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Improving Chromatographic Performance of Underivatised Anionic Polar Pesticides in Food to Overcome Renowned Analytical Challenges

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NEMC

2018

Introduction

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- Multiresidue analyses determine as many residues as possible in the smallest number of analyses
  - Generic extraction, no/limited cleanup, highly selective determination step (GC- and LC-MS/MS or HRMS)
  - A number of different very successful implementations
    - *e.g.* QuEChERS, mini Luke…
- Polar pesticides in many cases are not amenable to the generic multiresidue approach as they are challenging to analyse.
- The source of these difficulties arise from the physicochemical properties of these compounds, which impact and complicate each stage of the analysis.
- Historically these compounds have been analysed in a series of selective single residue methods (SRM), adding significant costs so were often excluded from surveillance.
- As well as glyphosate, EU screening labs also want to include AMPA (glyphosate metabolite) and a number of other challenging polar pesticides in a single method.

What and Why?

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Maximum Residue Limits (**MRL**s), are legislative limits on the concentrations of residual pesticides and their metabolites in fresh food.

Glyphosate used as a desiccant on cereal crops to aid harvest- results in a increased frequency of residues in cereal-based products such as bread and breakfast cereals and beer.

Ethephon – approved but frequent MRL violations 2016 figures from RASFF, 4 alerts and 3 information for attention notifications covering, grapes, tomatoes, peppers and figs

Chlorate – Biocide banned by EU in 2010 because of health risks
Perchlorate – EU established MRLS of 10 µg/kg for most foods in 2015

Ionic polar pesticides

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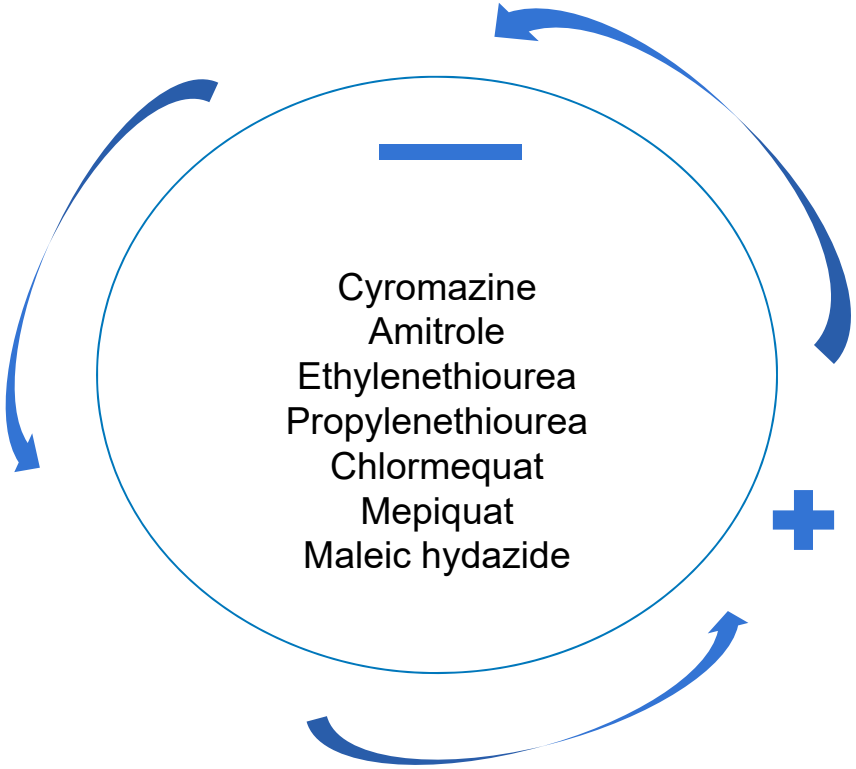

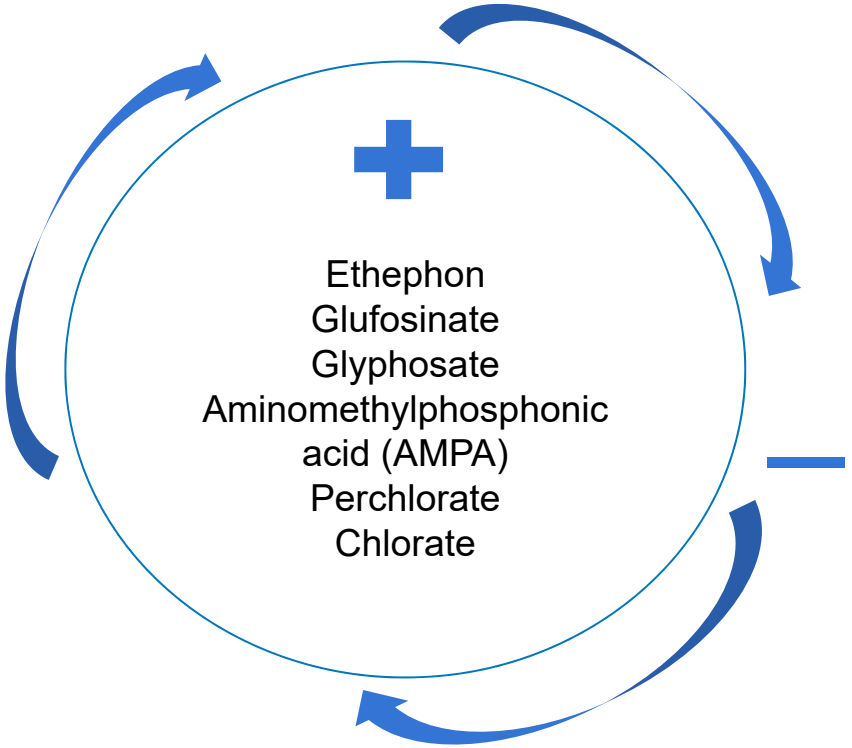

Options for Analysis and Sample preparation

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Sample preparation

- **Derivatization with FMOC?**
- **Extraction with aqueous – Narong's et al. method**
- **Extraction organic solvents – EURL Quppe**

Liquid Chromatography

- Reversed-phase (RP) LC
- Ion chromatography (IC)
- Porous graphitised/graphitic carbon (PGC)
- Hydrophilic interaction liquid chromatography (HILIC)
- Mixed mode

Derivatization - using FMOC reagent
Glyphosate, AMPA and Glufosinate

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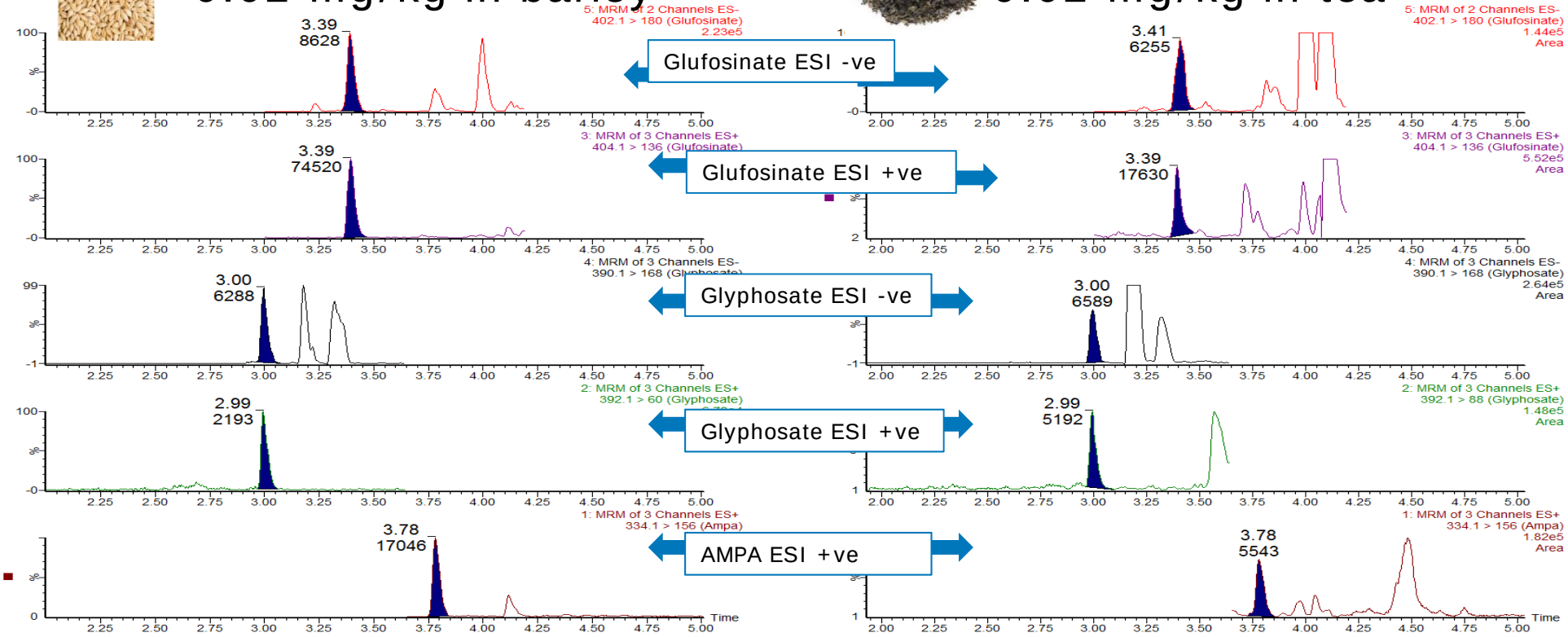
0.02 mg/kg in barley

