European Commission

EURL-FV

Dual channel chromatography for positive and negative mode

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Practical Training - New Advances in MRM (on-line training)
21-22/01/2021

Introduction

LC-MS analysis of pesticide residues

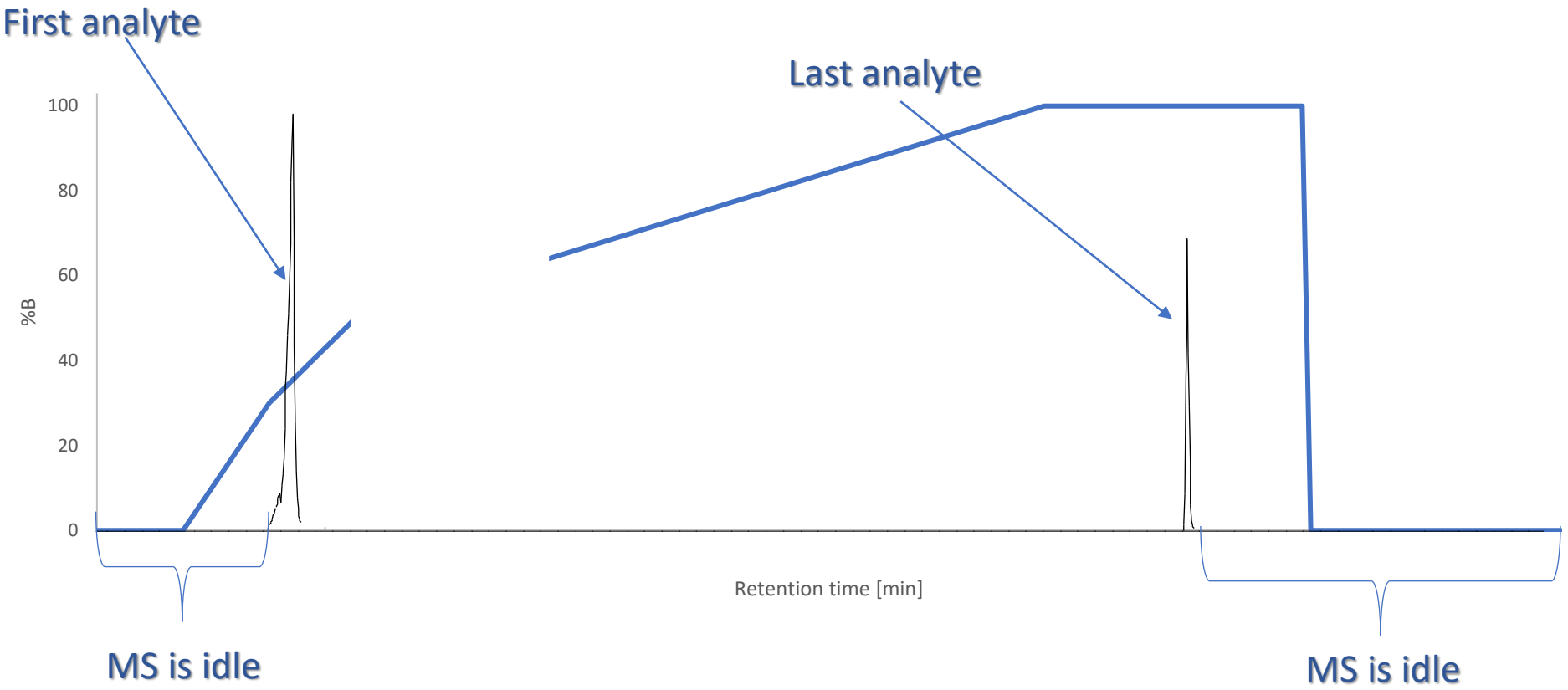
A typical LC-MS method:

- Column length 100 mm
- Particle size 2-3 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
    - More coeluting matrix (especially in “dirty matrices”)
      - Higher matrix effects -> lower sensitivity
      - Possible interferences

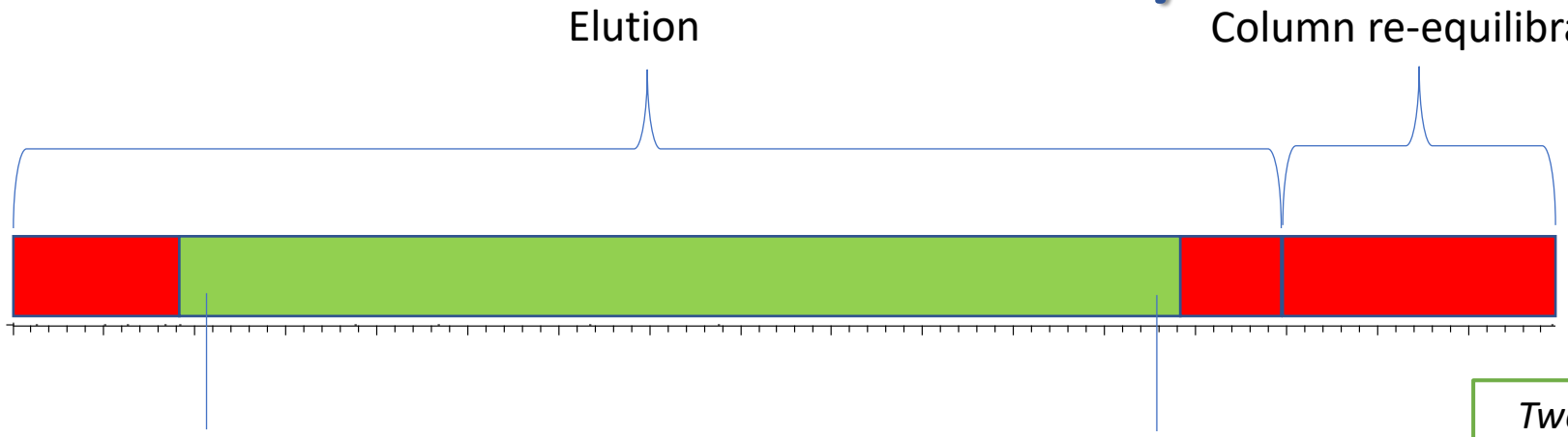
Another option to decrease the analysis time?



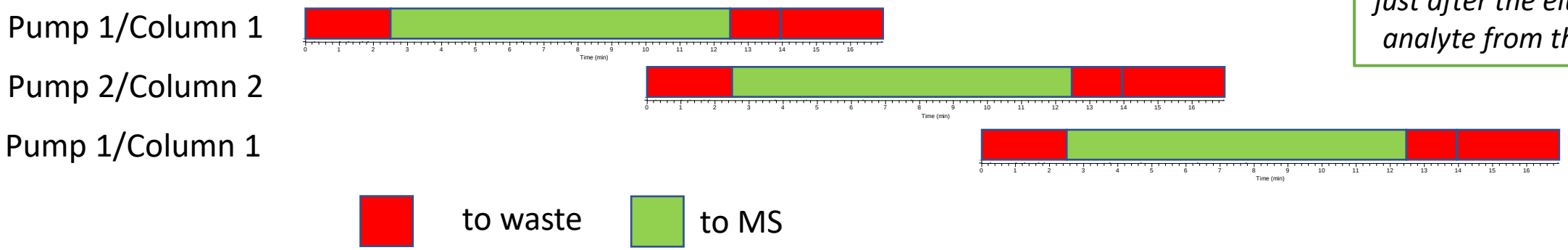
Analysis time can be decreased by the application of multi-channel chromatography and reduction of the idle time of the mass spectrometer

Hardware

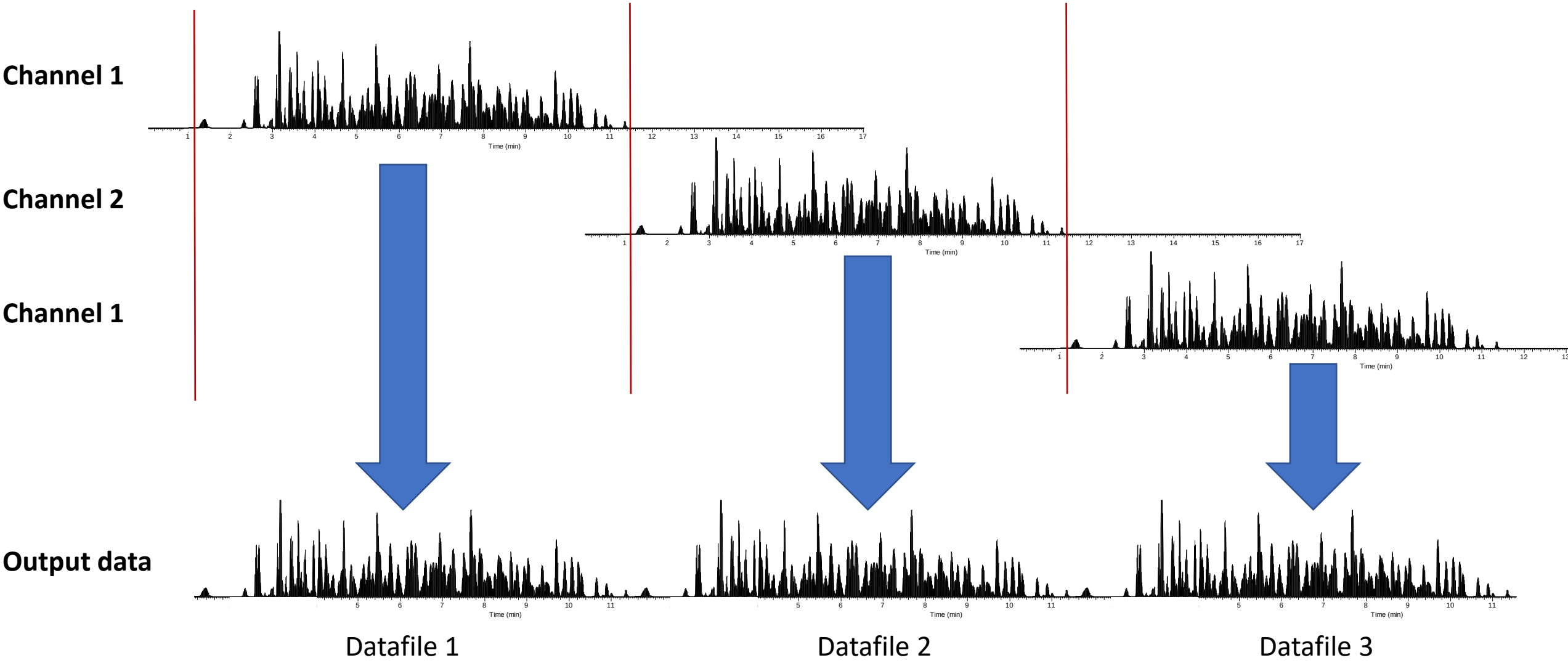
How does a dual-channel system work?



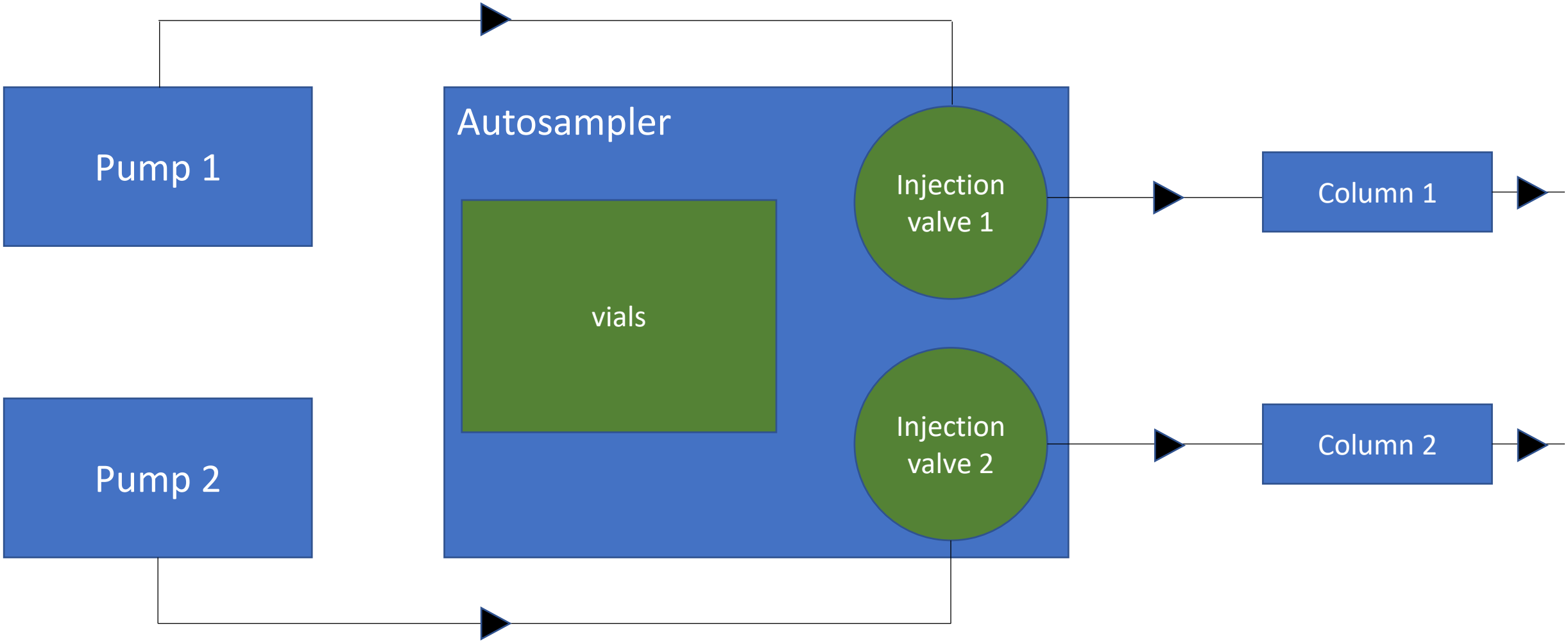
Two columns are operated in parallel. Then, consecutive injections are partially overlapped and synchronised in the way that the first analyte from the second column elutes just after the elution of the last analyte from the first column.



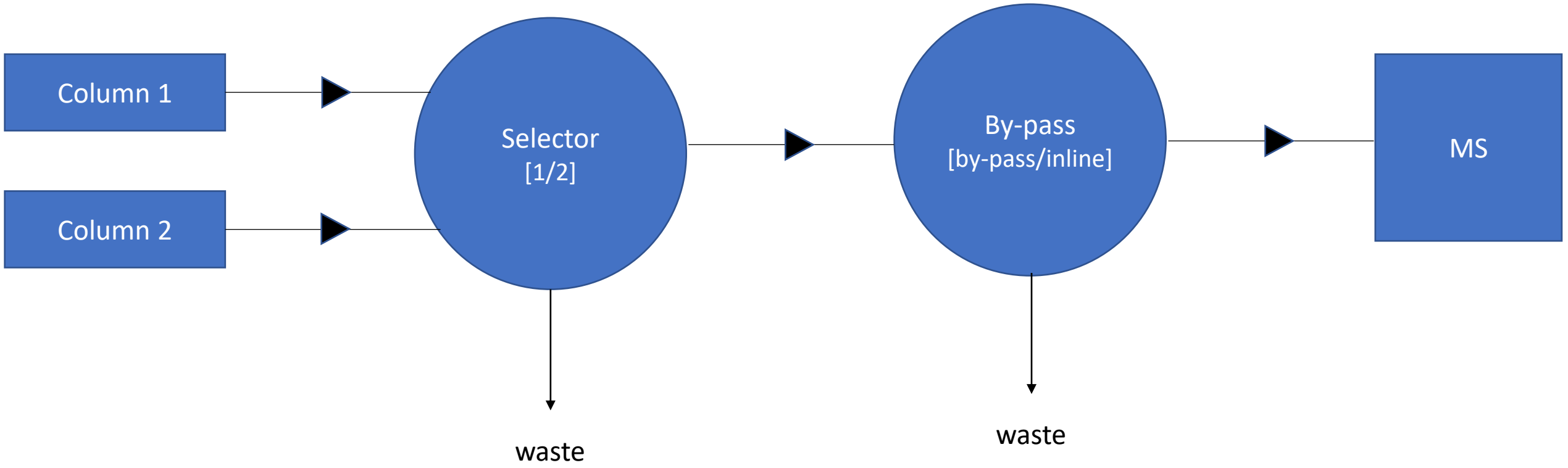
Chromatographic output from a dual-channel system



Schematic of the Dual Channel Configuration

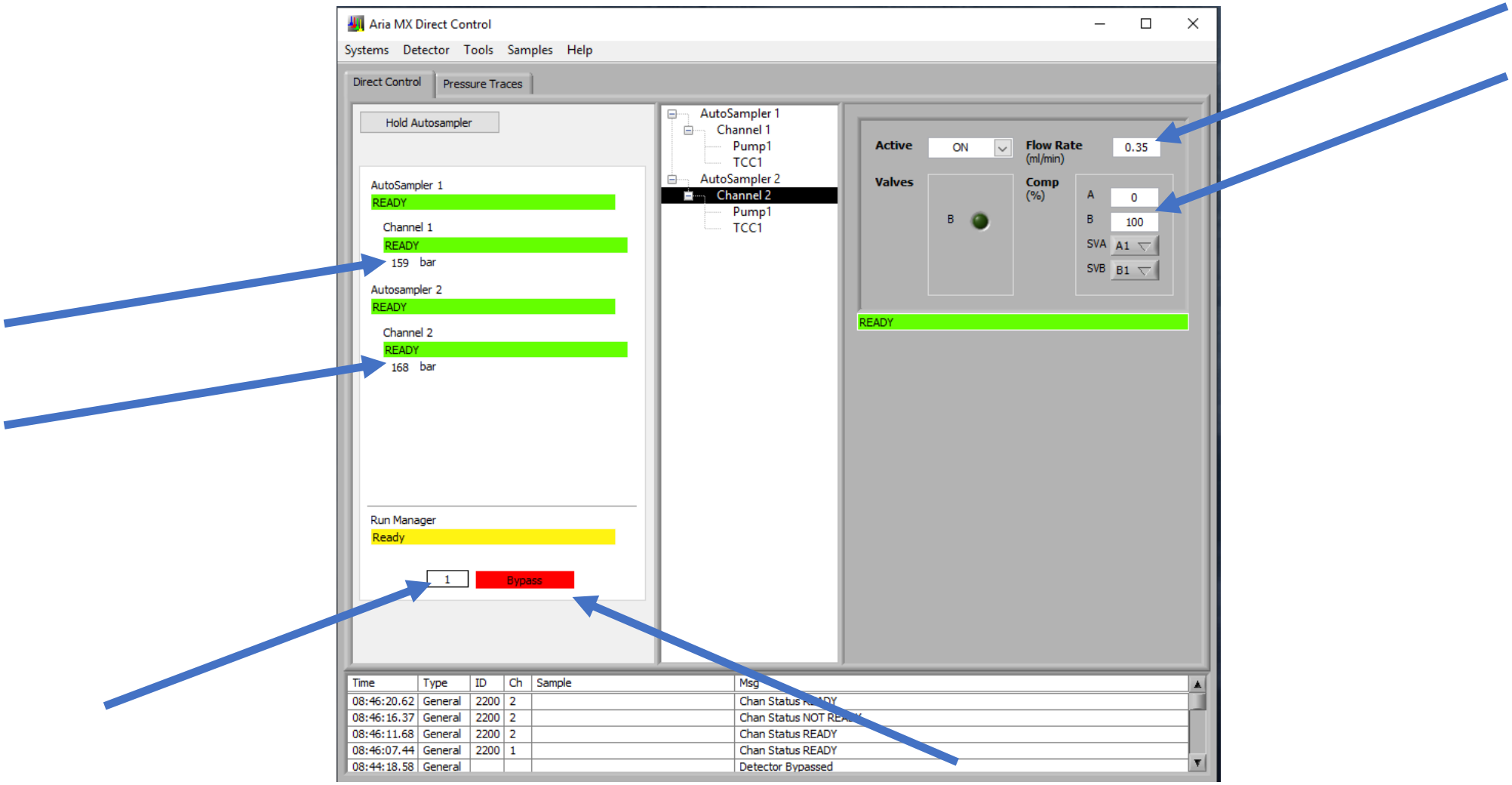


Transcend Duo LX-2 – channel selector



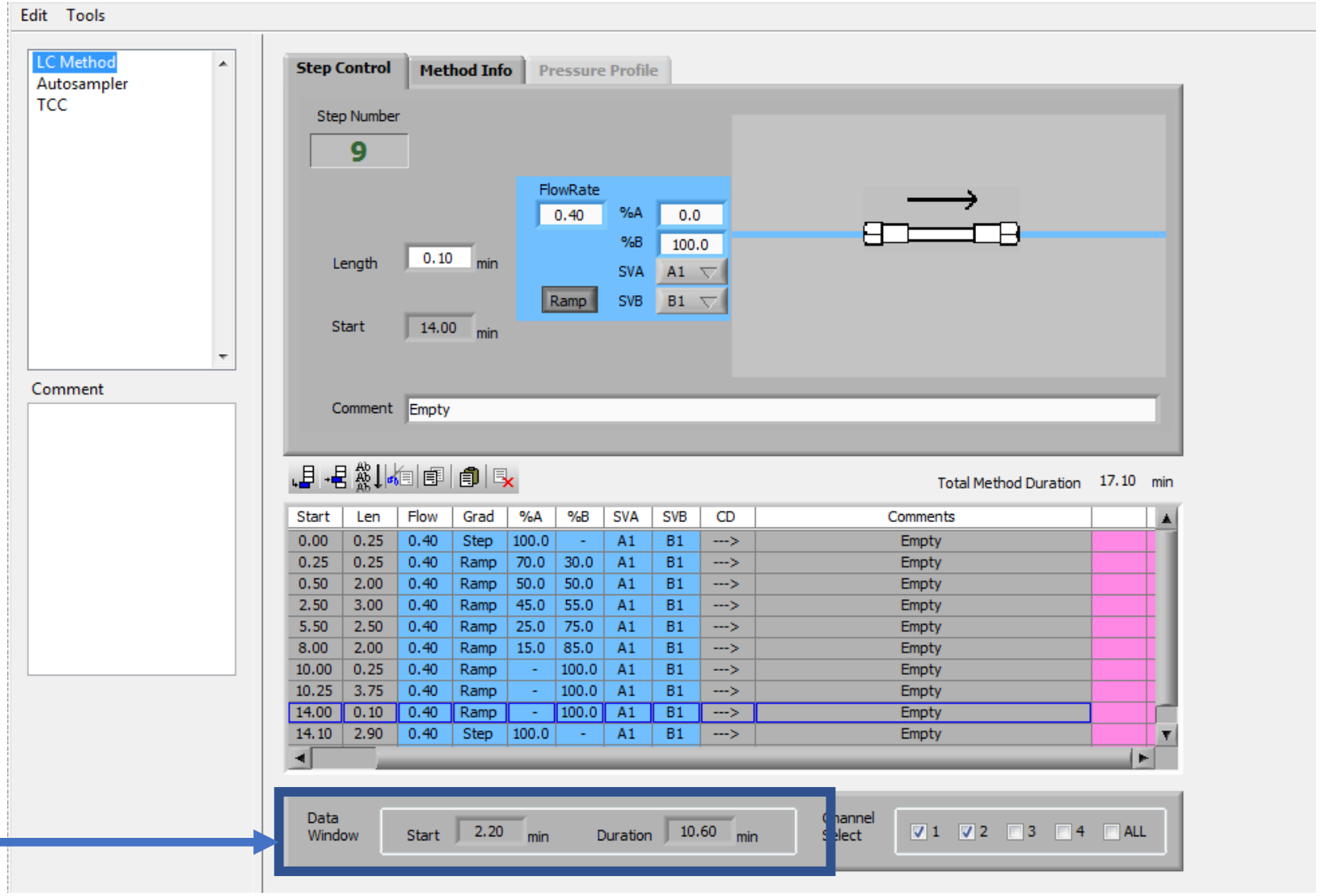
Software

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
Chan Status READY
08:46:16.37
General
2200
2
Chan Status NOT READY
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

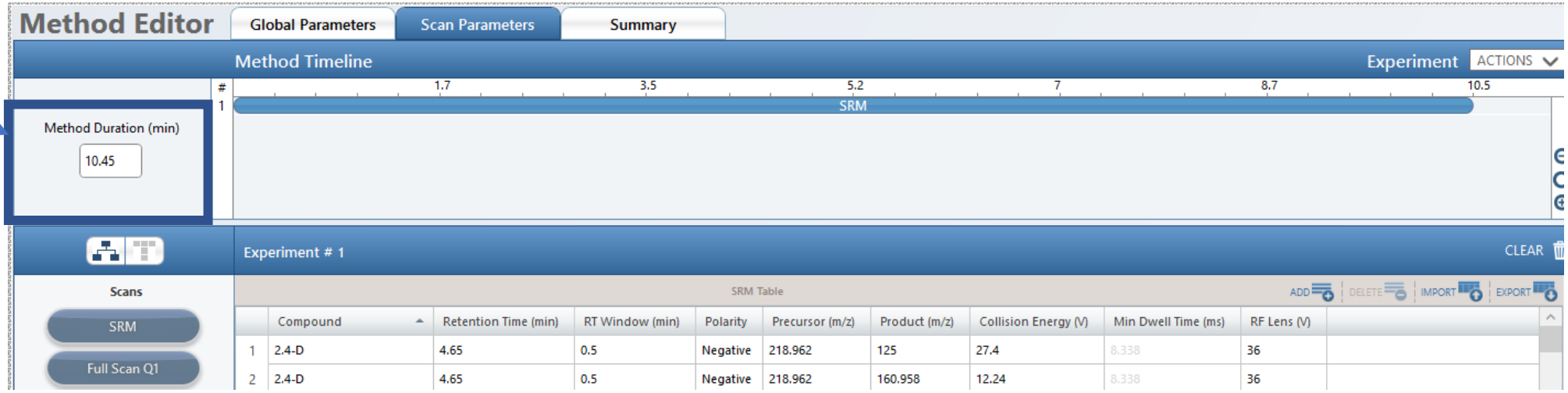
Aria MX Method setup

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.

