An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

Anionic Pesticide Residues in Food Samples

Anatte Margalit, Stephen Burrell, Michael Kelly and Jim Garvey

Summary

- Anionic Pesticides
- QuPPE Extraction - modified
- Mass Spectrometry data
- Validation data
  - Cereals
  - Milk / Infant Formula
  - Fruit and Vegetables
- Conclusion

Introduction

EURL-SRM — EU Reference Laboratories for Residues of Pesticides — Single Residue Methods

Table 6: *Scope of QuPPe-LC-Methods of analytes analyzed in the ESI-neg. mode Part.I*

QuPPe method code	M 1.1 (5.6.2)	M 1.2 (5.6.3)	M 1.3 (5.6.4)	M 1.4 (5.6.5)	M 1.5 (5.6.6)	M 1.6a/b (5.6.7)	M 1.7 a/b (5.6.8)	M 1.10 (5.6.9)	M 1.11 (5.6.10)	M 1.12 (5.6.11)
Separation principle	Anion Ex.	Anion Ex.	Carbon	Carbon	HILIC	HILIC	HILIC	HILIC	HILIC	HILIC
Column type	AS 11	AS 11-HC	Hypercarb	Hypercarb	Trinity Q1	a: Torus DEA/ b: APPC	a: Torus DEA/ b: APPC	APPC	Raptor PolarX	ObeliscN
ANALYTES COVERED BY LC-MS/MS IN THE ESI-NEGATIVE MODE										
Ethephon	✓	✓	✓	NT	✓	✓	(✓)		✓	✓
HEPA	✓	✓	✓	NT	✓	✓	(✓)		✓	✓
Glufosinate	✓	✓	✓	NT	✓	✓	(✓)		✓	✓
N-Acetyl-Glufosinate	✓	✓	✓	NT	✓	✓	(✓)		✓	✓
MPPA	✓	✓	✓	NT	✓	✓	(✓)		✓	✓
Glyphosate	✓	✓	✓	NT	✓	✓	(✓)		✓	✓
AMPA	✓	✓	✓	NT	✓	✓	(✓)		✓	(✓)
Phosphonic acid	(✓)	(✓)	✓	✓	✓	✓	✓		✓	(✓)***
N-Acetyl-AMPA	NT	✓	✓	NT	✓	✓	(✓)		NT	NT
Fosetyl-Al	-	✓	✓	NT	✓	✓	(✓)		✓	(✓)***
Maleic Hydrazide	-	-	✓	NT	-	-	-	(✓)	(✓)	(✓)
Perchlorate	NT	-	✓	✓	✓	(✓)**	✓	✓	✓	✓
Chlorate	NT	-	✓	✓	✓	(✓)**	✓	✓	(✓)	✓
Bialaphos	NT	NT	✓	NT	✓	NT	NT		✓	NT
Cyanuric acid	NT	NT	✓	NT	-	-	-	✓	(✓)	(✓)
Bromide	NT	NT	-	✓	✓	(✓)**	✓		✓	✓
Bromate	NT	NT	(✓)	✓	NT	NT	✓		NT	NT
N-Acetyl-Glyphosate	NT	NT	✓	NT	(✓)**	✓	(✓)		-	✓
Difluoroacetic acid	NT	NT	NT	NT	NT	NT	NT		✓	NT
Trifluoroacetic acid	NT	NT	NT	NT	NT	NT	NT		✓	✓
Thiocyanate	NT	NT	(✓)**	✓	NT	NT	NT		✓	NT
Desmethyl-Dimethoate	NT	NT	✓	-	NT	NT	NT		NT	NT

✓ = satisfactory chomatography and detection sensitivity achieved,
NT = Not tested under the conditions shown in the respective sections,
(✓) = possible but compromised due to matrix effects or lacking separation or limited sensitivity or limitations in the detection of qualifiers compromising identification.
"-" analysis was tested and found to be poor under the described conditions,
* Using a gradient (98% B -> 60% B in 5 min, hold 2 min)
** Different LC-conditions required to improve peaks (see M1.7 or M1.9)
*** Compromised quantitation of Phosphonic acid due to co-elution of Phosphonic acid and Fosetyl, see also General Hints **5.6.1**
**** Quality of analysis may strongly depend on instrument type and condition

Introduction

These molecules are small highly polar and in many cases acidic.

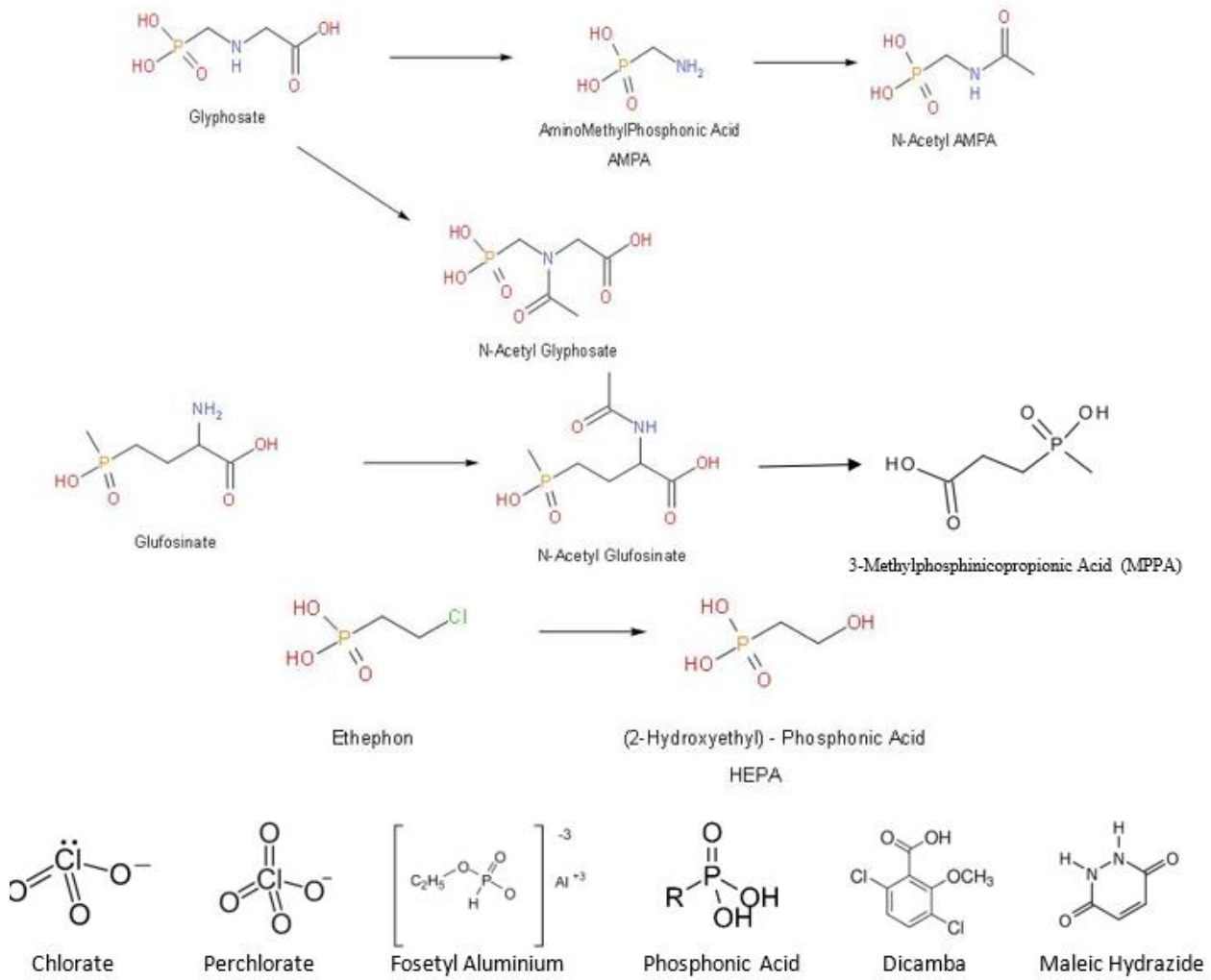
They are not retained on reverse phase columns

Methods to date include, normal phase, HILIC, multimode and ion exchange chromatography

Because of the similarity in structures they fragment to give common fragments

Mostly PO_2^- (m/z = 63) and PO_3^- (m/z = 79)

