NEW ADVANCES IN AUTOMATISATION FOR THE ANALYSIS OF PESTICIDE RESIDUES

SAX CLEAN-UP FOR IMPROVING THE ANALYSIS OF POLAR COMPOUNDS

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ISO 17025 accreditation for the analysis of highly polar pesticides

IC-HRMS

	Tomato	Orange	Grape	Coffe	Quince	Rice	Cocoa	Tea	Quinoa
PESTICIDES	LOQ (mg/kg)	LOQ (mg/kg)	LOQ (mg/kg)	LOQ (mg/kg)	LOQ (mg/kg)	LOQ (mg/kg)	LOQ (mg/kg)	LOQ (mg/kg)	LOQ (mg/kg)
AMPA	0.05	0.05	0.05	0.01*	0.05	0.05	0.01*	0.01*	0.05
Chlorate	0.05	0.05	0.05	0.01	0.05	0.01	0.01	0.01	0.01
Ethephon	0.05	0.05	0.05	0.01	0.05	0.05	0.01	0.05	0.05
Fosetyl- Al	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Glufosinate- ammonium	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Glyphosate	0.01	0.01	0.01	0.01*	0.01	0.05	0.01*	0.01*	0.05
Perchlorate	0.05	0.05	0.05	0.05	0.05	0.01*	0.05	0.01	0.01*
Phosphonic Acid		0.05							
N-Acetyl glufosinate		0.05							

ISO 17025 accreditation for the analysis of highly polar pesticides

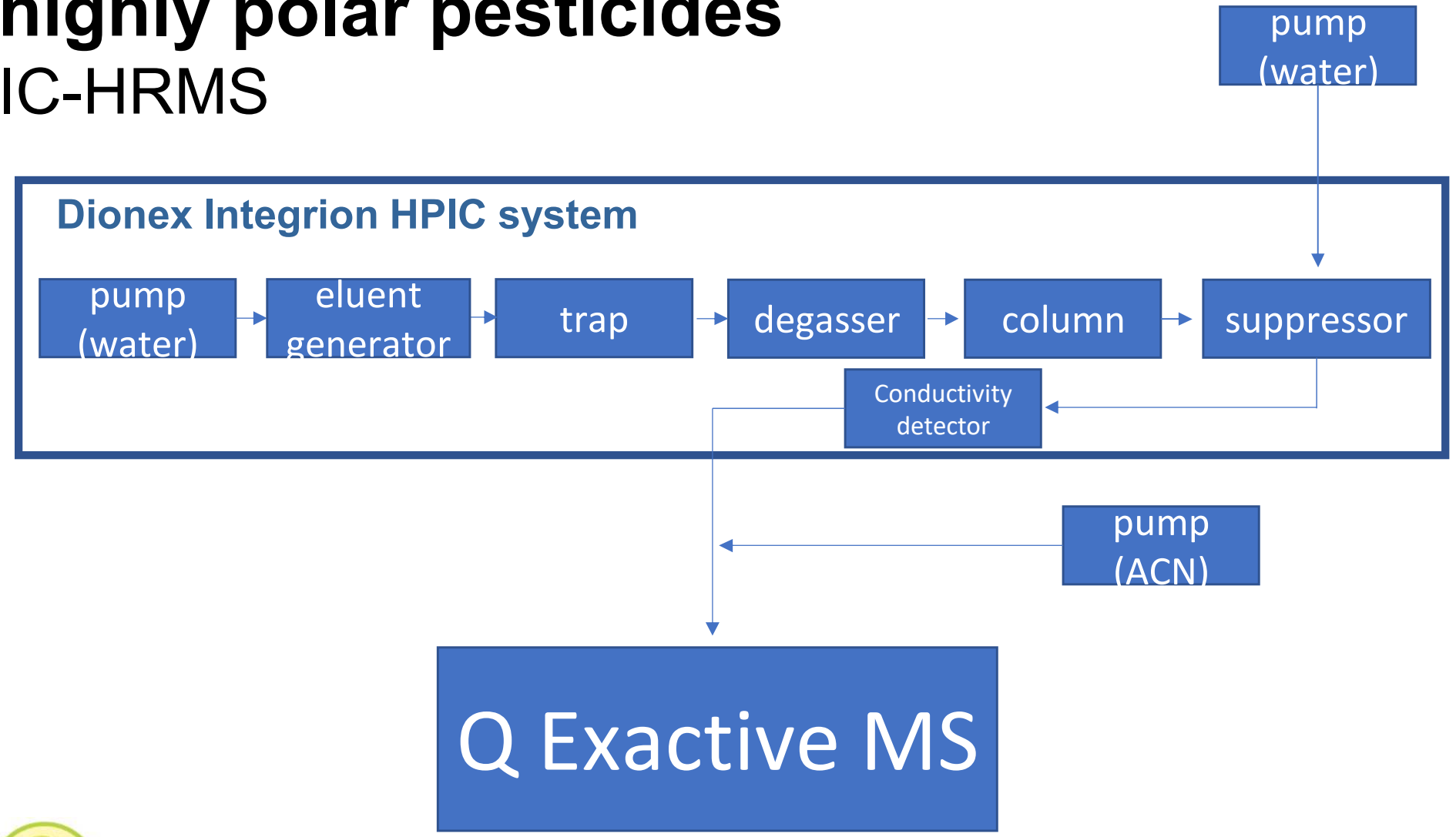
IC-HRMS

Thermo Scientific
Dionex Integrion
HPIC System

Thermo Scientific
Q Exactive Focus
MS System

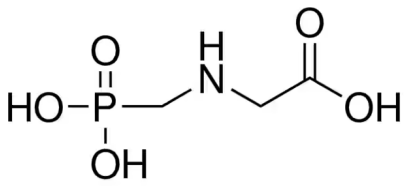
ISO 17025 accreditation for the analysis of highly polar pesticides

