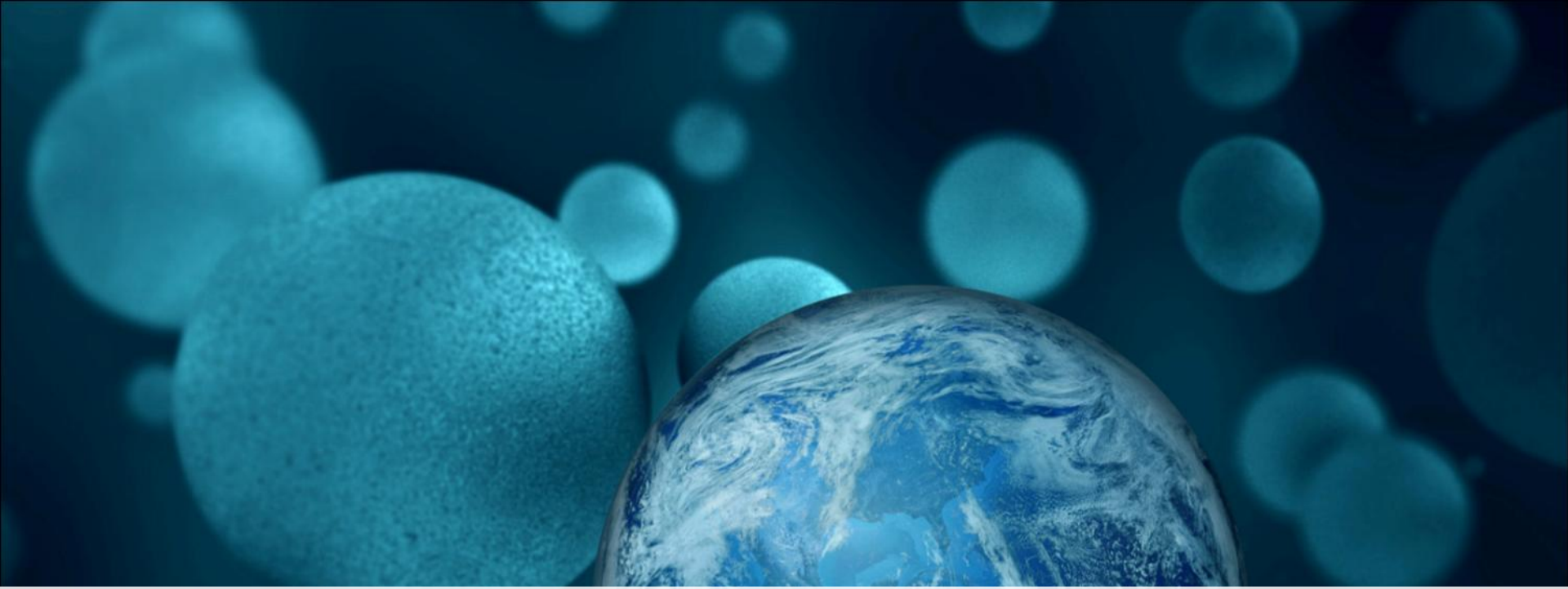

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The analysis of polar ionic pesticides using ion-exchange chromatography coupled to mass spectrometry: turning negatives into positives

Richard Fussell, Vertical Marketing Manager Food & Beverage

11th EPRW, Limassol Cyprus 24th-27th May 2016

Presentation Overview

- An overview of the Quick Polar Pesticides Extraction (QuPPe) Method
  - negative and positive aspects
- An update on the current Status of IC-MS/MS for the determination of polar ionic pesticides
- Recent developments and results from a collaboration between Thermo Fisher Scientific and Fera Science Ltd., York, UK
- Summary including further developments creating new possibilities
- Acknowledgement of co-authors
  - Stuart Adams, Jonathan Guest (Fera Science Ltd, UK)
  - Jonathan Beck and Frans Schoutsen (Thermo Fisher Scientific)

Polar Ionic Pesticides in the News

- Widely used in agricultural production
- 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

QuPPe-PO Method: An Imperfect Compromise

- Generic extraction using acidified methanol- no partition, no clean-up
- The QuPPe method developed by EURL-SRM is not perfect, but is your glass……..

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QuPPe Method: An Imperfect Compromise

Half Empty - negative point of view:

- 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

Half Full - positive point of view:

- Because of the nature of the analytes compromises are inevitable
- Cost effective compared to previous approaches (derivatisation etc.)
- QuPPe has enabled analysis of pesticides monitored infrequently in the past
- Labelled internal standards became commercially available

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

QuPPe-PO v 9.1-Positive Mode Compounds

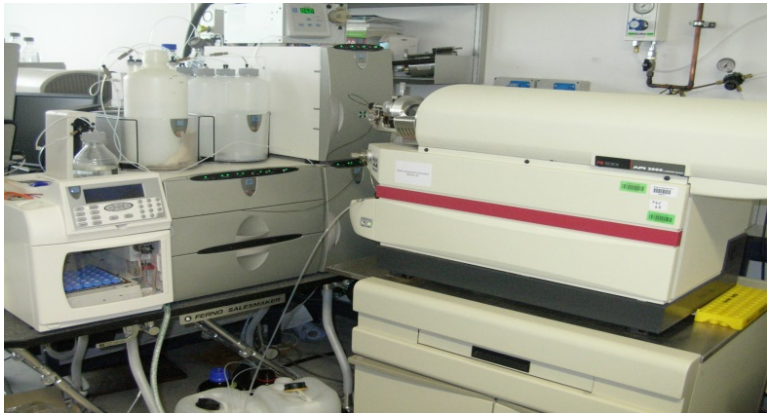
Table 3: Overview and scope of the methods proposed within this document for the QuPPe method:

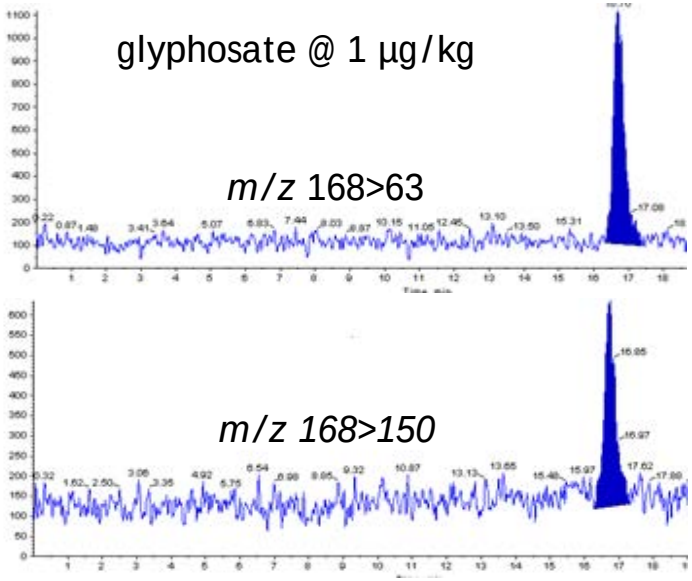
	M 1.1	M 1.2	M 1.3	M 1.4	M 2	M 3	M 4.1	M 4.2	M 5	M 6	M 7	M8
ESI-mode	Neg.	Neg.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
Separation principle	Anion Exchange	Anion Exchange	Carbon	Carbon	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	Carbon
Column type	AS 11	AS 11-HC	Hyper-carb	Hyper-carb	Obelisc-R	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

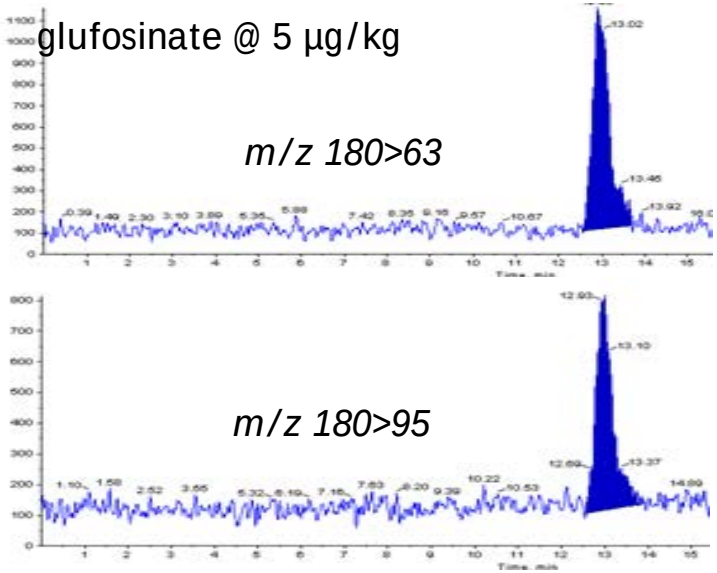
http://www.crl-pesticides.eu/userfiles/file/EurlSRM/meth_QuPPe-PO_EurlSRM.pdf

Can IC-MS/MS Help with the Analysis of Polar Ionic Pesticides?