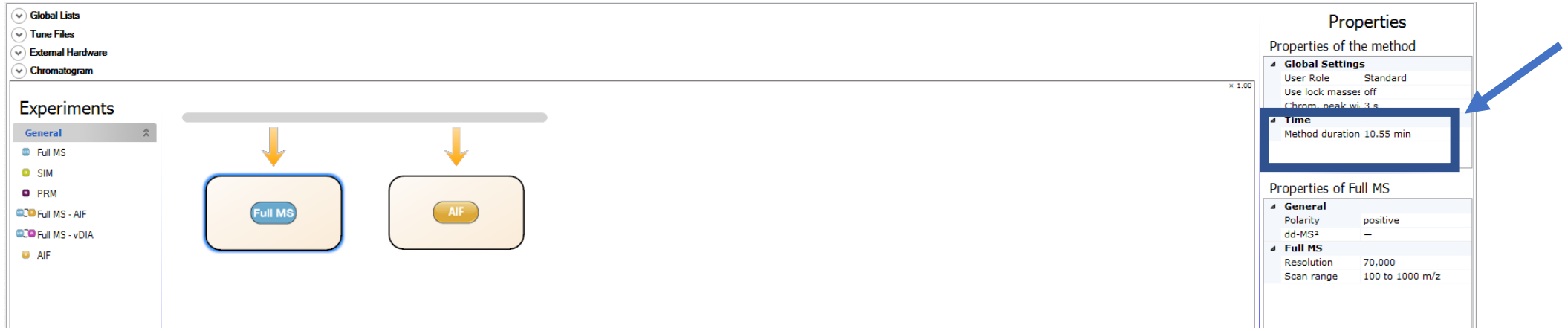


Method setup

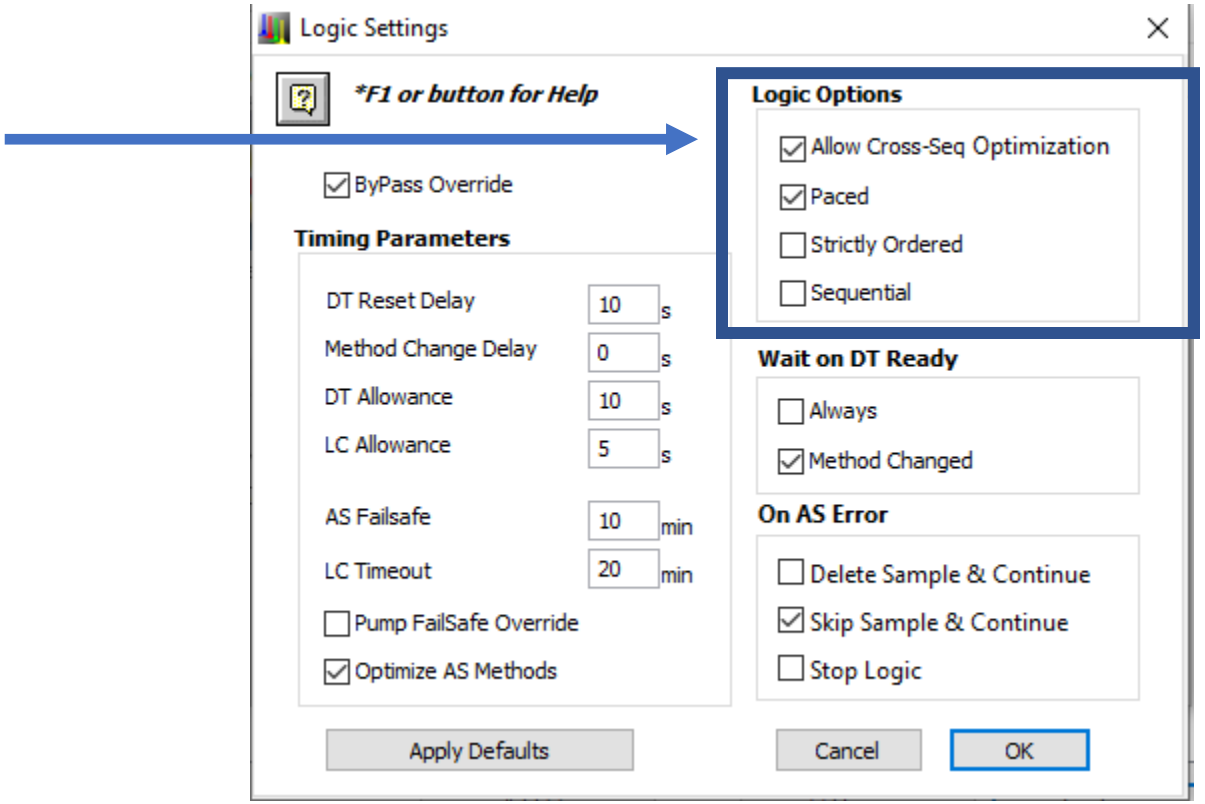
TSQ Altis



QE Focus



Aria MX Logic Settings

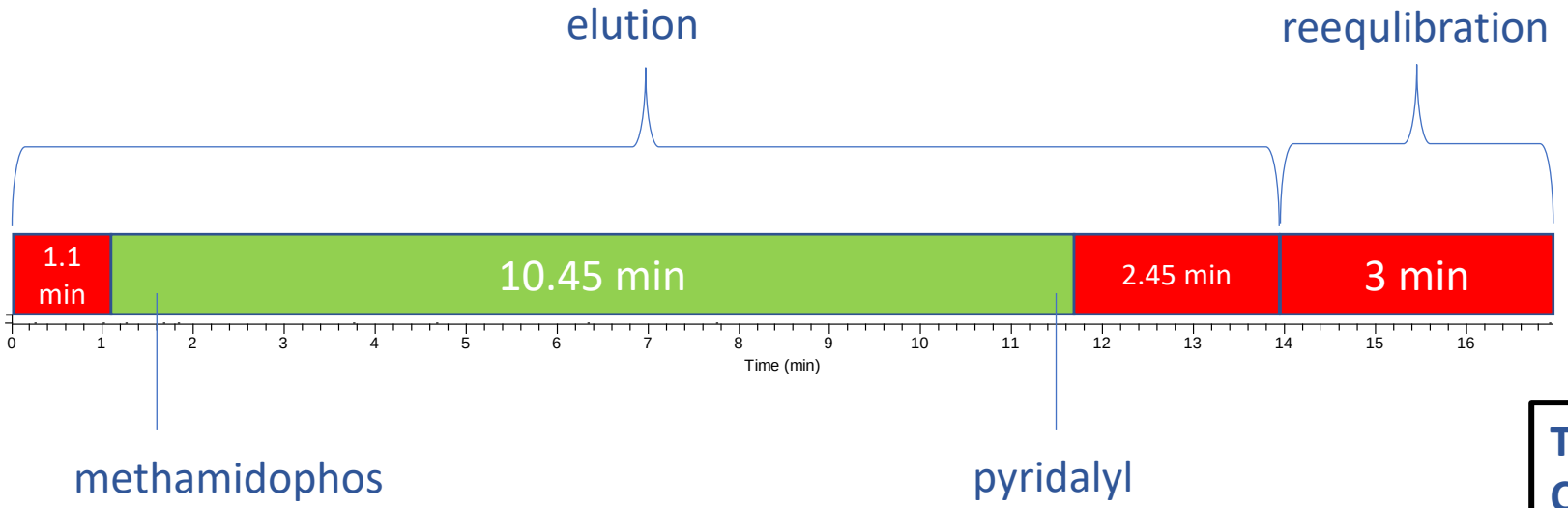


Sequence setup

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

Evaluation of 100 mm columns

Time segments in dual-channel chromatography

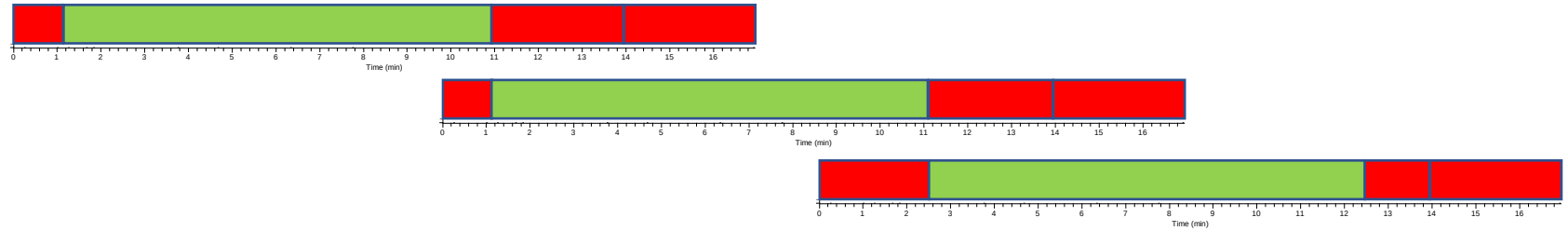


TSQ Altis
Column length 100 mm

Channel 1

Channel 2

Channel 1

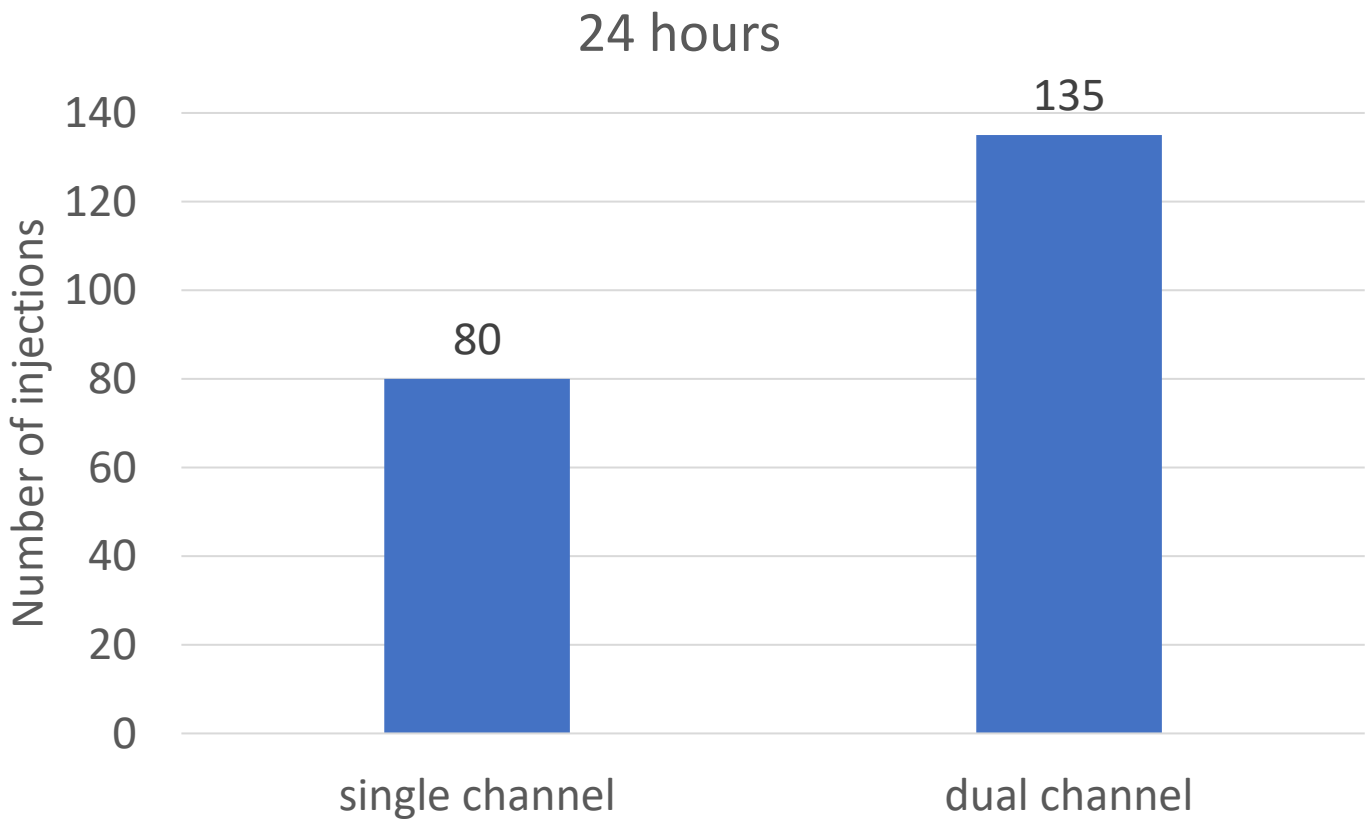


to waste

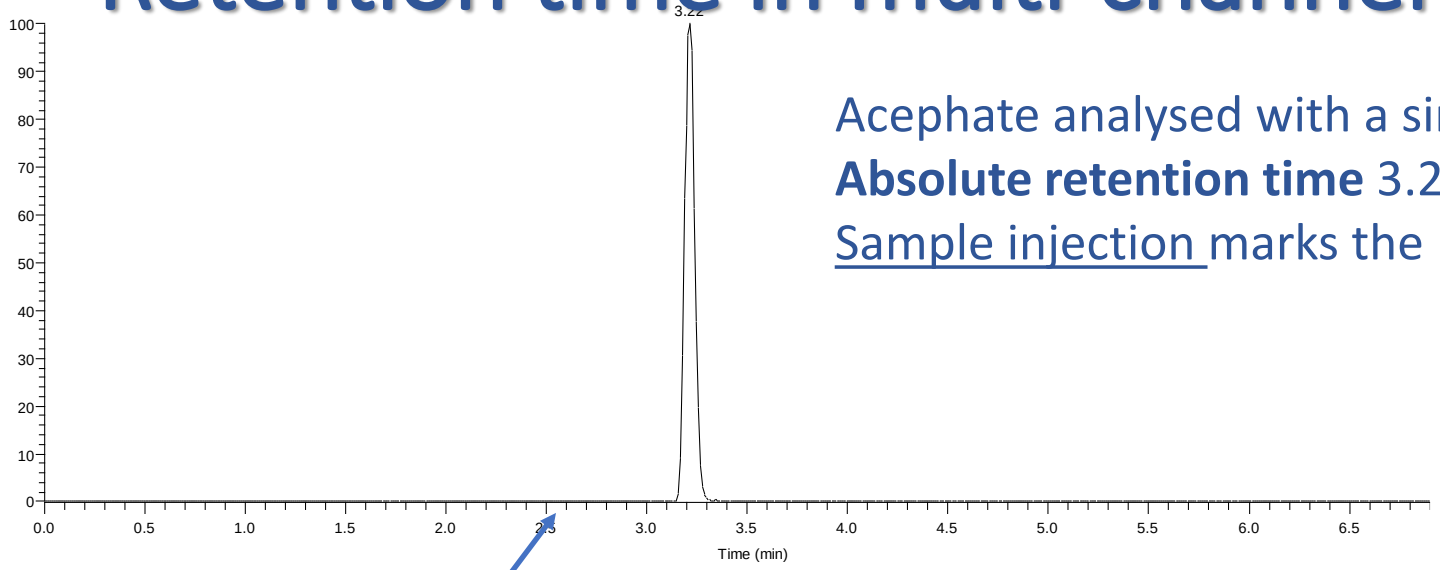
to MS (**acquisition time 10.45 min**)

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

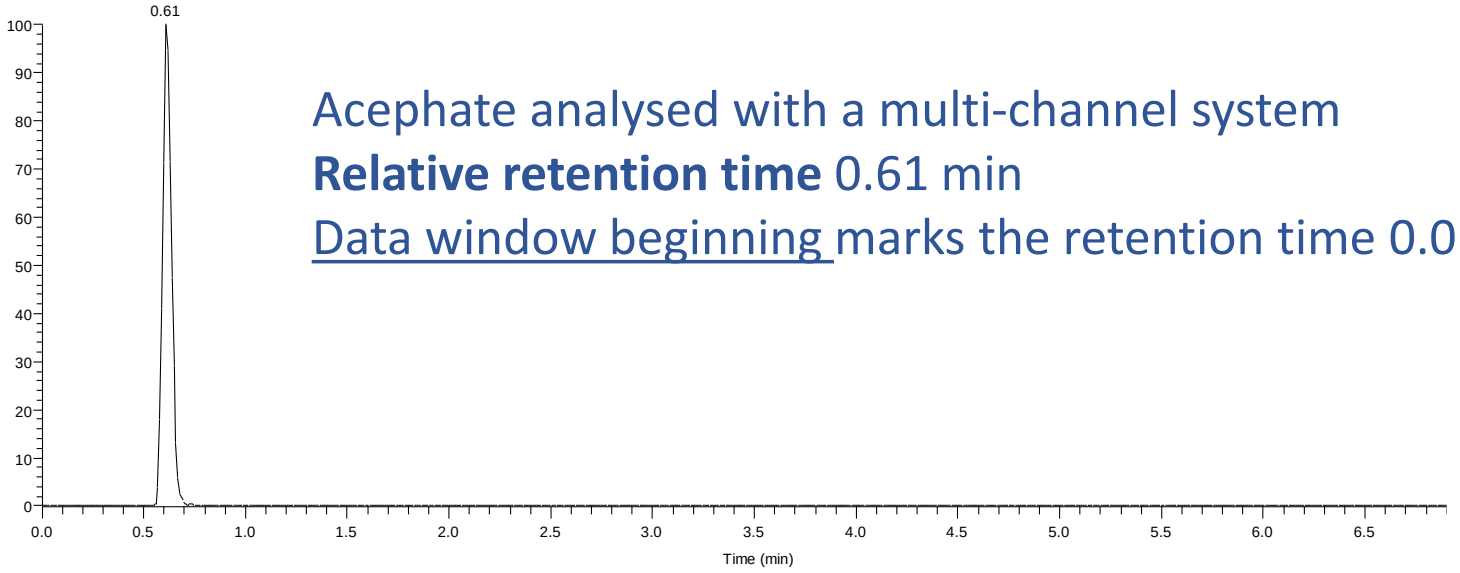
Improved sample throughput using a 100 mm column



Retention time in multi-channel chromatography



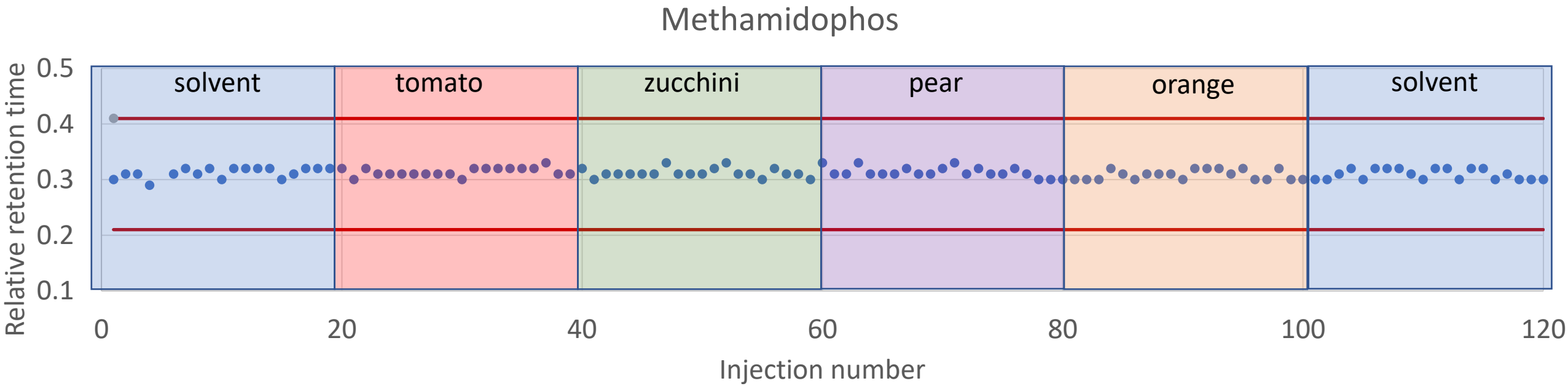
Acephate analysed with a single-channel system
Absolute retention time 3.22 min
Sample injection marks the retention time 0.0 min.



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

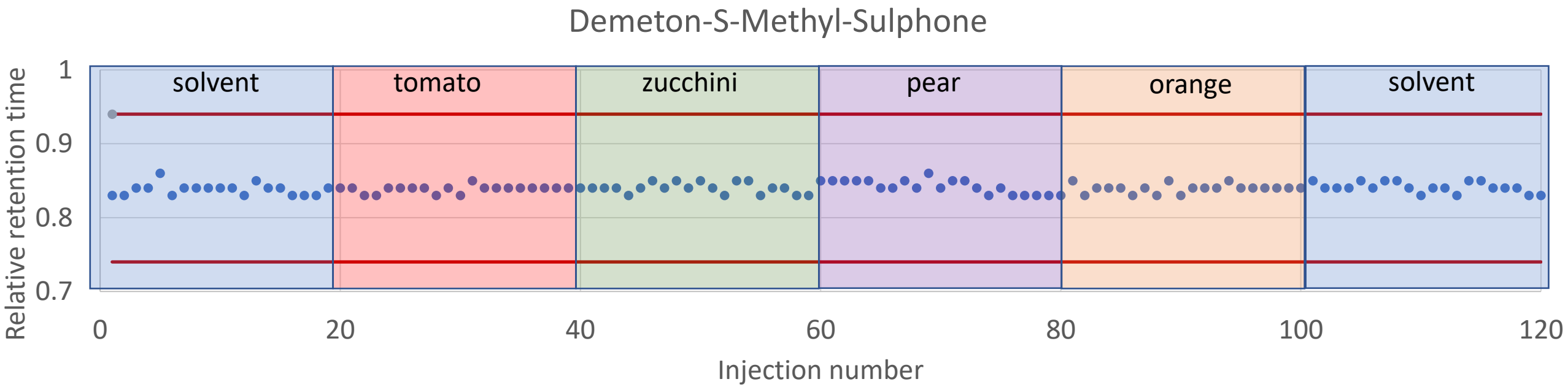
Retention time stability (methamidophos)



The red lines mark ± 0.1 min

120 injections alternating on column 1 & column 2

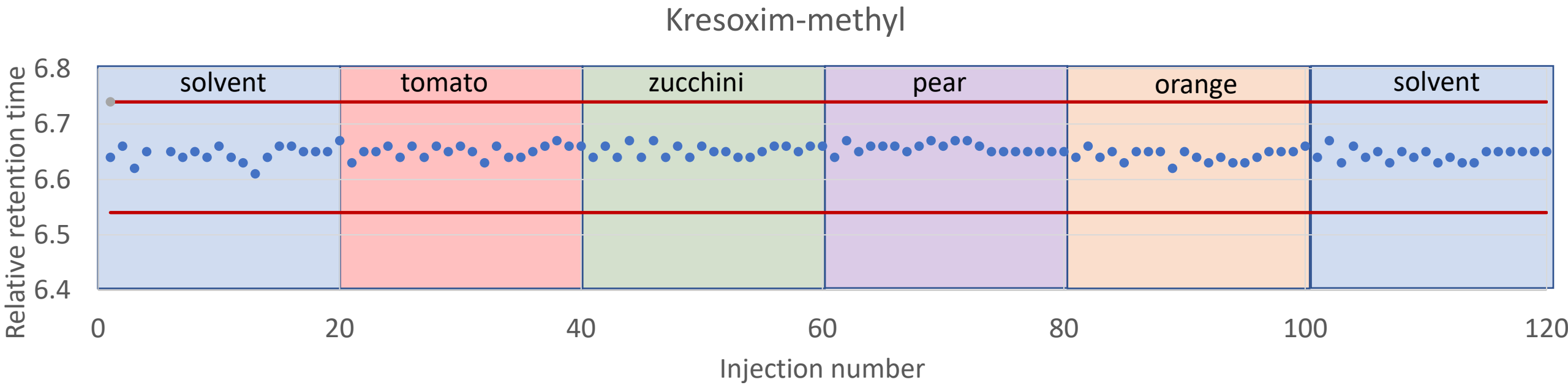
Retention time stability (demeton-S-methyl sulphone)



The red lines mark ± 0.1 min

120 injections alternating on column 1 & column 2

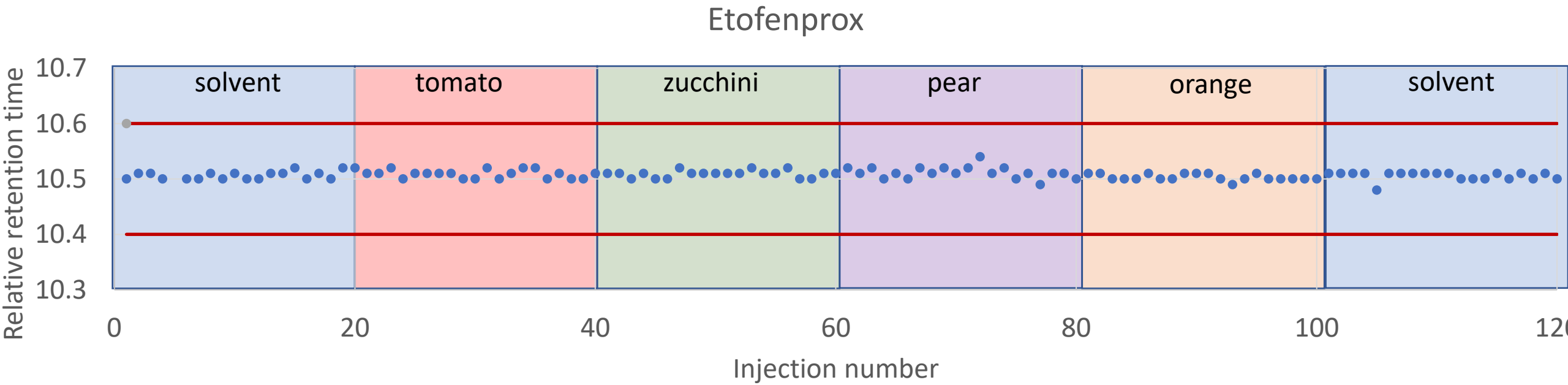
Retention time stability (kresoxim-methyl)



The red lines mark ± 0.1 min

120 injections alternating on column 1 & column 2

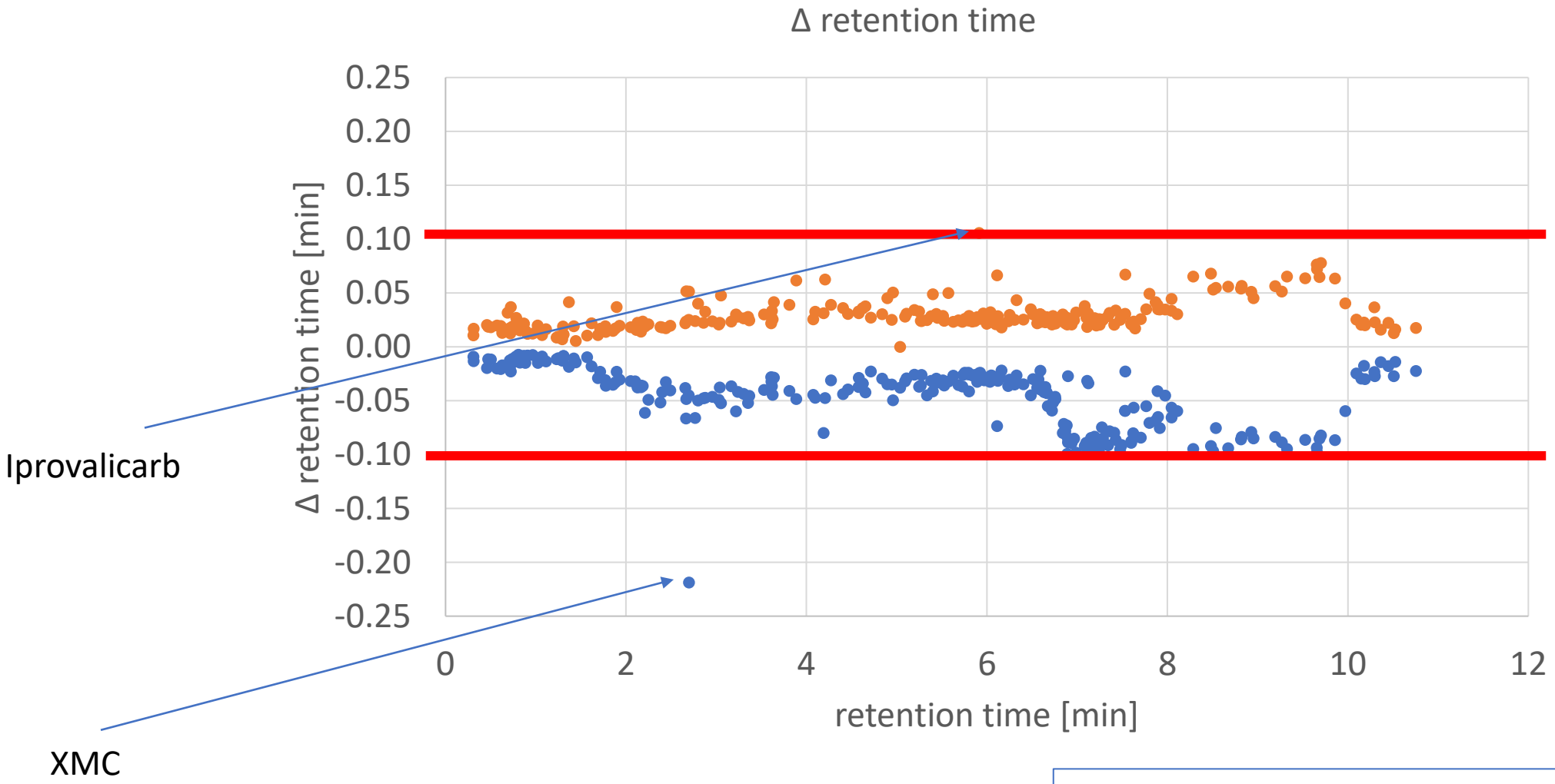
Retention time stability (Etofenprox)



The red lines mark ± 0.1 min

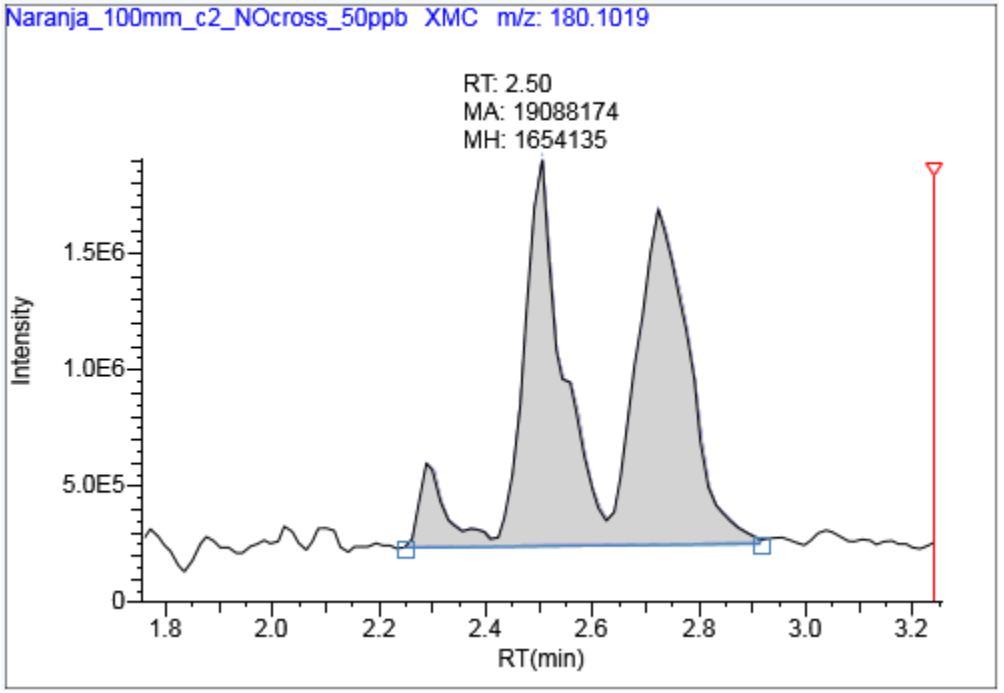
120 injections alternating on column 1 & column 2

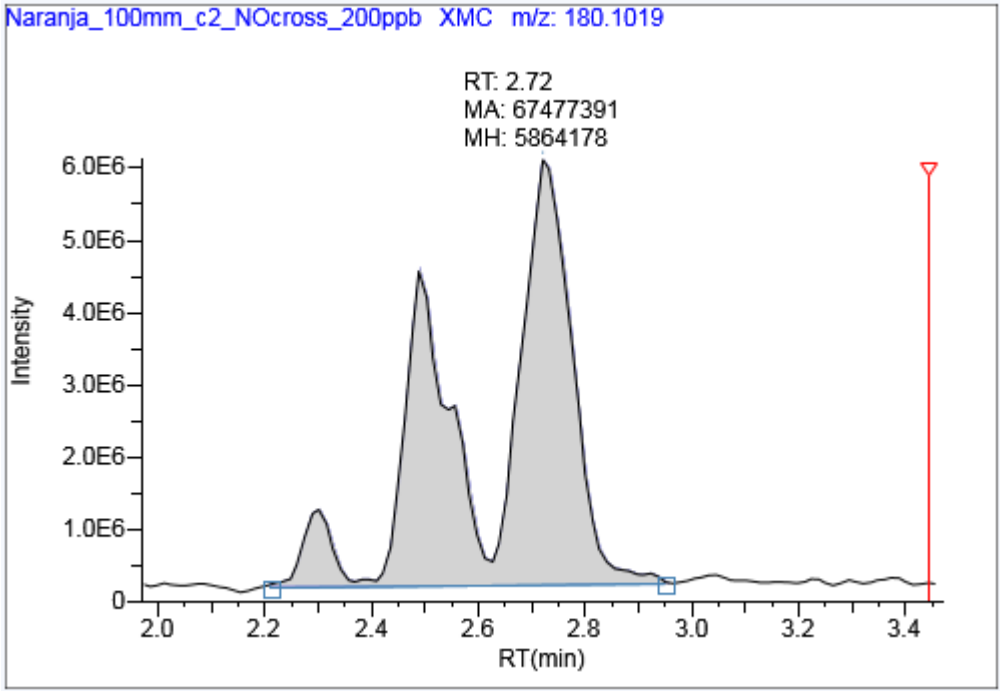
Retention time stability – outliers?



120 injections alternating on column 1 & column 2

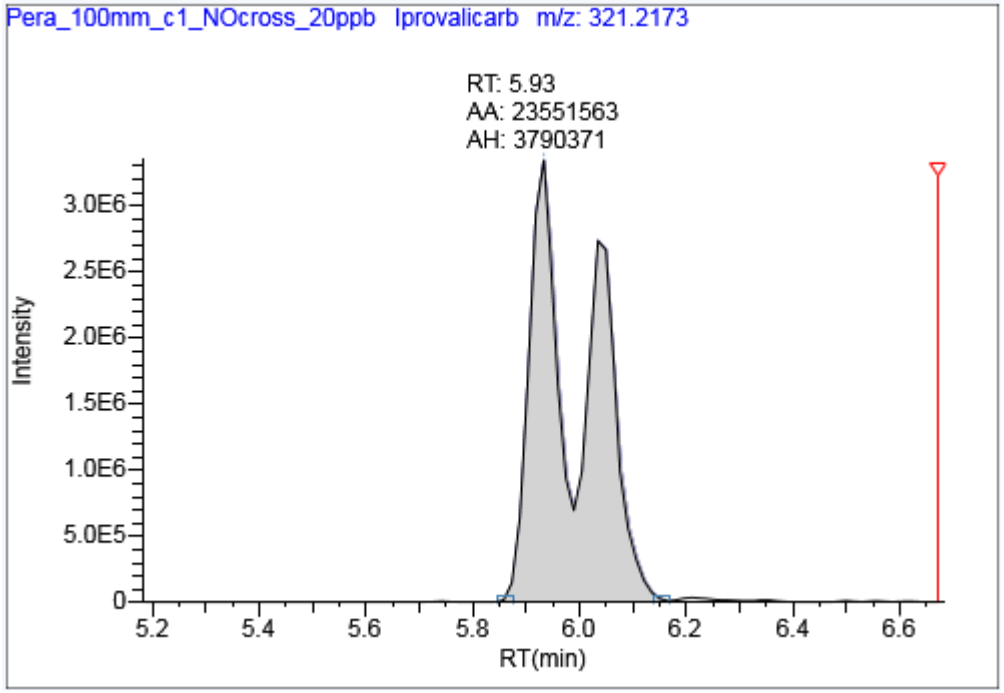
Retention time outlier? (XMC)

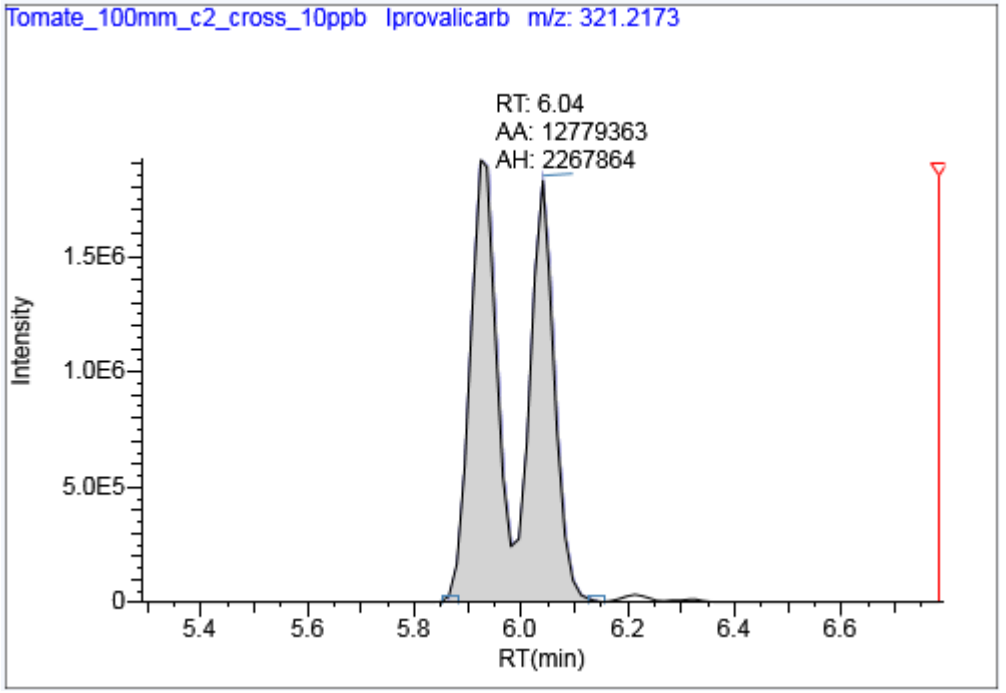




No misassignment of the peak when summed, the retention times are stable

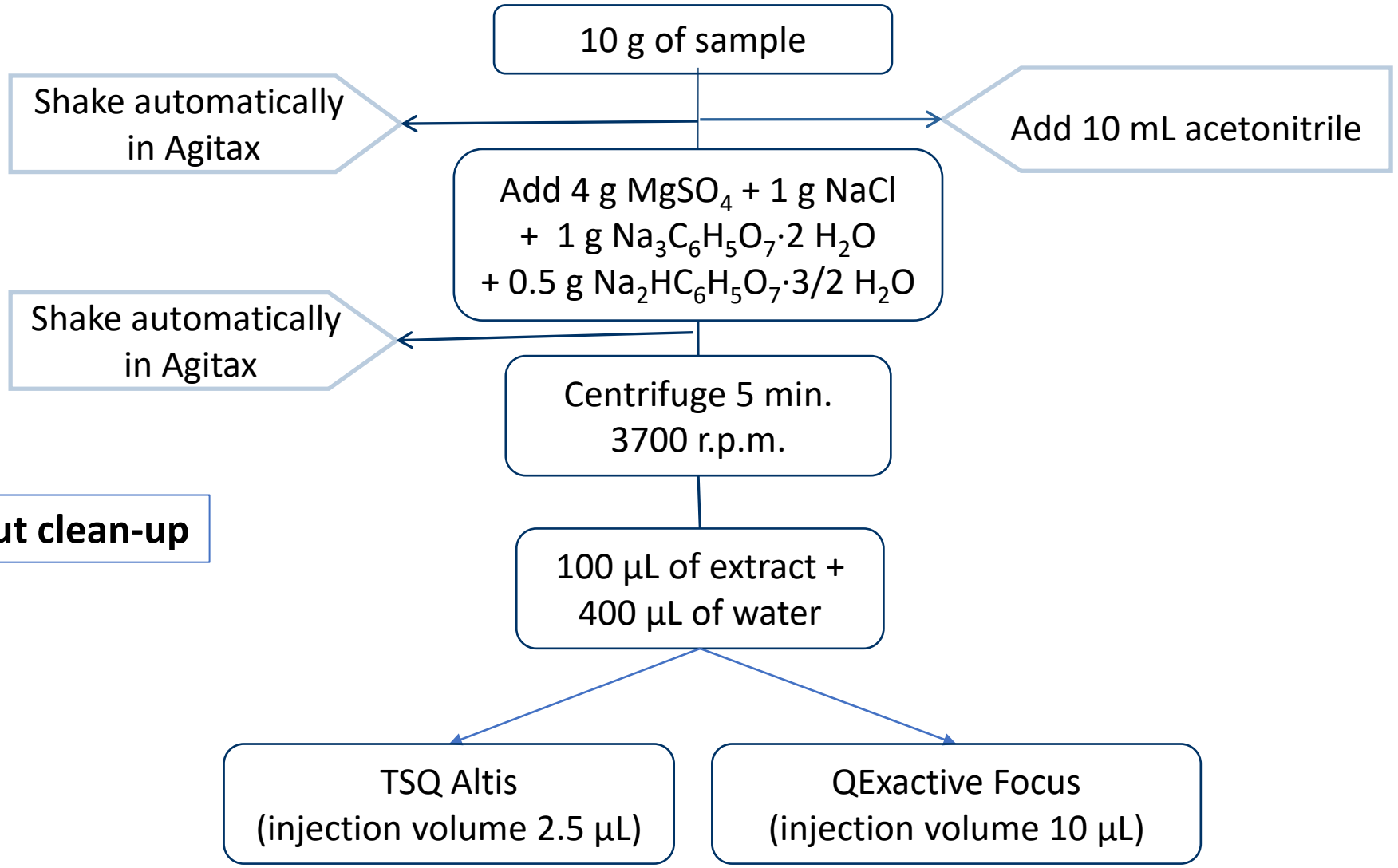
Retention time outlier? (Iprovalicarb)





