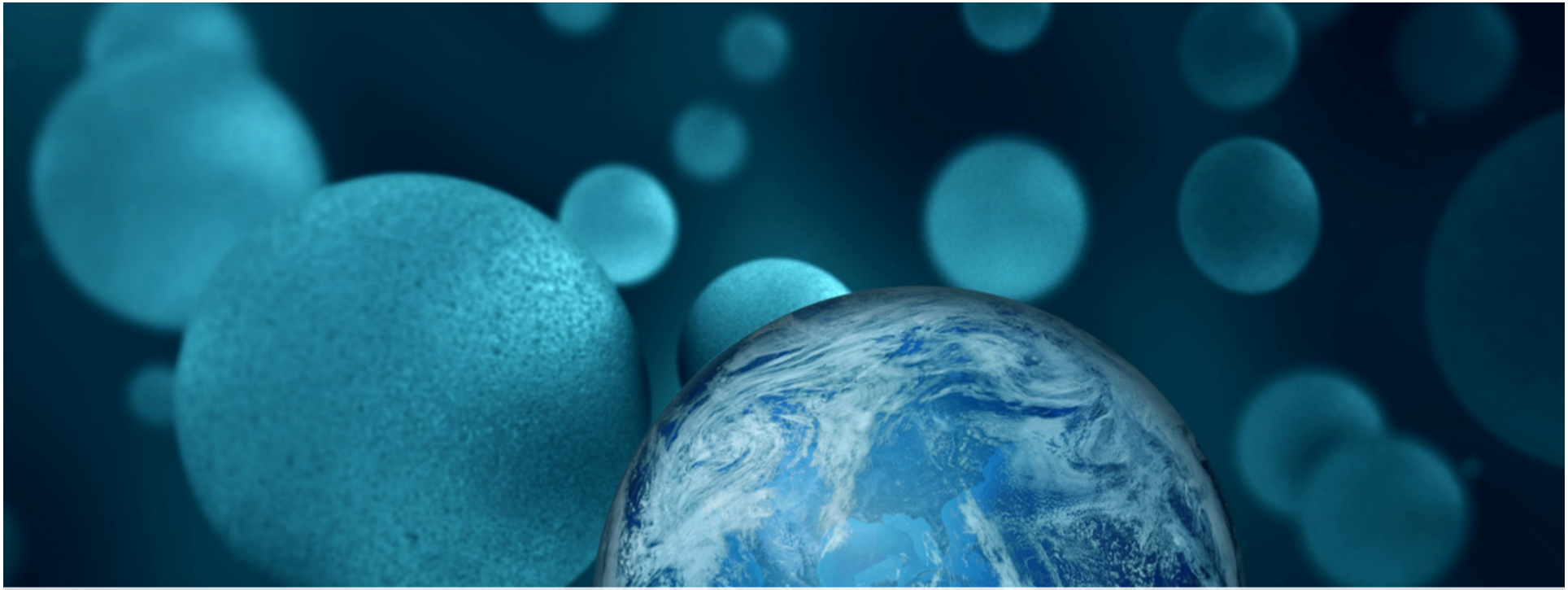

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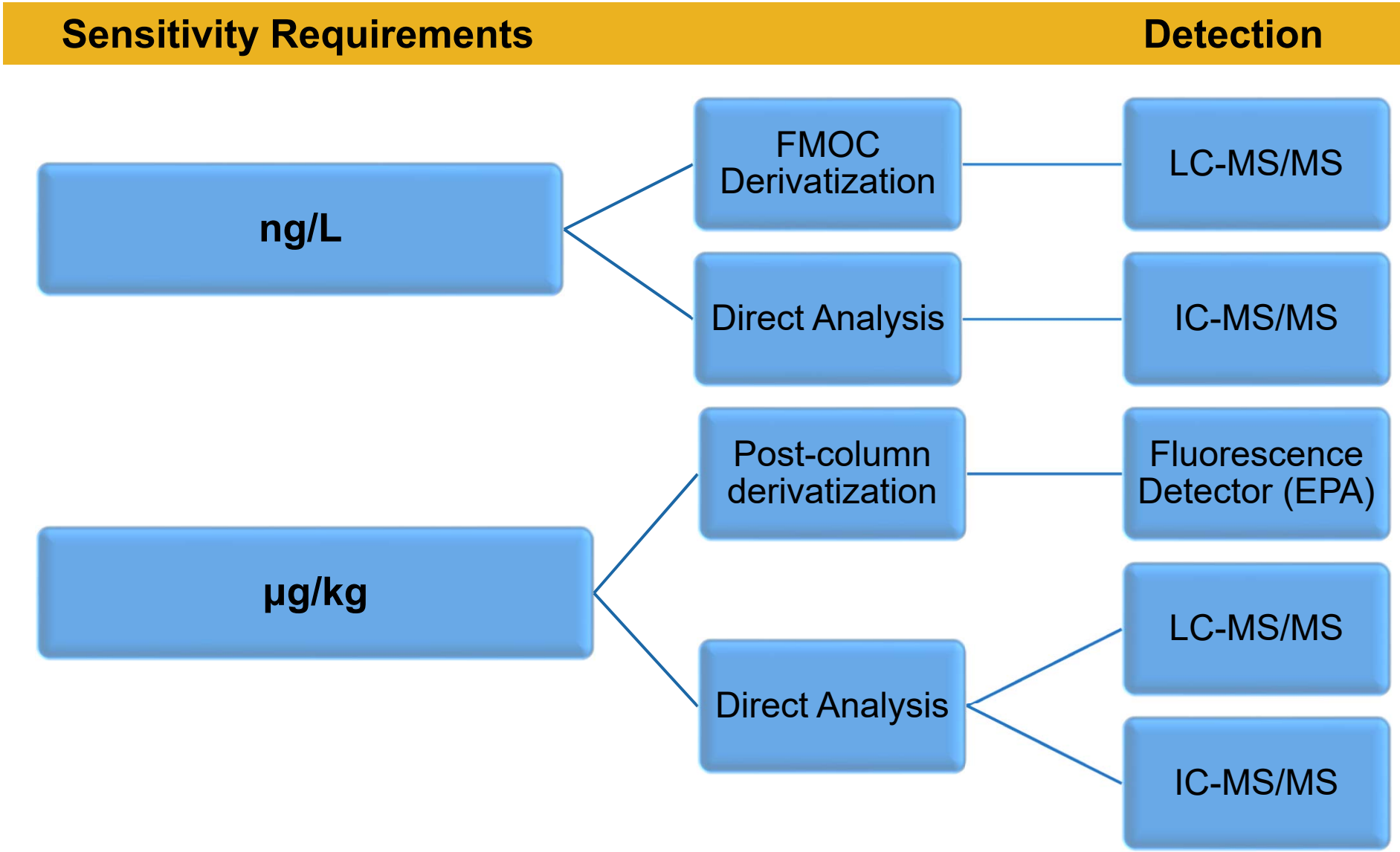
Improve Your Ability to Detect and Analyze Polar Pesticides using IC-MS/MS

Richard Jack and John Madden

Contents

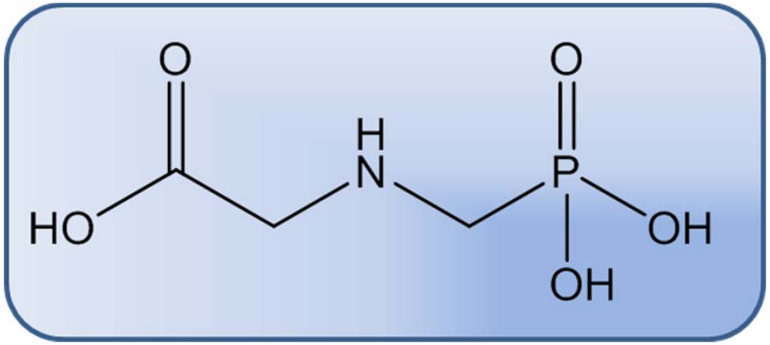
- Overview of polar pesticide analysis techniques
- Analysis of anionic polar pesticides in water
- Analysis of anionic polar pesticides in food
- Analysis of cationic polar pesticides
- Conclusions

Options for the analysis

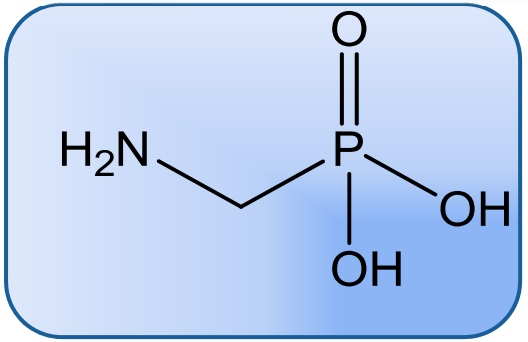


What is Glyphosate?

- **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



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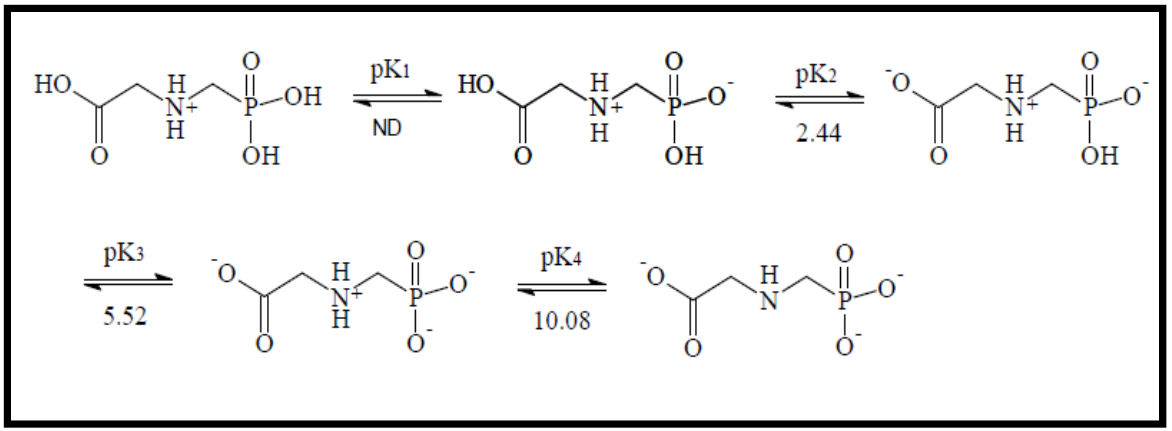
Glyphosate in the news

- **In 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. Then of 24 foods had detectable levels of glyphosate (86–1,327 µg/kg) (www.anh-usa.org)

Why is the analysis difficult?

- Zwitterionic compound
- Low volatility
- High solubility in water
- Low product ion masses
- Lack of chemical groups



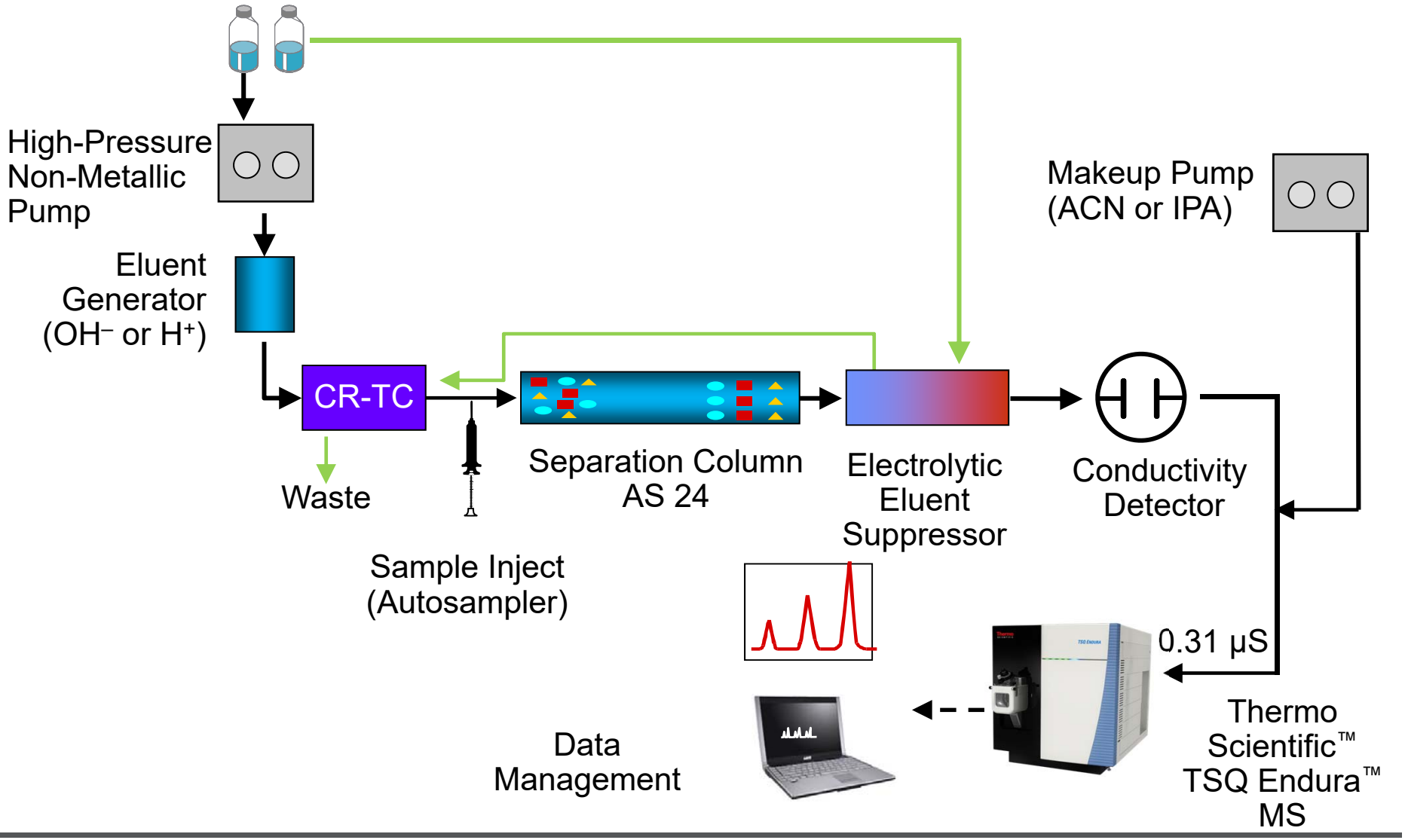
Current status for polar pesticide analysis in the EU

