UNIVERSIDAD DE ALMERÍA

Implementing new LC-MS technologies for the targeted and non-targeted analysis of pesticide residues and more

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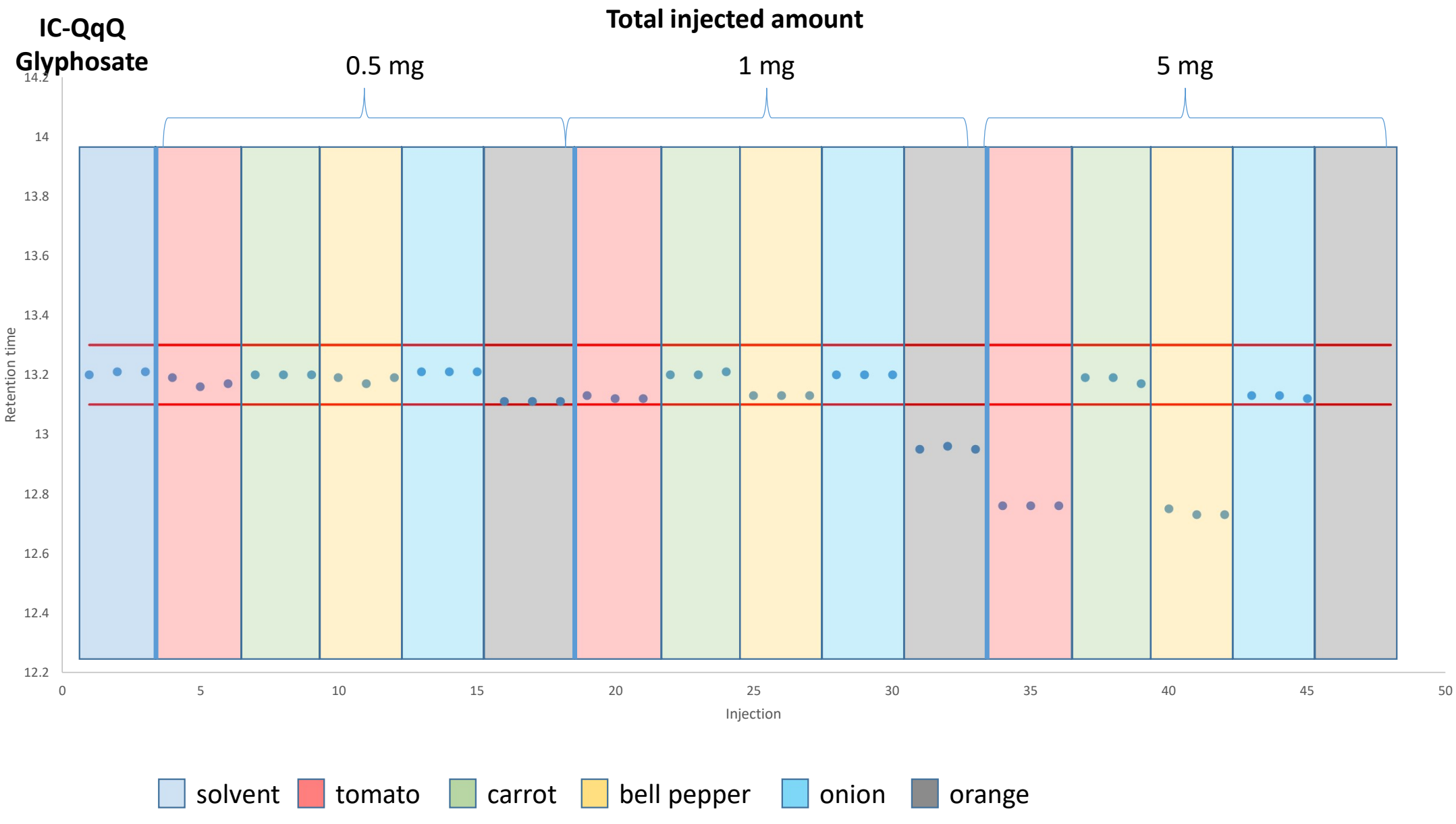
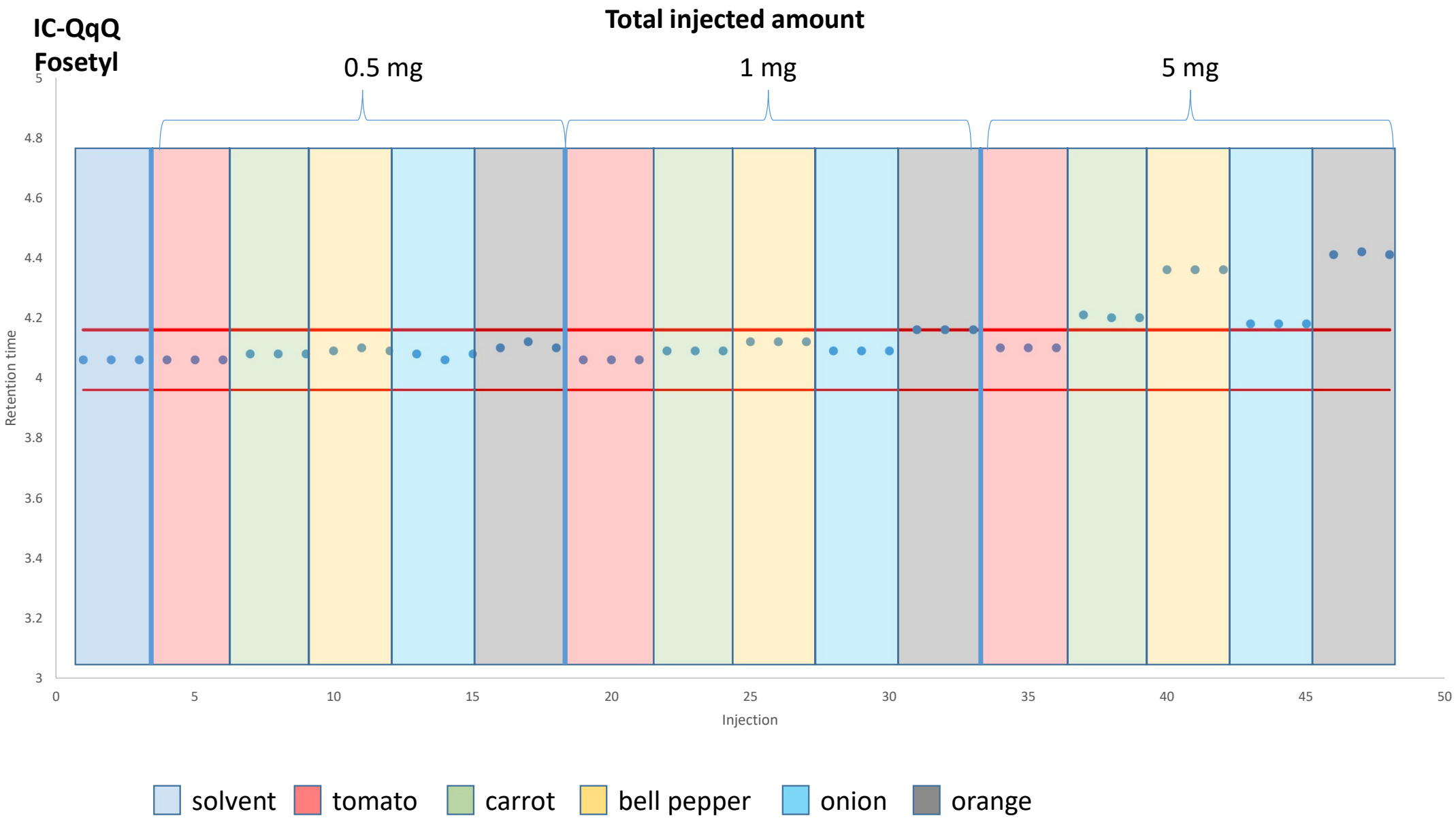
Current projects

Thermo Scientific™ Dionex™ Integrion™ HPIC™ System

Thermo Scientific™ Orbitrap Exploris™ 240 MS System


IC-QqQ
Glyphosate
Total injected amount
0.5 mg
1 mg
5 mg
Retention time
14.2
14
13.8
13.6
13.4
13.2
13
12.8
12.6
12.4
12.2
0
5
10
15
20
25
30
35
40
45
50
Injection
solvent
tomato
carrot
bell pepper
onion
orange


IC-QqQ
Fosetyl
Total injected amount
0.5 mg
1 mg
5 mg
Retention time
5
4.8
4.6
4.4
4.2
4
3.8
3.6
3.4
3.2
3
0
5
10
15
20
25
30
35
40
45
50
Injection
solvent
tomato
carrot
bell pepper
onion
orange

Current projects

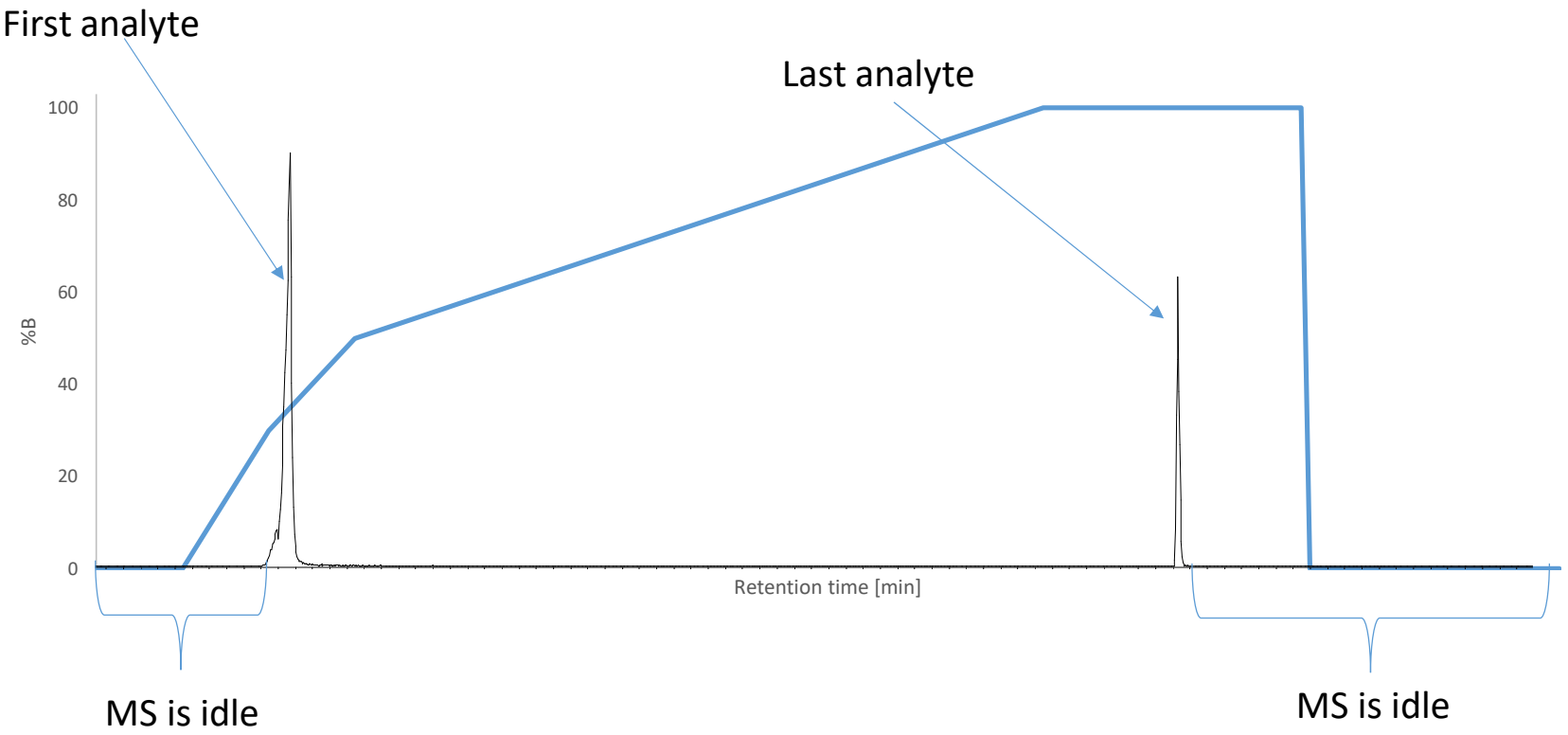
Thermo Scientific™
Transcend™ DUO LX-2
UHPLC System

Thermo Scientific™
TSQ Altis™
Triple Quadrupole Mass Spectrometer

How to decrease the analysis time?

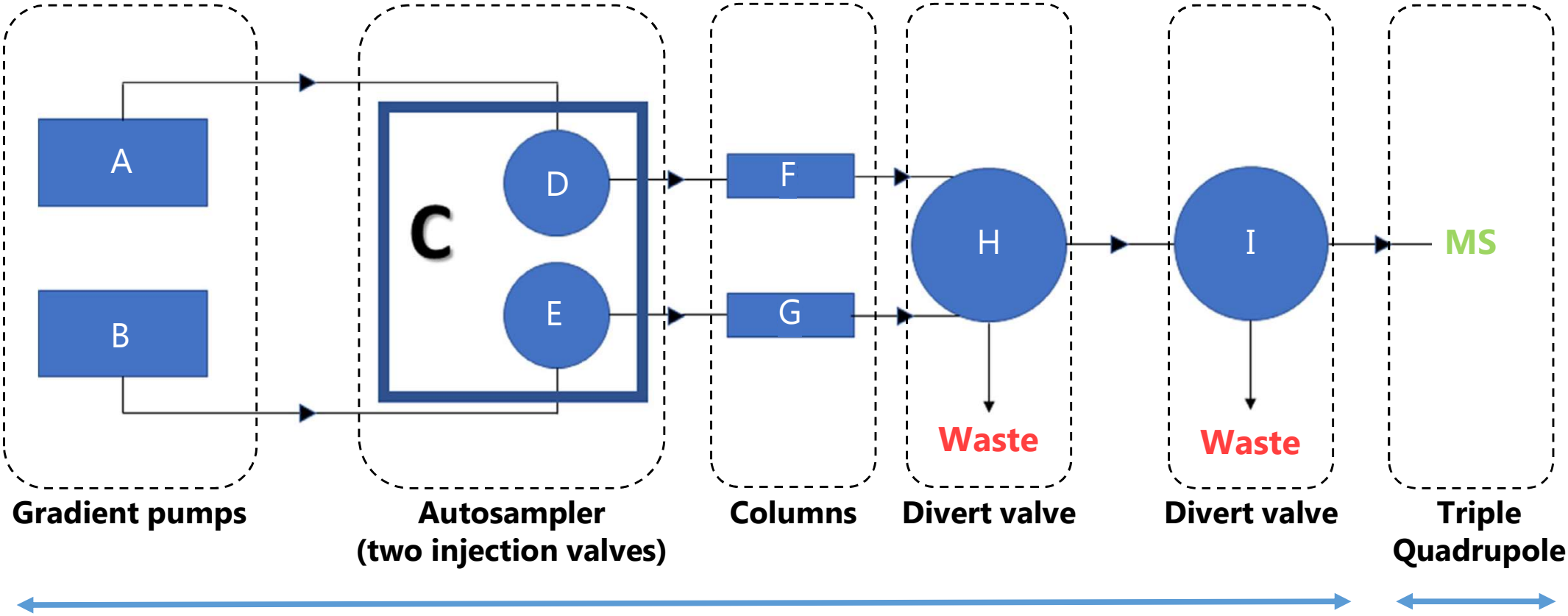
- Shorter column, steeper gradient, higher flow:
  - Compromised separation
    - More coeluting pesticides
      - Shorter dwell times -> lower sensitivity
      - Longer duty cycle -> Less data points per chromatographic peak -> worse peak area reproducibility
      - Common transitions
      - Possible cross-talk
    - More coeluting matrix (especially in “dirty matrices”)
      - Higher matrix effects -> lower sensitivity
      - Possible interferences

Another option to decrease the analysis time



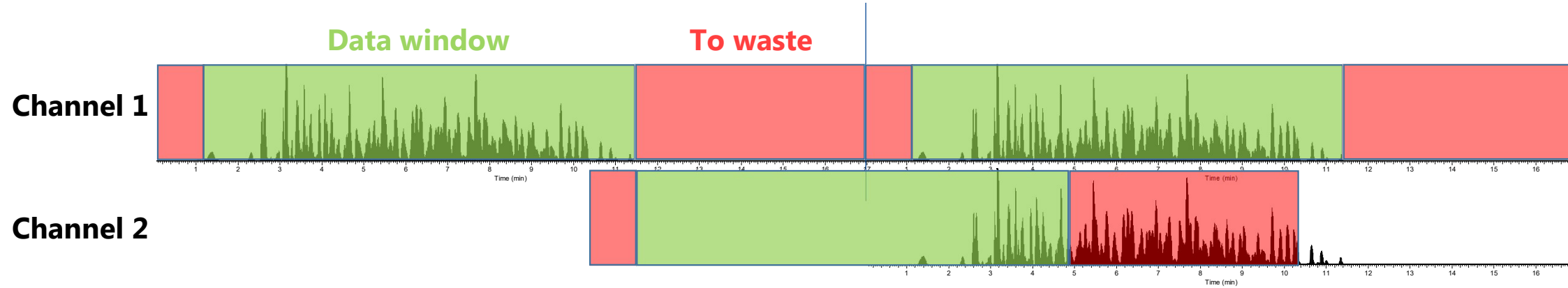
Analysis time can be decreased by the application of multi-channel chromatography and reduction of the idle time of the mass spectrometer