0.02 mg/kg in tea



Our experiences so far ...

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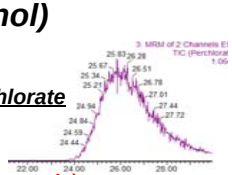
HILIC type column (polyvinyl alcohol)

+ Retention
+/- Separation of critical pairs
- Ret time stability (> 1min)
- Perchlorate compatibility (5min wide peak)

Perchlorate



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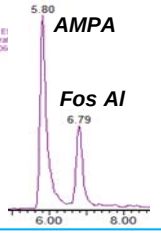
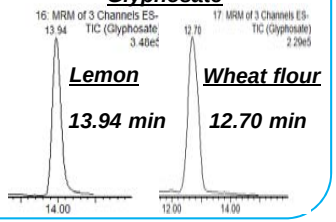


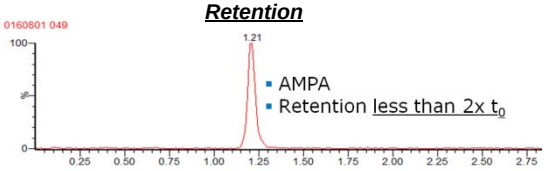
Retention time stability Glyphosate



Mixed mode column

- Retention
- Separation of critical pairs
- Ret time stability

Retention



RSDs > 20% in different commodities

Chromatographic separation:
HILIC Polyvinyl alcohol based LC column

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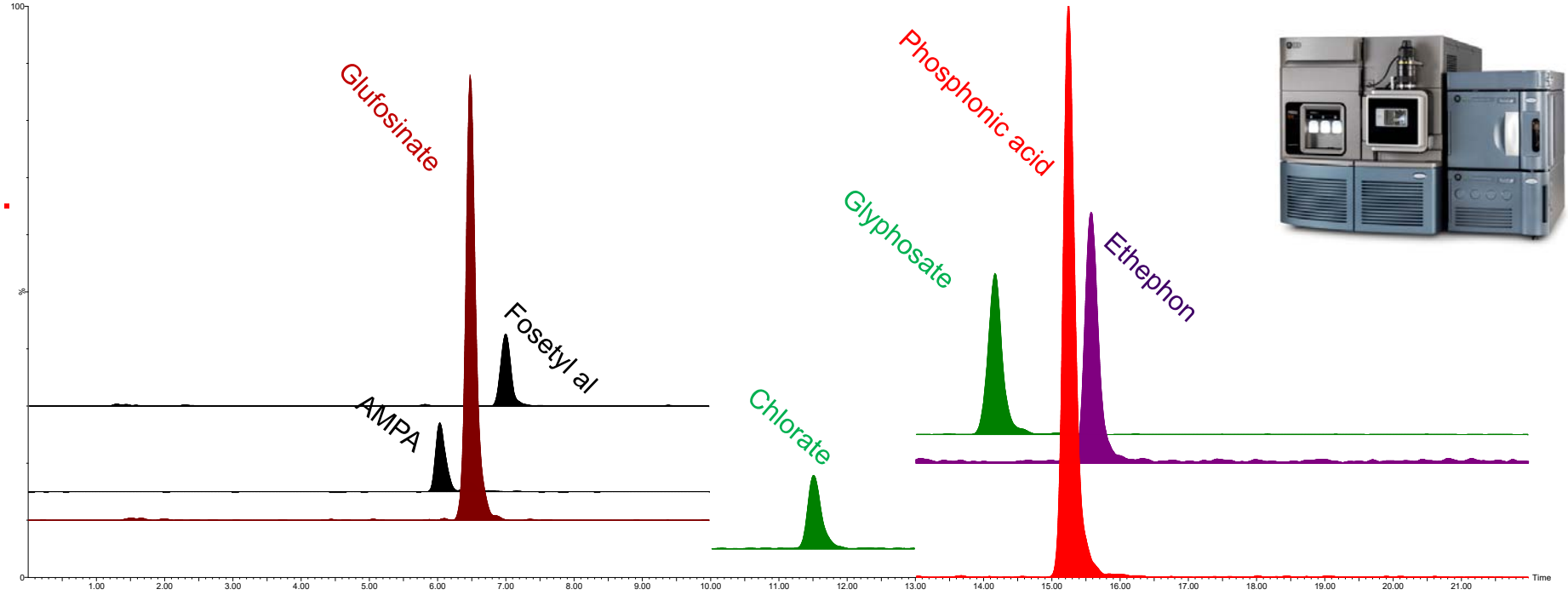
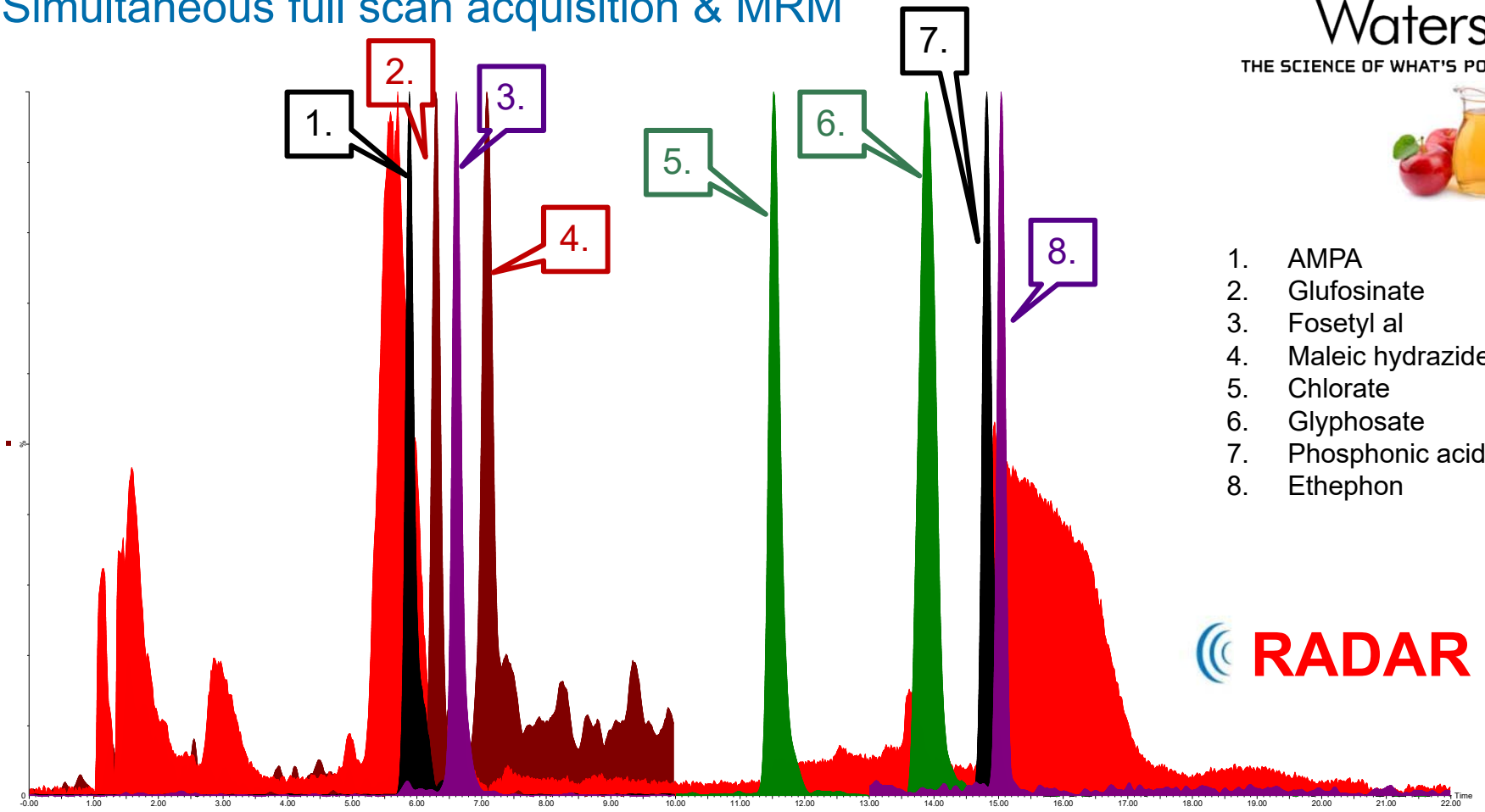
- 0.01 mg/kg in beer and extracted as per the EURL Quick Polar Pesticides Extraction method
- MP A: 68:12: 20 water: 45mM ammonium bicarbonate: MeCN; MP B: 50mM ammonium bicarbonate