- EURL-SRM Method 9.3 (August 2017)
- QuPPe LC-MS methods
- 13 different methods covering
  - 29 cations
  - 20 anions
- 7 different columns
- IC-MS/MS can reduce the number of methods and columns

	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	M 9
ESI-mode	Neg.	Neg.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Neg.
Separation princip-le	Anion Exch.	Anion Exch.	Carbon	Carbon	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	Carbon	HILIC
Column type	AS 11	AS 11-HC	Hyper-carb	Hyper-carb	Obe-lisc-R	Obe-lisc-R	Obe-lisc-R	BEH-Amide	PFP	Obe-lisc-R	Trini-ty P1	Hyper-carb	Trinity P1

Environmental Analysis: IC-MS/MS for Water Analysis

IC-MS flow diagram



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Configuration

- IC-System: Thermo Scientific™ Dionex™ Integrion™ HPIC™ system (PN: 22153-60208)
  - Eluent Source: Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge (PN: 075778)
  - Eluent: Potassium Hydroxide
  - Suppressor: Thermo Scientific™ Dionex™ ASRS™ 300 Suppressor – 2mm (PN: 064555)
    - External water mode regeneration
- External Pump 1 (for suppressor regeneration): Thermo Scientific™ Dionex™ AXP-MS Auxiliary pump (PN: 60684)
- External Pump 2 (for make-up flow): Dionex AXP-MS Auxiliary pump (PN: 60684)
- Autosampler: Thermo Scientific™ Dionex™ AS-AP Autosampler (PN: 074926)
- Mass Spectrometer: Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer

IC-MS conditions

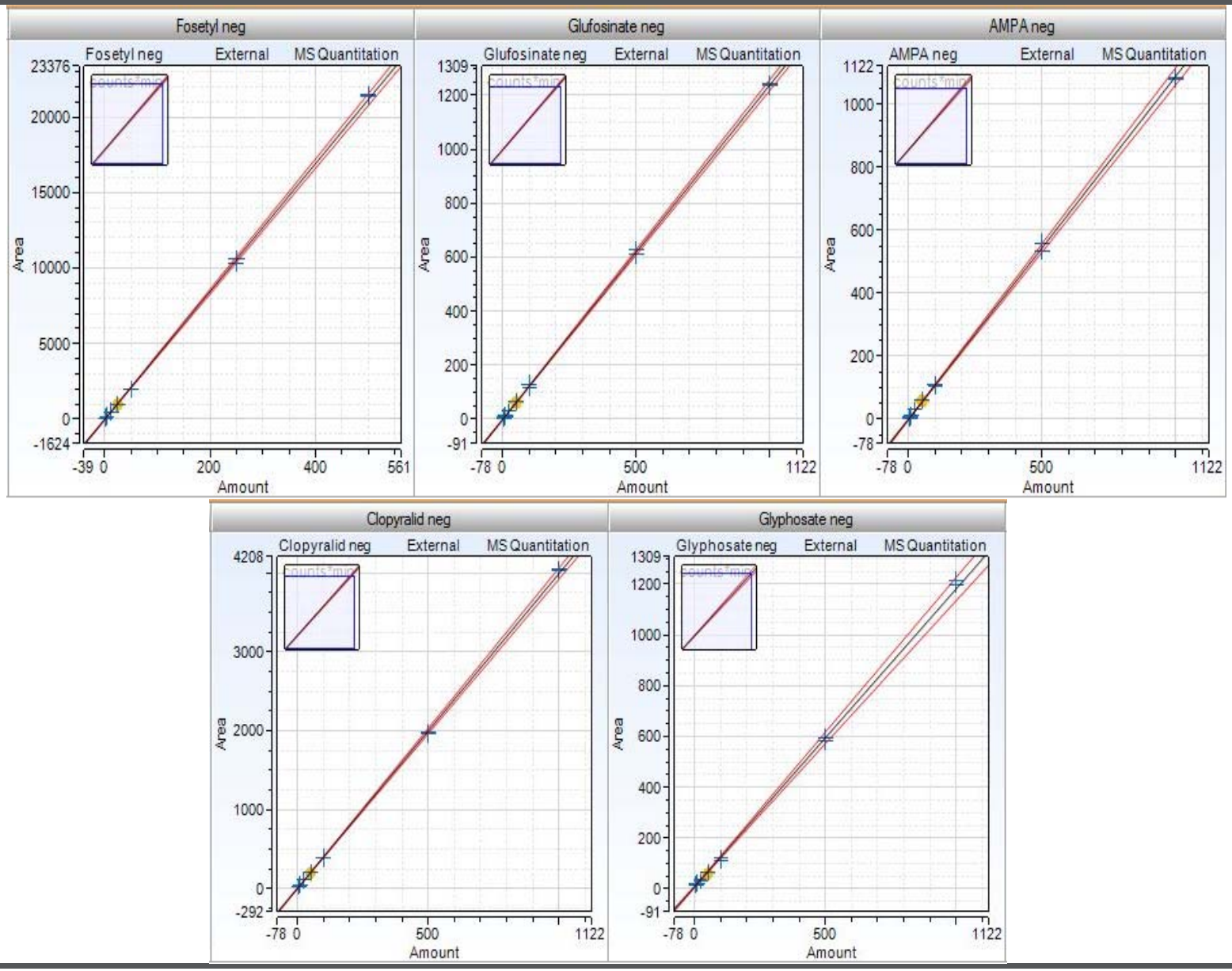
Column:	Thermo Scientific™ Dionex™ IonPac™ AS24 (2 x 250 mm)
Guard Column:	Thermo Scientific™ Dionex™ IonPac™ AG24 (2 x 50 mm)
Eluent:	KOH
Column Temperature:	30 ºC
Flow rate:	0.3 ml/min
Make-up flow:	0.1 ml/min
Make-up solvent:	IPA, 0.1 mL/min
Duration:	22 min
Injection volume:	100 µL
Injection Mode:	PushFull
Loop Overfill:	2.000

Ion Source Type	H-ESI
Spray Voltage (Neg)	2800 V
Sheath Gas (Arb)	30
Aux Gas (Arb)	12
Sweep Gas (Arb)	1
Ion Transfer Tube	340 ºC
Vaporizer Temperature	360 ºC
Cycle time (s)	0.5
Q1/Q3 Resolution (FWHM)	0.7
CID gas (mTorr)	1.5
Source Fragmentation (V)	0
Use calibrated RF Lens:	

IC method

Time (min)	Potassium hydroxide (KOH) (mM)	Suppressor current (mA)
0	22	25
7	25	25
7.1	40	25
9.5	40	25
9.6	80	25
10.6	80	75
14.5	80	75
14.6	100	75
17	100	75
17.1	22	75
18	22	25
20	22	25

Calibration drinking water



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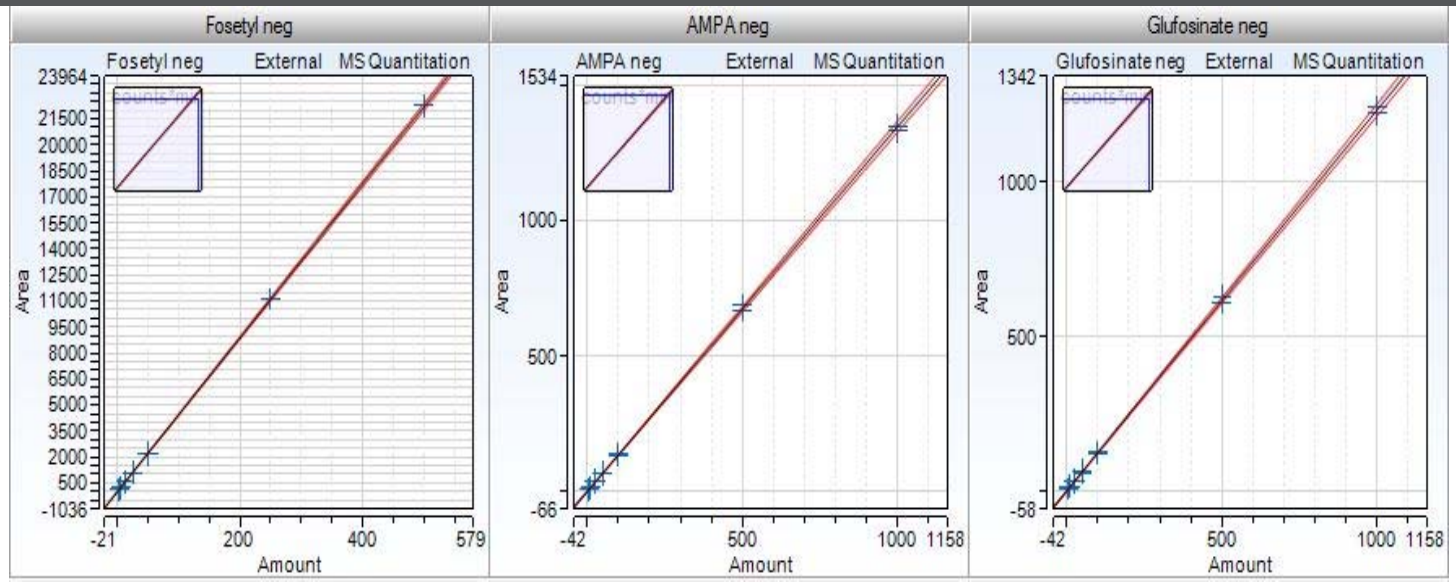
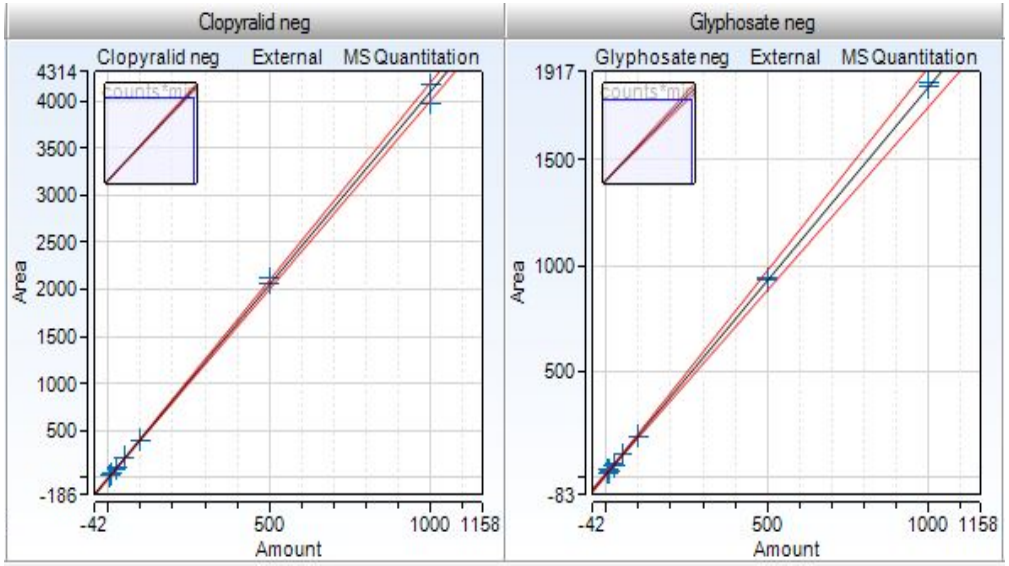
Calibration with bottled Evian™ water