Dual-Channel LC-MS/MS: general diagram

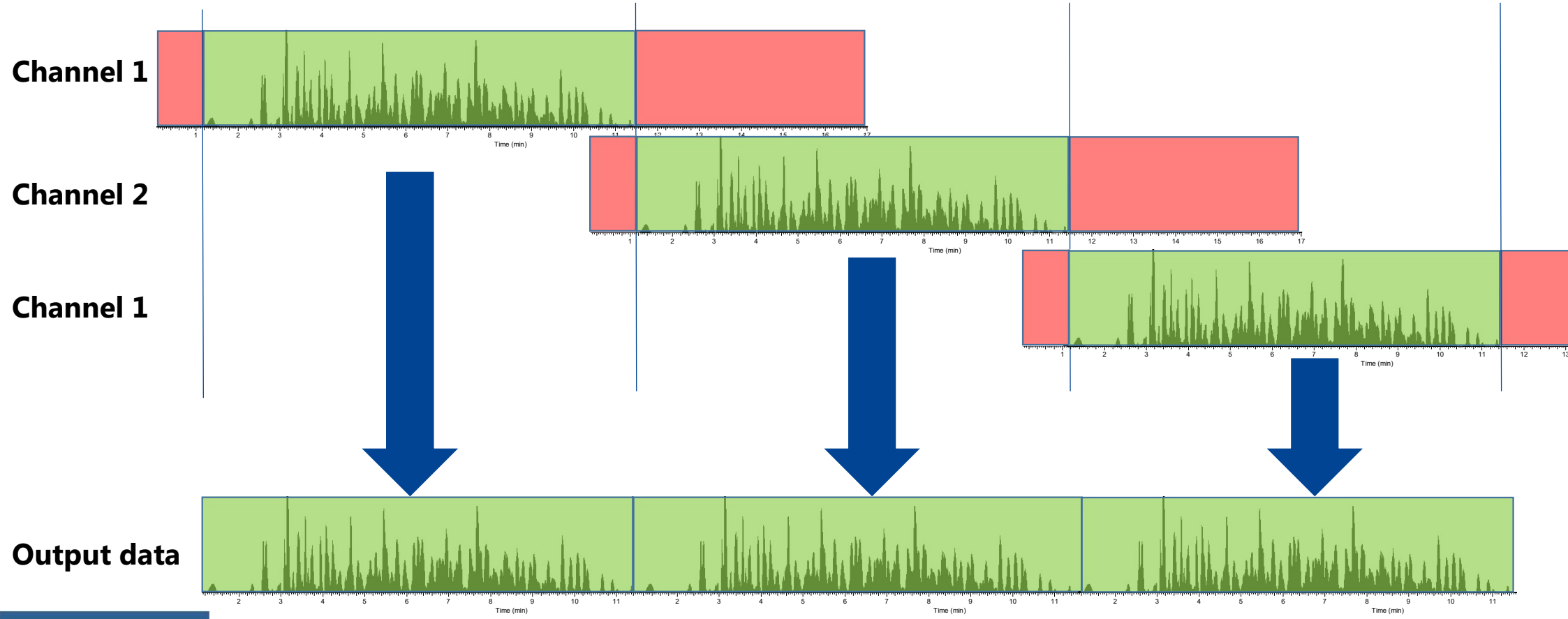


Thermo Scientific ™ Transcend™ Duo LX-2 UHPLC System

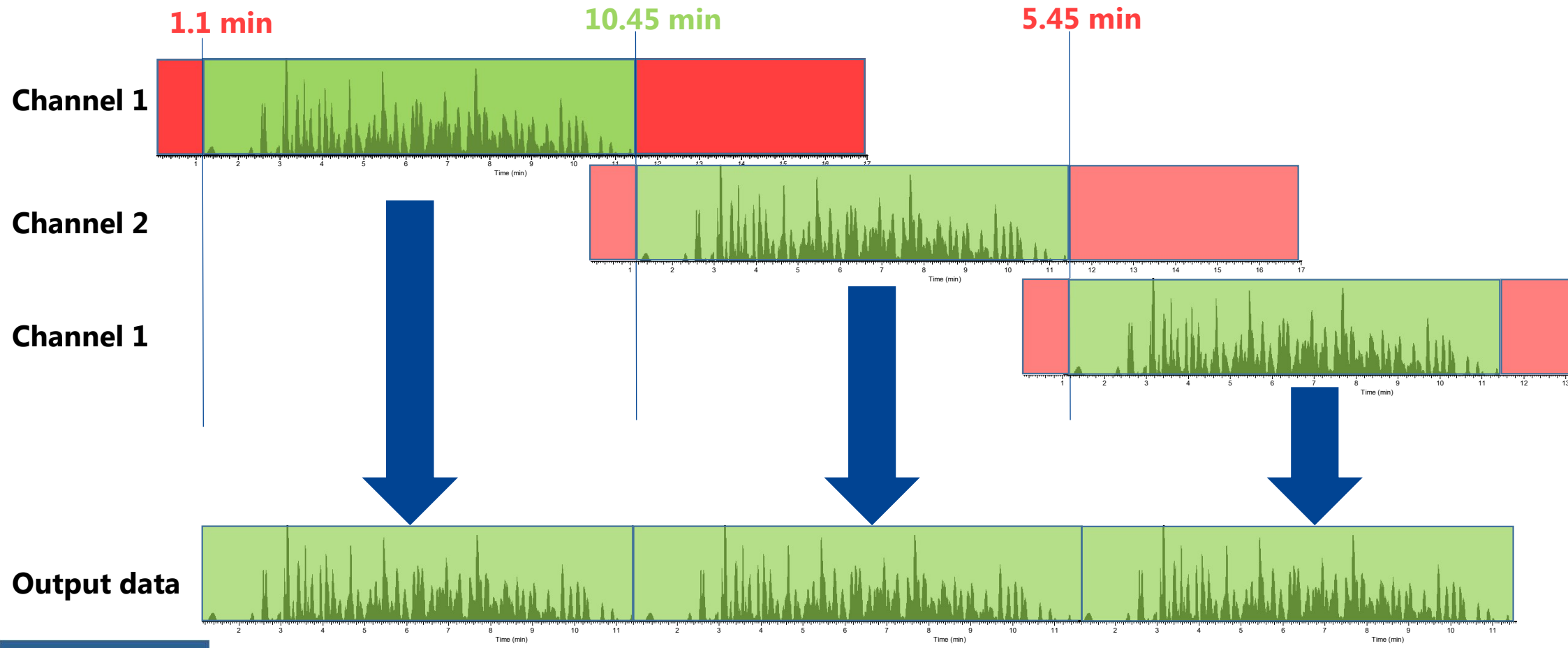
Dual-Channel LC-MS/MS: sample throughput



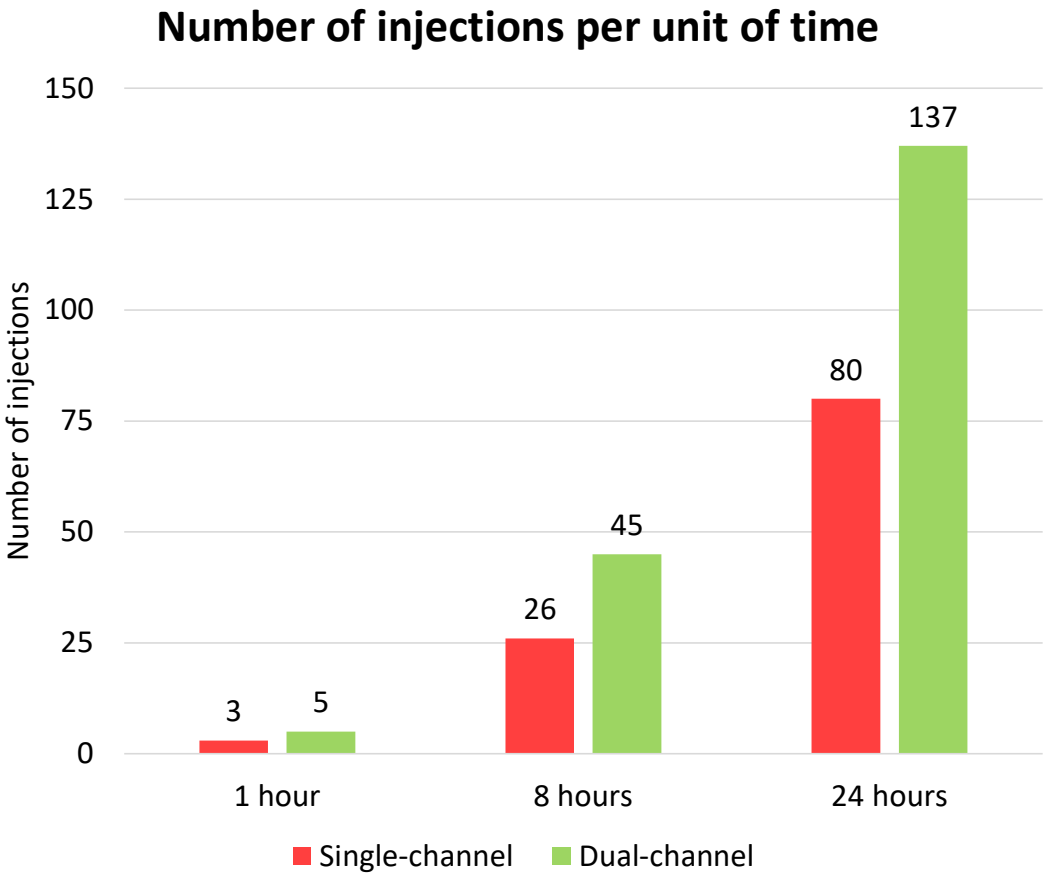
Dual-Channel LC-MS/MS: sample throughput



Dual-Channel LC-MS/MS: sample throughput



Dual-Channel LC-MS/MS: sample throughput



- With Dual-Channel chromatography, pre-acquisition and post-acquisition MS-idle times are removed
- Sample throughput is increased over 70 % (45 injections in an 8 hr period)

Benefits of a sensitive triple quadrupole MS

Thermo Scientific™ TSQ Altis™
Triple Quadrupole Mass Spectrometer

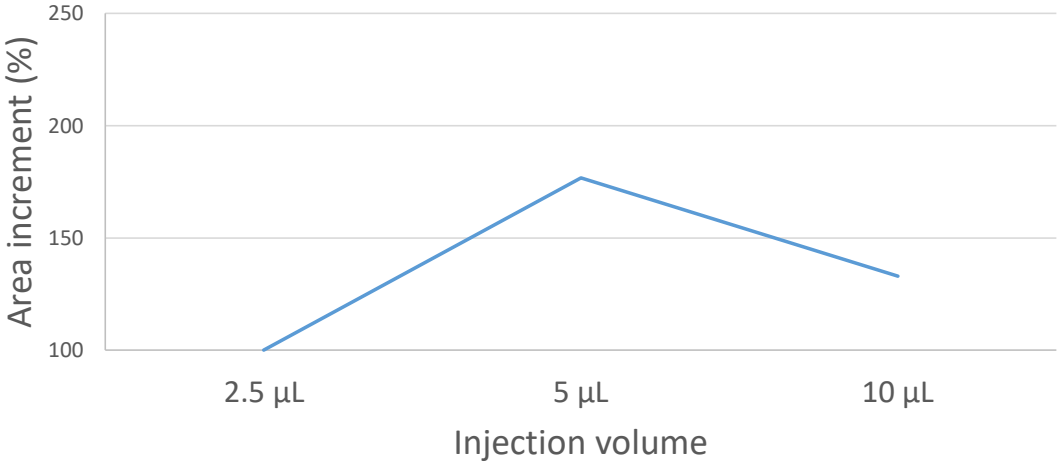
More sensitive triple quadrupole means:

- Lower injection volume
  - Reduced maintenance
  - Better peak shape
  - Lower matrix effects
    - More accurate quantitation
- Shorter dwell times
  - More concurrent transitions possible
  - Shorter duty cycle

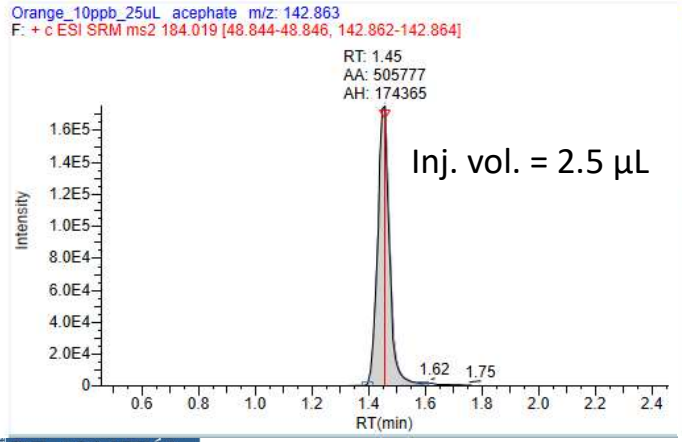
Sensitive triple quadrupole MS: reduction of injection volume

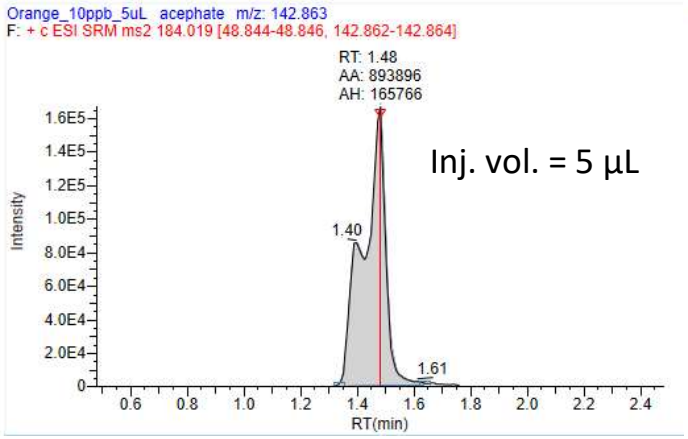
Acephate

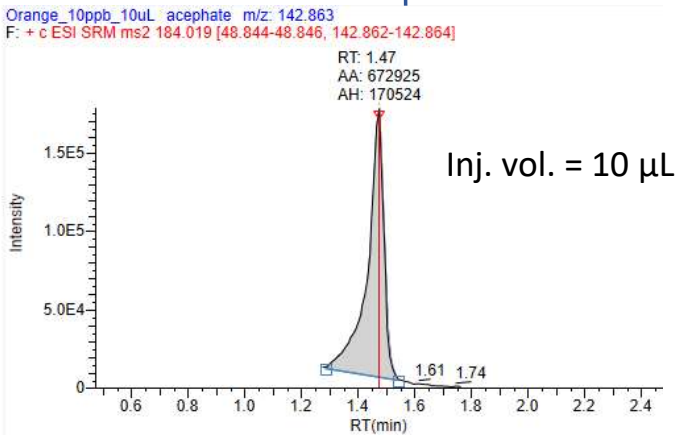
Retention time = 1.45 min



Peak splitting: compound **OUT** of acquisition window



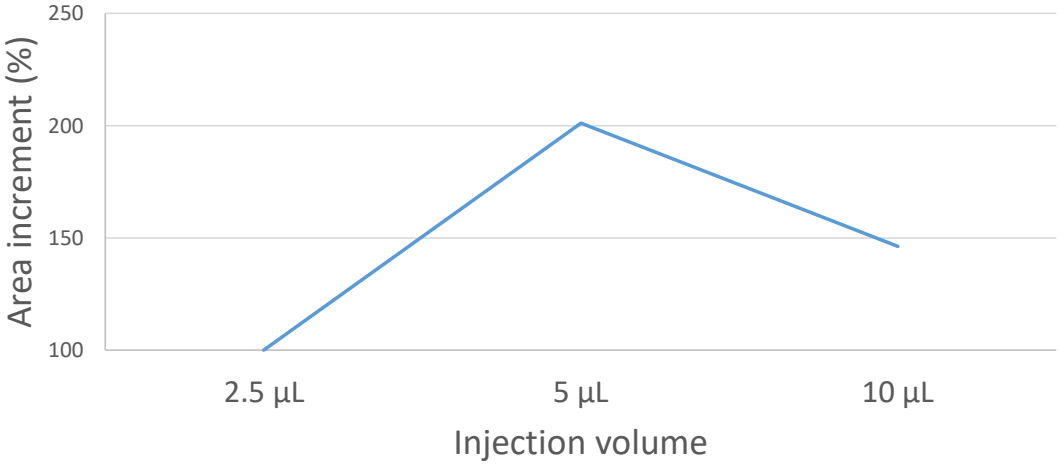




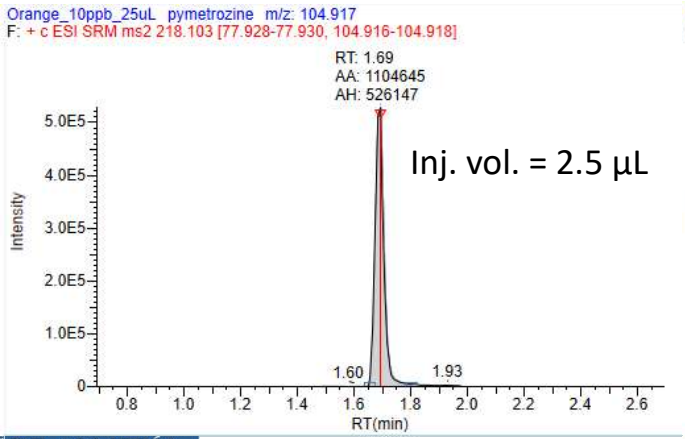
Sensitive triple quadrupole MS: reduction of injection volume