IC-HRMS

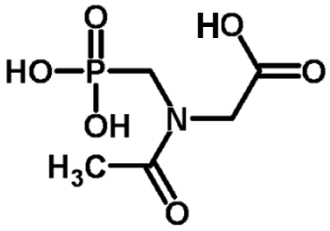


Alternative method for the analysis of highly polar pesticides

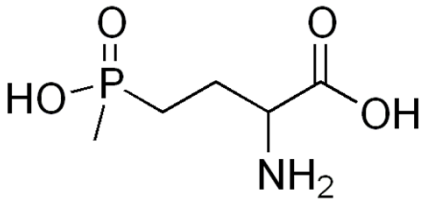
List of compounds



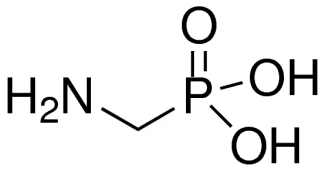
Glyphosate



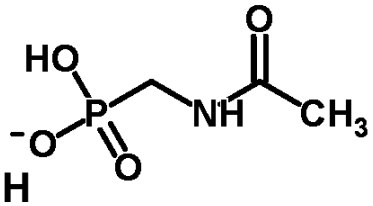
N-acetyl glyphosate



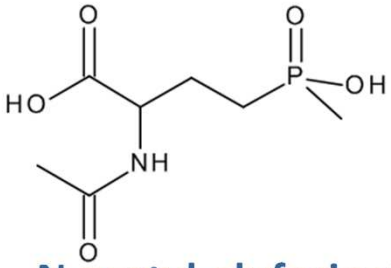
Glufosinate



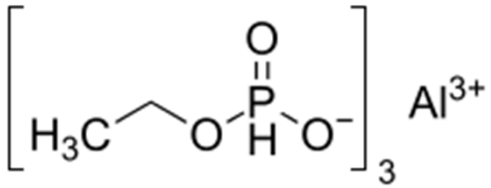
AMPA



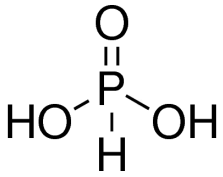
N-acetyl -AMPA



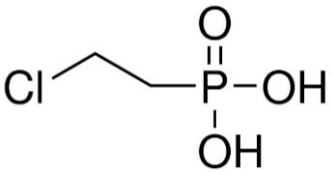
N-acetyl-glufosinate



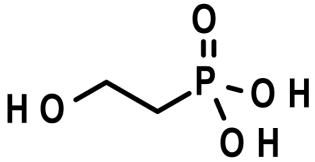
Fosetyl-aluminium



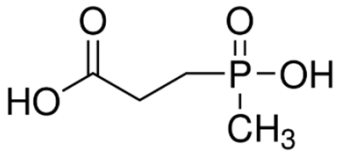
Phosphonic acid



Etephon



HEPA



MPPA

LC-MS conditions

Thermo Scientific™
Transcend™ DUO LX-2
UHPLC System

Thermo Scientific™
TSQ Altis™
Triple Quadrupole Mass Spectrometer

LC-MS conditions – HILIC Column

Waters™ **Anionic Polar Pesticide (APP)** Column
(100 mm x 2.1 mm, 5 µm)

HILIC

Stationary phase	Diethylamine
Particle shape	Spherical
Particle size	5 µm
Pore size	130 Å
Pore volume	0.7 cc/g
Surface area	185 m^2/g
Endcapped	Propietary

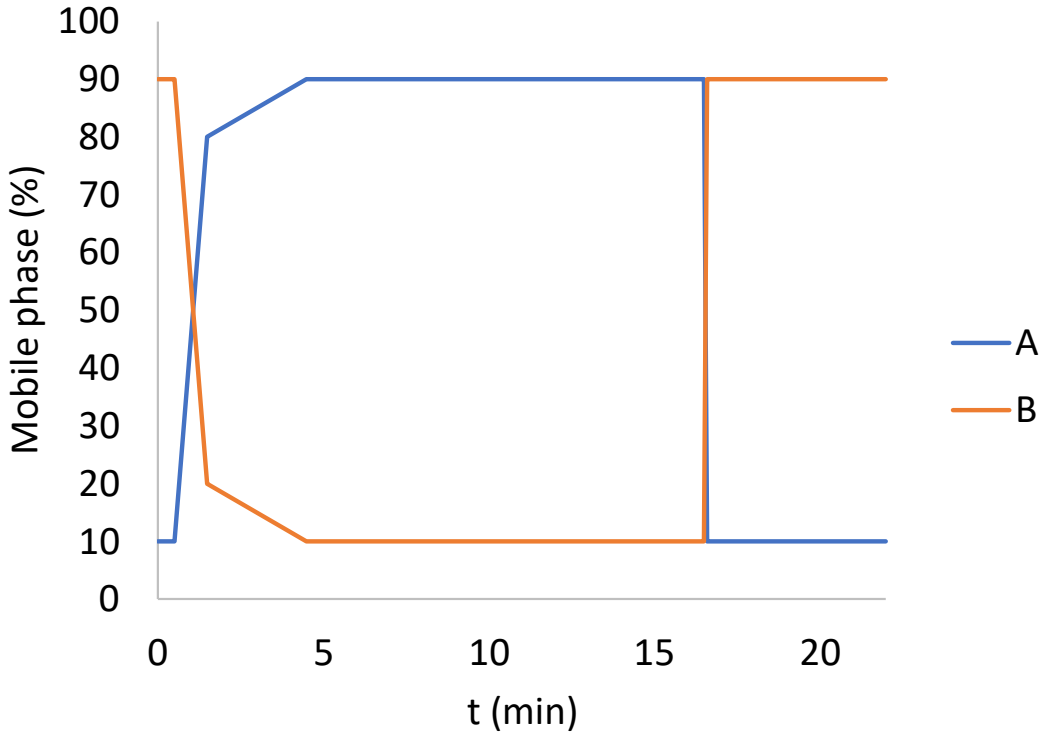
LC-MS conditions - Chromatographic method

Mobile phase A: Water 1.2% formic acid

Mobile phase B: Acetonitrile 0.5% formic acid

Flow: 0.5 mL/min

Injection volume: 10 µL



Extraction methods

5 g sample

9 mL H_2O
+ 10 mL MeOH

Shake & Centrifuge

2 g sample

10 mL H_2O

Shake & Centrifuge

ACN (1:1) + C18 (50 mg mL^{-1})

Shake & Centrifuge

Clean-up for highly polar pesticides

- Very low extractability in typical multiresidue methods
  - use of a more-polar solvent such as wáter, methanol or methanol plus water
    - co-extraction of a high level of polar matrix components
      - reduction of column lifetime, increase of instrument maintenance frequency
      - several isobaric interferences, reduced sensitivity, compromised accuracy and/or strong matrix effects, especially signal suppression

Evaluation of clean-up strategies

Hypersep SAX (Strong Anion eXchange) cartridges

Product Name	HyperSep SAX
Bed Weight	500 mg
Column Capacity	6 mL
Chemical	Quaternary Amine
Particle Size	40-63 µm
Average Pore Size	60 Å
Surface Area	504 m^2/g
Pore Volume	0.74 cm^3/g
Anion Exchange Capacity	0.239 meq/g

Extraction methods

Extraction

5 g sample	2 g sample
9 mL H_2O + 10 mL MeOH	10 mL H_2O
Shake & Centrifuge	Shake & Centrifuge
	ACN (1:1) + C18 (50 mg mL^{-1})
	Shake & Centrifuge

