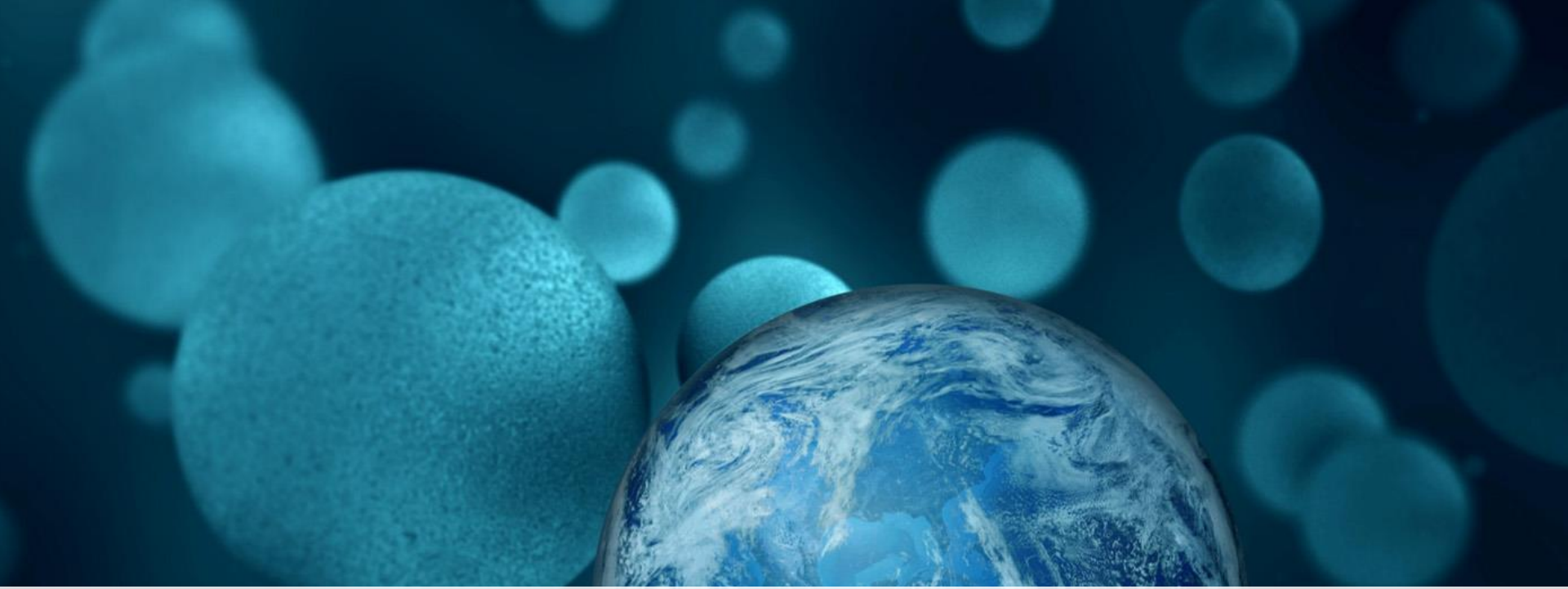


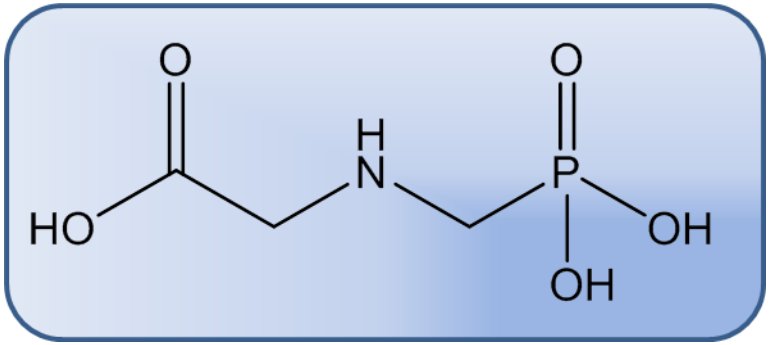
ThermoFisher SCIENTIFIC

The analysis of Polar Pesticides by LC/IC-MS

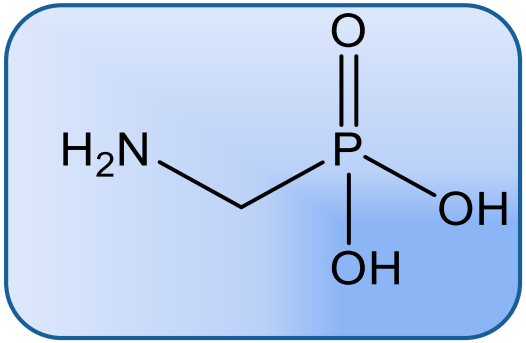
Michal Godula, Ph.D.
Thermo Fisher Scientific

What is Glyphosate

- **Glyphosate** (*N*-(phosphonomethyl)glycine) is a broad spectrum systemic herbicide commonly used as weed control.



- It is rapidly degraded to aminomethylphosphonic acid (**AMPA**) metabolite frequently found in plants, water and soil.
- Very polar, zwitterionic compound
- Difficult to retain on C18 LC column



Glyphosate in the news

- **In 2016**
  - The Munich Environmental Institute group - found glyphosate in 14 of Germany's most popular beers (0.46 – 29.74 µg/L)
  - Alliance for Natural Health USA tested 24 popular breakfast foods, 10 of 24 goods had detectable levels of glyphosate (86 – 1,327 µg/kg) (www.anh-usa.org)

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……..

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:

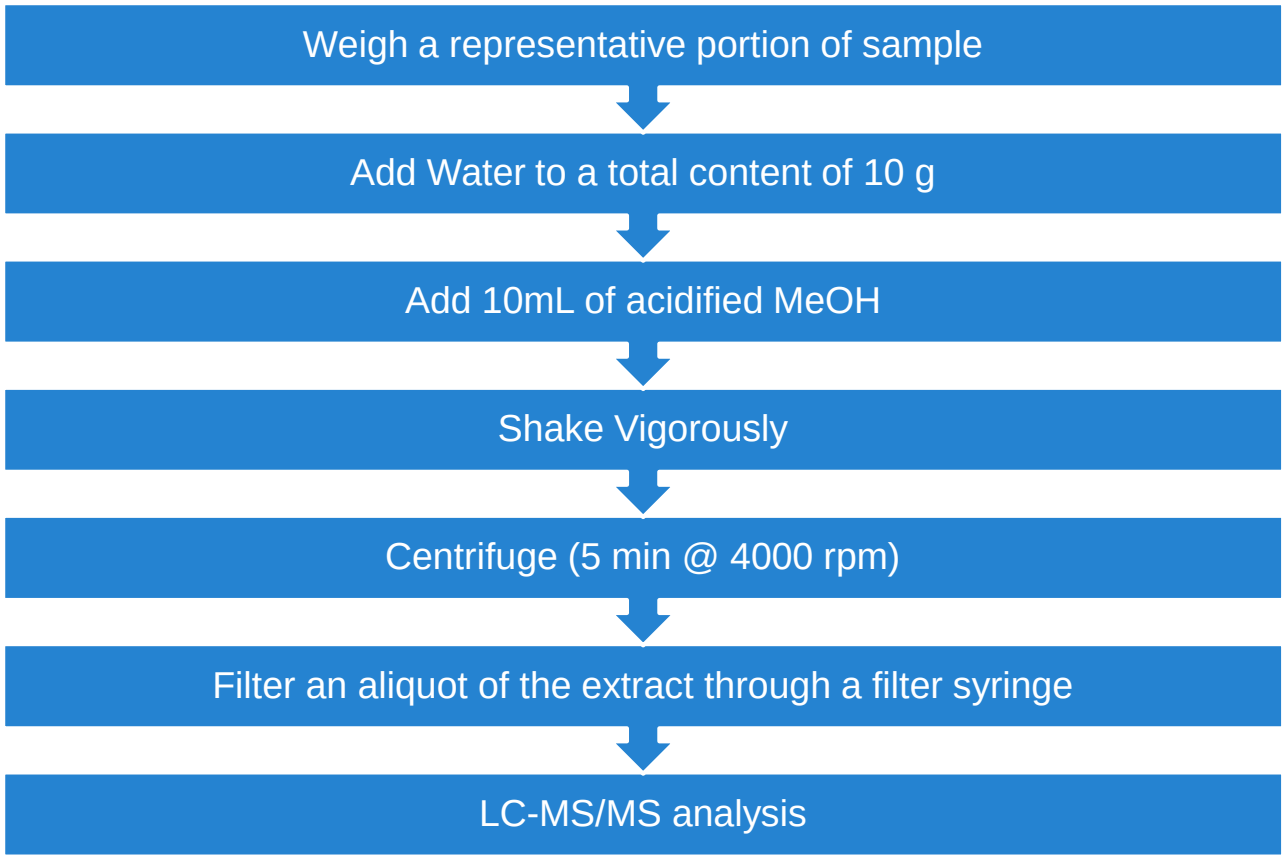
	M 1.1	M 1.2	M 1.3	M 1.4	M 2	M 3	M 4.1	M 4.2	M 5	M 6	M 7	M8
ESI-mode	Neg.	Neg.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
Separation principle	Anion Exchange	Anion Exchange	Carbon	Carbon	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC	Carbon
Column type	AS 11	AS 11-HC	Hyper-carb	Hyper-carb	Obelisc-R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

Principle

- The generic extraction approach is not without problems!
- There is no partitioning stage in the extraction method which can result in a large number of co-extractives in the final extract
- There is no clean-up suggested except for animal products
- There are many different column chemistries to cover the wide range of analytes included in the method scope, including unsuppressed IC-MS/MS

Our Sample Preparation



- The use of **plastic material** is highly recommended!

Configuration of the System

- LC-System Thermo Scientific™ UltiMate 3400 RSLC
- Mass Spectrometer Thermo Scientific™ TSQ Quantiva™ LC-MS/MS

Column

Hypercarb 2.1 x 100 mm, 5 µm

Guard Column

Hypercarb 2.1 x 10 mm, 5 µm

Eluent

A: 1% Acetic Acid in water + 5% MeOH

B: 1% Acetic Acid in Methanol

Injection volume **5 µL**

Column Temperature **40 ºC**

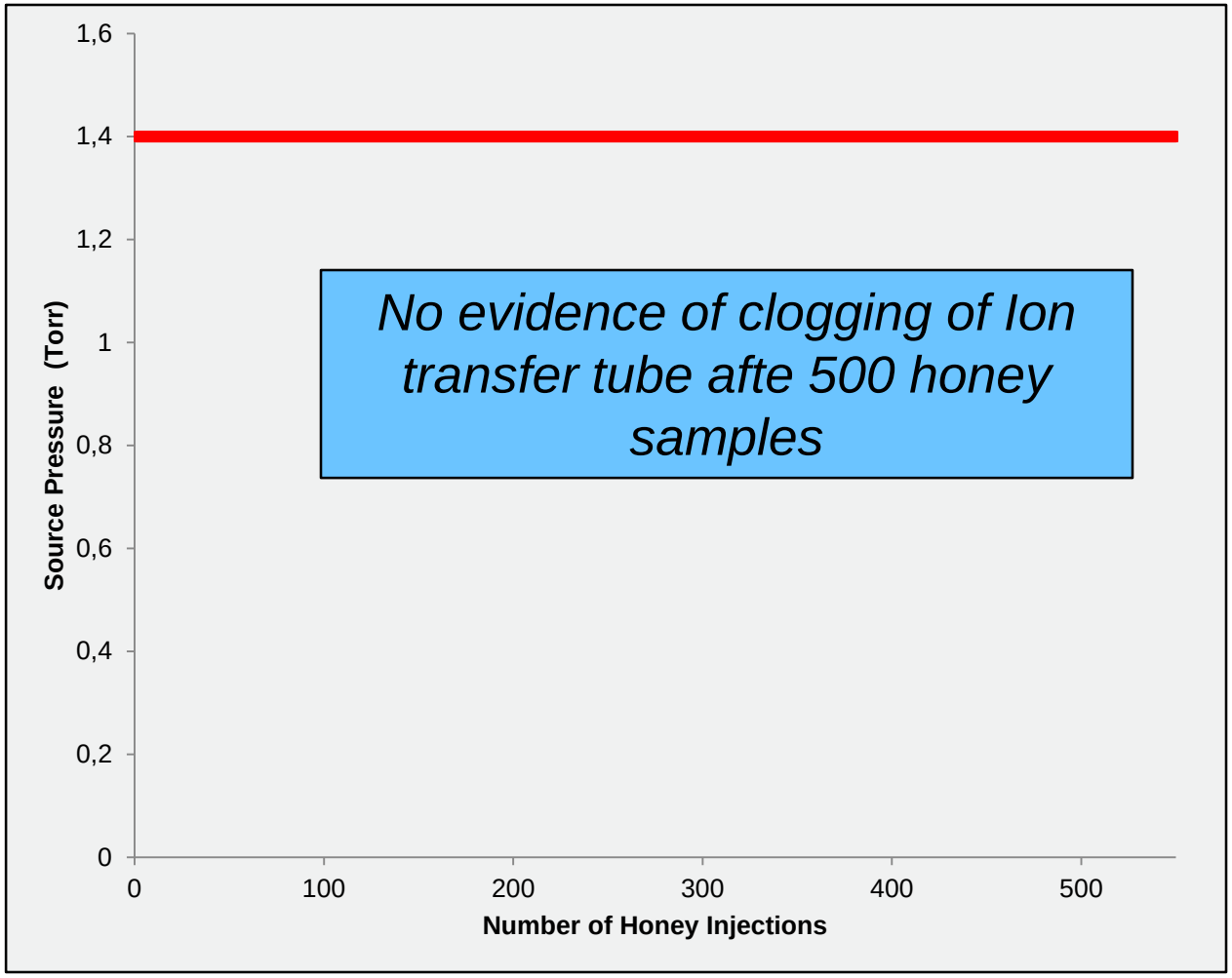
%A	Flow [mL/min]	Time [min]
100	0.2	0
70	0.2	10
70	0.4	11
70	0.4	18
10	0.4	19
10	0.4	22
100	0.2	22.1
100	0.2	30

