



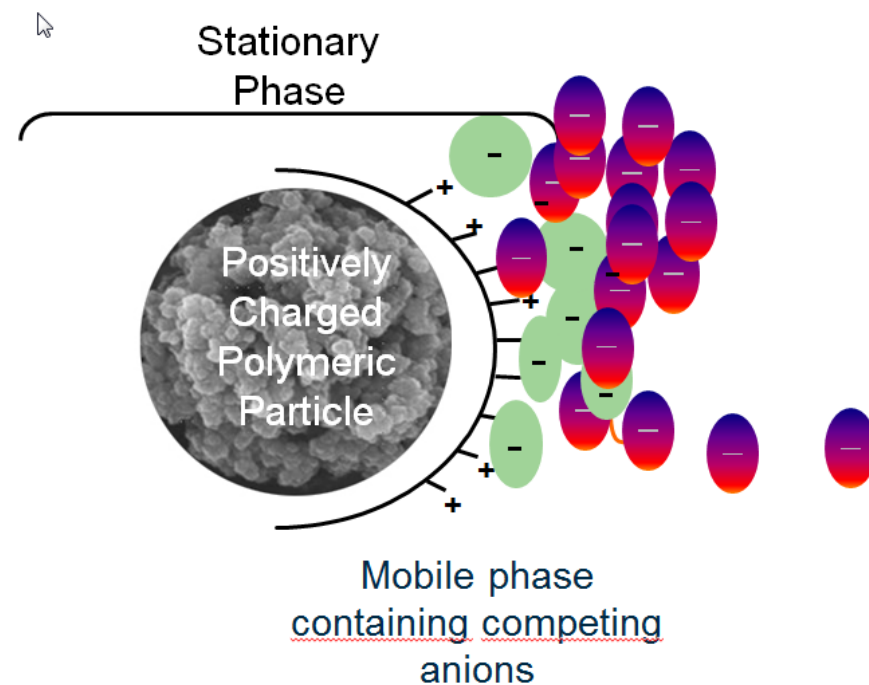
Why Can IC-MS Provide the Answer to the Problem of the Analysis of Polar Pesticides in Food

*Richard Fussell, Qilei Guo, Yingchen Li & Fausto Pigozzo

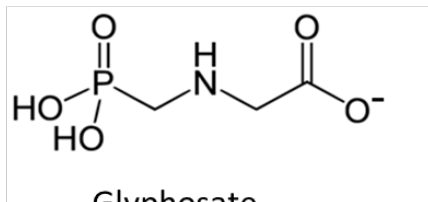
*Vertical Marketing Manager, Thermo Fisher Scientific, Hemel Hempstead, UK

NPRW 2019_Oslo June 12-13

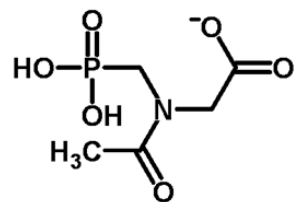
- Is IC-MS/MS a solution to the `hot topic` of analysis of polar pesticides?
- Benefits of using high-performance ion exchange chromatography coupled to mass spectrometry
- Anionic pesticide workflow development & validation
- Issues encountered during optimisation
- Summary



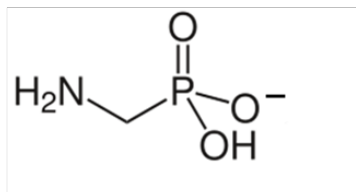
Anionic Pesticides Widely Used in Agricultural Production and Food Preparation



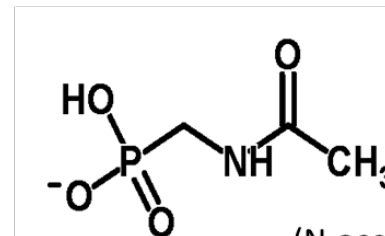
Glyphosate
(*m/z* 168.0067)



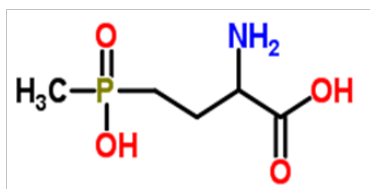
N-acetyl glyphosate
(*m/z* 210.0173)



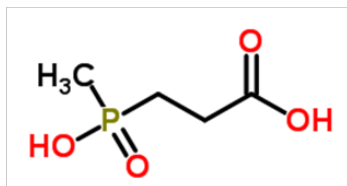
Aminomethyl phosphonic acid
(AMPA)
(*m/z* 110.0012)



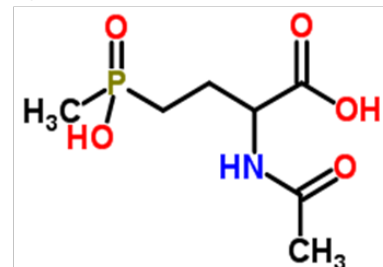
(N-acetyl AMPA)
(*m/z* 152.0118)



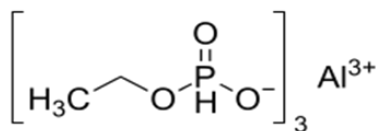
Glufosinate
(*m/z* 180)



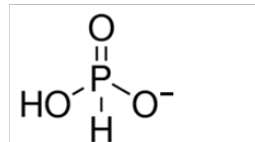
3-MPPA
(*m/z* 151)



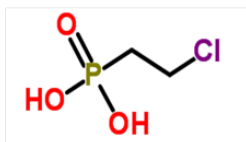
N-acetyl Glufosinate
(*m/z* 222)



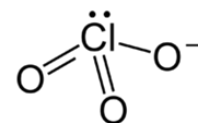
Fosetyl-aluminium
(*m/z* 109.0060)



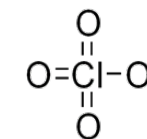
Phosphonic acid
(*m/z* 80.9747)



Ethephon
(*m/z* 143)



Chlorate
(*m/z* 82.9541)



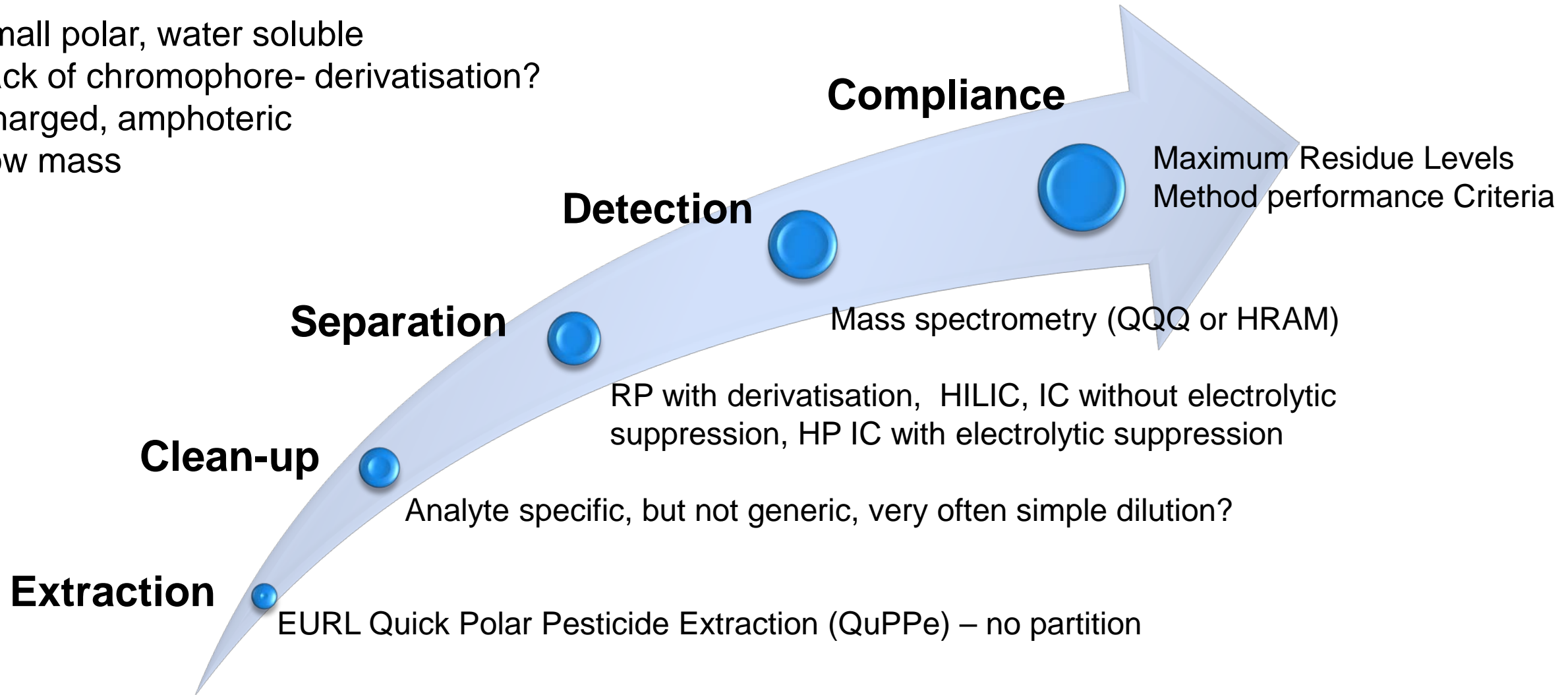
Perchlorate
(*m/z* 98.9491)

