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

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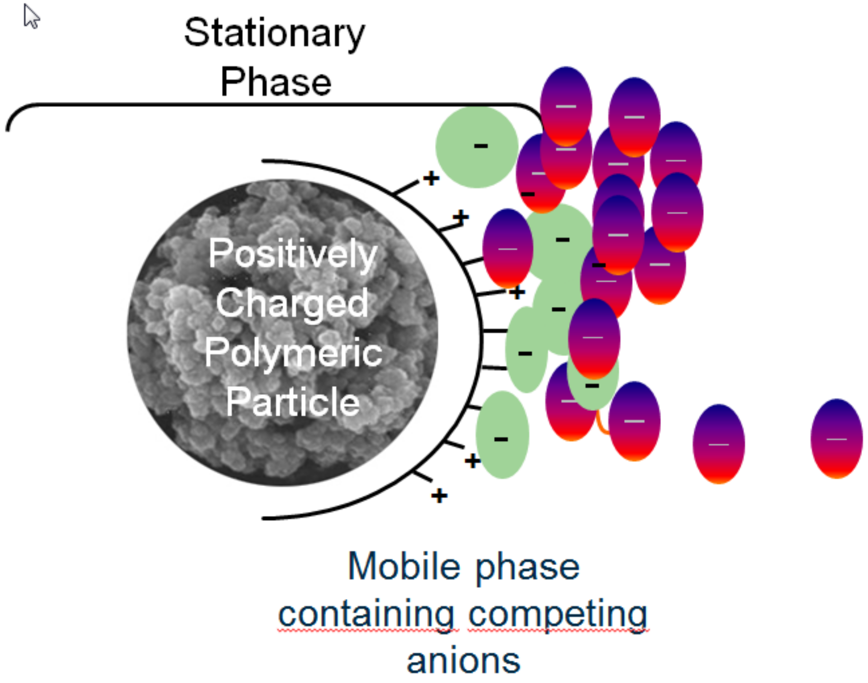
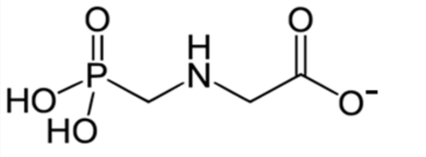
NPRW 2019_Oslo June 12-13

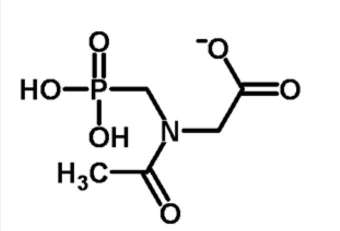
Outline

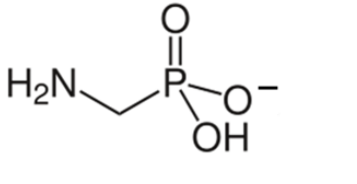
- Is IC-MS/MS a solution to the `hot topic` of analysis of polar pesticides?
- Benefits of using high-performance ion exchange chromatography coupled to mass spectrometry
- Anionic pesticide workflow development & validation
- Issues encountered during optimisation
- Summary

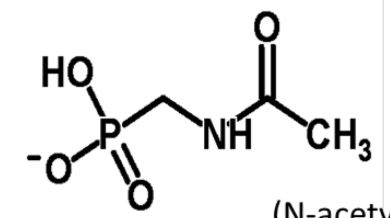


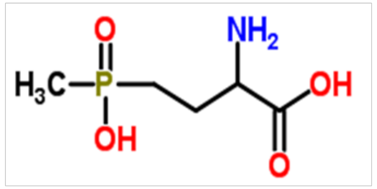
Anionic Pesticides Widely Used in Agricultural Production and Food Preparation

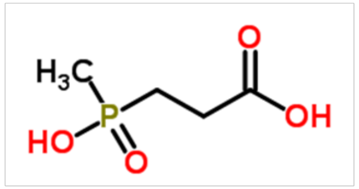

Glyphosate
(m/z 168.0067)

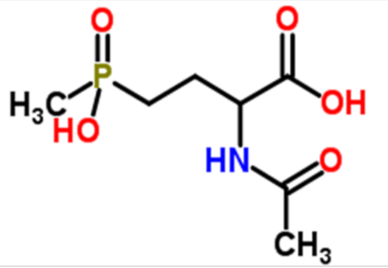

N-acetyl glyphosate
(m/z 210.0173)

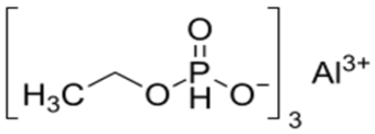

Aminomethyl phosphonic acid
(AMPA)
(m/z 110.0012)

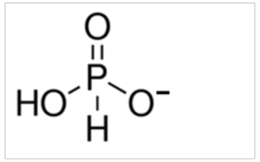

(N-acetyl AMPA)
(m/z 152.0118)

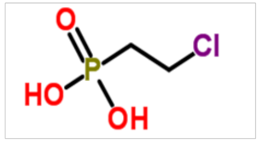

Glufosinate
(m/z *180*)

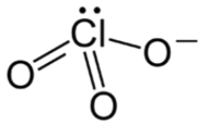

3-MPPA
(m/z 151)

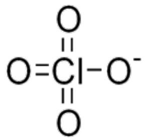

N- acetyl Glufosinate
(m/z *222)*


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)

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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

Compliance

Maximum Residue Levels
Method performance Criteria

Detection

Mass spectrometry (QQQ or HRAM)

Separation

RP with derivatisation, HILIC, IC without electrolytic suppression, HP IC with electrolytic suppression

Clean-up

Analyte specific, but not generic, very often simple dilution?