Summary of Performance - **Honey – TSQ Quantiva**

Compound	LOQ (pg on column)	LOD µg/kg	LOQ µg/kg
AMPA	50	5	10
Glyphosate	25	2	5
Ethephon	250	10	50
Fosetyl Aluminium	50	10	10
Glufosinate	25	2	5
Maleic Hydrazide	500	50	100
MPPA	2500	100	500
Phosphonic Acid	25	5	5
Sodium Chlorate	50	5	10

Estimation of **LOQ** based on: % RSD < 20%

Optimization for Robust Operation



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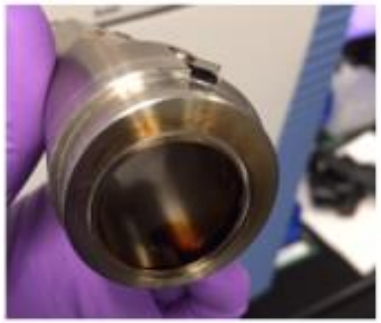
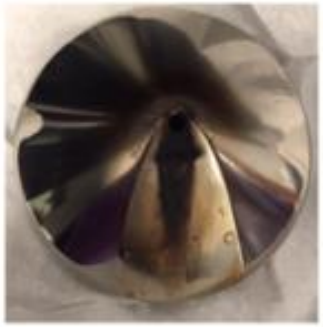
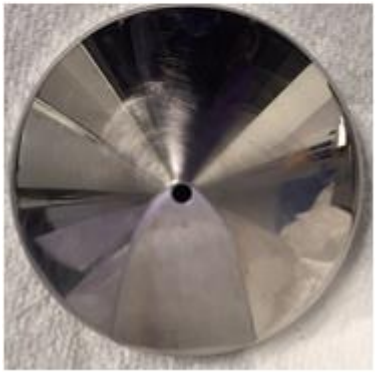
Maintenance performed after + 500 injections of honey

BEFORE

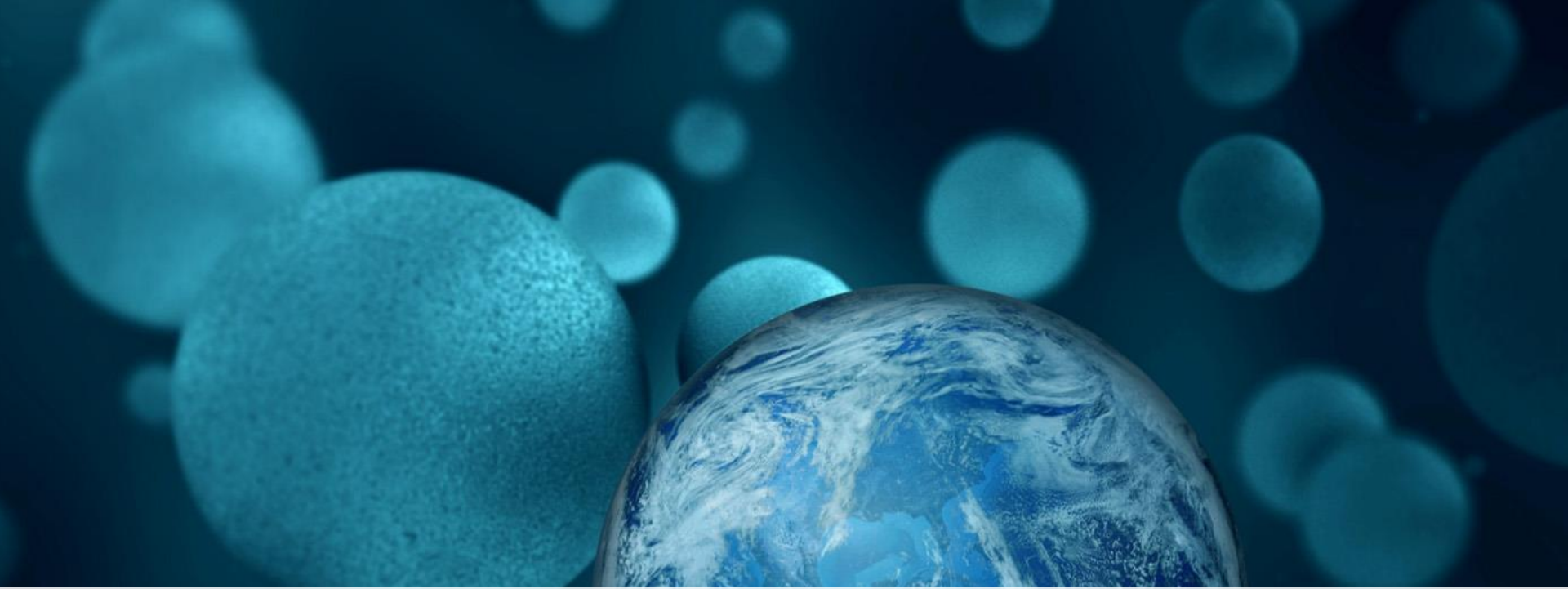




AFTER



Cleaning procedure focused on cone, ITT and drain
Flush with hot water
Sonication with Water:MeOH (1:1)
10 min Procedure



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Option for Food Analysis

QuPPe by IC-MS/MS

System used for analysis

Ion Chromatograph: Thermo Scientific™ Dionex Integrion™

Mass Spectrometer: Thermo Scientific™ TSQ Endura™ (TSQ Quantiva™)

Column: AS24 (2 x 250 mm)
Guard Column: AG24 (2 x 50 mm)
Eluent: KOH
Injection volume: 10 µL
Column Temperature: 21 ºC
Flow rate: 0.3 ml/min
Make-up flow: 0.1 ml/min
Make-up solvent: CH_3OH

Time (min)	Concentration of KOH in eluent (mM)
0	25
0.2	25
11	80
11.1	100
12.5	100
12.6	25
17.0	25

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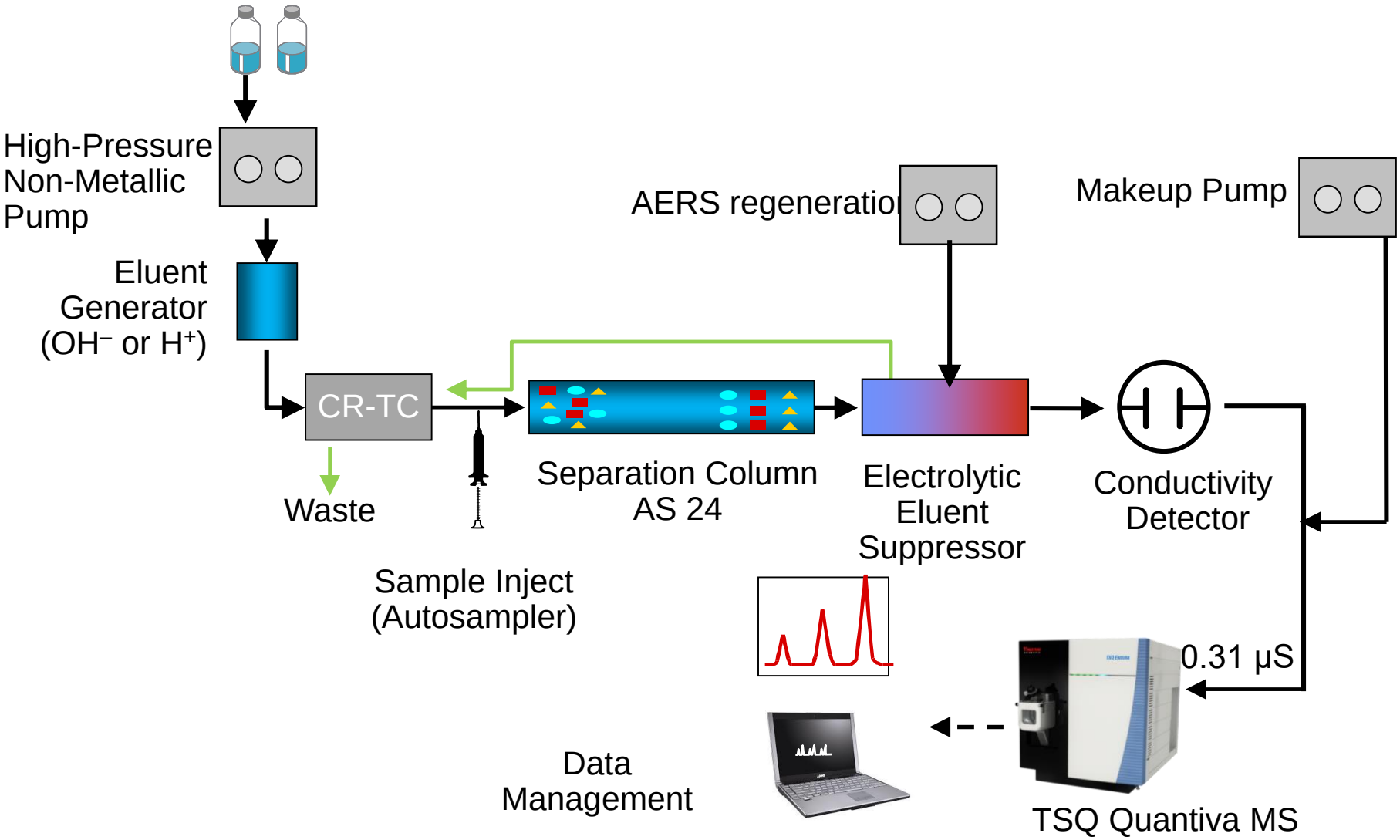
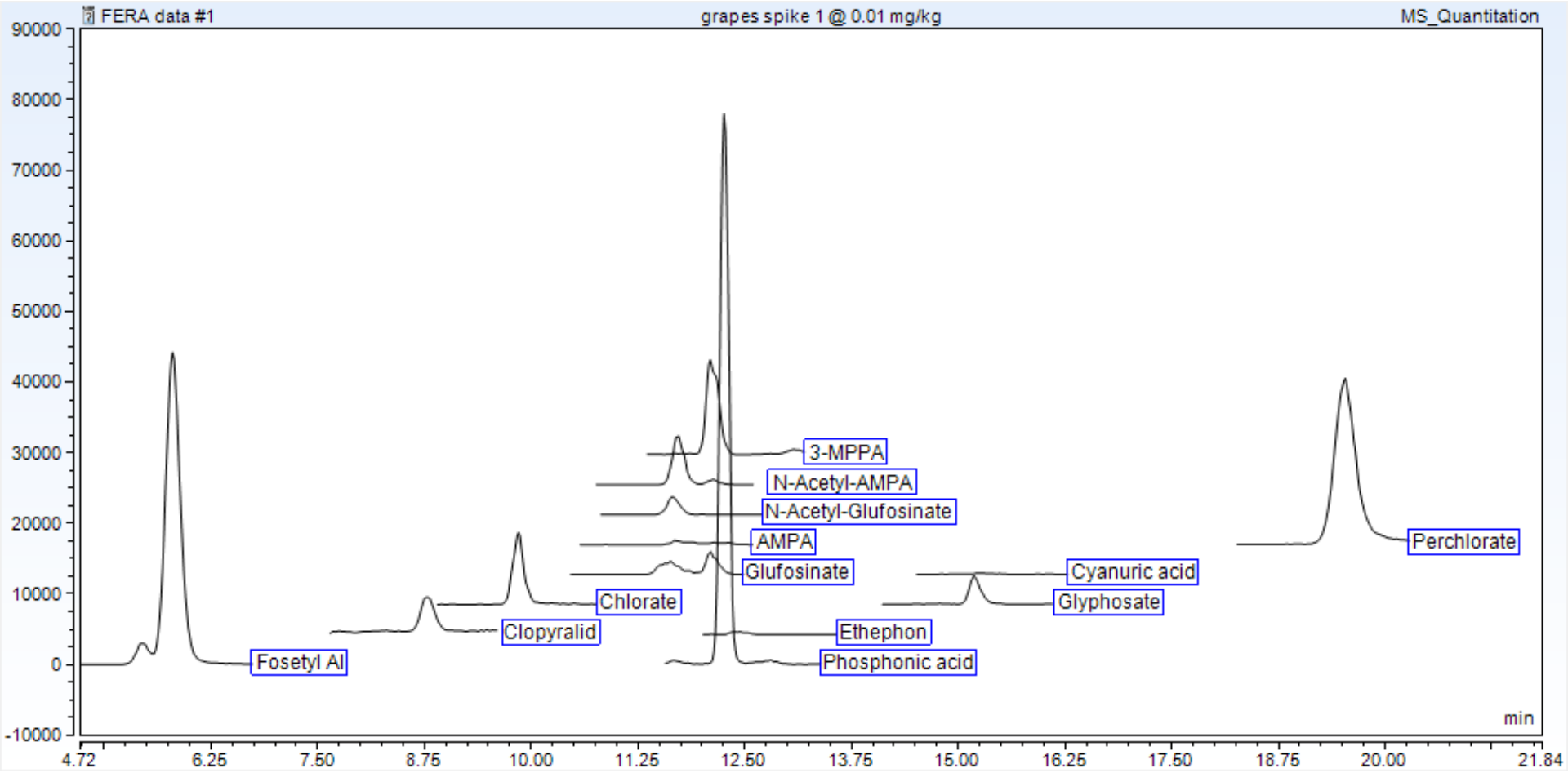
Scheme of IC-MS/MS



