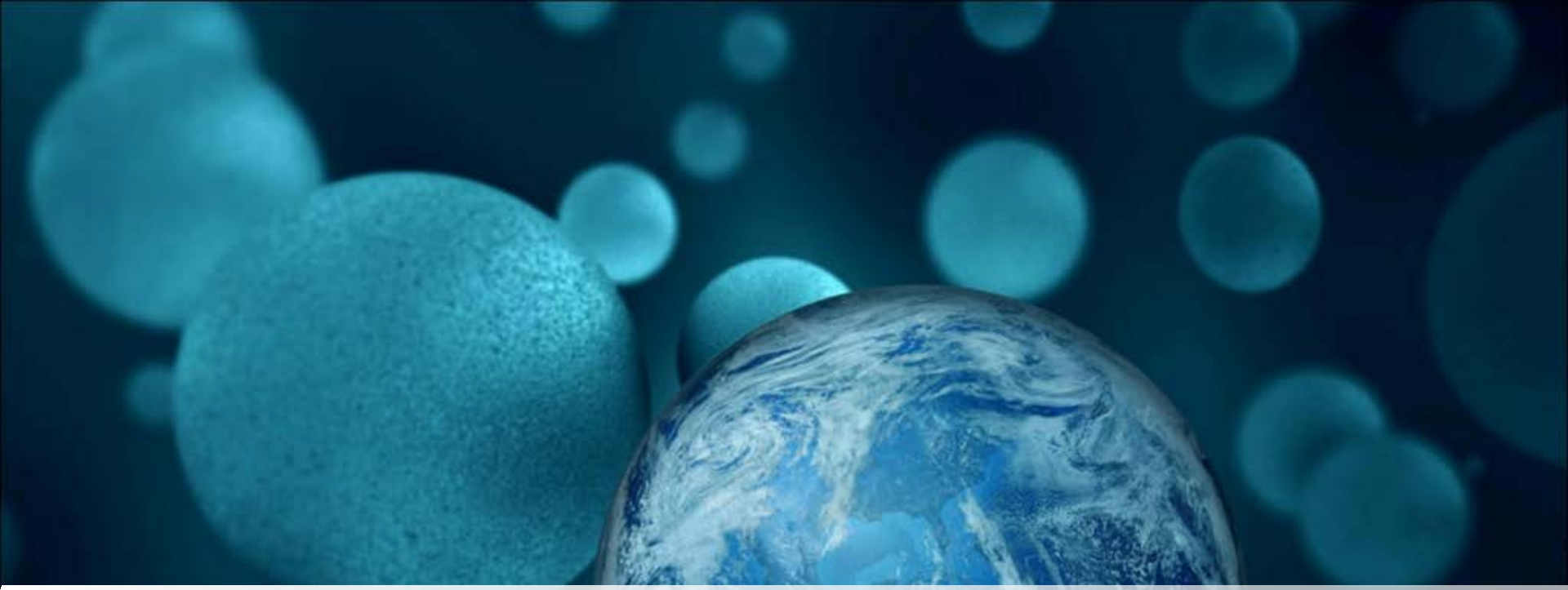

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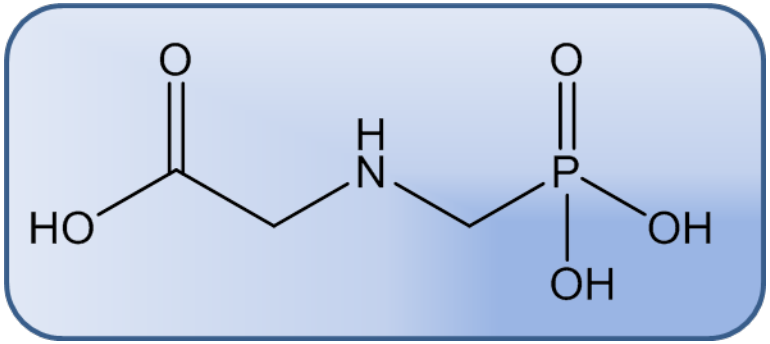
Ion Chromatography Coupled to MS, a Powerful Approach for Polar Pesticides Determination

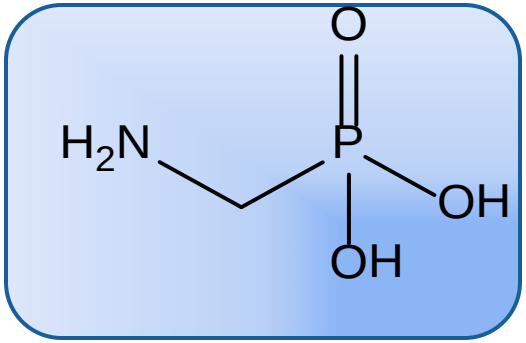
Dott. Luca Gerardo
IC/SP Application Specialist, Thermo Fisher Scientific, Rodano (MI)

What is Glyphosate?

- **Glyphosate** (*N*-(phosphonomethyl)glycine) is a broad spectrum systemic herbicide commonly used as weed control.



- It is rapidly degraded to aminomethylphosphonic acid (**AMPA**) metabolite frequently found in plants, water and soil.



- Very polar, zwitterionic compound
- Difficult to retain on C18 LC column

Glyphosate – Health Concerns Timeline

- In 2015, the World Health Organization's International Agency for Research on Cancer (IARC) classified glyphosate as "probably carcinogenic to humans."
  - International scientists found there was a particularly strong connection to non-Hodgkins lymphoma.

- 2015: The European Food Safety Authority and the European Chemicals Agency have said glyphosate is NOT likely to be carcinogenic to humans.
  - Under scrutiny for research conducted by chemical companies

- 2016: The World Health Organization/ United Nations Joint Meeting on Pesticide Residues also cleared glyphosate as unlikely to pose a risk to humans,
  - tarnished by conflicts of interest regarding ties to the International Life Sciences Institute, a food industry front group

- March, 2017: California Environmental Protection Agency's Office of Environmental Health Hazard Assessment confirmed that it would add glyphosate to list of cancer causing chemicals.

Glyphosate in the News

- **2016**
  - The Munich Environmental Institute group found glyphosate in 14 of Germany's most popular beers (0.46 – 29.74 µg/L).
  - Alliance for Natural Health USA tested 24 popular breakfast foods, 10 of 24 goods had detectable levels of glyphosate (86 – 1,327 µg/kg) (www.anh-usa.org).
  - FDA found glyphosate in US honey at double the levels allowed in the EU.
  - More than 50 lawsuits against glyphosate producers are pending in US District Court in San Francisco.
  - Several hundred similar actions are pending in state courts.

Analysis of Glyphosate

HPLC methods:

- Typically require extensive sample preparation
  - SPE
  - QuePP method
- Preparation of buffers/acidic mobile phases

Time and additional source of errors

- Non retentive on C18 columns
  - Pre or post column derivatisation
  - Speciality columns – poor resolving power
  - Fluorescence detection
- Matrix effects and interferences

Robustness issues

QuPPe-PO v 9.1-Negative Mode Compounds

- Method lists a total of 42 different (pos and neg mode) analytes.

Table 3: Overview and scope of the methods proposed within this document for the QuPPe method:

	M 1.1	M 1.2	M 1.3	M 1.4	M 2	M 3	M 4.1	M 4.2	M 5	M 6	M 7	M8
ESI-mode	Neg.	Neg.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
Separation principle	Anion Exchange	Anion Exchange	Carbon	Carbon	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	Carbon
Column type	AS 11	AS 11-HC	Hyper-carb	Hyper-carb	Obelisc-R	Obelisc-R	Obelisc-R	BEH-Amide	PFP	Obelisc-R	Trinity P1	Hyper-carb
NEGATIVE MODE												
Ethephon	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT
HEPA	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT
Glufosinate	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT
N-Acetyl-glufosinate	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT
MPPA	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT
Glyphosate	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT
AMPA	✓	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT
Phosphonic acid	(✓)	(✓)	✓	✓	NT	NT	NT	NT	NT	NT	-	NT
N-Acetyl-AMPA	NT	✓	✓	NT	NT	NT	NT	NT	NT	NT	-	NT
Fosetyl-Al	-	✓	✓	NT	✓	NT	NT	NT	NT	NT	✓*	NT
Maleic hydrazide	-	-	✓	NT	✓	NT	NT	NT	NT	NT	✓*	NT
Perchlorate	NT	-	✓	✓	✓	NT	NT	NT	NT	NT	✓*	NT
Chlorate	NT	-	✓	✓	NT	NT	NT	NT	NT	NT	✓*	NT
Bialaphos	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT	-	NT
Cyanuric acid	NT	NT	✓	NT	NT	NT	NT	NT	NT	NT	✓*	NT
Bromide	NT	NT	-	✓	NT	NT	NT	NT	NT	NT	NT	NT
Bromate	NT	NT	(✓)	✓	NT	NT	NT	NT	NT	NT	NT	NT

http://www.crl-pesticides.eu/userfiles/file/EurlSRM/meth_QuPPe-PO_EurlSRM.pdf

Why Couple Ion Chromatography (IC) to MS?

- IC columns provide great specificity and selectivity for ionic compounds.

- Metal-free flow path reduces fouling of ion-exchange columns.

- Just add water to prepare eluents for anions and cations analysis.

- MS and conductivity detection in series

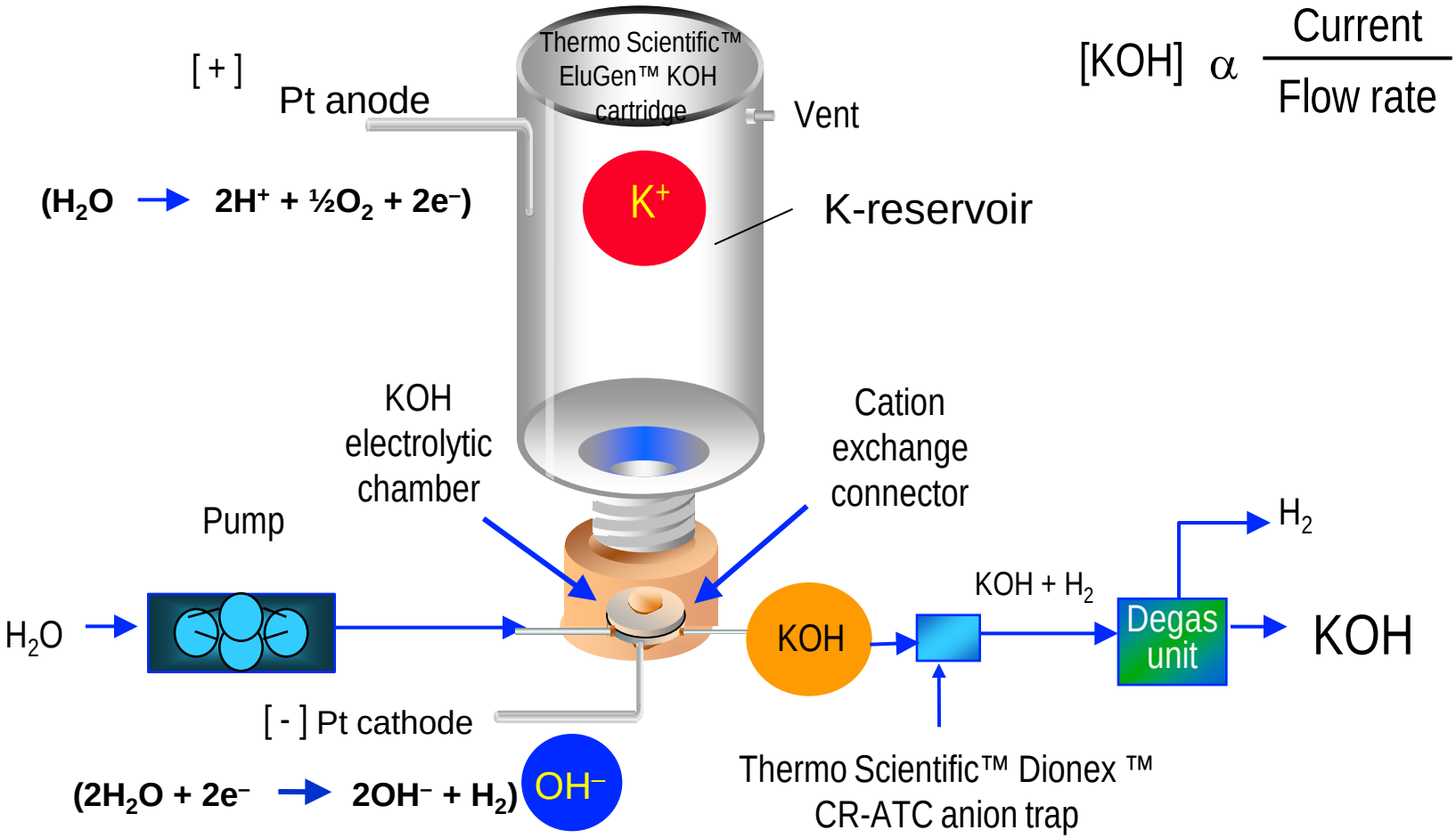
  Possibility to determine high concentration contaminants (Anions / Cations) and trace contaminants (Pesticides/Disinfection byproducts) in the same chromatographic run.

