European Commission

EURL-FV

Dual Channel instrumentation: sample throughput, identification advantages, and concurrent applications

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2023 Training for the NRLs-FV (online/on-site)
New advances in automatisation for the analysis of pesticide residues
19/09/2023

LC-MS analysis of pesticide residues

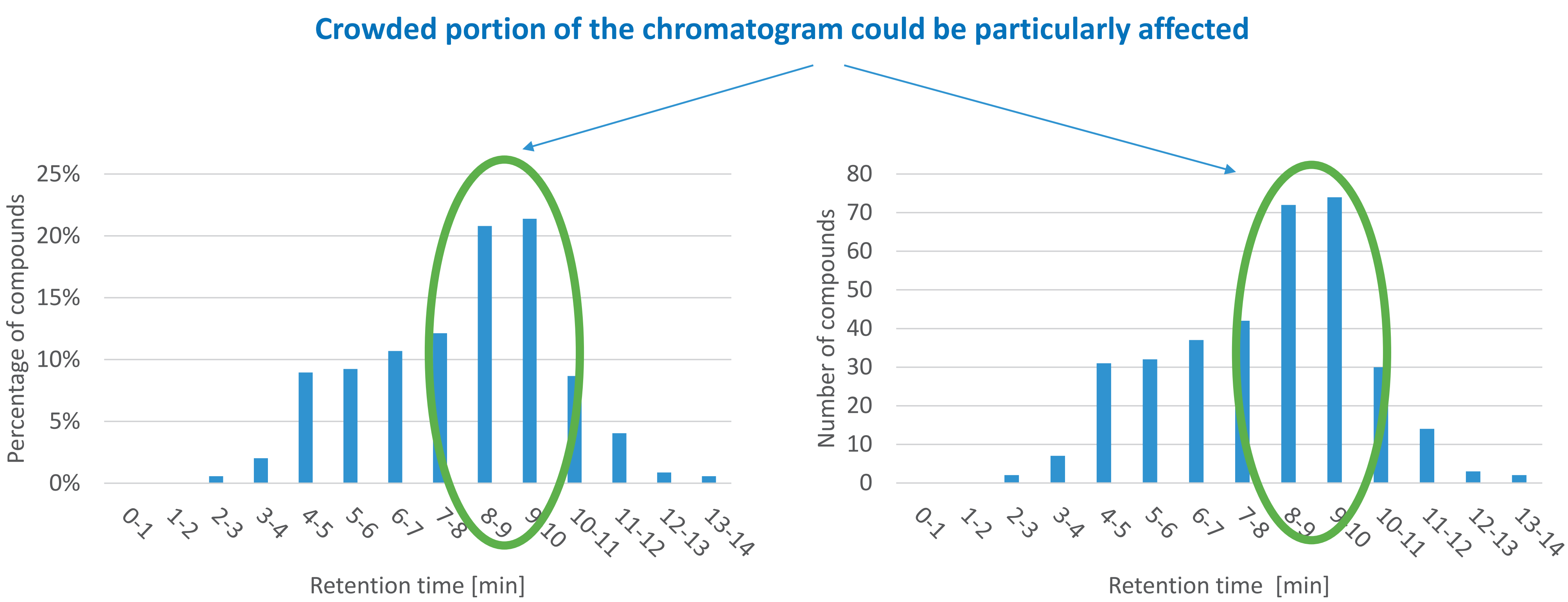
A typical LC-MS method:

- Column length 100 mm
- Particle size 1.7-3 µm
- Flow rate 300-400 µL/min
- Analysis time (gradient + column equilibration) 15-20 min
- Number of pesticides 150 – 400 (300 – 800 transitions)

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

The consequence of faster analysis times



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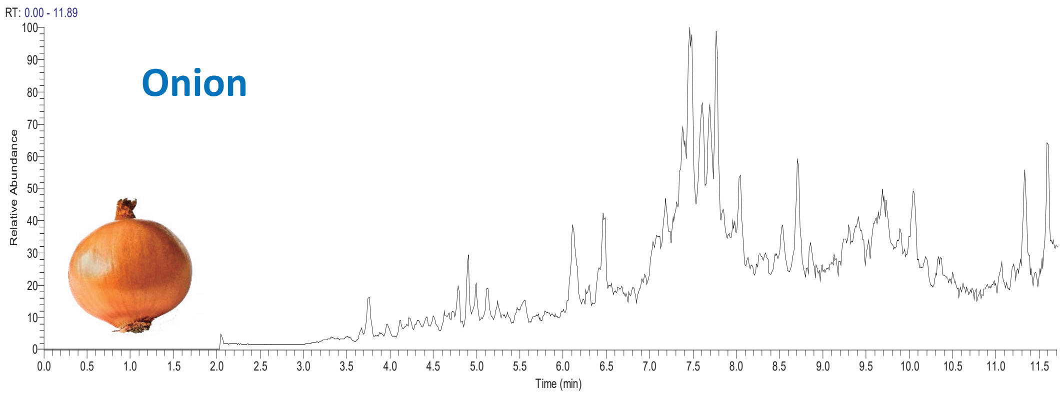
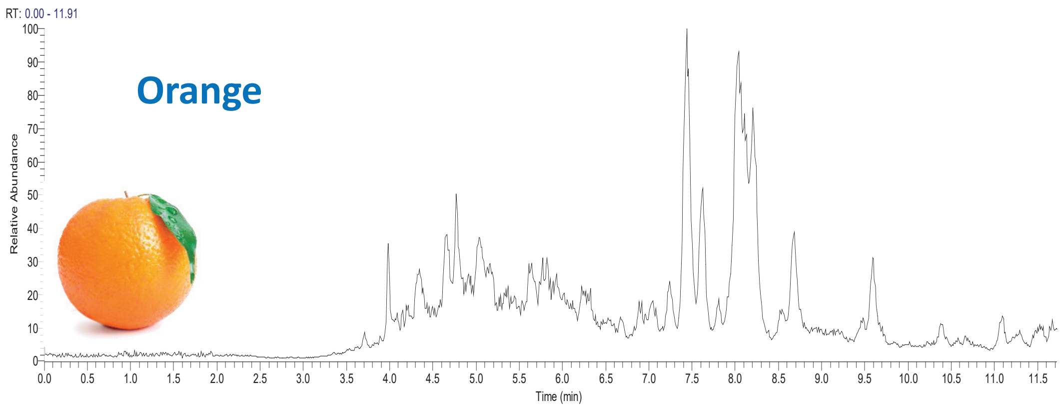
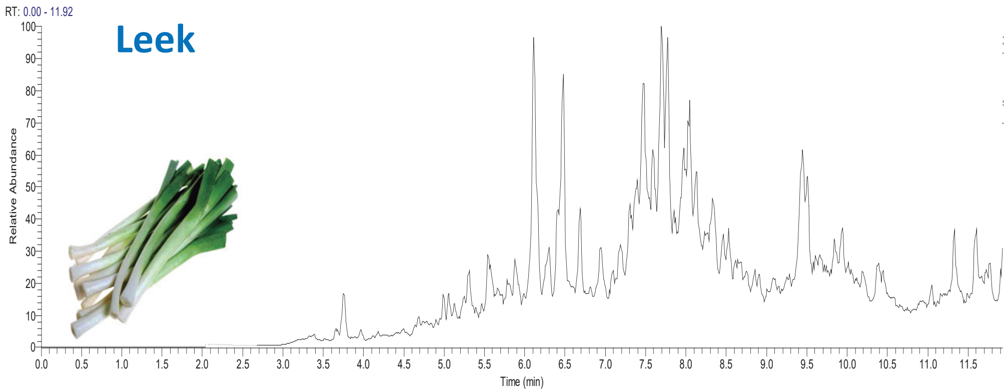
  - Compromised separation
    - More coeluting pesticides
      - Shorter dwell times -> lower sensitivity
      - Longer duty cycle -> Less data points per chromatographic peak -> worse peak area reproducibility
      - Common transitions
      - Possible cross-talk
    - More coeluting matrix (especially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

The consequence of faster analysis times

The crowded portion of the chromatogram is particularly affected







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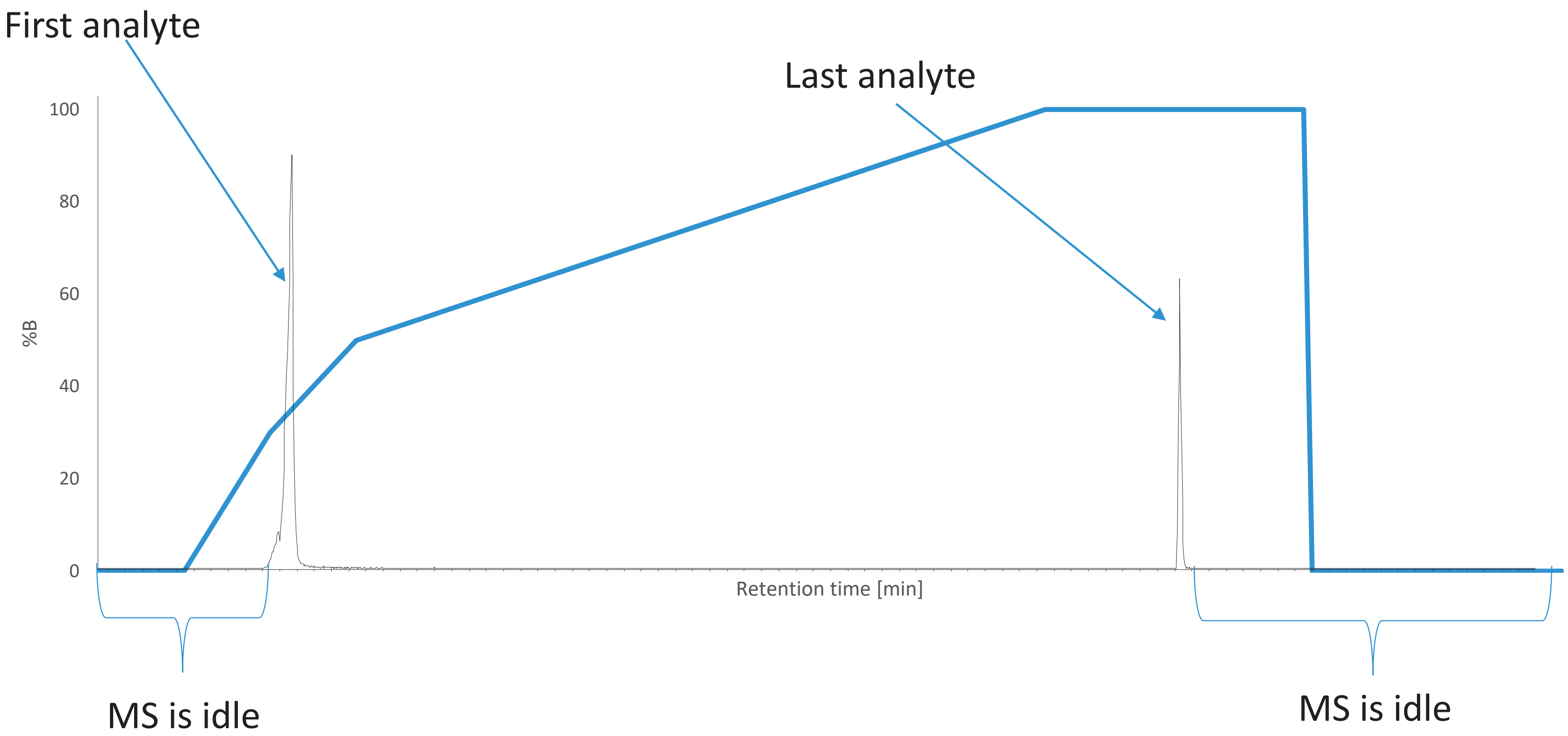
Another option to decrease the analysis time



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

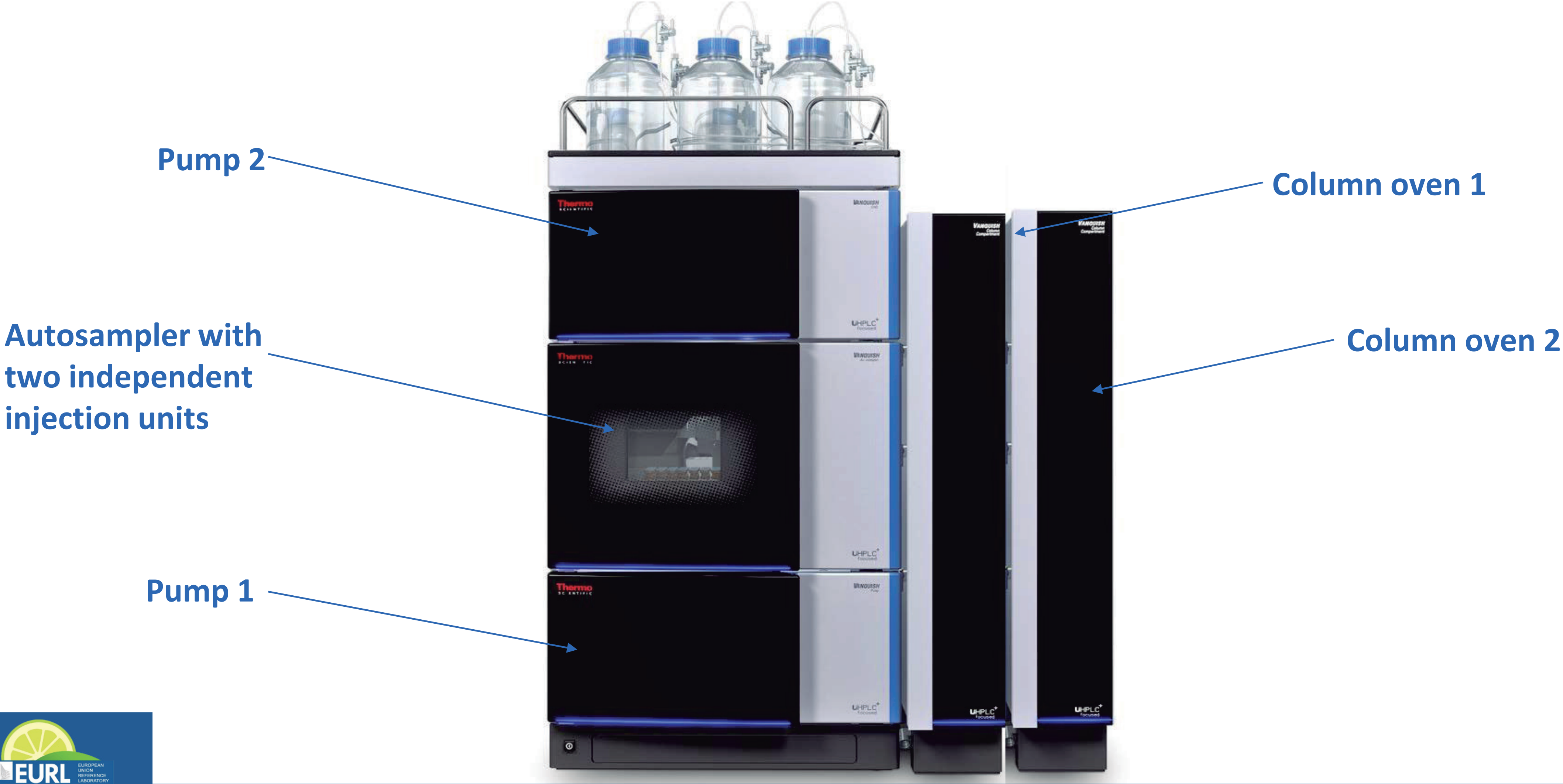
Hardware

Instrumentation

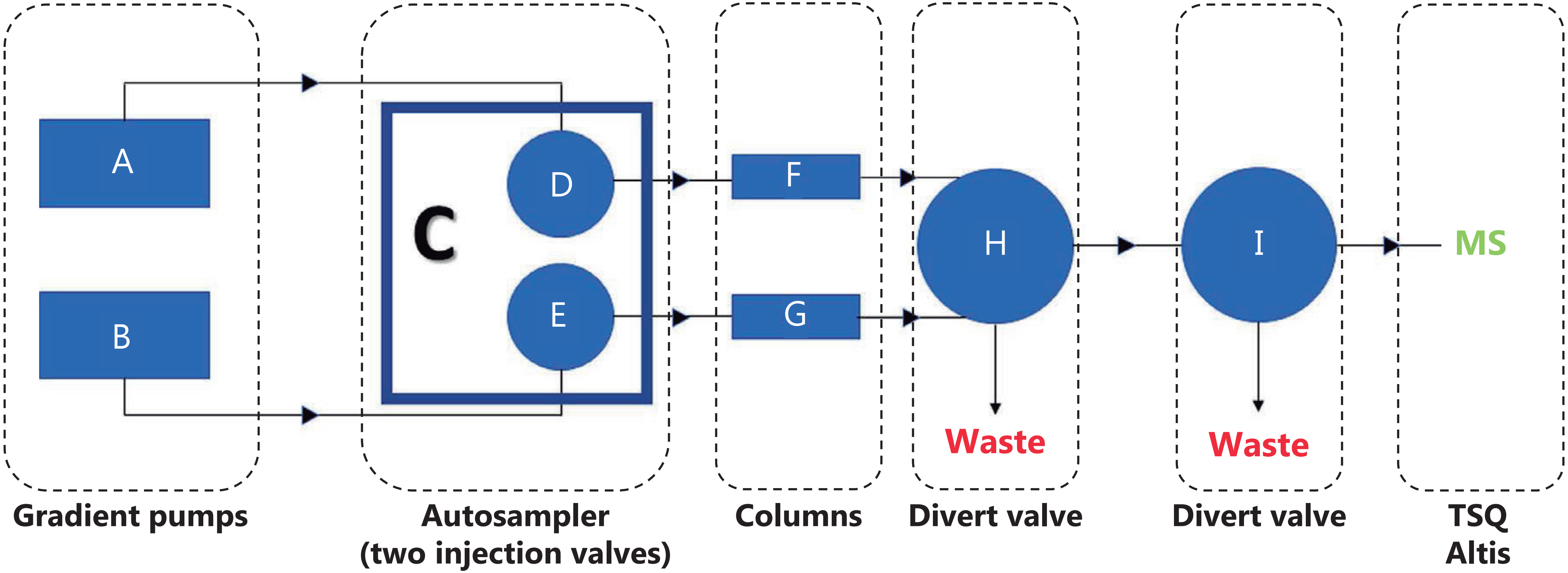
Thermo Scientific™
Transcend™ DUO LX-2
UHPLC System

Thermo Scientific™
TSQ Altis™
Triple Quadrupole Mass Spectrometer

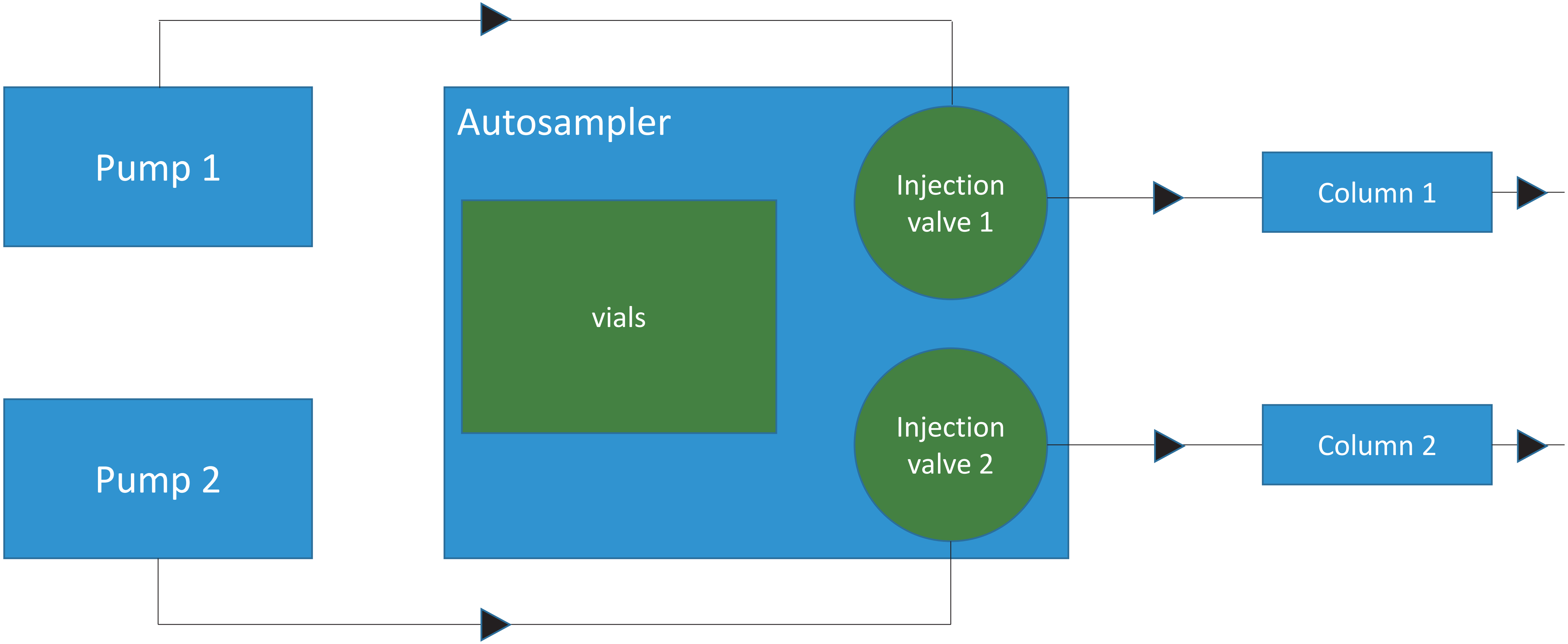
Instrumentation



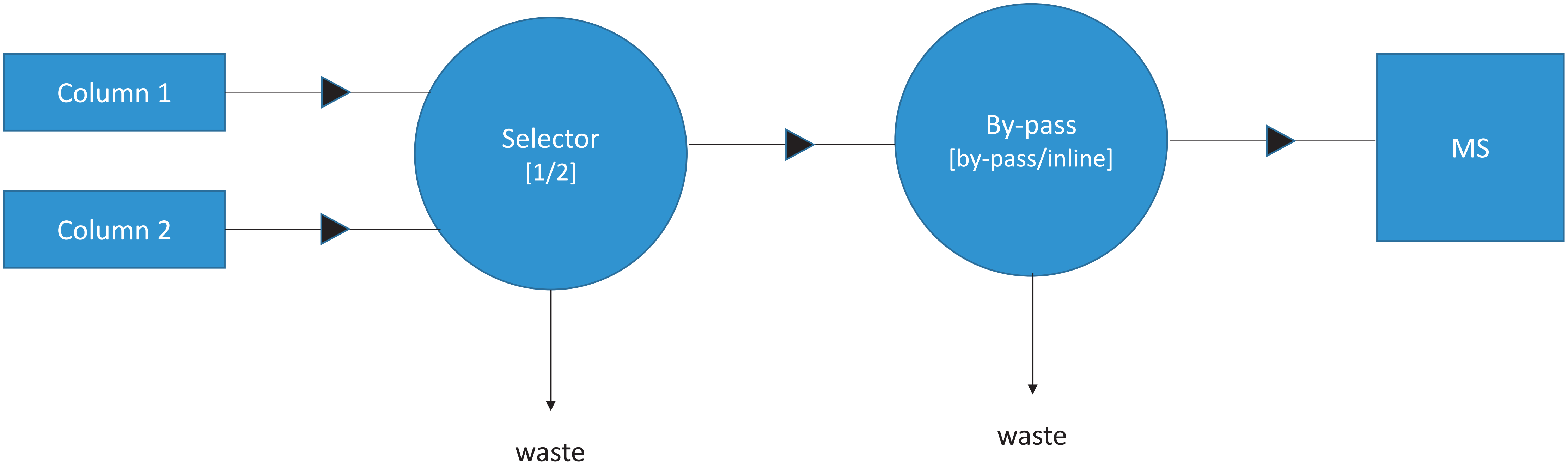
Dual-Channel LC-MS/MS: general diagram



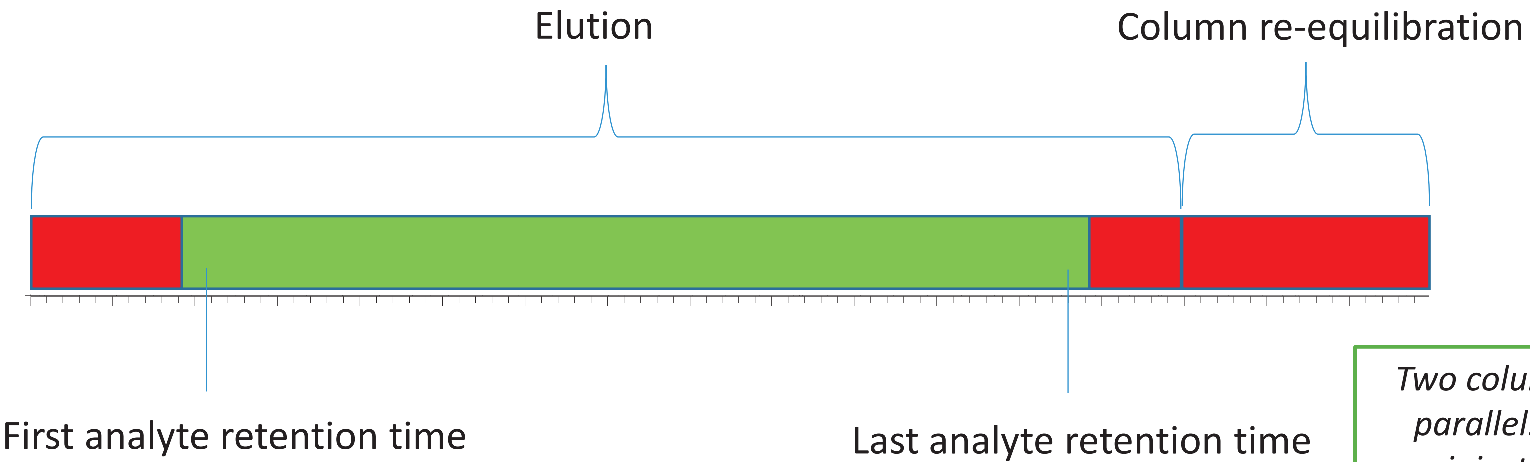
Schematic of the Dual Channel Configuration



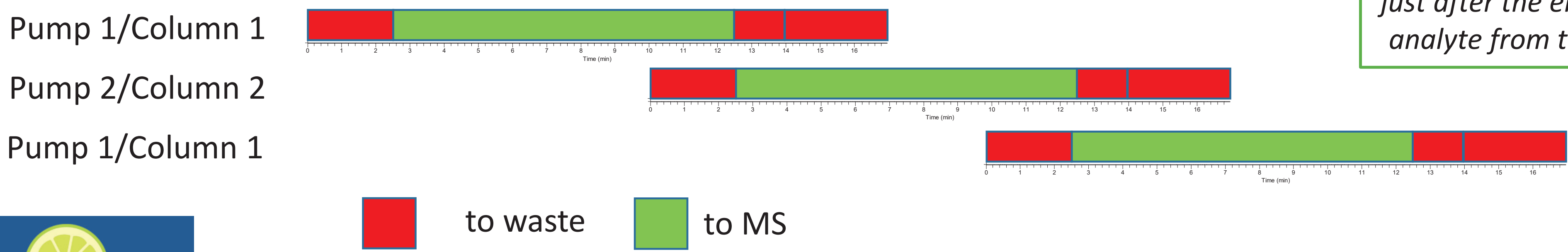
Transcend Duo LX-2 – channel selector



Dual-Channel LC-MS/MS: time segments

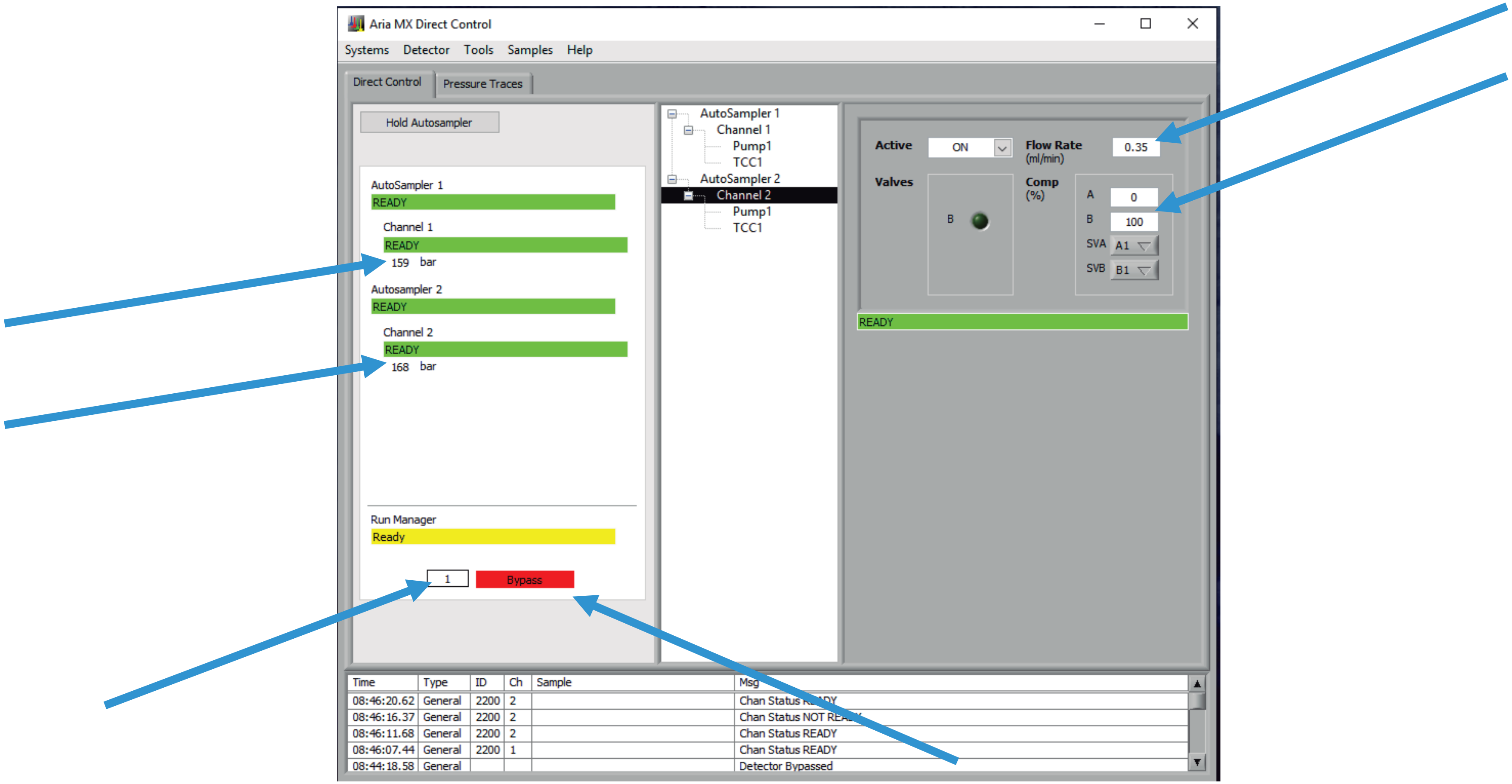


Two columns are operated in parallel. Then, consecutive injections are partially overlapped and synchronised in the way that the first analyte from the second column elutes just after the elution of the last analyte from the first column.



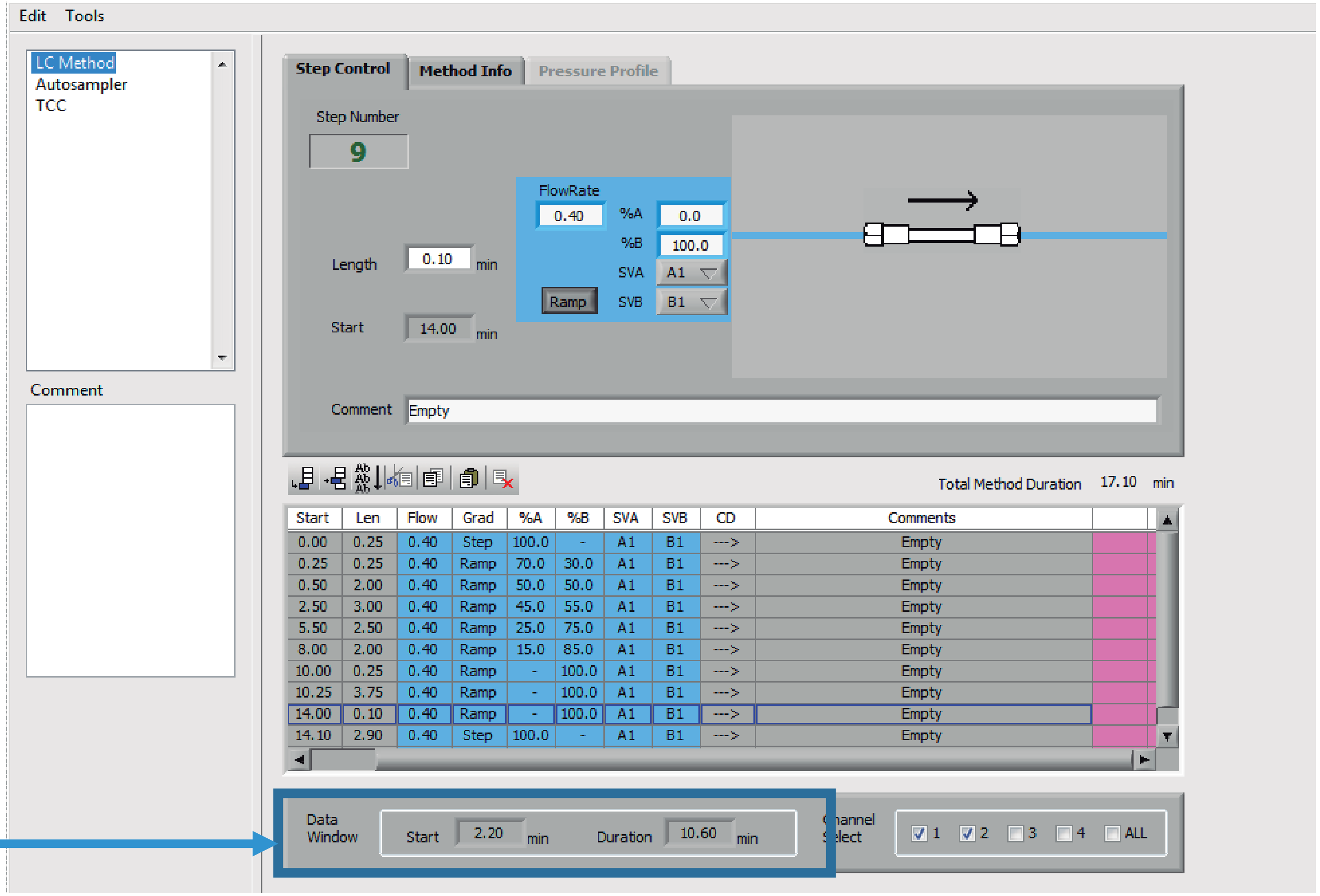
Software

Aria MX



Aria MX Direct Control
Systems Detector Tools Samples Help
Direct Control
Pressure Traces
Hold Autosampler
AutoSampler 1
READY
Channel 1
READY
159 bar
Autosampler 2
READY
Channel 2
READY
168 bar
Run Manager
Ready
1
Bypass
AutoSampler 1
Channel 1
Pump1
TCC1
AutoSampler 2
Channel 2
Pump1
TCC1
Active
ON
Flow Rate
(ml/min)
0.35
Valves
B
Comp
(%)
A
0
B
100
SVA
A1
SVB
B1
READY
Time Type ID Ch Sample Msg
08:46:20.62 General 2200 2
08:46:16.37 General 2200 2
08:46:11.68 General 2200 2 Chan Status READY
08:46:07.44 General 2200 1 Chan Status READY
08:44:18.58 General Detector Bypassed

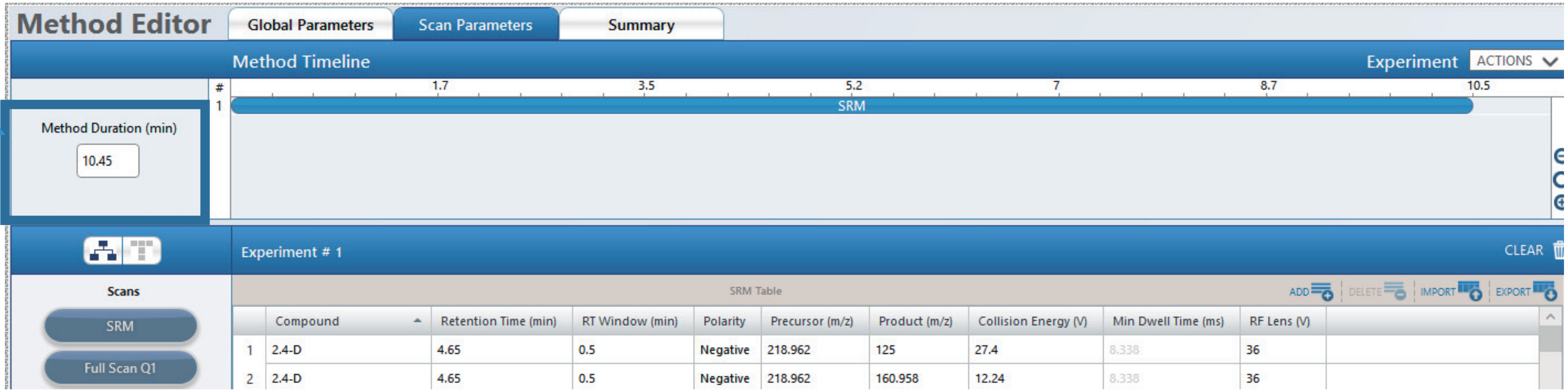
Aria MX – Method setup



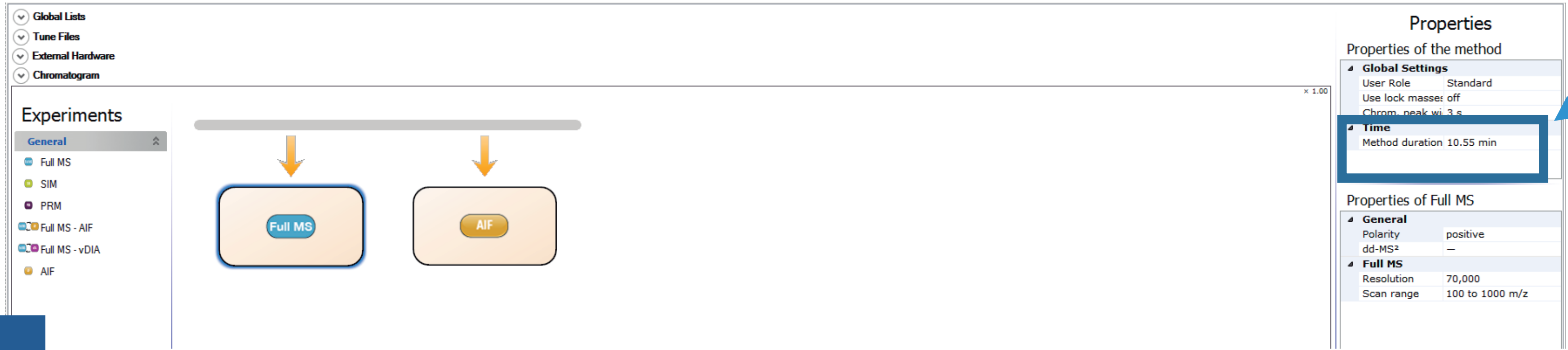
Method setup is very easy. The user has to specify only the retention time when the acquisition should start and how long it should take. Other parameters are the same as in a single-channel system.

