Practical Training - New Advances in MRM (on-line training)

Exploring new resources in column chromatography

EURL-FV

1. Fully porous vs Core-shell

2. HILIC/IonExchange Hybrid column

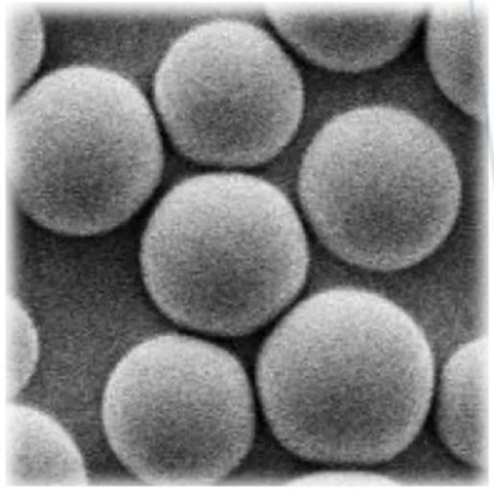
1. Fully porous vs Core-shell

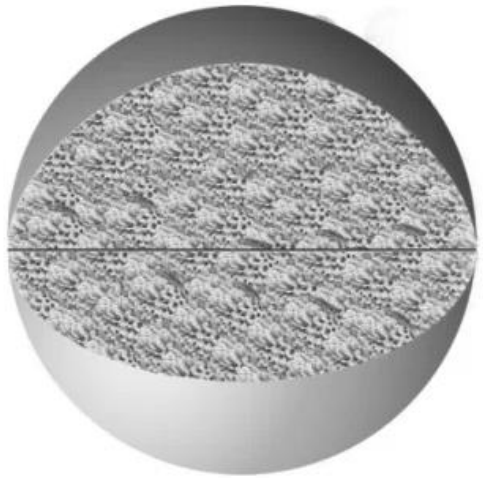
2. HILIC/IonExchange Hybrid column


Agilent Technologies
Agilent Technologies
Funnel
Agilent Technologies 6490 Triple Quad LC/MS
Z400

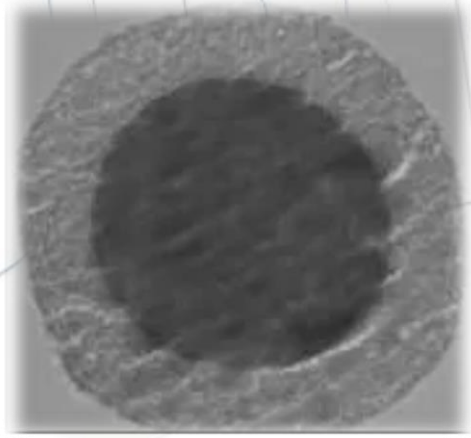
Fully porous vs Core-shell

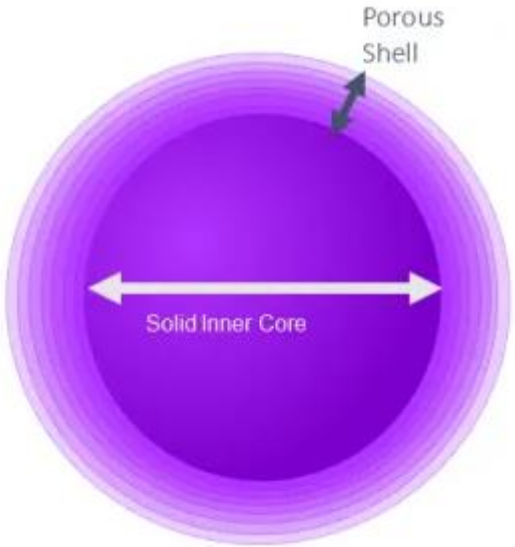
Fully Porous Particle





Core-shell Particle



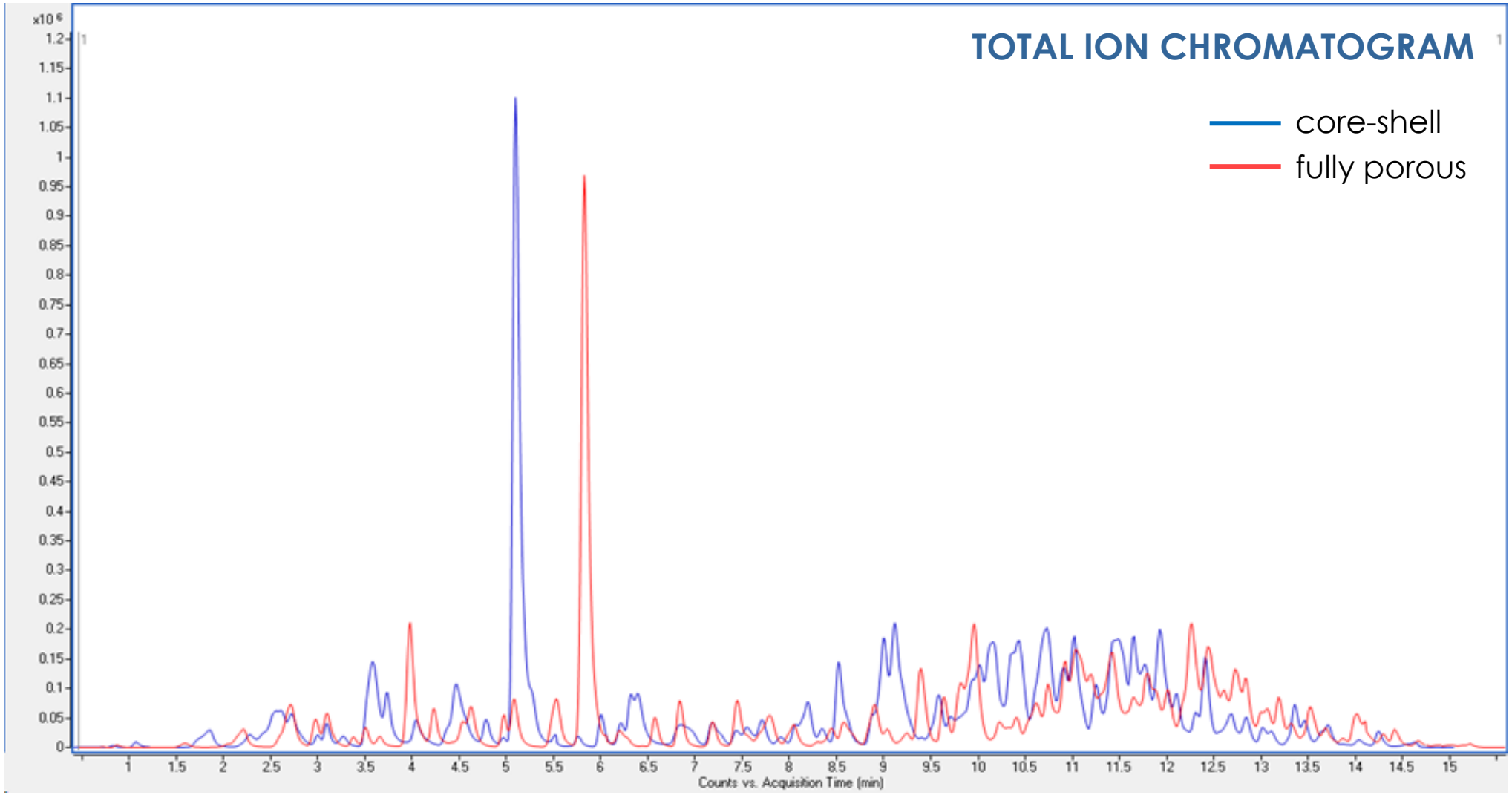


1290 UHPLC – 6490 TQ/MS(Agilent)

- Mobile phase A: Water (0.1 % formic acid, 5 mM ammonium formate, 2 % MeOH)
- Mobile phase B: Methanol (0.1 % formic acid, 5 mM ammonium formate, 2 % water)
- Column temperature: 35 °C
- Flow rate: 0.3 mL/min
- Injection volume: 5 µL