Application note 720006070en

Simultaneous full scan acquisition & MRM



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1. AMPA
2. Glufosinate
3. Fosetyl al
4. Maleic hydrazide
5. Chlorate
6. Glyphosate
7. Phosphonic acid
8. Ethephon

RADAR

Quantitation by LC-MS/MS: Glyphosate; 1 to 250 ppb

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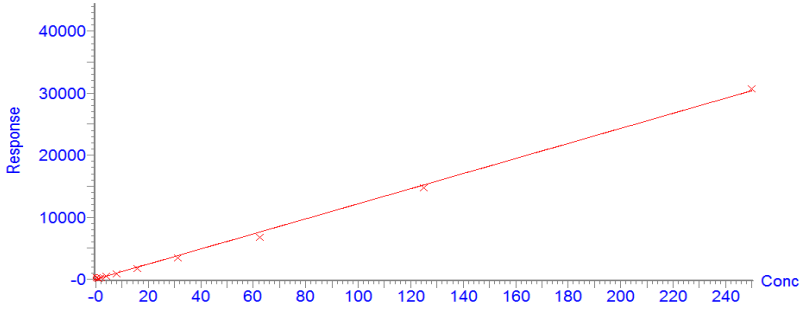
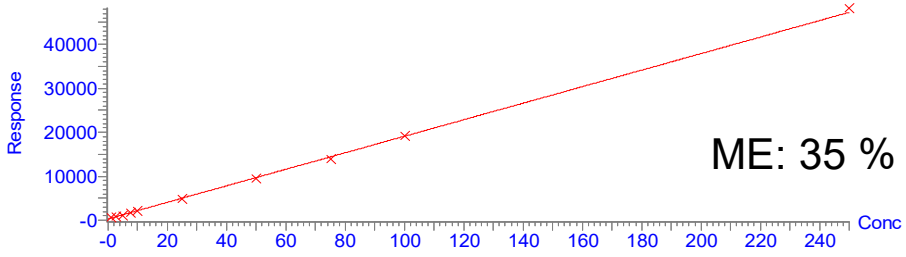
Solvent standard

Compound name: Glyphosate
Correlation coefficient: r = 0.999013, r^2 = 0.998027
Calibration curve: 121.528 * x + 18.5267
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Apple juice

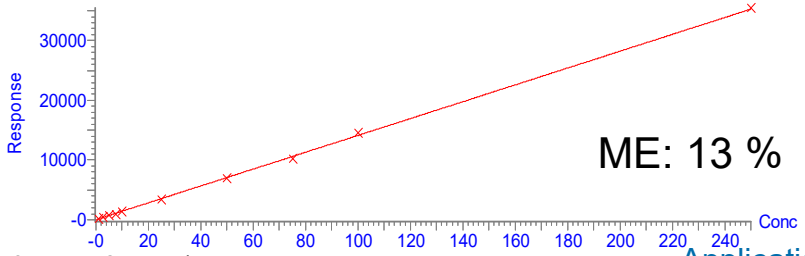
Compound name: Glyphosate
Correlation coefficient: r = 0.999543, r^2 = 0.999087
Calibration curve: 187.972 * x + 303.684
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



ME: 35 %

Tomato juice

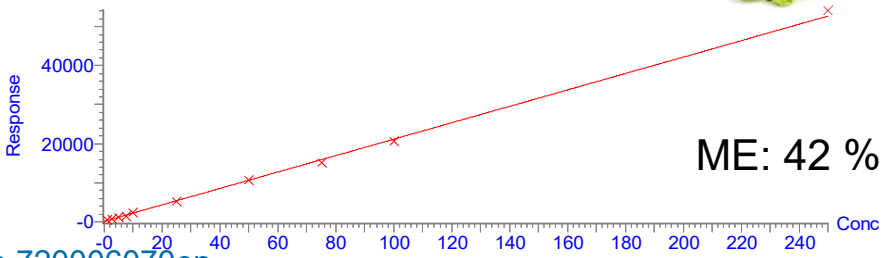
Compound name: Glyphosate
Correlation coefficient: r = 0.999464, r^2 = 0.998929
Calibration curve: 140.983 * x + 32.7736
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



ME: 13 %

Beer

Compound name: Glyphosate
Correlation coefficient: r = 0.999286, r^2 = 0.998572
Calibration curve: 210.302 * x + 132.7
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



ME: 42 %

Application note 720006070en

Recoveries and repeatability

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			Apple juice		Tomato juice		Beer	
	Conc (mg/kg)	n	Mean (mg/kg)	RSD (%)	Mean (mg/kg)	RSD (%)	Mean (mg/kg)	RSD (%)
Glufosinate	0.01 0.05 0.10	9 9 9	0.0092 0.0501 0.1039	9.8 4.9 4.6	0.0100 0.0521 0.0980	9.0 3.9 2.9	0.0099 0.0528 0.1047	5.7 4.0 3.6
Glyphosate	0.01 0.05 0.10	9 9 9	0.0099 0.0507 0.1046	8.5 6.1 6.0	0.0106 0.0508 0.0961	9.8 3.8 2.0	0.0107 0.0549 0.1068	4.5 5.7 3.1
Ethephon	0.01 0.05 0.10	9 9 9	0.0095 0.0457 0.0934	8.7 6.3 5.0	0.0097 0.0522 0.1006	7.7 3.4 3.5	0.0106 0.0541 0.1055	6.2 5.7 3.7
AMPA	0.01 0.05 0.10	9 9 9	0.0108 0.0498 0.1011	9.8 9.0 8.1	0.0094 0.0460 0.0912	2.1 5.4 2.8	0.0100 0.0542 0.1060	7.4 5.6 5.1
Fosetyl Al	0.01 0.05 0.10	9 9 9	0.0095 0.0518 0.1061	9.0 4.5 3.2	0.0090 0.0440 0.0900	6.6 1.6 1.5	0.0106 0.0548 0.1054	4.4 5.2 4.3

Application note 720006070en

Our experiences so far ...

