

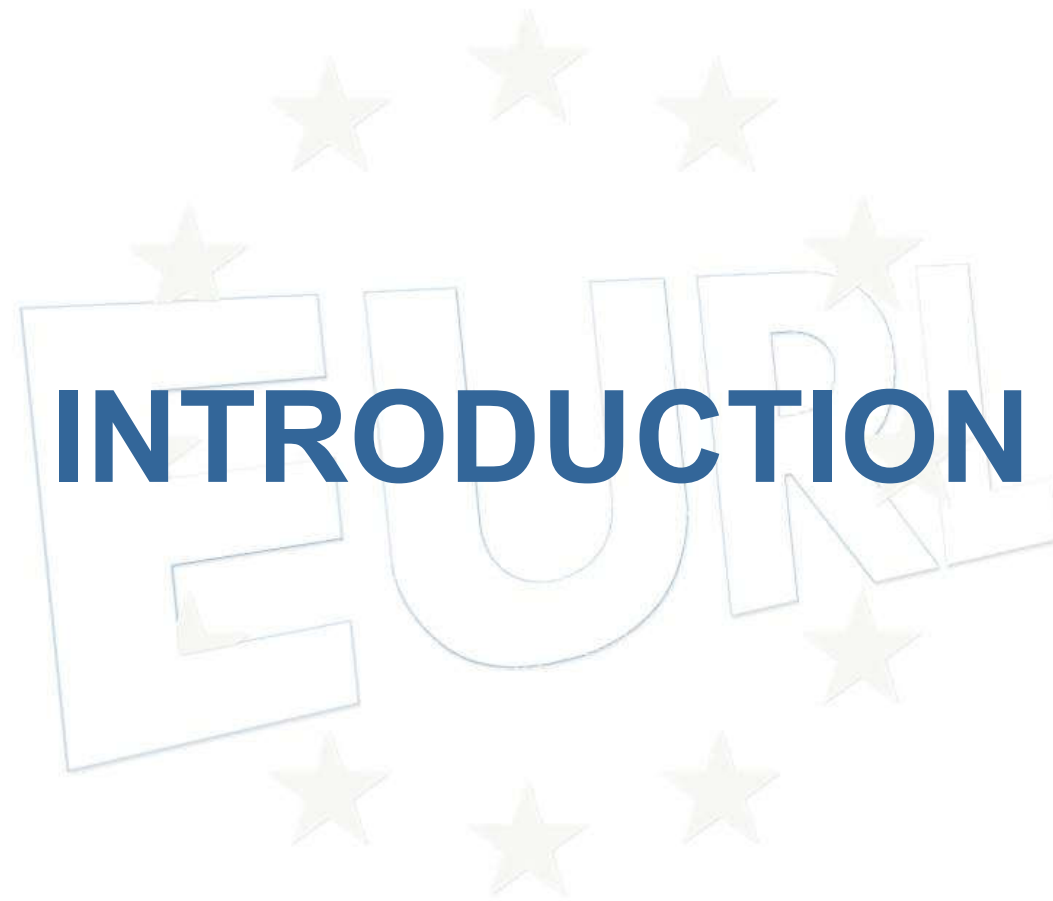


Analysis of Polar Ionic Pesticides using High Performance Ion Chromatography coupled with High Resolution Accurate Mass Spectrometry

Łukasz Rajski,

Francisco José Díaz Galiano, Víctor Manuel Cutillas Juárez,

Amadeo Rodríguez Fernández-Alba

A large, faint background graphic consisting of the letters 'EURL' in a stylized, outlined font, surrounded by a circle of twelve yellow stars, similar to the European Union flag.

INTRODUCTION

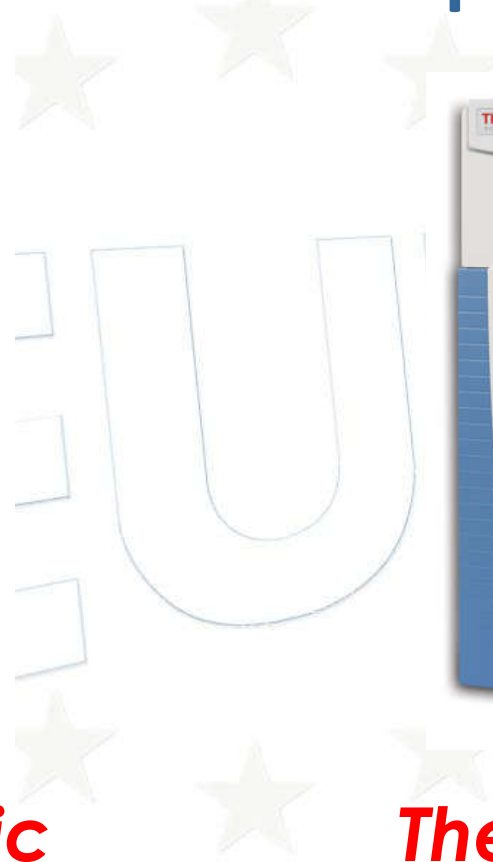
A large, faint watermark of the EURL logo, consisting of the letters 'EURL' in a stylized font surrounded by twelve yellow stars, is centered in the background.

INSTRUMENTATION

Ion chromatography coupled to high resolution mass spectrometry

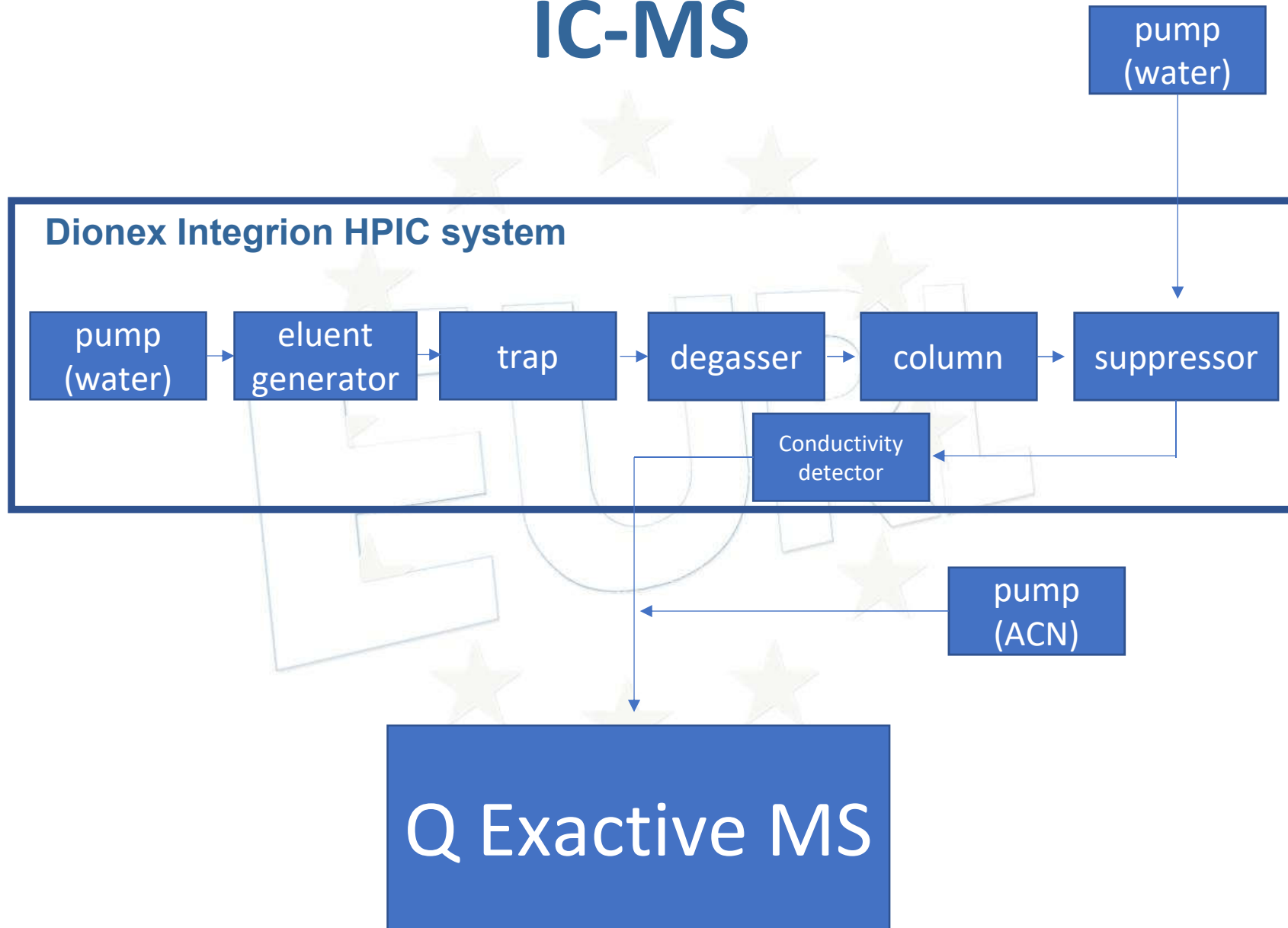


Thermo Scientific
Dionex Integrion
HPIC System

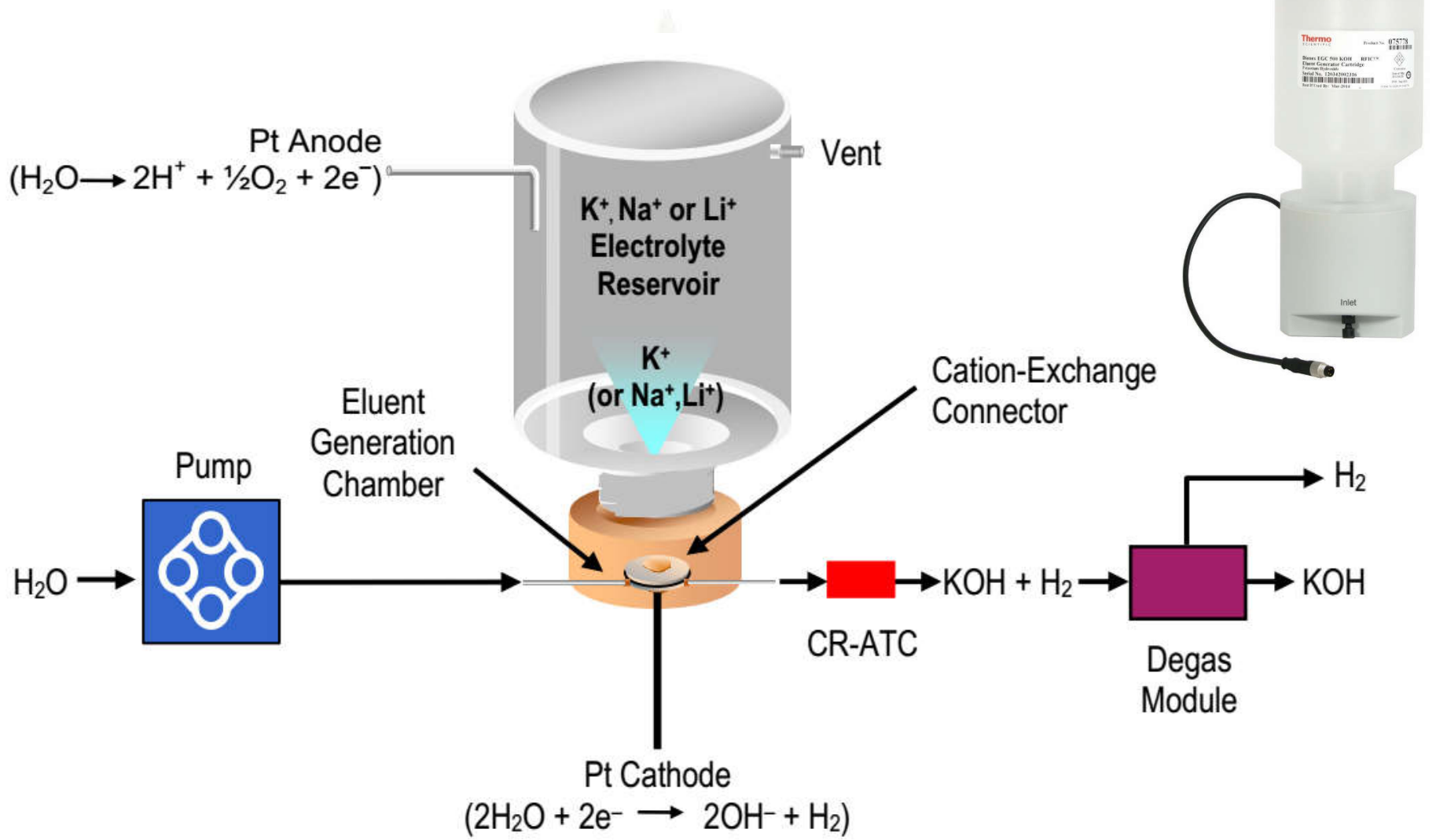


Thermo Scientific
Q Exactive
Focus MS System

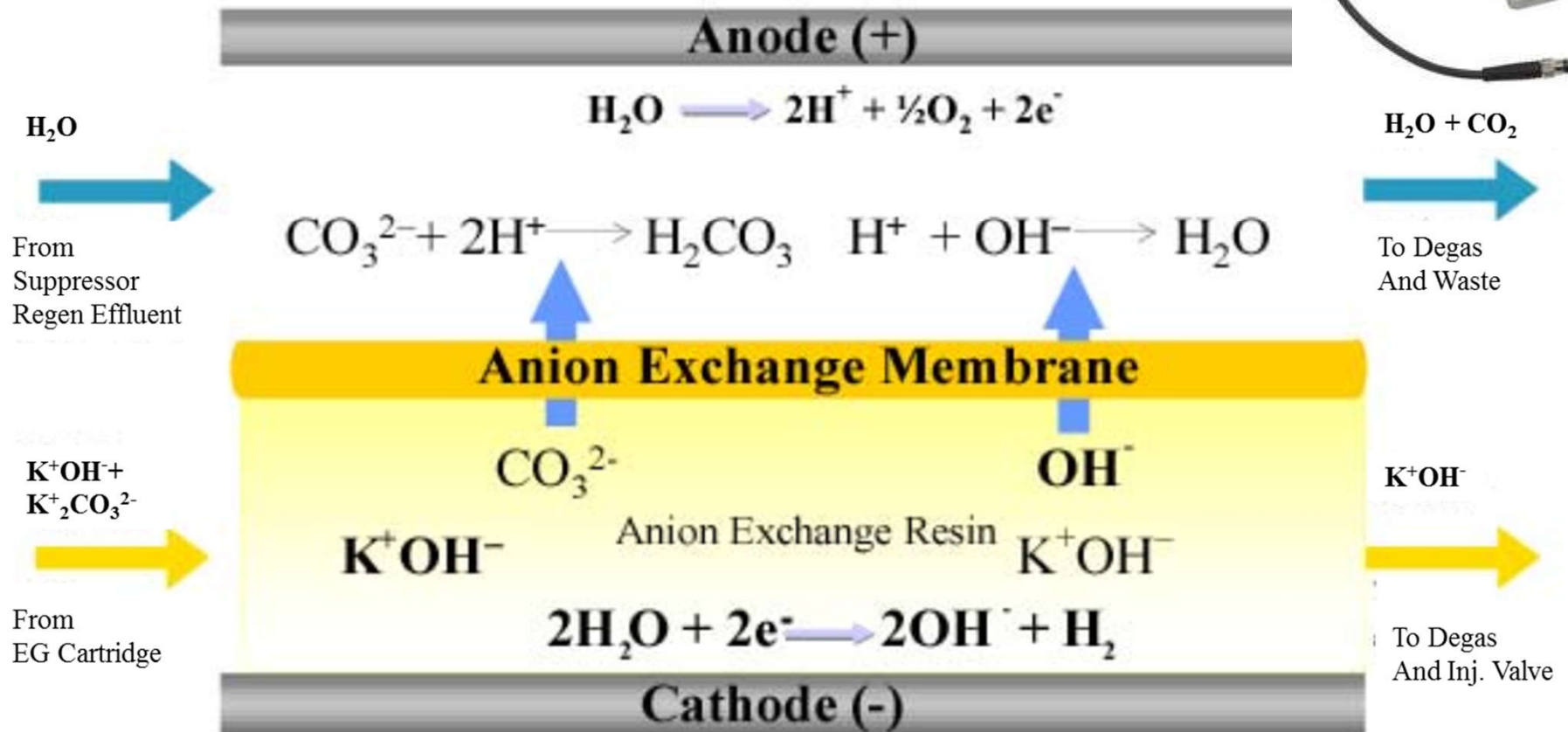
IC-MS



Eluent Generator Cartridge



Continuously regenerated trap column (CRTC)



Column

- Guard column **Thermo Scientific** Dionex IonPac AG19 (50mm x 2mm x 4 μ m)
- Column **Thermo Scientific** Dionex IonPac AS19 (250mm x 2mm x 4 μ m)

	Particle diameter [μ m]	Pore Size [Å]	Crosslinking [%DVB]	Resin	Functional Group	Capacity [μ eq/column]	Hydrophobicity
AG19	4	< 1	55%	Microporous	Alkanol quaternary ammonium	1.5	Ultralow
AS19	4	2000	55%	Supermacroporous	Alkanol quaternary ammonium	60	Ultralow

Column

- Guard column **Thermo Scientific** Dionex IonPac AG19 (50mm x 2mm x 4 μ m)
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- Guard column **Thermo Scientific** Dionex IonPac AG11-HC (50mm x 2mm x 4 μ m)
- Column **Thermo Scientific** Dionex IonPac AS11-HC (250mm x 2mm x 4 μ m)

	Particle diameter [μ m]	Pore Size [Å]	Crosslinking [%DVB]	Resin	Functional Group	Capacity [μ eq/column]	Hydrophobicity
AG11 HC	4	< 1	55%	?	Alkanol quaternary ammonium	1.75	Medium Low
AS11 HC	4	2000	55%	?	Alkanol quaternary ammonium	72.5	Medium Low

