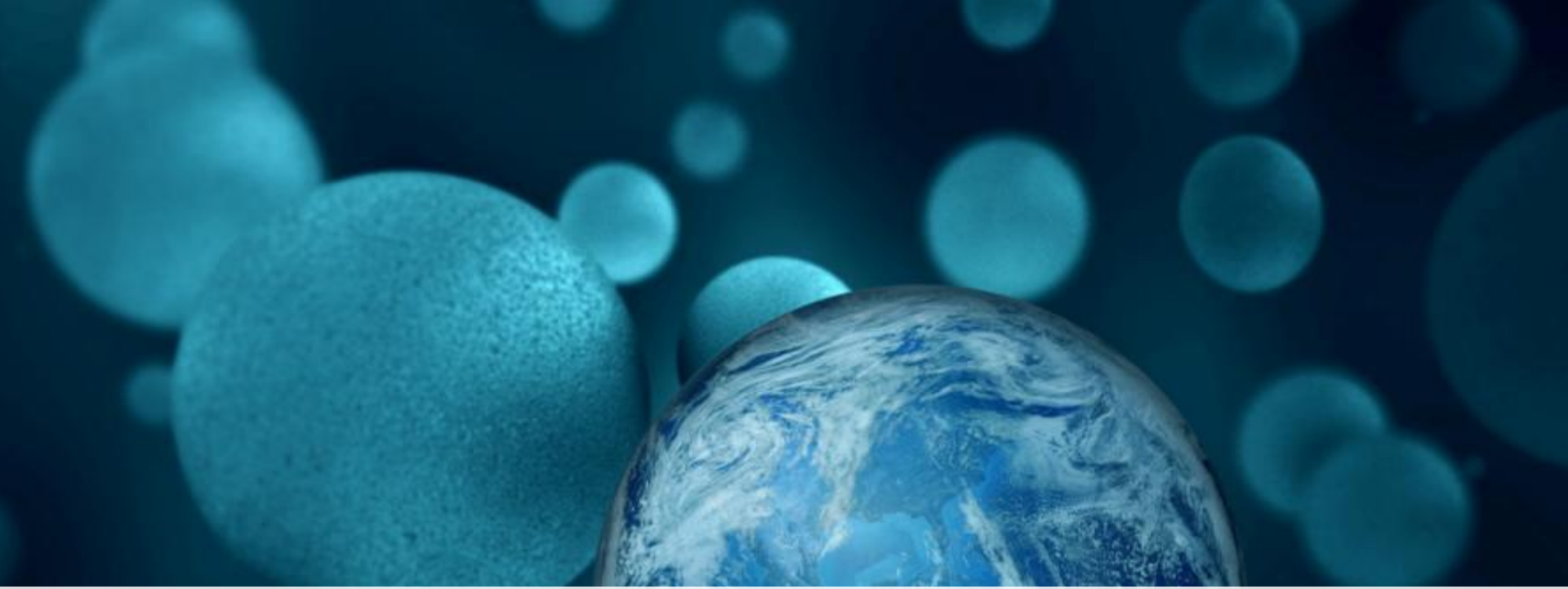


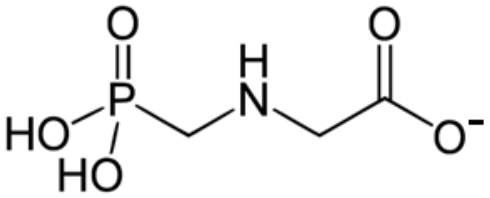
ThermoFisher SCIENTIFIC

Ion Chromatography for the Analysis of Polar Ionic Pesticides

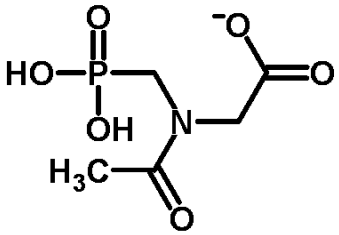
Polar Ionic Pesticides in the News

- Widely used in agricultural production; and occur during food preparation
- High frequency of residues of certain compounds detected in food
- EPRW 2014 – number of poster on residues of chlorate in leafy vegetables (and perchlorate residues from fertiliser use)
- 2016 Alliance for Natural Health USA: reported 10 of 24 breakfast foods had residues of glyphosate (86 – 1,327 µg/kg) (www.anh-usa.org)
- 2016: Glyphosate residues in German beers
- Glyphosate under scrutiny after the International Agency for Research on Cancer (IARC) that informs the World Health Organization (WHO) on cancer risk factors, classified glyphosate as a 'probable carcinogen' last March 2015
- Blog: Analysis of the Pesky Polar Pesticides: In the News, but What's the Answer?
  http://analyteguru.com/analysis-of-the-pesky-polar-pesticides-in-the-news-but-whats-the-answer

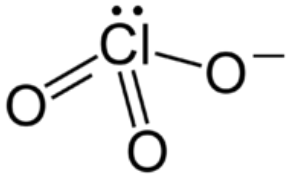
Some Polar Analytes of Interest



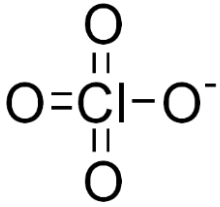
Glyphosate
(*m/z* 168.0067)



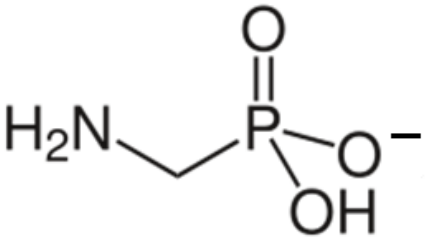
N-acetyl glyphosate
(*m/z* 210.0173)



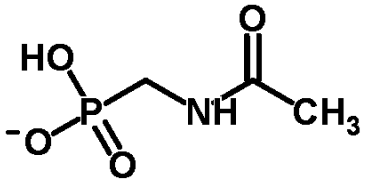
Chlorate
(*m/z* 82.9541)



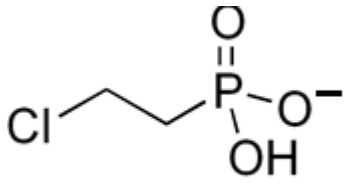
Perchlorate
(m/z 98.9491)



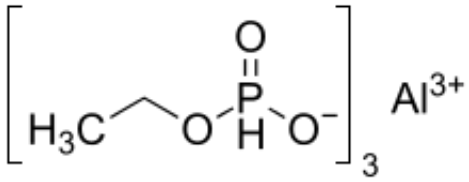
Aminomethylphosphonic acid
(AMPA)
(*m/z* 110.0012)



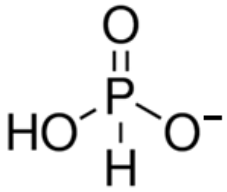
N-acetyl aminomethylphosphonic acid
(N-acetyl AMPA)
(m/z 152.0118)



Etephon
(m/z 142.9670)



Fosetyl-aluminium
(m/z 109.0060)



Phosphonic acid
(m/z 80.9747)

Br^-

Bromide ion
(m/z 78.9189)

Courtesy Prof. Amadeo Rodríguez Fernández-Alba, University of Almeria, Spain ,

QuPPe Method: Pros and Cons

- Extracts contain high amounts of co-extractives: contaminate columns and MS
- Observed variation in retention time (especially glyphosate)
- Variable recoveries/ precision (use labelled internal standards which are costly)
- A number of different column chemistries required
- Cost effective compared to previous approaches (derivatisation etc.)