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Torus Technology

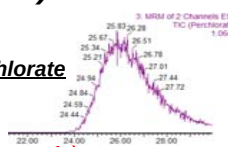
HILIC type column

+ Retention
+ Separation of critical pairs
+ Ret time stability
+ Perchlorate compatibility

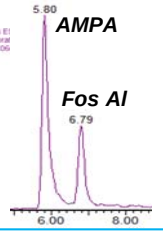
HILIC type column (polyvinyl alcohol)

+ Retention
+/- Separation of critical pairs
- Ret time stability (> 1min)
- Perchlorate compatibility (5min wide peak)

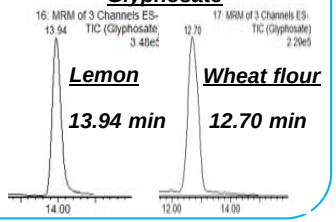
Perchlorate



Separation



Retention time stability Glyphosate



Mixed mode column

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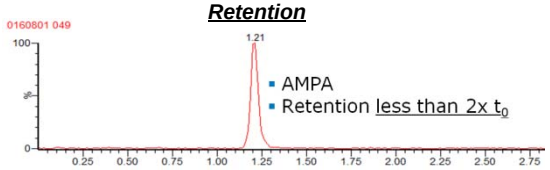
Retention



RSDs > 20% in different commodities

Calculating Column 'Dead' Volume t0
TORUS DEA 2.1 x 100mm

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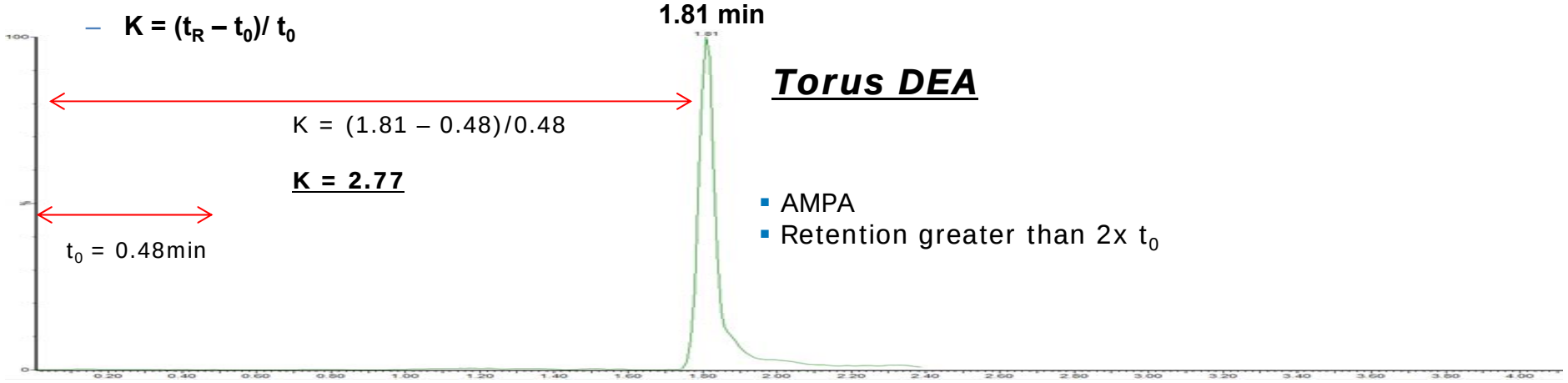
- Time required for one volume of mobilephase to exit the column
  - **No retention occurs before the t_0**
- t_0 is needed to calculate retention factor
  - **t_0=V/F**
- **Retention Factor (K)** is the measurement of a columns retention in relation to the column 'dead volume' (t_0)
  - **$K = (t_R - t_0)/ t_0$**

2.1mm x 100mm
V =0.24ml
F =0.50ml/min



Chromatography:
Example in spinach at 0.01 mg/kg

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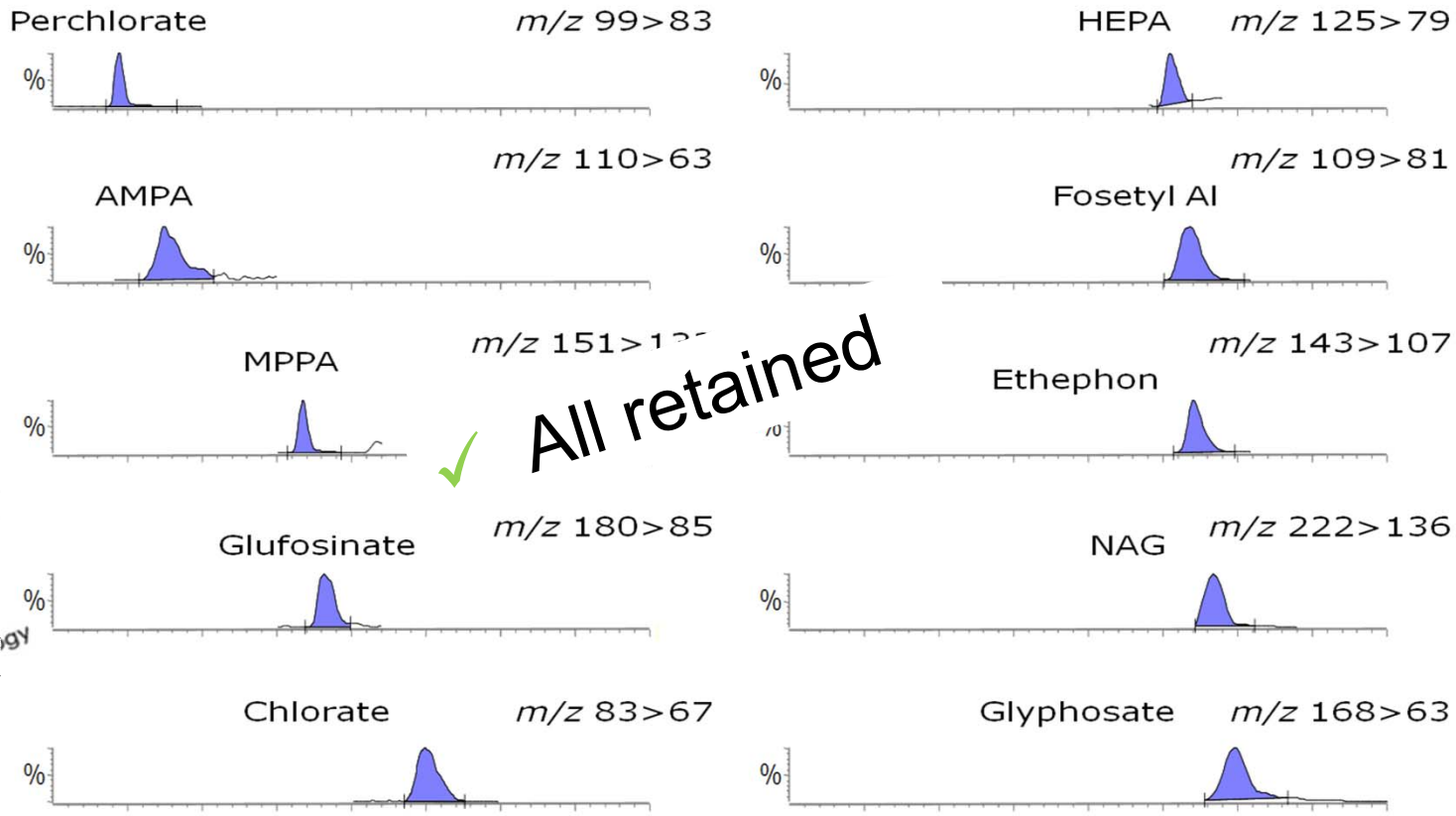