IC-MS/MS pesticide multi residue ion chromatogram

- 10 µg/kg spike in grape (Fosetyl & Phosphonic acid @ 100 µg/kg)



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Recovery and Repeatability

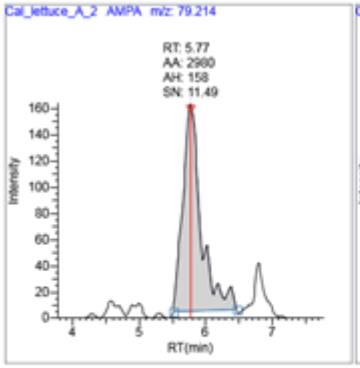
- 3 levels: 50, 200 and 500 µg/kg
- 6 repetitions at each level

Analyte	Level 1 (in ppb)		Level 2 (in ppb)		Level 3 (in ppb)	
	REC %	RSD %	REC %	RSD %	REC %	RSD %
AMPA	84	14	85	8	80	3
Ethephon	120	10	88	5	92	12
Fosetyl-Al	98	16	97	12	82	3
Glufosinate	101	4	93	8	86	3
Glyphosate	88	12	83	10	81	3
HEPA	118	7	93	9	81	4
MPPA	116	4	98	8	81	3
N-acetyl-AMPA	95	8	89	9	79	2
N-acetyl-glufosinate	93	8	91	8	84	2
Phosphonic acid	115	11	99	11	81	3

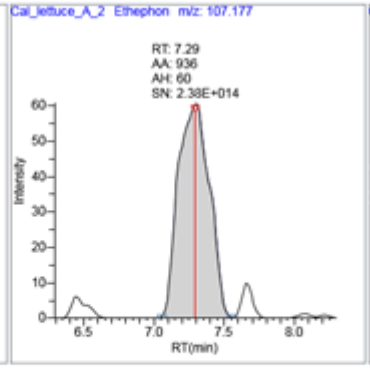
LOD and LOQ with TSQ Endura

Analyte	LOD (µg/kg)	LOQ (µg/kg)	LOQ (pg on column)
AMPA	10	20	200
Ethephon	10	20	200
Fosetyl-Al	10	20	200
Glufosinate	1	10	100
Glyphosate	5	10	100
HEPA	10	20	200
MPPA	1	10	100
N-acetyl-AMPA	1	10	100
N-acetyl-glufosinate	3	10	100
Phosphonic acid	1	10	100

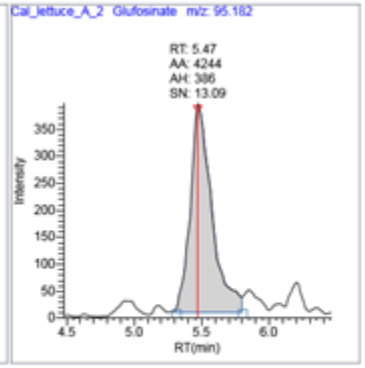
10 ppb in lettuce



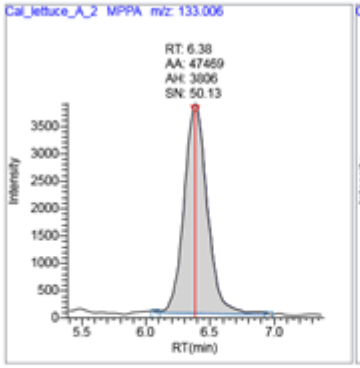
AMPA



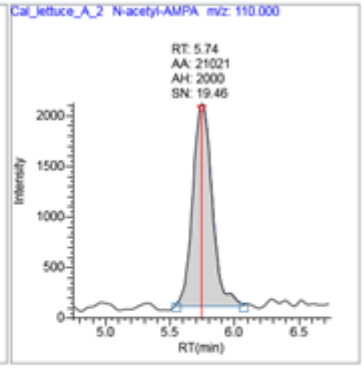
Ethephon



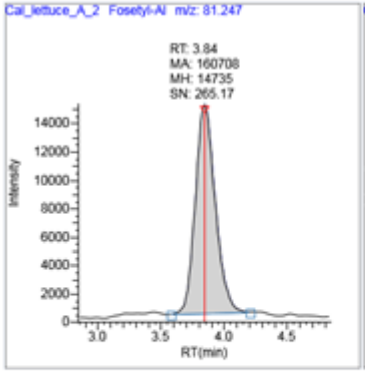
Glufosinate



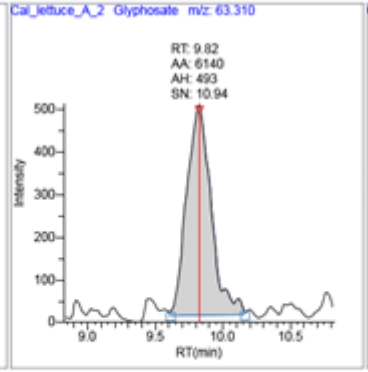
MPPA



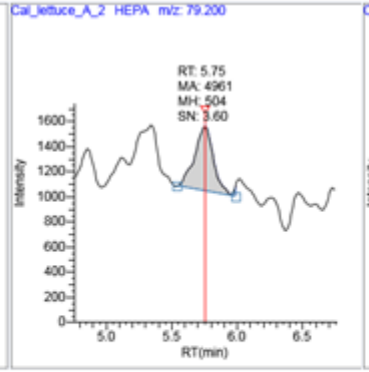
N-Acetyl-AMPA



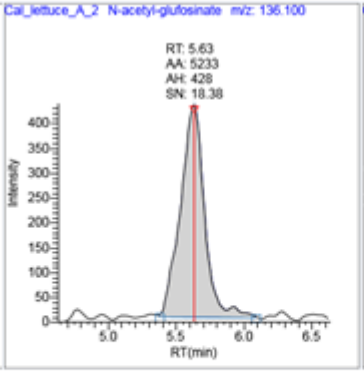
Fosetyl-Al



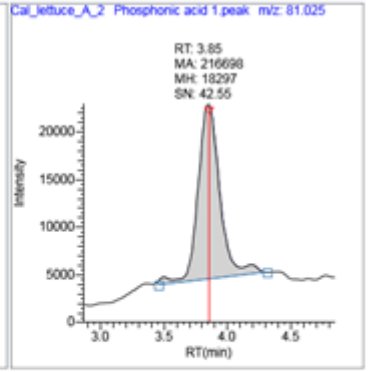
Glyphosate



HEPA

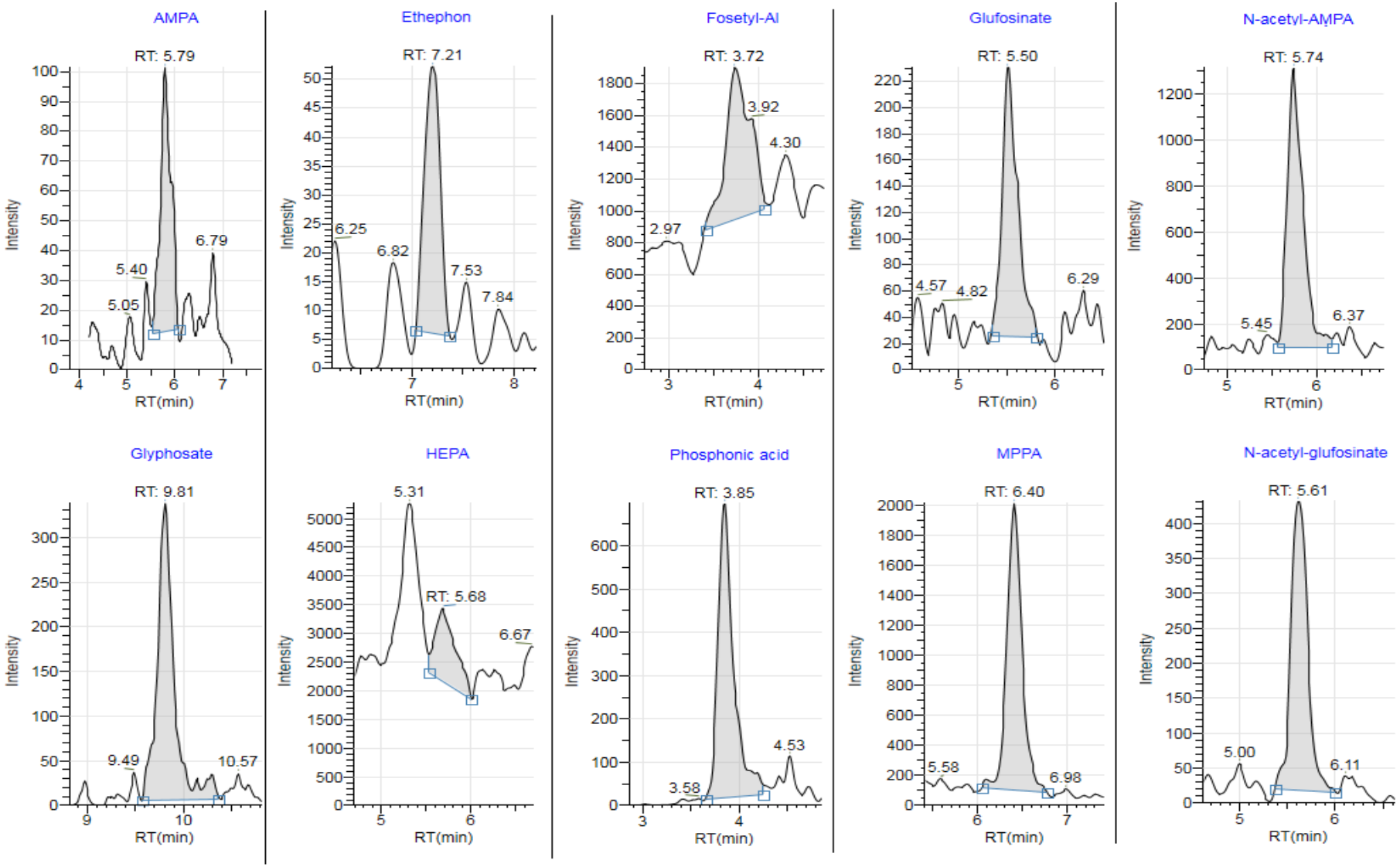


N-acetyl-Glu



Phosphonic acid

10 ppb in oranges



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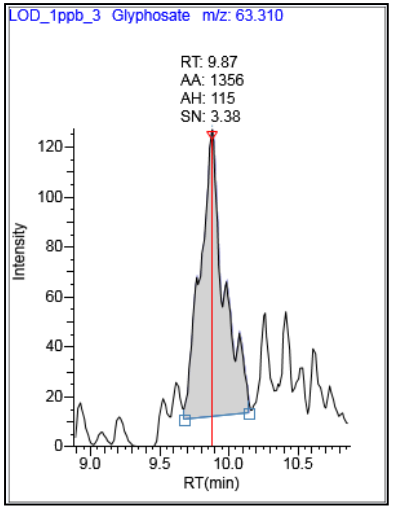
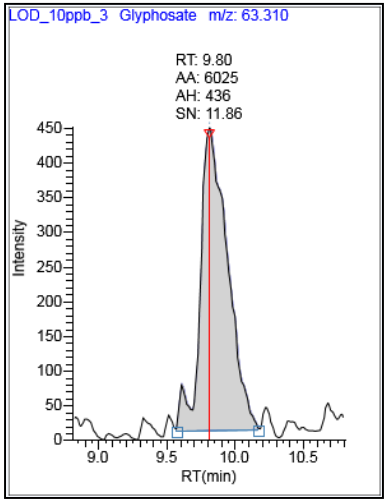
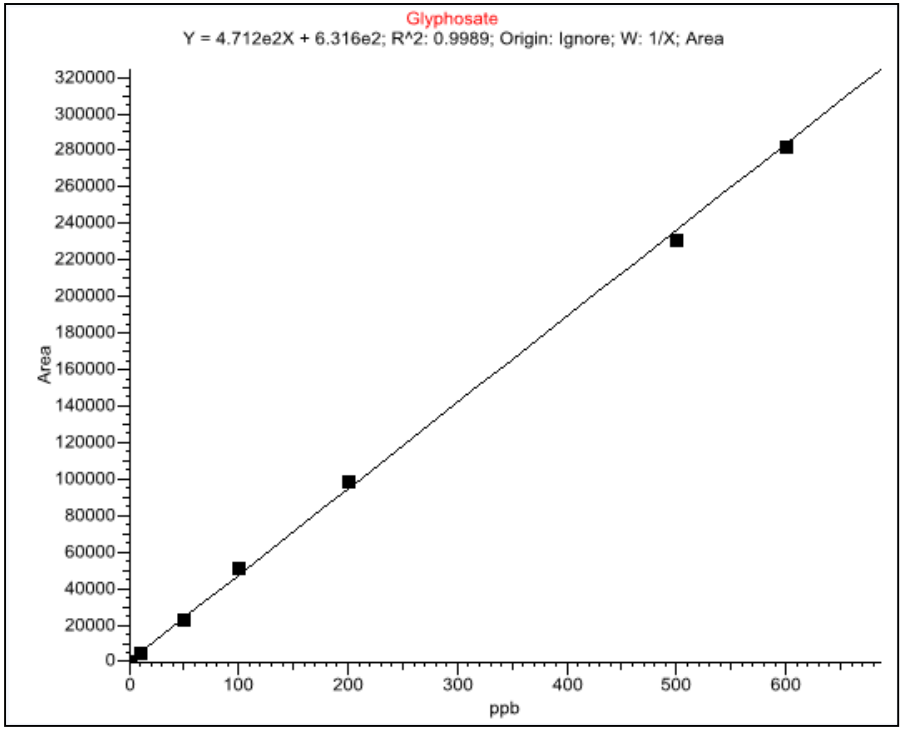
Quan Details Glyphosate in lettuce