Extraction

EURL Quick Polar Pesticide Extraction (QuPPe) – no partition

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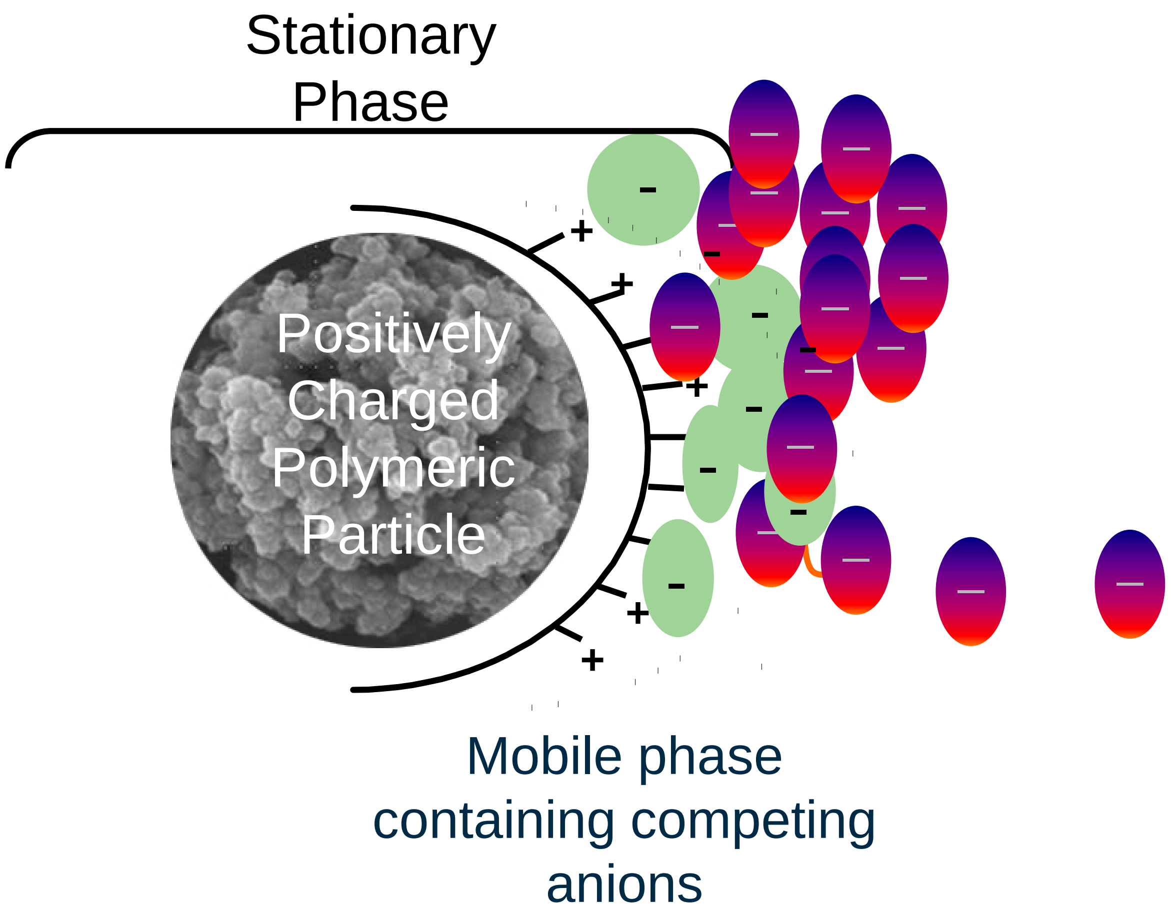
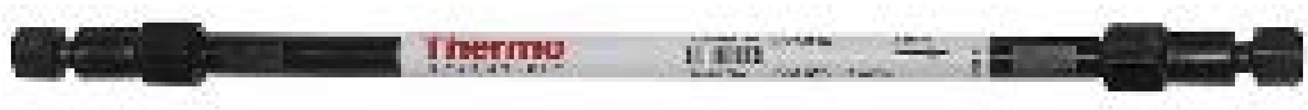


Advantages of ion chromatography

- ✓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™ Integrion™ HPIC™ system (single channel system)

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

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- ✓ PEEK – no metals
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- ✓ Electrolytic Eluent Generation
- ✓ Reproducible Gradients
- ✓ Reagent Free - Just add Water

- ✓ Flexible configuration
- ✓ Single or dual channel
- ✓ Concentration/inline sample cleanup
- ✓ 2D ion Chromatography

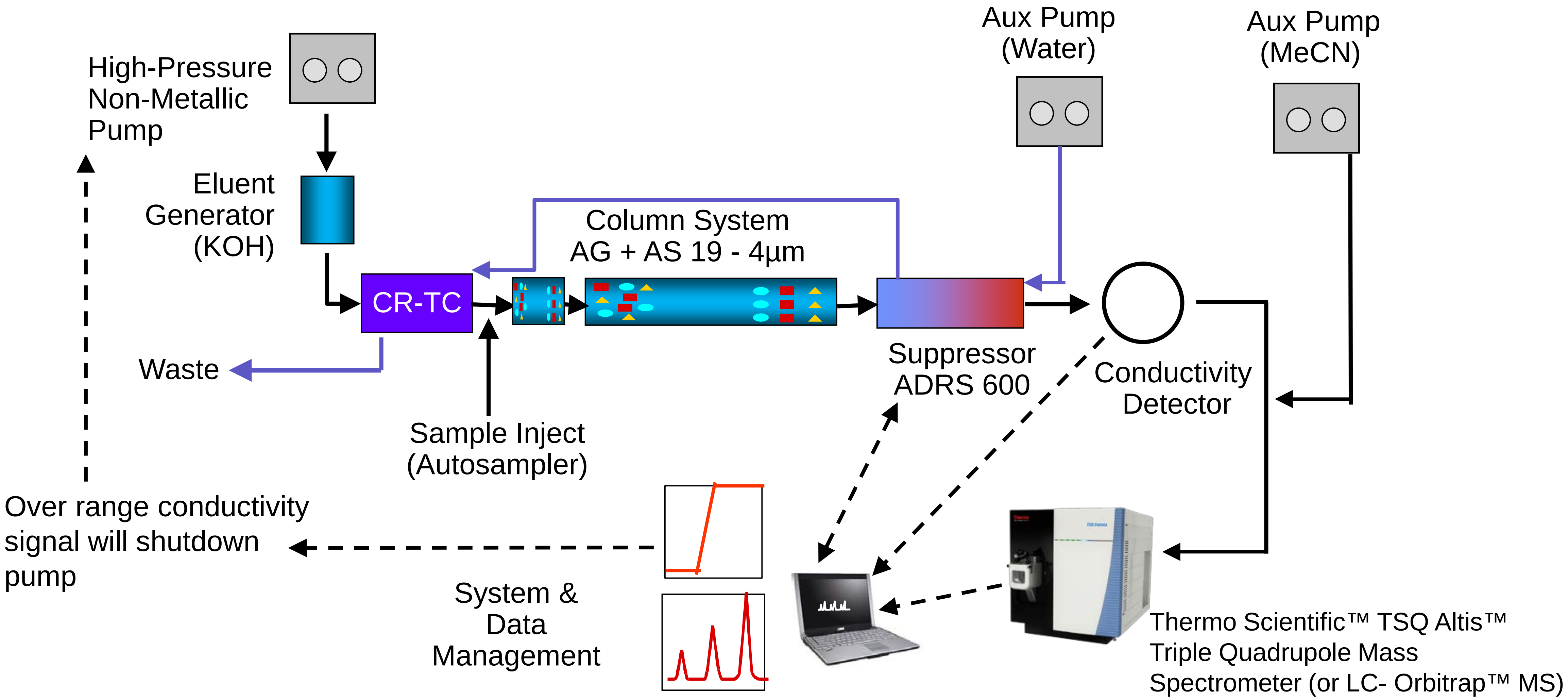
- ✓ Post Column AERS (Anion electrolytic regenerating suppressor)
- ✓ Enables use of high Ionic strength Mobile Phase
- ✓ Desalting of mobile phase
- ✓ Compatibility with MS