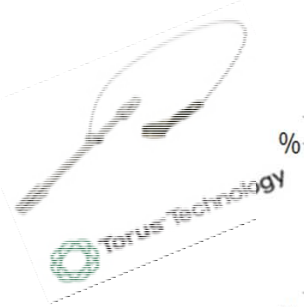

Chromatography: Critical compounds

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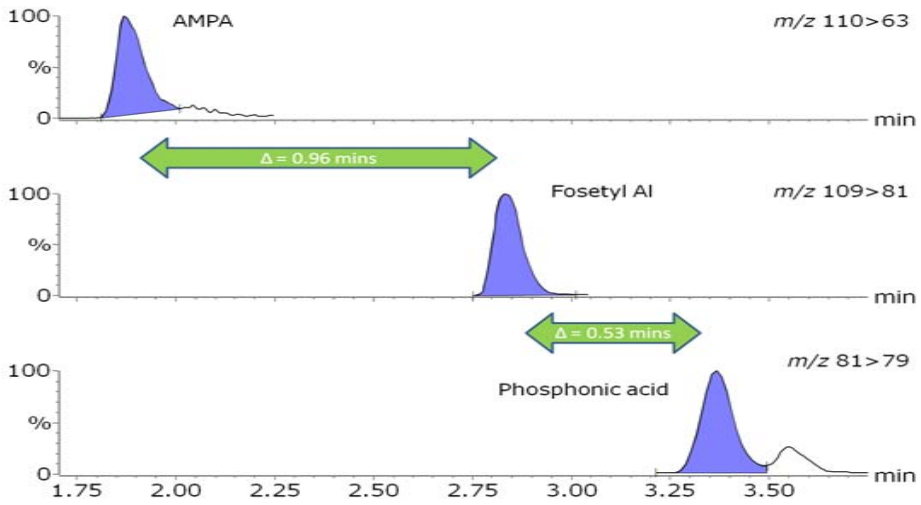
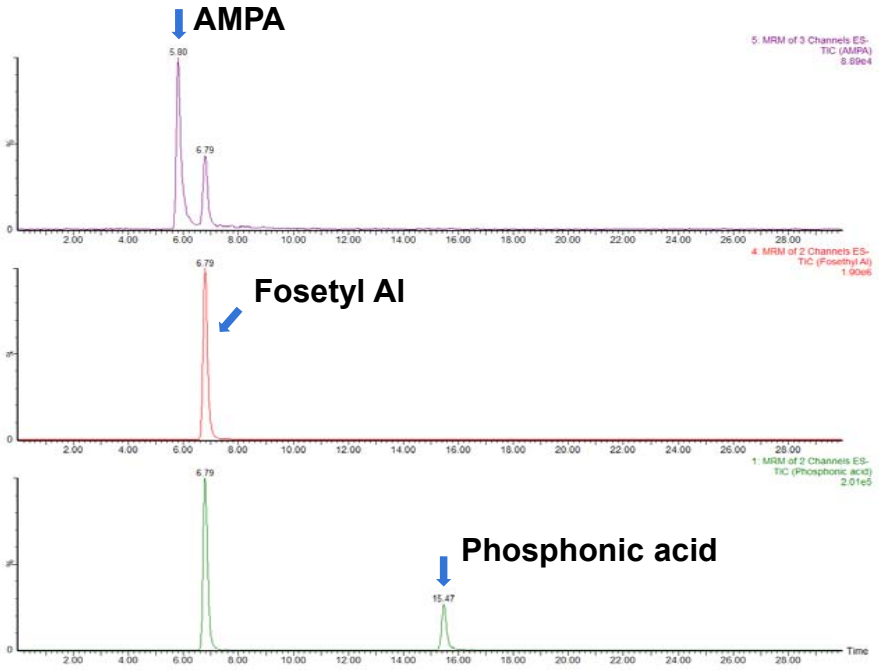
TORUS column

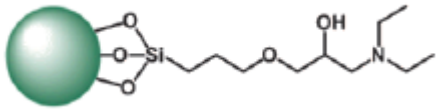


HILIC type column (polyvinyl alcohol)

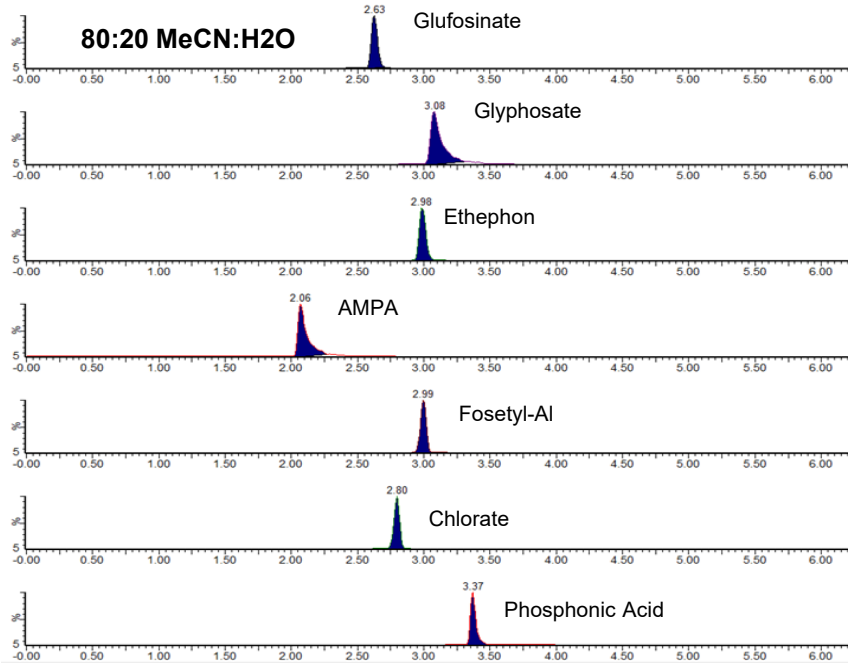


Torus DEA – Polar pesticides analysis
Some background

- **Torus DEA, 130Å, 1.7µm, 2.1 mm x 100 mm**
- DEA: Diethylamine - HILIC/WAX
- Ethylene bridged hybrid (BEH) particle
- Two stage functionalization
  - Control retention characteristics
  - Selectivity and peak shape



Patented Approved Technology



(methodology patent pending)

Experimental: MS parameters

Capillary voltage (kV)	2.4
Source temperature (°C)	150
Desolvation temperature (°C)	600
Cone gas flow (L/Hr)	300
Desolvation gas flow (L/Hr)	1000
Nebuliser (bar)	7

Compound	Ion mode	Transitions	Cone voltage (V)	Collision energy (eV)
Glyphosate	ESI-	**167.85 > 62.80** 167.85 > 80.80 167.85> 149.85	15	15 15 10
AMPA	ESI-	**109.80 > 62.80** 109.80 > 78.80 109.80 > 80.80	15	15 15 10
Glufosinate	ESI-	179.90 > 62.80 **179.90 > 84.85** 179.90 > 134.00	15	25 17 16
Chlorate	ESI-	**82.85 > 66.80** 82.85 > 50.80	15	14 15
Ethephon	ESI-	142.85 > 78.75 **142.85 > 106.85**	15	13 8
Fosethyl Aluminium	ESI-	108.85 > 62.80 **108.85 > 80.80**	15	16 10
Phosphonic acid	ESI-	80.80 > 62.80 **80.80 > 78.80**	15	15 10
Perchlorate	ESI-	98.80 > 66.90 **98.80 > 82.85**	20	45 18
Ethephon Hydroxy	ESI-	**124.80 > 78.80** 124.80 > 94.80 124.80 > 106.90	15	14 12 11
MPPA	ESI-	150.70 > 62.80 150.70 > 106.85 **150.70 > 132.85**	15	25 16 12
N-Acetyl-Glufosinate	ESI-	221.90 > 58.90 **221.90 > 135.90** 221.90 > 161.90	20	14 20 12

Experimental: LC conditions

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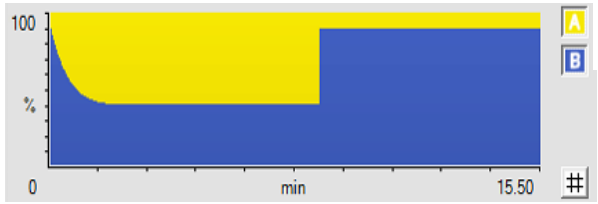
Column	Torus DEA column (130Å, 1.7 µm, 2.1 x 100 mm)
LC System	I Class FL
Solvent A	50 mM Ammonium Formate pH 2.9 (0.9% Formic Acid)
Solvent B	MeCN + 0.9% Formic Acid
Column Temp	50°C
Sample Temp	10°C
Injection Volume	10 µL
Flow rate	0.5 mL/min

Time (min)	%A	%B	Curve
0	10	90	-
4.50	60	40	2
8.50	60	40	6
15.50	10	90	1



(methodology patent pending)

Experimental:
Quick Polar Pesticides (QuPPe) extraction procedure

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Weighed homogenized sample (5g) into 50 ml tube