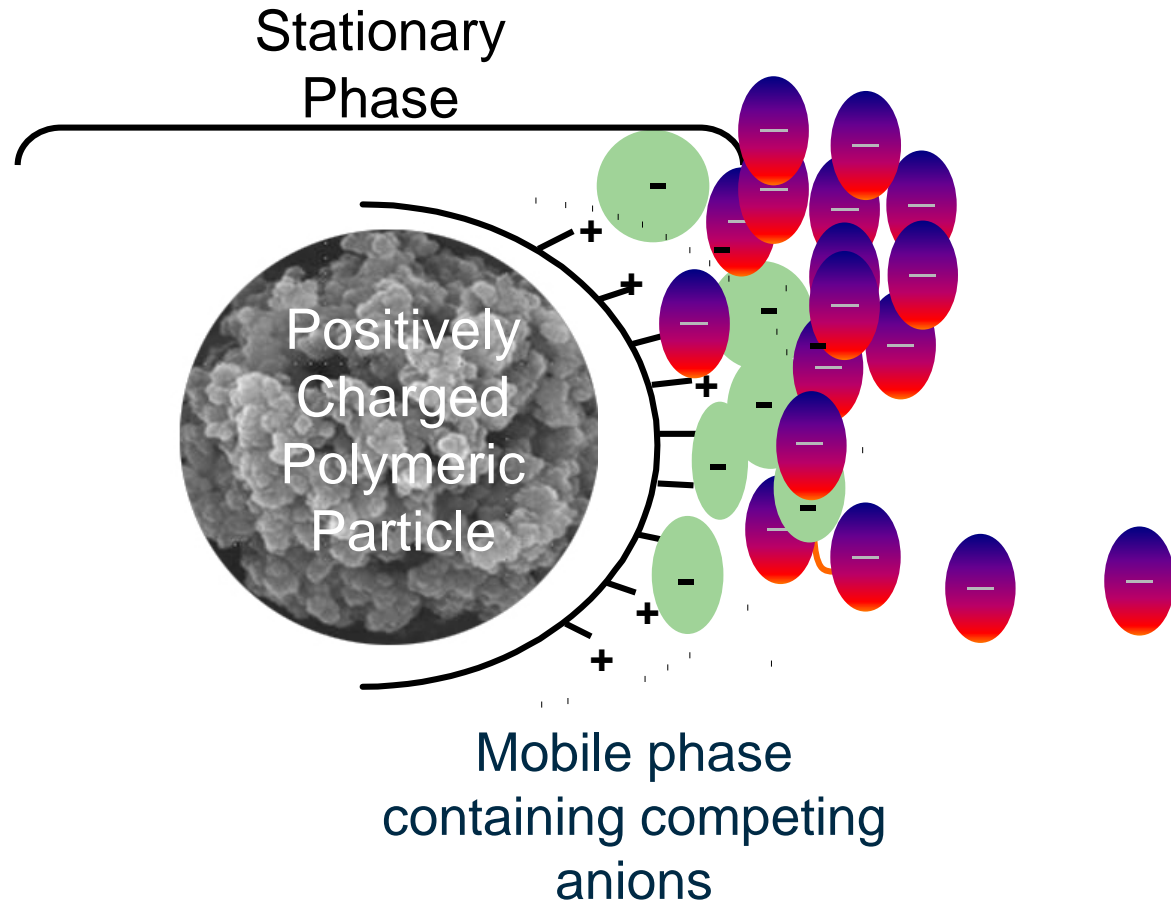
Glyphosate in the News

- Glyphosate is one of the highest use pesticides worldwide
- Used as a herbicide in agriculture, in parks and gardens and as a cereal crop desiccant
- Residues frequently detected in cereal and cereal products
- 2016 - low levels reported in German beers (and from other countries)
- 2018 - glyphosate reported in honey in Latin America
- 2015 - The [International Agency for Research on Cancer \(IARC\)](#) informed the World Health Organization (WHO) on cancer risk factors, and classified glyphosate as a 'probable carcinogen,
- EFSA derived a different conclusion
- EFSA have indicated the residue definition for glyphosate in risk assessment studies should be the sum of glyphosate, N-acetyl glyphosate, AMPA and N-acetyl AMPA
- EFSA & the European Commission have requested laboratories to provide more data

The Analytical Challenges for Multi-Analyte Anionic Pesticides Analysis

- Small polar, water soluble
- Lack of chromophore- derivatisation?
- Charged, amphoteric
- Low mass





Advantages of ion chromatography

- ✓ High Capacity of IC Columns
- ✓ High sensitivity
- ✓ Increased retention of polar pesticides
- ✓ Gradient elution
- ✓ Excellent selectivity
- ✓ Direct analysis
 - No chemical derivatization



High Performance Ion Chromatography (HPIC) provides many benefits

Benefits of Dionex Ion Chromatography Systems

- ✓ PEEK – no metals
- ✓ No metals contamination of column
- ✓ No chelation of analytes

- ✓ Electrolytic Eluent Generation
- ✓ Reproducible Gradients
- ✓ Reagent Free - Just add Water



Thermo Scientific™
Dionex™ Integriion™
HPIC™ system
(single channel system)



Thermo Scientific™
Dionex™ ICS-6000
(two channel system)

Benefits of Dionex Ion Chromatography Systems

- ✓ PEEK – no metals
- ✓ No metals contamination of column
- ✓ No chelation of analytes
- ✓ Electrolytic Eluent Generation
- ✓ Reproducible Gradients
- ✓ Reagent Free - Just add Water
- ✓ Flexible configuration
- ✓ Single or dual channel
- ✓ Concentration/inline sample cleanup
- ✓ 2D ion Chromatography
- ✓ Post Column AERS (Anion electrolytic regenerating suppressor)
- ✓ Enables use of high Ionic strength Mobile Phase
- ✓ Desalting of mobile phase
- ✓ Compatibility with MS

RFIC

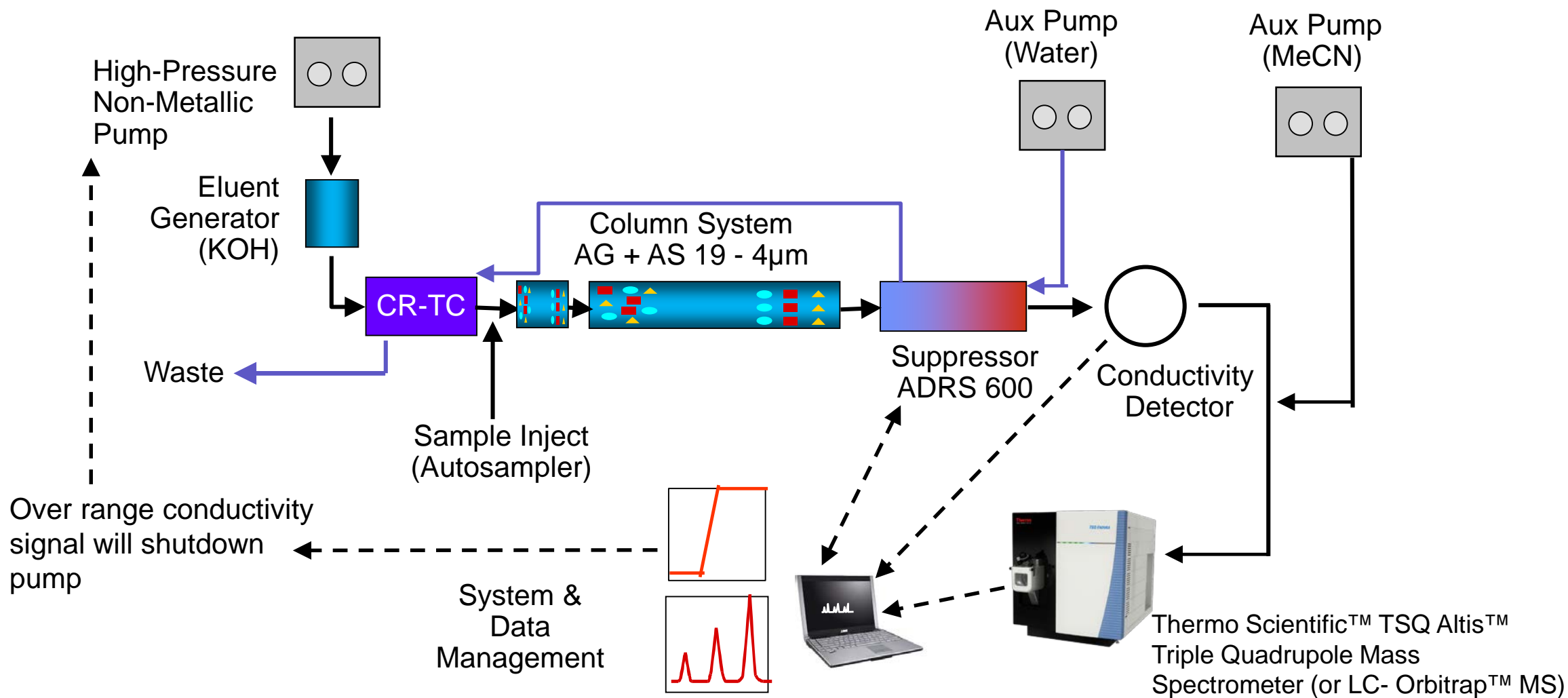


Dionex Integriion
(single channel system)