RFIC

Dionex Integrion (single channnel 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 ℃, 15 min

↓

Centrifuge, 8000 rpm 8 min

↓

Dilute×10 with water

↓

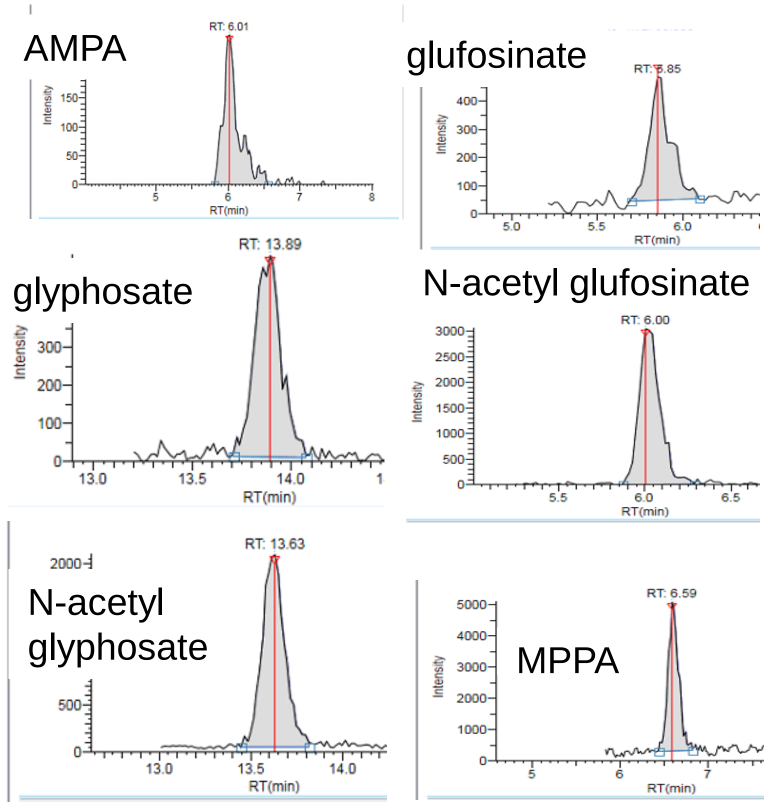
Push-through SPE -OnGuard RP cartridge

↓

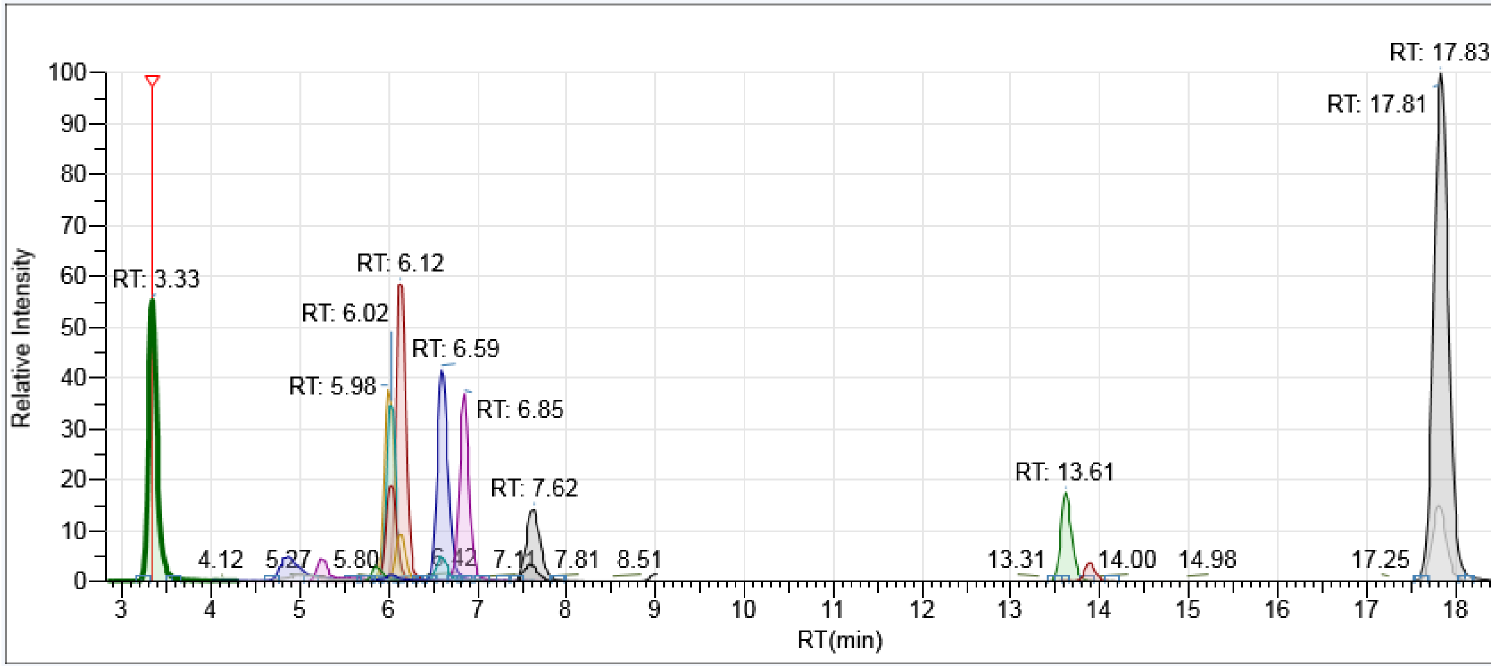
Filter for IC-MS/MS → Inj 25µL

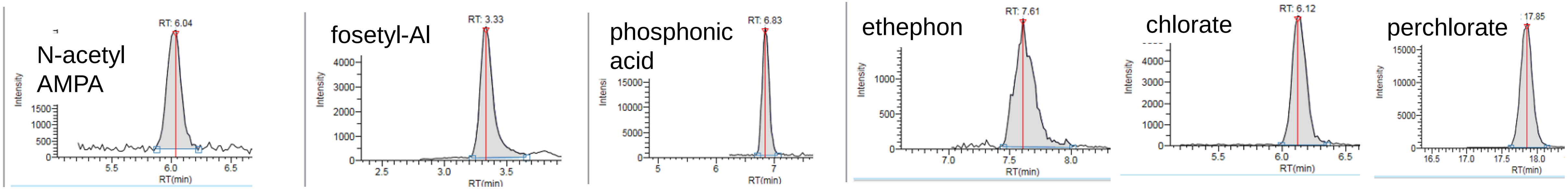
SPE & Filtration

Polar Anionic Pesticides Explorer - Response in Wheat Extracts



TIC in wheat flour (15 anionic pesticides)at 200 ng/g





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

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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)	
Concentration ➡	10 ng/g	50 ng/g	10 ng/g	50 ng/g	10 ng/g	50 ng/g
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)