Method was originally set up with HRAM system



Introduction

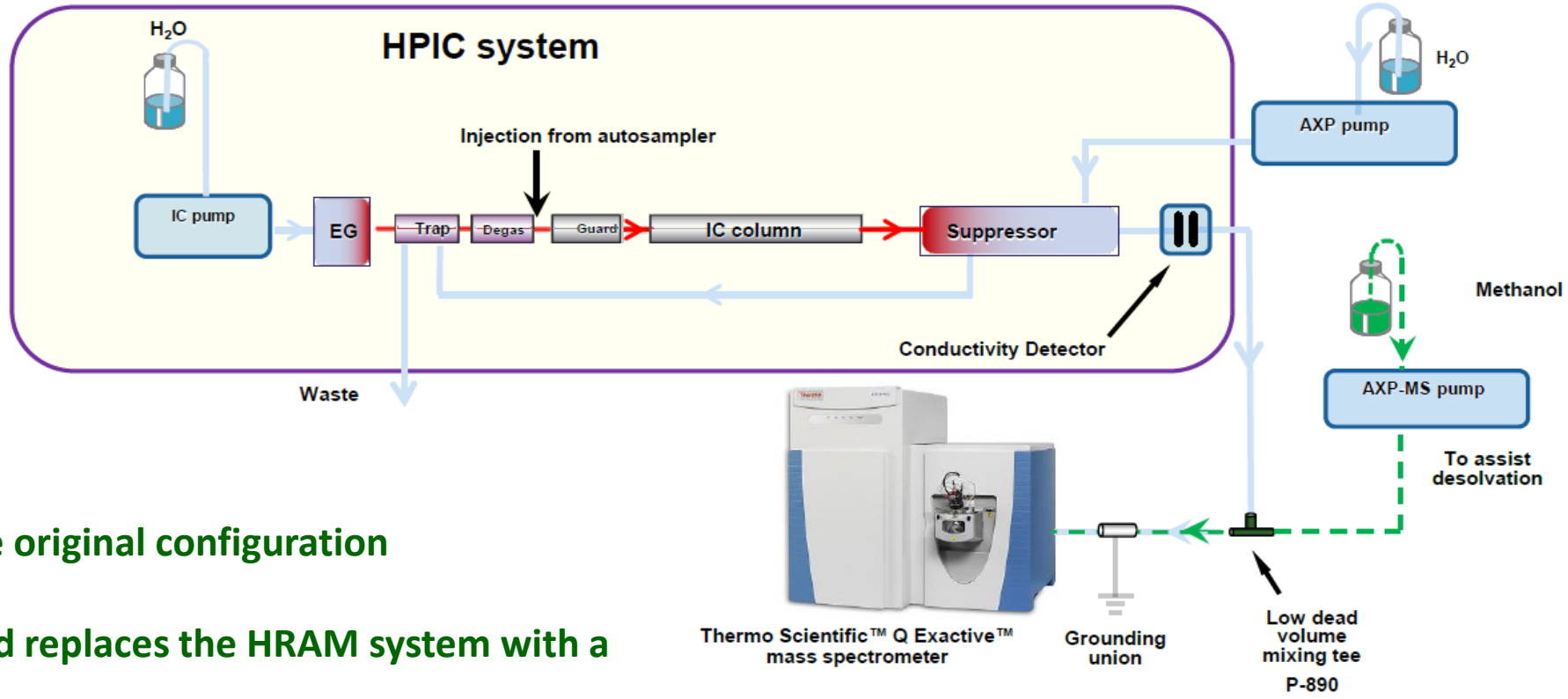
	Pros	Cons
Normal Phase – Hypercarb	Covers the full range of analytes	Needs excessive conditioning
HILIC / Multimode	Covers most of the analytes	Needs a lot of conditioning System needs to be deactivated regularly
Ion Chromatography	Covers full range of analytes with scope for expansion Very little conditioning and no deactivation required	Requires dedicated system

New versus old

QE Focus		Altis+
There is no advantage to using a high resolution system with these molecules because we have a lot of common fragments – also we were using PRM mode		MS/MS with lower resolution and lower mass accuracy is more than adequate for these molecules
HRAM System was shared with an LC		Now have a dedicated IC system
Sensitivity did not allow us to dilute out matrix effects		Much higher sensitivity allows us to use dilution to our advantage
Ion source cleaning was required after every batch		Higher dilution also means much less cleaning and maintenance on the system
10 hour bake out after maintenance meant a lot of system down time		Down time minimised

An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

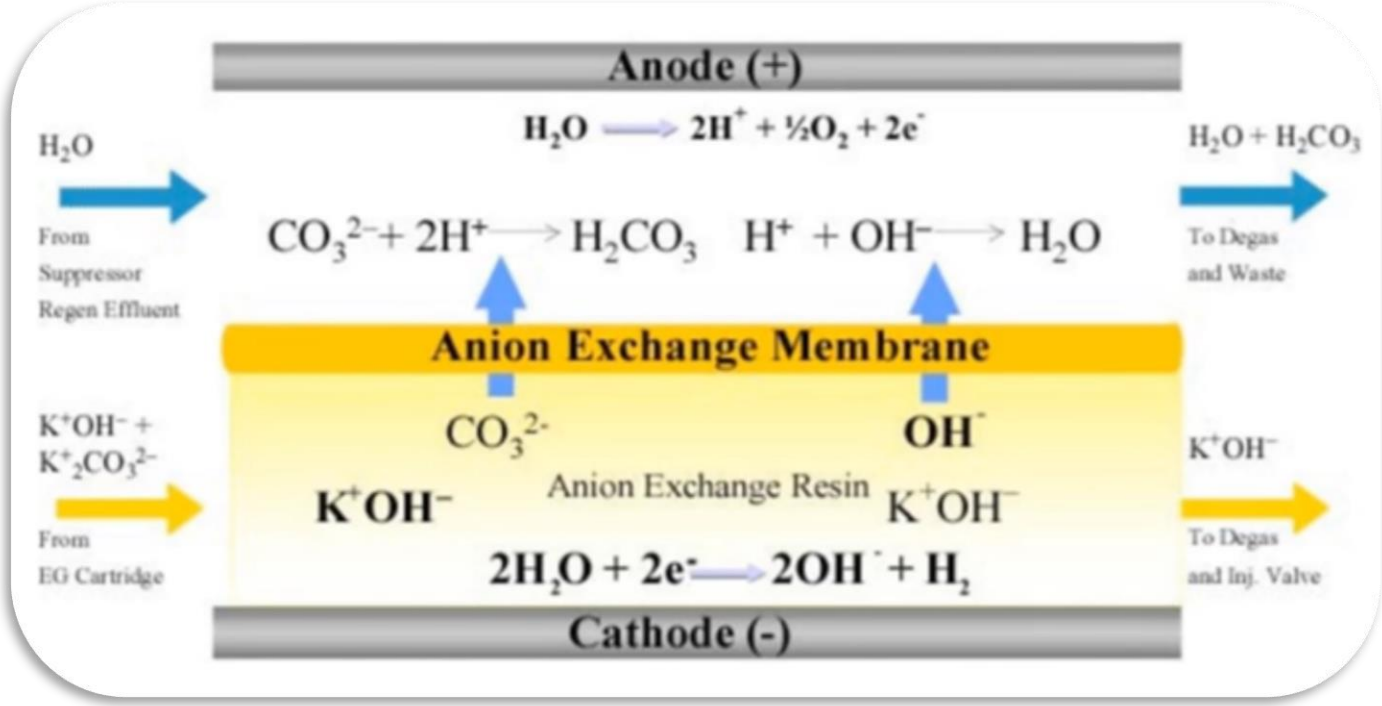
Ion Chromatograph Configuration



This was the original configuration

New method replaces the HRAM system with a triple quad

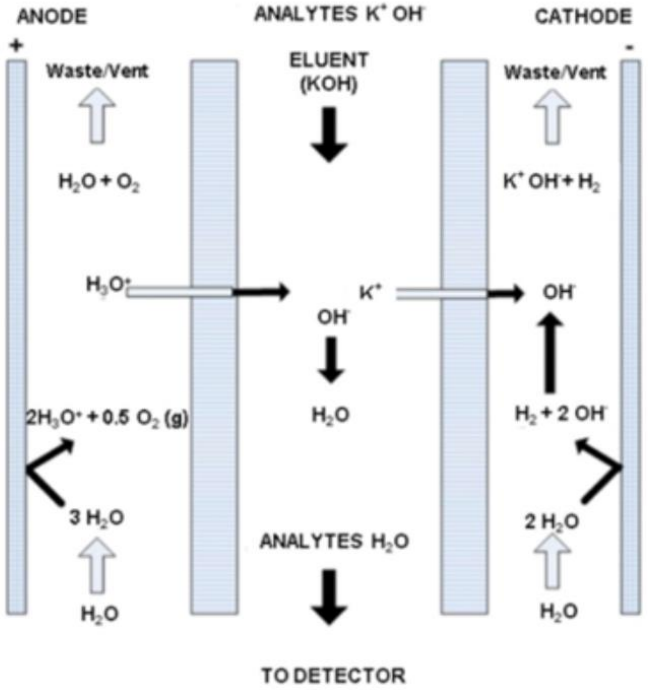
Continuously Regenerated Trap Cartridge



- The trap removes CO_2 in the form of Carbonic acid and produces OH^- and H_2 from the hydrolysis of water.

Suppressor

- The suppressor consists of a membrane with a cathode and an anode
- KOH eluent enters the suppressor and the K^+ ions migrate towards the cathode, react hydrolytically with water to form KOH which goes to waste
- The anode produce H^+ which crosses the membrane and combines with OH^- to form water.
- The net effect is to remove KOH from the eluent and replace it with water
- This is critical to protect the ion source and MS



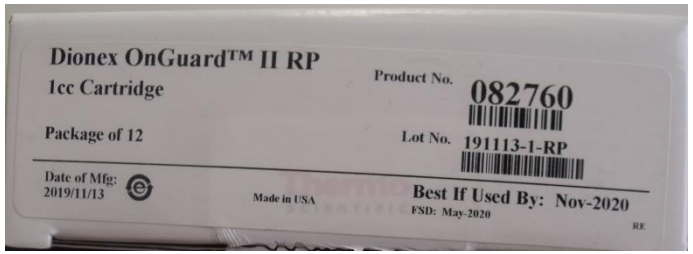
An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

Extraction and Clean-up

	Cereals	Milk / IF	Fruit & Veg
Sample	2g	4ml	10g
Sample Prep	Add water to the sample	IF made up as consumed	Frozen and homogenised
Extraction	Methanol: Water 50:50	Methanol (4ml) + 3% acetic acid	Methanol (10ml)
	Centrifuge	Centrifuge	Centrifuge
Clean-up	Activated ICC cartridge and 0.2µ filter	Activated ICC cartridge and 0.2µ filter	Activated ICC cartridge and 0.2µ filter
Final dilution	Dilute 1/10 for analysis	Dilute 1/10 for analysis	Dilute 1/5 for analysis
Total dilution	1/100	1/20	1/10
	Inject 30µl	Inject 30µl	Inject 30µl