- Eluent suppression to Water

  Enable rapid switching from anion to cation analysis sharing MS device.

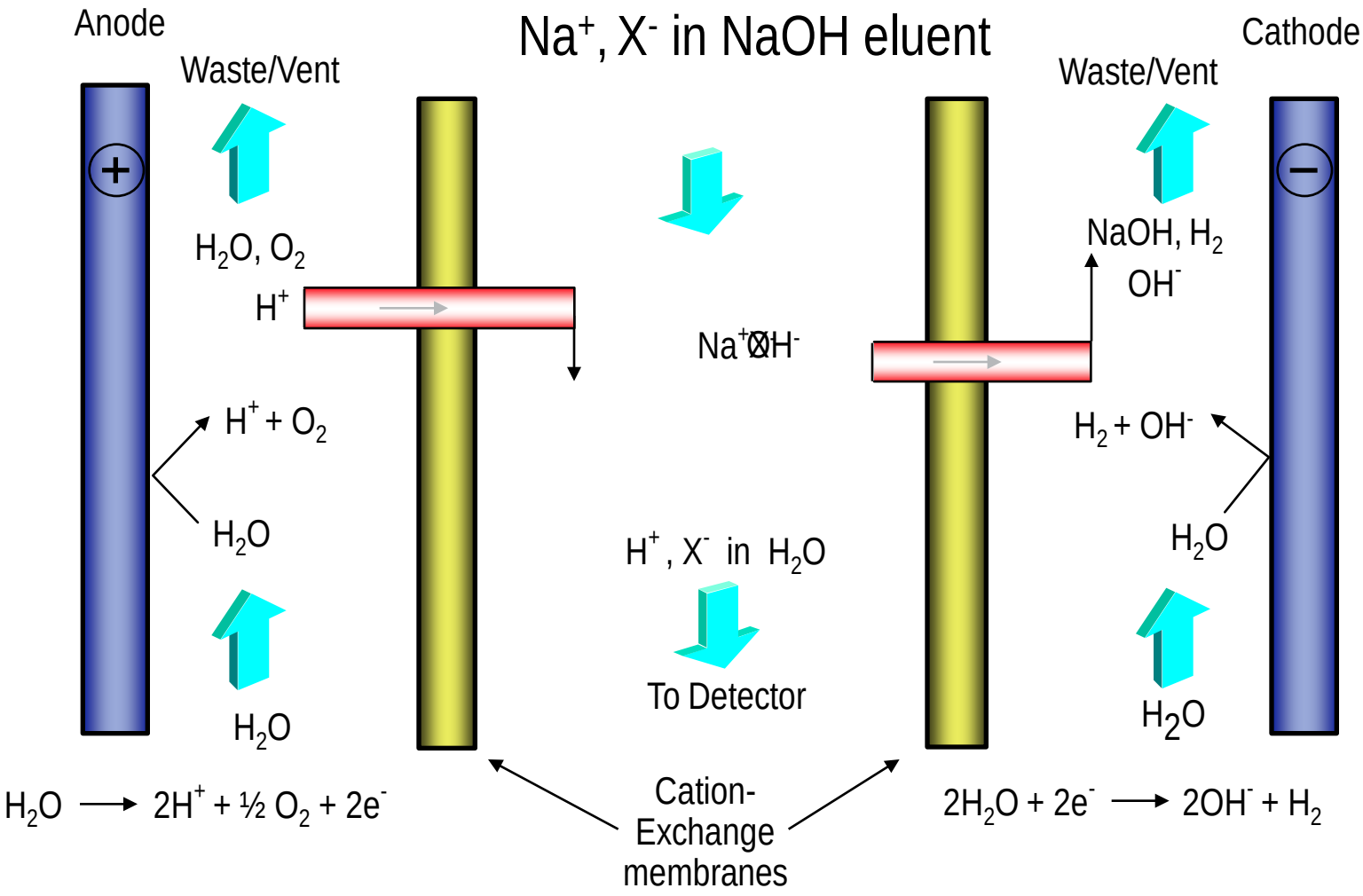
Principles of RFIC - Eluent Generation

Thermo Scientific™ RFIC technology - Reagent-free IC without manual eluent preparation