Column

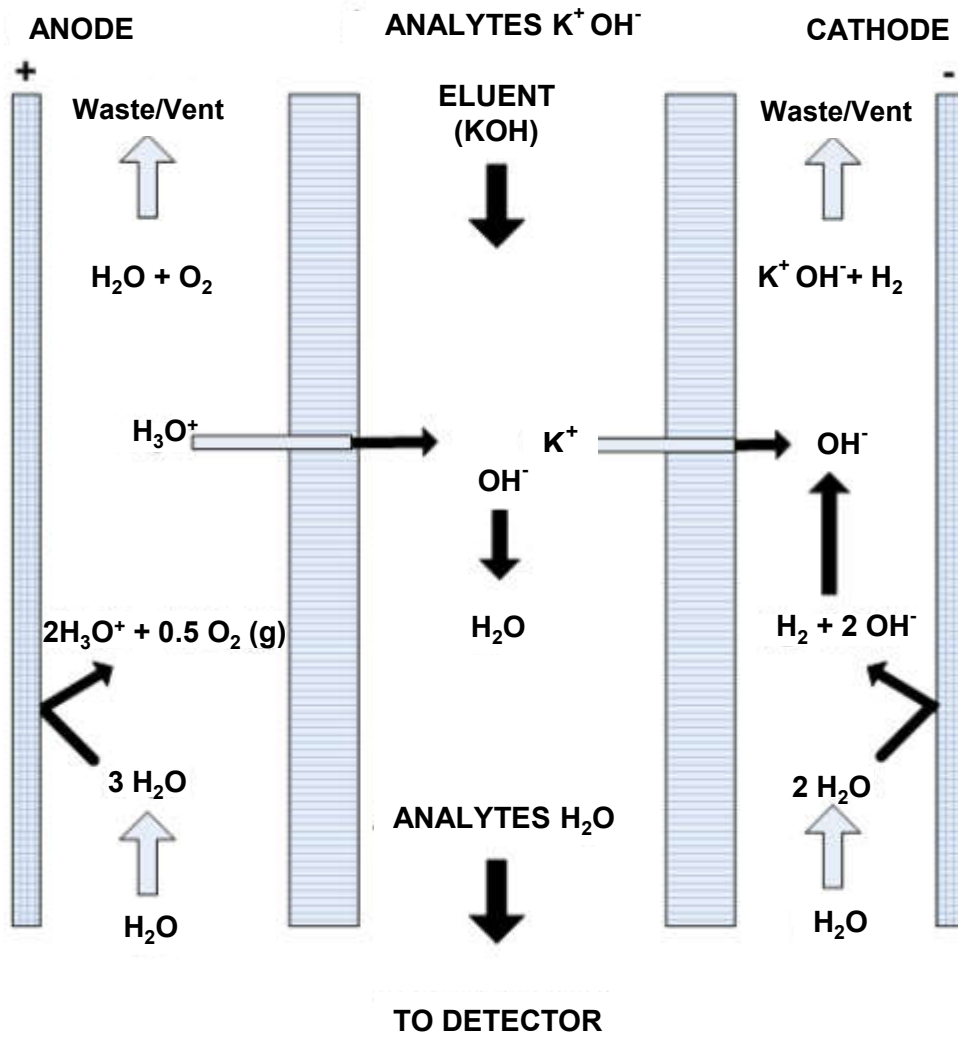
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AS19	4	2000	55%	Supermacroporous	Alkanol quaternary ammonium	60	Ultralow

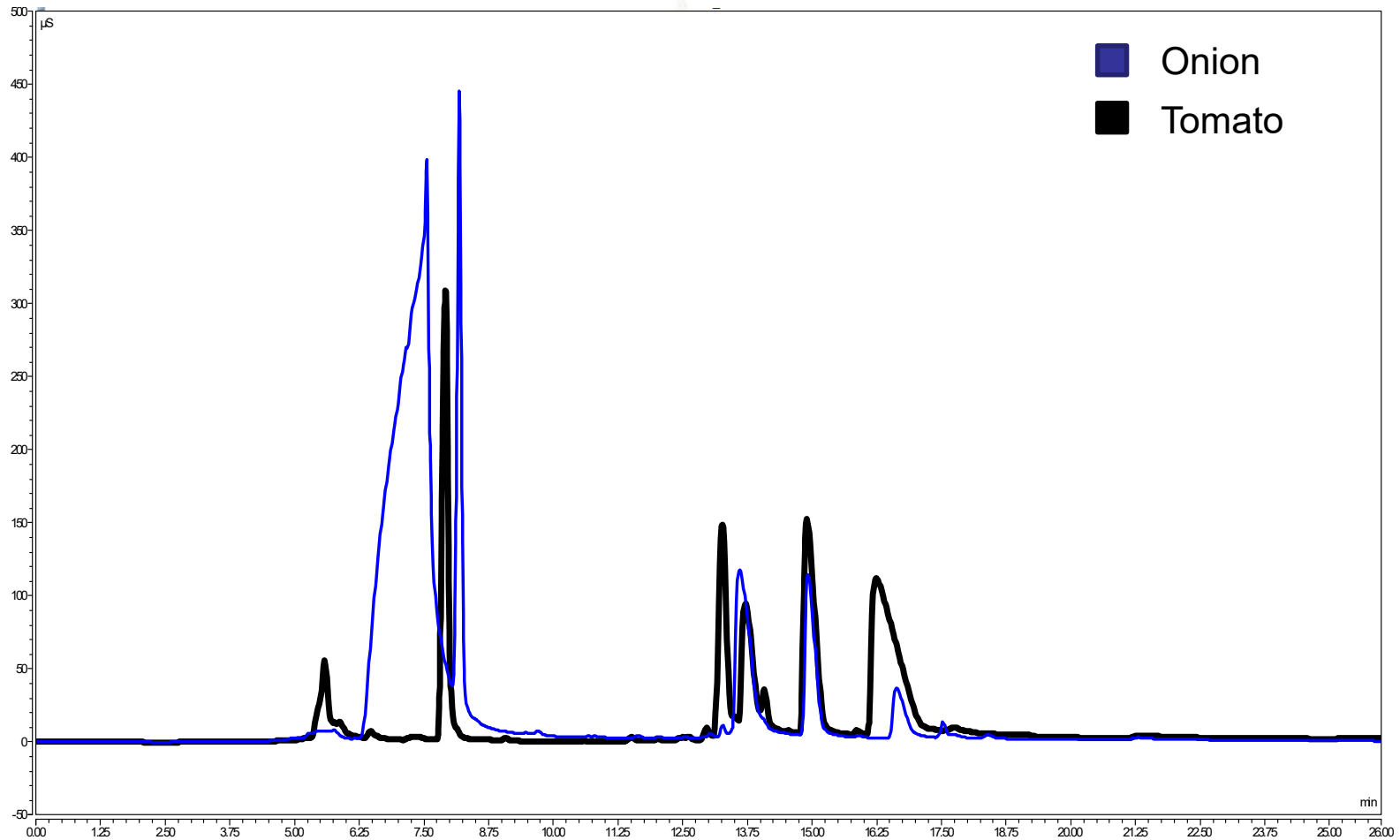
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	Particle diameter [µm]	Pore Size [Å]	Crosslinking [%DVB]	Resin	Functional Group	Capacity [µeq/column]	Hydrophobicity
AG11 HC	4	< 1	55%	?	Alkanol quaternary ammonium	1.75	Medium Low
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Suppressor

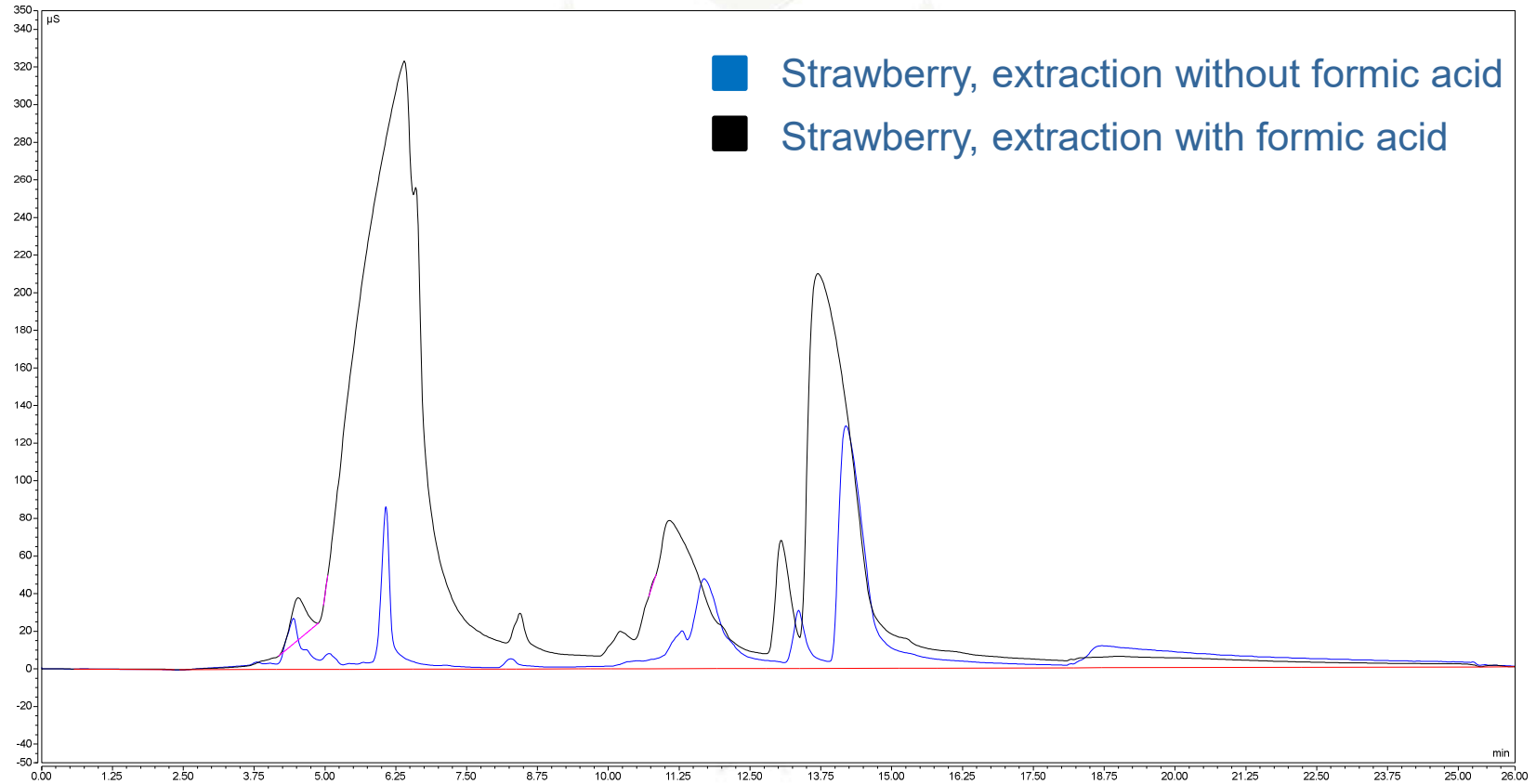


Conductivity detector



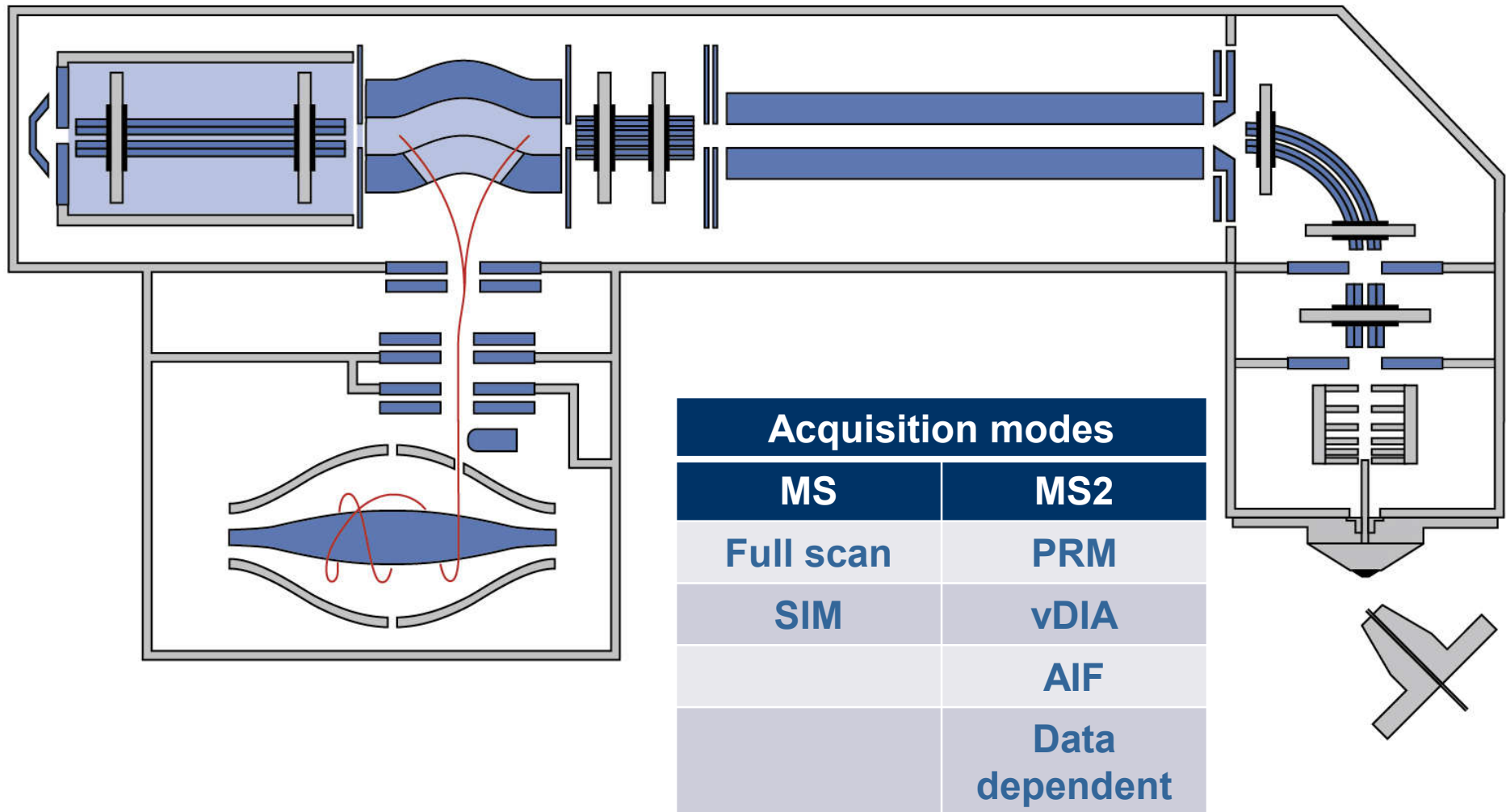
Chromatogram from conductivity detector

Conductivity detector



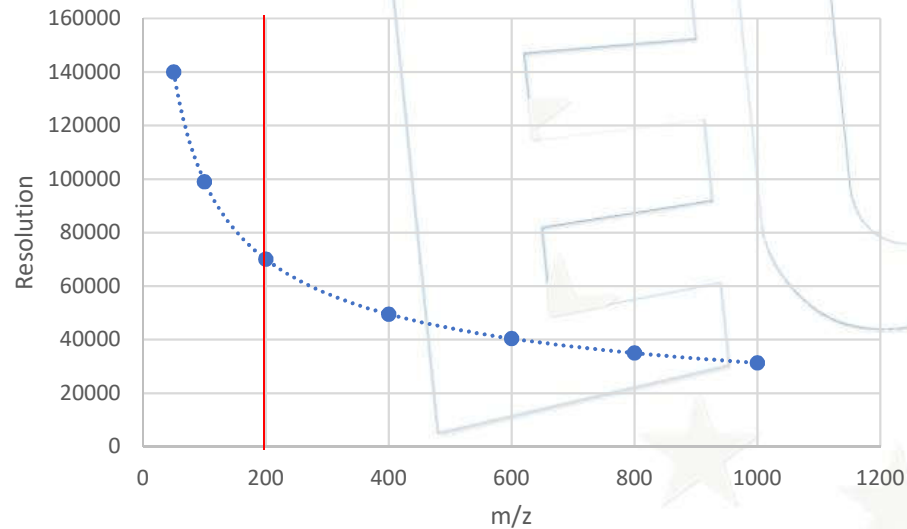
Chromatogram from conductivity detector

Q Exactive MS

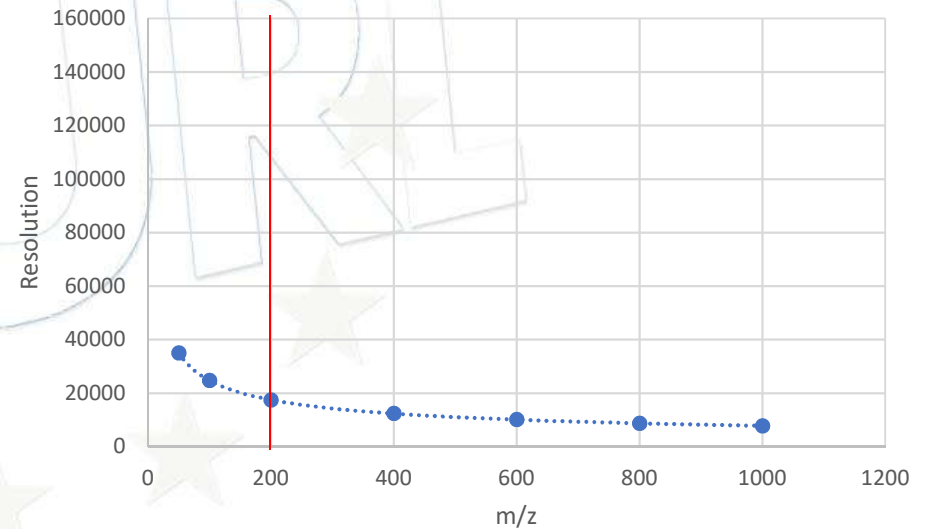


Resolution

Resolution 70,000 at m/z 200
3.7 scans/s
Applied for MS

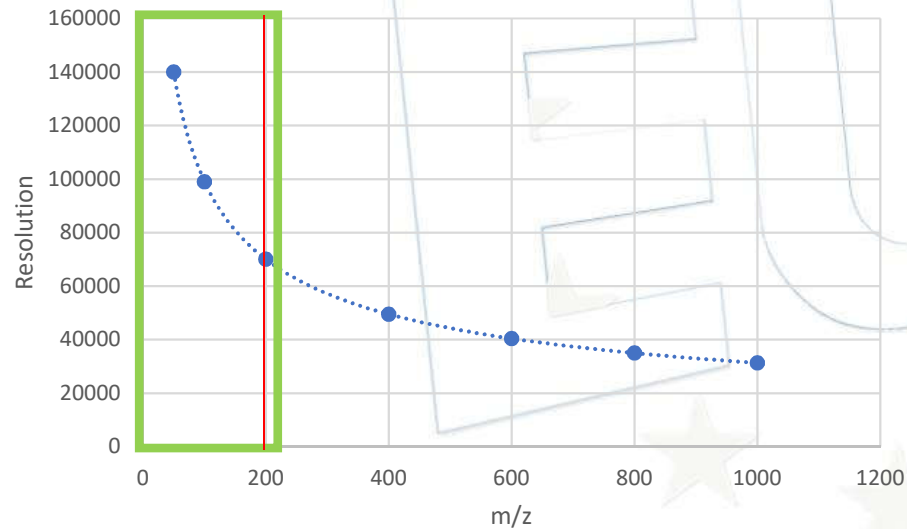


Resolution 17,500 at m/z 200
12 scans/s
Applied for MS²

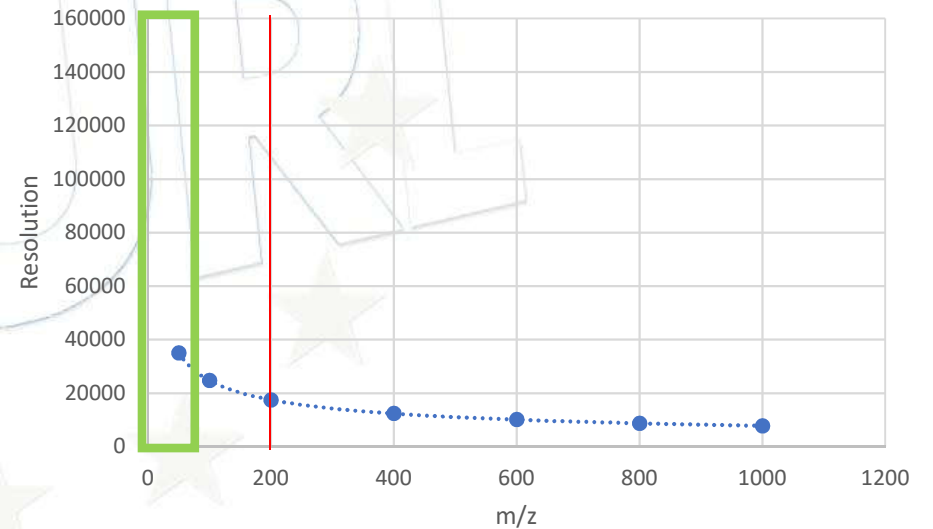


Resolution

Resolution 70,000 at m/z 200
3.7 scans/s
Applied for MS

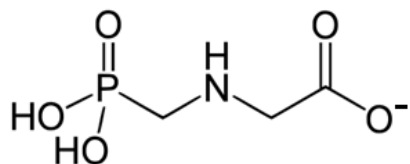


Resolution 17,500 at m/z 200
12 scans/s
Applied for MS²

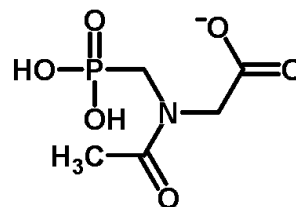




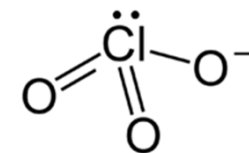
List of Pesticides (n=10)