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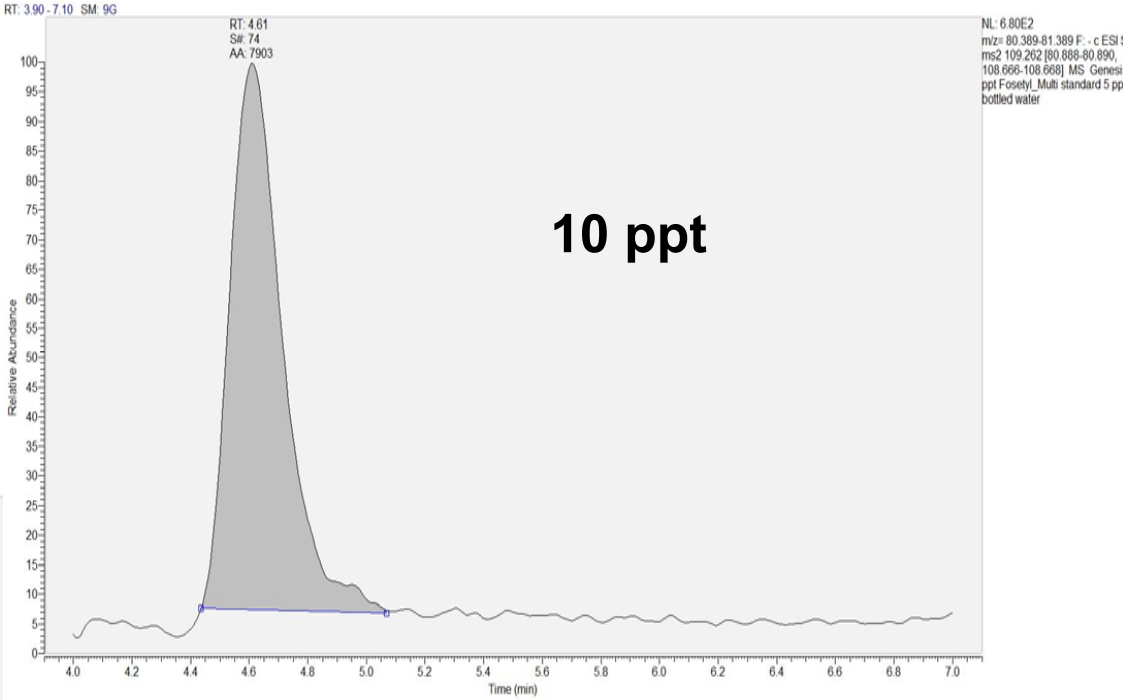
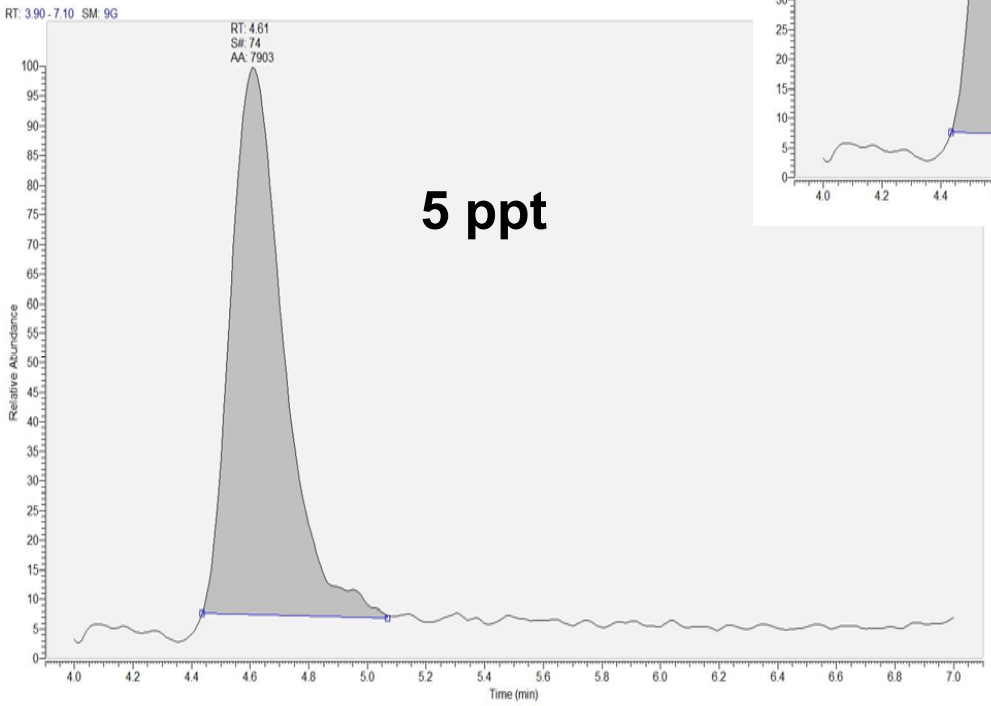
Bottled Evian™ water performance

Name	LOD [ppt]	LOQ [ppt]	RSD % (10 ppt) level
Fosetyl-Al	1	2.5	5
Clopyralid	10	50	9
AMPA	2	5	9
Glyphosate	5	10	15
Glufosinate	2	5	4

Bottled water — Fosetyl Al

Fosetyl	LOD [ppt]	LOQ [ppt]
Bottled water	1	2.5





	5 ppt	50 ppt	500 ppt
RSD [%]	2.4	0.86	1.12
REC [%]	100	101	106

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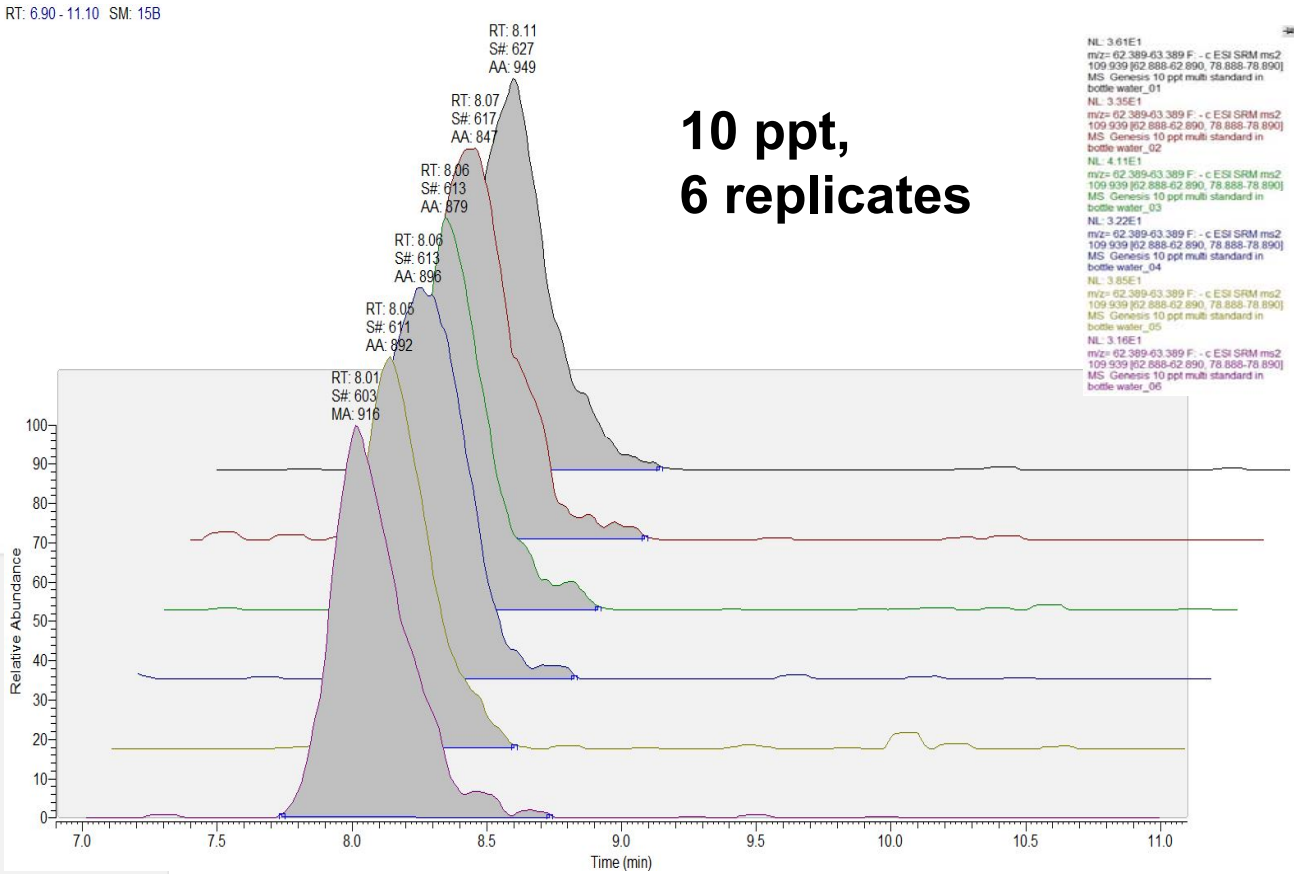
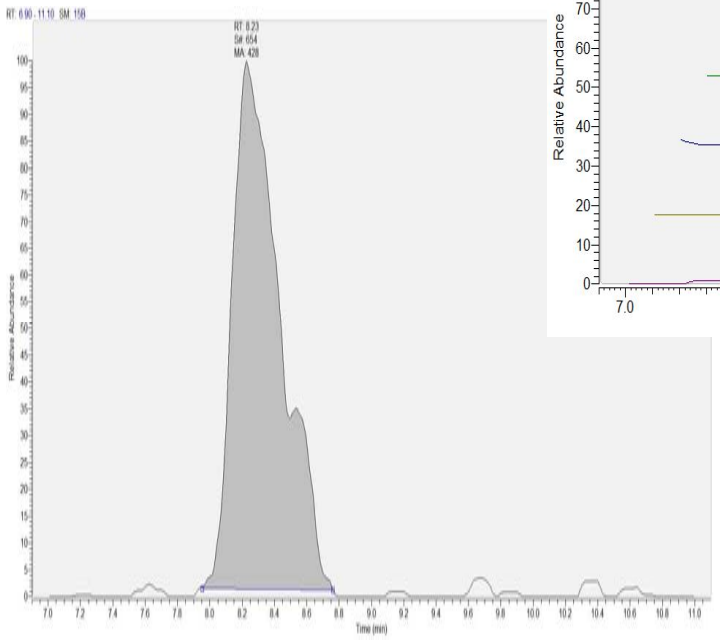
Bottled water — AMPA

10 ppt,
6 replicates



5 ppt



* 6 vials in a row

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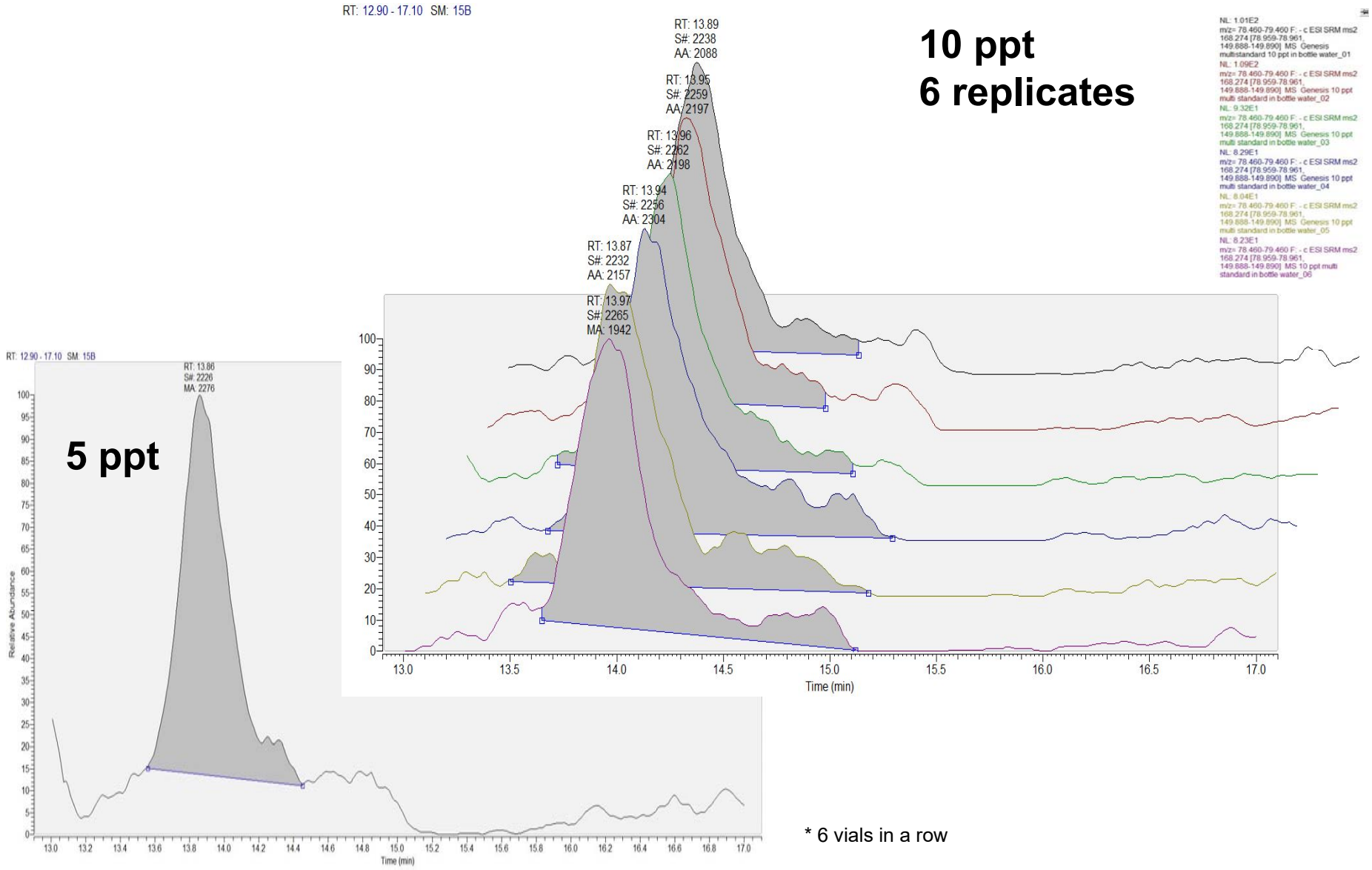
Bottled water — Glyphosate