1 ppb



LOQ – 10 ppb





Calibration range in matrix: 10 – 600 ppb

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CRM measurement

- Fapas T19186 – Red Grape Purée - **Ethephon**
  - Assigned value: 629 µg/kg
  - Satisfactory range: 413 – 845 µg/kg

25 Years fapas Accredited Laboratory Proficiency Testing

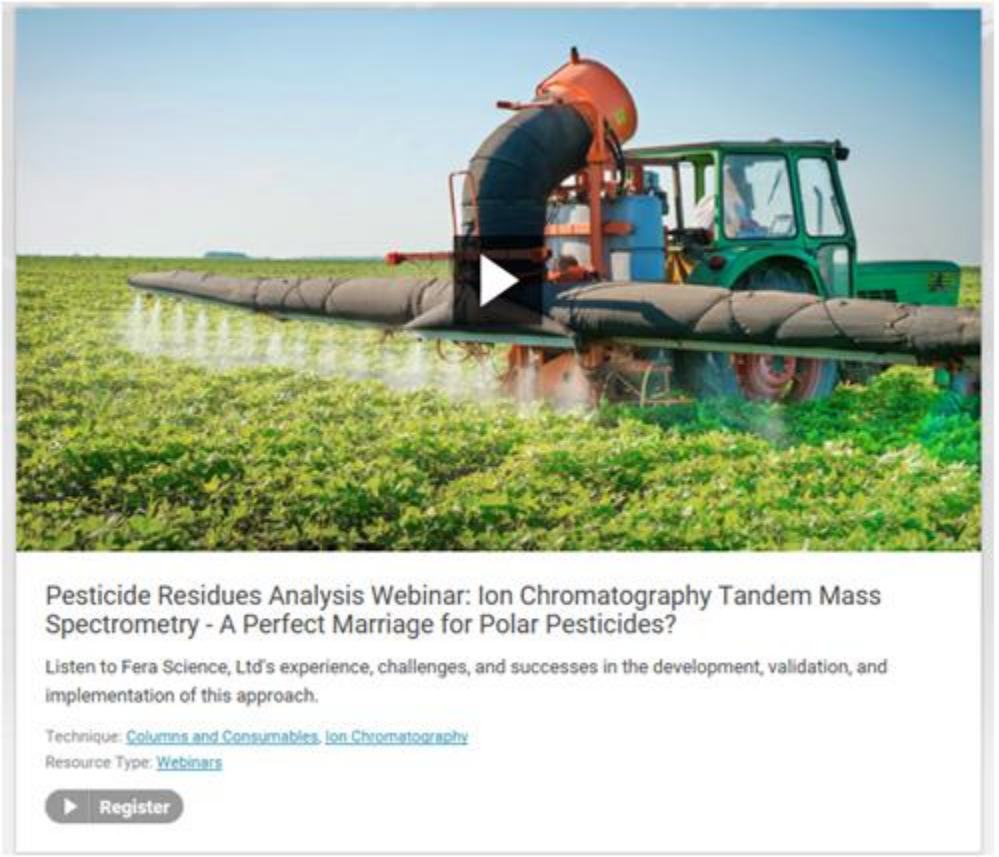
Sample	Result (µg/kg)
Fapas/19186 - 1	535
Fapas/19186 - 2	525
Fapas/19186 - 3	598

STDEV	Average (µg/kg)	RSD (%)	REC (%)
40	553	7	88

Recent cooperation with FERA (UK)

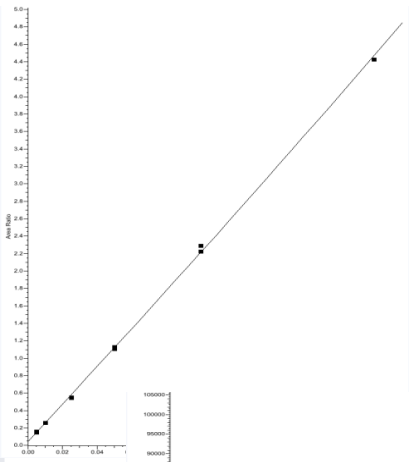
- http://analyteguru.com/webinars/pesticide-residues-analysis-webinar-ion-chromatography-tandem-mass-spectrometry-a-perfect-marriage-for-polar-pesticides/

- **IC-TSQ Quantiva used**

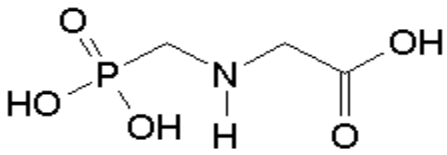


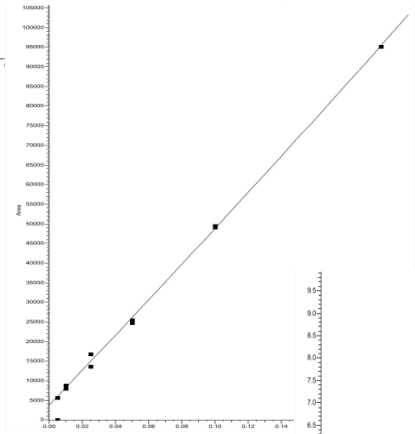
Summary of Validation Results (Wheat Flour, TSQ Quantiva)

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

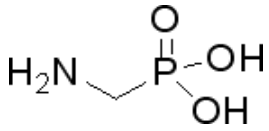


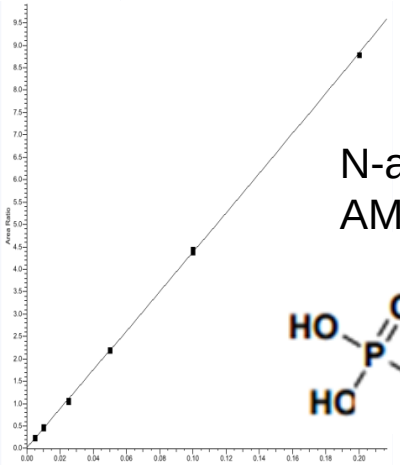
glyphosate



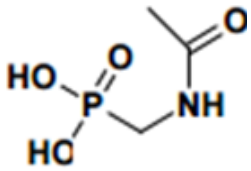


AMPA



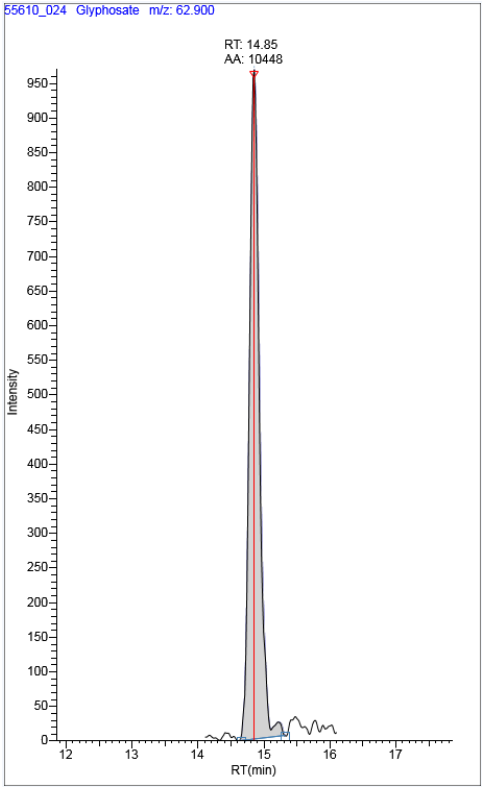


N-acetyl AMPA

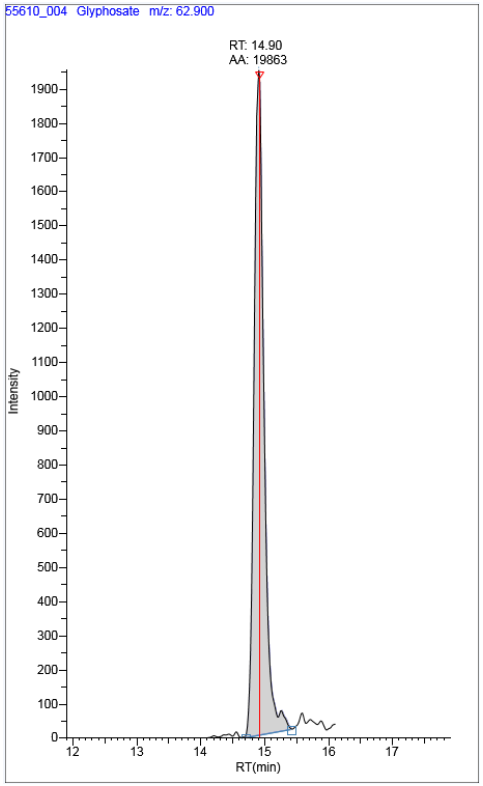


Glyphosate in Beer – No Extraction Required (TSQ Quantiva)

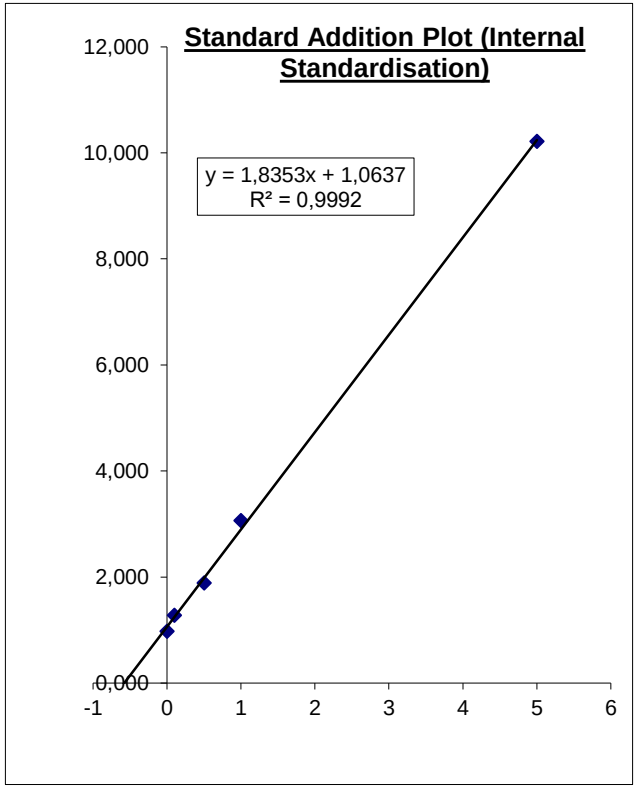
- Glyphosate incurred residue @ 0.58 µg/L



Glyphosate spike @ 0.5 µg/L



Calibration plot 0.1 – 5 µg/L spikes

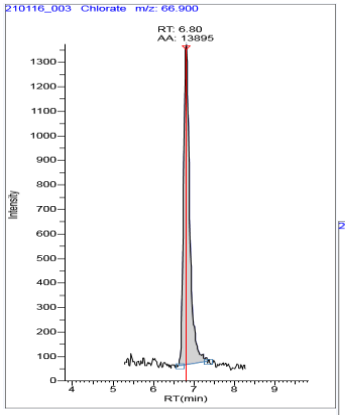


- 1/10 dilution with water and internal standard added

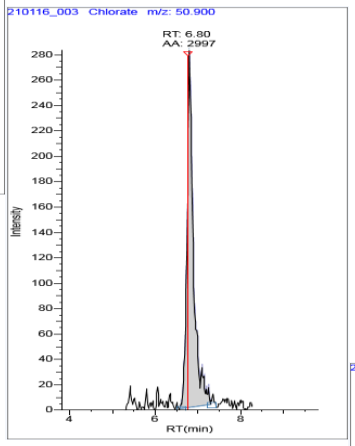
Courtesy of Fera Science Ltd UK

ThermoFisher SCIENTIFIC

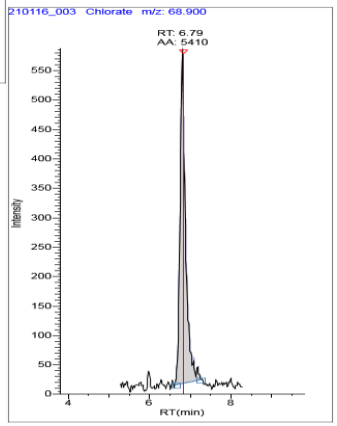
Determination of Chlorate in Dairy Produce @ 5 µg/kg