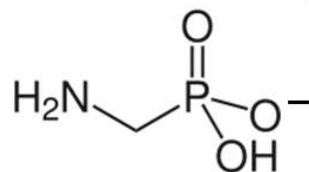
Glyphosate
(*m/z* 168.0067)



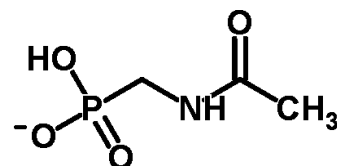
N-acetyl glyphosate
(*m/z* 210.0173)



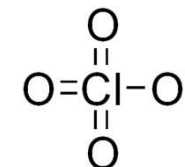
Chlorate
(*m/z* 82.9541)



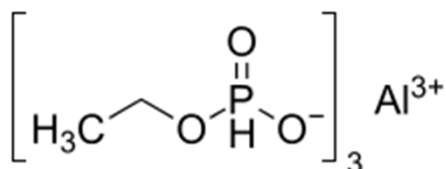
**Aminomethylphosphonic acid
(AMPA)**
(*m/z* 110.0012)



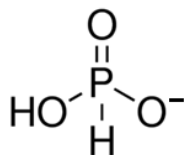
**N-acetyl aminomethylphosphonic acid
(N-acetyl AMPA)**
(*m/z* 152.0118)



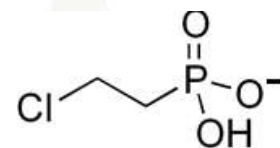
Perchlorate
(*m/z* 98.9491)



Fosetyl-aluminium
(*m/z* 109.0060)



Phosphonic acid
(*m/z* 80.9747)



Etephon
(*m/z* 142.9670)



Bromide ion
(*m/z* 78.9189)

SPECIAL GUEST EDITOR SECTION

Coupling Ion Chromatography to Q-Orbitrap for the Fast and Robust Analysis of Anionic Pesticides in Fruits and Vegetables

ŁUKASZ RAJSKI, FRANCISCO JOSÉ DÍAZ GALIANO, VÍCTOR CUTILLAS, and AMADEO R. FERNÁNDEZ-ALBA¹

University of Almería, Department of Chemistry and Physics, Agrifood Campus of International Excellence (CeiA3), European Union Reference Laboratory for Pesticide Residues in Fruits and Vegetables, 04120 Almería, Spain

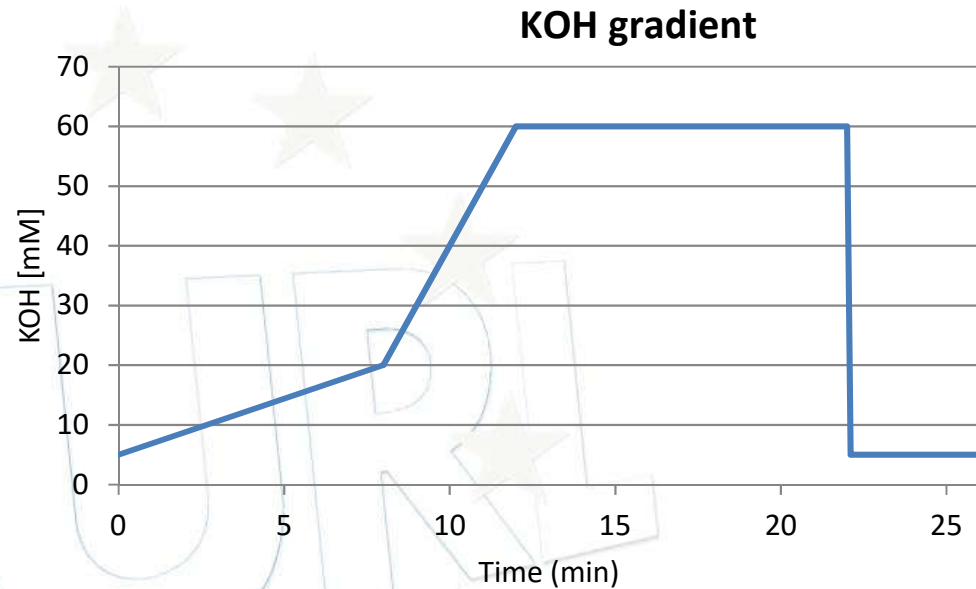
Ion chromatography coupled to a quadrupole Orbitrap mass analyzer was used to develop a multiresidue method for the determination of highly polar pesticides and their metabolites (chlorate, perchlorate, fosetyl-aluminum, glyphosate, aminomethylphosphonic acid (AMPA), phosphonic acid, *N*-acetyl AMPA, and *N*-acetyl glyphosate) in fruits and vegetables. After extraction with methanol, samples were diluted 5× with water. No

responsible for the retention of the analytes (2). Since the introduction of IC, column technology has improved. Current columns are characterized by higher ion-exchange capacity, higher column efficiency, reductions in column diameters, and a new chemistry of bonded functional groups (3).

Various detectors are used with IC. Examples of the application of conductivity (4, 5), UV (6), and mass detectors (7, 8), can be found in the literature. Because of the typical high content of nonvolatile salts, an ion chromatograph cannot be

IC-MS Conditions

- Column Dionex IonPac AS19 (250mm x 2mm x 4 μ m)
- Guard column Thermo Scientific™ Dionex™ IonPac™ AG19 (50mm x 2mm x 4 μ m)
- Column temperature 40°C
- Mobil phase flow 0.35 ml/min
- Suppressor flow 0.60 ml/min
- Suppressor current 52 mA
- Make-up solvent: acetonitrile
- Make-up solvent flow: 0.40 ml/min
- Injection volume: 50 μ L
- Dilution factor: 5



Workflow: FS-MS + SIM-MS + PRM-MS²

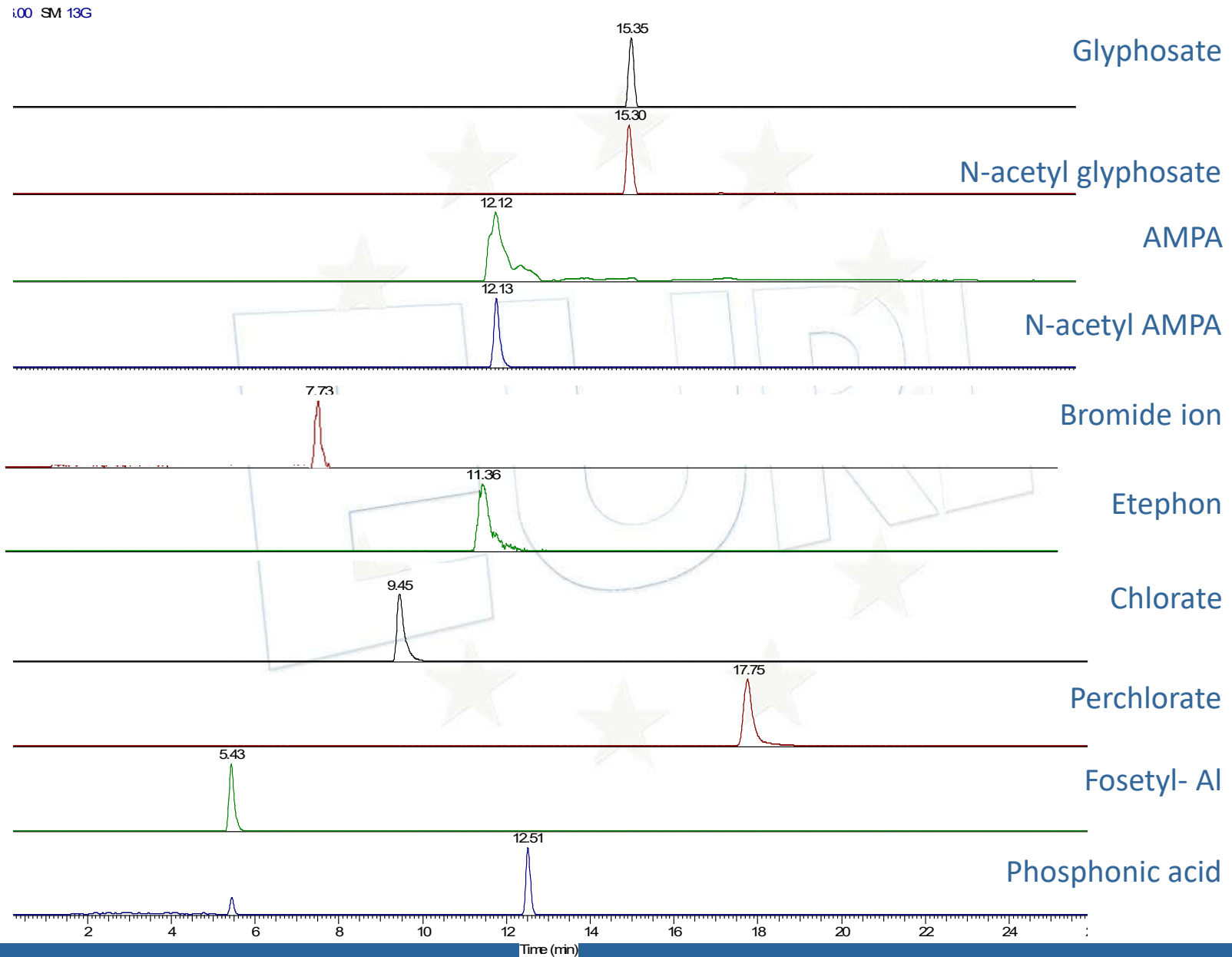
• MS:

- Range 1: m/z 78 – 212
- Range 2: m/z 109.5 – 110.5 (for AMPA)
- Resolution 70,000 (at m/z 200)
- AGC target 1e6
- Max IT auto

• MS²:

- PRM
- Resolution 17,500 (at m/z 200)
- Quadrupole isolation window 1Da
- AGC target 1e6
- Max IT auto

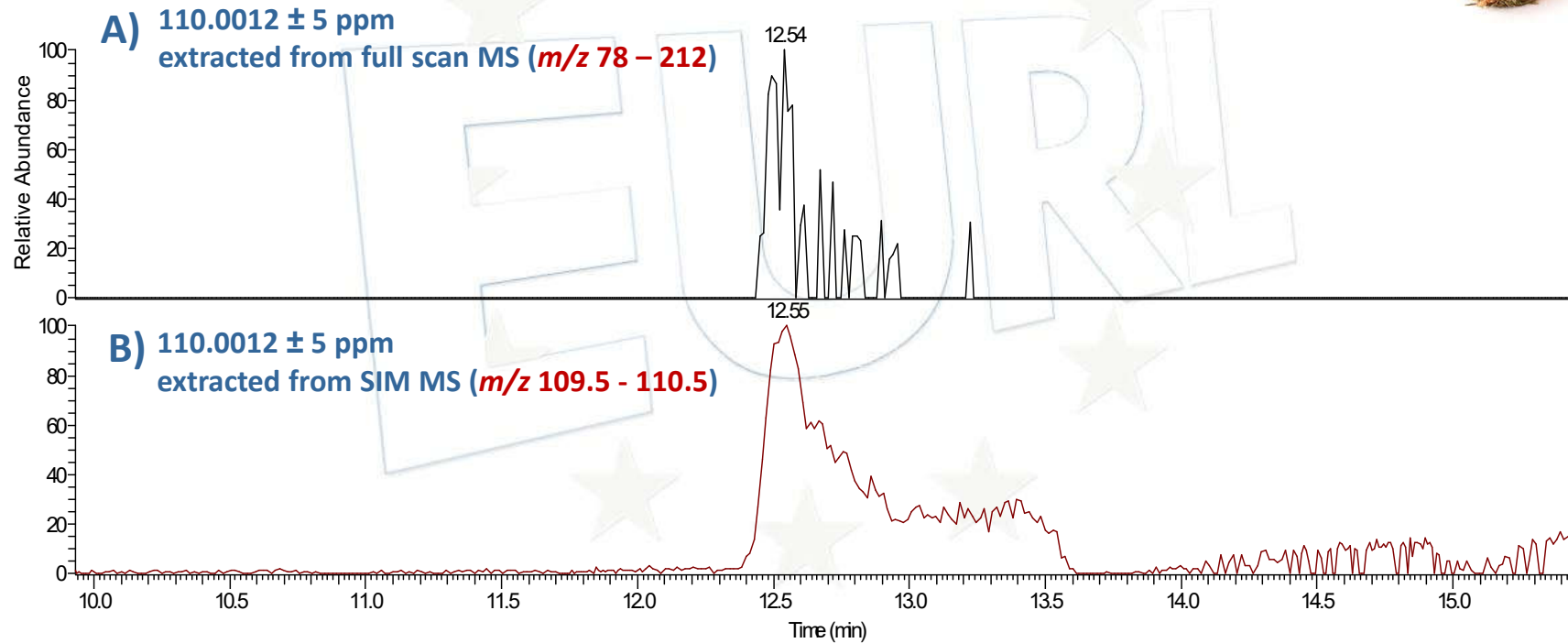
Extracted ion chromatograms (precursor ions)



AMPA

Sensitivity improvement in SIM

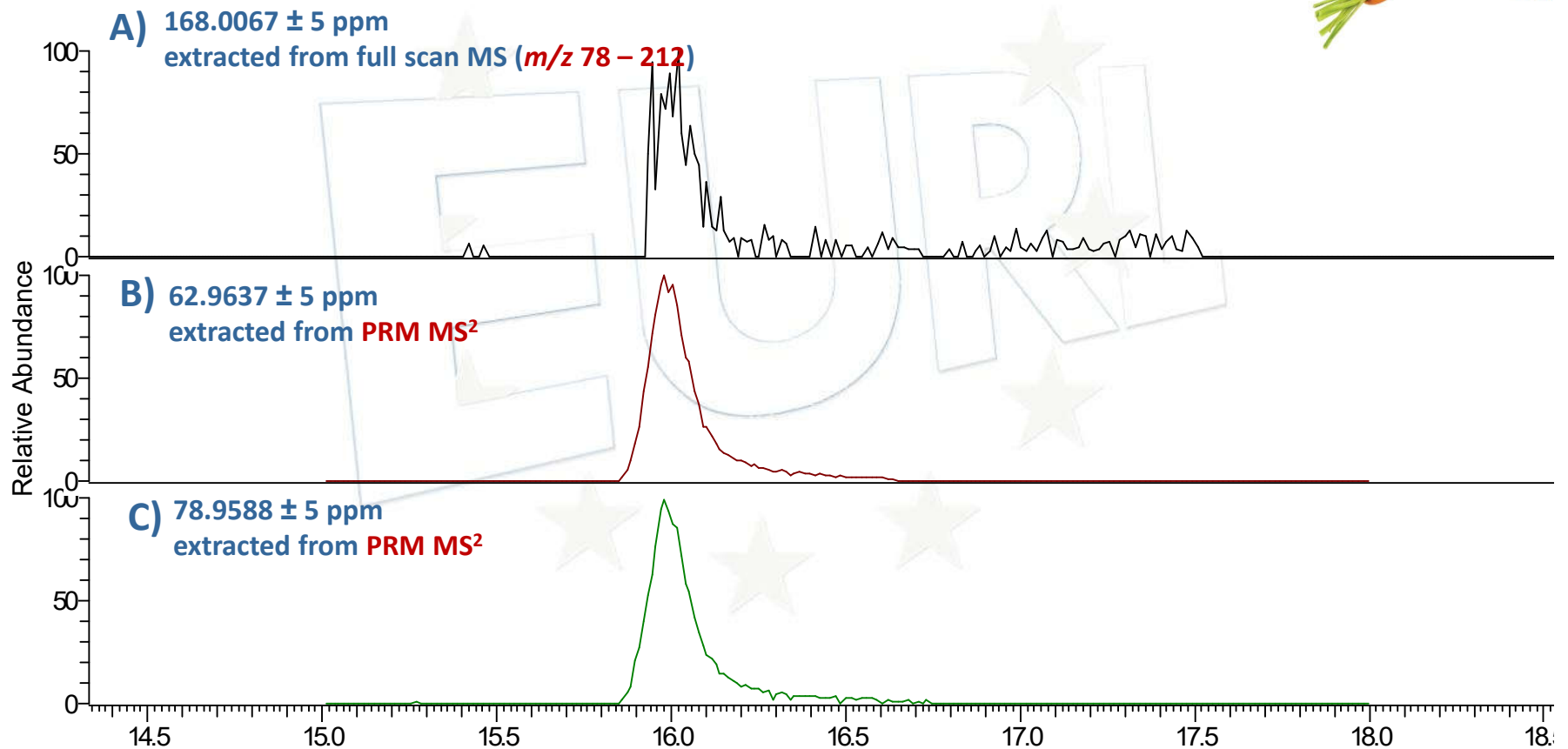
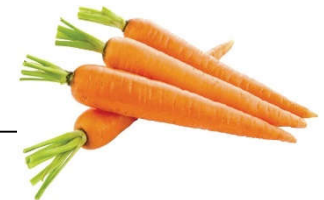
0.01 mg/kg of AMPA in onion extract after 5-fold dilution.



Glyphosate

Sensitivity improvement in MS²

0.01 mg/kg of glyphosate in carrot extract after 5-fold dilution.