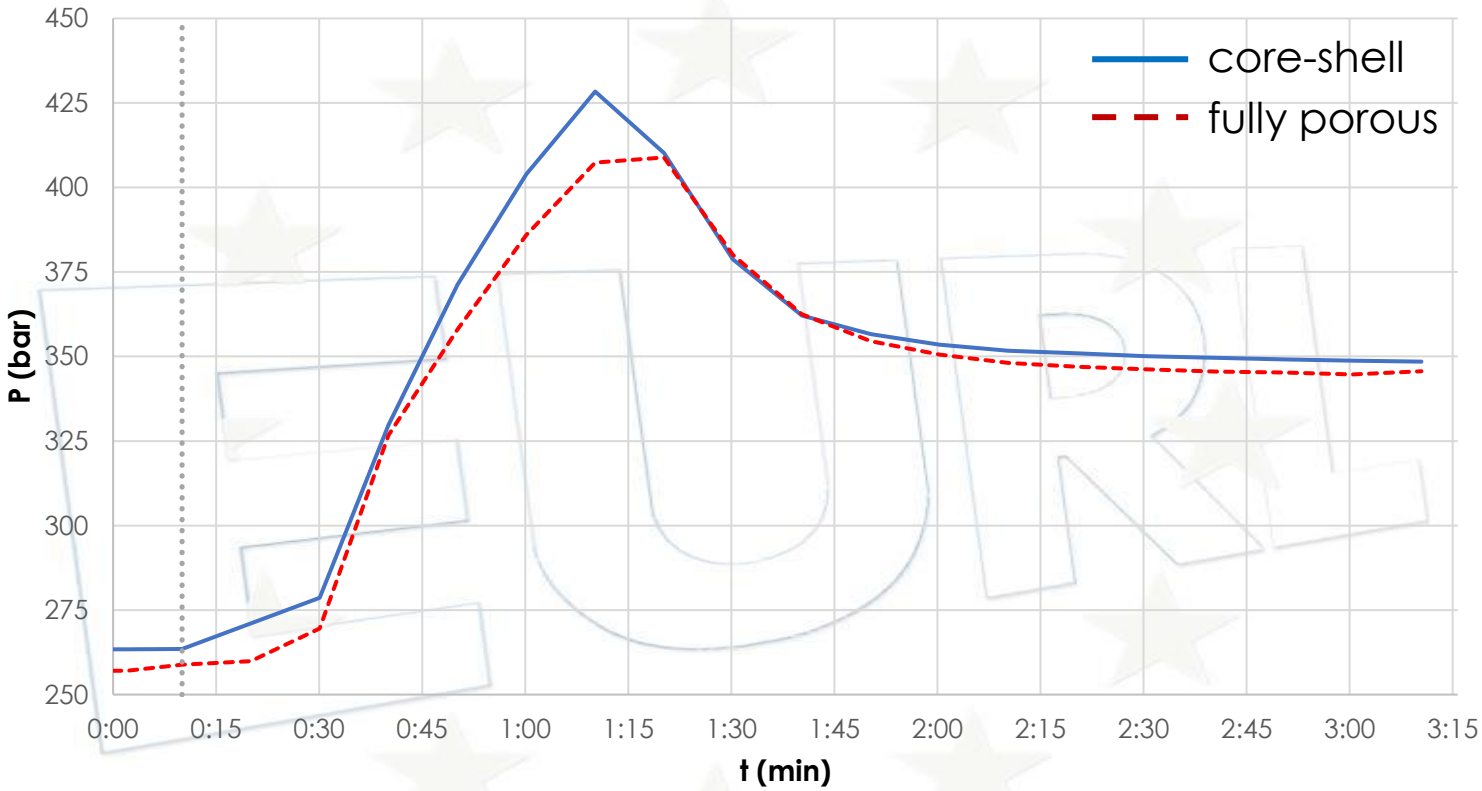
Time [min]	Mobile phase A	Mobile phase B
0	100 %	0 %
2	80 %	20 %
15	0 %	100 %
18	0 %	100 %

- Column A: **core-shell** C8 2.1x100 mm and 1.7 µm particle size
- Column B: **fully porous** C8 2.1x100 mm and 1.8 µm particle size


TOTAL ION CHROMATOGRAM
core-shell
fully porous
x10 6
1.2
1.15
1.1
1.05
1
0.95
0.9
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7
7.5
8
8.5
9
9.5
10
10.5
11
11.5
12
12.5
13
13.5
14
14.5
15
Counts vs. Acquisition Time (min)

Column **A**: **core-shell**
Column **B**: **fully porous**

RE-EQUILIBRATION TIME



Pressure values for column A (blue) and B (red) after changing the mobile phase composition from 0 % [A] to 100 % [A]

MATRICES

Tomato

Orange

Avocado

Tea

Column **A**: **core-shell**
Column **B**: **fully porous**

RETENTION TIME

Matrix	Column	Average t_R (min)	Median t_R (min)	Minimum t_R (min)	Maximum t_R (min)
Tomato	A	9.64	10.46	1.50	14.63
	B	10.35	11.25	1.85	15.23
Orange	A	9.64	10.44	1.50	14.63
	B	10.36	11.25	1.85	15.23
Avocado	A	9.64	10.45	1.50	14.63
	B	10.35	11.25	1.85	15.24
Tea	A	9.64	10.46	1.50	14.63
	B	10.35	11.25	1.85	15.22

Band Broadening

MassHunter® Data

PEAK SYMETRY

Perfect value

1.0

1.0 < X (Peak tailing)

PEAK WIDTH

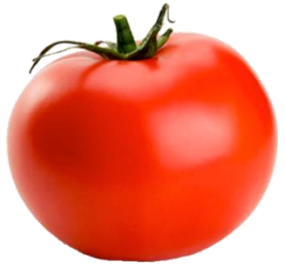
Perfect value

1.0

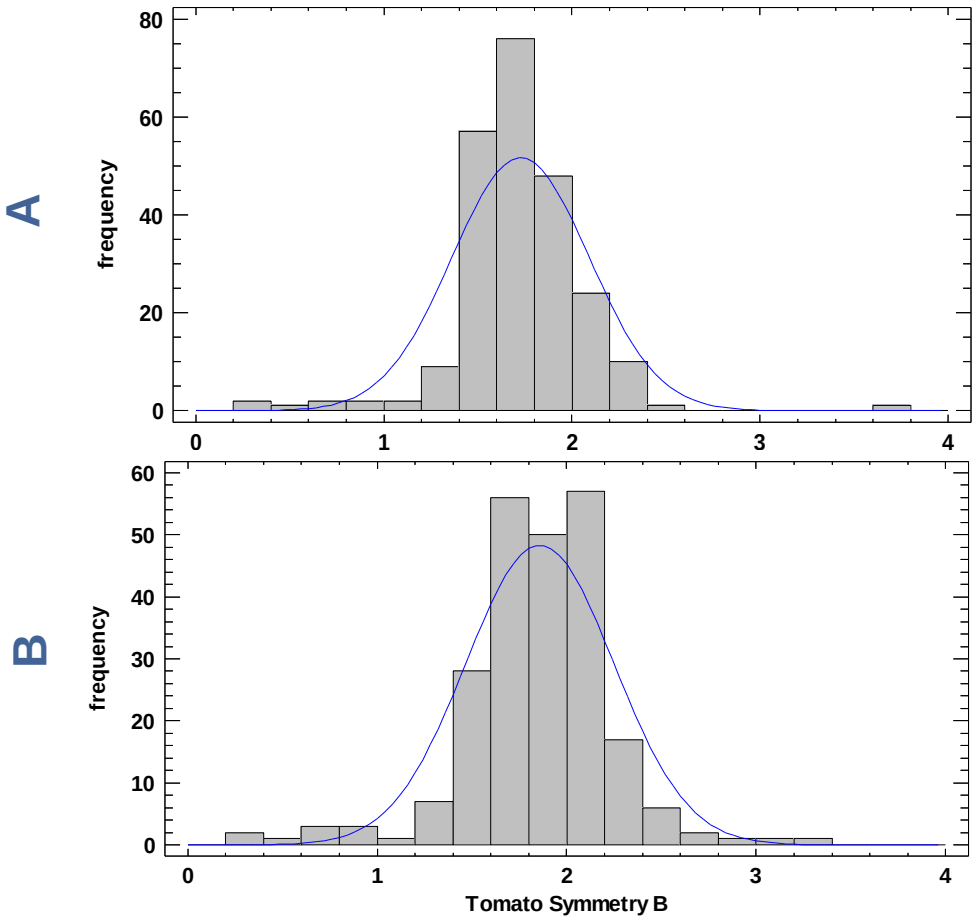
1.0 > X (Narrower peak)