Extraction

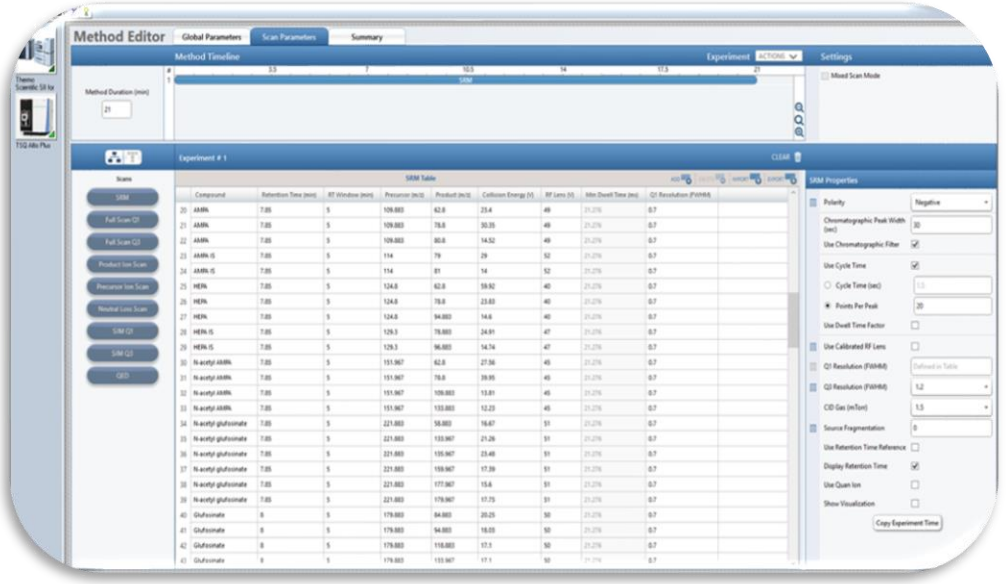
- Extraction – Water – Water / Methanol (50:50) followed by SPE clean-up and filtration
- After extraction samples are cleaned-up using a Dionex OnGuard ICC cartridge
- This is followed by filtration through a 0.2µm filter and then this extract is analysed



An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

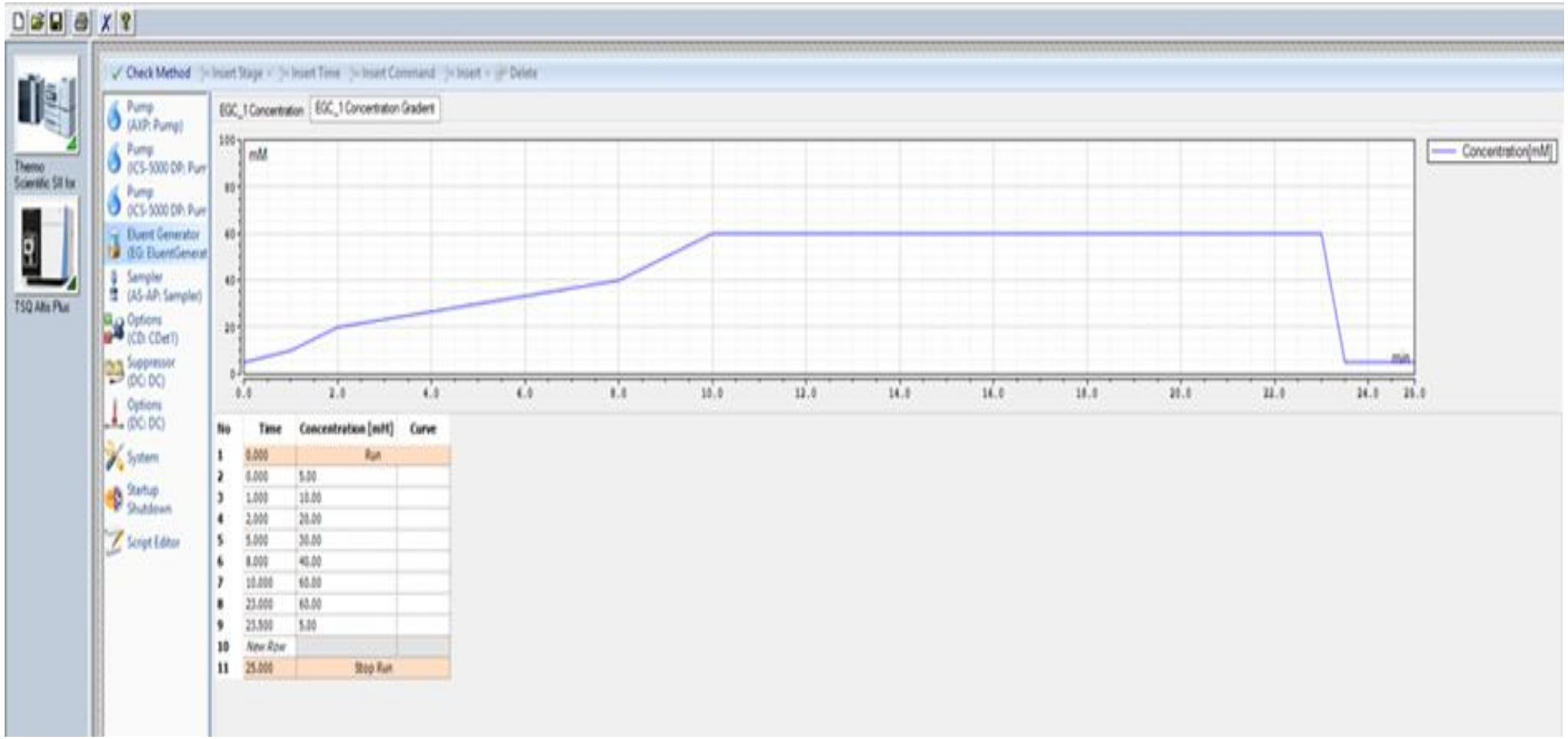
Mass Spectrometry Data

	Transitions		
Compound	Parent ion	Quan	Confirming
Chlorate	82.9	66.8	50.9, 68.8
Dicamba	218.8	174.9	144.8, 34.9
Ethephon	142.9	106.9	62.9, 78.9
Fosetyl Al	108.9	80.9	62.8, 78.8
Glufosinate	179/9	94.9	136.0, 118.8, 134.0, 84.9
Glyphosate	167.8	80.8	62.8, 149.9, 123.9, 78.8
HEPA	124.8	78.8	94.9, 62,8
Maleic Hydrazide	110.9	81.9	82.9, 54.9
MPPA	150.9	132.9	1-6/9, 62.8, 77.9
N-acetyl-AMPA	152.0	109.9	62.8, 78.8,133.9
N-acetyl-glufosinate	221.9	134.0	137.0, 177.0 58.9, 160.0
N-acetyl-glyphosate	209.9	123.9	147.9, 62.8, 78.8 80.8, 149.9
Perchlorate	98.8	82.8	66.9, 50.9
Phosphonic acid	80.7	78.7	78.8, 62,9



An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

Ionic Strength Gradient



An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

Validation plan

- Matrices chosen were wheat and infant formula

- 6 Recovery spikes at concentrations covering the linear range

- Evaluate Linearity, precision, accuracy, ion ratio's and matrix effects
- Look at performance with PT samples

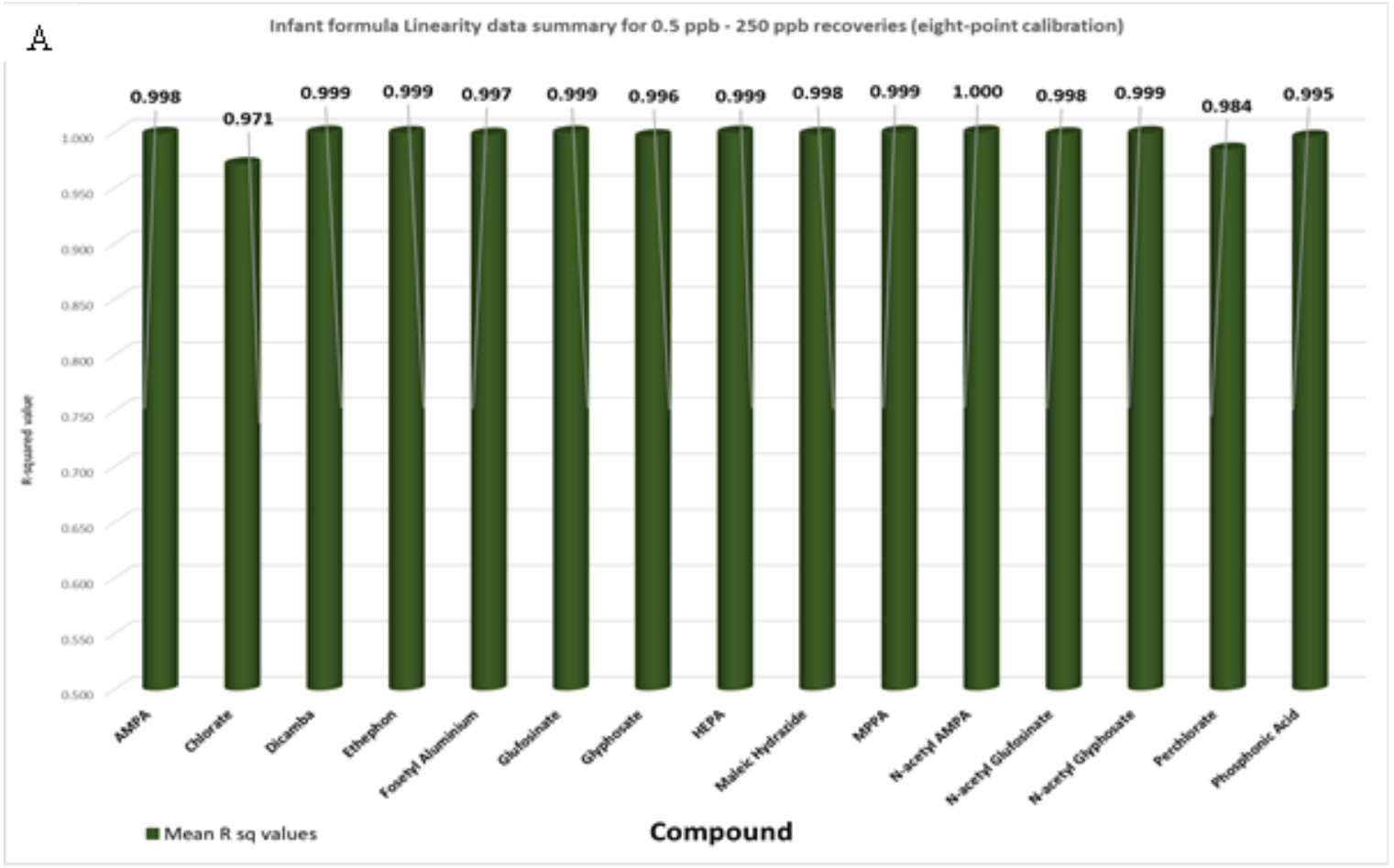
- For fruit and veg the same things were covered
- Matrices chosen:

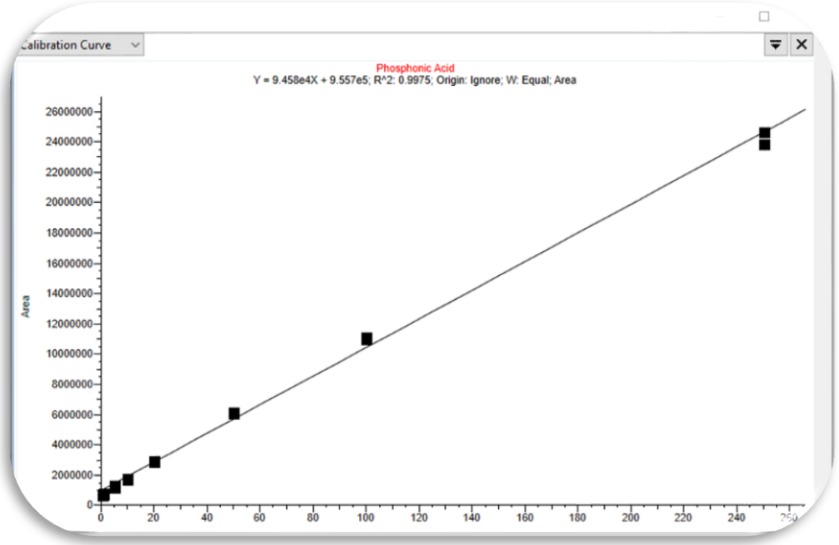
  - High water - Tomato
  - High Acid - Oranges