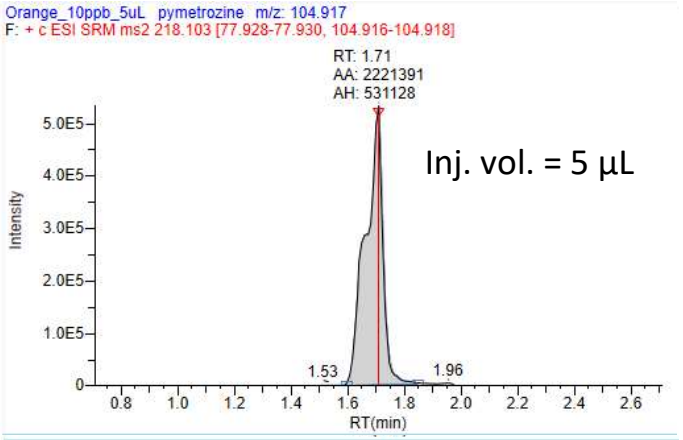
Pymetrozine

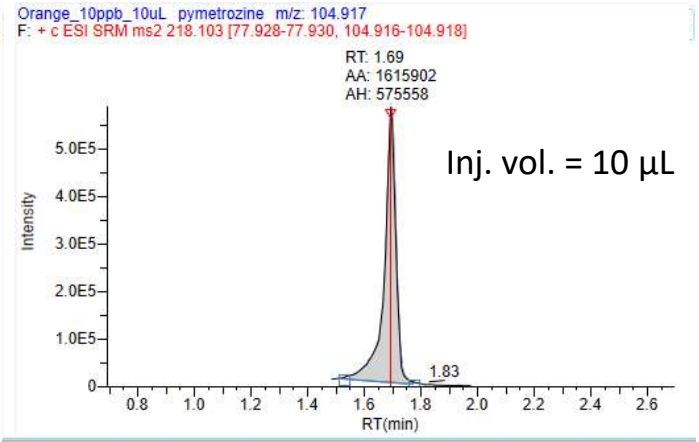
Retention time = 1.69 min



Peak splitting: compound **OUT** of acquisition window





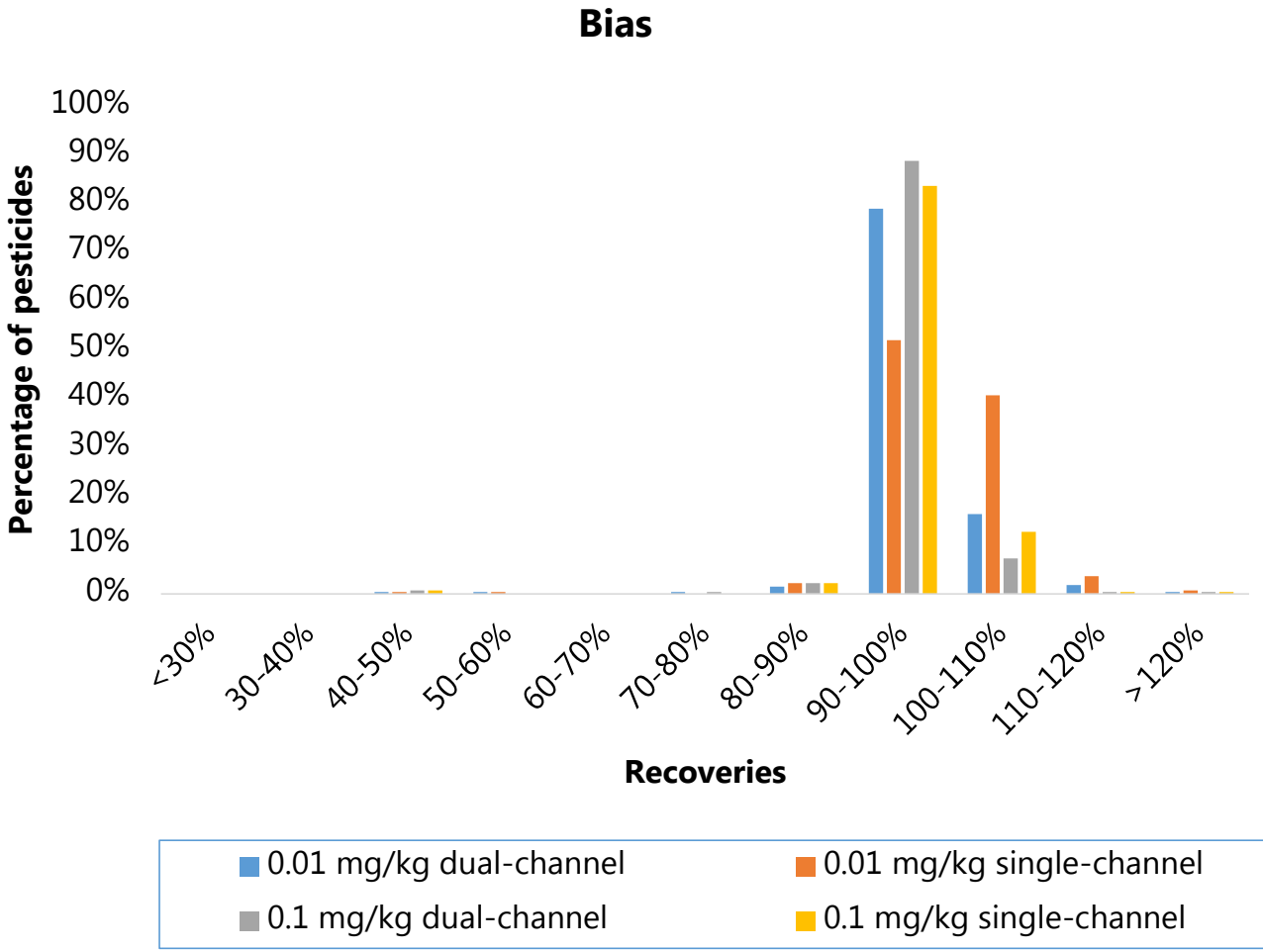


Single and Dual-Channel validation

- Validation experiments were performed employing **single channel** and **Dual-Channel**
- A total of **273 LC-amenable pesticide residues** were evaluated
- **Three matrices** belonging to two different commodity groups were studied
- Samples were extracted employing citrate-buffered QuEChERS method
- Validation criteria as per the Document Nº SANTE/12682/2019

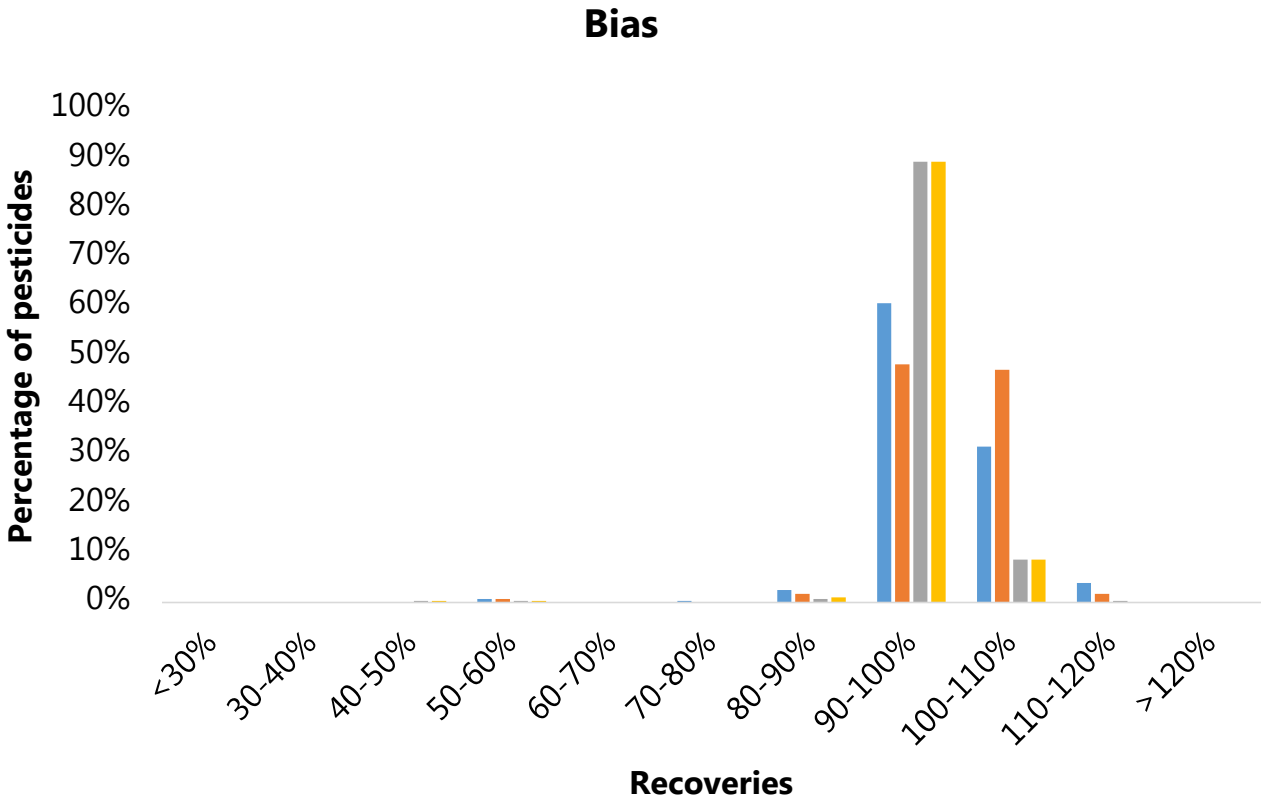
Single and Dual-Channel validation: apple

Technique	<70%	70-120%	>120%
Single channel 0.01 mg/kg	2	269	2
Dual-Channel 0.01 mg/kg	2	270	1
Single channel 0.1 mg/kg	2	270	1
Dual-Channel 0.1 mg/kg	2	270	1



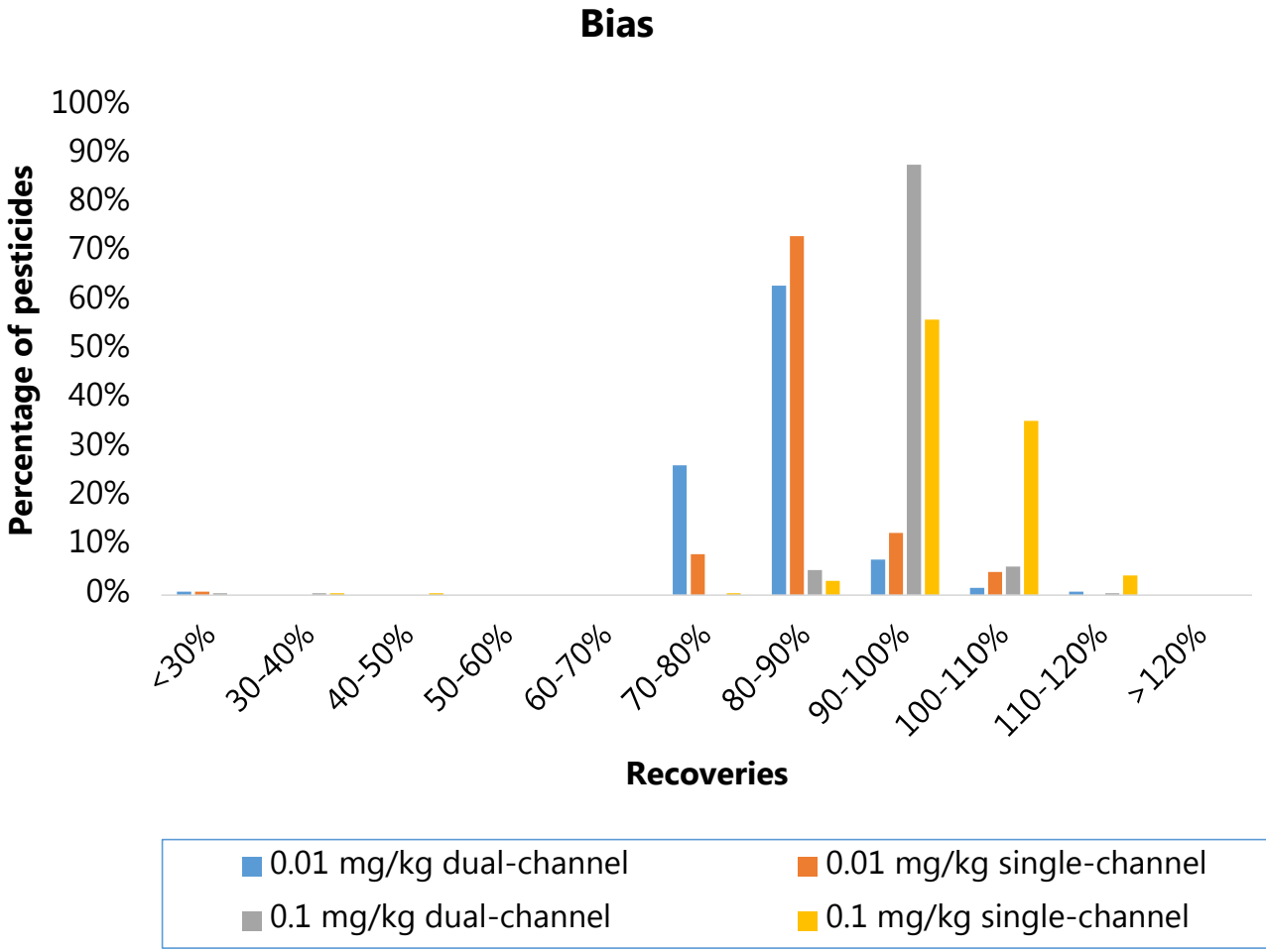
Single and Dual-Channel validation: bell pepper

Technique	<70%	70-120%	>120%
Single channel 0.01 mg/kg	2	271	-
Dual-Channel 0.01 mg/kg	2	271	-
Single channel 0.1 mg/kg	2	271	-
Dual-Channel 0.1 mg/kg	2	271	-



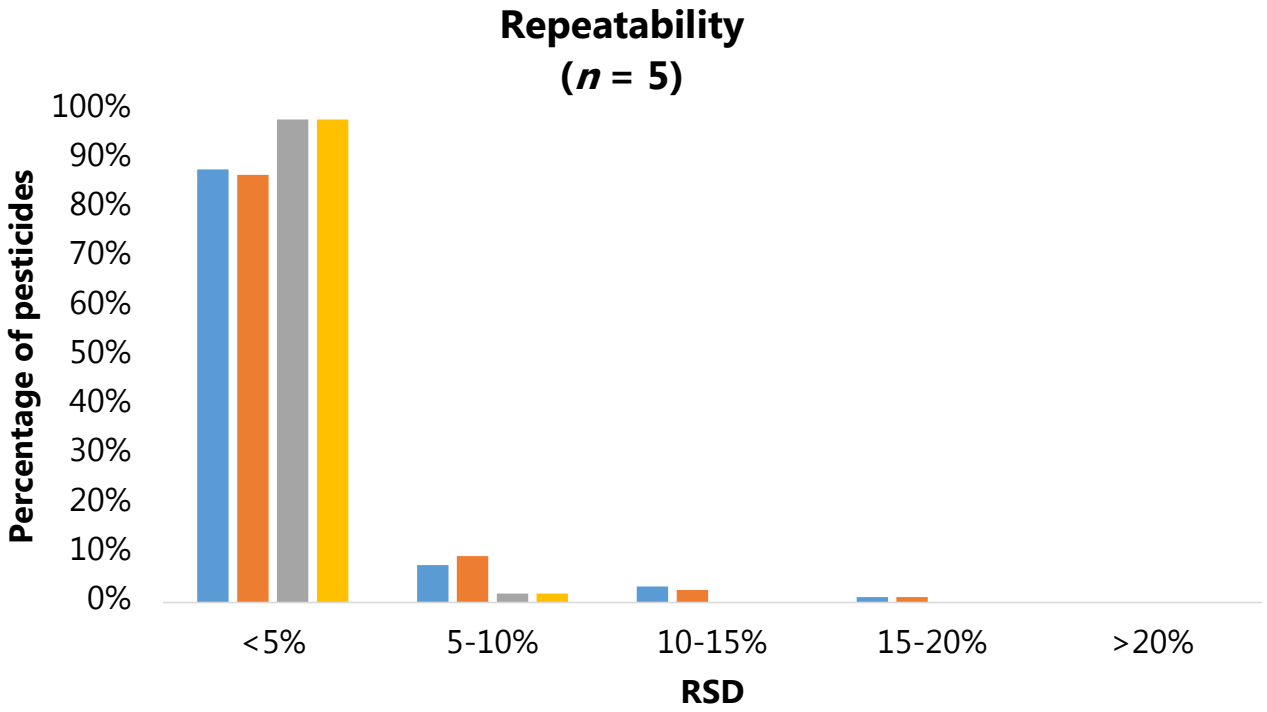
Single and Dual-Channel validation: orange

Technique	<70%	70-120%	>120%
Single channel 0.01 mg/kg	2	271	-
Dual-Channel 0.01 mg/kg	2	271	-
Single channel 0.1 mg/kg	2	271	-
Dual-Channel 0.1 mg/kg	2	271	-



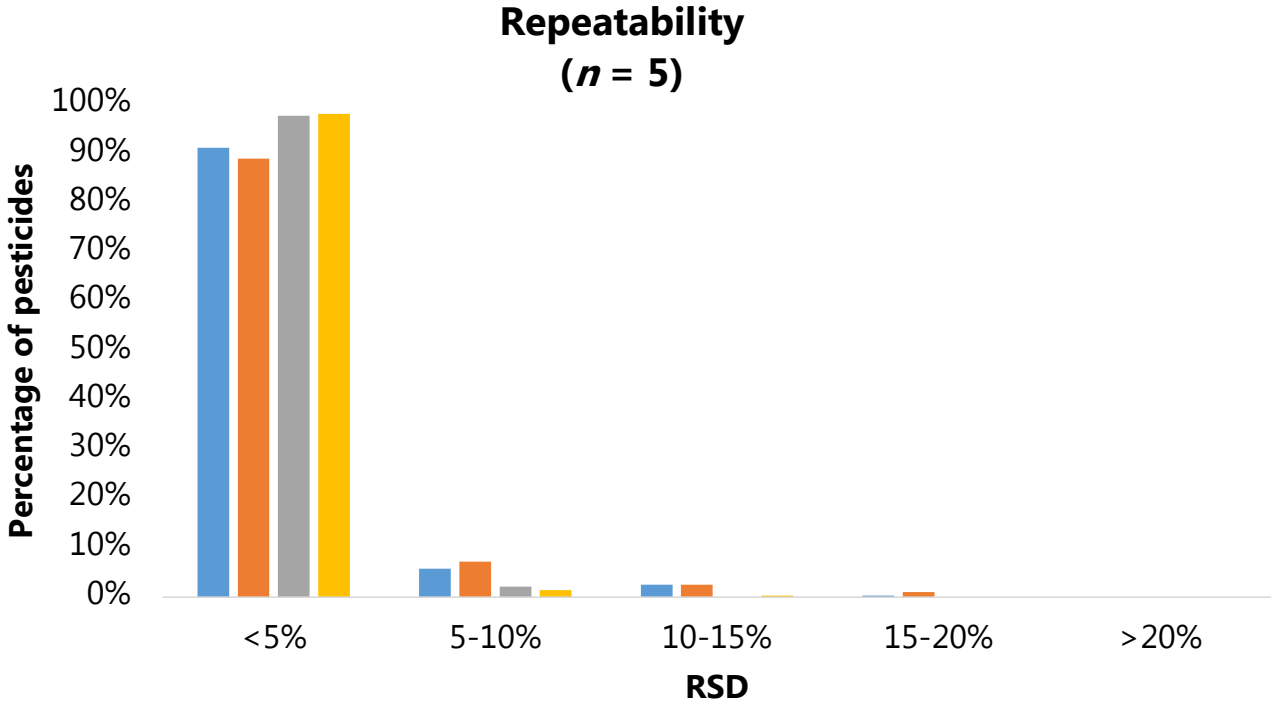
Single and Dual-Channel validation: apple

Technique	<5%	5-20%	>20%
Single channel 0.01 mg/kg	87%	13%	-
Dual-Channel 0.01 mg/kg	88%	12%	-
Single channel 0.1 mg/kg	98%	2%	-
Dual-Channel 0.1 mg/kg	98%	2%	-