Column **A**: **core-shell**
Column **B**: **fully porous**
243 Pesticides

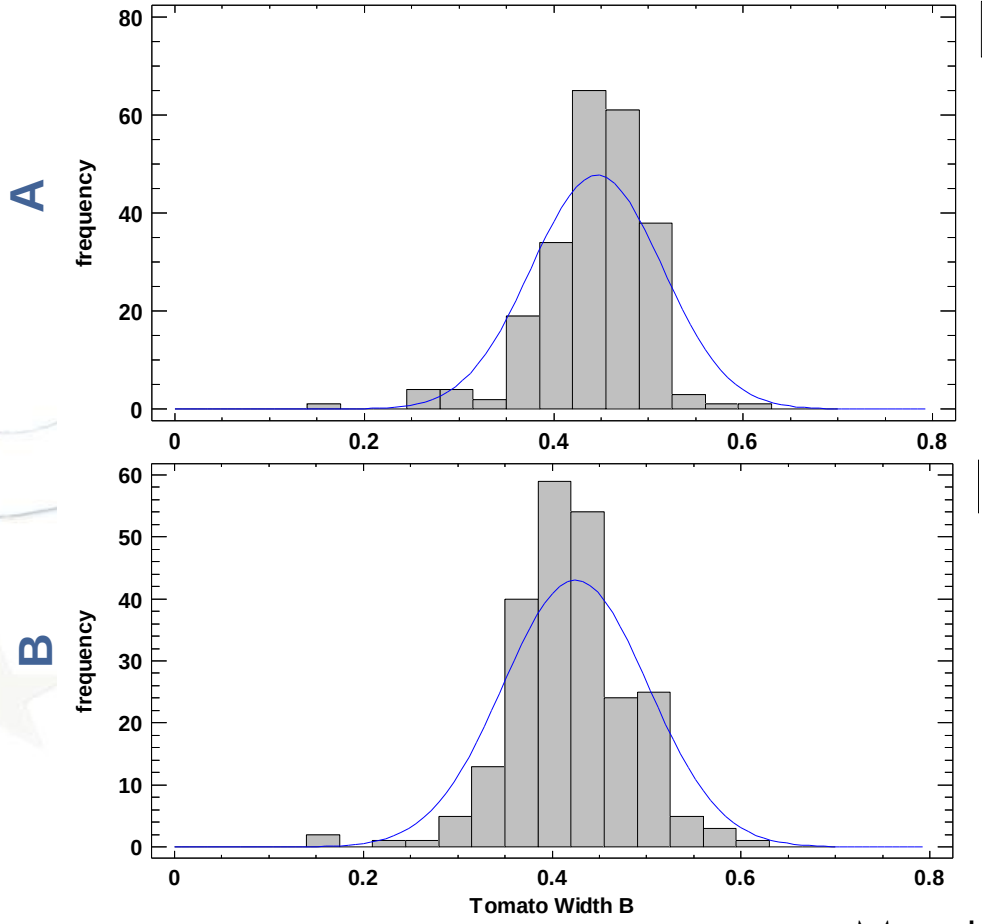
TOMATO



PEAK SYMETRY



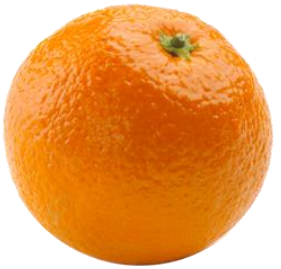
PEAK WIDTH



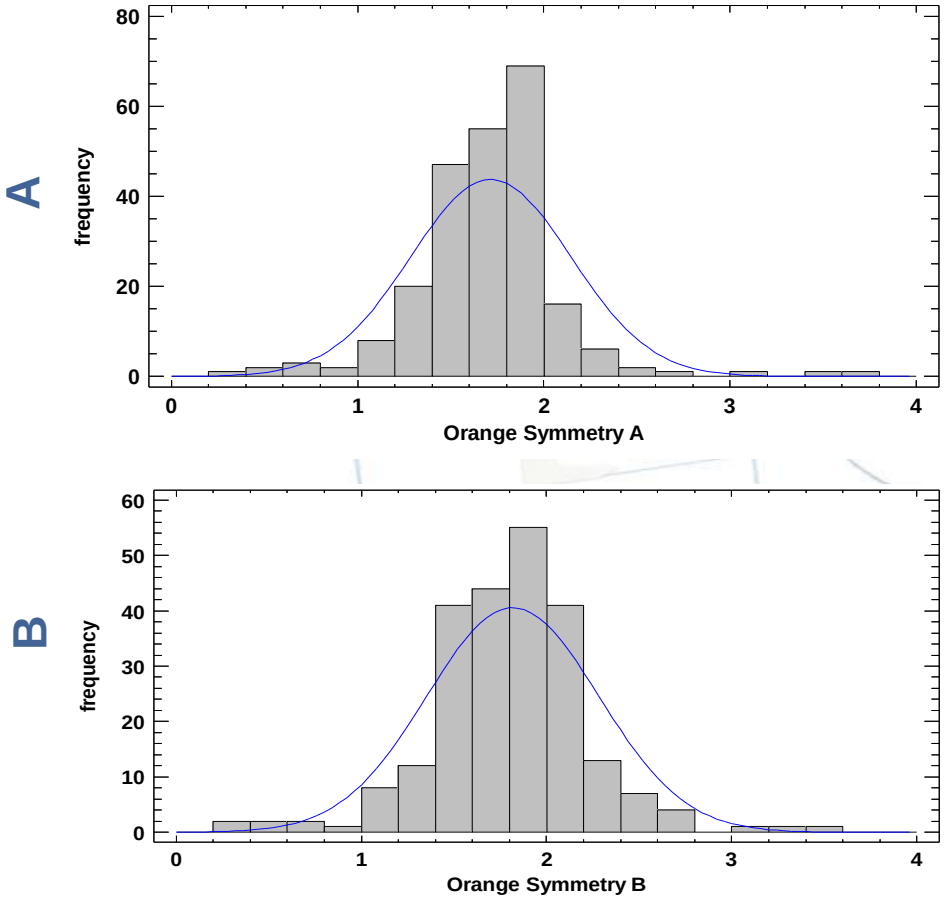
MassHunter® Data

Column **A**: **core-shell**
Column **B**: **fully porous**
243 Pesticides

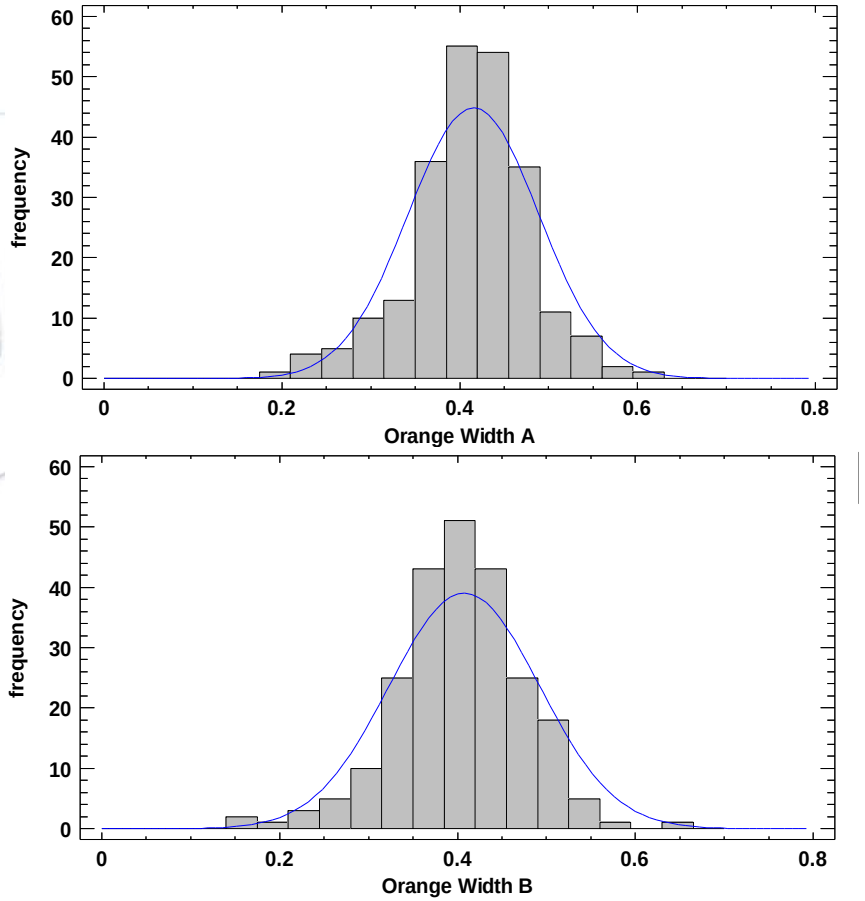
ORANGE



PEAK SYMETRY



PEAK WIDTH

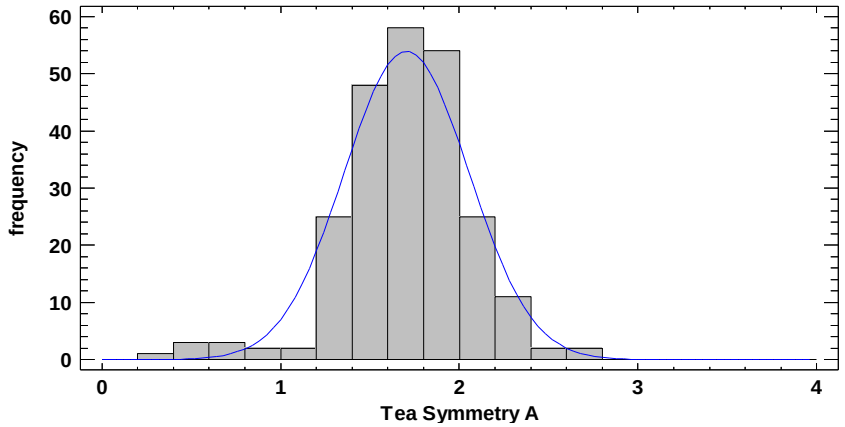


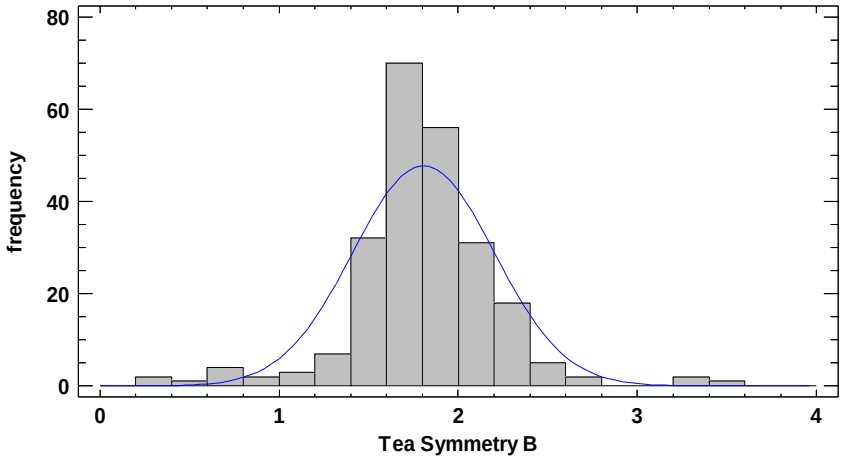
MassHunter® Data

Column **A**: **core-shell**
Column **B**: **fully porous**
243 Pesticides

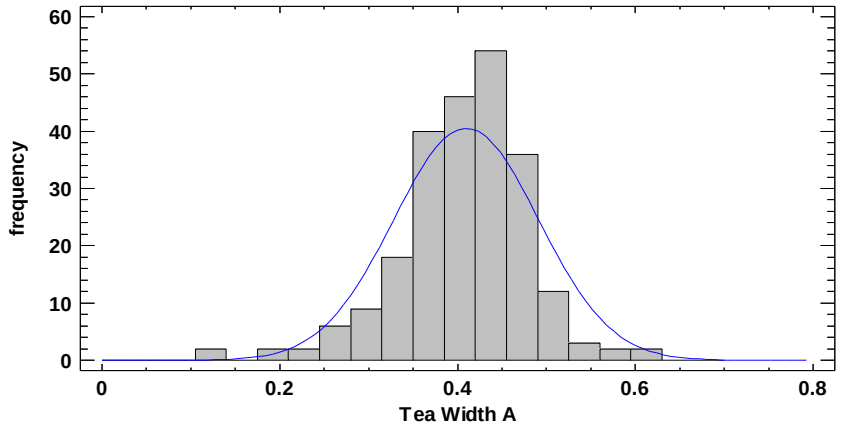
TEA

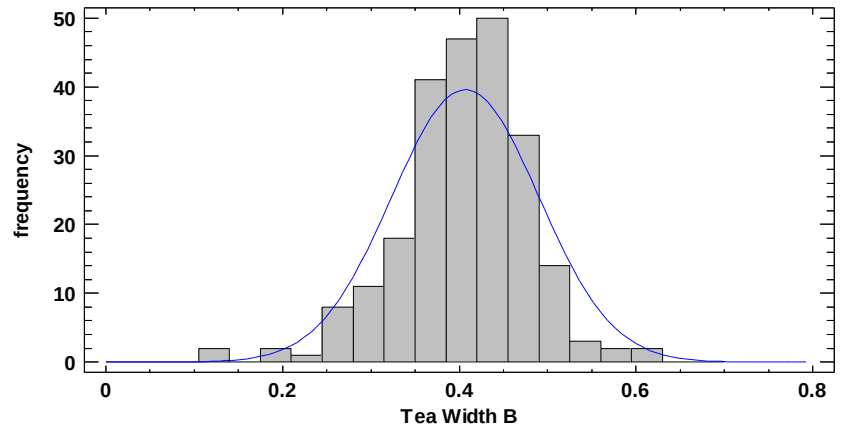
PEAK SYMETRY





PEAK WIDTH





MassHunter® Data

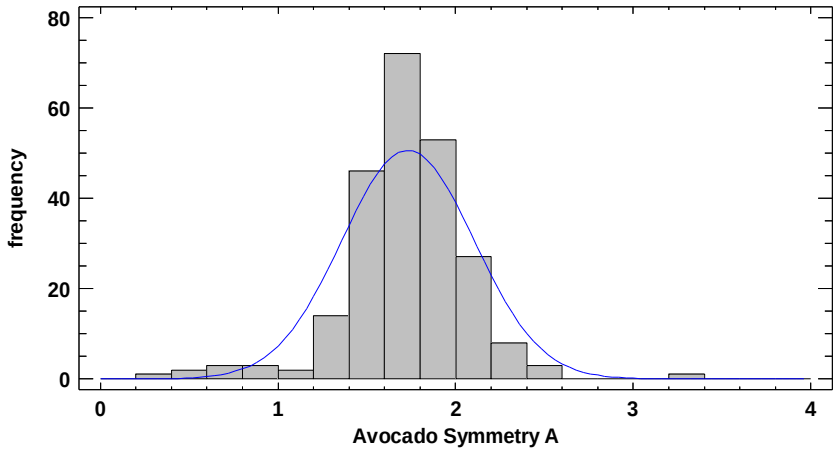
Column **A**: **core-shell**