  - Root vegetable - Carrots

An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

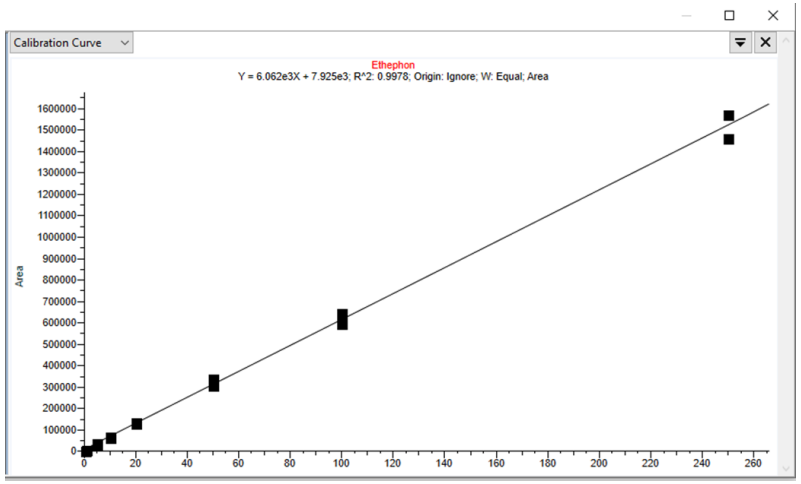
Linearity



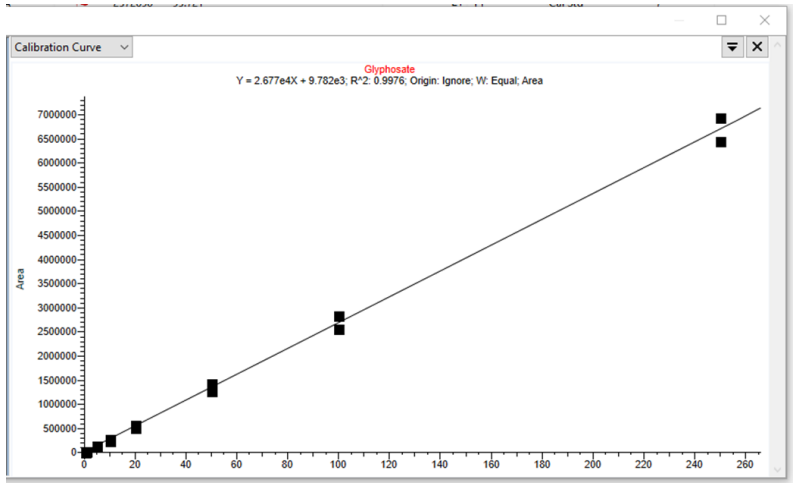


An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

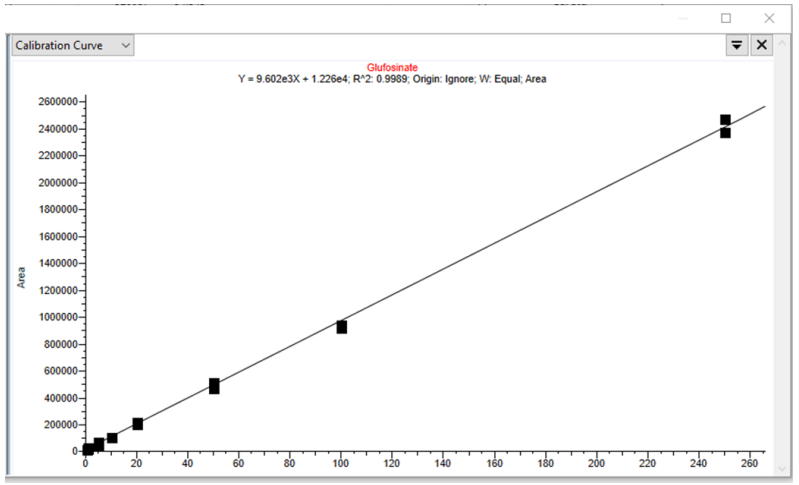
Linearity



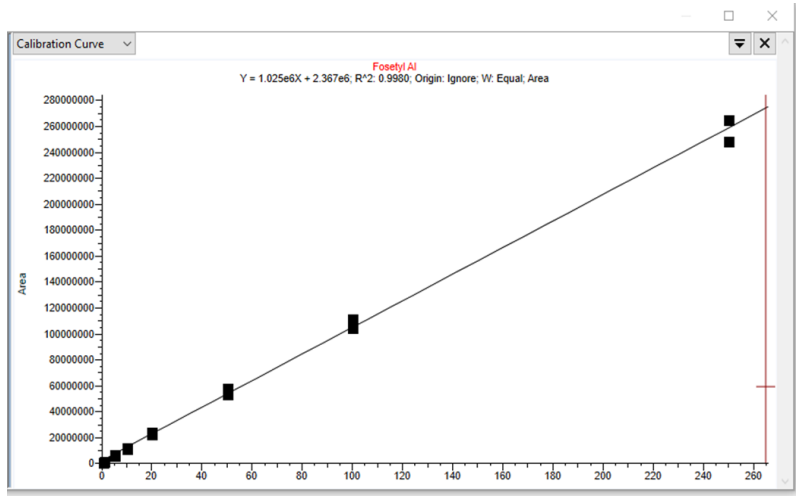
Ethephon



Glyphosate



Glufosinate

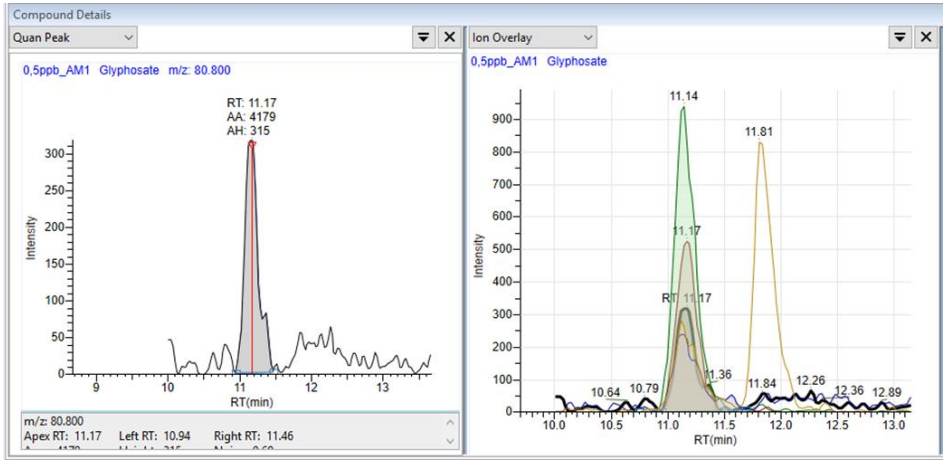


Fosetyl - Al

An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

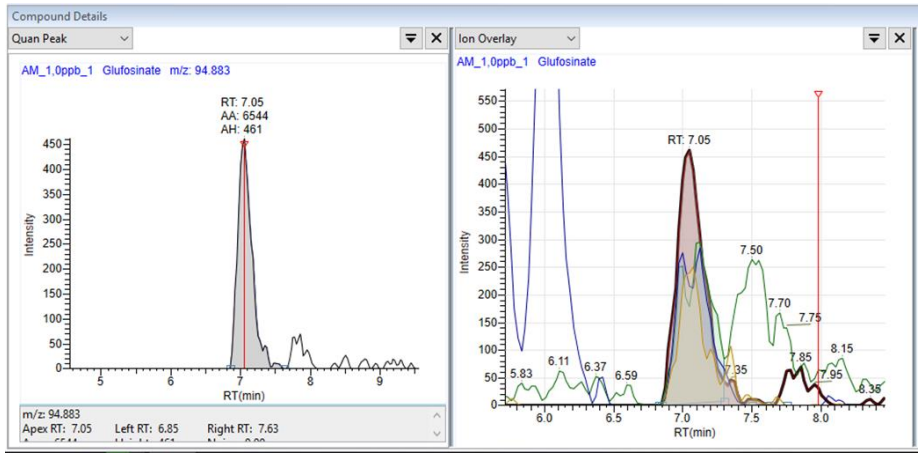
Data Examples

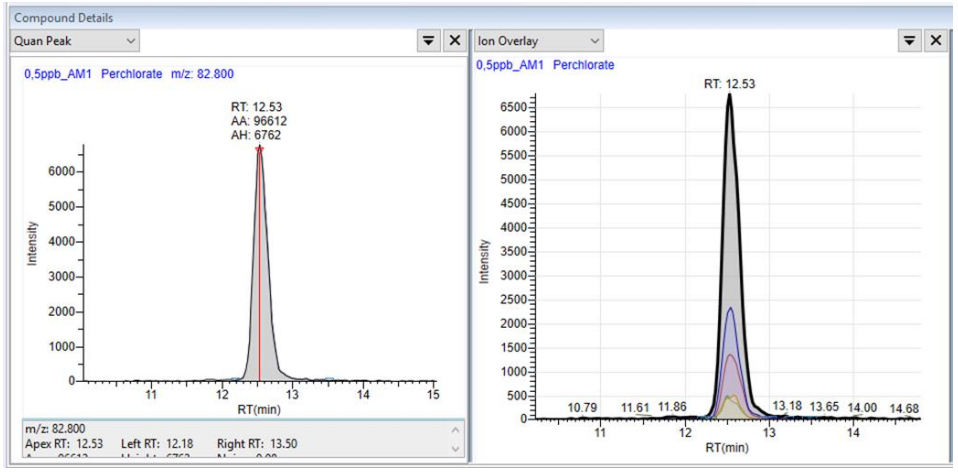
0.5µg/kg corresponding to 10µg/kg in IF



Glyphosate

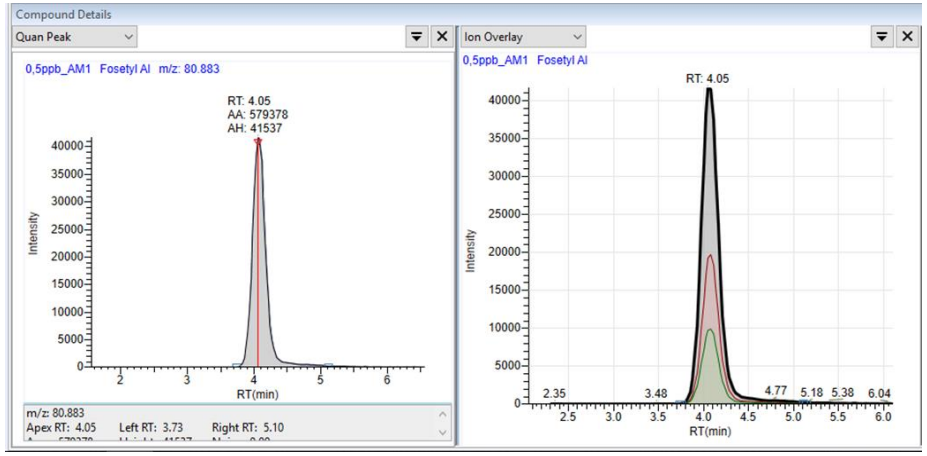
Glufosinate 1µg/kg





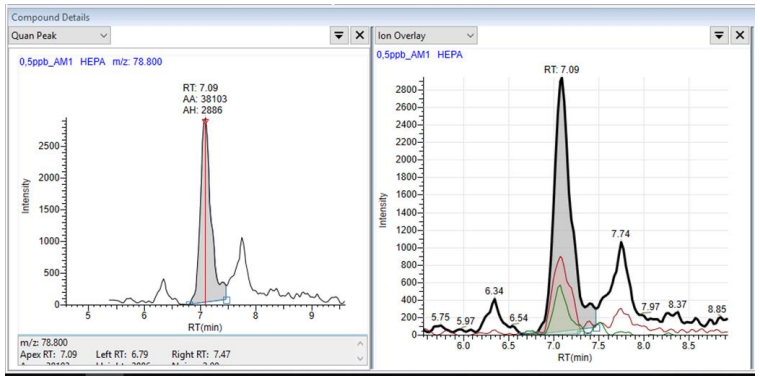
Perchlorate

Fosetyl - Al



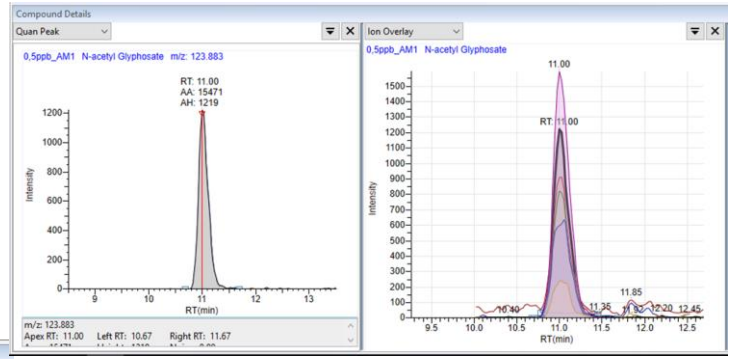
An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