Dionex ICS-6000
(two channel system)

Optimisation of IC-QQQ MS/MS Configuration for Automated Routine Analysis



Modified QuPPE Extraction- Analysis of Wheat

Wheat samples (5 ± 0.1 g)

Add 10 mL water, Shake vigorously for 1 min
and stand to soak for 10 min

Add 10 mL methanol, Shake vigorously for 10 min

Freeze in -20 °C, 15 min

Centrifuge, 8000 rpm 8 min

Dilute $\times 10$ with water

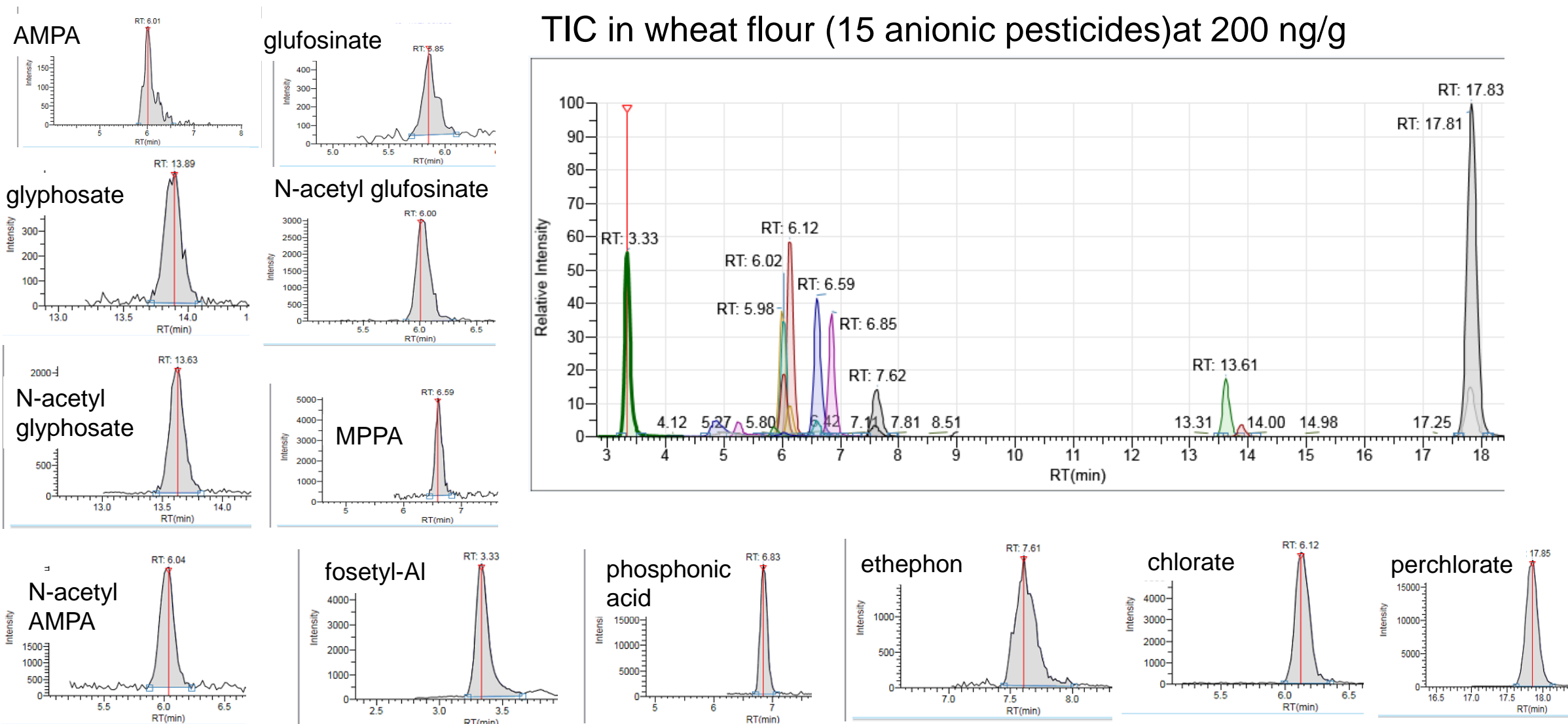
Push-through SPE -OnGuard RP cartridge

Filter for IC-MS/MS \rightarrow Inj 25 μ L

SPE & Filtration



Polar Anionic Pesticides Explorer - Response in Wheat Extracts



Extracted Ion chromatograms for analytes at 4 ng/g in sample

Anionic Pesticides in Wheat—Different Calibration Approaches

Calibration Approach →	Matrix-matched (no ILIS) %Recovery (%RSD)		Matrix-matched (+ ILIS) % Apparent Recovery (%RSD)		Procedural (No ILIS) % Apparent Recovery (%RSD)	
	10 ng/g	50 ng/g	10 ng/g	50 ng/g	10 ng/g	50 ng/g
Concentration →						
Analyte ↓						
Fosetyl-Al	93 (2.7)	89 (1.9)	-	-	96 (2.7)	94 (1.9)
Bialaphos	96 (6.4)	90 (3.2)	-	-	95 (5.7)	83 (3.2)
Glufosinate	85 (12)	76 (4.5)	92 (8.6)	94 (3.0)	87 (12)	82 (4.5)
AMPA	65 (6.6)	61 (4.9)	115 (6.1)	108 (9.0)	104 (6.5)	97 (4.9)
HEPA	86 (2.4)	80 (0.7)	-	-	96 (2.6)	94 (0.7)
N-acetyl AMPA	85 (1.0)	81 (0.6)	-	-	98 (1.1)	96 (0.6)
N-Acetyl-Glufosinate	79 (2.4)	72 (2.9)	-	-	87 (2.8)	95 (3.0)
Chlorate	77 (2.2)	73 (2.0)	96 (1.7)	92 (0.8)	100 (2.3)	101 (2.1)
MPPA	71 (1.0)	63 (2.5)	96 (1.4)	94 (1.9)	95 (1.1)	93 (2.6)
Phosphonic acid	36 (25)	69 (3.4)	-	-	84 (14)	96 (3.1)
Ethephon	79 (1.4)	74 (0.9)	97 (2.1)	92 (0.3)	100 (1.4)	98 (0.8)
Cyanuric acid	87 (12)	89 (1.8)	-	-	95 (12)	101 (1.8)
N-Acetyl-Glyphosate	60 (2.9)	53 (1.7)	-	-	100 (3.0)	94 (1.8)
Glyphosate	40 (4.5)	34 (2.0)	111 (2.2)	101 (1.5)	104 (5.4)	98 (2.1)
Perchlorate	66 (4.2)	63 (3.0)	100 (0.9)	97 (0.7)	90 (5.2)	99 (3.1)

Wheat Flour Data Summary- Procedural Standards

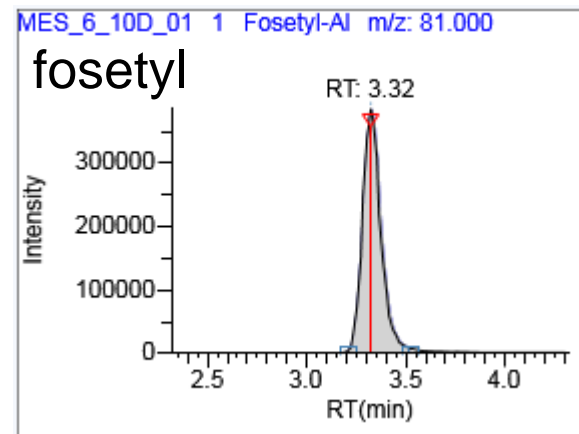
Sample No.7 used as calibration curve matrix	PS Curve	Spiked level (10 ng/g)							
		Sample No. 7 (n=5)		No. 4 (n=3)		No. 6 (n=3)		No. 9 (n=3)	
		Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
AMPA	ISTD	108	6.5	114	7.6	86	6.5	111	4.1
Chlorate	ISTD	98	1.7	84	2.0	87	2.4	77	0.8
Ethephon	ISTD	97	2.2	103	3.7	100	7.6	103	1.5
Glufosinate	ISTD	98	8.7	88	8.7	95	5.5	100	7.3
Glyphosate	ISTD	101	2.4	90	4.1	93	9.9	99	7.3
MPPA	ISTD	96	1.4	102	3.1	116	2.2	97	1.7
Perchlorate	ISTD	86	1.0	95	1.1	88	4.0	77	2.0
Cyanuric acid	No ILIS	95	12		1.0	69	2.1	67	2.7
Bialaphos	No ILIS	95	5.7	67	0.3	58	4.5	68	2.0
Fosetyl-Al	No ILIS	96	2.7	85	1.8	75	2.0	49	1.3
HEPA	No ILIS	95	2.6	85	2.6	80	6.5	87	4.6
N-acetyl AMPA	No ILIS	97	1.1	95	4.0	79	1.0	91	0.9
N-Acetyl-Glufosinate	No ILIS	87.	2.8	94	0.9	68	2.4	92	1.6
N-Acetyl-Glyphosate	No ILIS	100	3.0	68	3.7	59	2.7	87	2.3
Phosphonic acid	No ILIS	84	14	87	1.7	79	1.9	93	2.2