Added methanol (10 ml) containing 1 % formic acid

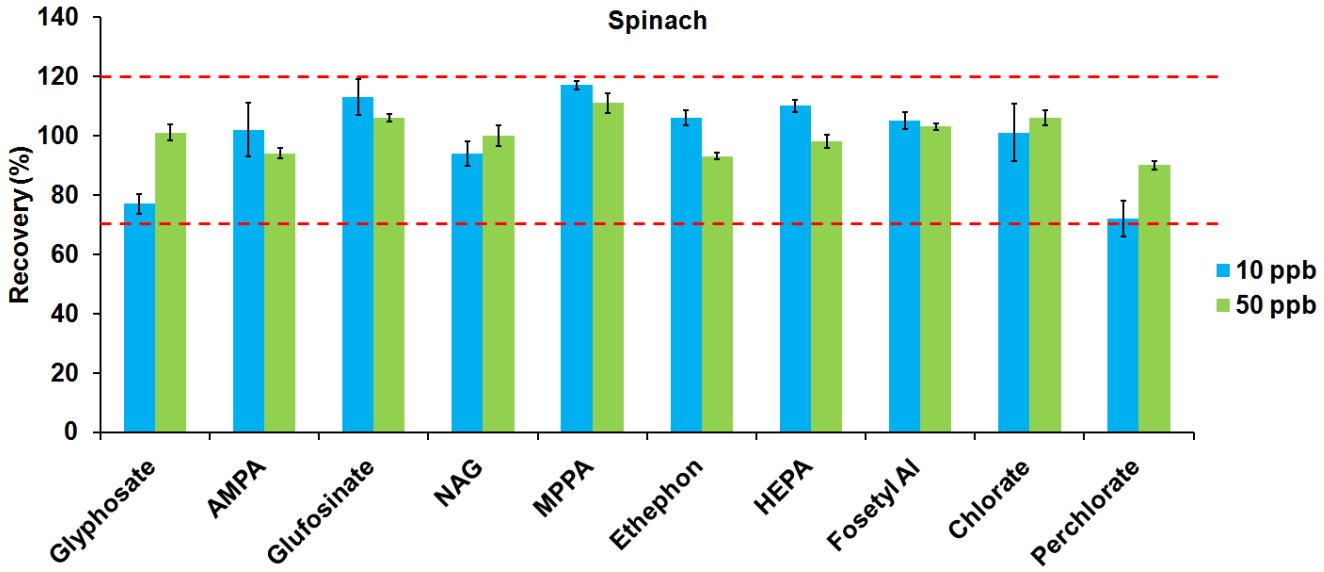
Vortexed thoroughly for 2 minutes

Centrifuged at 6000 rpm for 5 minutes

Filtered supernatant (0.25µm, PVDF, spin filters) into a plastic vial

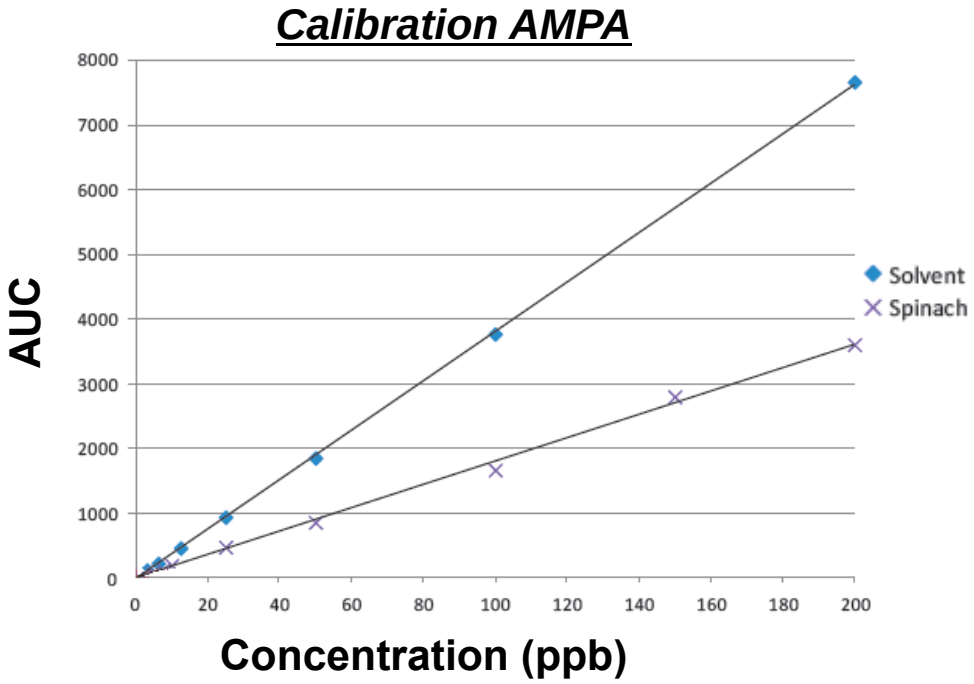
http://www.eurl-pesticides.eu/userfiles/file/EurlSRM/meth_QuPPe-PO_EurlSRM.pdf

Method performance:
Recoveries and repeatability



Incurred residue of phosphonic acid was detected and so has been omitted from the results

Matrix effects

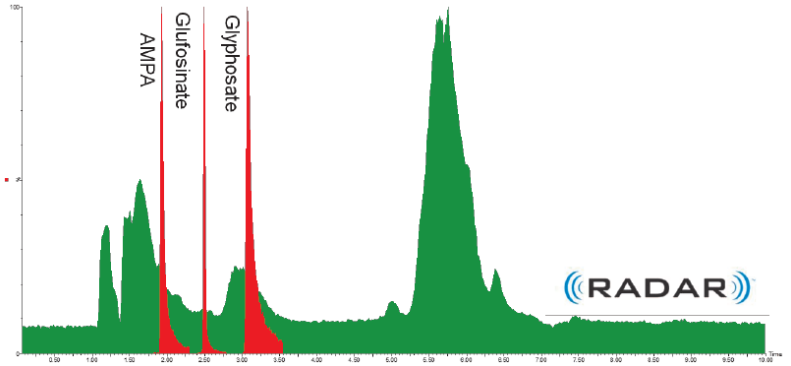


	Spinach (%)
N-Acetyl-Glufosinate	233
Glufosinate	99
Glyphosate	104
MPPA	488
Ethephon	287
Fosetyl Al	107
AMPA	47
Perchlorate	494
Chlorate	245
Ethephon hydroxy	156
Phosphonic acid	151

Enhancement

No effect

Suppression



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Chlorate and Perchlorate Analysis

Chlorate – Biocide banned by EU in 2010 because of health risks

– EU established MRLs of 10 µg/kg

Update: Perchlorate and Chlorate

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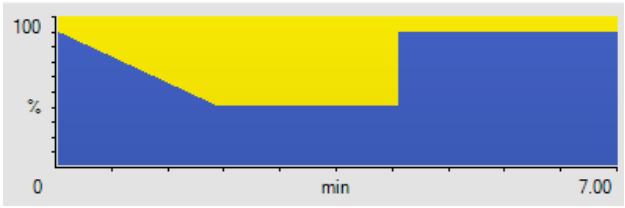
- **How**
  - An alternative LC-MS/MS method with chromatographic separation achieved on the TORUS DEA column, applying an ammonium formate mobile phase gradient.
- **Benefits**
  - Short seven minute run time
  - UPLC technology providing optimal peak shapes
  - Excellent retention and separation, separation is key due to isobaric interference of perchlorate in chlorates transition.
  - Excellent linearity and sensitivity

Torus DEA
Chlorate and Perchlorate analysis - LC conditions

Column	Torus DEA column (130Å, 1.7 µm, 2.1 x **50** mm)
LC System	I Class FL
Solvent A	50 mM Ammonium Formate pH 2.9 (0.9% Formic Acid)
Solvent B	MeCN + 0.9% Formic Acid
Column Temp	50°C
Sample Temp	10°C
Injection Volume	**3.0 µL**
Flow rate	0.5 mL/min