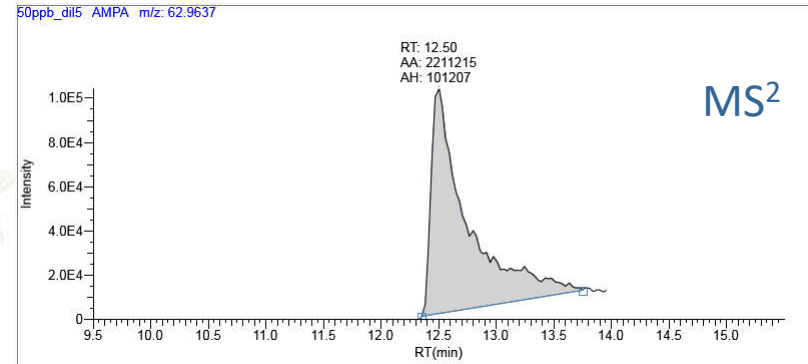
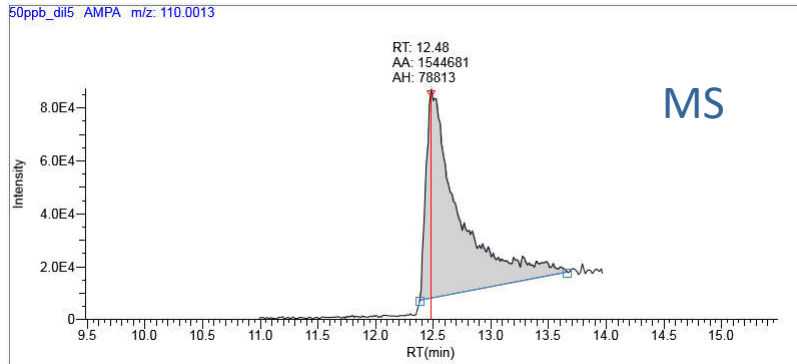


Analytes and their ions

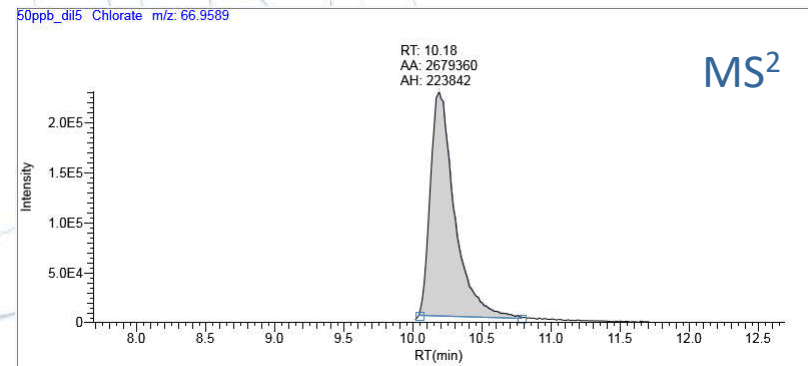
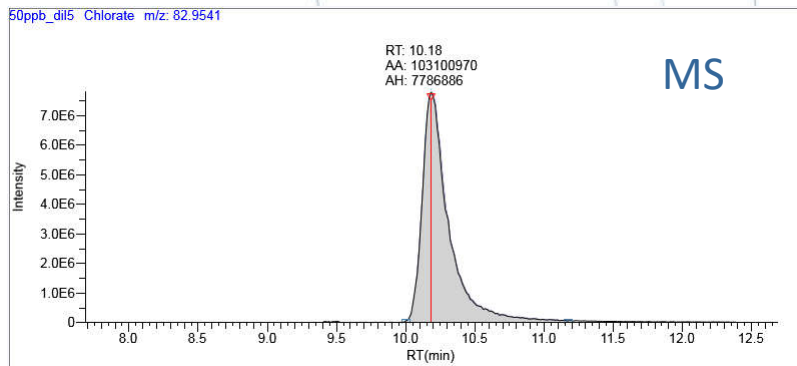
Compound	Quantifier	Qualifier	CE [eV]
AMPA	110.0012	62.9641	25
Bromide ion	78.9189	80.9168	-
Chlorate	82.9541	66.9592	55
Glyphosate	62.9641	78.9590	25
Etephon	142.9670	78.9590	35
N-acetyl AMPA	152.0118	78.9590	25
N-acetyl glyphosate	210.0173	78.9590	45
Perchlorate	98.9491	82.9541	25
Fosetyl-Al	62.9641	78.9590	25
Phosphonic acid	62.9641	78.9590	25

MS; MS²

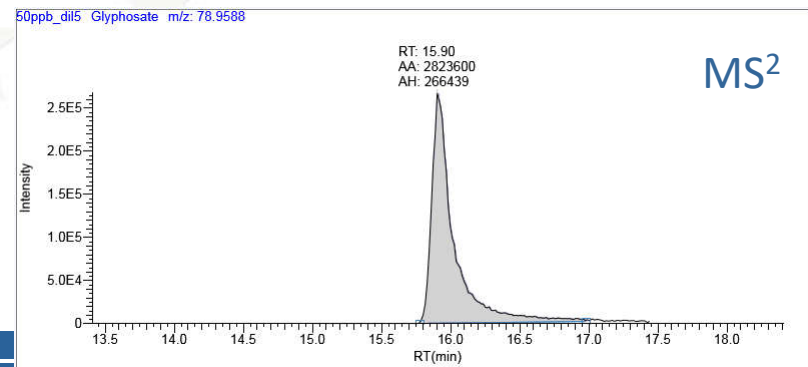
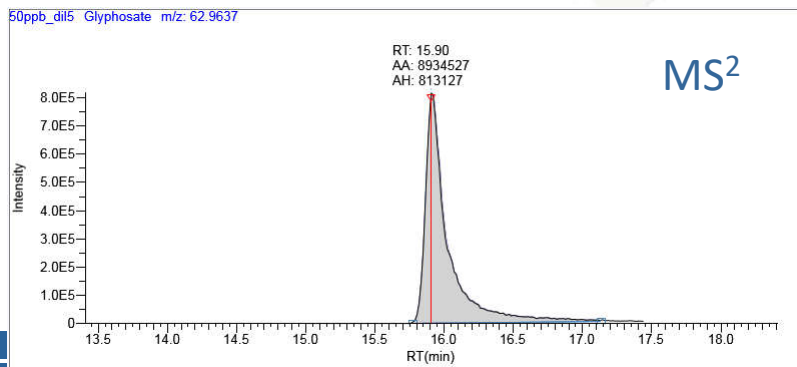
AMPA



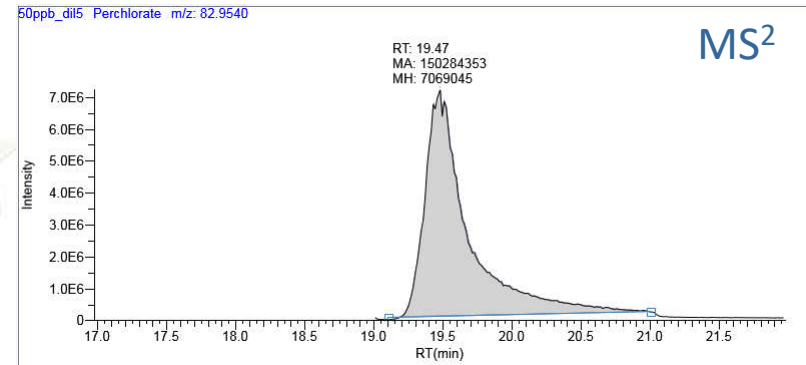
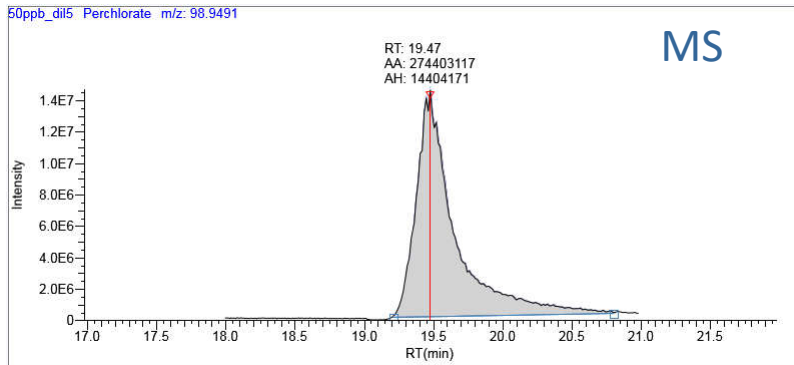
Chlorate



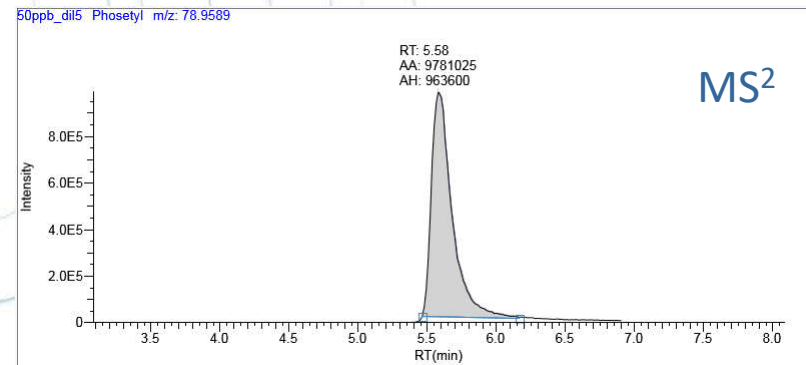
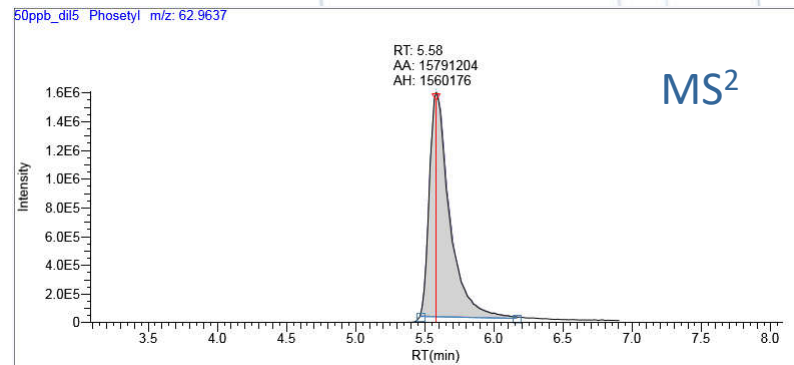
Glyphosate



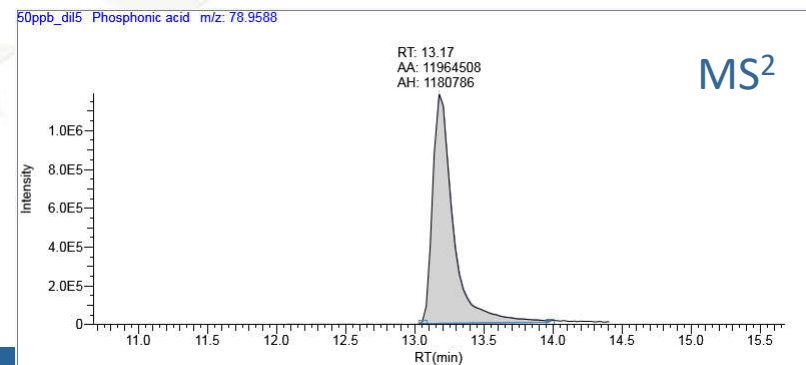
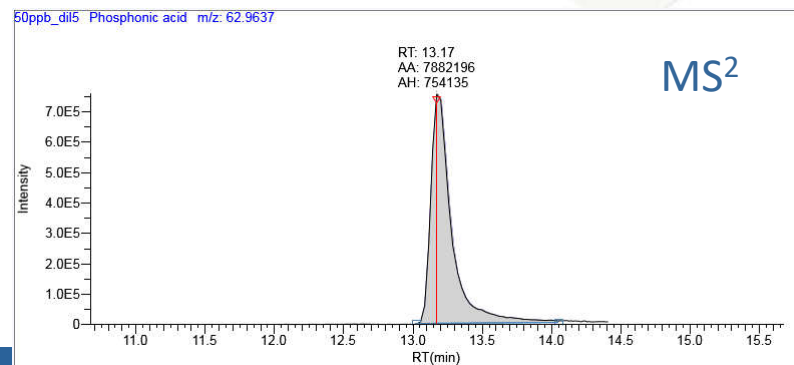
Perchlorate



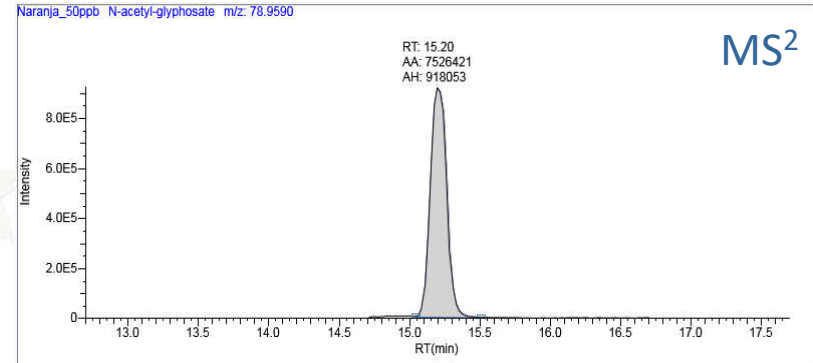
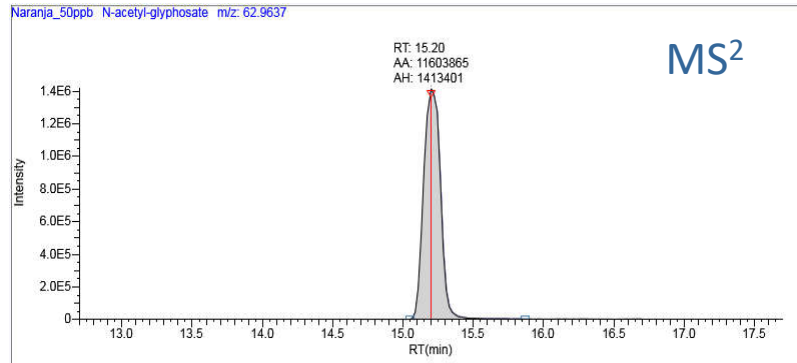
Fosetyl- Al



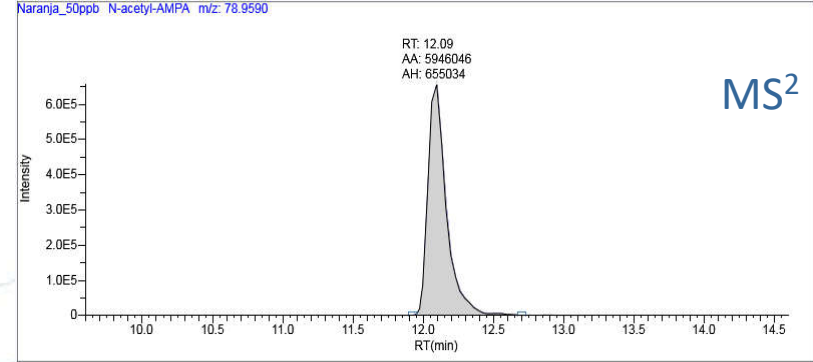
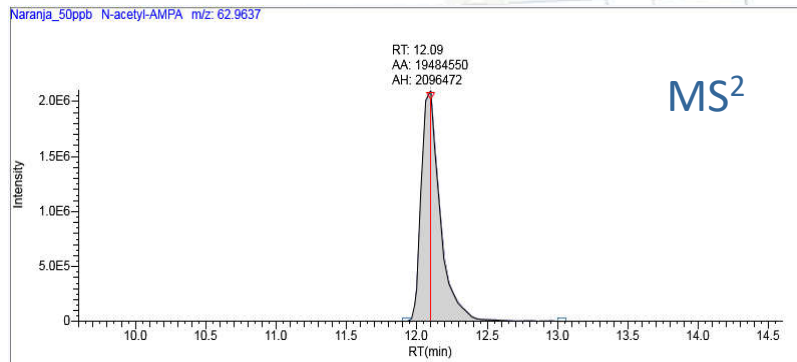
Phosphonic acid



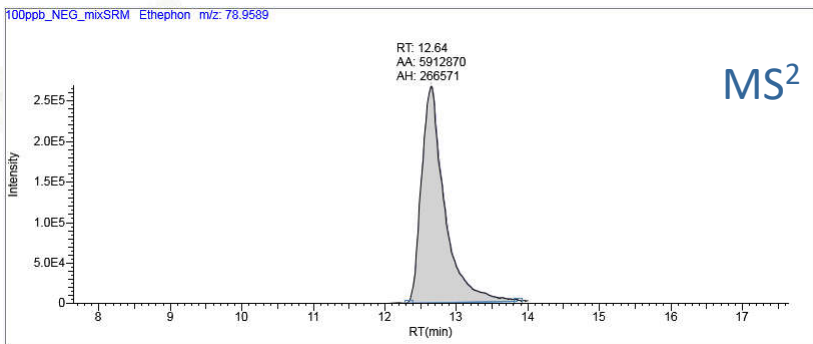
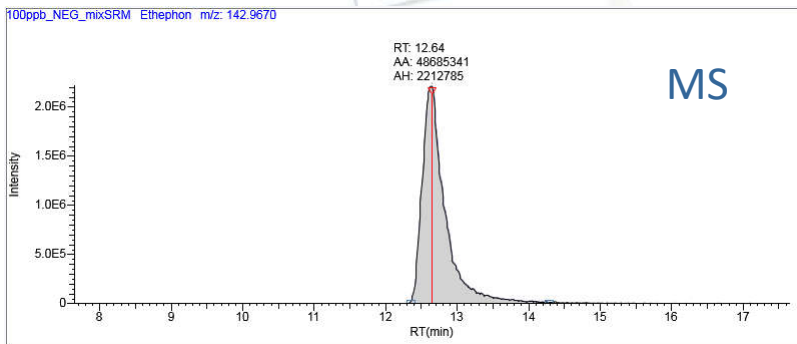
N-acetyl glyphosate



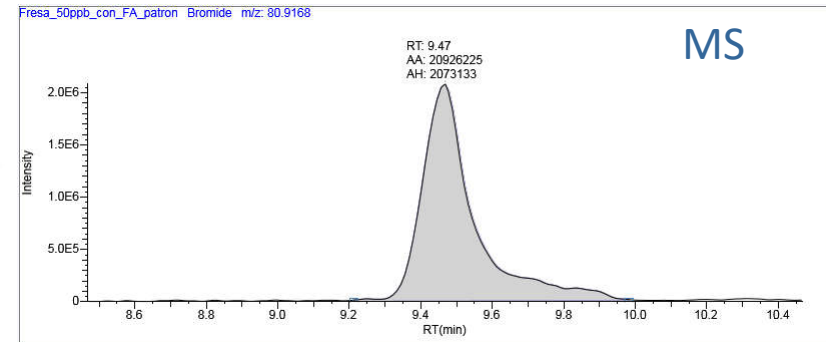
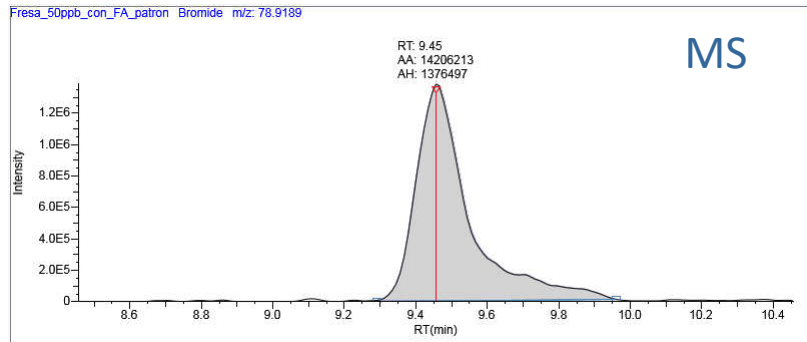
N-acetyl AMPA