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-P

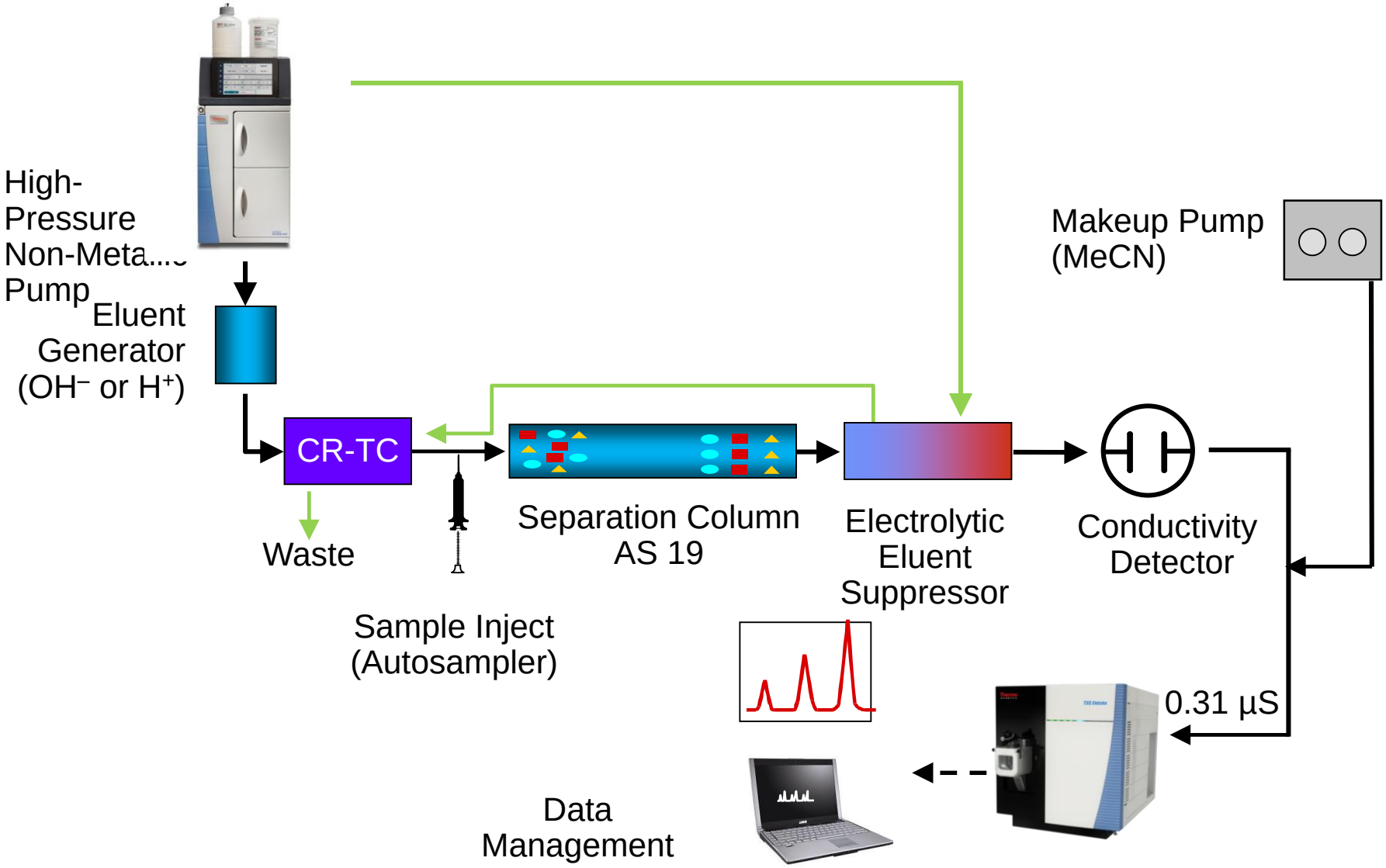
QuPPe-PO v 9.1-Positive Mode Compounds

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

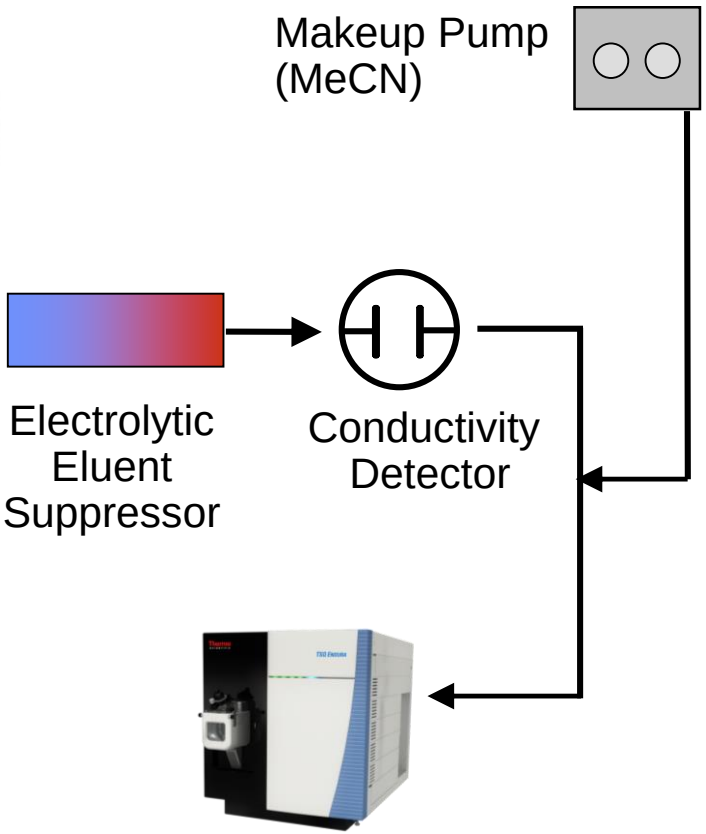
	M 1.1	M 1.2	M 1.3	M 1.4	M 2	M 3	M 4.1	M 4.2	M 5	M 6	M 7	M8
ESI-mode	Neg.	Neg.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
Separation principle	Anion Exchange	Anion Exchange	Carbon	Carbon	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	Carbon
Column type	AS 11	AS 11-HC	Hyper-carb	Hyper-carb	Obelisc-R	Obelisc-R	Obelisc-R	BEH-Amide	PFP	Obelisc-R	Trinity P1	Hyper-carb
POSITIVE MODE												
Amitrole	NT	NT	-	NT	NT	✓	-	✓	NT	NT	NT	NT
ETU	NT	NT	✓	NT	NT	✓	-	✓	✓	NT	NT	NT
PTU	NT	NT	✓	NT	NT	✓	-	✓	✓	NT	NT	NT
Cyromazine	NT	NT	NT	NT	NT	✓	✓	✓	NT	NT	NT	NT
Trimesium	NT	NT	NT	NT	NT	✓	✓	✓	NT	NT	NT	NT
Daminozide	NT	NT	NT	NT	NT	✓	✓	✓	NT	NT	NT	NT
Chlormequat	NT	NT	✓	NT	NT	✓	✓	✓	✓	NT	NT	NT
Mepiquat	NT	NT	✓	NT	NT	✓	✓	✓	✓	NT	NT	NT
Difenzoquat	NT	NT	-	NT	NT	✓	✓	✓	✓	NT	NT	NT
Propamocarb	NT	NT	NT	NT	NT	✓	✓	✓	NT	NT	NT	NT
Melamine	NT	NT	NT	NT	NT	NT	✓	✓	NT	NT	NT	NT
Diquat	NT	NT	-	NT	NT	NT	✓	-	NT	NT	NT	NT
Paraquat	NT	NT	-	NT	NT	NT	✓	-	NT	NT	NT	NT
N,N-Dimethylhydrazine	NT	NT	-	NT	NT	NT	✓	-	NT	NT	NT	NT
Nereistoxin	NT	NT	✓	NT	NT	NT	✓	✓	NT	NT	NT	NT
Streptomycin	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓	NT	NT
Kasugamycin	NT	NT	NT	NT	NT	NT	NT	NT	NT	✓	NT	NT
Morpholine	NT	NT	NT	NT	NT	NT	(✓)	(✓)	NT	NT	✓	NT
Diethanolamine	NT	NT	NT	NT	NT	NT	(✓)	(✓)	NT	NT	✓	NT
Triethanolamine	NT	NT	NT	NT	NT	NT	(✓)	(✓)	NT	NT	✓	NT
1,2,4-Triazole	NT	NT	NT	NT	NT	NT	(✓)	-	NT	NT	NT	✓
Triazole-alanine	NT	NT	NT	NT	NT	NT	(✓)	-	NT	NT	NT	✓
Triazole-acetic acid	NT	NT	NT	NT	NT	NT	(✓)	-	NT	NT	NT	✓
Triazole-lactic acid	NT	NT	NT	NT	NT	NT	NT	-	NT	NT	NT	✓
Aminocyclopyrachlor	NT	NT	NT	NT	NT	NT	NT	✓	NT	NT	NT	NT

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

IC-MS/MS Configuration



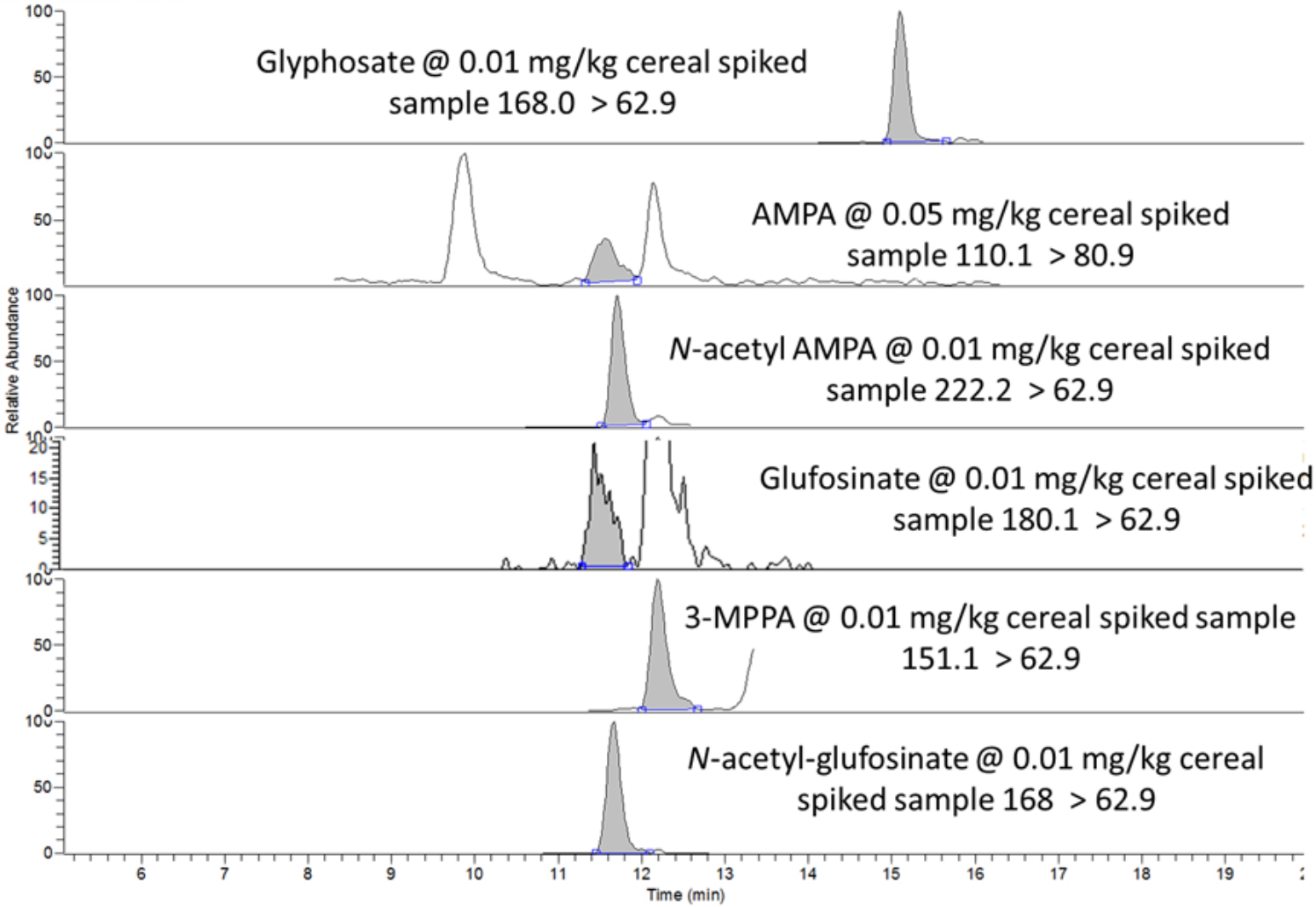
Post Column Suppression and Addition of Organic Modifier