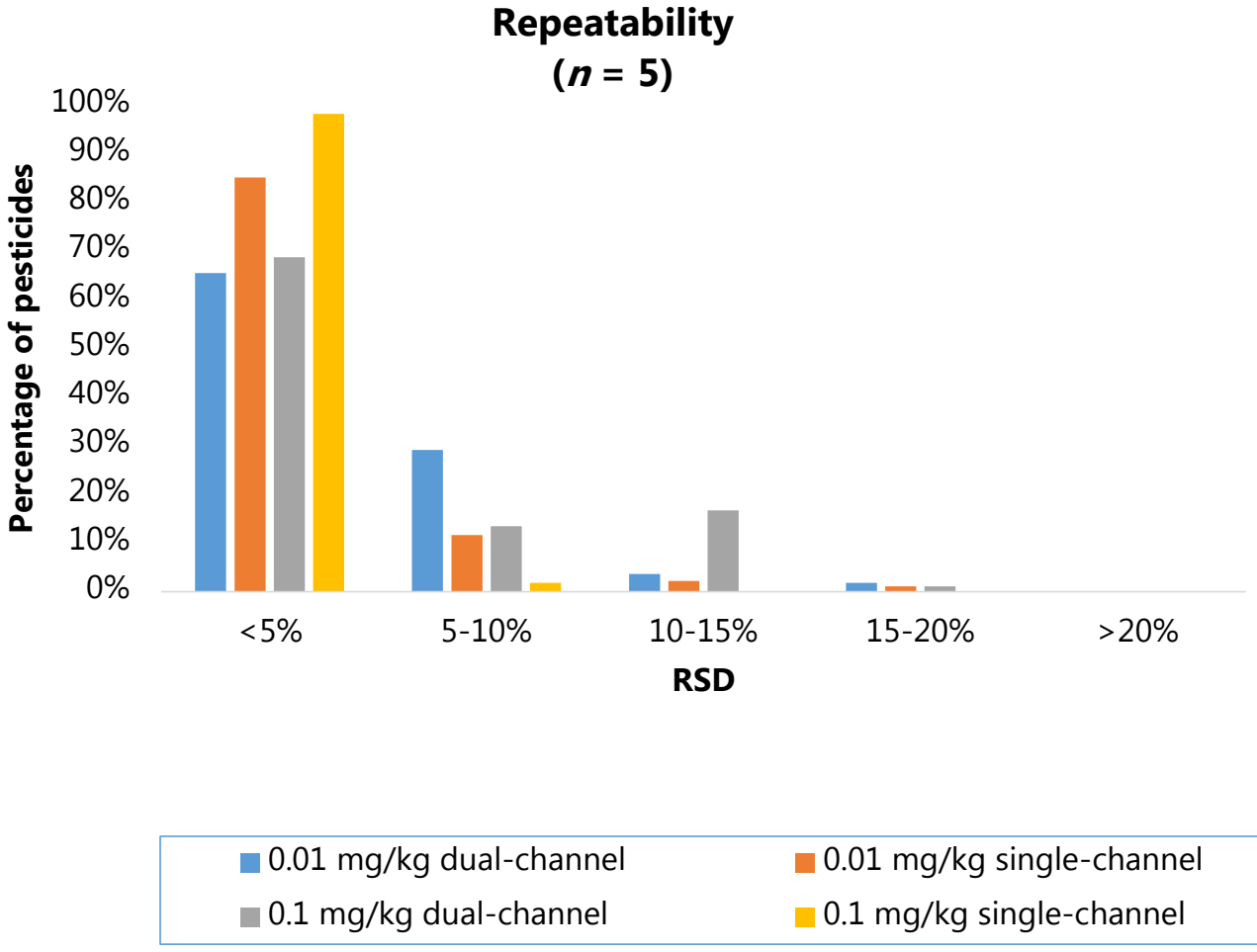
Single and Dual-Channel validation: bell pepper

Technique	<5%	5-20%	>20%
Single channel 0.01 mg/kg	89%	11%	-
Dual-Channel 0.01 mg/kg	91%	9%	-
Single channel 0.1 mg/kg	98%	2%	-
Dual-Channel 0.1 mg/kg	98%	2%	-



Single and Dual-Channel validation: orange

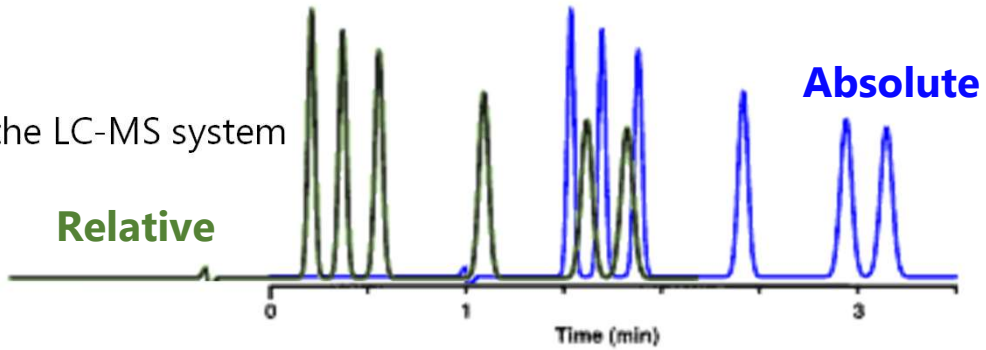
Technique	<5%	5-20%	>20%
Single channel 0.01 mg/kg	85%	15%	-
Dual-Channel 0.01 mg/kg	65%	35%	-
Single channel 0.1 mg/kg	98%	2%	-
Dual-Channel 0.1 mg/kg	69%	31%	-



Dual-Channel LC-MS/MS: retention time

- Retention time is **measured differently** in single channel compared to Dual-Channel

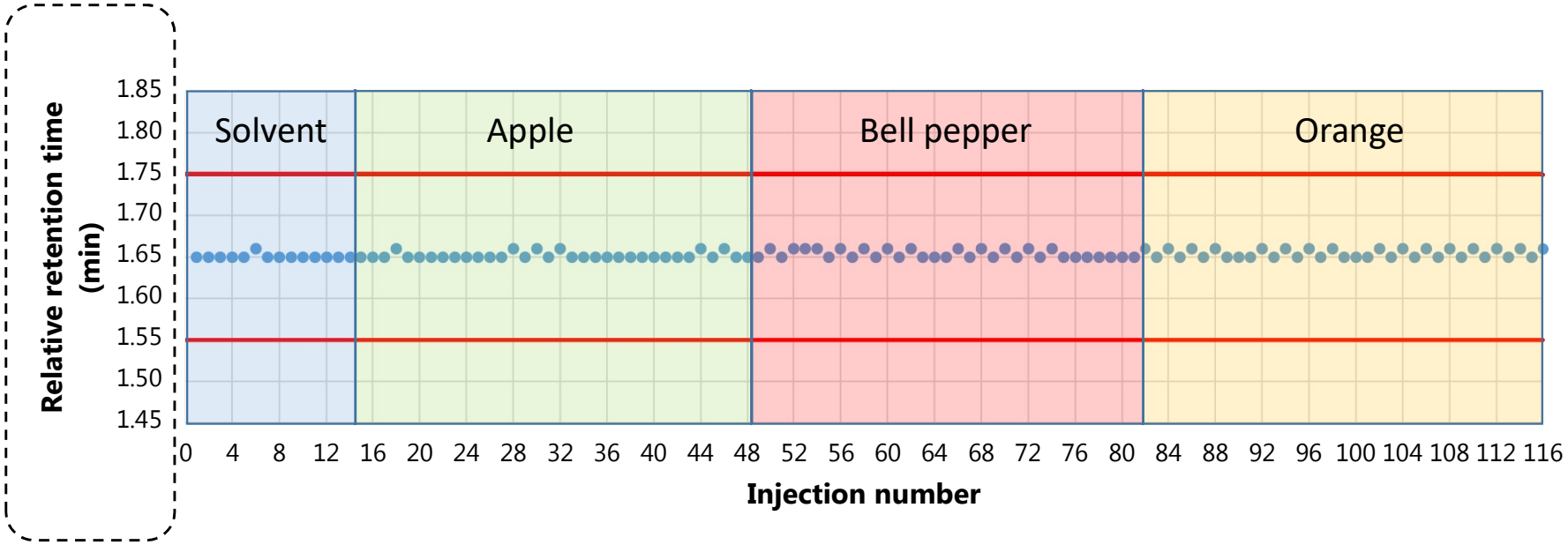
- **Single channel:**
  - Sample injection → 0.0 min
  - Data window beginning → 0.0 min
  - Retention time (absolute) → time an analyte spends on the LC-MS system



- **Dual-Channel**:
  - Sample injection → 0.0 min
  - Data window beginning → > 0.0 min
  - Retention time (relative) → time an analyte spends on the LC-MS system since the start of the data window

- The chromatographic process is the same in both cases

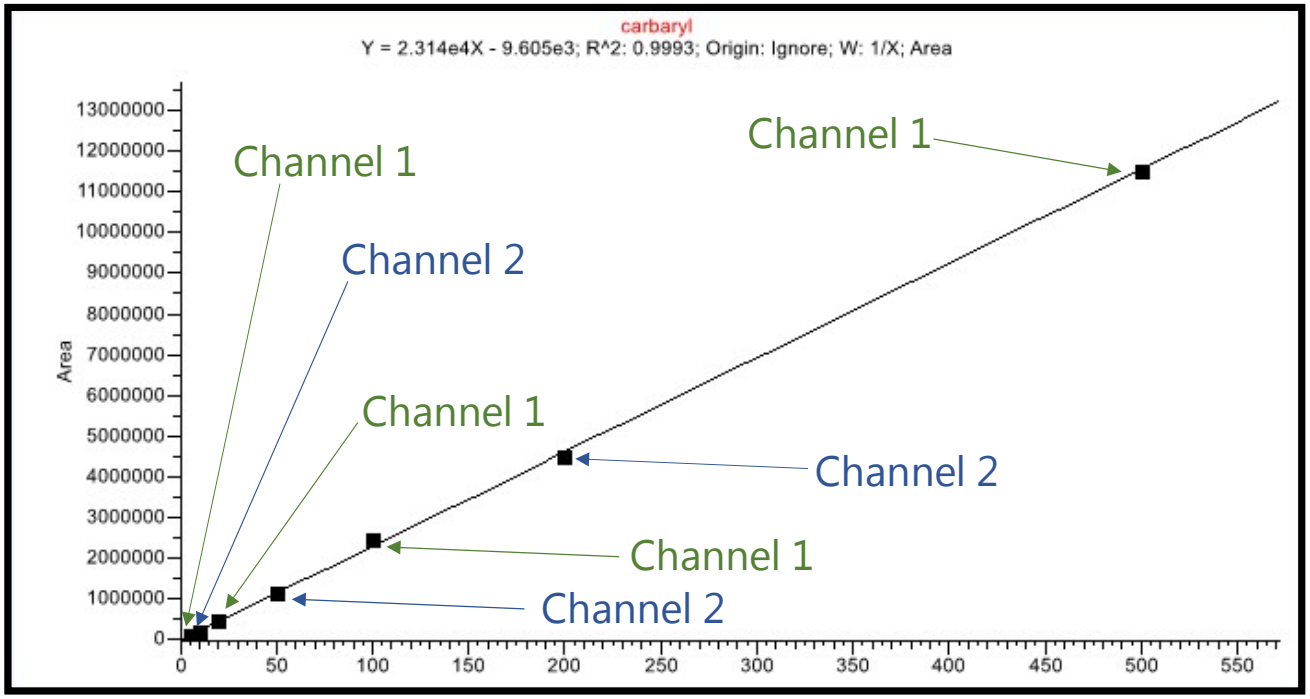
Dual-Channel LC-MS/MS: retention time stability



Retention time stability of pymetrozine. A sequence of 116 injections, alternate injections on channel 1 and channel 2. Red horizontal lines represent the ±0.1 min tolerance specified in the DG SANTE Document.

Dual-Channel LC-MS/MS: (cross-channel) calibration

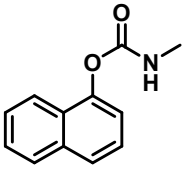
- Calibration standards can be injected using one channel, two channels, or either channel



Channel 1
R^2 = 0.9995

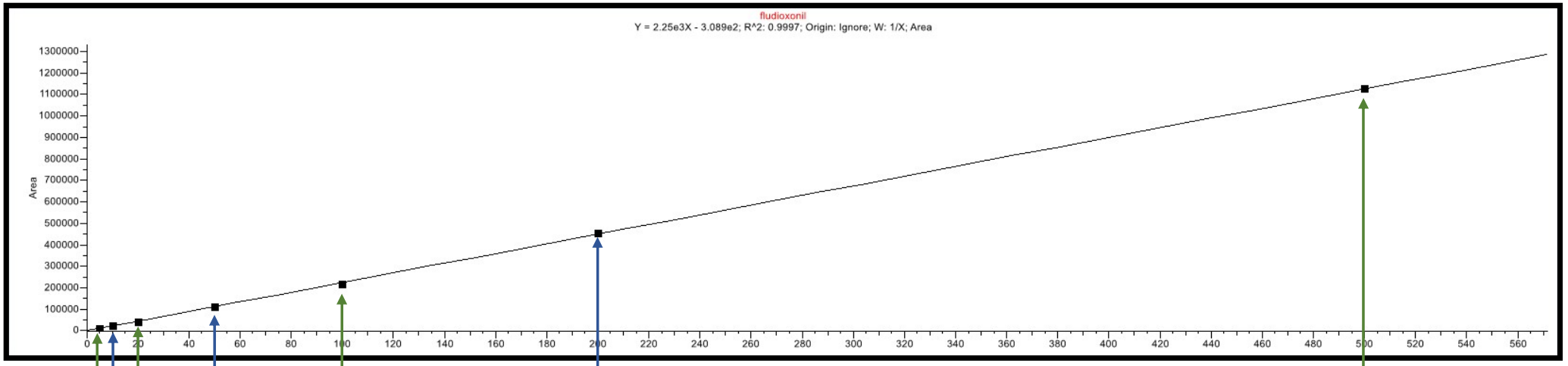
Channel 1 & 2
R^2 = 0.9993

Channel 2
R^2 = 0.9997



Carbaryl

Dual-Channel LC-MS/MS: cross-channel calibration



Channel 1

Channel 2

Channel 1

Channel 2

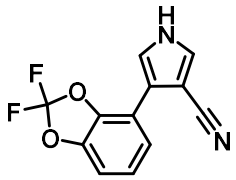
Channel 1

Channel 2

Channel 1

Channel 1 & 2

R^2 = 0.9997



Fludioxonil

Dual-Channel LC-MS/MS: proficiency test samples

EUPT-FV 17
(broccoli)

Z-score

Compound	In-channel calibration/sample injected on channel 1	In-channel calibration/sample injected on channel 2	Cross-channel calibration/sample injected on channel 1	Cross-channel calibration/sample injected on channel 2
Bupirimate	0.2	0.1	0.2	0.2
Carbendazim	0.0	0.1	0.0	0.0
Diazinon	0.5	0.0	0.5	0.0
Difenoconazole	0.2	0.4	0.4	0.2
Diflubenzuron	0.2	0.2	0.1	0.3
Methoxyfenozide	0.7	1.0	0.8	0.9
Pendimethalin	0.5	0.1	0.6	0.1
Permethrin	0.7	0.7	0.6	1.0
Spinosad	0.6	0.6	0.1	0.0
Thiabendazole	0.5	0.4	0.5	0.4
Trifloxystrobin	0.0	0.2	0.3	0.1

Cal.: Channel 1 **Sample:** Channel 1

Cal.: Channel 2 **Sample:** Channel 2

Cal.: Cross-Channel **Sample:** Channel 1

Cal.: Cross-Channel **Sample:** Channel 2

Dual-Channel LC-MS/MS: proficiency test samples

EUPT-FV 13 (mandarin)

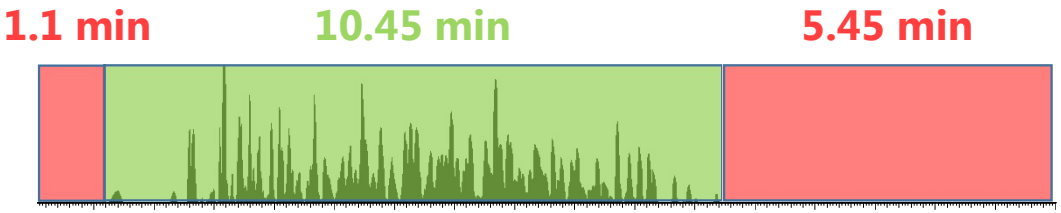
Z-score

Compound	In-channel calibration/sample injected on channel 1	In-channel calibration/sample injected on channel 2	Cross-channel calibration/sample injected on channel 1	Cross-channel calibration/sample injected on channel 2
Carbendazim	0.7	0.7	0.7	0.6
Chlorpyrifos	0.5	0.6	0.4	0.5
Diazinon	0.6	0.7	0.6	0.7
EPN	0.1	0.2	0.0	0.3
Imazalil	0.1	0.1	0.1	0.1
Indoxacarb	0.7	0.5	0.7	0.5
Malathion	0.6	0.5	0.5	0.5
Methidathion	0.4	0.3	0.4	0.3
Methomyl	0.2	0.2	0.2	0.3
Oxamyl	1.3	1.6	1.4	1.6
Pendimethalin	0.2	0.3	0.2	0.3
Phosalone	0.7	0.8	0.6	0.7
Prochloraz	0.7	0.8	0.8	0.9
Pyriproxifen	0.5	0.4	0.4	0.3
Spinosad	0.7	0.6	0.8	0.6
Thiabendazole	0.1	0.2	0.1	0.3

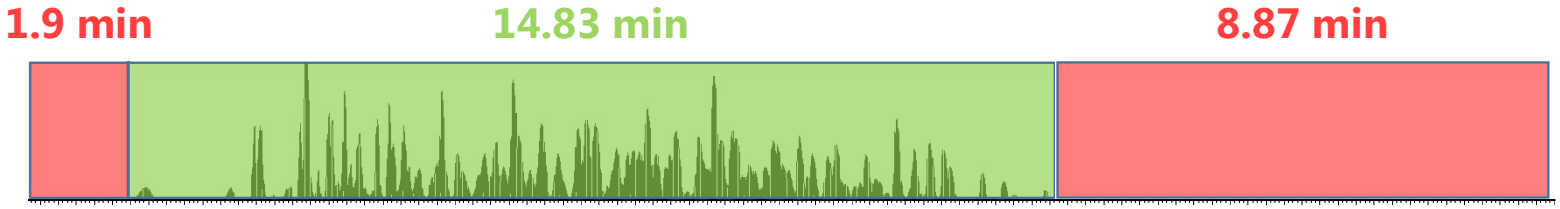
Dual-Channel LC-MS/MS: increased column length

- Chromatographic columns of **100 mm** and **150 mm** in length were compared
- **Remaining properties** were kept identical (porosity, particle size, type)
- 1.5x length → 1.5x increase in each **gradient** step
- **Elution time** also increased 1.5x, 14 min → 21 min
- **Data window** 14.83 min (TSQ Altis) and 15.85 min (Thermo Scientific™ Q Exactive™ Focus system)
- **Longer analysis** time of longer columns compensated by Dual-Channel time savings