Time (min)	%A	%B	Curve
0	10	90	-
2.00	60	40	6
4.25	60	40	6
7.00	10	90	1



Red Grape:
0.01 mg/kg spiked post QuPPe extraction

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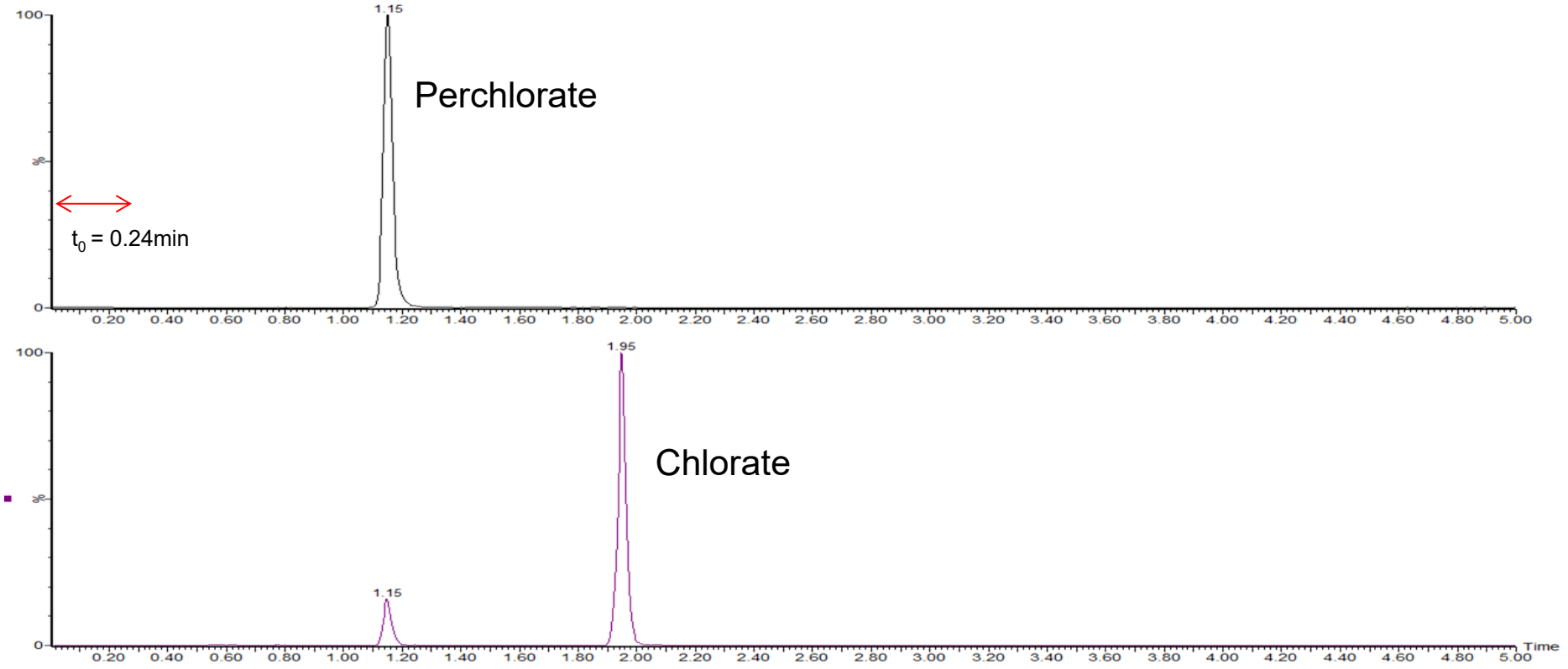

Grape QuPPe Extraction: Post Spike Matrix Matched 0.010mg/kg – 0.2mg/kg

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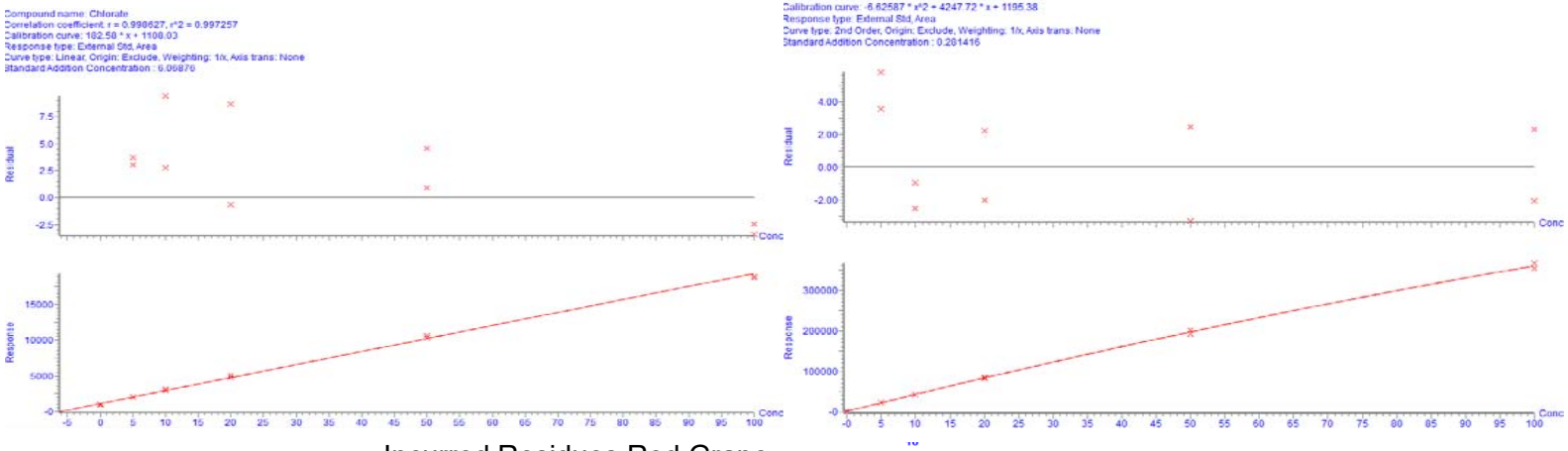
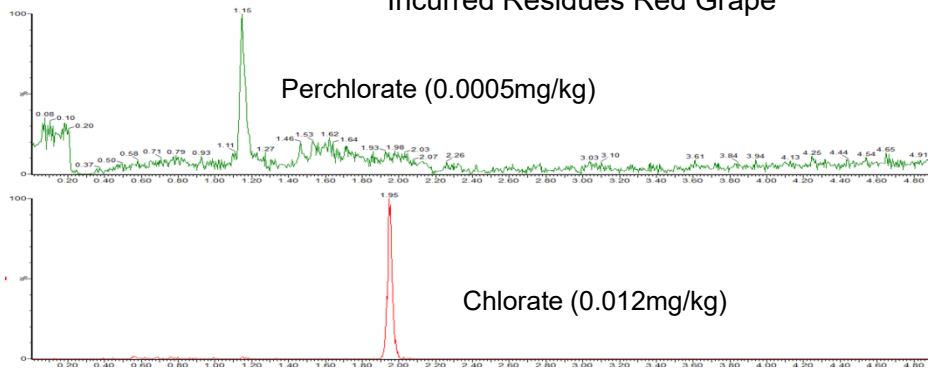


Incurred Residues Red Grape



	Perchlorate %RSD n=6	Chlorate %RSD n=6
0.010mg/kg Post Spike	1.30	2.73*
0.020mg/kg Post Spike	0.96	2.87

Cucumber:
Spiked post QuPPe extraction

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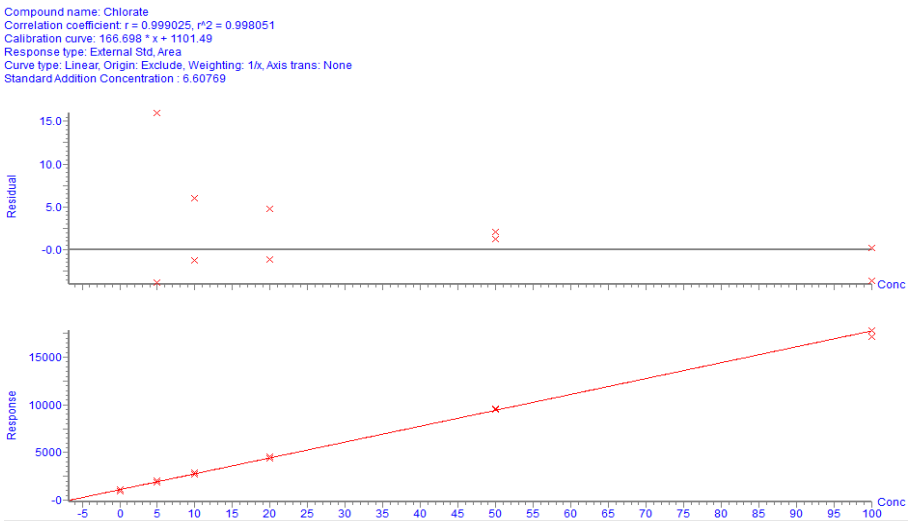