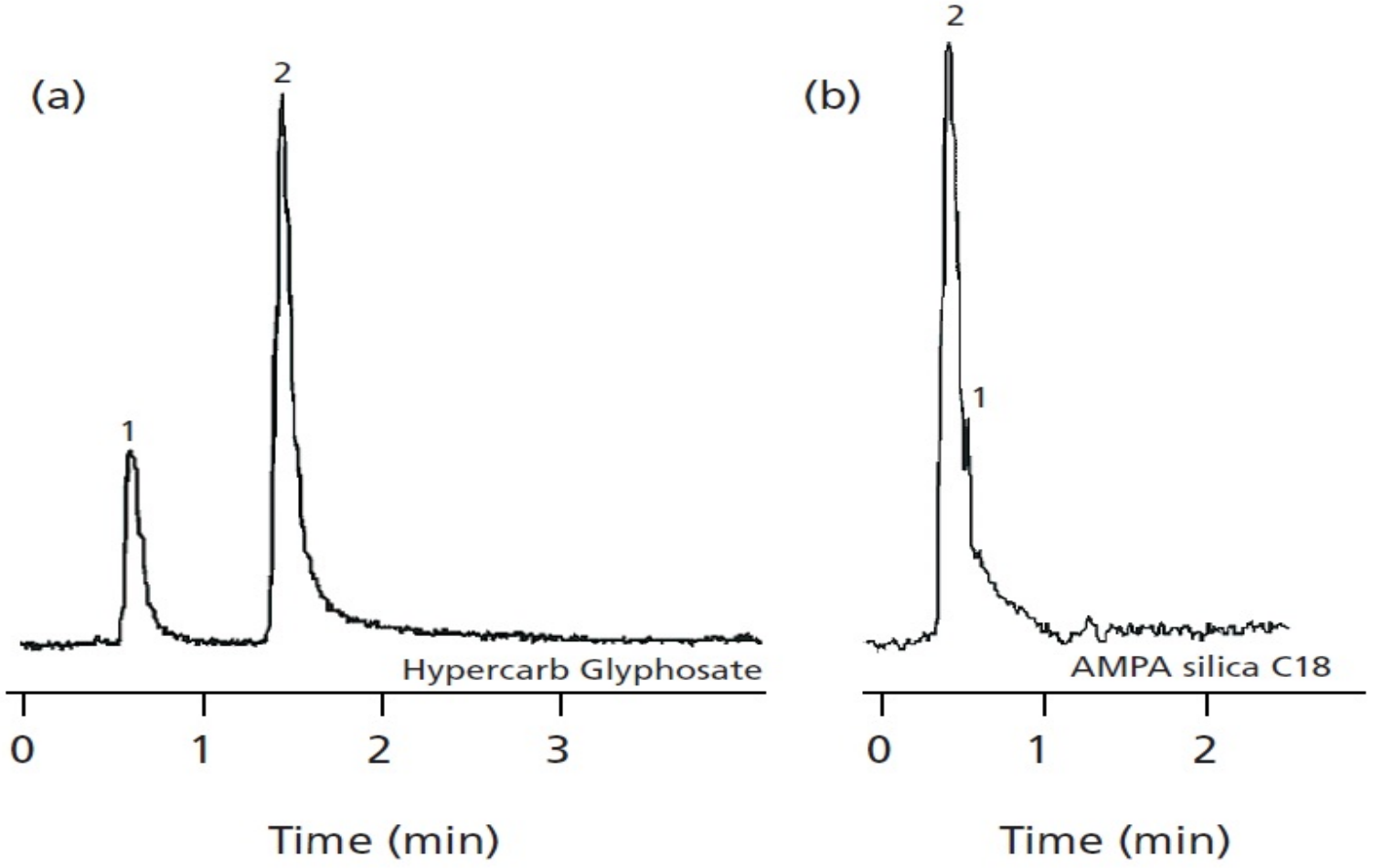
Chemistry and Ion Movement …

… in an Thermo Scientific™ Dionex™ ERS™ electrolytically regenerated suppressor



HPLC Separation

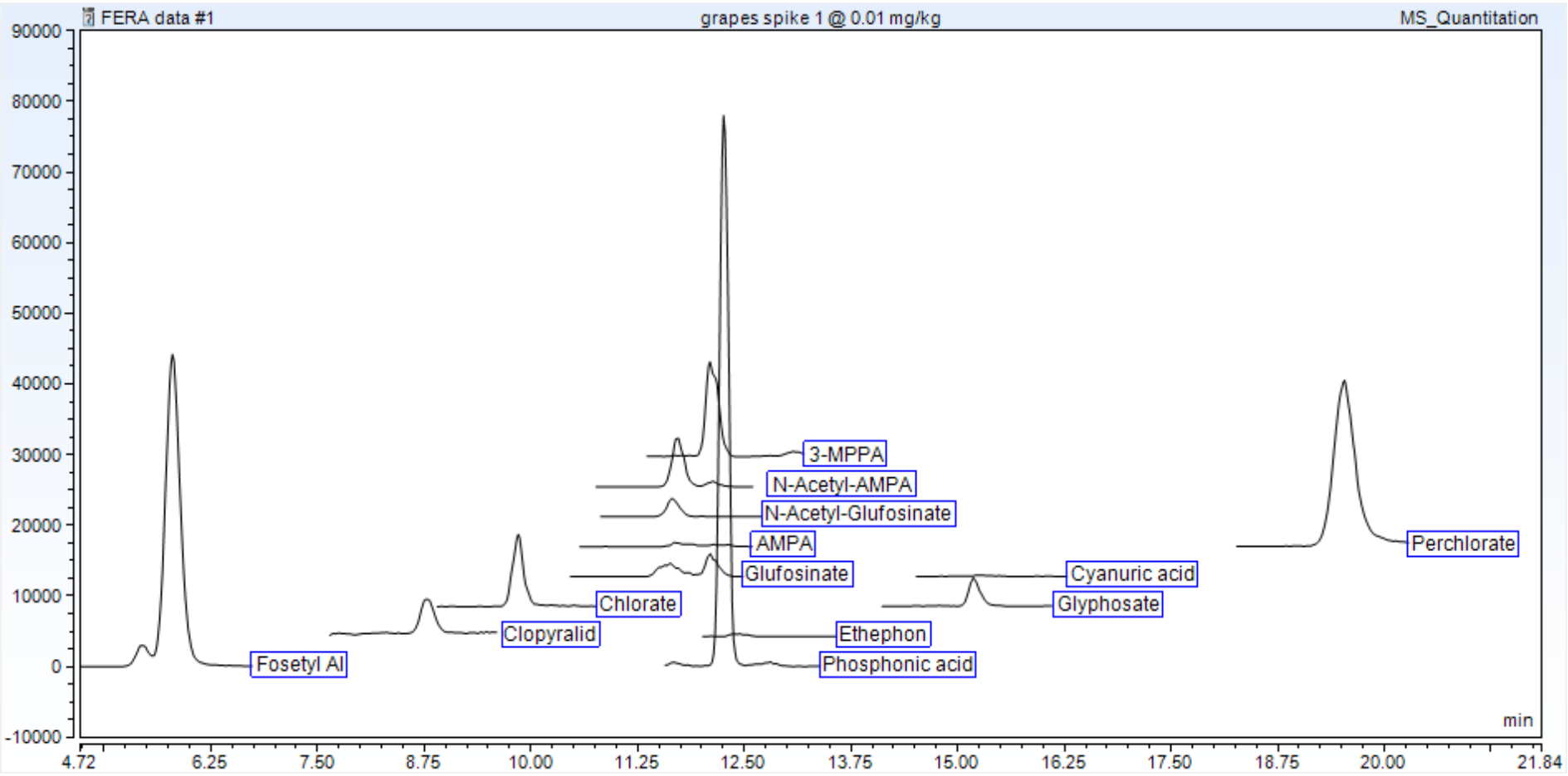
… Thermo Scientific™ Hypercarb™ and Silica C18 columns



Peaks: 1 = AMPA, 2 = glyphosate

IC-MS/MS Pesticide Multi Residue Ion Chromatogram

- 10 µg/kg spike in grapes (Fosetyl & Phosphonic acid @ 100 µg/kg)



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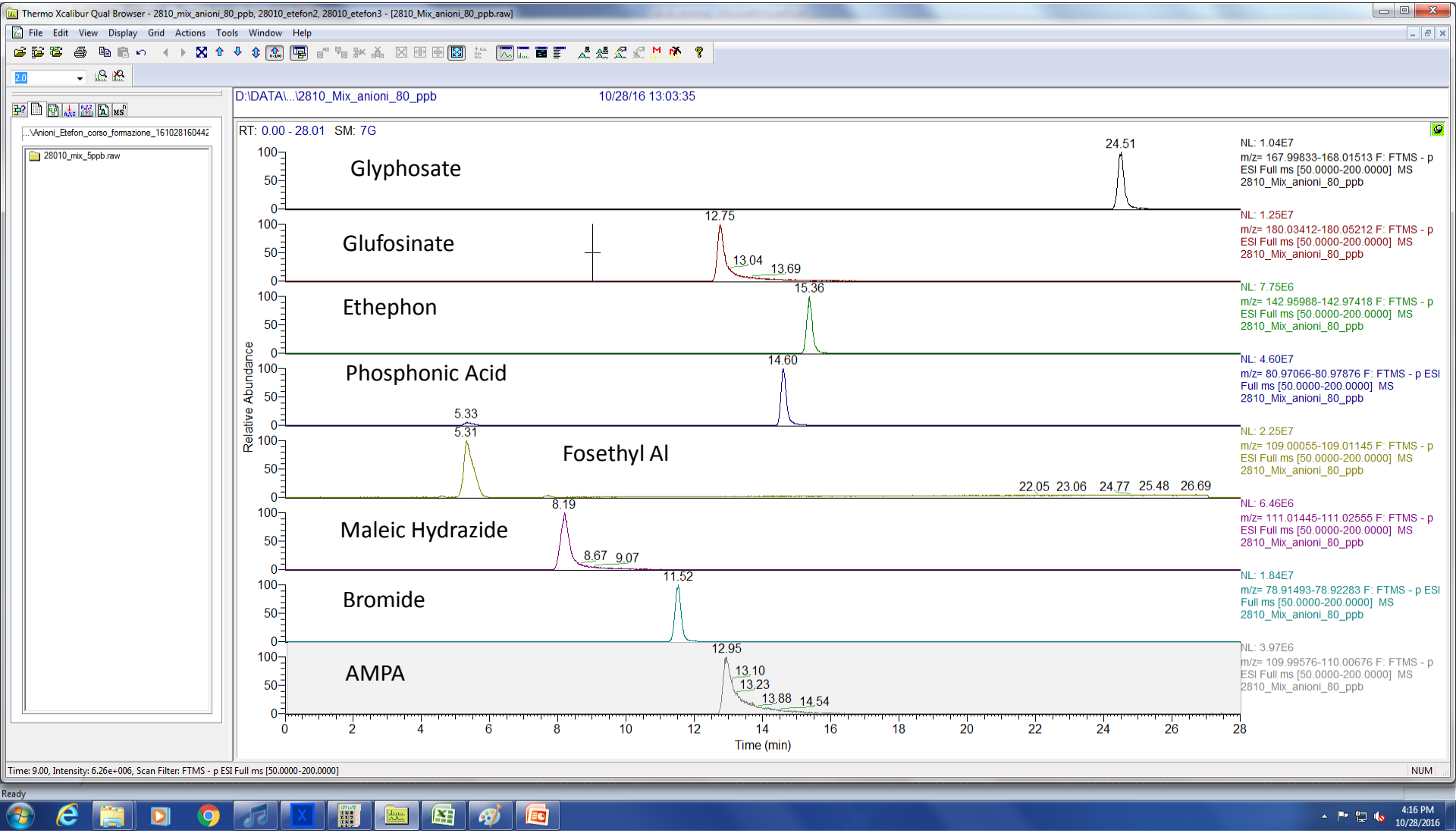
IC Separations - Polar Pesticides …

… on a Thermo Scientific™ Dionex™ IonPac™ AS19 4 µm column



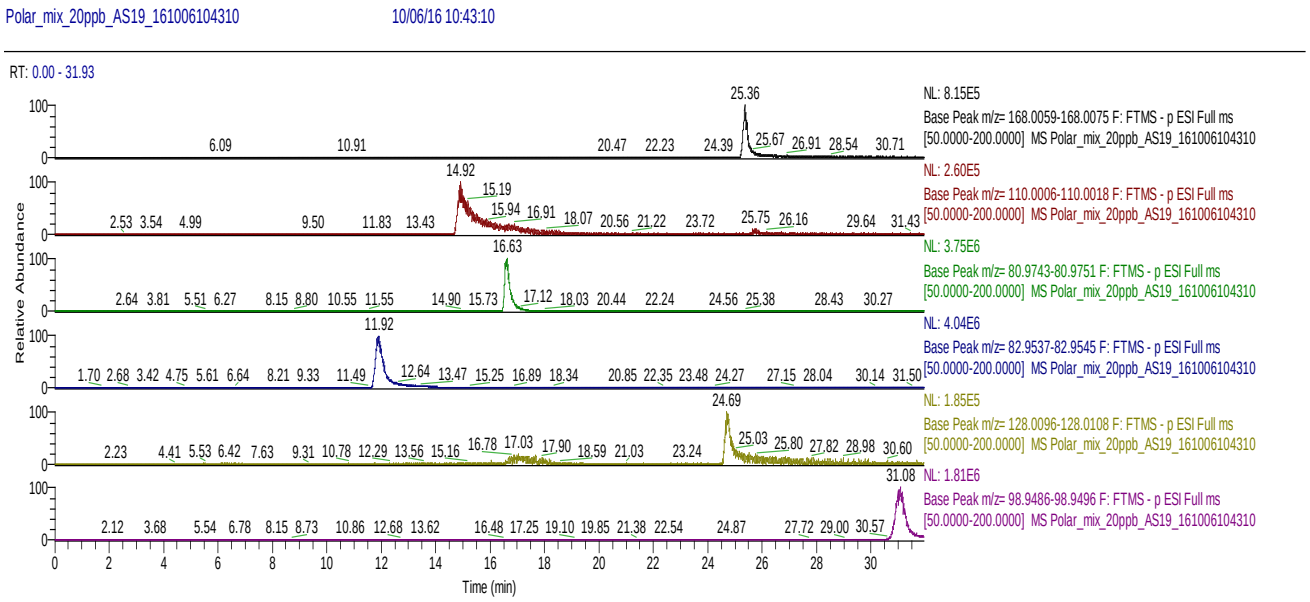
Ion Chromatography Selectivity

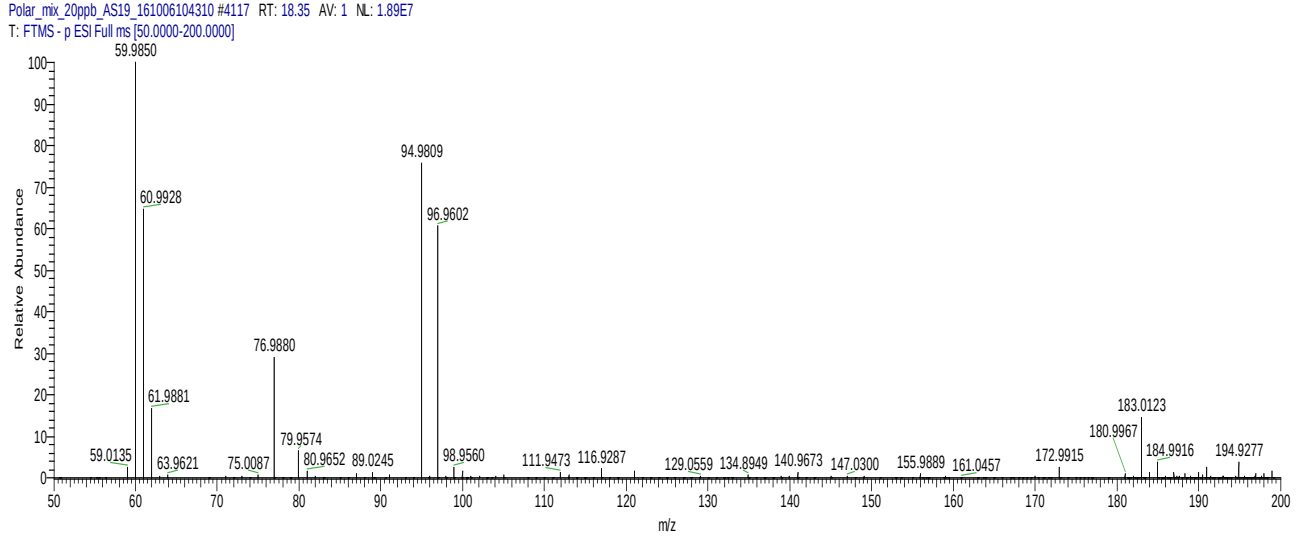
- 0- 12 minutes - Matrix interferences in conductivity trace can be sent to waste to preserve the ion source.
- 12 minutes +/- analytes of interest directed to MS