MS Method setup

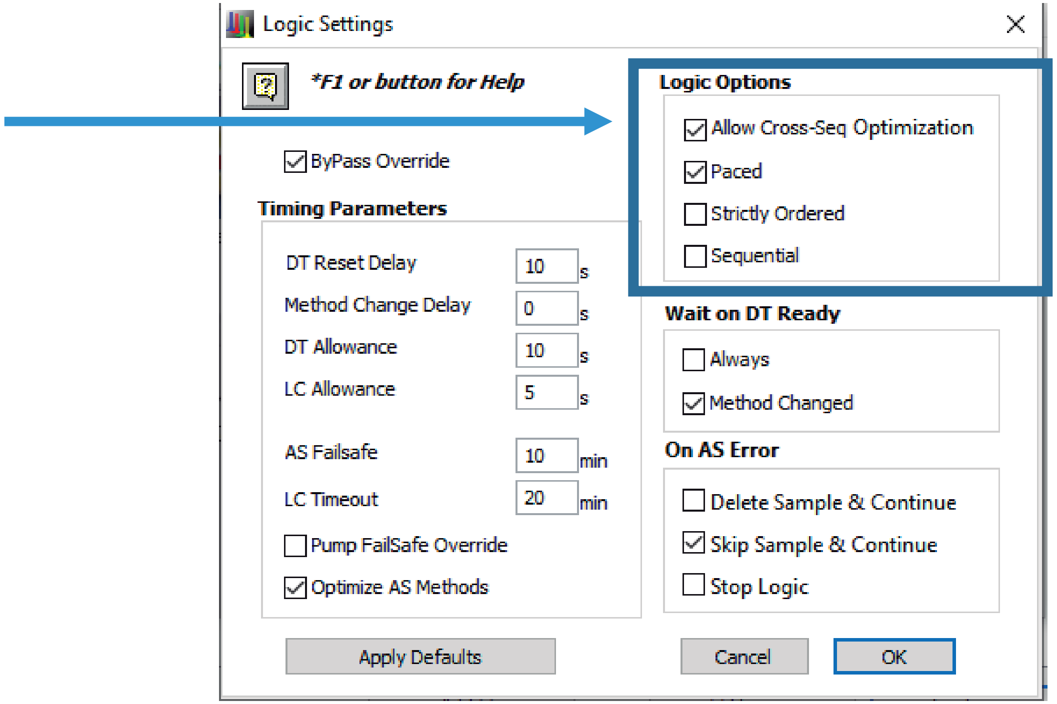
TSQ Altis



QE Focus



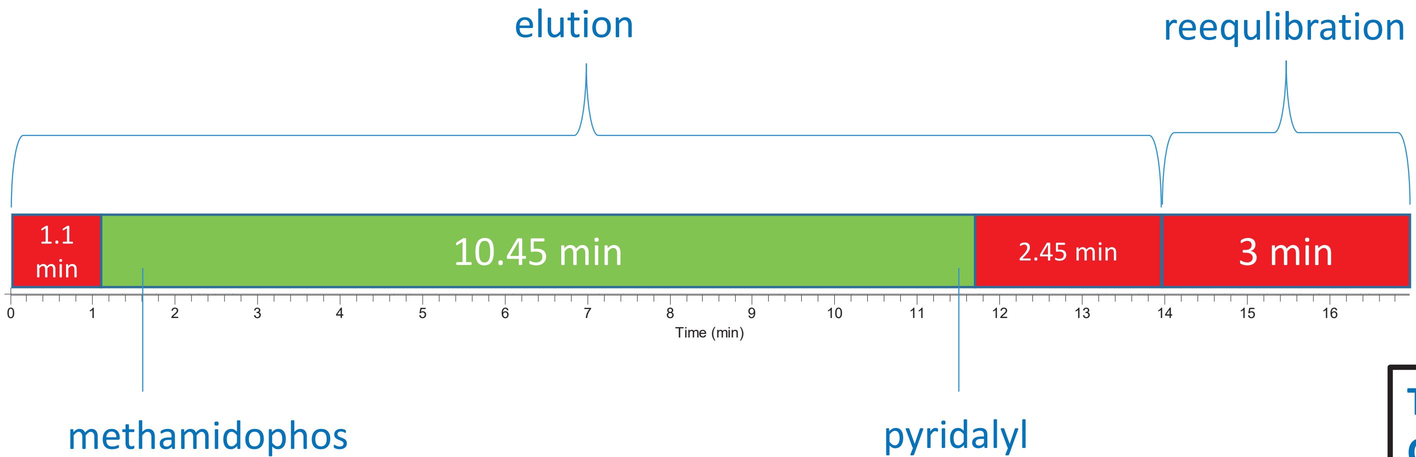
Aria MX – Logic settings



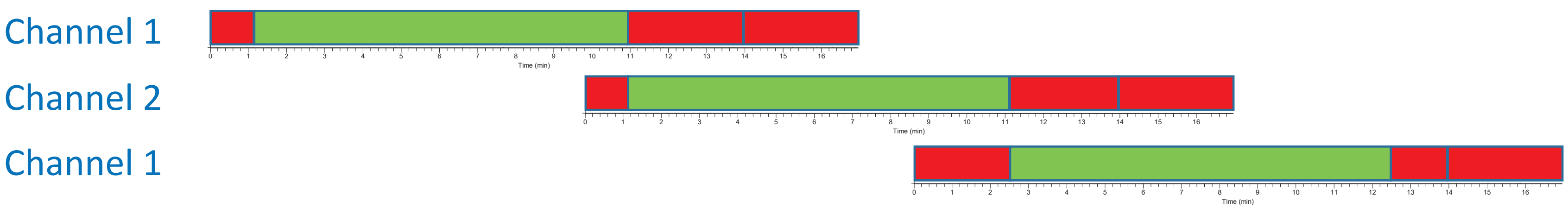
Xcalibur – sequence setup

File Name	Path	Inst Meth	Position	Inj Vol	ChannelSelect
Dvte_c1_03	C:\Xcalibur\data\2020_01\3001_multiplexing_100r	C:\TraceFinderData\InstrumentMethods\Vanquish\Mulplx_100mm_vDIA	R:A1	10.00	1
Dvte_c2_03	C:\Xcalibur\data\2020_01\3001_multiplexing_100r	C:\TraceFinderData\InstrumentMethods\Vanquish\Mulplx_100mm_vDIA	R:A1	10.00	2
Tomate_100mm_c1_5ppb	C:\Xcalibur\data\2020_01\3001_multiplexing_100r	C:\TraceFinderData\InstrumentMethods\Vanquish\Mulplx_100mm_vDIA	R:B1	10.00	1
Tomate_100mm_c2_5ppb	C:\Xcalibur\data\2020_01\3001_multiplexing_100r	C:\TraceFinderData\InstrumentMethods\Vanquish\Mulplx_100mm_vDIA	R:B1	10.00	2
Tomate_100mm_c1_10ppb	C:\Xcalibur\data\2020_01\3001_multiplexing_100r	C:\TraceFinderData\InstrumentMethods\Vanquish\Mulplx_100mm_vDIA	R:B2	10.00	1
Tomate_100mm_c2_10ppb	C:\Xcalibur\data\2020_01\3001_multiplexing_100r	C:\TraceFinderData\InstrumentMethods\Vanquish\Mulplx_100mm_vDIA	R:B2	10.00	2
Tomate_100mm_c1_50ppb	C:\Xcalibur\data\2020_01\3001_multiplexing_100r	C:\TraceFinderData\InstrumentMethods\Vanquish\Mulplx_100mm_vDIA	R:B3	10.00	1
Tomate_100mm_c2_50ppb	C:\Xcalibur\data\2020_01\3001_multiplexing_100r	C:\TraceFinderData\InstrumentMethods\Vanquish\Mulplx_100mm_vDIA	R:B3	10.00	2
Tomate_100mm_c1_100ppb	C:\Xcalibur\data\2020_01\3001_multiplexing_100r	C:\TraceFinderData\InstrumentMethods\Vanquish\Mulplx_100mm_vDIA	R:B4	10.00	1
Tomate_100mm_c2_100ppb	C:\Xcalibur\data\2020_01\3001_multiplexing_100r	C:\TraceFinderData\InstrumentMethods\Vanquish\Mulplx_100mm_vDIA	R:B4	10.00	2
Tomate_100mm_c1_500ppb	C:\Xcalibur\data\2020_01\3001_multiplexing_100r	C:\TraceFinderData\InstrumentMethods\Vanquish\Mulplx_100mm_vDIA	R:B5	10.00	1
Tomate_100mm_c2_500ppb	C:\Xcalibur\data\2020_01\3001_multiplexing_100r	C:\TraceFinderData\InstrumentMethods\Vanquish\Mulplx_100mm_vDIA	R:B5	10.00	2

Dual-Channel LC-MS/MS: time segments



TSQ Altis
Column length 100 mm

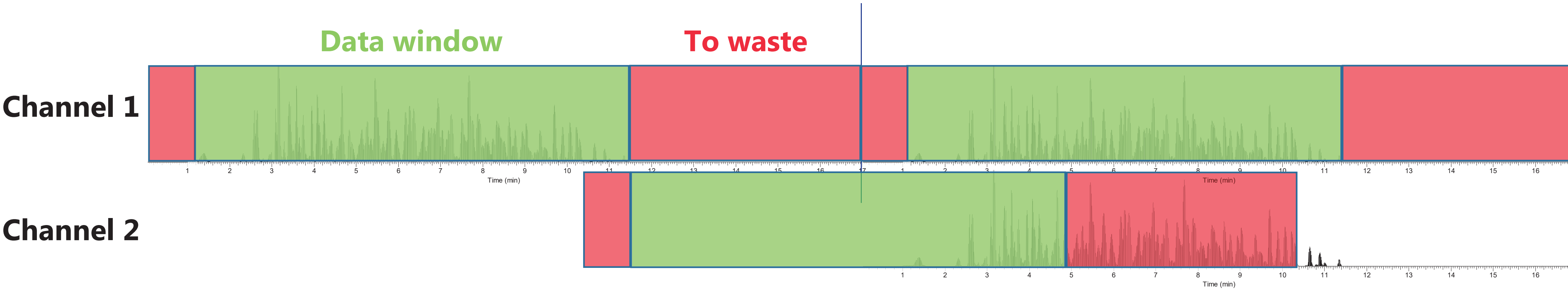


to waste

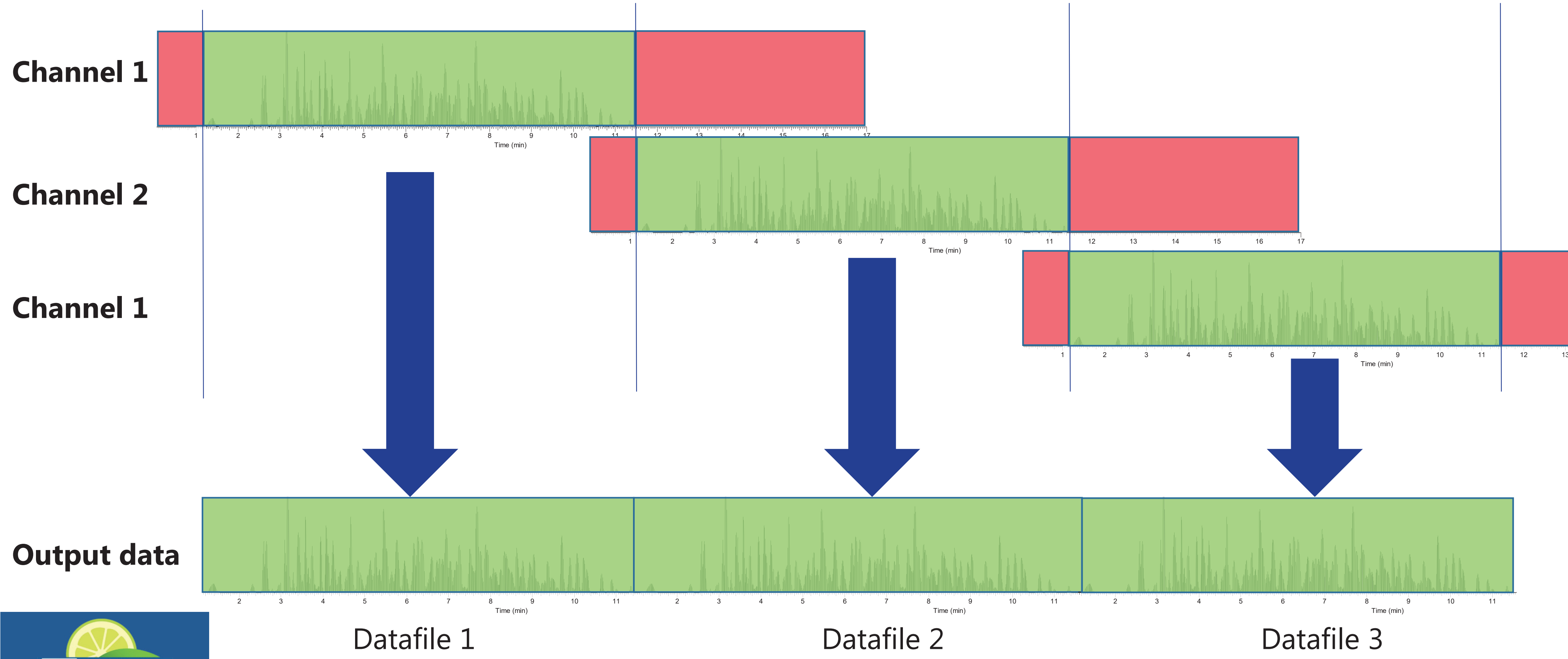
to MS (**acquisition time 10.45 min**)

Total time in a single-channel system: 17 min
(+ 1 minute for needle wash, sample aspiration, etc.)

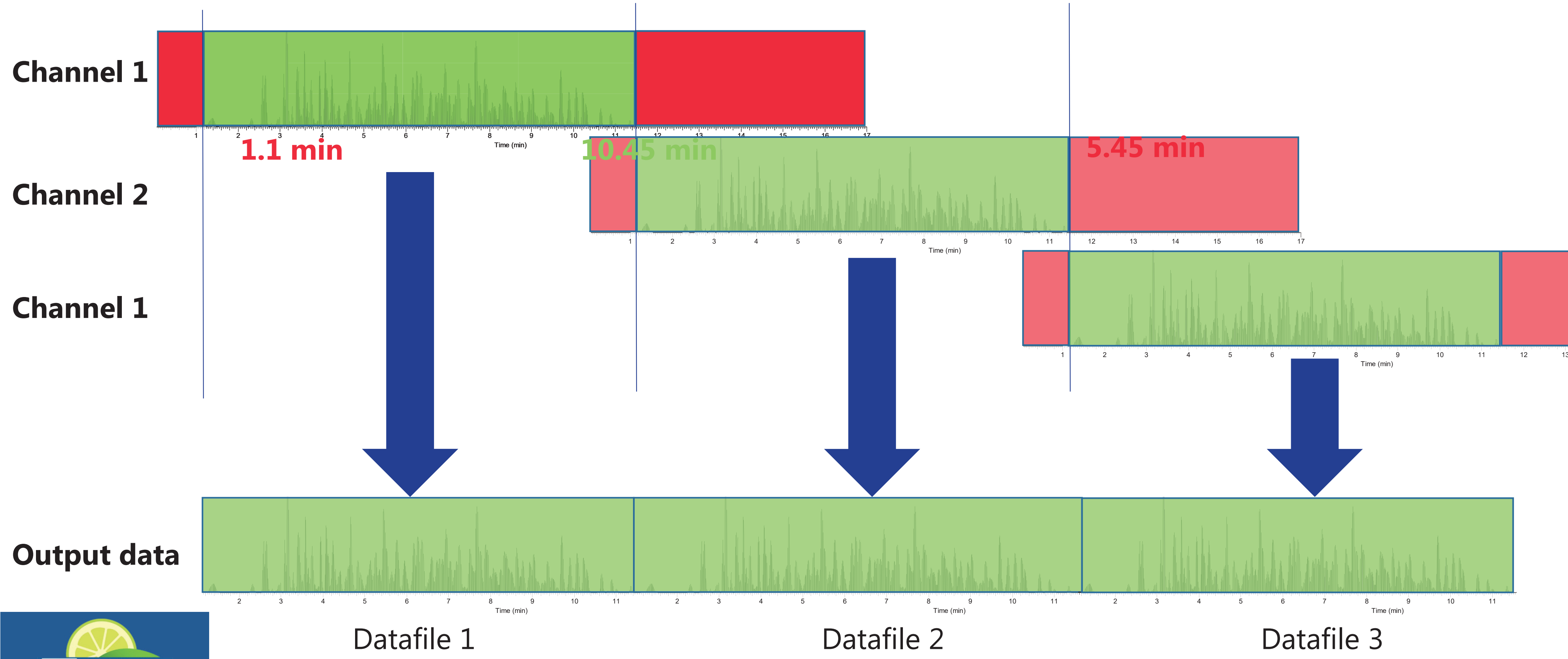
Dual-Channel LC-MS/MS: sample throughput



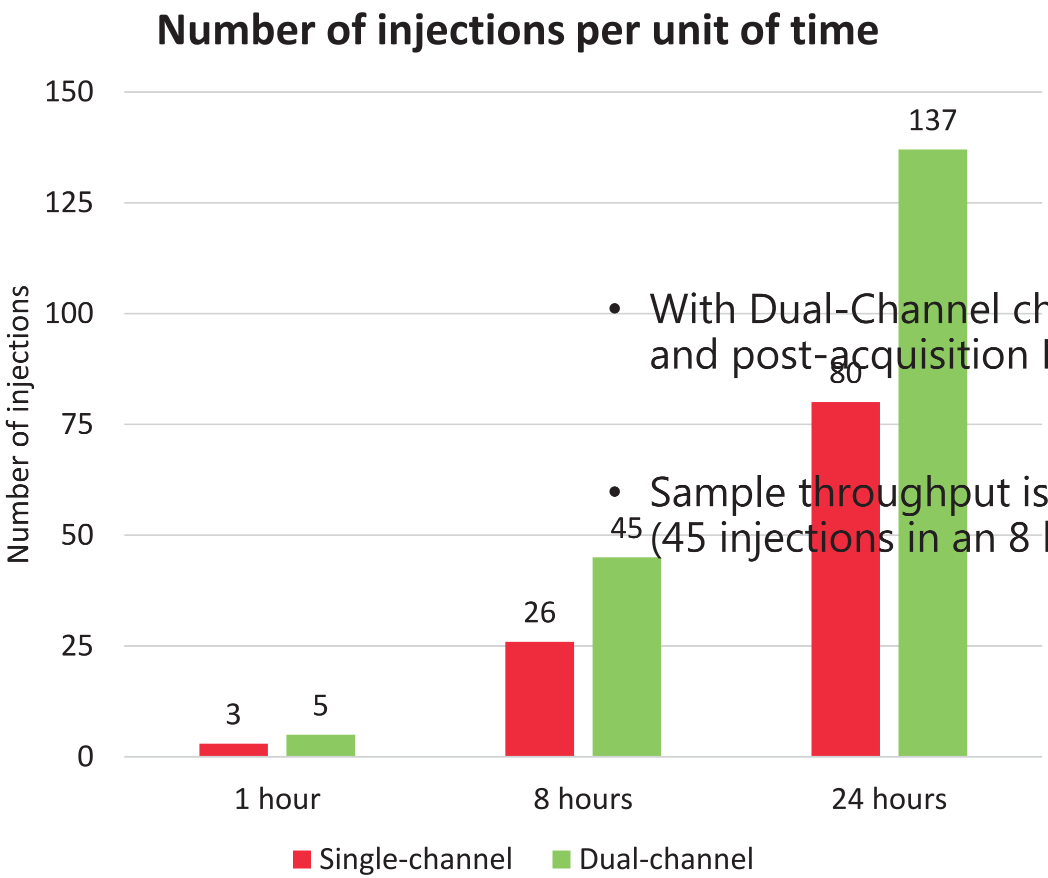
Dual-Channel LC-MS/MS: sample throughput



Dual-Channel LC-MS/MS: sample throughput



Dual-Channel LC-MS/MS: sample throughput

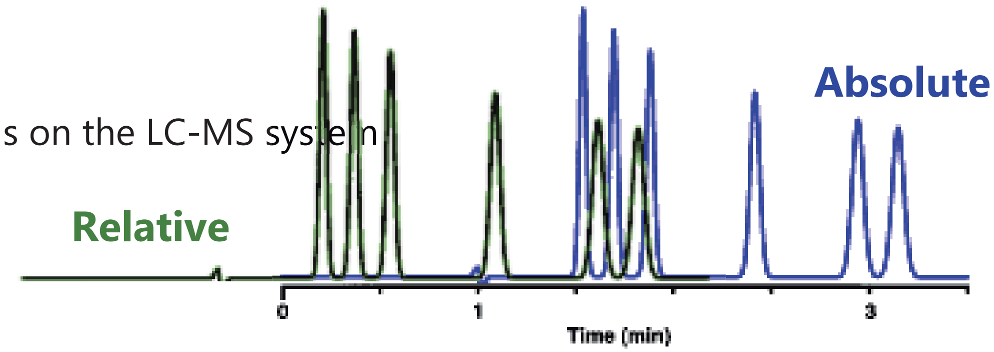


- With Dual-Channel chromatography, pre-acquisition and post-acquisition MS-idle times are removed
- Sample throughput is increased over 70 % (45 injections in an 8 hr period)

Dual-Channel LC-MS/MS: retention time

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

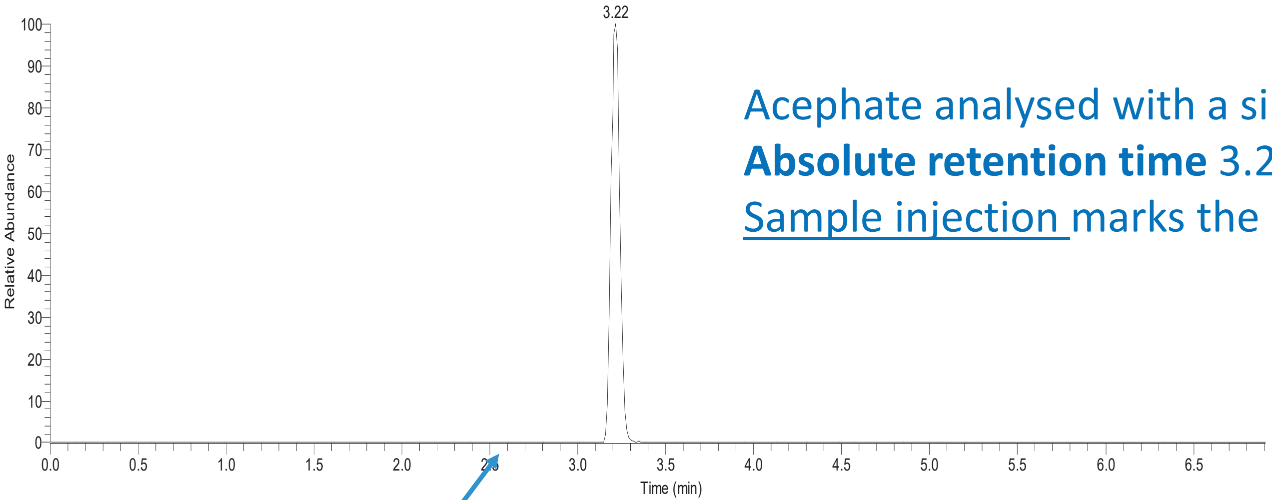
- **Single channel:**
  - Sample injection → 0.0 min
  - Data window beginning → 0.0 min
  - Retention time (absolute) → time an analyte spends on the LC-MS system



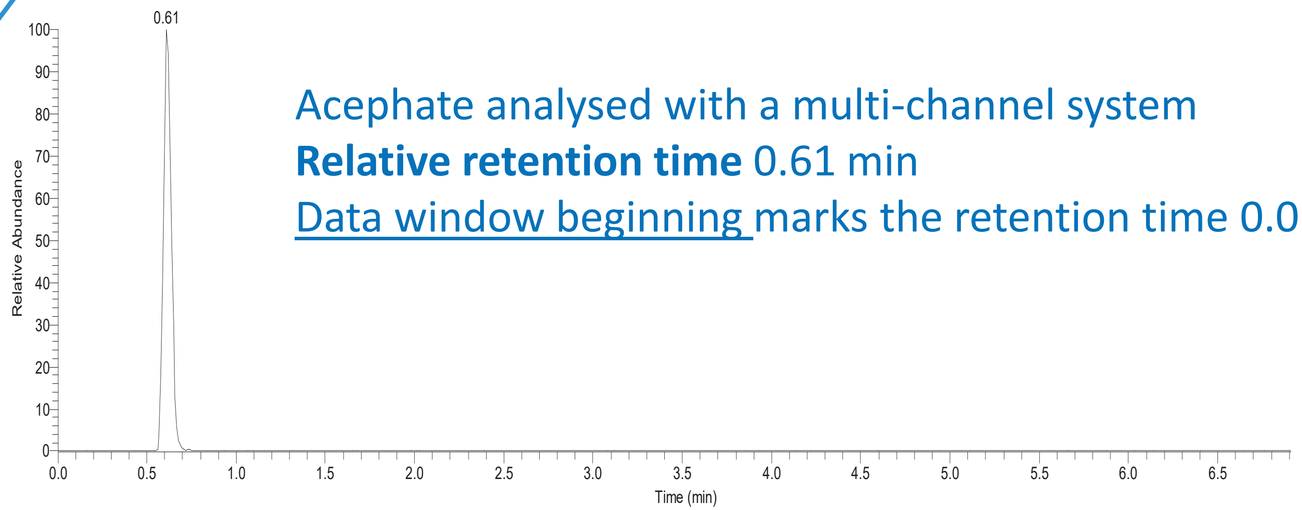
- **Dual-Channel**:
  - Sample injection → 0.0 min
  - Data window beginning → > 0.0 min
  - Retention time (relative) → time an analyte spends on the LC-MS system since the start of the data window

- The chromatographic process is the same in both cases

Dual-Channel LC-MS/MS: retention time



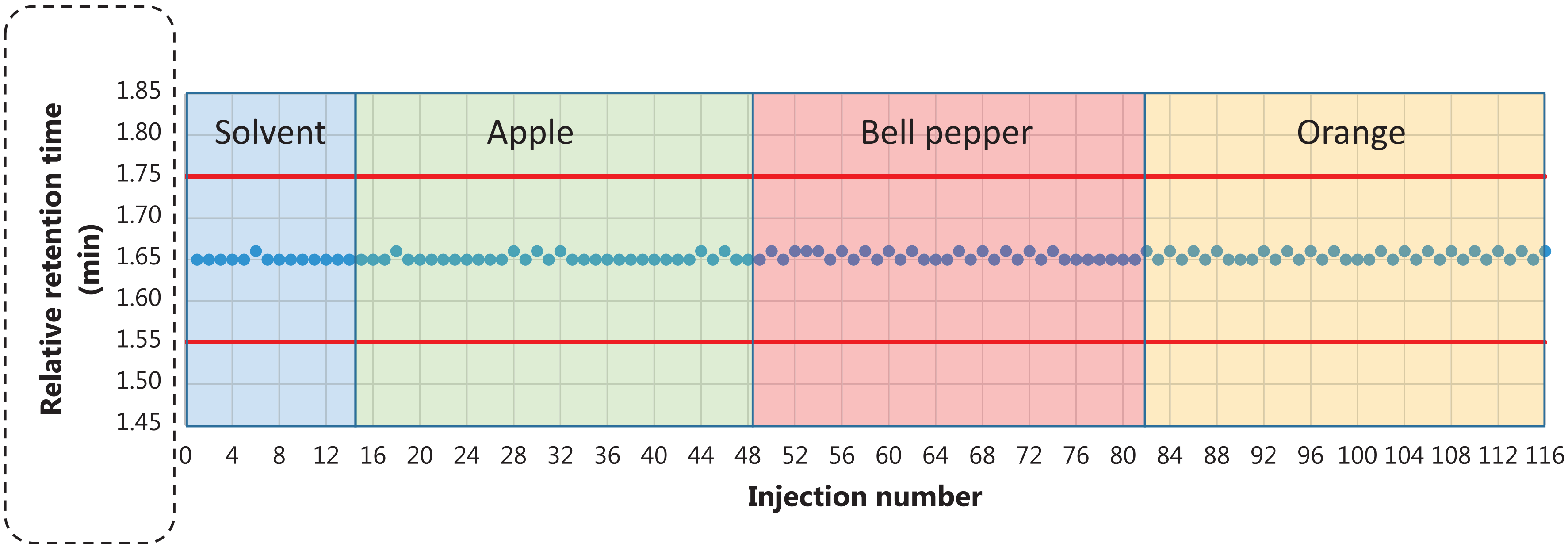
Acephate analysed with a single-channel system
Absolute retention time 3.22 min
Sample injection marks the retention time 0.0 min.



Acephate analysed with a multi-channel system
Relative retention time 0.61 min
Data window beginning marks the retention time 0.0 min.

In the multi-channel analysis the data window starts here

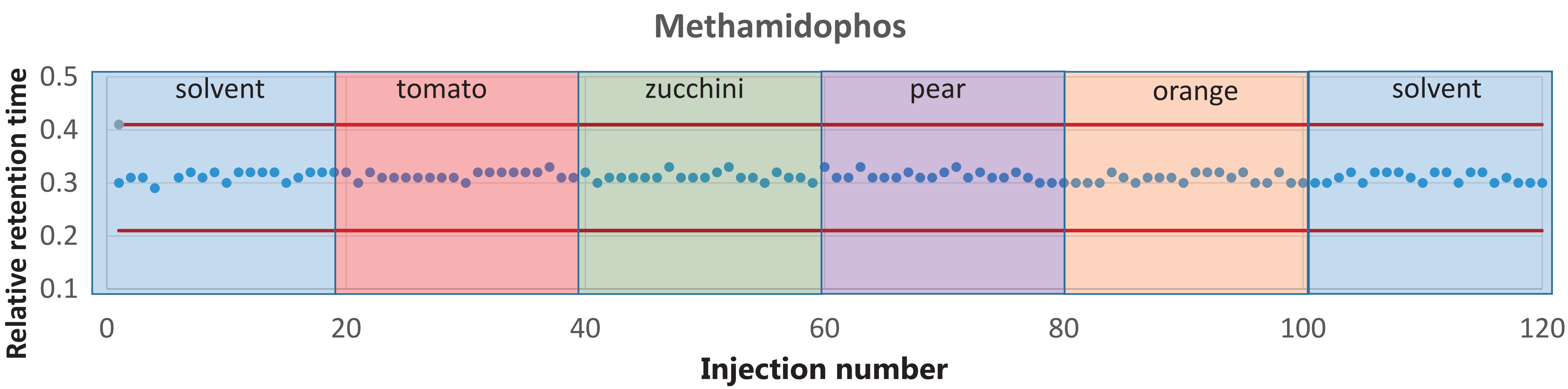
Dual-Channel LC-MS/MS: retention time stability



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.