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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

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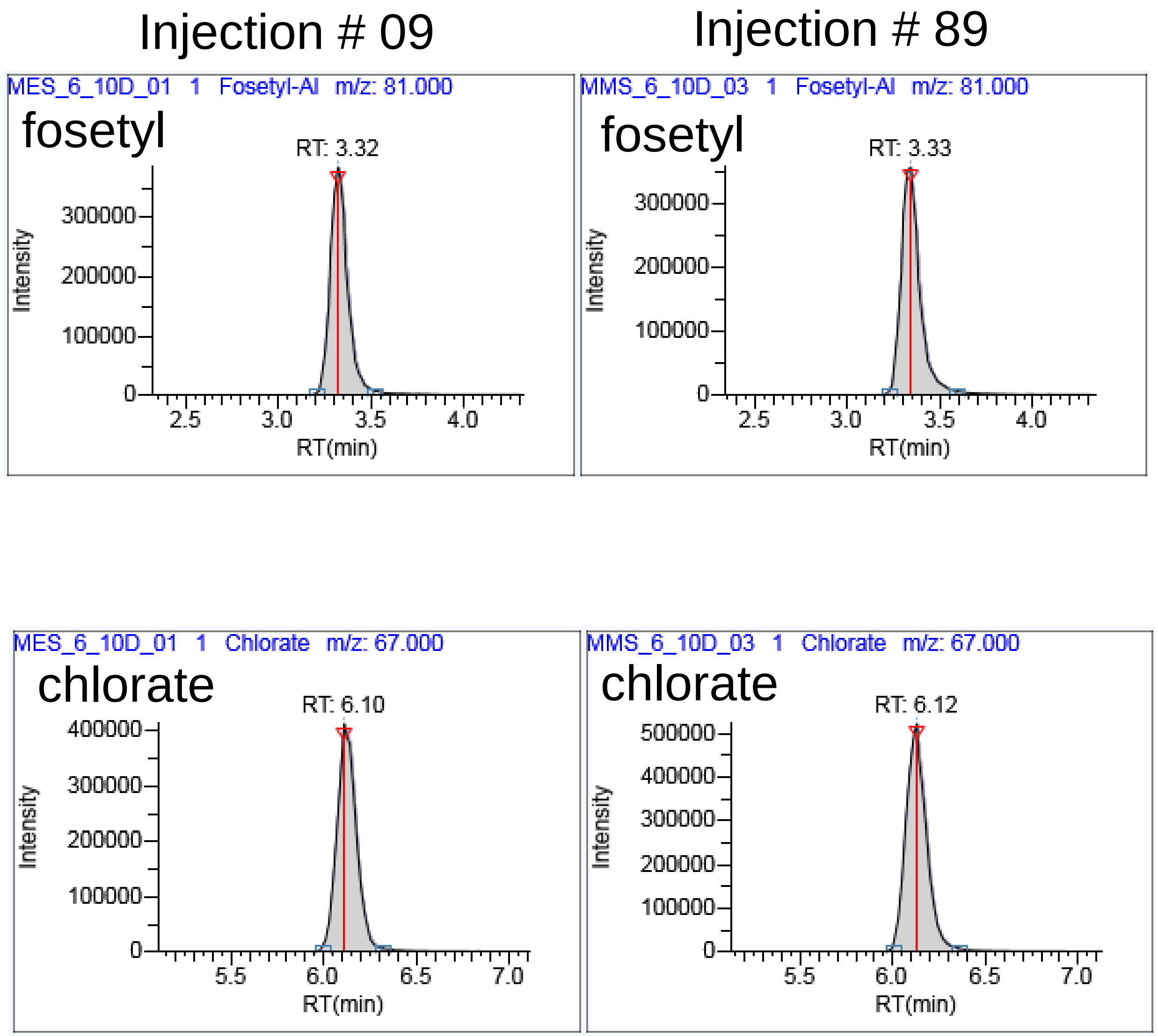
Robustness – with OnGuard-RP SPE

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



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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!

Baby Food samples (10 ± 0.1 g)

↓

Add 1 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 ℃, 15 min

↓

Centrifuge, 8000 rpm 8 min

↓

Dilute ×10 with water

↓

Experimental SPE - clean-up

↓

Filter for IC-MS/MS → Inj 50µL

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

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Preliminary Method Validation at 2.5 ng/g – Baby Foods

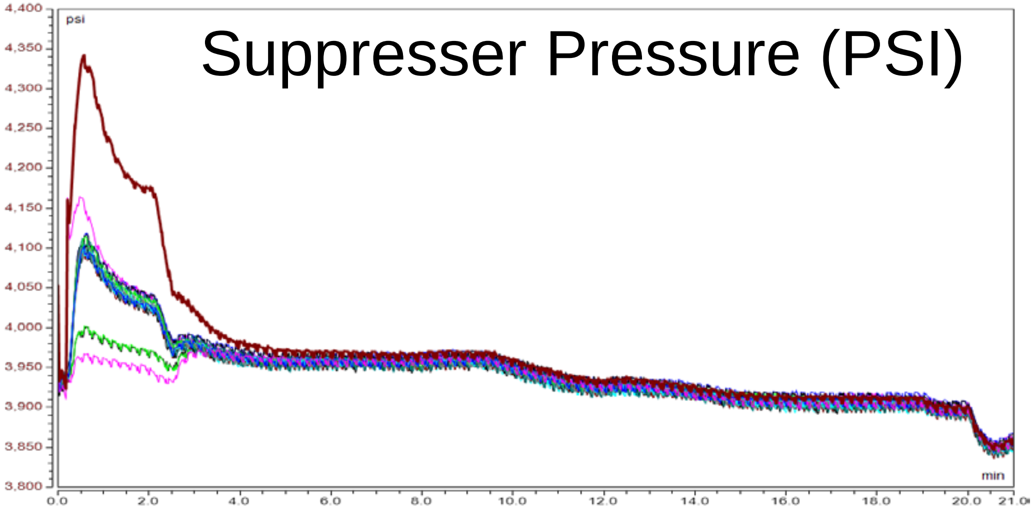
Recoveries of 10 anionic pesticides in 9 different baby food (spiked 2.5 ng/g)

	9 different baby food matrices										
Compounds	**1#**	**5#**	**6#**	**7#**	**8#**	**9#**	**10#**	**11#**	**12#**	**Average**	**RSD %**
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