SAX clean-up

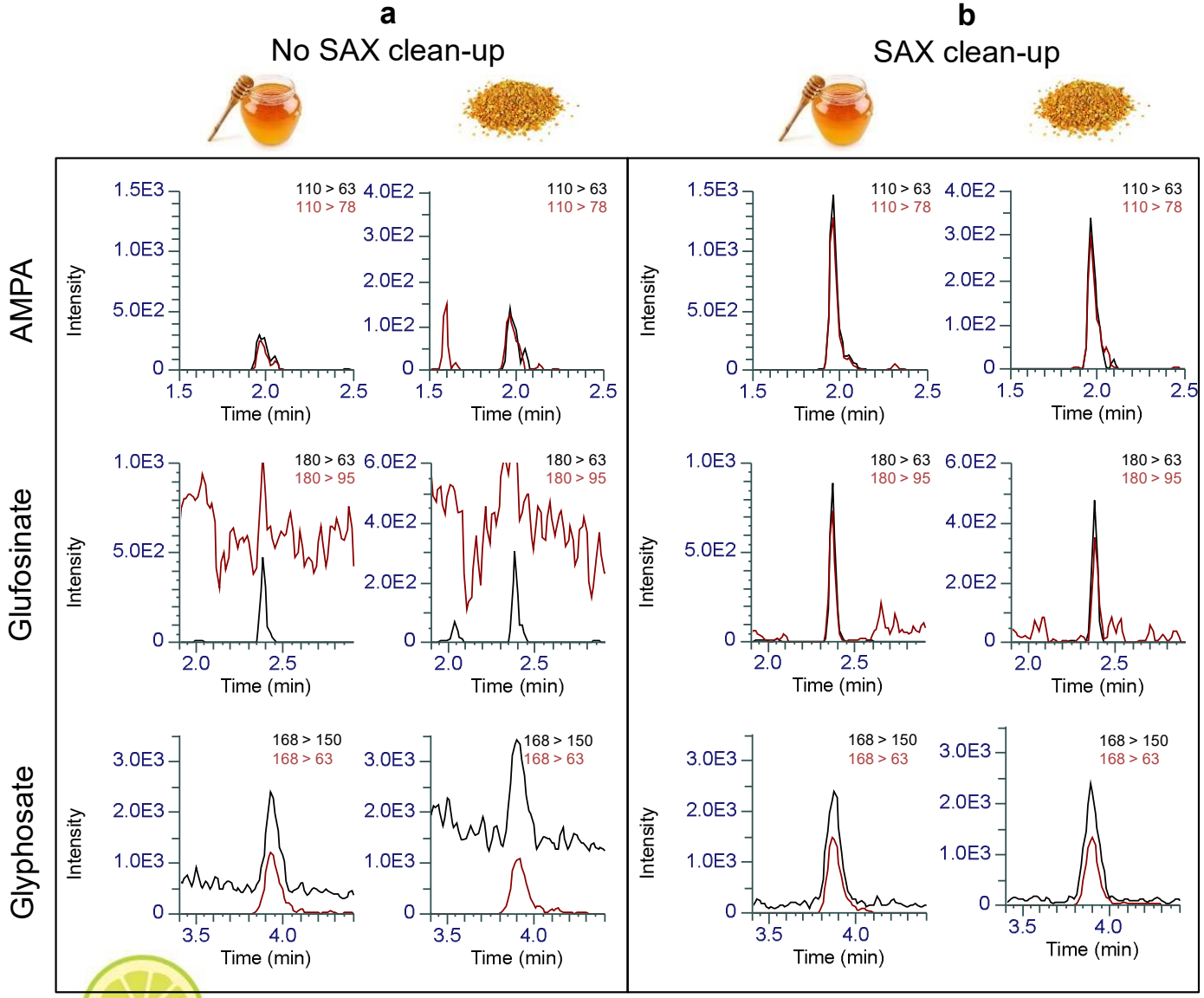
	Manual SPE 500 mg SAX	**Automated µSPE** 50 mg SAX
Condi-tioning	10 mL MeOH	1 mL MeOH
Washing	10 mL (MeOH-diluted extract)	1 mL (MeOH-diluted extract)
Sample Loading	6 mL MeOH	0.6 mL MeOH
Elution	3 mL MeOH : HCl (1M) (90:10)	0.4 mL MeOH : HCl (1M) (90:10)

SAX clean-up

SPE workflow

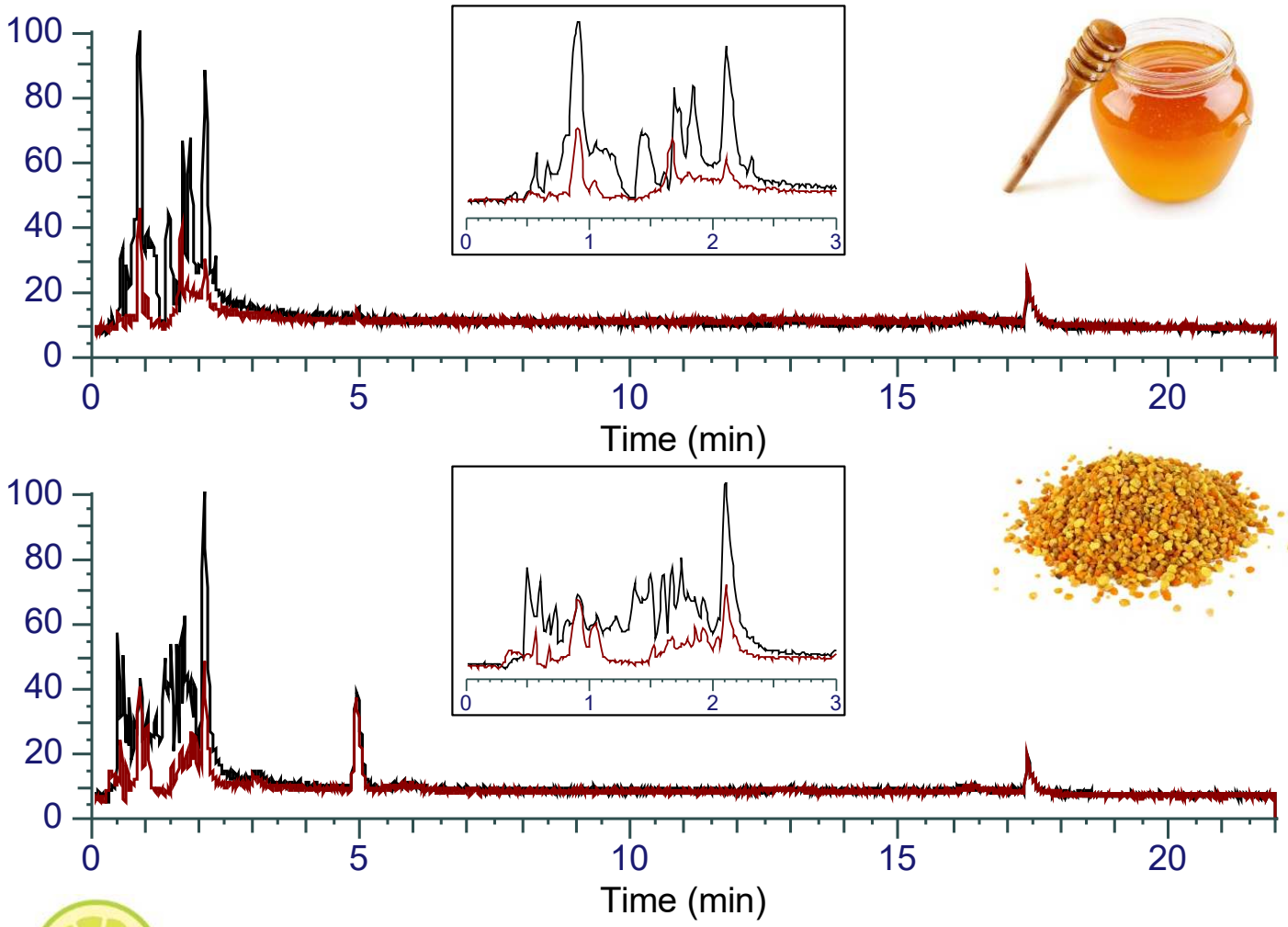
Conditioning	10 mL MeOH
Sample Loading	10 mL (MeOH-diluted extract)
Washing	6 mL MeOH
Elution	3 mL MeOH : HCl (1M) (9:1)

SAX clean-up evaluation



Extracted ion chromatograms of standards of AMPA, glufosinate and glyphosate at 0.010 mg kg^{-1} (0.002 mg L^{-1}) in honey and pollen extracts, **(a)** before and **(b)** after performing SAX clean-up