The red lines mark ± 0.1 min

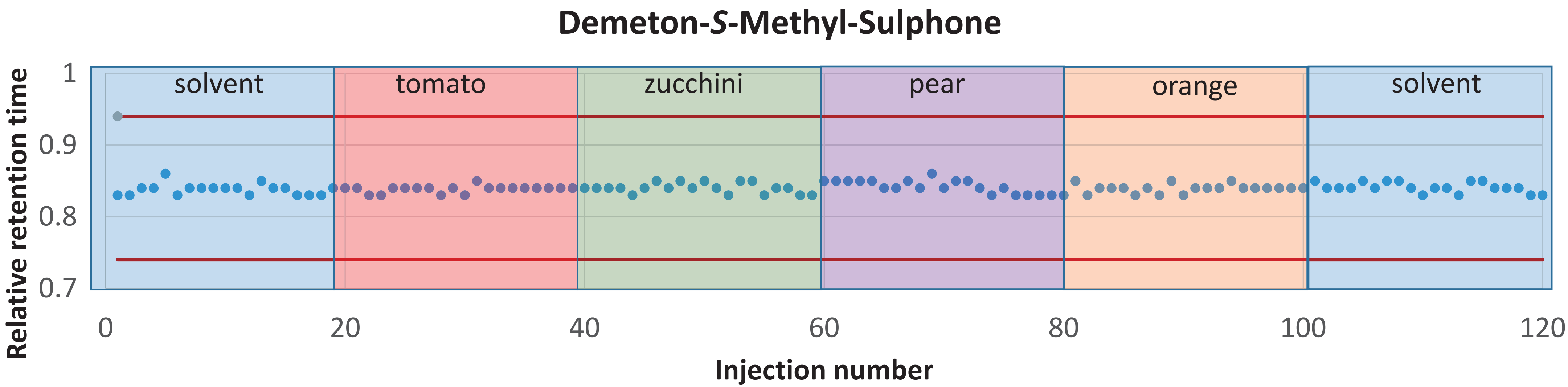
Dual-Channel LC-MS/MS: retention time stability



120 injections alternating on column 1 & column 2

The red lines mark ± 0.1 min

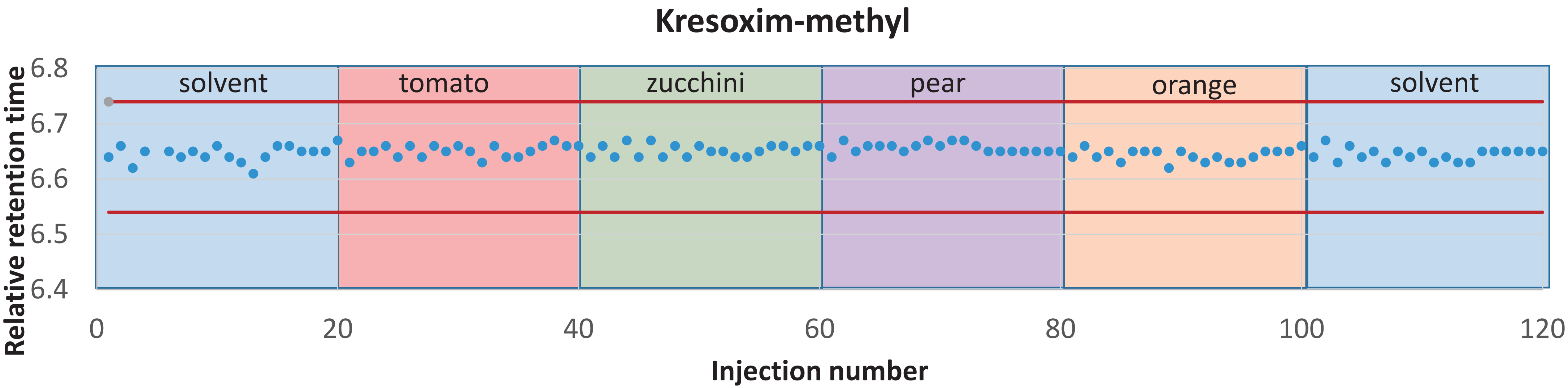
Dual-Channel LC-MS/MS: retention time stability



120 injections alternating on column 1 & column 2

The red lines mark ± 0.1 min

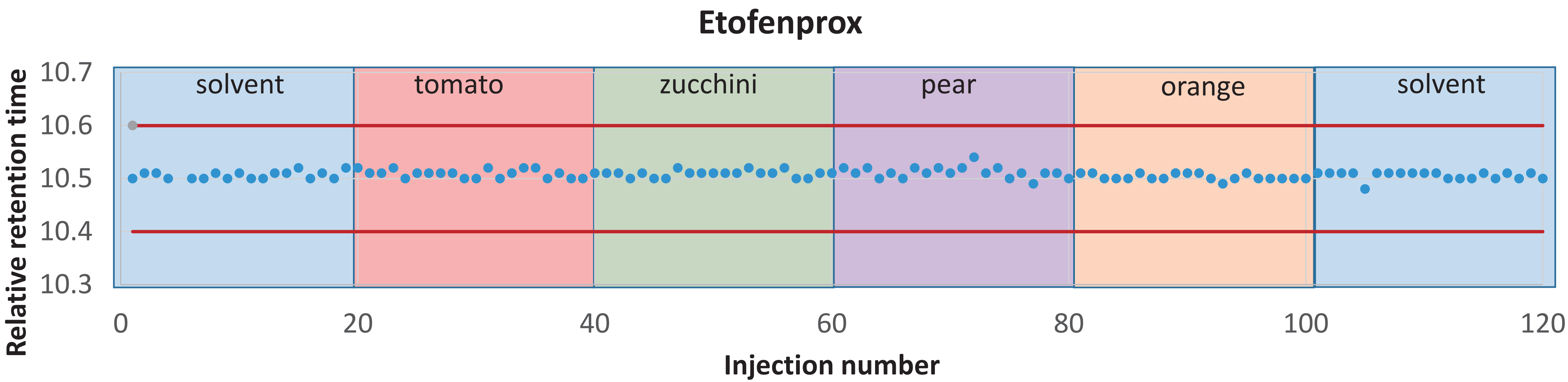
Dual-Channel LC-MS/MS: retention time stability



120 injections alternating on column 1 & column 2

The red lines mark ± 0.1 min

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



120 injections alternating on column 1 & column 2

The red lines mark ± 0.1 min

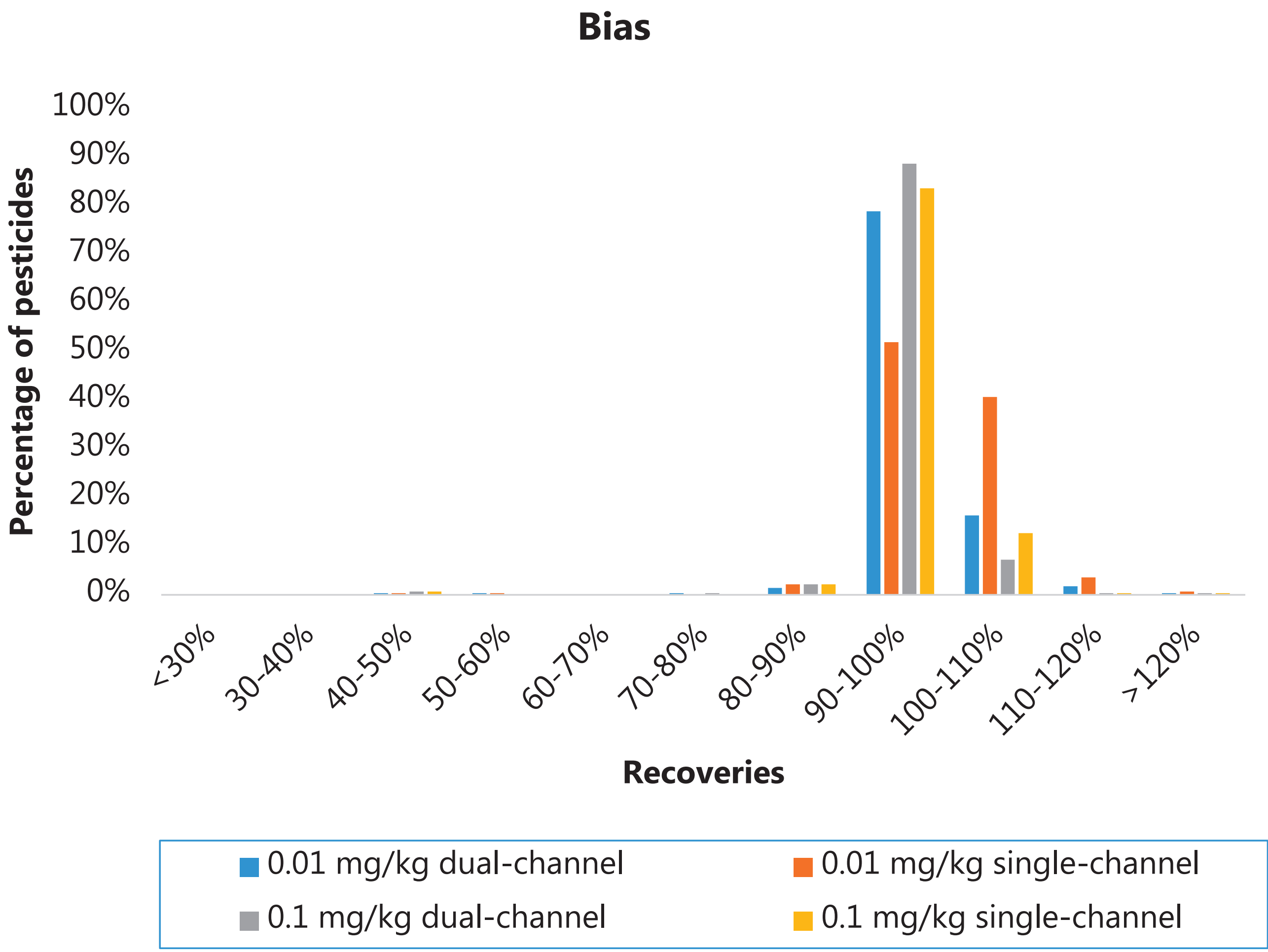
Single and Dual-Channel validation

- Validation experiments were performed employing **single channel** and **Dual-Channel**
- A Thermo **TSQ Altis** Triple Quadrupole Mass Spectrometer was used for the measurements
- A total of **273 LC-amenable pesticide residues** were evaluated
- **Three matrices** belonging to two different commodity groups were studied
- Validation criteria as per the Document Nº SANTE/12682/2019

Single and Dual-Channel validation: apple

Both channels were used simultaneously

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: apple

10 g of sample

Add 10 mL acetonitrile

Shake automatically in Agitax

Add 4 g $MgSO_4$ + 1 g NaCl + 1 g $Na_3C_6H_5O_7 \cdot 2\ H_2O$ + 0.5 g $Na_2HC_6H_5O_7 \cdot 3/2\ H_2O$

Shake automatically in Agitax

Centrifuge 5 min. 3700 r.p.m.

100 µL of extract + 400 µL of water

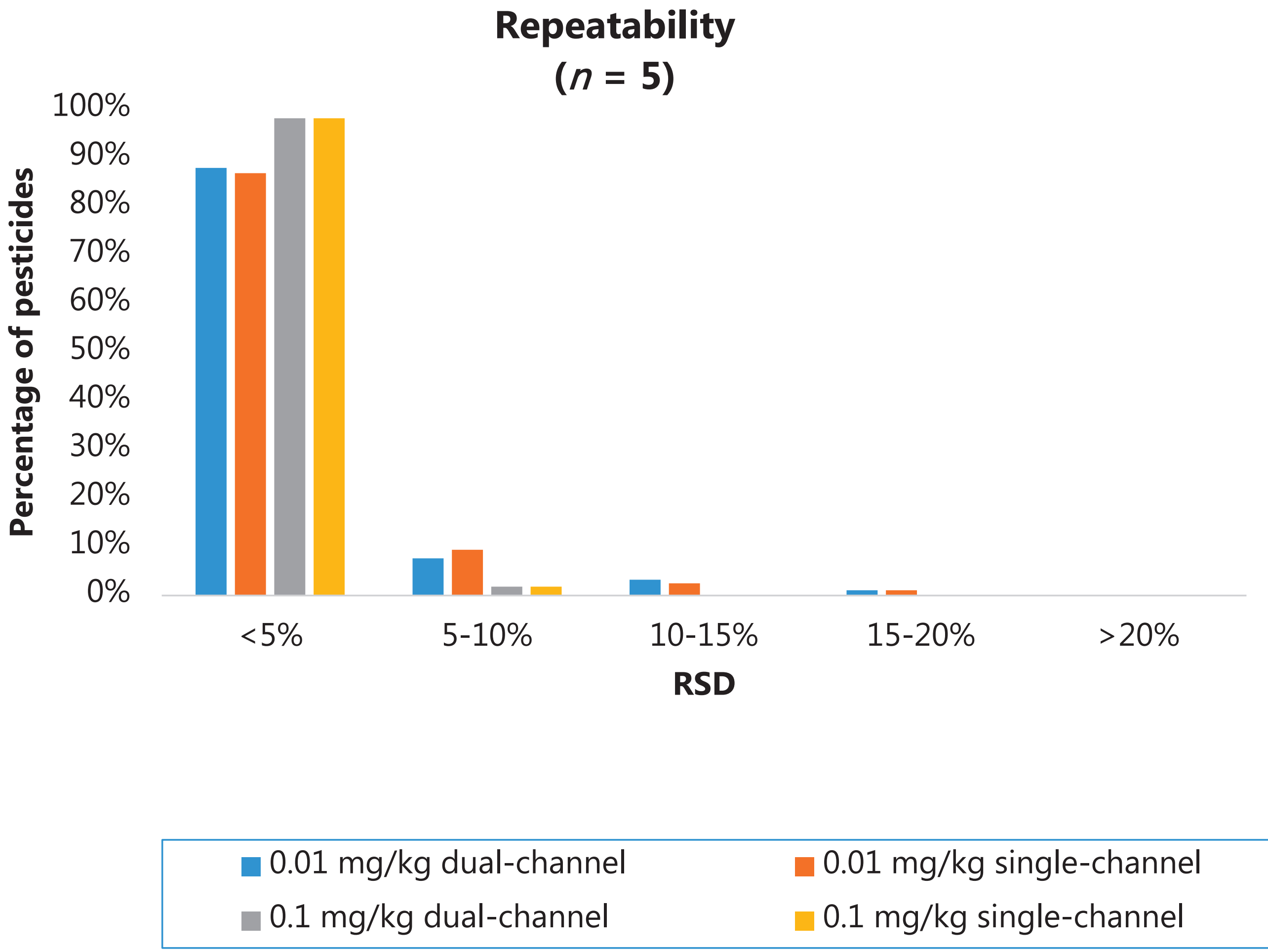
TSQ Altis (injection volume 2.5 µL)

QExactive Focus (injection volume 10 µL)

QuEChERS without clean-up

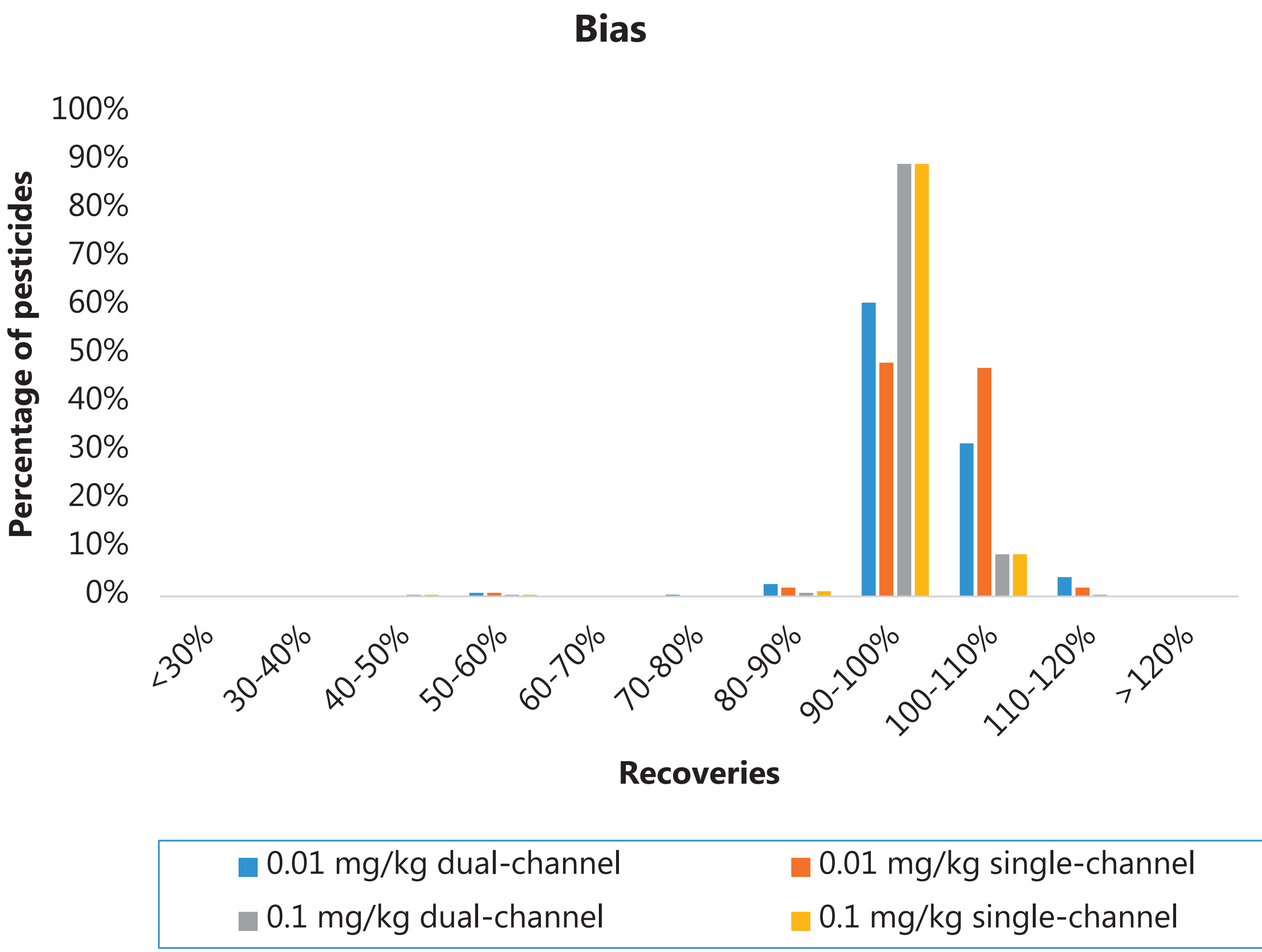
Single and Dual-Channel validation: apple

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%	-



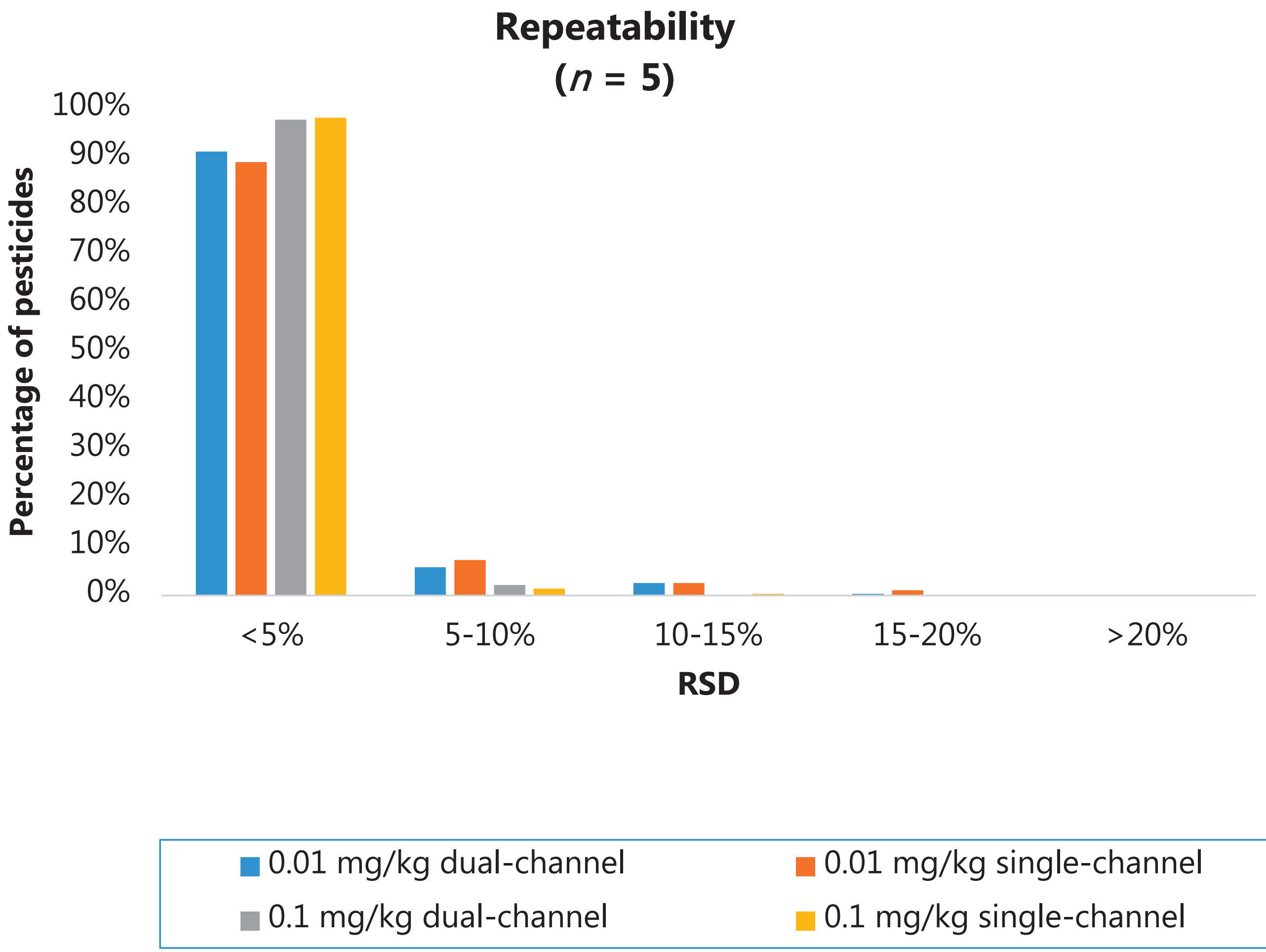
Single and Dual-Channel validation: bell pepper

Technique	<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	-



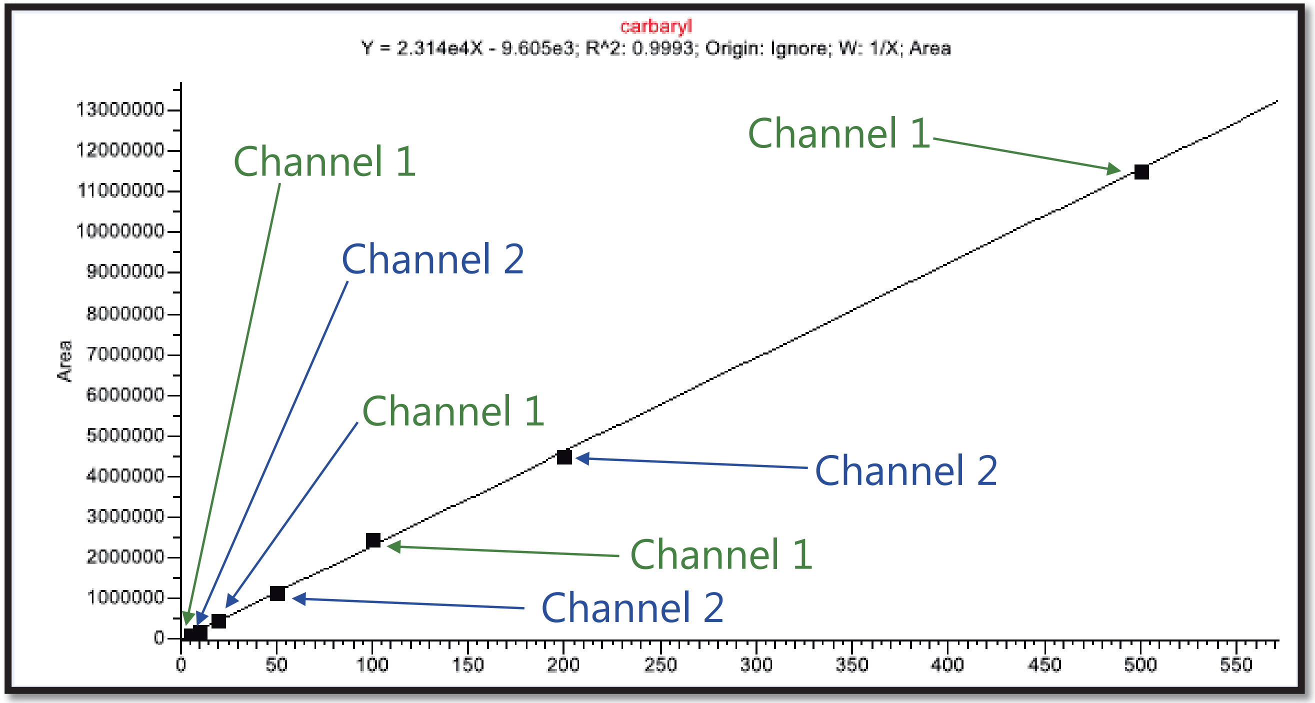
Single and Dual-Channel validation: bell pepper

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%	-



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

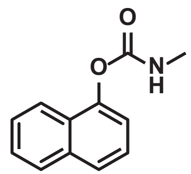
- Calibration curves can be injected using one channel, two channels, or either channel



Channel 1
R^2 = 0.9995

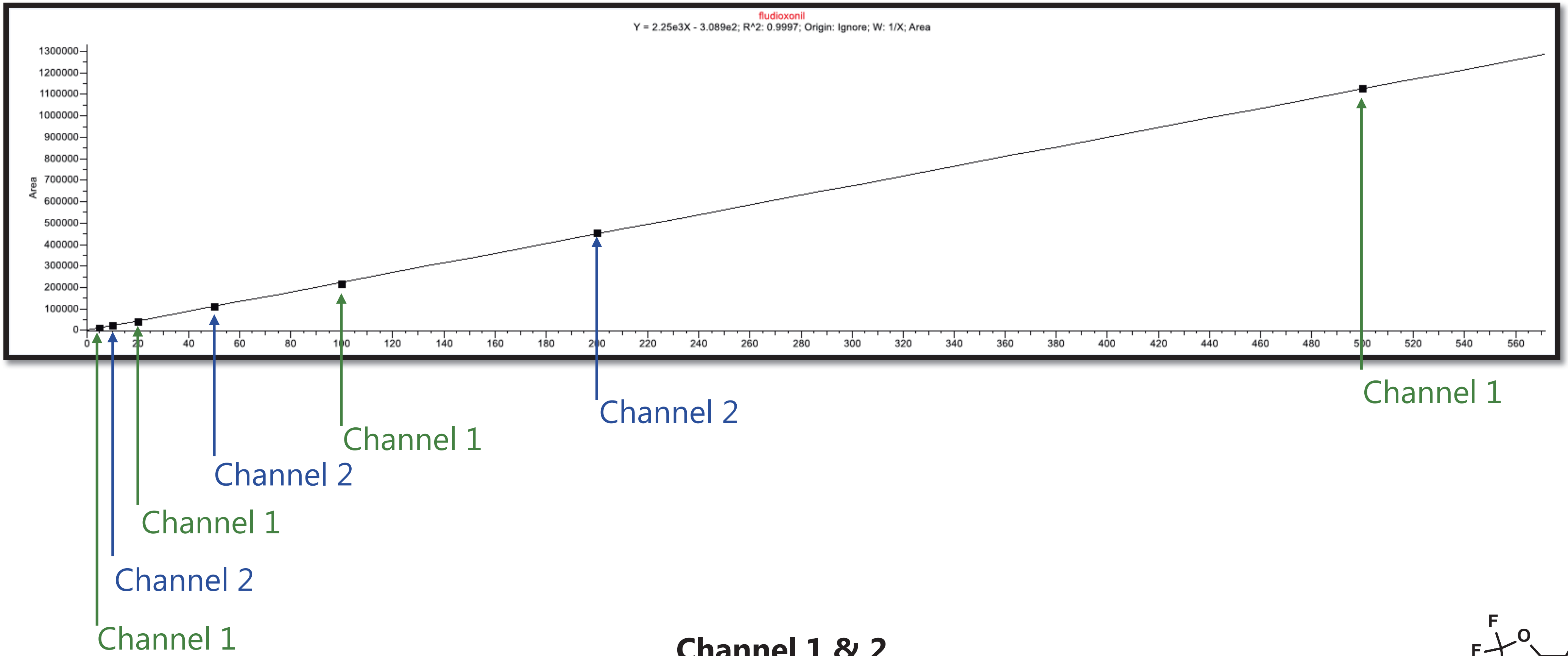
Channel 1 & 2
R^2 = 0.9993

Channel 2
R^2 = 0.9997



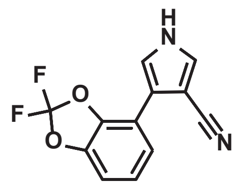
Carbaryl

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



Channel 1 & 2

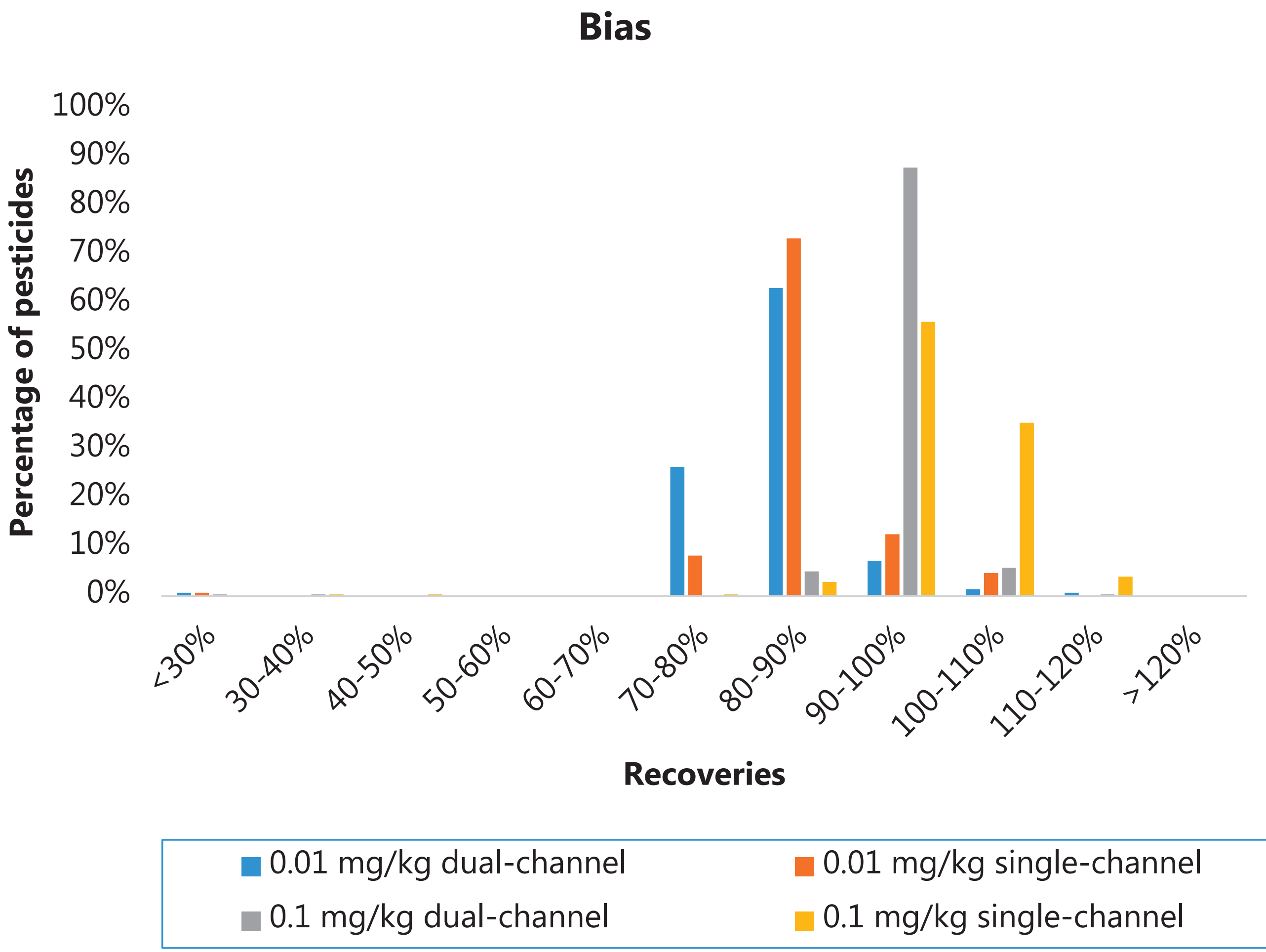
R^2 = 0.9997



Fludioxonil

Single and Dual-Channel validation: orange

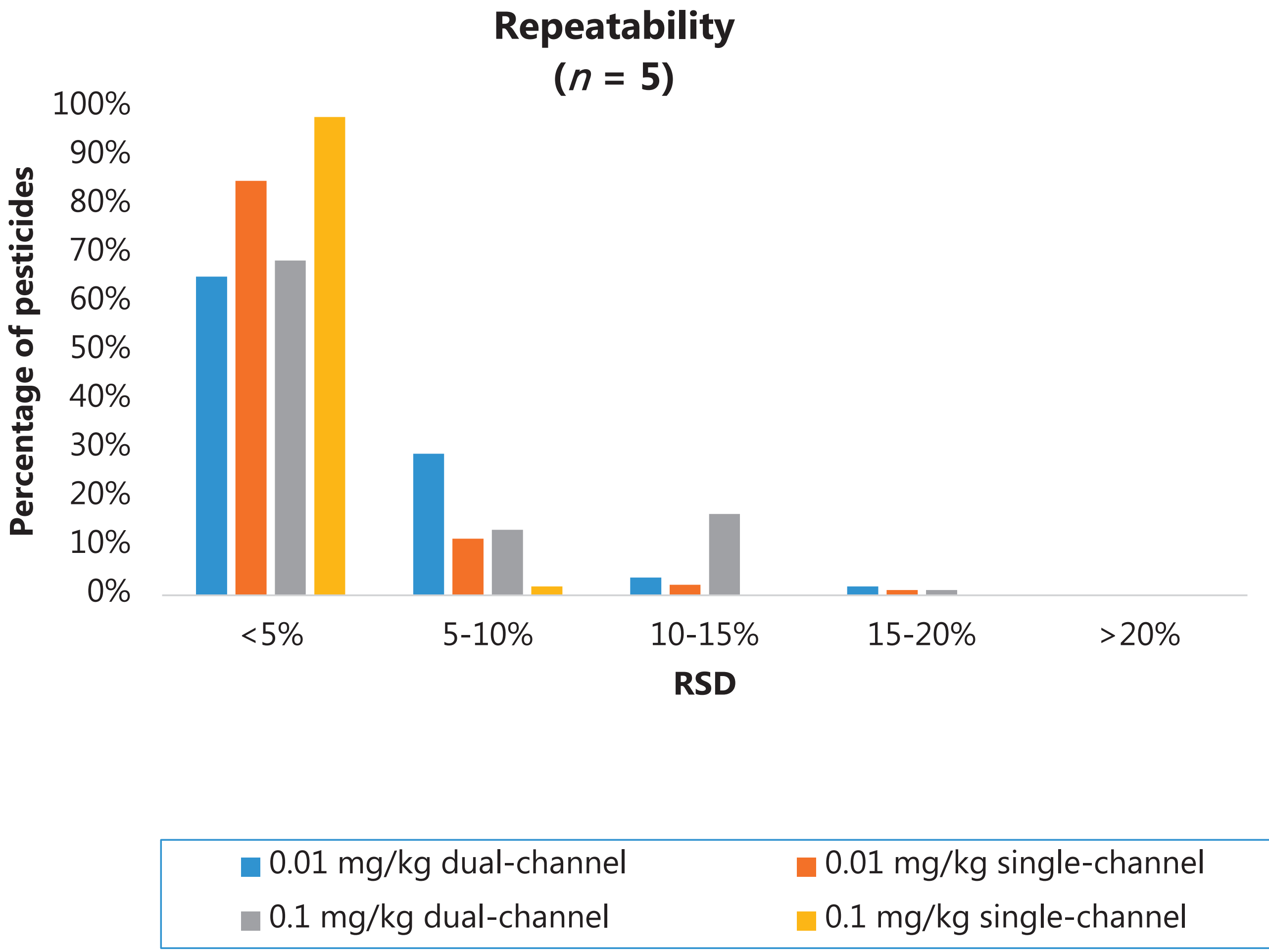
Technique	<70%	70-120%	>120%
Single channel 0.01 mg/kg	2	271	-
Dual-Channel 0.01 mg/kg	2	271	-
Single channel 0.1 mg/kg	2	271	-
Dual-Channel 0.1 mg/kg	2	271	-



Single and Dual-Channel validation: orange

We don't think it is a consequence of DC use

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: proficiency test samples

FAPAS FCPM2-VEG76 (tomato)

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
Cymoxanil	0.6	0.7	0.6	0.7
Dimethomorph	0.9	1.2	1.1	1.1
Fluopyram	0.1	0.1	0.1	0.0
Methoxyfenozide	0.7	0.8	0.8	0.8
Oxadixyl	0.3	0.2	0.2	0.2
Pirimicard	0.4	0.3	0.4	0.4
Pyridalyl	compound detected/standard not injected -> not quantified			
Teflubenzuron	1.0	1.1	1.0	1.0
	Cal.: Channel 1 **Sample:** Channel 1	**Cal.:** Channel 2 **Sample:** Channel 2	**Cal.:** Cross-Channel **Sample:** Channel 1	**Cal.:** Cross-Channel **Sample:** Channel 2