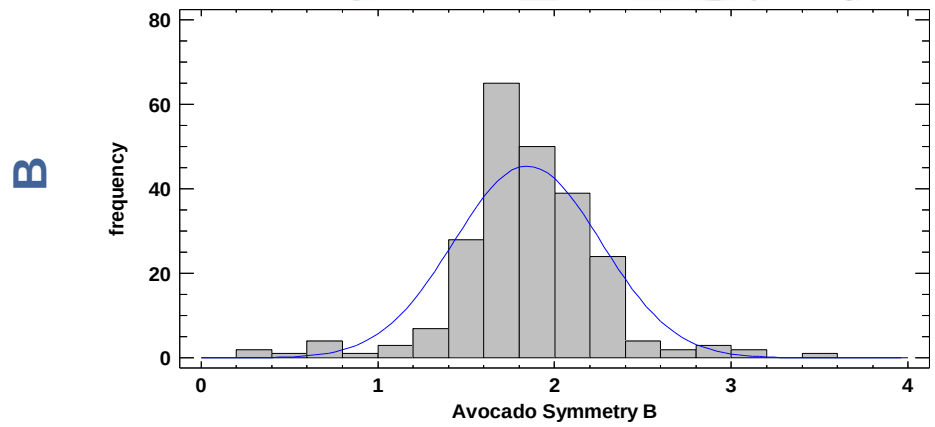
Column **B**: **fully porous**

243 Pesticides

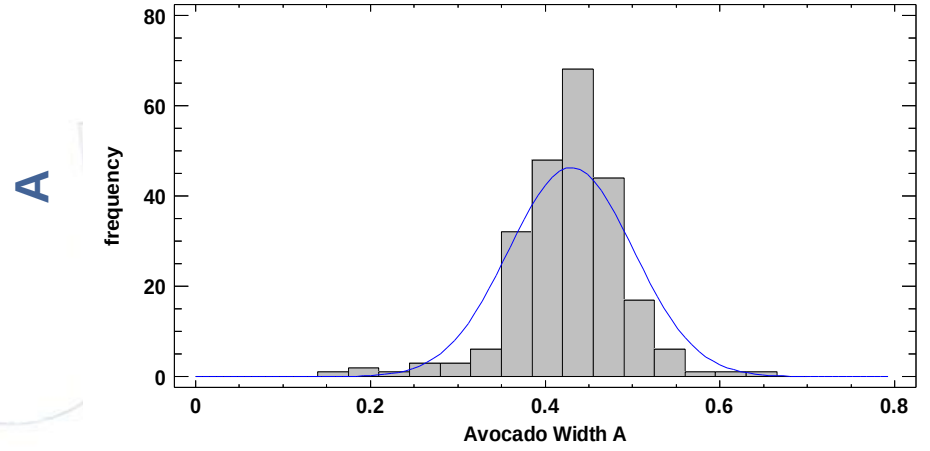
AVOCADO

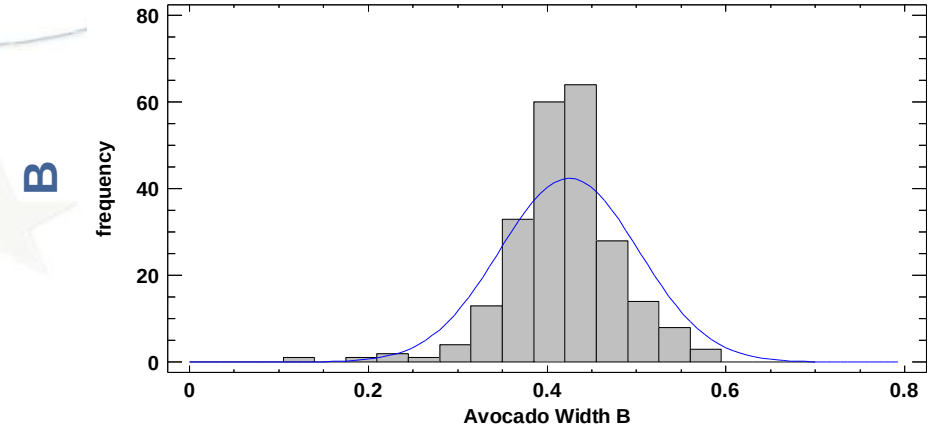
PEAK SYMETRY





PEAK WIDTH





MassHunter® Data

CONCLUSIONS

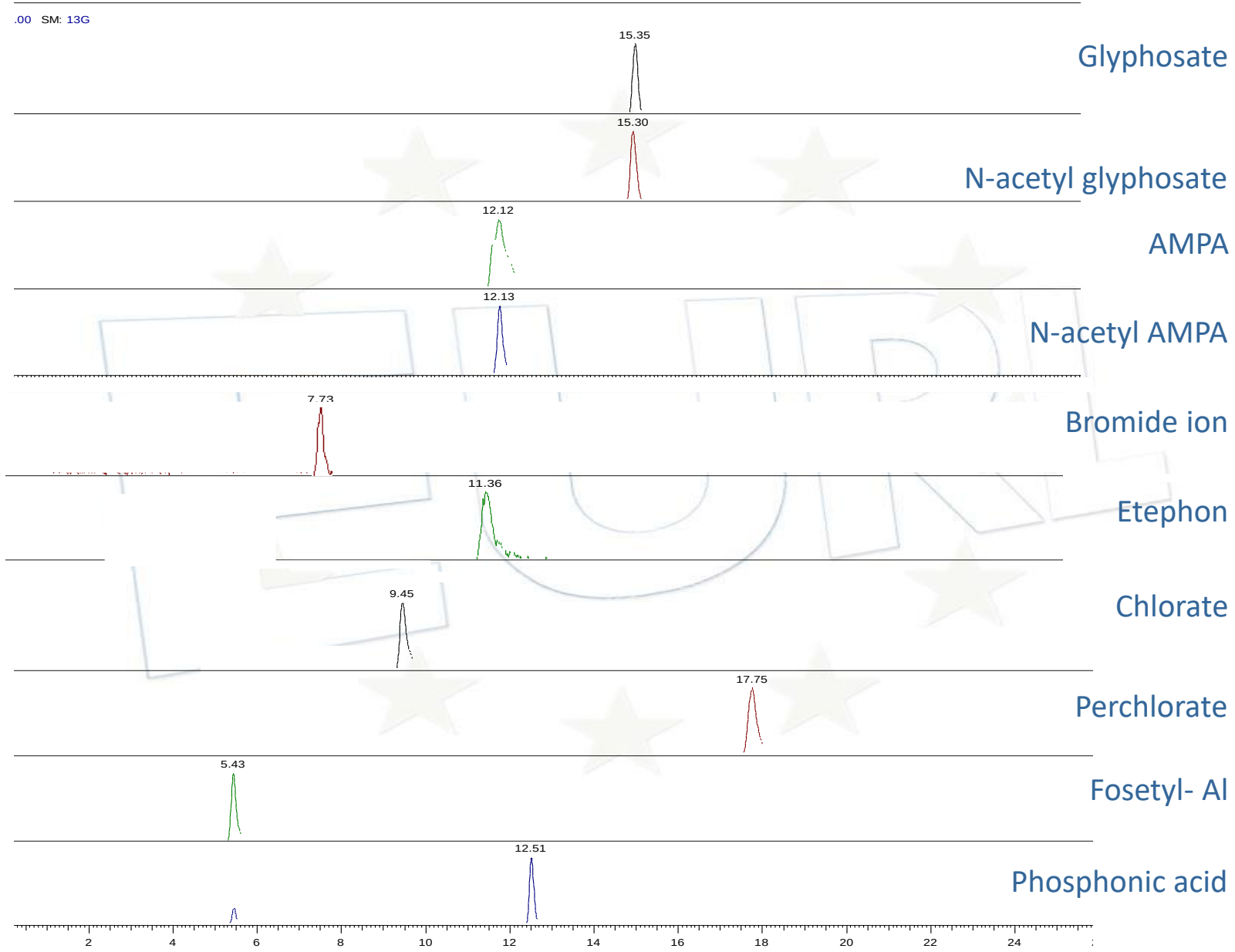
- Retention times were shorter (7%) using core shell column compared to fully porous column.
- Re-equilibration times were equal between both columns, so no advantage on using either column could be determined with this parameter.
- Chromatographic peaks were slightly more symmetric when using the core-shell column.
- Regarding time savings, using core shell column with the tested compounds, a 3.5 % time savings of the total run time is achieved compared to fully porous column .