SAX clean-up evaluation

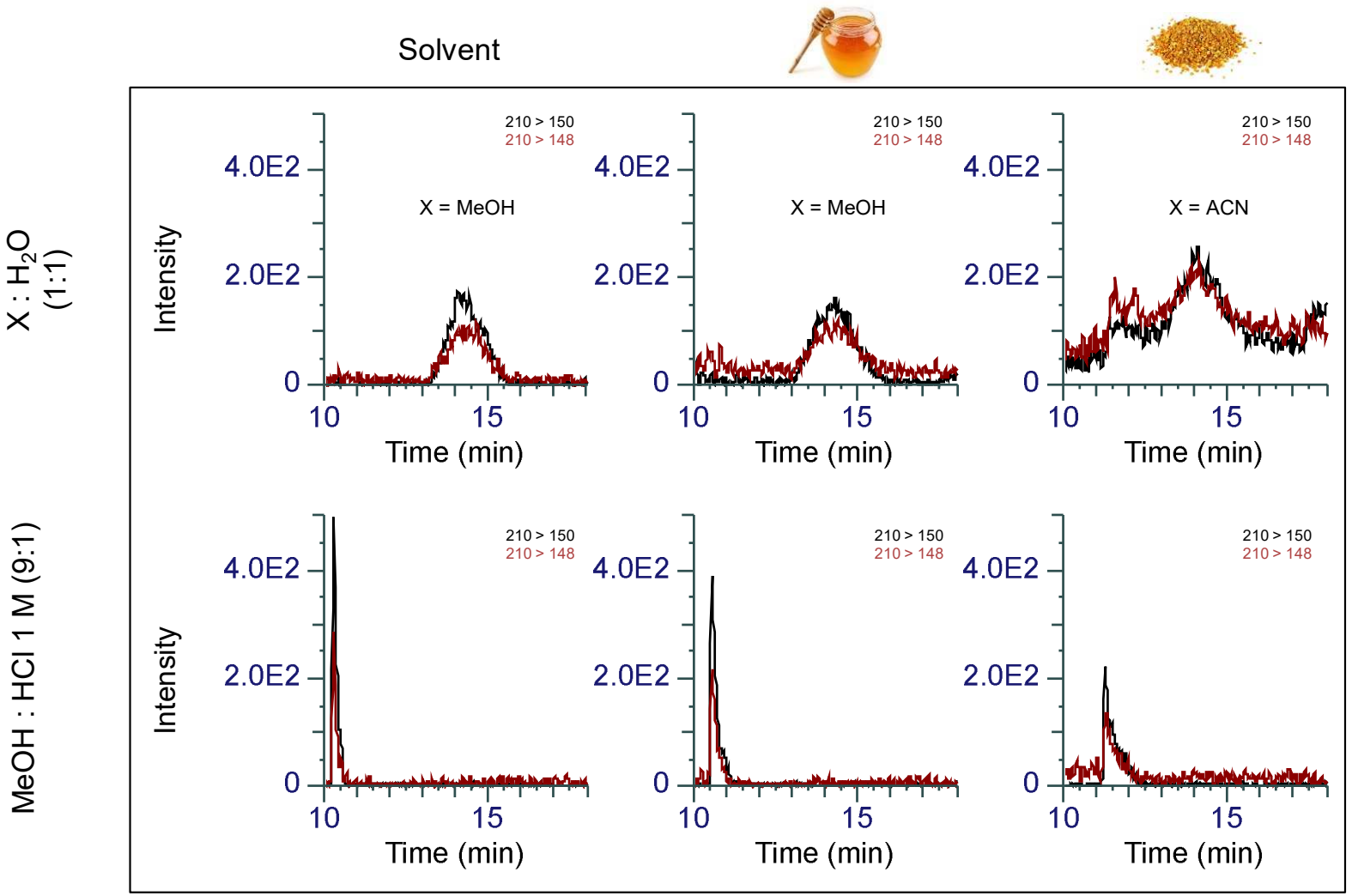


Full-scan (m/z 50-700) total ion current (TIC) chromatograms obtained for honey and pollen in negative polarity ionisation mode

—— Before SAX clean-up

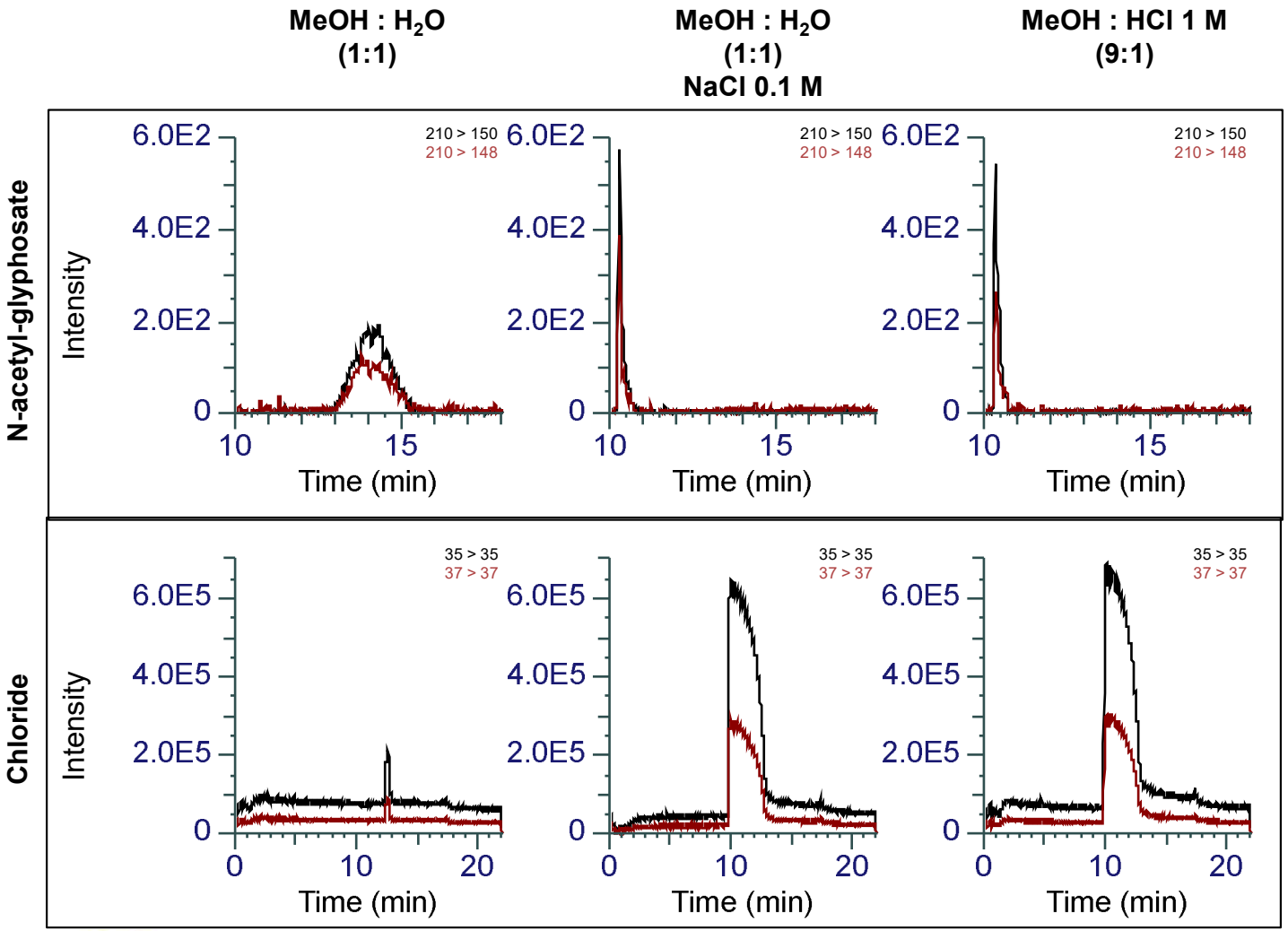
—— After SAX clean-up

SAX clean-up evaluation



Extracted ion chromatograms of standard of N-acetyl-glyphosate at 0.010 mg kg^{-1} (0.002 mg L^{-1}) in solvent and in honey and pollen extracts, before and after performing SAX clean-up