- 2007: analysis of glyphosate and glufosinate in sugar, dextrins, maltodextrins
- Thermo Scientific™ Dionex™ ICS-3000 system coupled to a Sciex API 2000 MS
- Using large volume injections (up to 4.7 mL) with online concentration and 'clean-up' cartridge







Courtesy of Fera Science Ltd UK

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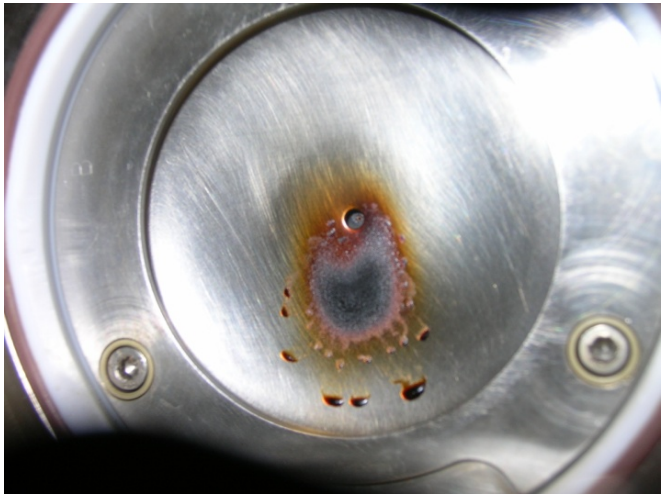
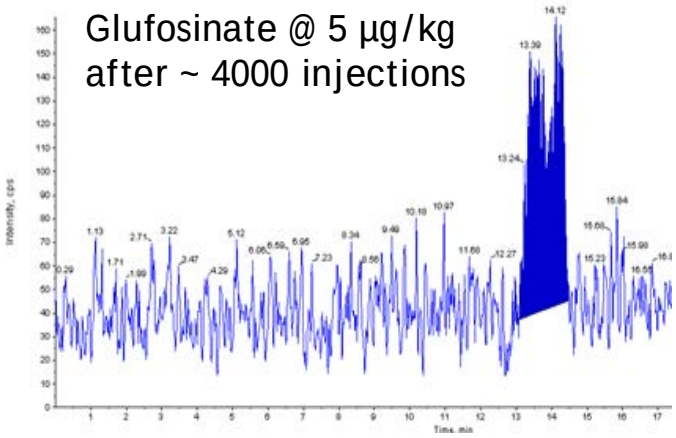
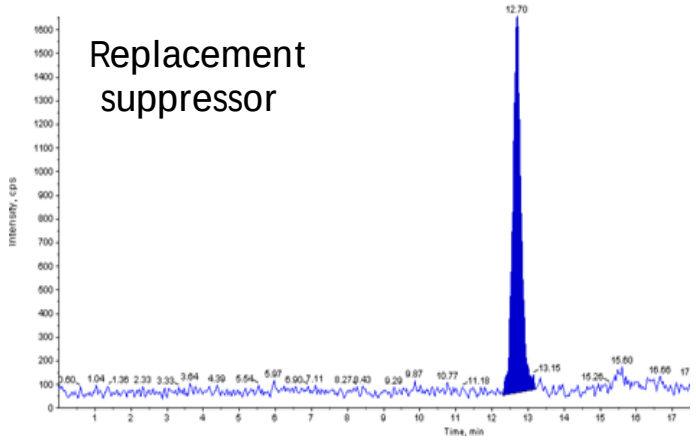
Realities of Using Large Volume Injection

- Pros and Cons
- Enables low limits of quantification
- Faster contamination of columns requiring regular offline cleaning
- Contamination of the suppressor
- Contamination of the MS system



after 100 injections



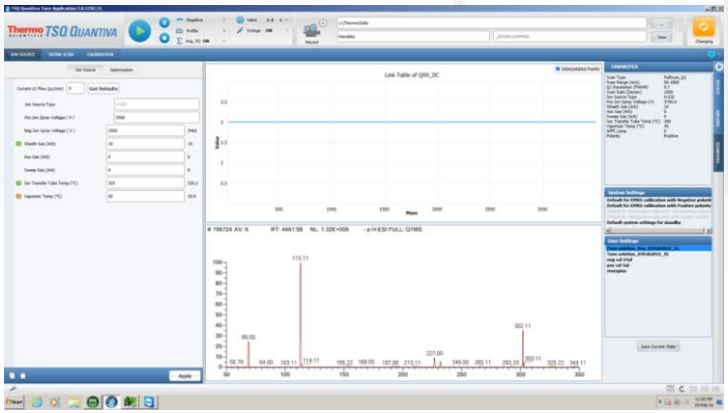


Courtesy of Fera Science Ltd UK

ThermoFisher SCIENTIFIC

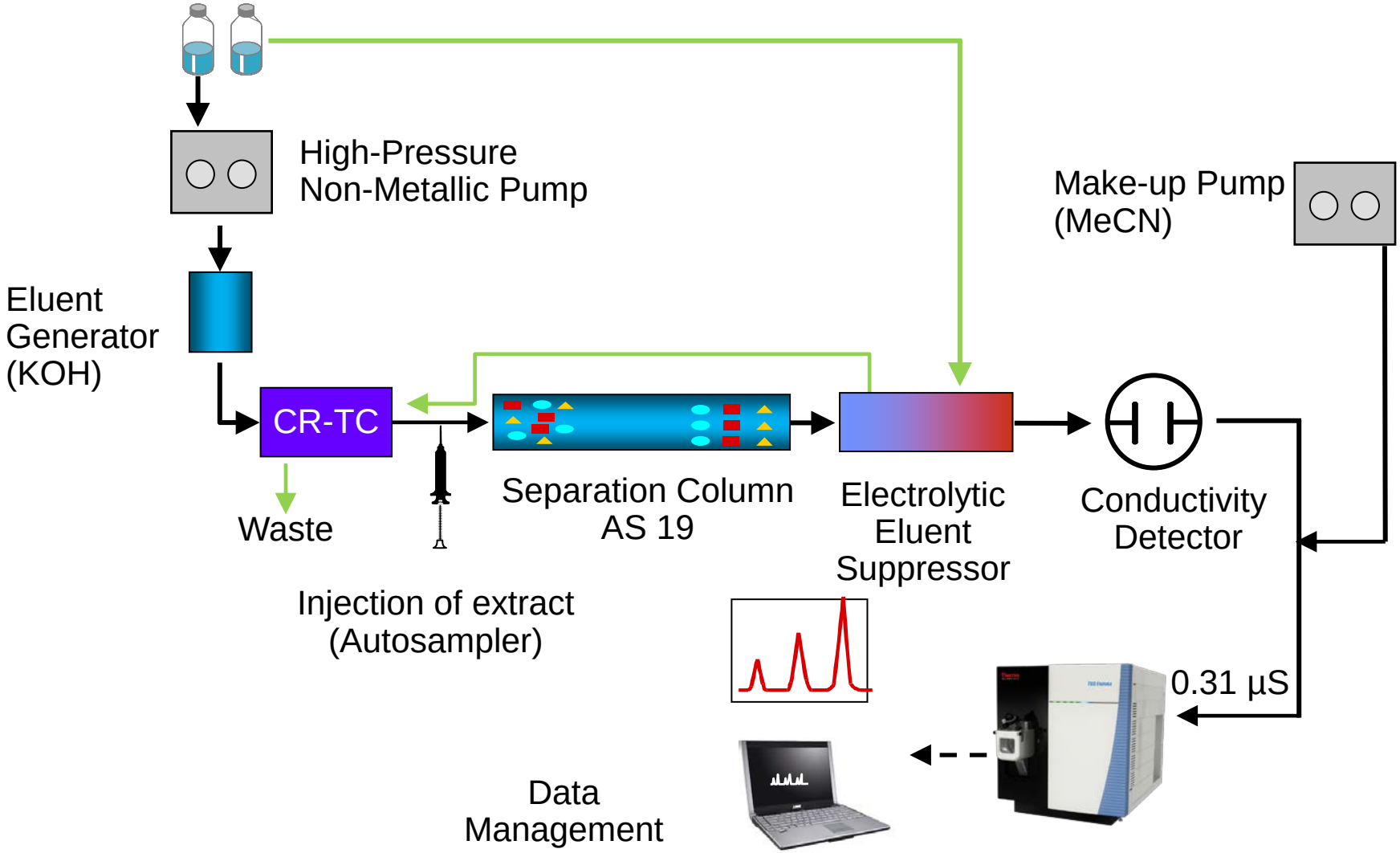
The Latest High Sensitivity IC-MS/MS (2016)

- Collaboration with Fera Science Ltd UK
- Currently evaluating the Thermo Scientific™ Dionex™ ICS-5000 HPIC™ system
- Fully integrated with Thermo Scientific™ TSQ™ Quantiva™ Triple Quadrupole MS
- Integrated system control via a single software package – Thermo Scientific™ TraceFinder™ 3.2 software
- Multi-residue analysis of polar ionic pesticides in 'QuPPe' extracts



- New generation IC columns with 4 µm particle size
- MS tune optimised for low mass
- High sensitivity allowing lower volume injections
- Ability to change ion transfer tube while the system under vacuum

IC-MS/MS Configuration



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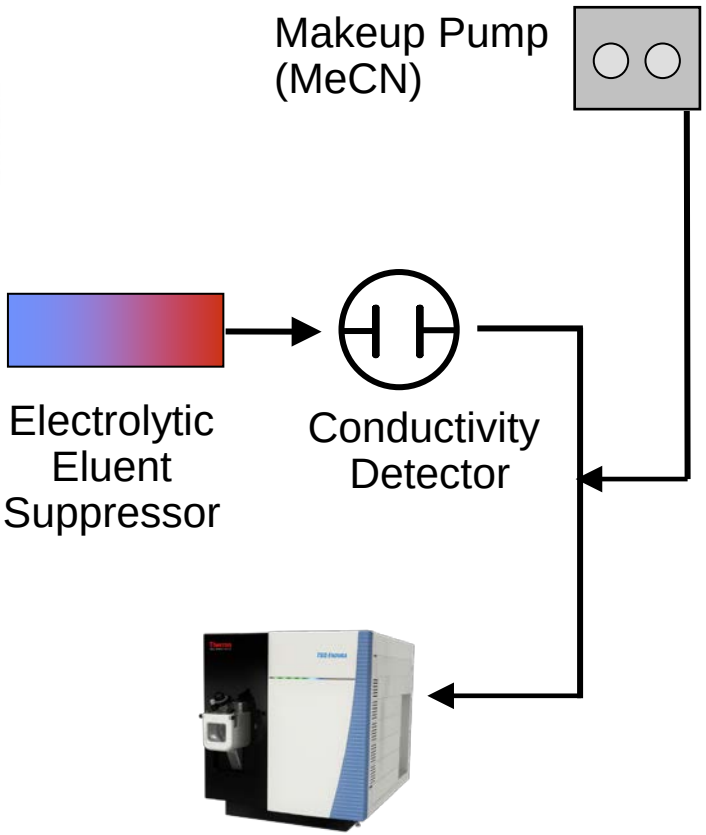
Column Capacity and Robustness – Key for Success

- 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.