1. Fully porous vs Core-shell

2. HILIC/IonExchange Hybrid column

ION CHROMATOGRAPHY

Extracted ion chromatograms (precursor ions)




SHIMADZU
LCMS-8060
Izasa Scientific

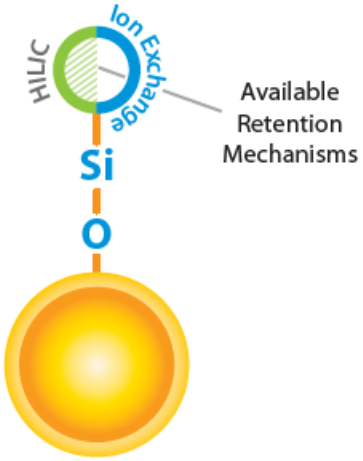
Nexera UC– 8060 TQ/MS(Shimadzu)

- Mobile phase A: Water (0.5 % formic acid)
- Mobile phase B: ACN (0.5 % formic acid)
- Column temperature: 35 °C
- Flow rate: 0.6 mL/min
- Injection volume: 5 µL

Time [min]	Mobile phase A	Mobile phase B
0	35 %	65 %
2	90 %	10 %
9	90 %	10 %
10	35 %	65 %

Column: Restek Raptor Polar 2.7µm 50x2.1mm

A single ligand capable of HILIC *and* Ion-Exchange retention for maximum versatility when separating polar compounds.



Available Retention Mechanisms

Column:
Restek Raptor Polar
2.7µm 50x2.1mm

High Organic

HILIC

IonEx

High Water

By changing between simple mobile phase conditions, retention can be tuned to retain a wide variety of polar analytes.

LC Passivation Solution

Methylenediphosphonic acid (Medronic Acid) (1984-15-2)

Description	CAS #	Conc. in Solvent	cat.#
Methylenediphosphonic acid (Medronic Acid)	1984-15-2	1,760 µg/mL, Methanol (HPLC grade)/Water (50:50), 1mL/ampul	32475 (ea.)

MATRICES

Tomato

Orange

Onion

Quince

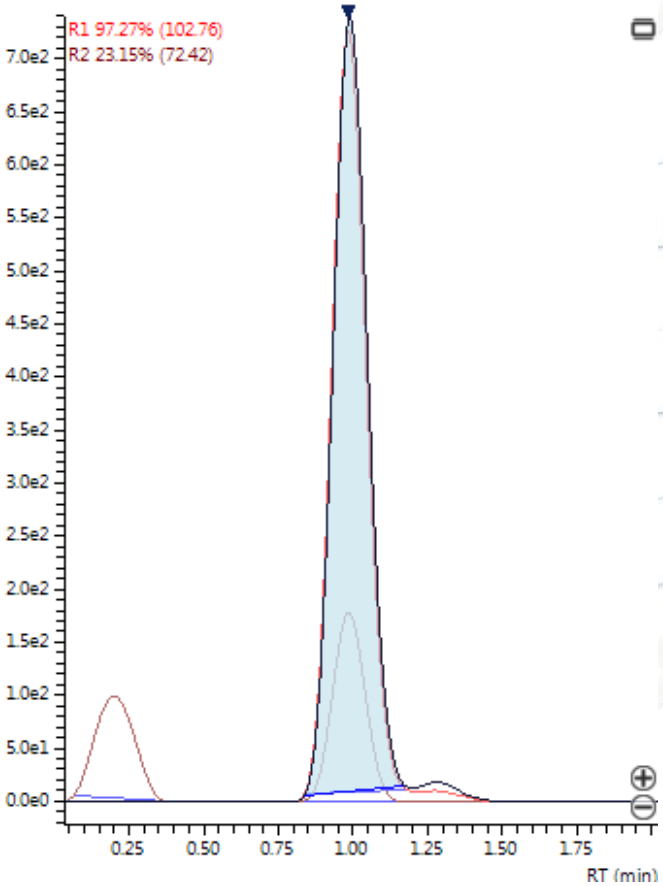
METHOD

		$R_{t\ (min)}$
AMPA	110.1>79/110.1>63	0.98
Ethephon	143.2>107/145.3>107	4.937
Fosetyl	109.1>81/109.1>63	7.176
Glufosinate	180.1>62.95/180.1>85.1	2.662
Glyphosate	167.9>63/167.9>79	4.775
Glyphosate_C13N15	171>63	4.766
N-acetyl-AMPA	152.1>63/152.1>110.1	6.492
Phosphonic acid	81.1>79/81.1>62.95	7.016
Chlorate	83.1>67/83.1>51	4.599
Perchlorate	99.2>83.05/99.2>67	3.22