Matrix Interferences Removed

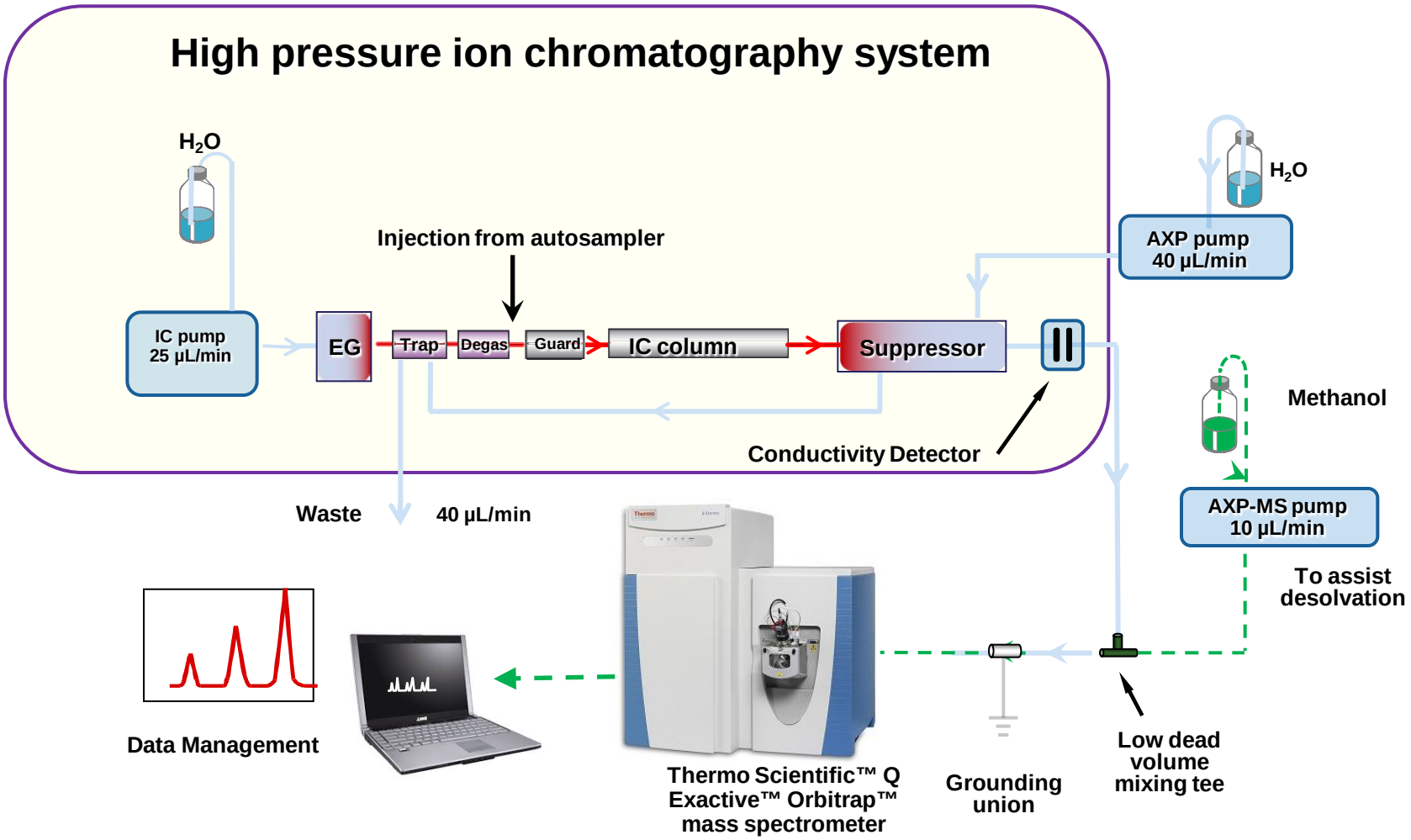
TIC showing 20ppb:

*Glyphosate
*AMPA
*Phosphonic acid
*Chlorate
*Cyanuric acid
*Perchlorate

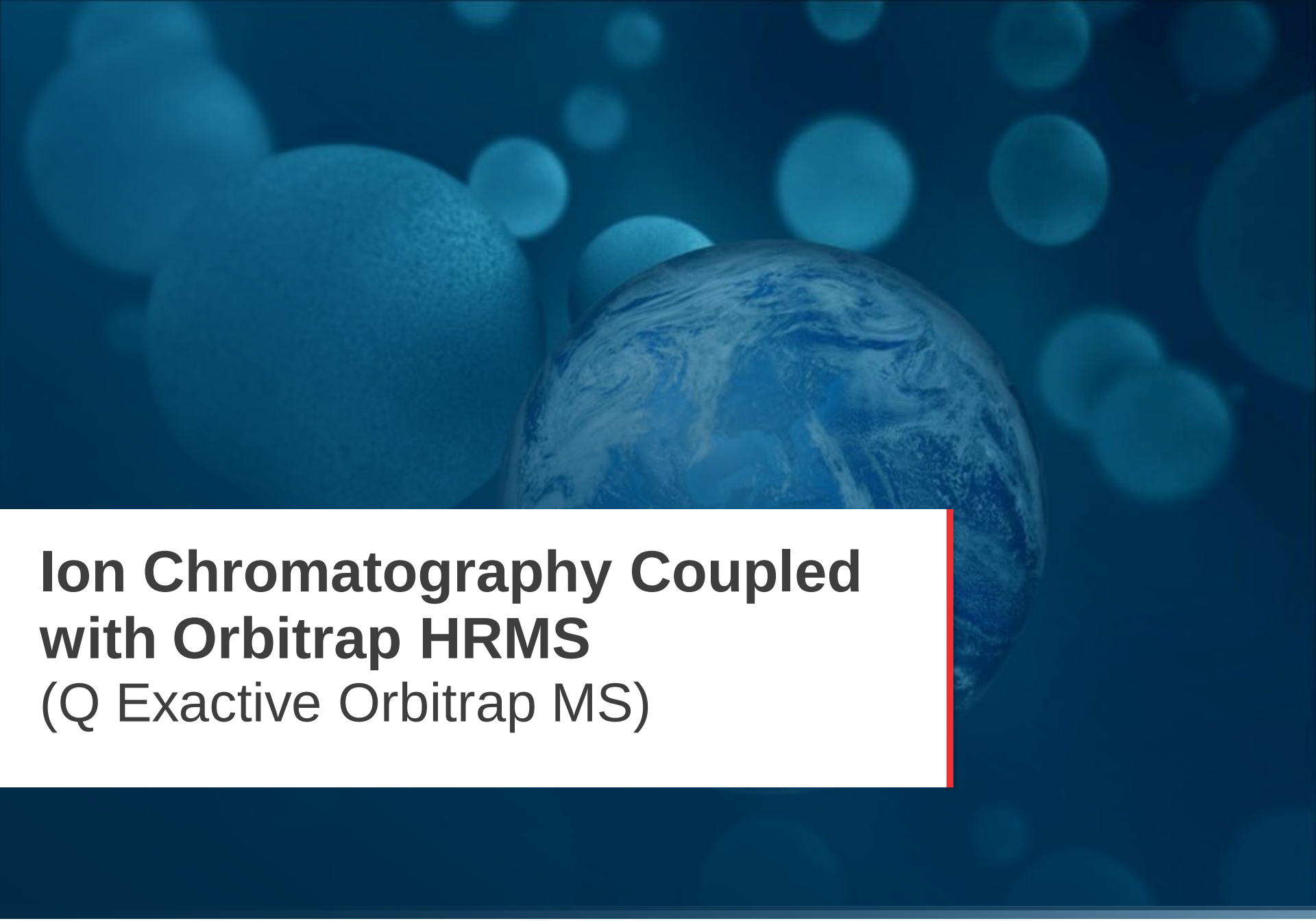




Flow Diagram— IC-HR/AM MS System



Wang, J., Christison, T., *et al.* Submitted to Analytical Chemistry



Ion Chromatography Coupled with Orbitrap HRMS

(Q Exactive Orbitrap MS)