Etephon



Bromide ion

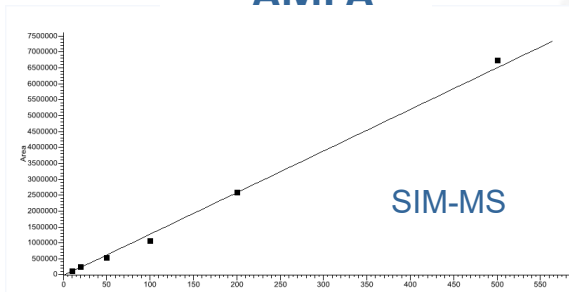


Linearity in orange

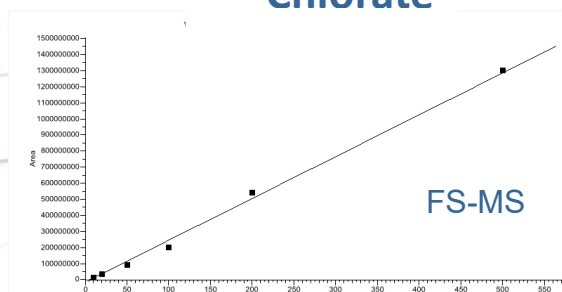
Range 0.01 – 0.5 mg/kg



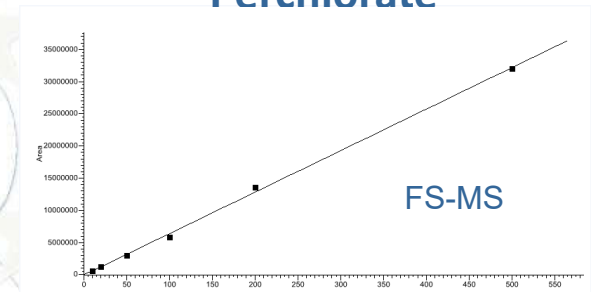
AMPA



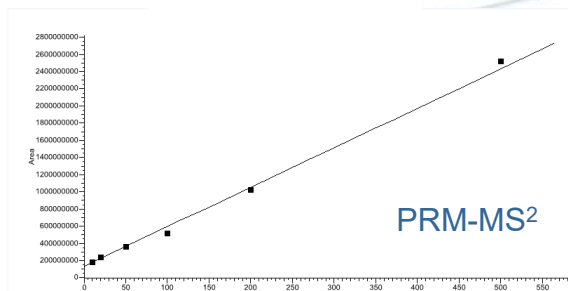
Chlorate



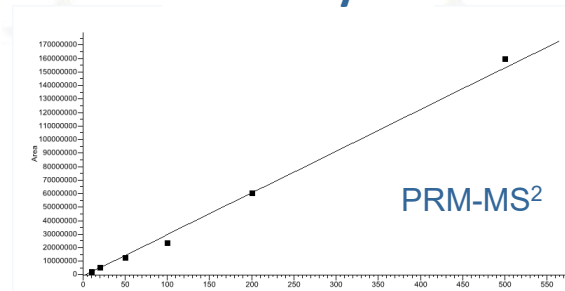
Perchlorate



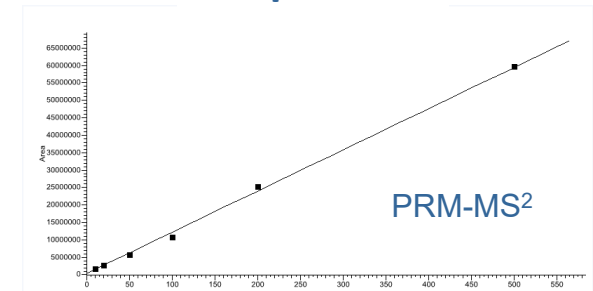
Glyphosate



Fosetyl- Al



Phosphonic acid

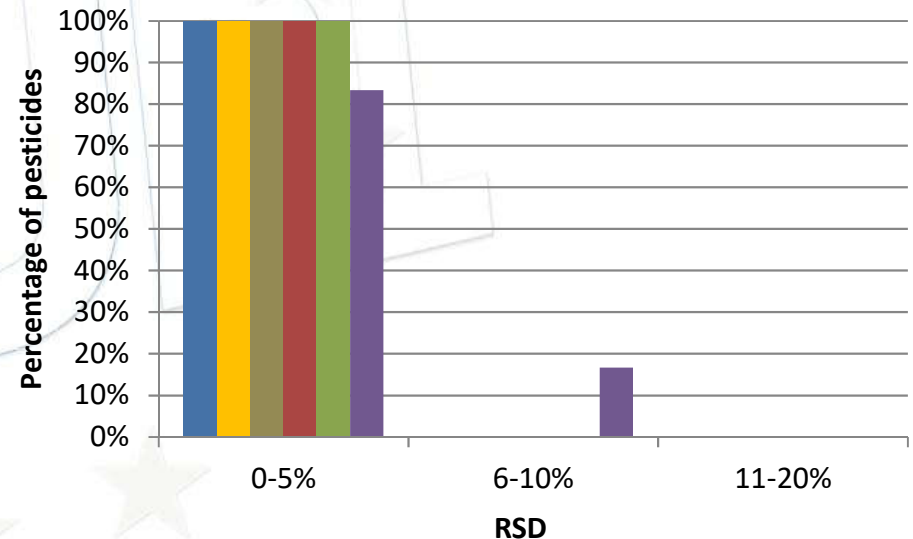
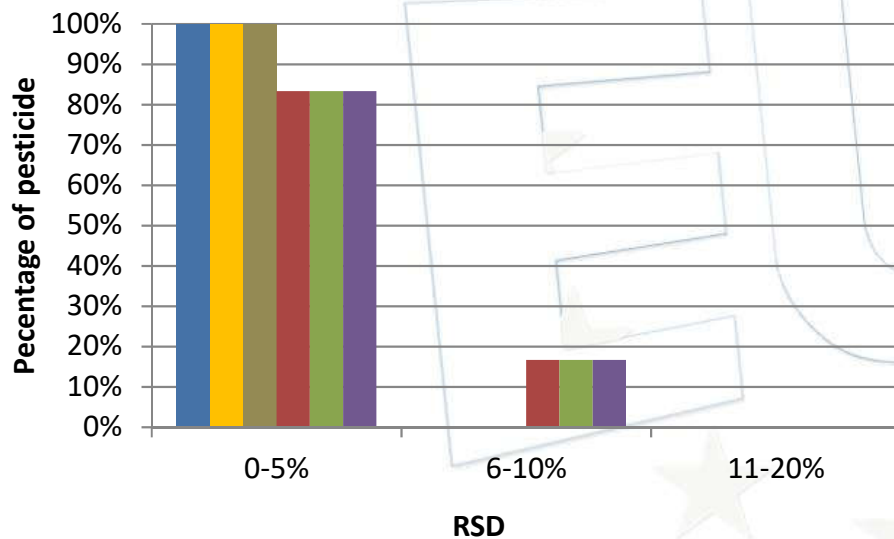


Peak area repeatability

(n = 5)

0.01 mg/kg

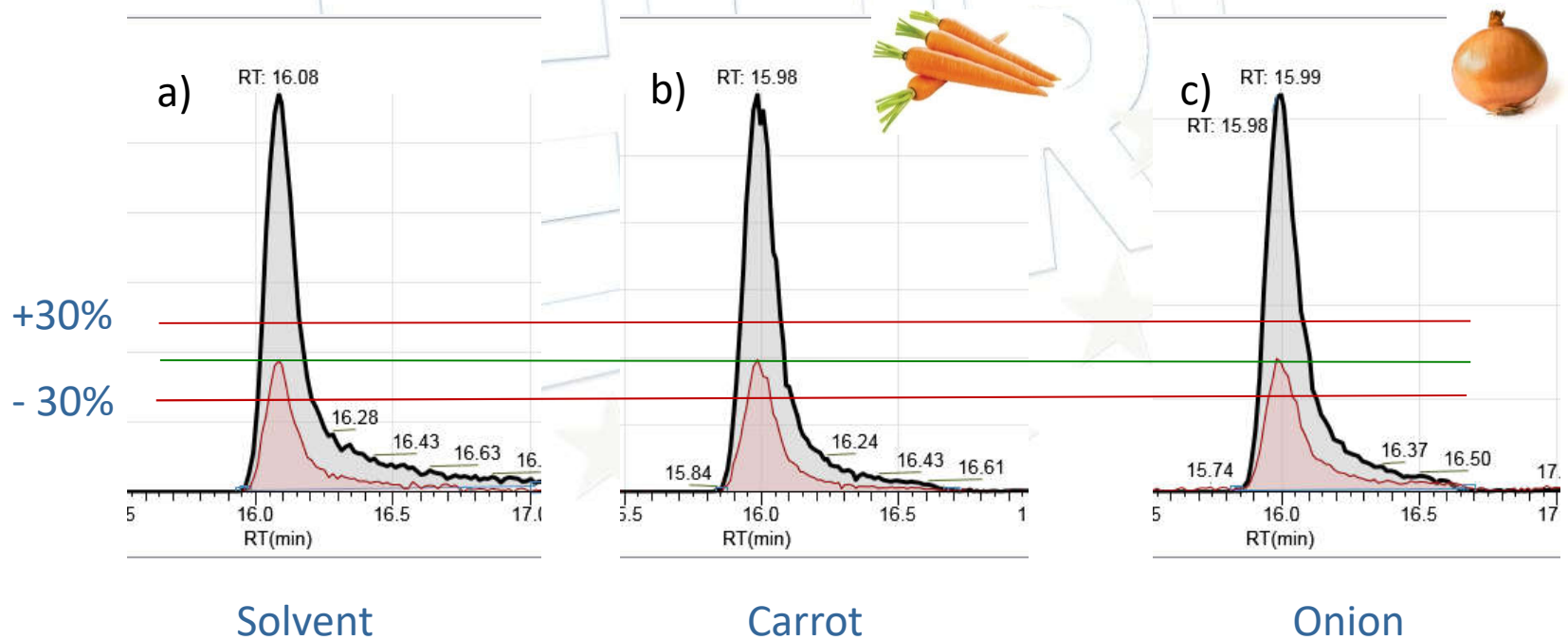
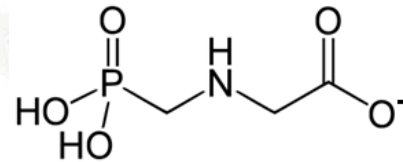
0.05 mg/kg



■ solvent
 ■ orange
 ■ onion
 ■ tomato
 ■ melon
 ■ carrot

Ion ratio robustness

0.01 mg/kg of glyphosate



Mass accuracy

Glyphosate in orange
MS² ion



m/z (Expected)	m/z (Apex)	m/z (Delta)
62.9637	62.9638	.0794 (mmu)
62.9637	62.9638	.0680 (mmu)
62.9637	62.9638	.0833 (mmu)
62.9637	62.9638	.0833 (mmu)
62.9637	62.9638	.0718 (mmu)
62.9637	62.9638	.0718 (mmu)
62.9637	62.9638	.0680 (mmu)
62.9637	62.9638	.0794 (mmu)
62.9637	62.9638	.0756 (mmu)
62.9637	62.9638	.0756 (mmu)
62.9637	62.9638	.0871 (mmu)
62.9637	62.9638	.0718 (mmu)

Chlorate in onion
MS ion



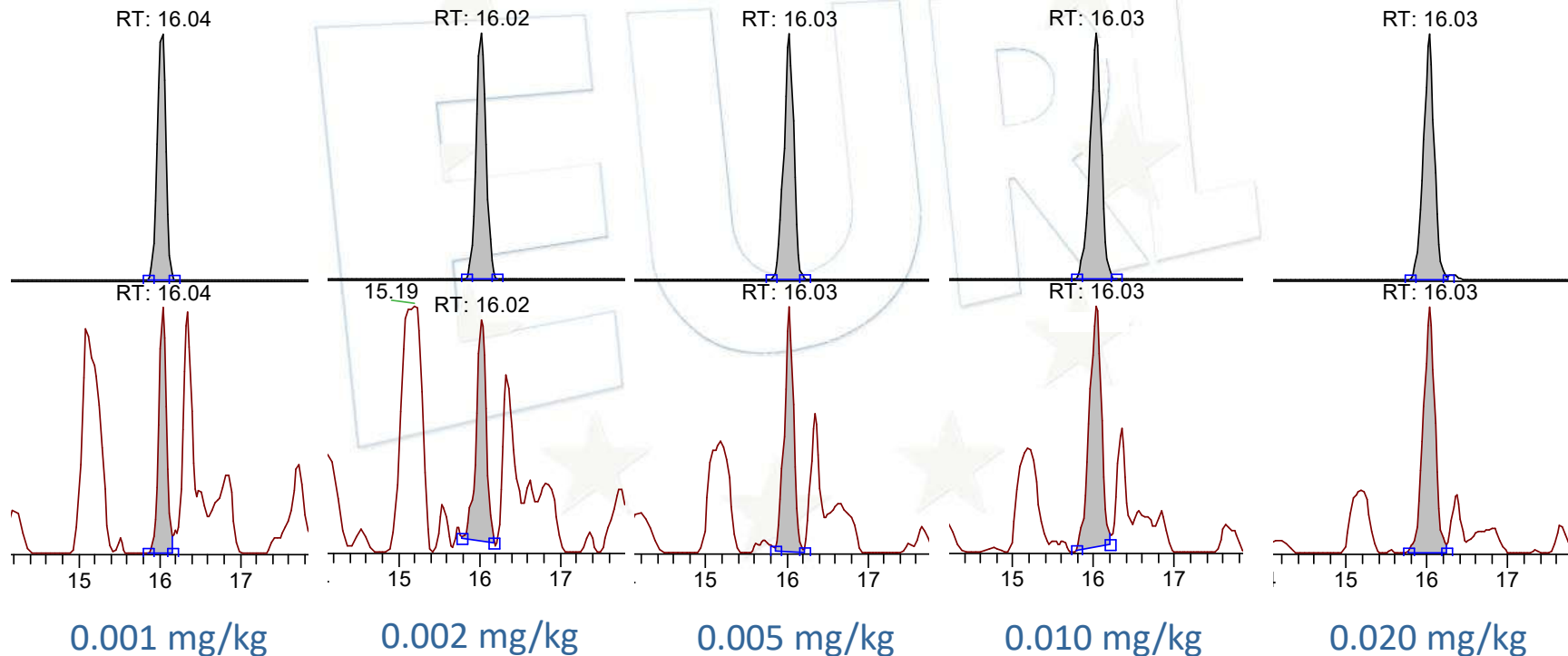
m/z (Expected)	m/z (Apex)	m/z (Delta)
82.9541	82.9541	-.0079 (mmu)
82.9541	82.9541	-.0308 (mmu)
82.9541	82.9541	-.0384 (mmu)
82.9541	82.9541	-.0384 (mmu)
82.9541	82.9541	-.0461 (mmu)
82.9541	82.9541	-.0384 (mmu)
82.9541	82.9541	-.0461 (mmu)
82.9541	82.9541	-.0384 (mmu)
82.9541	82.9541	-.0537 (mmu)
82.9541	82.9541	-.0308 (mmu)
82.9541	82.9541	-.0537 (mmu)
82.9541	82.9541	-.0461 (mmu)
82.9541	82.9541	-.0079 (mmu)

Glyphosate in baby food



Extract diluted 5 times

PRM MS²: 62.9641 ± 5ppm
78.9590 ± 5ppm

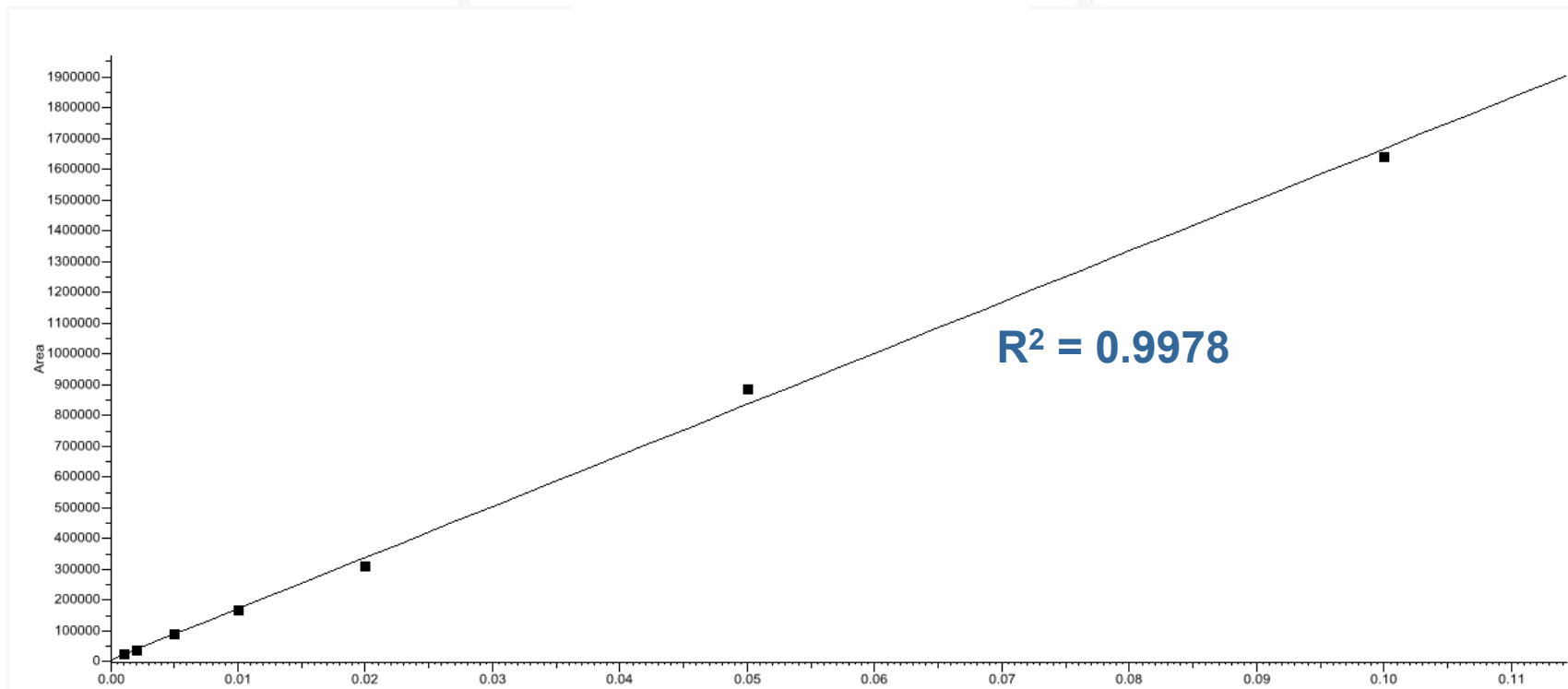


(composition: peach, apple, banana, apricot, orange juice, grape juice)