✗ N-acetyl-glyphosate

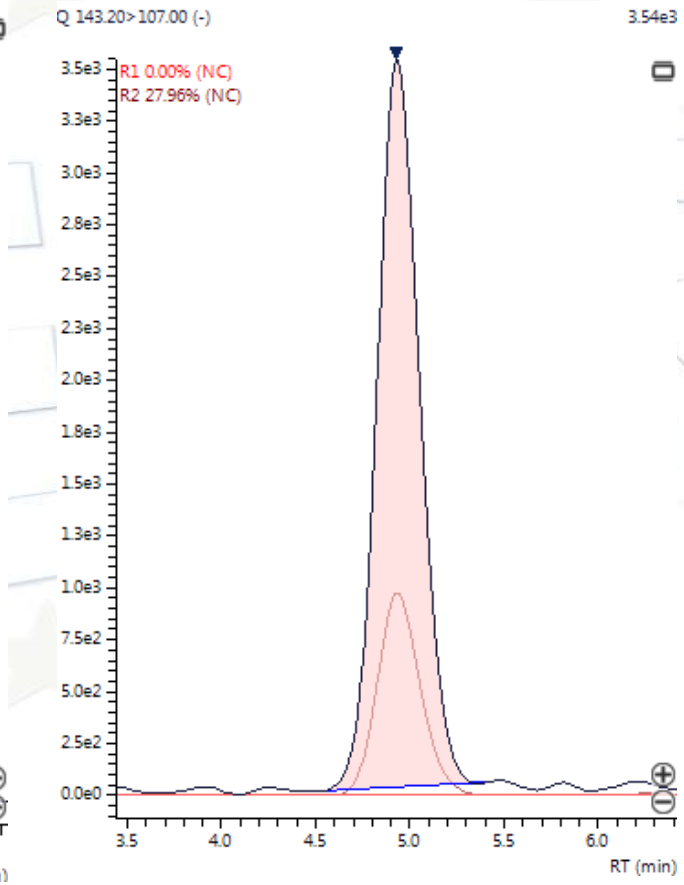
Orange
50µg/Kg

AMPA



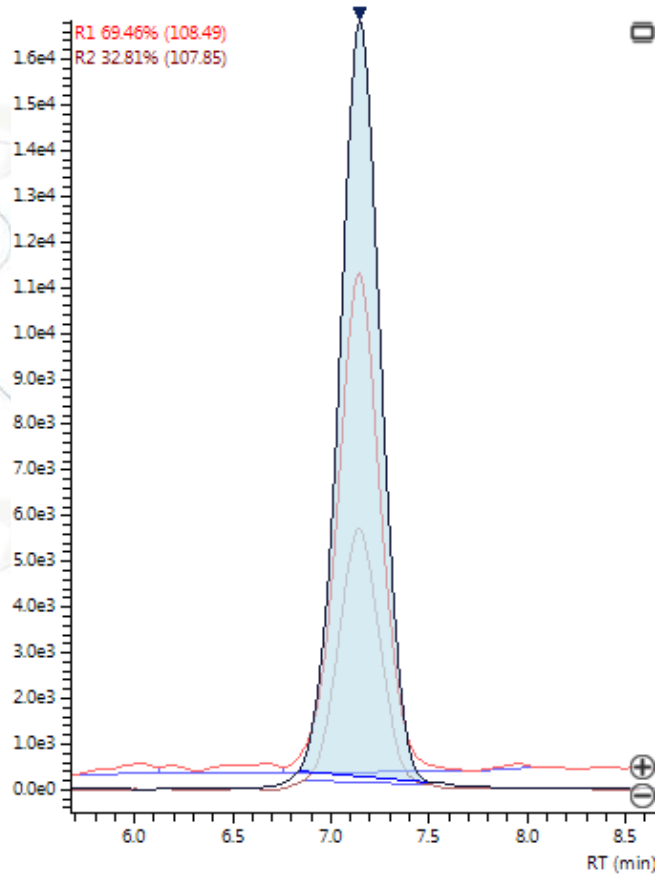
Rt: 0.95 min

ETHEPHON



Rt: 4.94 min

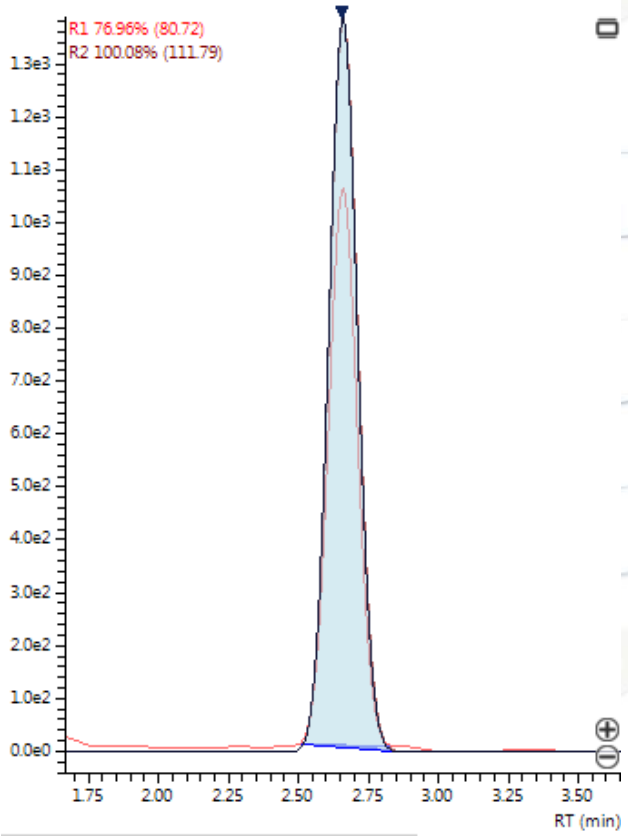
FOSETYL



Rt: 7.18 min

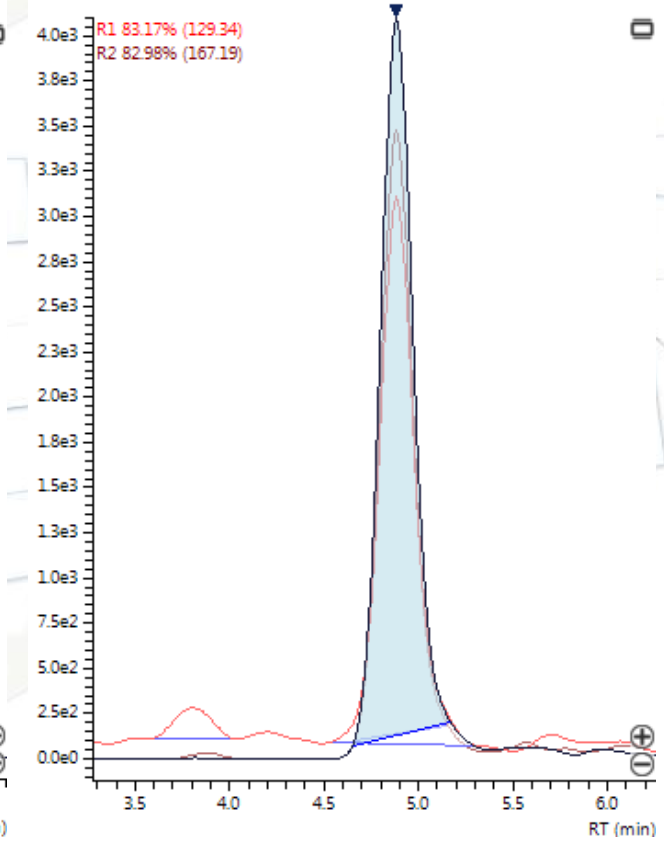
Orange
50µg/Kg

GLUFOSINATE



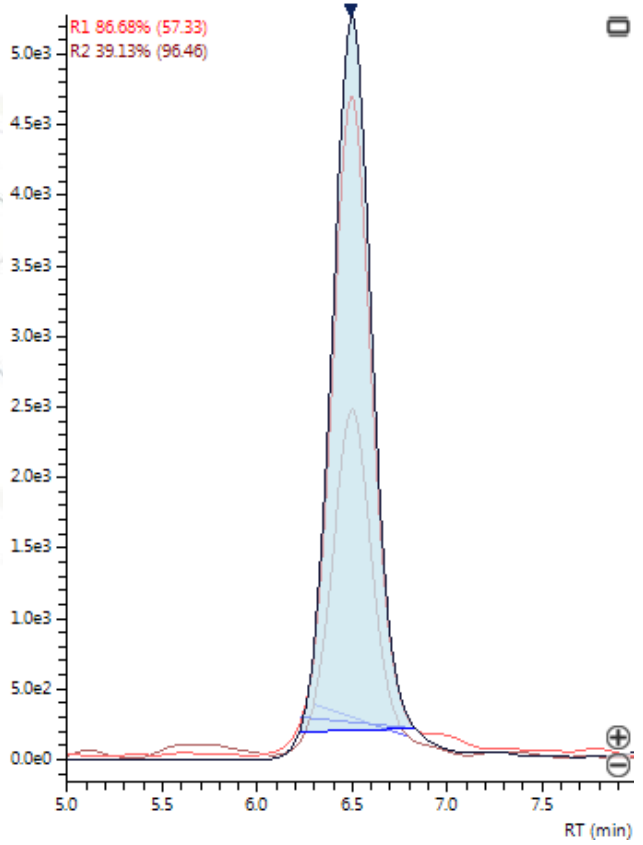
Rt: 2.66 min

GLYPHOSATE



Rt: 4.78 min

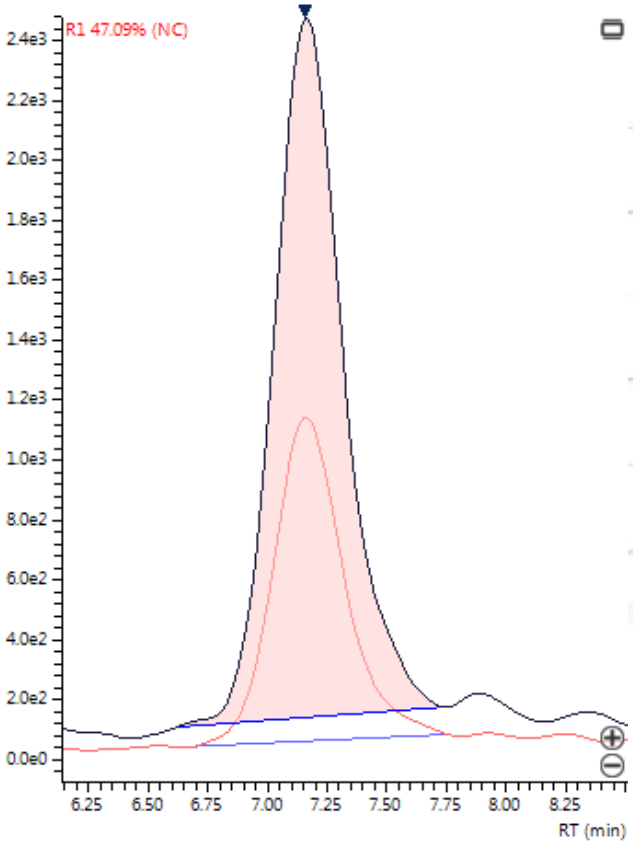
N-Acetyl-AMPA



Rt: 6.49 min

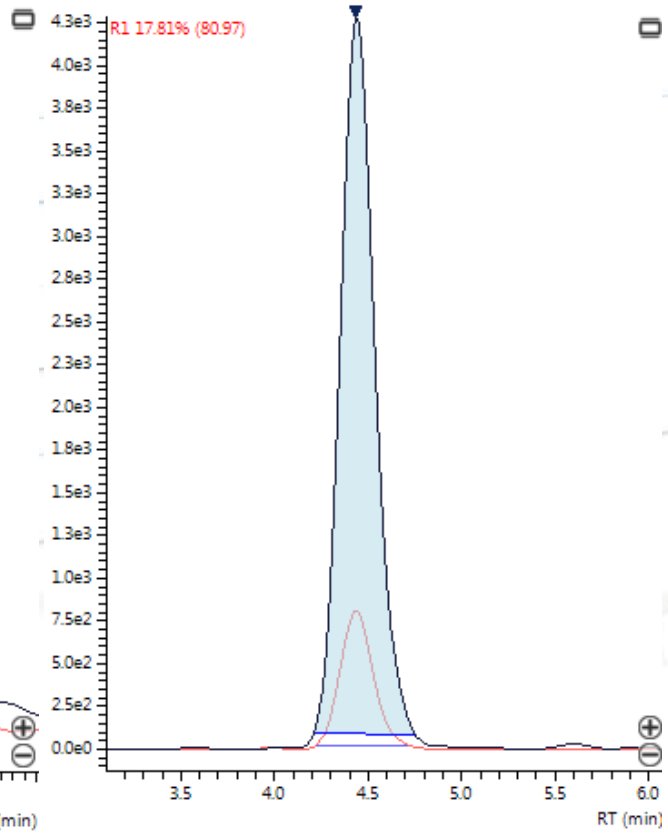
Orange
50µg/Kg

PHOSPHONIC ACID



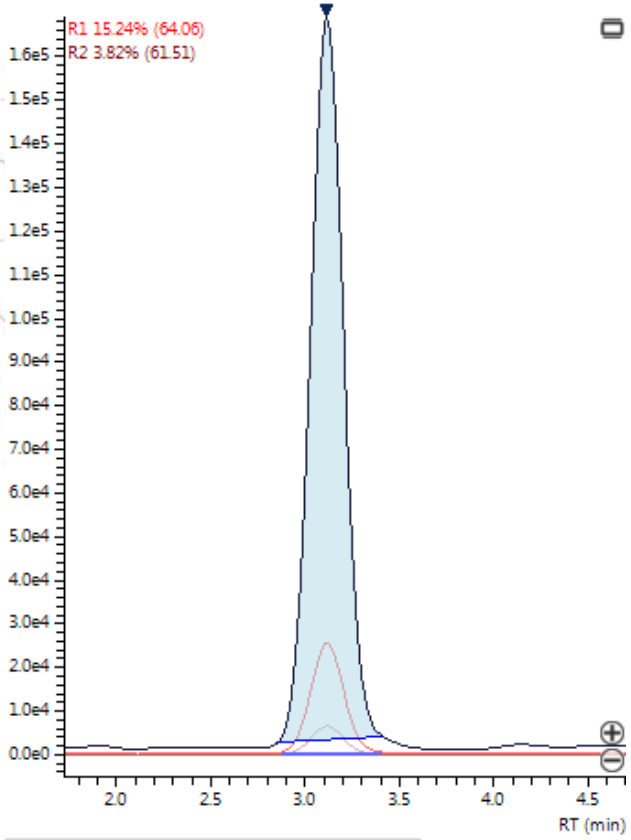
Rt: 7.02 min

CHLORATE



Rt: 4.60 min

PERCHLORATE



Rt: 3.22 min

LIMITS OF QUANTIFICATION (µg/Kg)