No misassignment of the peak when summed, the retention times are stable

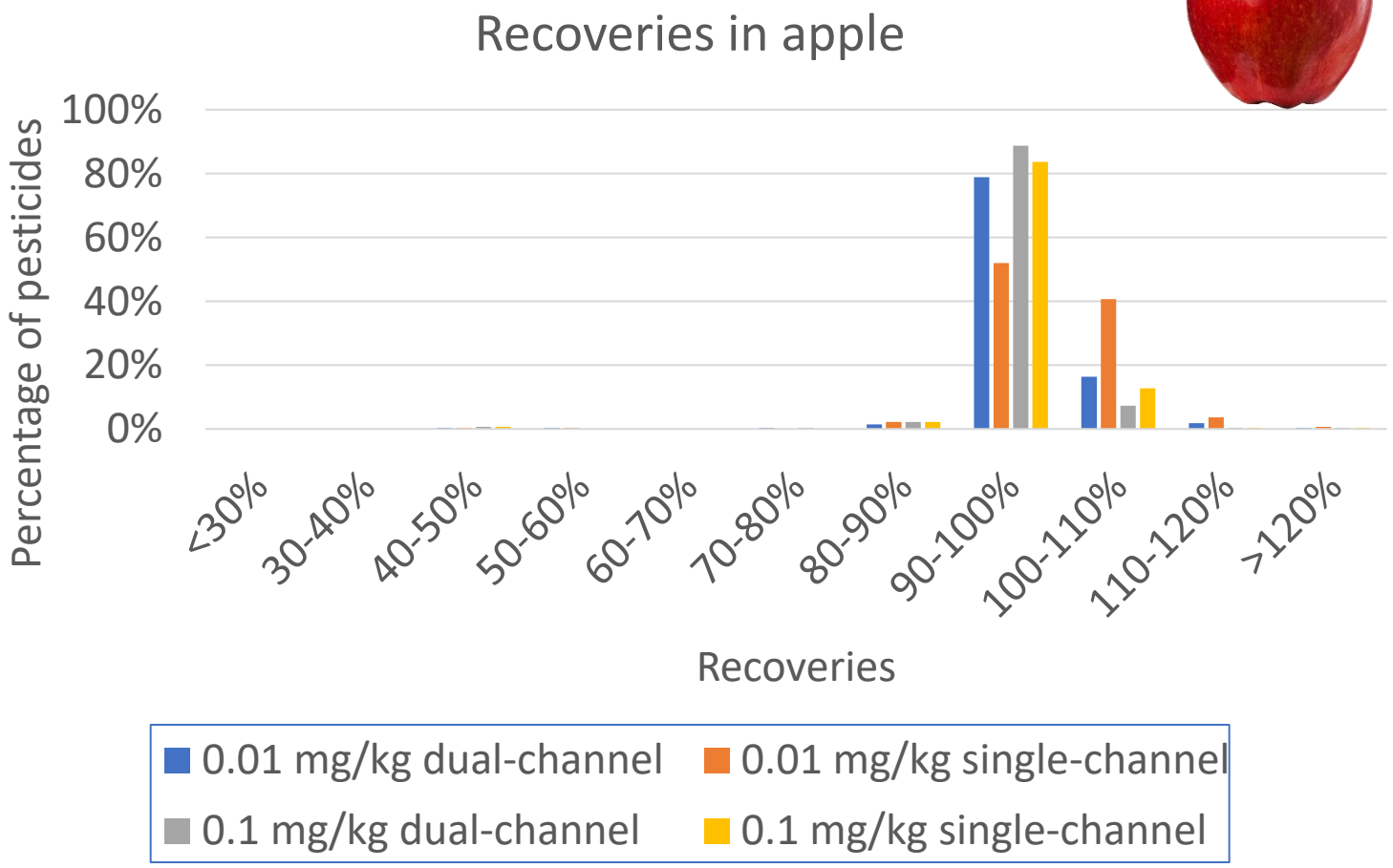
Recovery studies – extraction method



QuEChERS without clean-up

Recovery data (apple): single-channel vs dual-channel

	<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

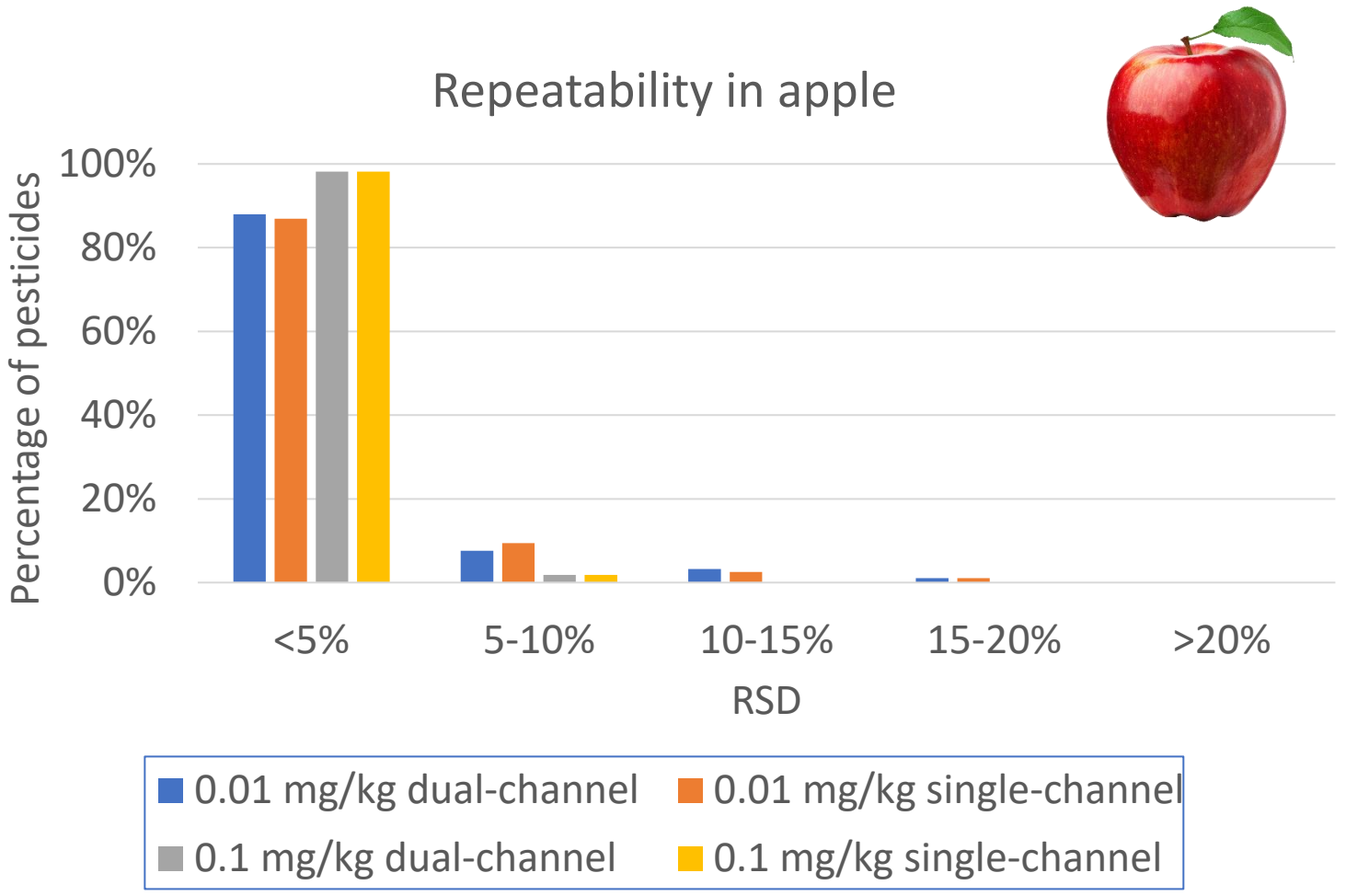


Results obtained using triple quadrupole

Number of compounds: 273

Repeatability data (apple): single-channel vs dual-channel

	<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%	-



Results obtained using triple quadrupole

Number of compounds: 273

Recovery data (bell pepper): single-channel vs dual-channel

	<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	-

Results obtained using triple quadrupole

Number of compounds: 273

Repeatability data (bell pepper): single-channel vs dual-channel

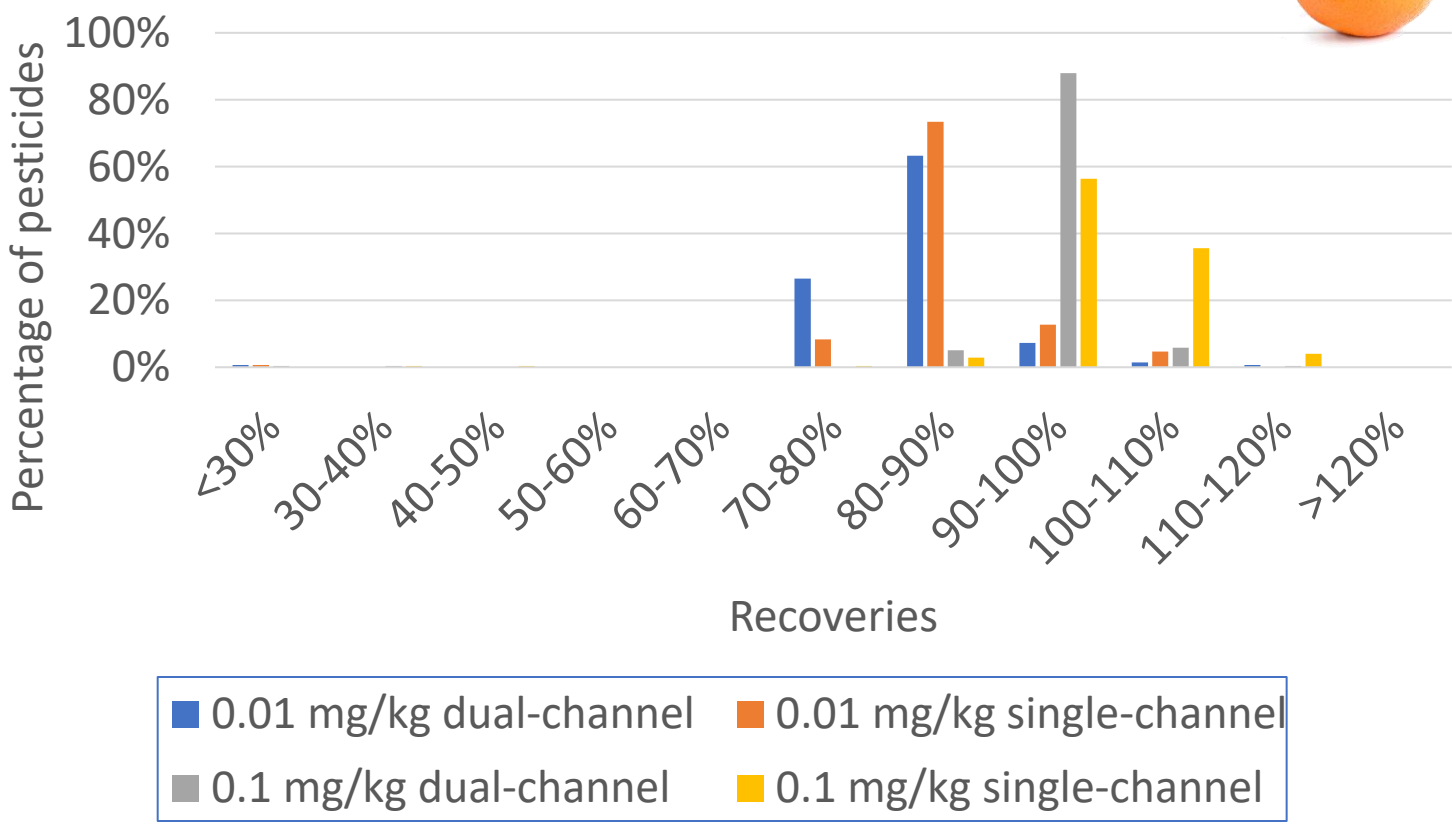
	<5%	5-20%	>20%
Single channel 0.01 mg/kg	89%	11%	-
Dual channel 0.01 mg/kg	91%	9%	-
Single channel 0.1 mg/kg	98%	2%	-
Dual channel 0.1 mg/kg	98%	2%	-

Results obtained using triple quadrupole

Number of compounds: 273

Recovery data (orange): single-channel vs dual-channel

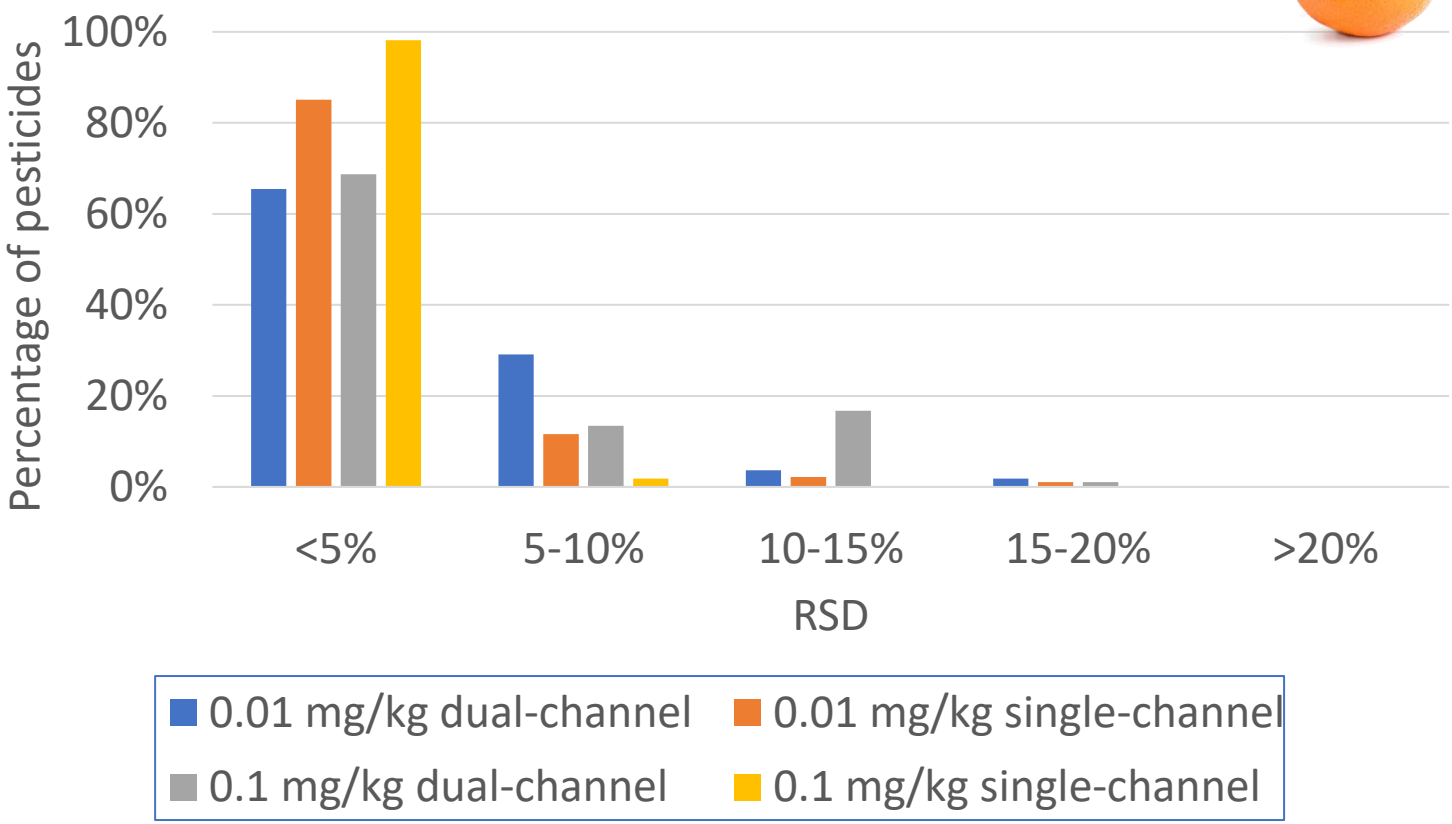
	<70%	70-120%	>120%
Single channel 0.01 mg/kg	2	271	-
Dual channel 0.01 mg/kg	2	271	-
Single channel 0.1 mg/kg	2	271	-
Dual channel 0.1 mg/kg	2	271	-



Results obtained using triple quadrupole

Repeatability data (orange): single-channel vs dual-channel

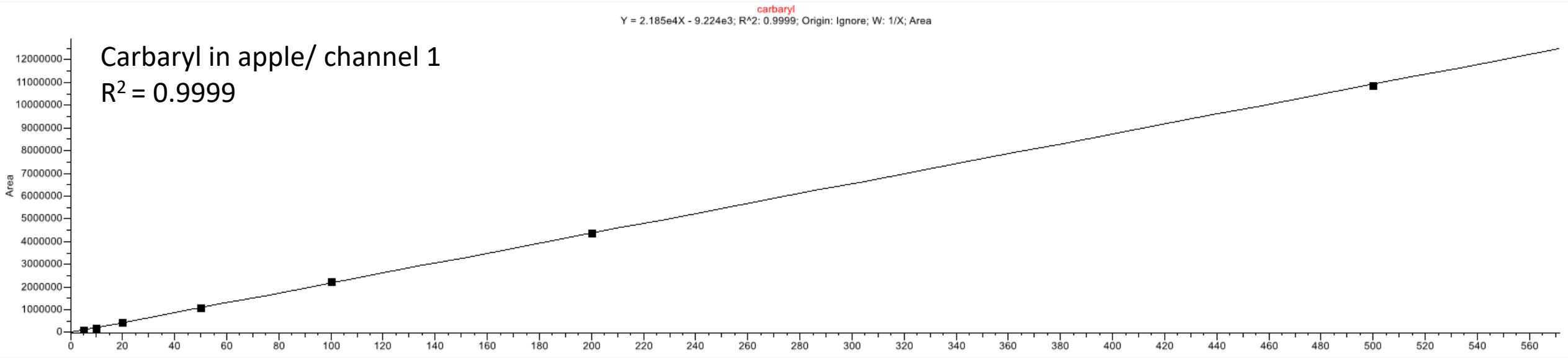
	<5%	5-20%	>20%
Single channel 0.01 mg/kg	85%	15%	-
Dual channel 0.01 mg/kg	65%	35%	-
Single channel 0.1 mg/kg	98%	2%	-
Dual channel 0.1 mg/kg	69%	31%	-

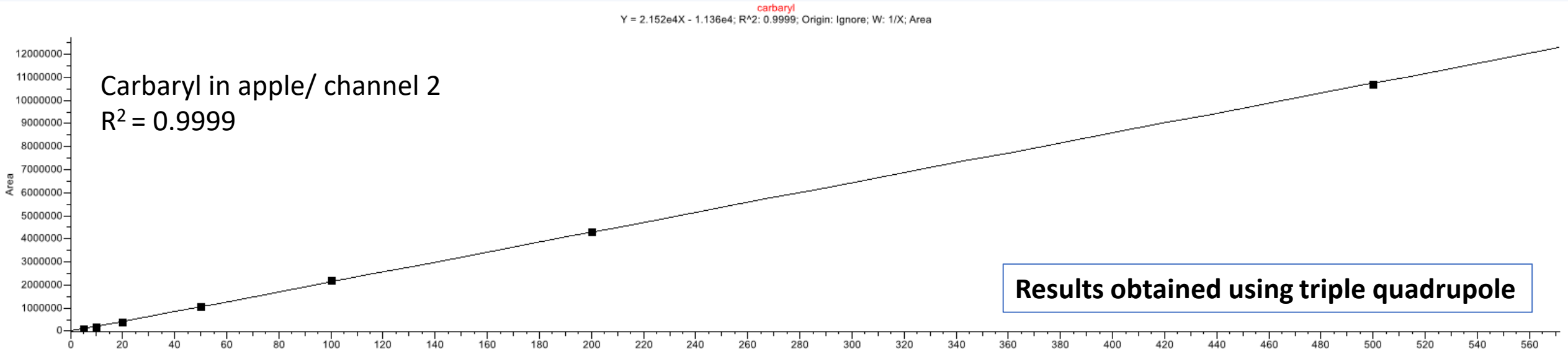


Results obtained using triple quadrupole

Number of compound: 273

Single-channel calibration for carbaryl in apple

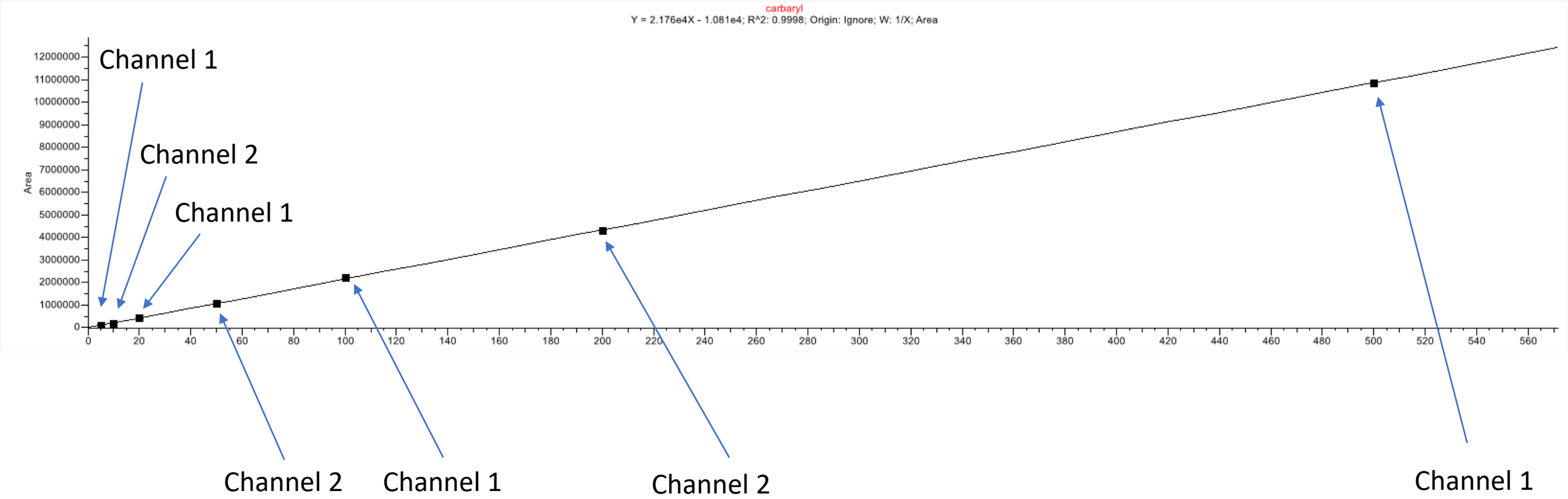




Results obtained using triple quadrupole

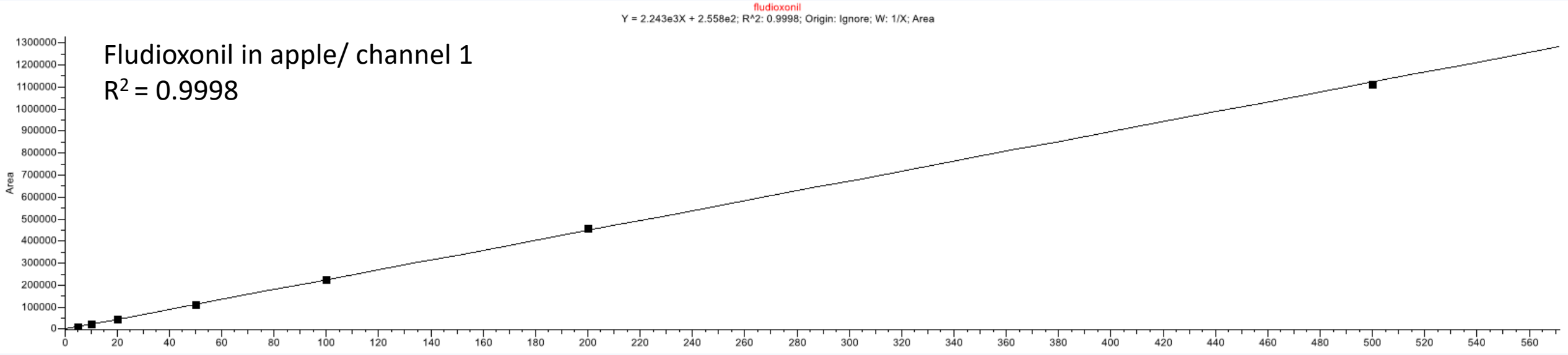
Cross-channel calibration for carbaryl in apple

Carbaryl in apple/ **cross-channel**
R^2 = 0.9998



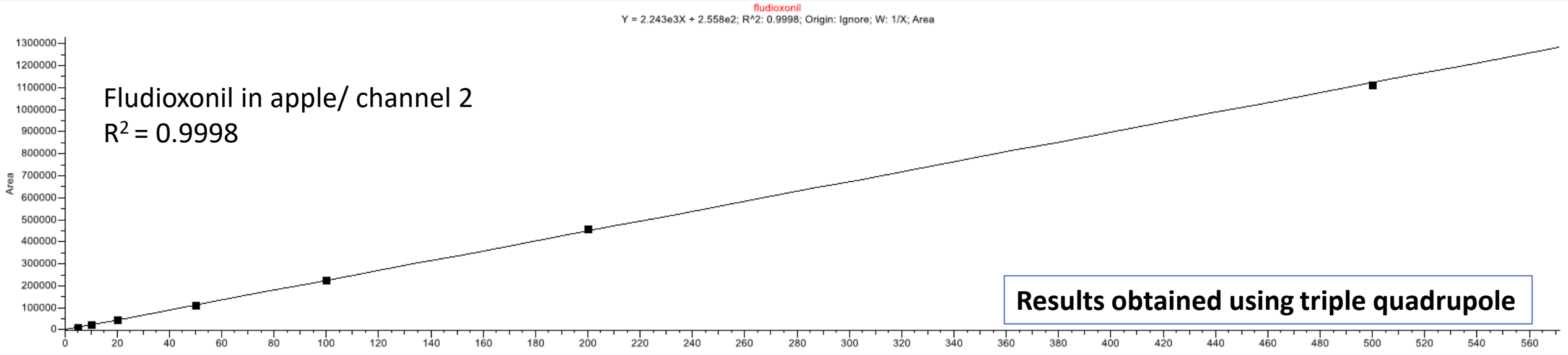
Results obtained using triple quadrupole