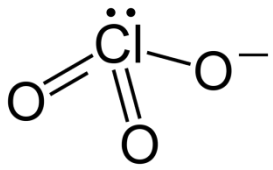
83.1>66.9

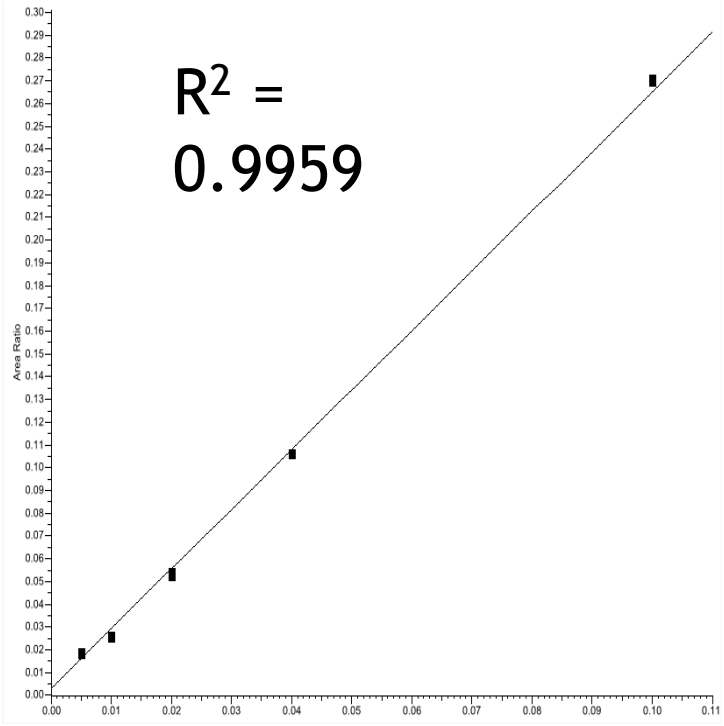


83.1>50.9



85.1>68.9





R^2 = 0.9959

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

Courtesy of Fera Science Ltd UK

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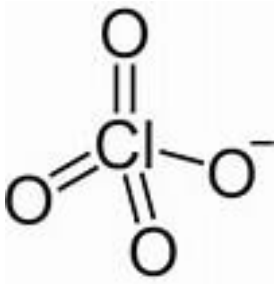
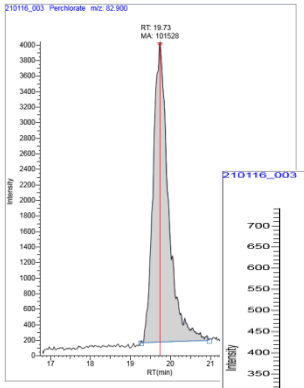
Determination of Perchlorate in Dairy Products @ 5 µg/kg





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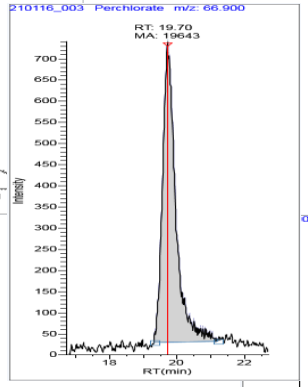

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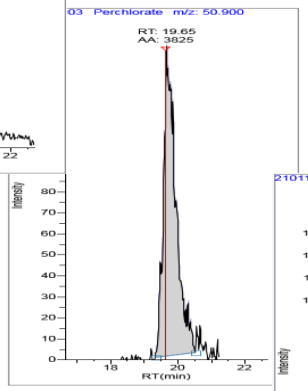

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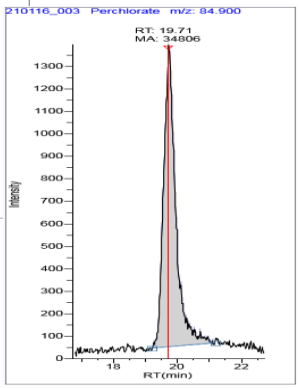

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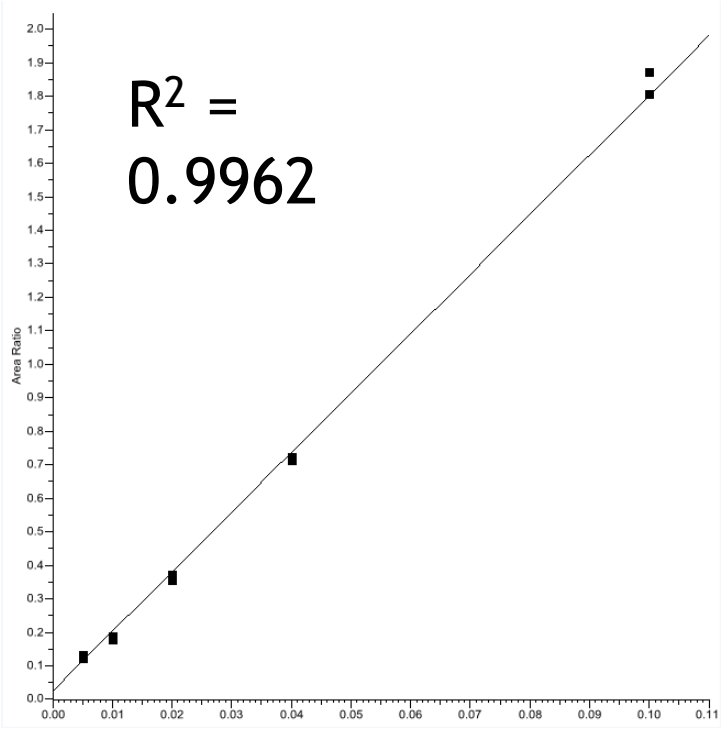

- Perchlorate in dairy products calibration 5 – 100 µg/kg

Courtesy of Fera Science Ltd UK

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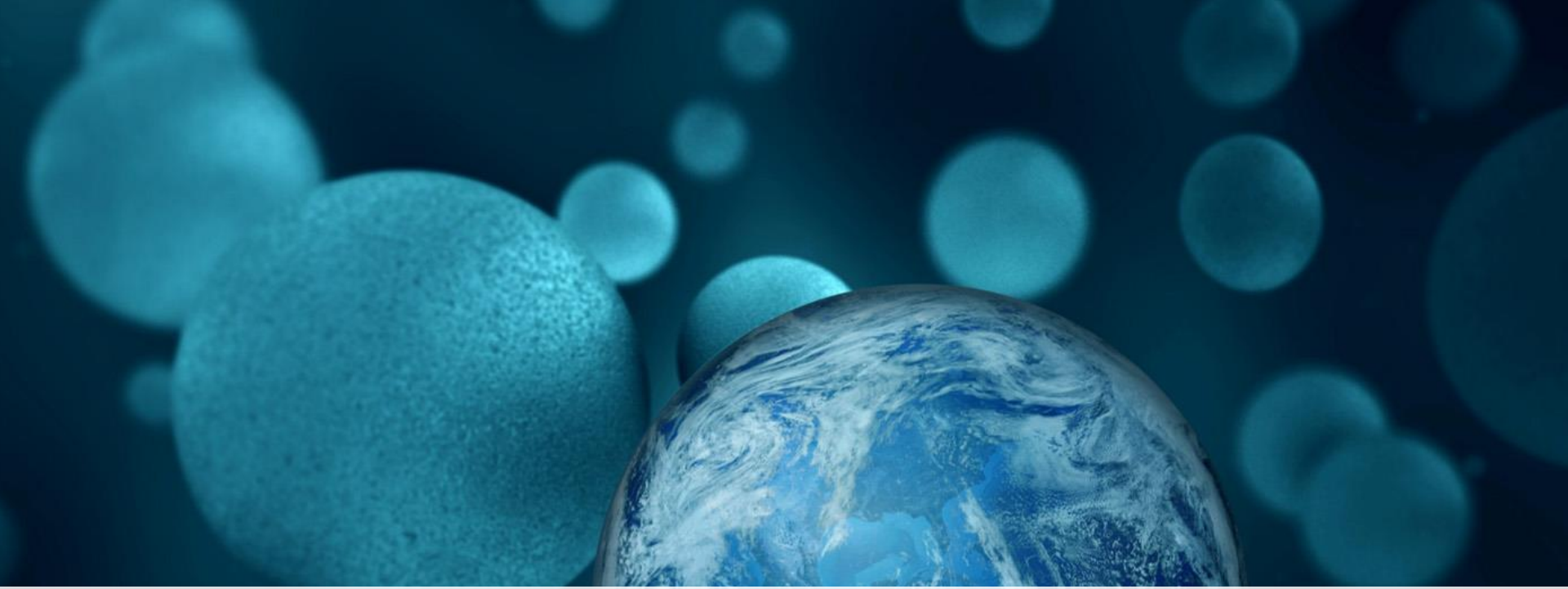

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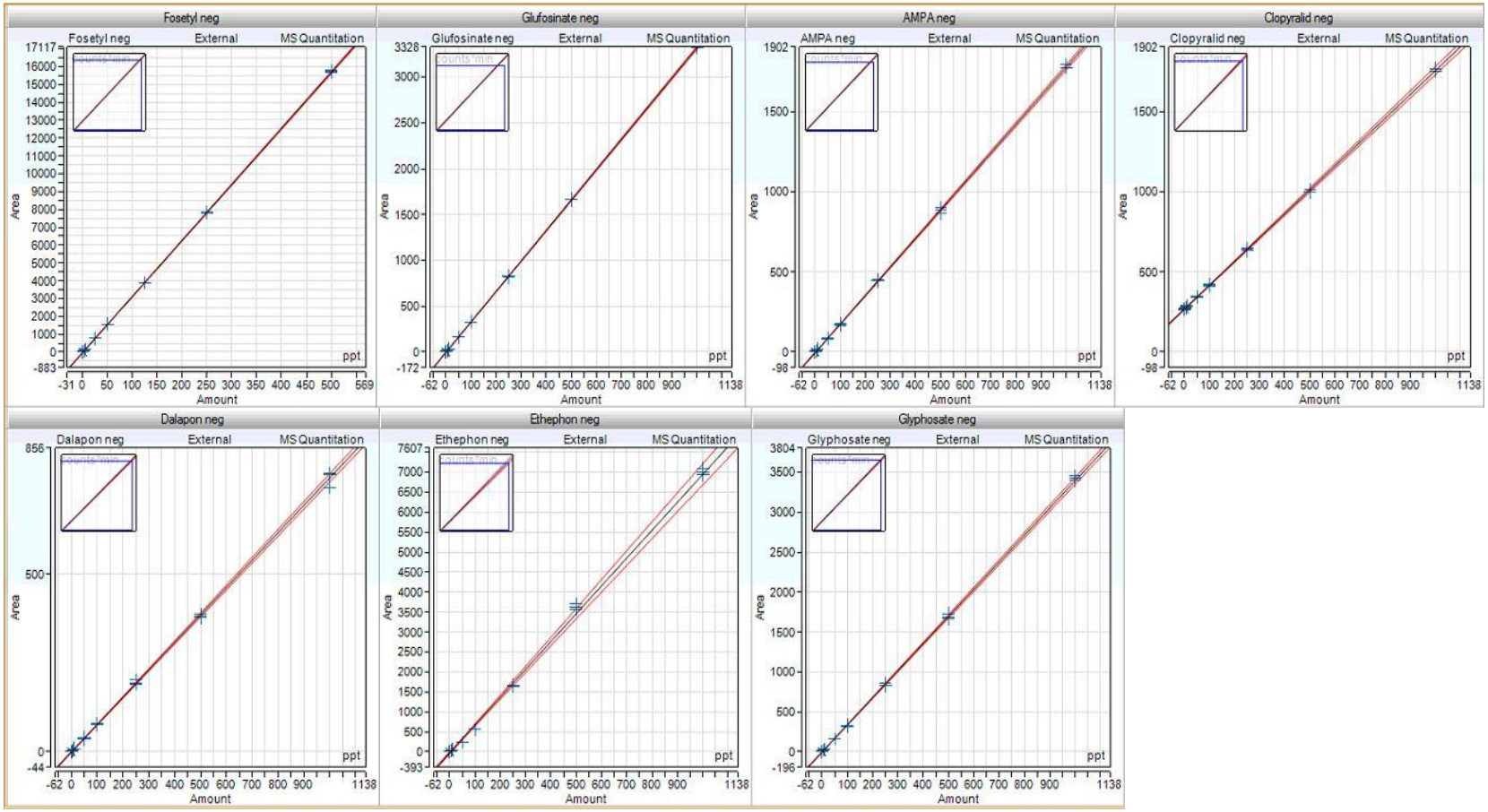
Water analysis by IC-MS/MS