- Over-spiking or standard addition is the only option without availability of ILIS

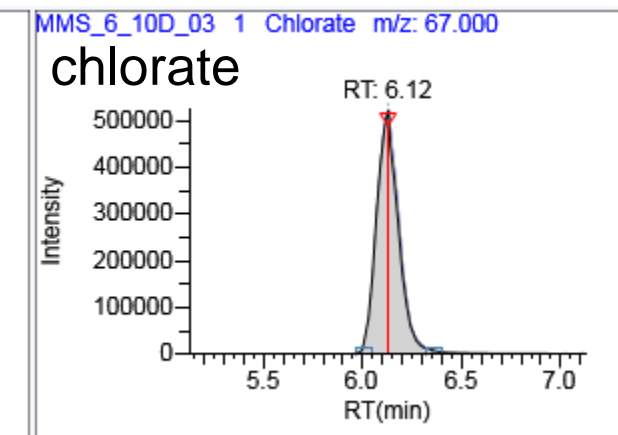
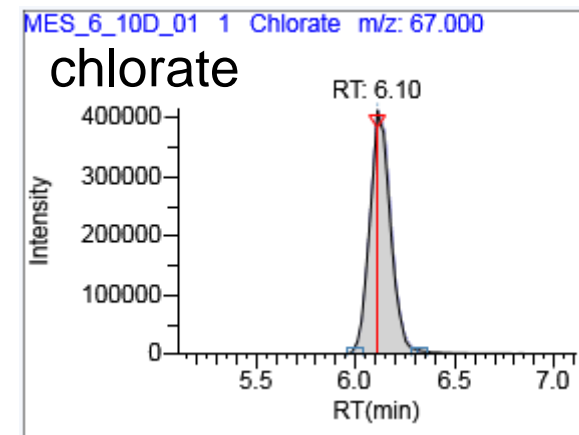
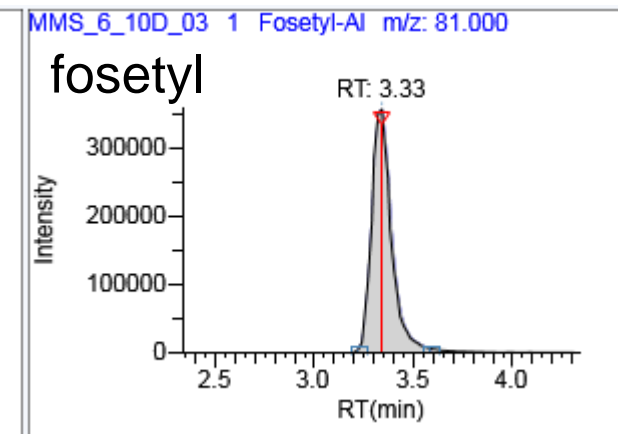
Robustness – with OnGuard-RP SPE

- After a sequence of 80 injections of wheat flour extracts:
- The ion source remained clean
- Peak shapes & retention times remained stable
- Analyte response remained stable

Injection # 09



Injection # 89

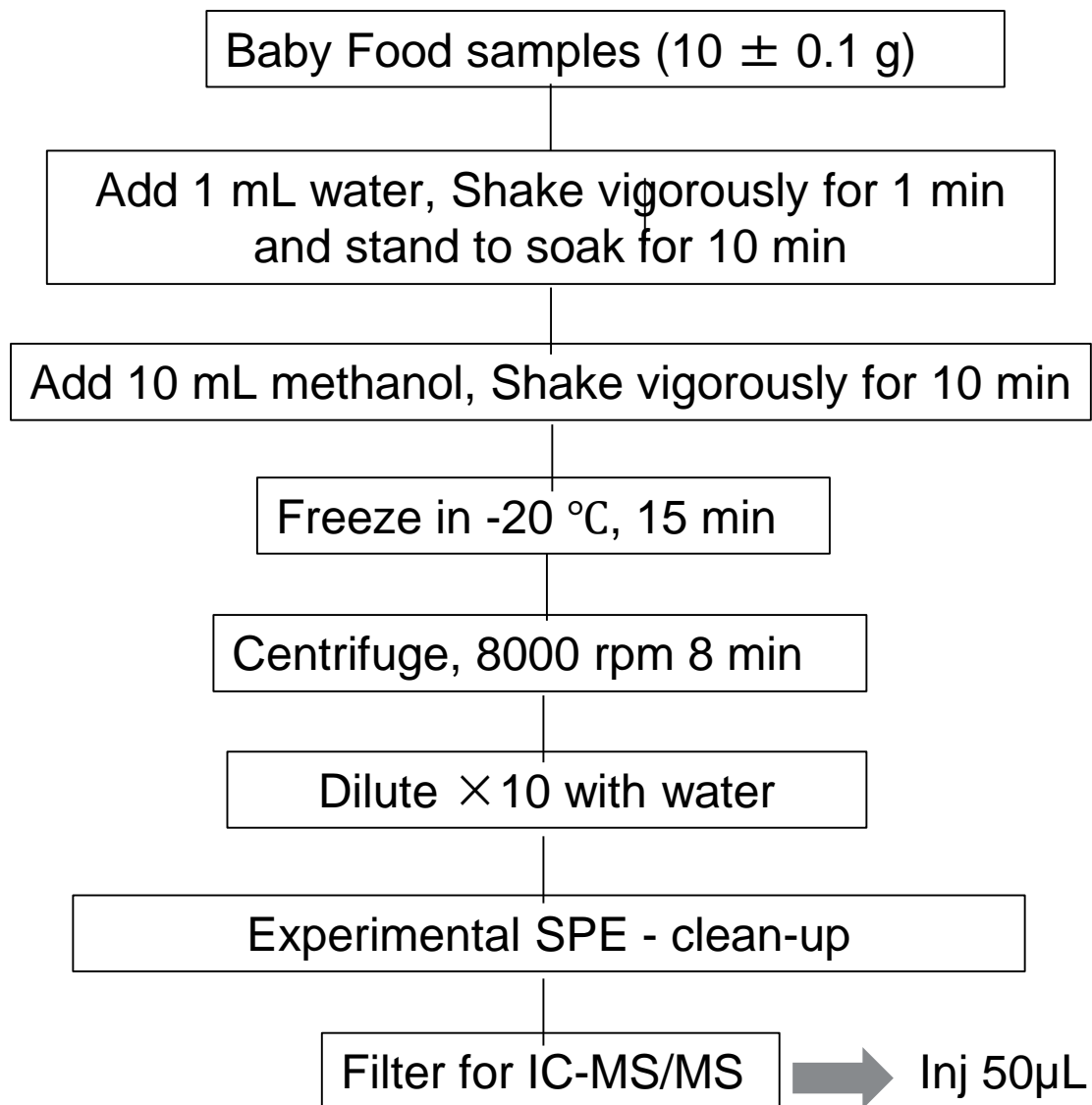


Leek Validation Data – Procedural Standards

No.3 as calibration curve matrix	PS Curve	Spiked level 1 (10 ng/g)					
		Sample No. 1 (n=5)		No. 2 (n=5)		No. 3 (n=5)	
		Recovery%	RSD%	Recovery%	RSD%	Recovery%	RSD%
AMPA	ISTD	118	2.0	108	6.5	94	9.6
Chlorate	ISTD	70	1.5	70	1.1	105	1.80
Ethephon	ISTD	104	2.7	104	1.4	102	2.8
Glufosinate	ISTD	108	8.4	90	8.1	119	8.0
Glyphosate	ISTD	95	1.4	95	2.4	94	1.6
MPPA	ISTD	94	2.8	93	1.9	92	1.8
Perchlorate	ISTD	85	0.6	84	0.3	98	0.4
Cyanuric acid	No ILIS	101	5.8	115	8.2	104	4.4
Bialaphos	No ILIS	101	5.7	95	7.6	82	9.0
Fosetyl-Al	No ILIS	97	0.9	93	3.2	93	0.8
HEPA	No ILIS	97	8.9	104	7.5	87	6.1
N-acetyl AMPA	No ILIS	73	1.6	75	1.9	100	0.7
N-Acetyl-Glufosinate	No ILIS	98	1.7	78	2.1	88	1.6
N-Acetyl-Glyphosate	No ILIS	104	0.7	114	1.2	103	0.8
Phosphonic acid	No ILIS	79	1.3	83	1.7	85	8.1

Recoveries within the range of 70% to 120%, RSD% are all below 10%

Analysis of Polar Pesticides in Baby-Food- A Challenging Matrix!