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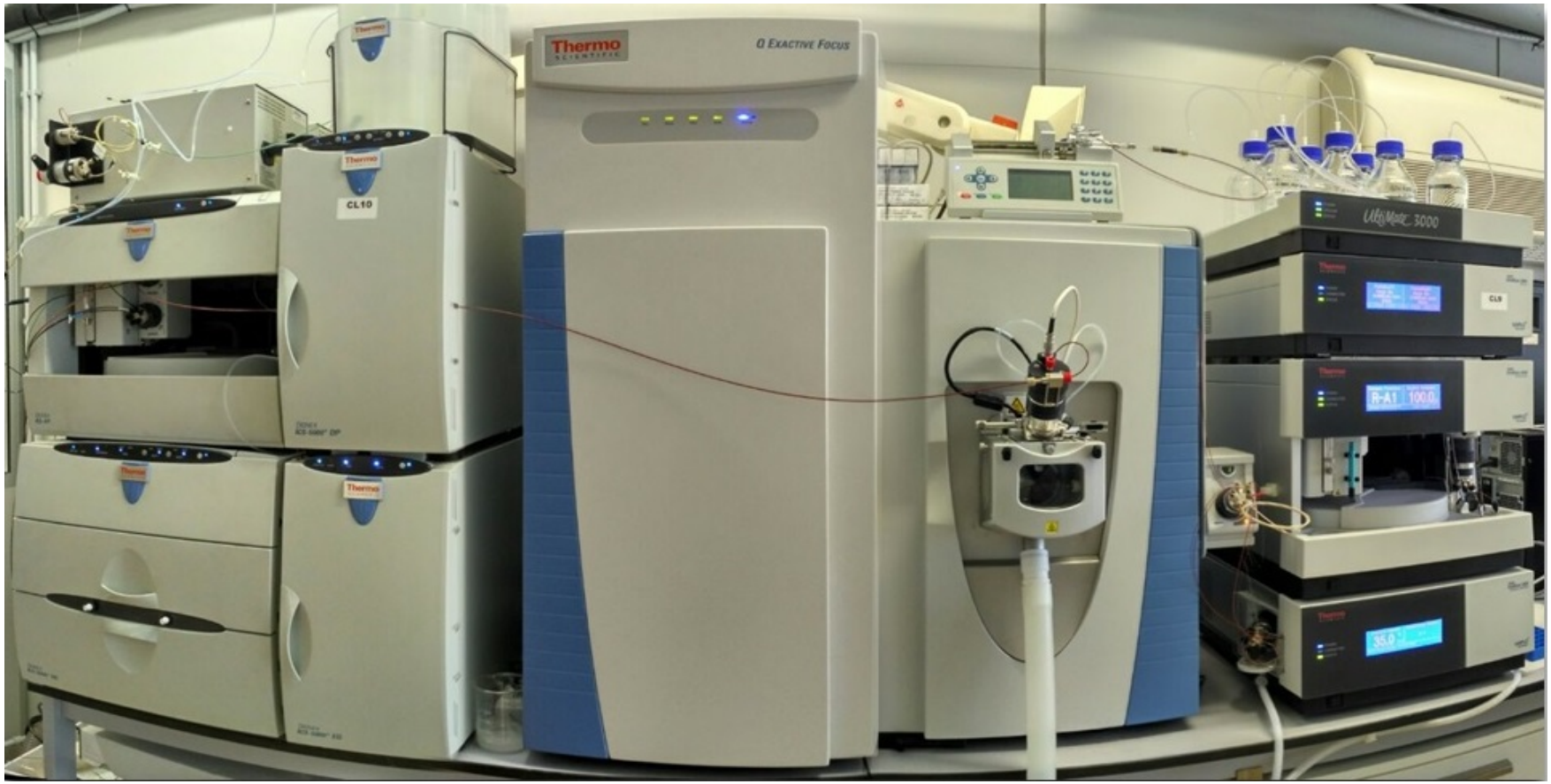
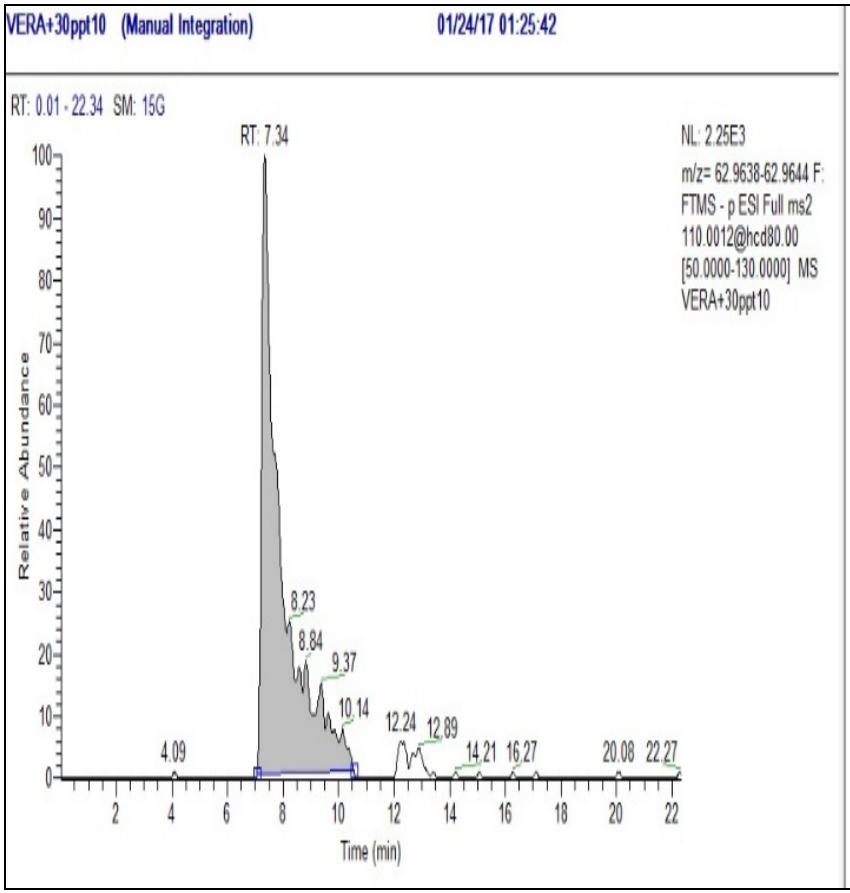
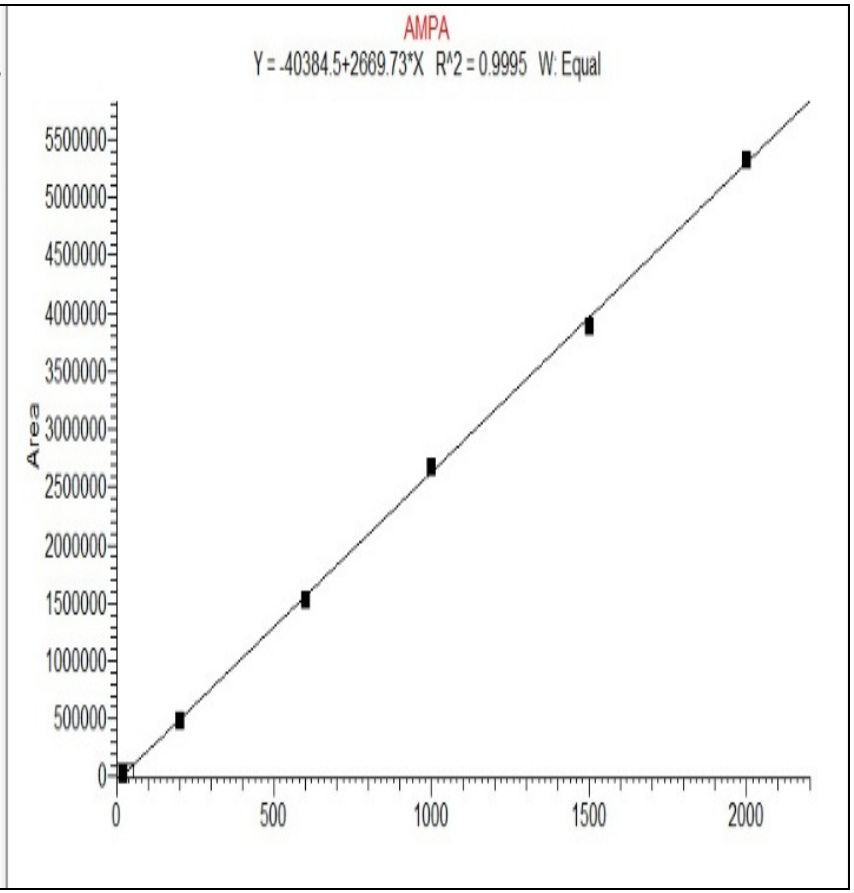
Comprehensive solution for pesticide analysis

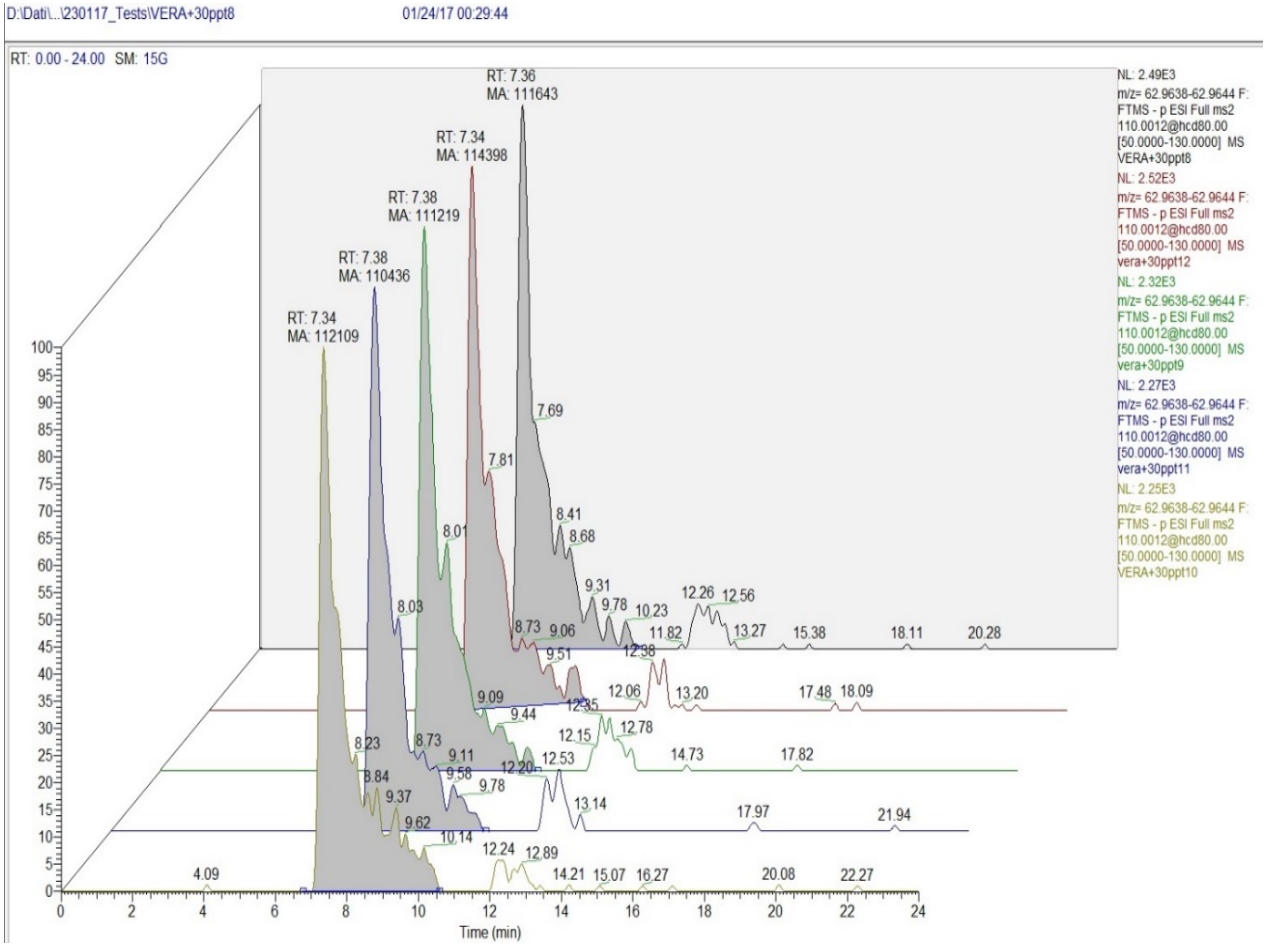


AMPA Linearity from 20 to 2000 ppt in Drinking Water





AMPA Repeatability at 30 ppt in Drinking Water



Picco	Area
1	112109
2	110436
3	111219
4	114398
5	111643
Media	111961
StDev	1494.84
CV%	**1.3**

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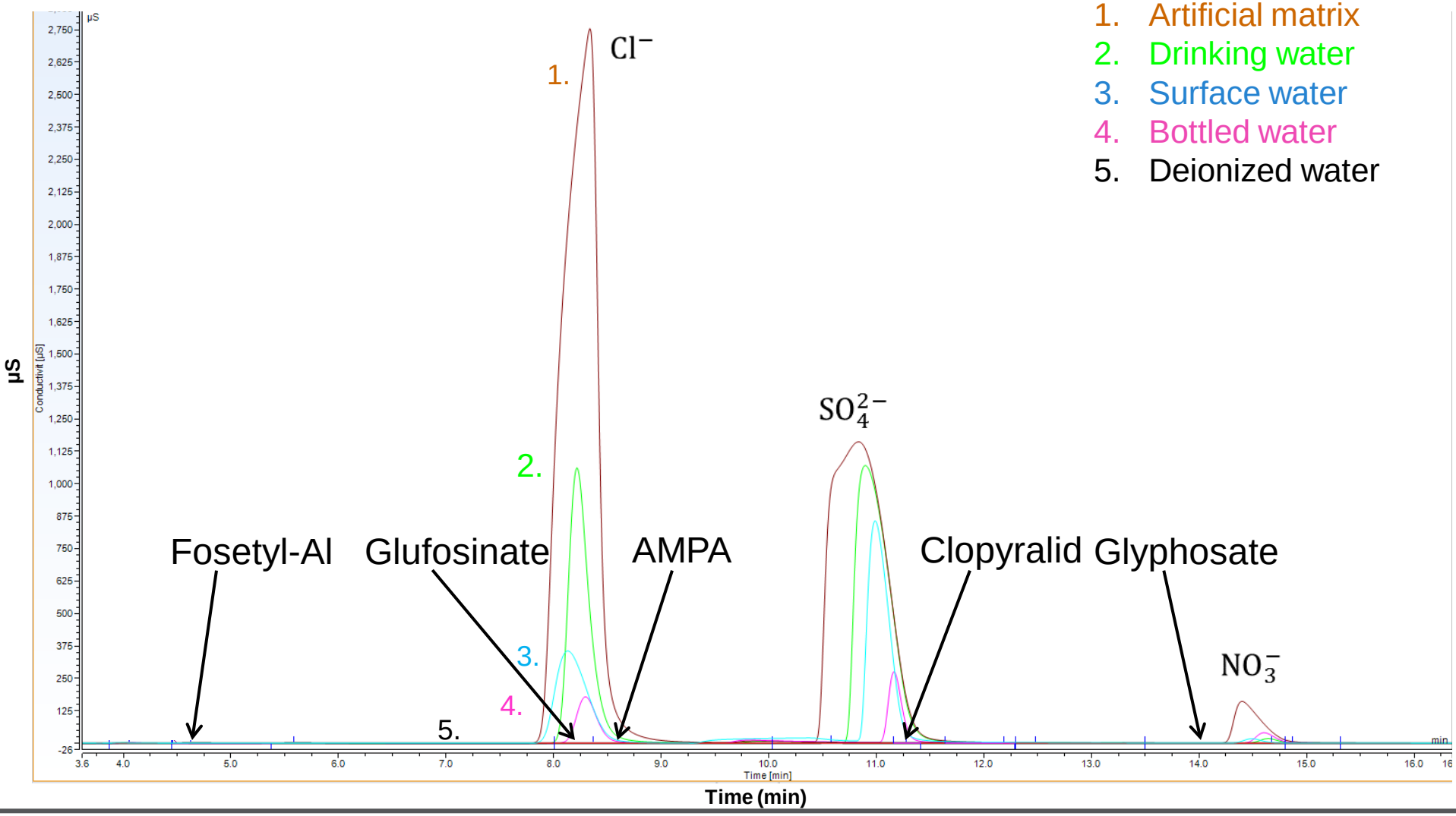
Conductivity Traces of Anions Present in Different Matrices