Calibration deionized water – calibration plots



Peak Name	Ret.Time min	Cal.Type	Number of Points	Rel.Std.Dev. %	Coeff.of Determination	C0 (Intercept)	C1 (Slope)	C2 (Curve)
Fosetyl neg	*4.78*	*Lin, WithOffset, 1/A*	*21*	*2.3632*	*1.0000*	*-1.992948091*	*31.37508104*	*0*
AMPA neg	8.43	Lin, WithOffset, 1/A	21	6.3759	0.9997	-2.382828757	1.777517548	0
Clopyralid neg	11.42	Lin, WithOffset, 1/A	21	1.0821	0.9992	267.9471671	1.487049519	0
Ethephon neg	11.91	Lin, WithOffset, 1/A	21	28.7637	0.9959	-26.48187711	6.976503359	0
Dalapon neg	12.05	Lin, WithOffset, 1/A	21	10.0368	0.9993	-0.8829762292	0.7728082764	0
Glyphosate neg	14.12	Lin, WithOffset, 1/A	21	8.1252	0.9995	-1.492587864	3.395639022	0
Glufosinate neg	n.a.	Lin, WithOffset, 1/A	21	2.9959	0.9999	-1.935000084	3.322568994	0

* Levels 5, 10, 50, 100, 250, 500, 1000 ppt, 3 injections each

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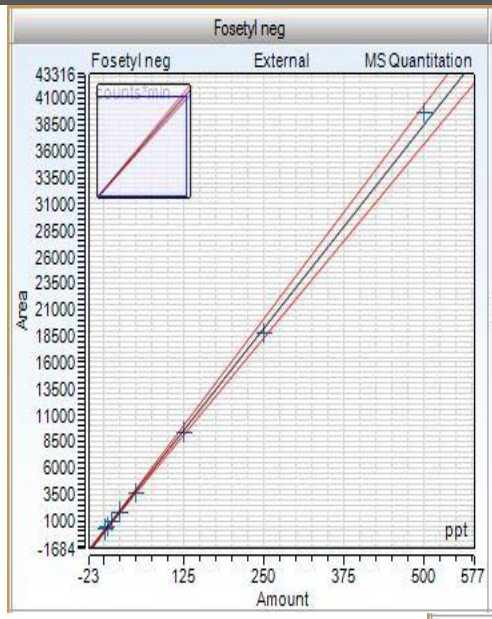
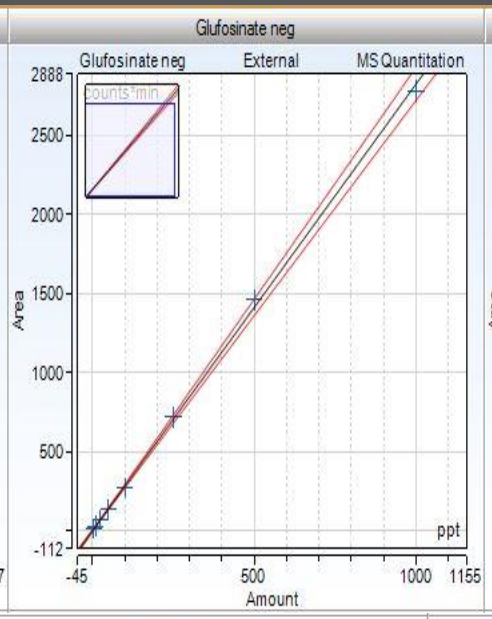
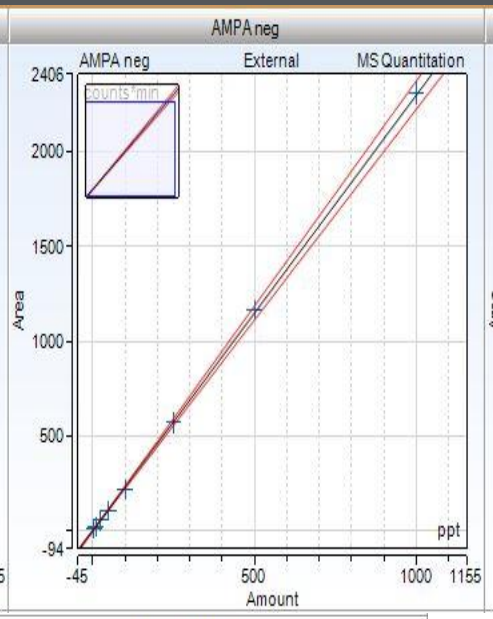
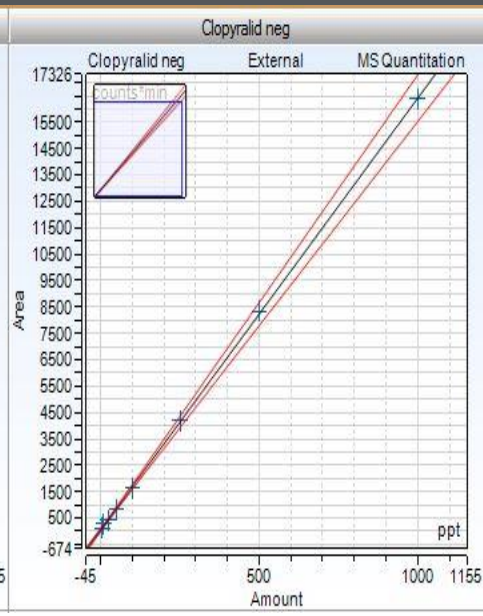
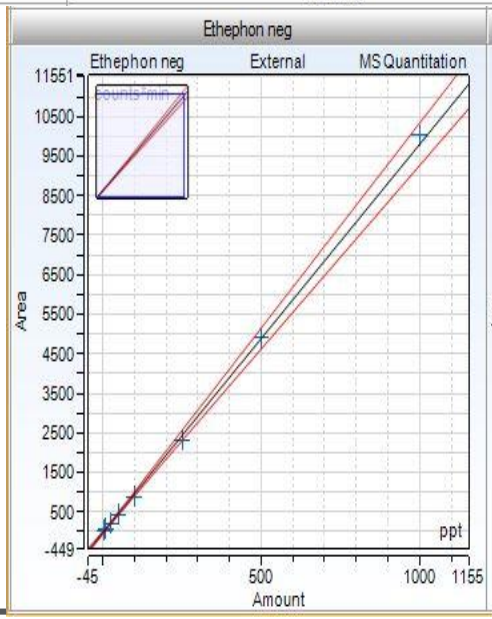
Calibration Surface water – calibration plots

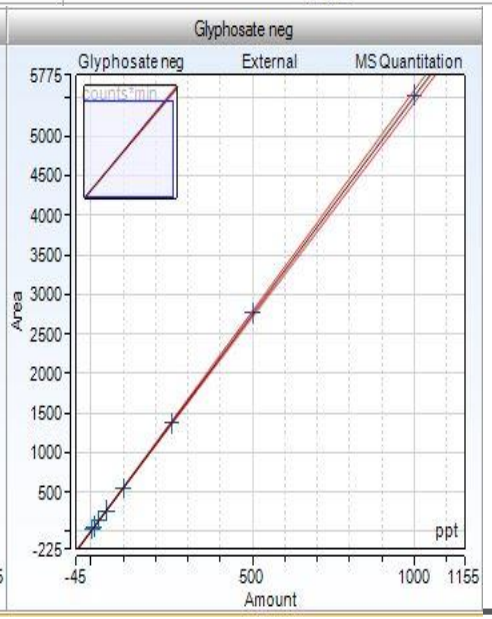












* Levels 5, 10, 25, 50, 100, 500, 1000 ppt, 1 injections each

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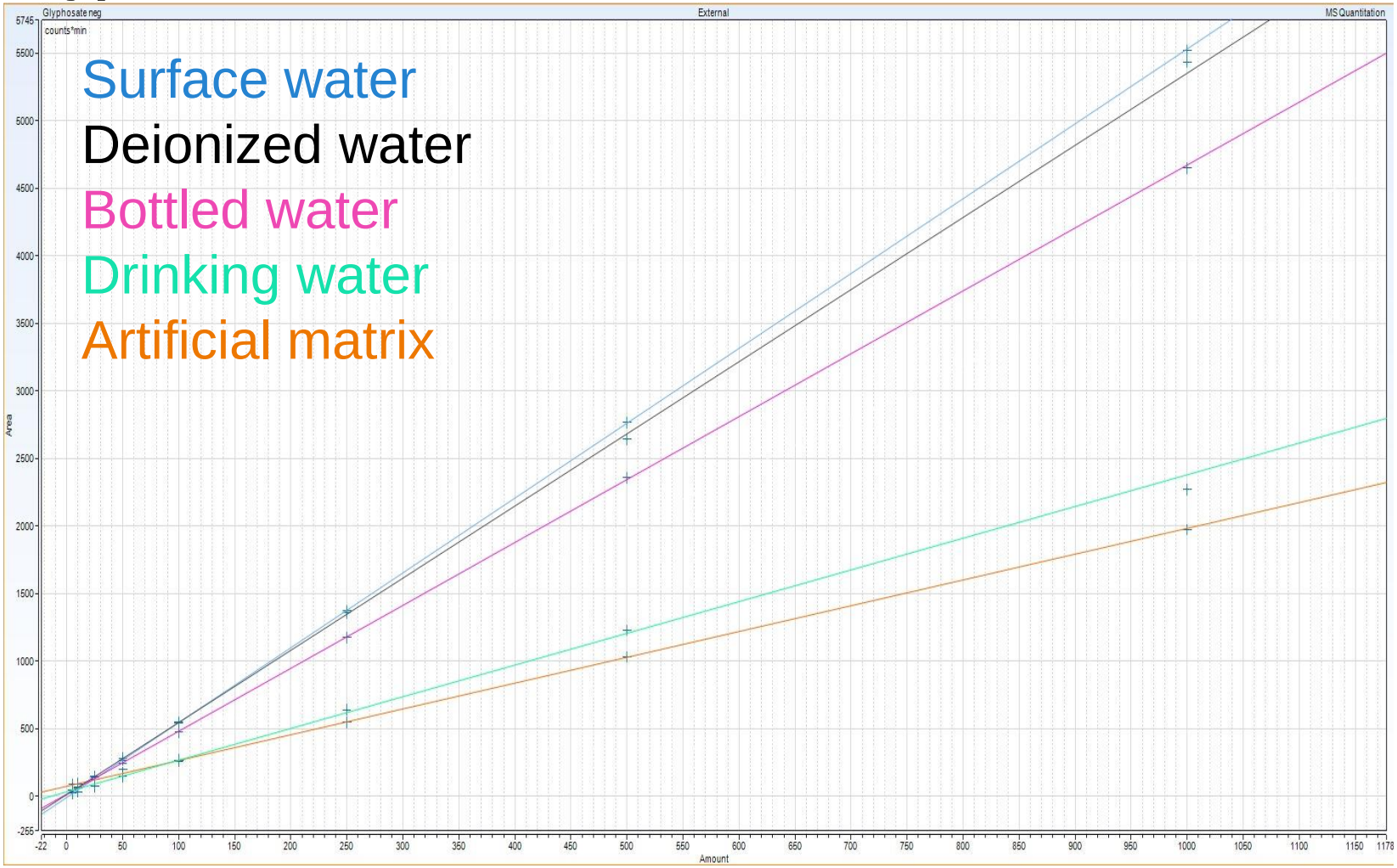
Comparison of calibrations in different matrices