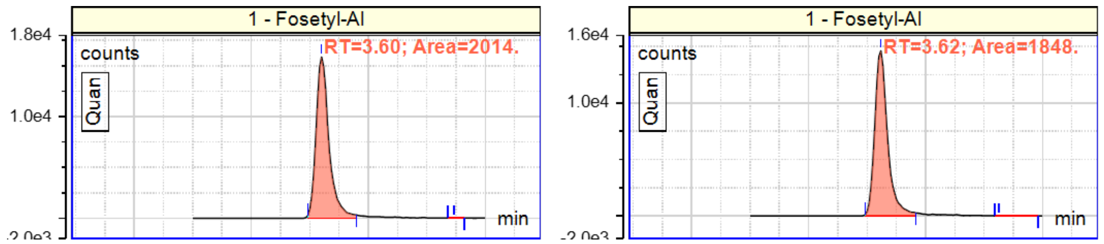
Because of high blank, chlorate, perchlorate, and phosphonic acid could not evaluate recoveries. The sensitivity was not sufficient validate HEPA at 2.5 ng/g

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

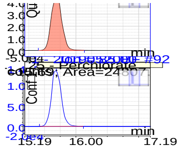


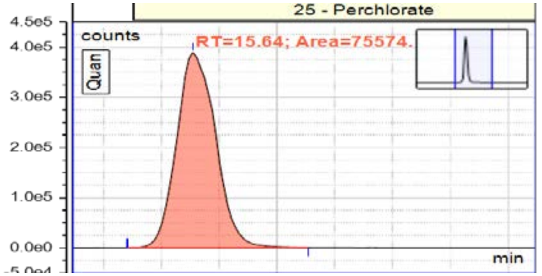
- AG19 guard and AS19 analytical column have been used for about 1300 injections, including 870 injections of baby food matrix

Peak shape after 90 injections



Fosetyl-Al

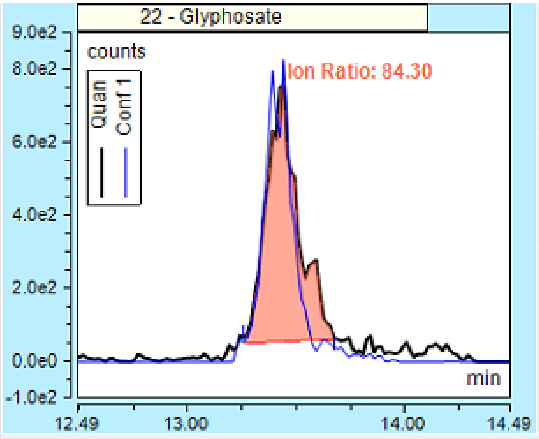




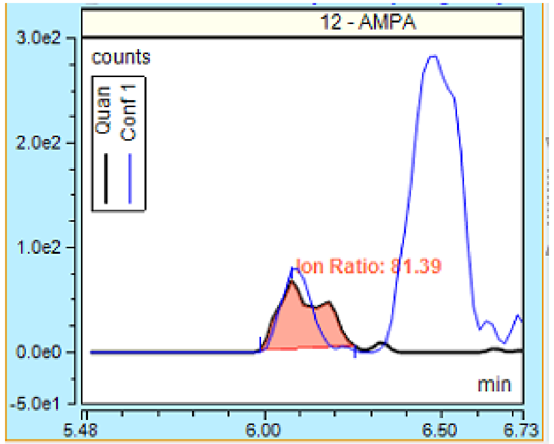
Perchlorate

LOQ of Glyphosate / Glufosinate and Metabolites in Baby Food Matrix

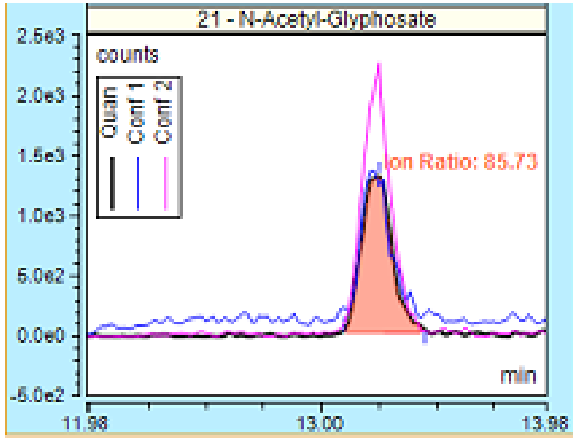
Glyphosate (2.5 ng/g)



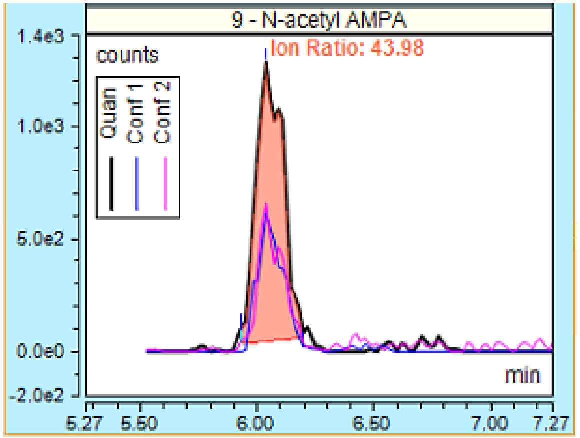
AMPA (2.5 ng/g)



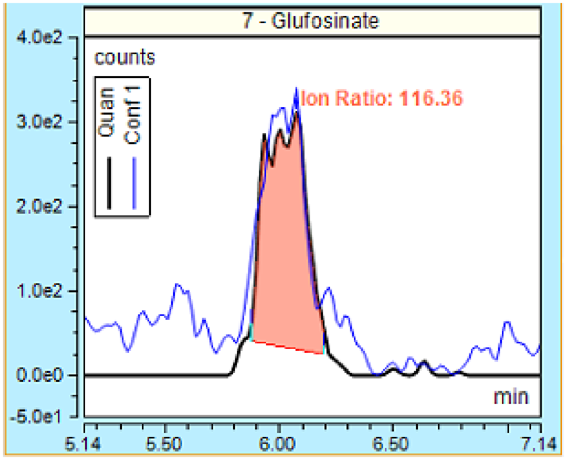
N-acetyl glyphosate(2.5 ng/g)



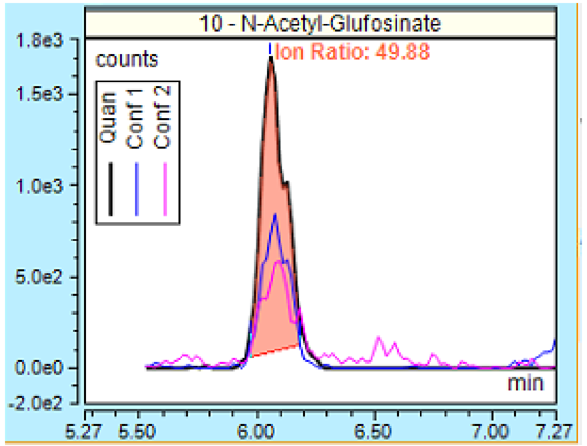
N-acetyl AMPA (2.5 ng/g)