Effect of using post suppressor modifier MeCN	
Analyte	% Increase in response
3-MPPA	391
Chlorate	458
Clopyralid	284
Glufosinate	365
Glyphosate	421
N-acetyl-Glufosinate	360

- Ideal operating back pressure for suppressor is around 100-150 psi
- Monitor conductivity signal

Chromatogram (part A) of Polar Ionic Pesticides in Cereal



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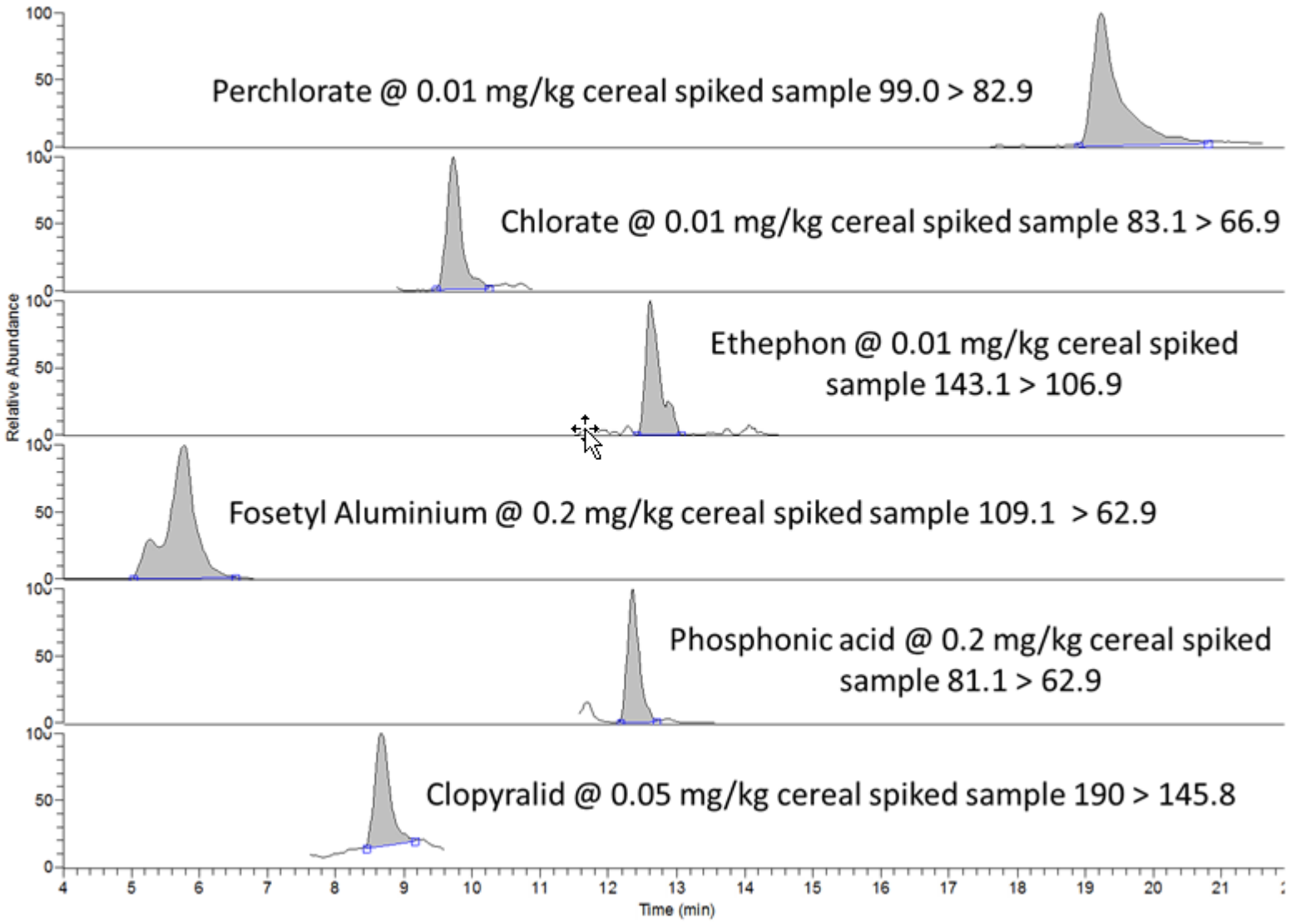
Chromatogram (part B) of Polar Ionic Pesticides in cereal

59



60

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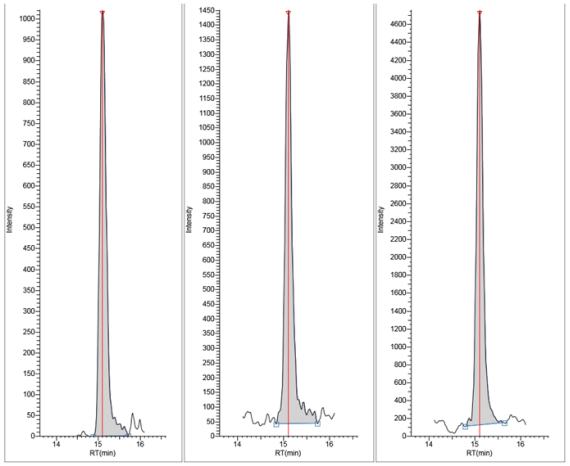
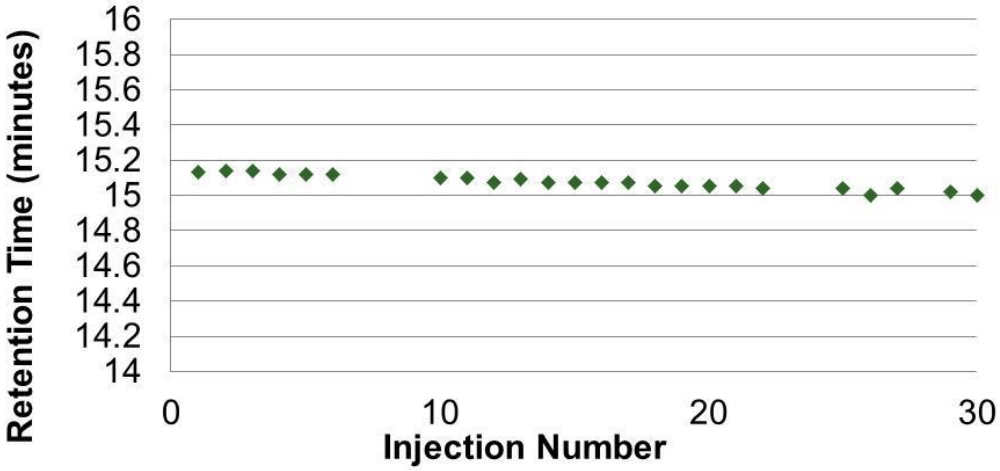
Glyphosate: IC-QQQ Analysis of QuPPe Extracts of Wheat Flour

Glyphosate spiked @ 10 µg/kg

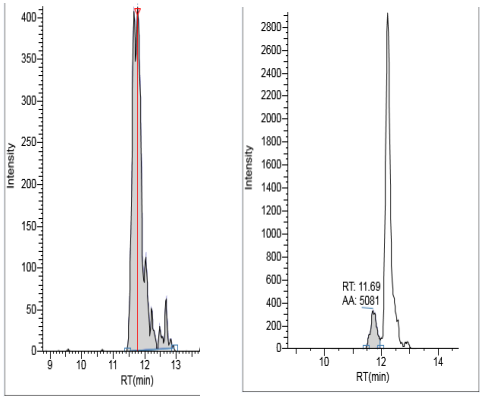


168 >62.9 168 >78.9 168 >149.9

- Stable retention times



AMPA spiked @ 10 µg/kg



110.1>78.9 110.1>62.9

- Multi-analyte capability

Glyphosate	Glufosinate	**Fosetyl**
N-acetyl glyphosate	*N*-acetyl glufosinate	Phosphonic acid
AMPA	3-MPPA	chlorate
N-acetyl AMPA	ethephon	perchlorate