Glyphosate



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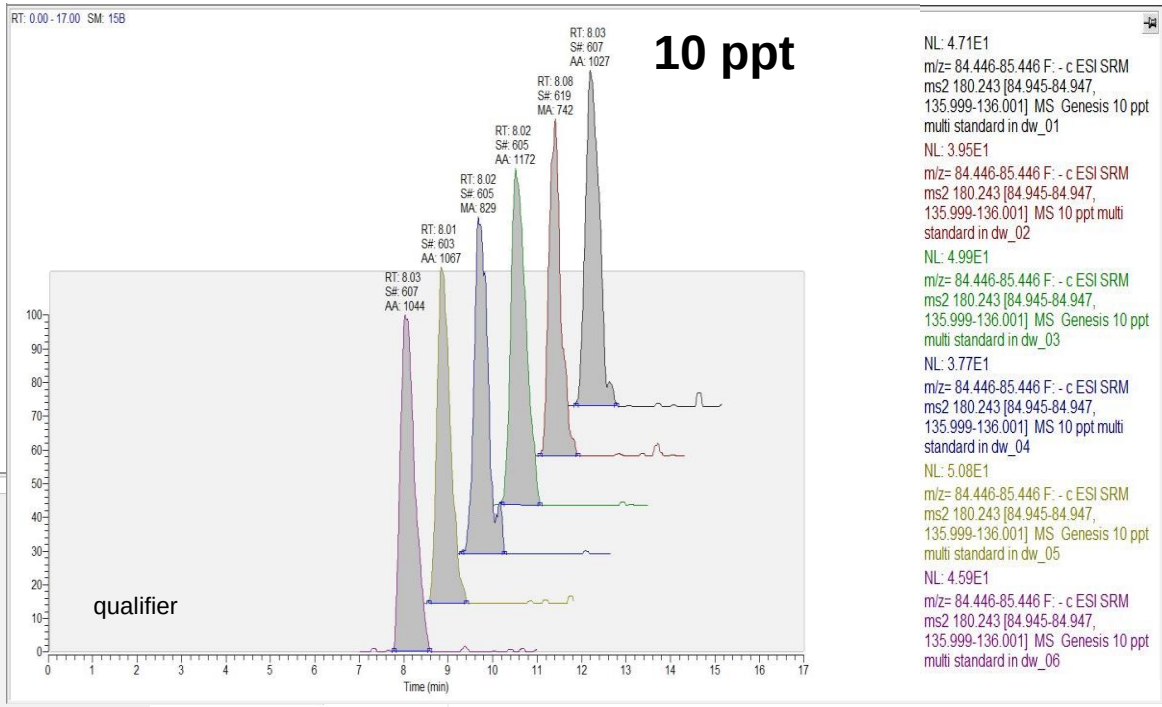
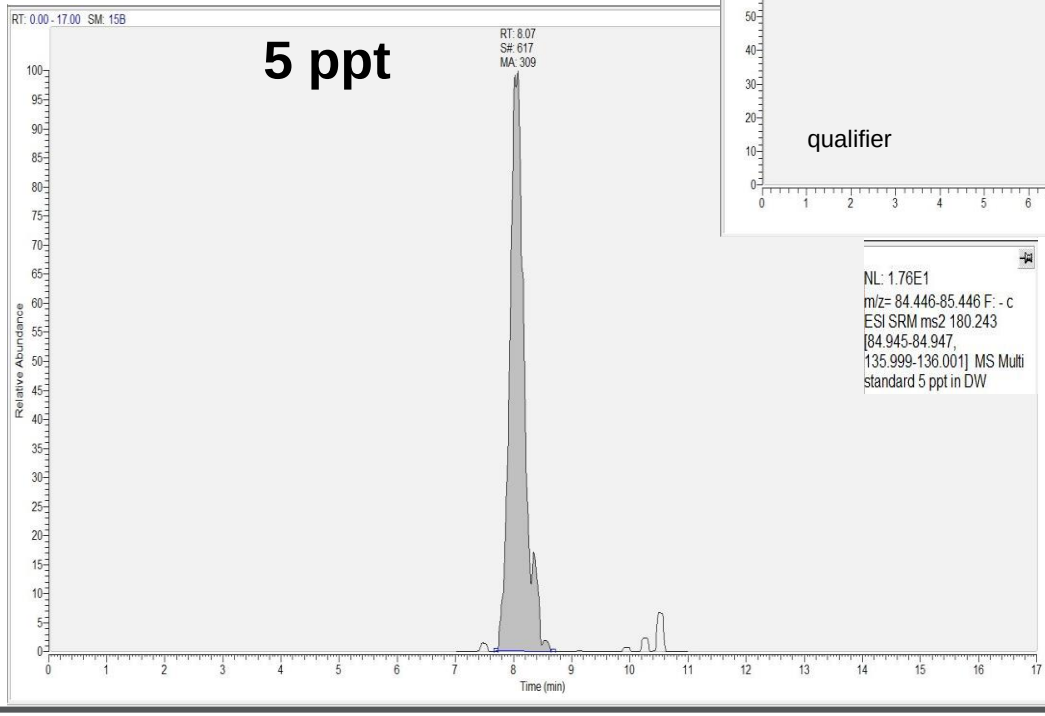
Calibration DW - components

	LOD [ppt]	LOQ [ppt]
Glufosinate	5	10





	10 ppt	20 ppt	50 ppt
REC [%]*	133	121	95

	10 ppt	100 ppt	1000 ppt
RSD [%]**	9.27	12.39	0.93

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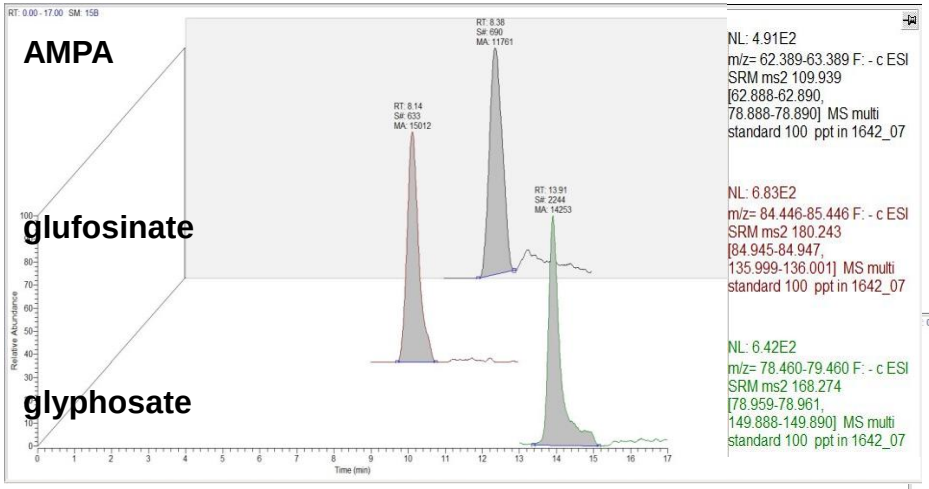
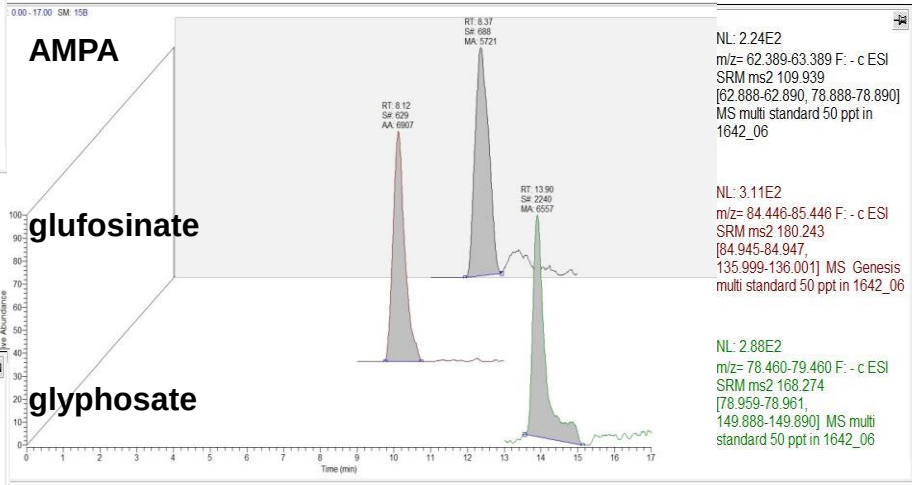
Overview - AMPA, Glu, Gly in Surface water

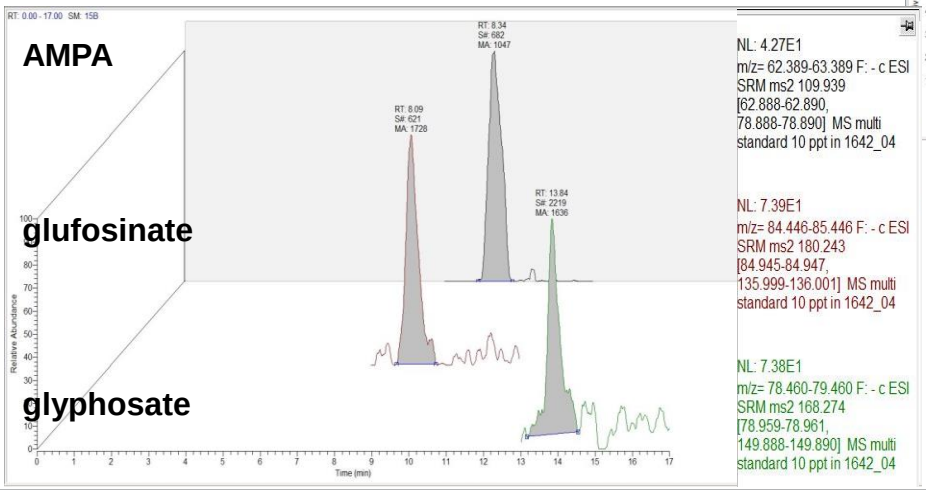
100 ppt



50 ppt



10 ppt



* Levels 5, 10, 25, 50, 100, 500, 1000 ppt, 2 injections each

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Validation results

Component	Matrix	LOD [ppt]]	LOQ [ppt]	REC [%]			RSD [%]		
				10 ppt	20 ppt	50 ppt	10 ppt	100 ppt	1000 ppt
Fosetyl	DW	2.5	5	133	122	132	8.95	1.29	0.74
	Bottled water	1	2.5	121	123	128	2.04	0.86	1.12
	SW	2.5	5	105	105	104	1.96	1.14	0.64
Glufosinate	DW	5	10	133	121	95	9.27	2.39	0.93
	Bottled water	5	10	56	112	96	4.05	3.39	0.83
	SW	5	10	124	111	93	2.17	1.64	1.15
AMPA	DW	5	10	91	93	83	9.14	2.28	0.75
	Bottled water	5	10	109	107	95	8.69	2.10	0.83
	SW	5	10	91	100	98	4.50	3.70	0.98
Clopyralid	DW	10	50	110	88	90	11.94	1.42	0.98
	Bottled water	5	10	53	89	86	9.07	1.42	0.92
	SW	5	10	109	114	140	9.95	1.43	0.59
Glyphosate	DW	10	50	89	106	83	6.28	2.29	1.25
	Bottled water	10	50	66	108	105	14.43	1.73	3.37
	SW	10	50	82	101	90	5.03	10.12	1.76

Comparison of methods

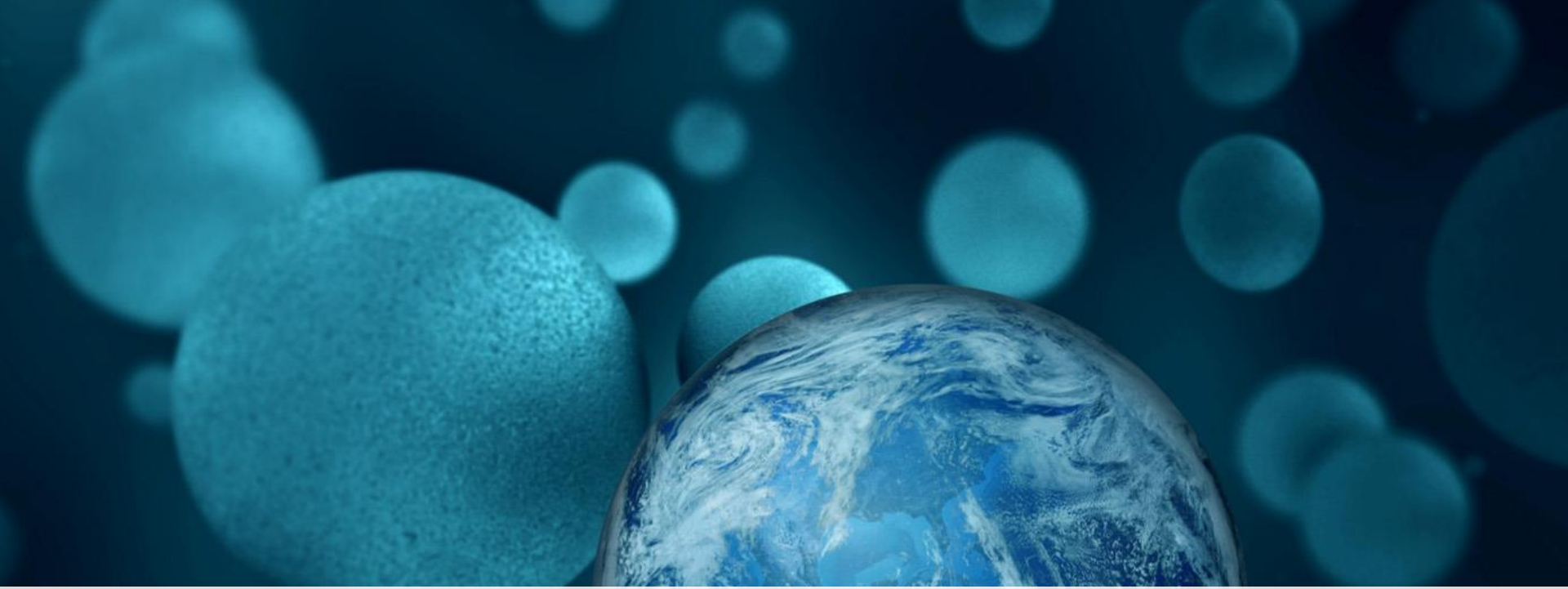
Comparison of two methods for the trace determination of glufosinate, AMPA and glyphosate in Czech SW via HPLC-MS and IC-MS*