Post Column Suppression and Addition of Organic Modifier

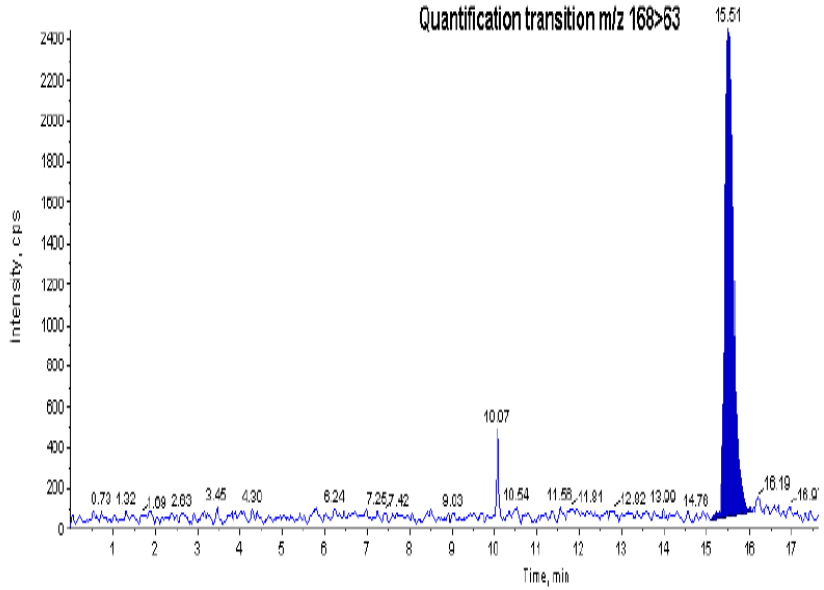


Effect of using post suppressor modifier MeCN	
Analyte	% Increase in response
3-MPPA	391
chlorate	458
clopyralid	284
glufosinate	365
glyphosate	421
N-acetyl-glufosinate	360

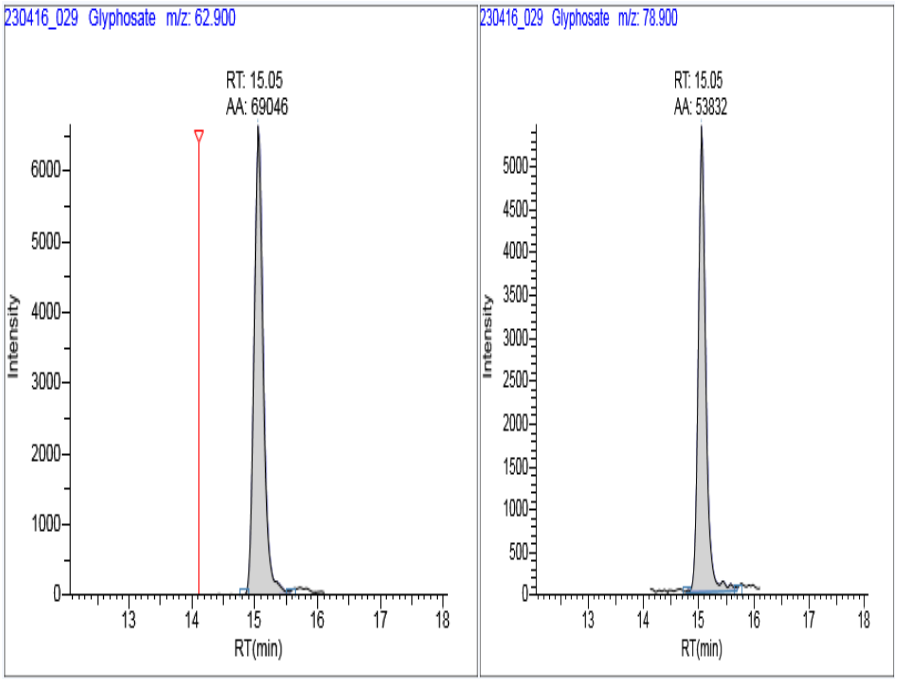
- Ideal operating back pressure for suppressor is around 100-150 psi
- Monitor conductivity signal

From the Past to the Present; What a Difference!

- 2007: glyphosate @ 100 µg/kg - in cereal with 2500 µL injection and inline concentration



- 2016: glyphosate @ 100 µg/kg- 1/10 extraction dilution of QuPPE extracts of wheat flour, 100 µL loop injection
- Equivalent to 10 µL of extract

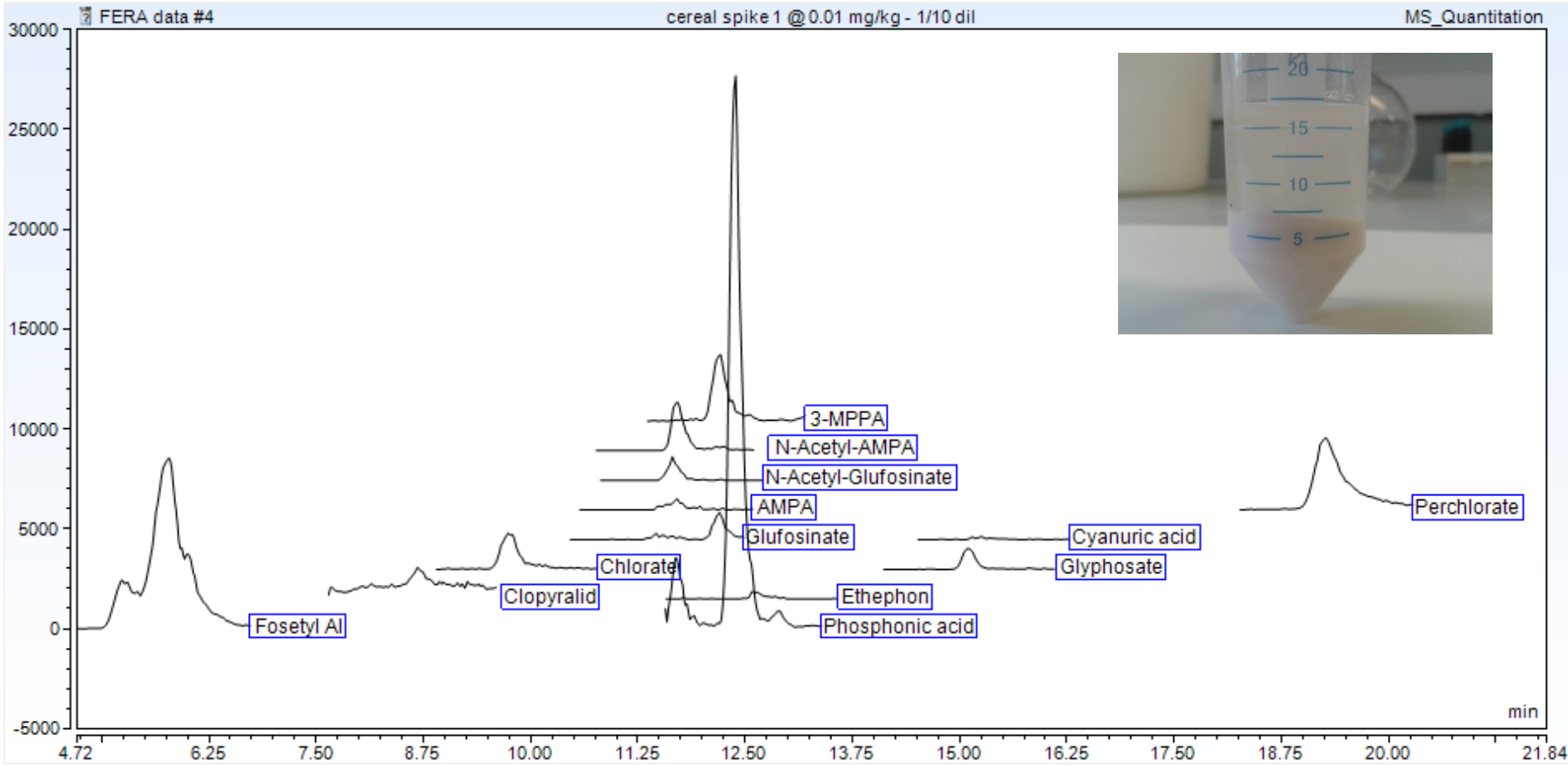


Courtesy of Fera Science Ltd UK

ThermoFisher SCIENTIFIC

IC-MS/MS Multiresidue RIC for Cereal (Wheat Flour)

- All analytes at 10 µg/kg except fosetyl & phosphonic acid (@ 200 µg/kg)



Courtesy of Fera Science Ltd UK

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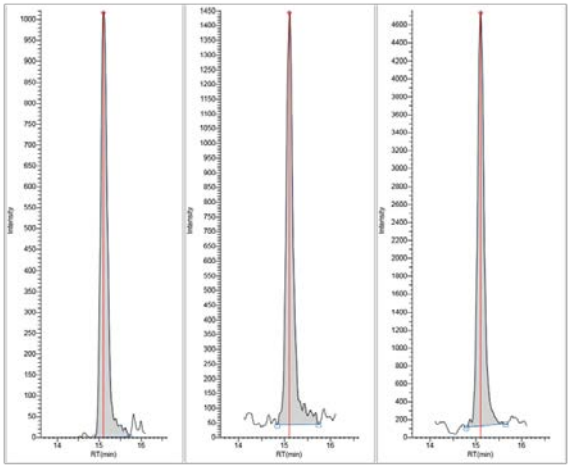
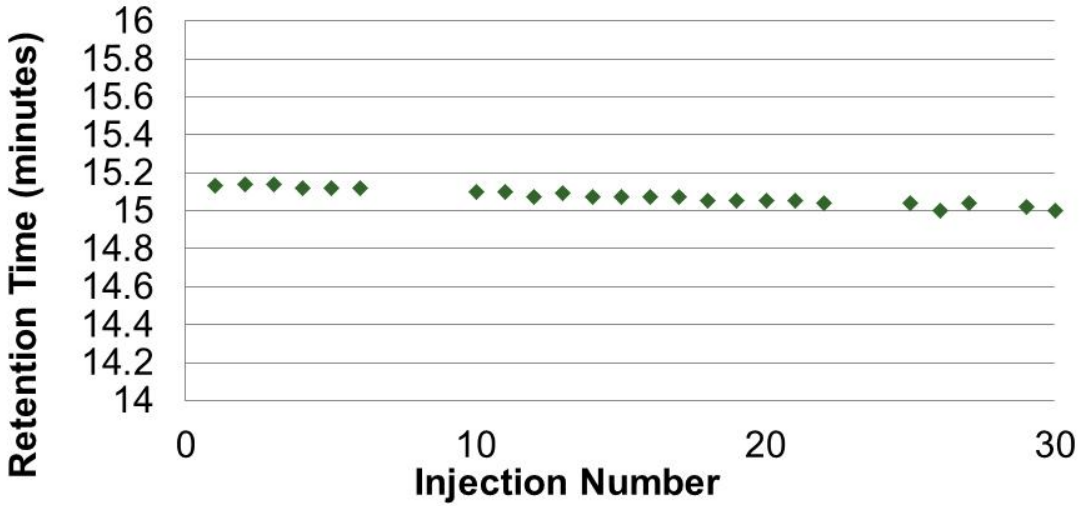
Glyphosate and AMPA in QuPPe Extracts of Wheat Flour