***z*-scores**

$|z| \leq 2.0$ → Acceptable

$2.0 < |z| \leq 3.0$ → Questionnable

$|z| \geq 3.0$ → Inacceptable

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

EUPT-FV 13
(mandarin)

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
Chlorpyriphos	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
Pendimethanil	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: proficiency test samples

EUPT-FV 14
(pear)

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
Boscalid	0.5	0.4	0.5	0.4
Cyprodinil	0.2	0.1	0.1	0.1
Diazinon	0.5	0.5	0.5	0.5
Fludioxonil	0.5	0.5	0.6	0.6
Flufenoxuron	0.5	0.6	0.8	0.5
Indoxacarb	0.5	0.3	0.5	0.4
Methoxyfenozide	0.1	0.0	0.0	0.0
Phosemt	0.4	0.4	0.3	0.4
Pyraclostrobin	0.7	0.7	0.8	0.7
Pyrimethanil	0.4	0.4	0.4	0.4
Spirodiclofen	0.2	0.3	0.3	0.3
Thiabendazole	0.7	0.5	0.7	0.5
Thiacloprid	0.5	0.5	0.7	0.5
Triflumuron	0.6	0.7	0.7	0.6

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

EUPT-FV 16
(bell pepper)

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
Acetamiprid	0.2	0.2	0.2	0.1
Buprofezin	0.0	0.0	0.1	0.0
Chlorpyriphos	0.3	0.2	0.3	0.2
Cyprodinil	0.4	0.2	0.4	0.2
Diazinon	0.2	0.3	0.2	0.4
Difenoconazol	0.3	0.4	0.4	0.4
Fenamiphos	0.8	0.9	0.8	1.0
Fenamiphos Sulfone	0.6	0.5	0.6	0.5
Fenamiphos Sulfoxide	0.6	0.5	0.7	0.5
Fenhexamid	0.5	0.5	0.6	0.6
Fludioxonil	0.5	0.6	0.4	0.6
Methoxyfenozide	0.5	0.5	0.5	0.5
Pirimicarb	0.1	0.1	0.1	0.1
Pyridaben	0.3	0.4	0.4	0.4
Spinosad	0.3	0.3	0.2	0.1
Tetraconazole	0.1	0.2	0.0	0.1

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

EUPT-FV 17 (broccoli)

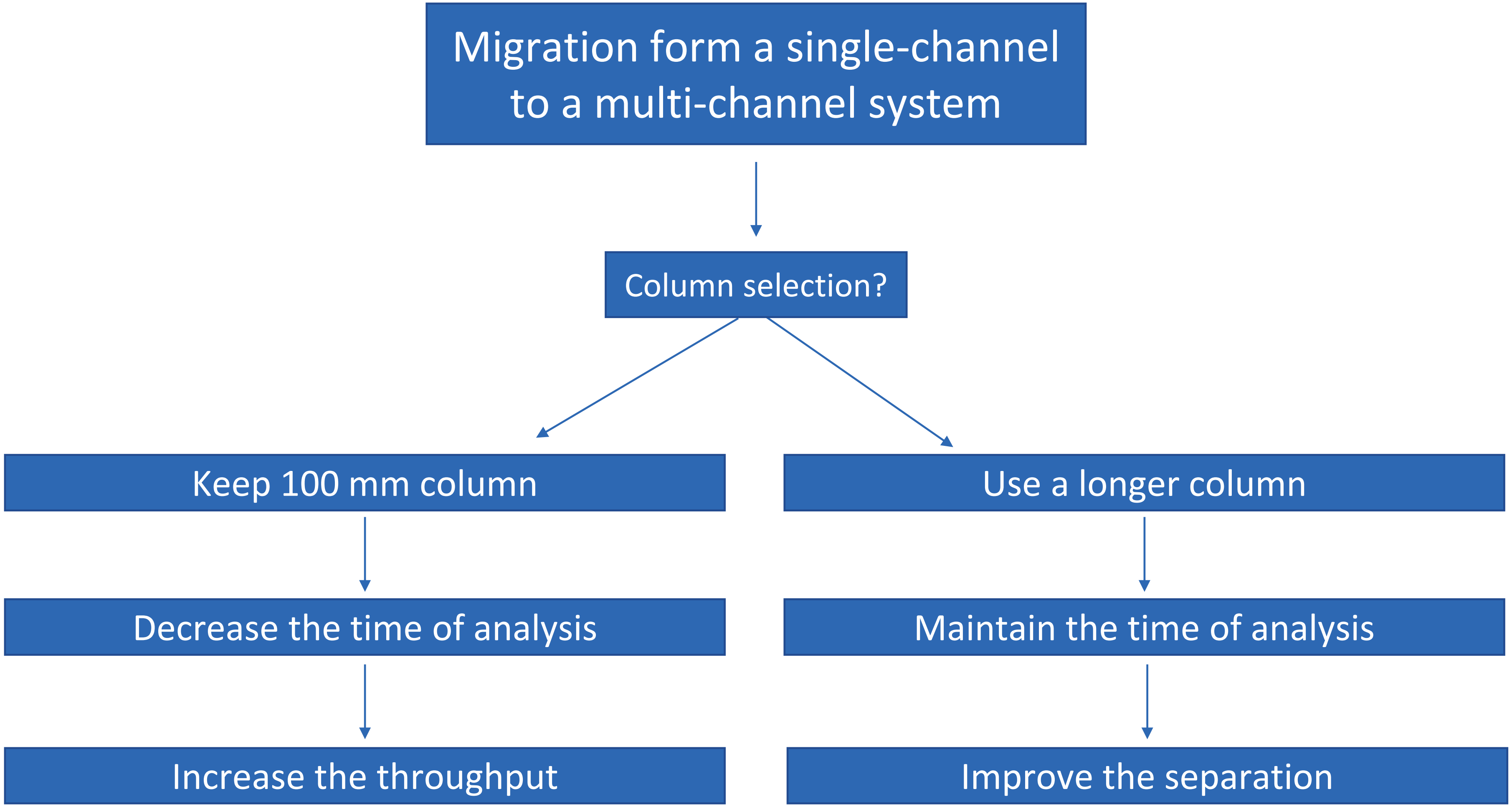
Compound	In-channel calibration/sample injected on channel 1	In-channel calibration/sample injected on channel 2	Cross-channel calibration/sample injected on channel 1	Cross-channel calibration/sample injected on channel 2
Bupirimate	0.2	0.1	0.2	0.2
Carbendazim	0.0	0.1	0.0	0.0
Diazinon	0.5	0.0	0.5	0.0
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

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

EUPT-FV 18
(spinach)

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
Chlorantraniliprole	0.4	0.4	0.4	0.4
Difenoconazole	0.3	0.4	0.3	0.5
Diflubenzuron	0.6	0.2	0.0	0.2
Dimethoate	0.3	0.3	0.7	0.7
Dimethoate (dimethoate+omethoate)	*1.1*	*1.1*	*1.1*	*1.1*
Famoxadone	0.2	0.4	0.1	0.5
Fluopyram	0.3	0.2	0.3	0.2
Imidacloprid	0.4	0.7	0.4	0.6
Indoxacarb	0.3	0.3	0.3	0.5
Metalaxyl	0.1	0.1	0.3	0.1
Omethoate	0.9	0.9	0.9	0.9
Thiaclorpid	0.3	0.3	0.3	0.3
Triadimenol	0.5	0.3	0.4	0.4

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

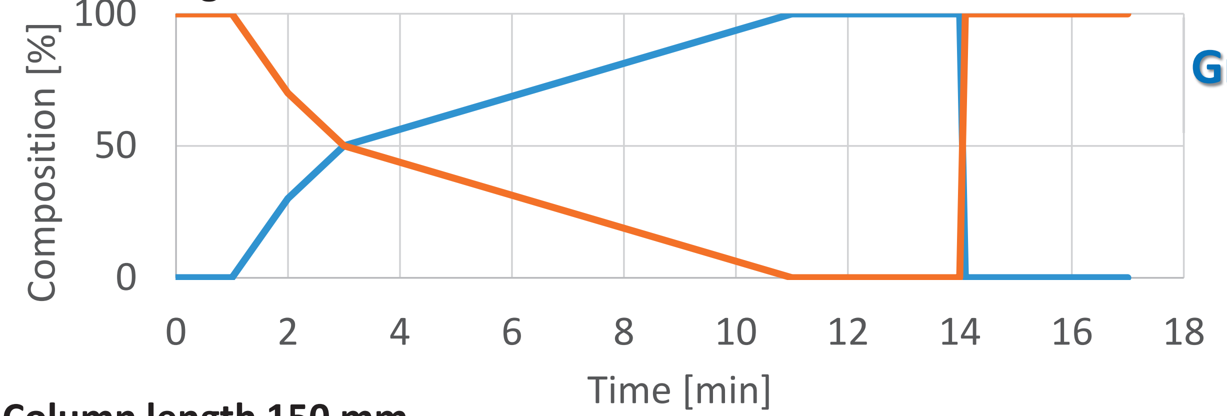


Dual-Channel LC-MS/MS: increased column length

- 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 (QOrbitrap)
- **Longer analysis** time of longer columns compensated by Dual-Channel time savings

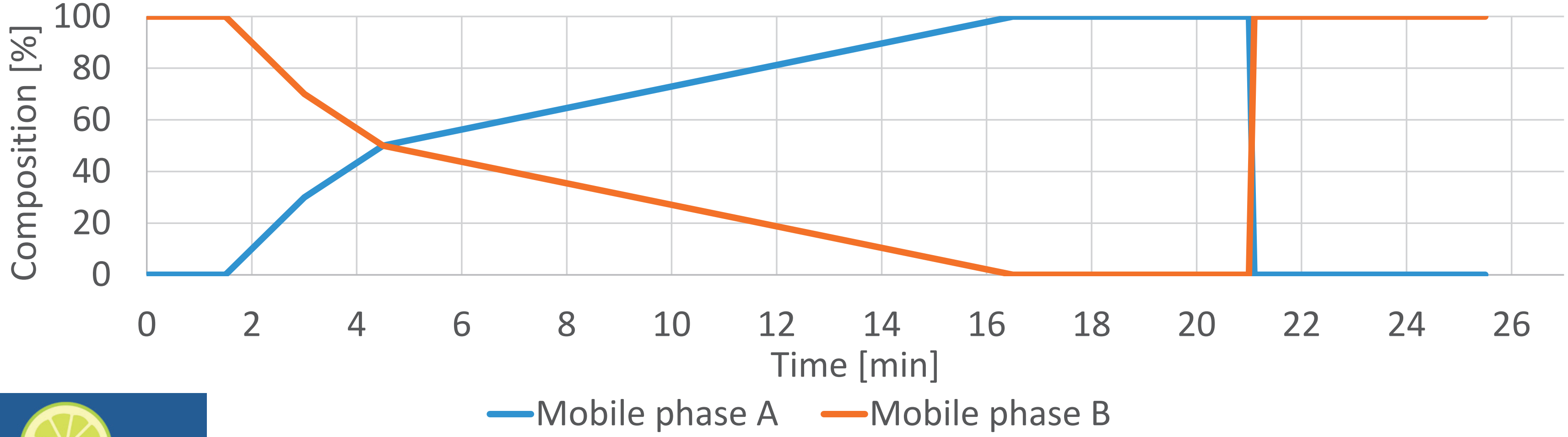
Dual-Channel LC-MS/MS: increased column length

Column lenghth 100 mm



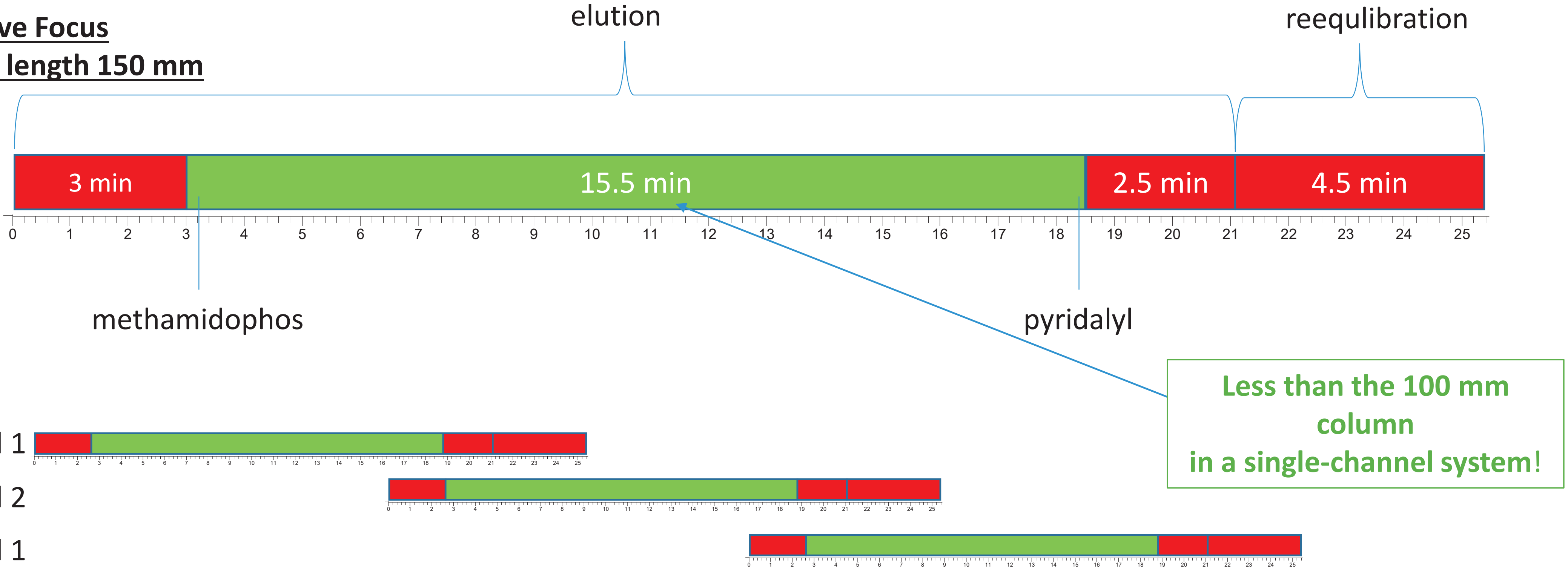
Gradient used with the triple quadrupole

Column length 150 mm



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

QExactive Focus
Column length 150 mm

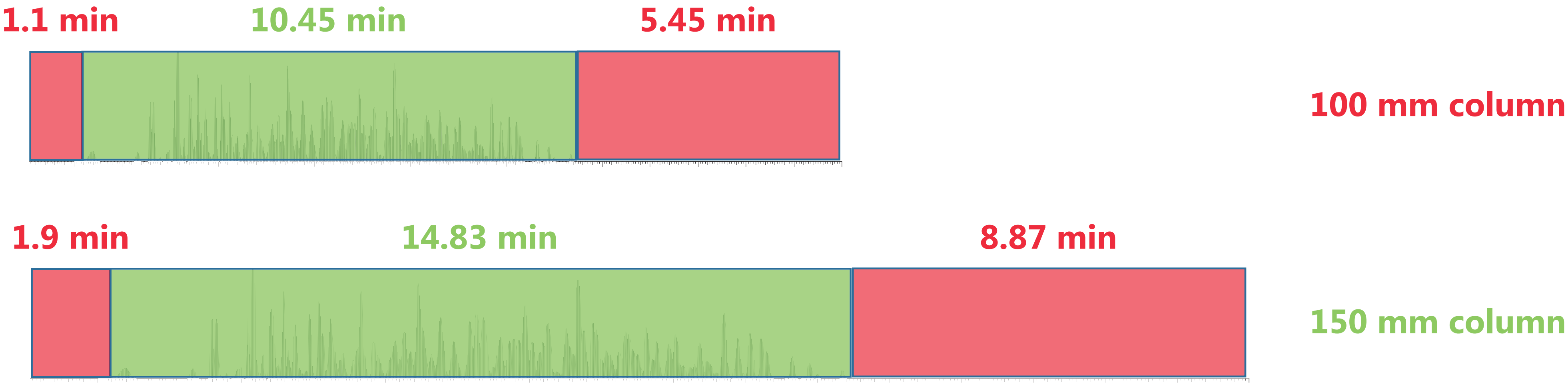


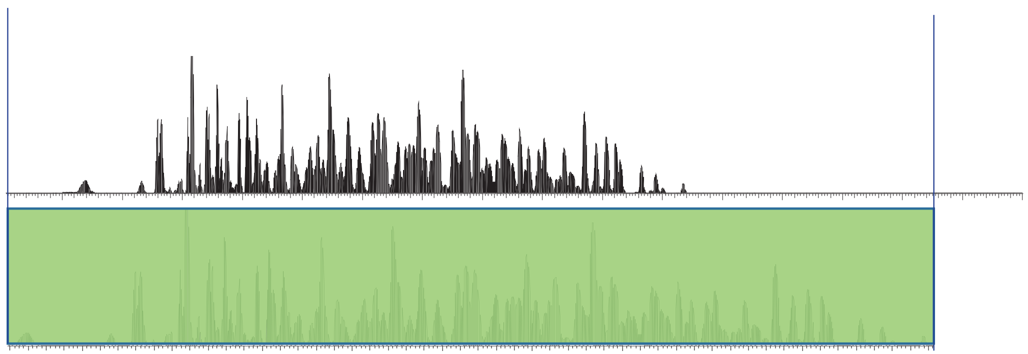
to waste

to MS (**acquisition time 15.5 min**)

Total time in a single-channel system 25.5 min
(+ 1 minute for needle wash, sample aspiration, etc.)

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

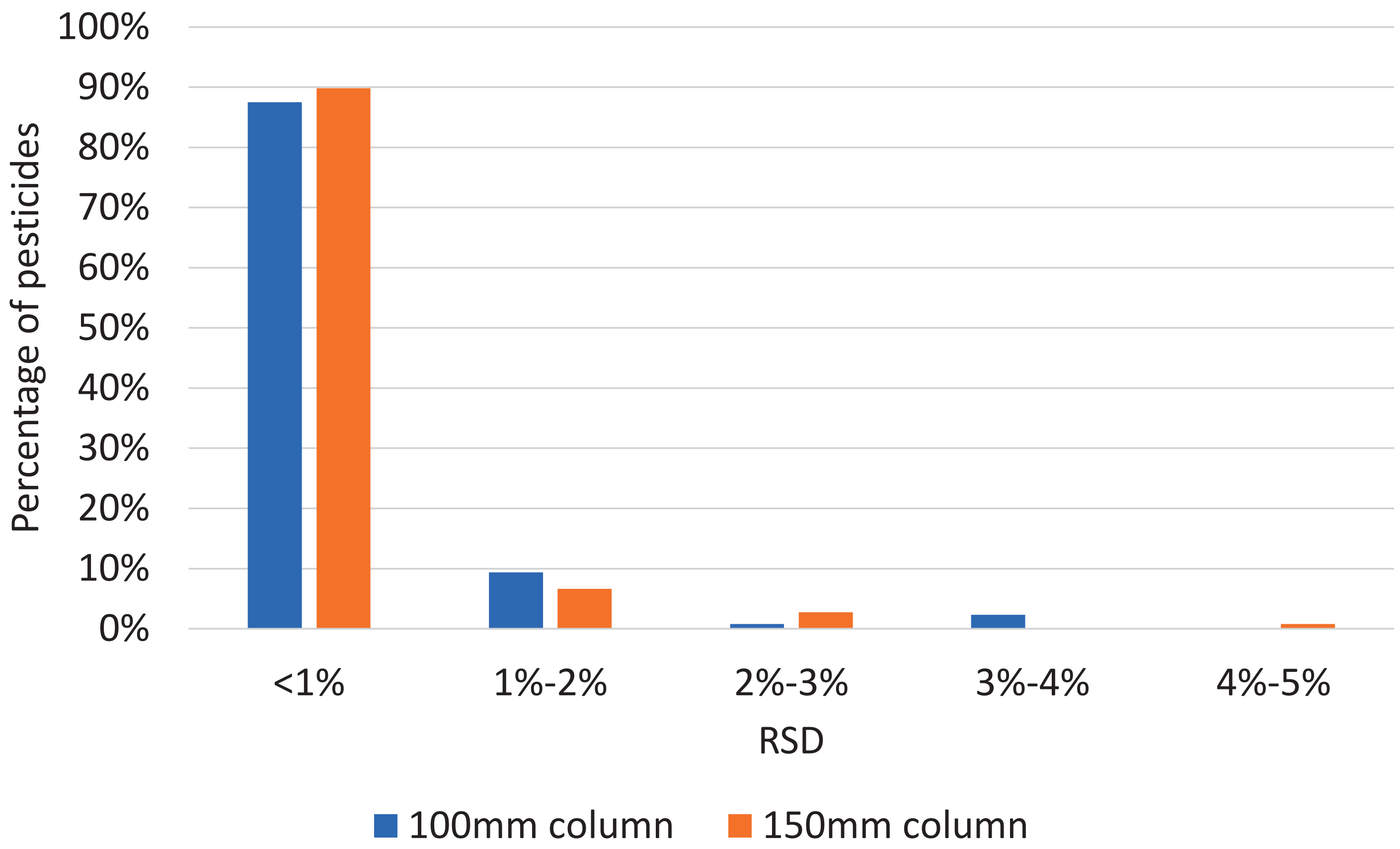




Using Dual-Channel chromatography and a 150 mm column results in shorter analysis times per sample compared to a single channel analysis on a 100 mm column

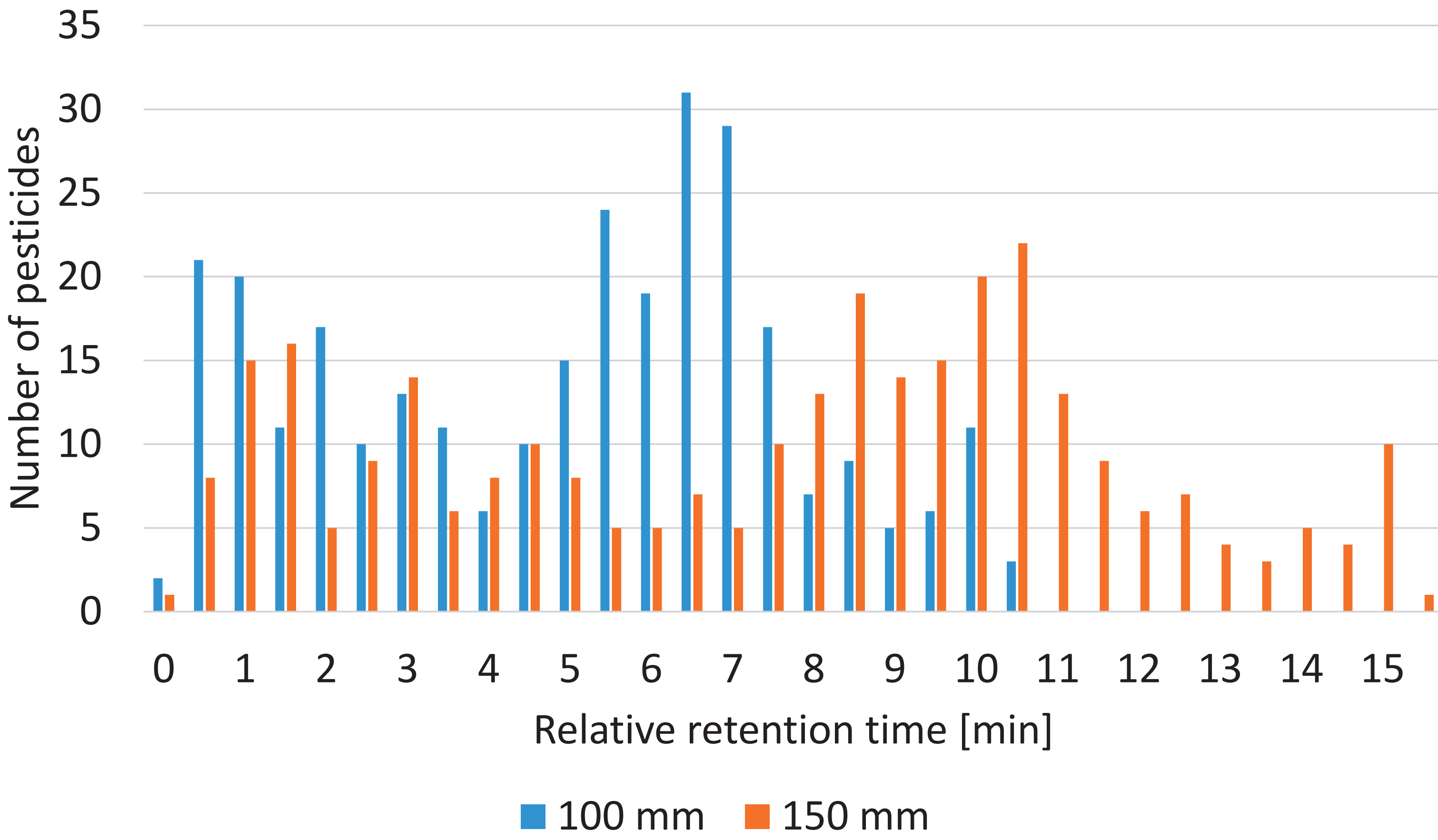
The use of a longer column results in improved separation, increasing selectivity and sensitivity without compromising analysis time

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



n = 25

Dual-Channel LC-MS/MS: pesticide distribution

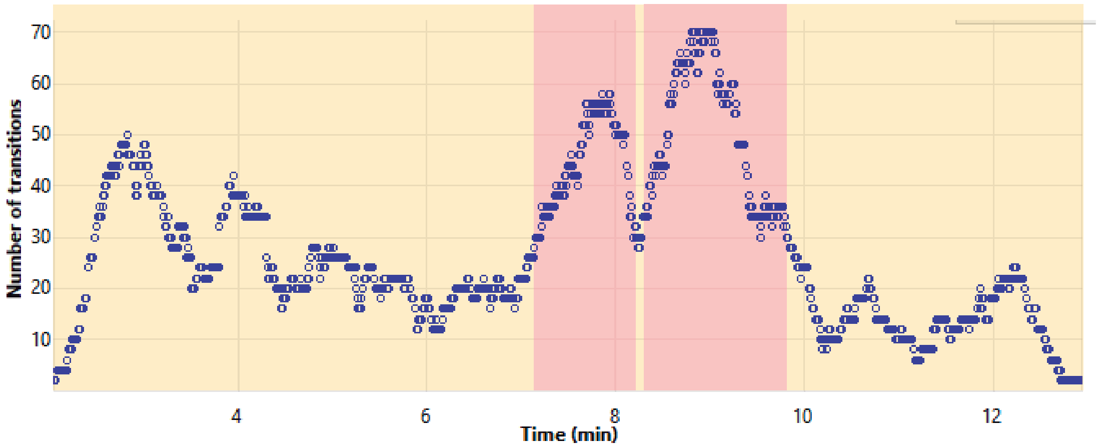


Dual-Channel LC-MS/MS: pesticide distribution

300 pesticides / 600 transitions

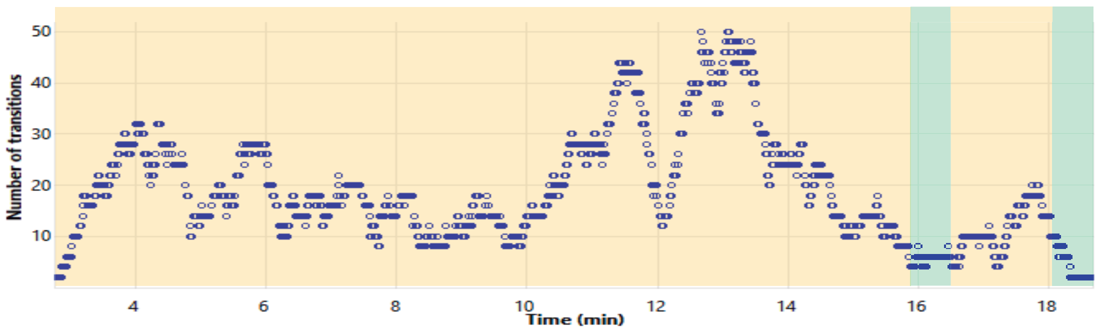
100 mm column

Number of transitions per cycle



150 mm column

Number of transitions per cycle



Dwell time < 10 ms

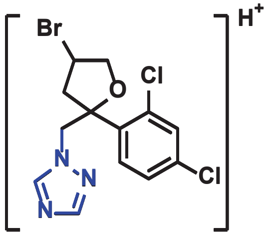
Dwell time 10 – 50 ms

Dwell time > 50 ms

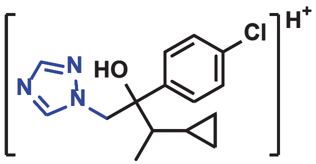
A longer column separates better the analytes. The dwell times can be increased without increasing the duty cycle.

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

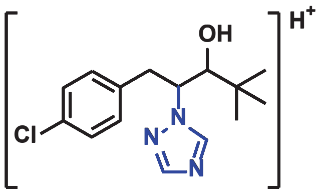
Bromuconazole (first peak)
Full scan MS
375.9614 ± 5 ppm

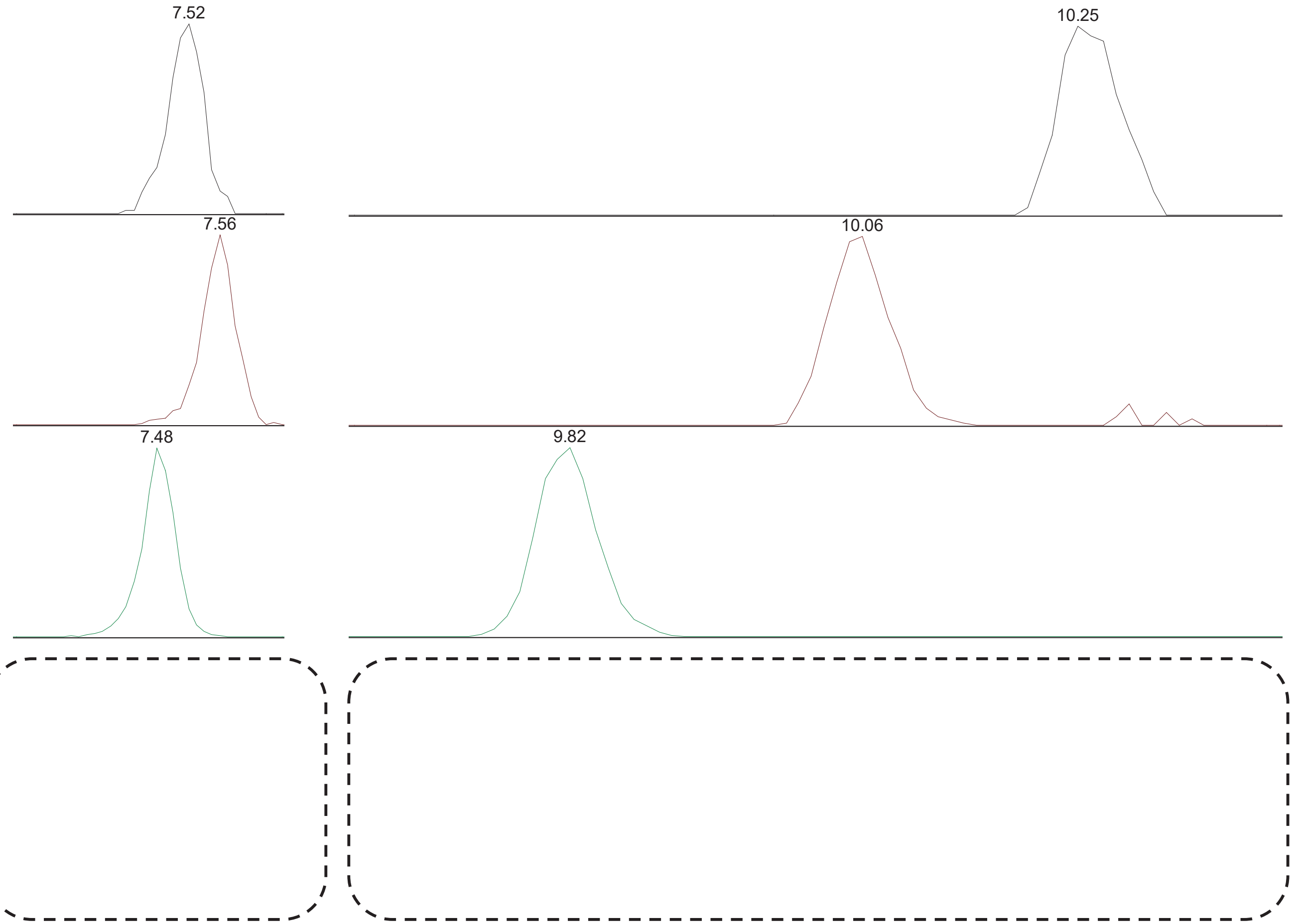


Cyproconazole (first peak)
Full scan MS
292.1211 ± 5 ppm



Paclobutrazole
Full scan MS
294.1368 ± 5 ppm





100 mm column

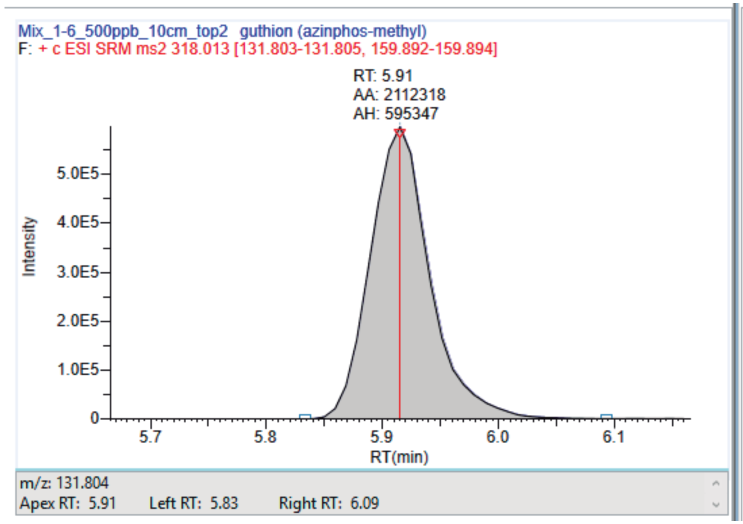
150 mm column

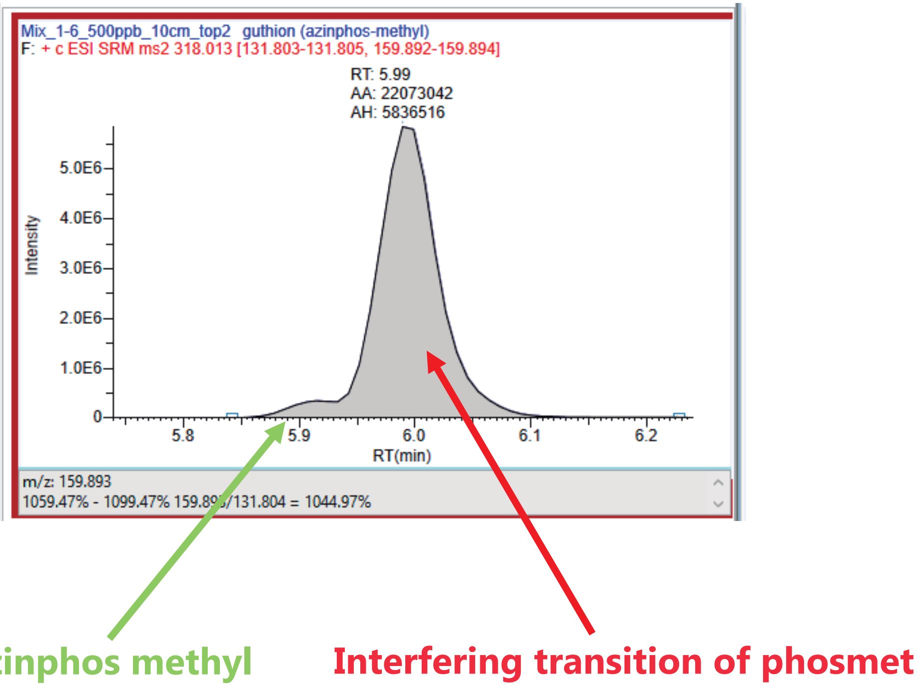
Dual-Channel LC-MS/MS: solving analyte coelution

Azinphos methyl & phosmet coelution

TSQ Altis
Triple quadrupole
100 mm column

m/z **318 -> 132** *m/z* **318 -> 159**





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

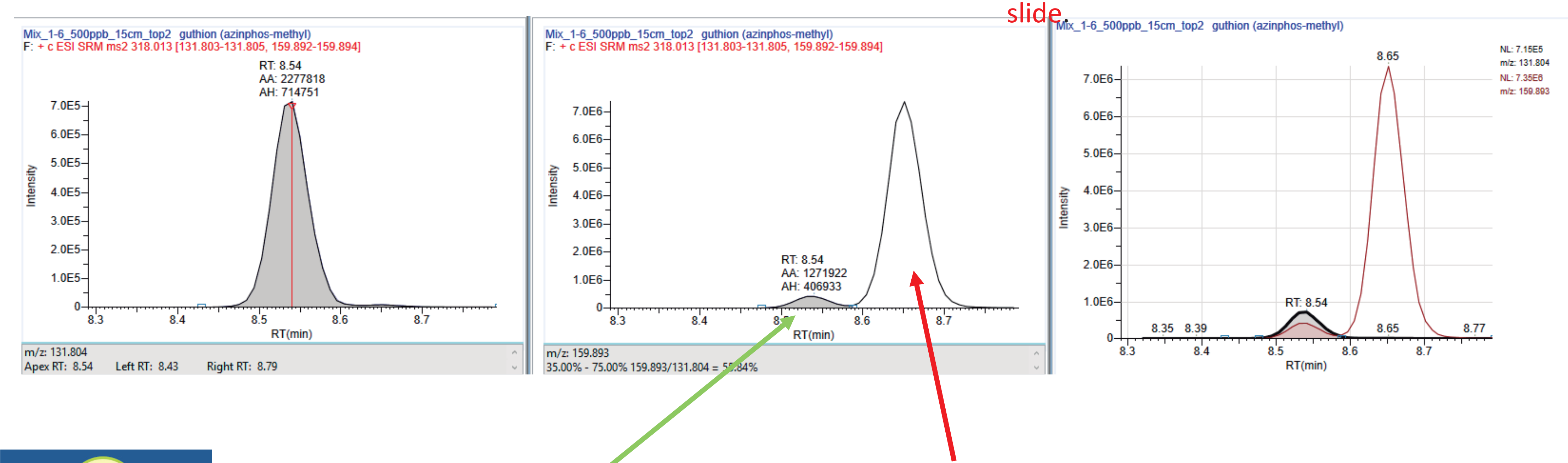
Azinphos methyl & phosmet coelution

TSQ Altis
Triple quadrupole
150 mm column

Combine slide 29 and 30 in one single slide.