Glyphosate in baby food



Linearity 0.001 – 0.100 mg/kg



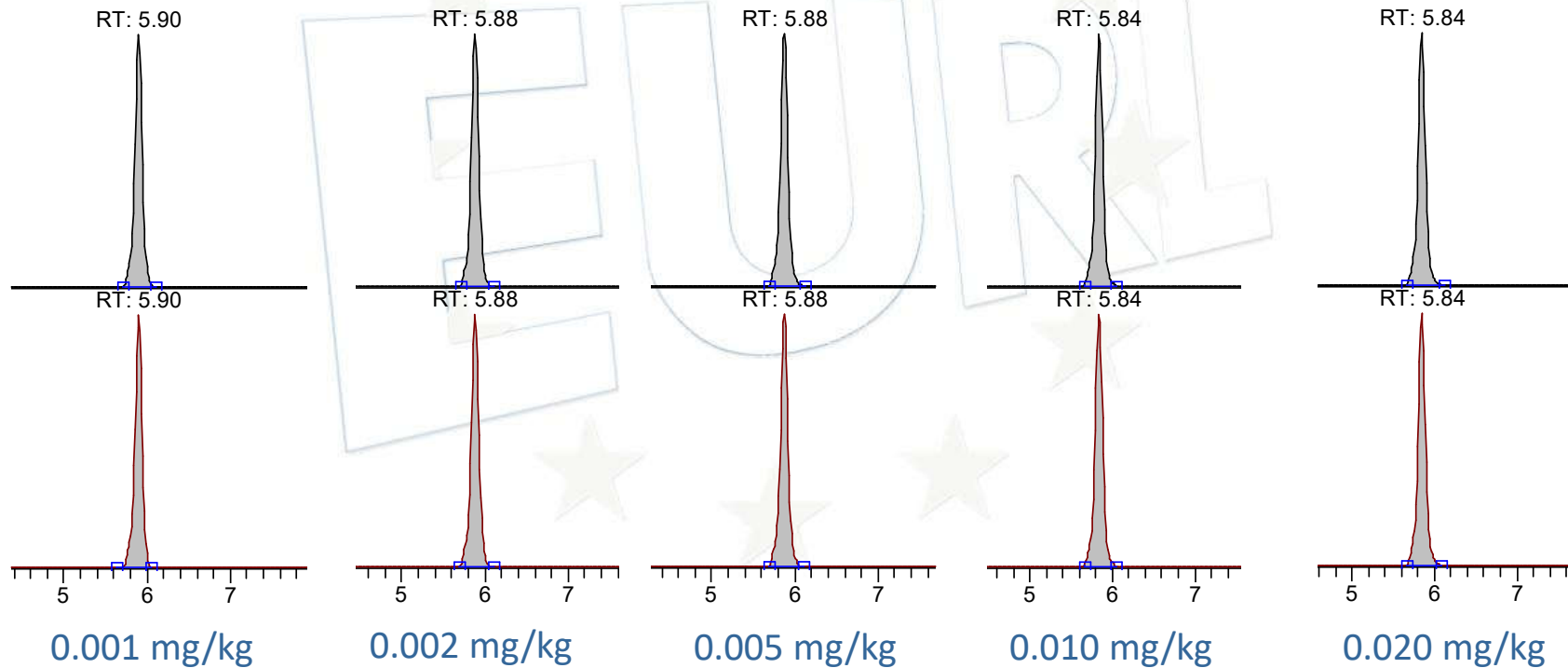
(composition: peach, apple, banana, apricot, orange juice, grape juice)

Fosetyl-aluminum in baby food



Extract diluted 5 times

PRM MS²: 62.9641 ± 5ppm
78.9590 ± 5ppm

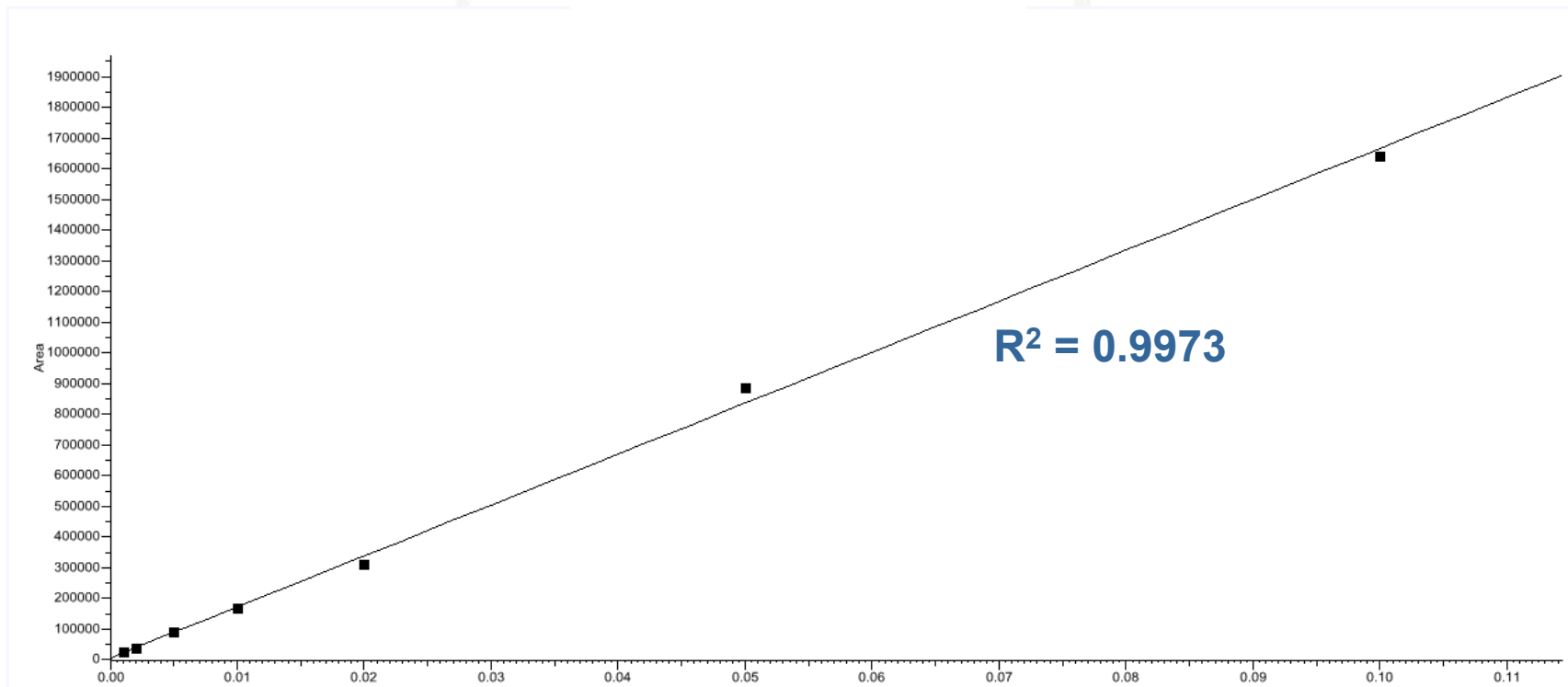


(composition: peach, apple, banana, apricot, orange juice, grape juice)

Fosetyl-aluminum in baby food



Linearity 0.001 – 0.100 mg/kg



(composition: peach, apple, banana, apricot, orange juice, grape juice)

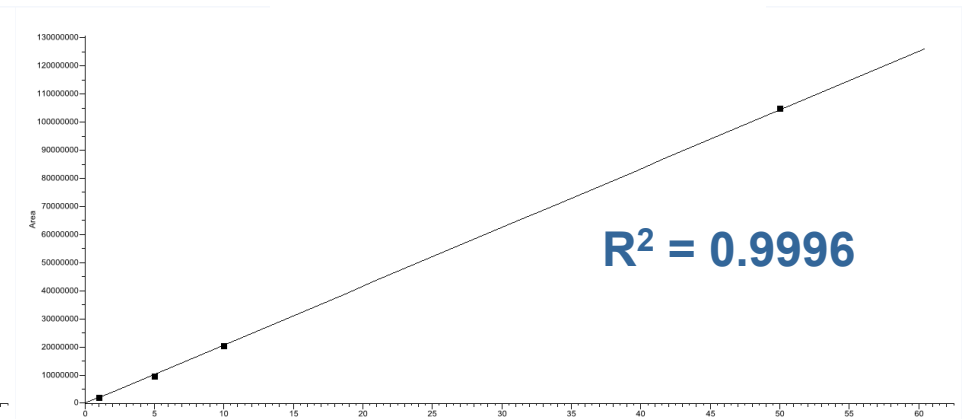
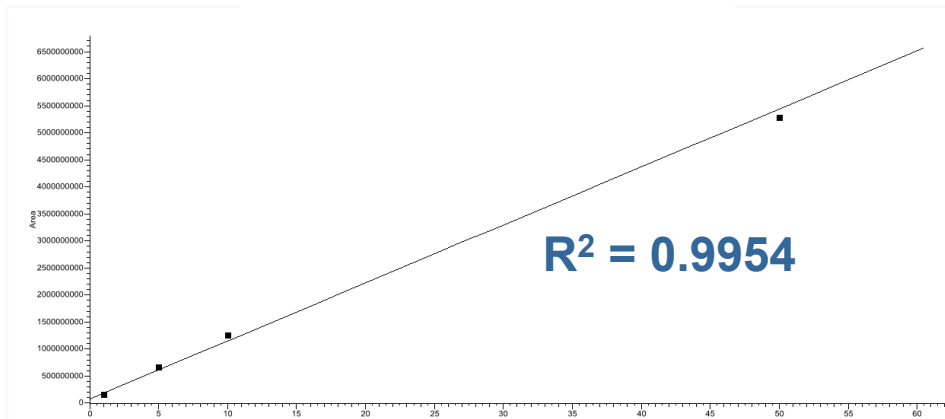
Phosphonic acid in strawberry



Linearity 1.0 – 50 mg/kg

5-fold dilution

250-fold dilution



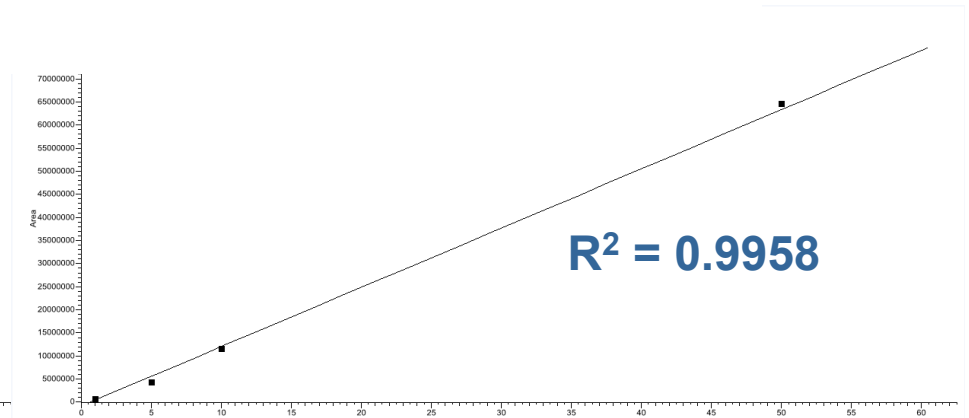
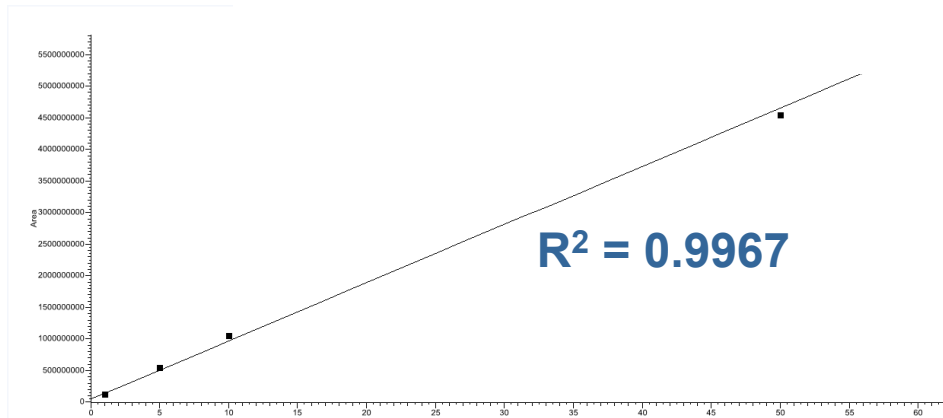
Bromide ion in strawberry



Linearity 1.0 – 50 mg/kg

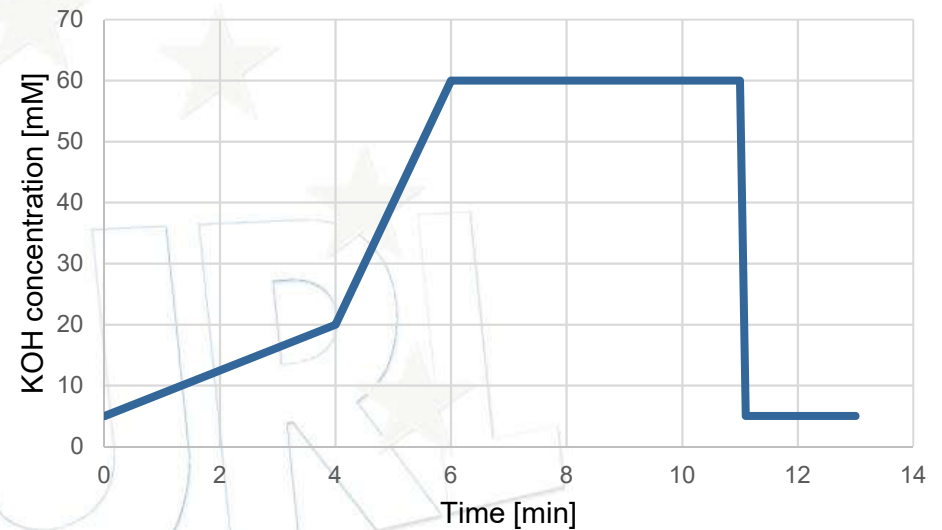
5-fold dilution

250-fold dilution



IC-MS Conditions

KOH gradient



- Column Dionex IonPac AS19 (100mm x 2mm x 4µm)
- Guard column Thermo Scientific™ Dionex™ IonPac™ AG19 (50mm x 2mm x 4µm)
- Column temperature 40°C
- Mobil phase flow 0.35 ml/min
- Suppressor flow 0.60 ml/min
- Suppressor current 52 mA
- Make-up solvent: acetonitrile
- Make-up solvent flow: 0.20 ml/min
- Injection volume: 50 µL
- Dilution factor: 5

Workflow: FS-MS + SIM-MS + PRM-MS²

• MS:

- Range 1: m/z 150 – 212
- Range 2: m/z 109.5 – 110.5 (for AMPA)
- Resolution 70,000 (at m/z 200)
- AGC target 1e6
- Max IT auto

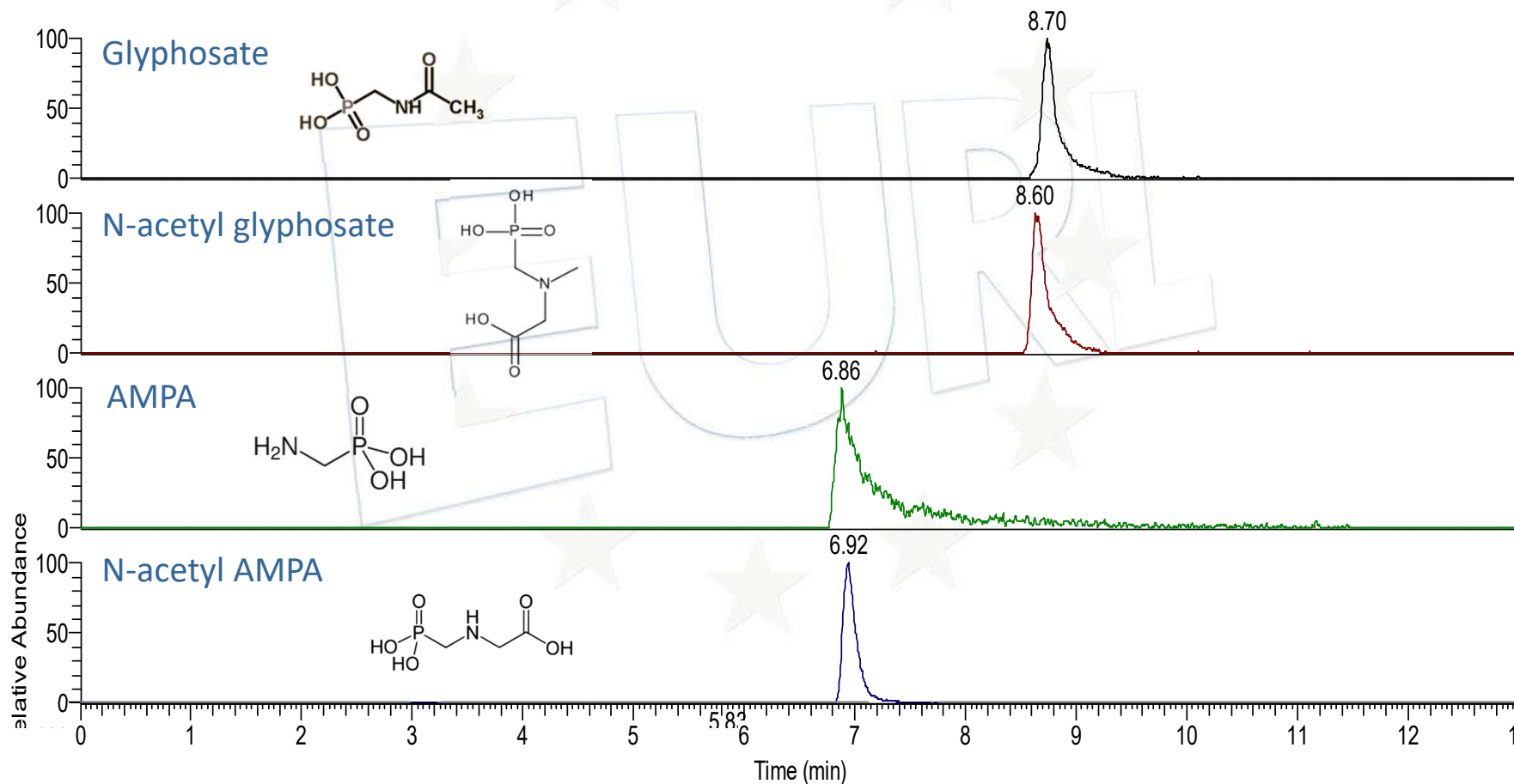
• MS²:

- PRM
- Resolution 17,500 (at m/z 200)
- Quadrupole isolation window 1Da
- AGC target 1e6
- Max IT auto

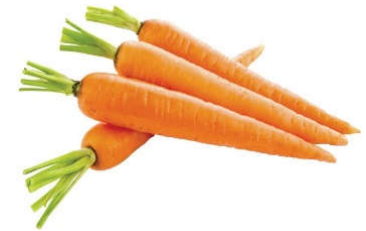
Extracted ion chromatograms (precursor ions)