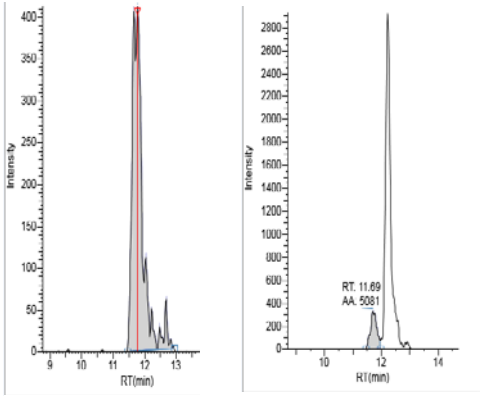
Glyphosate spiked @ 10 µg/kg



168 >62.9 168 >78.9 168 >149.9



AMPA spiked @ 10 µg/kg



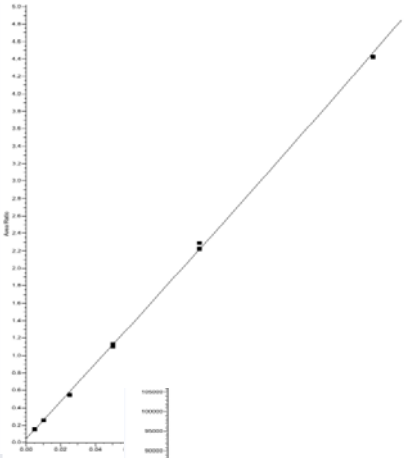
110.1>78.9 110.1>62.9

- Retention time for glyphosate decreased 0.13 minutes after 30 injections of QuPPE extracts of wheat flour

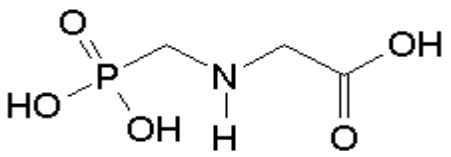
Courtesy of Fera Science Ltd UK

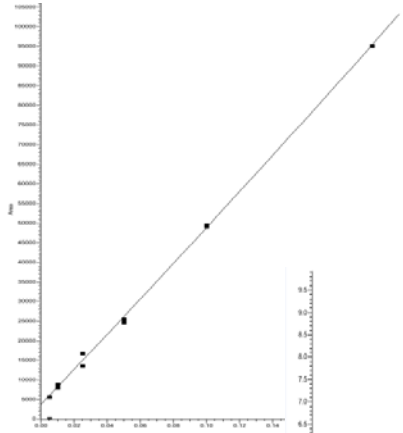
Summary of Validation Results (Wheat Flour)

Compound	Conc^{n} (µg/kg)	Mean Rec (%) (n=5)	RSD (%)
Glyphosate (IS)	10	112	15
	50	108	12
	100	111	7
AMPA (no IS)	10	92	22
	50	98	13
	100	97	3
N-acetyl-AMPA (no IS)	10	85	7
	50	82	10
	100	86	2

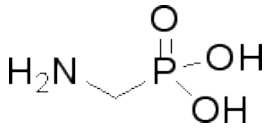


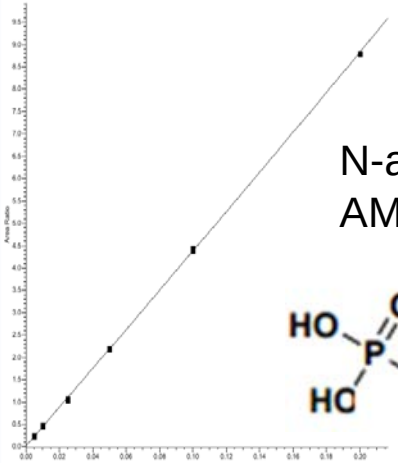
glyphosate



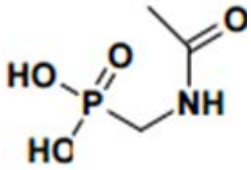


AMPA





N-acetyl AMPA



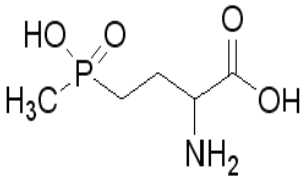
Note: N-acetyl glyphosate standard not available

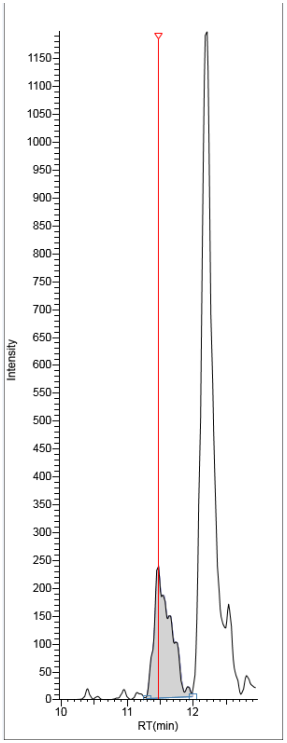
Courtesy of Fera Science Ltd UK

Glufosinate & Metabolites in Cereal (Wheat Flour)

Compound	Concn (µg/kg)	Mean Rec (%) (n=5)	RSD (%)
Glufosinate (IS)	10	100	16
	50	109	11
	100	109	8
3-MPPA (IS)	10	106	17
	50	108	13
	100	111	7
N-Acetyl-Glufosinate (IS)	10	88	6
	50	88	9
	100	91	3

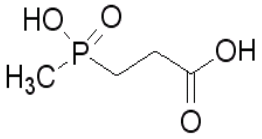
Glufosinate

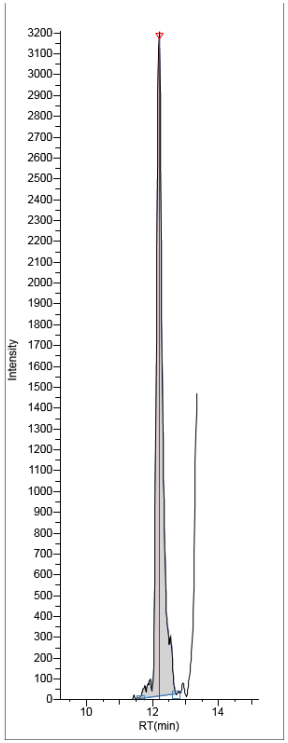




80.1>62.9

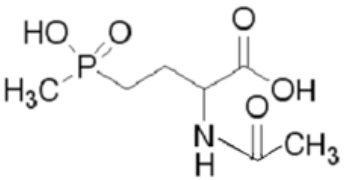
3-MPPA

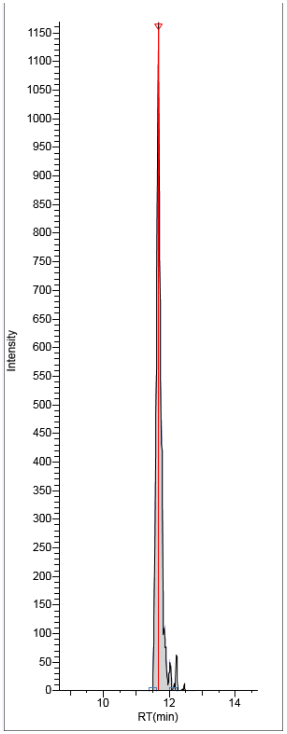




151.1 >62.9

N-Acetyl Glufosinate





222.2 >62.9

Courtesy of Fera Science Ltd UK

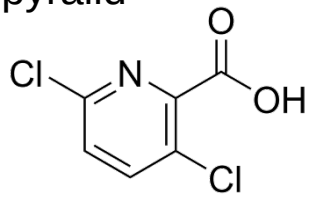
ThermoFisher SCIENTIFIC

Summary of Validation Results (II): Wheat Flour

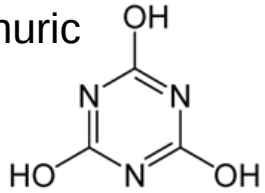
Analyte	Conc^{n} (µg/kg)	Mean Rec (%) (n=5)	RSD (%)
Perchlorate (IS)	10	95	6
	50	90	7
	100	92	9
Chlorate (IS)	10	93	5
	50	88	2
	100	87	4
Ethephon (IS)	10	95	11
	50	86	4
	100	85	4
Clopyralid	10	Insufficient S/N	
	50	70	5
	100	89	6