Metabolites

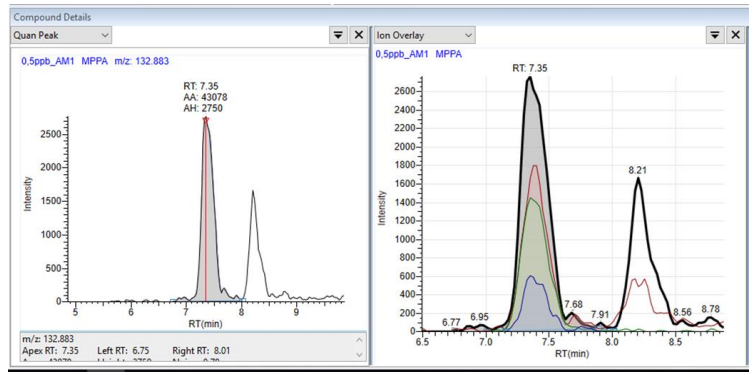


HEPA

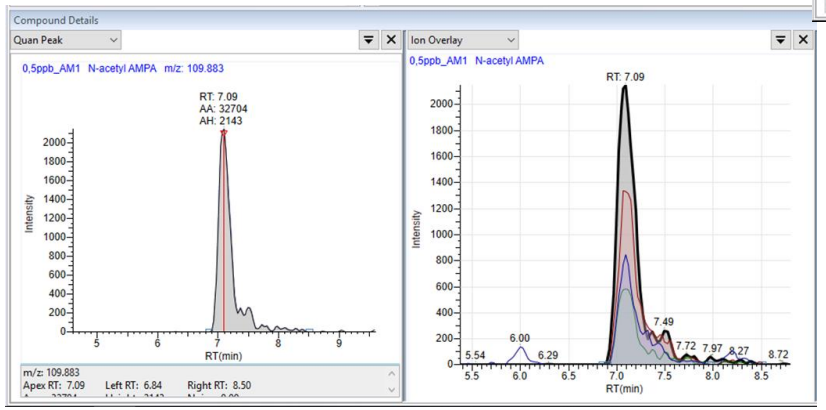
0.5µg/kg



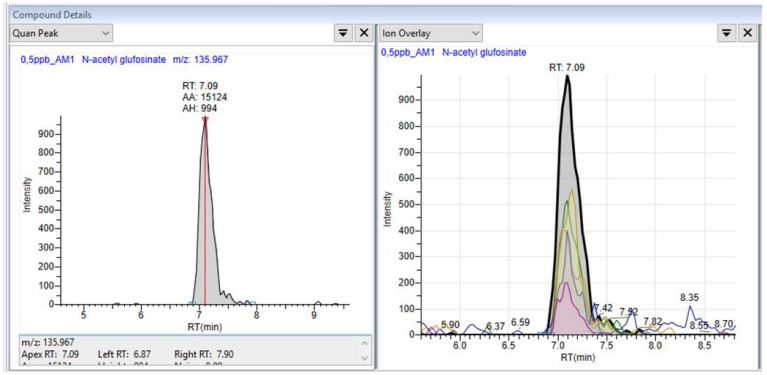
MPPA



N-Acetyl Glyphosate



N-Acetyl AMPA



N-Acetyl Glufosinate

An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

Reproducibility across all batches

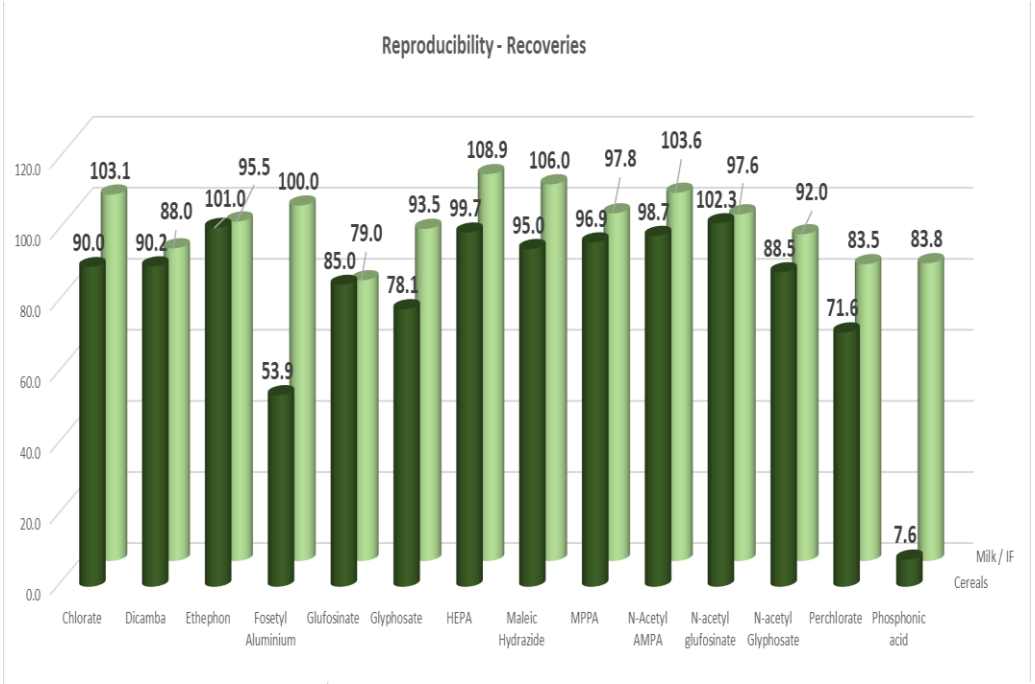
Recovery used as an estimate of accuracy and precision used to calculate repeatability and inter-lab reproducibility

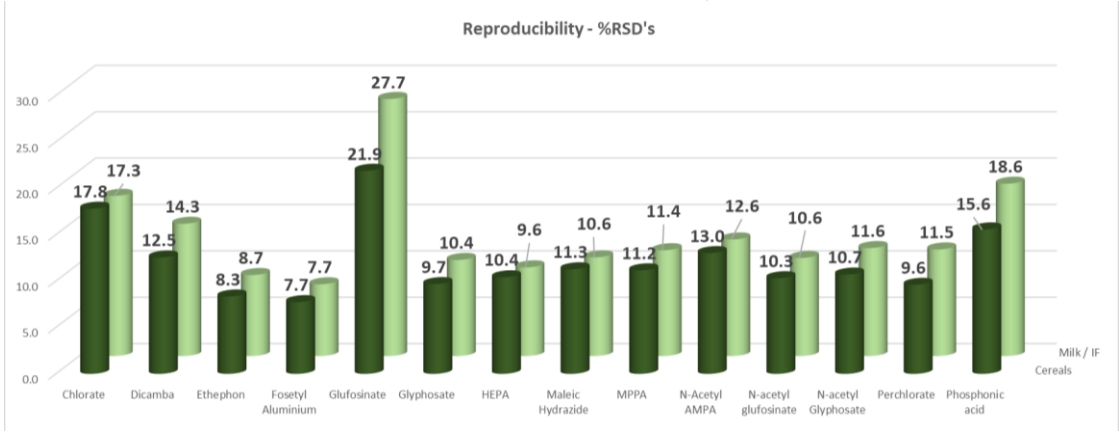
6 Repeatability spikes carried out at each concentration level

Repeatability calculated as mean and %RSD at each concentration

The mean of each repeatability experiment is used as one point in the calculation of the inter-Lab reproducibility

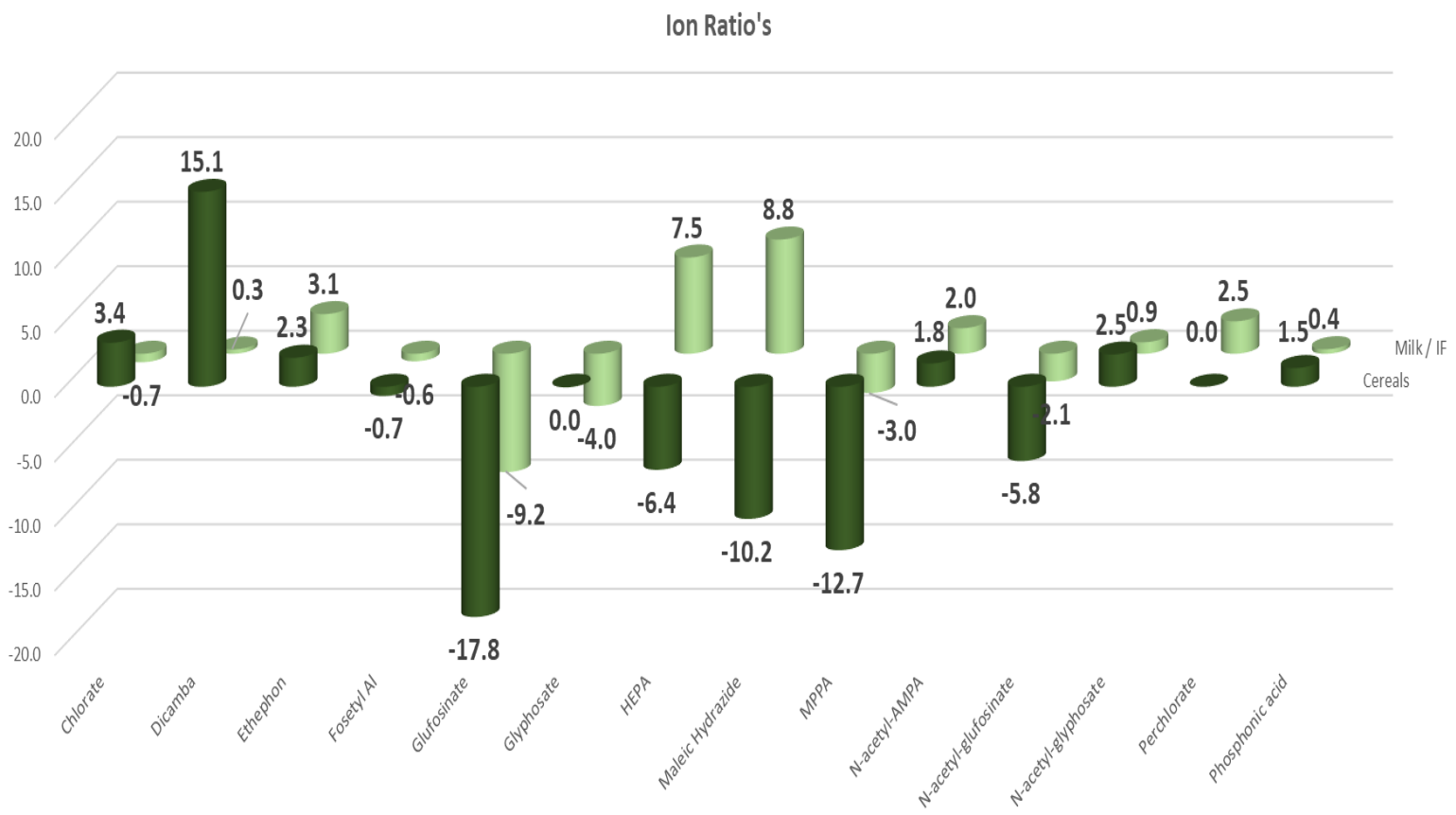
Reproducibility mean and %RSD calculated from this data





An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

Ion Ratio's



Comparison of ion ratio's of standards versus spikes at each level

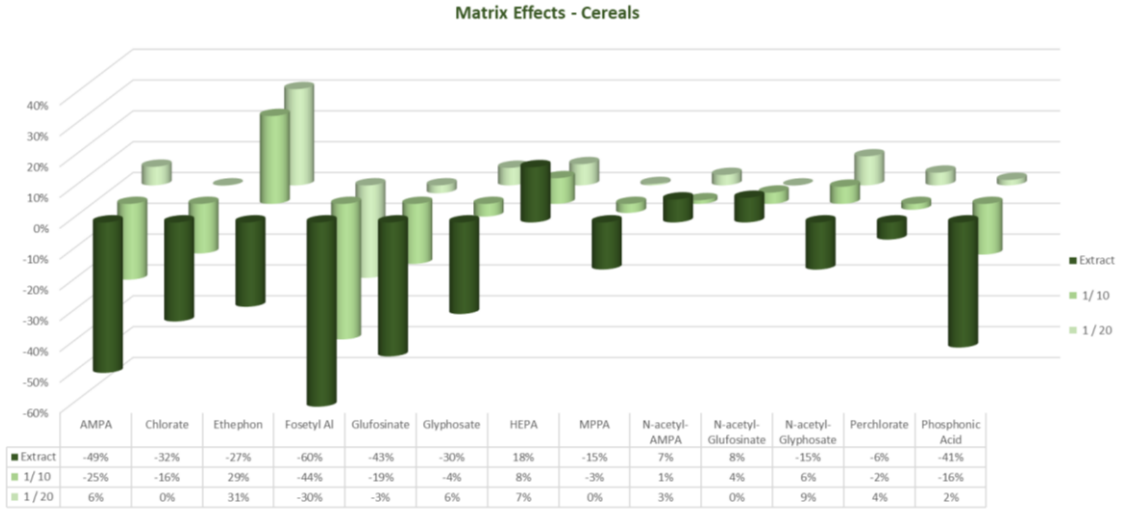
Data here is for the first confirmation transition in each case

Chart shows the ion ratio's of the spikes averaged across all concentration levels.

