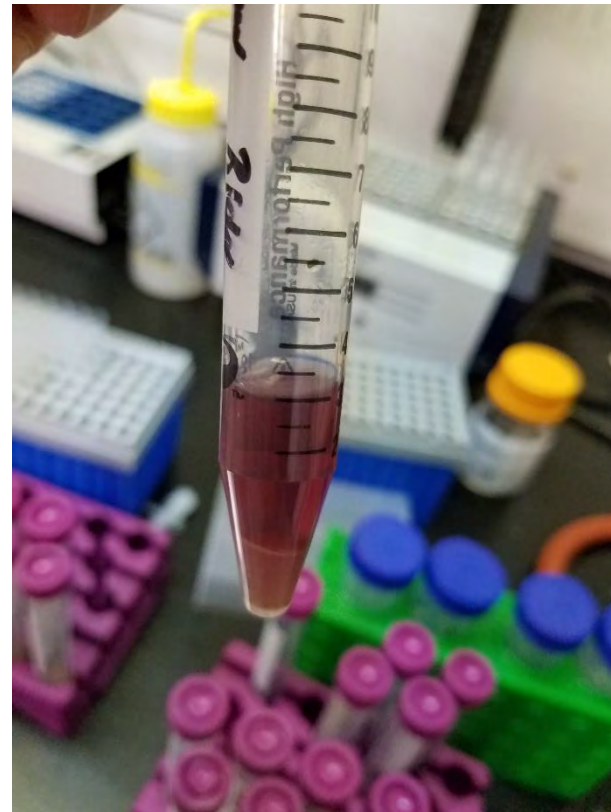
Samples

Wheat Flour and Organic Strawberries

- Water extraction- no cleanup
 - Compounds not detected
 - Pass through C8-
 - Wheat samples, glyphosate and AMPA detected
 - Strawberry samples, not detected
- Buffered (pH 8.2 bicarbonate) extraction
 - 50 mM bicarb
 - Ok for wheat
 - pH still < 5 for strawberry
 - 200 mM bicarb
 - Ok wheat
 - OK strawberry

Extraction of Wheat and Strawberry

- 1 gram sample
- 9 mL of buffer
- Vortex 20 min
- Spin at 4K rpm for 10 min

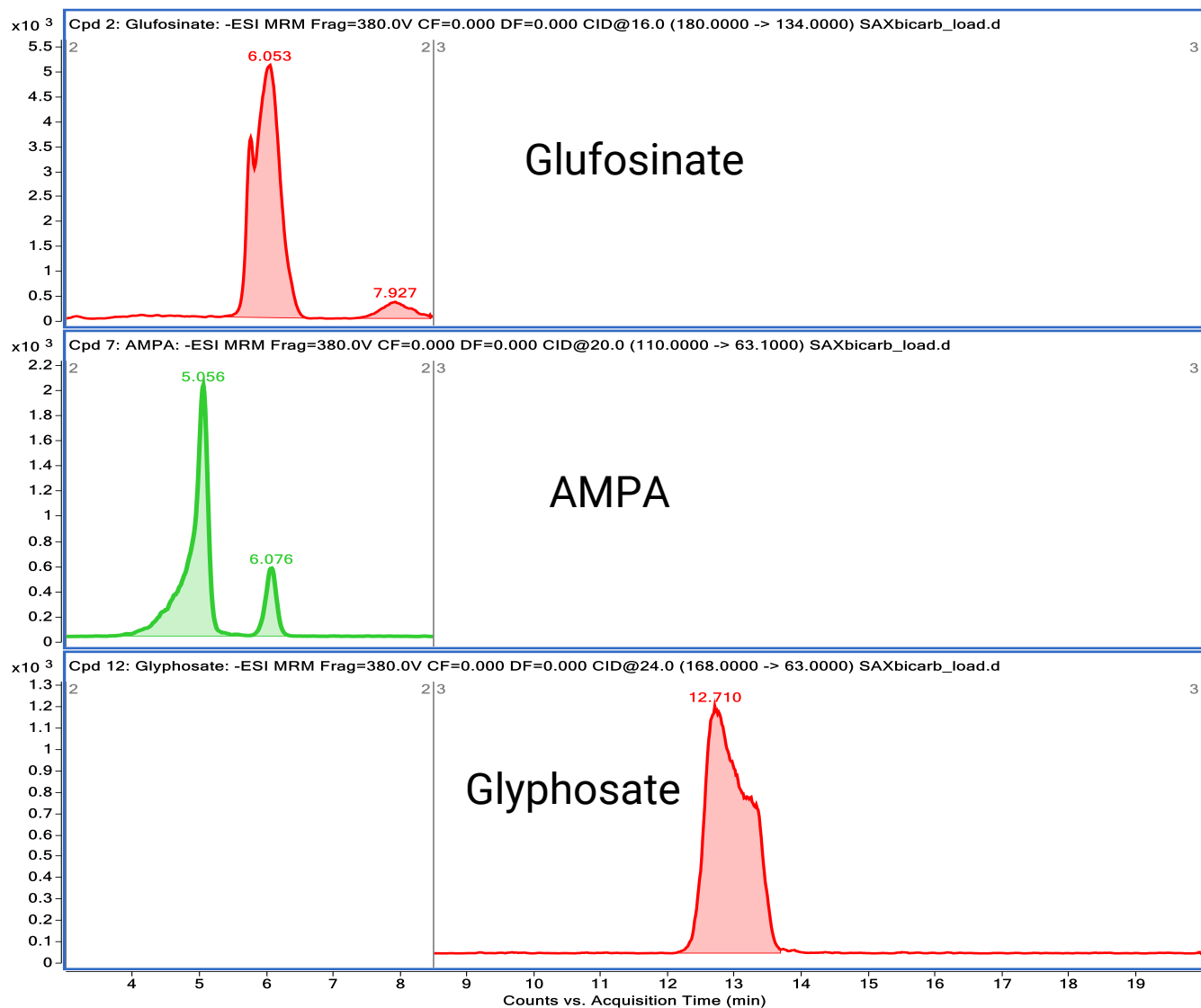


Cleanup on PAX



- Take 4 mL of extract load onto cartridge
- Wash with water 2 mL
- Wash with methanol 2 mL
- Elute with 1 mL 1% formic acid- neutralize with NH_4OH – run on IC/MS

Load- 200 mM bicarbonate buffer extract- 50 μ L injection



Final Discussions