* 6 vials in a row

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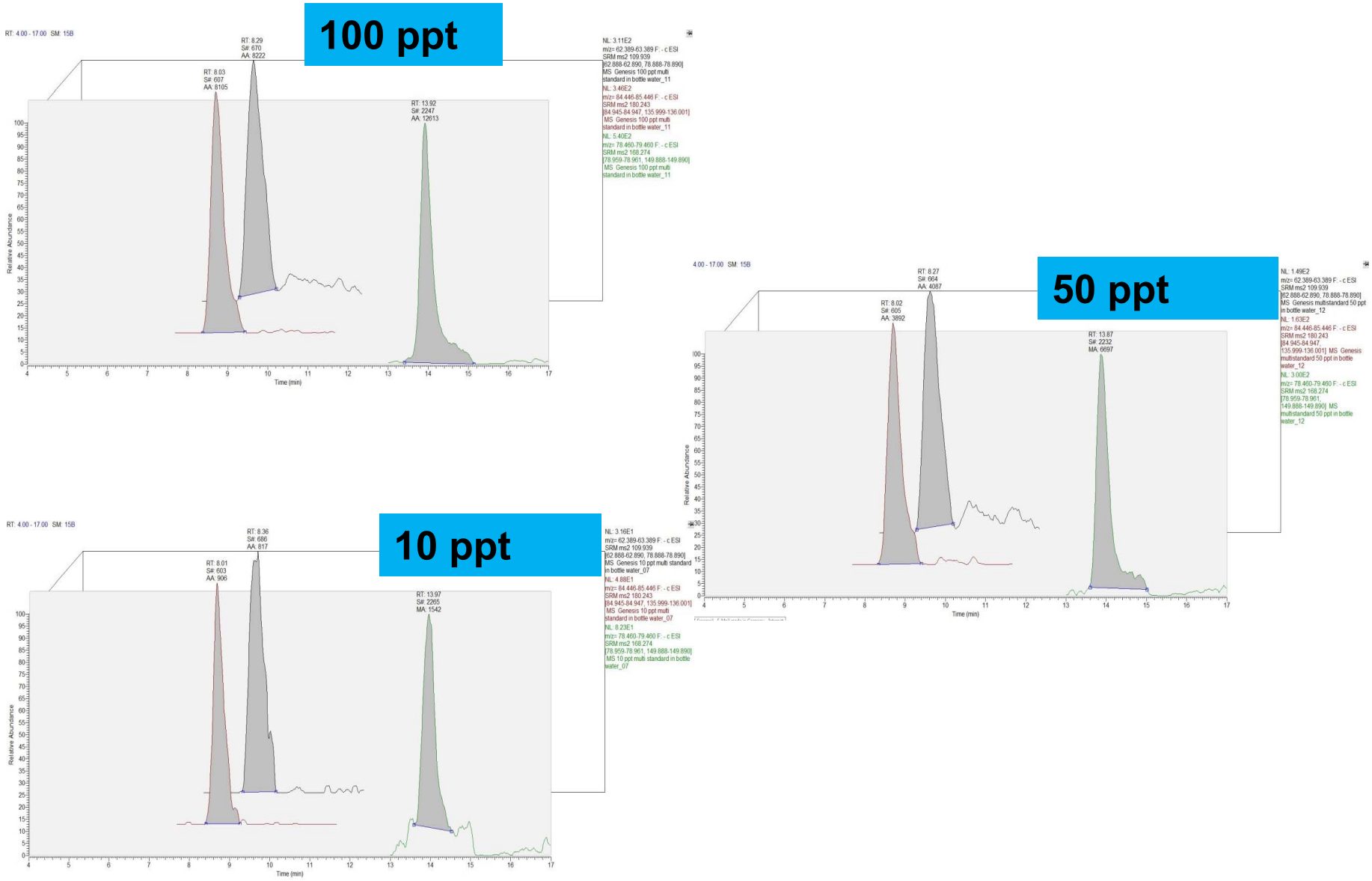
Overview — AMPA, Glu, Gly spiked into bottled Evian™ water



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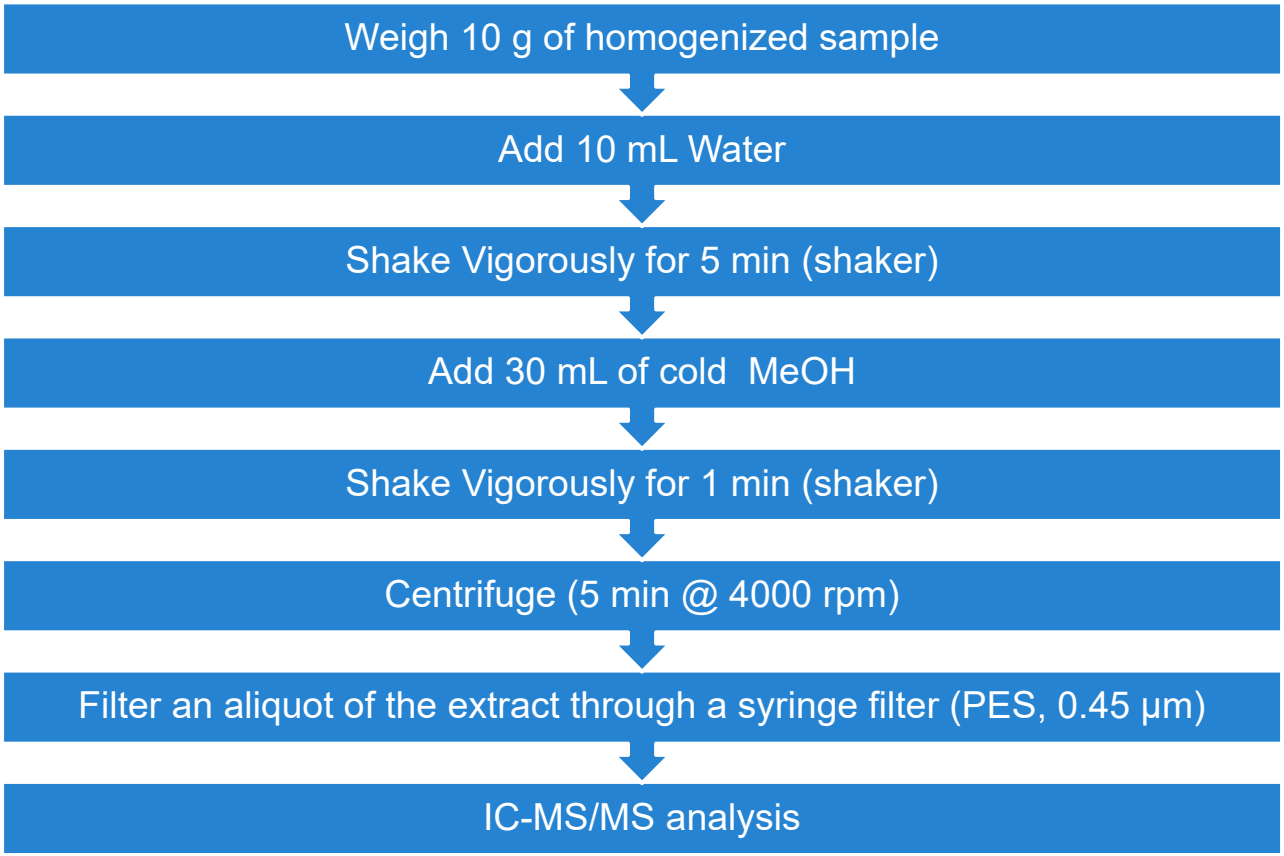
Food Analysis—QuPPe by IC-MS/MS

Sample preparation



- The use of **plastic material** is highly recommended!

Configuration

- IC-System: Thermo Scientific™ Dionex™ ICS-2100 IC system
  - Eluent Source: Thermo Scientific™ Dionex™ EGC III KOH Eluent Generator Cartridge
  - Eluent: Potassium Hydroxide
  - Suppressor: Thermo Scientific™ Dionex™ AERS 500 Anion Electrolytically Regenerated Suppressor – 2mm
    - External water mode regeneration
- External Pump 1 (for suppressor regeneration): Ismatec Peristaltic Pump
- External Pump 2 (for make-up flow): Dionex AXP-MS Auxiliary pump
- Autosampler: Thermo Scientific™ Dionex™ WPS-3000 TRS–AS Autosample
- Mass Spectrometer: TSQ Endura MS





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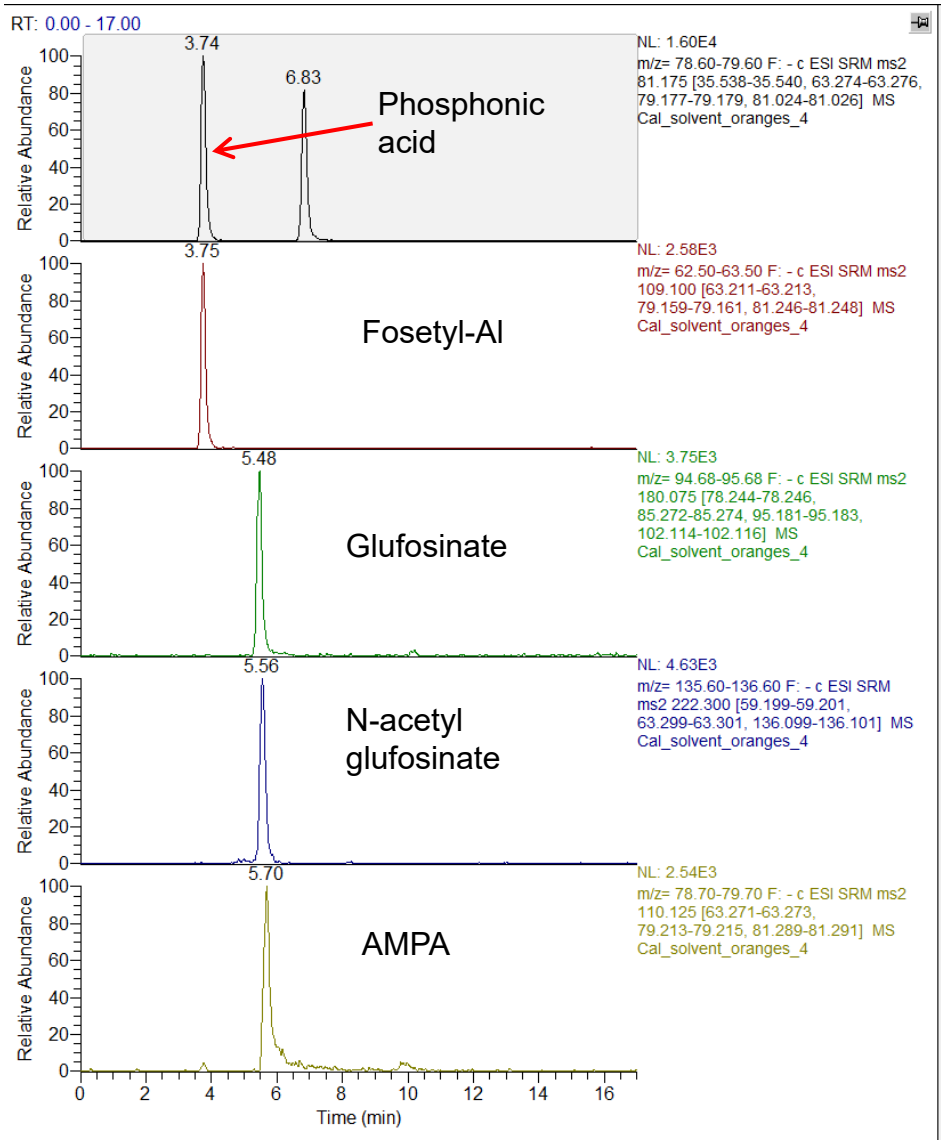
IC-MS/MS conditions

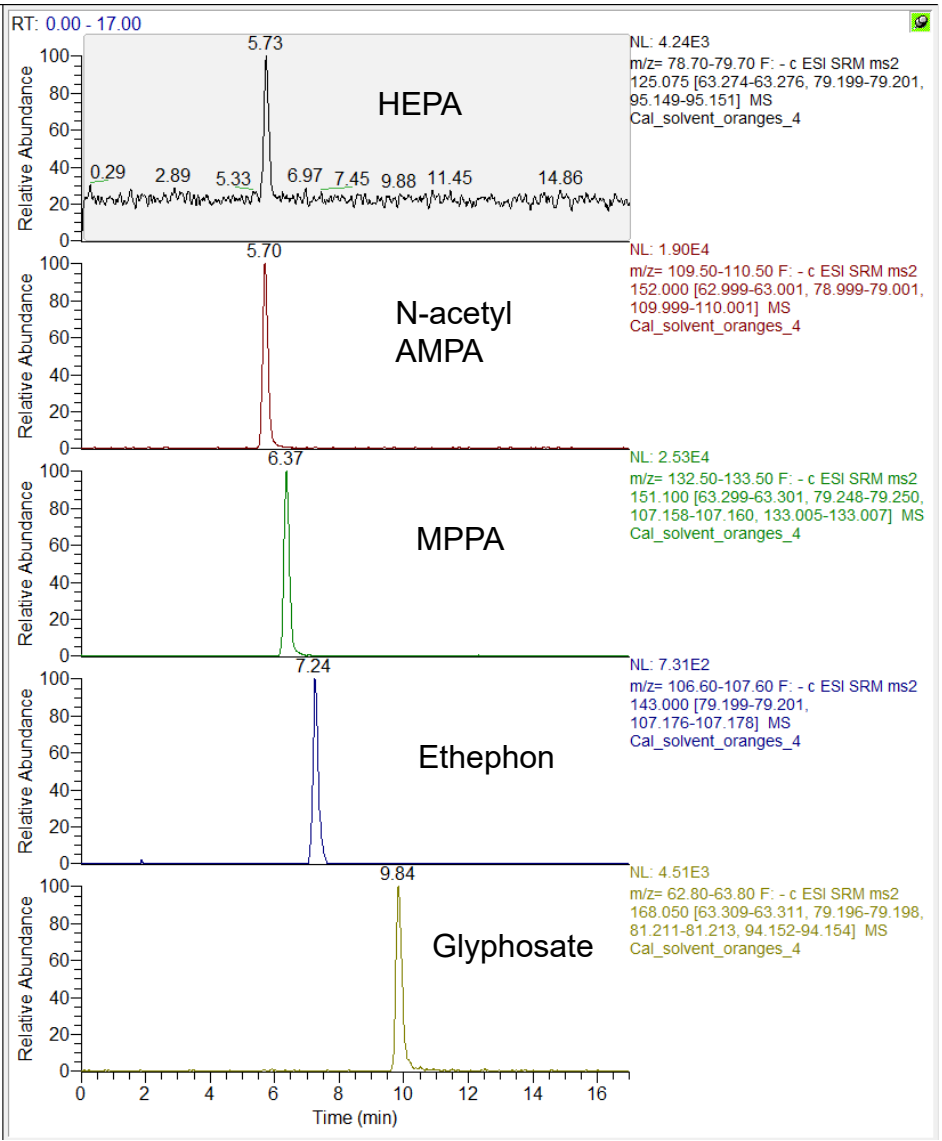
- Column: Dionex IonPac AS24 (2 x 250 mm)
- Guard Column: Dionex IonPac AG24 (2 x 50 mm)
- Eluent: KOH
- Injection volume: 10 µL
- Column Temperature: 21 ºC
- Flow rate: 0.3 mL/min
- Make-up flow: 0.1 ml/min
- Make-up solvent: CH_3OH