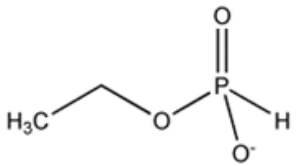
Analyte	Conc^{n} (µg/kg)	Mean Rec (%) (n=5)	RSD (%)
Fosetyl Al	200	60	4
	1,000	71	4
	2,000	72	2
Phosphonic acid	200	106	5
	1,000	94	4
	2,000	97	2
Cyanuric acid (IS)	10	Insufficient S/N	
	50	75	31
	100	88	13

clopyralid

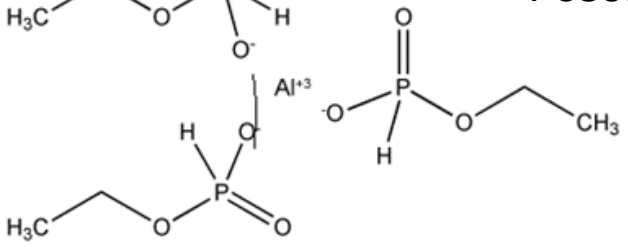


cyanuric acid





Fosetyl-Al

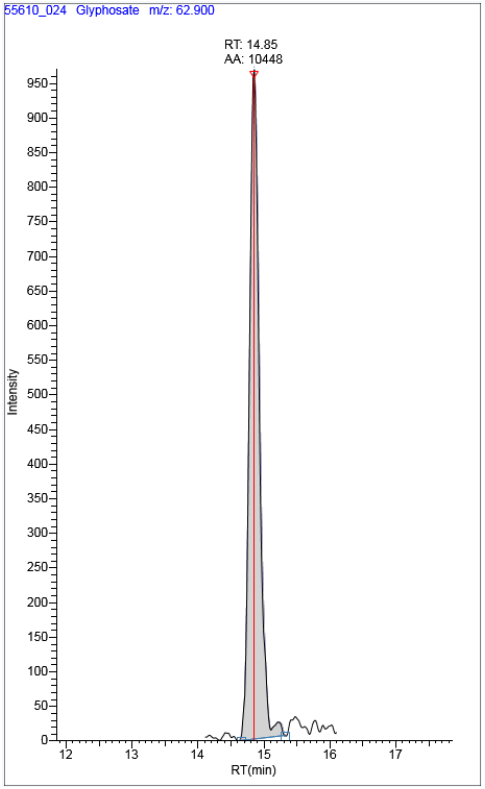


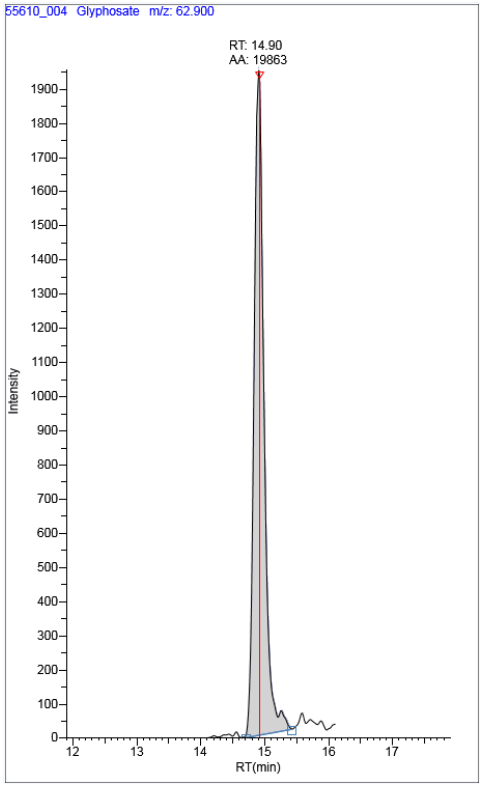
Courtesy of Fera Science Ltd UK

Glyphosate in Beer – No Extraction Required

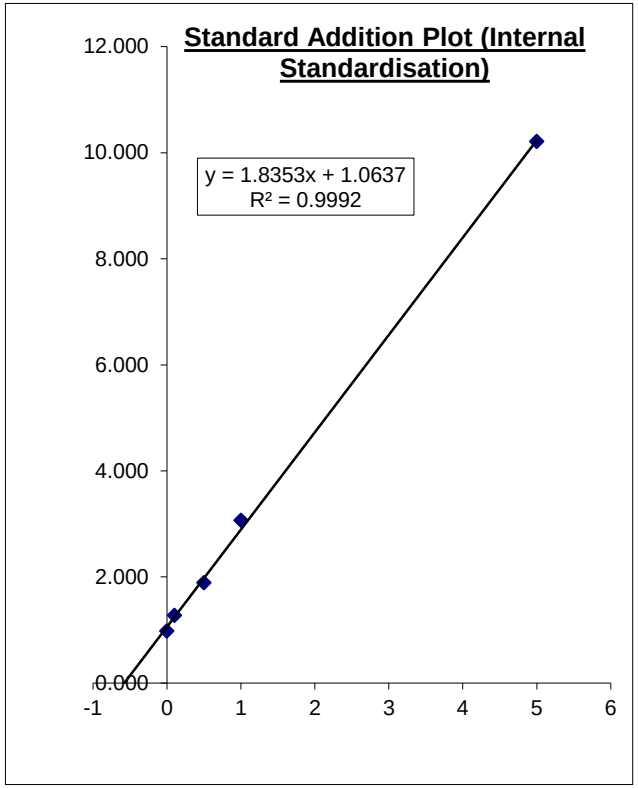
- Glyphosate incurred residue @ 0.58 µg/L

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

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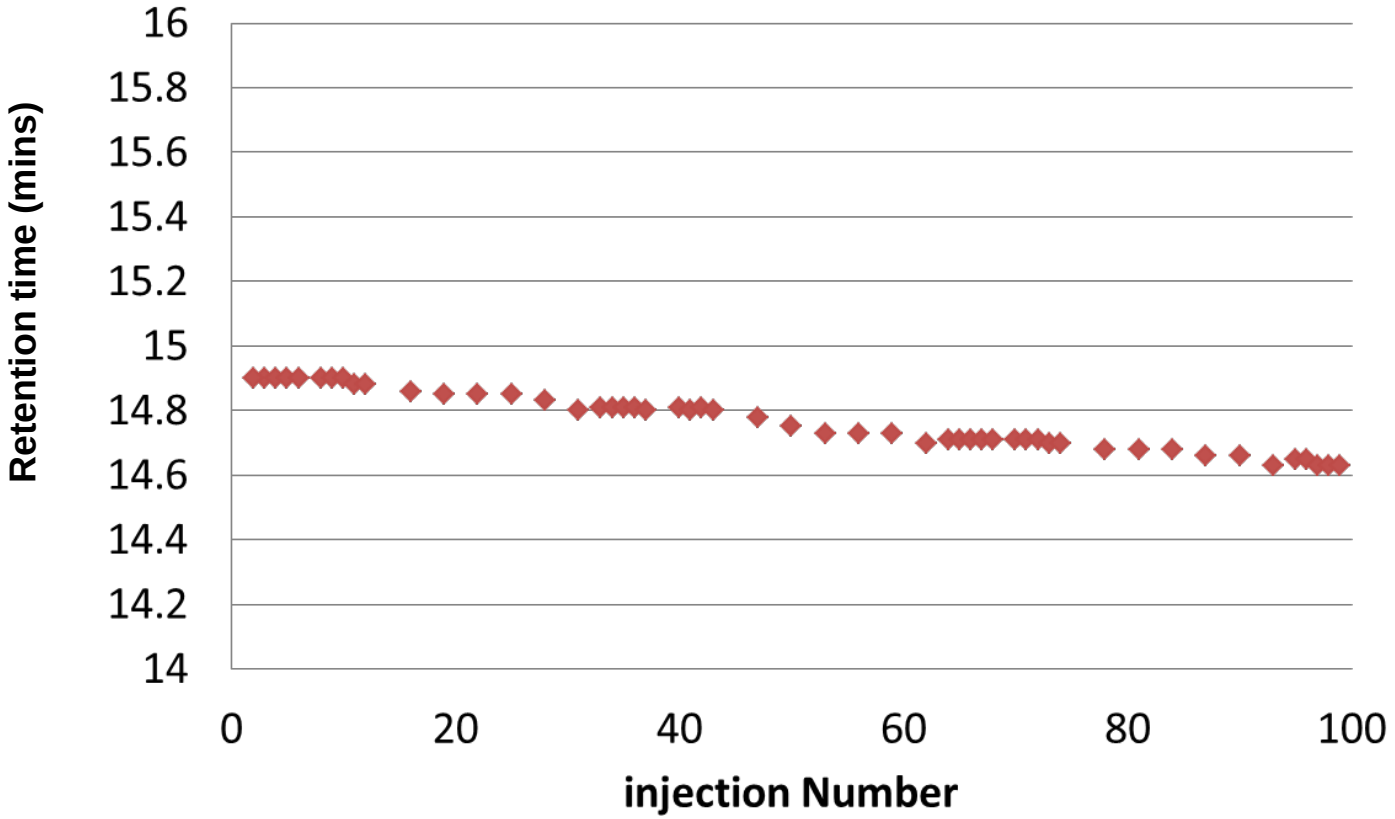
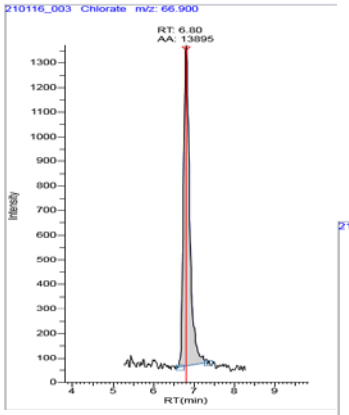
Glyphosate in Beer - Retention Time Stability

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

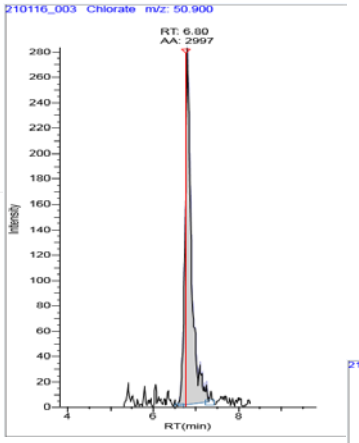


Courtesy of Fera Science Ltd UK

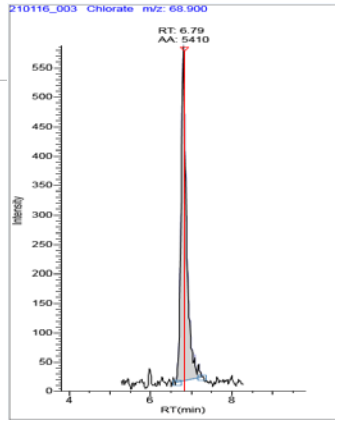
Determination of Chlorate in Dairy Produce @ 5 µg/kg