- The most challenging matrix tested
- Can cause pressure increase!

Preliminary Method Validation at 2.5 ng/g – Baby Foods

Recoveries of 10 anionic pesticides in 9 different baby food (spiked 2.5 ng/g)

Compounds	9 different baby food matrices									Average	RSD %
	1#	5#	6#	7#	8#	9#	10#	11#	12#		
Fosetyl-Al	113.7	109.4	113.2	114.6	109.4	114.0	113.7	111.0	105.8	111.6	2.64
Bialaphos	109.1	109.9	108.8	108.7	109.2	101.2	107.8	110.7	110.5	108.4	2.65
N-acetyl AMPA	103.2	118.7	109.7	108.1	111.7	111.8	112.8	97.1	107.5	108.9	5.65
N-Acetyl-Glufosinate	113.1	112.1	117.8	108.1	115.2	104.0	120.8	116.3	103.4	112.3	5.38
N-Acetyl-Glyphosate	105.3	105.4	110.5	107.9	109.8	109.4	118.9	115.3	111.3	110.4	4.00
AMPA	151.8	91.4	93.9	75.9	107.3	130.6	170.3	116.3	104.3	115.8	26.22
Glufosinate	111.6	106.3	100.5	121.4	115.5	104.5	120.4	113.2	91.1	109.4	8.97
MPPA	118.9	115.7	98.1	106.0	115.2	114.5	125.5	104.8	95.3	110.4	9.06
Ethephon	97.2	97.9	120.3	108.8	103.2	100.1	117.0	107.2	97.6	105.5	8.13
Glyphosate	98.8	109.0	117.2	116.3	106.1	132.5	106.6	101.6	108.9	110.8	9.13

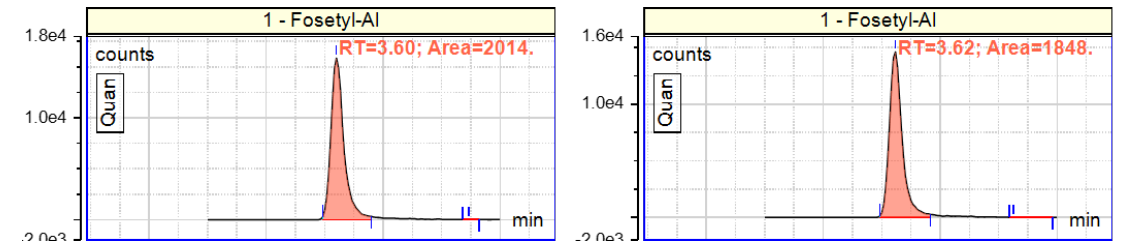
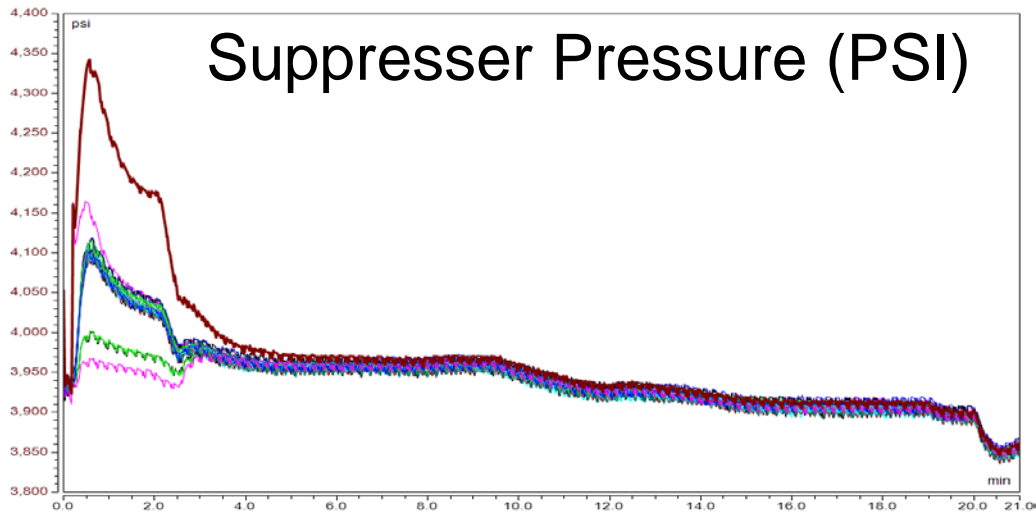
Spiked recoveries were calculated using the matrix matched standard in the corresponding sample

Because of high blank, chlorate, perchlorate, and phosphonic acid could not evaluate recoveries. The sensitivity was not sufficient validate HEPA at 2.5 ng/g

Robustness of IC-MS/MS System

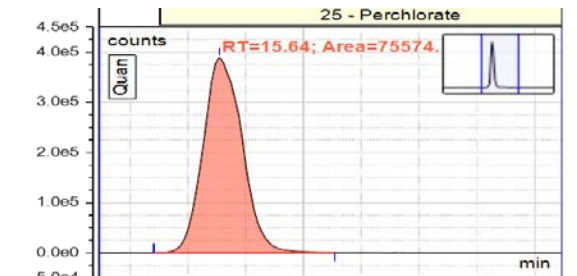
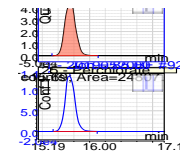
- Suppressor ADRS 600 (2mm) has been subjected to 300 injections of baby food matrix- cleaned up by SPE

Peak shape after 90 injections



Fosetyl-Al

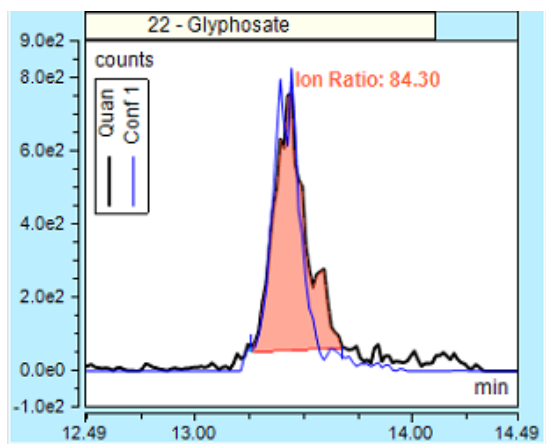
- AG19 guard and AS19 analytical column have been used for about 1300 injections, including 870 injections of baby food matrix



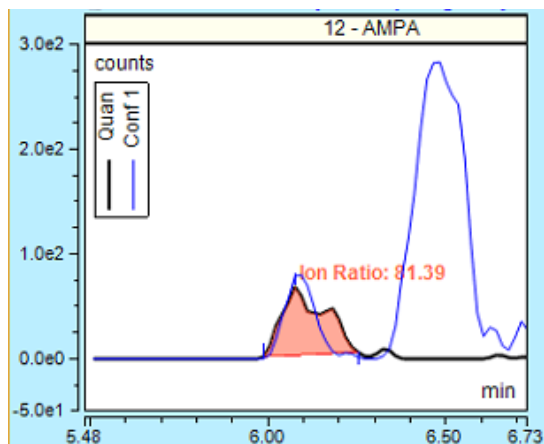
Perchlorate

LOQ of Glyphosate / Glufosinate and Metabolites in Baby Food Matrix

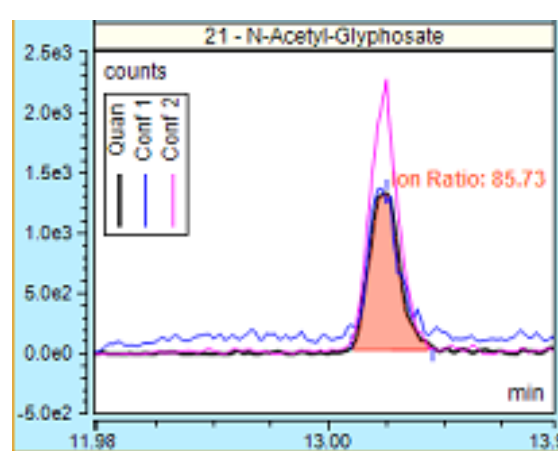
Glyphosate (2.5 ng/g)