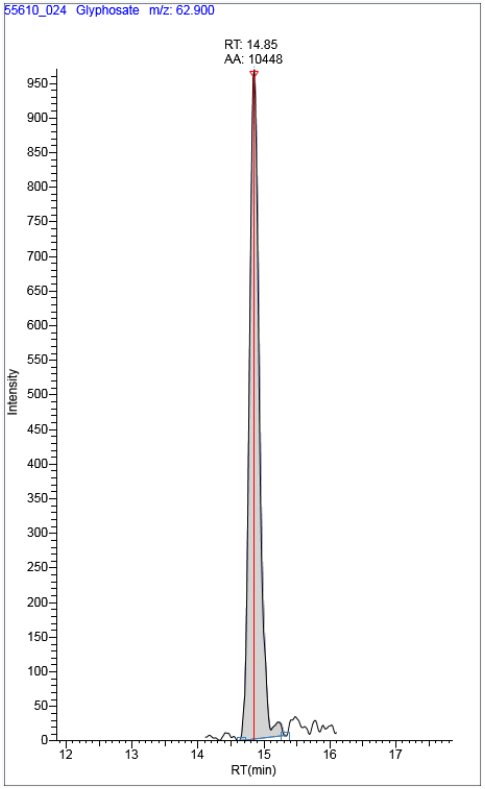
Data Courtesy of Fera Science Ltd UK

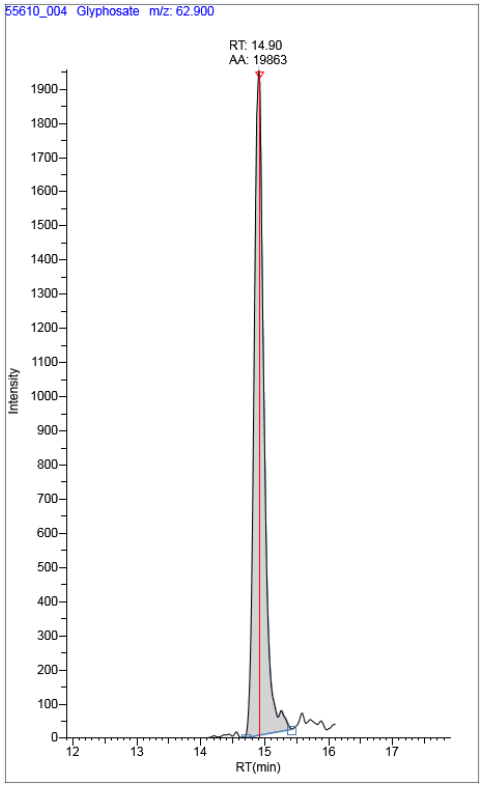
Glyphosate in Beer – No Extraction Required!

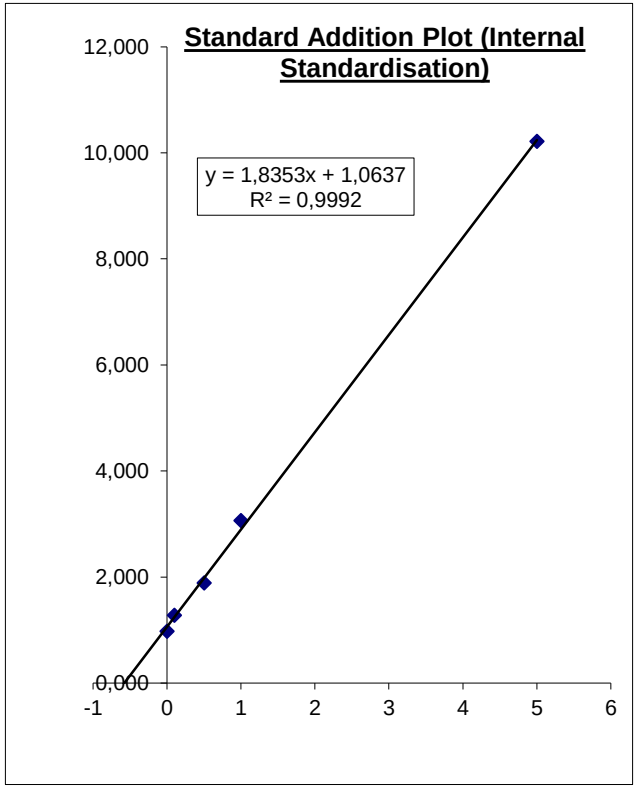
- Glyphosate incurred residue @ 0.58 µg/L

Glyphosate spike @ 0.5 µg/L

Calibration plot 0.1 – 5 µg/L spikes





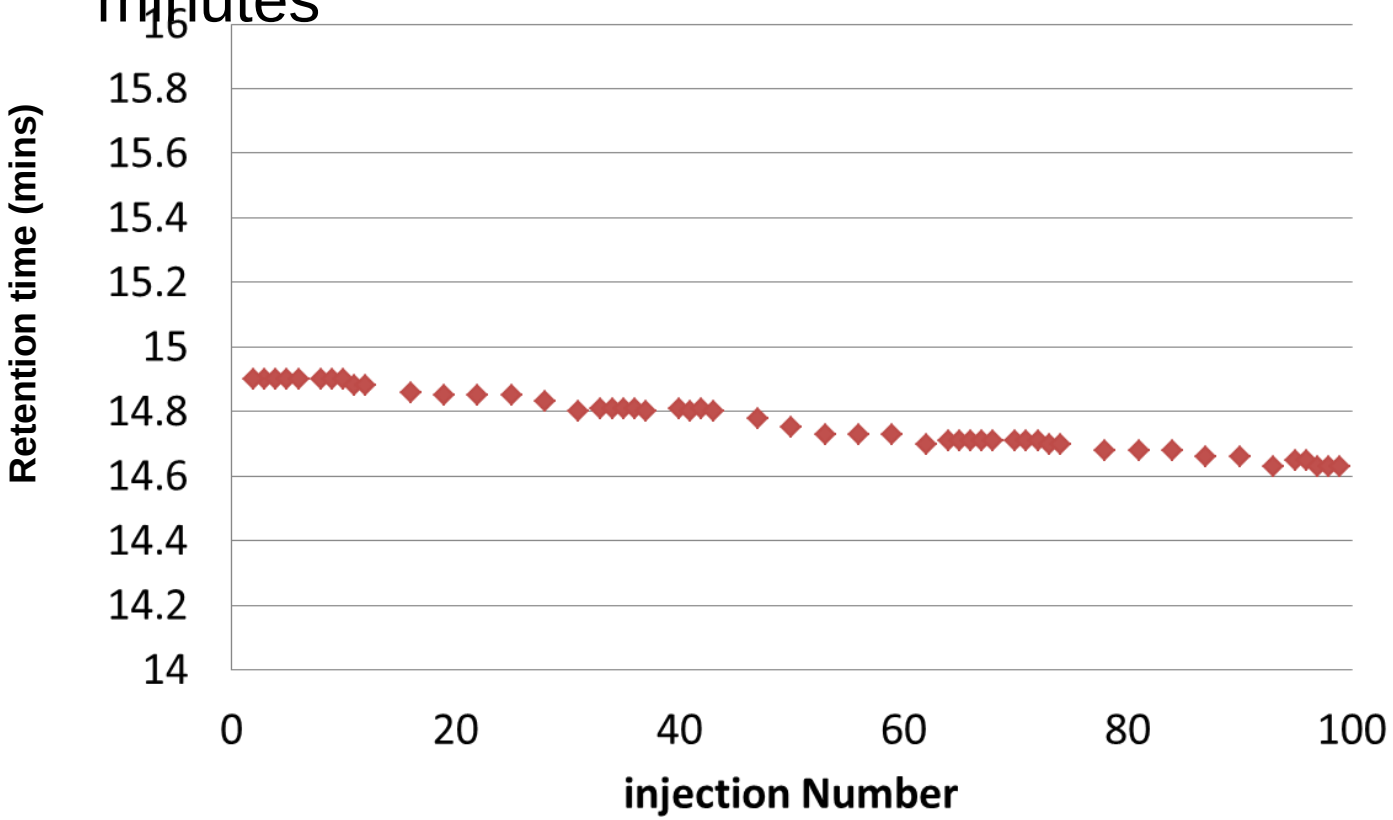


- 1/10 dilution with water and internal standard added

Courtesy of Fera Science Ltd UK

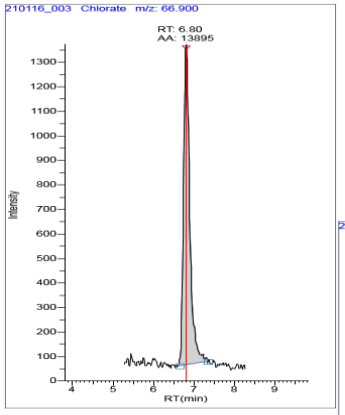
Glyphosate in Beer - Retention Time Stability

- Over 100 injections over 2.5 days, retention time of glyphosate starts at 14.90 minutes and moves to 14.63 minutes

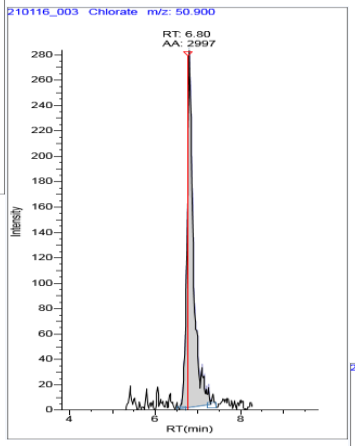


Courtesy of Fera Science Ltd UK

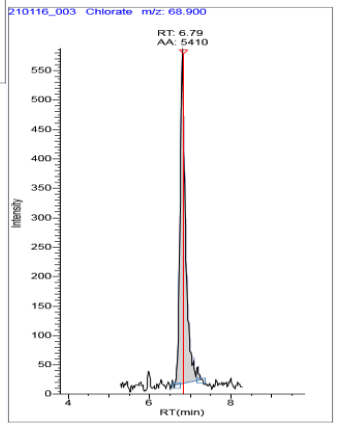
Determination of Chlorate in Dairy Produce @ 5 µg/kg