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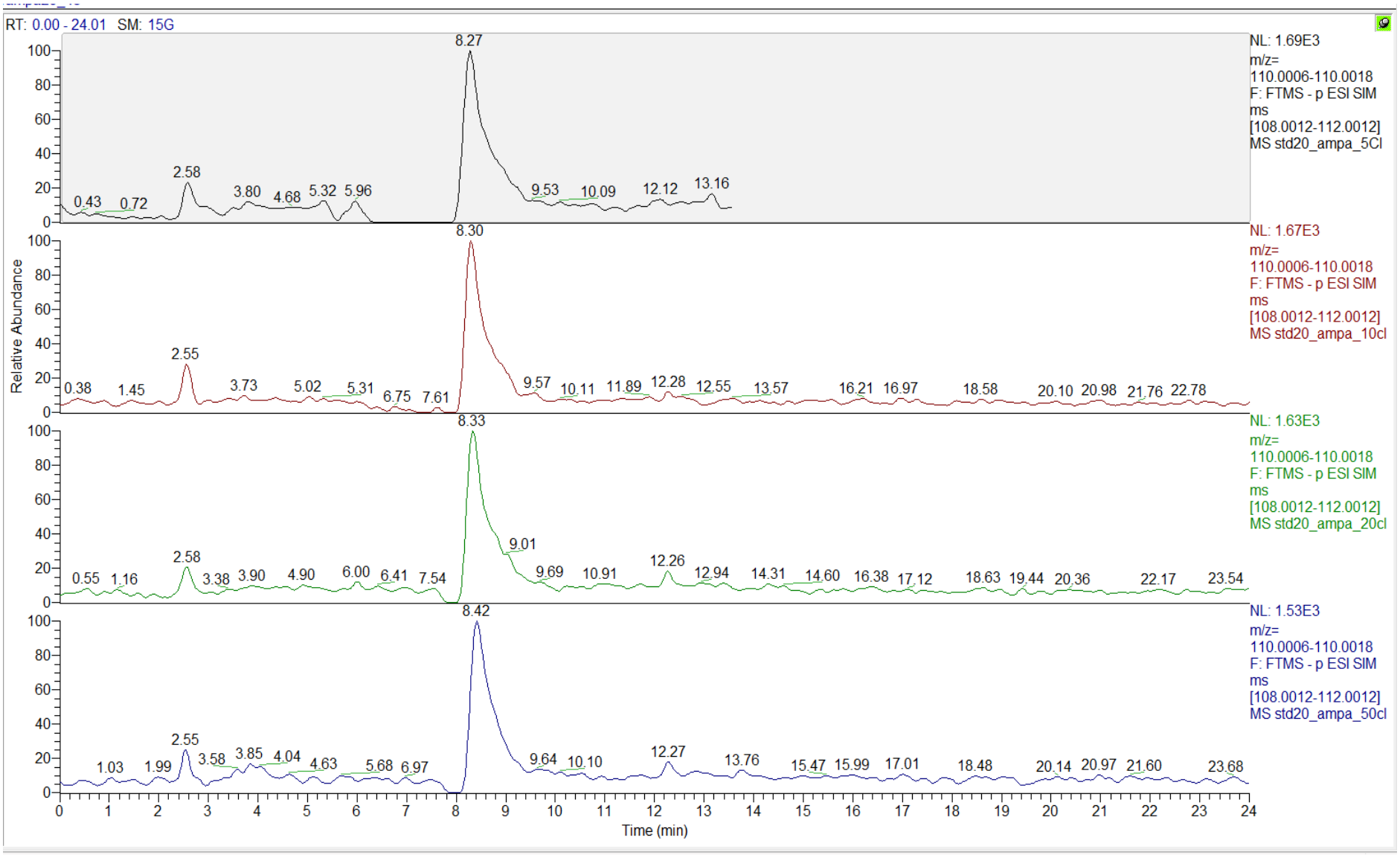
20 ppt AMPA Spiked in Water Containing Cl^- (5 to 50 ppm)



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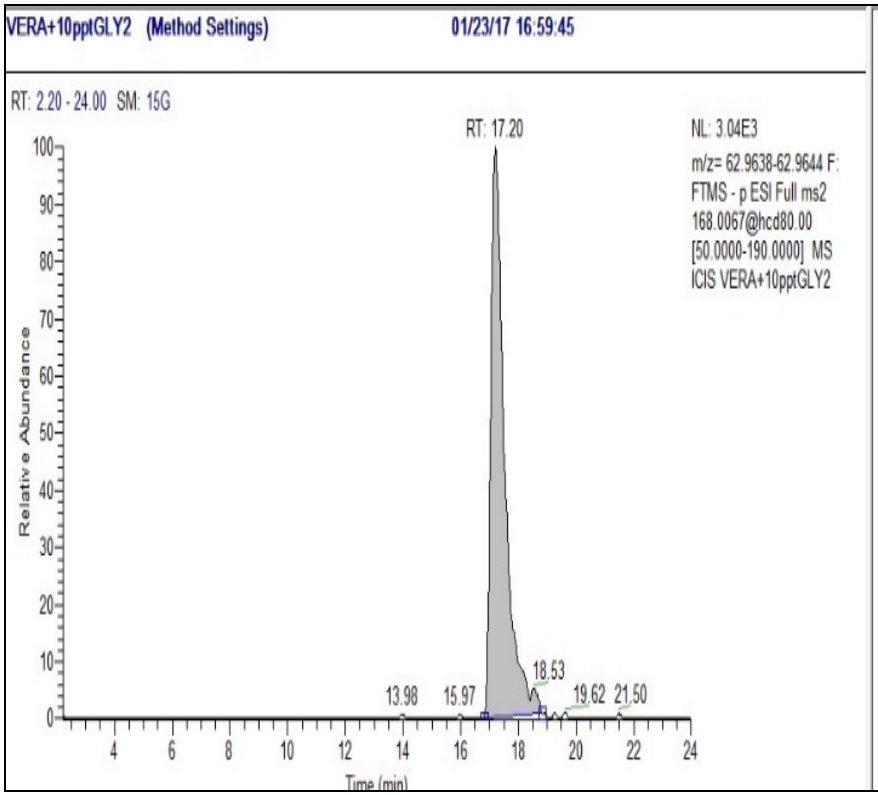
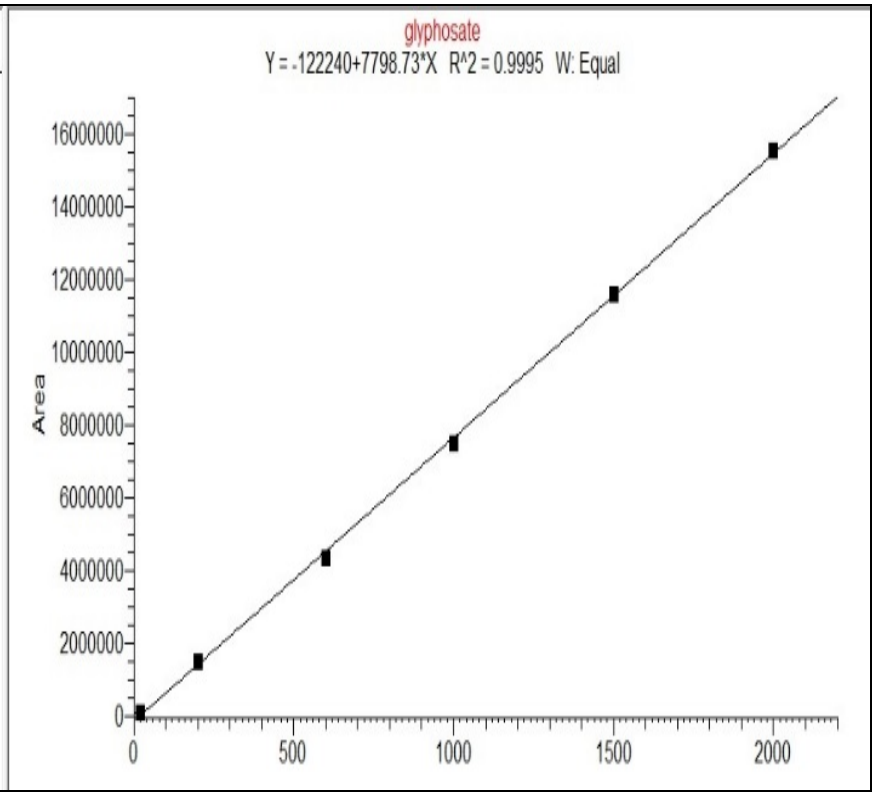
Glyphosate Lineatity from 20 to 2000 ppt