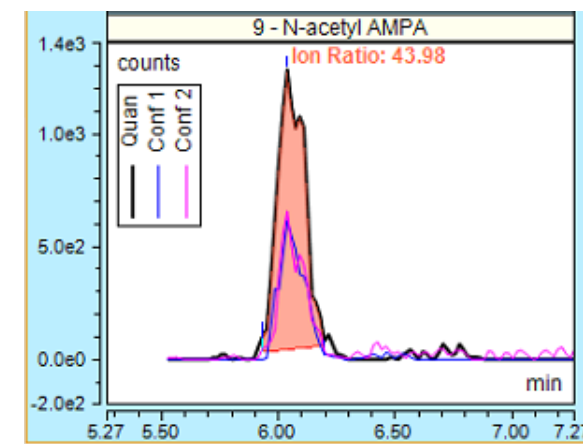
AMPA (2.5 ng/g)



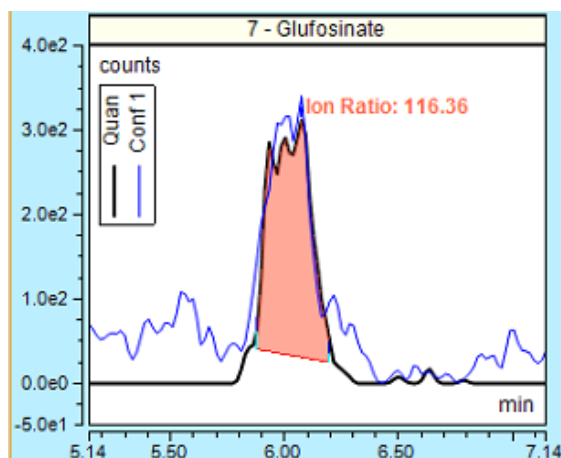
N-acetyl glyphosate(2.5 ng/g)



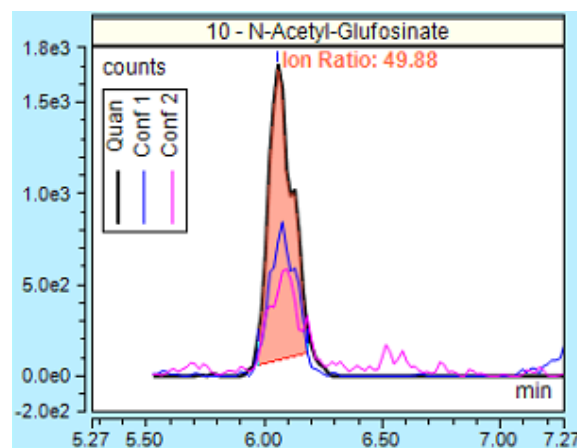
N-acetyl AMPA (2.5 ng/g)



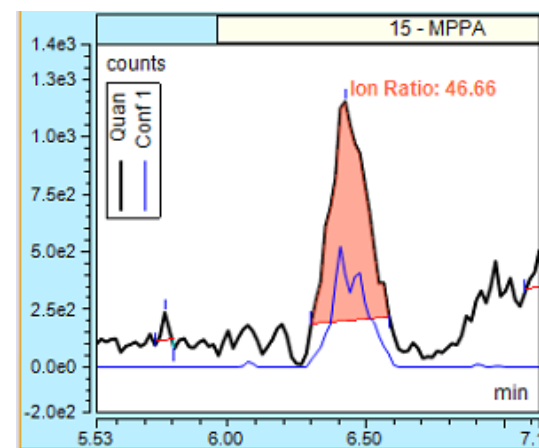
Glufosinate (5 ng/g)



N-acetyl glufosinate (2.5 ng/g)



MPPA (2.5 ng/g)



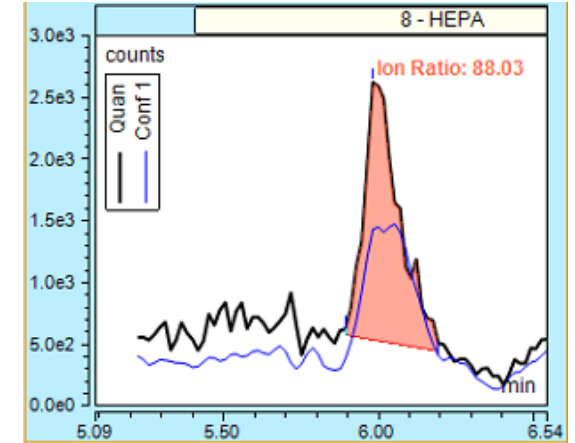
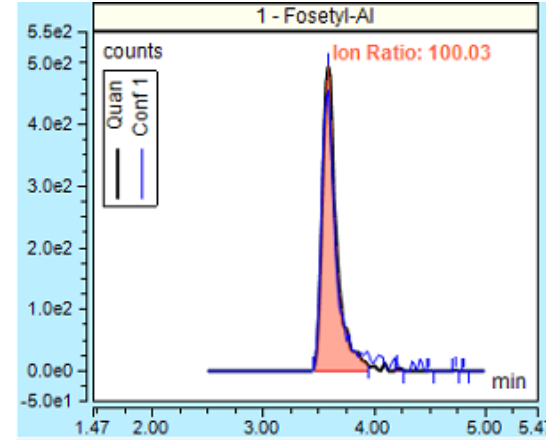
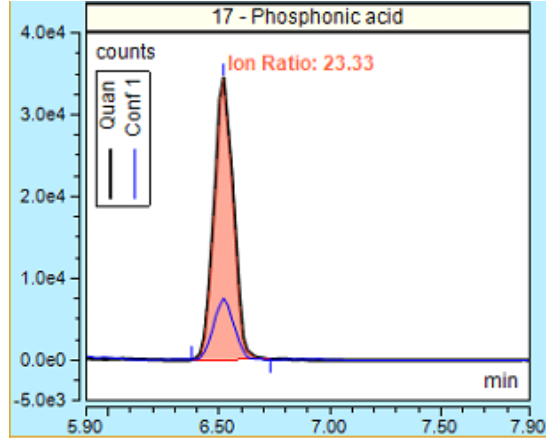
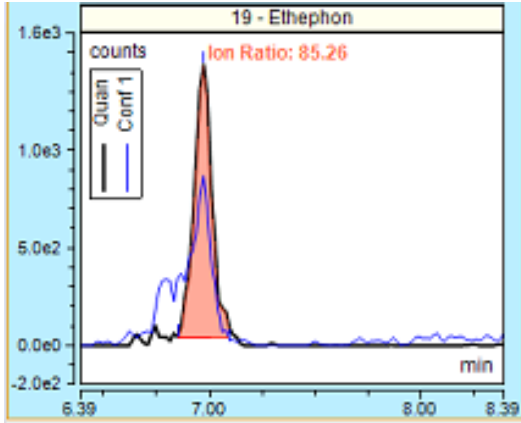
Anionic Pesticides LOQ in Baby Food- Matrix-Matched Standards

Fosetyl (2.5 ng/g)

Phosphonic acid (2.5 ng/g)

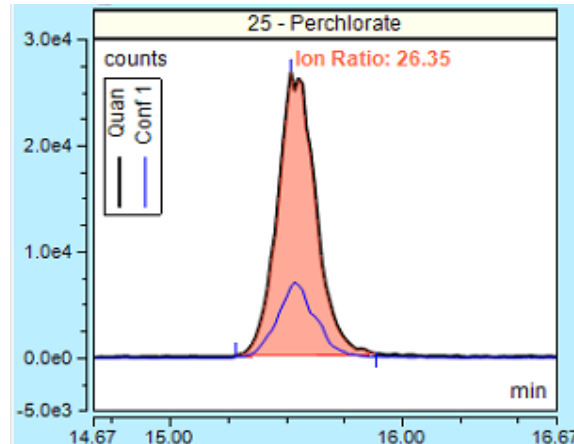
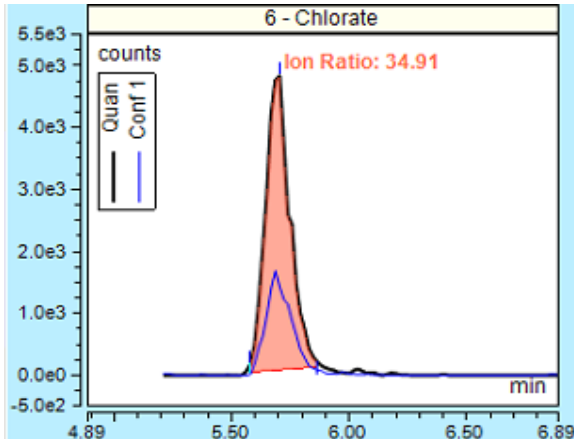
Ethephon (5 ng/g)

Hepa (5 ng/g)