Tomato matrix

	LOQ	MRL
AMPA	10	
Ethephon	20	2000
Fosetyl	10	100000
Glufosinate	10	100
Glyphosate	20	100
N-acetyl-AMPA	20	
Phosphonic acid	50	
Chlorate	20	100
Perchlorate	20	10

LIMITS OF QUANTIFICATION (µg/Kg)

Orange matrix

	LOQ	MRL
AMPA	50	
Ethephon	50	50
Fosetyl	10	75000
Glufosinate	10	50
Glyphosate	10	500
N-acetyl-AMPA	20	
Phosphonic acid	50	
Chlorate	20	50
Perchlorate	10	10

LIMITS OF QUANTIFICATION (µg/Kg)

Onion matrix

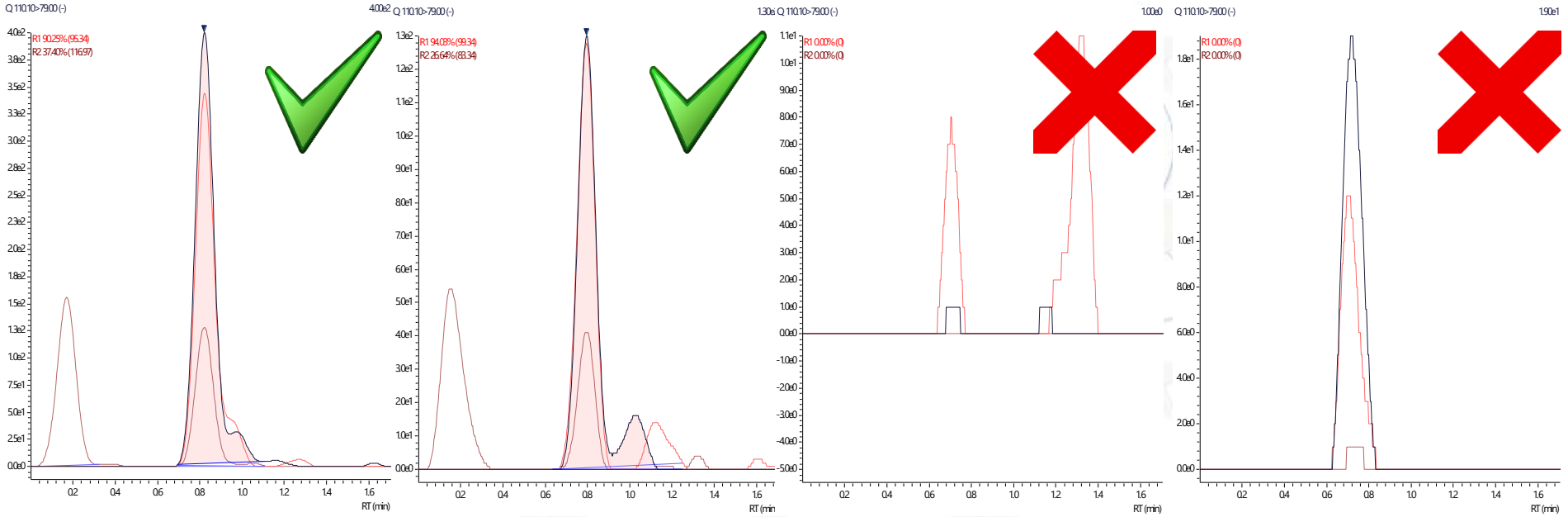
	LOQ	MRL
AMPA	20	
Ethephon	50	50
Fosetyl	10	50000
Glufosinate	10	50
Glyphosate	10	100
N-acetyl-AMPA	20	
Phosphonic acid	50	
Chlorate	10	50
Perchlorate	10	10

LIMITS OF QUANTIFICATION (µg/Kg)

Quince matrix

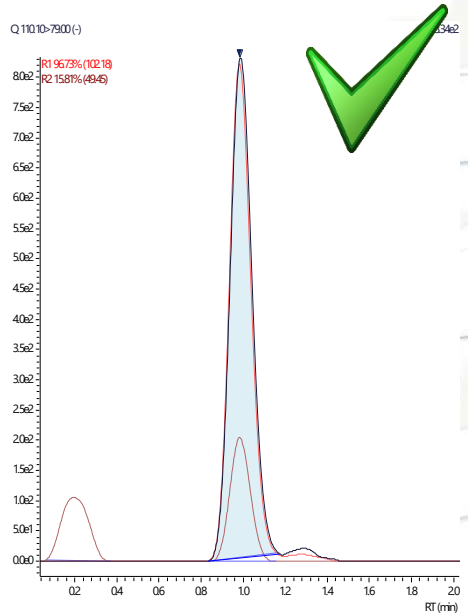
	LOQ	MRL
AMPA	20	
Ethephon	50	50
Fosetyl	10	150000
Glufosinate	10	100
Glyphosate	20	100
N-acetyl-AMPA	10	
Phosphonic acid	50	
Chlorate	20	50
Perchlorate	10	10