Time (min)	Concentration of KOH in eluent (mM)
0	25
0.2	25
11	80
11.1	100
12.5	100
12.6	25
17.0	25

- Ion Source Type: H-ESI
- Spray Voltage (Neg): 2500V
- Sheath Gas (Arb): 20
- Aux Gas (Arb): 5
- Sweep Gas (Arb): 0
- Ion Transfer Tube: 329ºC
- Vaporizer Temperature: 400 ºC
- Dwell Time (ms): 10
- Q1/Q3 Resolution (FWHM): 0.7
- CID gas (mTorr): 1.5
- Source Fragmentation (V): 0
- Use calibrated RF Lens: YES

Chromatogram – 100 ppb in solvent





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Results for lettuce

Recovery and Repeatability

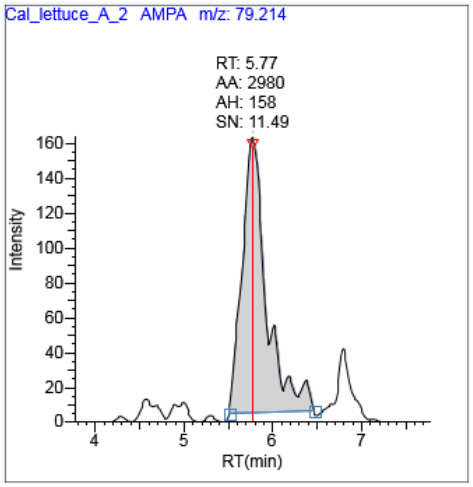
- 3 levels: 50, 200 and 500 µg/kg
- 6 repetitions at each level

Analyte	Level 1 (in ppb)		Level 2 (in ppb)		Level 3 (in ppb)	
	REC %	RSD %	REC %	RSD %	REC %	RSD %
AMPA	84	14	85	8	80	3
Ethephon	120	10	88	5	92	12
Fosetyl-Al	98	16	97	12	82	3
Glufosinate	101	4	93	8	86	3
Glyphosate	88	12	83	10	81	3
HEPA	118	7	93	9	81	4
Maleic hydrazide	51	245	0	-	40	117
MPPA	116	4	98	8	81	3
N-acetyl-AMPA	95	8	89	9	79	2
N-acetyl-glufosinate	93	8	91	8	84	2
Phosphonic acid	115	11	99	11	81	3

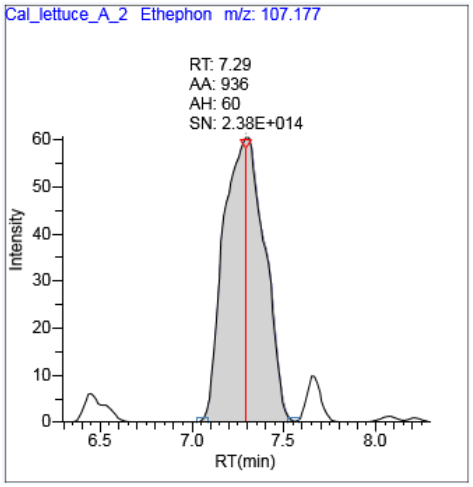
LOD and LOQ

Analyte	LOD (µg/kg)	LOQ (µg/kg)	LOD (pg on column)	LOQ (pg on column)
AMPA	10	20	100	200
Ethephon	10	20	100	200
Fosetyl-Al	10	20	100	200
Glufosinate	1	10	10	100
Glyphosate	5	10	50	100
HEPA	10	20	100	200
Maleic hydrazide	100	200	1000	2000
MPPA	1	10	10	100
N-acetyl-AMPA	1	10	10	100
N-acetyl-glufosinate	3	10	30	100
Phosphonic acid	1	10	10	100

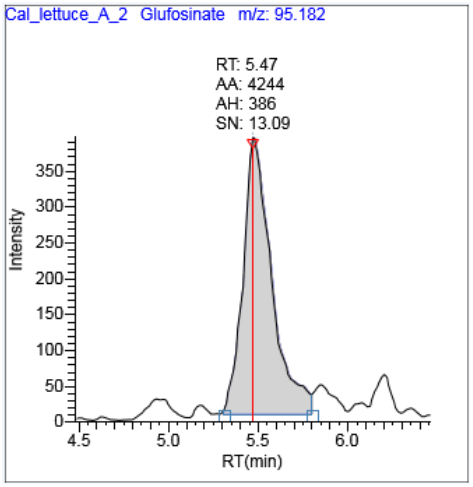
10 ppb in lettuce



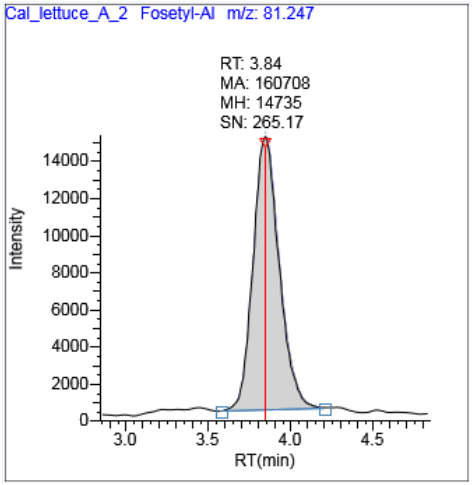
AMPA



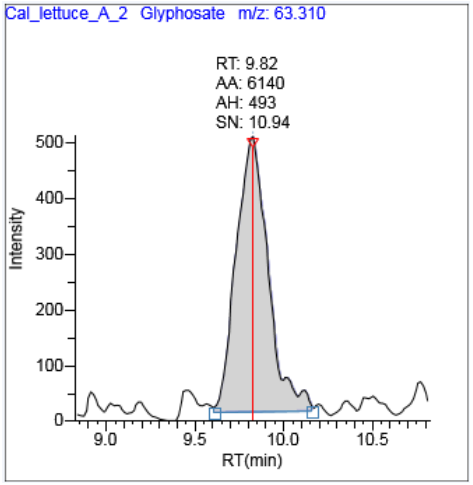
Ethephon



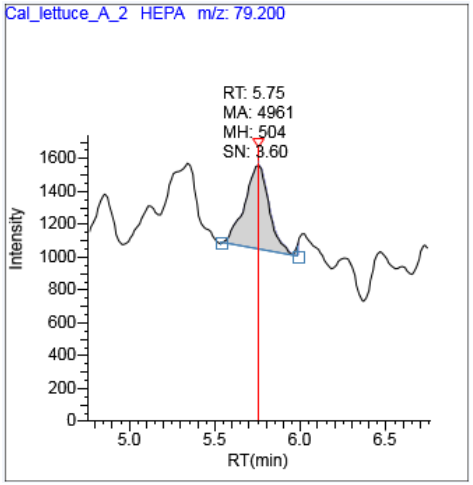
Glufosinate



Fosetyl-Al



Glyphosate



HEPA

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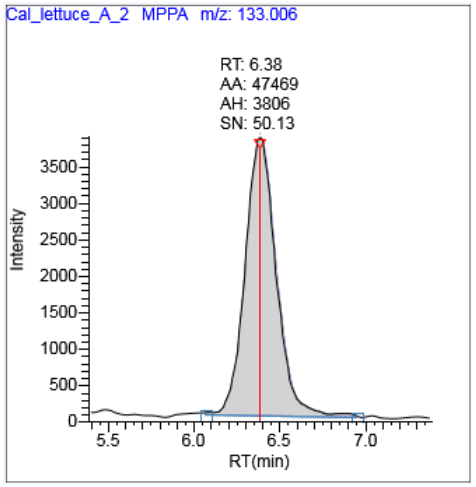
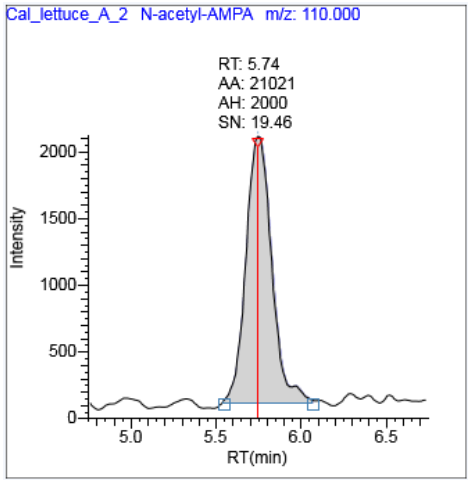
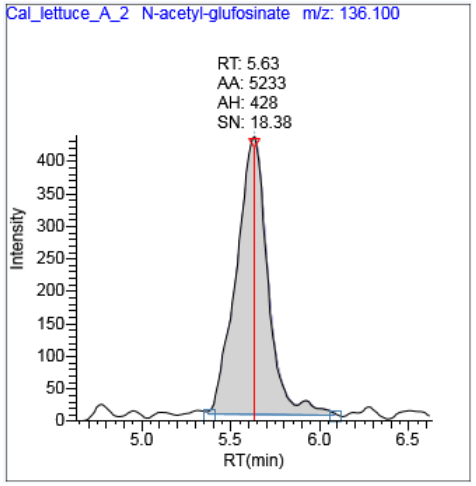
Pesticides 10 ppb in lettuce



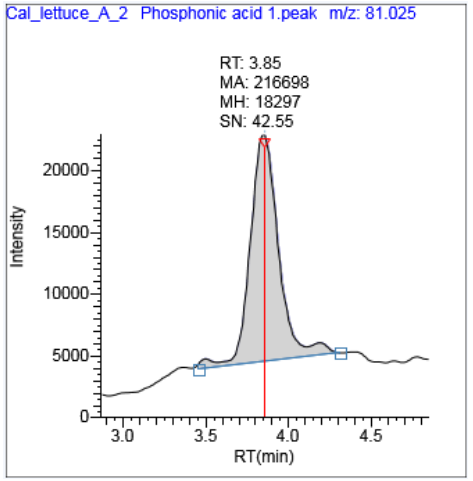
MPPA



N-Acetyl-AMPA



N-acetyl-glufosinate



Phosphonic acid

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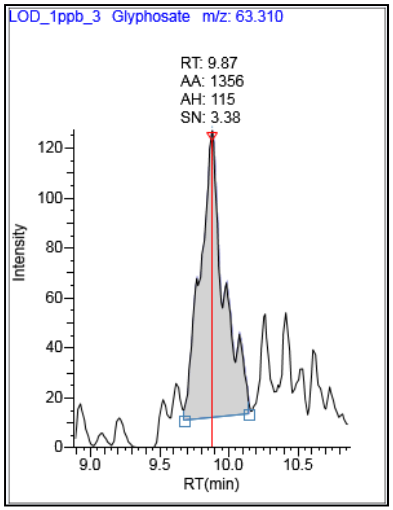
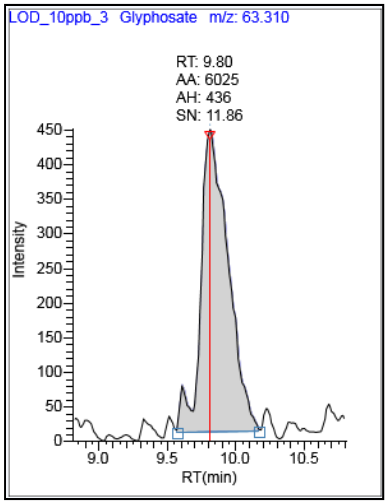
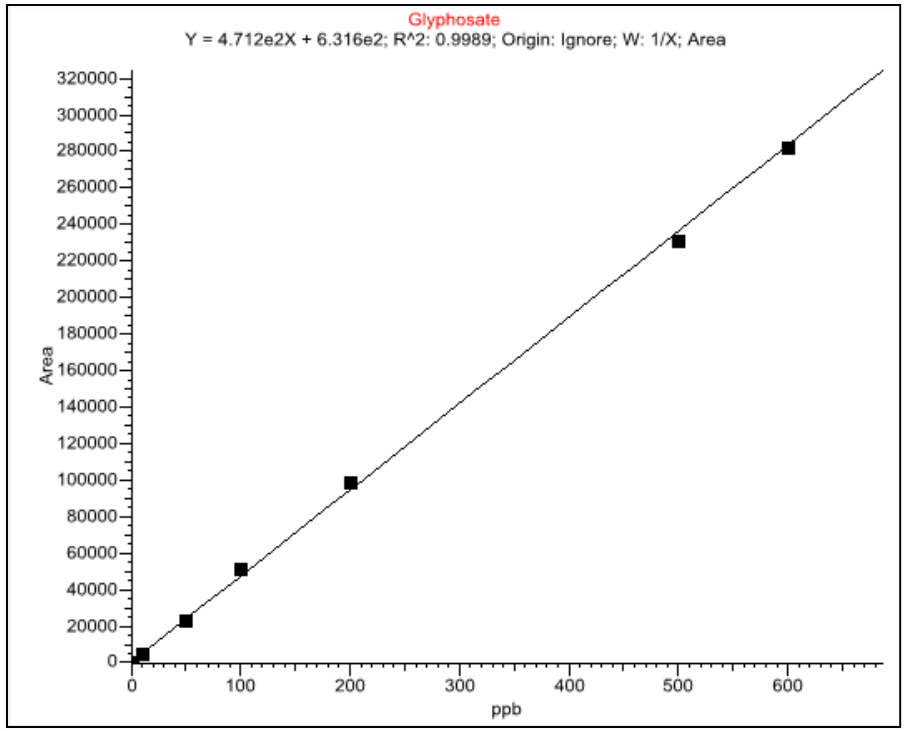
Quan details for Glyphosate