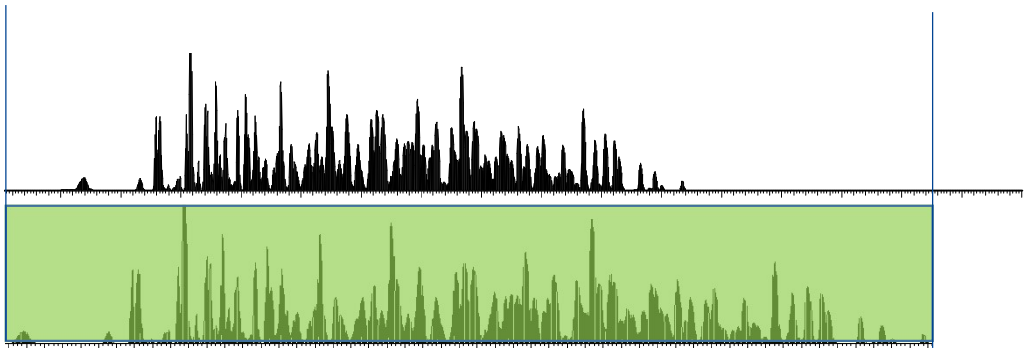
Dual-Channel LC-MS: increased column length



100 mm column



150 mm column



Using Dual-Channel chromatography and a 150 mm column results in shorter analysis times per sample compared to a single channel analysis on a 100 mm column

The use of a longer column results in improved separation, increasing selectivity and sensitivity without compromising analysis time

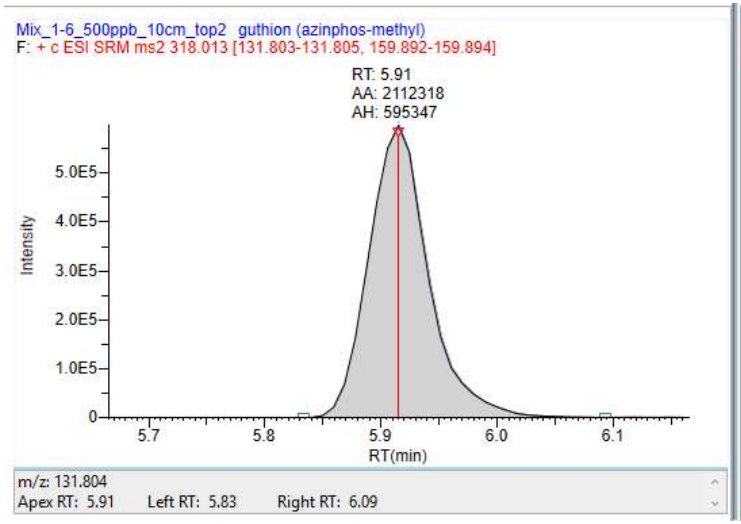
Dual-Channel LC-MS/MS: solving analyte coelution

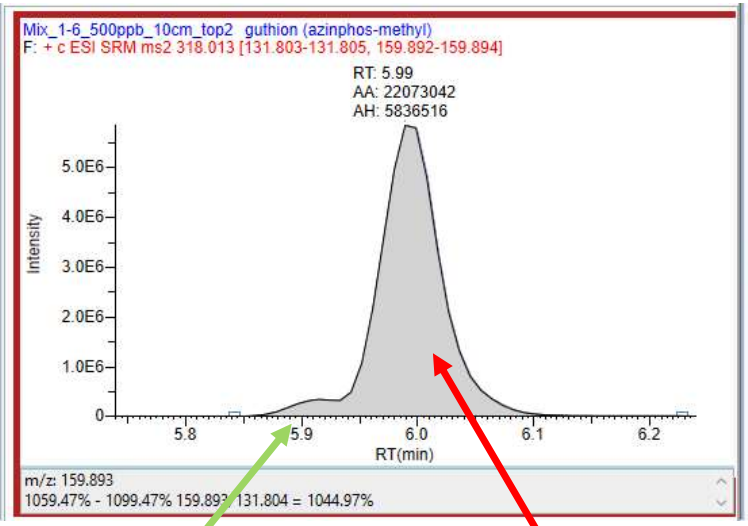
Azinphos methyl & phosmet coelution

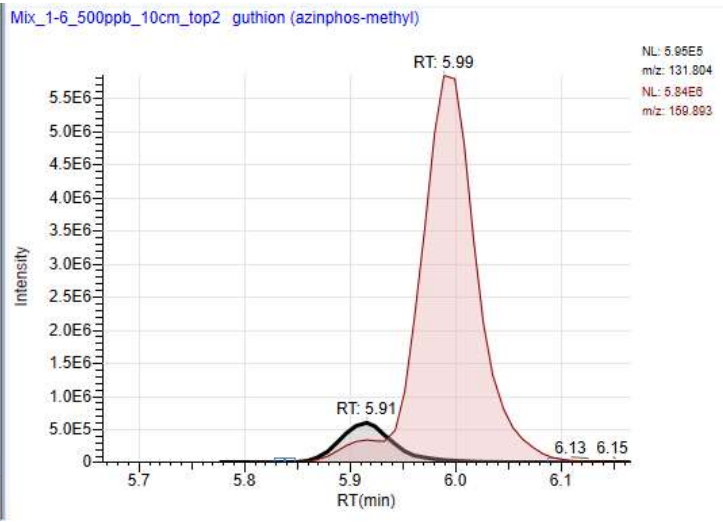
TSQ Altis
Triple quadrupole
100 mm column

***m/z* 318 -> 132**

***m/z* 318 -> 159**







Azinphos methyl

Interfering transition of phosmet

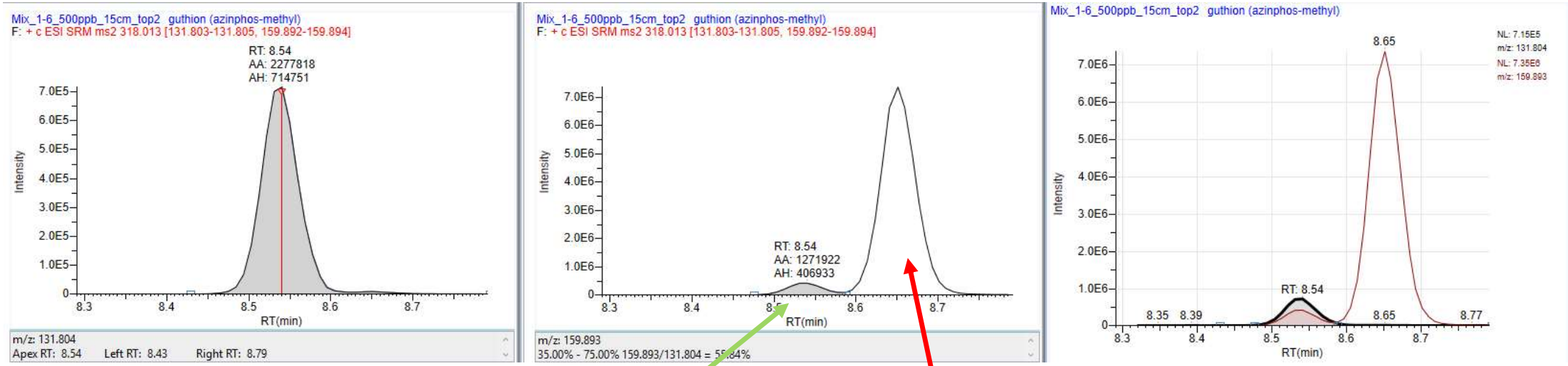
Dual-Channel LC-MS/MS: solving analyte coelution

Azinphos methyl & phosmet coelution

TSQ Altis
Triple quadrupole
150 mm column

***m/z* 318 -> 132**

***m/z* 318 -> 159**

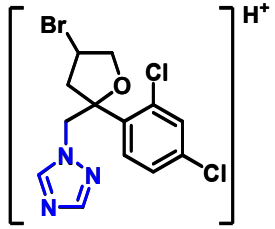


Azinphos methyl

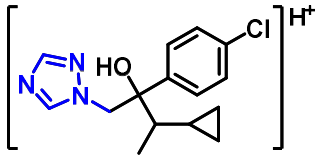
Phosmet is separated from azinphos methyl

Dual-Channel LC-HRMS: increased column length

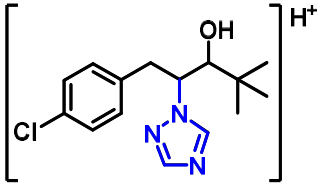
Bromuconazole (first peak)
Full scan MS
375.9614 ± 5 ppm



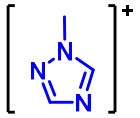
Cyproconazole (first peak)
Full scan MS
292.1211 ± 5 ppm

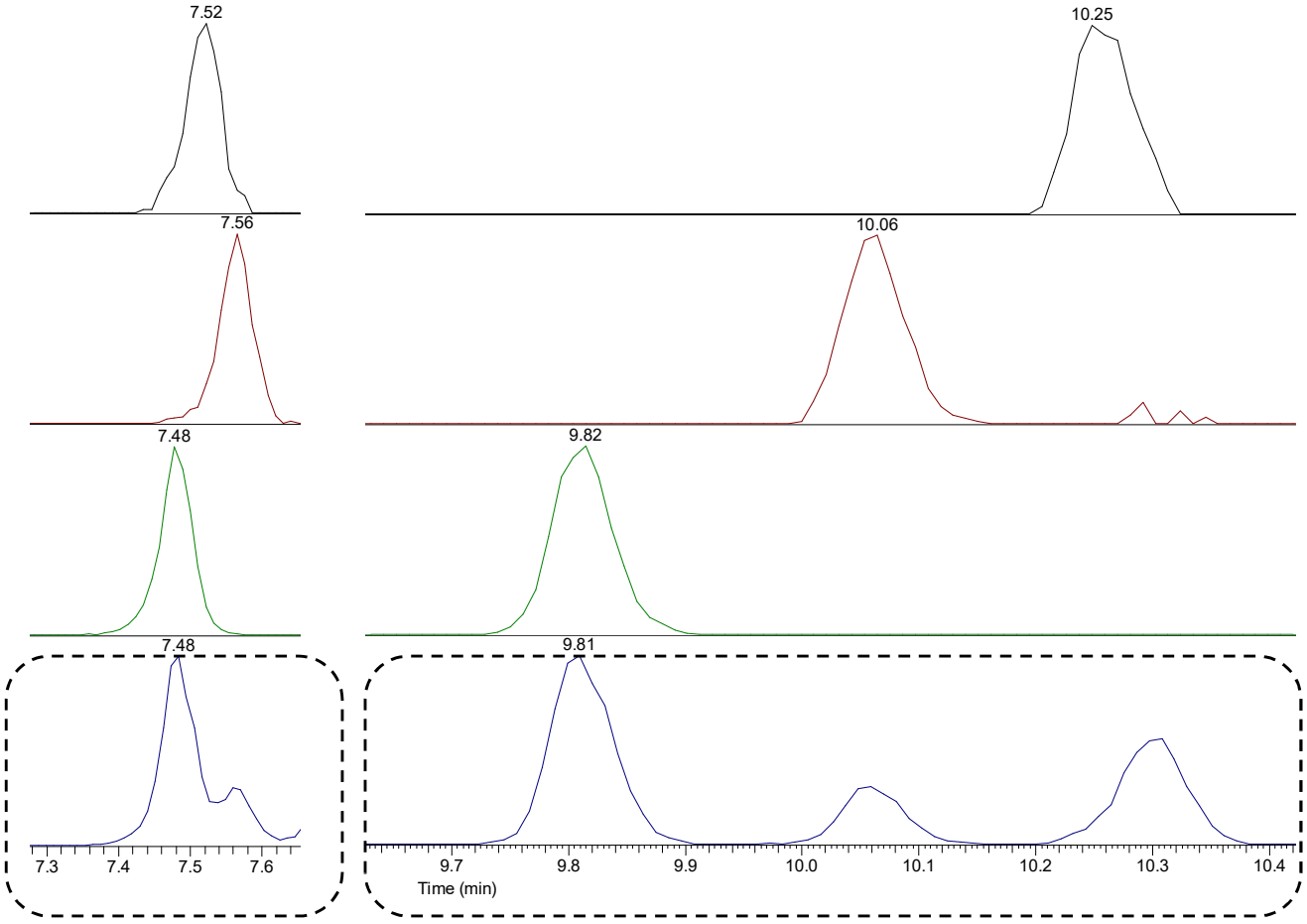


Paclobutrazol
Full scan MS
294.1368 ± 5 ppm



Common fragment ion
AIF MS²
70.03997 ± 5 ppm
(Most sensitive fragment)

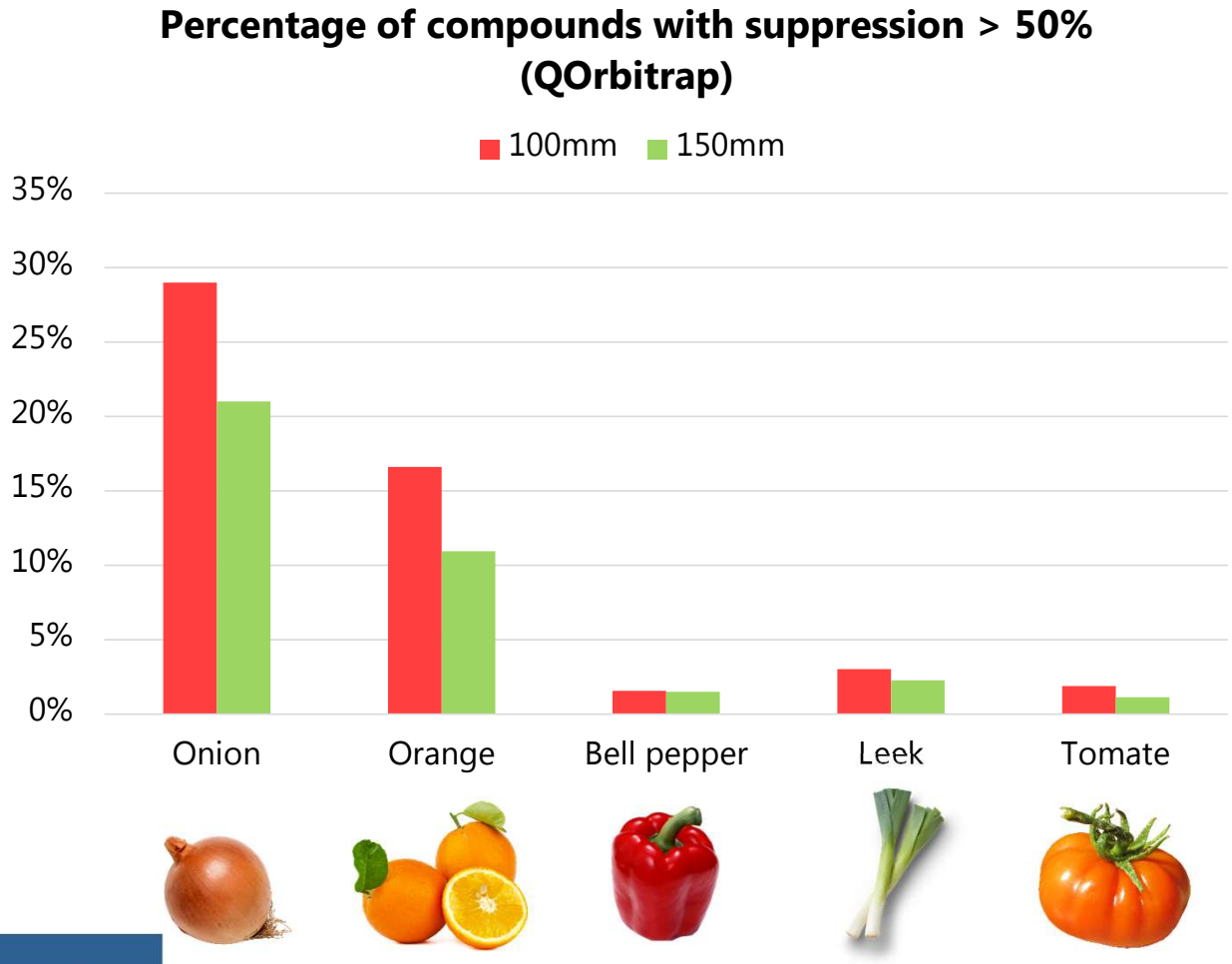




100 mm column

150 mm column

Dual-Channel LC-HRMS: increased column length



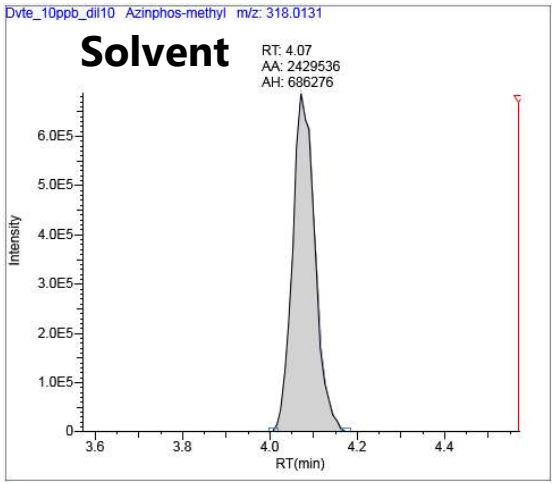
$$ME(\%) = \frac{slope\ in\ solvent - slope\ in\ matrix}{slope\ in\ matrix} \cdot 100$$

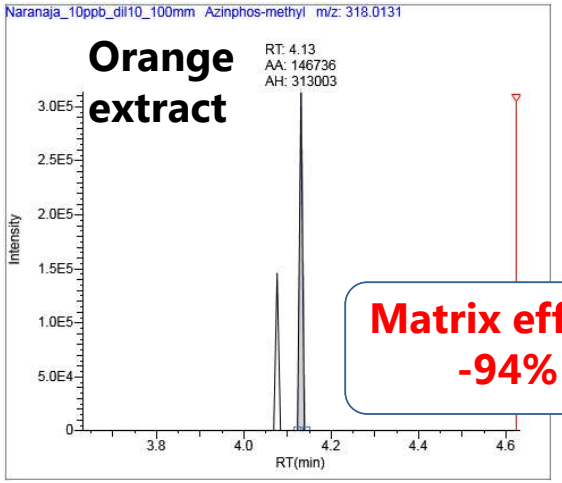
- Matrix effects were more pronounced on QOrbitrap than in TSQ
- Reducing ion suppression achieves greater sensitivity and better quantitation
- Particularly important for matrix-matched calibration

Dual-Channel LC-HRMS: increased column length

QExactive Focus
High-resolution MS
100 mm column

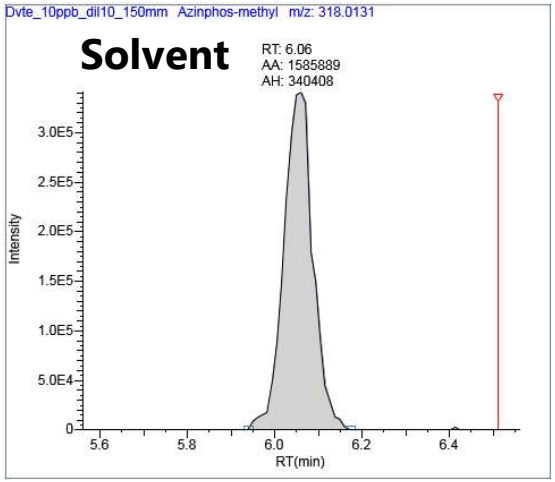
0.01 mg/kg of azinphos-methyl
Full Scan MS
***m/z* 318.0131 ± 5 ppm**