AMPA 50µg/Kg
30mm column

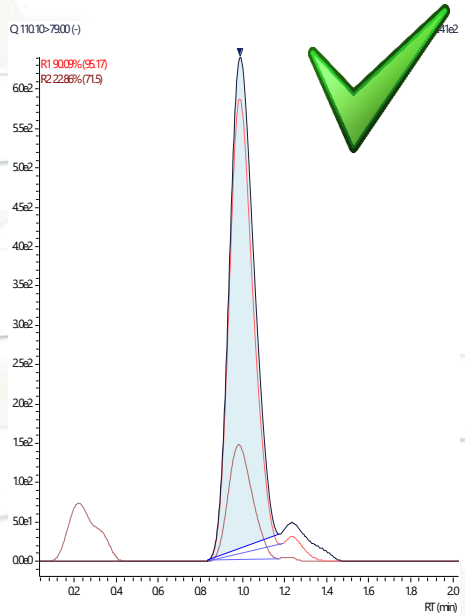


Tomato Onion Quince Orange

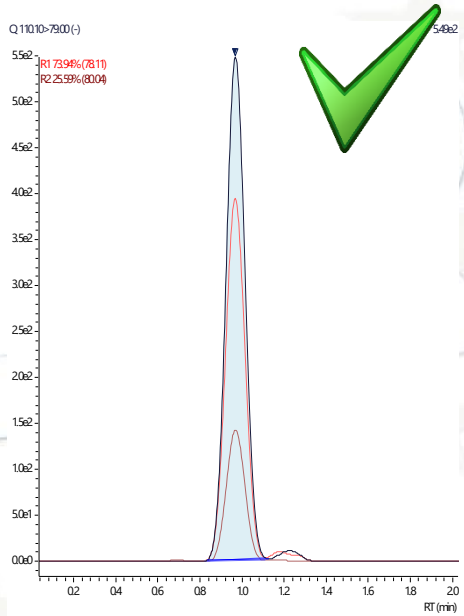
AMPA 50ug/L
50mm column



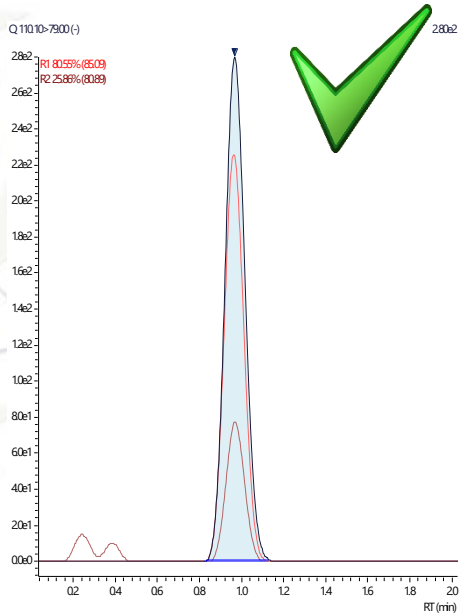
Tomato



Onion

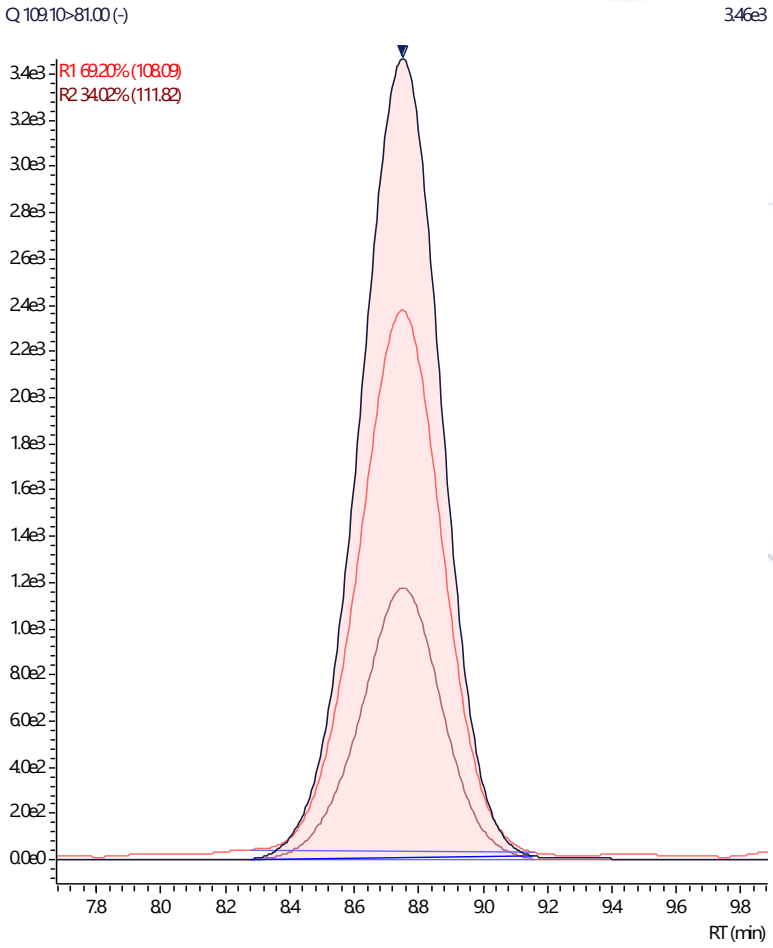


Quince



Orange

FOSETYL Retention time 50µg/L tomato



Tomato_50ppb_R1		Unknown	8.593
Tomato_50ppb_R2		Unknown	8.591
Tomato_50ppb_R3		Unknown	8.586
Tomato_50ppb_R4		Unknown	8.574
Tomato_50ppb_R5		Unknown	8.577
Tomato_50ppb_R6		Unknown	8.557
Tomato_50ppb_R7		Unknown	8.549
Tomato_50ppb_R8		Unknown	8.548
Tomato_50ppb_R9		Unknown	8.540
Tomato_50ppb_R10		Unknown	8.534
Tomato_50ppb_R11		Unknown	8.527
Tomato_50ppb_R12		Unknown	8.526
Tomato_50ppb_R13		Unknown	8.517
Tomato_50ppb_R14		Unknown	8.511
Tomato_50ppb_R15		Unknown	8.510
Tomato_50ppb_R16		Unknown	8.500
Tomato_50ppb_R17		Unknown	8.497
Tomato_50ppb_R18		Unknown	8.492
Tomato_50ppb_R19		Unknown	8.492
Tomato_50ppb_R20		Unknown	8.484
Tomato_50ppb_R21		Unknown	8.475
Tomato_50ppb_R22		Unknown	8.470
Tomato_50ppb_R23		Unknown	8.462
Tomato_50ppb_R24		Unknown	8.451
Tomato_50ppb_R25		Unknown	8.451

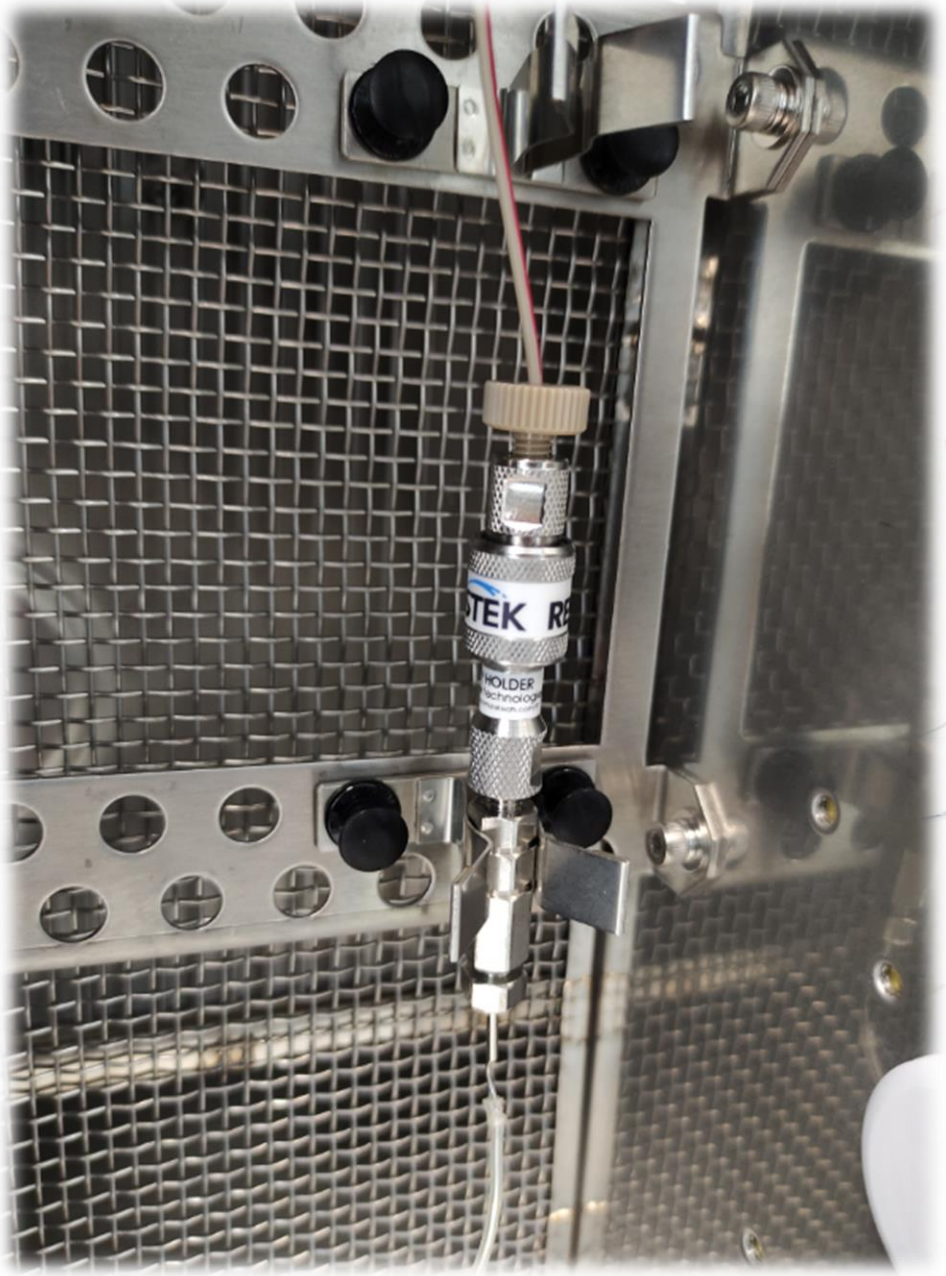
FOSETYL Retention time 50µg/L tomato

BEFORE CHANGING GUARD-COLUMN CARTRIDGE

Tomato_50ppb_R1		Unknown	8.750
Tomato_50ppb_R2		Unknown	8.742
Tomato_50ppb_R3		Unknown	8.733
Tomato_50ppb_R4		Unknown	8.733
Tomato_50ppb_R5		Unknown	8.721
Tomato_50ppb_R6		Unknown	8.722
Tomato_50ppb_R7		Unknown	8.711
Tomato_50ppb_R8		Unknown	8.706
Tomato_50ppb_R9		Unknown	8.710
Tomato_50ppb_R10		Unknown	8.701
Tomato_50ppb_R11		Unknown	8.707
Tomato_50ppb_R12		Unknown	8.706
Tomato_50ppb_R13		Unknown	8.710
Tomato_50ppb_R14		Unknown	8.695
Tomato_50ppb_R15		Unknown	8.705

AFTER CHANGING GUARD-COLUMN CARTRIDGE

Tomato_50ppb_R1		Unknown	8.787
Tomato_50ppb_R2		Unknown	8.782
Tomato_50ppb_R3		Unknown	8.762
Tomato_50ppb_R4		Unknown	8.758
Tomato_50ppb_R5		Unknown	8.755
Tomato_50ppb_R6		Unknown	8.738
Tomato_50ppb_R7		Unknown	8.742
Tomato_50ppb_R8		Unknown	8.728
Tomato_50ppb_R9		Unknown	8.720
Tomato_50ppb_R10		Unknown	8.716
Tomato_50ppb_R11		Unknown	8.711
Tomato_50ppb_R12		Unknown	8.703
Tomato_50ppb_R13		Unknown	8.698
Tomato_50ppb_R14		Unknown	8.695
Tomato_50ppb_R15		Unknown	8.688



BACKFLUSHING:

WATER (1% Formic acid)

Flow: 1mL/min (60 min)

RETENTION TIME
30 Injections
FOSETYL 50ppb Onion

BACKFLUSHING WITH WATER
(1% Formic acid)
Flow: 1mL/min (60 min)

7.83	7.773
7.826	7.77
7.826	7.765
7.82	7.766
7.817	7.763
7.805	7.764
7.809	7.756
7.805	7.748
7.803	7.753
7.799	7.751
7.8	7.752
7.798	7.742
7.796	7.751
7.795	7.75
7.794	7.754
7.786	7.751
7.786	7.75
7.787	7.756
7.779	7.763
7.774	7.758
7.773	7.759
7.772	7.754
7.775	7.757
7.772	7.755
7.766	7.756
7.766	7.754
7.765	7.757
7.767	7.756
7.759	7.754
7.758	7.749

BF: 60mL

Rt diff. (1st-30th)	Rt diff. (1st-30th)
0.072	**0.024**

CONCLUSIONS

- A method of 10 polar analytes was optimized using this new hybrid column technology
- All the compounds were identified at the same or below MRL levels in the four matrices studied (tomato, orange, onion, and quince)
- N-acetyl-glyphosate cannot be detected due to the properties of the stationary phase and at least 50mm column size is necessary to detect AMPA in some matrices.
- A backflushing method is being developed to reduce the decrease of the compound's retention time after multiple injections.

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Thank You for Your Attention

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