1 ppb (LOD = 5 ppb)



LOQ – 10 ppb





Calibration range in matrix: 10 – 600 ppb

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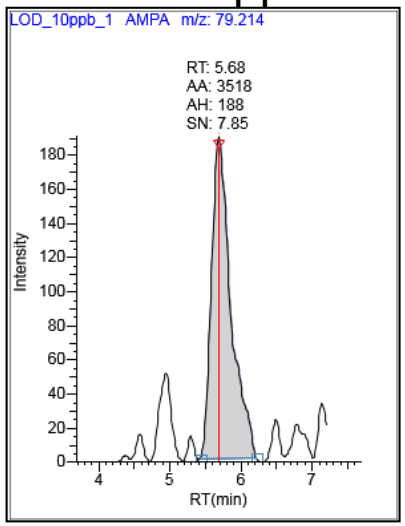
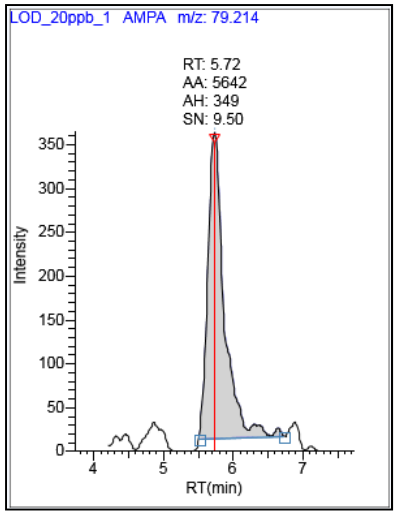
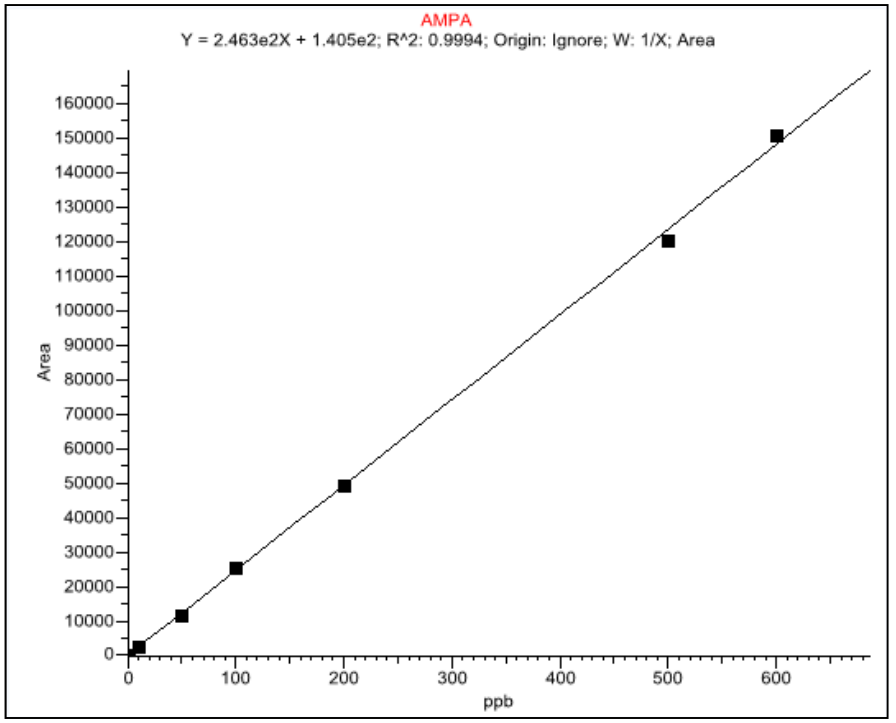
Quan details for AMPA

LOD – 10 ppb



LOQ – 20 ppb





Calibration range in matrix: 10 – 600 ppb

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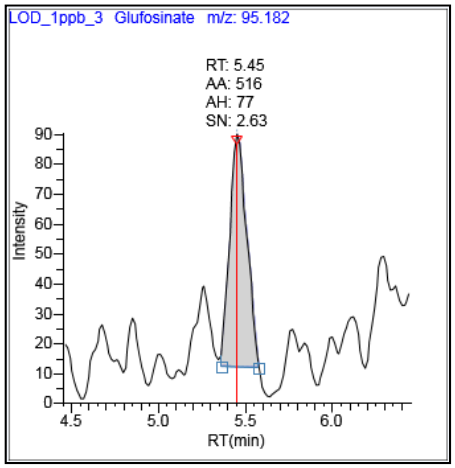
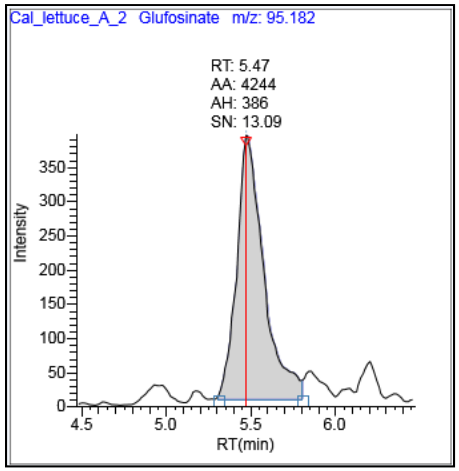
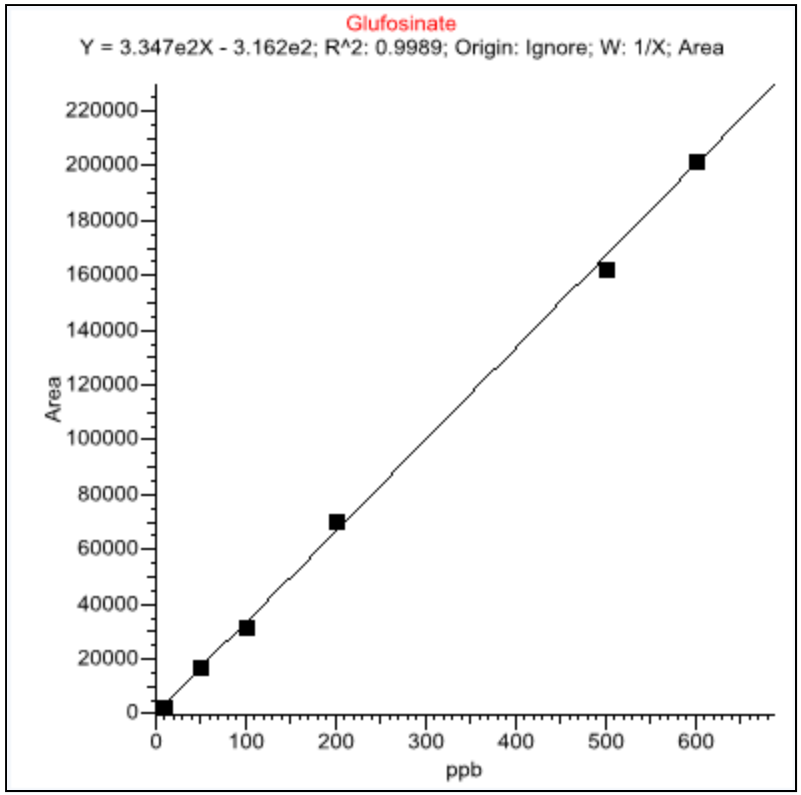
Quan details for Glufosinate

LOD – 1 ppb



LOQ – 10 ppb





Calibration range in matrix: 10 – 600 ppb

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Analysis of Cationic Polar Pesticides by IC-MS/MS

Cationic Pesticides of Interest

- *Triazole-acetic acid*
- Diethanolamine
- N,N-Dimethylhydrazine
- Triethanolamine
- Morpholine
- *1,2,4-Triazole*
- Trimethylsulfonium
- Melamine
- **Chlormequat**
- **Mepiquat**
- Nereistoxin
- Streptomycin
- **Diquat**
- *Kasugamycin*
- **Paraquat**
- Propamocarb
- *Daminozide*
- Cyromazine
- *Cyanuric Acid*
- *Maleic Hydrazide*
- Ethylene Thiourea
- *Triazole Alanine*
- *Amitrol*

IC analysis – suppressed MS (SRM) detection conditions

IC Conditions

Parameter	Setting
Mobile Phase:	RFIC MSA (See Gradient Conditions)
Analytical Column:	Dionex IonPac CS17 (2 x 250 mm)
Guard Column:	None
Suppressor:	Dionex CERS 500es (2 mm)
Pump Flow:	0.40 mL/min
Injection Volume:	10 µL
Column Temp:	40°C
Suppressor Current:	71 mA
Detector Comp. Temp:	20°C
Conductivity Cell Temp:	35°C

Eluent Gradient Conditions

Time (min)	Concentration of MSA (mM)
-4.000	0.50
0.000	0.50
4.000	3.20
10.000	15.00
14.000	60.00
18.000	60.00
18.000	0.50

Mass Spectrometer Conditions (SIM mode)

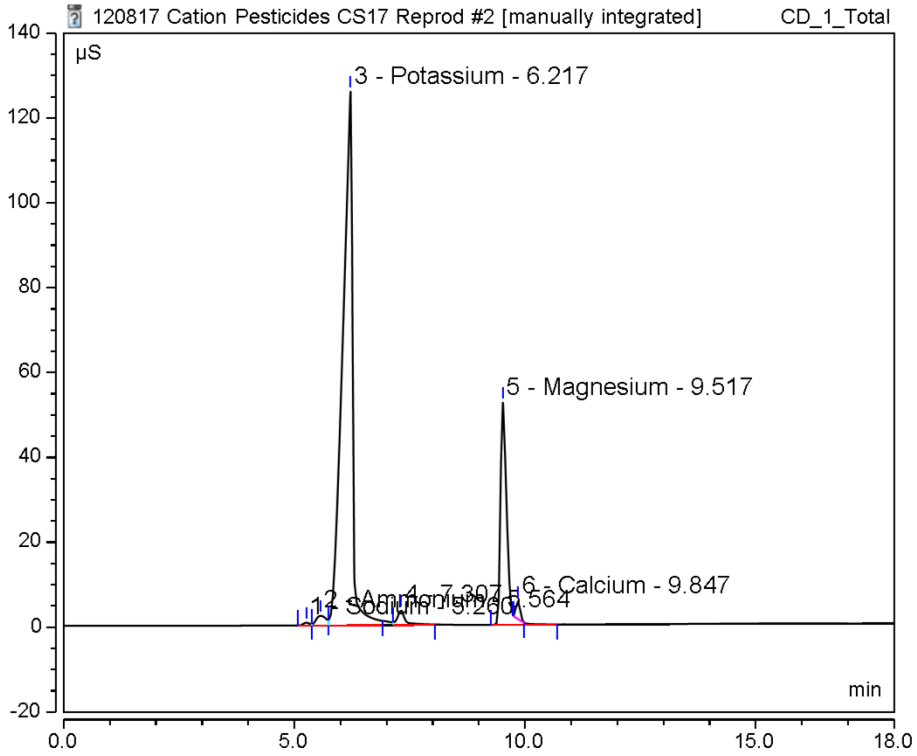
Parameter	Setting
Ionization Mode:	Heated Electrospray (H-ESI)
Scan Type:	SRM
Polarity:	Positive Ion Mode
Spray Voltage:	3500 V
Sheath Gas Pressure:	35 Arb
Aux Gas Pressure:	15 Arb
Ion Sweep Gas Pressure:	1 Arb
Ion Transfer Tube Temp:	310°C
Vaporizer Temp:	250°C
Cycle Time:	0.8 s
Q1 Resolution:	0.7
Q3 Resolution:	1.2
CID Gas:	1.5 mTorr
Source Fragmentation:	10 V
Use Calibrated RF Lens:	Yes

IC analysis –quantitative analysis results (µg/L)

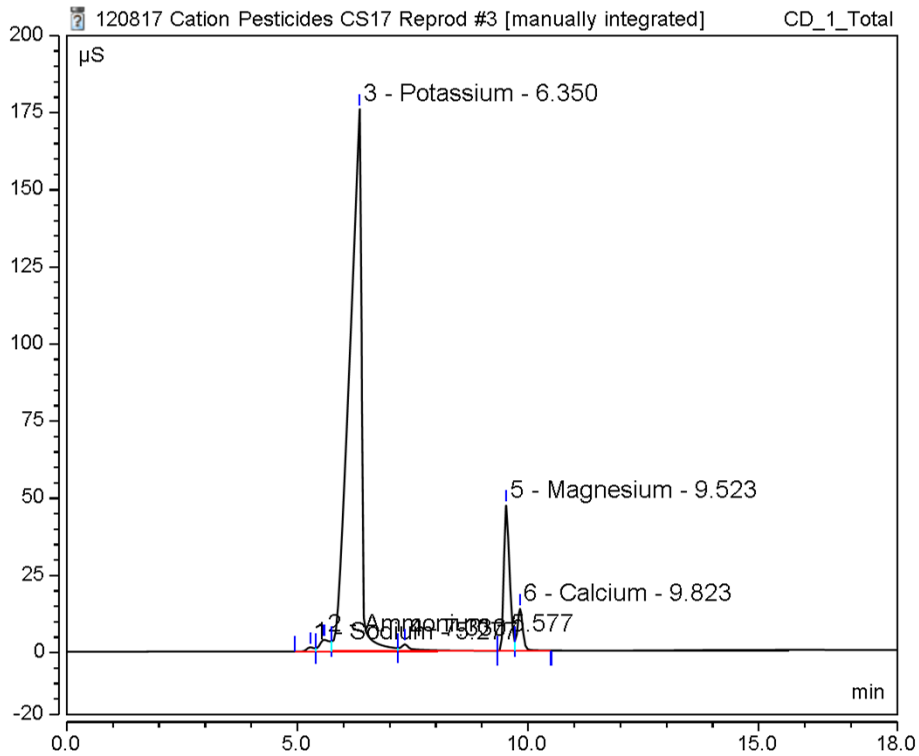
Analyte	SANTE LOQ	LOQ	LOD
Ethylene Thiourea			
Diethanolamine	< 0.5 (8.7%)	0.68	0.22
N,N-Dimethylhydrazine			
Triethanolamine	< 0.5 (2.9%)	0.26	0.086
Morpholine	< 0.5 (7.9%)	0.37	0.12
Trimethylsulfonium	< 0.5 (8.0%)	0.37	0.12
Melamine	< 0.5 (3.6%)	0.26	0.086
Chlormequat	< 0.5 (4.5%)	0.20	0.065
Mepiquat	< 0.5 (2.0%)	0.094	0.031
Nereistoxin	< 0.5 (8.6%)	0.52	0.17
Cyromazine	> 0.5 (21.8%)	0.82	0.27
Streptomycin			
Diquat	> 0.5 (20.8%)	1.08	0.36
Paraquat	< 10 (3.6%)	4.74	1.56
Propamocarb	< 0.5 (1.9%)	0.088	0.029