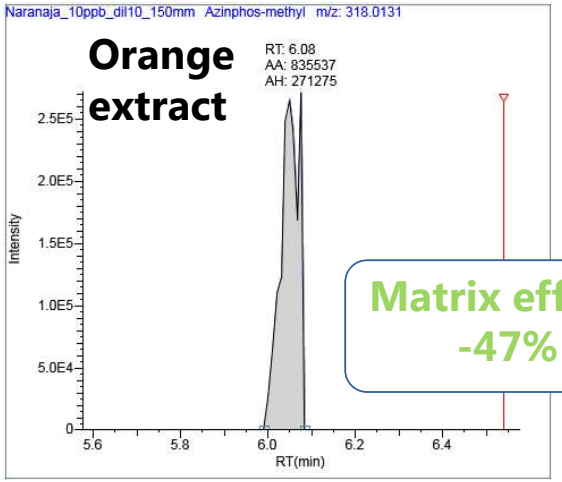




Matrix effects -94%

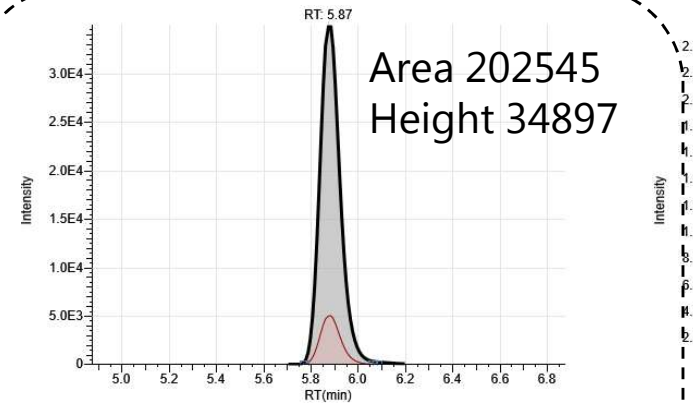
QExactive Focus
High-resolution MS
150 mm column



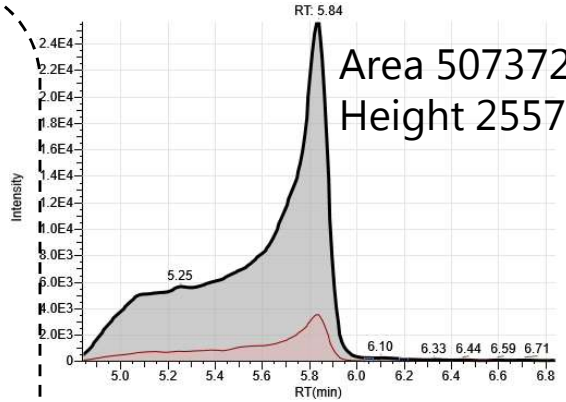


Matrix effects -47%

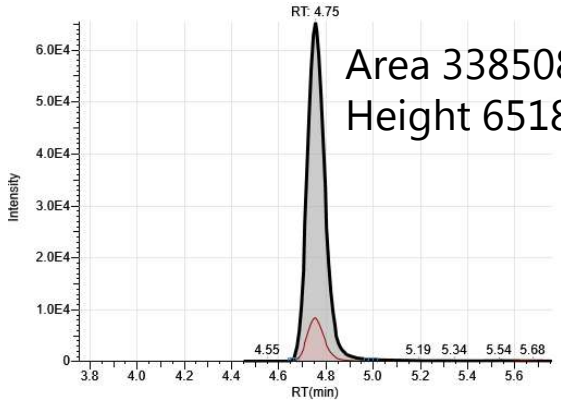
Dual-Channel LC-MS/MS: Mobile phase optimization- negative mode