All values meet the < 30% of the standards criteria

An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

Matrix Effects



	AMPA	Chlorate	Ethephon	Fosetyl Al	Glufosinate	Glyphosate	HEPA	MPPA	N-acetyl-AMPA	N-acetyl-Glufosinate	N-acetyl-Glyphosate	Perchlorate	Phosphonic Acid
Extract	-49%	-32%	-27%	-60%	-43%	-30%	18%	-15%	7%	8%	-15%	-6%	-41%
1/ 10	-25%	-16%	29%	-44%	-19%	-4%	8%	-3%	1%	4%	6%	-2%	-16%
1 / 20	6%	0%	31%	-30%	-3%	6%	7%	0%	3%	0%	9%	4%	2%

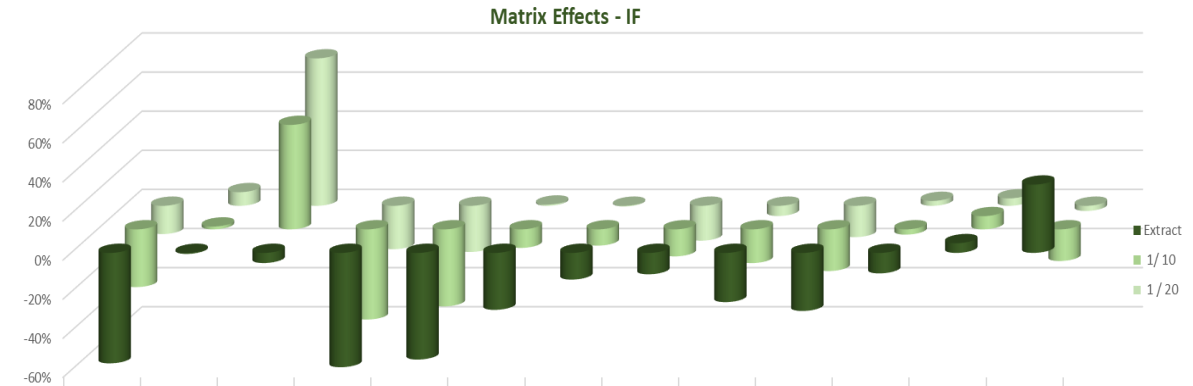
Matrix effects reduced but not eliminated by dilution

Fosetyl Aluminium is the only one with suppression above 20% in each case.

Ethephon goes from a low level of suppression to a high level of enhancement

Matrix matched standards are used for calibration

The matrix concentration is the same as for samples

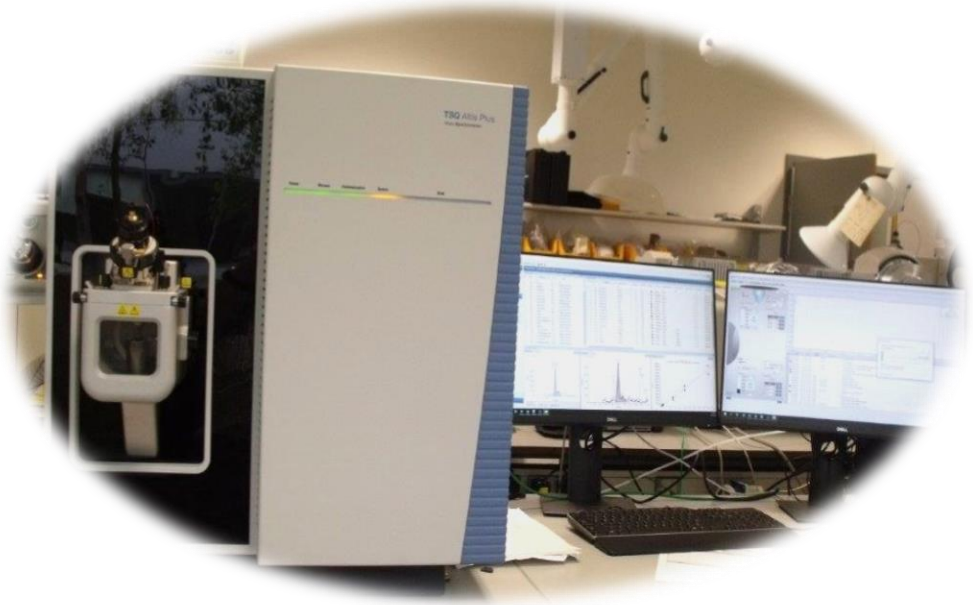


	AMPA	Chlorate	Ethephon	Fosetyl Al	Glufosinate	Glyphosate	HEPA	MPPA	N-acetyl-AMPA	N-acetyl-Glufosinate	N-acetyl-Glyphosate	Perchlorate	Phosphonic Acid
Extract	-56%	-1%	-5%	-58%	-55%	-29%	-14%	-11%	-25%	-30%	-10%	5%	35%
1/ 10	-30%	1%	53%	-46%	-39%	-9%	-8%	-14%	-17%	-21%	-3%	7%	-16%
1 / 20	-14%	7%	75%	-22%	-24%	1%	0%	-18%	-5%	-16%	3%	4%	-3%

An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

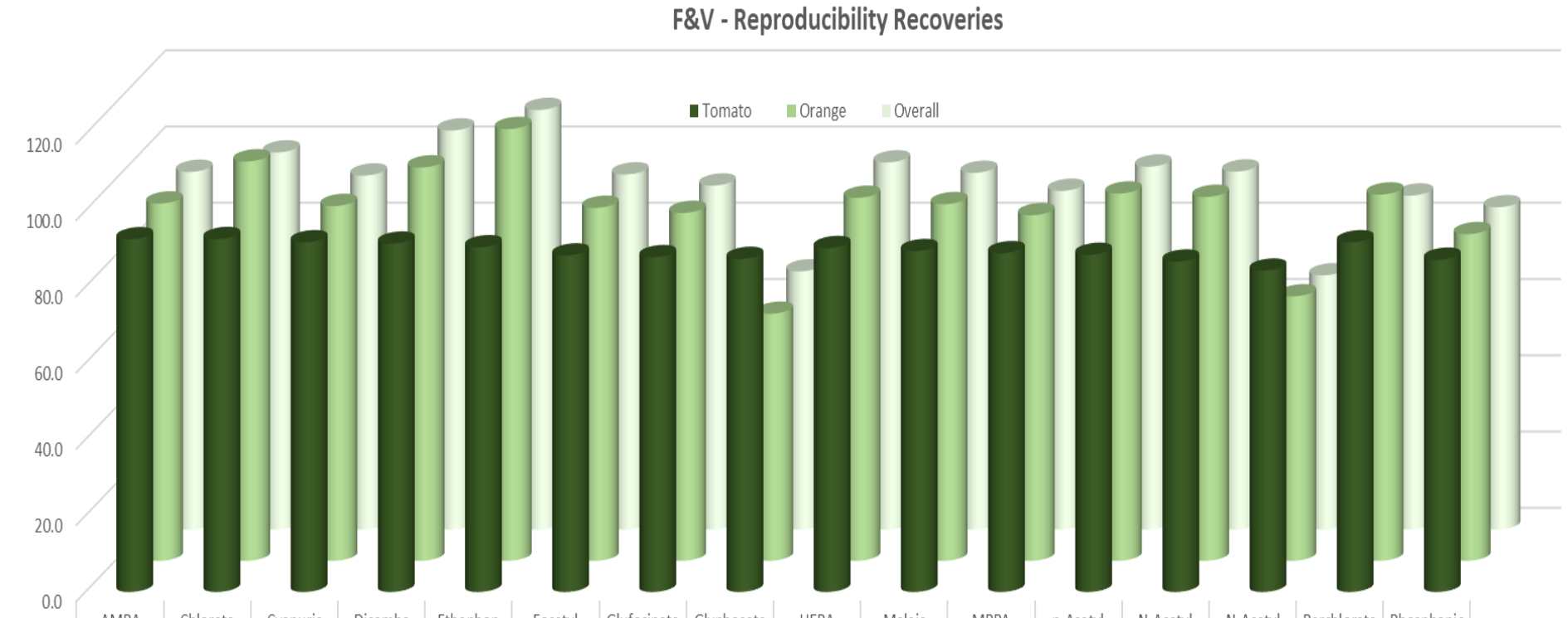
Analytical Issues

- For AMPA the data obtained for both these methods was not good and is not included here
- Fosetyl Aluminium and Phosphonic Acid gave low recovery in Cereals but were fine in Milk/IF.
- The low recoveries were consistent - %RSD's are very good.
- Struggled to get good data for Glufosinate at 10µg/kg for Infant Formula



An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

Fruit and Vegetables



	AMPA	Chlorate	Cyanuric Acid	Dicamba	Ethephon	Fosetyl Aluminium	Glufosinate	Glyphosate	HEPA	Maleic Hydrazide	MPPA	n-Acetyl AMPA	N-Acetyl Glufosinate	N-Acetyl Glyphosate	Perchlorate	Phosphonic Acid
Tomato	92.5	92.5	91.7	91.3	90.4	88.2	87.8	87.3	90.2	89.4	88.8	88.4	86.7	84.3	91.7	87.1
Orange	93.7	104.7	93.0	103.2	113.2	92.6	91.2	64.8	95.2	93.5	90.6	96.3	95.4	69.3	96.0	85.6
Overall	93.8	98.9	92.8	104.6	110.0	93.1	90.2	67.7	96.3	93.5	88.8	95.2	93.9	66.7	87.6	84.5