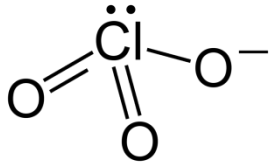
83.1>66.9

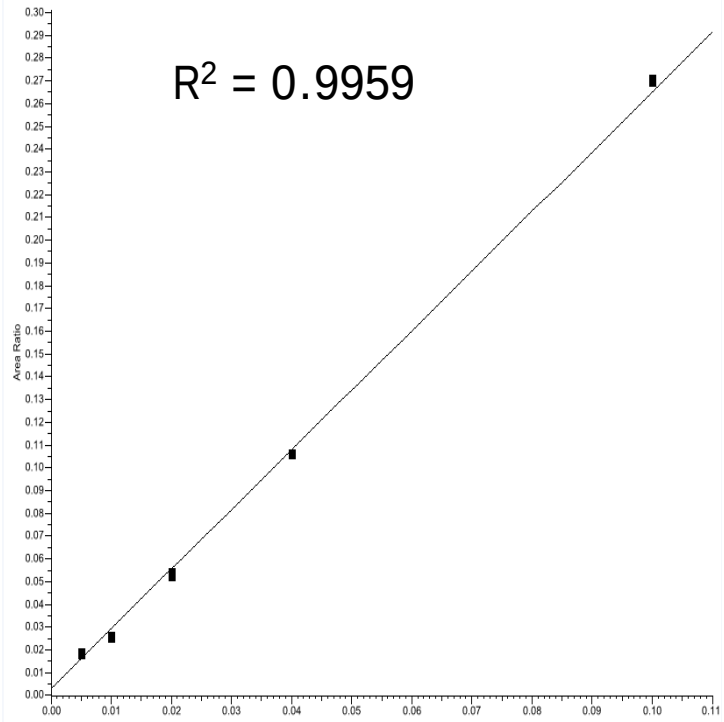


83.1>50.9



85.1>68.9





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

Courtesy of Fera Science Ltd UK

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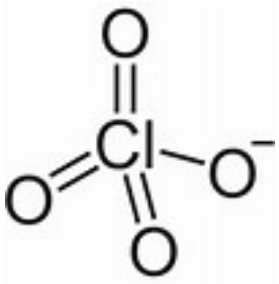
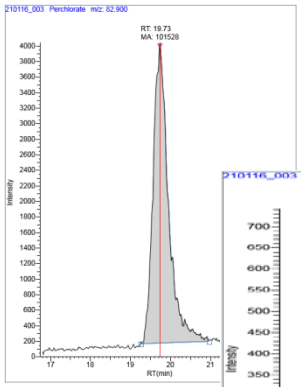
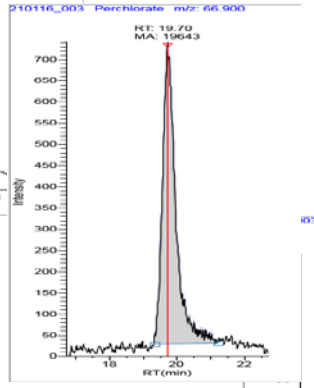
Determination of Perchlorate in Dairy Products @ 5 µg/kg





99>82.9



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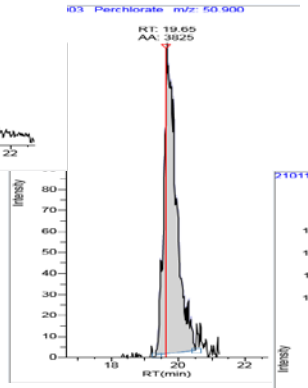

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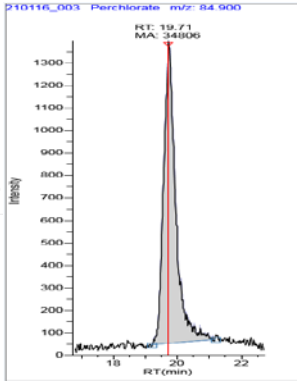

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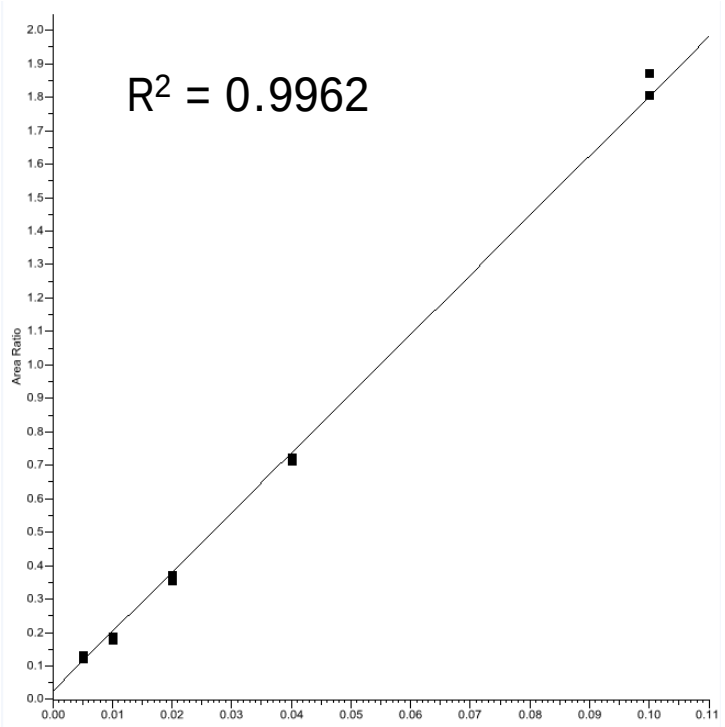

- Perchlorate in dairy products calibration 5 – 100 µg/kg

Courtesy of Fera Science Ltd UK

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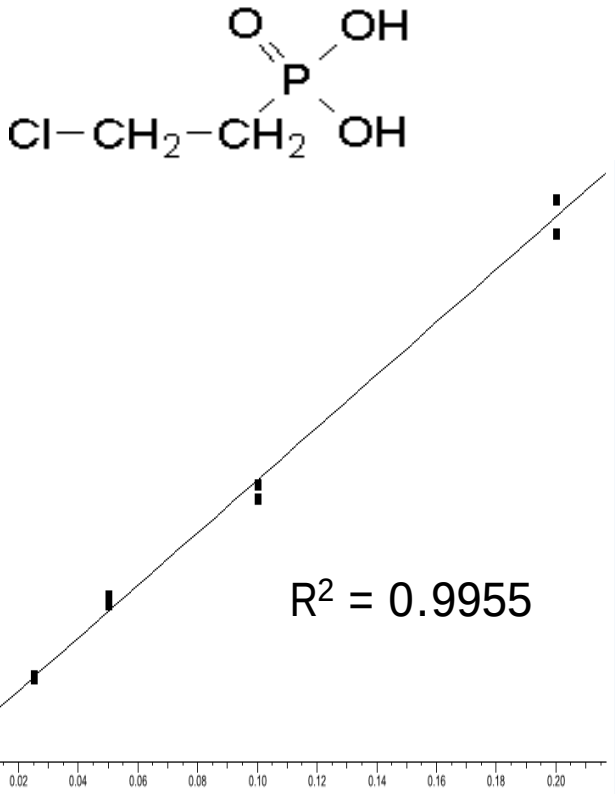
Determination of Ethephon @ 50 µg/kg in Grapes

Analyte	Conc^{n} (µg/kg)	Mean Rec (%) (n=5)	RSD (%)
Ethephon (IS)	10	114	17
	50	95	14
	100	102	10



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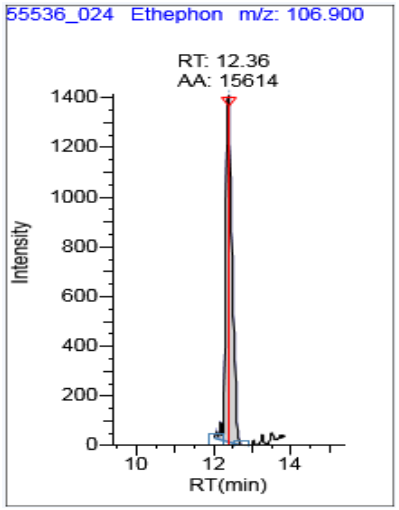

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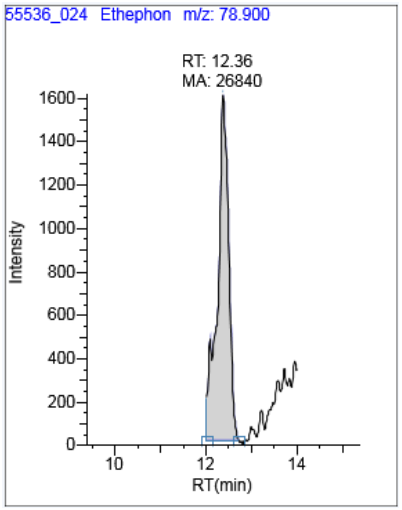

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Summary of Validation Results for Grapes

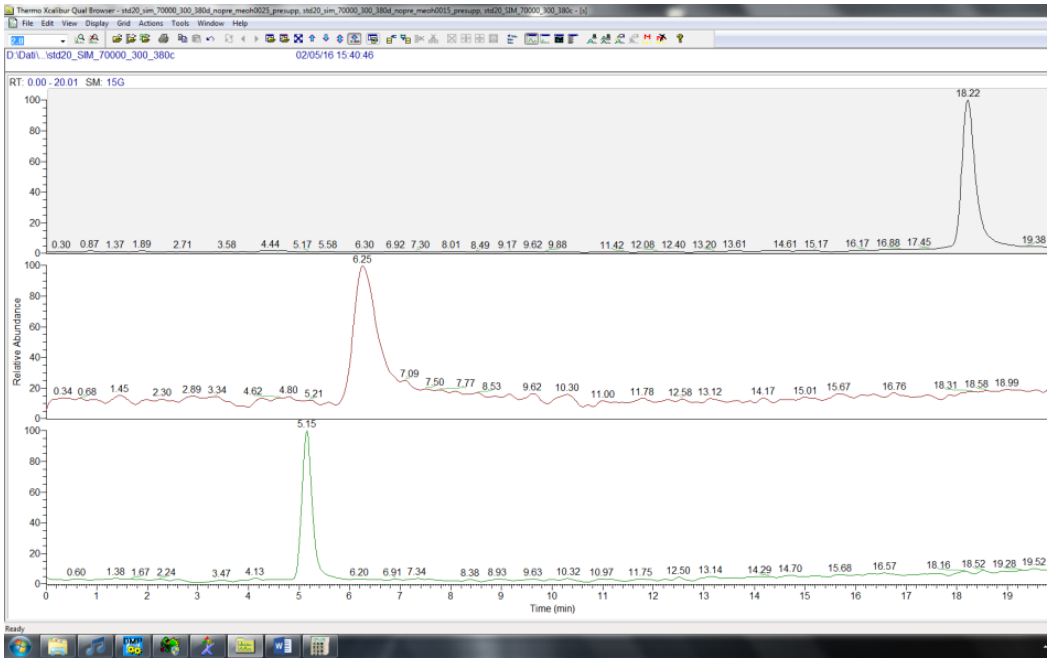
Analyte	Conc^{n} (µg/kg)	Mean Rec (%) (n=5)	RSD (%)
Perchlorate (IS)	10	110	17
	50	110	12
	100	113	6
Chlorate (IS)	10	112	19
	50	111	12
	100	115	6
Clopyralid	10	90	1
	50	91	1
	100	97	1

