“Incredibly Powerful, Remarkably Efficient Quantitation”

Patrick M. Jeanville, Ph.D.
LC/TQ Product Manager

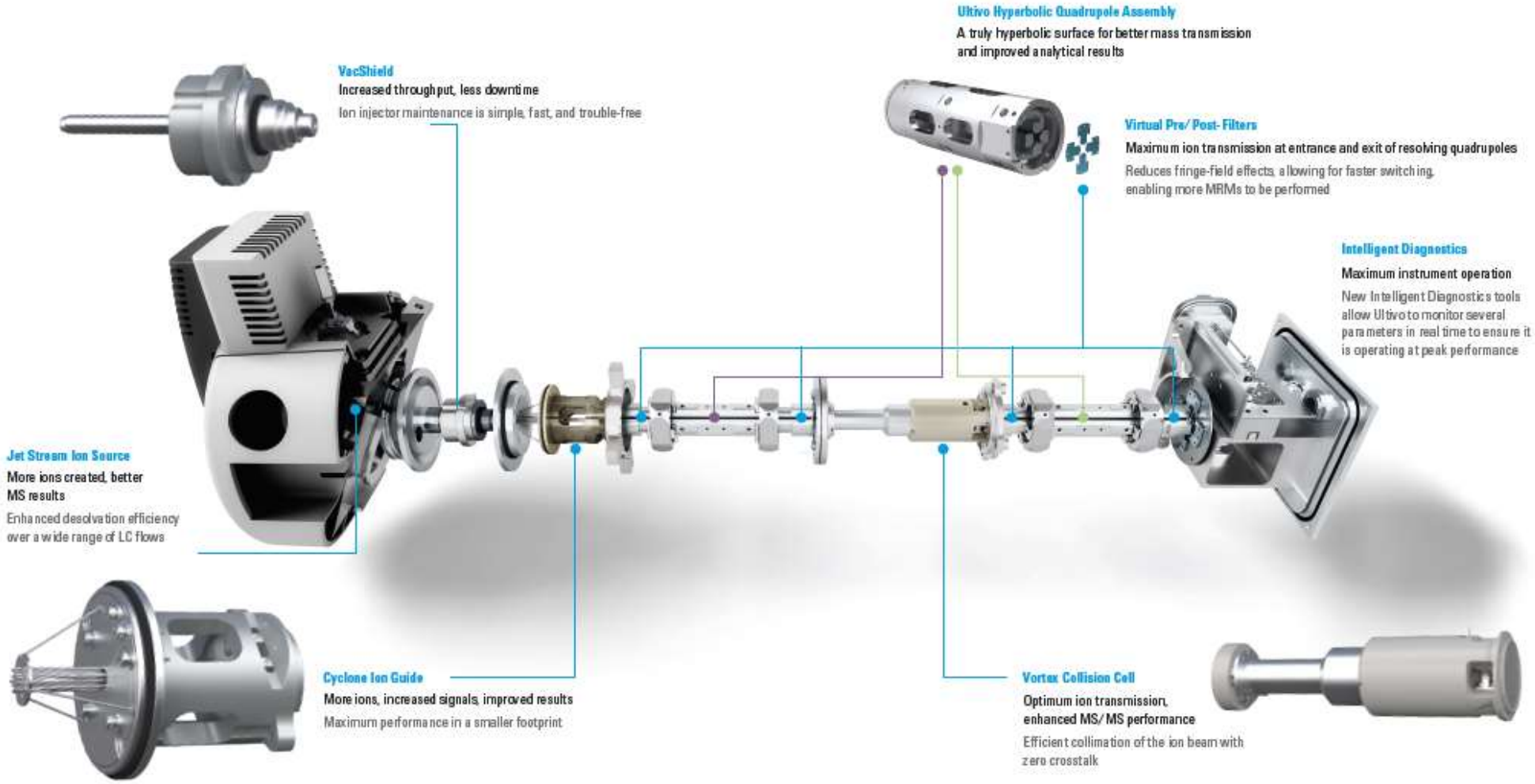
Ruben Garnica, Ph.D.
MS Solutions Product Manager

Yanan Yang, Ph.D.
Application Scientist

Jennifer Hitchcock, Ph.D.
Application Scientist

Agilent
Trusted Answers

Ultivo: Innovation with Purpose!



Trade Shows/Conferences

- HPLC 2017, Prague
- ASMS 2017
- NACRW 2017
- Taiwan MS Society
- ANZSMS
- RACI 100
- British MS Society (September)
- JASIS
- LabAfrika (Johannesburg)
- Korea MS Society (August)
- LabThailand (September)
- LabAsia (October)
- Analitica, Brazil (September)
- SETAC, Brazil (September)
- India
  - Analytika-Anacon (September)
  - Customer events (Jim Yano)
- BCEIA, China (October)
- RAFA (CZ)
- Intl POP conference Japan (sept)
- AOAC
- SETAC (No. Am)

Press Events

- HPLC 2017, Prague
- ASMS 2017
- China (Beijing)
- VIP Event, London
- Singapore (June)
- VIP Event, Mexico City
- India (Sept)

Seminars

- EMEAI – Manchester
- AFO
  - Environmental Seminar (Houston)
  - Lunch and learn events (Aug-Oct)
- Gr. China – Shanghai, Taiwan, Hong Kong. MS Tech seminar series throughout Gr. China
- Japan – 6X seminars (August/Sept)
- SAPK – MS Technical Seminars (4X) July
  - Indonesia (September)
- Korea (August)
- India (August-September)