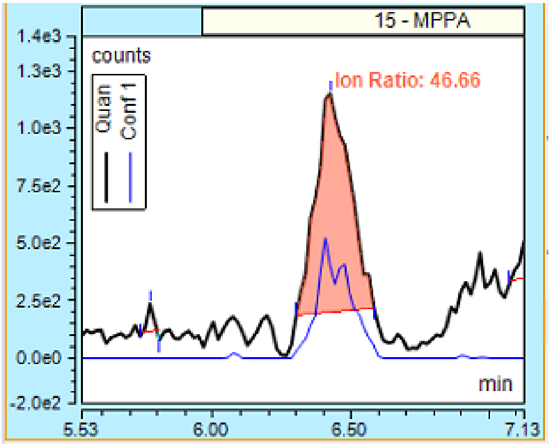
Glufosinate (5 ng/g)



N-acetyl glufosinate (2.5 ng/g)

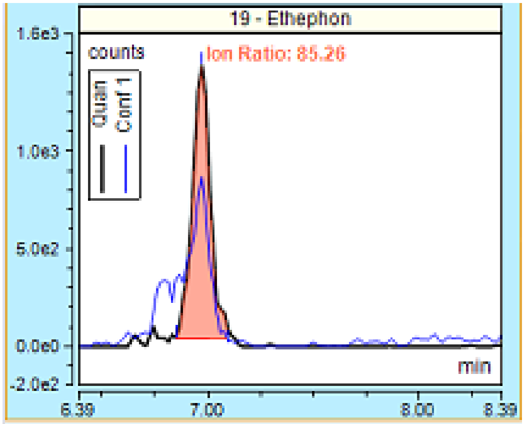


MPPA (2.5 ng/g)

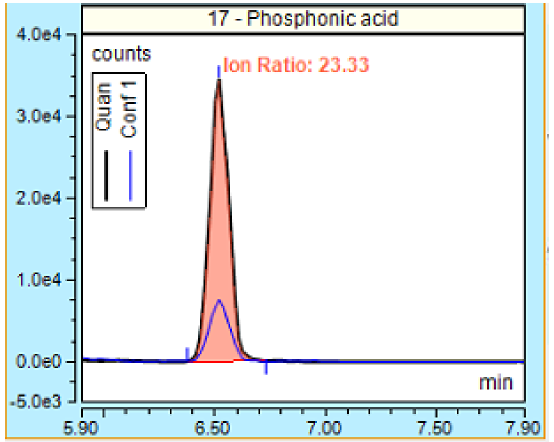


Anionic Pesticides LOQ in Baby Food- Matrix-Matched Standards

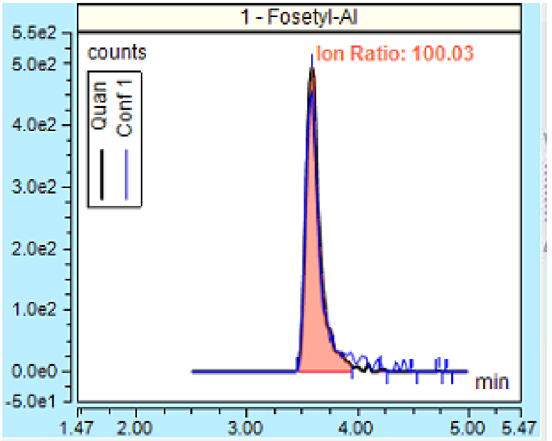
Fosetyl (2.5 ng/g)



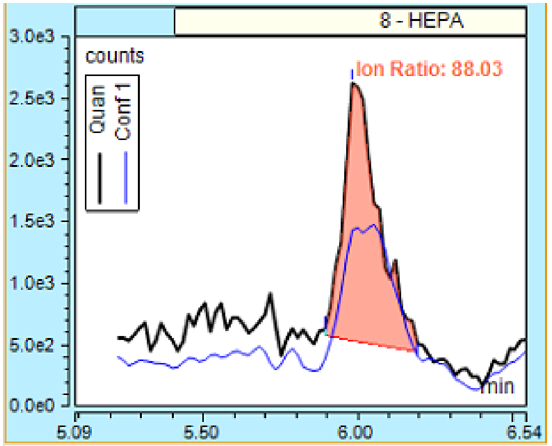
Phosphonic acid (2.5 ng/g)



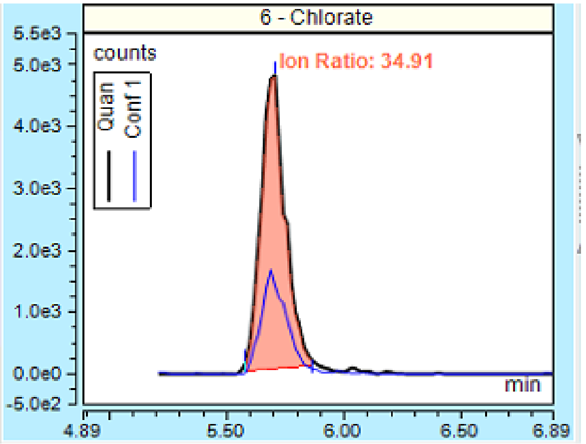
Ethephon (5 ng/g)



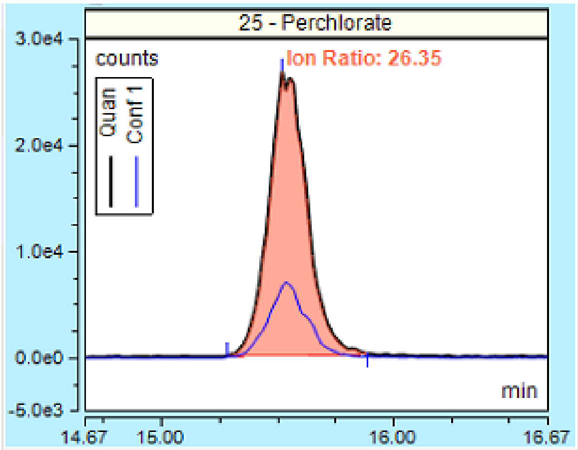
Hepa (5 ng/g)