Analyte	Conc^{n} (µg/kg)	Mean Rec (%) (n=5)	RSD (%)
Fosetyl Al	100	98	3
	500	92	2
	1,000	90	2
Phosphonic acid	100	102	2
	500	97	7
	1,000	103	2
Cyanuric acid (IS)	10	Insufficient S/N	
	50	116	12
	100	113	8

Courtesy of Fera Science Ltd UK

IC Coupled to Thermo Scientific Q Exactive Focus MS

- Glyphosate, AMPA and Glufosinate at 20 ppt in surface water



VERITAS

Courtesy Veritas Laboratory – Venice

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The Dionex™ Integrion™ HPIC™ System

40 YEARS of Ion Chromatography

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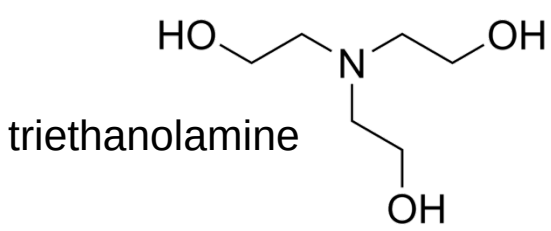
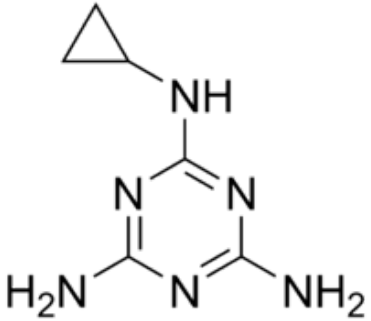
Candidates for Cation Exchange Chromatography

Analytes
aminomethylphosphonic acid (AMPA
amitrole
chlormequat
cyanuric acid
cyromazine
daminozide
diethanolamine
difenzoquat
ETU / PTU
fosetyl-AL
maleic hydrazide
melamine
mepiquat

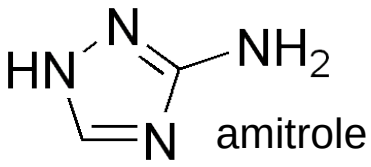
- A diversity of chemical structures



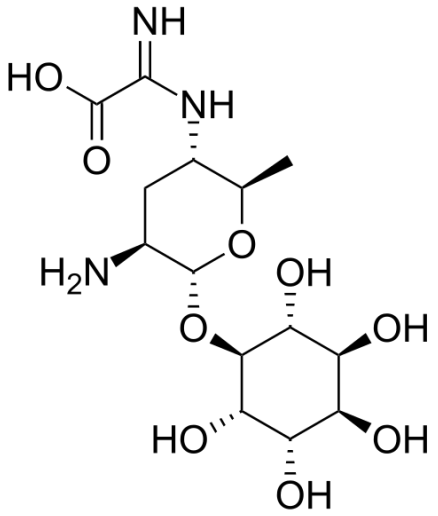
triethanolamine



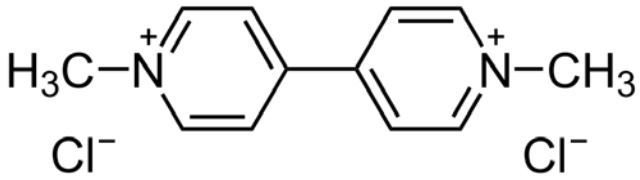
cyromazine



amitrole



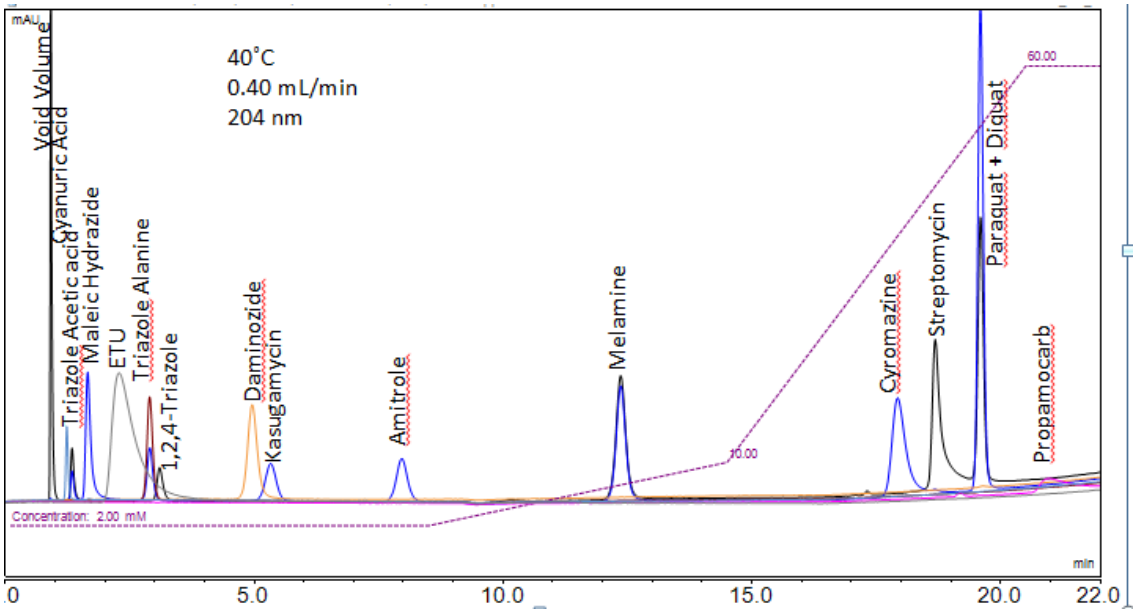
kasugamycin

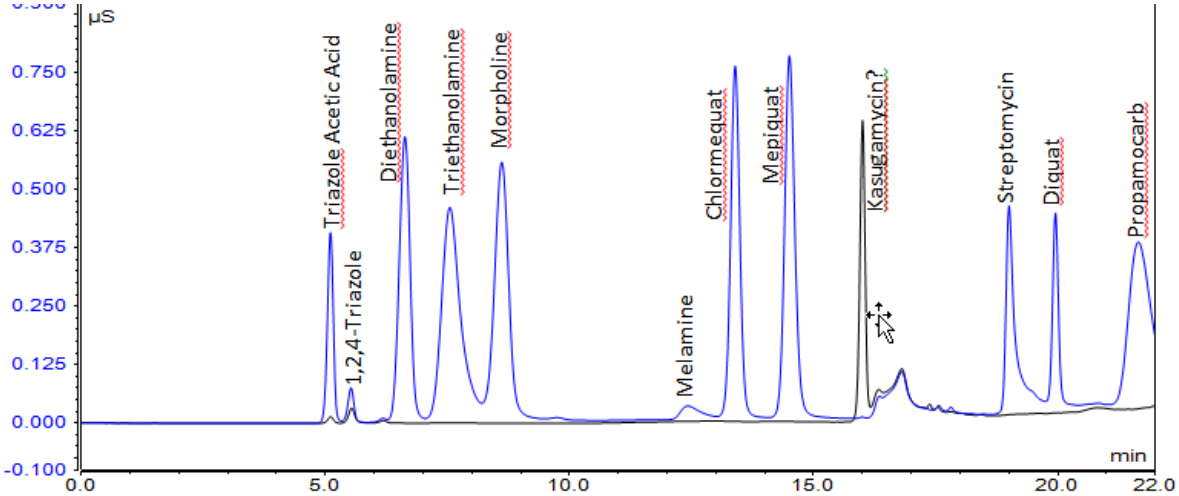


paraquat (Note: requires different extraction)

Plus, aminoglycosides, N,N' dimethylhydrazine, morpholine, triethanolamine, nereistoxin, triazole metabolites

Potential for the IC Separation of Cations





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Potential for Other Applications