Single-channel calibration for fludioxinil in apple



Fludioxonil in apple/ channel 1

$R^2 = 0.9998$



Fludioxonil in apple/ channel 2

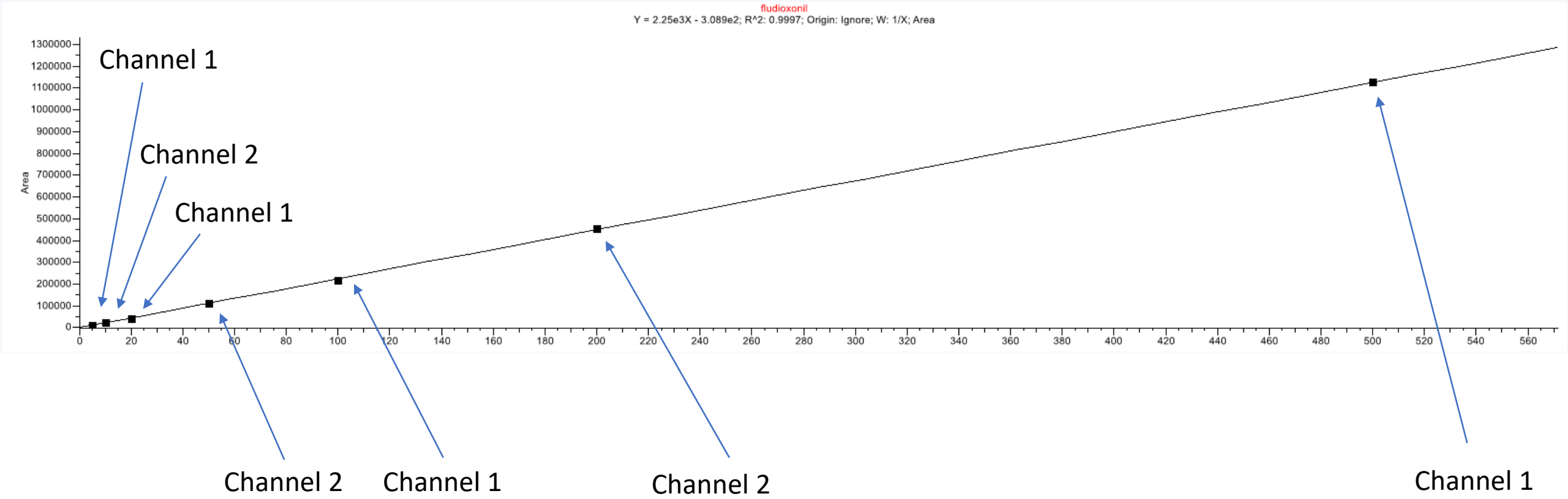
$R^2 = 0.9998$

Results obtained using triple quadrupole

Cross-channel calibration for fludioxinil in apple

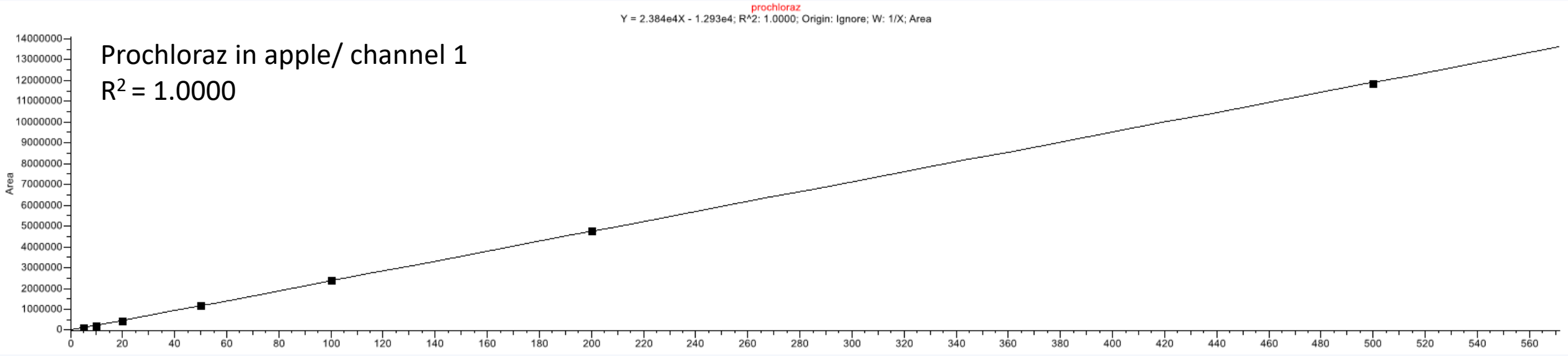
Fludioxonil in apple/ **cross-channel**

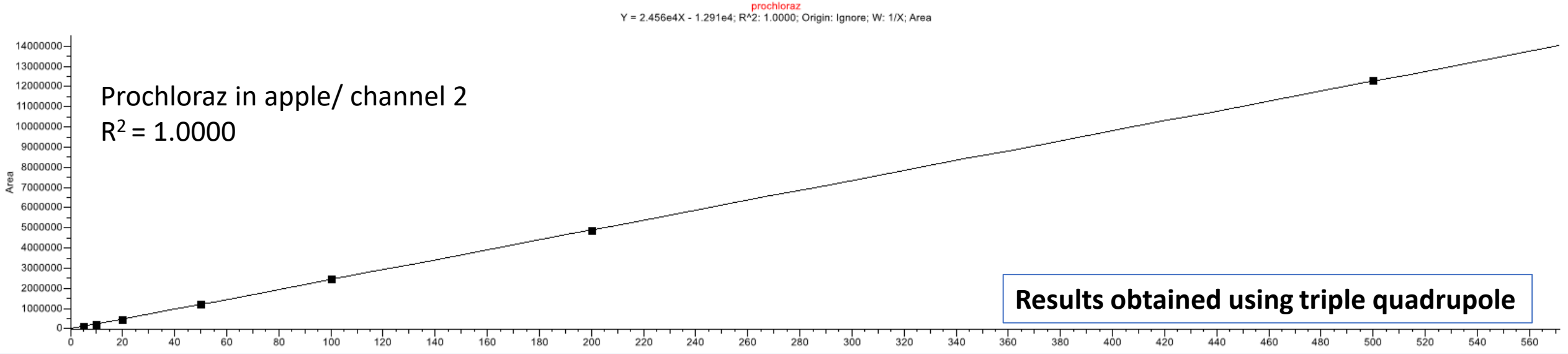
$R^2 = 0.9997$



Results obtained using triple quadrupole

Single-channel calibration for prochloraz in apple



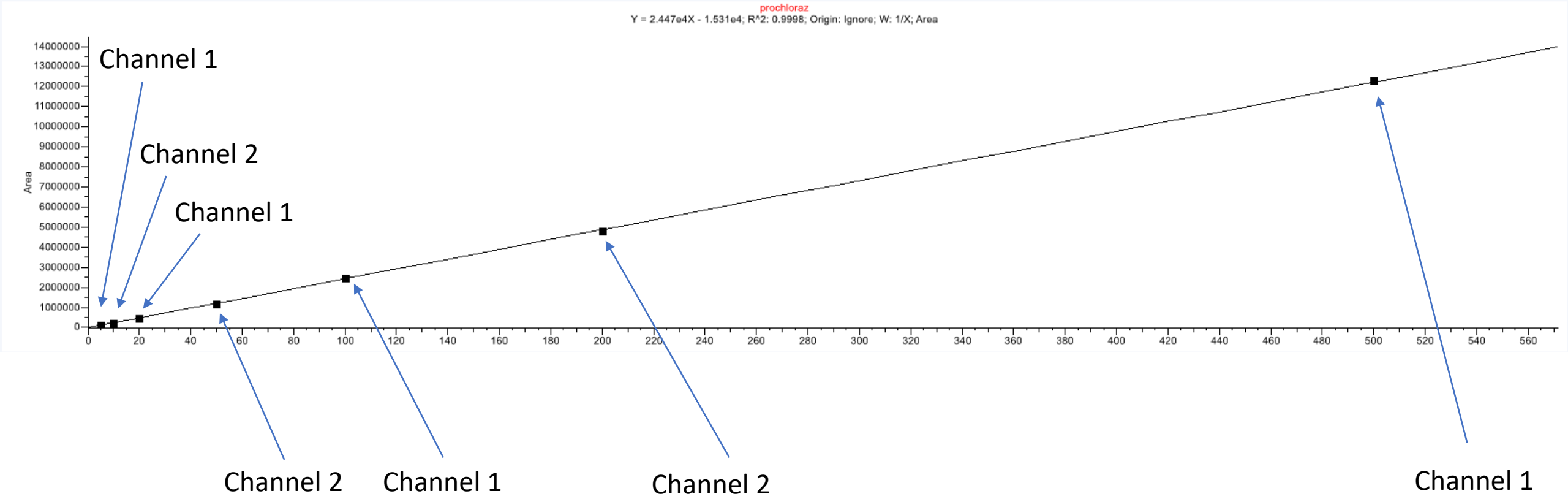


Results obtained using triple quadrupole

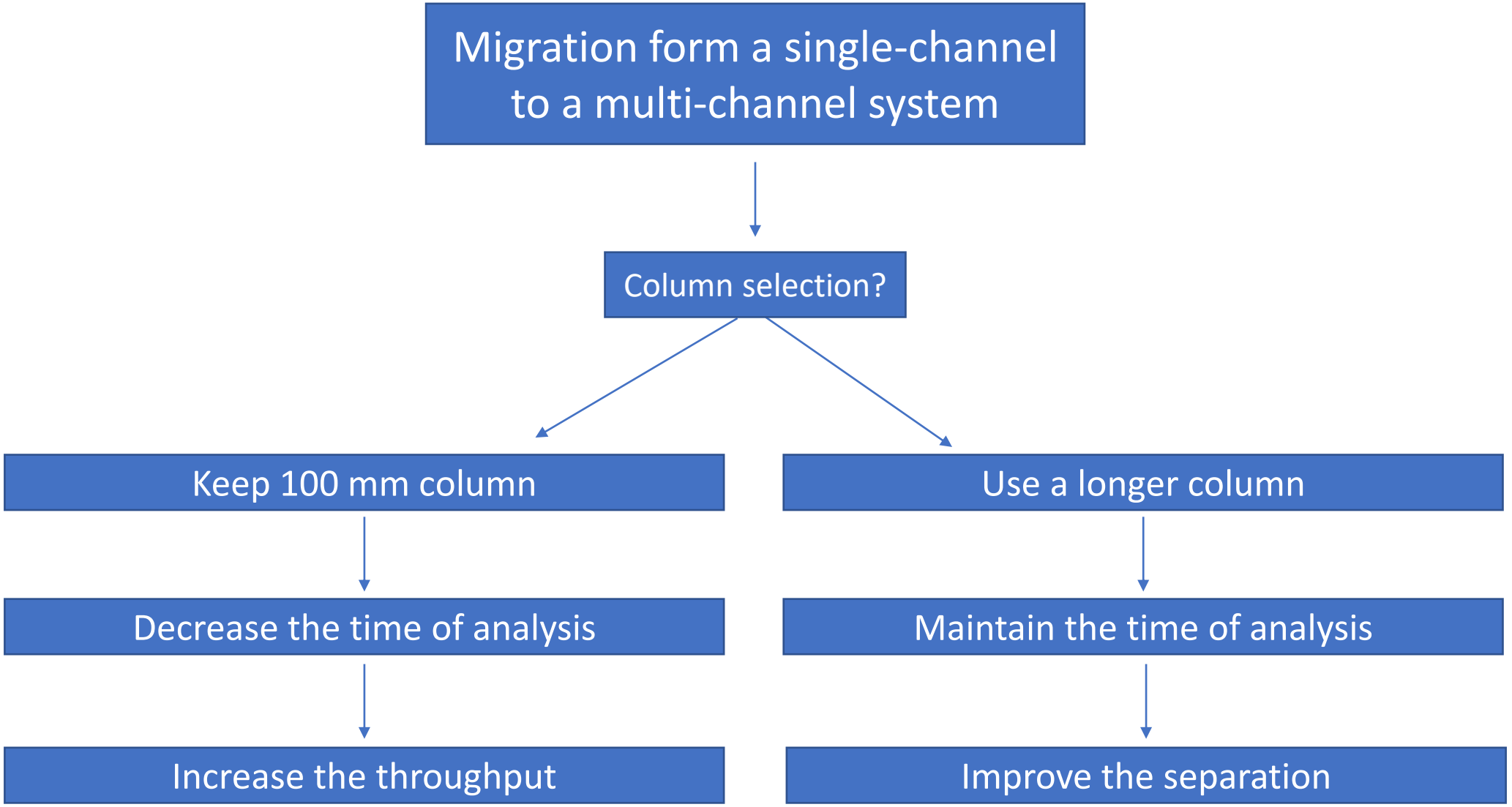
Cross channel calibration for prochloraz in apple

Prochloraz in apple/ **cross channel**

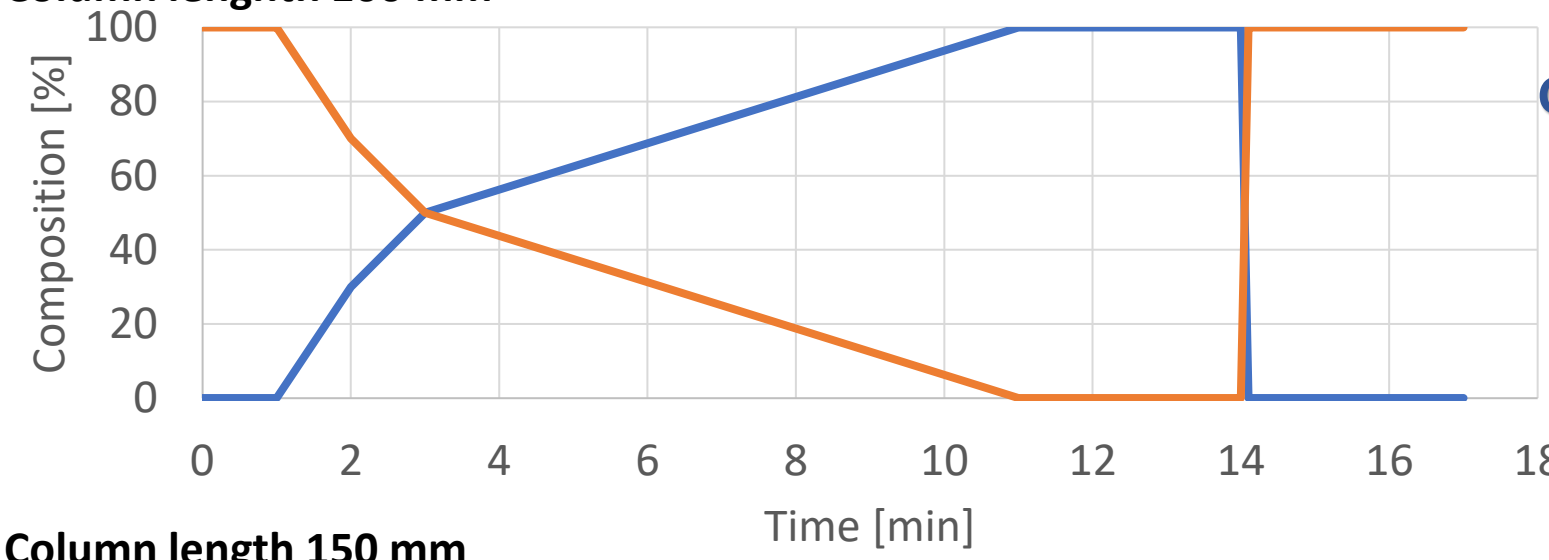
$R^2 = 0.9998$



Results obtained using triple quadrupole

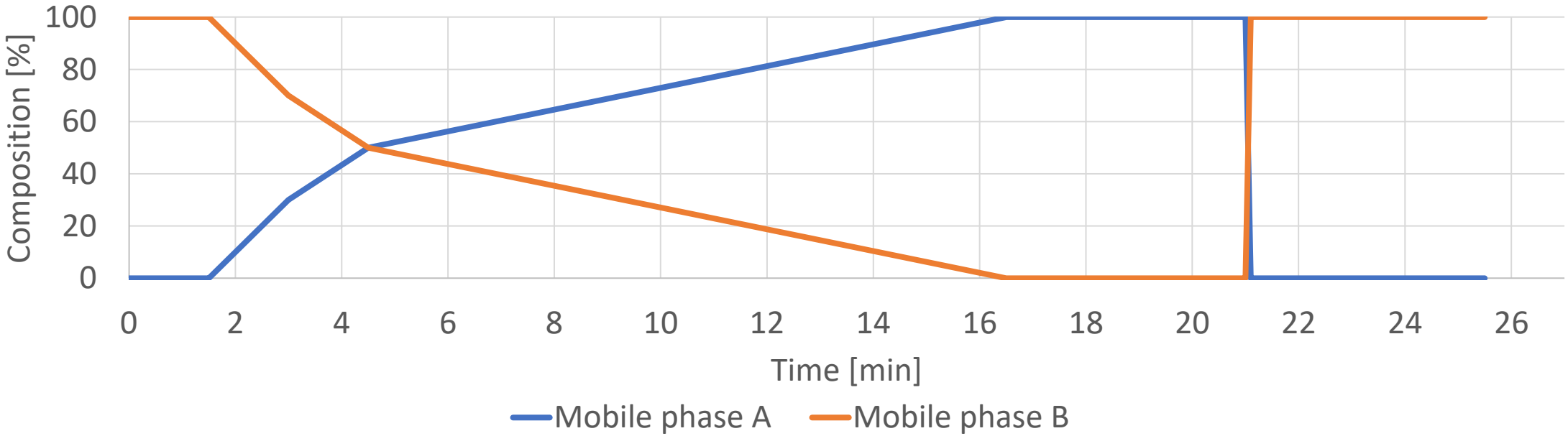

Migration form a single-channel to a multi-channel system
Column selection?
Keep 100 mm column
Use a longer column
Decrease the time of analysis
Maintain the time of analysis
Increase the throughput
Improve the separation

Column lenghth 100 mm



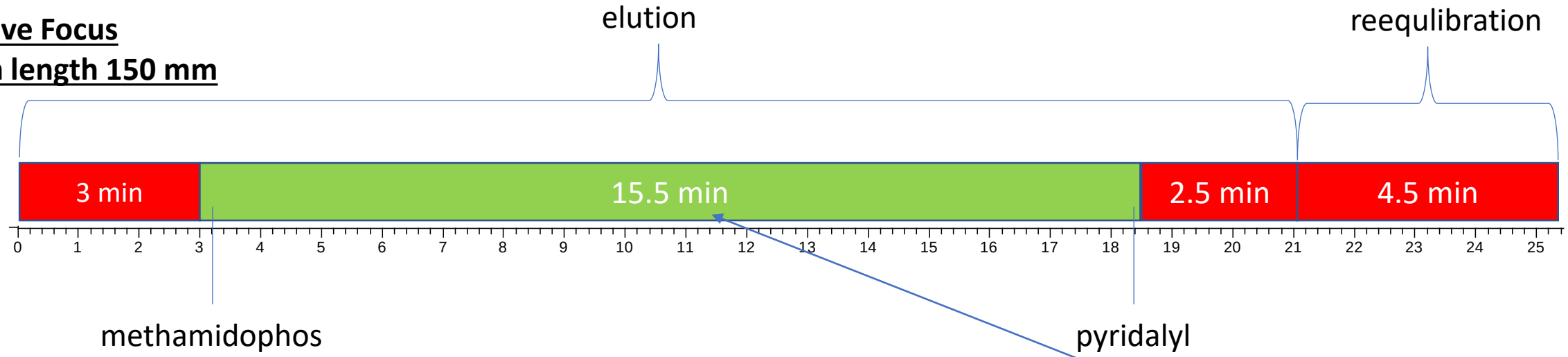
Gradient used with the triple quadrupole

Column length 150 mm



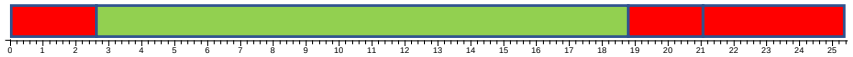
Time segments in dual-channel chromatography

QExactive Focus
Column length 150 mm

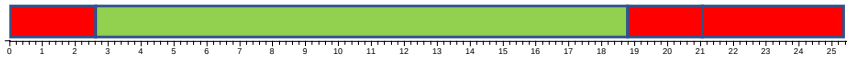


Less than the 100 mm column in a single-channel system!

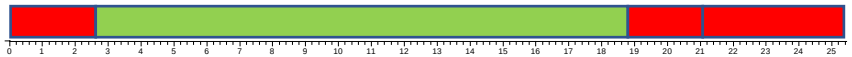
Channel 1



Channel 2



Channel 1

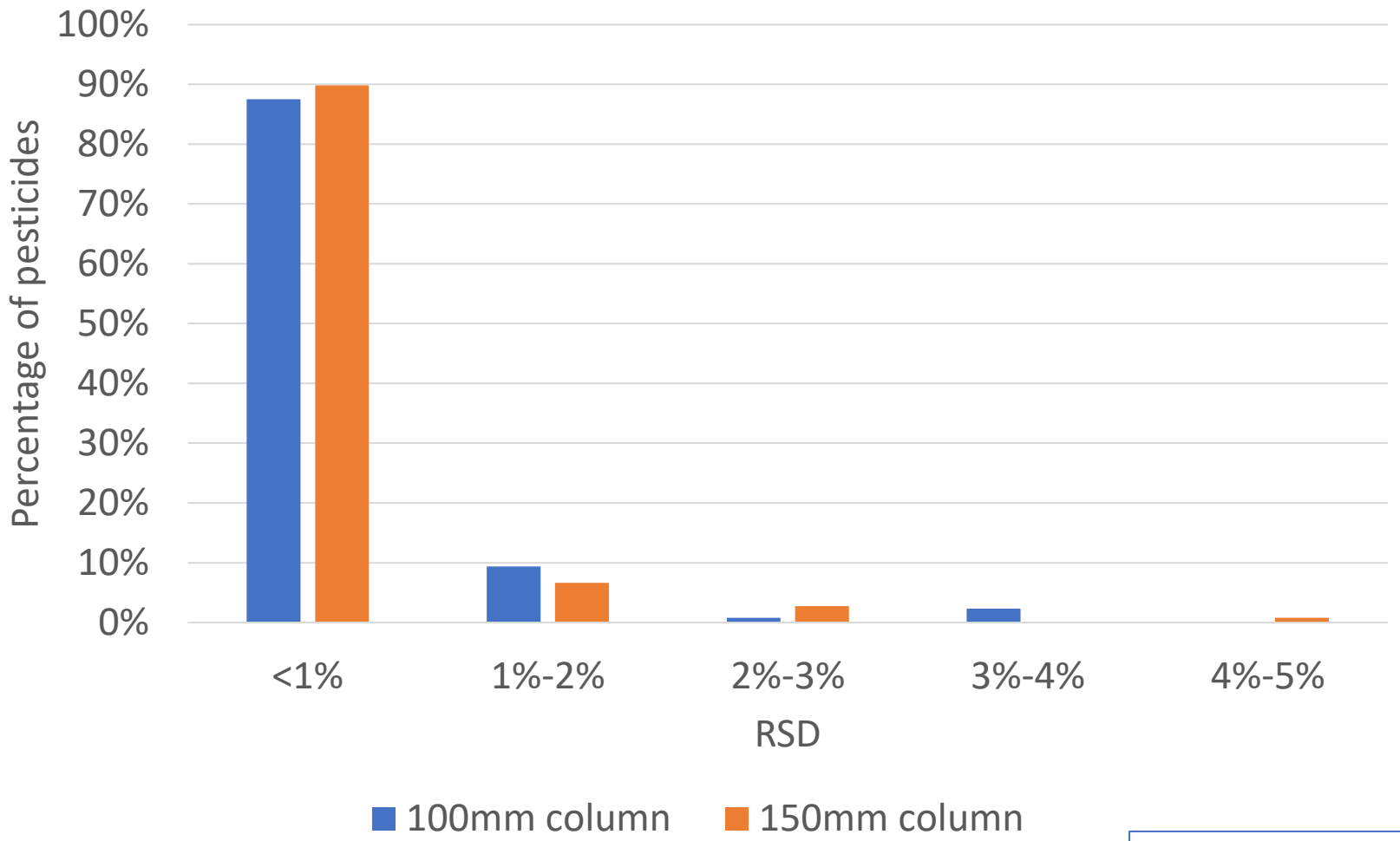


to waste

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

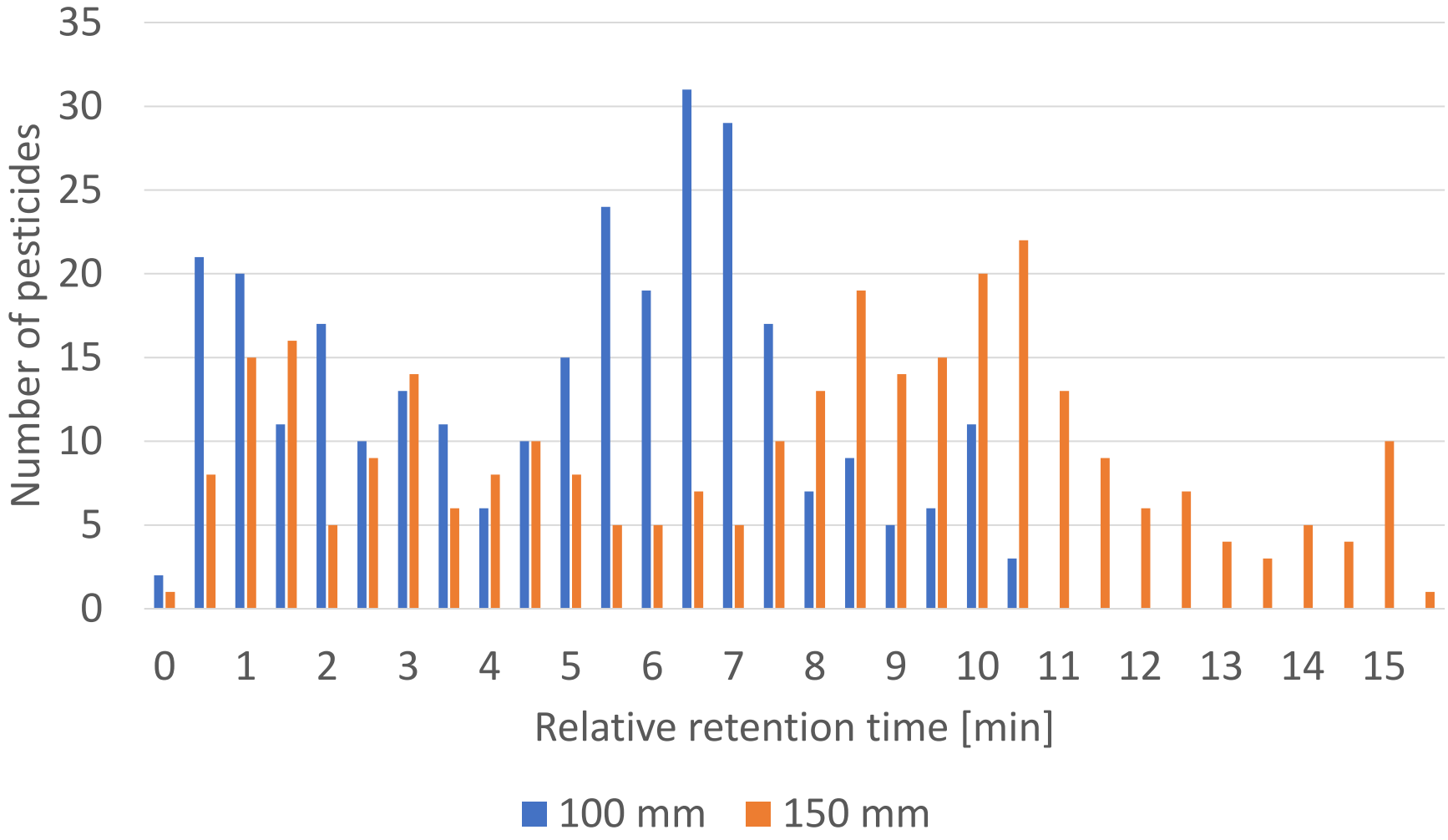
Total time in a single-channel system 25.5 min
(+ 1 minute for needle wash, sample aspiration, etc.)

Retention time variability (n = 25)



Results obtained using QE Focus

Distribution of pesticides



Results obtained using QE Focus

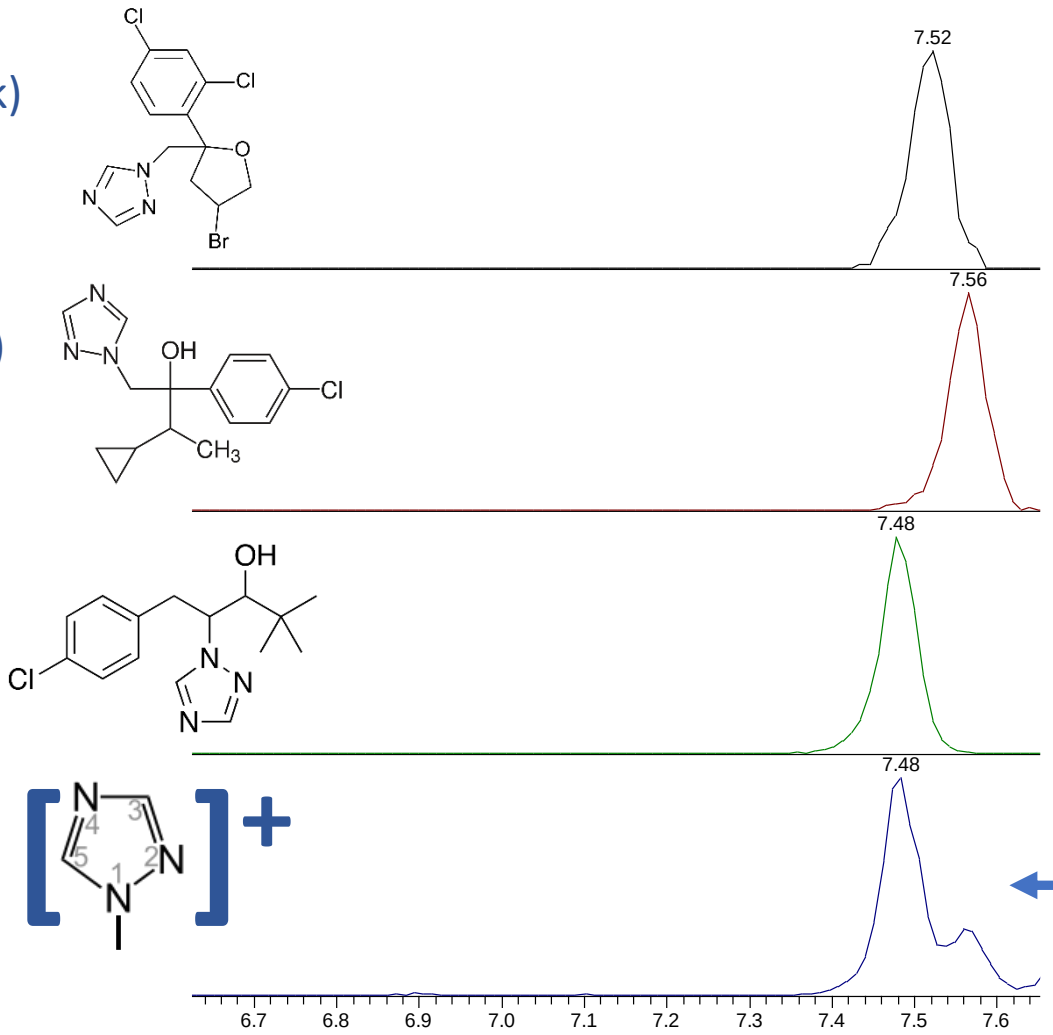
Co-elution of analytes with a 100 mm column

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

Commune fragment ion
AIF MS2
70.03997 ± 5 ppm



QExactive Focus
High-resolution MS
100 mm column

This fragment ion cannot be used for the identification because of the coelutions.

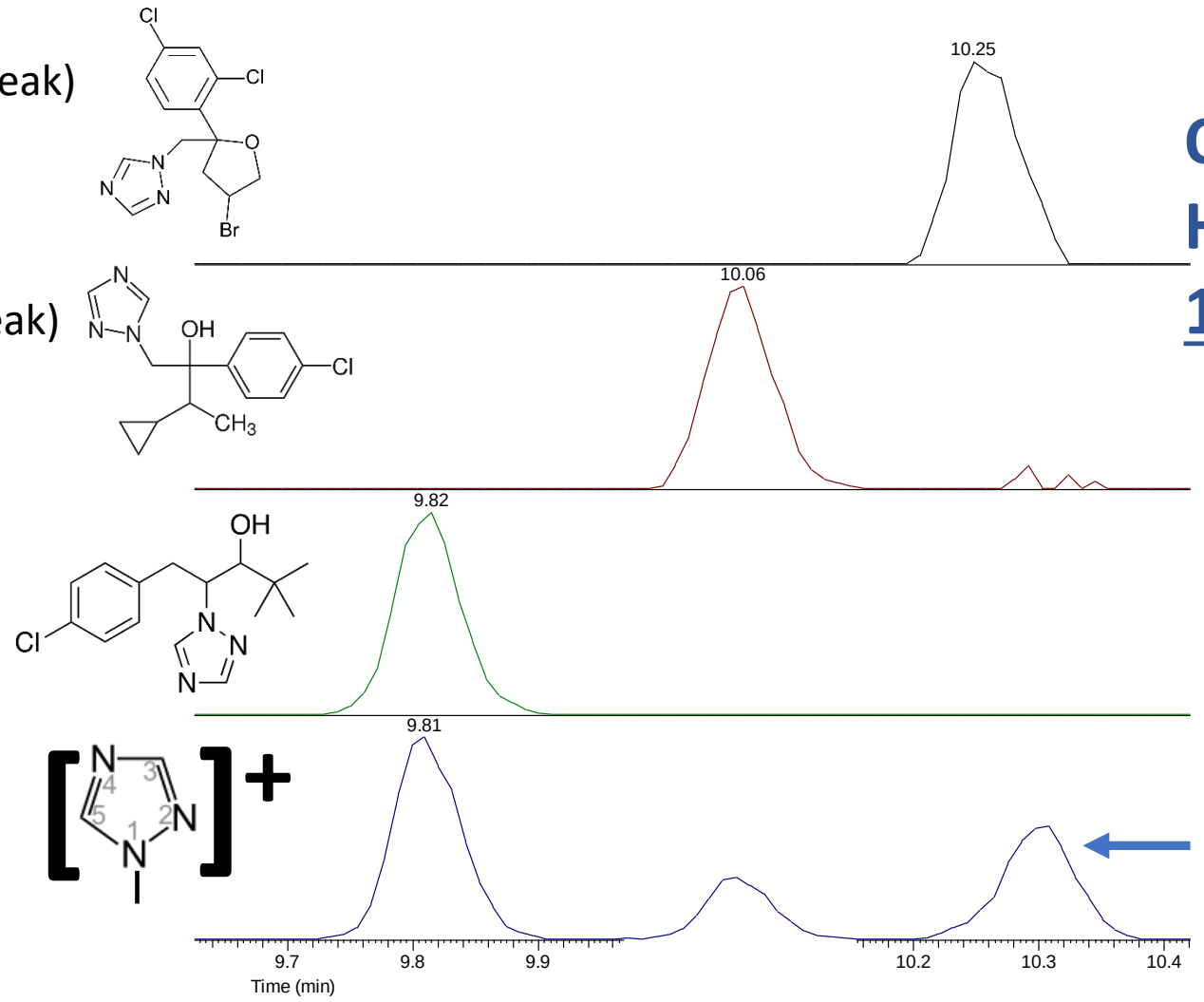
Using a longer column to overcome co-elution of analytes

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

Comune fragment ion
AIF MS2
70.03997 ± 5 ppm



QExactive Focus
High-resolution MS
150 mm column

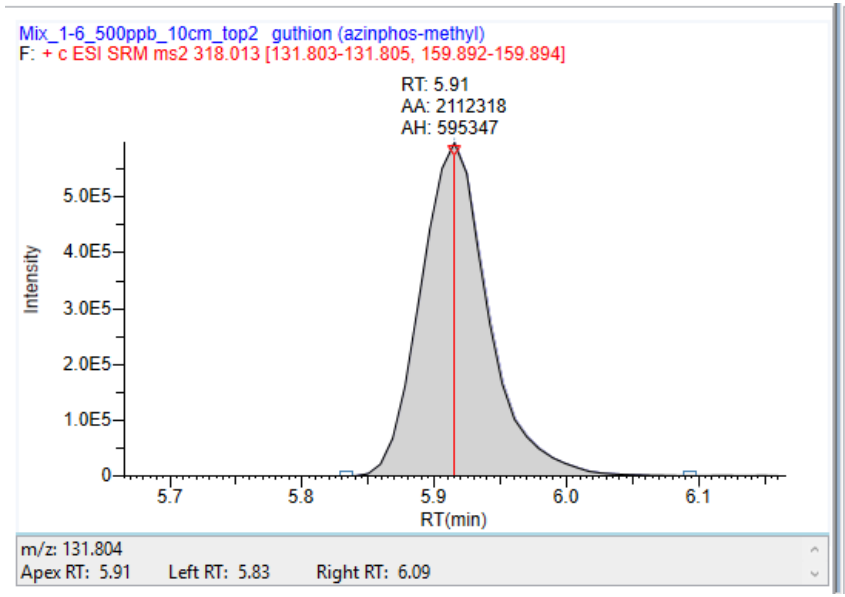
With the longer column, this fragment ion can be used for the identification of bromuconazole, cyproconazole, and paclobutrazole.