Chlorate at 2.5 ng/g



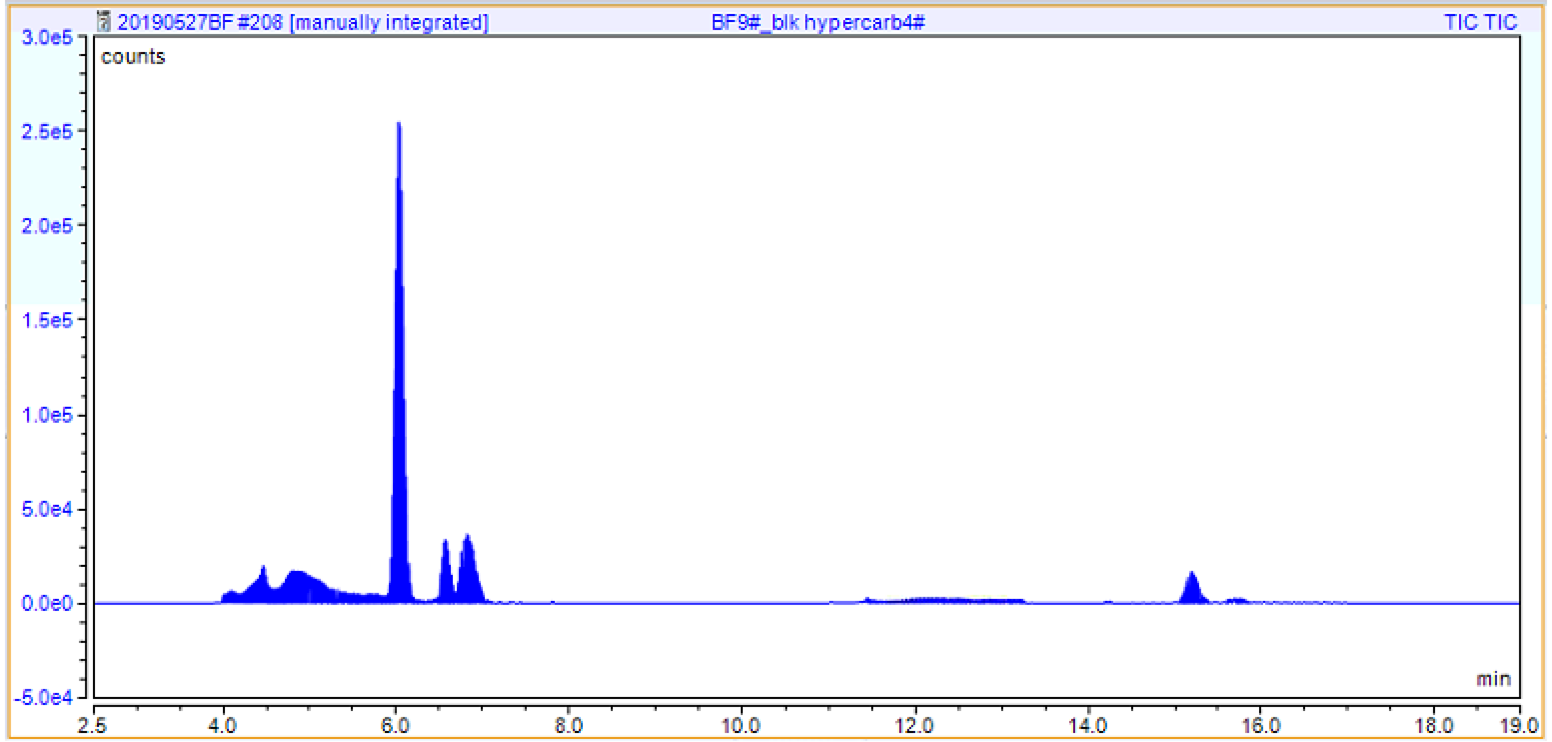
Perchlorate (2.5ng/g ng/g

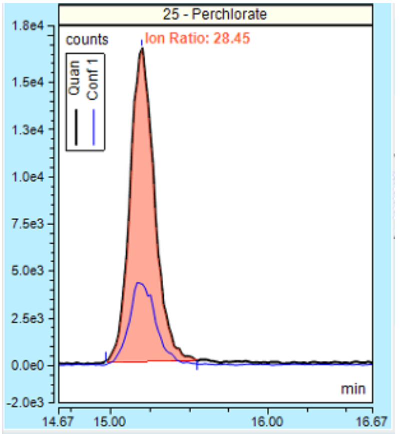


- All ion ratios are within the expected range compared to standards

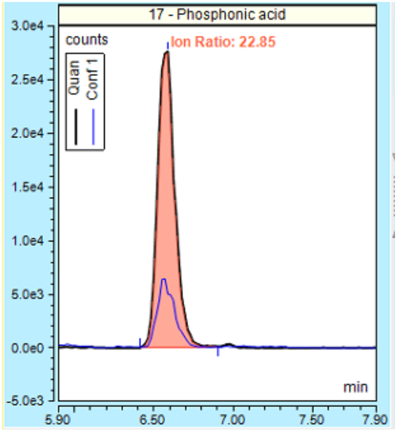
Residues Detected in Retail Samples

- TIC of baby food sample #9





Perchlorate in sample



Phosphonic acid in sample

- Concentration too high to determine LOQ

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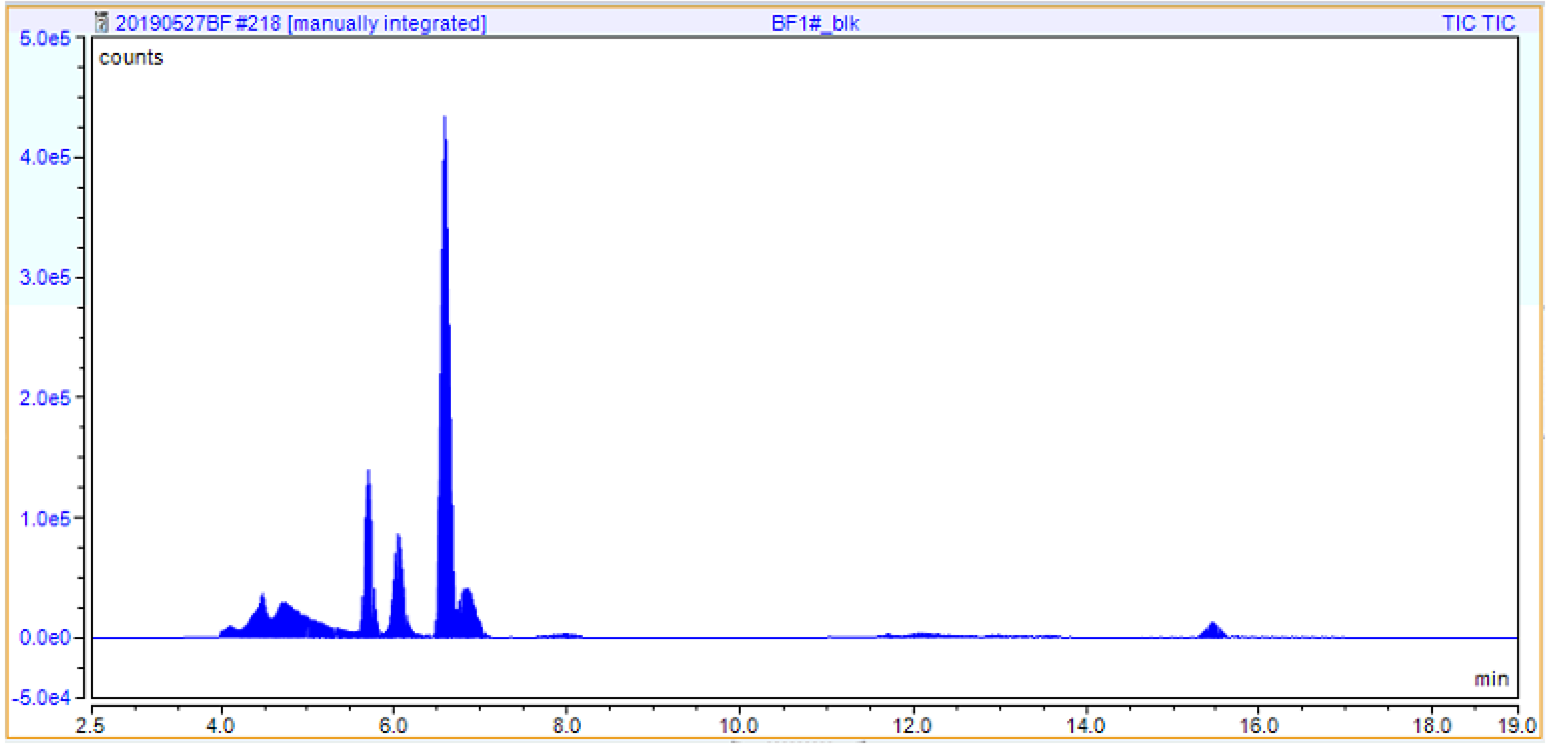
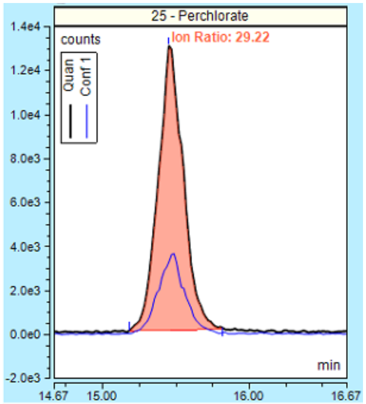