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

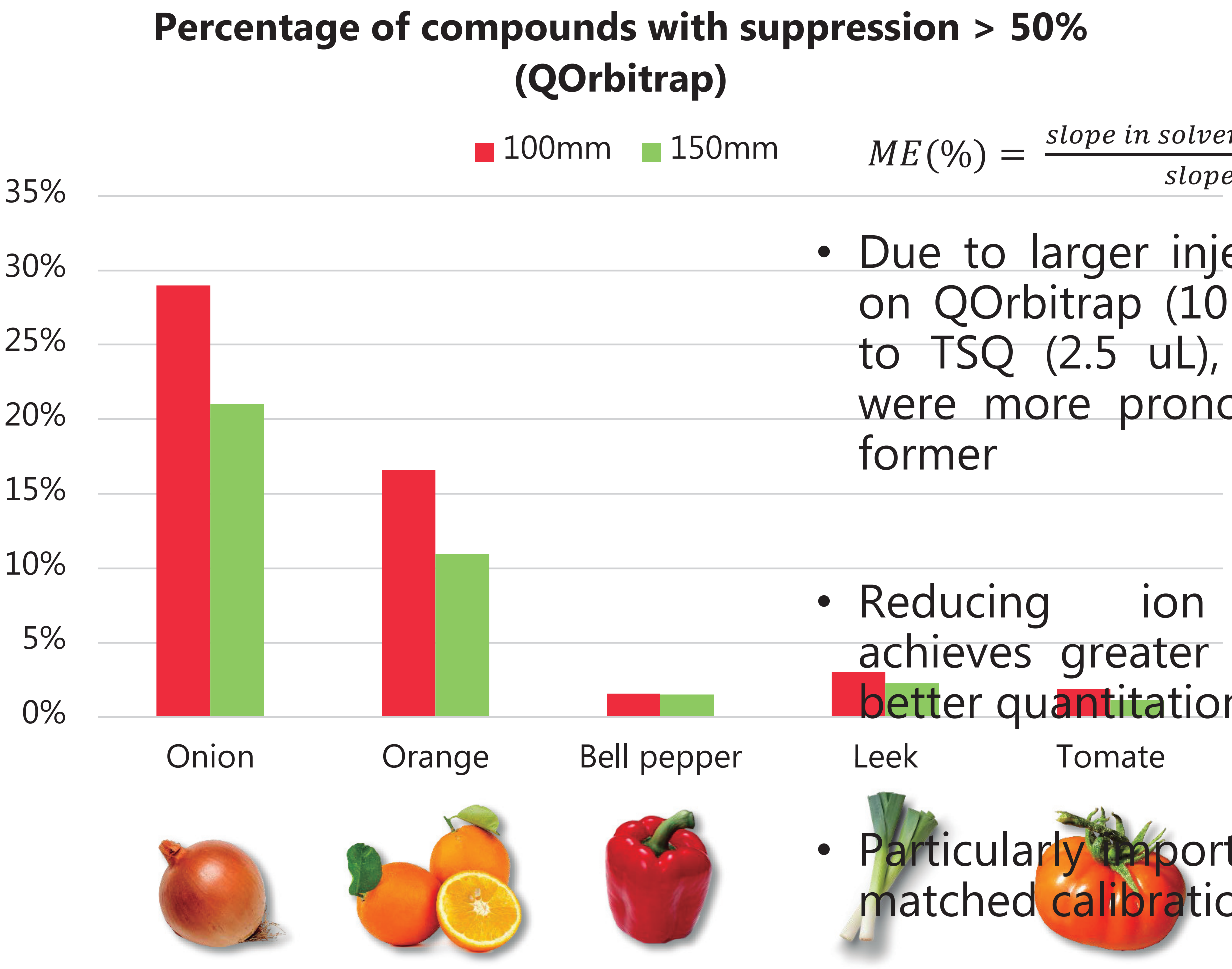
***m/z* 318 -> 159**



Azinphos methyl

Phosmet is separated from azinphos methyl

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



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

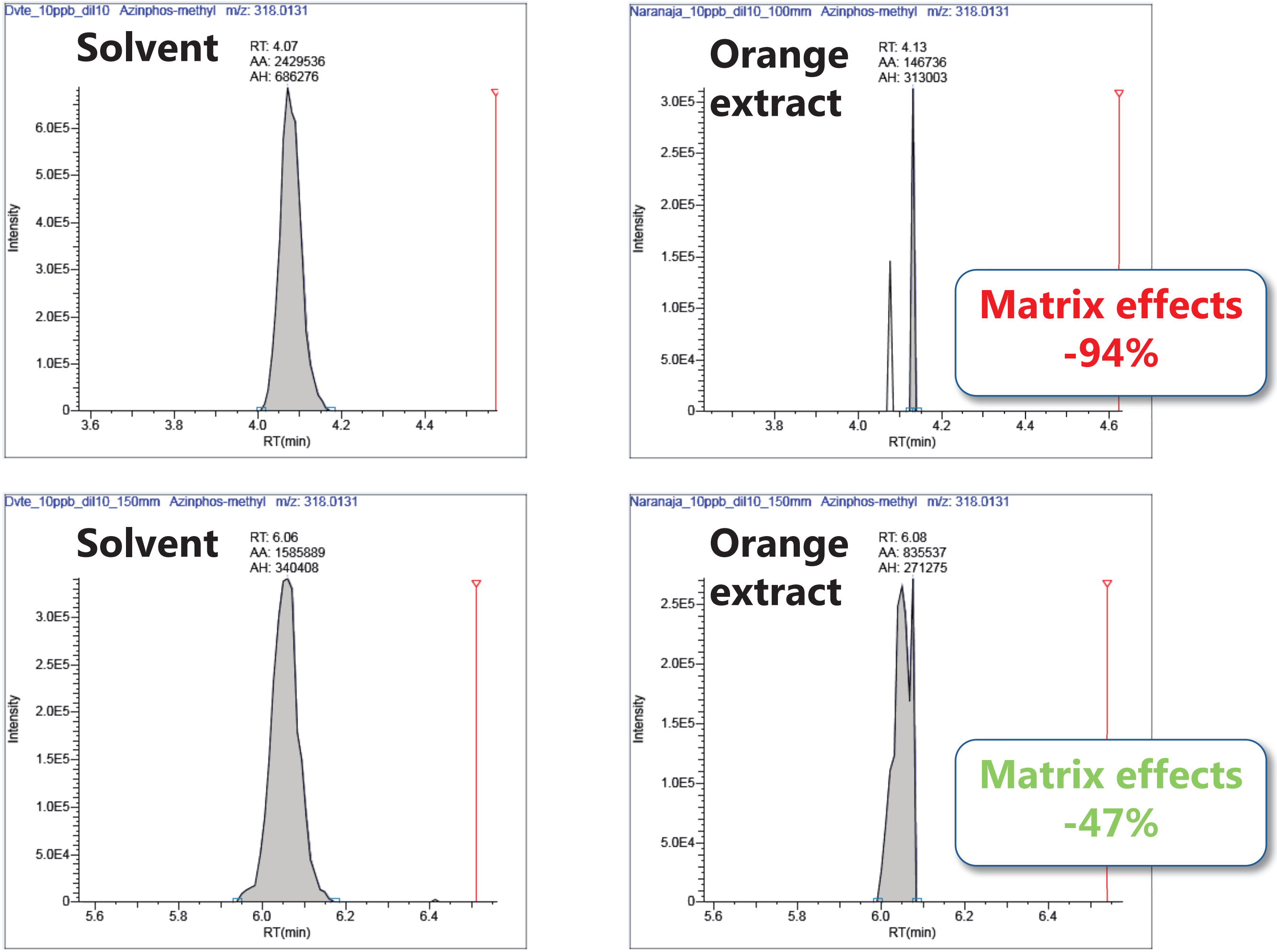
- Due to larger injection volumes on QOrbitrap (10 uL) compared to TSQ (2.5 uL), matrix effects were more pronounced on the former
- Reducing ion suppression achieves greater sensitivity and better quantitation
- Particularly important for matrix-matched calibration

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

QExactive Focus
High-resolution MS
100 mm column

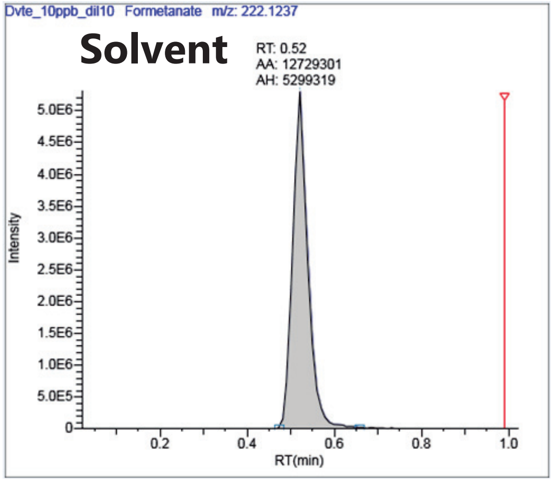
0.01 mg/kg of azinphos-methyl
Full Scan MS
***m/z* 318.0131 ± 5 ppm**

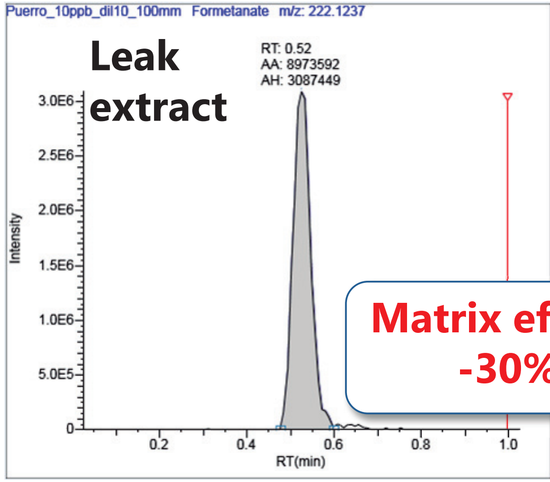
QExactive Focus
High-resolution MS
150 mm column



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

QExactive Focus
High-resolution MS
100 mm column

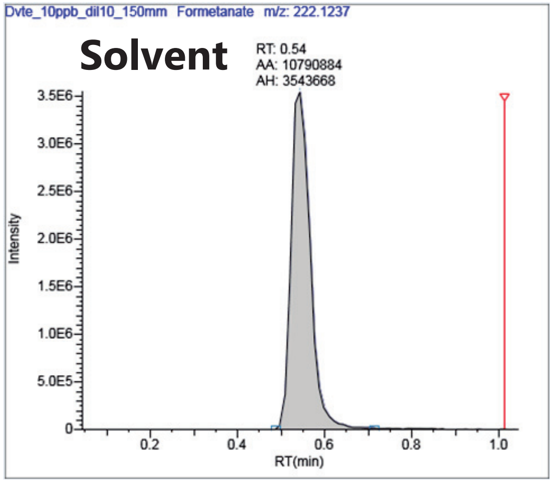


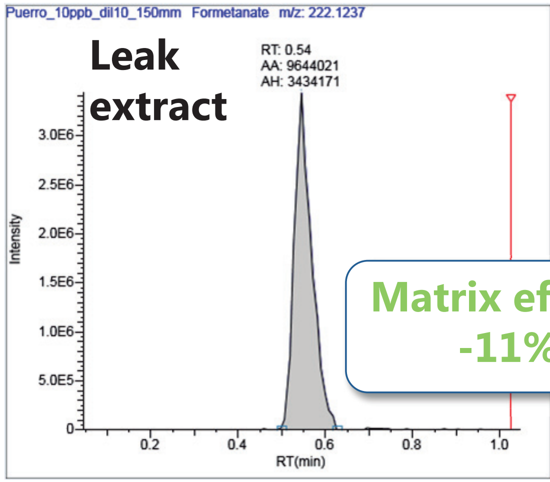


Matrix effects -30%

0.01 mg/kg of formetanate
Full Scan MS
***m/z* 222.1237 ± 5 ppm**

QExactive Focus
High-resolution MS
150 mm column





Matrix effects -11%

Dual-Channel LC-MS/MS: scientific paper

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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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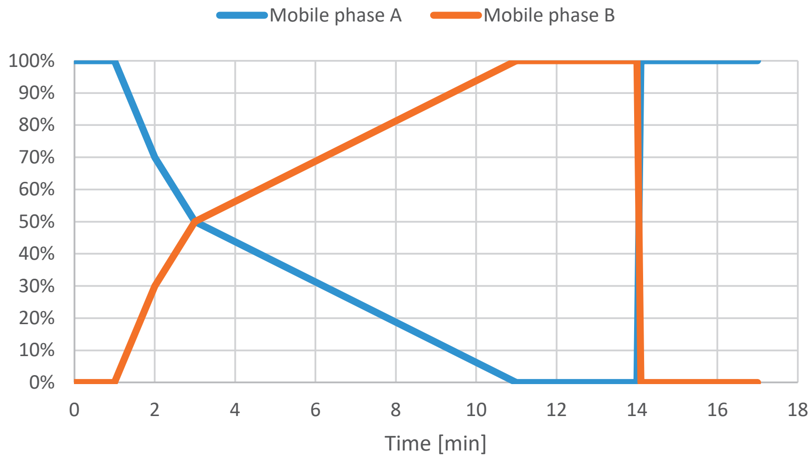
ABSTRACT

Dual-channel chromatography was evaluated for pesticide residue analysis in fruits and vegetables and for unknown compounds detection. A dual-channel system was tested coupled to triple quadrupole and high-resolution mass spectrometry. The first part of the investigation was related to the improvement of the sample throughput with a 100 mm column. The dual-channel system provided the same analytical results as the single-channel system, however, with the throughput higher of about 70% (80 injections vs 137 injections in 24 h). Two types of calibration (in-channel and cross-channel) were checked. In the article, also solvent consumption is discussed. Six proficiency test samples were analysed to assess the quality of the results. Nor false positives neither false negatives were found. Calculated z-scores were typically <1. In the second part, a different approach was investigated. The 100 mm column was replaced by a 150 mm column keeping shorter run times than single channel system and 100 mm. The longer column improved the sensitivity and selectivity what was demonstrated in the target pesticide residue analysis. Additionally, the 150 mm column was compared with the 100 mm column in the analysis of unknown natural matrix compounds by high resolution mass spectrometry. The longer column allowed to detect up to 26% unknown compounds more than the shorter column.

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

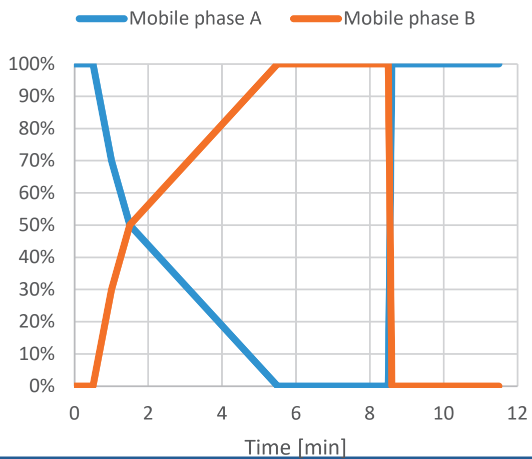
Positive polarity



Mobile phase A:
98 % water
2 % methanol
0.1% formic acid
5 mM ammonium format

Mobile phase B:
98 % methanol
2 % water
0.1 % formic acid
5 mM ammonium format

Negative polarity

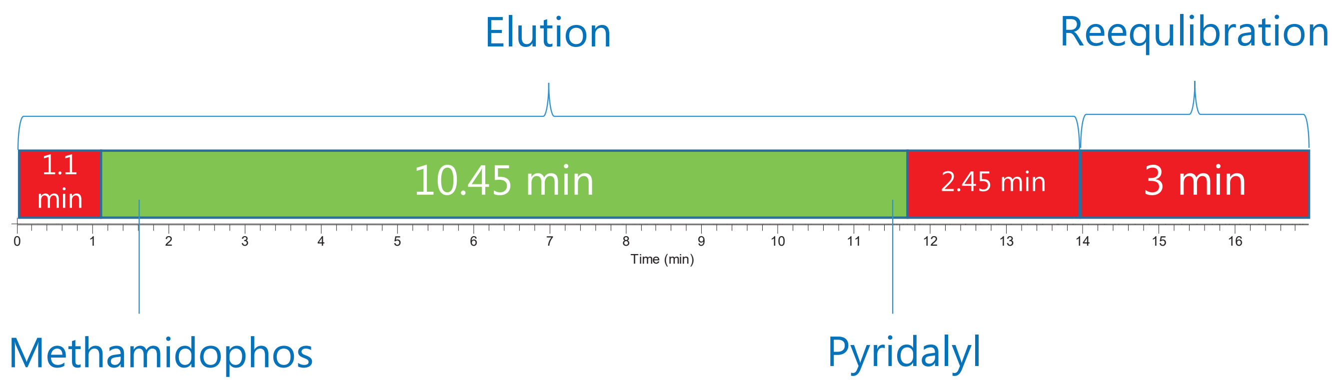


Mobile phase A:
Water + 0.05 % acetic acid

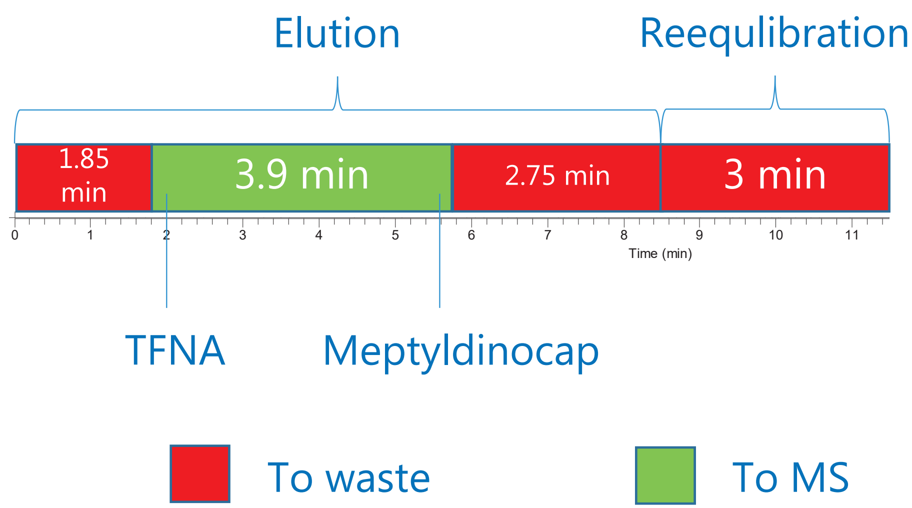
Mobile phase B:
Acetonitrile + 0.05 % acetic acid

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

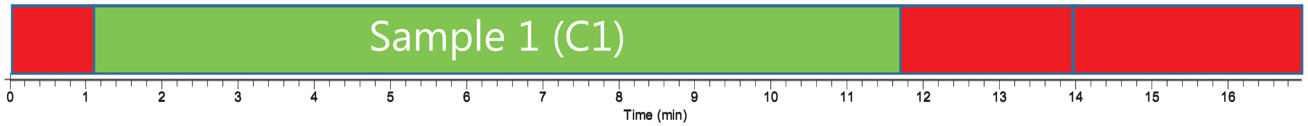
Gradient 1
Water:MeOH
Formic acid (0.1 %)
Ammonium formate (5 mM)

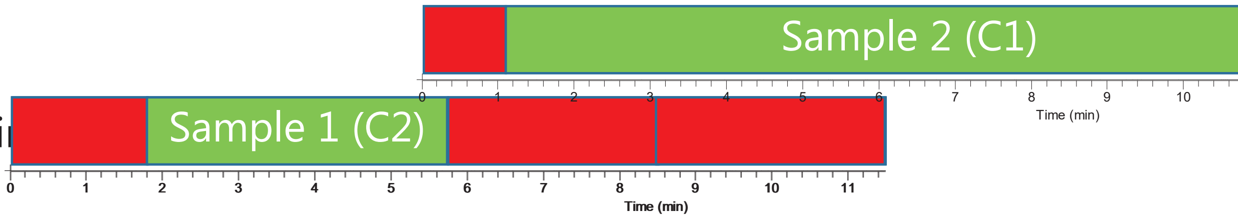


Gradient 2
Water:AcN
Acetic acid (0.05 %)



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





On a single-channel instrument with polarity switchi

On a dual-channel instrument with optimized mobile phases, **sample analysis is 18 min**

Improved method efficiency without time loss – no compromises

To waste

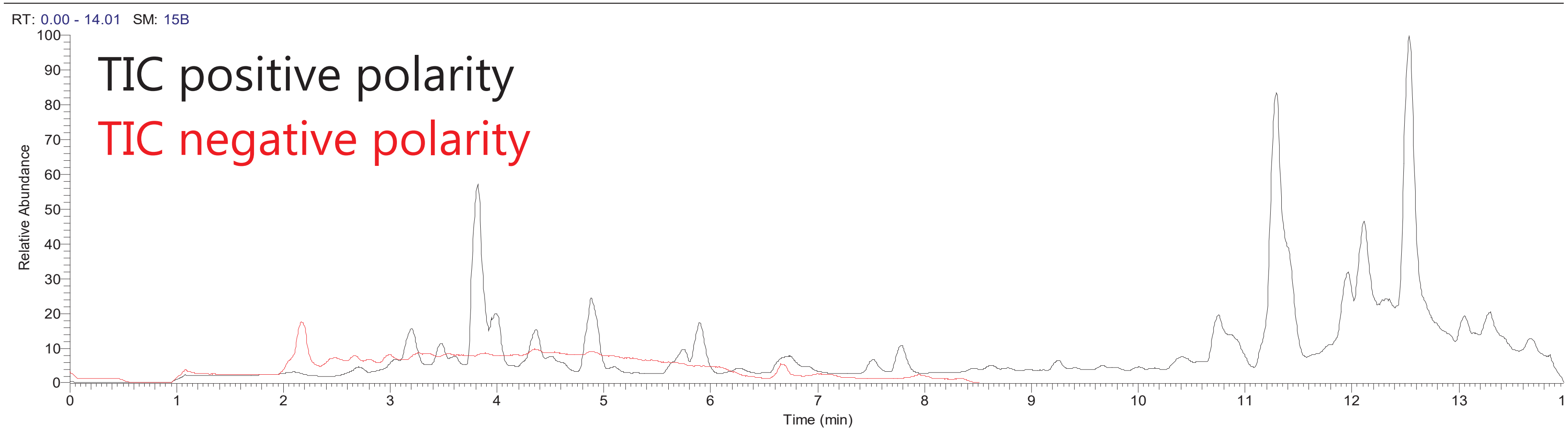
To MS

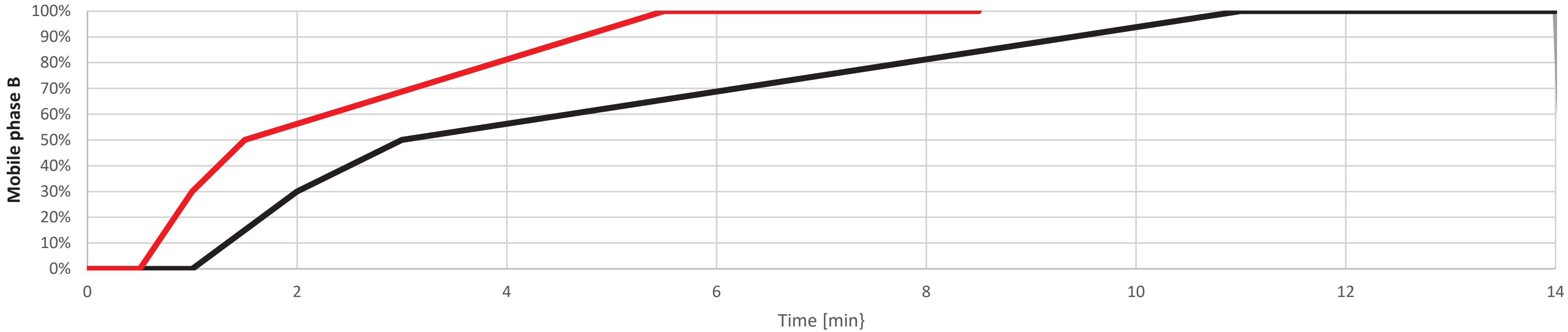
Dual-Channel LC-MS/MS: independent mobile phases

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

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

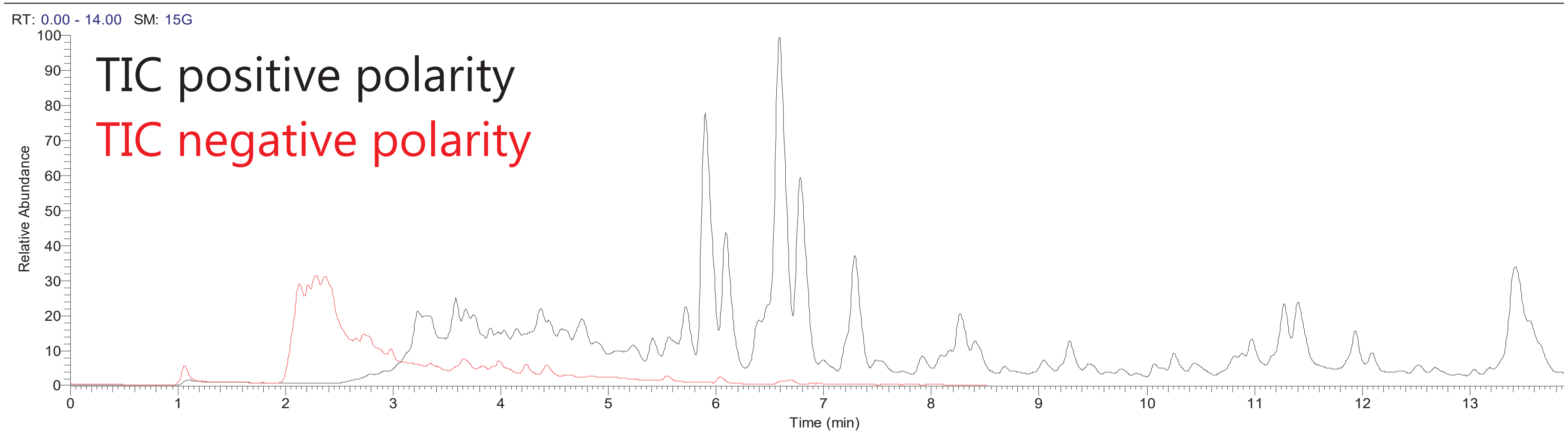
Tomato

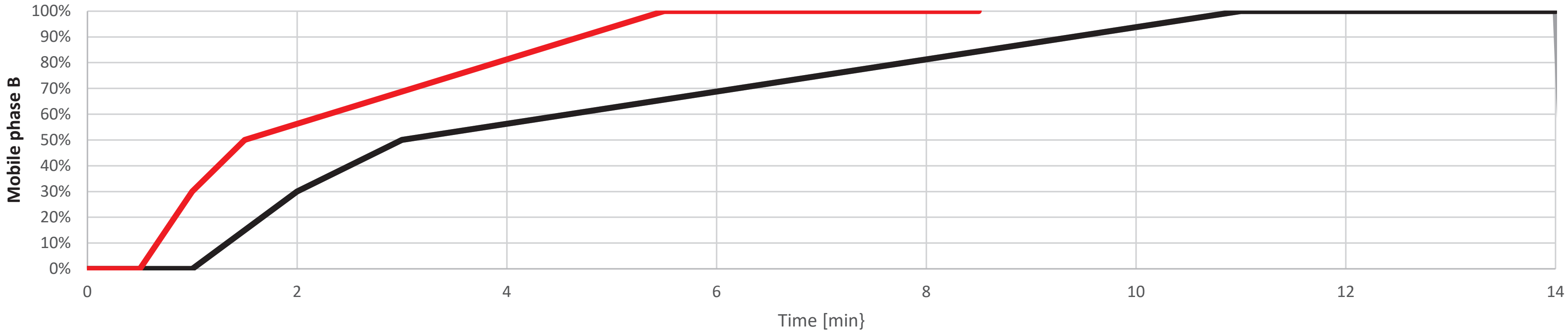




Dual-Channel LC-MS/MS: total ion chromatograms

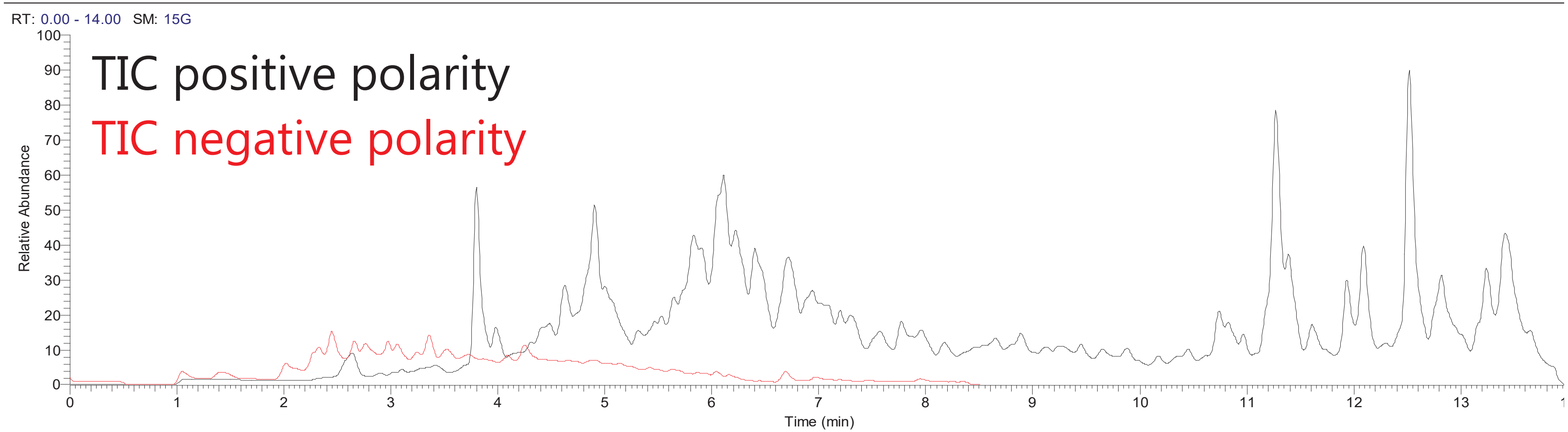
Orange

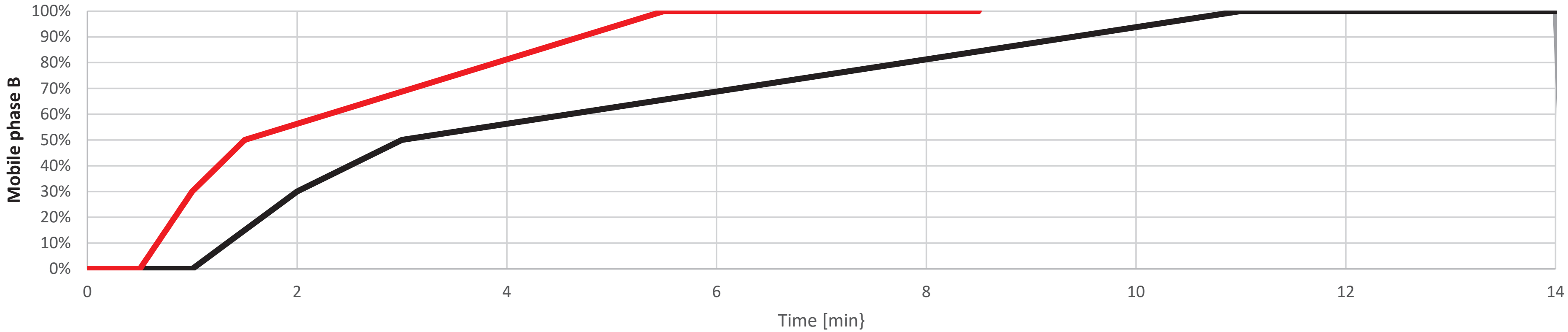




Dual-Channel LC-MS/MS: total ion chromatograms

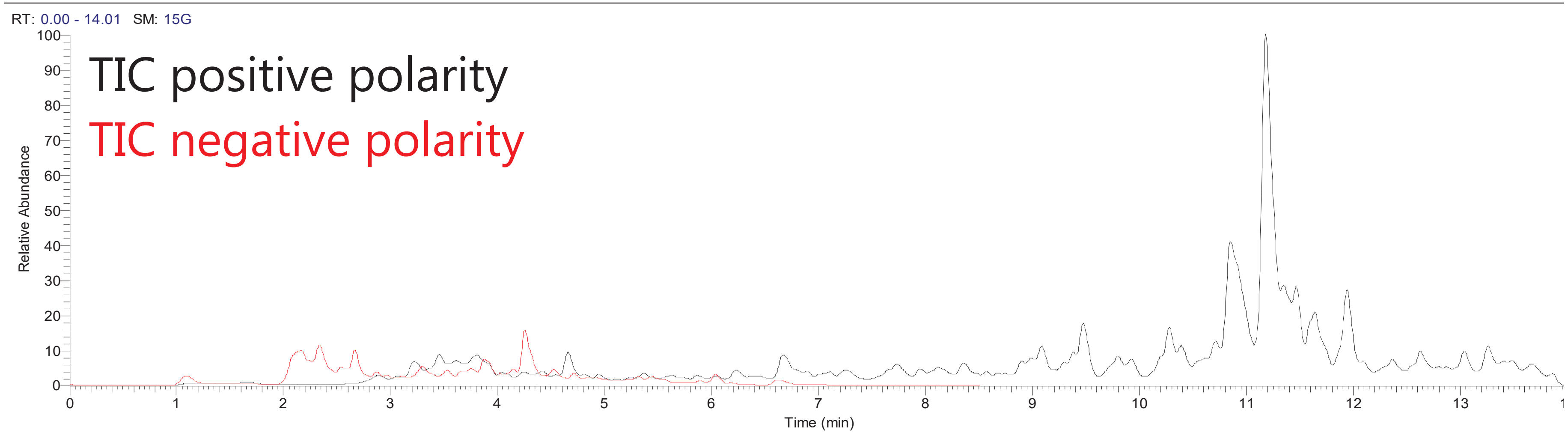
Onion

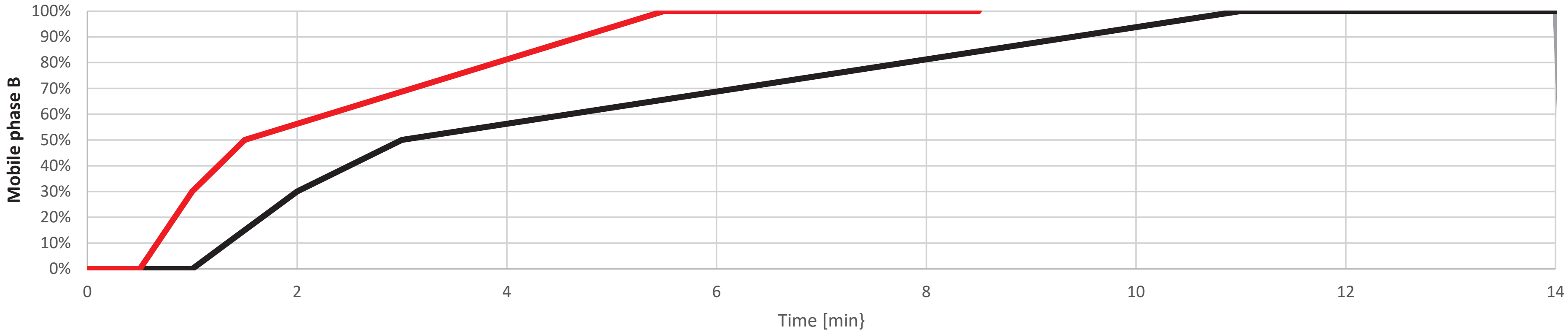




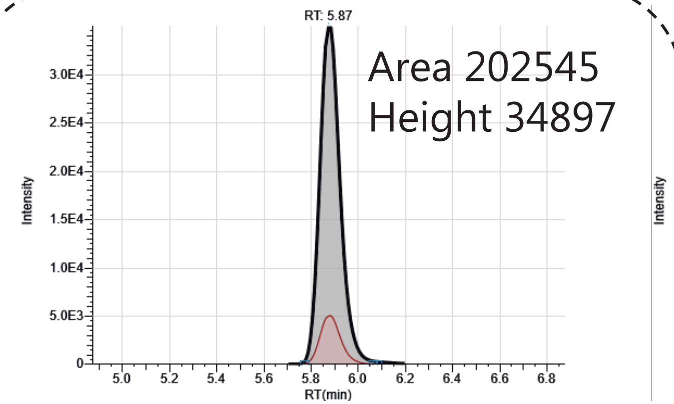
Dual-Channel LC-MS/MS: total ion chromatograms