Food matrix

QuPPe extracted ‘Gerber®’ Green Beans



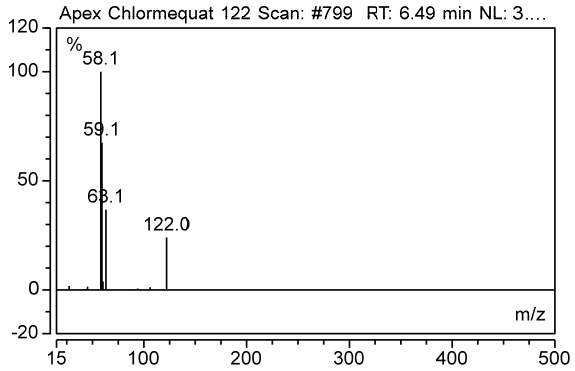
QuPPe extracted ‘Gerber®’ Prunes

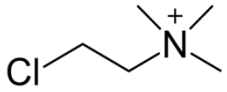


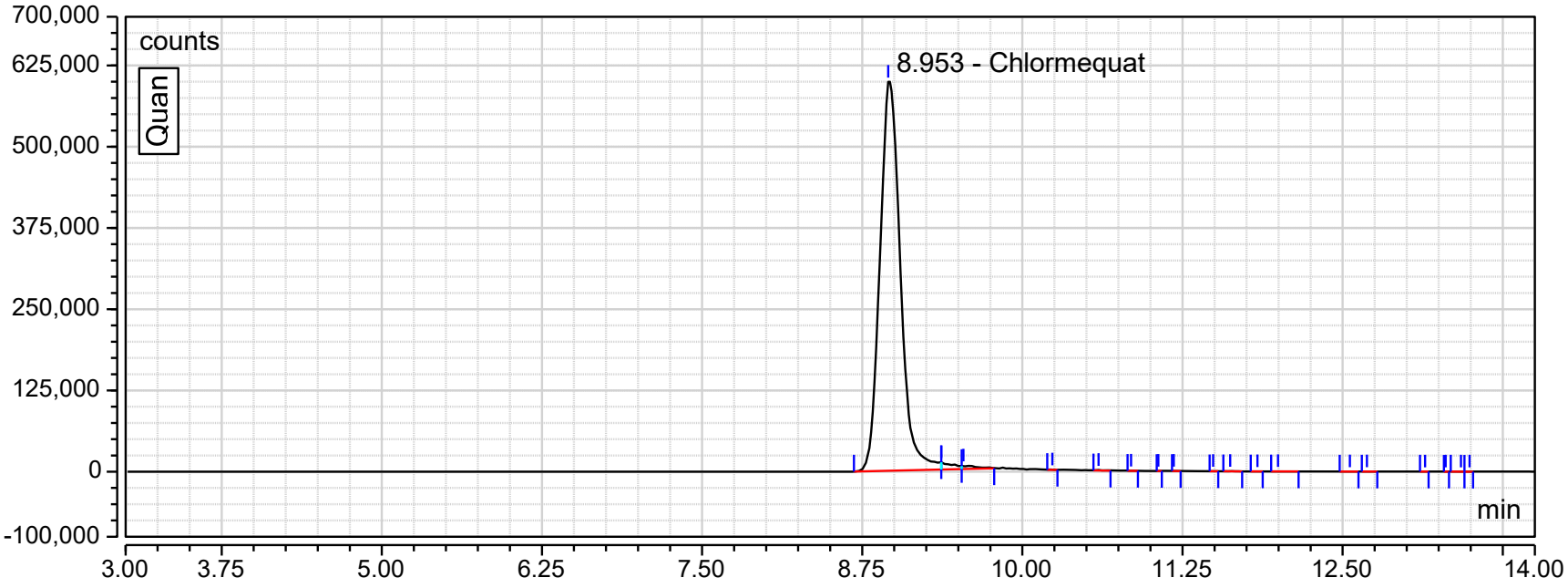
ThermoFisher SCIENTIFIC

Chlormequat

- Chemical Formula: $C_5H_{13}Cl\ N$
- Molar Mass: 122.62 g/mol
- SRM: 122 > 58
- Collision Energy: 30 V



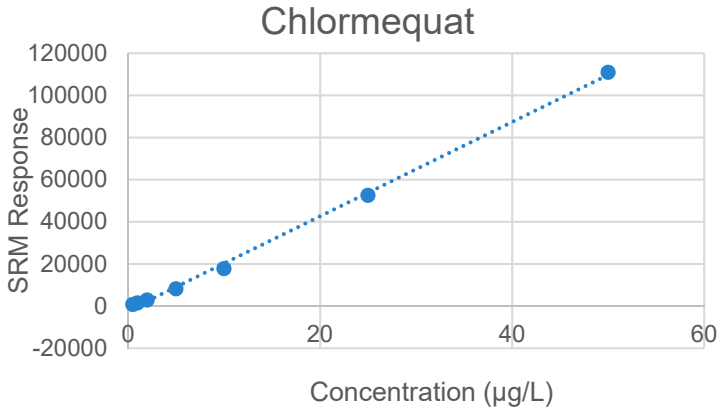


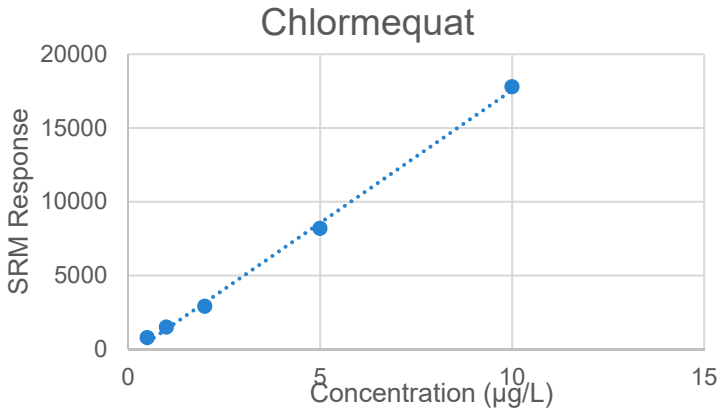


ThermoFisher SCIENTIFIC

Chlormequat

- SANTE LOQ < 0.5 µg/L
  - According to SANTE guidelines, LOQ's are established by identifying the lowest level having RSD < 20%
  - RSD @ 0.5 µg/L across 7 injections: 4.5%
- LOQ: 0.20 µg/L
  - t-test method based on standard deviation of the response and the slope
  - LOQ = 10 σ / Slope
- LOD: 0.065 µg/L
  - t-test method
  - LOD = 3.3 σ / Slope

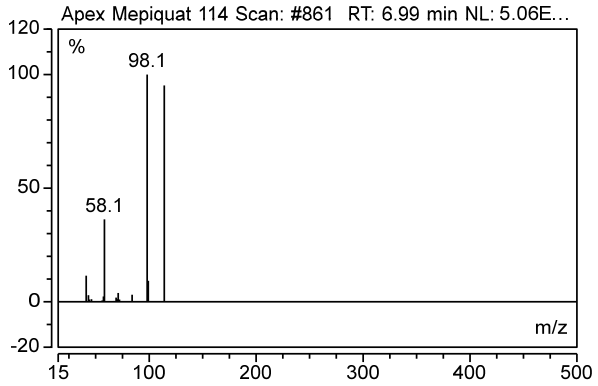


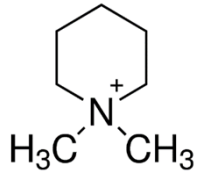


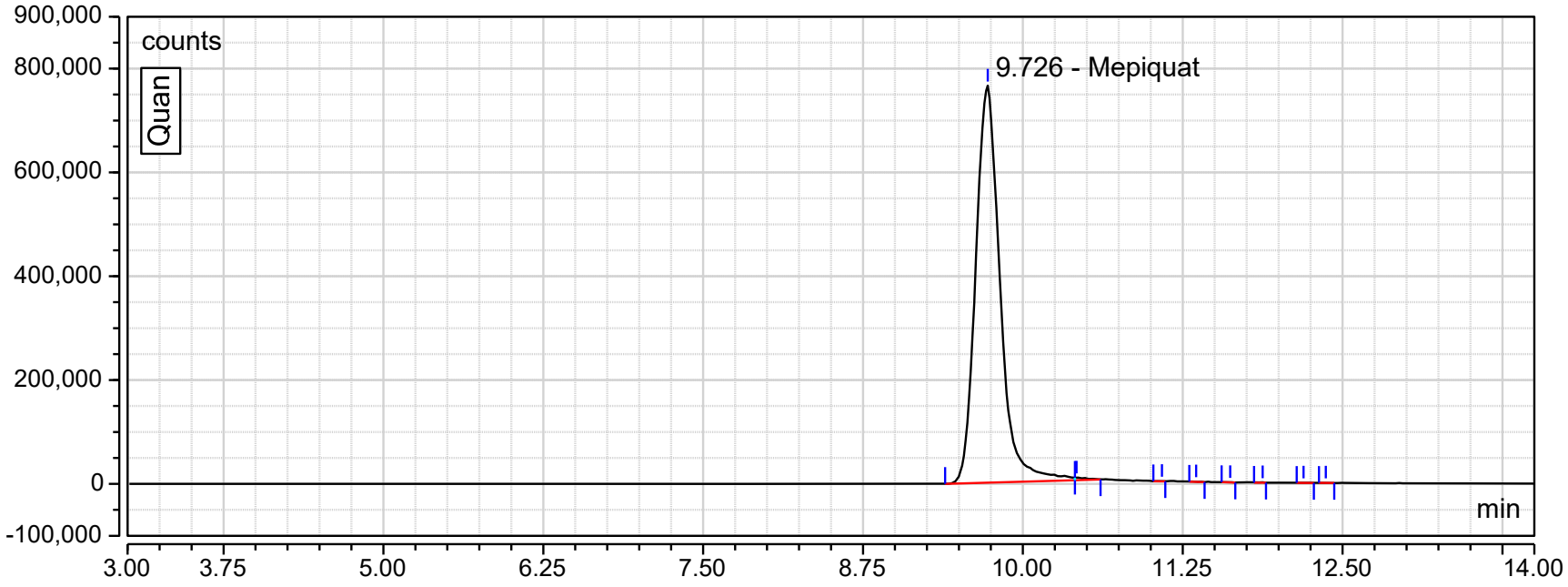
ThermoFisher SCIENTIFIC

Mepiquat

- Chemical Formula: $C_7H_{16}N$
- Molar Mass: 114.21 g/mol
- SRM: 114 > 98
- Collision Energy: 30 V



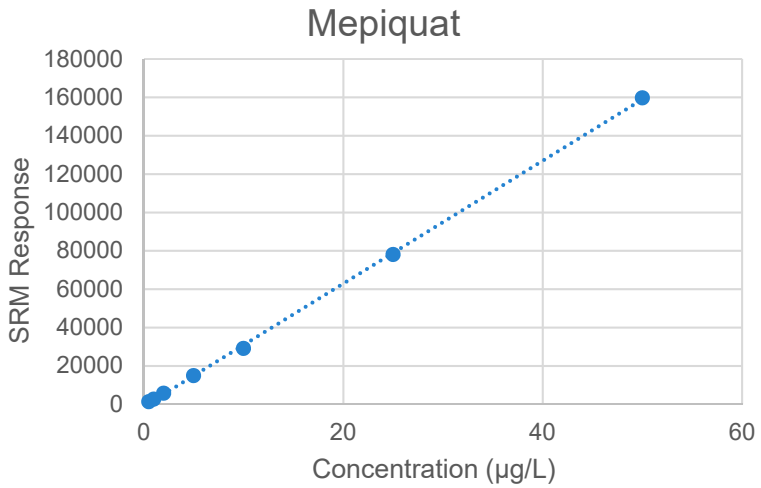


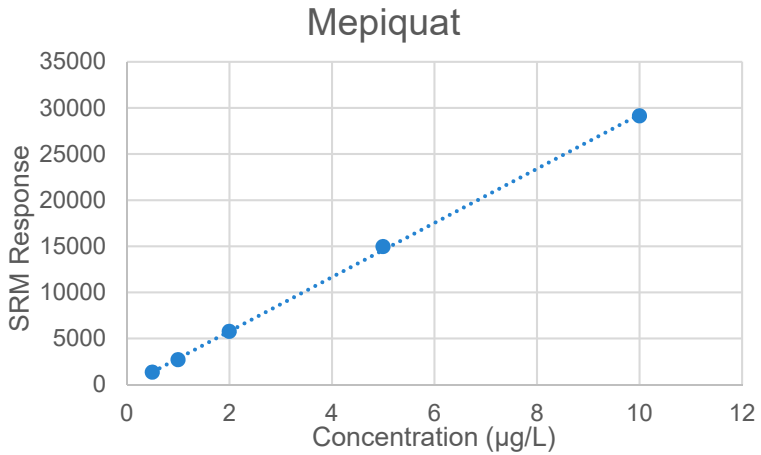


ThermoFisher SCIENTIFIC

Mepiquat

- SANTE LOQ < 0.5 µg/L
  - RSD @ 0.5 µg/L across 7 injections: 1.98%
- LOQ: 0.094 µg/L
  - LOQ = 10 σ / Slope
- LOD: 0.031 µg/L
  - LOD = 3.3 σ / Slope