Column Dionex IonPac AS19 (**100mm** x 2mm x 4 μ m) - prototype

RT: 0.00 - 13.01 SM: 7G

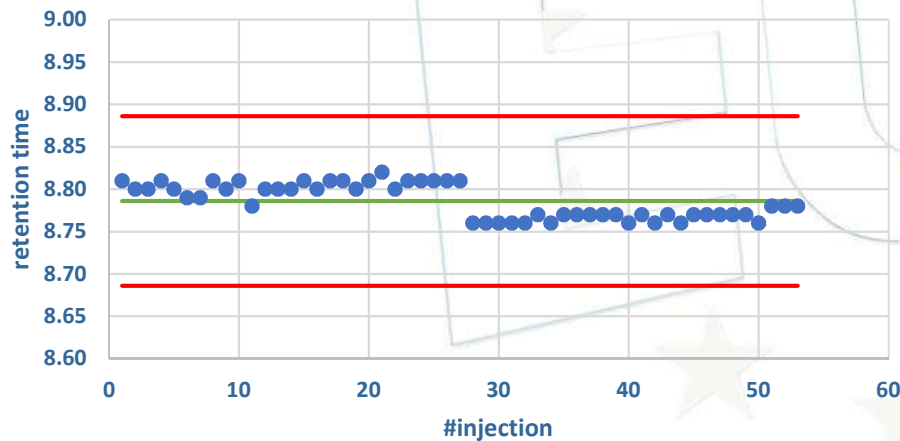


Retention time stability

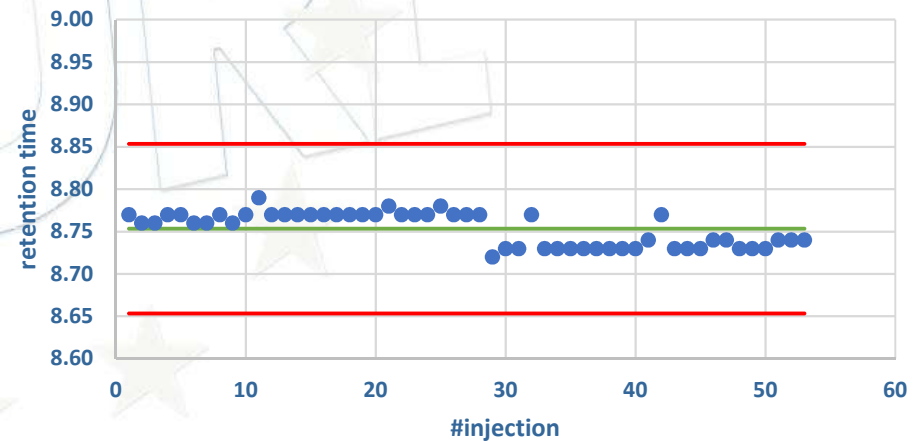


0.05 mg/kg in carrot
(0.005 mg/kg in the vial)

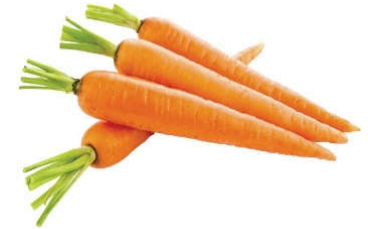
Glyphosate



N-acetyl-glyphosate

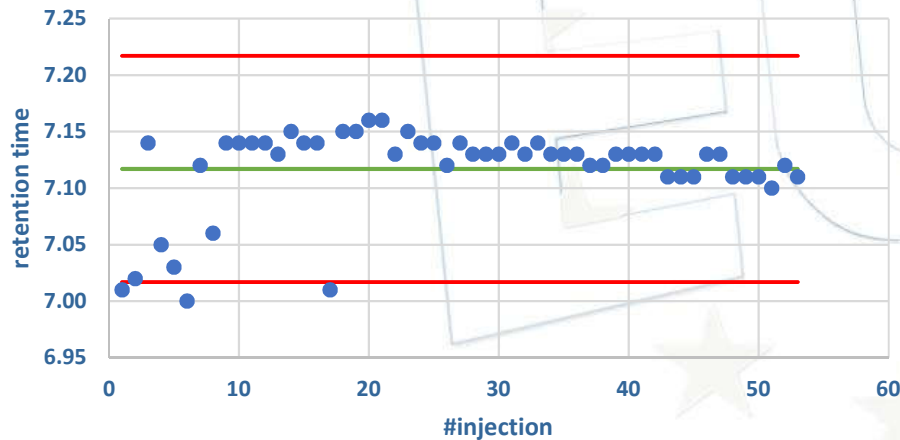


Retention time stability

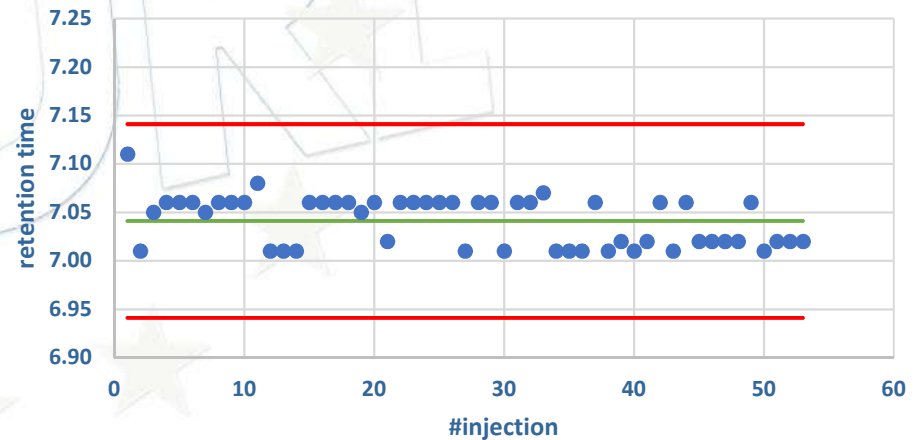


0.05 mg/kg in carrot
(0.005 mg/kg in the vial)

AMPA

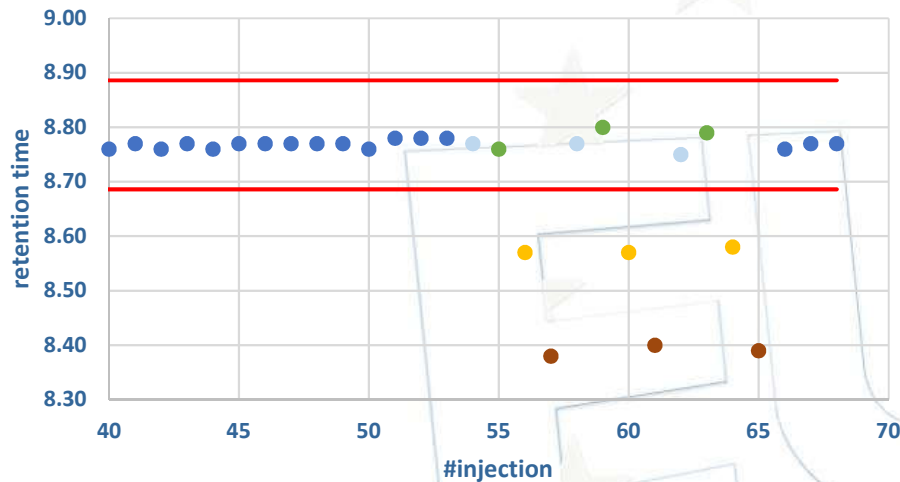


N-acetyl-AMPA



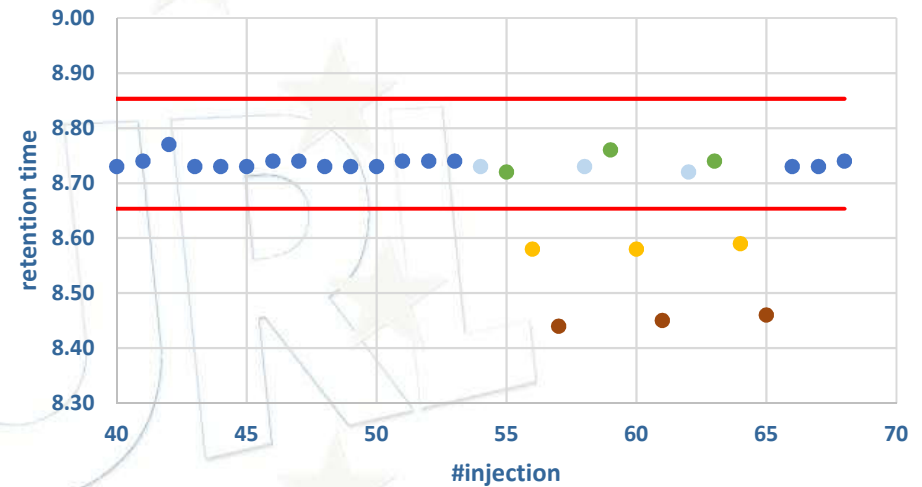
Retention time stability

Glyphosate



- Carrot 0.05 mg/kg
- Carrot 0.005 mg/kg
- Apple 0.05 mg/kg
- Mandarin 0.05 mg/kg
- Strawberry 0.05 mg/kg

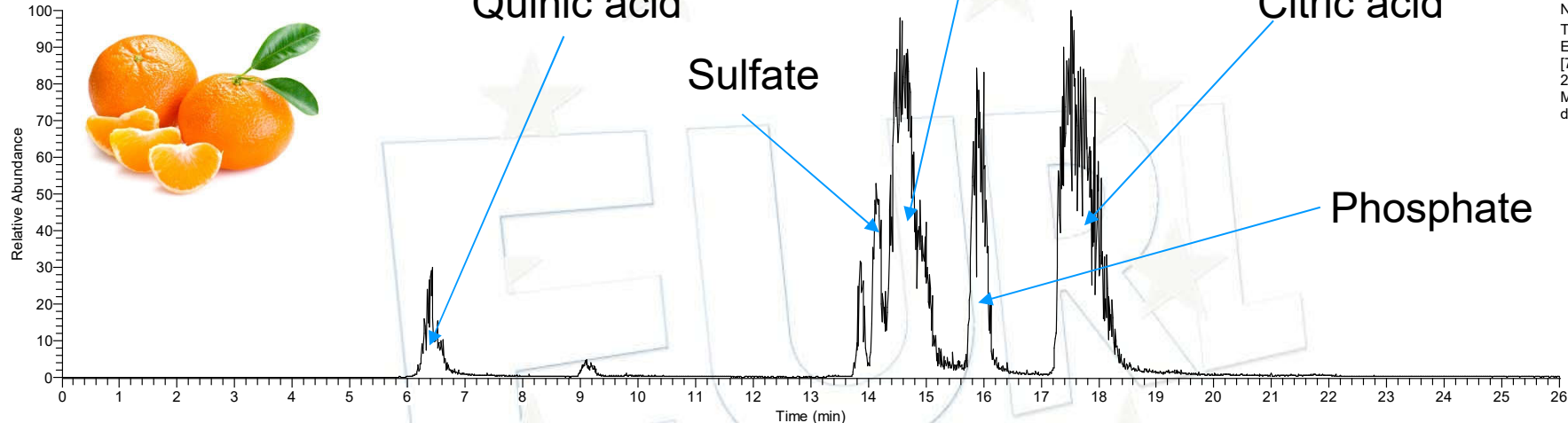
N-acetyl-glyphosate



- Carrot 0.05 mg/kg
- Carrot 0.005 mg/kg
- Apple 0.05 mg/kg
- Mandarin 0.05 mg/kg
- Strawberry 0.05 mg/kg

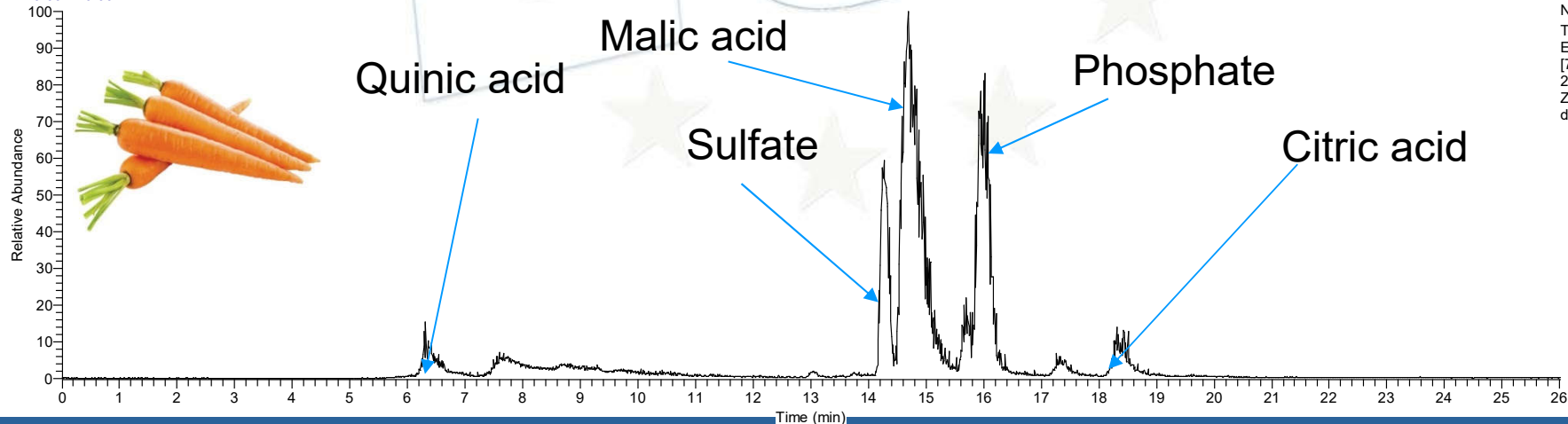
Matrix interferences

RT: 0.00 - 26.01



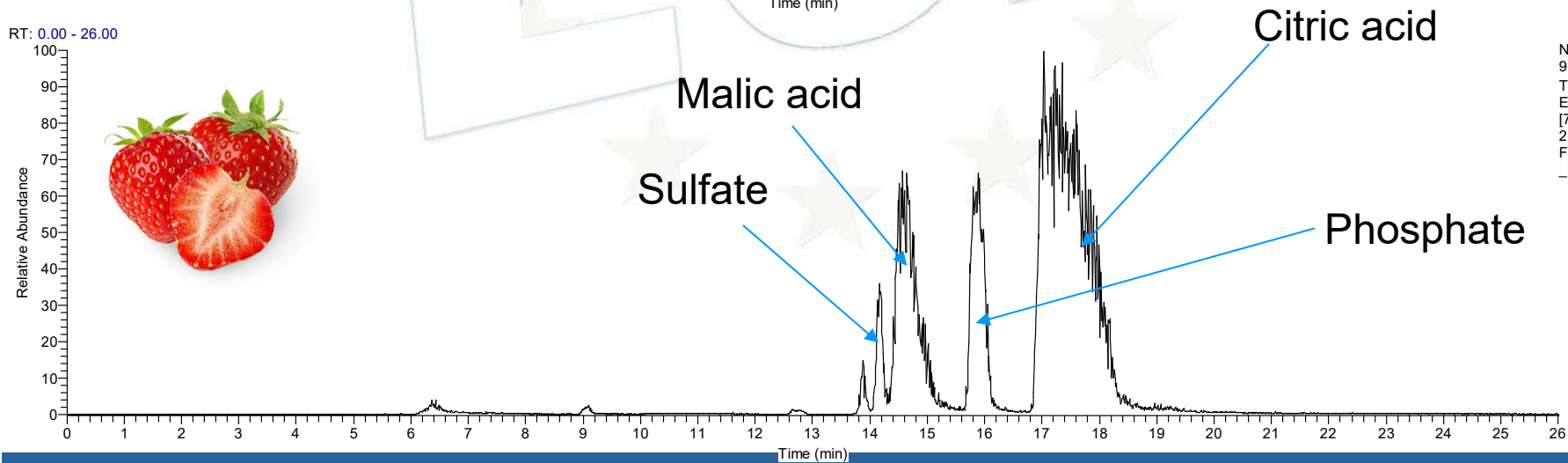
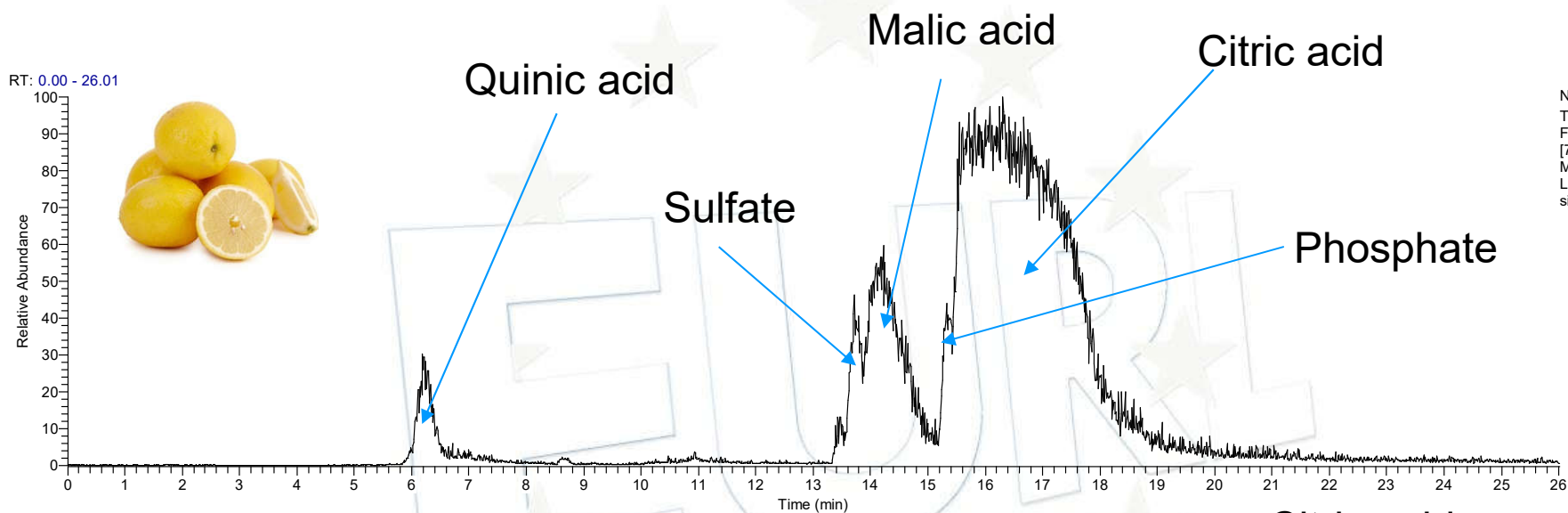
NL: 7.48E9
 TIC F: FTMS - p
 ESI Full ms
 [78.0000-
 212.0000] MS
 Mandarina_20ppb_
 dil5_R3