Method	Name	Fosetyl [ppt]	Glufosinate [ppt]	AMPA [ppt]	Clopyralid [ppt]	Glyphosate [ppt]	AMPA STDEV[%]	GLY STDEV[%]
Own method	1475	<2.5	<10	145	<50	62.5	4.26	7.09
Reference		n.a.	<20	154	n.a.	69.1		
Own method	1489	<2.5	<10	157	<50	48.4	2.29	
Reference		n.a.	<20	152	n.a.	<50		
Own method	1502	<2.5	<10	164	<50	29.4	3.54	
Reference		n.a.	<20	156	n.a.	<50		
Own method	1520	<2.5	9.2	60.1	<50	29.4	5.65	
Reference		n.a.	<20	65.1	n.a.	<50		
Own method	1521	<2.5	<10	787	<50	183	2.38	17.6
Reference		n.a.	<20	761	n.a.	235		
Own method	1524	<2.5	<10	1801	<50	17.5	3.04	
Reference		n.a.	<20	1880	n.a.	<50		
Own method	1528	<2.5	<10	<10	<50	<10		
Reference		n.a.	<20	60.8	n.a.	<50		
Own method	1613	<2.5	<10	114	<50	15.4	25.0	
Reference		n.a.	<20	163	n.a.	<50		
Own method	1618	<2.5	<10	421	<50	86	23.1	76.9
Reference		n.a.	<20	585	n.a.	291		
Own method	1622	<2.5	<10	217	<50	45.2	15.6	
Reference		n.a.	<20	174	n.a.	<50		
Own method	1624	<2.5	<10	<10	<50	<10		
Reference		n.a.	<20	<50	n.a.	<50		
Own method	1642	<2.5	<10	59.0	<50	60.6		

IC vs. LC arguments

	Ion Chromatography		Liquid Chromatography	
Matrix	Food	Water	Food	Water
Sample preparation	Extraction (QuPPe)	None (filtration)	Extraction (QuPPe) (optional FMOC* derivatization)	FMOC* Derivatization (SPE cleanup) (automation possible)
Separation quality	Excellent	Excellent	Poor (Hypercarb)	Very good (Reversed Phase)
LOQs	1-10 ppb	5 -10 ppt	1 – 10 ppb	5 – 10 ppt
MS/MS	TSQ Endura	TSQ Quantiva	TSQ Quantiva (Endura not suitable)	TSQ Quantiva
Matrix tolerance	Very good	Very good	Limited	Good
Time consumption	Low	Low	Low - medium	Medium – High (automation possible)

** FMOC derivatization applicable only for Gly, AMPA, Glu!*



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Automated Glyphosate FMOC Derivatization

Data courtesy of
Klaus Wilmers, Chemical and Veterinary Analytical Institute Muensterland-Emscher- Lippe (CVUA-MEL) Joseph-Koenig-Str. 40, D-48147 Muenster/Germany
Short communication: Trace analysis of glyphosate in milk by zirconium precipitation and LC/high resolution MS
Deutsche Lebensmittel Rundschau, 112. Jahrgang Juli 2016 Behr's Verlag l Hamburg l ZKZ 9982

Method Description

- Analysis of Glyphosate in milk samples at sup –ppb levels using zirconium chloride precipitation and automated FMOC derivatization followed by HRAM determination using Thermo Scientific Q Exactive System

Benefits

- Possibility to monitor glyphosate in milk samples below 1 ug/L
- Automated approach to derivatization
- Selectivity and sensitivity of Q Exactive high resolution and accurate mass spectrometer

Data courtesy of
Klaus Wilmers, Chemical and Veterinary Analytical Institute Muensterland-Emscher- Lippe (CVUA-MEL) Joseph-Koenig-Str. 40, D-48147 Muenster/Germany
Short communication: Trace analysis of glyphosate in milk by zirconium precipitation and LC/high resolution MS
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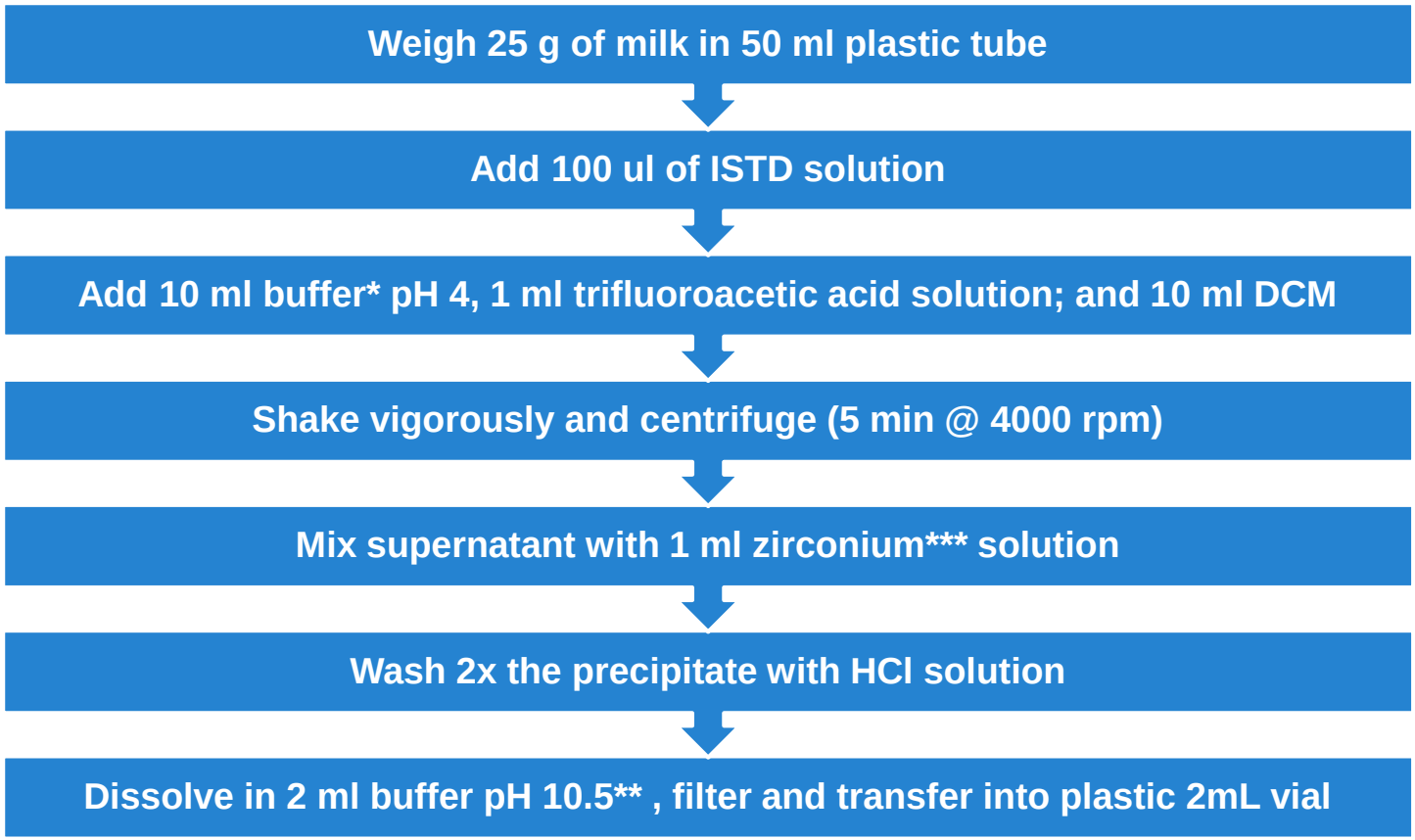
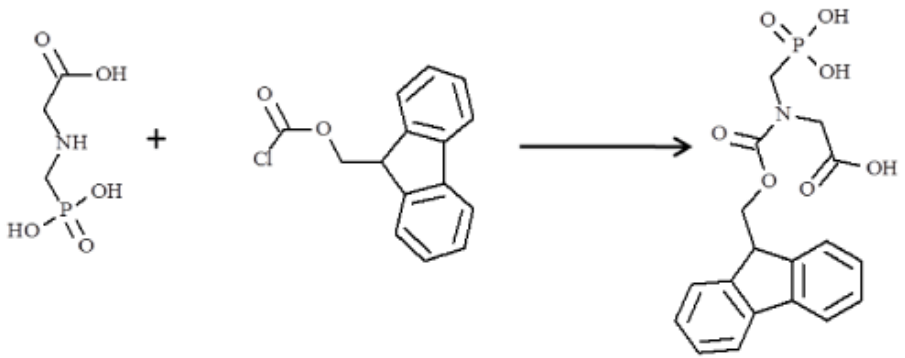
Sample preparation principle



* Buffer pH 4 0.1 m McIlvaine citrate-phosphate buffer, *Sigma-Aldrich*
** Buffer pH 10.5 Sodium tetraborate decahydrate, 3 % in water; *Merck*
***Zirconium solution Zirconium(IV) oxide chloride $ZrOCl_2 \cdot 8 H_2O$, 4 % in water/fuming hydrochloric acid (1:1) *Merck*

Glyphosate Derivatization

Principle of Reaction



Glyphosate FMOC-Cl Glyphosate-FMOC derivative

- Add 50 µL sample to 10 µL OPA reagent
- Mix for 2 min
- Add 20 µL acetonitrile and 10 µL FMOC
- Mix for 4 min
- Add 10 µL concentrated formic acid (stop reaction)
- Dilute with 100 µL formic acid (0.1 %)
- 100 ul injection into LC-MS

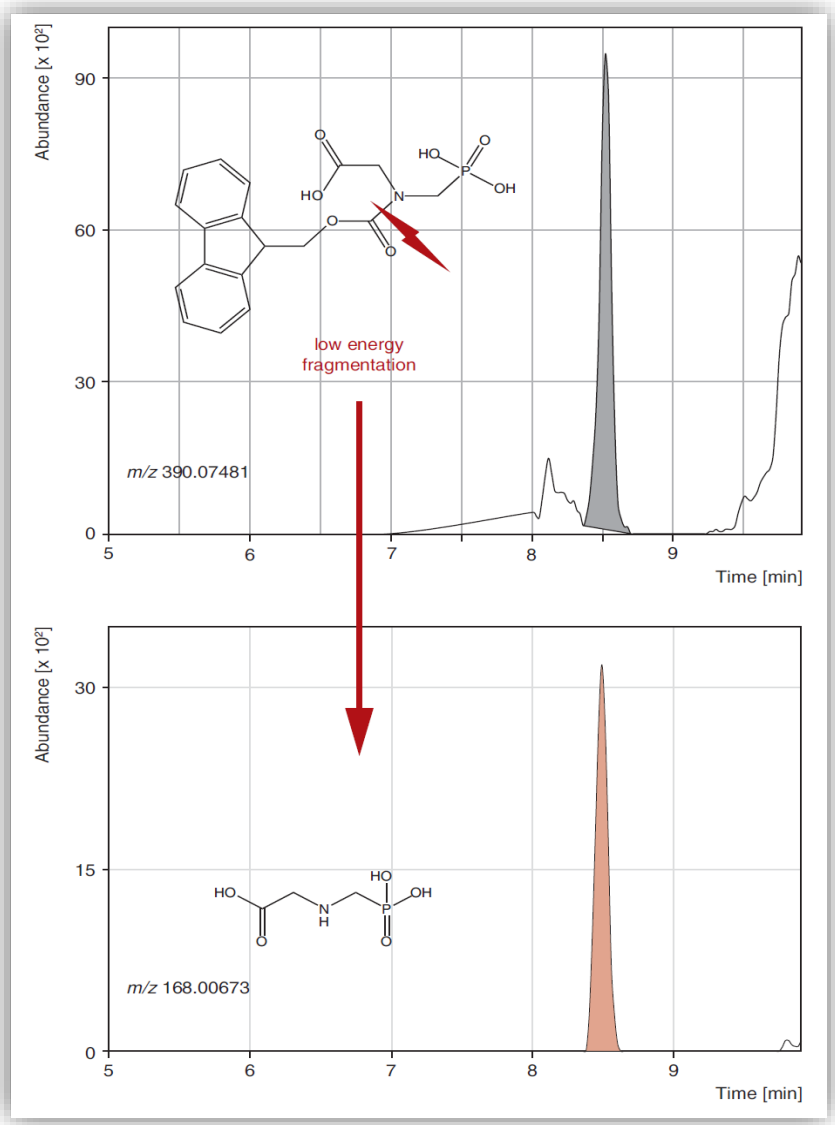
LC-MS System Configuration

Thermo Scientific™ Transcend II
coupled to Q Exactive™ MS

Technical Details

- Separation on PhenylHexyl column (2.1 × 150 mm, 3.5 µm)
- Water-acetonitrile gradient program, flow rate of 250 µL/min
- Full Scan/All Ion Fragmentation used at 140.000 FWHM resolving power

Results



- LOQ: 0.050 µg/kg of milk
- Linearity coefficient: 0.99
- Carry over < 0.05%
- OPA derivatization step eliminated strongly interfering coeluent of FMOC – Glyphosate
- Fragmentation in Q Exactive collision cell provides confirmation information

Conclusions

- Current IC/LC-MS/MS portfolio allows determination of polar pesticides in both food and environmental samples well below regulatory limits
- Simple sample preparation for IC separation – no FMOC needed!
- Good separation efficiency of IC makes it a suitable method for most polar pesticides
- TSQ Quantiva is the recommended MS/MS for water analysis @ ppt levels
- IC-TSQ Endura is suitable for food sample analysis @ ppb levels (LC-TSQ Quantiva an option if more sensitivity or more difficult matrix is analyzed)
- Full application support is provided for the methods by EU Sales Support Team