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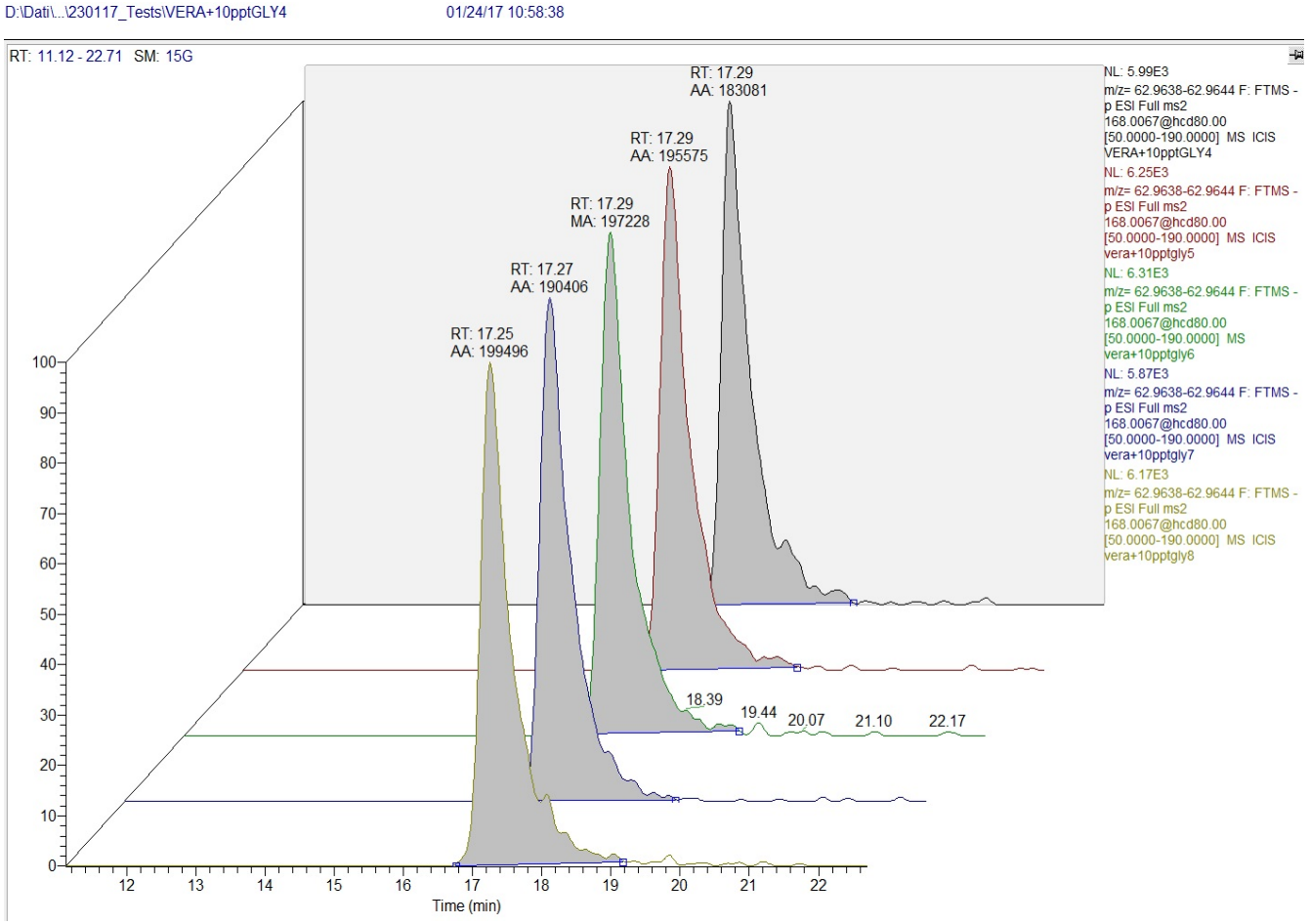
Glyphosate Repeatability at 10 ppt in Drinking Water



Picco	Area
1	199496
2	190406
3	197228
4	195575
5	183081
Media	193157.2
StDev	6551.6
CV%	**3.4**

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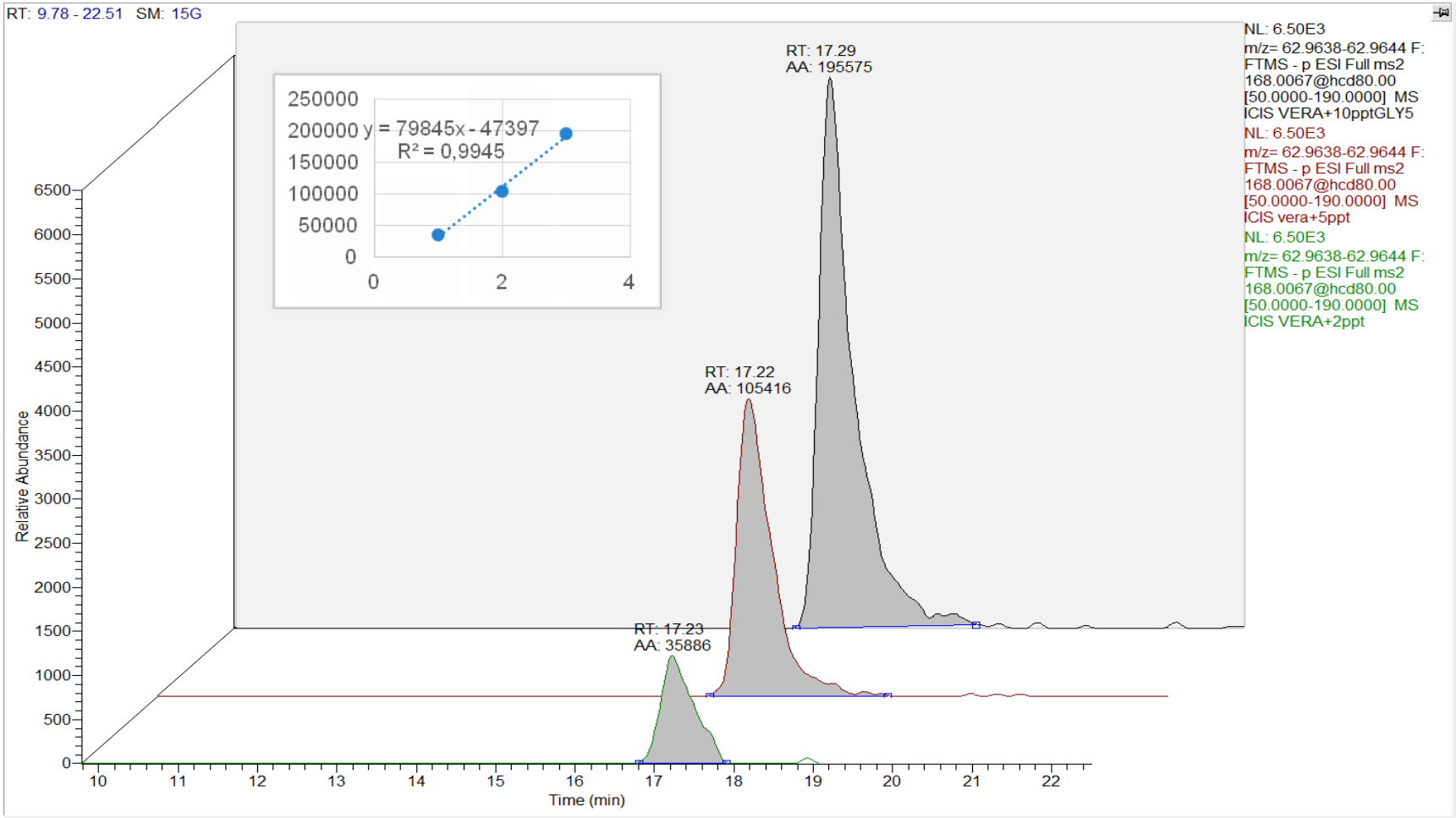
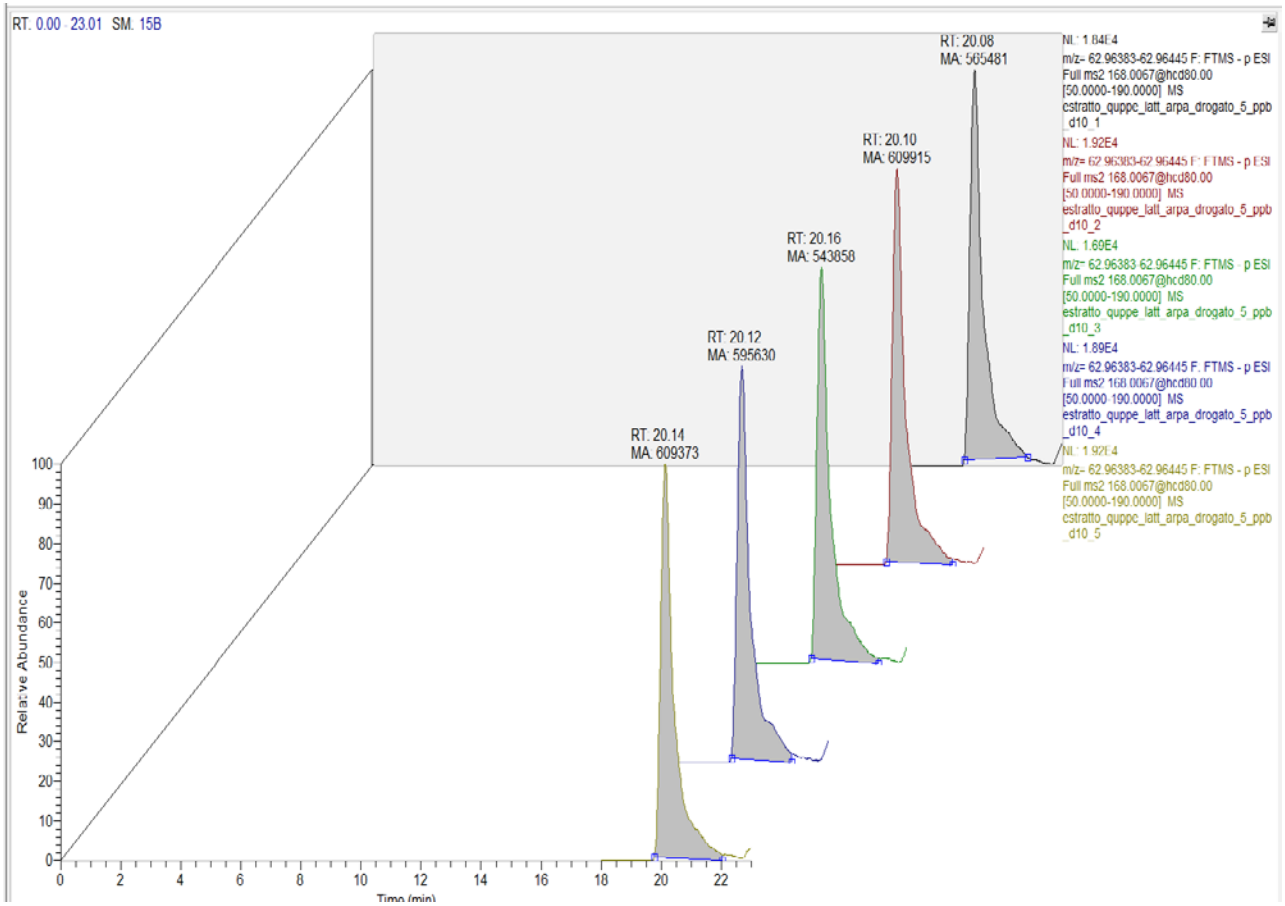
2 – 5 – 10 ppt of Glyphosate in Drinking Water



Repeatability of Glyphosate 500 ppt on Lettuce Extract



Picco	Area
1	565481
2	609915
3	543858
4	595630
5	609373
Media	584851.4
StDev	29160.51
CV%	**4.99**