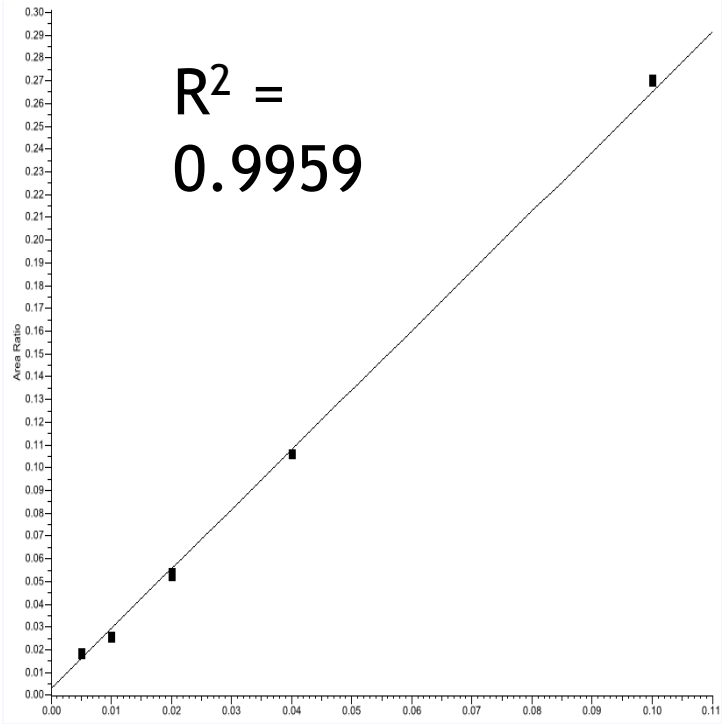
83.1>66.9



83.1>50.9



85.1>68.9



R^2 = 0.9959

- Chlorate in dairy products calibration 5 - 100 µg/kg

Courtesy of Fera Science Ltd UK

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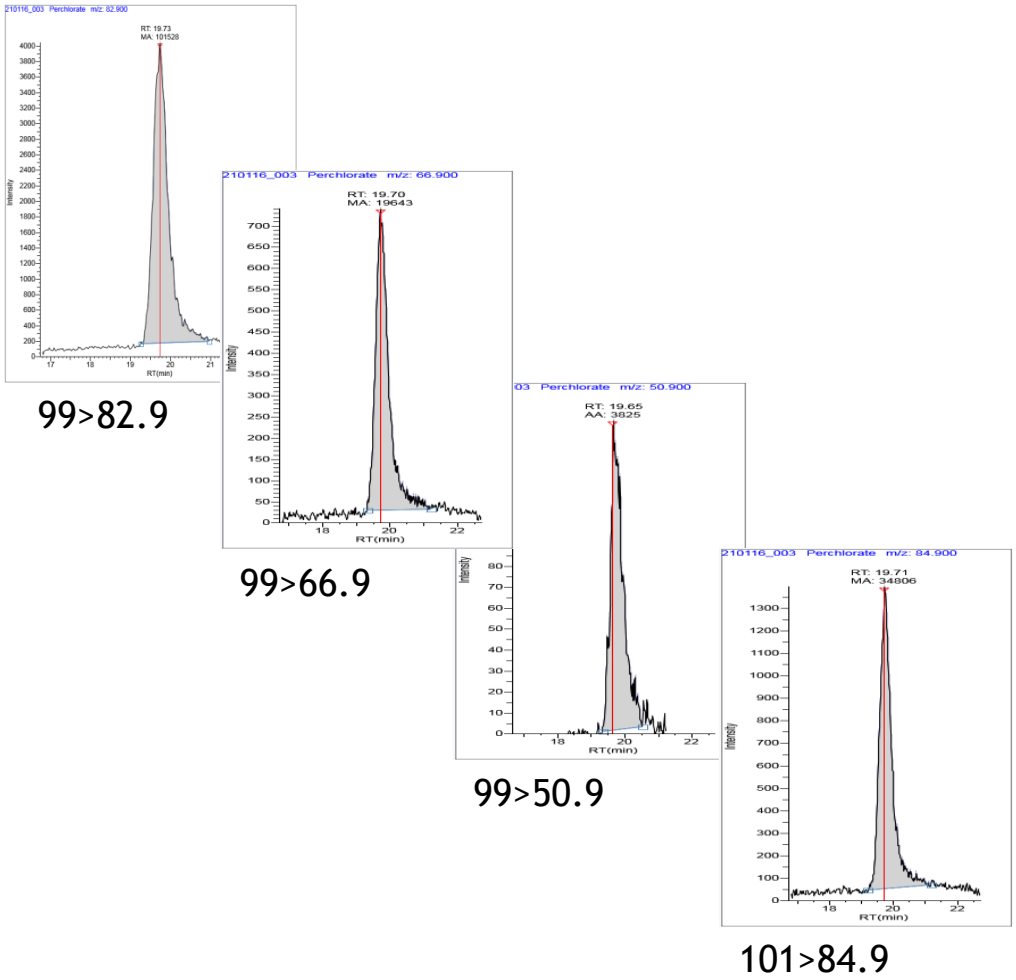
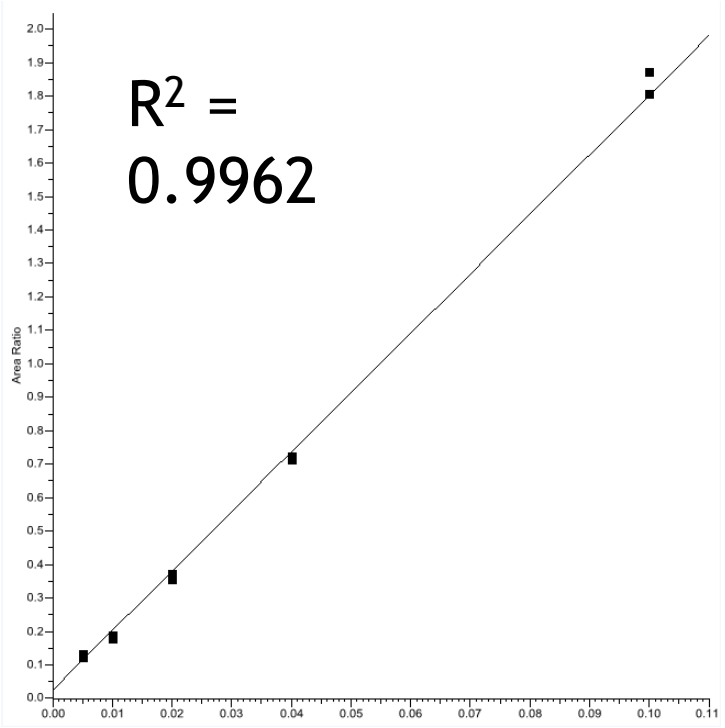
Determination of Perchlorate in Dairy Products @ 5 µg/kg





- Perchlorate in dairy products calibration 5 – 100 µg/kg

Courtesy of Fera Science Ltd UK

Polar Ionic Pesticides - Grape: QuPPE w/wo IS Correction

		IS Corrected		Not corrected	
Compound	Conc^{n} (ng/g)	Mean % Rec (n=5)	Mean % RSD	Mean % Rec (n=5)	Mean % RSD
Glyphosate	10	112	15	96	1
	100	111	7	94	1
AMPA†	10	121	10	92	22
	100	108	8	97	3
***N*-acetyl AMPA**	10	100	2	97	6
	100	99	2	96	2
Glufosinate	10	100	16	108	2
	100	109	8	94	2
3-MPPA	10	106	17	90	2
	100	111	7	96	2
***N*-acetyl Glufosinate**	10	104	15	92	4
	100	110	7	98	2
Perchlorate	10	110	17	94	11
	100	113	6	96	1
Chlorate	10	112	19	92	6
	100	115	6	100	2
Ethephon	0.01	114	17	104	7
	0.1	102	10	95	5
Fosetyl-Al	0.2	**IS not available**		98	3
	2			90	2
Phosphonic acid	0.2			102	2
	2			103	2

Polar Ionic Pesticides – Wheat Flour: QuPPE w/wo IS Correction

		IS Corrected		Not corrected	
Compound	Conc^{n} (ng/g)	Mean % Rec (n=5)	Mean % RSD	Mean % Rec (n=5)	Mean % RSD
Glyphosate	10	104	3	56	9
	100	92	2	48	3
AMPA†	10	69	11	85	4
	100	89	4	80	3
***N*-acetyl AMPA**	10	89	5	97	6
	100	84	2	74	1
Glufosinate	10	120	5	42	31
	100	91	8	104	9
3-MPPA	10	94	5	95	5
	100	88	2	80	4
***N*-acetyl Glufosinate**	10	88	7	98	7
	100	88	2	78	3
Perchlorate	10	107	1	66	2
	100	93	3	56	5
Chlorate	10	91	5	74	3
	100	88	1	67	2
Ethephon	0.01	104	10	66	9
	0.1	87	7	95	6
Fosetyl-Al	0.2	**IS not available**		60	4
	2			72	2
Phosphonic acid	0.2			106	5
	2			97	2

Procedural Standards

- What if Internal Standards are not available /too expensive?
  - Consider use of procedural standards
- What if sample blanks are not available?
  - Consider Standard addition – requires more injections
  - Will provide accurate results- perfect matrix matching
- What if you are dealing with a difficult matrix?
  - Consider ‘over-spiking’ analyse sample twice:
  - once with spiking and once without spiking to check response of eah analyte in matrix