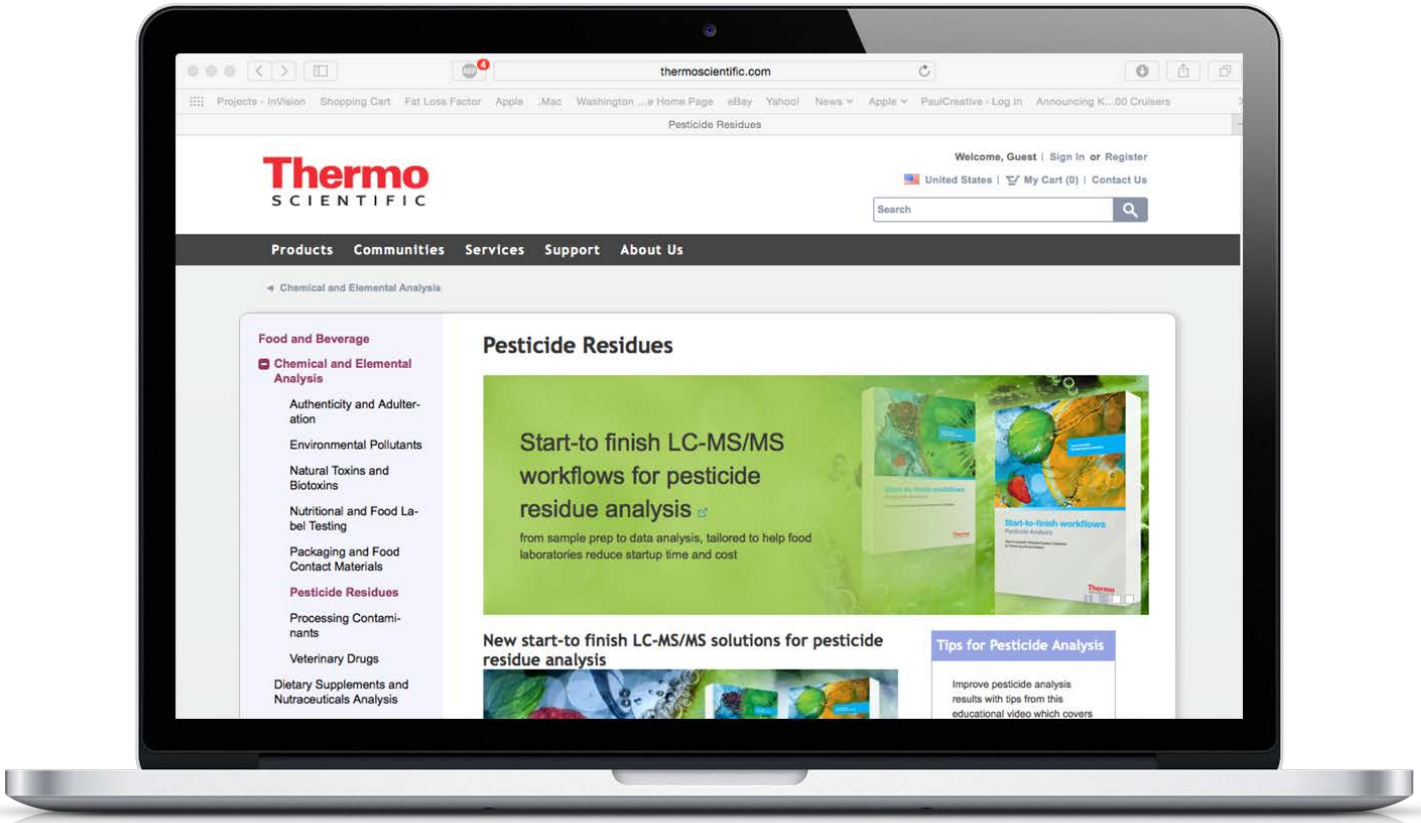
- The potential to analyse other analytes not just pesticides
  - Bromates
  - Halo acetic acids
  - Metals speciation
  - Anions
  - Organic acids
  - Carbohydrates
  - Amines

Summary

- Method validated for 13 polar pesticides (mostly at 10 µg/kg) in a single run without issues of variation in retention times
- More cost effective compared to analysing a single extract by a number of different chromatographic approaches
- There are good possibilities to make improvements to the performance of the system
- Ion chromatography is proven to be robust for the analysis of ‘dirty’ sample extracts
- Possibility to analyse cations by IC-MS to extend the scope of the analysis
- Use of IC coupled to Orbitrap technology is in place and preliminary results are promising

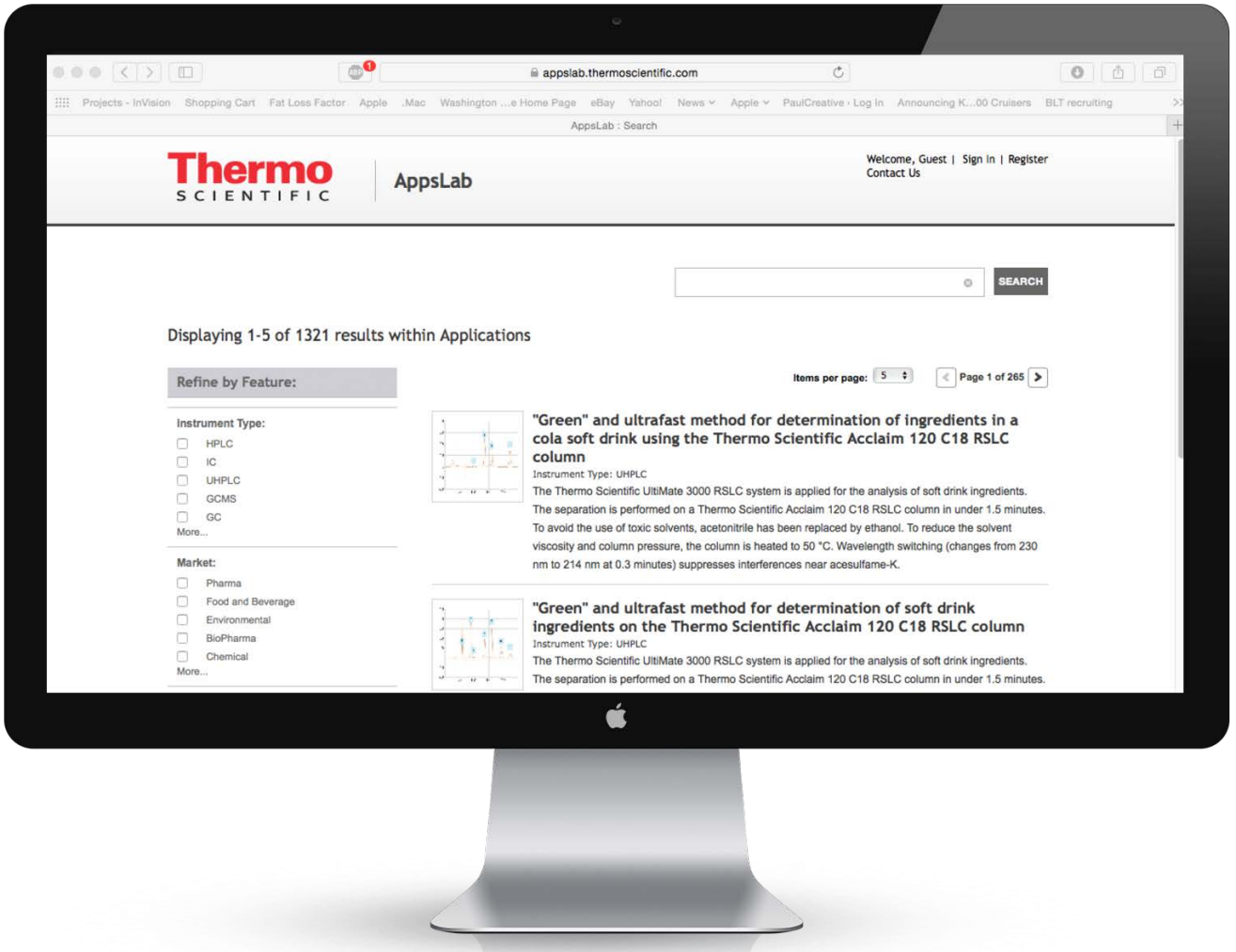
Thermo Fisher Scientific Food and Beverage Community

- Dedicated pesticide residues page



http://www.thermofisher.com/uk/en/home/industrial/food-beverage/food-analytical-testing/pesticide-residues-analysis.html

Thermo Scientific AppsLab Library of Analytical Applications



www.thermofisher.com/appslab

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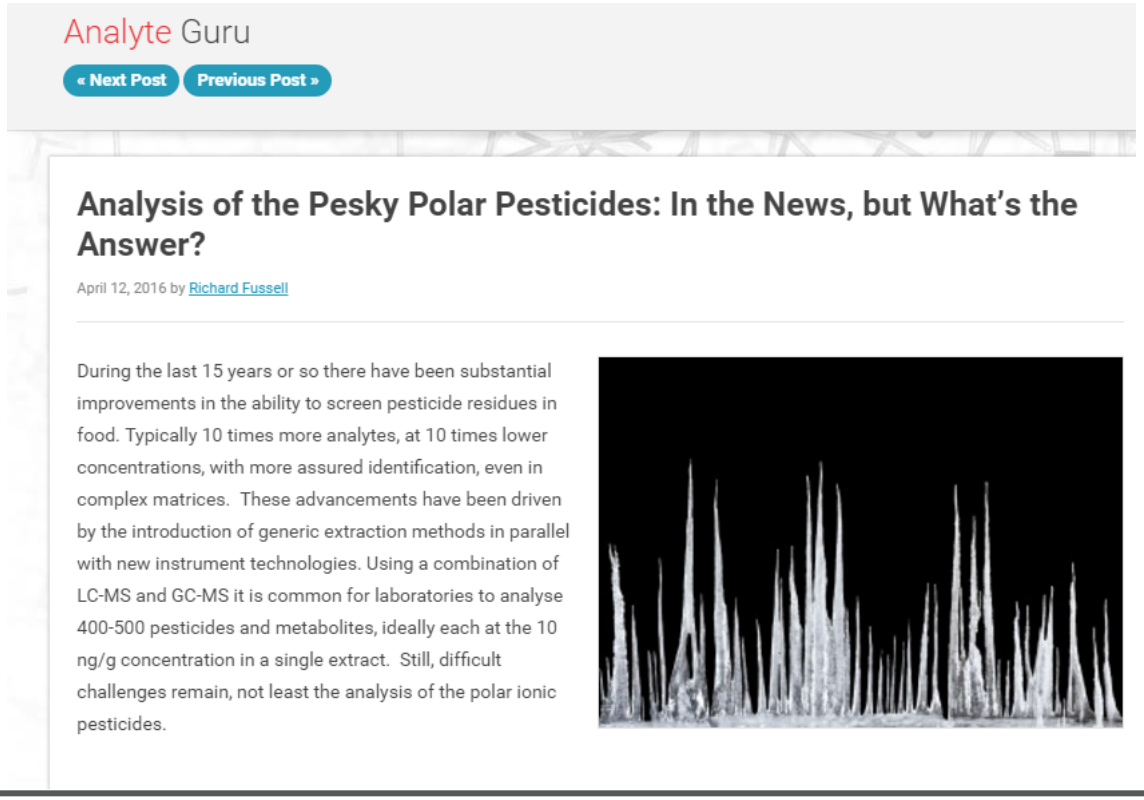

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