SAX clean-up evaluation



Extracted ion chromatograms of standard of N-acetyl-glyphosate at 0.002 mg L^{-1} and chloride in different injection solvents, confirming the influence of this anion in the decrease of N-acetyl-glyphosate retention and peak shape improvement on APP column

Automation and miniaturization of SAX clean- up

µSPE workflow

µSPE cartridge
50 mg SAX

Conditioning	1 mL MeOH
Sample Loading	1 mL (MeOH-diluted extract)
Washing	0.6 mL MeOH
Elution	0.4 mL MeOH : HCl (1M) (9:1)

Automation and miniaturization of SAX clean- up

µSPE workflow

	Syringe wash (3 cycles) with MeOH
Conditioning	Load **1000 µL MeOH**
	Condition µSPE cartridge at **5 µL/s** (to waste)
Loading	**1000 µL** of sample at **5 µL/s (to waste)**
	Syringe wash (3 cycles) with MeOH
Washing	Load **600 µL MeOH**
	Wash the cartridge at **5 µL/s** (to waste)
	Syringe wash (3 cycles) with MeOH
Elution	Load **400 µL** of **MeOH : HCl (1M) (9:1)**
	Elution step at **5 µL/s (Collection Vial)**
	Syringe wash (3 cycles) with MeOH

µSPE workflow - Setup

Mode	Offline as Local Script
µSPE Tool	LS 3
AP/Standards Tool	LS 2
Injection Tool	none
Fast Wash Module	Fast Wash 1
Use Wash1 during Sample Prep	No
Use Wash2 during Sample Prep	Yes
µSPE Cartridge Tray	Cartridge Tray
Dispose Cartridge	No
Cartridge Waste Container	none
µSPE Elution Tray	Elution Tray
Eluate Tray	Eluate Tray
Bottom Sense	Off

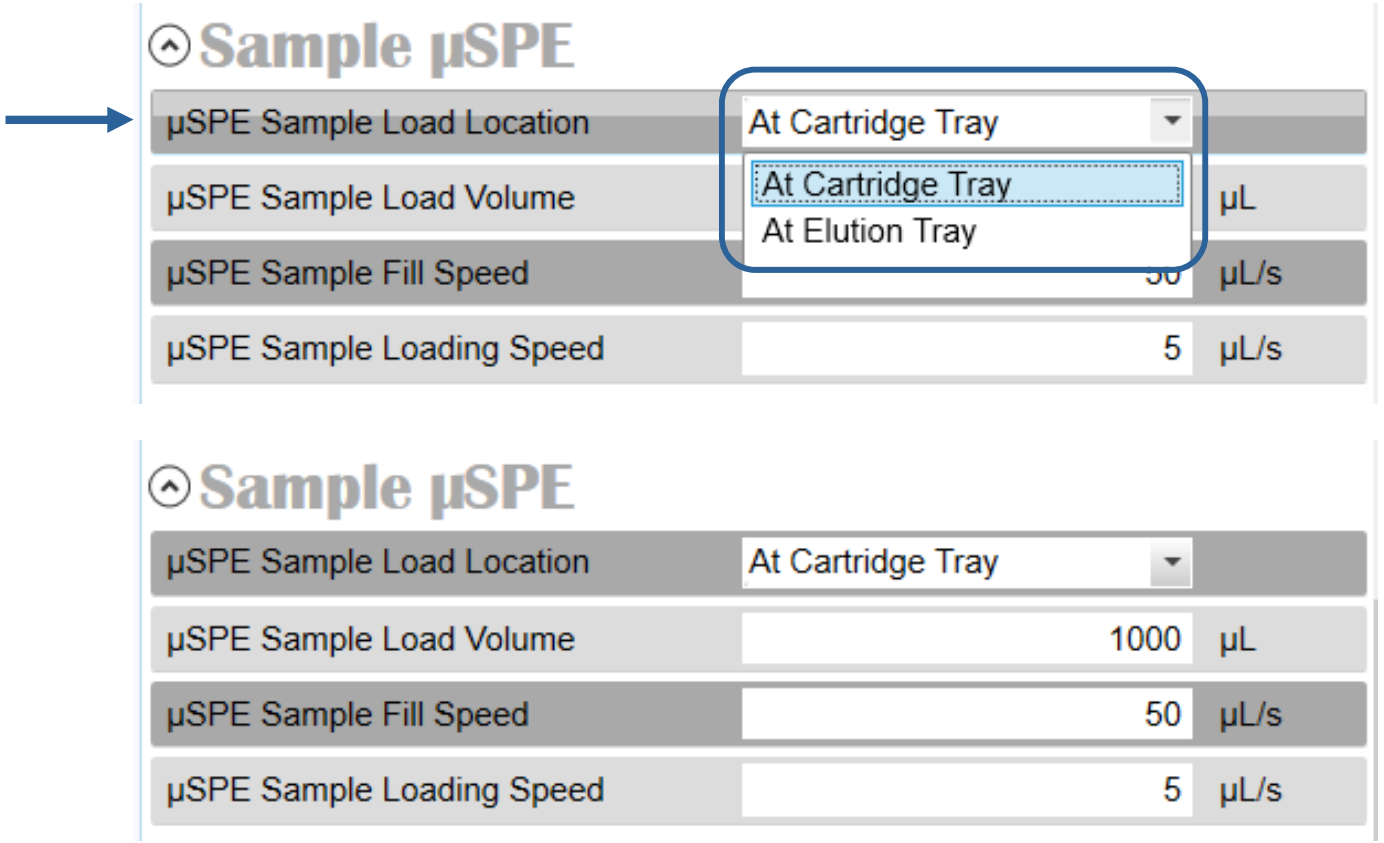
µSPE workflow – Conditioning step

Conditioning solvent: MeOH

Conditioning

Conditioning Solvent Source	Solvent Module1	
Conditioning Solvent Index	1	
Conditioning Solvent Volume	1000	µL
Conditioning Solvent Fill Speed	100	µL/s
Conditioning Solvent Loading Speed	5	µL/s

µSPE workflow – Sample loading



µSPE workflow – Washing step

Washing solvent: MeOH

Wash µSPE

Wash µSPE Solvent Source	Solvent Module1	
Wash µSPE Solvent Index	1	
Wash µSPE Volume	600	µL
Wash µSPE Solvent Fill Speed	100	µL/s
Wash µSPE Solvent Loading Speed	5	µL/s

µSPE workflow – Elution step

Elution solvent: MeOH : HCl (1M) (9:1)