Chlorate at 2.5 ng/g

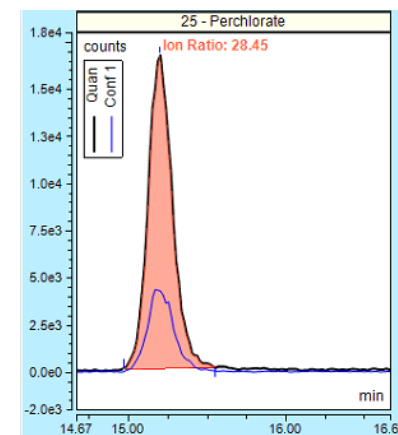
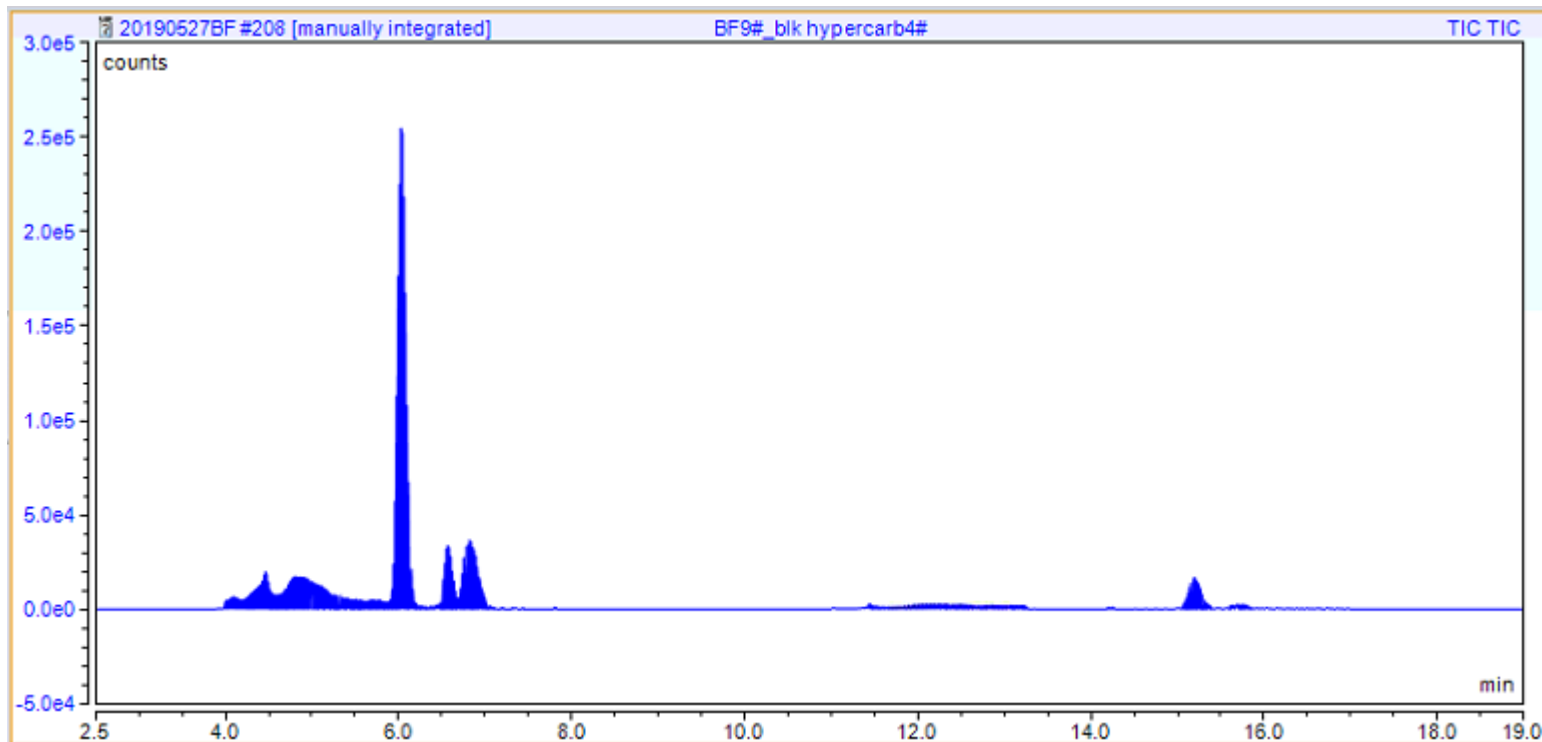
Perchlorate (2.5ng/g ng/g)



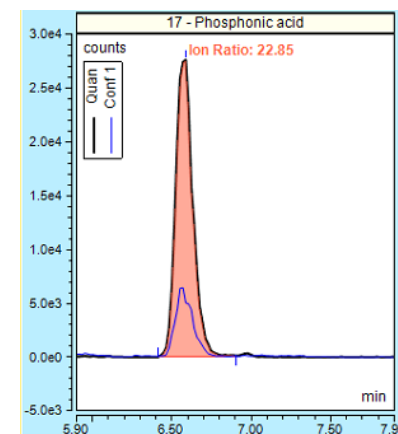
- All ion ratios are within the expected range compared to standards

Residues Detected in Retail Samples

- TIC of baby food sample #9



Perchlorate
in sample

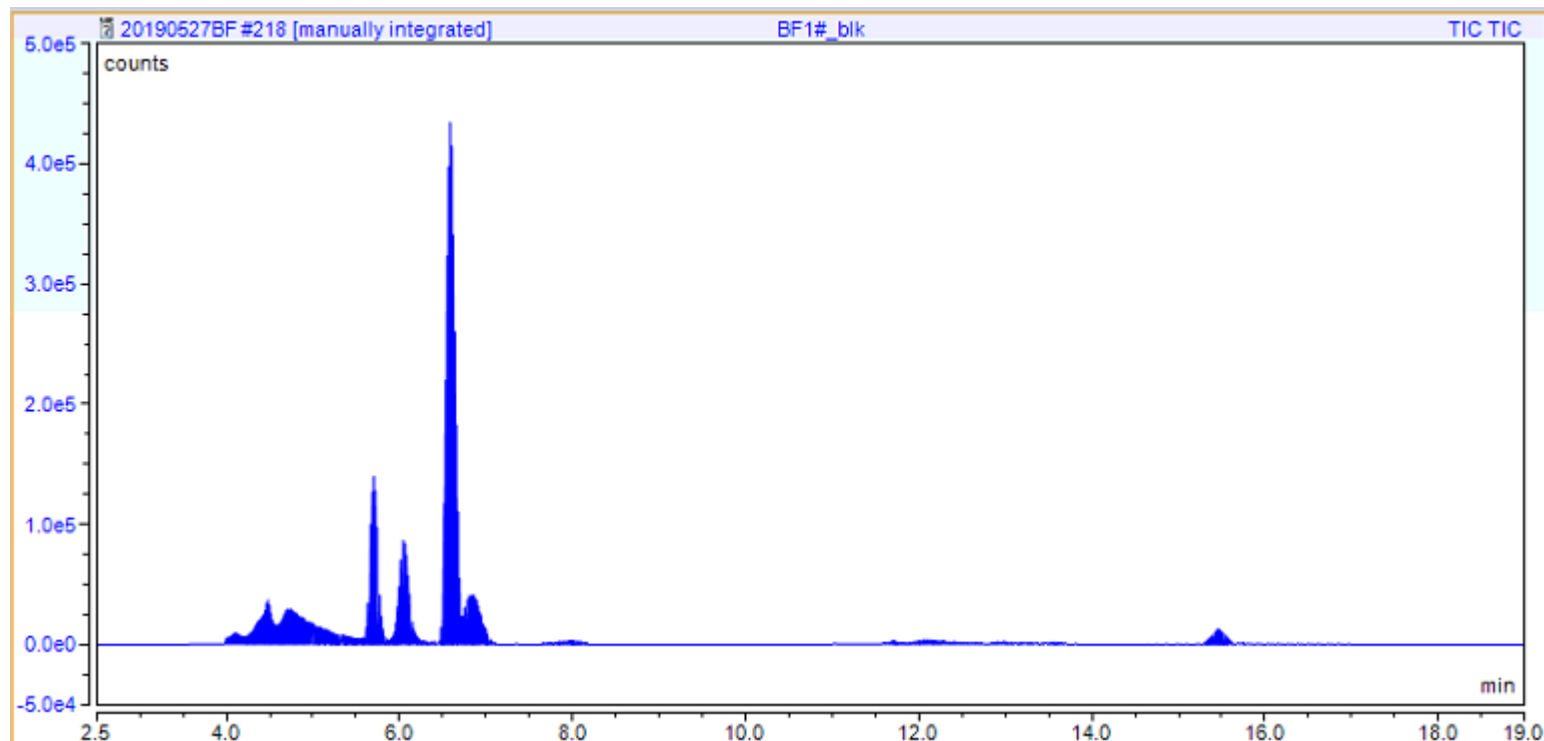


Phosphonic acid
in sample

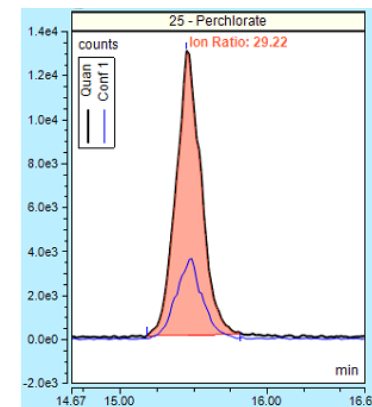
- Concentration too high to determine LOQ