RT: 0.00 - 26.00

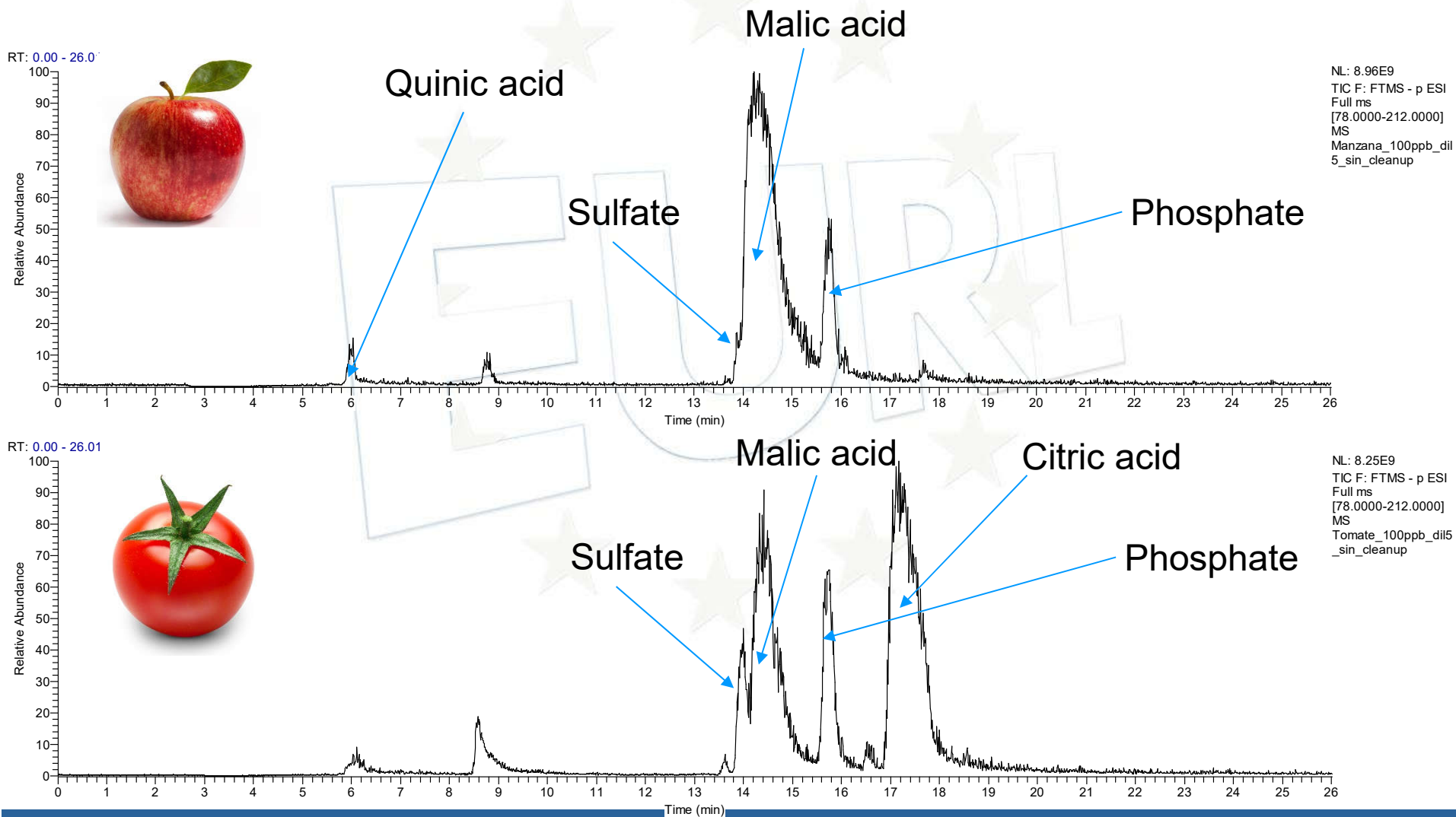


NL: 7.09E9
 TIC F: FTMS - p
 ESI Full ms
 [78.0000-
 212.0000] MS
 Zanahoria_20ppb_
 dil5_R1

Matrix interferences

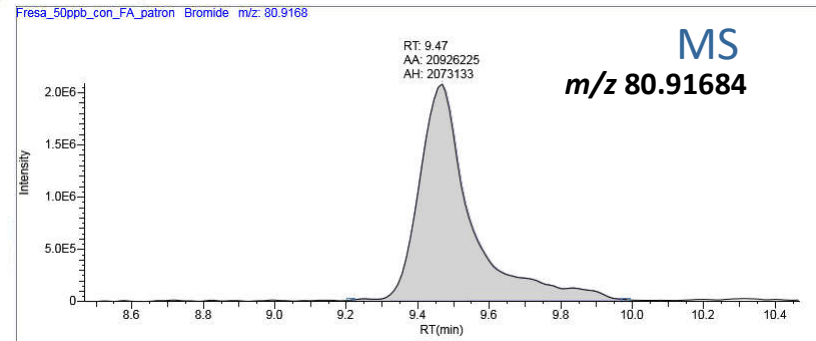
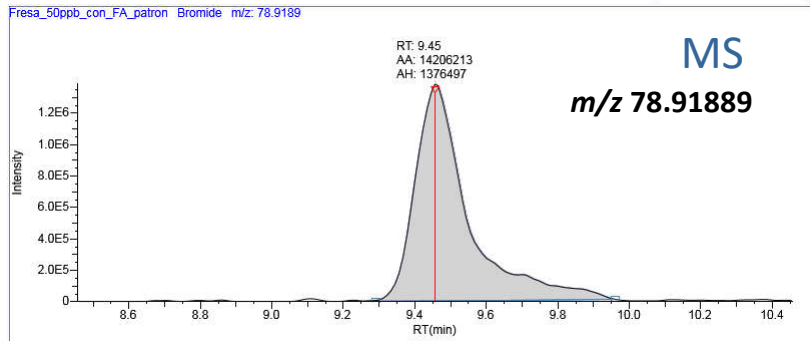


Matrix interferences



Advantages of high resolution mass spectrometry

Bromide ion



Triple quadrupole:

Transition (?) 1: $^{79}\text{Br}^- \rightarrow ^{79}\text{Br}^-$

Transition (?) 2: $^{81}\text{Br}^- \rightarrow ^{81}\text{Br}^-$

No fragmentation

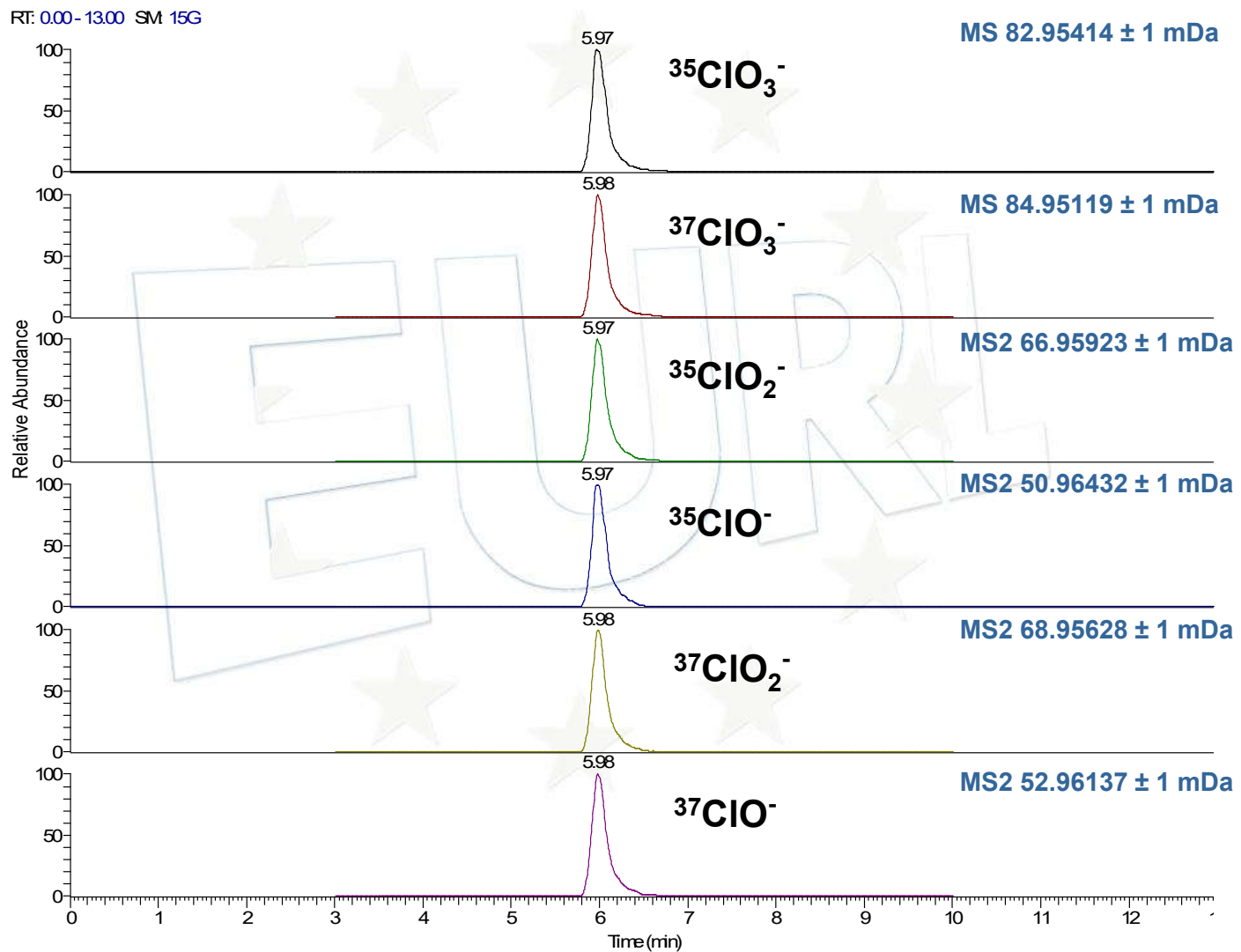
High resolution:

Ion 1: 78.91889

Ion 2: 80.91684

Very high selectivity

Advantages of high resolution mass spectrometry



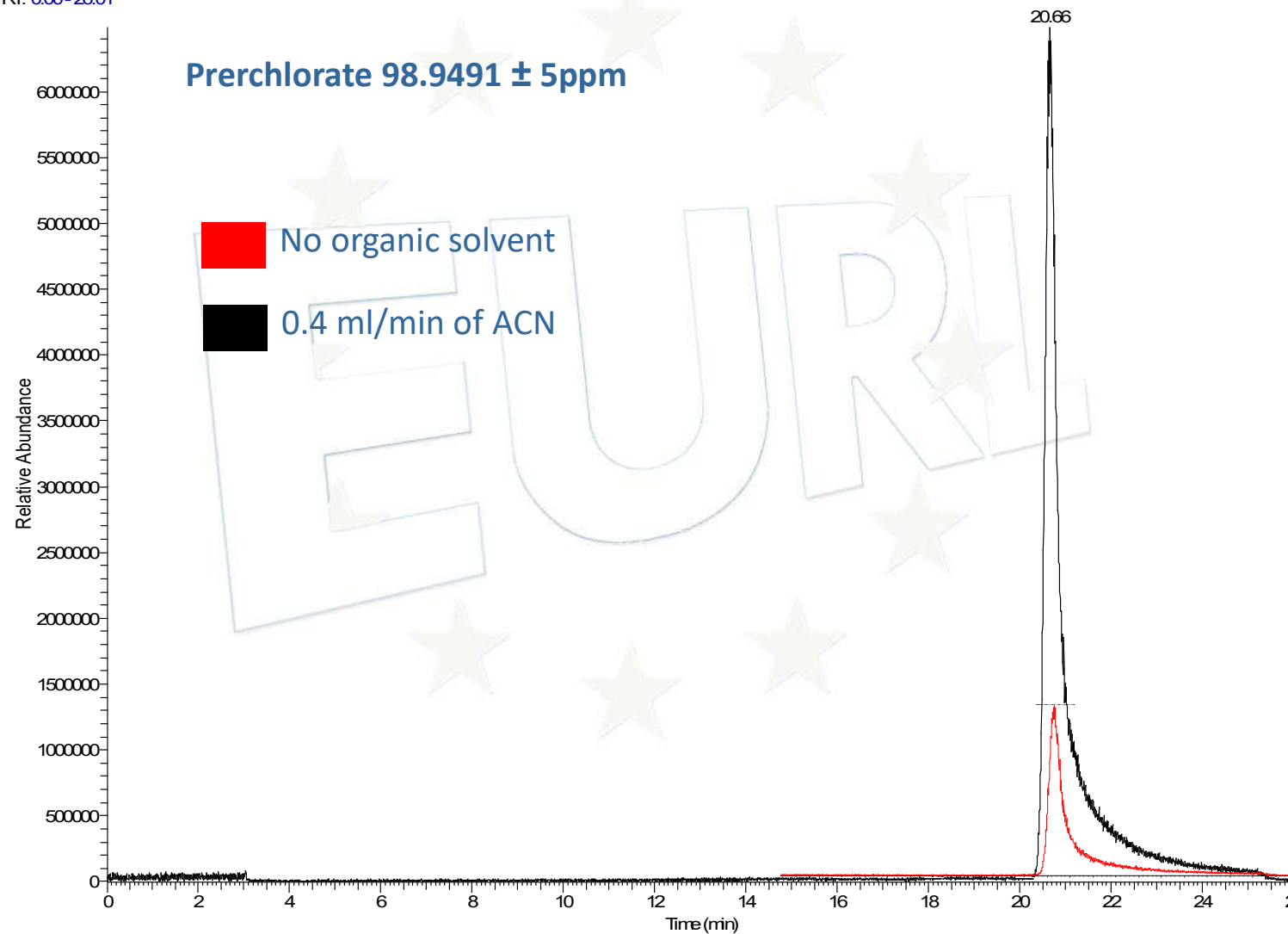
Post-column addition of organic solvent – sensitivity improvement

100% - peak area without organic solvent

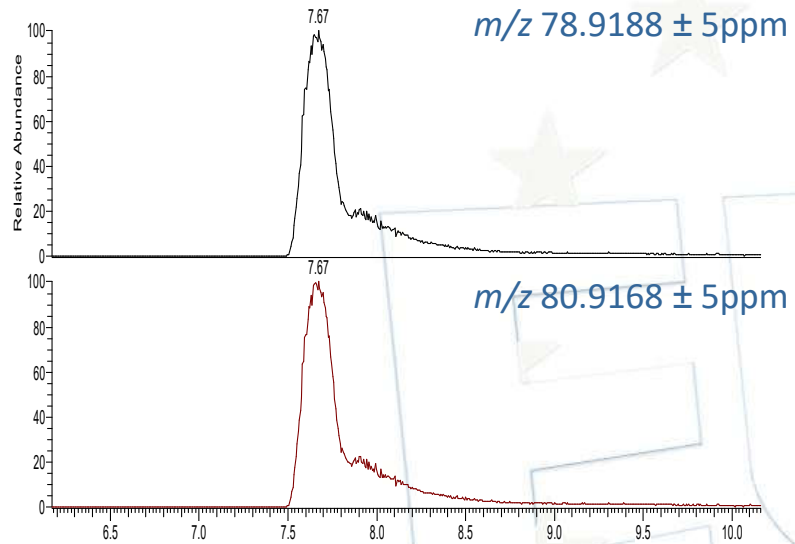
	MeOH 0.2 ml/min	ACN 0.2 ml/min	ACN 0.4 ml/min
AMPA	169%	269%	254%
Chlorate	121%	381%	434%
Glyphosat	145%	269%	235%
Perchlorate	132%	365%	454%
Fosetyl-Al	242%	347%	339%
Phosphonic acid	139%	280%	283%
N-acetyl AMPA	147%	254%	250%
N-acetyl glyphosate	138%	268%	255%

Post-column addition of organic solvent – sensitivity improvement

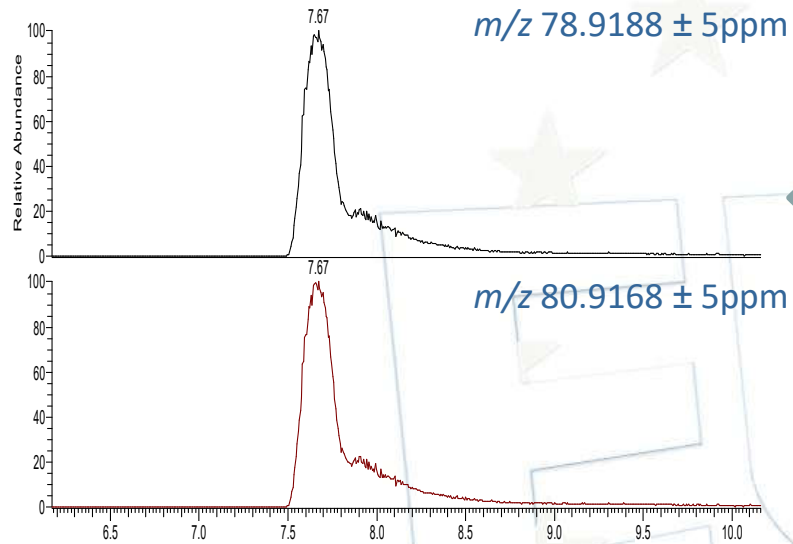
RT: 0.00-26.01



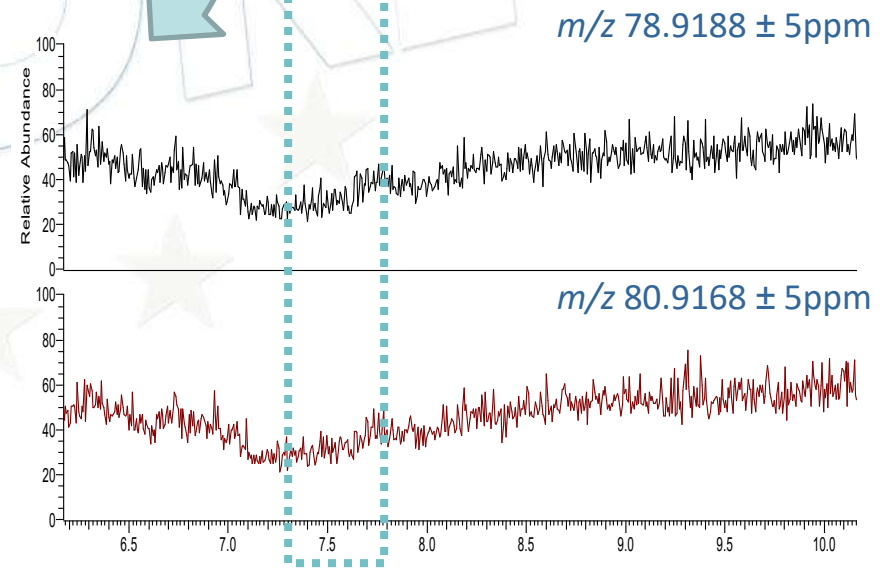
Bromide ion **50 mg/kg** in strawberry (dilucion 5x)



Bromide ion 50 mg/kg in strawberry (dilution 5x)



Blank solvent injected after the high concentration sample





SUMMARY

Advantages of IC-MS/MS

- **Good retention of very polar compounds**
- **Reproducible retention times**
- **Good sensitivity**
- **Automatic mobile phase preparation**
- **Easy and fast HPLC-IC change**

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**Thank You
for Your Attention**



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