Avocado



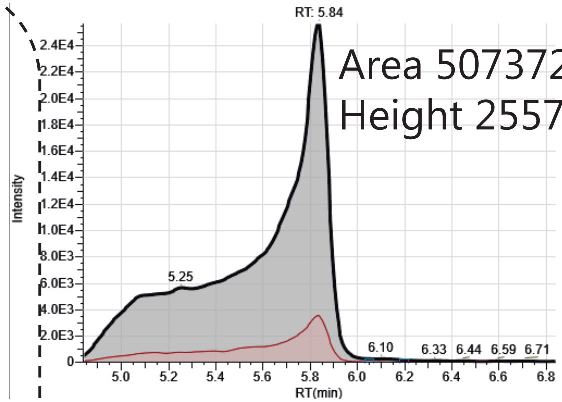


Dual-Channel LC-MS/MS: improved ionisation

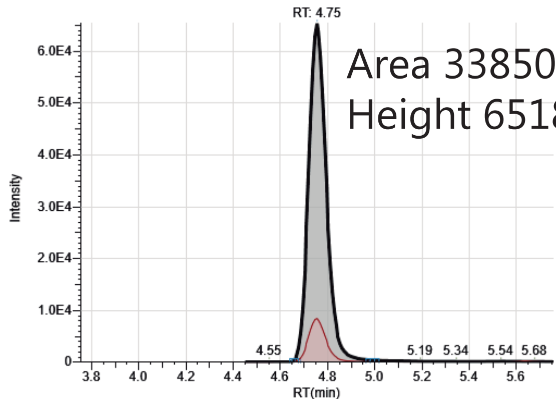


Water/MeOH/formic acid/ammonium formate

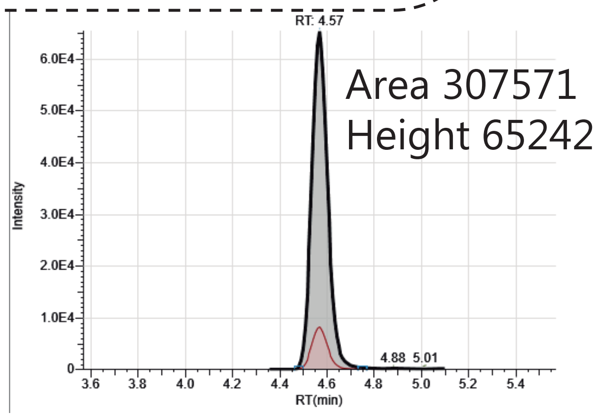
Gradient 1



Water/AcN

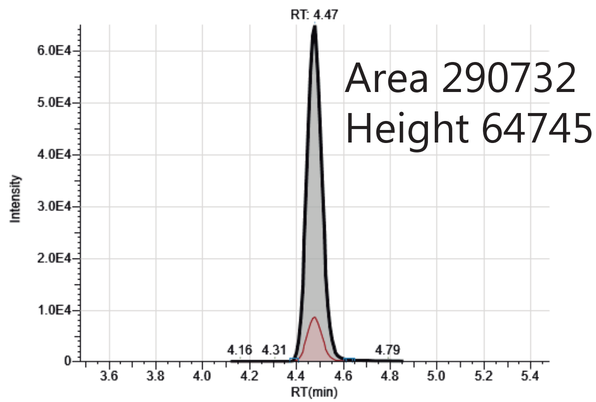


Water/AcN + 0.01 % acetic acid

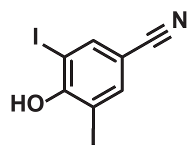


Water/AcN + 0.02 % acetic acid

Gradient 2



Water/AcN + 0.05 % acetic acid

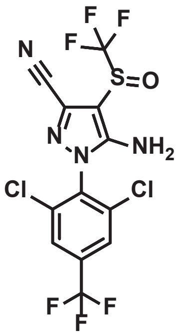


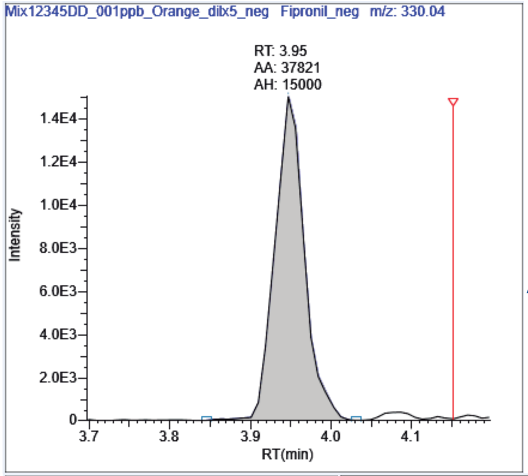
Ioxynil

ESI (-)

Dual-Channel LC-MS/MS: improved ionisation

Fipronil



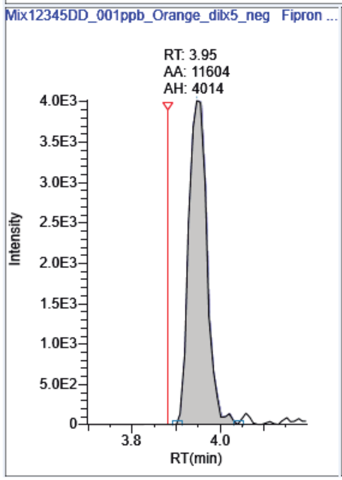


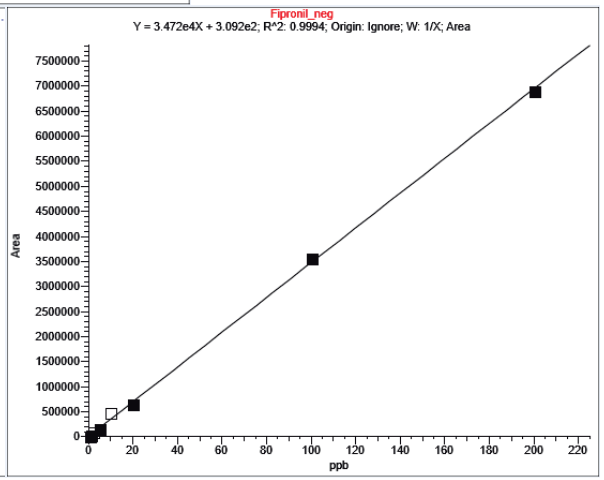
0.001 mg/L

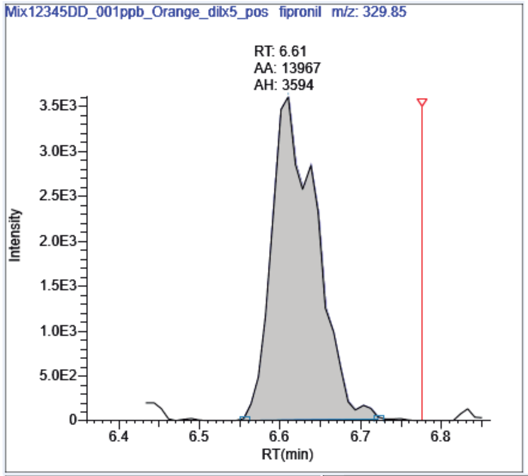
Orange extract

ACN/AA/H_2O

Negative polarity





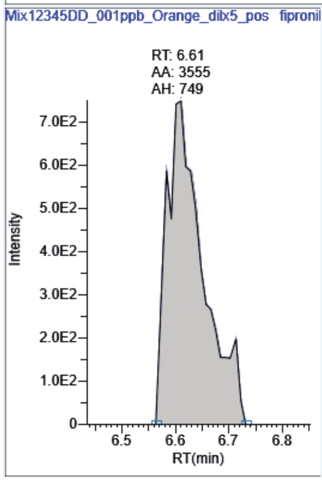


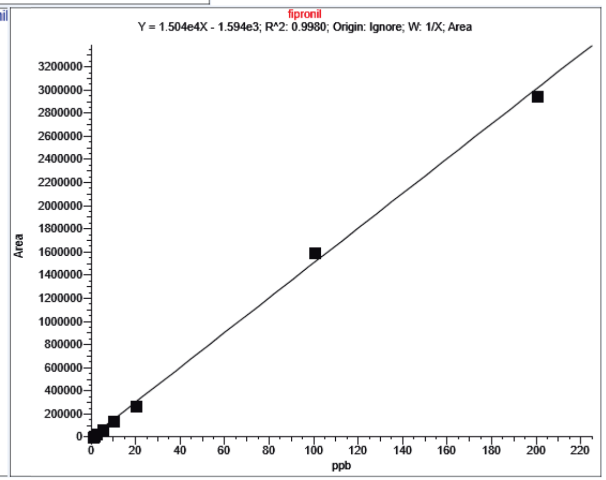
0.001 mg/L

Orange extract

MeOH/FA/AF/H_2O

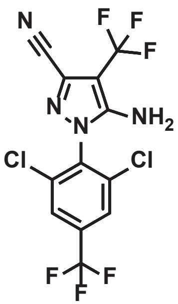
Negative polarity

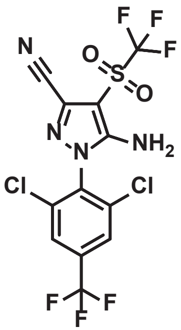


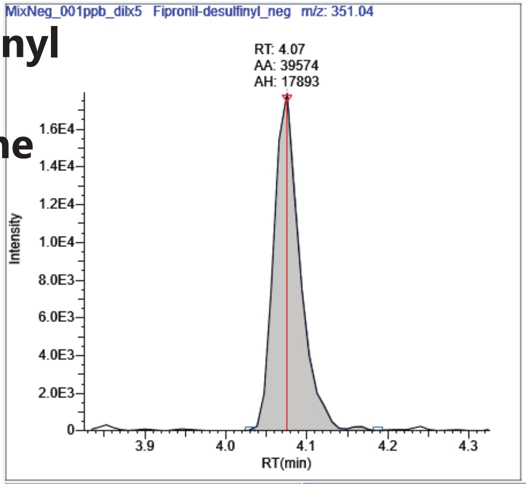


Dual-Channel LC-MS/MS: improved ionisation

Fipronil desulfinyl & fipronil sulfone





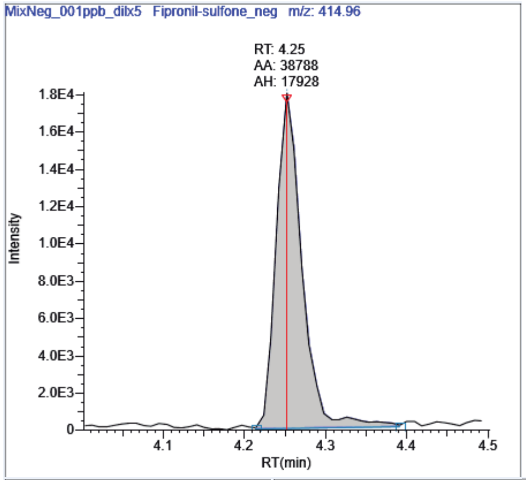


0.001 mg/L

Solvent

$ACN/AA/H_2O$

Negative polarity

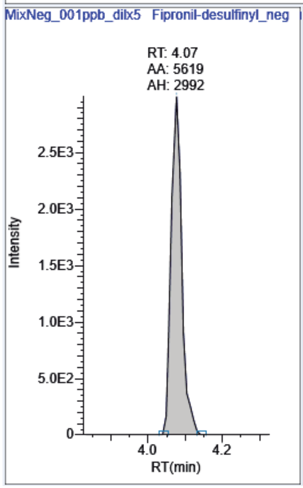


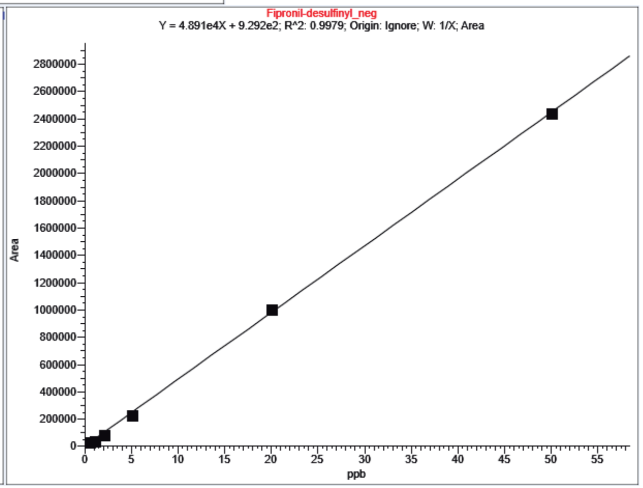
0.001 mg/L

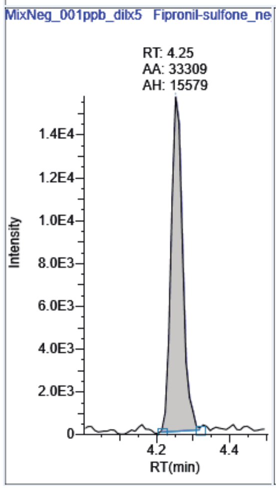
Solvent

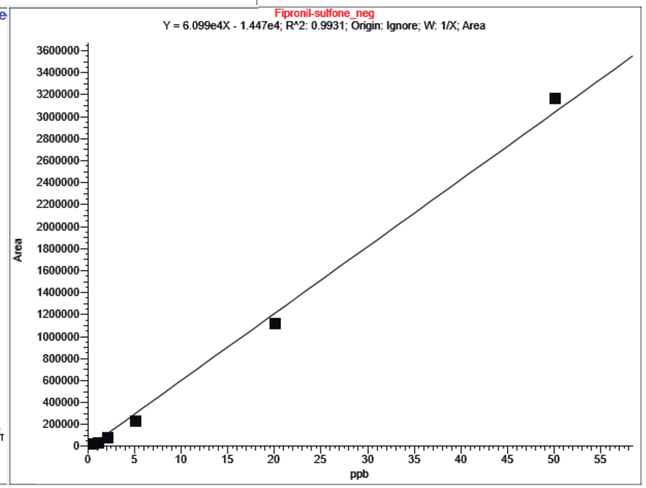
$ACN/AA/H_2O$

Negative polarity









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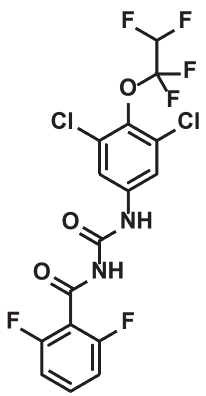
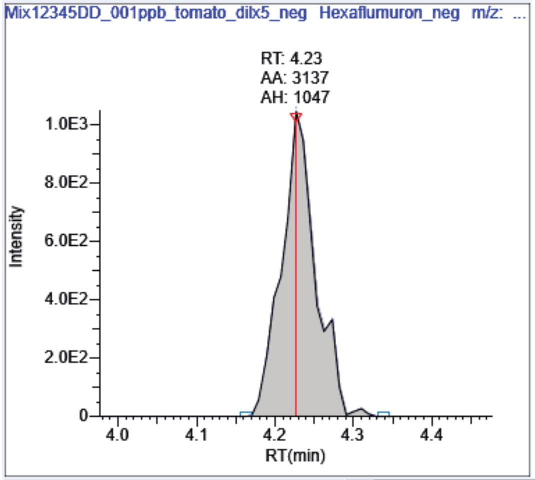
Dual-Channel LC-MS/MS: improved ionisation

Hexaflumuron



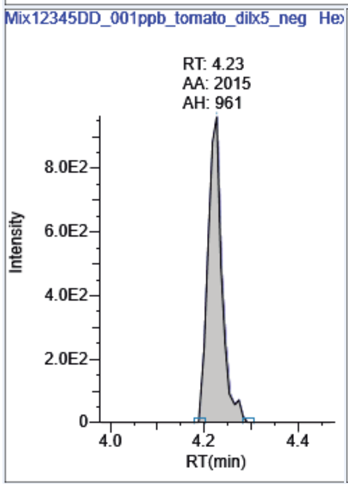


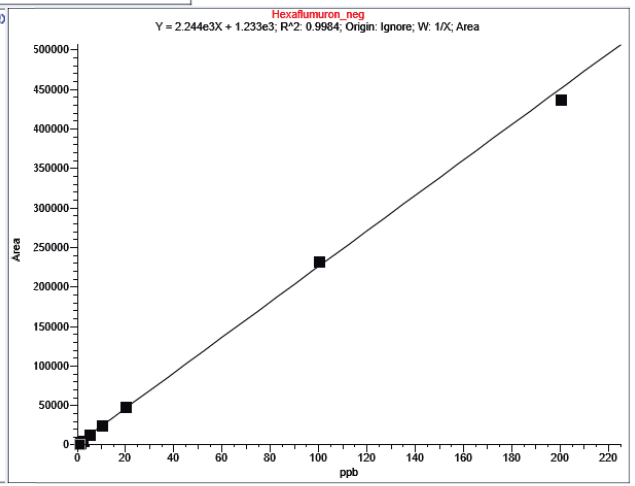
0.001 mg/L

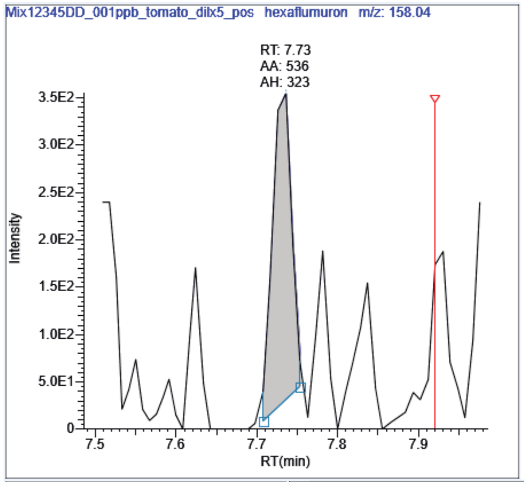
Tomato extract

ACN/AA/H_2O

Negative polarity





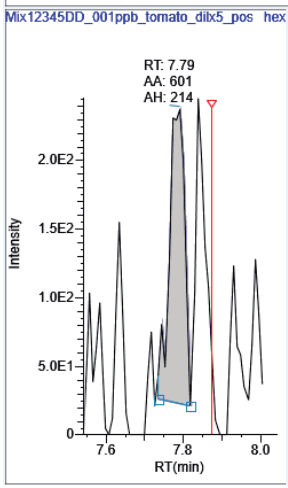


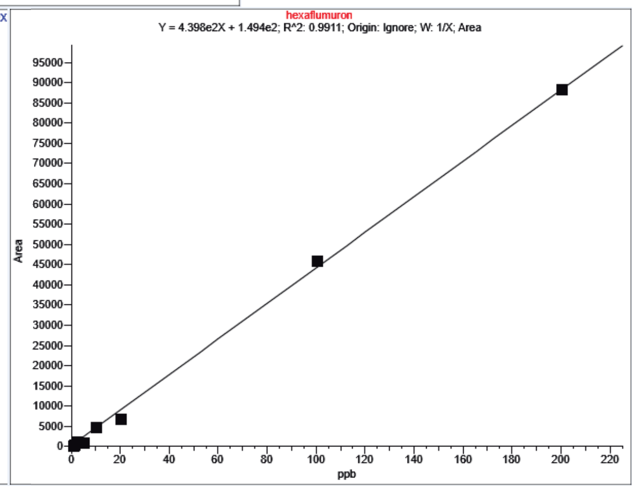
0.001 mg/L

Tomato extract

MeOH/FA/AF/H_2O

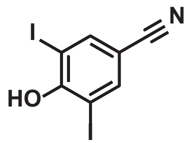
Positive polarity

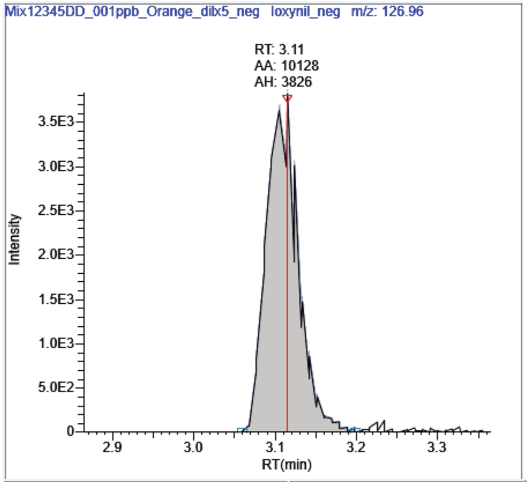




Dual-Channel LC-MS/MS: improved ionisation

Ioxynil



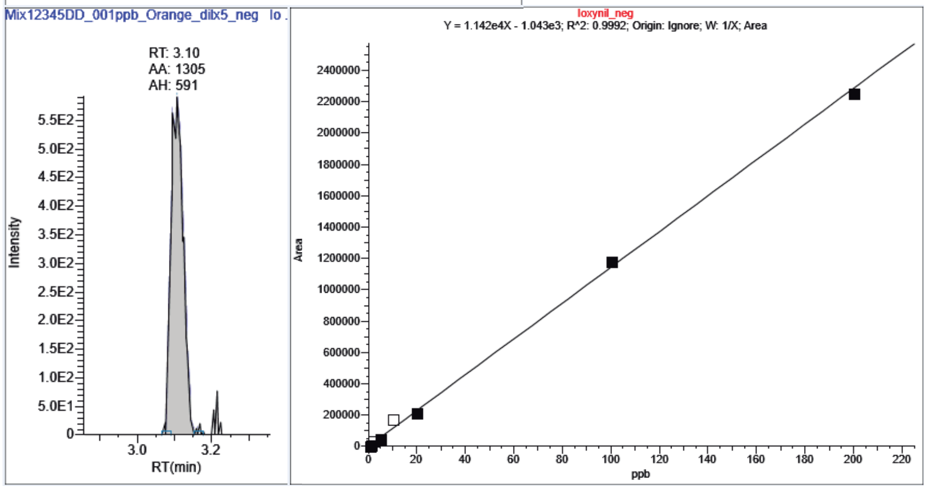


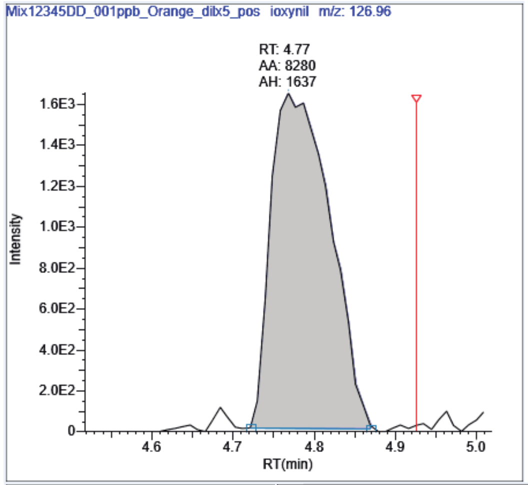
0.001 mg/L

Orange extract

ACN/AA/H_2O

Negative polarity



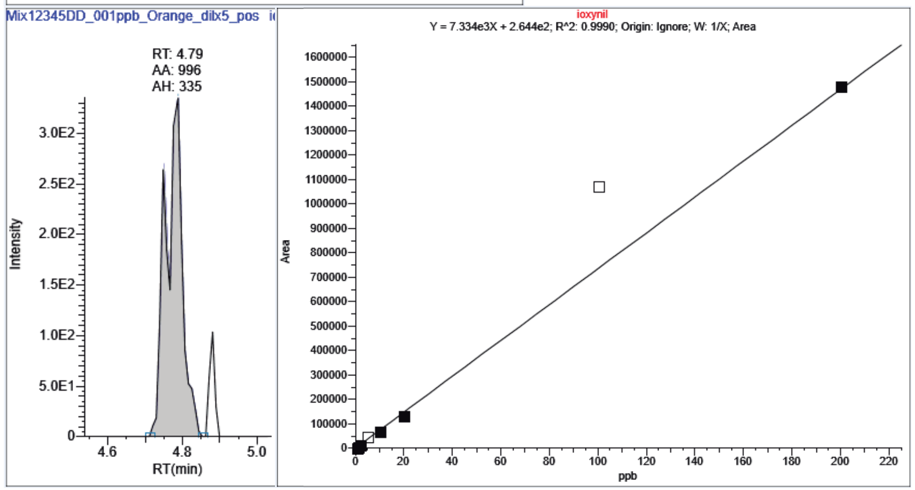


0.001 mg/L

Orange extract

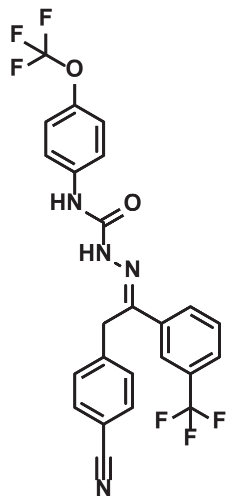
MeOH/FA/AF/H_2O

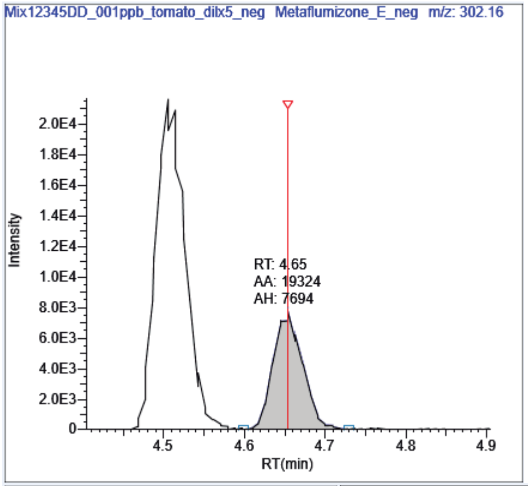
Negative polarity



Dual-Channel LC-MS/MS: improved ionisation

(*E*)-Metaflumizone



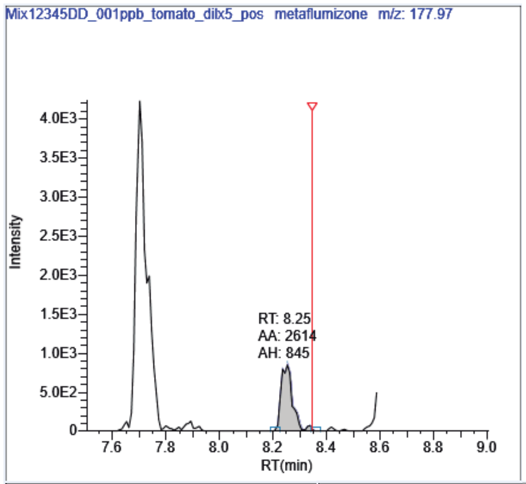


0.001 mg/L

Tomato extract

ACN/AA/H_2O

Negative polarity

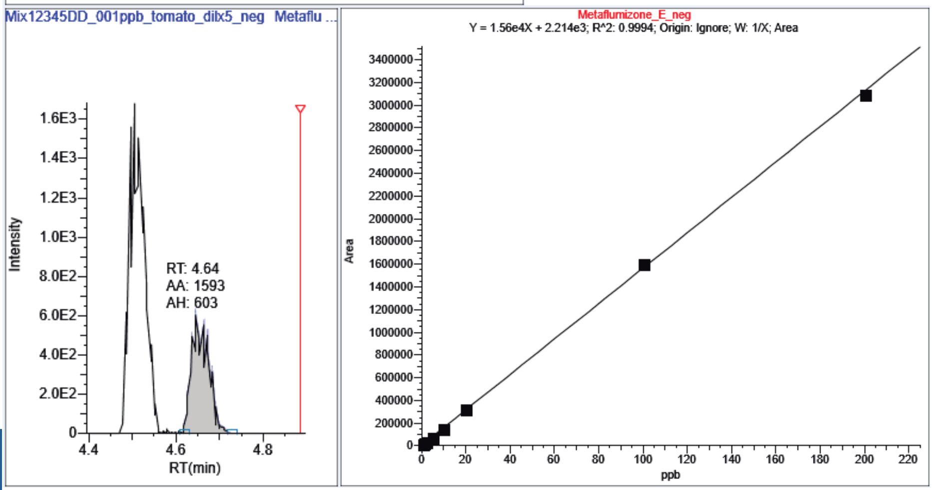


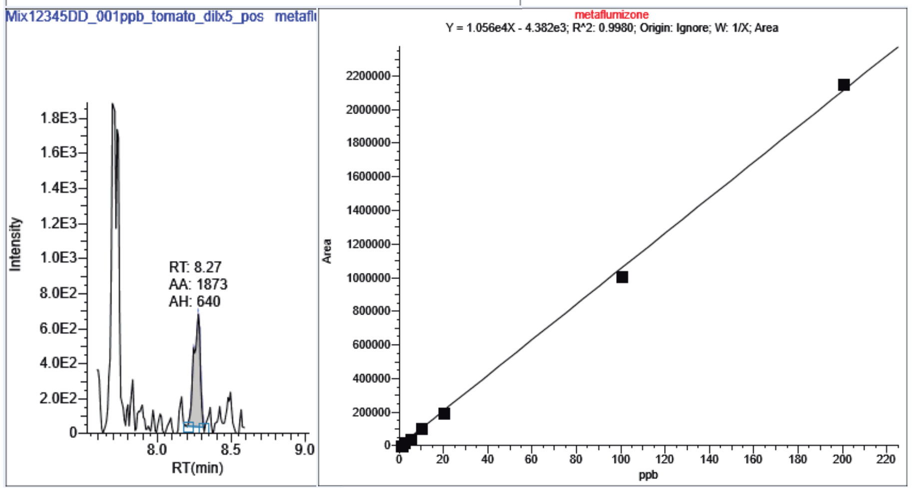
0.001 mg/L

Tomato extract

MeOH/FA/AF/H_2O

Negative polarity





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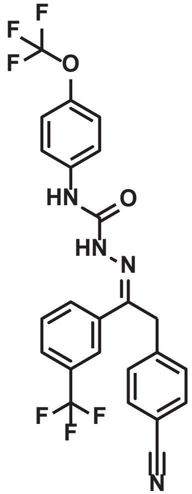
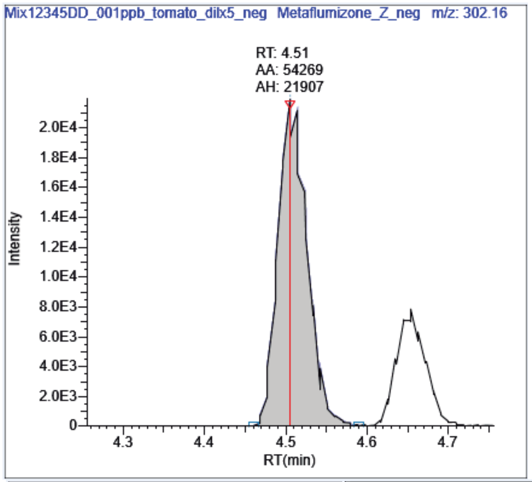
Dual-Channel LC-MS/MS: improved ionisation