Preliminary results very good

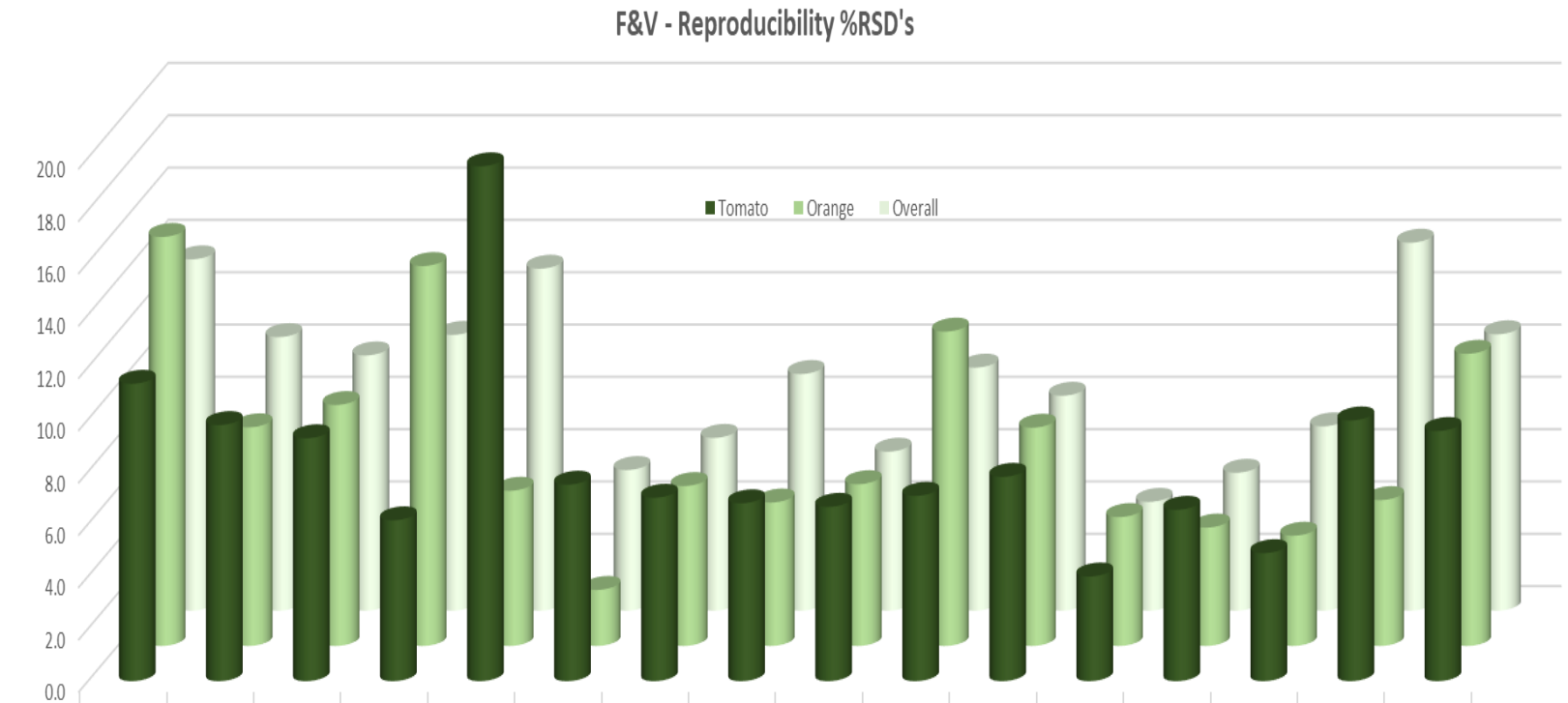
AMPA working very well in F&V

Cyanuric Acid added to the method

Glyphosate a little low

An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

Fruit and Vegetables



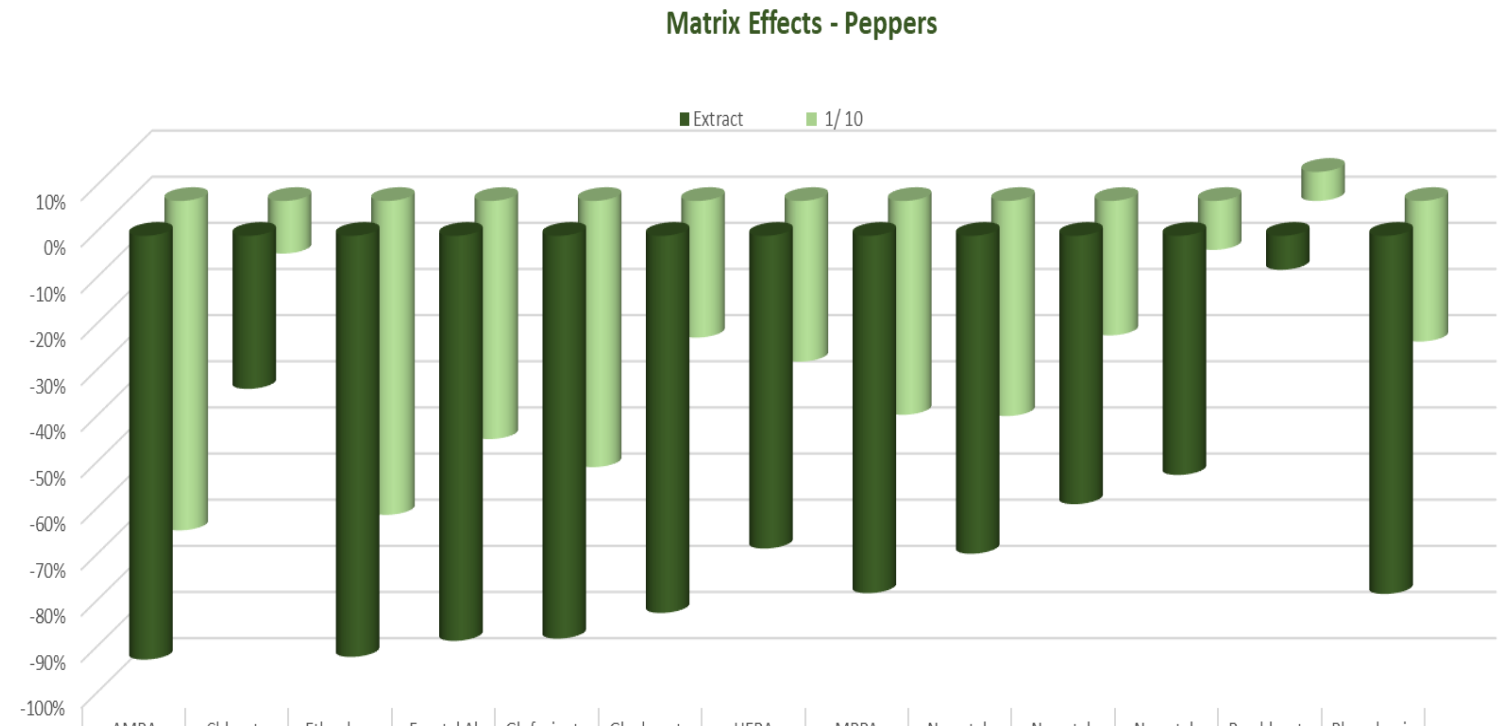
	AMPA	Chlorate	Cyanuric Acid	Dicamba	Ethephon	Fosetyl Aluminium	Glufosinate	Glyphosate	HEPA	Maleic Hydrazide	MPPA	n-Acetyl AMPA	N-Acetyl Glufosinate	N-Acetyl Glyphosate	Perchlorate	Phosphonic Acid
Tomato	11.4	9.8	9.3	6.1	19.7	7.5	7.0	6.8	6.7	7.1	7.8	4.0	6.6	4.9	10.0	9.6
Orange	15.6	8.4	9.2	14.5	5.9	2.1	6.1	5.5	6.2	12.0	8.3	4.9	4.5	4.2	5.6	11.2
Overall	13.4	10.5	9.8	10.5	13.1	5.4	6.6	9.0	6.1	9.3	8.2	4.2	5.3	7.0	14.1	10.6

Results average across all concentration levels

Inter-lab reproducibility < 20% in all cases

An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

Matrix Effects - Peppers



	AMPA	Chlorate	Ethephon	Fosetyl Al	Glufosinate	Glyphosate	HEPA	MPPA	N-acetyl-AMPA	N-acetyl-Glufosinate	N-acetyl-Glyphosate	Perchlorate	Phosphonic Acid
Extract	-92%	-33%	-91%	-88%	-87%	-82%	-68%	-77%	-69%	-58%	-52%	-7%	-78%
1/10	-71%	-11%	-68%	-52%	-58%	-30%	-35%	-46%	-47%	-29%	-11%	6%	-30%

Matrix effects very significant in Fruit and Veg

An exact matrix match is not possible in normal batches.

Need to look at further dilution to see if this will reduce the matrix effects to an acceptable level

An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

PT Results

EUPT 2021 SRM 16 Results

	Current Method				Previous Method			
	Result	AV	FFP s	z	Result	AV	FFP s	z
Chlorate	1.092	1.030	0.258	0.24	1.320	1.030	0.258	1.13
Ethephon	0.212	0.228	0.057	-0.28	0.987	0.228	0.057	13.32
Glufosinate	0.213	0.216	0.054	-0.06	0.232	0.216	0.054	0.30
Glyphosate	0.504	0.510	0.128	-0.05	0.562	0.510	0.128	0.41
Phosphonic Acid	0.550	0.676	0.169	-0.75	0.578	0.676	0.169	-0.58

EUPT 2021 SRM 17 Results

	Current Method				Previous Method			
	Result	AV	FFP s	z	Result	AV	FFP s	z
Maleic Hydrazide	0.646	0.544	0.136	0.75	0.33	0.544	0.136	-1.57

The Future

- Biggest priority is to develop a method for cations
- Instrument to be reconfigured to turn it into a two channel system
- The second channel will be used for cations including the Quats
- We have looked at some liver and fat samples on this system and these have worked well
- Will look to expand these methods to cover other analytes

An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

SRM Team

- Annate Margalit
- Stephen Burrell
- Michael Kelly


QUESTIONS
ANSWERS