Elution

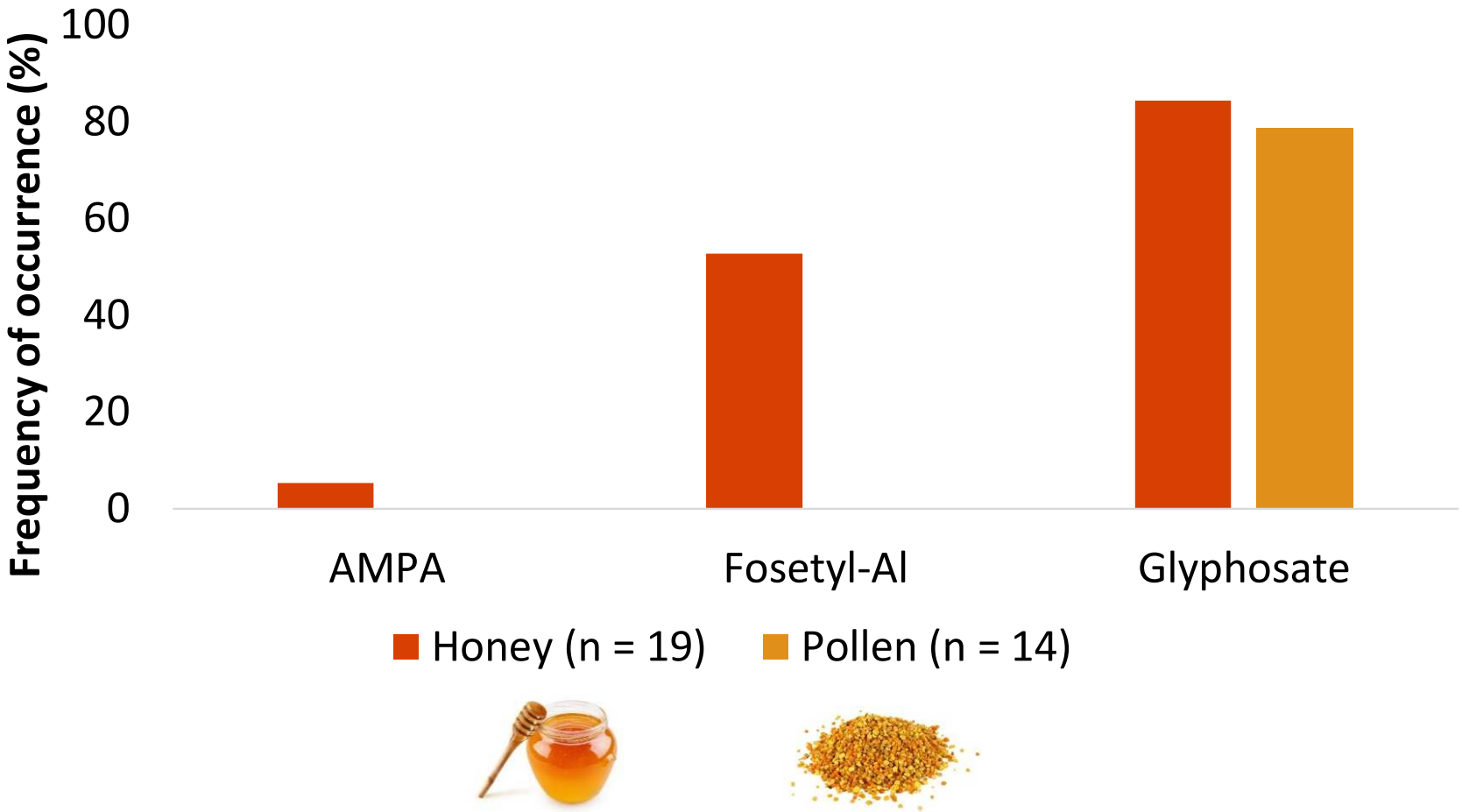
Elution Solvent Source	Solvent Module1	
Elution Solvent Index	2	
Elution Volume	400	µL
Elution Solvent Fill Speed	100	µL/s
Elution Solvent Loading Speed	5	µL/s

Method validation

Spiking levels: 0.005, 0.010, 0.020, 0.050 mg kg^{-1}

Compound	LOQ (mg kg^{-1})	Linear range (mg kg^{-1})	ME (%)		LOQ (mg kg^{-1})	Linear range (mg kg^{-1})	ME (%)	
			SPE	µSPE			SPE	µSPE
AMPA	0.005	0.002 – 0.200	-41	-71	0.010	0.010 - 0.200	-87	-88
Ethephon	0.005	0.002 – 0.200	-8	6	0.005	0.002 – 0.200	12	4
Fosetyl-Al	0.005	0.002 – 0.200	-2	9	0.005	0.002 – 0.200	13	-9
Glufosinate	0.010	0.010 – 0.200	-41	-30	0.010	0.010 - 0.200	-57	-52
Glyphosate	0.005	0.002 – 0.200	-12	21	0.005	0.002 – 0.200	-11	-9
HEPA	0.010	0.010 – 0.200	-12	-3	0.020	0.010 - 0.200	-24	-35
MPPA	0.005	0.002 – 0.200	-30	-10	0.005	0.002 – 0.200	31	-20
N-acetyl-AMPA	0.005	0.002 – 0.200	-14	2	0.005	0.002 – 0.200	-31	-37
N-acetyl-glufosinate	0.005	0.002 – 0.200	-21	-3	0.010	0.010 - 0.200	-34	-41
N-acetyl-glyphosate	0.005	0.002 – 0.200	-15	24	0.005	0.002 – 0.200	0	9
Phosphonic acid	0.005	0.002 – 0.200	-5	4	0.005	0.002 – 0.200	4	-16

Real sample analysis



Analysis of coffee beans without clean-up

High ion suppression for AMPA and Glufosinate

Evaluation of different clean-up steps

Extraction

2 g sample

9 mL H_2O + 10 mL MeOH + 1 mL EDTA (10% m/V)

Shake & Centrifuge

Evaluated clean-up cartridges

Dionex On Guard II RP | Strata-X | Oasis HLB | Hypercarb

SPE

5 mL MeOH + 5 mL H_2O — **Conditioning**

6 mL (Two-fold diluted with water) — **Sample loading**

Discard first 2 mL

Collect eluate (Around 4 mL)

Clean-up for coffee beans

SPE

Dionex Onguard II RP — 1

Strata-X — 2

Oasis-HLB — 3

Hypercarb — 4

SAX µSPE

Automated µSPE clean-up (SAX cartridges)

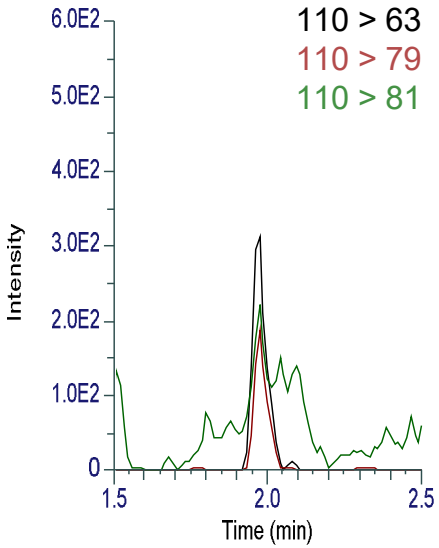
SAX cartridge colour after passing through coffee extract