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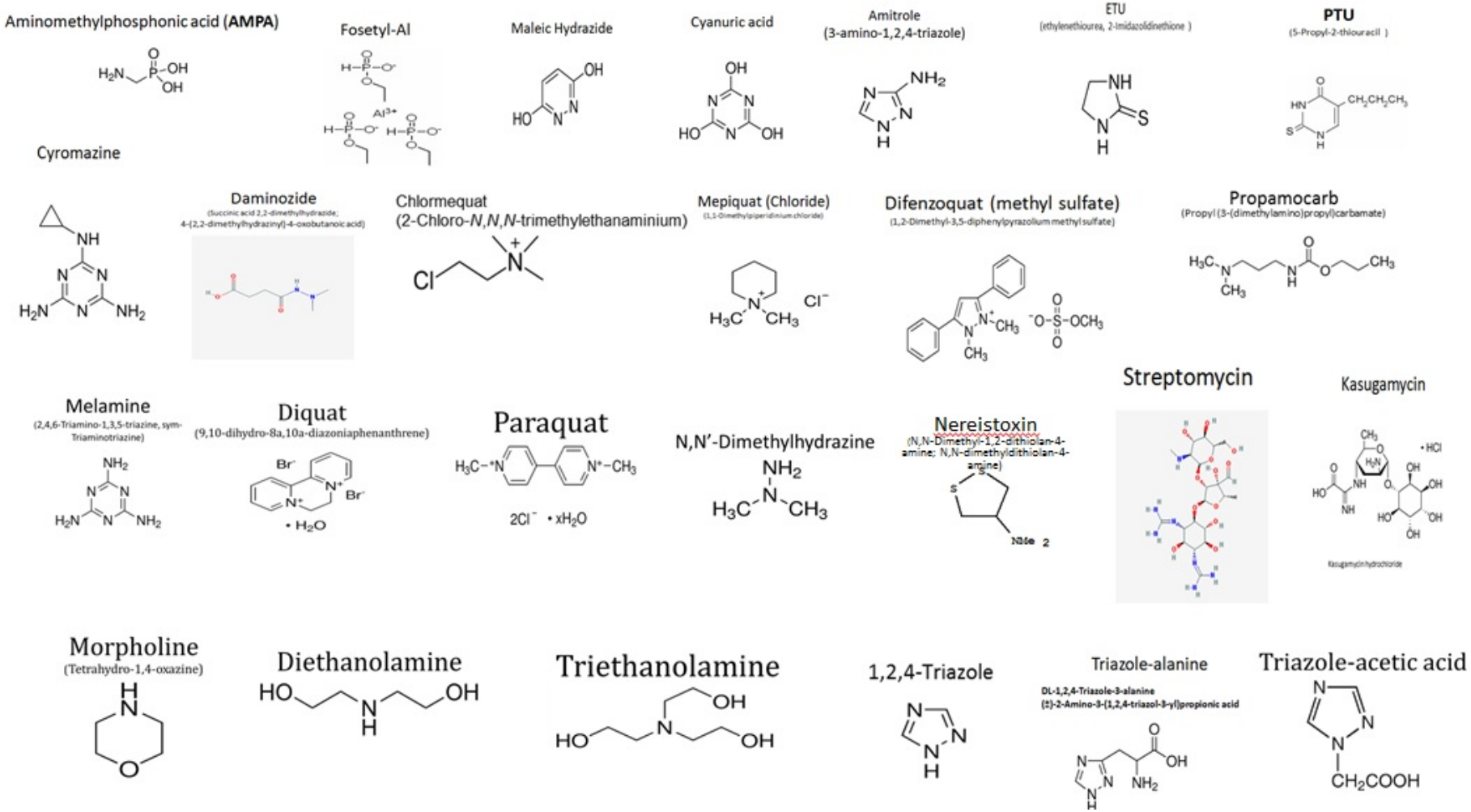
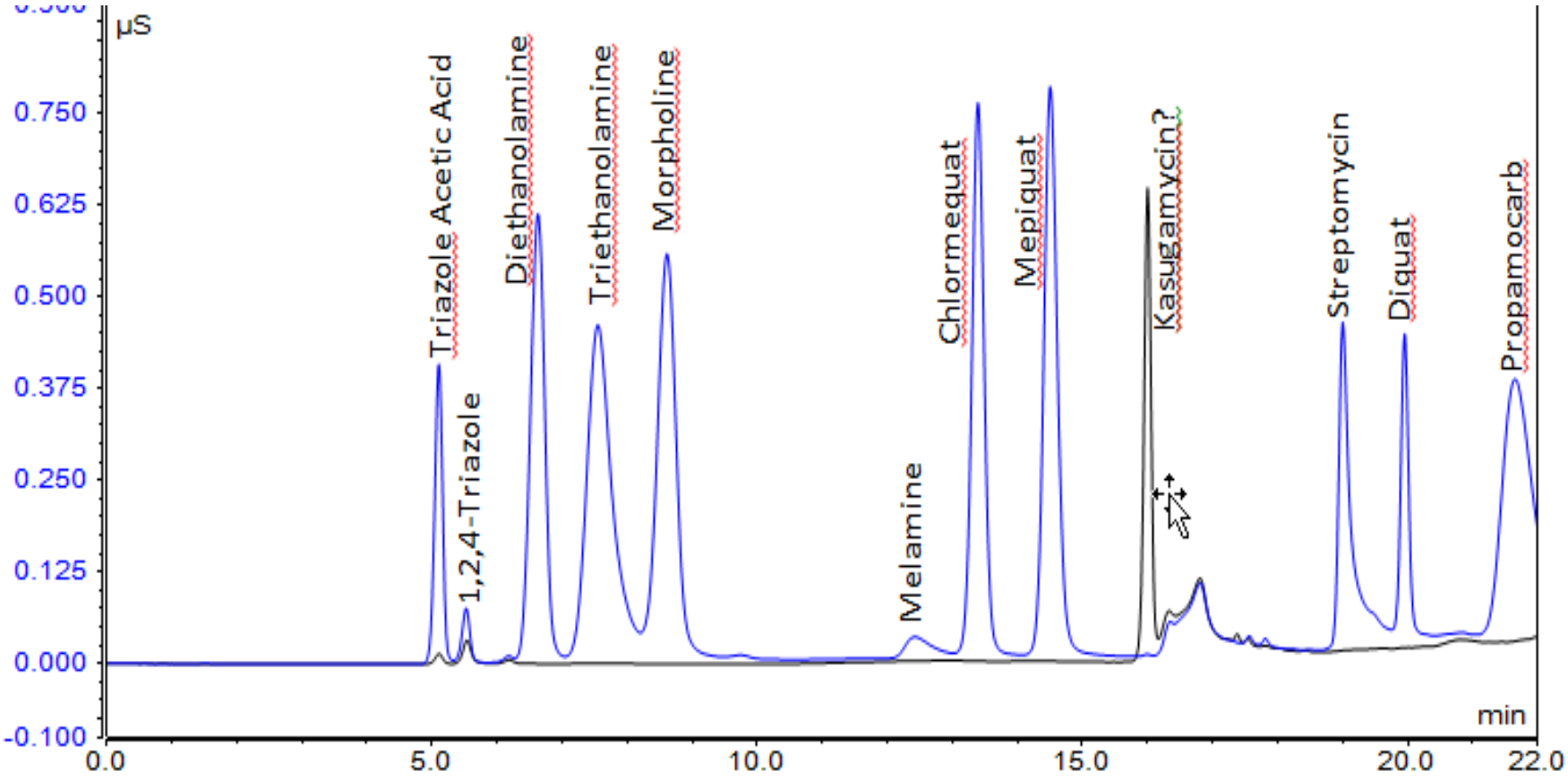
Cation Polar Pesticides



Note: Triazole-lactic acid missing

Courtesy of **Dr. Richard Fussell**

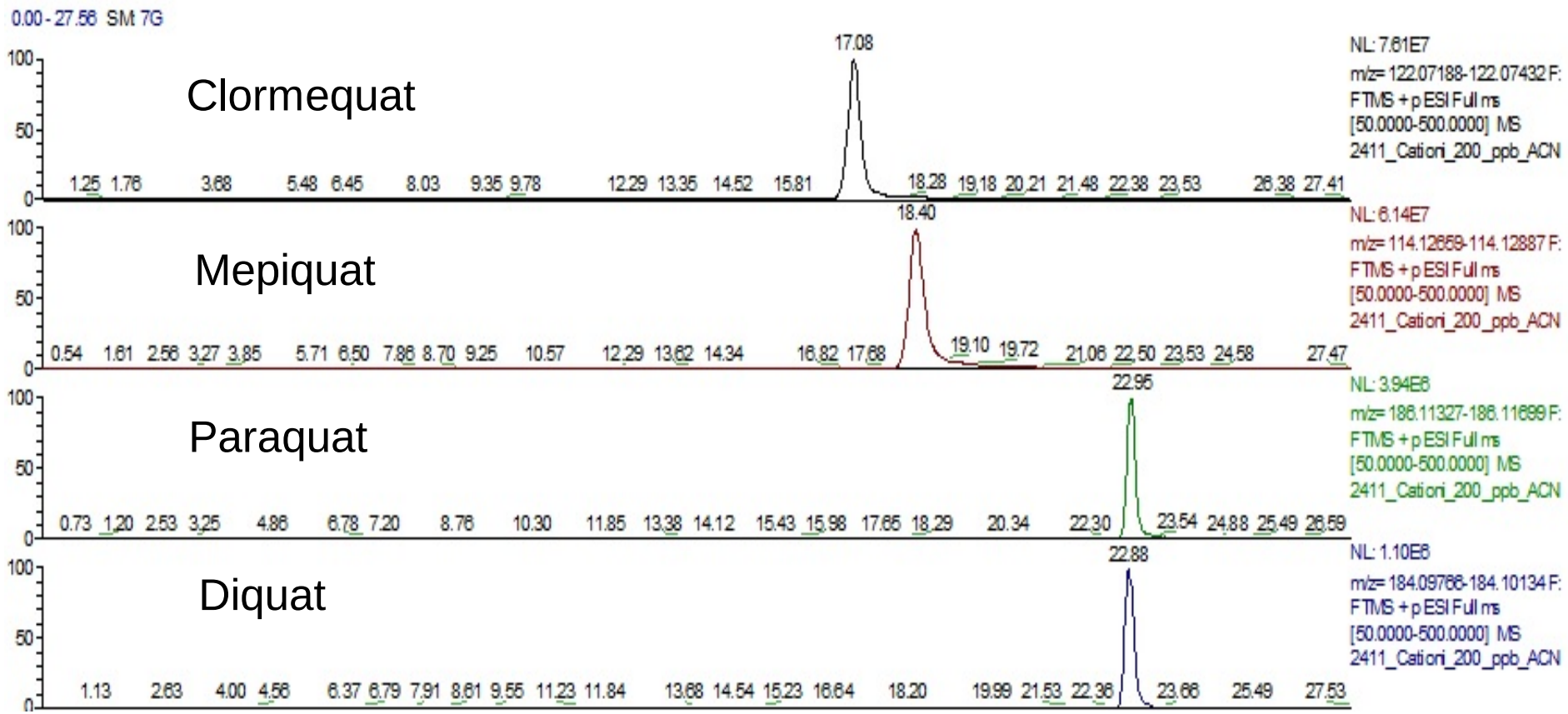
Cation Polar Pesticides on Dionex IonPac CS17 Column



Courtesy of **Dr. Richard Fussell**

Paraquat – Mepiquat – Chlormequat - Diquat

Dionex IonPac CS17 column full scan determination of cation polar pesticides



IC-MS Advantages

- Determination of several analytes in a single anionic run:
  - Glyphosate, AMPA, Glufosinate, anionic polar pesticides
  - Haloacetic Acid HAA9
  - Bromate (at ppt level)
  - Perclorate (at ppt level)
  - Organic ccids
  - Anions
- Determination of several analytes in a single cationic run:
  - Diquat, Paraquat, Chlormequat, cationic polar pesticides
  - Amines
  - Cations

Do you have any questions?

Do you have additional questions or do you want to talk to an expert from Thermo Fisher Scientific?

Please send an E-Mail to **analyze.eu@thermofisher.com**
and we will get back to you.