Co-elution of analytes with a 100 mm column and QQQ MS

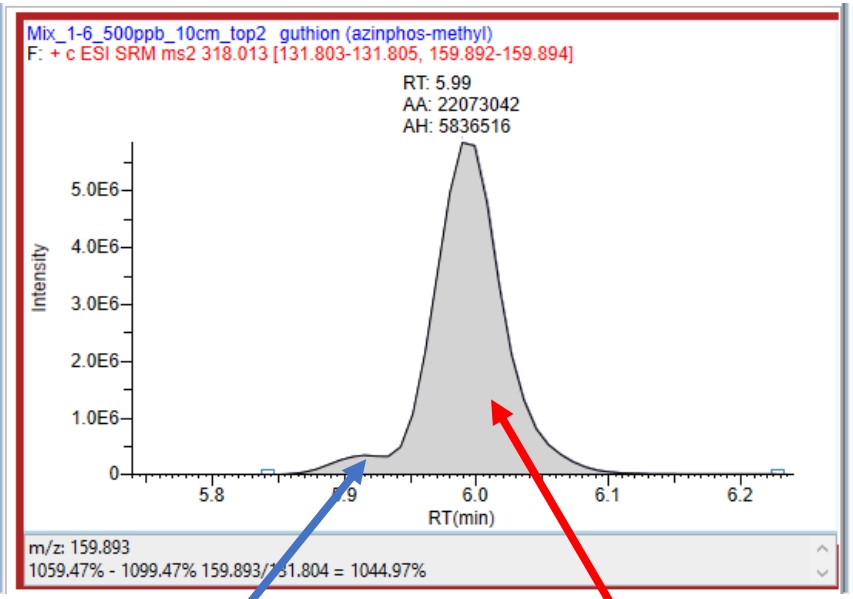
Triple quadrupole
100 mm column

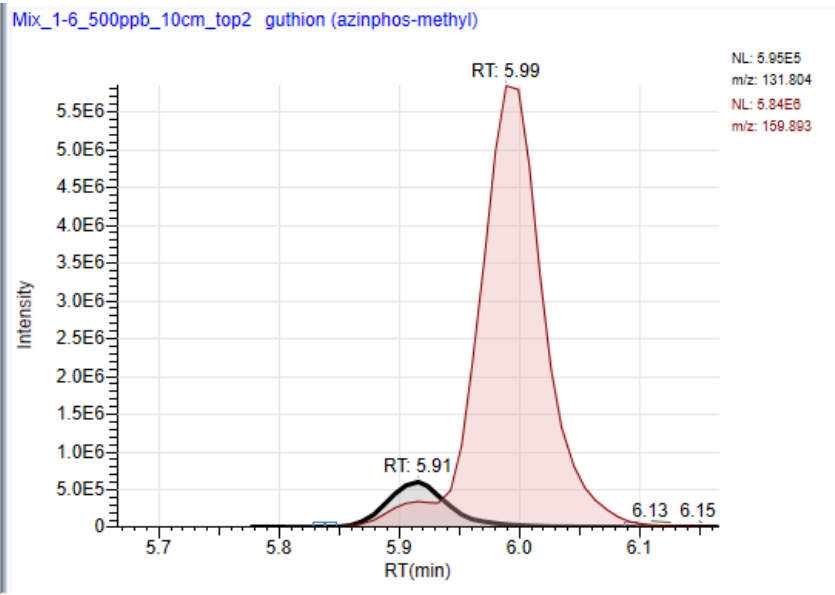
Azinphos methyl

m/z 318 -> 132



m/z 318 -> 159





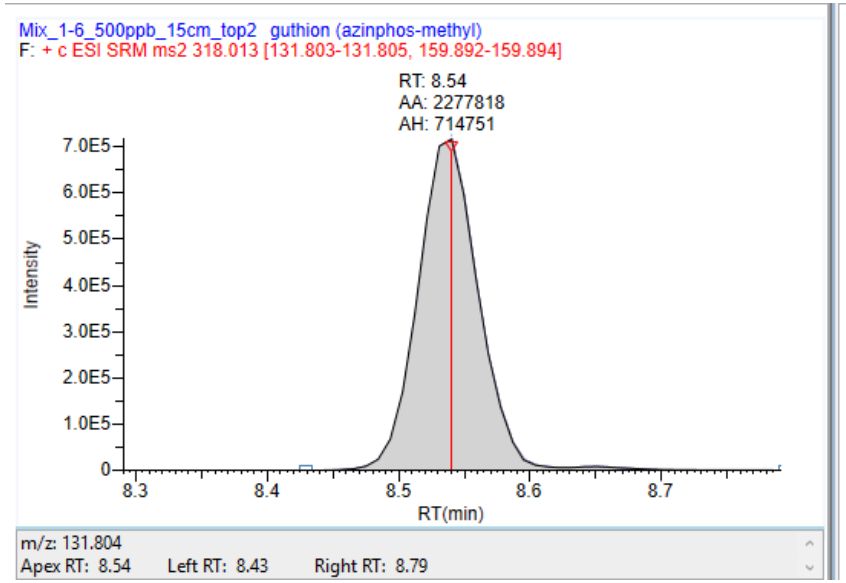
Azinphos methyl **Interfering transition of phosmet**

Using a longer column to resolve co-elution in QQQ MS

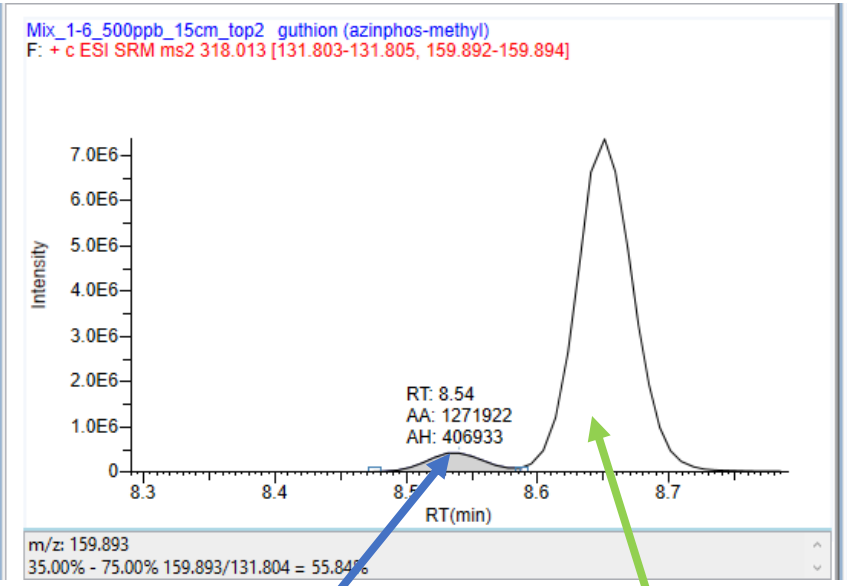
Triple quadrupole
150 mm column

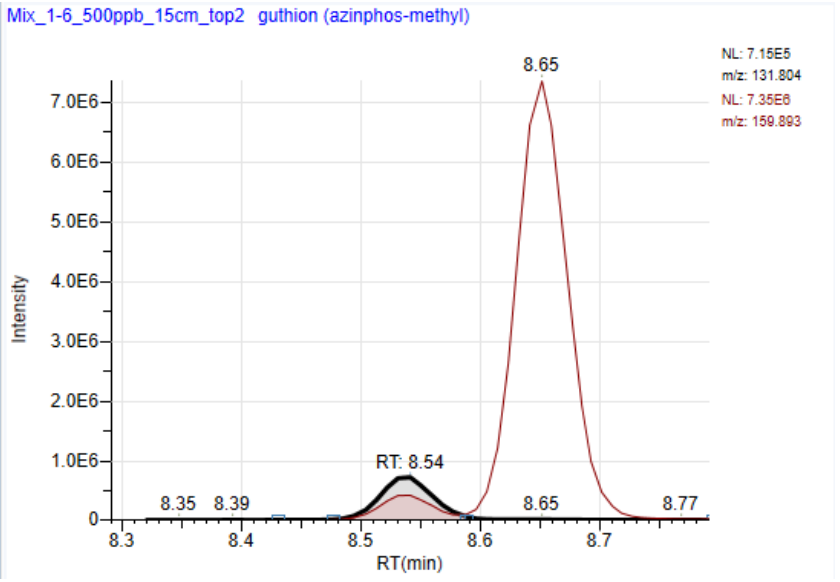
Azinphos methyl

m/z 318 -> 132



m/z 318 -> 159





Azinphos methyl

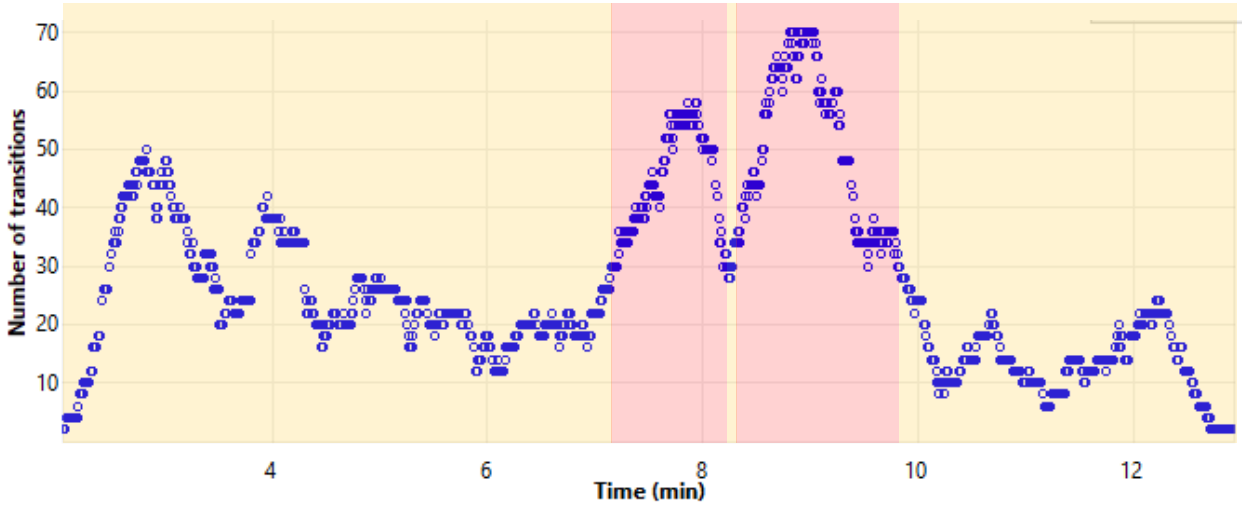
Phosmet is separated form azinphos methyl

Use of a longer column can increase the sensitivity of QQQ MS

300 pesticides / 600 transitions

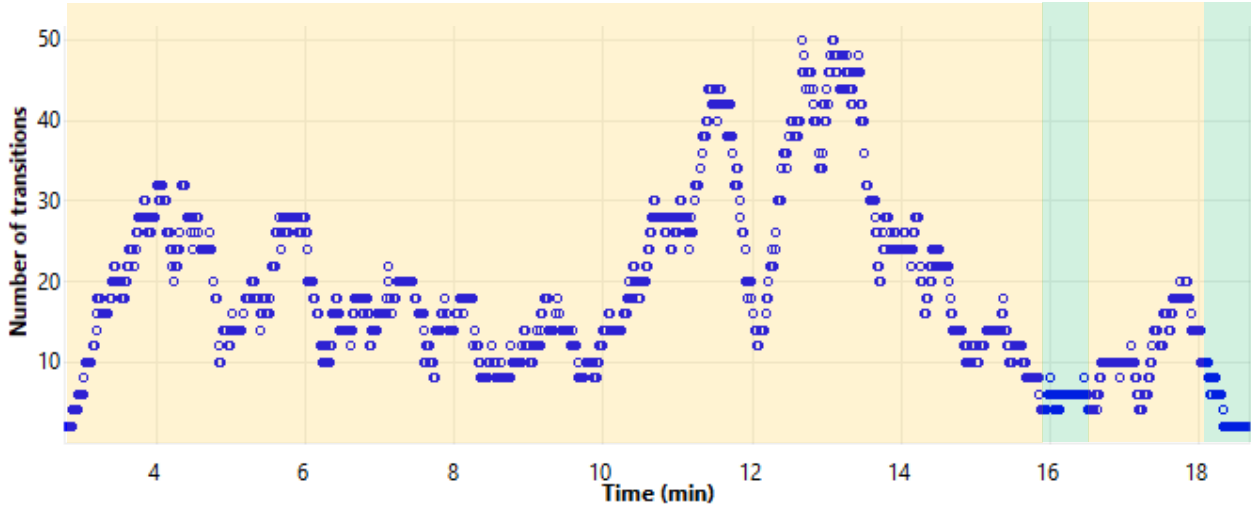
100 mm column

Number of transitions per cycle



150 mm column

Number of transitions per cycle



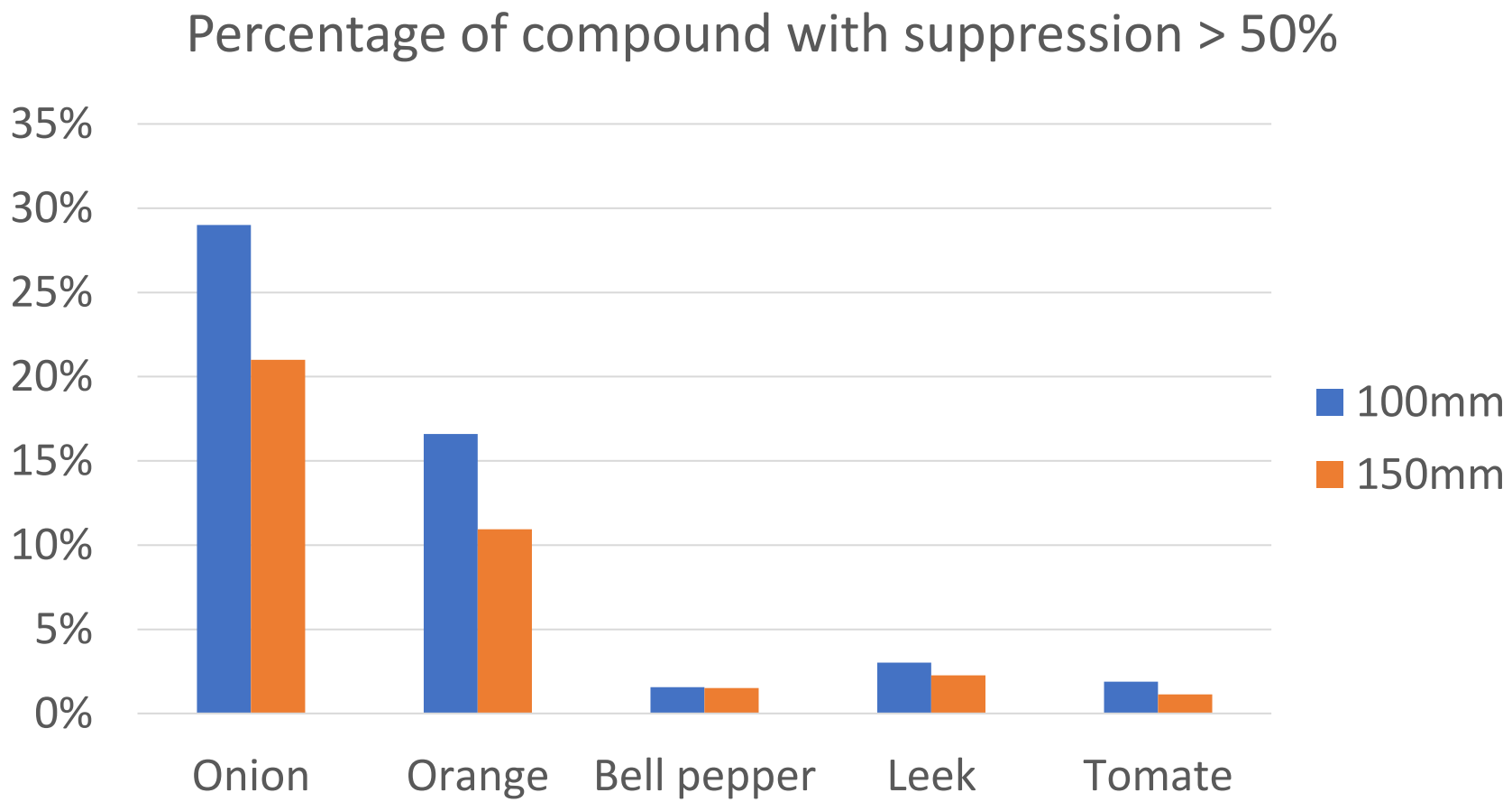
Dwell time < 10 ms

Dwell time 10 – 50 ms

Dwell time > 50 ms

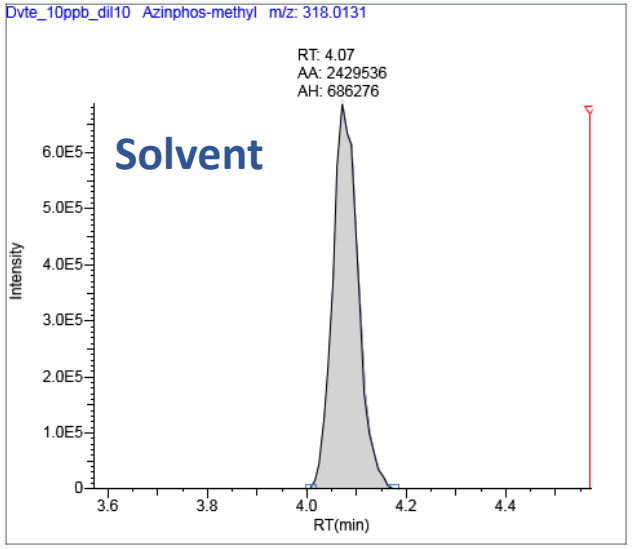
A longer column separates better the analytes. The dwell times can be increased without increasing the duty cycle.

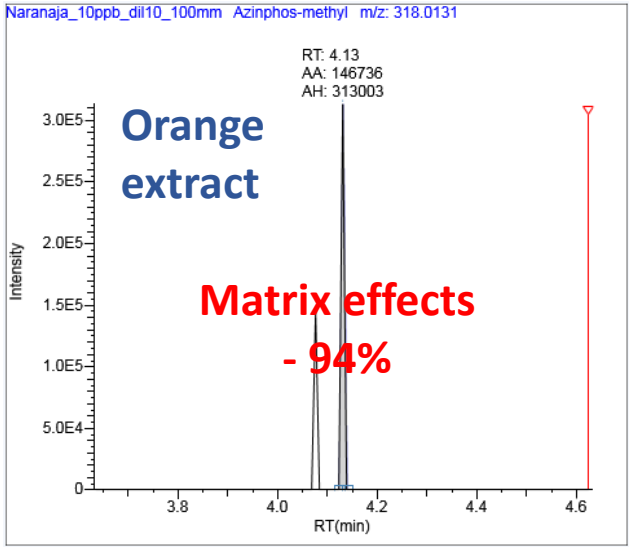
Use of a longer column can decrease matrix effects



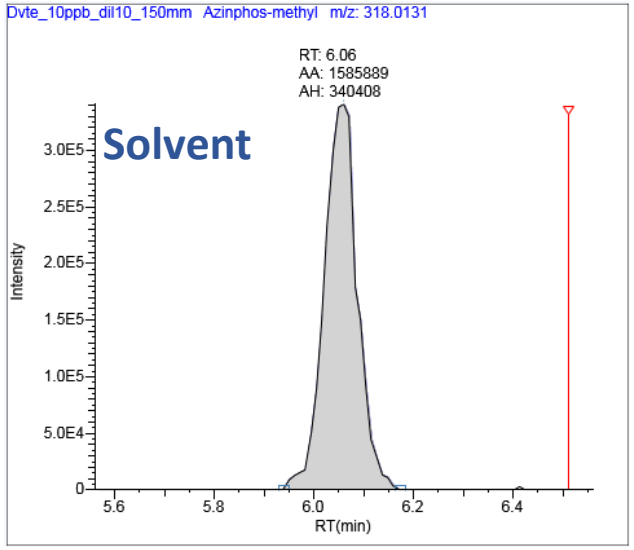
Use of a longer column to decrease matrix effects - orange

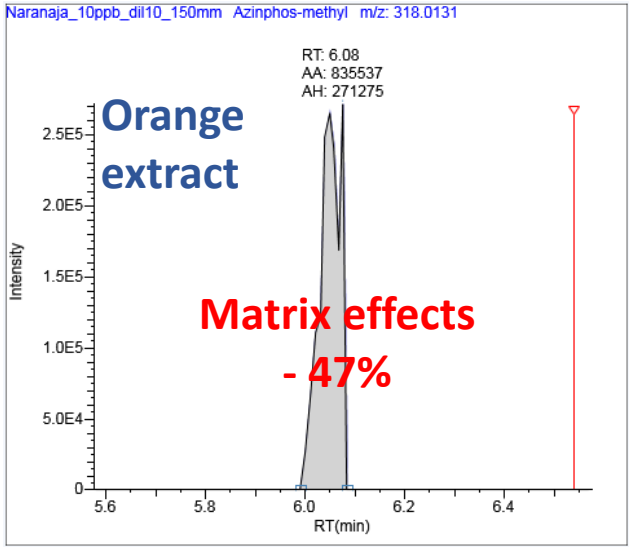
0.01 mg/kg of azinphos-methyl Full scan MS *m/z* 318.0131





QExactive Focus
High-resolution MS
100 mm column

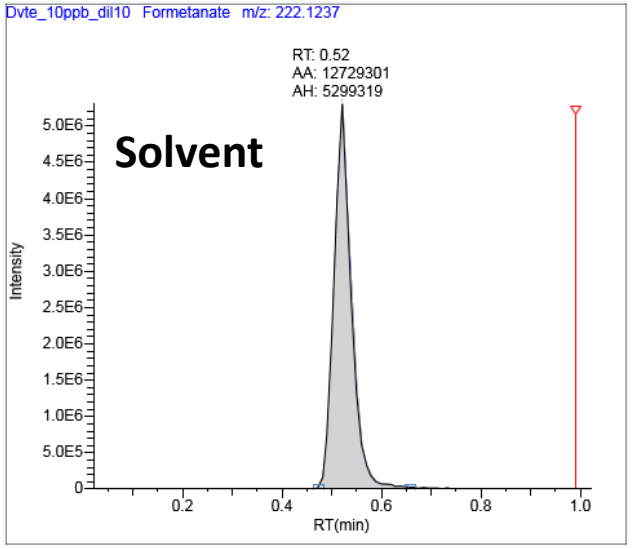


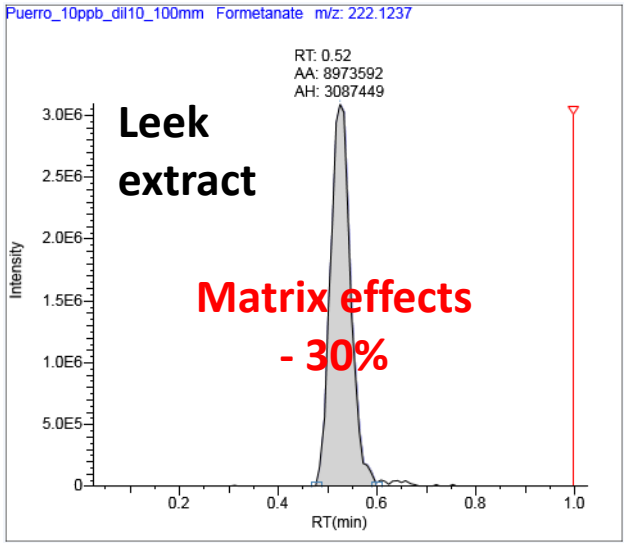


QExactive Focus
High-resolution MS
150 mm column

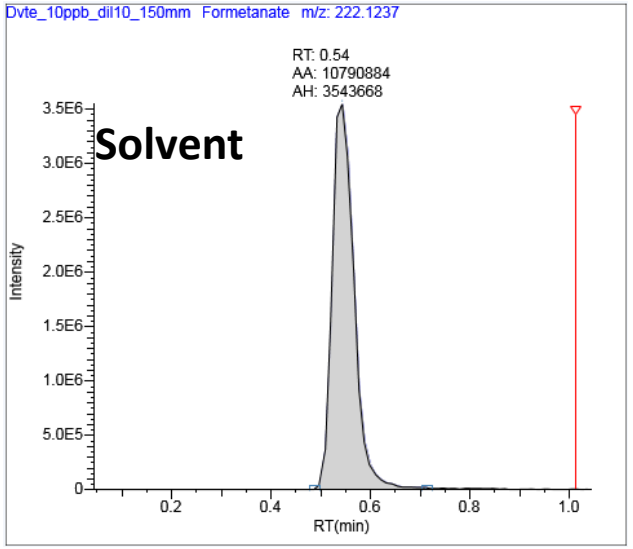
Use of a longer column to decrease matrix effects - leek

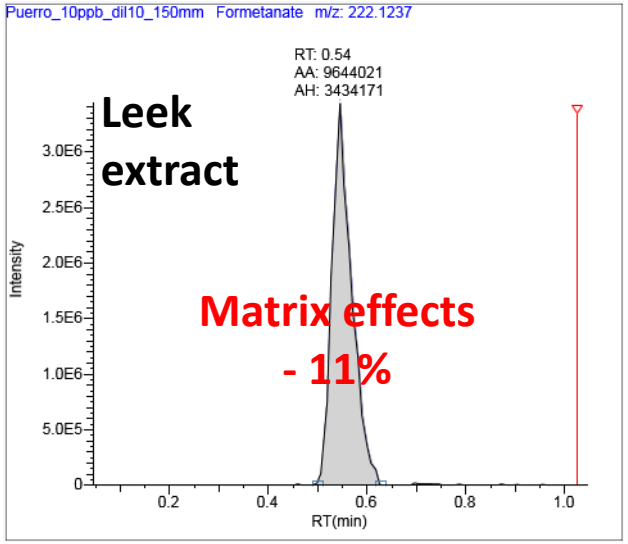
0.01 mg/kg of formetanate Full scan MS *m/z* 222.1237





QExactive Focus
High-resolution MS
100 mm column

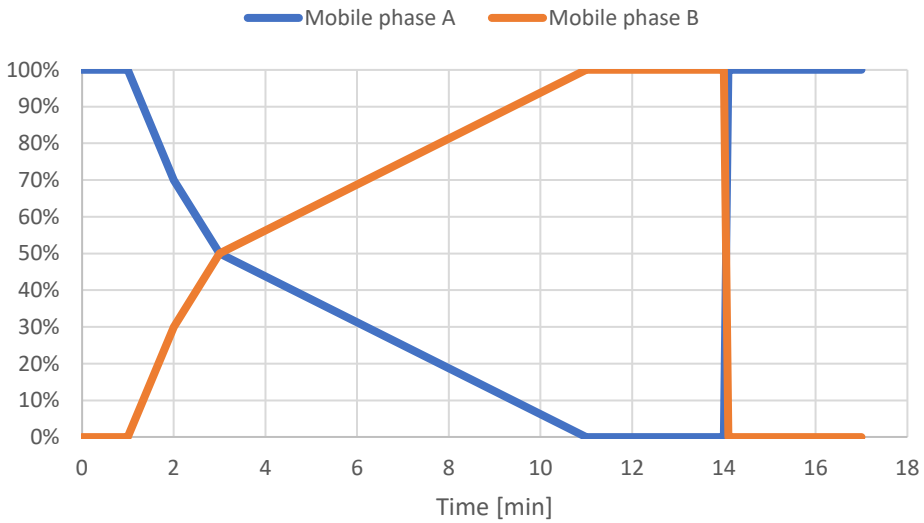




QExactive Focus
High-resolution MS
150 mm column

Gradient and mobil phase

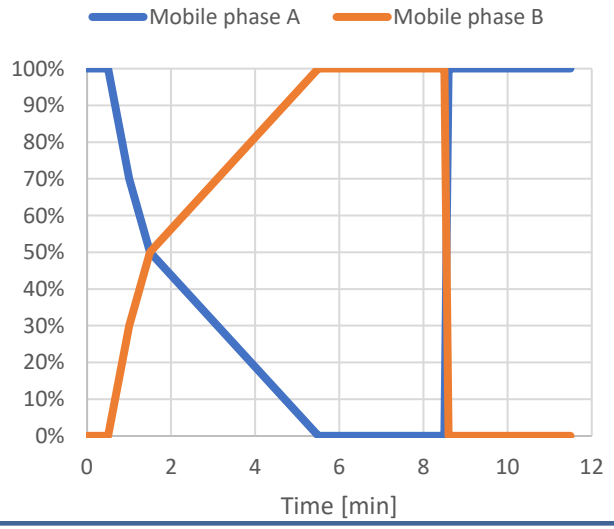
Positive polarity



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

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

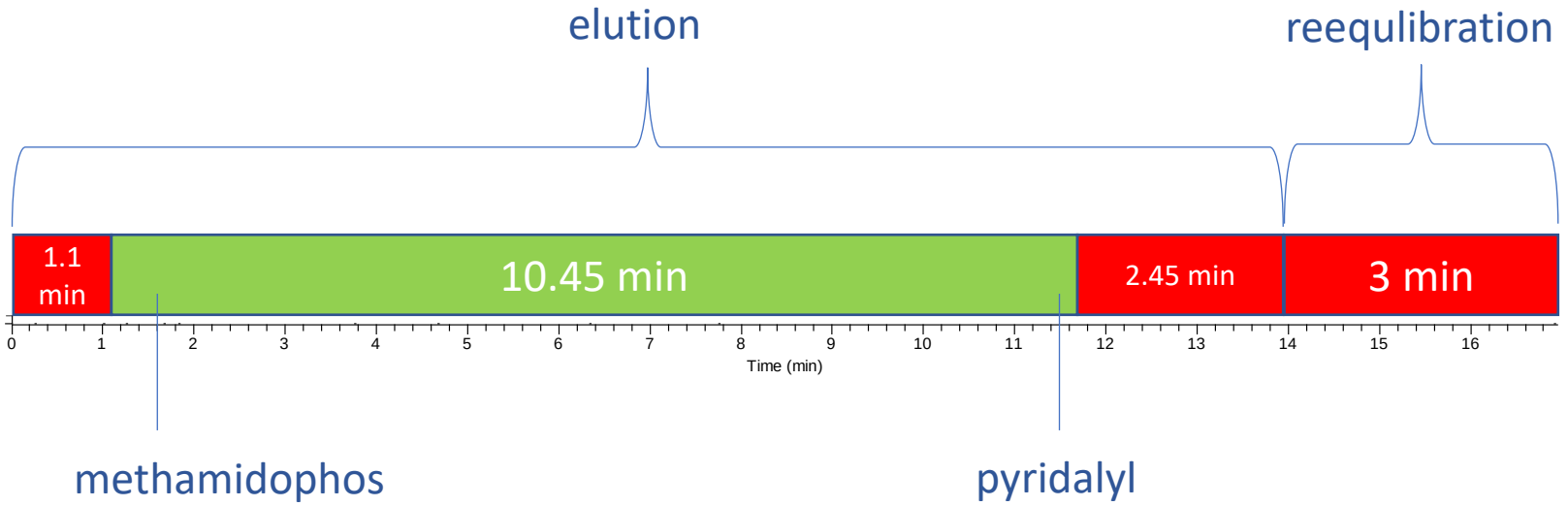
Negative polarity



Mobil phase A:
Water + 0.05% acetic acid

Mobil phase B:
Acetonitrile + 0.05% acetic acid

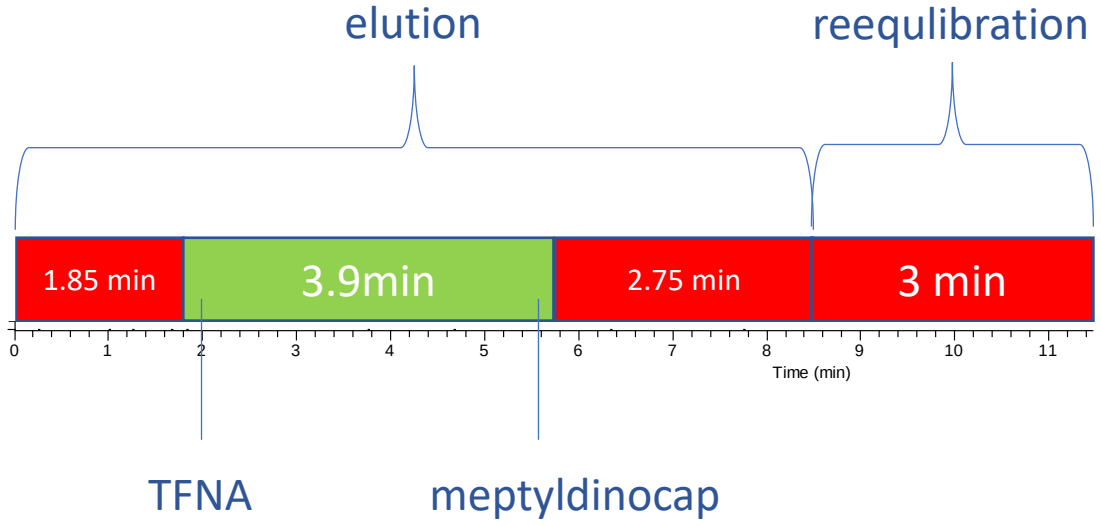
Time segments in dual-channel chromatography



Positive polarity

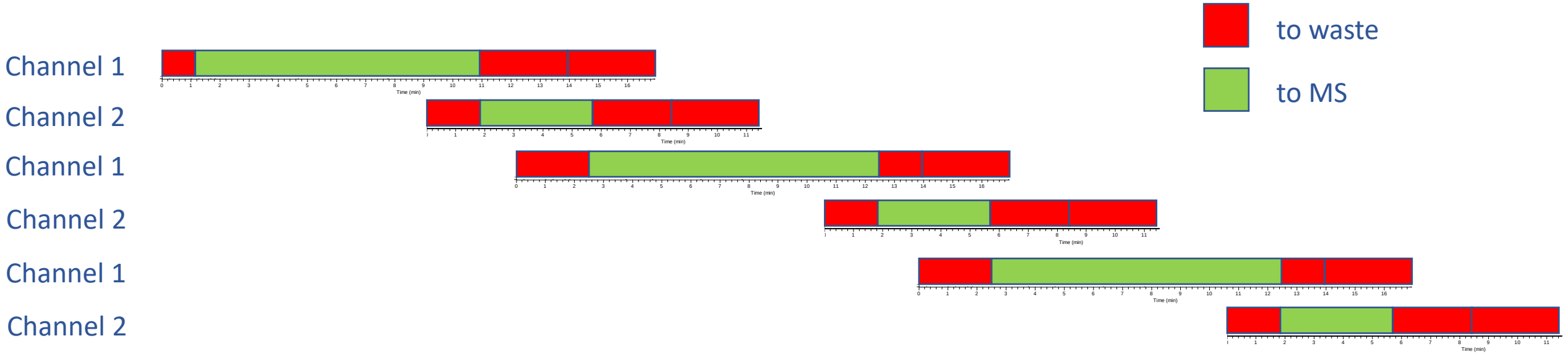
to waste

to MS



Negative polarity

Time segments in dual-channel chromatography



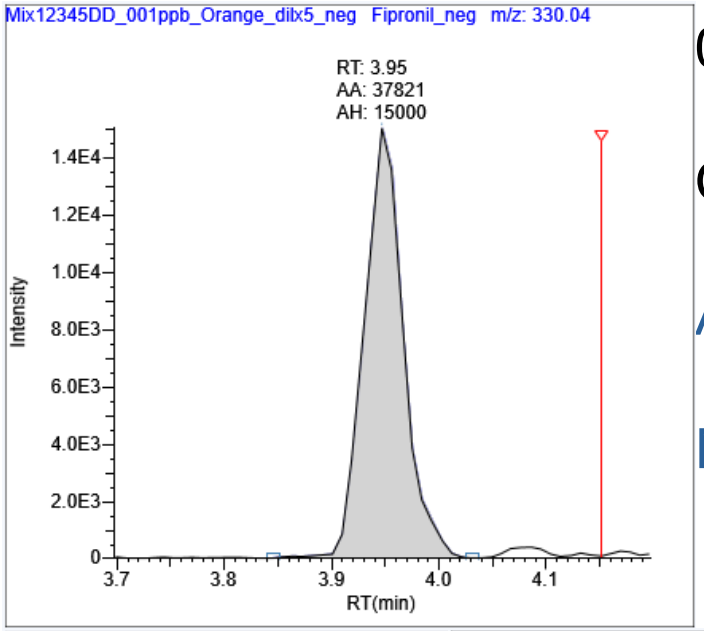
Total time of analysis in a dual channel system: 14.35 min

(10.45 min in pos + 3.9 min in neg)

Total time of analysis in a single channel system using polarity switching: 17 min

(+ 1 minute for needle wash, sample aspiration, etc.)