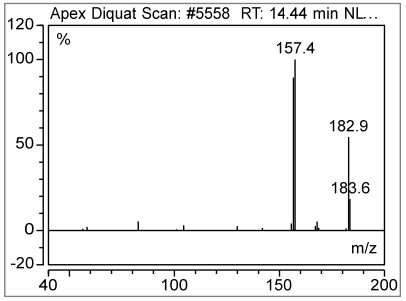


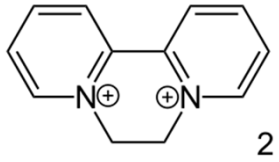


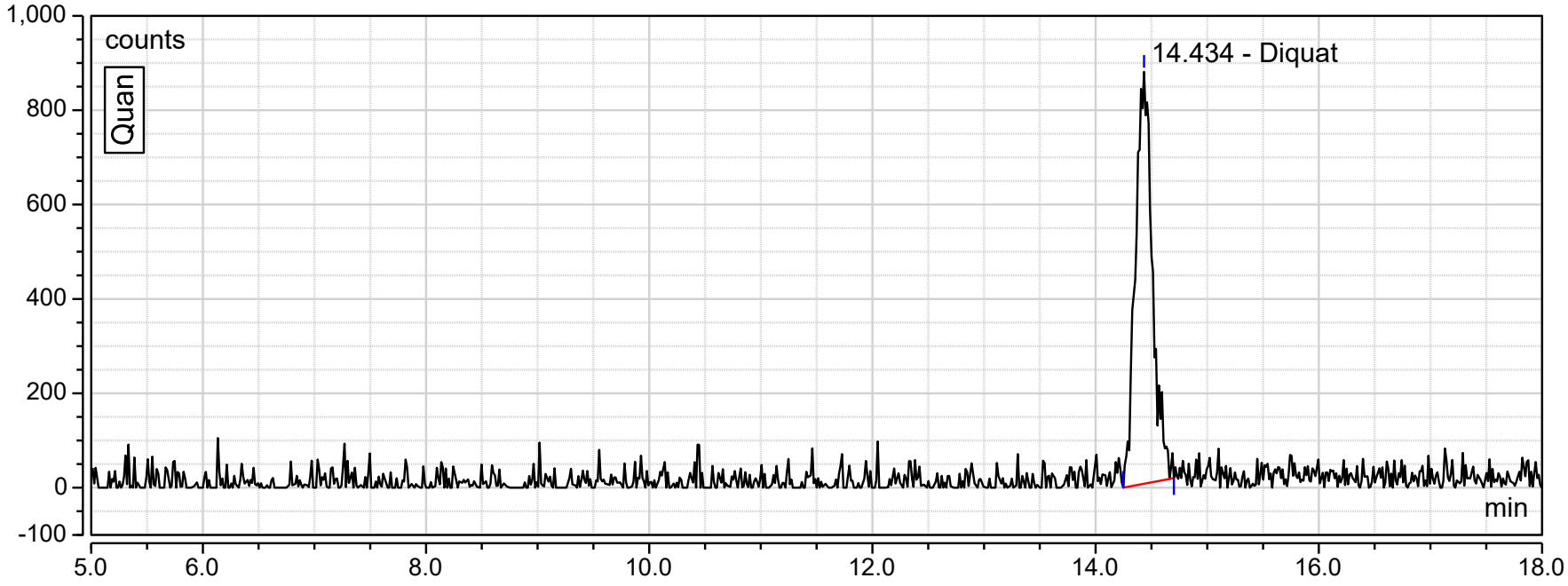
ThermoFisher SCIENTIFIC

Diquat

- Chemical Formula: $C_{12}H_{12}N_2$
- Molar Mass: 184.24 g/mol
- SRM: 183 > 157
- Collision Energy: 20 V



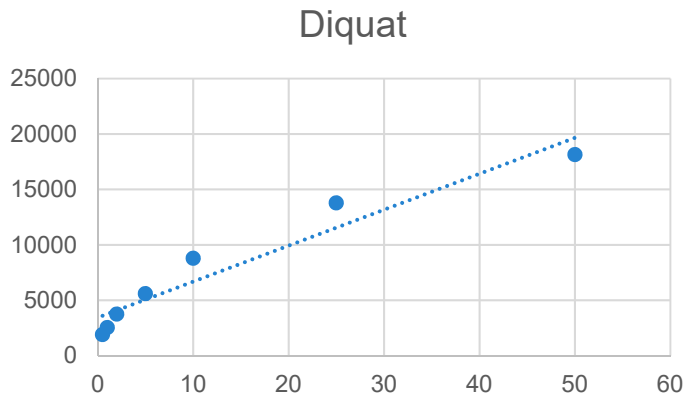


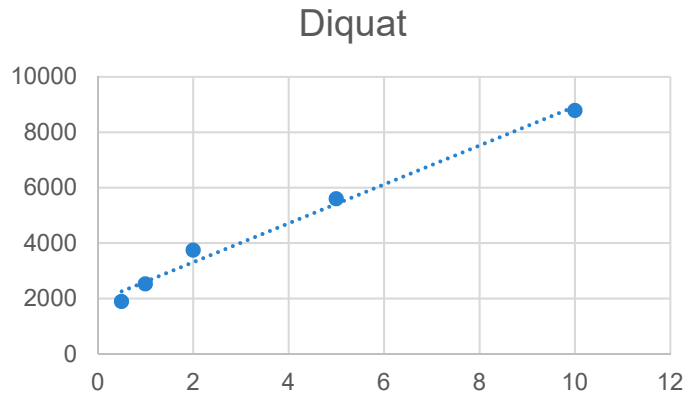


ThermoFisher SCIENTIFIC

Diquat

- SANTE LOQ > 5 µg/L
  - RSD @ 5 µg/L across 7 injections: 20.77%
- LOQ: 1.08 µg/L
  - LOQ = 10 σ / Slope
- LOD: 0.36 µg/L
  - LOD = 3.3 σ / Slope

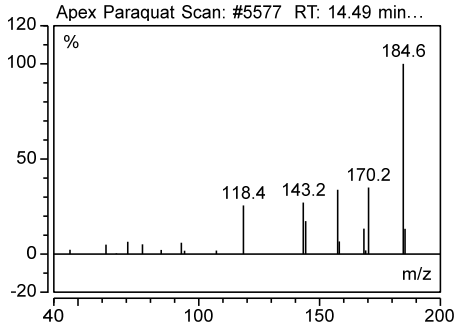


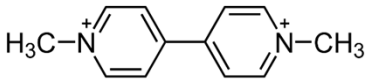


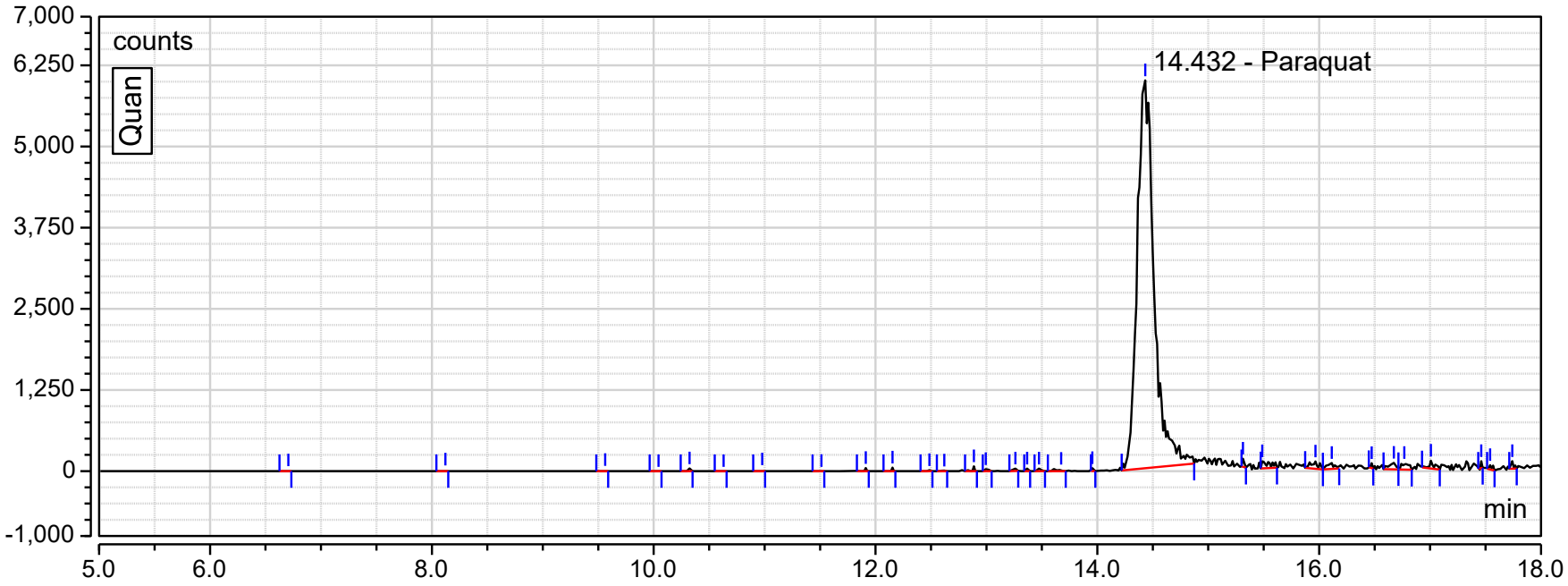
ThermoFisher SCIENTIFIC

Paraquat

- Chemical Formula: $C_{12}H_{14}N_2$
- Molar Mass: 186.25 g/mol
- SRM: 185 > 170
- Collision Energy: 19 V



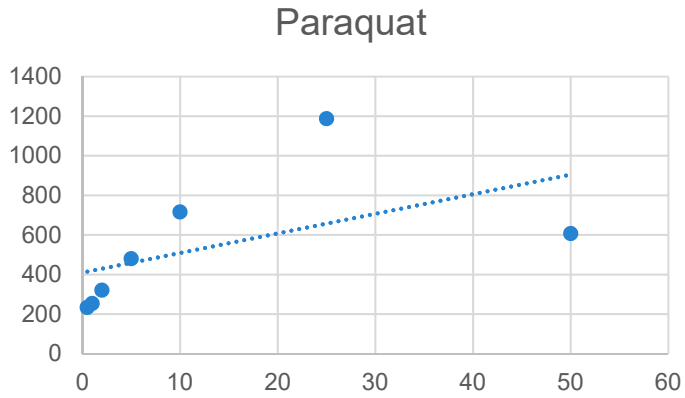


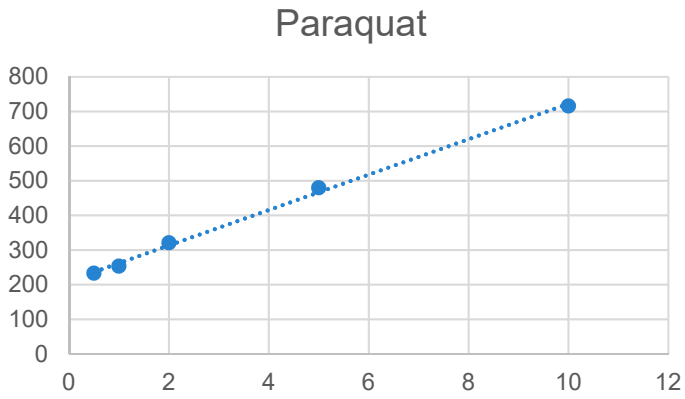


ThermoFisher SCIENTIFIC

Paraquat

- SANTE LOQ < 10 µg/L
  - RSD @ 10 µg/L across 7 injections: 3.56%
- LOQ: 4.74 µg/L
  - LOQ = 10 σ / Slope
- LOD: 1.56 µg/L
  - LOD = 3.3 σ / Slope





Why are paraquat and diquat difficult?

- On the Thermo Scientific™ Dionex™ IonPac™ CS17 column, paraquat and diquat co-elute
  - With parent ions at 185 and 183 respectively, a nominal mass (quadrupole) MS lacks sufficient resolution to separate these compounds
  - PQ and DQ are prone to ion suppression in the HESI probe
  - Better ionization may be possible in a 100% aqueous mobile phase
- Coelution has been demonstrated on other Dionex IonPac columns
  - The Dionex IonPac CS14 column does separate paraquat and diquat, but has poor resolution of other cationic pesticides
- A new Dionex IonPac column is under development to resolve the four major quaternary amine pesticides
  - No coelution of species with similar masses
  - Elimination of ion suppression

Conclusions

- Current IC/LC-MS/MS portfolio allows determination of polar pesticides in both food and environmental samples
- Good separation efficiency of IC makes it the recommended method for polar pesticides
- A Thermo Scientific™ Dionex™ ICS-6000 HPIC™ or Thermo Scientific™ Dionex™ Integrion™ system is recommended for separation of polar pesticides
- A Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer is the recommended MS/MS for water analysis @ ppt levels
- A TSQ Quantis triple quadrupole MS is recommended for food sample analysis @ ppb levels (TSQ Altis MS is an option if more sensitivity is required or a more difficult matrix is analysed)
- A Thermo Scientific™ Q Exactive™ Focus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer is a good alternative for complex matrices and sensitivity
- Although the Dionex IonPac CS17 can do most cationic polar pesticides, work is progressing to address PQ/DQ issue with a new stationary phase
  - The Q Exactive Focus MS can address resolution issue of these two compounds