Water/MeOH/formic acid/ammonium formate



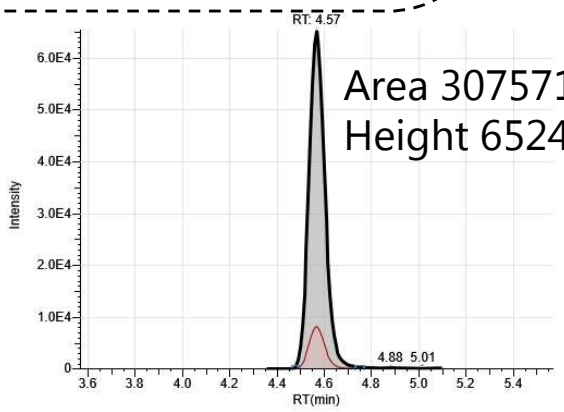
Water/AcN



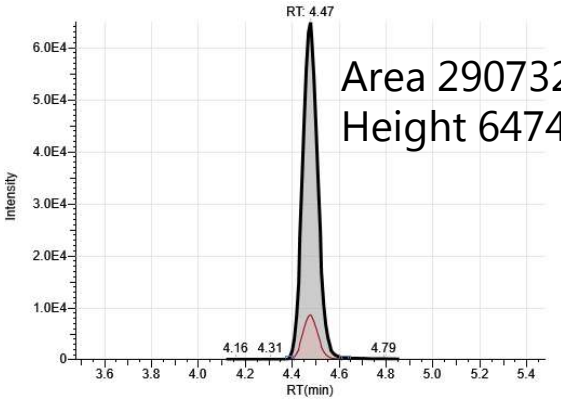
Water/AcN + 0.01 % acetic acid

Gradient 1

Gradient 2

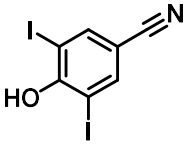


Water/AcN + 0.02 % acetic acid



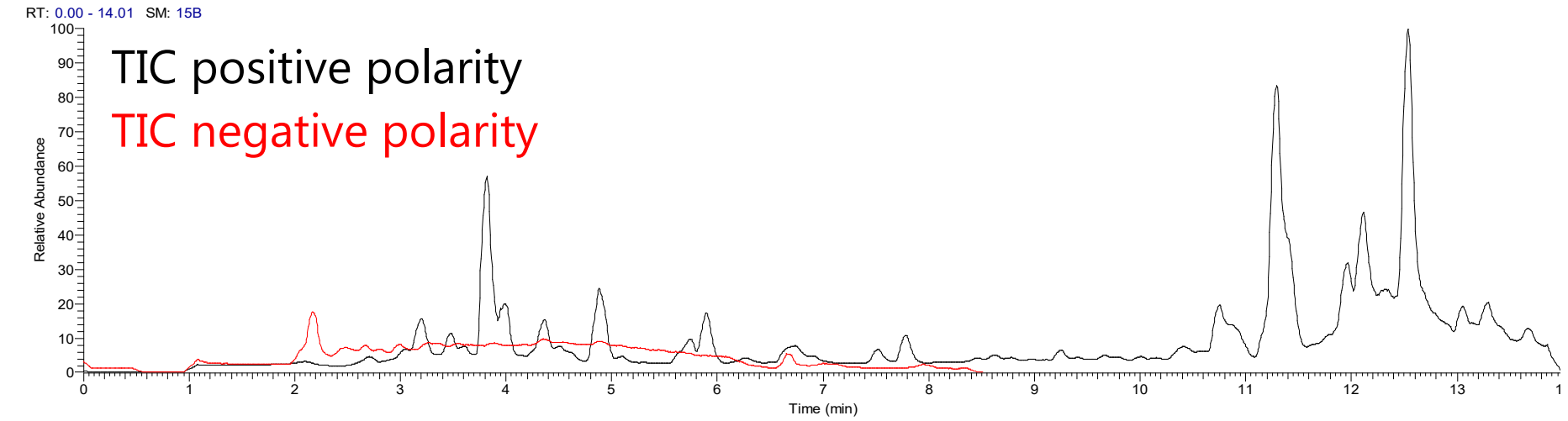
Water/AcN + 0.05 % acetic acid

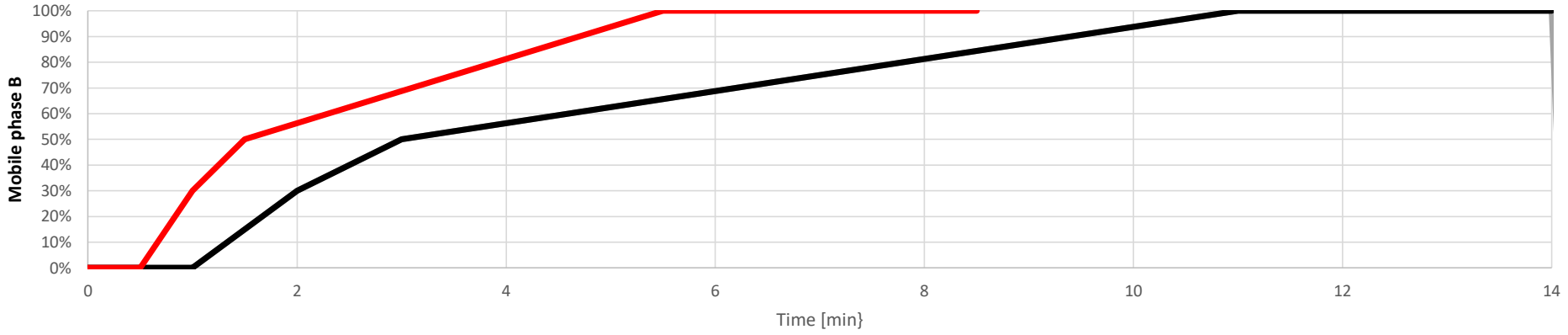
Ioxynil



Dual-Channel LC-MS/MS: total ion chromatograms

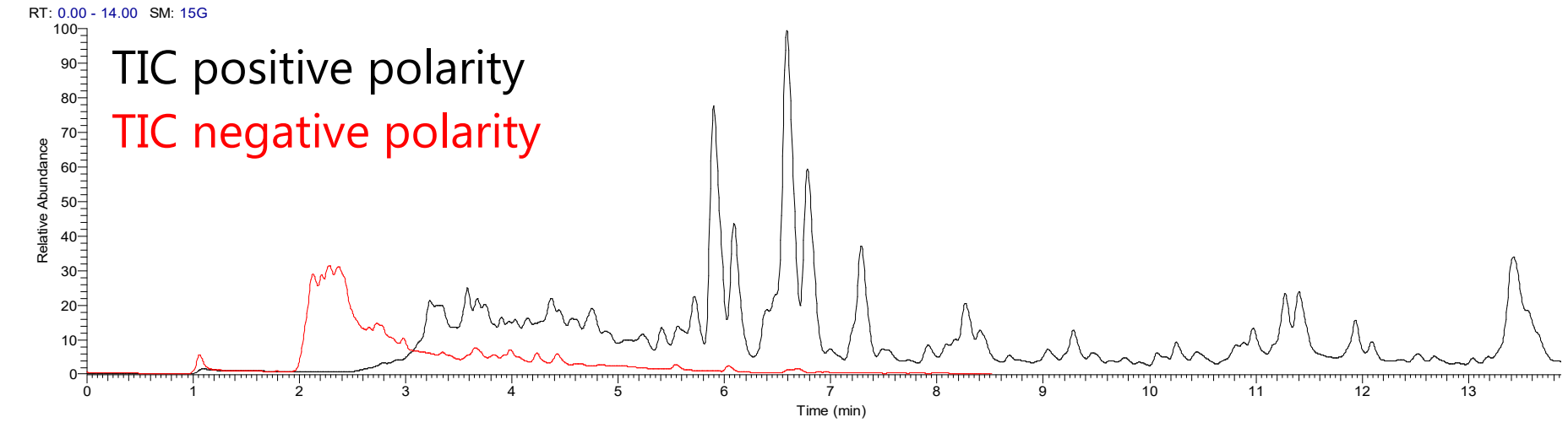
Tomato

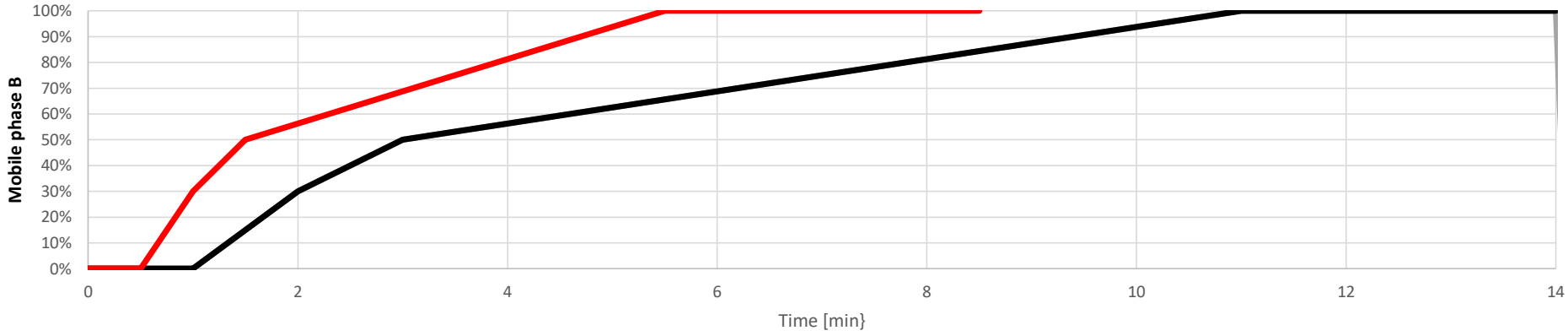




Dual-Channel LC-MS/MS: total ion chromatograms

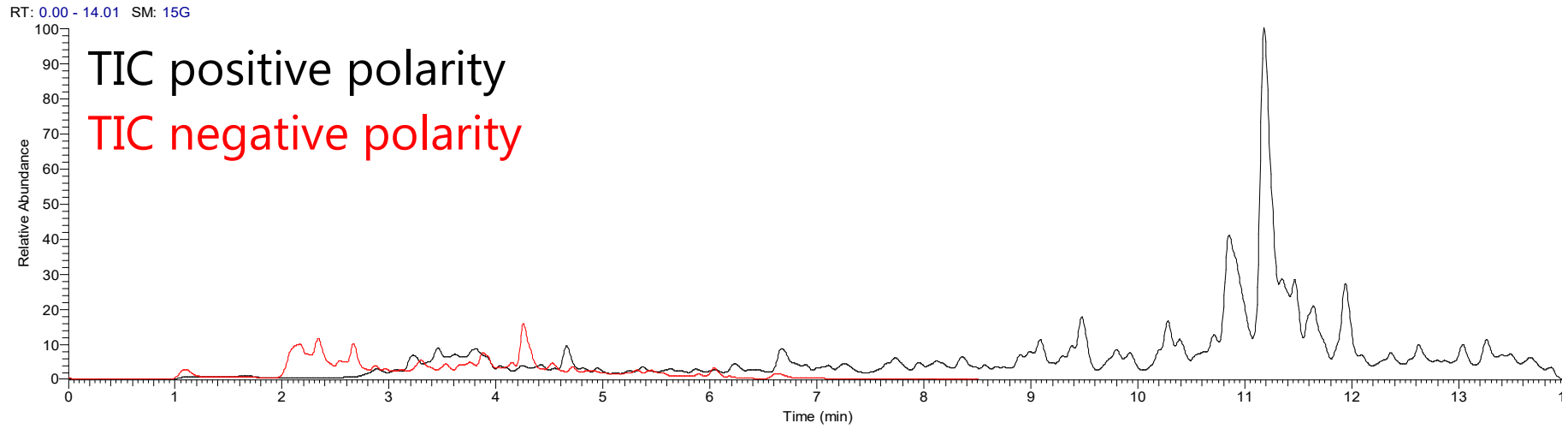
Orange

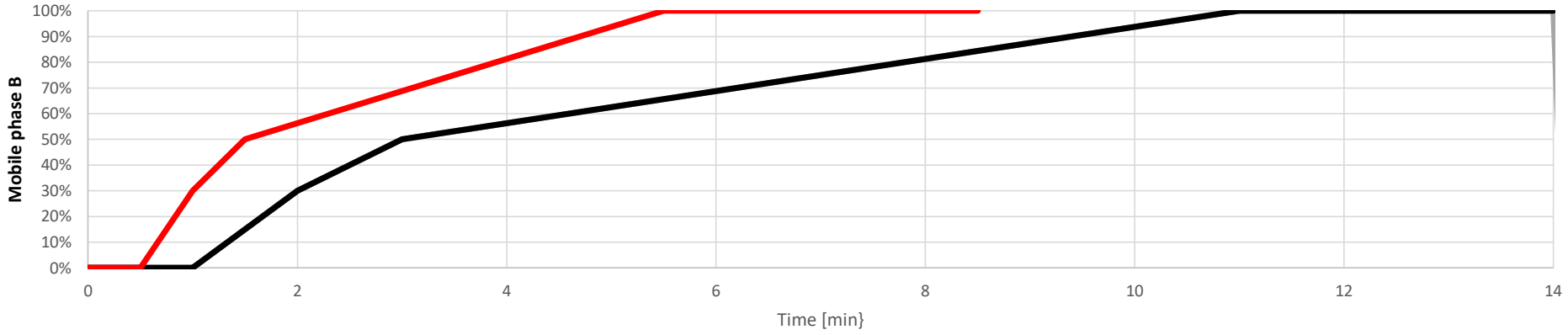




Dual-Channel LC-MS/MS: total ion chromatograms

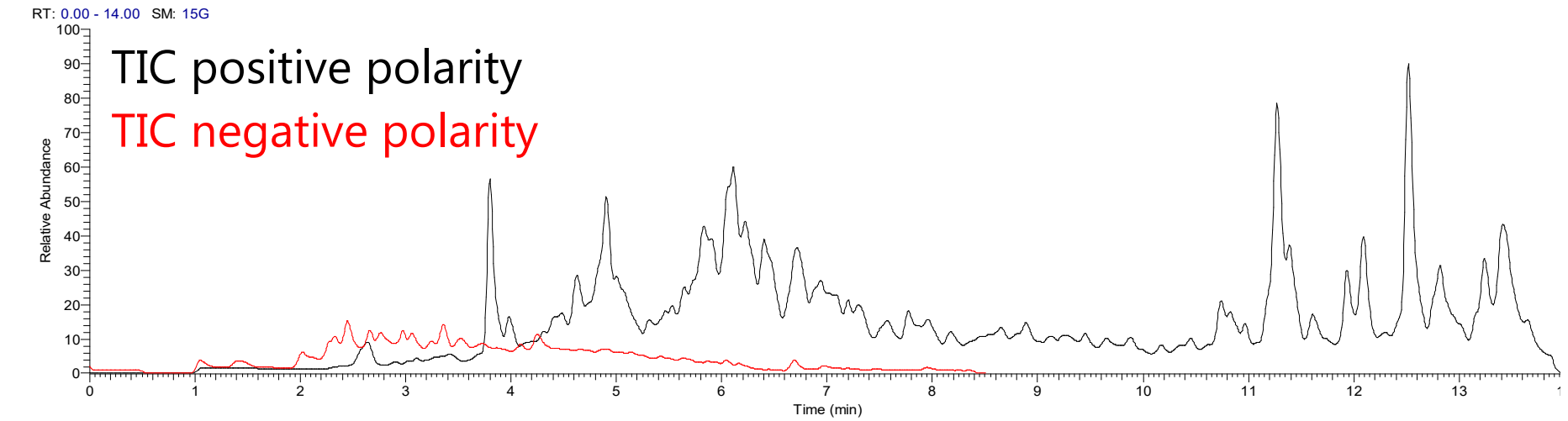
Avocado

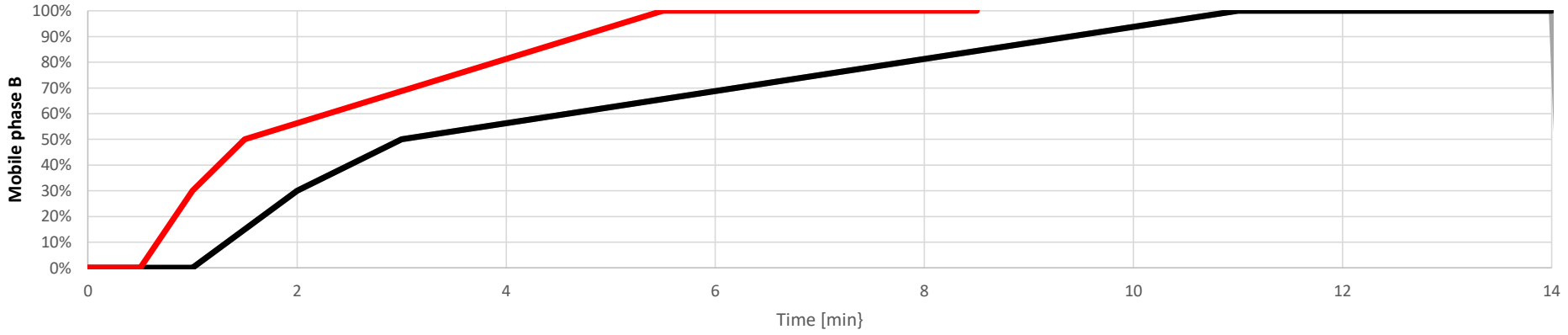




Dual-Channel LC-MS/MS: total ion chromatograms

Onion

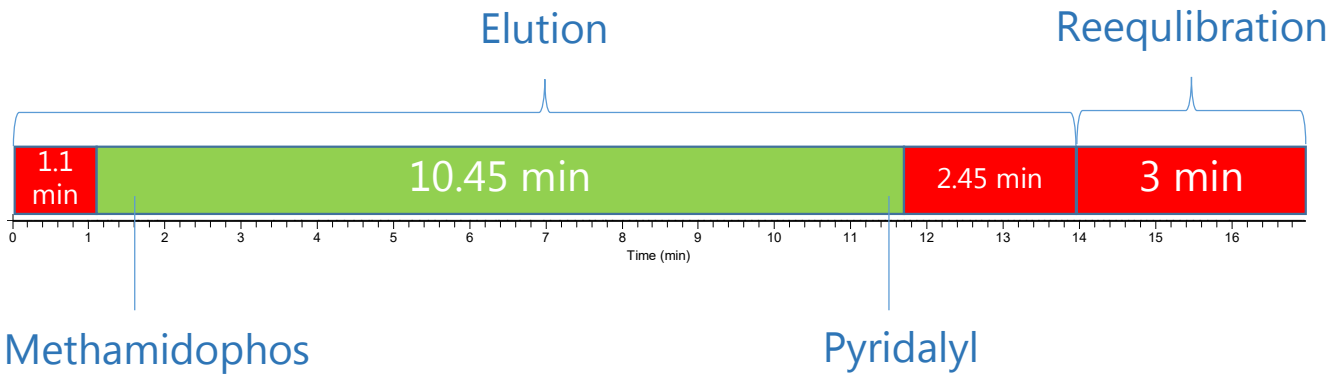




Dual-Channel LC-MS/MS: independent mobile phases

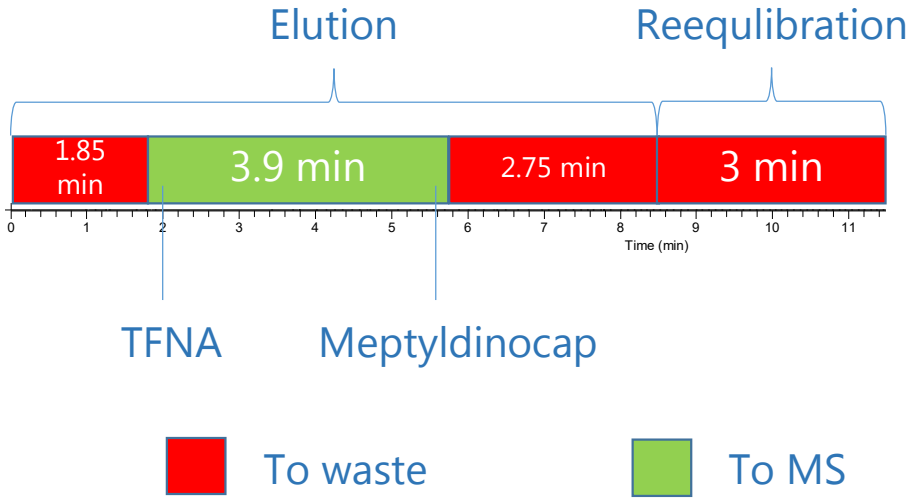
Gradient 1

Water:MeOH
Formic acid (0.1 %)
Ammonium formate (5 mM)

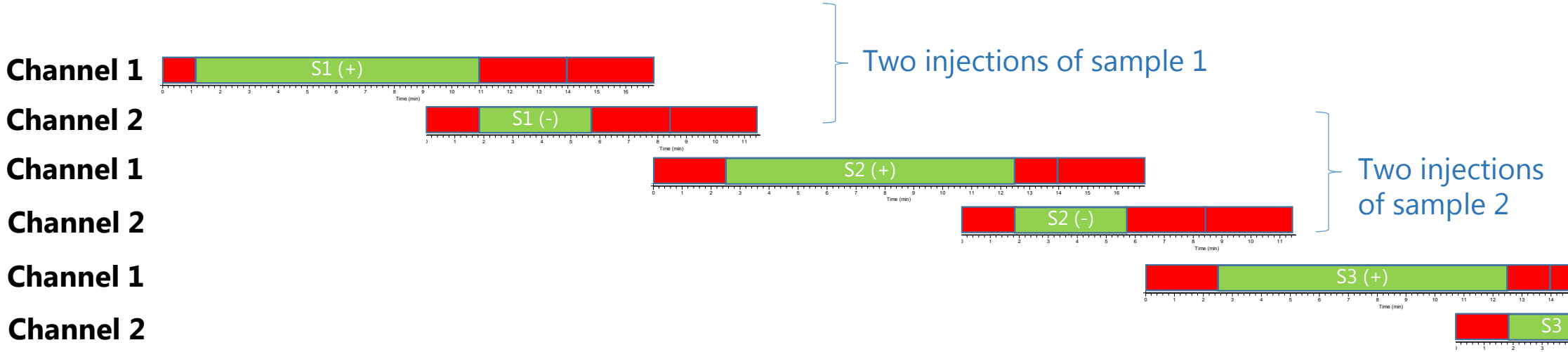


Gradient 2

Water:AcN
Acetic acid (0.05 %)

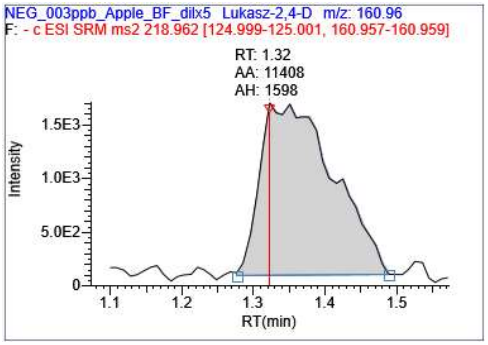


Dual-Channel LC-MS/MS: independent mobile phases

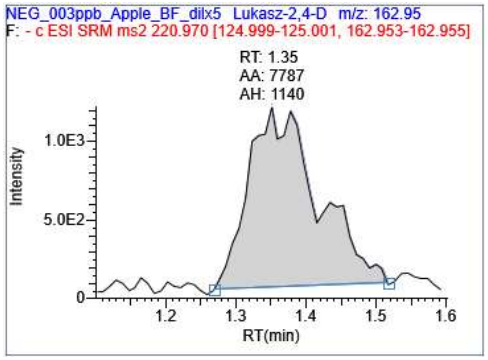


On a single channel instrument with polarity switching only one analysis in 18 min

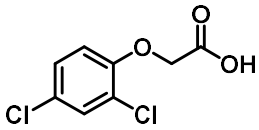
Dual-Channel LC-MS/MS: improved ionisation

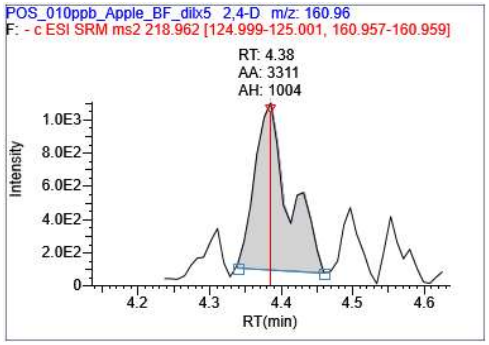


0.003 mg/kg

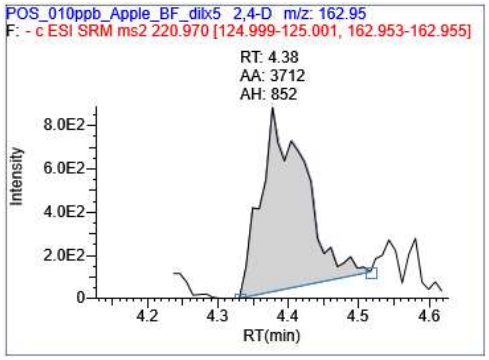


2,4-D





0.010 mg/kg



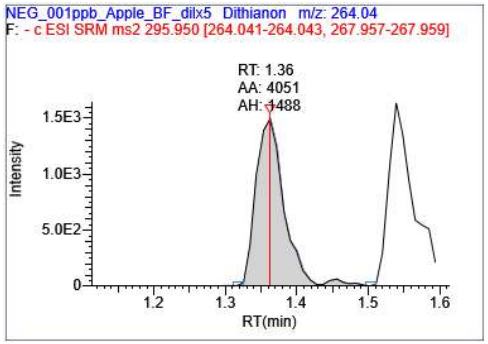
Gradient 2

Water:AcN
Acetic acid (0.05 %)

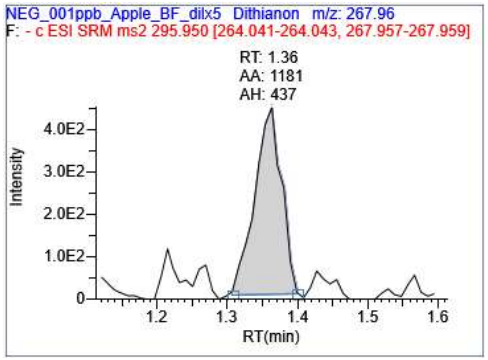
Gradient 1

Water:MeOH
Formic acid (0.1 %)
Ammonium formate (5 mM)

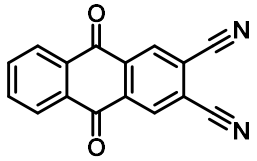
Dual-Channel LC-MS/MS: improved ionisation

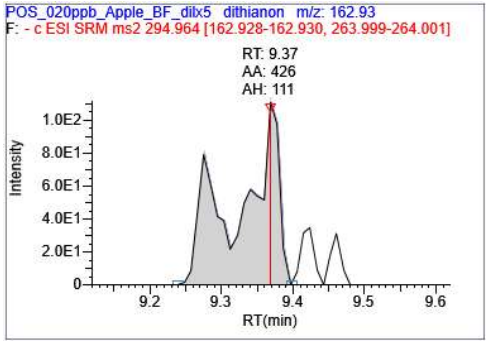


0.001 mg/kg

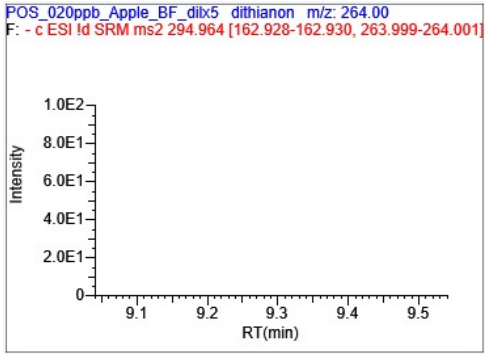


Dithianon





0.020 mg/kg

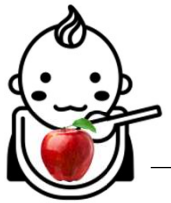


Gradient 2

Water:AcN
Acetic acid (0.05 %)

Gradient 1

Water:MeOH
Formic acid (0.1 %)
Ammonium formate (5 mM)



Dual-Channel LC-MS/MS: apple baby food validation

Gradient 1

Water:MeOH
Formic acid (0.1 %)
Ammonium formate (5 mM)

Gradient 2

Water:AcN
Acetic acid (0.05 %)

Commission Directive 2006/125/EC

Compound	Recoveries 0.003 mg/kg	RSD 0.003 mg/kg	Recoveries 0.006 mg/kg	RSD 0.006 mg/kg	Lowest calibration level (mg/kg)	Highest calibration level (mg/kg)
2,4-D	97 %	11 %	109 %	6 %	0.006	0.02
Bromacil	100 %	5 %	104 %	4 %	0.0005	0.02
Dithianon	96 %	3 %	96 %	3 %	0.0005	0.02
Diuron	103 %	3 %	101 %	2 %	0.0005	0.02
Fensulfothion	102 %	3 %	104 %	3 %	0.0005	0.02
Fensulfothion-oxon-sulfone	104 %	4 %	103 %	4 %	0.0005	0.02
Fipronil	100 %	4 %	102 %	3 %	0.0005	0.02
Fipronil-desulfinyl	101 %	2 %	100 %	2 %	0.0005	0.02
Fipronil-sulfone	103 %	2 %	108 %	2 %	0.0005	0.02
Flubendiamide	104 %	21 %	98 %	12 %	0.0005	0.02
Fludioxonil	105 %	6 %	103 %	2 %	0.0005	0.02
Haloxyfop	97 %	13 %	101 %	6 %	0.003	0.02
Hexaflumuron	94 %	5 %	104 %	18 %	0.0005	0.02
Ioxynil	103 %	2 %	103 %	6 %	0.0005	0.02
Lufenuron	108 %	26 %	105 %	30 %	0.0005	0.02
MCPA	114 %	7 %	99 %	13 %	0.003	0.02
MCPB	-	-	115 %	10 %	0.006	0.02
Meptyldinocap	86 %	14 %	118 %	26 %	0.006	0.02
(*E*)-Metaflumizone	103 %	2 %	95 %	6 %	0.0005	0.02
(*Z*)-Metaflumizone	104 %	1 %	102 %	2 %	0.0005	0.02
Penthiopyrad	100 %	2 %	101 %	1 %	0.0005	0.02
Prothioconazole	107 %	12 %	110 %	15 %	0.0005	0.02
Prothioconazole-desthio	106 %	8 %	104 %	2 %	0.0005	0.02
Teflubenzuron	107 %	9 %	105 %	2 %	0.0005	0.02
TFNA	-	-	98 %	7 %	0.006	0.02
TFNG	103 %	28 %	101 %	53 %	0.003	0.02

UNIVERSIDAD DE ALMERÍA

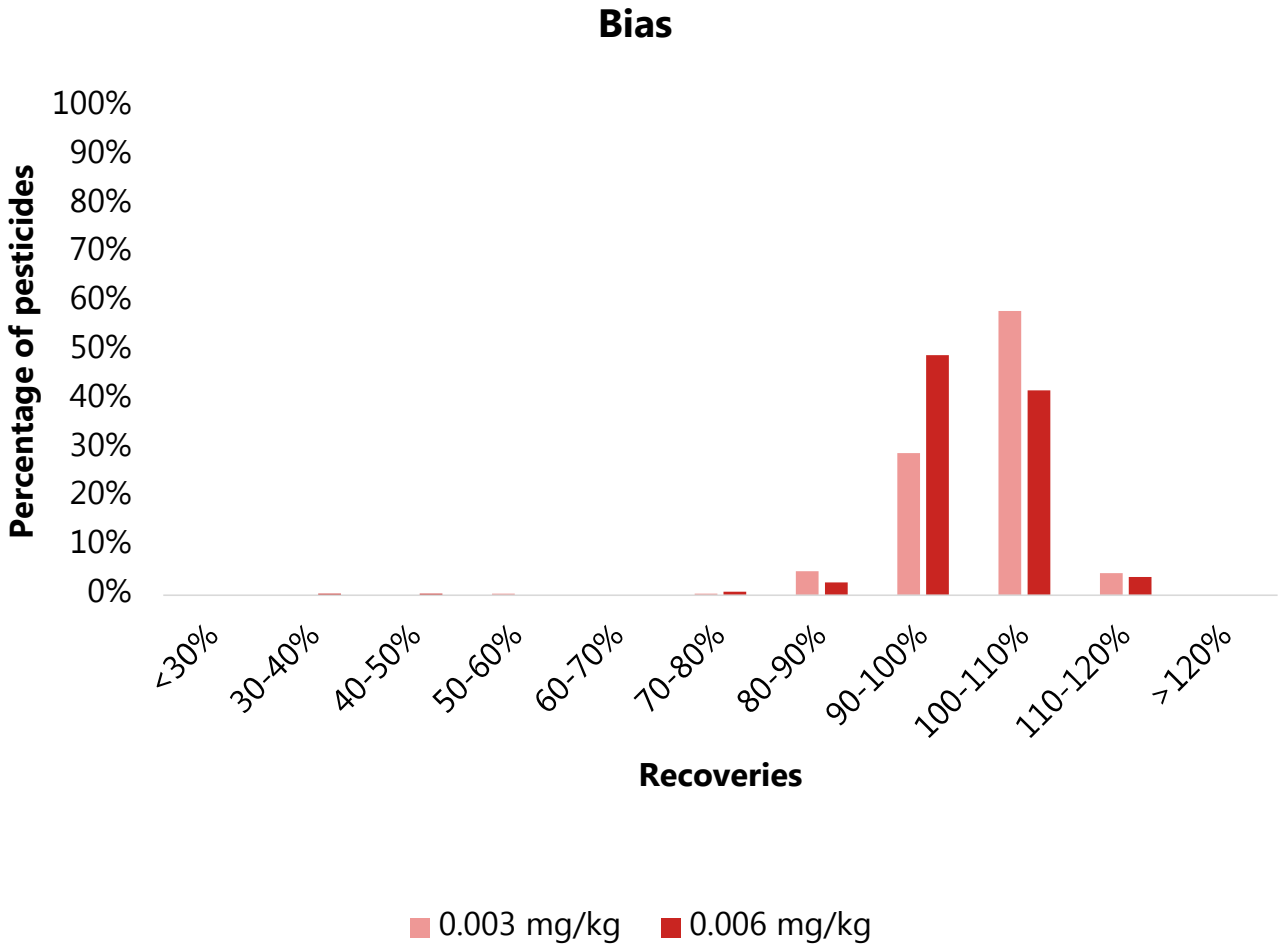
Dual-Channel LC-MS/MS: apple baby food validation