Fipronil

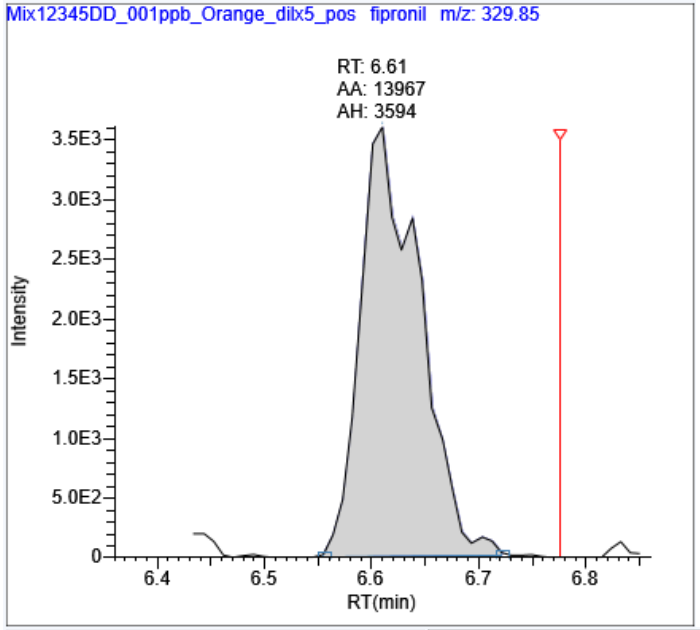


0.001 mg/L

Orange extract

ACN/AA/H_2O

Negative polarity

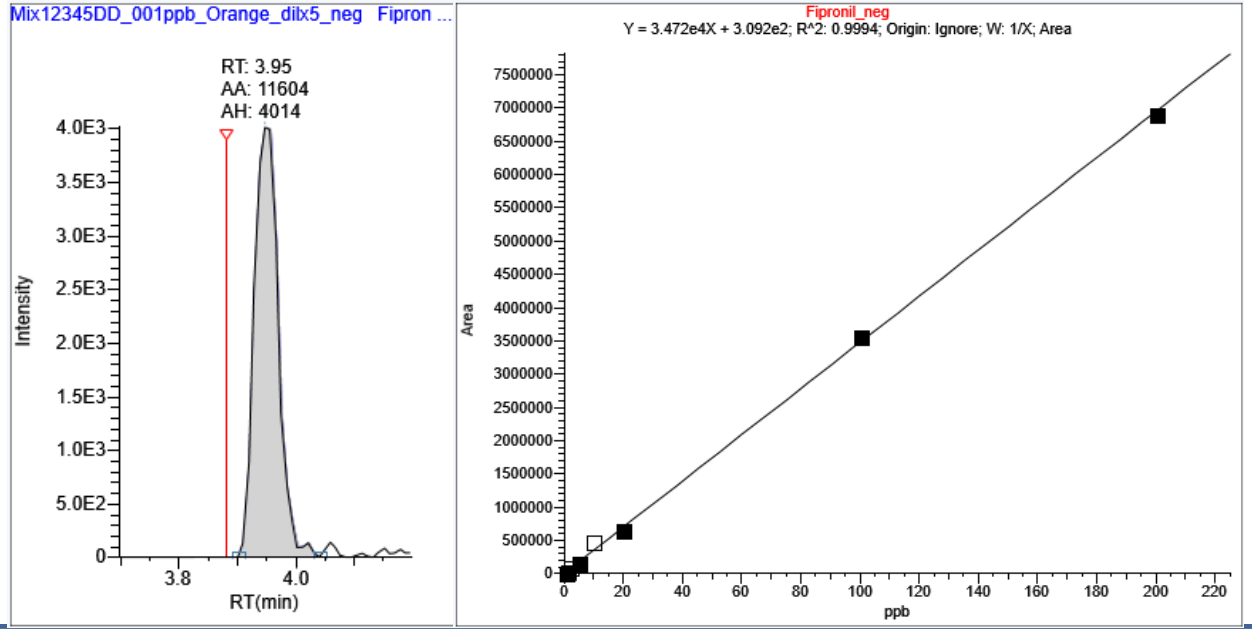


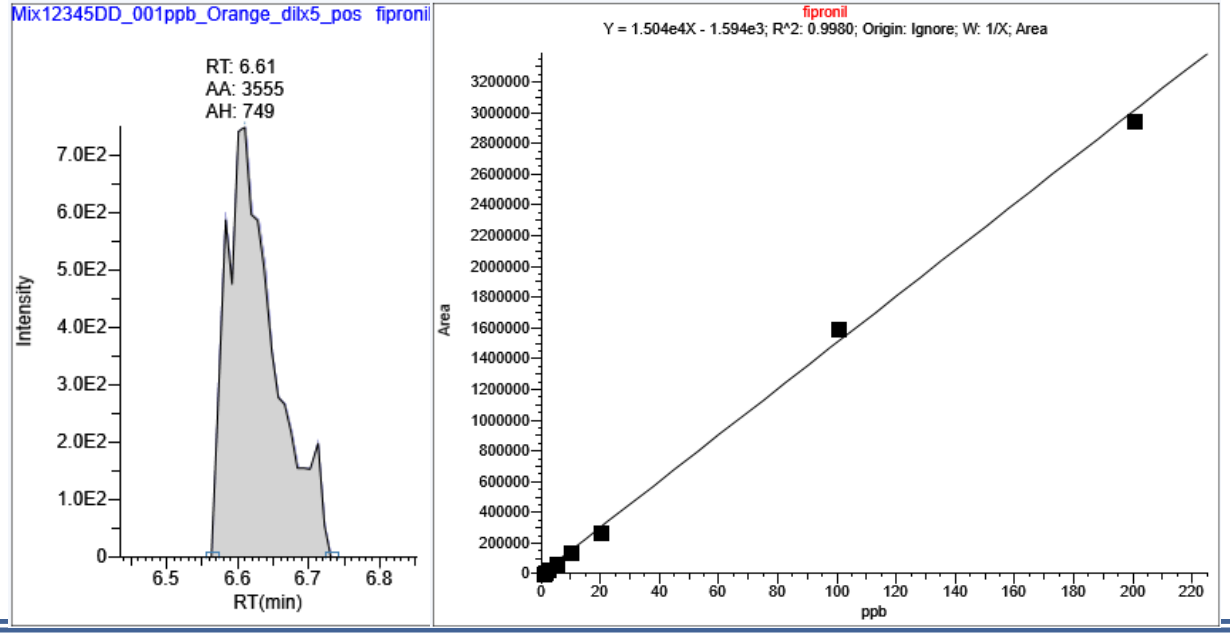
0.001 mg/L

Orange extract

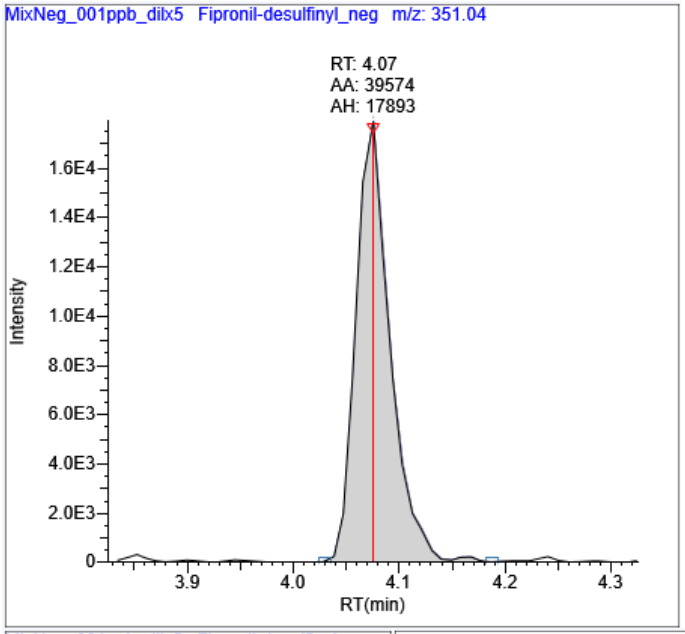
MeOH/FA/AF/H_2O

Negative polarity





Fipronil-desulfinyl & Fipronil-sulfone

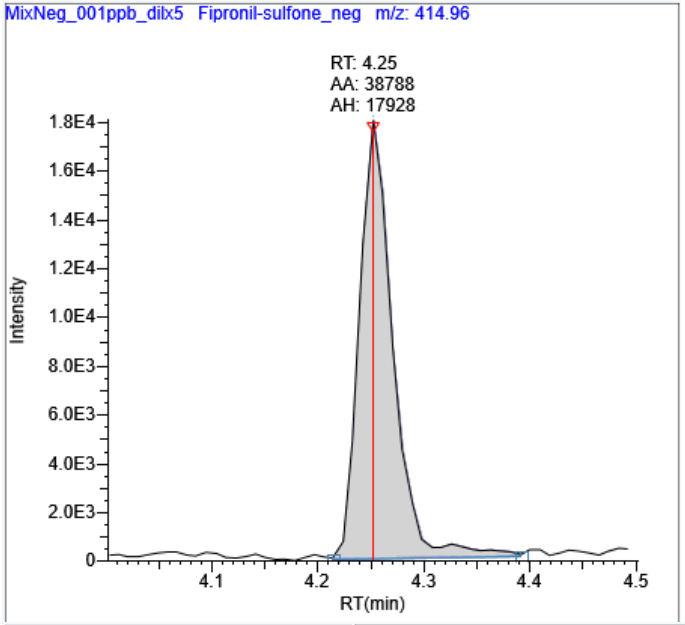


0.001 mg/L

Solvent

ACN/AA/H_2O

Negative polarity

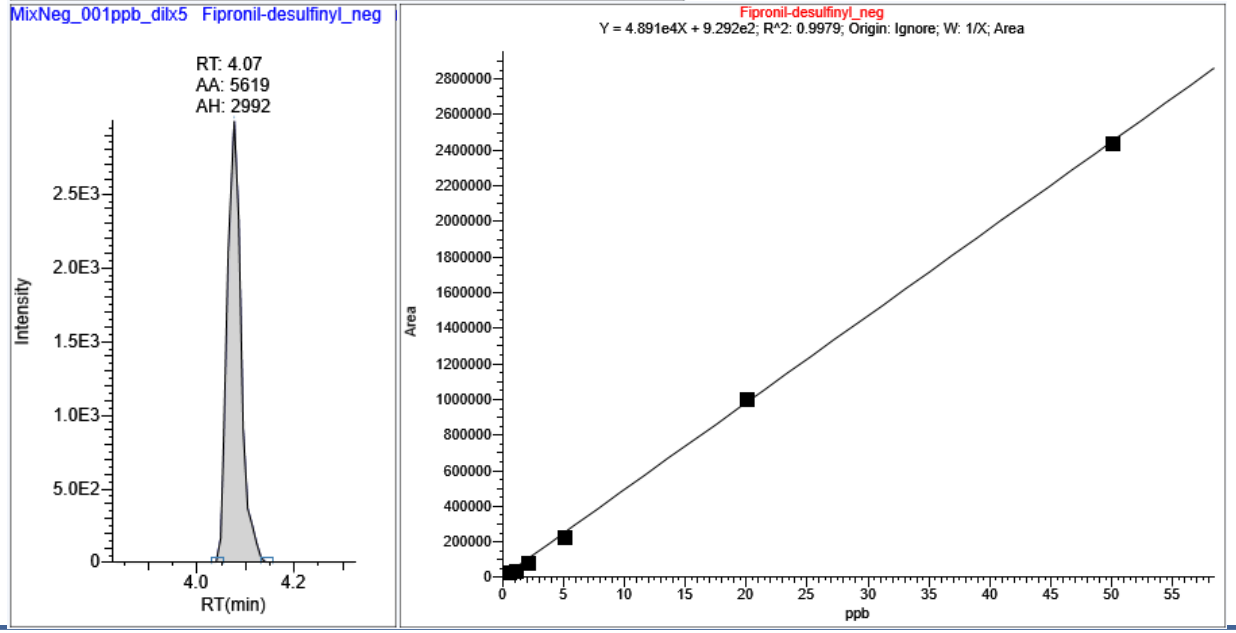


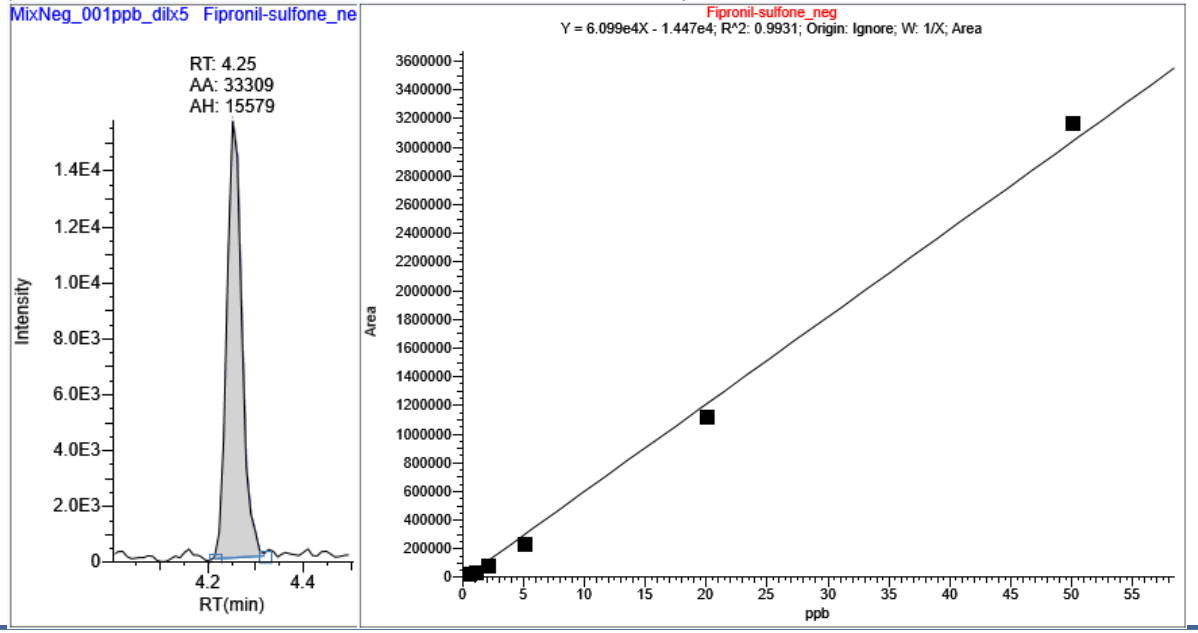
0.001 mg/L

Solvent

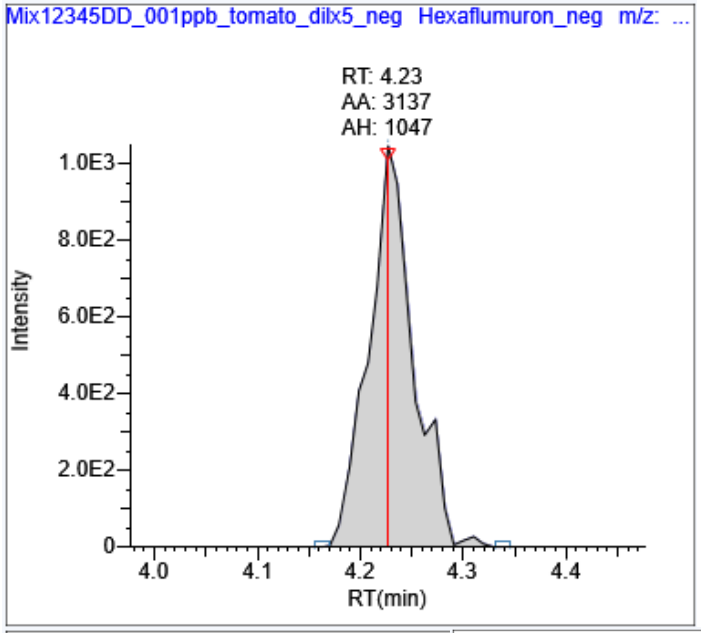
ACN/AA/H_2O

Negative polarity





Hexaflumuron

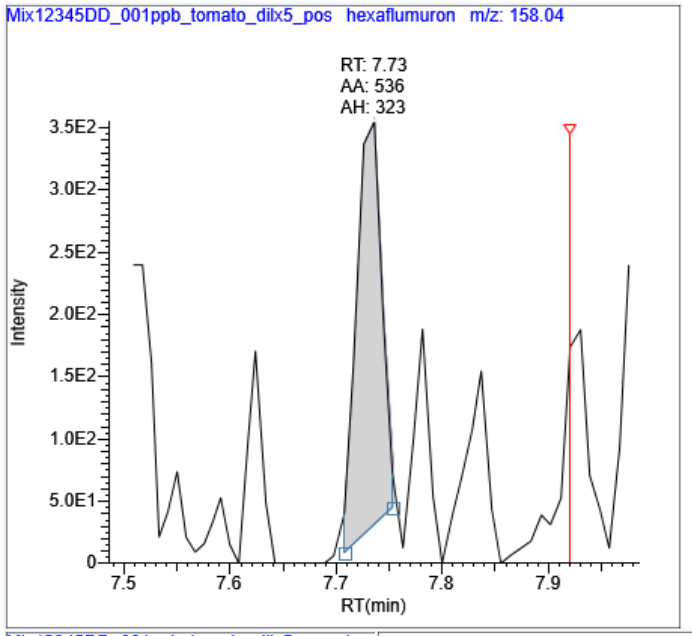


0.001 mg/L

Tomato extract

ACN/AA/H_2O

Negative polarity

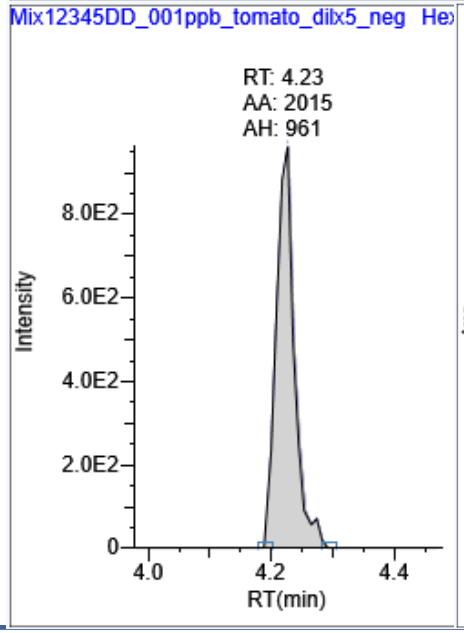


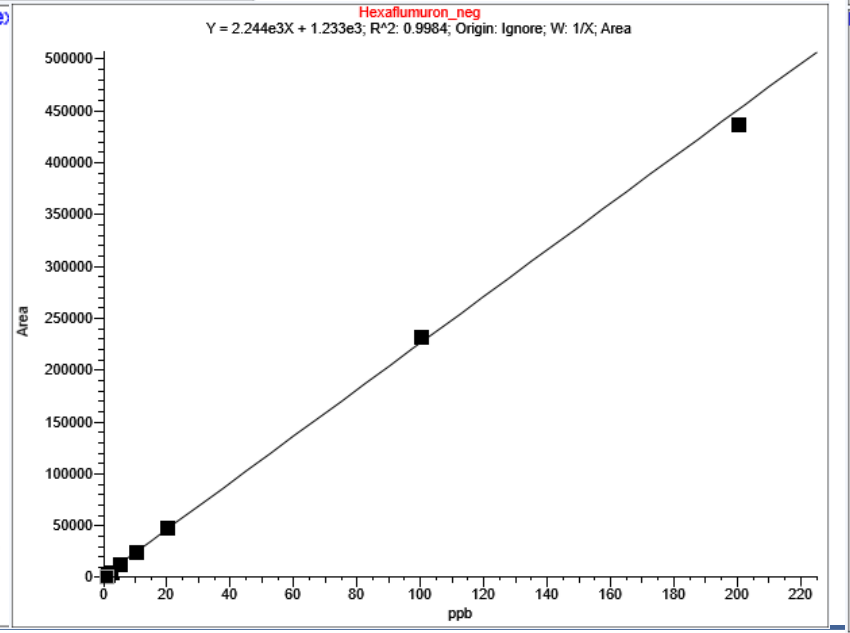
0.001 mg/L

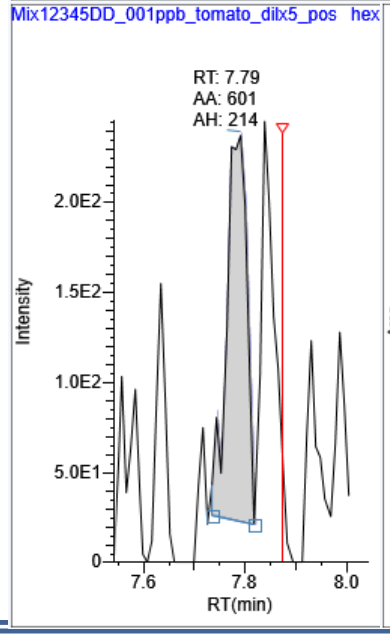
Tomato extract

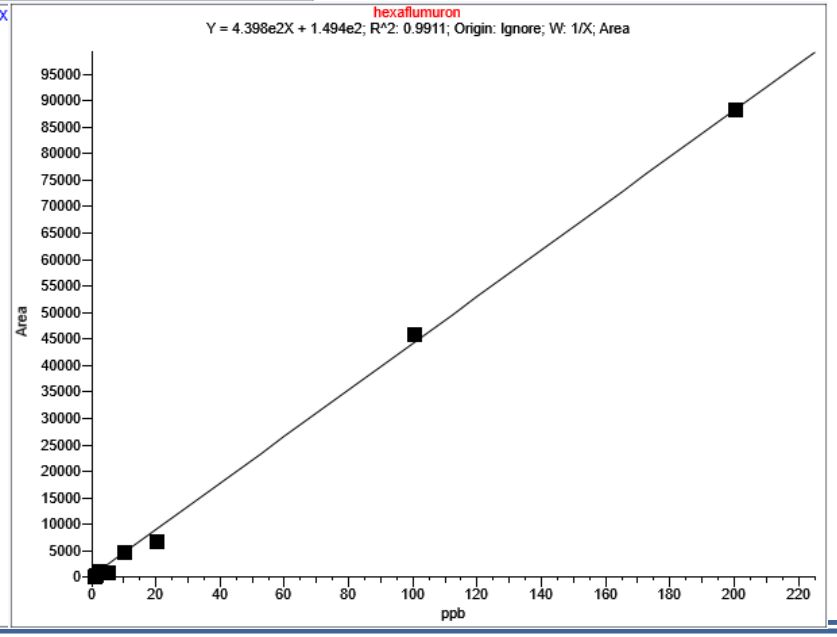
MeOH/FA/AF/H_2O

Positive polarity

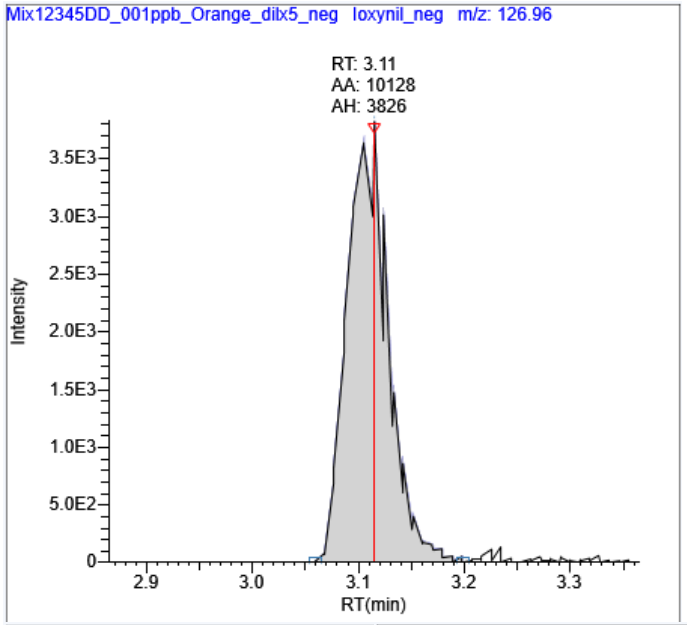








Ioxynil

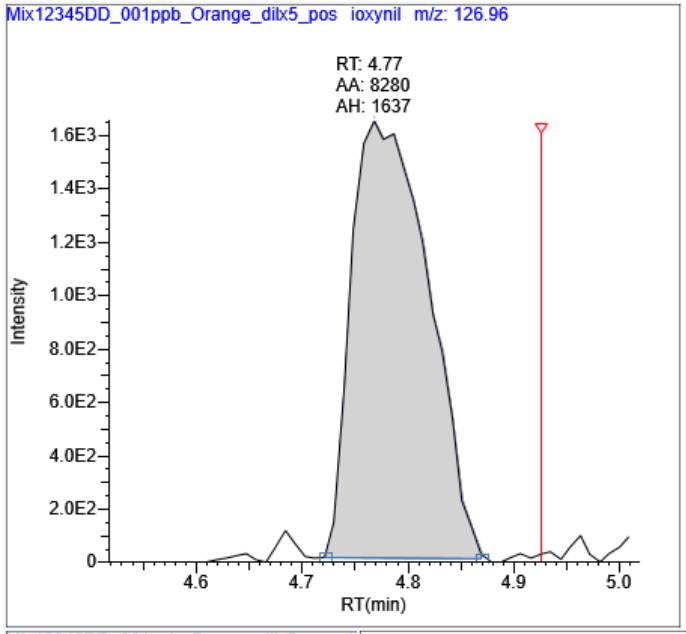


0.001 mg/L

Orange extract

ACN/AA/H_2O

Negative polarity

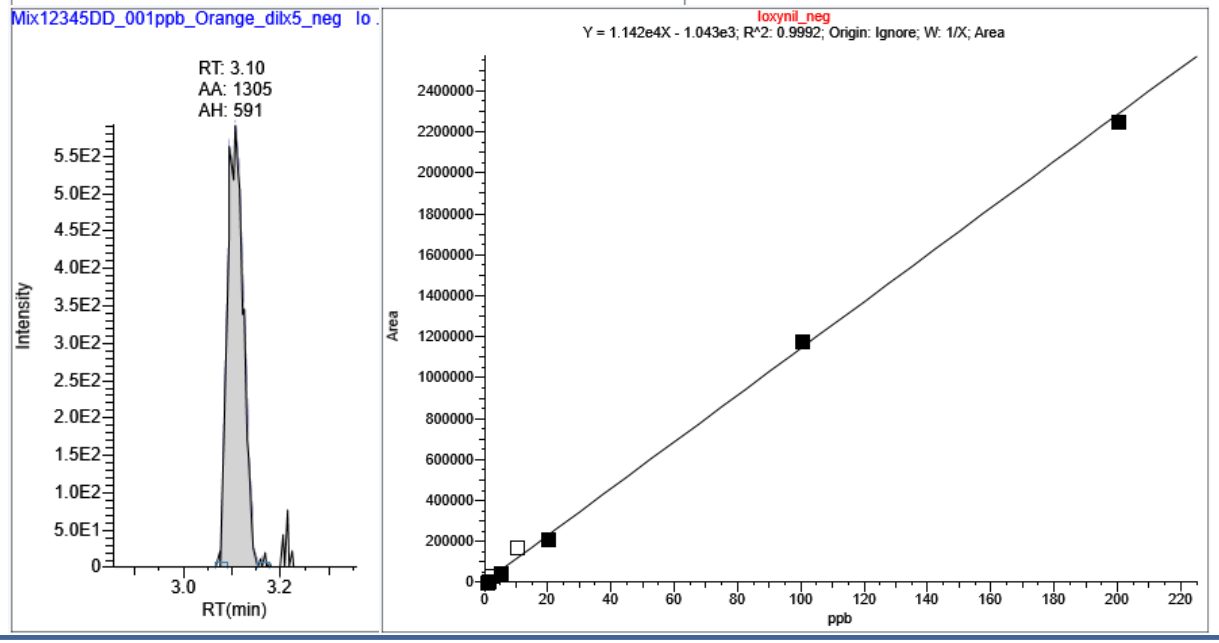


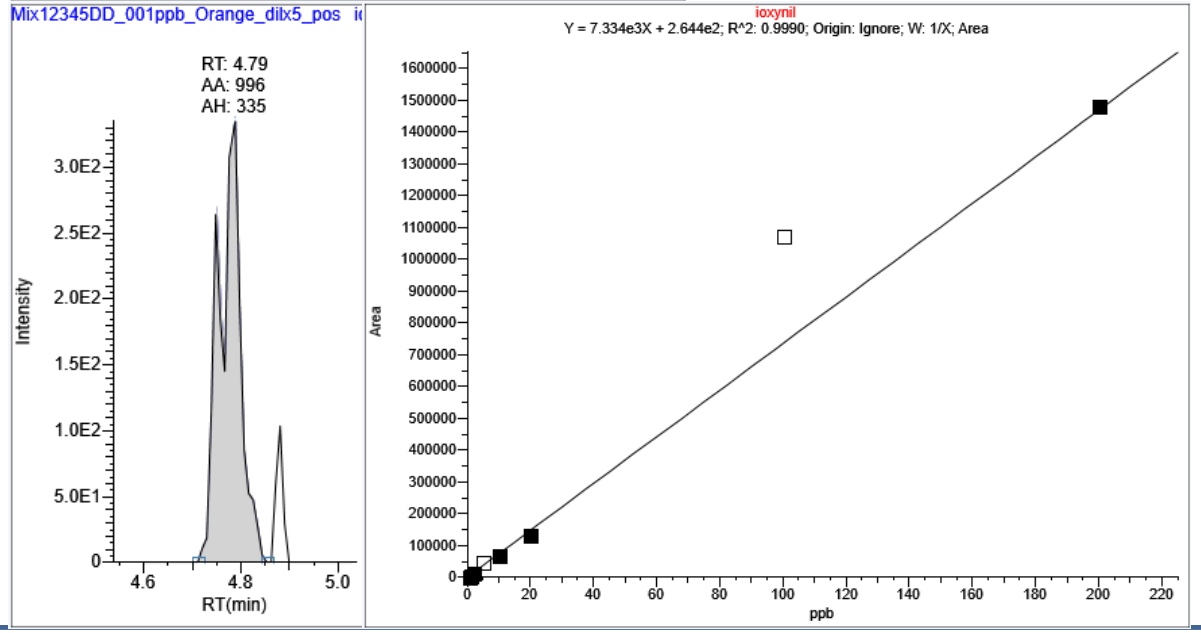
0.001 mg/L

Orange extract

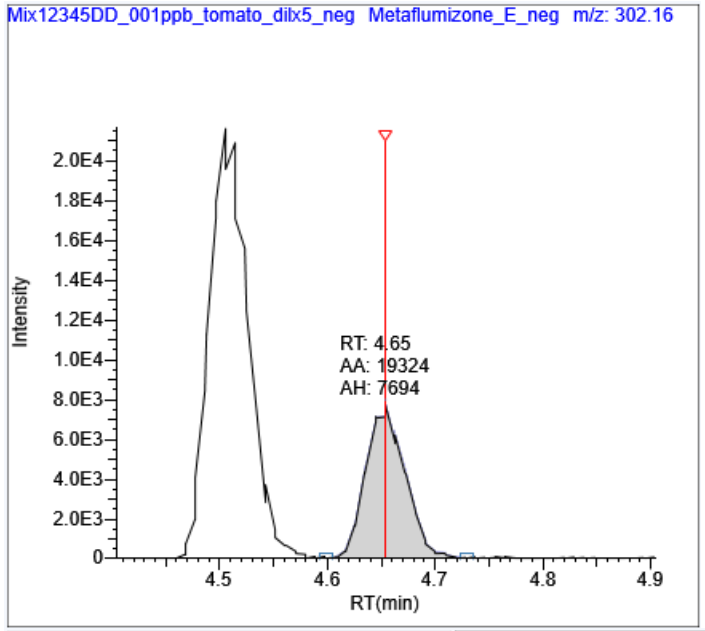
MeOH/FA/AF/H_2O

Negative polarity





Metaflumizone (E)