(*Z*)-Metaflumizone



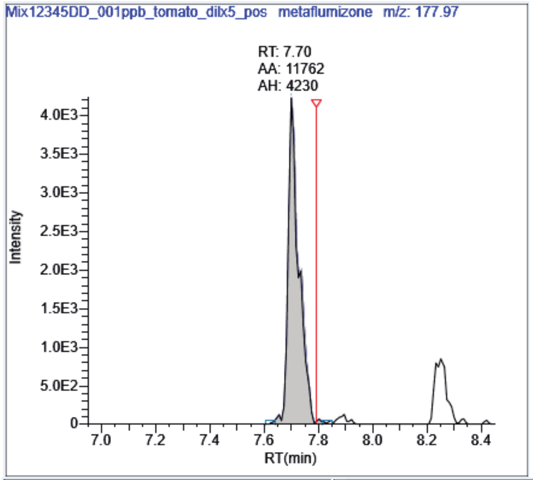


0.001 mg/L

Tomato extract

ACN/AA/H_2O

Negative polarity

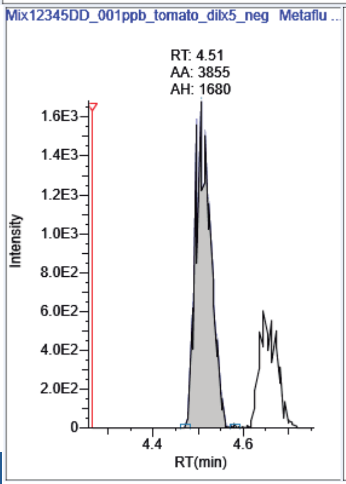


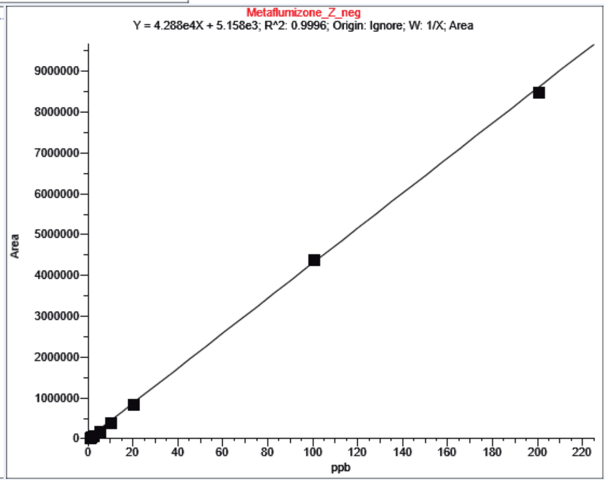
0.001 mg/L

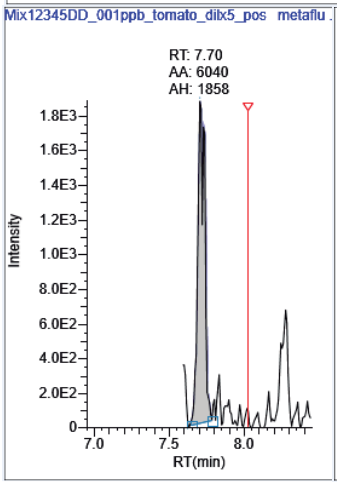
Tomato extract

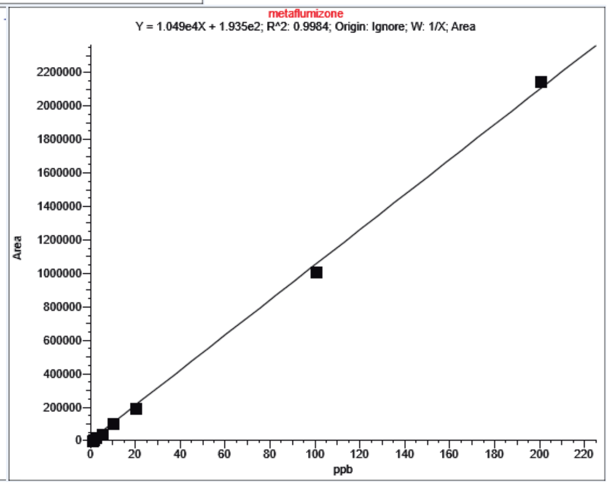
MeOH/FA/AF/H_2O

Negative polarity



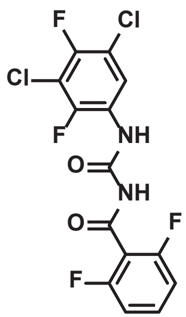


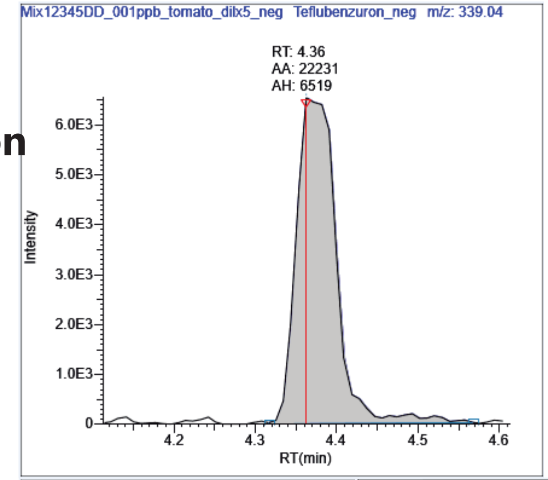




Dual-Channel LC-MS/MS: improved ionisation

Teflubenzuron



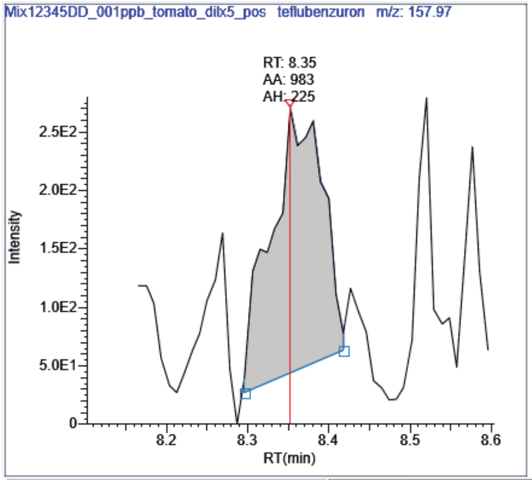


0.001 mg/L

Tomato extract

ACN/AA/H_2O

Negative polarity

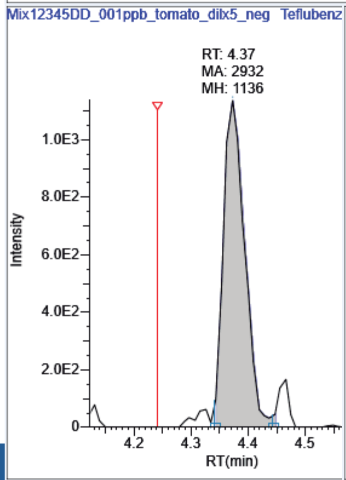


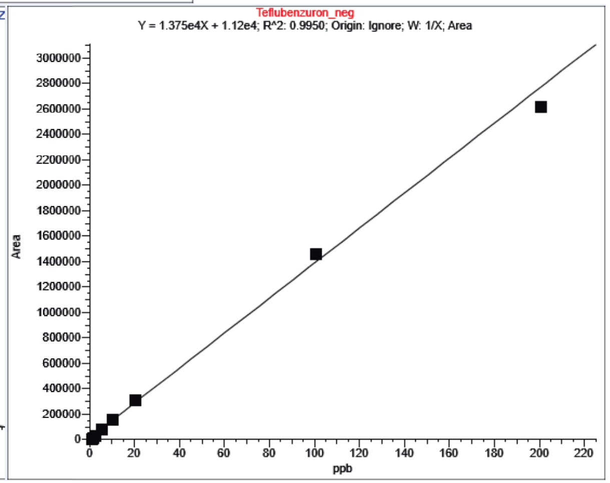
0.001 mg/L

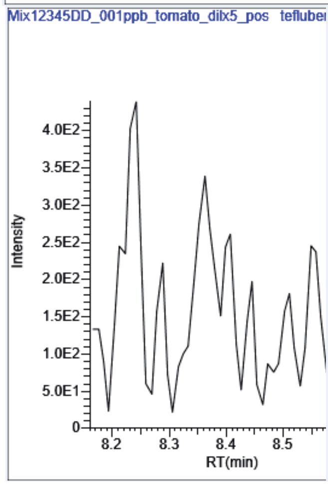
Tomato extract

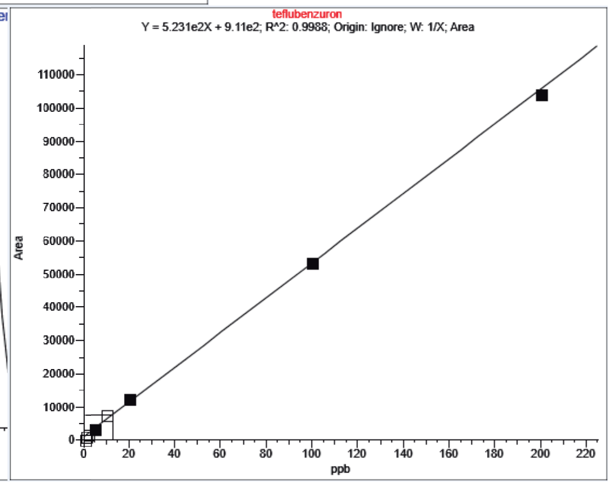
MeOH/FA/AF/H_2O

Negative polarity



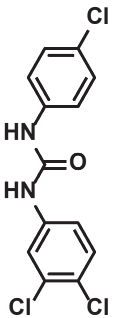


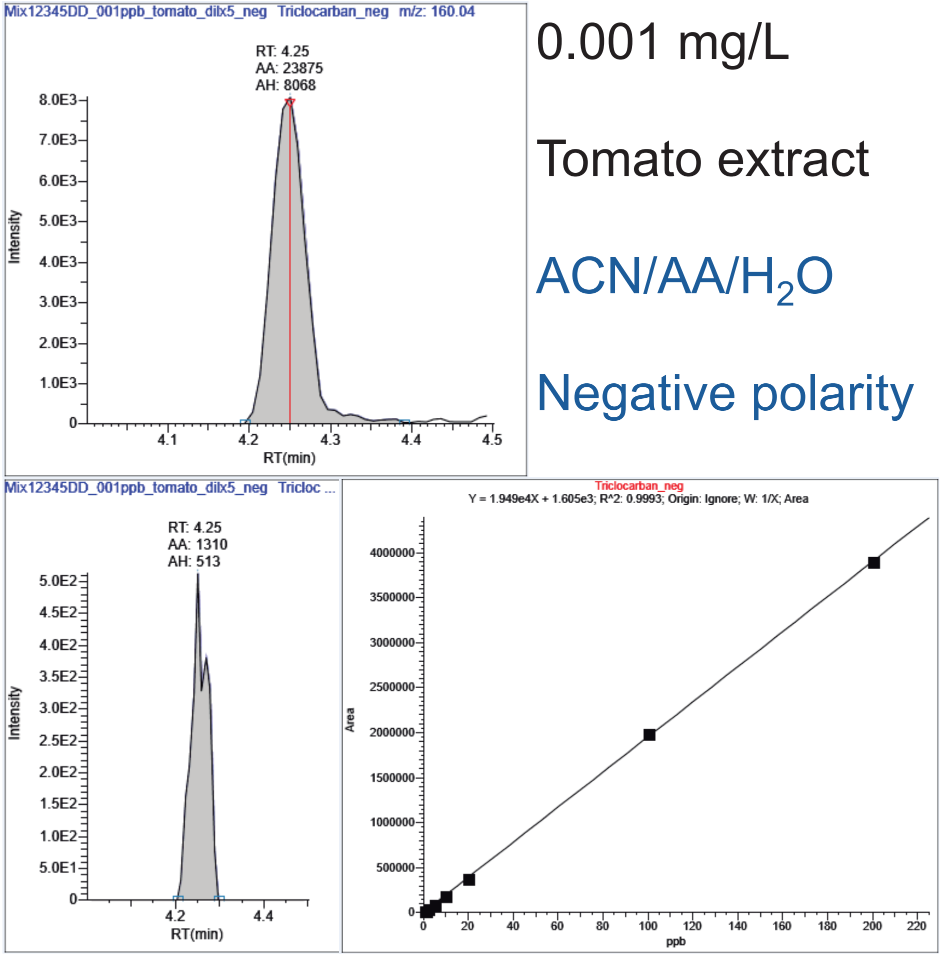




Dual-Channel LC-MS/MS: improved ionisation

Triclocarban



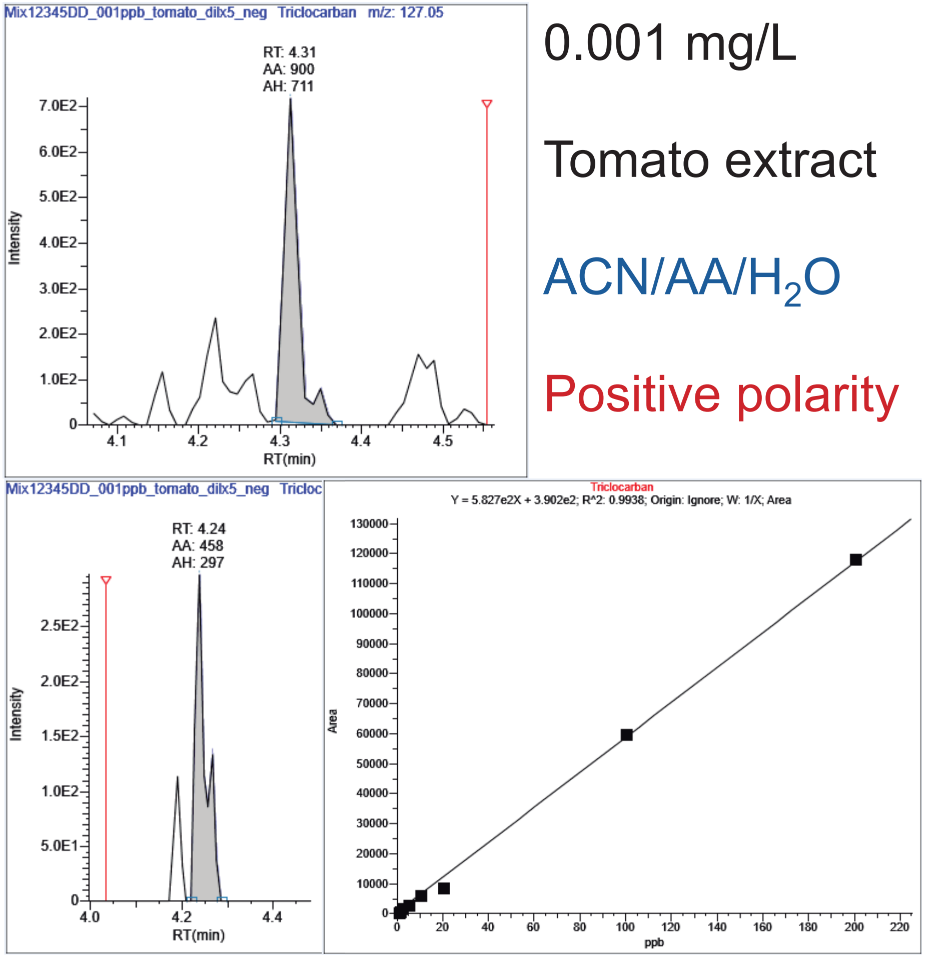


0.001 mg/L

Tomato extract

ACN/AA/H_2O

Negative polarity



0.001 mg/L

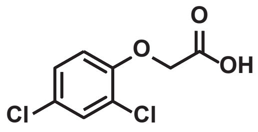
Tomato extract

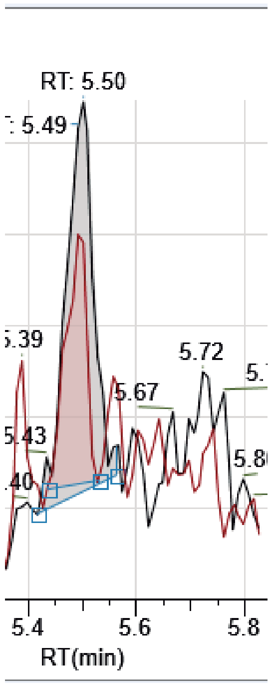
ACN/AA/H_2O

Positive polarity

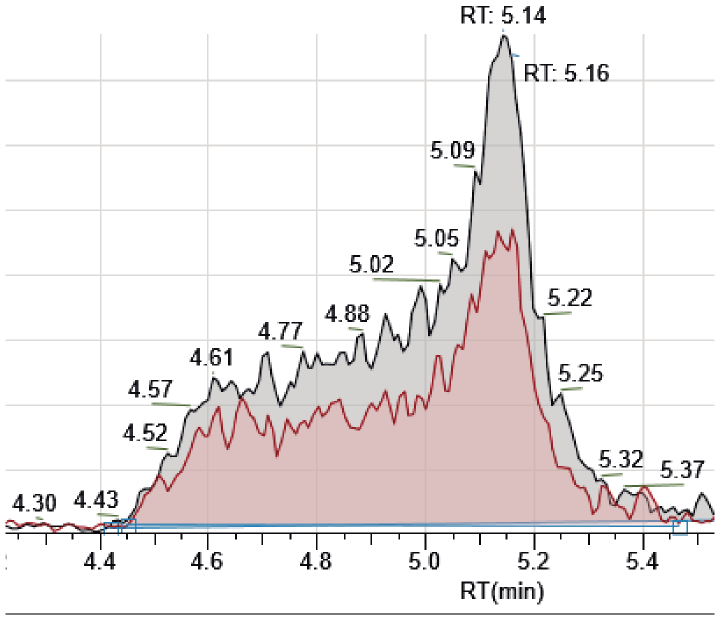
Dual-Channel LC-MS/MS: improved ionisation

2,4-D

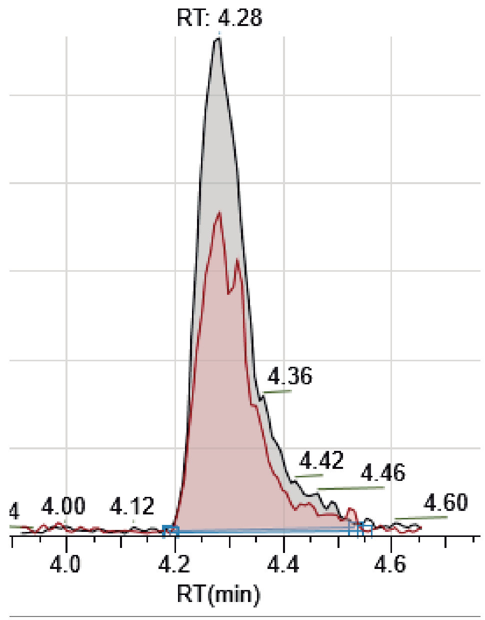




MeOH/FA/AF/H_2O
Negative polarity
Peak area 7 606



ACN/H_2O
Negative polarity
Peak area 74 079



ACN/AA/H_2O
Negative polarity
Peak area 36 193

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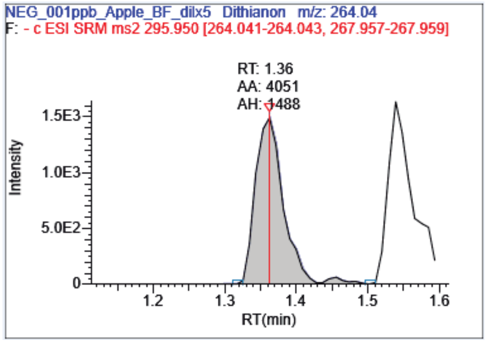
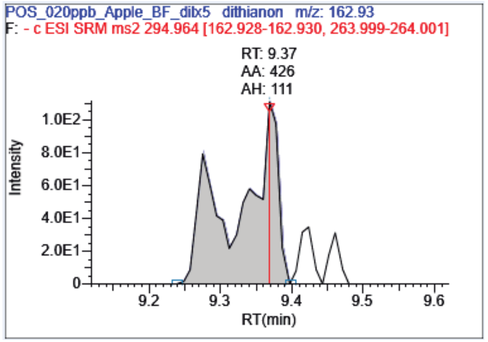
Dual-Channel LC-MS/MS: improved ionisation

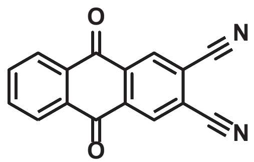


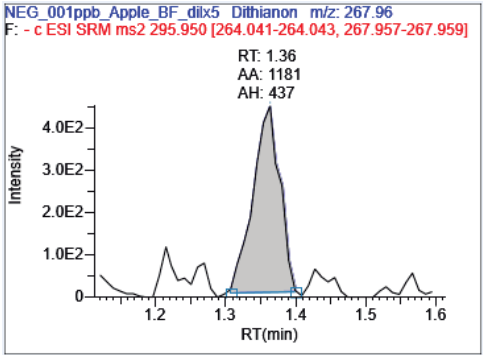
0.001 mg/kg

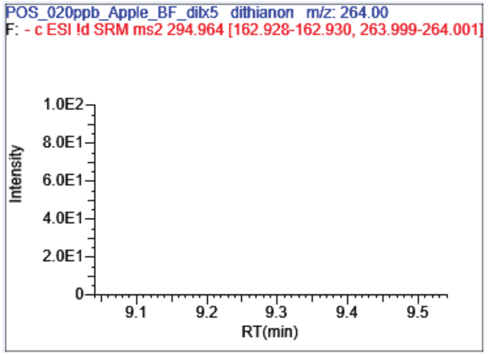


0.020 mg/kg

Dithianon







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 validation

Gradient 1

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

Gradient 2

Water:AcN
Acetic acid (0.05 %)

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 %	0.001	0.02
Bromacil	100/101 %	5/6 %	97/104 %	3/4 %	0.0005	0.02
Dithianon	96 %	3 %	96 %	3 %	0.0005	0.02
Diuron	99/103 %	3/5 %	99/101 %	2/3 %	0.0005	0.02
Fensulfothion	99/102 %	3/5 %	100/104 %	3 %	0.0005	0.02
Fensulfothion-oxon-sulfone	100/104 %	4/5 %	95/103 %	2/4 %	0.0005	0.02
Fipronil	100/103 %	1/4 %	92/101 %	1/3 %	0.0005	0.02
Fipronil-desulfinyl	99/101 %	2/7 %	100 %	2/3 %	0.0005	0.02
Fipronil-sulfone	98/103 %	2/3 %	100/108 %	2/3 %	0.0005	0.02
Flubendiamide	104/25 %	21/24 %	97/98 %	12/14 %	0.0005/0.003	0.02
Fludioxonil	93/105 %	6 %	102/103 %	2/6 %	0.0005	0.02
Haloxyfop	96/97 %	13/15 %	93/101 %	3/6 %	0.003	0.02
Hexaflumuron	94 %	5 %	104/106 %	11/18 %	0.0005/0.003	0.02
Ioxynil	101/108 %	2/3 %	103/105 %	3/6 %	0.0005/0.001	0.02
Lufenuron	83/108 %	6/22 %	75/102 %	3/30 %	0.0005/0.003	0.02
MCPA	114 %	7 %	94/99 %	3/13 %	0.001/0.003	0.02
MCPB	-	-	115 %	10 %	0.006	0.02
Meptyldinocap	86 %	14 %	113/118 %	6/29 %	0.003/0.006	0.02
(*E*)-Metaflumizone	83/104 %	2/5 %	85 %	1/6 %	0.0005/0.001	0.02
(*Z*)-Metaflumizone	104/109 %	1/3 %	102/105 %	2/5 %	0.0005/0.001	0.02
Penthiopyrad	100/103 %	2/3 %	100/101 %	1 %	0.0005	0.02
Prothioconazole	106/107 %	12/13 %	100/110 %	11/15 %	0.0005/0.003	0.02
Prothioconazole-desthio	101/106 %	2/8 %	94/100 %	2/5 %	0.0005	0.02
Teflubenzuron	100/107 %	3/9 %	95/105 %	2 %	0.0005/0.001	0.02
TFNA	-	-	98 %	7 %	0.006	0.02
TFNG	103/26 %	28/24 %	101/47 %	5/93 %	0.003	0.02

EURL EUROPEAN UNION REFERENCE LABORATORY FRUITS AND VEGETABLES

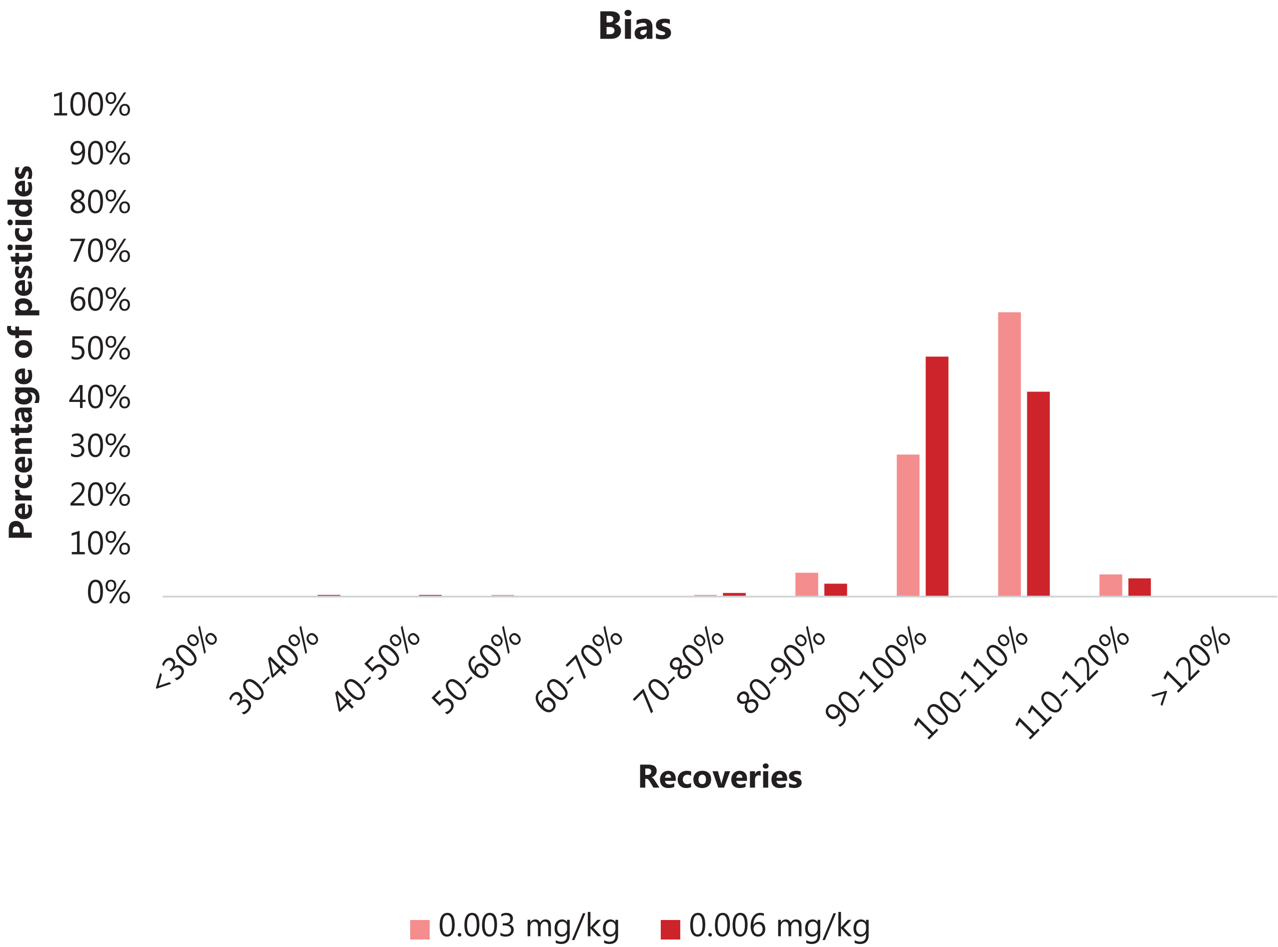
Dual-Channel LC-MS/MS: apple validation

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

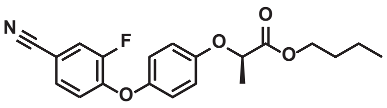
260 pesticide residues validated at 0.006 mg/kg



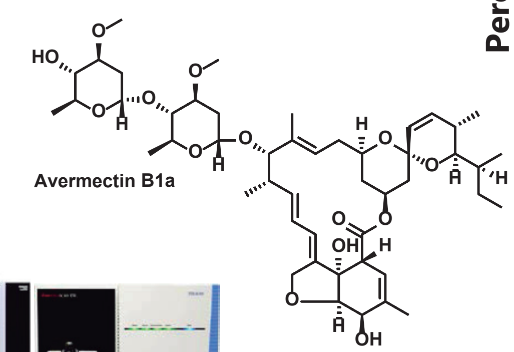
Dual-Channel LC-MS/MS: apple validation

264 total pesticide residues (ESI+ and ESI-)

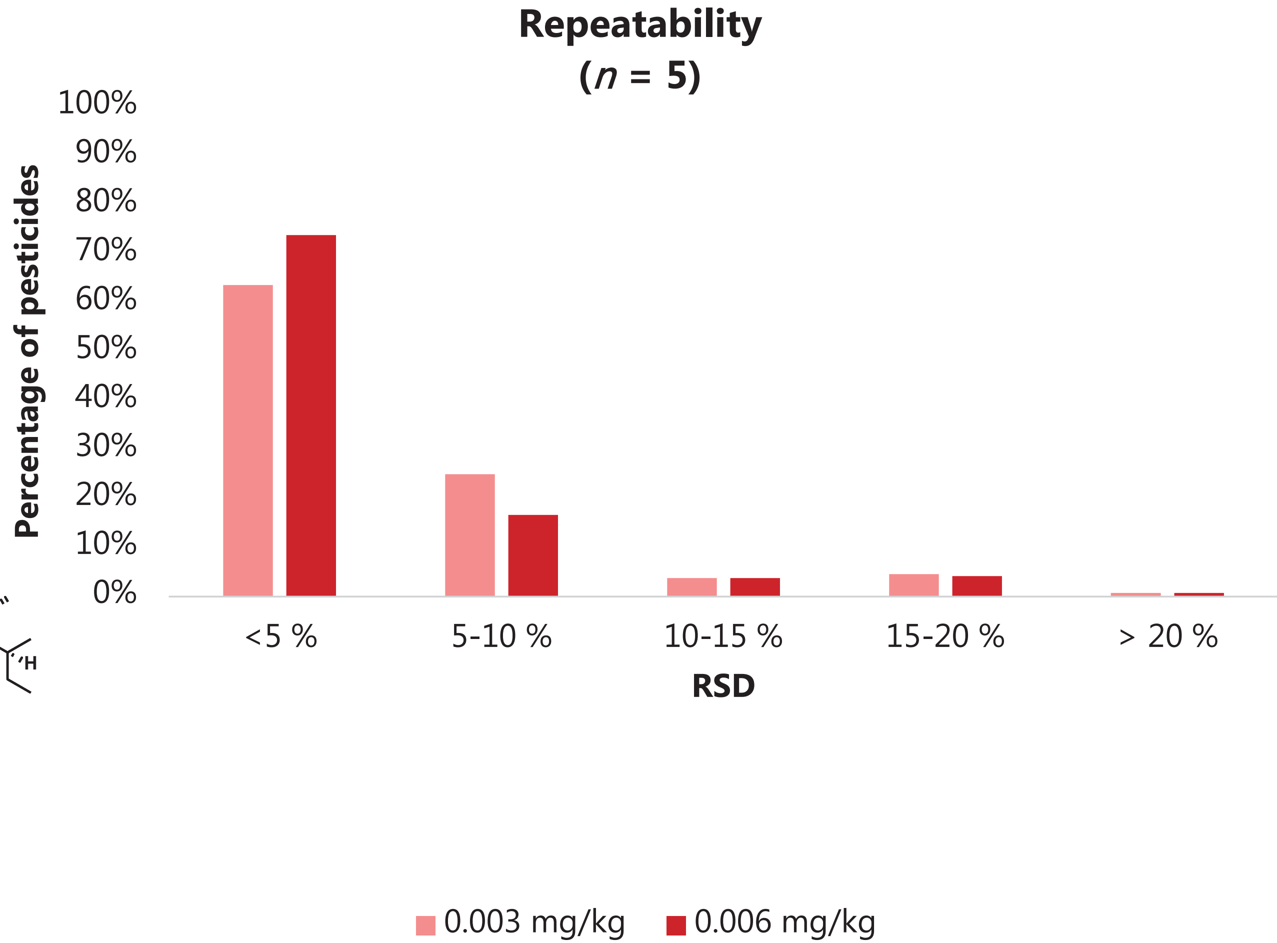
Technique	<5%	5-20%	>20%
Dual-Channel 0.003 mg/kg	64%	33%	1%
Dual-Channel 0.006 mg/kg	74%	25%	1%



Cyhalofop-butyl



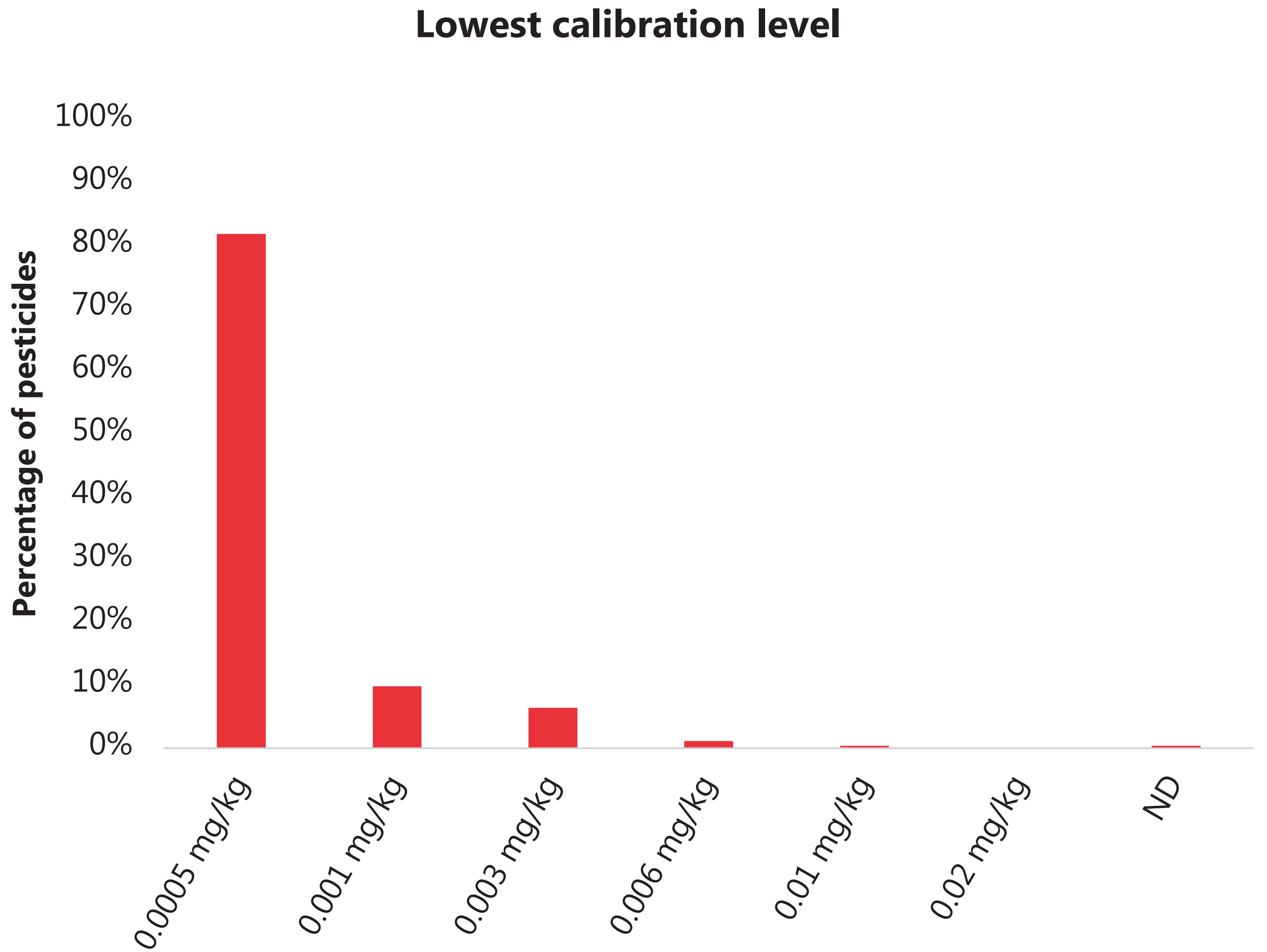
Avermectin B1a



Dual-Channel LC-MS/MS: apple validation

264 total pesticide residues (ESI+ and ESI-)

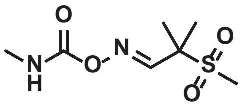
Calibration level (mg/kg)	No. of analytes
0.0005	216
0.001	26
0.003	17
0.006	3
0.010	1
0.020	0
ND	1



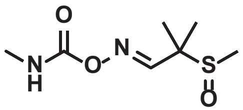
Dual-Channel LC-MS/MS: apple ND

Non detections

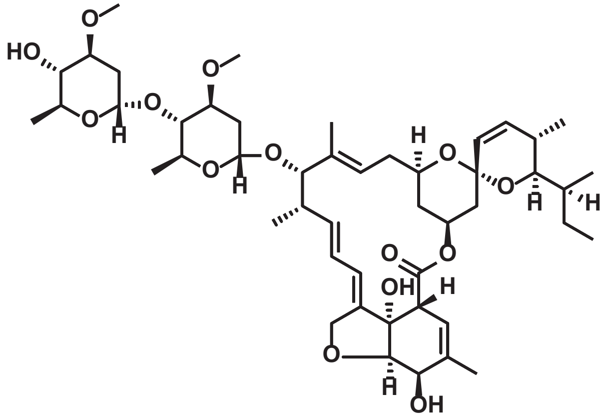
0.006 mg/kg



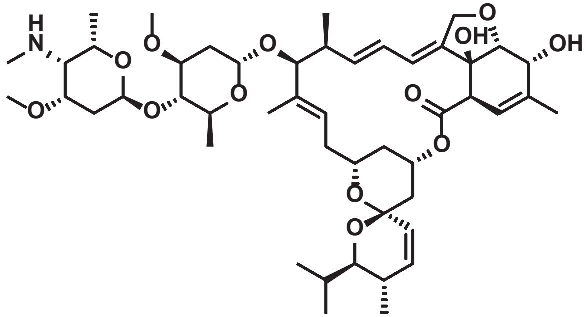
Aldicarb-sulfone



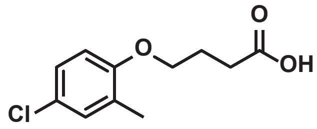
Aldicarb-sulfoxide



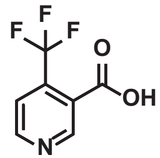
Avermectin B1a



Emamectin B1b



MCPB



TFNA

Dual-Channel LC-MS/MS: banana & orange validation

Gradient 1

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

Gradient 2

Water:AcN
Acetic acid (0.05 %)