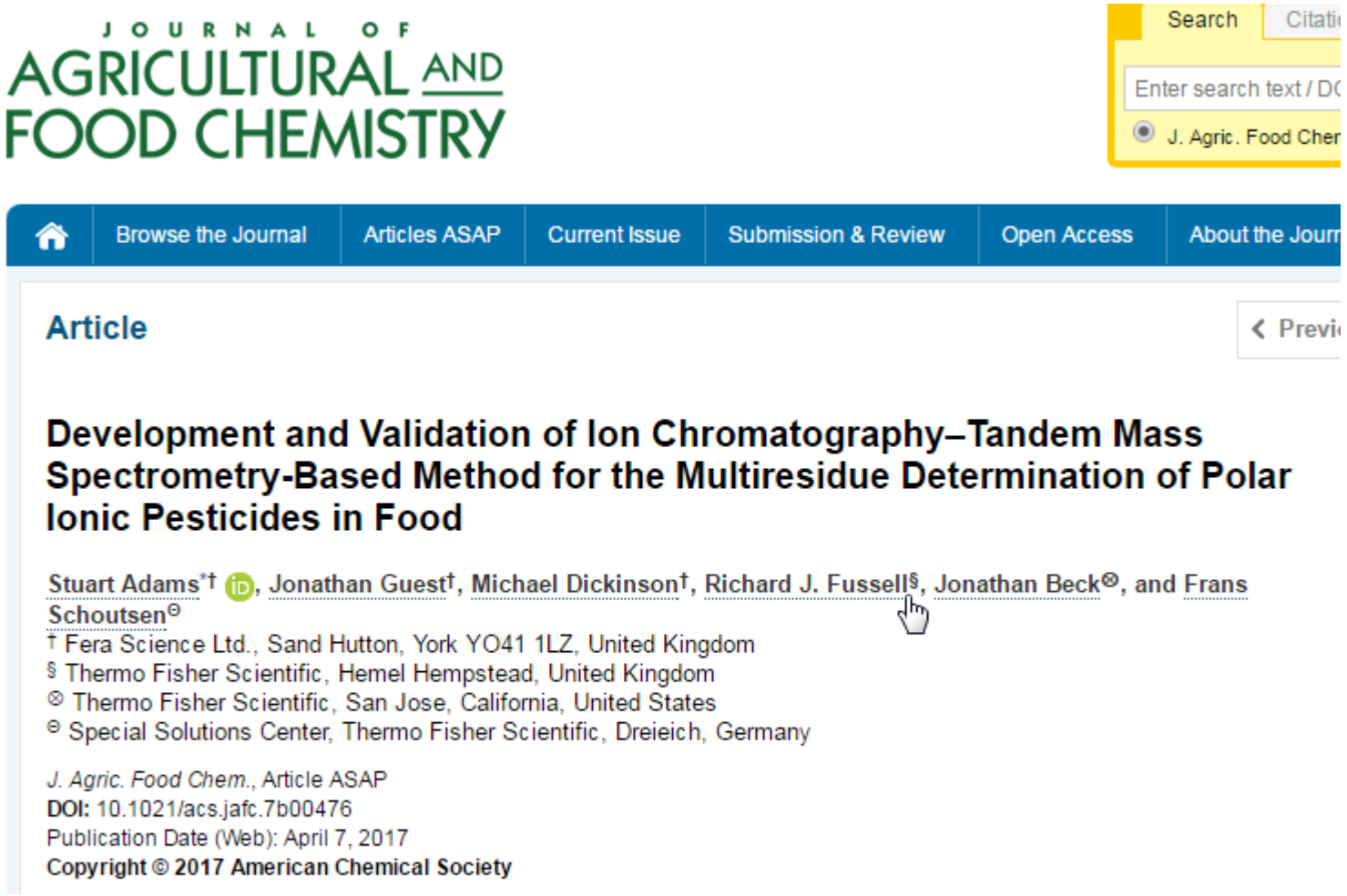
Column Capacity and Robustness – Key for Success

- More than 40 years of history of manufacturing Ion-exchange columns
- Backpressure a good indication of the condition of the column
- Columns are robust and can be cleaned
- 1M KOH (aq) overnight at a low flow rate, then 200 mM H_2SO_4 in 80% acetonitrile at a low flow rate
- Post column suppression is needed to realise the benefits of using high capacity ion-exchange columns.

IC-MS: The Solution to the Problem Analysis of Polar Pesticides?

- The High Capacity of Dionex Ion-exchange Columns
- The Robustness of Ion-exchange Columns
- Compatibility with Mass Spectrometers
- Independent Results in Compliance with Accepted Guideline Criteria
- A Commitment to Continuous Method Improvement

IC-MS Publication



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Previ

Development and Validation of Ion Chromatography–Tandem Mass Spectrometry-Based Method for the Multiresidue Determination of Polar Ionic Pesticides in Food

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Stuart Adams *et al*
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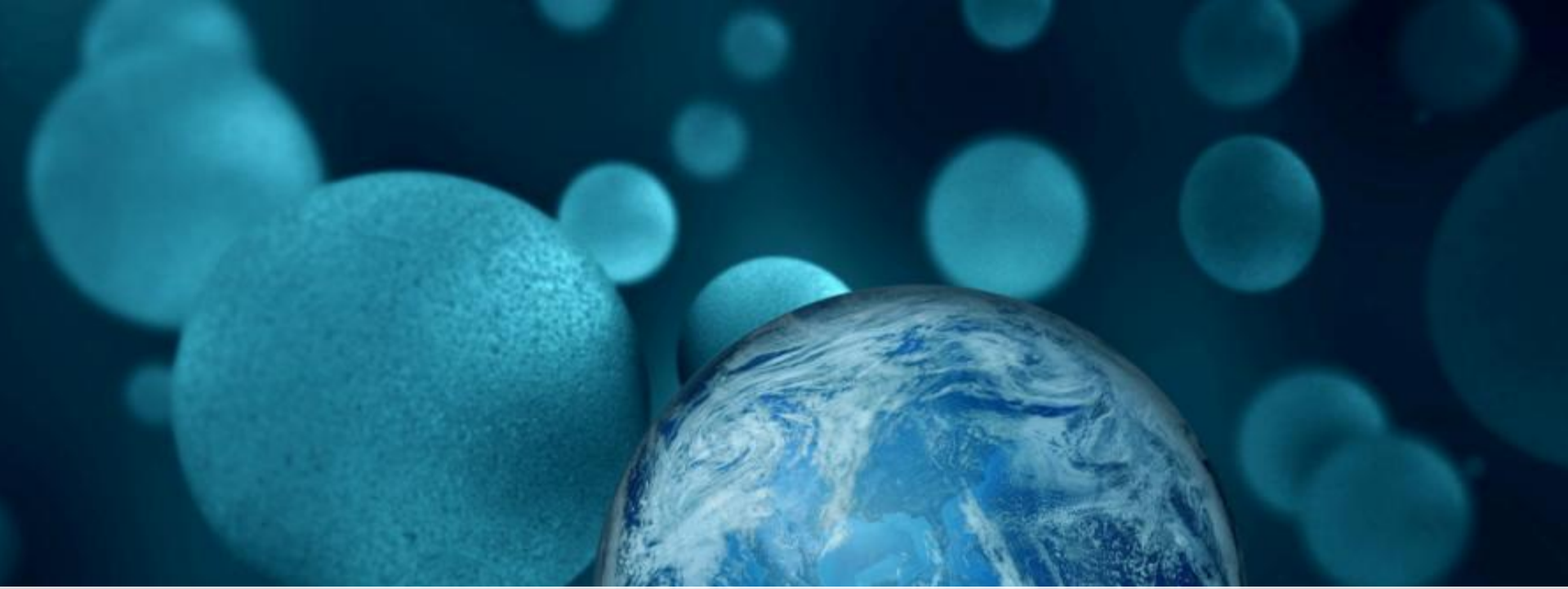
ThermoFisher SCIENTIFIC



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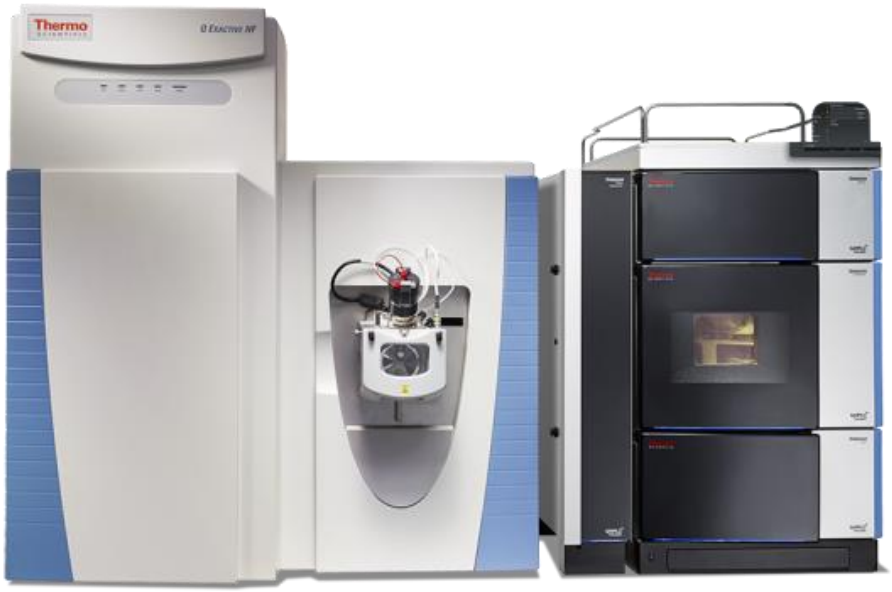
Ion Chromatography: polar pesticides: UHPLC-Orbitrap