1 2 3 4

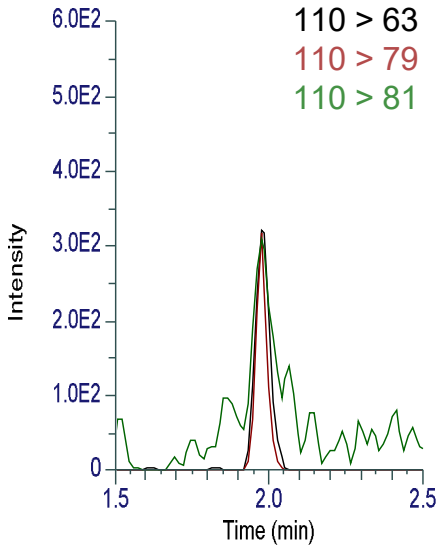
Final sample extract (SPE + SAX µSPE)

Clean-up for coffee beans

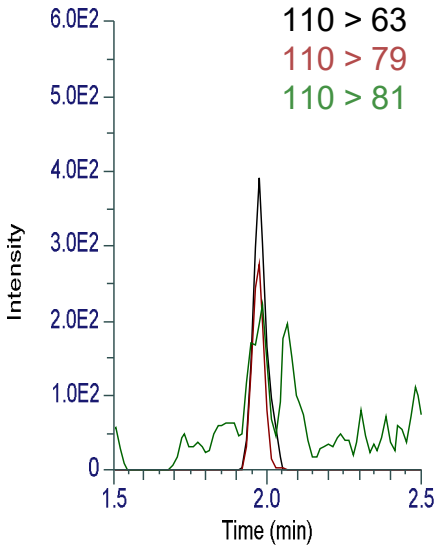
AMPA std in coffee beans extract spiked at 0.020 mg kg^{-1}



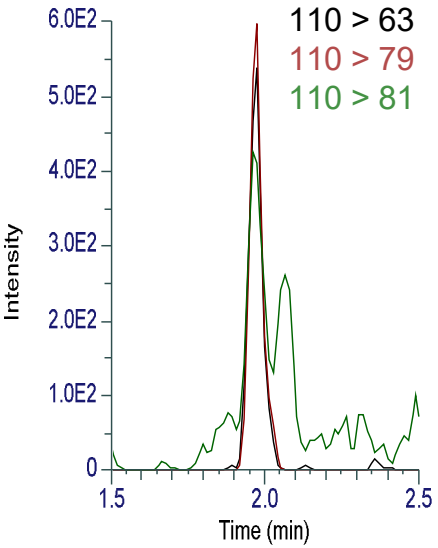
On Guard II RP + SAX
Area 983



Strata-X + SAX
Area 1084



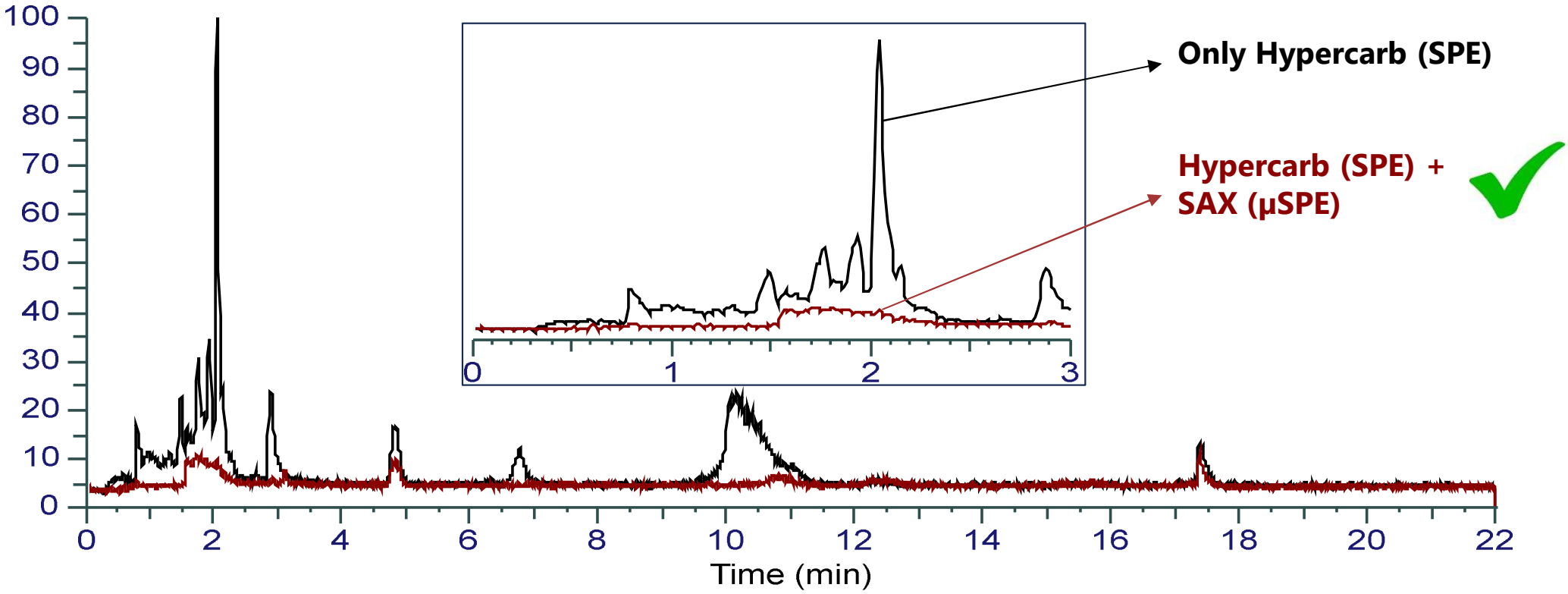
OASIS-HLB + SAX
Area 1196



Hypercarb + SAX
Area 1666

Clean-up for coffee beans

Full-scan (m/z 50-700) total ion current (TIC)