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	94 %	10 %	9243 %	6 %	0.003	0.02
Bromacil	1950 %	6 %	1950 %	4 %	0.0005	0.02
Dithianon	81 %	6 %	86 %	6 %	0.003	0.02
Diuron	106 %	4 %	100 %	2 %	0.0005	0.02
Fensulfothion	93 %	4 %	104 %	2 %	0.0005	0.02
Fensulfothion-oxon-sulfone	1925 %	4 %	101 %	3 %	0.0005	0.02
Fipronil	97 %	2 %	1943 %	6 %	0.0005	0.02
Fipronil-desulfinyl	96 %	6 %	1955 %	4 %	0.0005	0.02
Fipronil-sulfone	99 %	6 %	1912 %	3 %	0.0005	0.02
Flubendiamide	98 %	134 %	1954 %	8 %	0.0005	0.02
Fludioxonil	107 %	160 %	1932 %	8 %	0.0005	0.02
Haloxyfop	1742 %	4 %	1983 %	130 %	0.003	0.02
Hexaflumuron	95 %	6 %	84 %	36 %	0.0005	0.02
Ioxynil	103 %	2 %	103 %	2 %	0.0005	0.02
Lufenuron	95 %	167 %	103 %	250 %	0.0005	0.02
MCPA	1962 %	743 %	94 %	170 %	0.003	0.02
MCPB	-	-	115 %	18 %	0.006	0.02
Meptyldinocap	93 %	16 %	119 %	14 %	0.006	0.02
(*E*)-Metaflumizone	84 %	420 %	96 %	138 %	0.0005	0.02
(*Z*)-Metaflumizone	1962 %	3 %	1988 %	8 %	0.0005	0.02
Penthiopyrad	99 %	2 %	104 %	2 %	0.0005	0.02
Prothioconazole	98 %	5 %	108 %	172 %	0.0005	0.02
Prothioconazole-desthio	1927 %	3 %	106 %	2 %	0.0005	0.02
Teflubenzuron	1945 %	145 %	109 %	147 %	0.0005	0.02
TFNA	-	-	99 %	3 %	0.006	0.02
TFNG	96 %	9 %	95 %	5 %	0.003	0.02

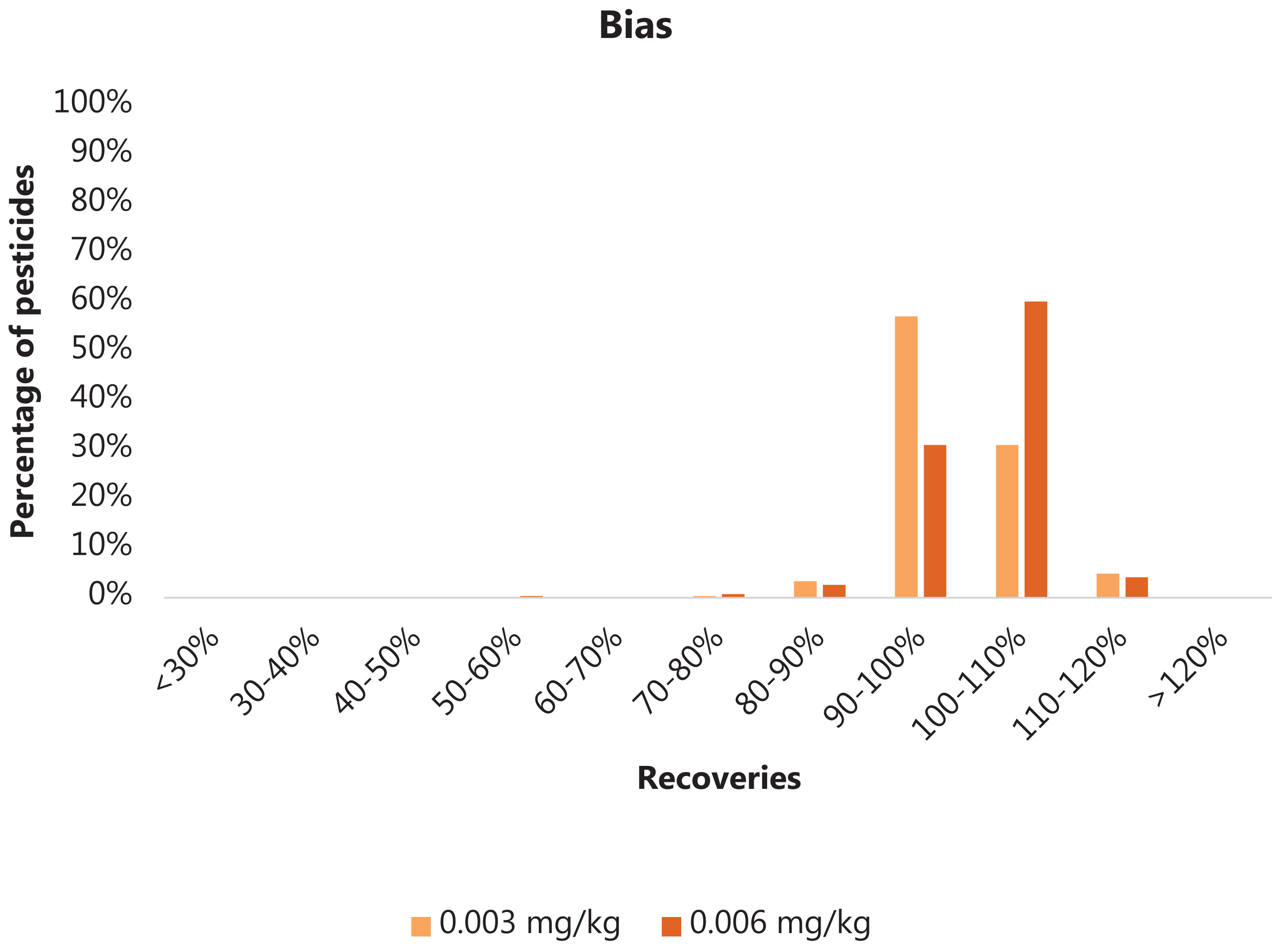
Dual-Channel LC-MS/MS: banana & orange validation

264 total pesticide residues (ESI+ and ESI-)

Technique	<70%	70-120%	>120%
Dual-Channel 0.003 mg/kg	0	256	-
Dual-Channel 0.006 mg/kg	1	261	-

256 pesticide residues validated at 0.003 mg/kg

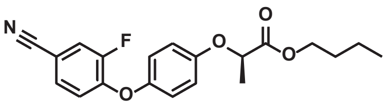
260 pesticide residues validated at 0.006 mg/kg



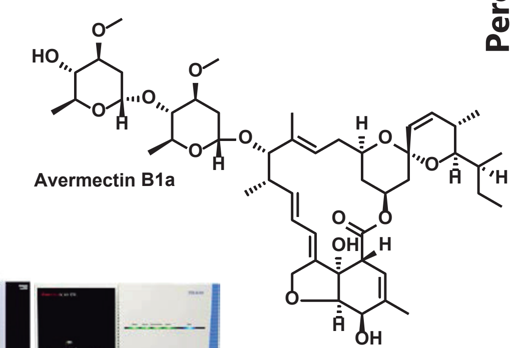
Dual-Channel LC-MS/MS: banana & orange validation

264 total pesticide residues (ESI+ and ESI-)

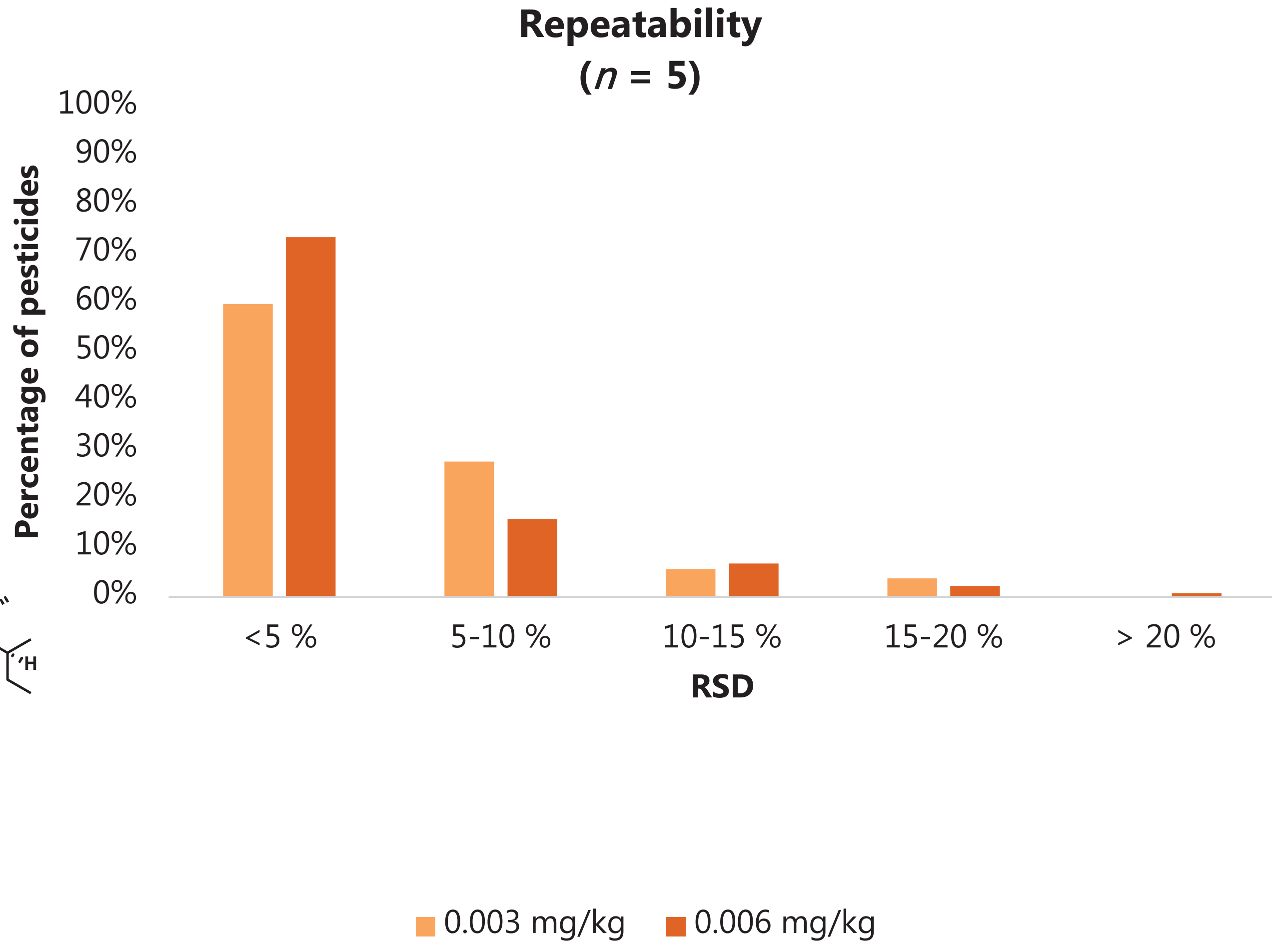
Technique	<5%	5-20%	>20%
Dual-Channel 0.003 mg/kg	60%	37%	-
Dual-Channel 0.006 mg/kg	73%	25%	<1%



Cyhalofop-butyl



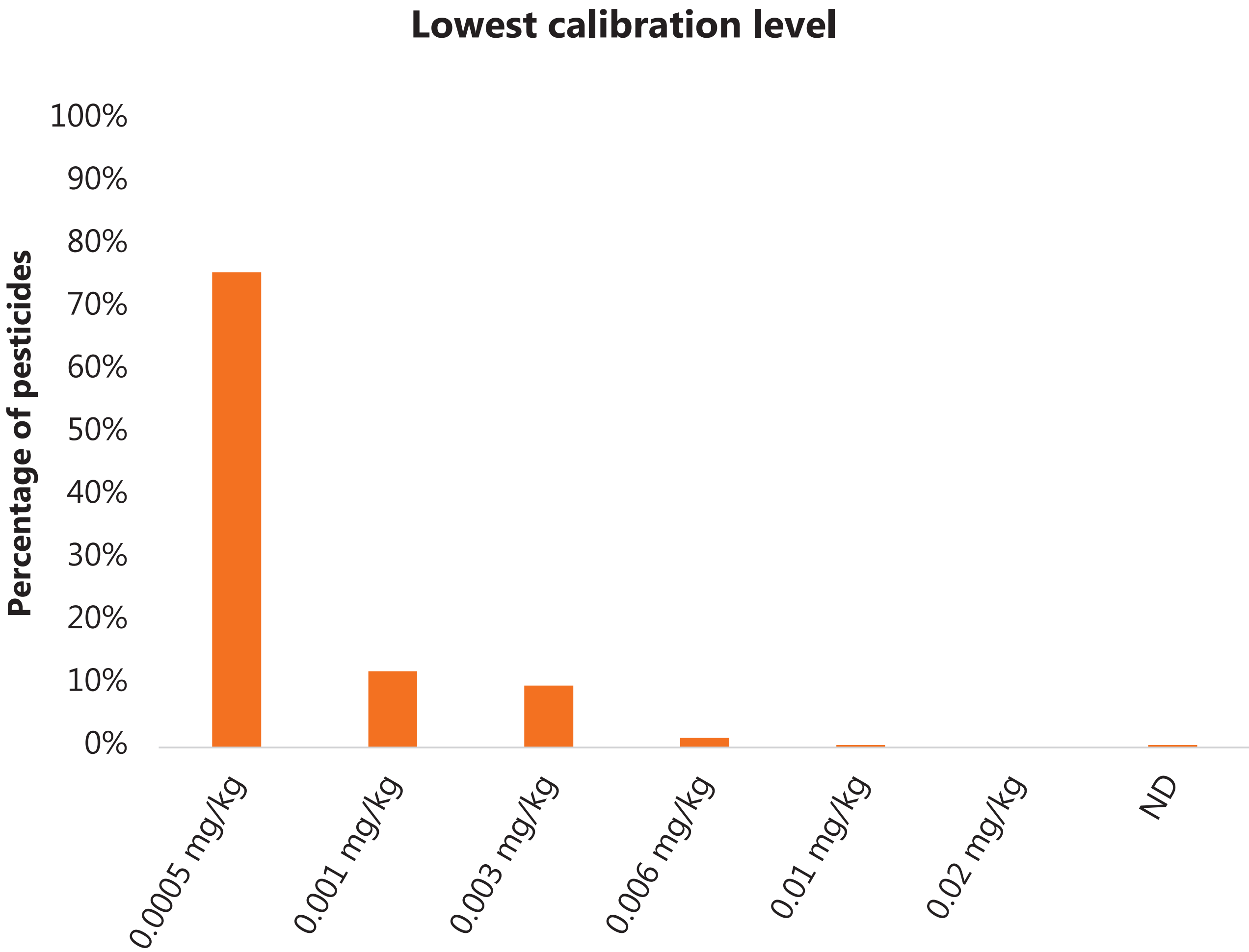
Avermectin B1a



Dual-Channel LC-MS/MS: banana & orange validation

264 total pesticide residues (ESI+ and ESI-)

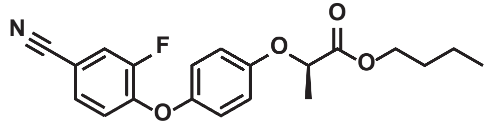
Calibration level (mg/kg)	No. of analytes
0.0005	200
0.001	32
0.003	26
0.006	4
0.010	1
0.020	0
ND	1



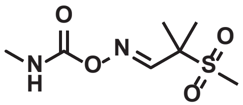
Dual-Channel LC-MS/MS: banana & orange ND

Non detections

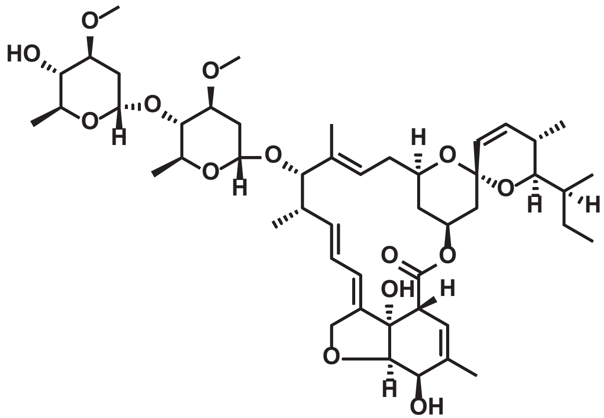
0.006 mg/kg



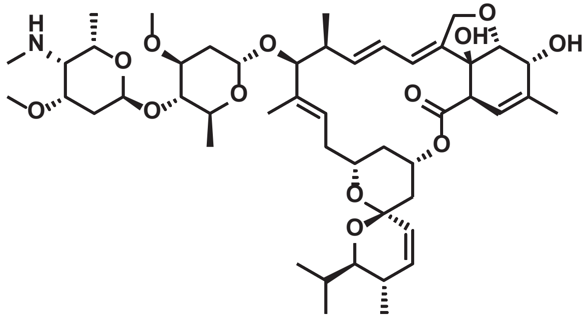
Cyhalofop-butyl



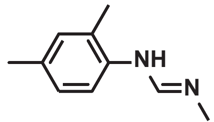
Aldicarb-sulfone



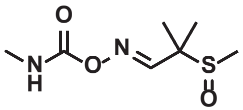
Avermectin B1a



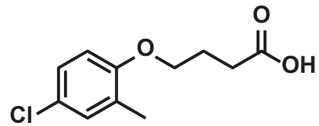
Emamectin B1b



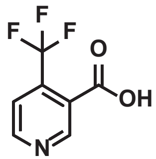
DMPF



Aldicarb-sulfoxide



MCPB



TFNA

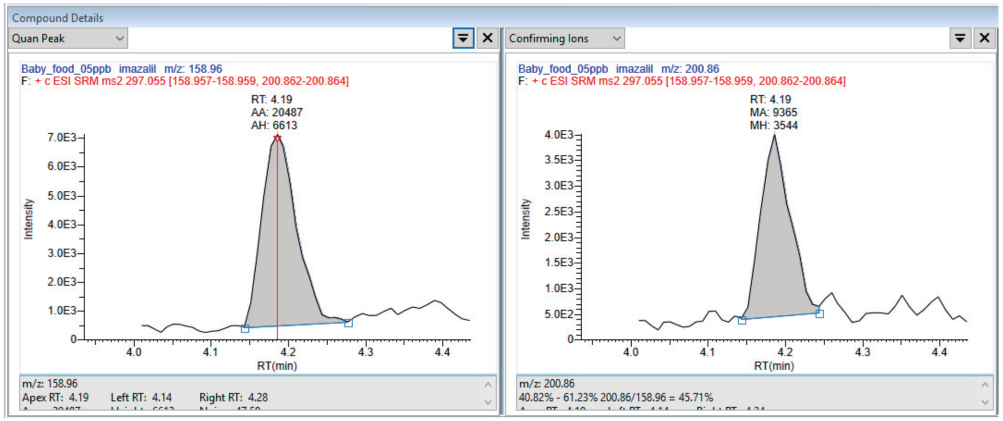
Dual-Channel LC-MS/MS: carry over test

Sequence:

1. Calibration curve 0.0005 – 0.020 mg/kg (ppb)
2. 3 injections at 0.020 mg/kg
3. Baby food blank
4. 6 injections at 0.020 mg/kg
5. Baby food blank
6. 12 injections at 0.020 mg/kg
7. Baby food blank

Compound	**Carry over after 3 injections [ppb]**	**Carry over after 3 injections [%]**	**Carry over after 6 injections [ppb]**	**Carry over after 6 injections [%]**	**Carry over after 12 injections [ppb]**	**Carry over after 12 injections [%]**
Acetamiprid	0.2	1.0 %	0.2	1.0 %	0.2	1.1 %
Deet	0.6	2.8 %	0.5	2.7 %	0.5	2.6 %
Demeton-*S*-methyl	0.9	4.6 %	0.9	4.6 %	1.0	5.1 %
Fluometuron	0.7	3.4 %	0.6	3.1 %	0.7	3.3 %
Dimethomorph	0.2	1.1 %	0.2	1.2 %	0.2	0.9 %
Fenamiphos-sulfoxide	0.5	2.6 %	0.5	2.6 %	0.5	2.7 %
Imazalil	0.3	1.3 %	0.2	0.8 %	0.2	0.9 %
Imidacloprid	0.4	2.2 %	0.5	2.4 %	0.4	2.1 %
Oxadixyl	0.2	1.1 %	0.3	1.3 %	0.2	1.1 %
Pendimethalin	3.6	18.2 %	3.2	16.2 %	2.9	14.7 %
2,4-D [neg]	0.8	4.1 %	1.1	5.3 %	1.1	5.4 %

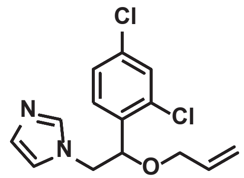
Dual-Channel LC-MS/MS: carry over test

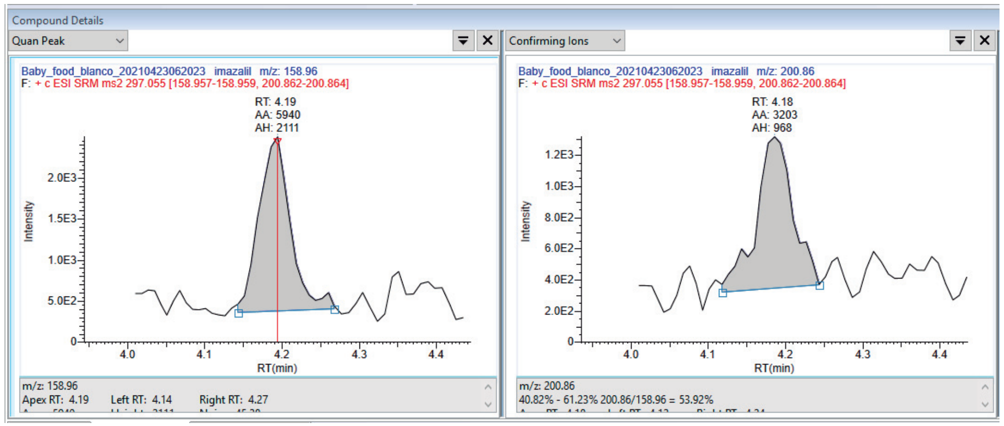


Imazalil

<u>0.5 ppb in baby food</u>

Quant ion peak area 2.0E4



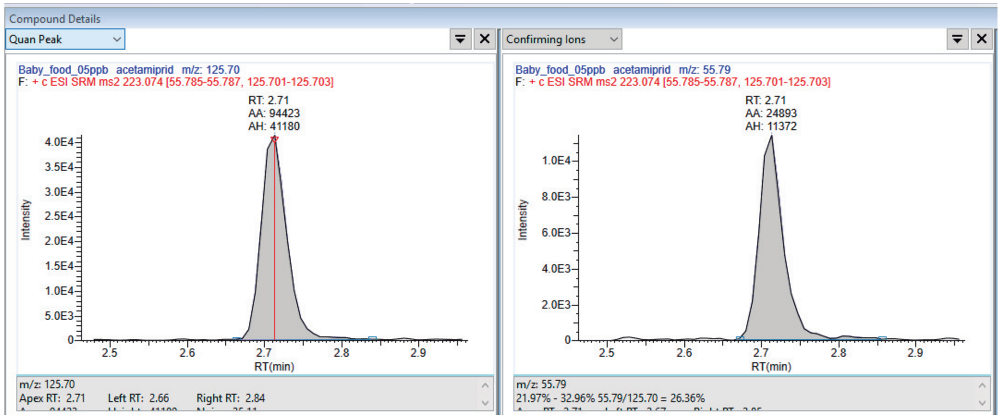


Imazalil

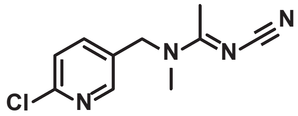
<u>Blank baby food after 12 injections of 20 ppb standard</u>

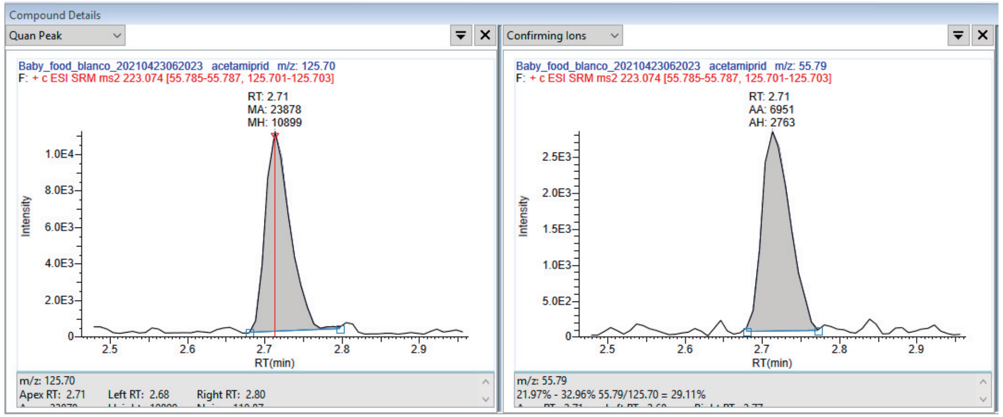
Quant ion peak area 6.0E3

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



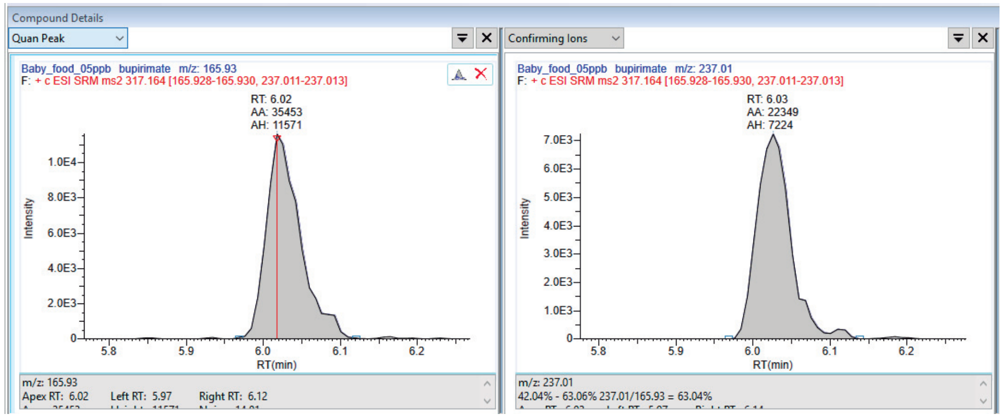
Acetamiprid
<u>0.5 ppb in baby food</u>
Quant ion peak area 9.4E4





Acetamiprid
<u>Blank baby food after 12 injections of 20 ppb standard</u>
Quant ion peak area 2.4E4

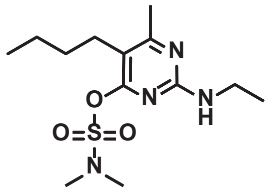
Dual-Channel LC-MS/MS: carry over test

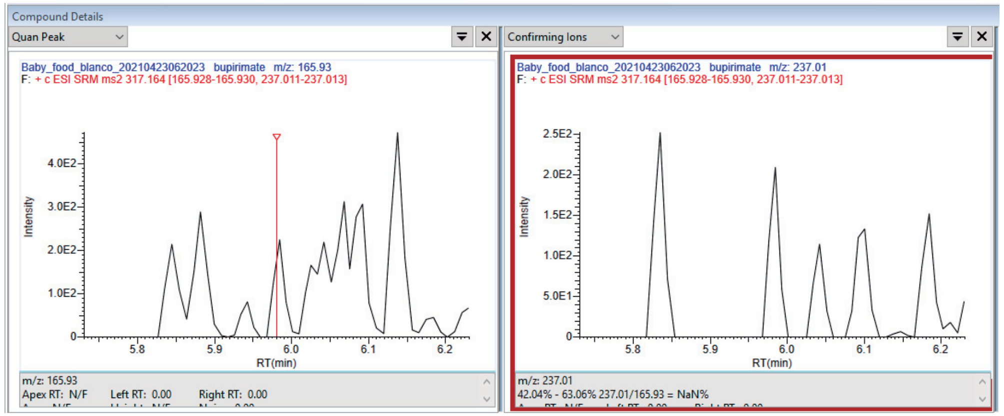


Bupirimate

<u>0.5 ppb in baby food</u>

Quant ion peak area 1.2E4



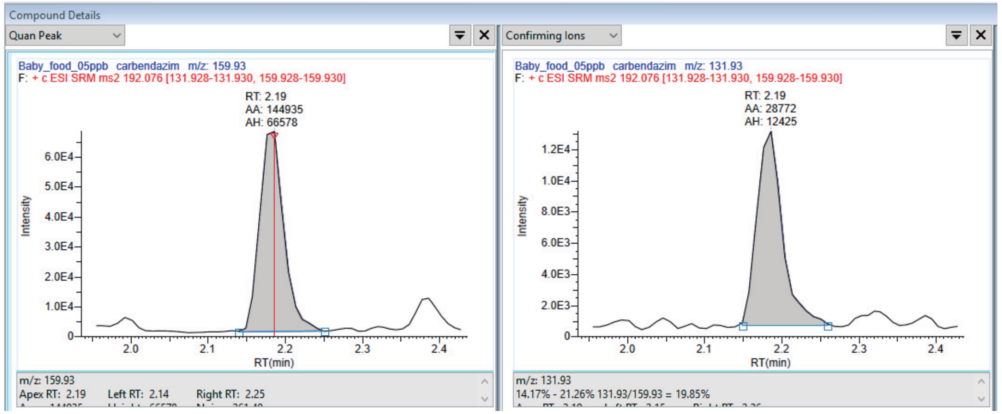


Bupirimate

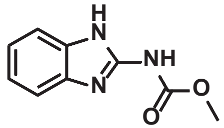
<u>Blank baby food after 12 injections of 20 ppb standard</u>

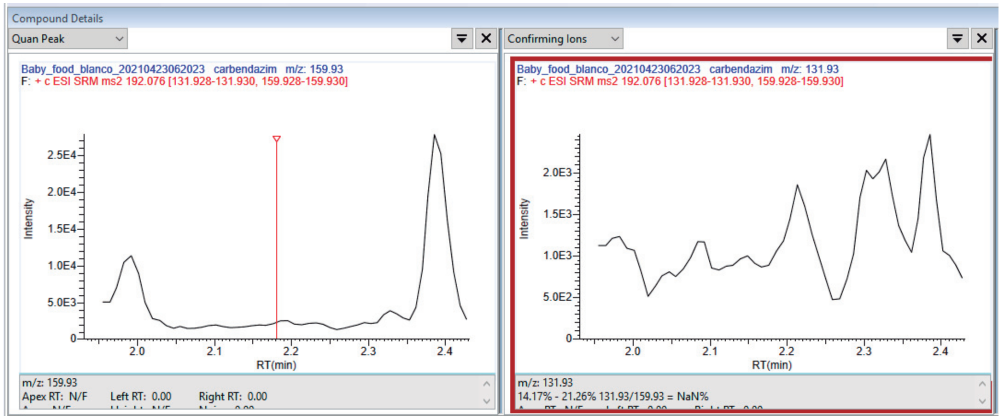
Quant ion peak area 0.0E0

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



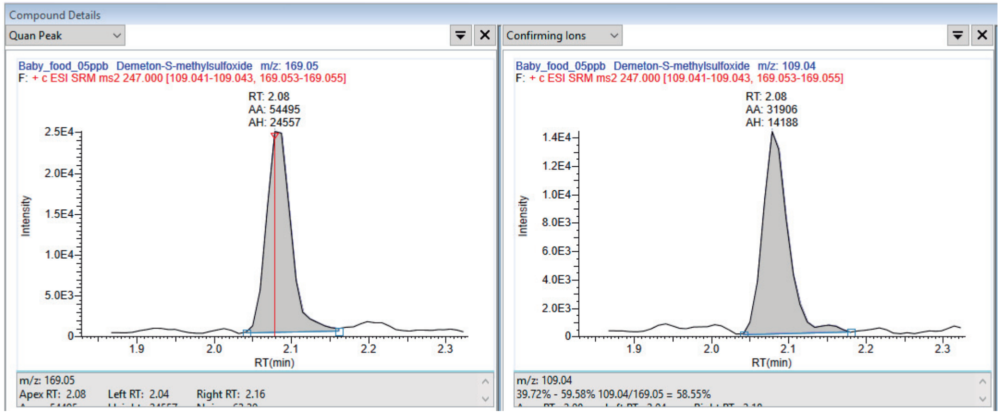
Carbendazim
<u>0.5 ppb in baby food</u>
Quant ion peak area 1.4E5





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

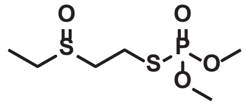
Dual-Channel LC-MS/MS: carry over test

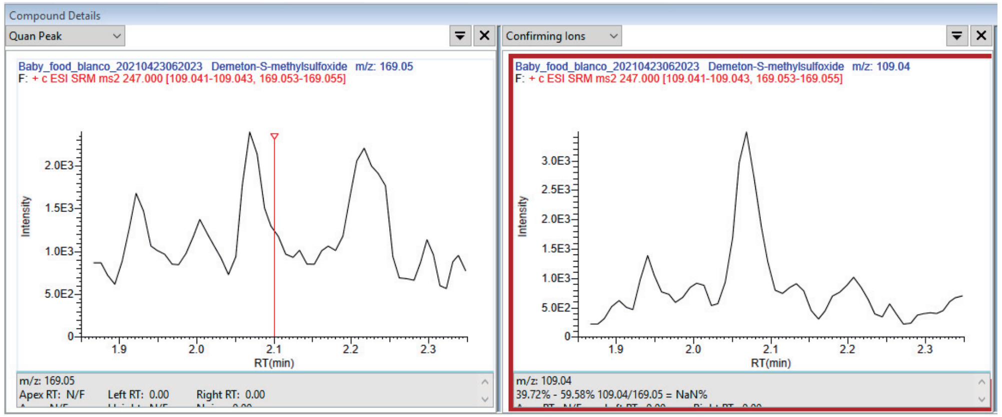


Demeton-*S*-methylsulfoxide

0.5 ppb in baby food

Quant ion peak area 5.5E4





Demeton-*S*-methylsulfoxide

Blank baby food after 12 injections of 20 ppb standard

Quant ion peak area 0.0E0

Dual-Channel LC-MS/MS reference

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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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European Union Reference Laboratory for Pesticide Residues in Fruit & Vegetables, University of Almería, Agrifood Campus of International Excellence (ceiA3), Ctra. Sacramento S/n. La Cañada de San Urbano, 04120, Almería, Spain

Conclusions

- Dual-Channel LC-QqQ-MS/MS can be used to increase **sample throughput** 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

- Rajski, Ł., Jesús, F., Díaz-Galiano, F.J., Fernández-Alba, A.R. Dual-channel chromatography a smart way to improve the analysis efficiency in liquid chromatography coupled to mass spectrometry. *J. Chrom. A* **2020**, *1633*, 461614.

- Cutting-edge approach using dual-channel chromatography to overcome the sensitivity issues associated with polarity switching in pesticide residues analysis. Díaz-Galiano, F.J., Rajski, Ł., Parrilla, P.; Ferrer, C.; Fernández-Alba, A.R. *Anal. Chim. Acta* **2021**, *1180*, 338875.

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