Redefining Routine LC-MS Analysis of Pesticides

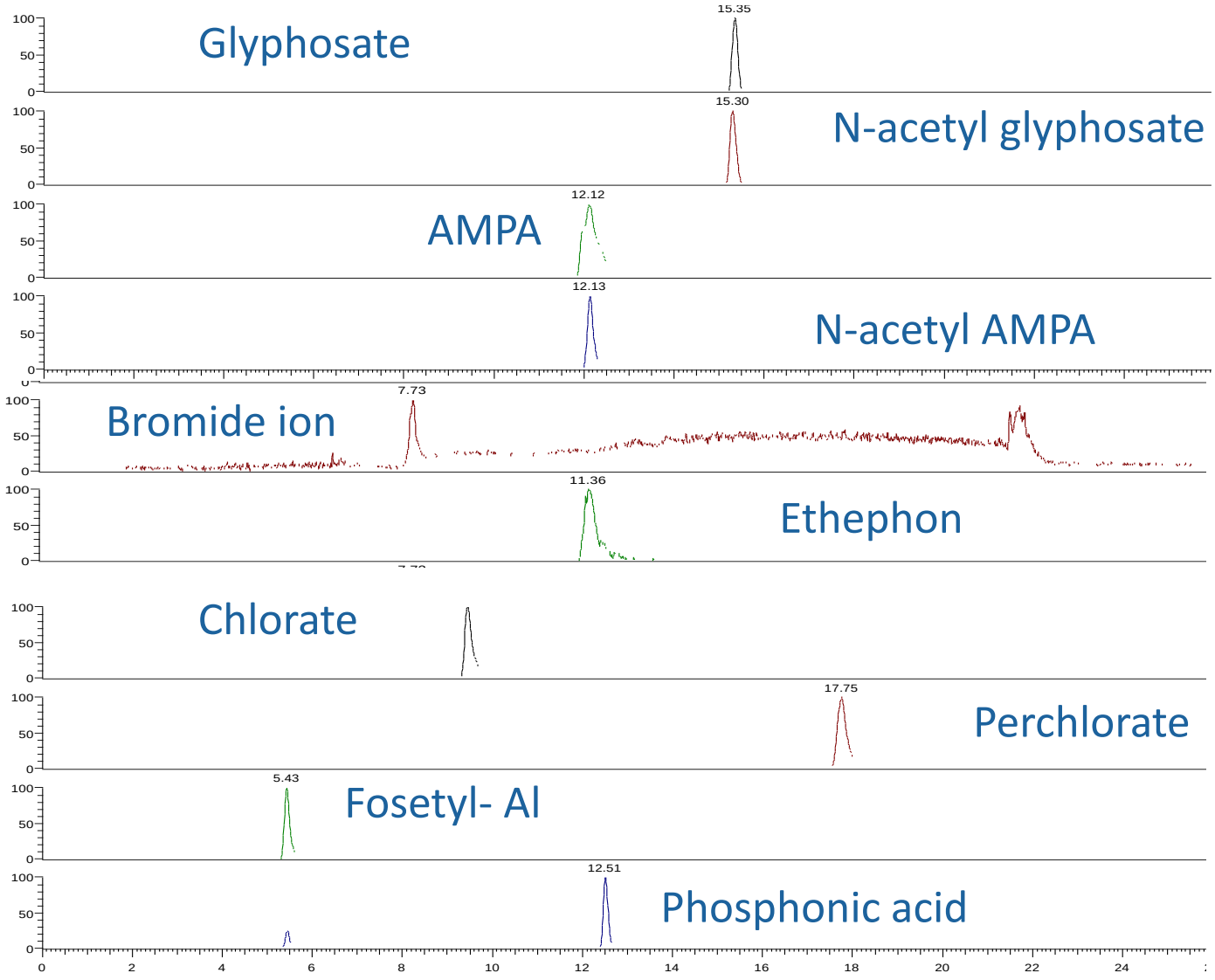
Q Exactive™ Focus hybrid quadrupole-Orbitrap MS



Mass Range	50 < m/z < 2,000
Resolution @ m/z 200	17,500 at 12Hz 35,000 at 6 Hz 70,000 at 3 Hz
Top N	2
Mass Accuracy	< 1ppm RMS, Internal Calibration < 3ppm RMS, External Calibration
Polarity Switching	one full cycle –ve/+ve @ 35.000 RP in <1sec
Flexible Acquisition	Full scan dd-MS2 AIF vDIA PRM (SIM)

vDIA is not available in the U.S.

Extracted Ion Chromatograms of Precursor ions @10 ng/g



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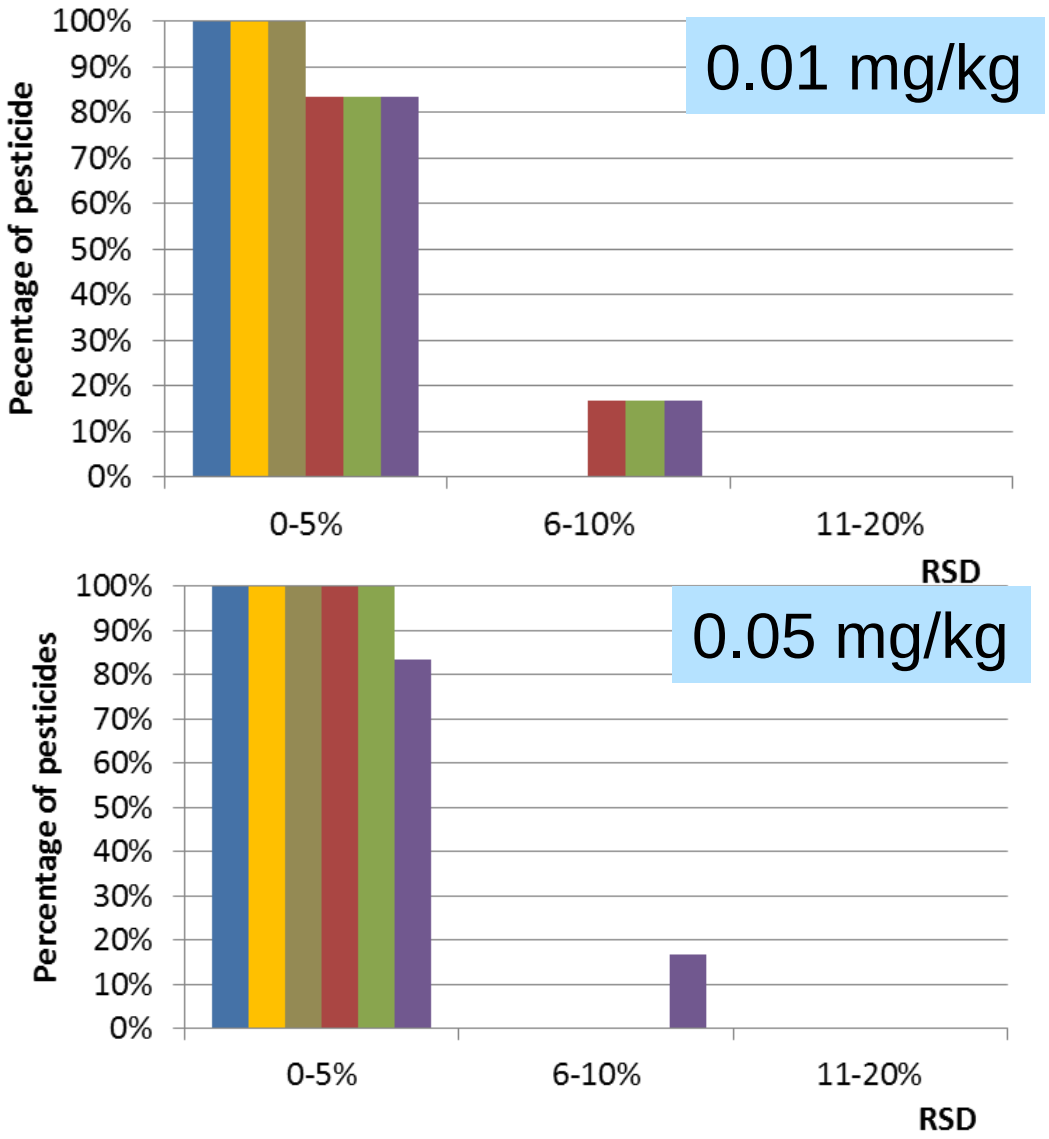
Validation IC Orbitrap Method: Peak Area Repeatability



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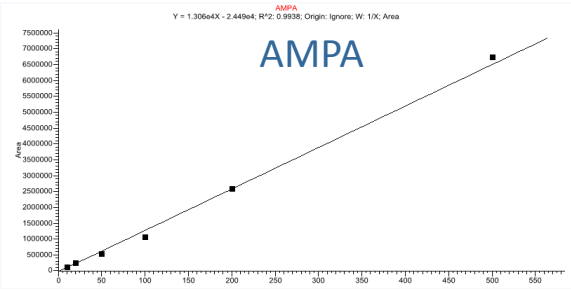
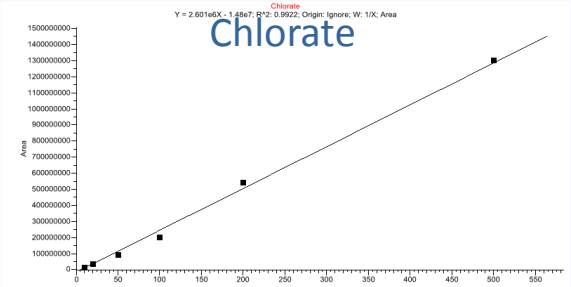
Validation IC Orbitrap Method : Linearity