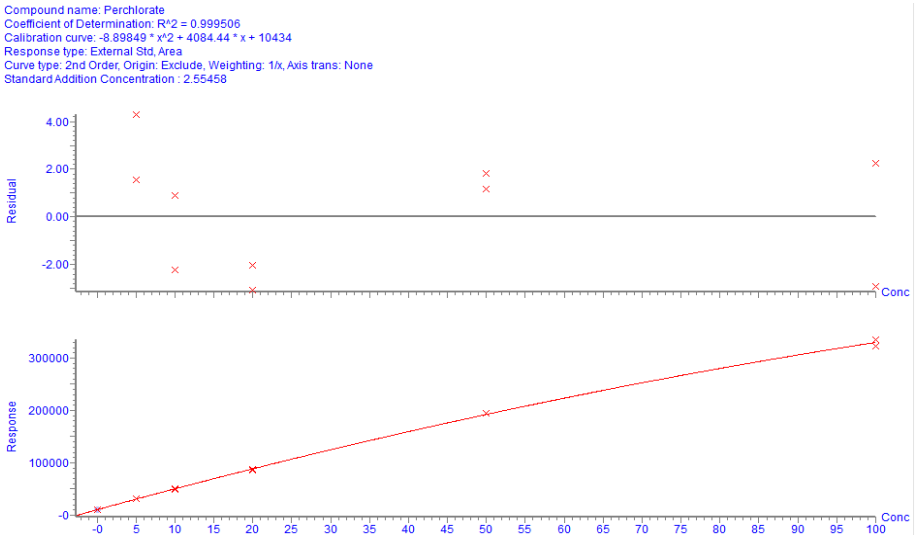

	Perchlorate %RSD n=6	Chlorate %RSD n=6
0.005 mg/kg	0.78	3.28
0.01 mg/kg	1.49	2.63

Infant Food QuPPe Extraction: Post Spike Matrix Matched 0.002mg/kg – 0.2mg/kg

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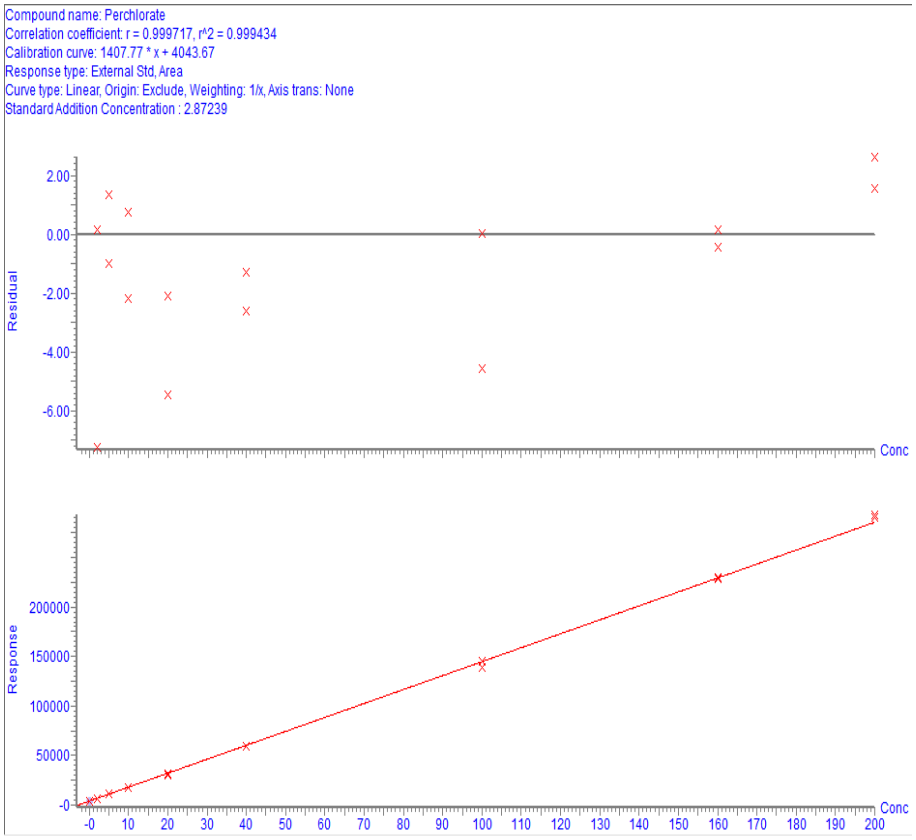
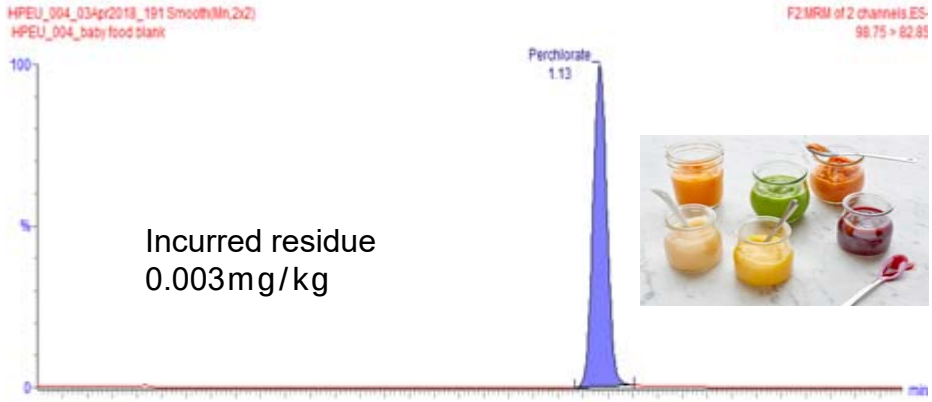
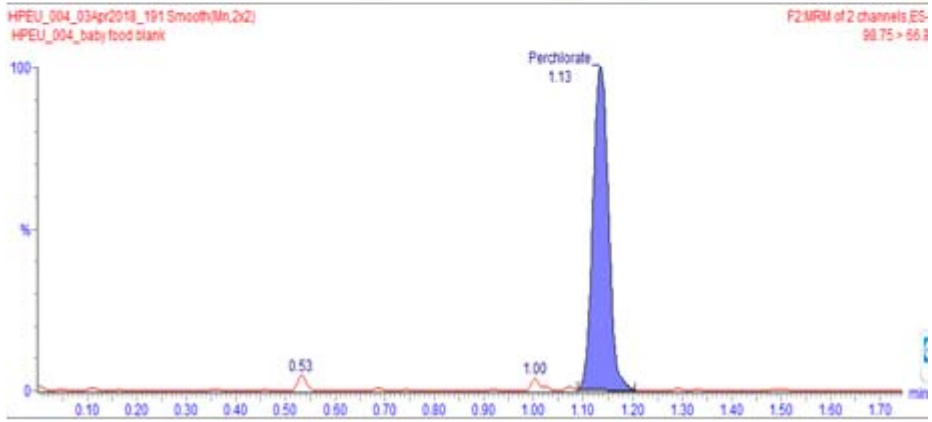




Incurred residue
0.003mg/kg



Conclusions

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- Expanding on previous LC-MS/MS methods, initial work using the Torus DEA has demonstrated excellent performance for the reliable analysis of polar pesticides in food

- Key benefits include:
  - Improved chromatographic performance for a broad scope of anionic pesticides in a single injection
  - Suitable application for checking compliance with **EU MRLs** and in agreement with **SANTE** guidelines
  - Maintained system sensitivity with LOQs < 0.01 mg/kg in different commodities
  - Repeatable quantitative analysis, with RSDs < 10% achieved at 0.01 mg/kg in spinach without isotopically labelled internal standards
  - Incurred residues of analytes accurately quantified using standard addition calibration

Acknowledgements

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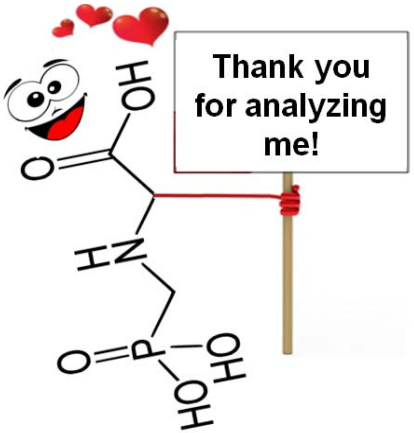
Acknowledgements

- **Customers**
  - UGRL, Turkey
  - Primoris, Belgium
  - Galab, Germany
- **Waters Wilmslow**
  - Euan Ross
  - Benjamin Wyuts
  - Eimear McCall
  - Simon Hird
- **Waters Milford**
  - Gareth Cleland
  - Dimple Shah



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Thank you for your attention

Any questions

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