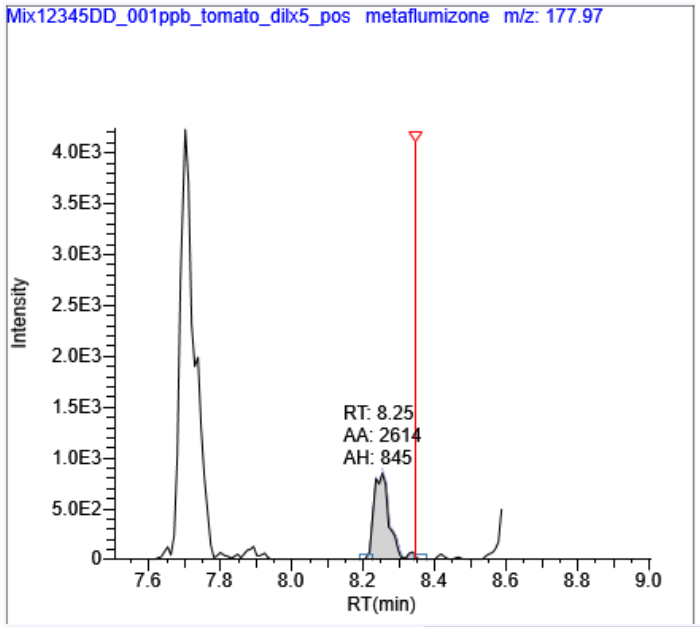


0.001 mg/L

Tomato extract

ACN/AA/H_2O

Negative polarity

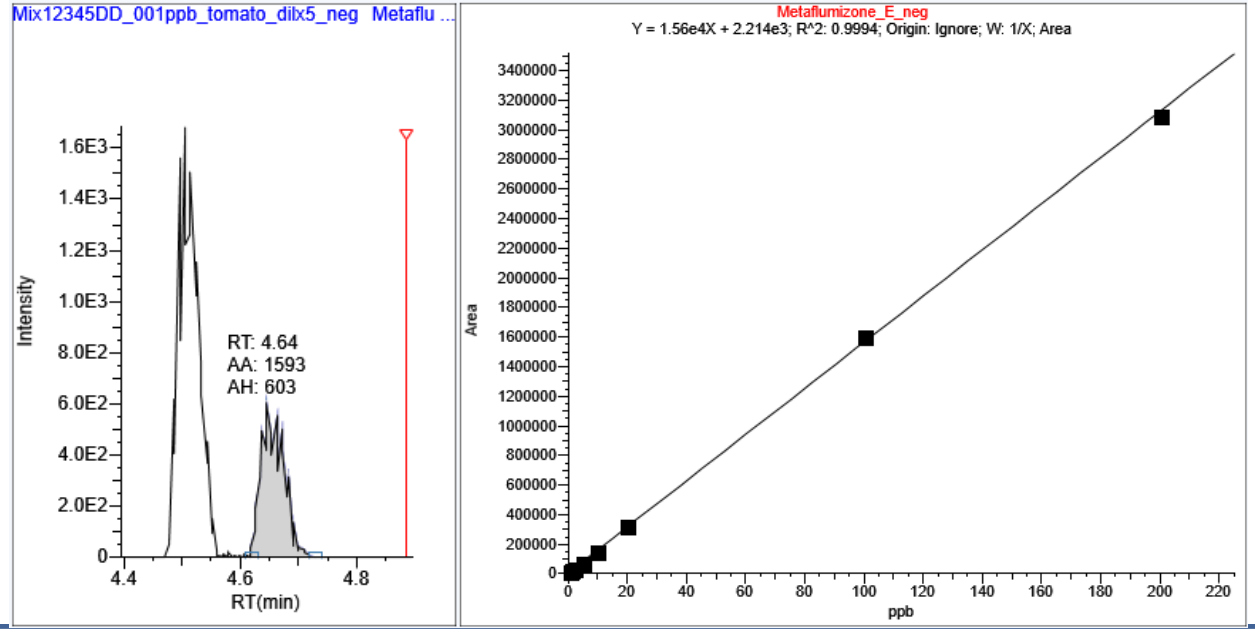


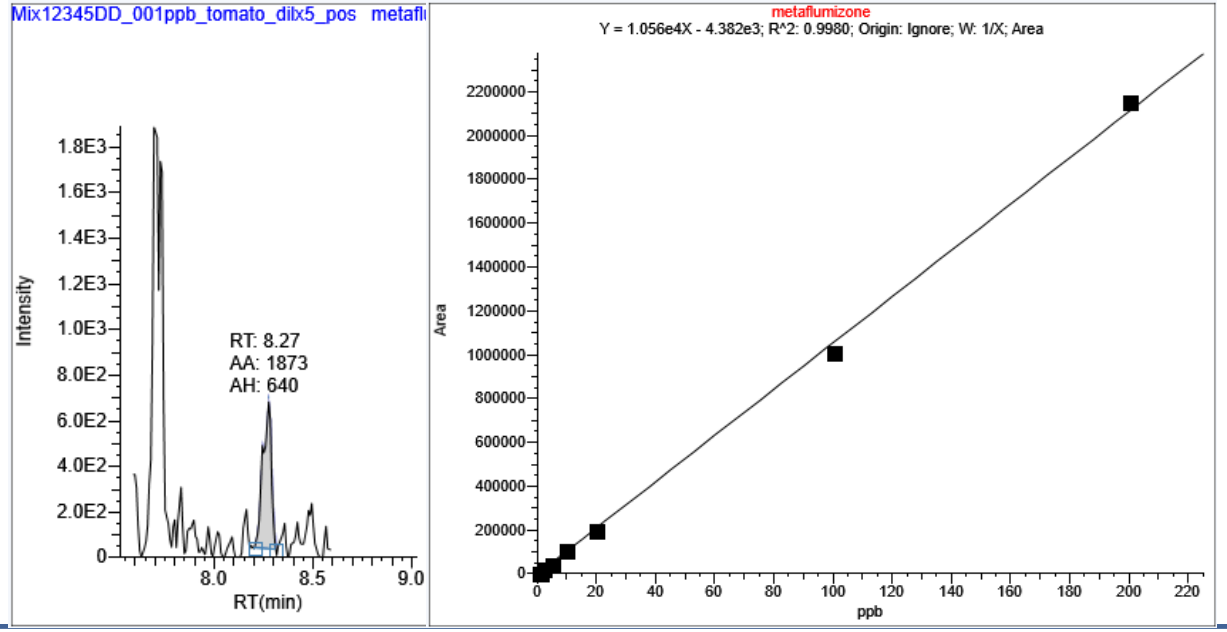
0.001 mg/L

Tomato extract

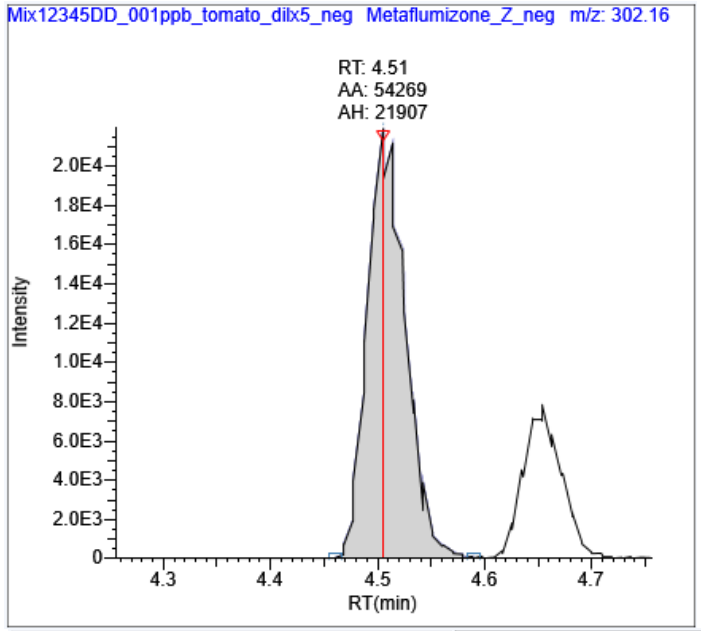
MeOH/FA/AF/H_2O

Negative polarity





Metaflumizone (Z)