0.01-0.05 mg/kg

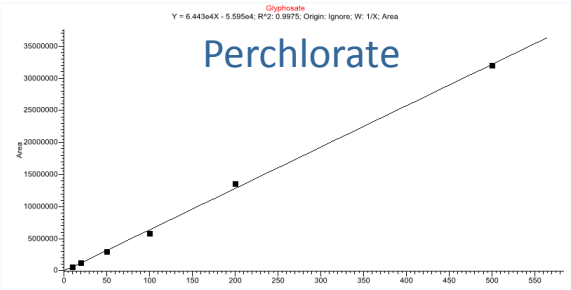
AMPA



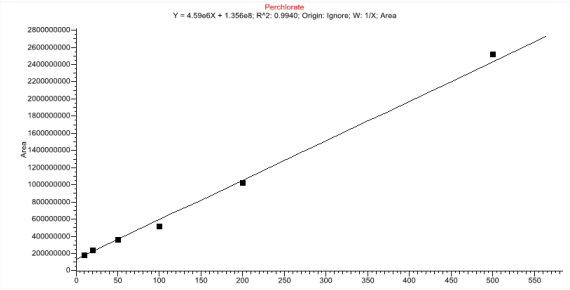
Chlorate



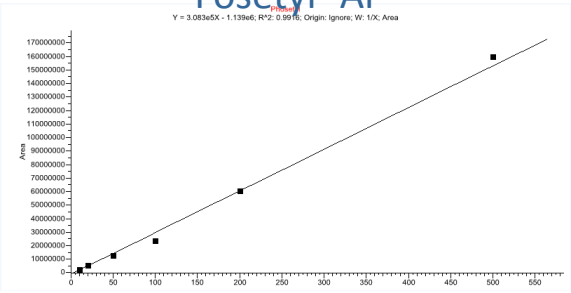
Perchlorate



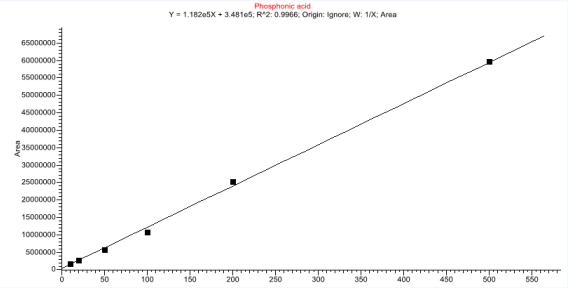
Glyphosate



Fosetyl- Al



Phosphonic acid



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Modified QuPPe-PO

Weigh sample homogenate in 50 mL centrifuge tube

Fresh fuits and vegetables (high water content):

10 ± 0.1 g

↓

Adjust water content of sample to 10 mL

↓

Add 10 mL MeOH

↓

Add 25 µL IS (20 ppm)

(^{13}C-Glyphosate & $^{18}O_3$-Chlorate)

↓

Shake automatically 5 min

↓

Centrifuge at 4000 rpm for 5 min

↓

Transfer 4 mL supernatant into a plastic vial

↓

IC-MS analysis

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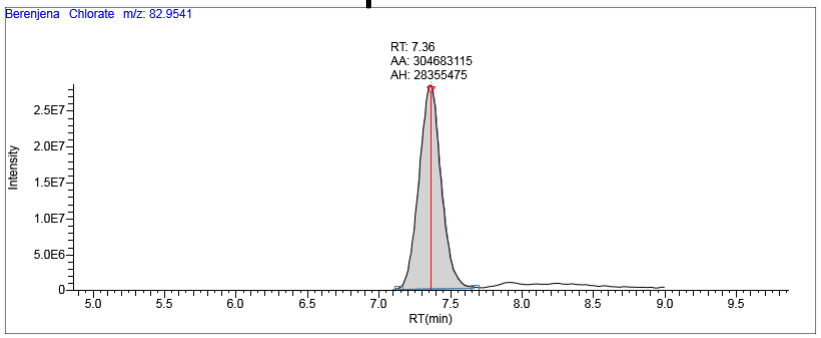
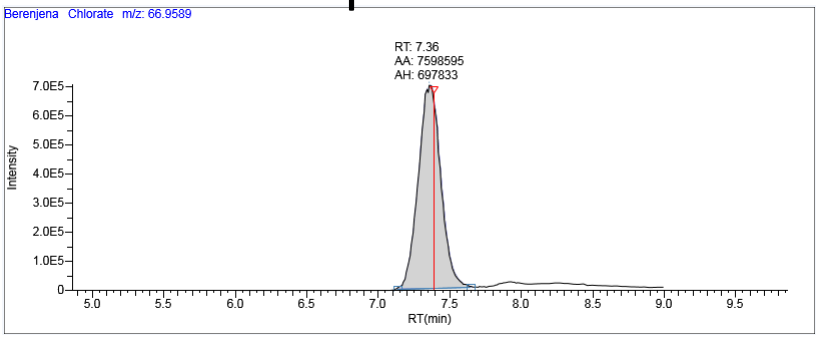
Analysis of Real Samples

Aubergine 0.51 mg/kg of chlorate

quantifier

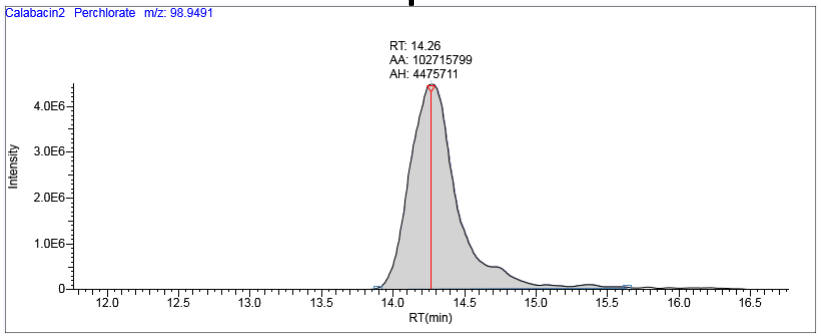


qualifier

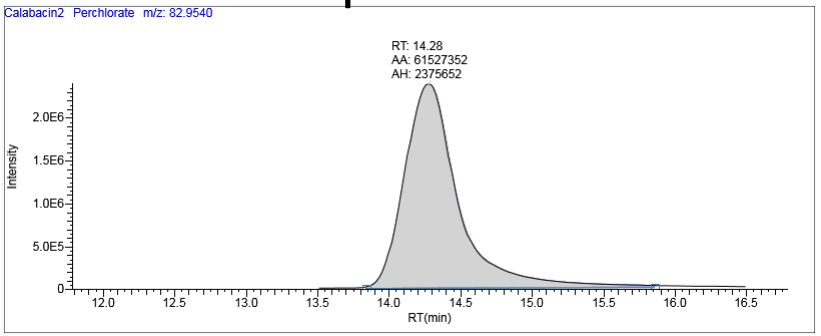


Zucchini 0.075 mg/kg of perchlorate

quantifier



qualifier



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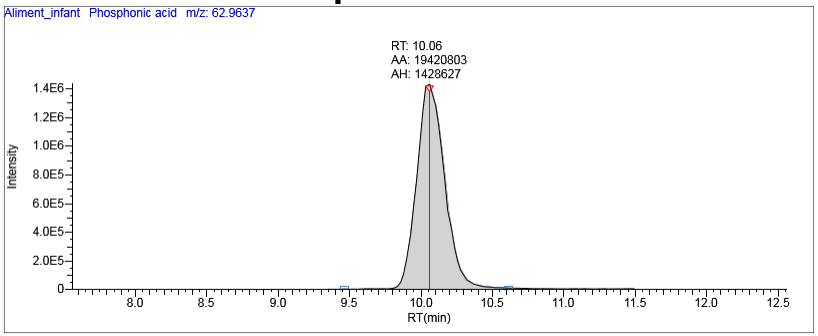
Analysis of Real Samples: More Examples

Baby food 0.26 mg/kg of phosphonic acid

quantifier



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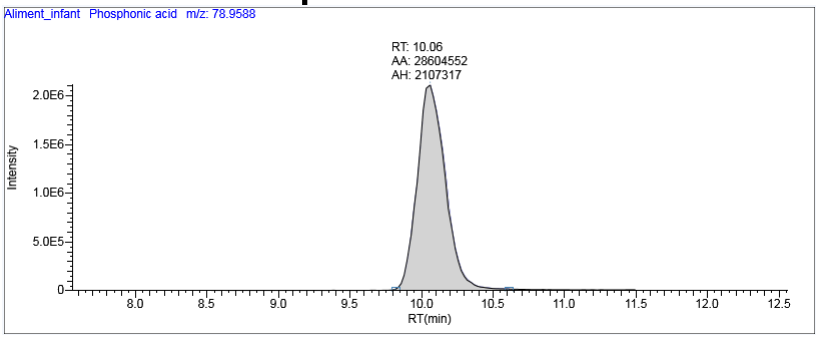
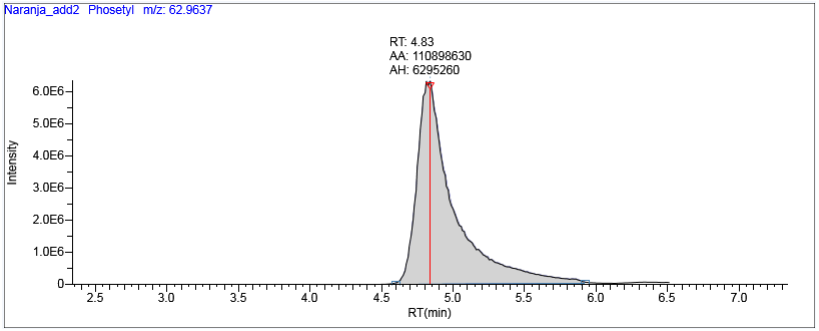


Orange 0.239 mg/kg of fosetyl-Al



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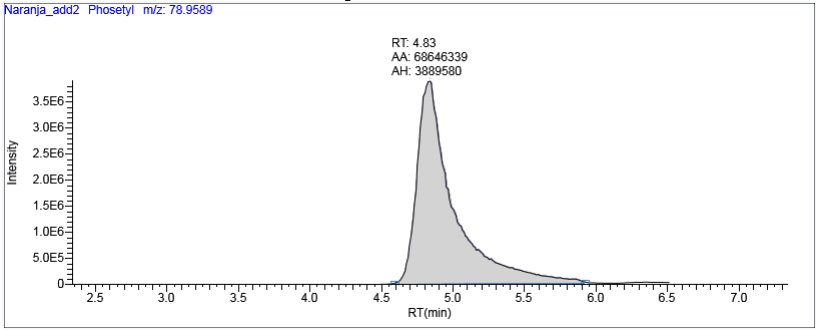

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Summary

- The benefits of ion chromatography: add a new capability to the workflow facilitating the migration of ionic pesticides into multi-residue method
- High capacity columns to cope with high matrix load to provide more resolution and retention time stability
- Robust columns and technology proven over 40 years of development

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