Potential Residues in Baby Food Samples (2)

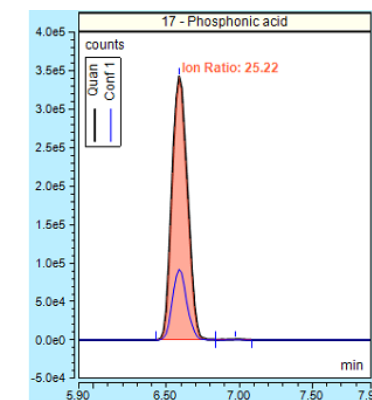
- TIC of blank sample #1



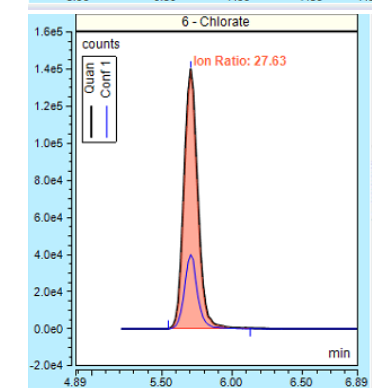
- Concentration too high to determine LOQ



Perchlorate
in sample



Phosphonic acid
in sample



Chlorate
in sample

Analysis of Glyphosate – Now Foods

now Liquid Chromatography-Tandem Mass Spectrometry vs. Ion Chromatography-Tandem Spectrometry for Glyphosate Residues Analysis in Botanical Matrices
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Introduction
Glyphosate is the widely adopted herbicide for agricultural and domestic use. It was first commercialized by Monsanto under a trade name, "Roundup". The widespread use of glyphosate and its application as a desiccant on crops results in a constantly recurring human exposure. Although the World Health Organization (WHO) classifies glyphosate as "safe" based on toxicological evidence of carcinogenicity in humans, epidemiological evidence supports the correlation between glyphosate usage and cancer occurrence.

Challenges
Due to growing healthy concerns of the general public, the analysis of glyphosate and its derivatives in foods and dietary supplements is needed.

Experimental
Materials:
Analytical standards of Glyphosate, 3-(13C)-15N-Glyphosate, AMPA, 13C-15N-AMPA, MPPA, glufosinate were obtained from commercial sources.
Reagents were obtained from commercial vendors and were reagent grade.
Matrices (Alfalfa (insecticide treated), rice, oat, soy powder and alfalfa).

IC-MS/MS
Apparatus:
• Dionex Integrator HPLC
• Thermo Micro Spectrometer
• Dionex HPLC AS 18 (4 unit, 2 x 250 Column)
• Dionex IonPac AG 19 (4 unit, 2 x 50 Guard Column)
• Dionex ASRS 500 (200mL Suppressor)

IC Conditions:
• Column Temp: 30°C
• Injection Volume: 100 µL
• Eluent Flow Rate: 0.33 mL/min
• Acetate buffer introduced at 5.1 min into the MS source from I.C.
• Injection Time: 30 min
• Gradient: 5 mM KOH (aq) to 20 mM KOH (aq) at 8 min, 60 mM KOH (aq) at 12 min, back at 50 mM KOH (aq) at 22 min, back at 5 mM KOH (aq) at 25 min. Cycle time 28 min.

MS Conditions:
• Polarity: Negative HESI
• Negative Ion Voltage: 2600V
• Sheath Gas (SG): 37
• Aux Gas (AG): 7
• Sweep Gas (SG): 1
• Ion Transfer Tube: 325°C
• Vaporizer Temp: 300°C

LC-MS/MS
Apparatus:
• HPLC (HPLC) Picoval
• Thermo Micro Spectrometer
• Shodex HLC802 VR-2D Column

HPLC Conditions:
• Column Temp: 40°C
• Injection Volume: 10 µL
• Mobile Phase A: 85:20:12 (Water:Acetonitrile:Ethanol:Ammonium Bicarbonate)
• Mobile Phase B: 20 mM Ammonium Bicarbonate
• Flow Rate: 0.5 mL/min
• Time: 5 min, 15 min
• Gradient: 0 100% B, 15 0 100 B, 21 100 0, 24 100 0

MS Conditions:
• Polarity: Negative HESI
• Negative Ion Voltage: 2600V
• Sheath Gas (SG): 37
• Aux Gas (AG): 7
• Sweep Gas (SG): 1
• Ion Transfer Tube: 325°C
• Vaporizer Temp: 300°C

Sample Preparation:
• Weigh 0.5 g sample in a clean vial.
• Add 10 mL of water.
• Allow to hydrate for 30 min.
• Add 33 mL of cold methanol.
• Shake on orbital shaker for 10 min.
• Centrifuge @ 10,000 rpm for 5 min.
• Dilute in solvent 1:5 with DI water before analysis.

Standard Preparation:
• Analytical standards were prepared in water.
• Standards were prepared in the range of 0.2 ng/mL - 500 ng/mL.

IC-MS/MS Results
Chromatographic Separation and Linearity
Figure 1: Chromatographic separation of glyphosate and metabolites with their corresponding mass spectra from IC-MS/MS.
Figure 2: Linear responses of glyphosate, AMPA, glufosinate and 16:1N-D-200 (ng/mL).
Figure 3: Peak responses of glyphosate and AMPA at 10 ng/mL.
Figure 4: 10 ng/mL recoveries of glyphosate and AMPA in rice.
Figure 5: 10 ng/mL recoveries of glyphosate and AMPA in oats.

LC-MS/MS Results
Chromatographic Separation and Linearity
Figure 6: Chromatographic separation of glyphosate and metabolites with their corresponding mass spectra from LC-MS/MS.
Figure 7: Quantitative peak and linear curve fit of glyphosate, AMPA, glufosinate and 16:1N-D-200 (ng/mL).
Figure 8: Peak responses of glyphosate and AMPA at 10 ng/mL.
Figure 9: 10 ng/mL recoveries of glyphosate in rice a lot of water.
Discussion:
• Two chromatographic approaches were evaluated for the analysis of glyphosate and related compounds in selected botanical matrices.
• The IC-MS/MS approach resulted in good peak responses and curves with wide linear responses with LODs below 0.2 ng/mL and LOQs below 1 ng/mL, except for AMPA with LOQ at 7 ng/mL.
• The LC-MS/MS approach is similar to the above, demonstrating all 10 target analytes that could be analyzed in the same run.
• The peak shape of AMPA was not optimal when analyzing it by IC-MS/MS. Broadening of the peak resulted in decreased sensitivity.
• The LC-MS/MS approach also resulted in good peak responses, wide range linear curves, LODs below 0.2 ng/mL, and LOQs below 1 ng/mL, except for glufosinate, with LOQ at 8 ng/mL.
• Although the peaks were sharper compared to those from IC-MS/MS, the response of glyphosate was lower than anticipated and peak detection was observed at lower concentrations.
• Matrix spike recoveries from both techniques were between 75-105% with the exception of AMPA (IC-MS/MS) and glufosinate (LC-MS/MS), which were between 50-100% recoveries, when corrected with corresponding ISDs.
References:
• S. Adame, J. Guedes, M. Oliveira, S. C. Soares, J. Lopes, F. Duarte, Development and Validation of the Chromatography to Analyze Residues of Glyphosate, Malathion, and Carbaryl. Food Control 2014, 48, 1-10.
• B. J. E. P. F. M., Overcoming the Challenges of Analyzing Analyte Pairs in Food.

- Poster at AOAC International meeting 2018
- Analysis of glyphosate, AMPA, Glufosinate and MPPA in Macca, Silymarin, Cats Claw
- Quantification well below 10 ng/g using Altis
- Compared IC-MS/MS and HPLC.
- IC-MS preferred because of higher sample capacity and lower detection limits

Summary of IC-MS/MS Workflow Optimisation and Benefits

- **Compliance** - with current EU MRL residue definitions/levels & EU SANTE guidelines for method validation and ongoing quality control
- **Productivity** - Aggregation of 2-3 methods into a single Analysis
- **Robustness** – proven for complex matrices such as Wheat and Baby Foods

- Soon available as an off-the shelf-analytical workflow including pre-loaded acquisition and data processing methods, and system suitability check standard solution
- Ease of Implementation- all instrument & software parameters optimised and documented to assist operator to maintain high system sensitivity
- Standardised configuration for improved customer support
- If ILIS are not available then standard addition is an option for complex matrices

- **And YES – for all of the benefits above IC-MS/MS can provide an answer to the analysis of Anionic pesticides**

Thanks for your attention



Thank You

For further Information look out for Separation Science Webinar – July 17th 2019

<https://webinar.sepscience.com/form/a-new-integrated-sample-to-result-analytical-workflow-for-the-sensitive-and-reliable-analysis-of-polar-anionic-pesticides-and-metabolites>