264 total pesticide residues (ESI+ and ESI-)

Technique	<70%	70-120%	>120%
Dual-Channel 0.003 mg/kg	1	257	-
Dual-Channel 0.006 mg/kg	2	260	-

256 pesticide residues validated at 0.003 mg/kg

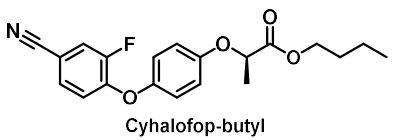
260 pesticide residues validated at 0.006 mg/kg



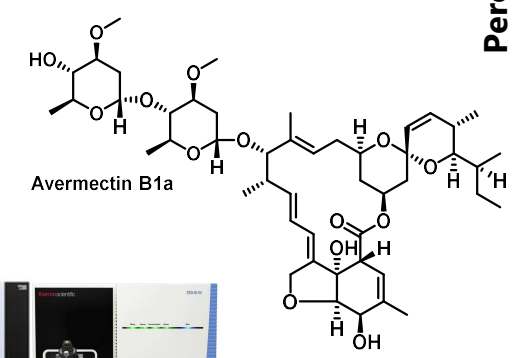
Dual-Channel LC-MS/MS: apple baby food validation

264 total pesticide residues (ESI+ and ESI-)

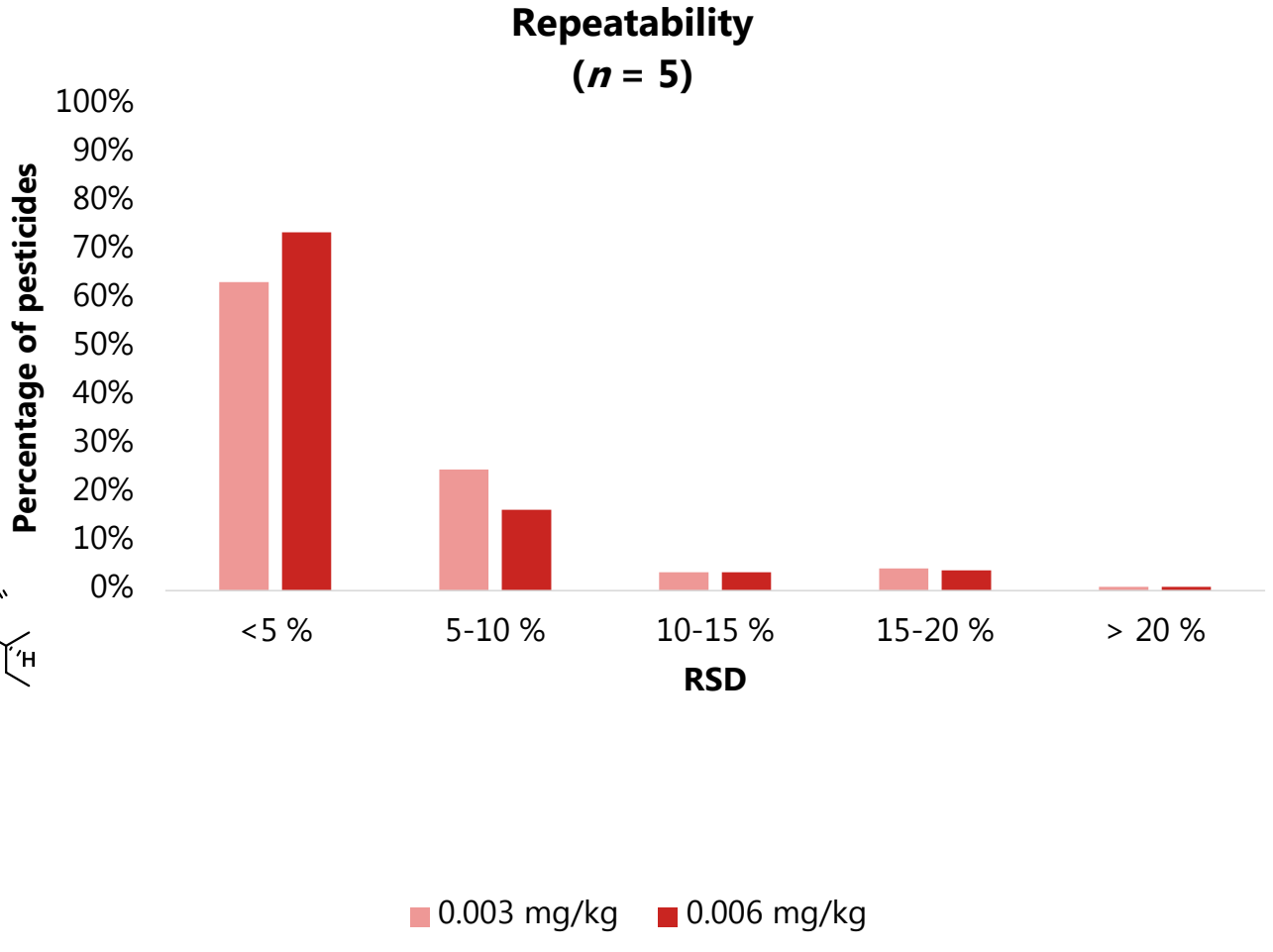
Technique	<5%	5-20%	>20%
Dual-Channel 0.003 mg/kg	64%	33%	1%
Dual-Channel 0.006 mg/kg	74%	25%	1%



Cyhalofop-butyl



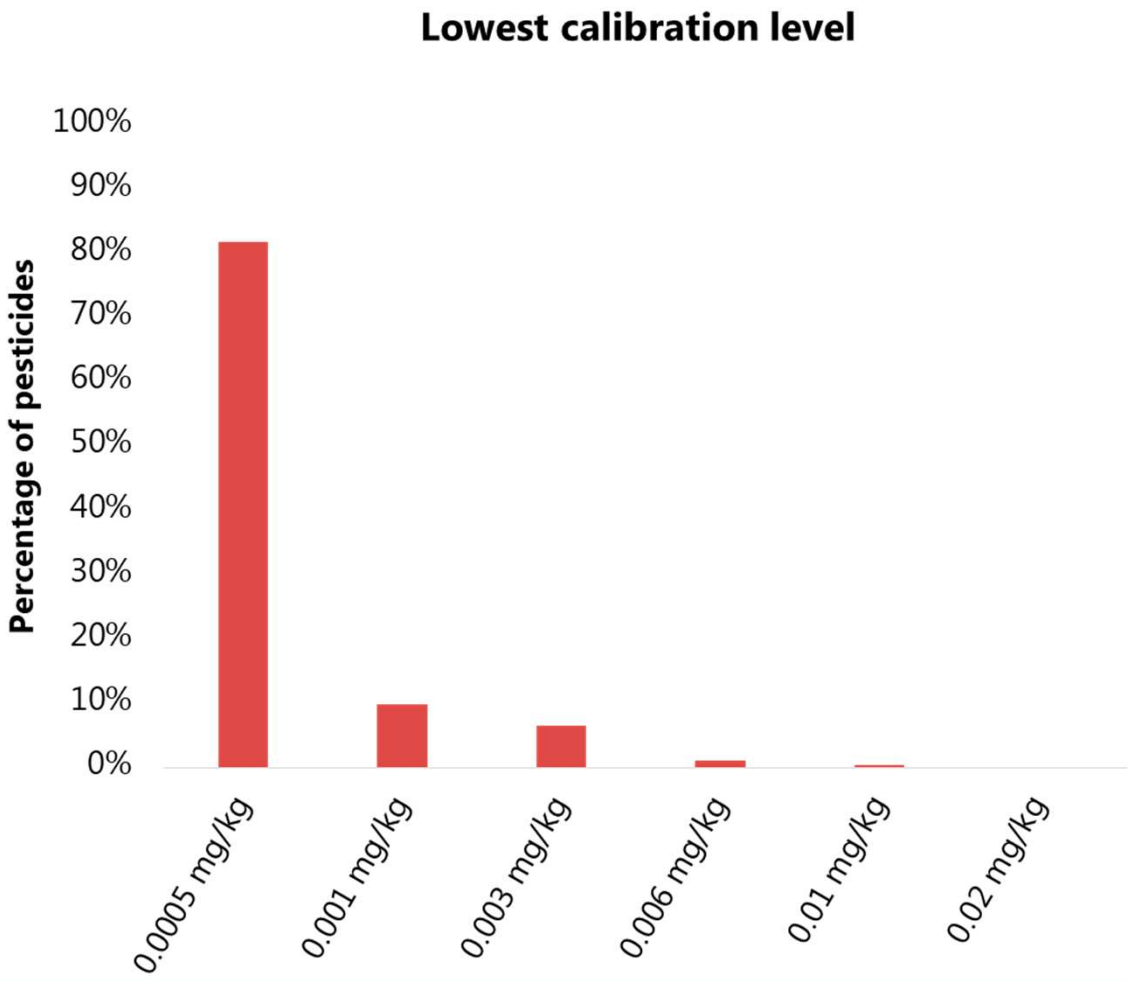
Avermectin B1a



Dual-Channel LC-MS/MS: apple baby food validation

264 total pesticide residues (ESI+ and ESI-)

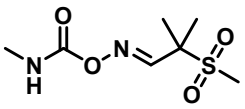
Calibration level (mg/kg)	Percentage of analytes
0.0005	82%
0.001	10%
0.003	6%
0.006	1%
0.010	1%
0.020	0%



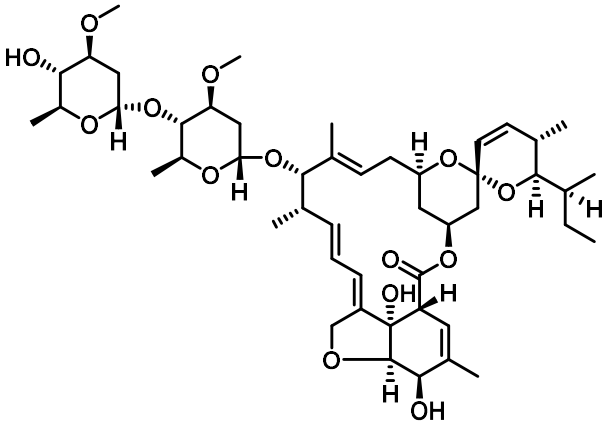
Dual-Channel LC-MS/MS: apple baby food- ND

Non detections

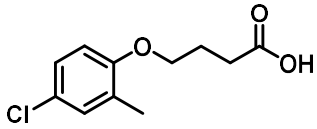
0.006 mg/kg



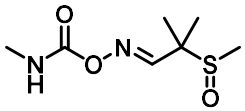
Aldicarb-sulfone



Avermectin B1a

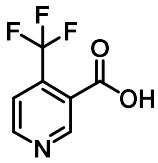


MCPB



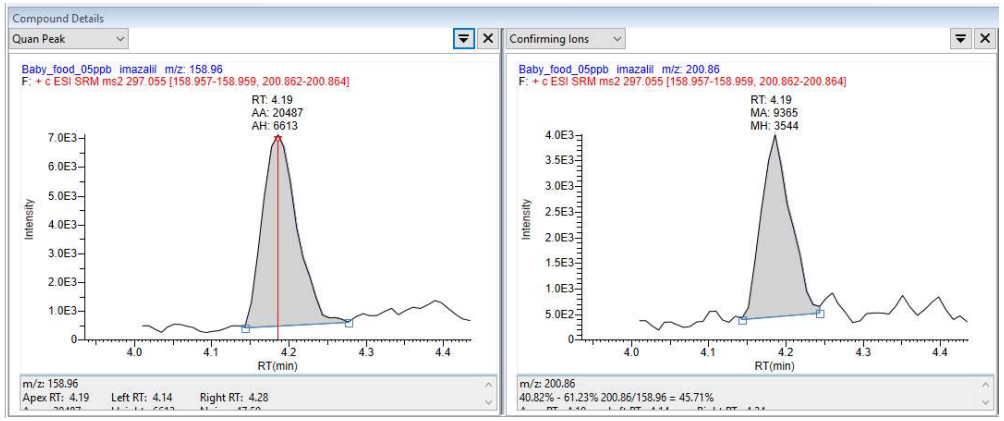
Aldicarb-sulfoxide

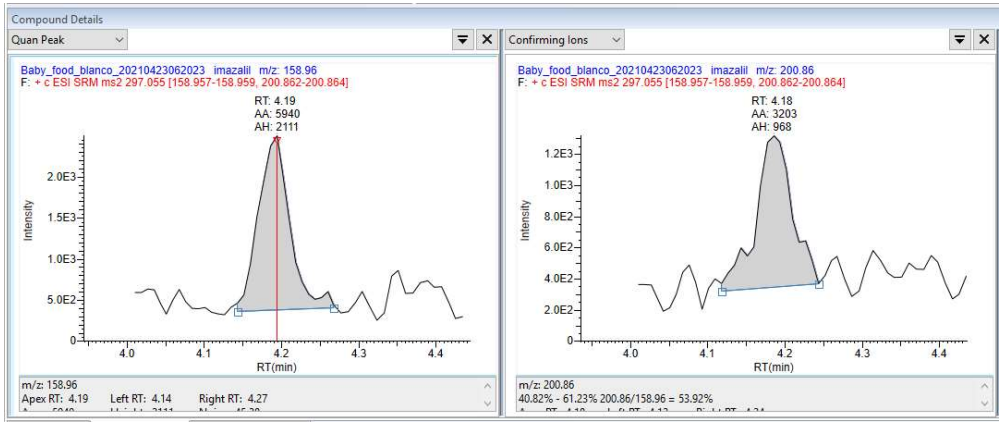
→ detected at **0.010 mg/kg**



TFNA

Dual-Channel LC-MS/MS: carry over test

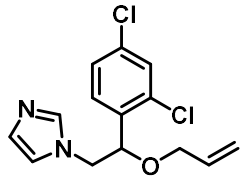




Imazalil

0.5 ppb in baby food

Quant ion peak area 2.0E4

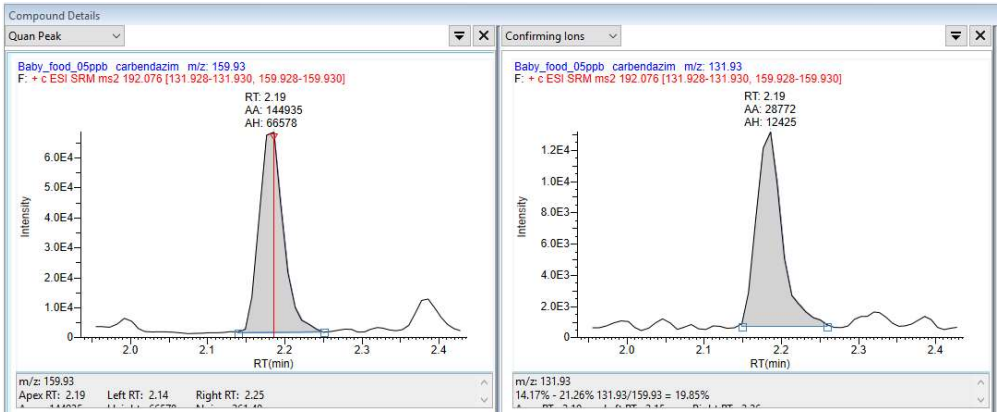


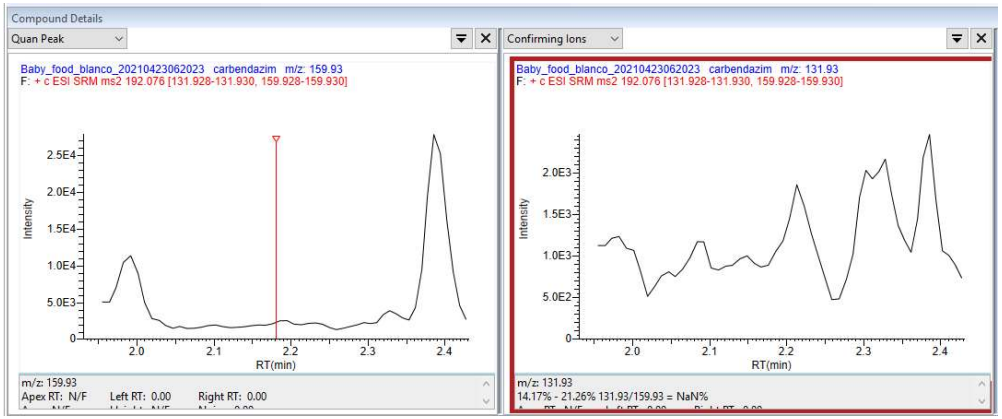
Imazalil

Blank baby food after 12 injections of 20 ppb standard

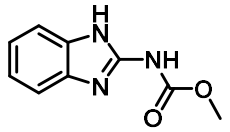
Quant ion peak area 6.0E3

Dual-Channel LC-MS/MS: carry over test





Carbendazim
0.5 ppb in baby food
Quant ion peak area 1.4E5



Carbendazim
Blank baby food after 12 injections of 20 ppb standard
Quant ion peak area 0.0E0

Conclusions

- Dual-Channel LC-MS can be used to increase **sample throughput** up to 70 %
- This technique can also be used to **improve selectivity** without sacrificing analysis time
- Furthermore, **two different mobile phases** can be employed simultaneously
- Most compounds could be **validated** on baby food at **0.003 mg/kg**, with minimum carry over
- In summary, Dual-Channel instrumentation provides laboratories advantages in **analysis time**, **selectivity**, and **sensitivity**

References

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Dual-channel chromatography a smart way to improve the analysis efficiency in liquid chromatography coupled to mass spectrometry

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Cutting-edge approach using dual-channel chromatography to overcome the sensitivity issues associated with polarity switching in pesticide residues analysis

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Thank you!