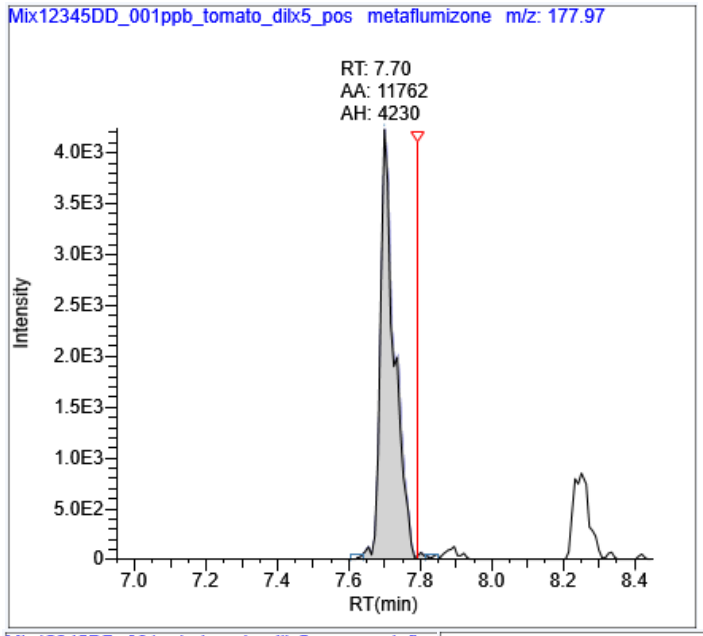


0.001 mg/L

Tomato extract

ACN/AA/H_2O

Negative polarity

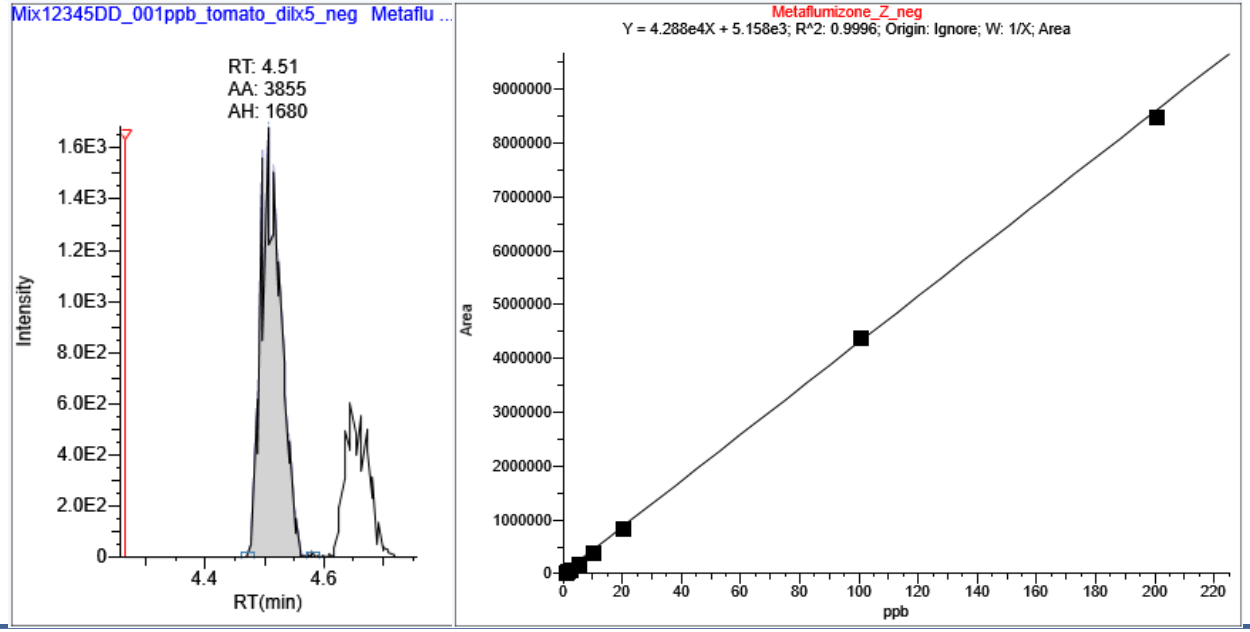


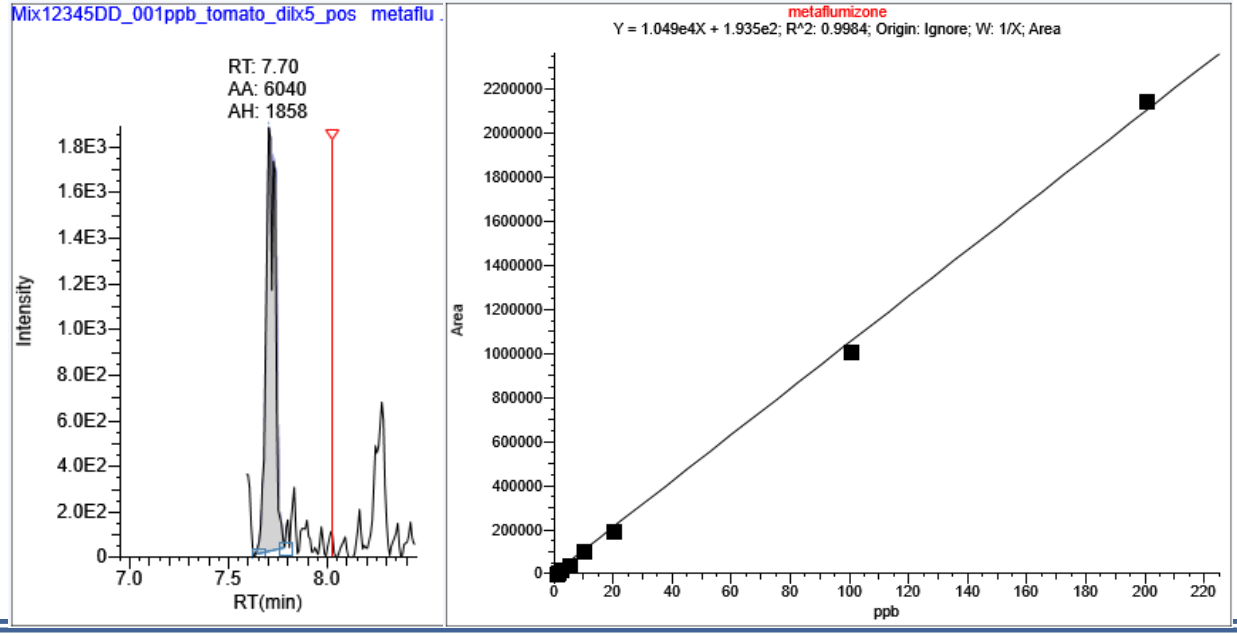
0.001 mg/L

Tomato extract

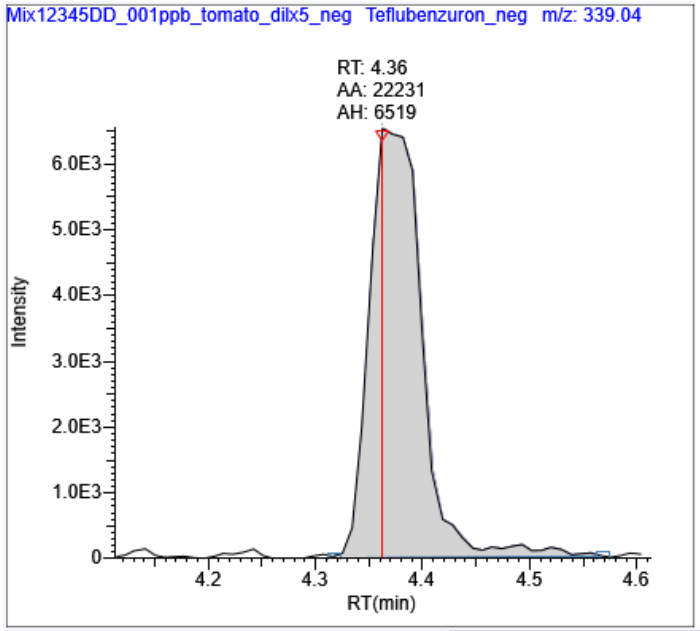
MeOH/FA/AF/H_2O

Negative polarity





Teflubenzuron

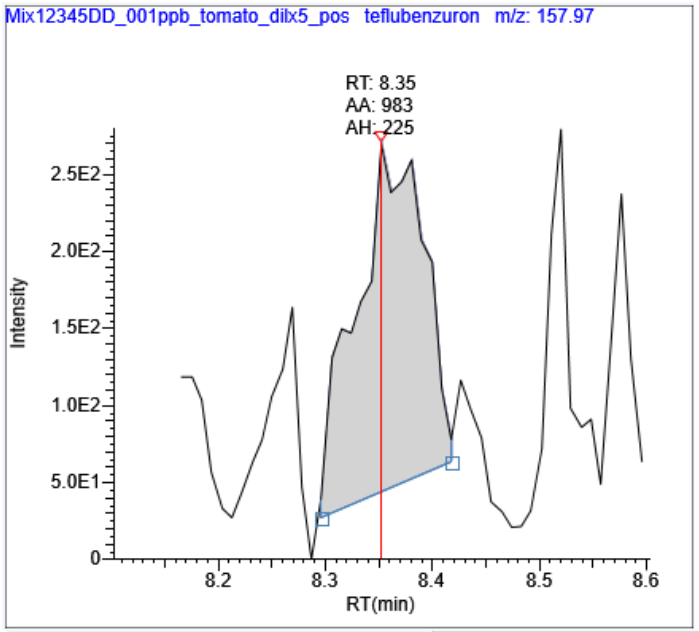


0.001 mg/L

Tomato extract

ACN/AA/H_2O

Negative polarity

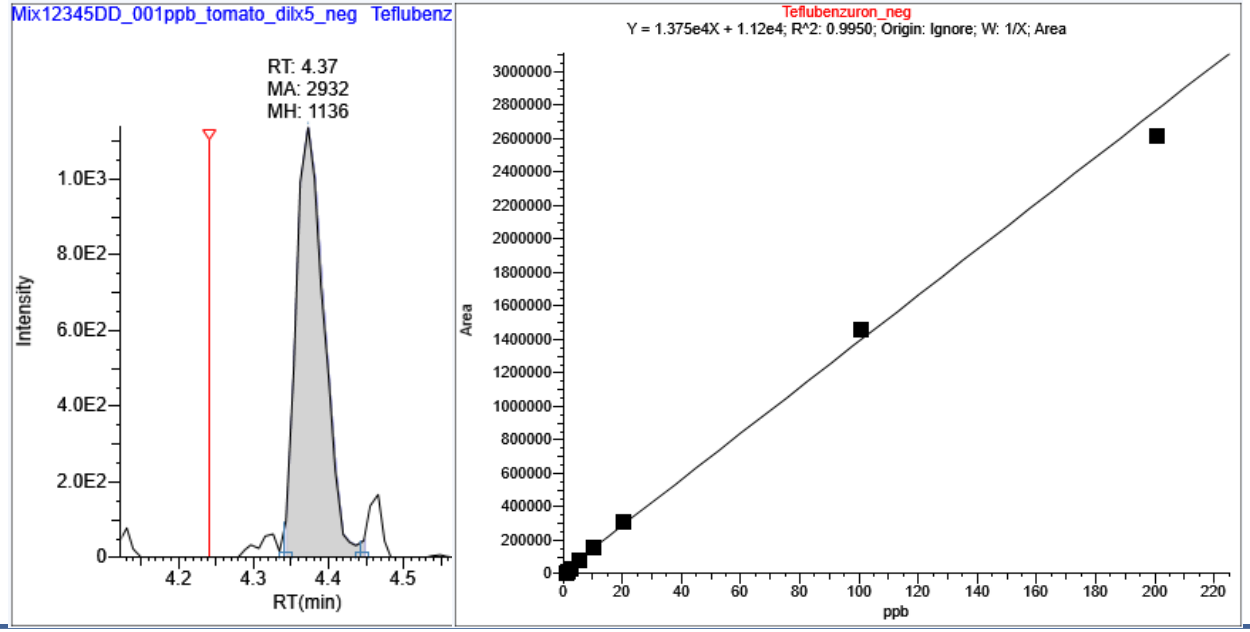


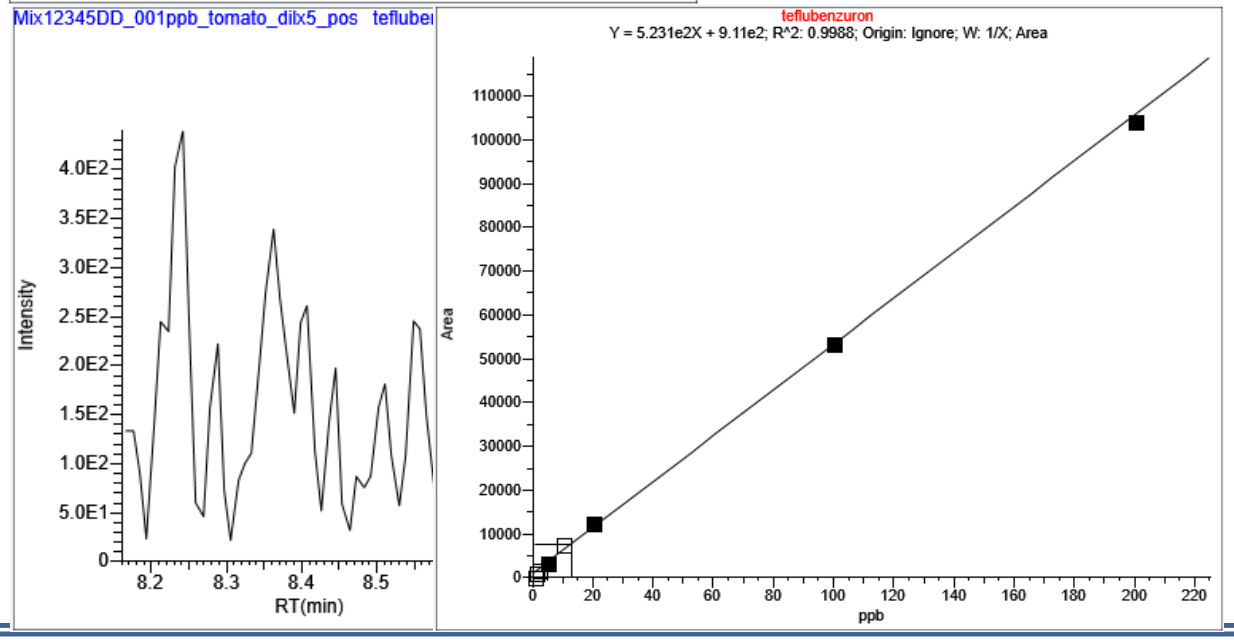
0.001 mg/L

Tomato extract

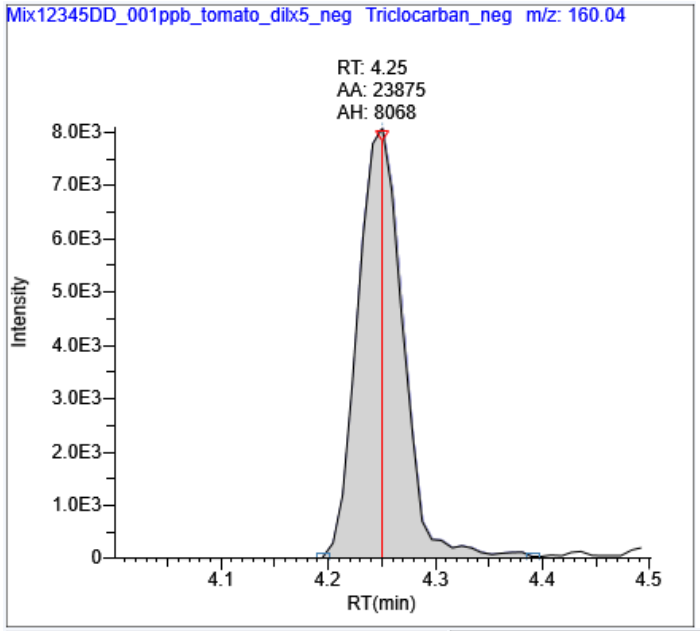
MeOH/FA/AF/H_2O

Negative polarity





Triclocarban

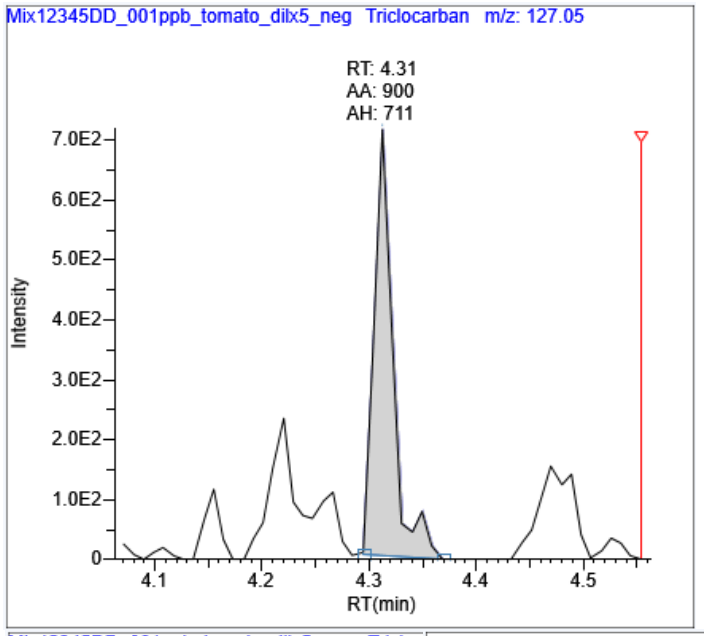


0.001 mg/L

Tomato extract

ACN/AA/H_2O

Negative polarity

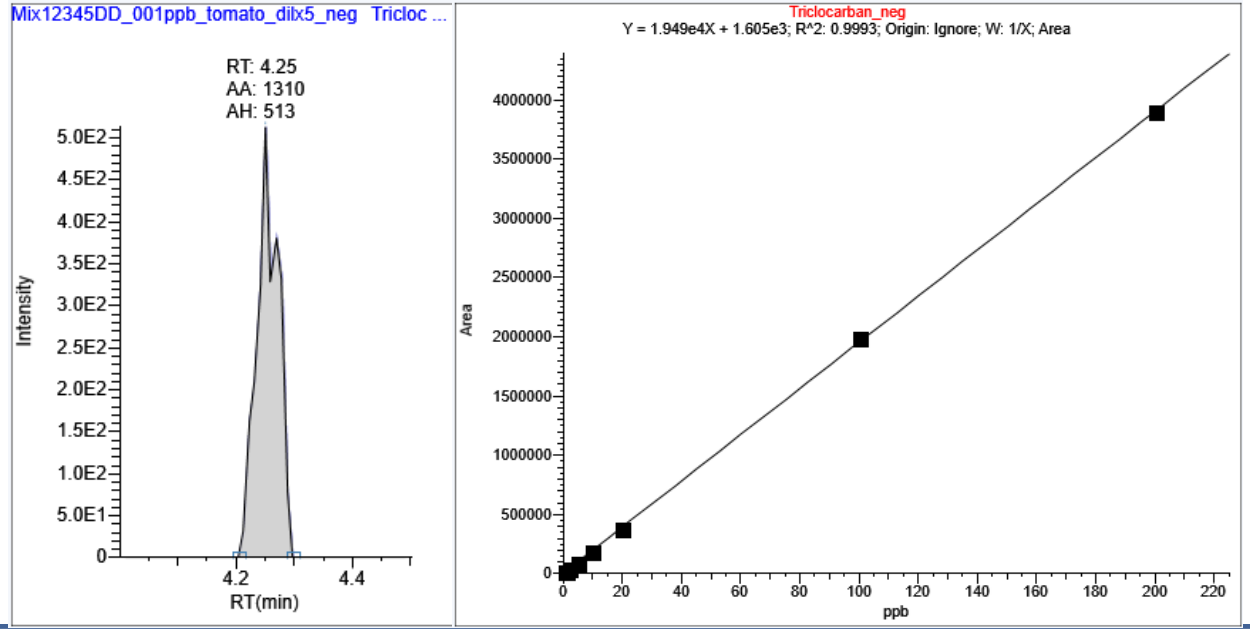


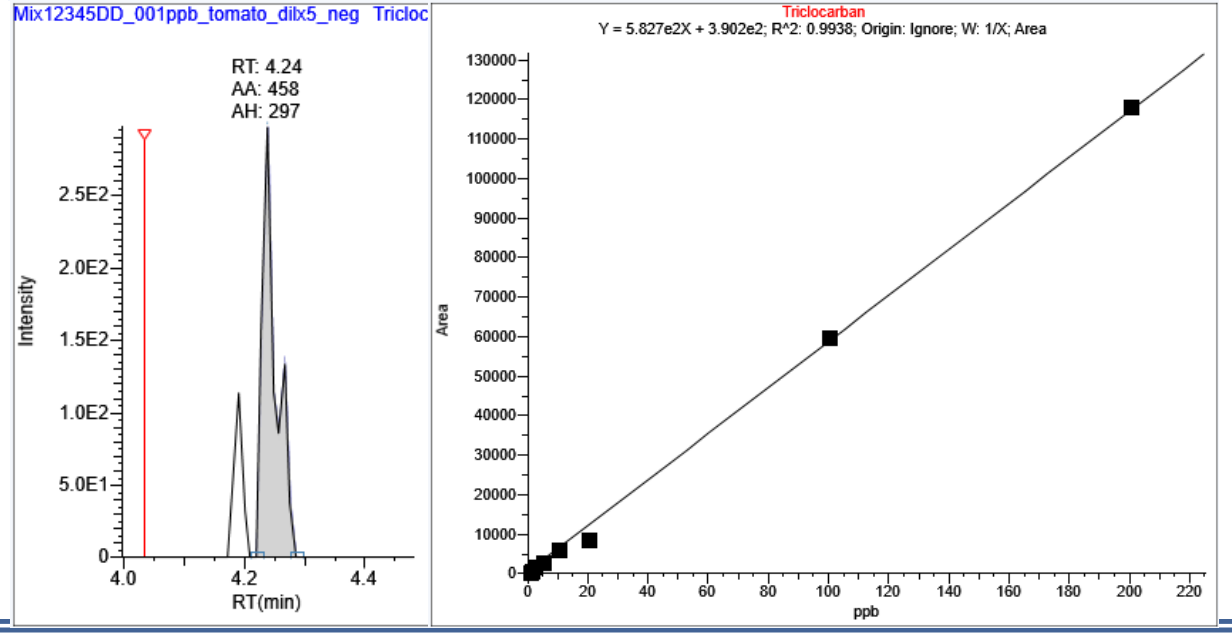
0.001 mg/L

Tomato extract

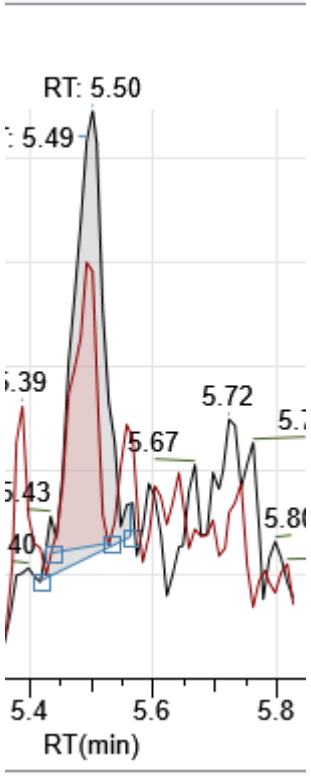
ACN/AA/H_2O

Positive polarity

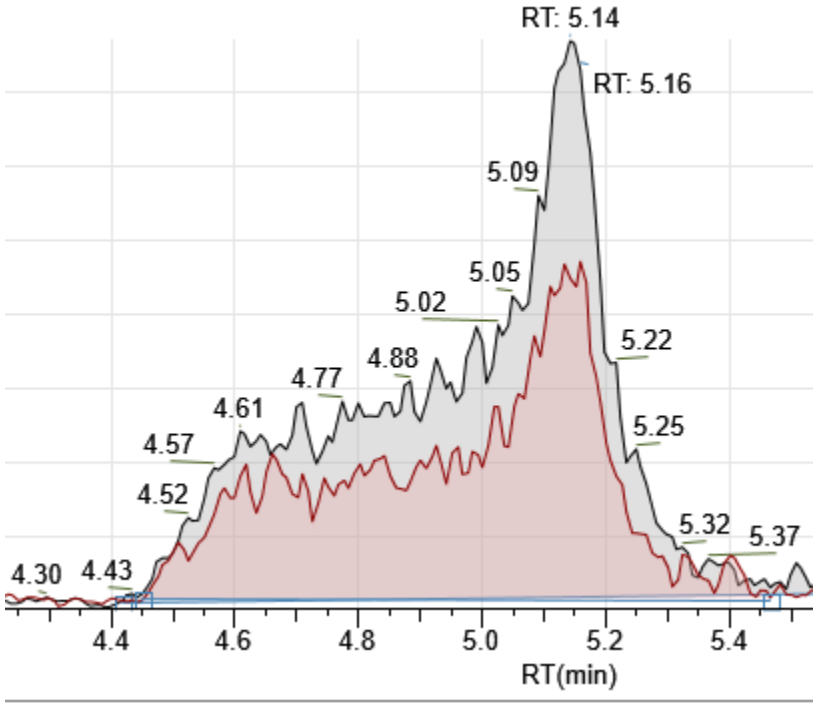




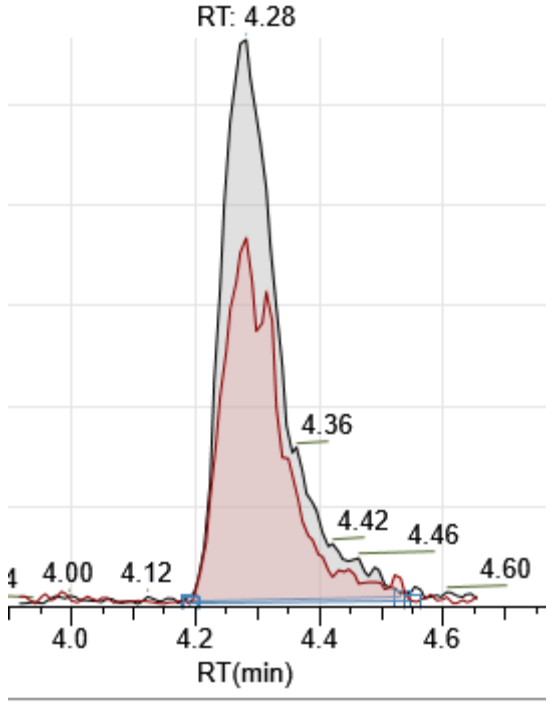
2,4-D



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

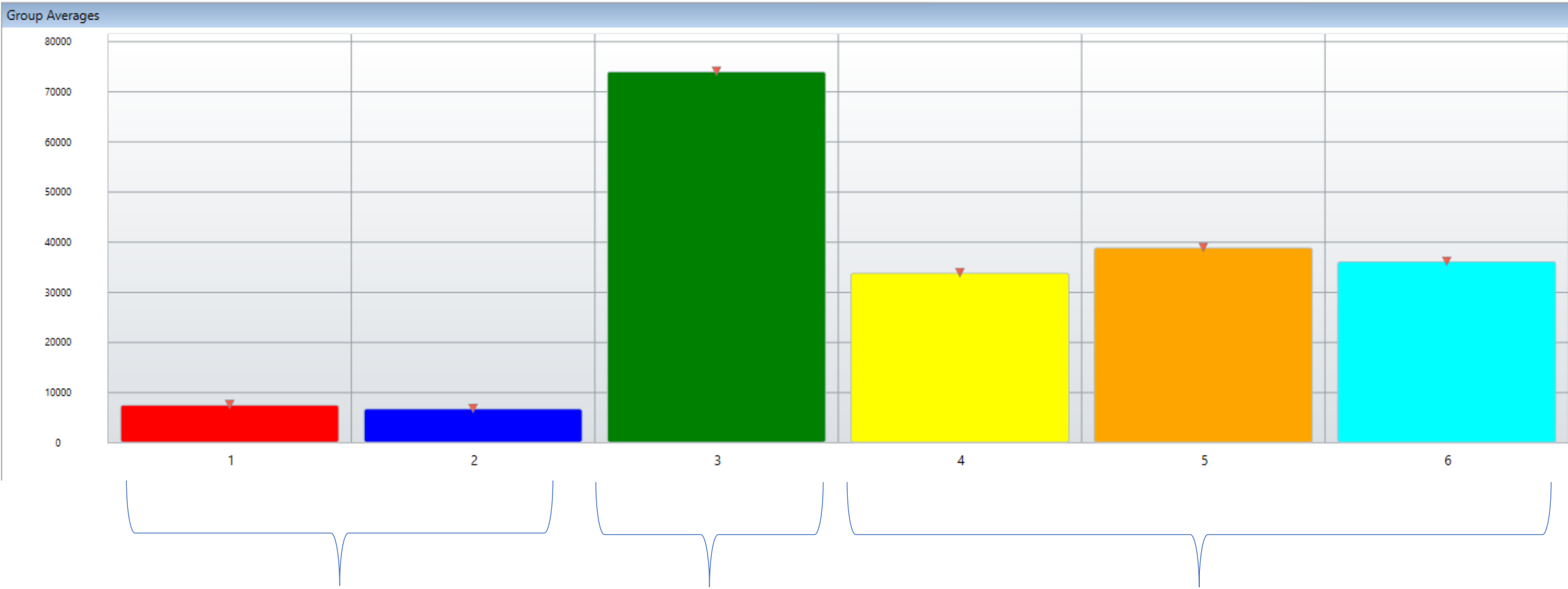


ACN/H_2O
Negative polarity
Peak area 74 079



ACN/AA/H_2O
Negative polarity
Peak area 36 193

2,4-D

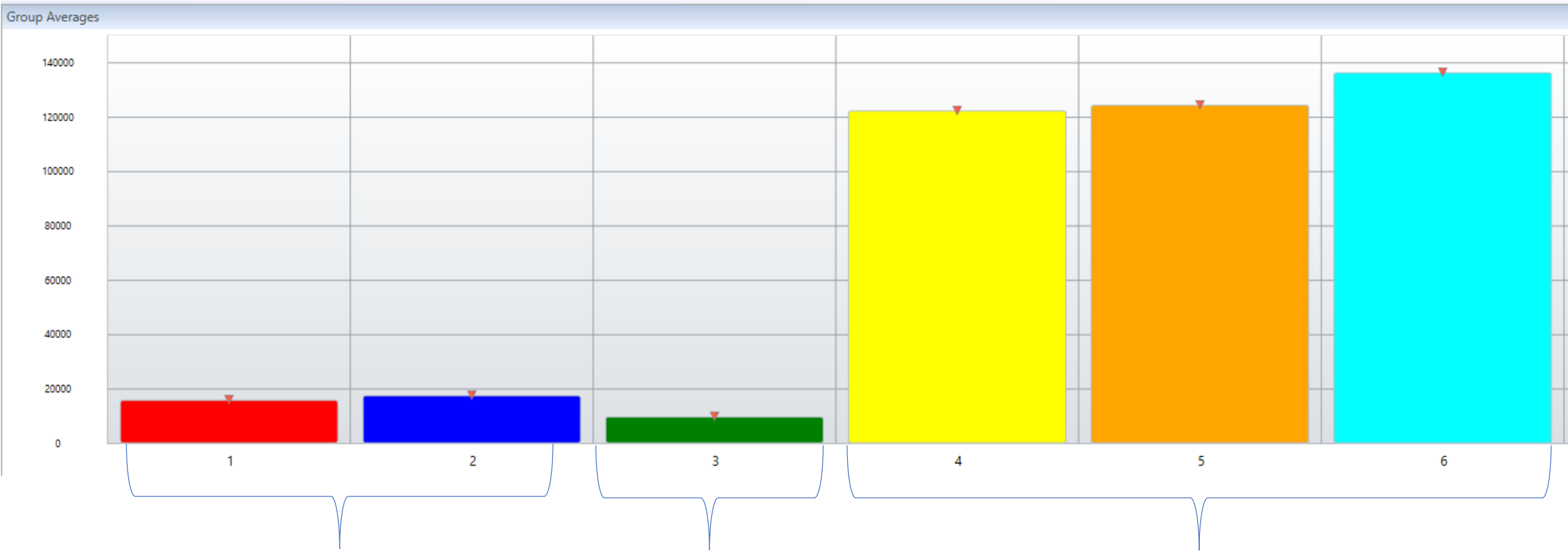


MeOH/H2O/formic acid/ammonium formate

ANC/H2O

ANC/H2O/acetic acid

Ethiprole

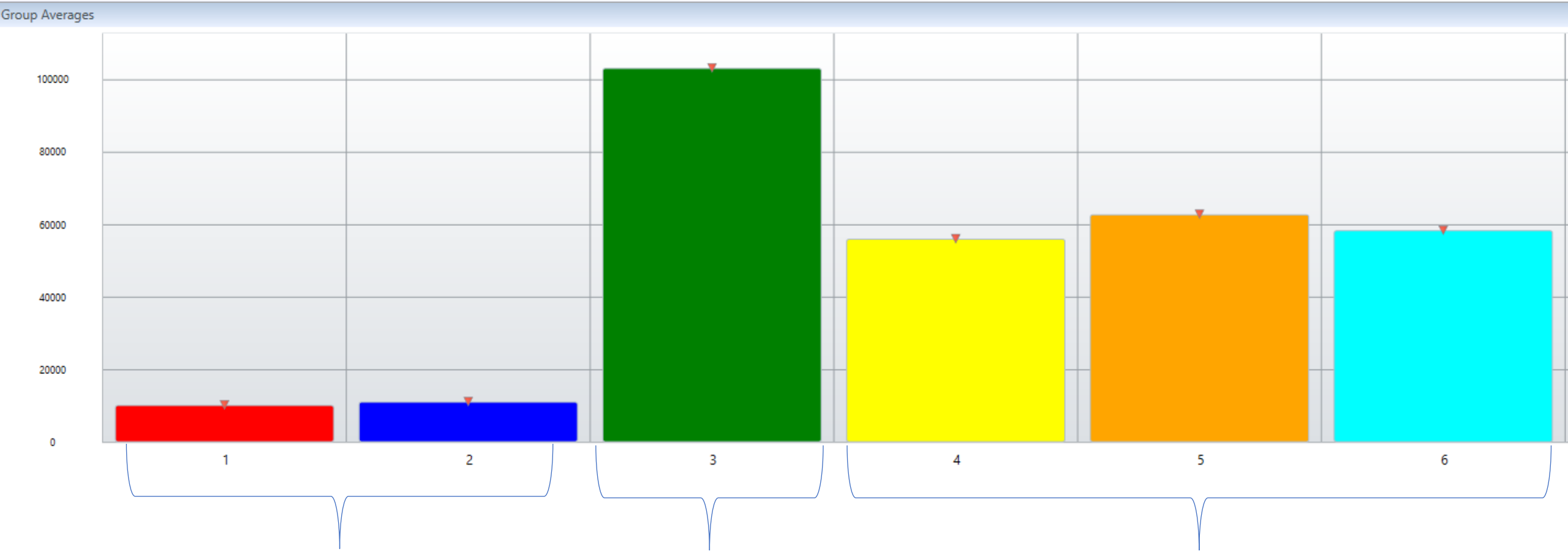


MeOH/H2O/formic acid/ammonium formate

ANC/H2O

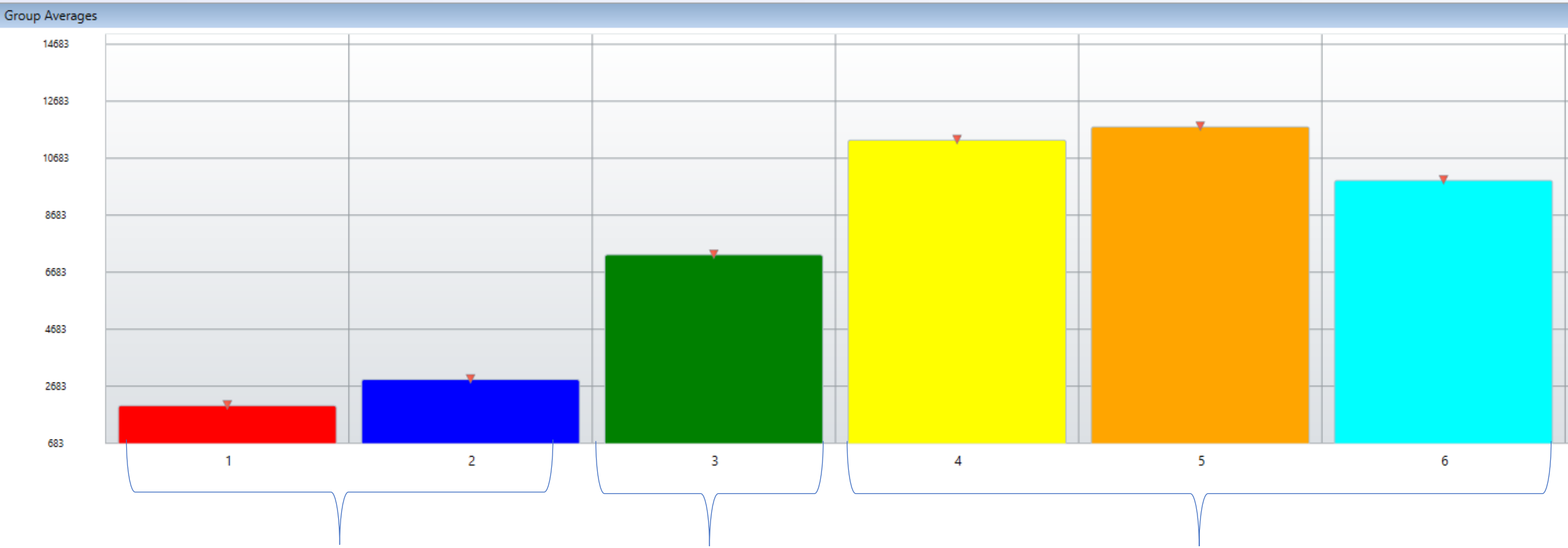
ANC/H2O/acetic acid

MCPA



MeOH/H2O/formic acid/ammonium formate ANC/H2O ANC/H2O/acetic acid

Dithianon

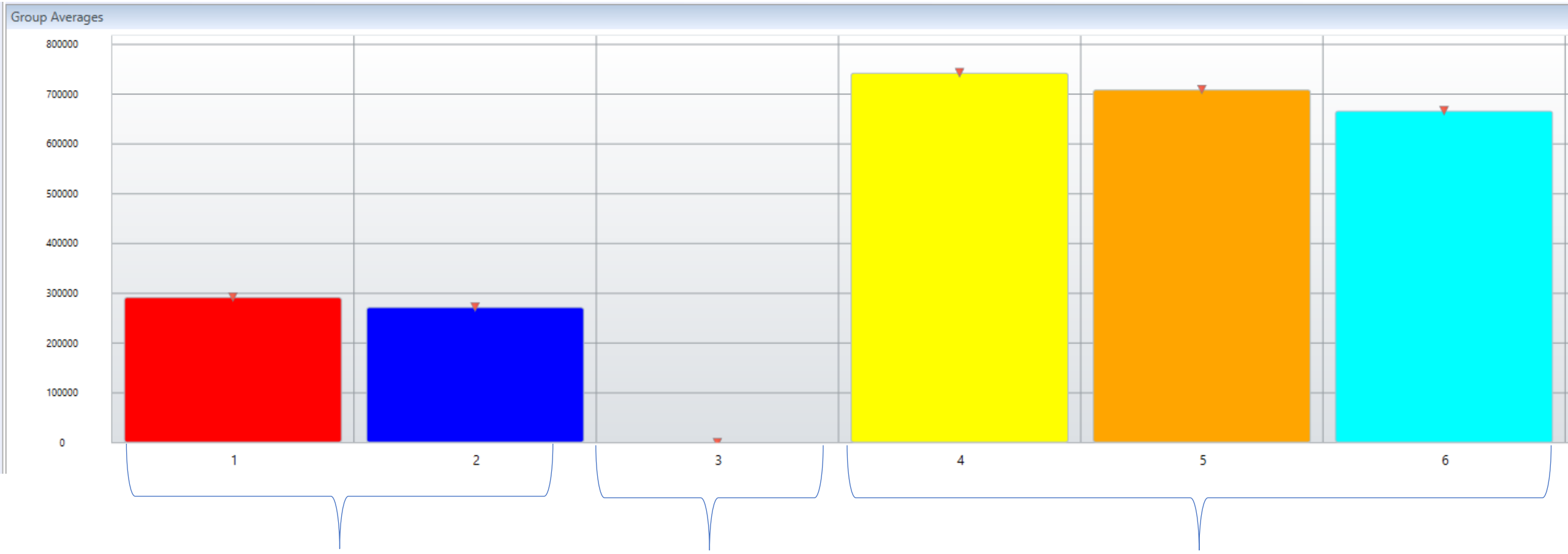


MeOH/H2O/formic acid/ammonium formate

ANC/H2O

ANC/H2O/acetic acid

Fipronil

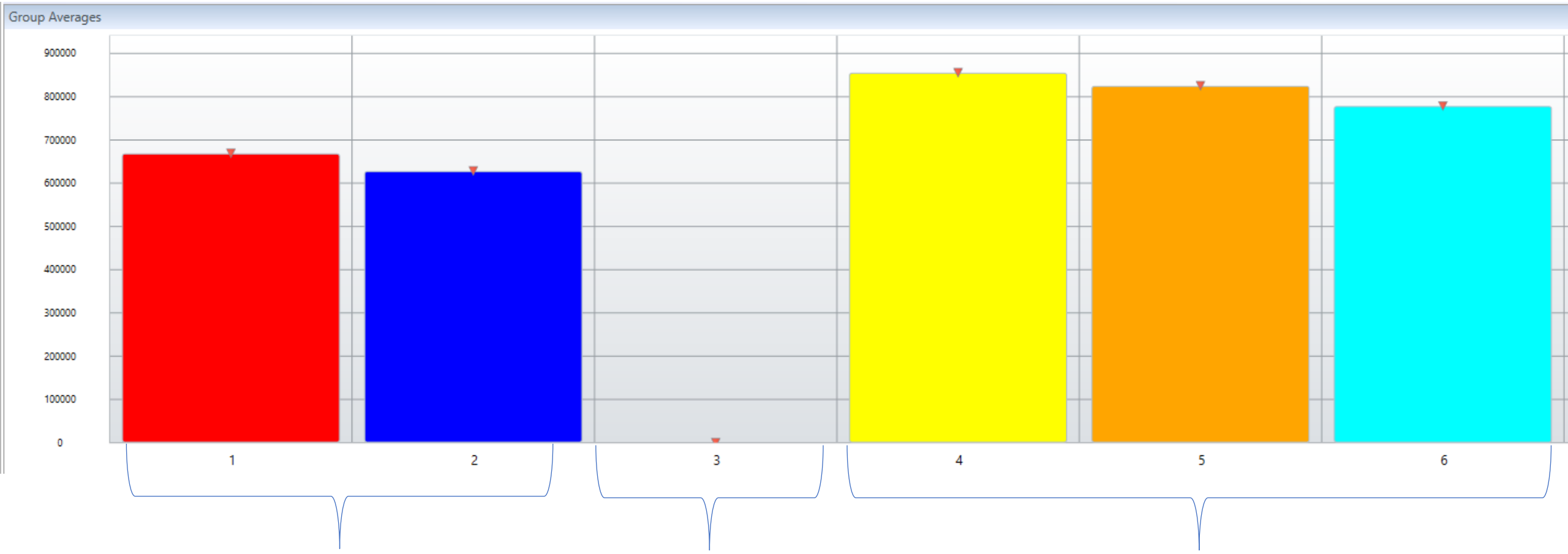


MeOH/H2O/formic acid/ammonium formate

ANC/H2O

ANC/H2O/acetic acid

Fipronil-sulfone

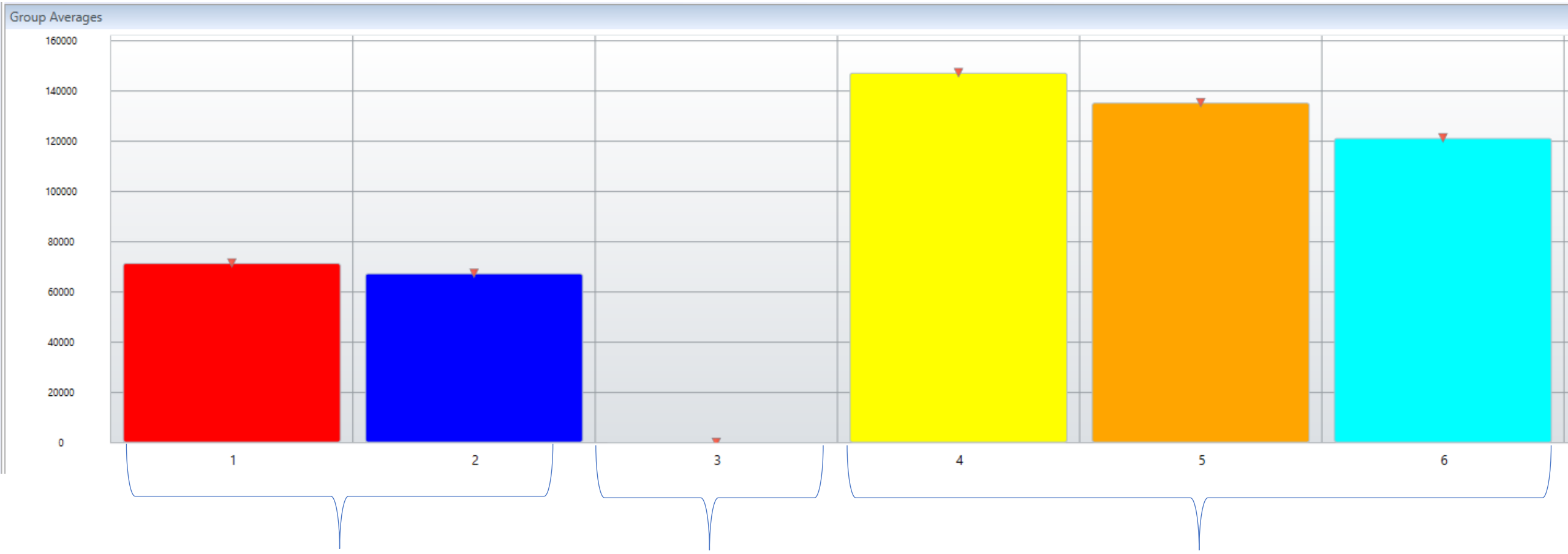


MeOH/H2O/formic acid/ammonium formate

ANC/H2O

ANC/H2O/acetic acid

Fludioxonil

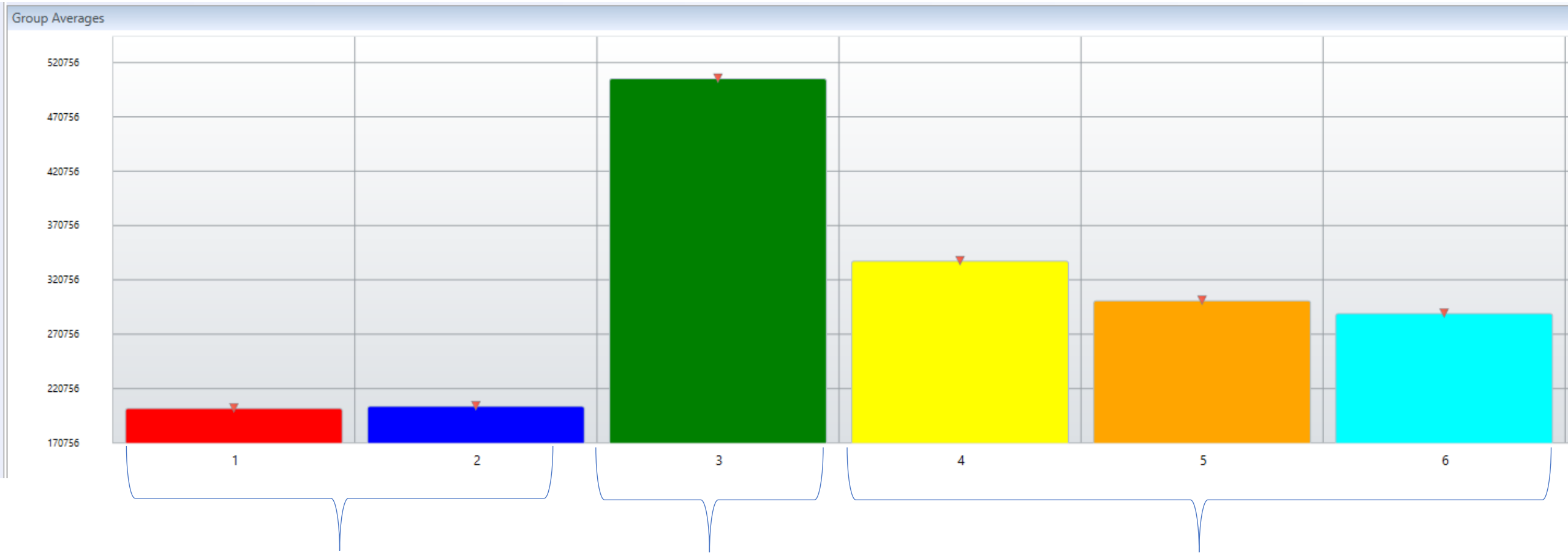


MeOH/H2O/formic acid/ammonium formate

ANC/H2O

ANC/H2O/acetic acid

Ioxinil

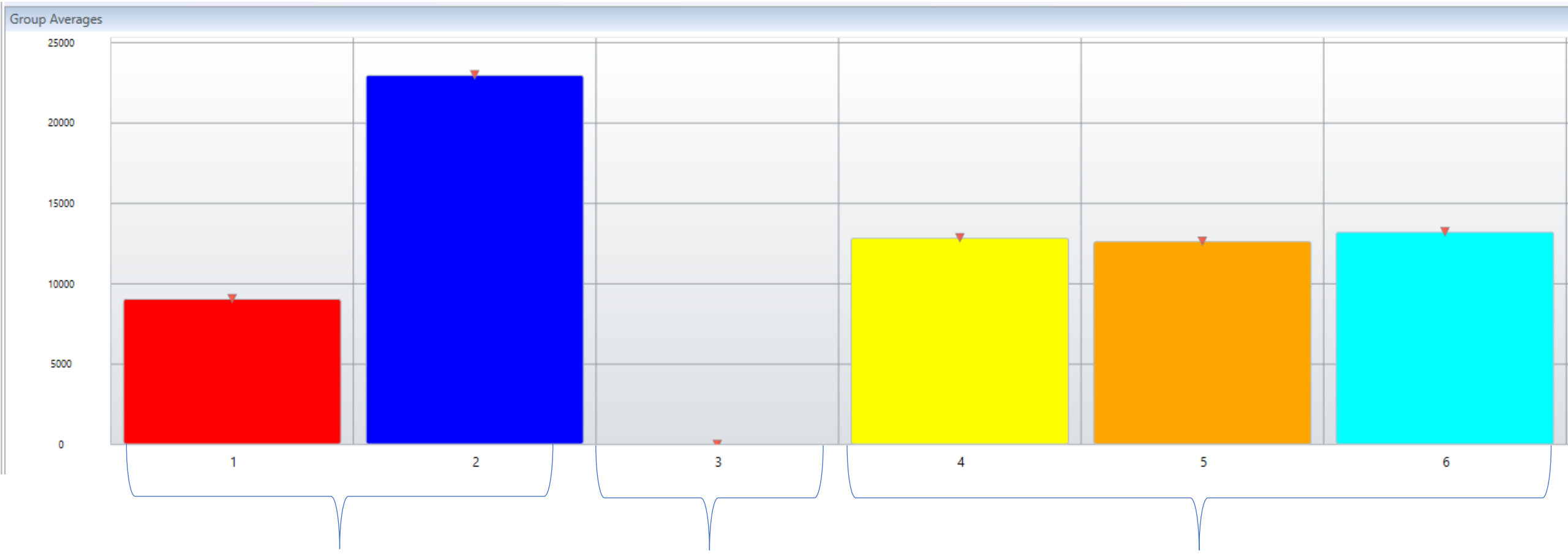


MeOH/H2O/formic acid/ammonium formate

ANC/H2O

ANC/H2O/acetic acid

Lufenuron

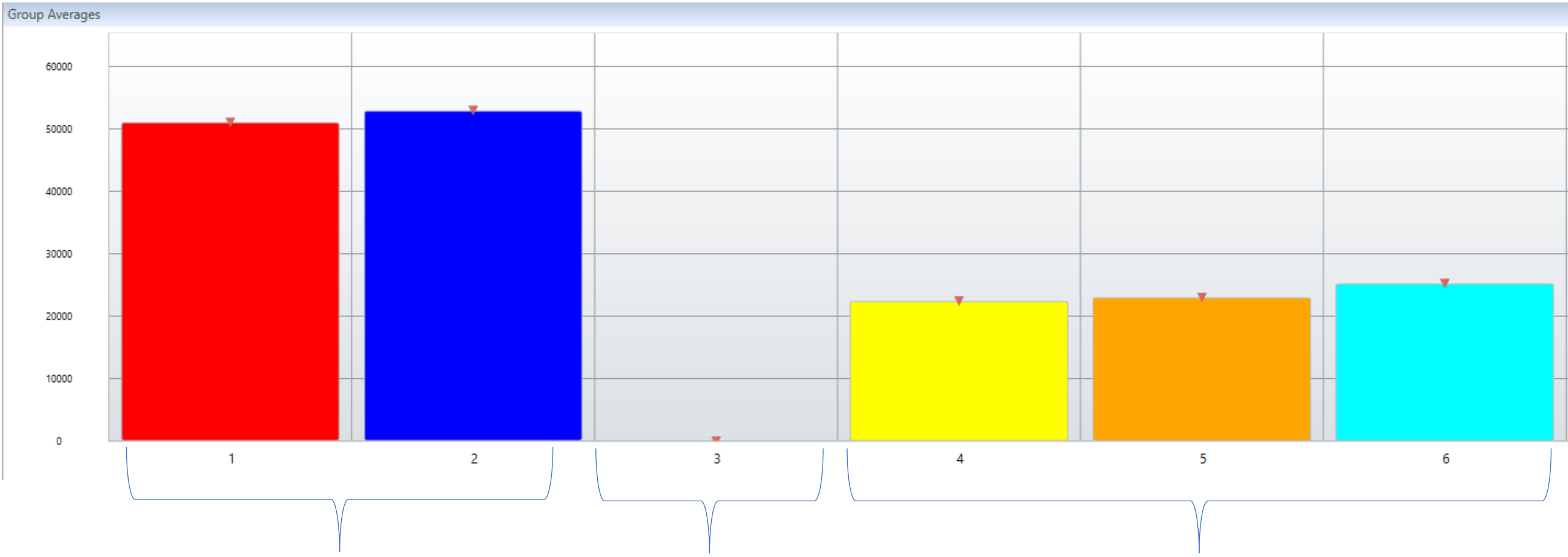


MeOH/H2O/formic acid/ammonium formate

ANC/H2O

ANC/H2O/acetic acid

Novaluron

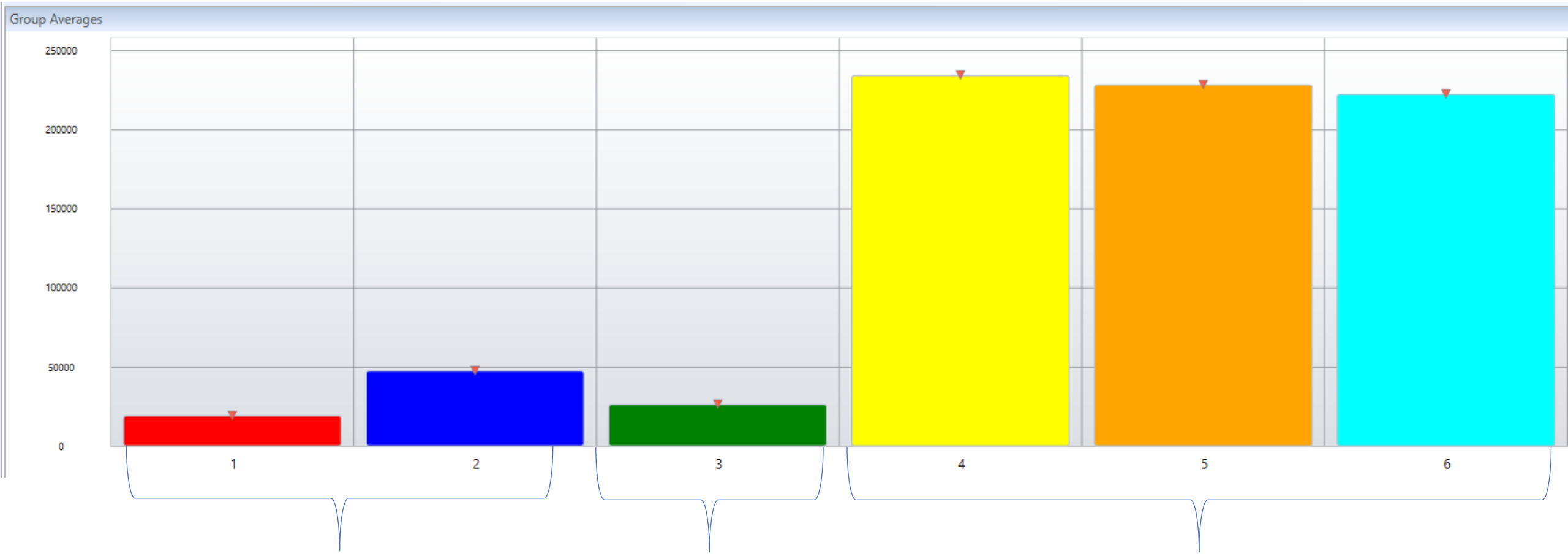


MeOH/H2O/formic acid/ammonium formate

ANC/H2O

ANC/H2O/acetic acid

Teflubenzuron

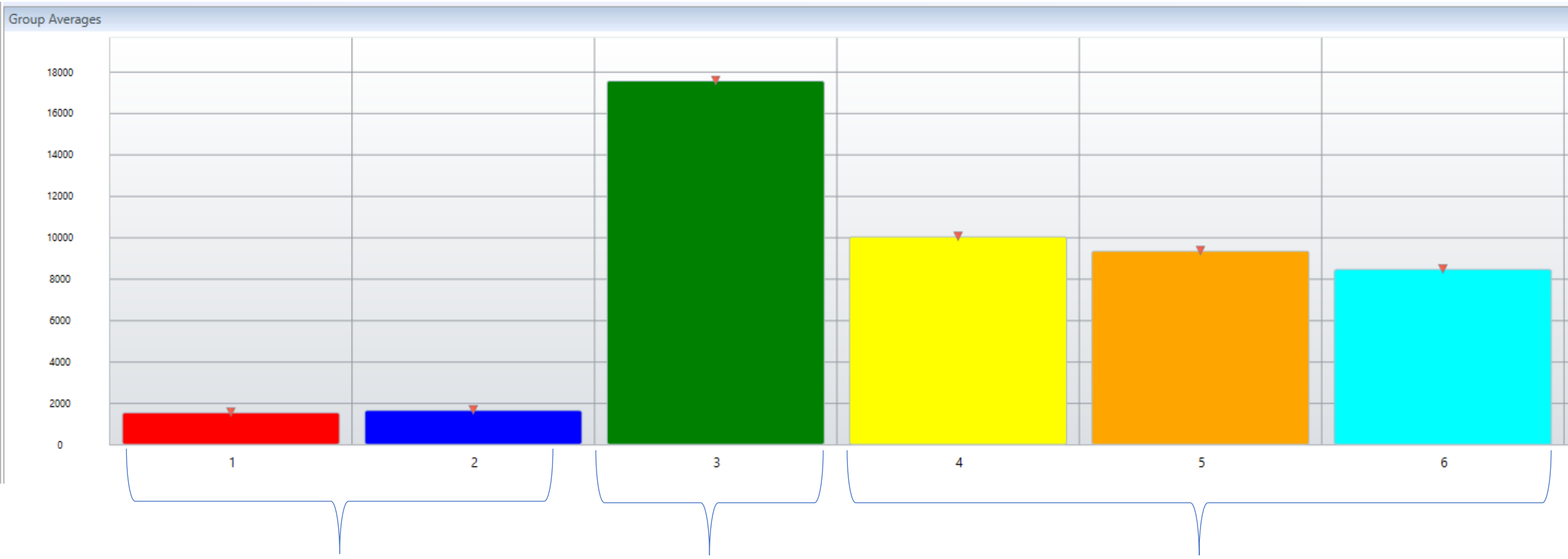


MeOH/H2O/formic acid/ammonium formate

ANC/H2O

ANC/H2O/acetic acid

TFNG



MeOH/H2O/formic acid/ammonium formate

ANC/H2O

ANC/H2O/acetic acid

Summary

- Dual-channel chromatography improves the utilisation of the mass spectrometer
  - 100 mm columns increase the sample throughput
  - 150 mm columns enhance sensitivity and selectivity

- Dual-channel mode provides results equivalent to single-channel mode
- Retention times in the dual-channel system are very stable
- Conversion of a single-channel chromatographic method into a dual-channel method is fast and simple
- In dual-channel chromatography positive and negative polarity compunds can be analysed separatly
- Aria MX software is user-friendly and reliable

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Thank You
for Your Attention