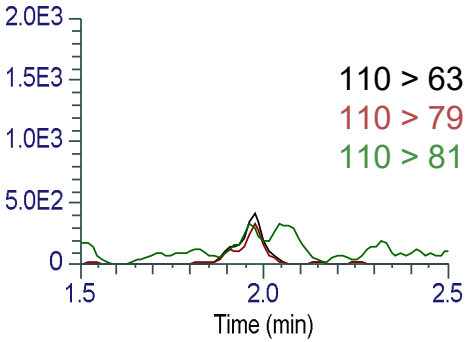


Clean-up for coffee beans: Hypercarb+SAX

AMPA

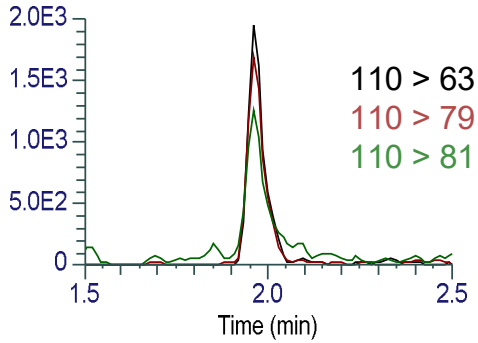
0.020 mg kg^{-1}

Hypercarb



Area: 1765

Hypercarb+SAX

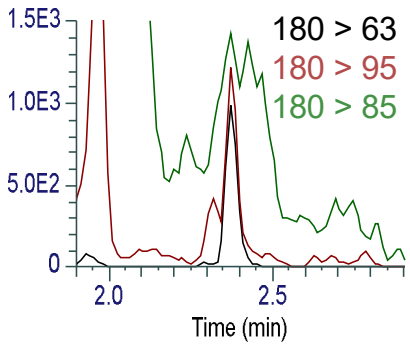


Area: 5907

Glufosinate

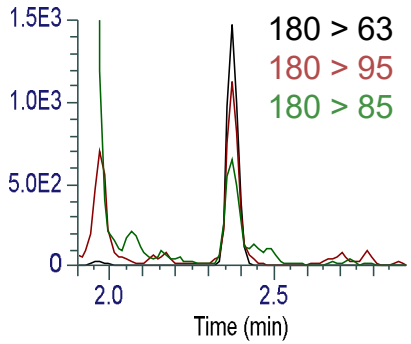
0.050 mg kg^{-1}

Hypercarb



Area: 2446

Hypercarb+SAX

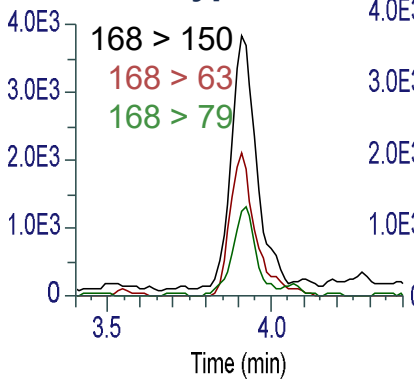


Area: 3323

Glyphosate

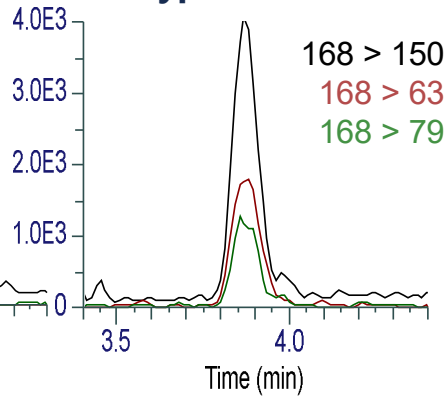
0.010 mg kg^{-1}

Hypercarb



Area: 19716

Hypercarb+SAX

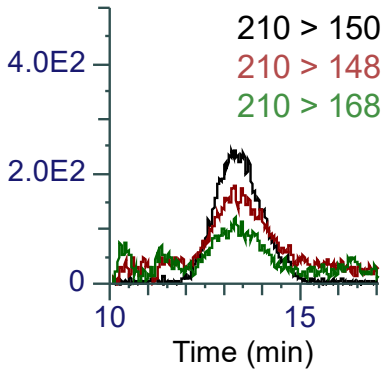


Area: 20705

N-acetyl-glyphosate

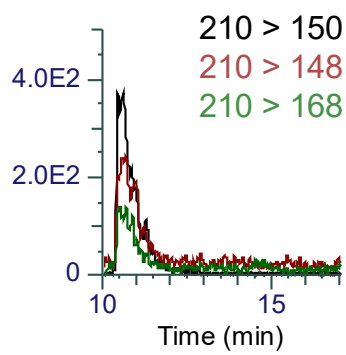
0.050 mg kg^{-1}

Hypercarb



Area: 21131

Hypercarb+SAX



Area:12806

Method validation

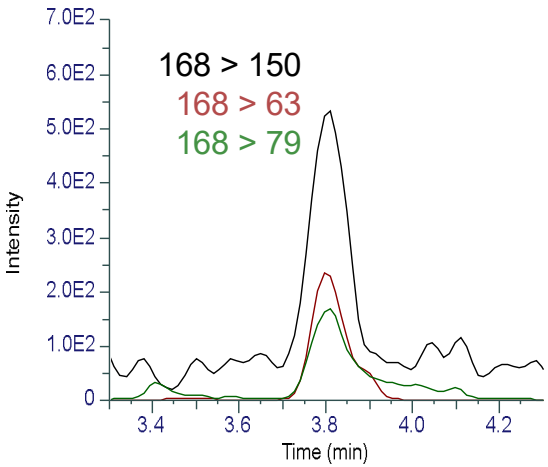
Spiking levels: 0.010, 0.020, 0.050, 0.100 mg kg^{-1}

Compound	LOQ (mg kg^{-1})	Linear range (mg kg^{-1})	ME (%)
AMPA	0.020	0.020 - 0.500	-65
Ethephon	0.010	0.010 - 0.500	5
Fosetyl-Al	0.010	0.005 - 0.500	-5
Glufosinate	0.050	0.050 - 0.500	-40
Glyphosate	0.010	0.010 - 0.500	-20
HEPA	0.050	0.050 - 0.500	-23
MPPA	0.020	0.010 - 0.500	-40
N-acetyl-AMPA	0.010	0.005 - 0.500	10
N-acetyl-glufosinate	0.020	0.020 - 0.500	2
N-acetyl-glyphosate	0.050	0.050 - 0.500	11
Phosphonic acid	0.050	0.050 - 0.500	-12

Real sample analysis

	Concentration (mg kg^{-1})	
Sample ID	Glyphosate	Phosphonic acid
01	0.014	
02	< LOQ	
03		0.095
04	< LOQ	0.070
05		
06		0.220
07		0.330
08		
09		0.243
10	0.016	
11		
12		
13		

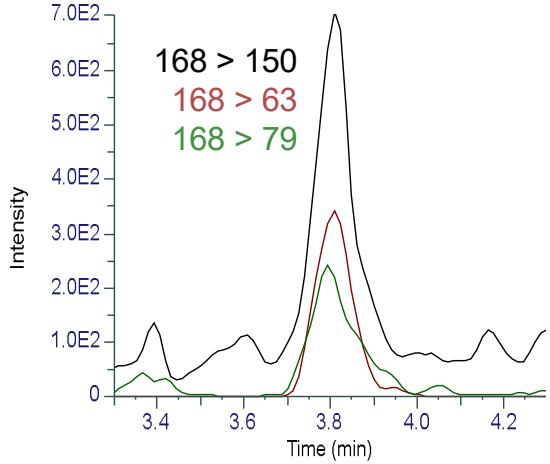
Glyphosate std in coffee beans extract 0.010 mg kg^{-1}



Area: 2674

Sample ID: 01

Glyphosate in coffee beans sample 0.014 mg kg^{-1}



Area: 3793

- LOQ Glyphosate: 0.010 mg kg^{-1}
- MRL Glyphosate: 0.100 mg kg^{-1}

- LOQ Phosphonic acid: 0.050 mg kg^{-1}
- MRL Phosphonic acid: 5 mg kg^{-1} expressed as fosetyl

Conclusions

- SAX clean-up method validated for the analysis of 11 highly polar anionic pesticides in honey, pollen and coffee beans.
- This clean-up showed to be very effective in terms of removal of matrix components, leading to improved selectivity and sensitivity, particularly for AMPA and glufosinate
- The analysis of N-acetyl-glyphosate at low concentrations in the same chromatographic run was possible due to the positive influence of the injection solvent derived from the clean-up step on the retention, peak shape and intensity of this compound.
- The automation and miniaturization of the clean-up step using SAX µSPE cartridges were achieved.
- The automated µSPE method offered benefits such as reduced manual labour, increased sample throughput, improved reproducibility, and decreased solvent consumption.

Thank You
for Your Attention