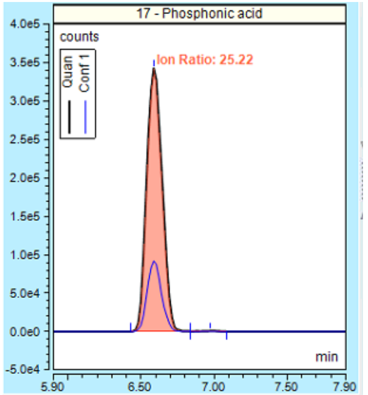
Potential Residues in Baby Food Samples (2)

- TIC of blank sample #1

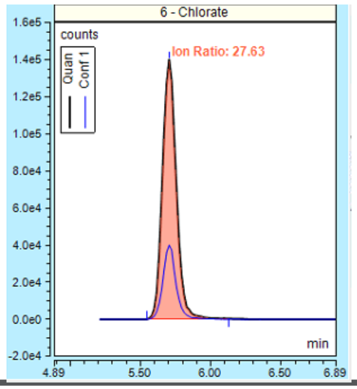




Perchlorate in sample



Phosphonic acid in sample



Chlorate in sample

- Concentration too high to determine LOQ

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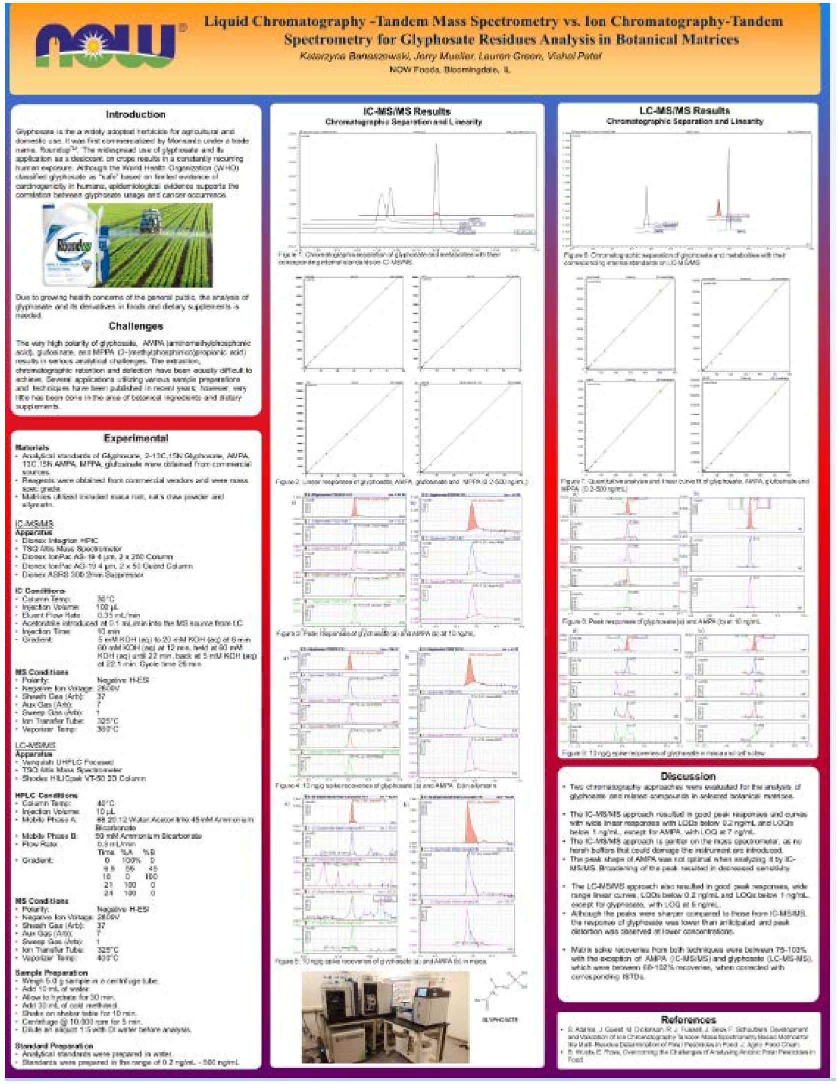
Analysis of Glyphosate – Now Foods



- 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 Hilic.
- 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