Training Events

- EMEAI – IDO, Waldbronn AE/PS
- AFO – ASMS, WebEx
- LATAM – AE, PS, IDO
- Gr. China – PS/AE, WebEx (2), F-t-F
- Japan – PS/AE, WebEx, F-t-F
- SAPK – SMT-IDO, ANZ (TP)/WebEx
- Korea – Direct and DR training August
- India – Field training (July)
- ANZ – Software & apps (M. Tischler, T Anumol.
- AE Training @ SCS
  - Oct 2-6, Oct 9-18 (all regions ex. China)
  - Nov 6-10 (CN specific)

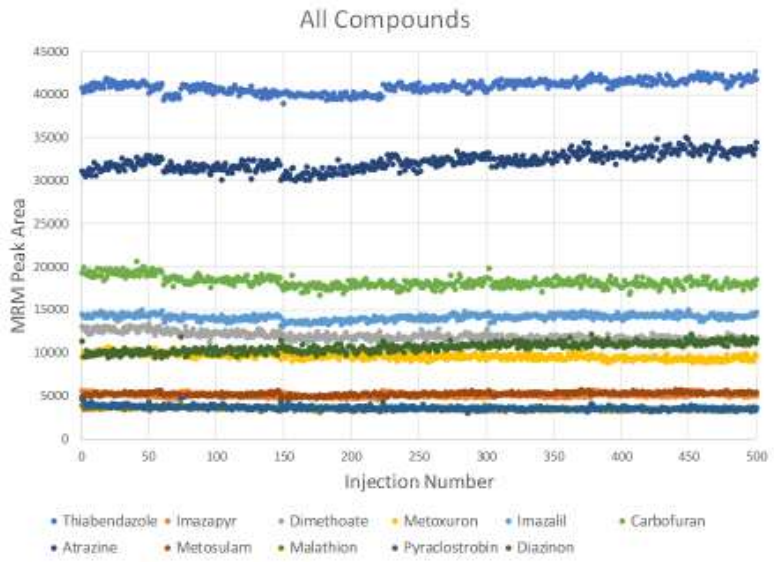
VIP Events

- EMEAI
  - HPLC 2017, Prague
  - Uppsala, Sweden
  - London, England
  - Basel, Switzerland
  - Milan, Italy
- AFO
  - Santa Clara, CA
  - Little Falls, DE
  - Chicago, IL
  - LATAM
    - Mexico City (August)
    - Sao Paulo (August)
    - Argentina (September)
    - Colombia (September)
- Gr. China
  - Beijing
- Japan
  - JASIS (September)
- SAPK
  - Singapore X2 (Monty, Mike McMullen)
  - Melbourne (RACI)
- ASMS 2017
- China (BCEIA)
- VIP Event, London
- Korea
  - Seoul (August)
- India (September, two locations)

Ultivo LC/TQ: Small, Robust, Easy-to-Use with EMF Feedback

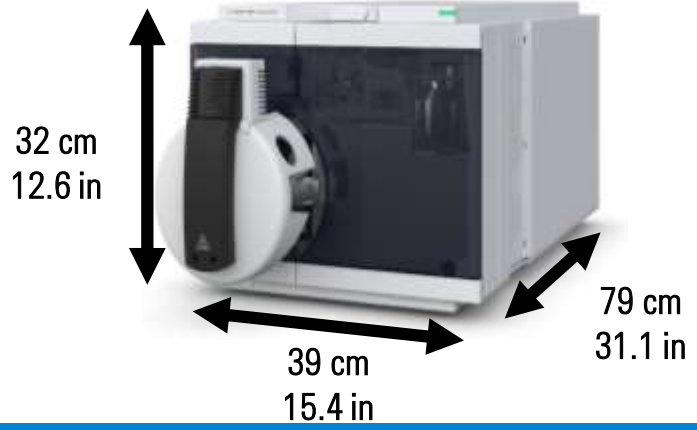
Ultivo System Robustness

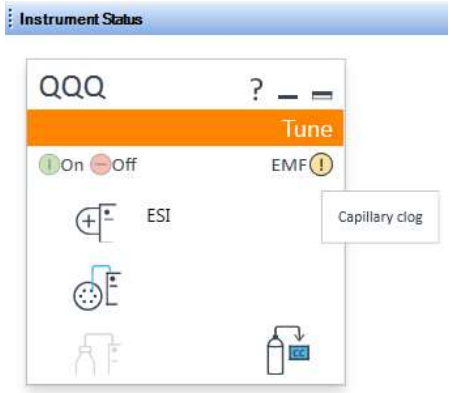
20 ppb Pesticide Basic Checkout Mix spiked into Avocado QuEChERS-EMR



Compound	% RSD (n=500)
Thiabendazole	1.8%
Imazapyr	4.0%
Dimethoate	4.1%
Metoxuron	3.6%
Imazalil	2.3%
Carbofuran	3.3%
Atrazine	3.0%
Metosulam	3.5%
Malathion	4.3%
Pyraclostrobin	6.1%
Diazinon	5.0%

Data collected by Mark Sartain





Infinity II HPLC Stack

Ultivo Triple Quad

Agilent

Incredibly Powerful, Remarkably Efficient Quantitation

MassHunter Productivity Software Applications

Ruben Garnica, Ph. D.
MS Solutions Product Manager
Agilent Technologies, Inc.
Wilmington, DE

Agilent
Trusted Answers

Ultivo LC/TQ – Reducing Pain Points

Diagnostics – Provide “real-time” information to customer concerning stability/usability of the system

Form Factor – Size/ Footprint, stackability, external appearance

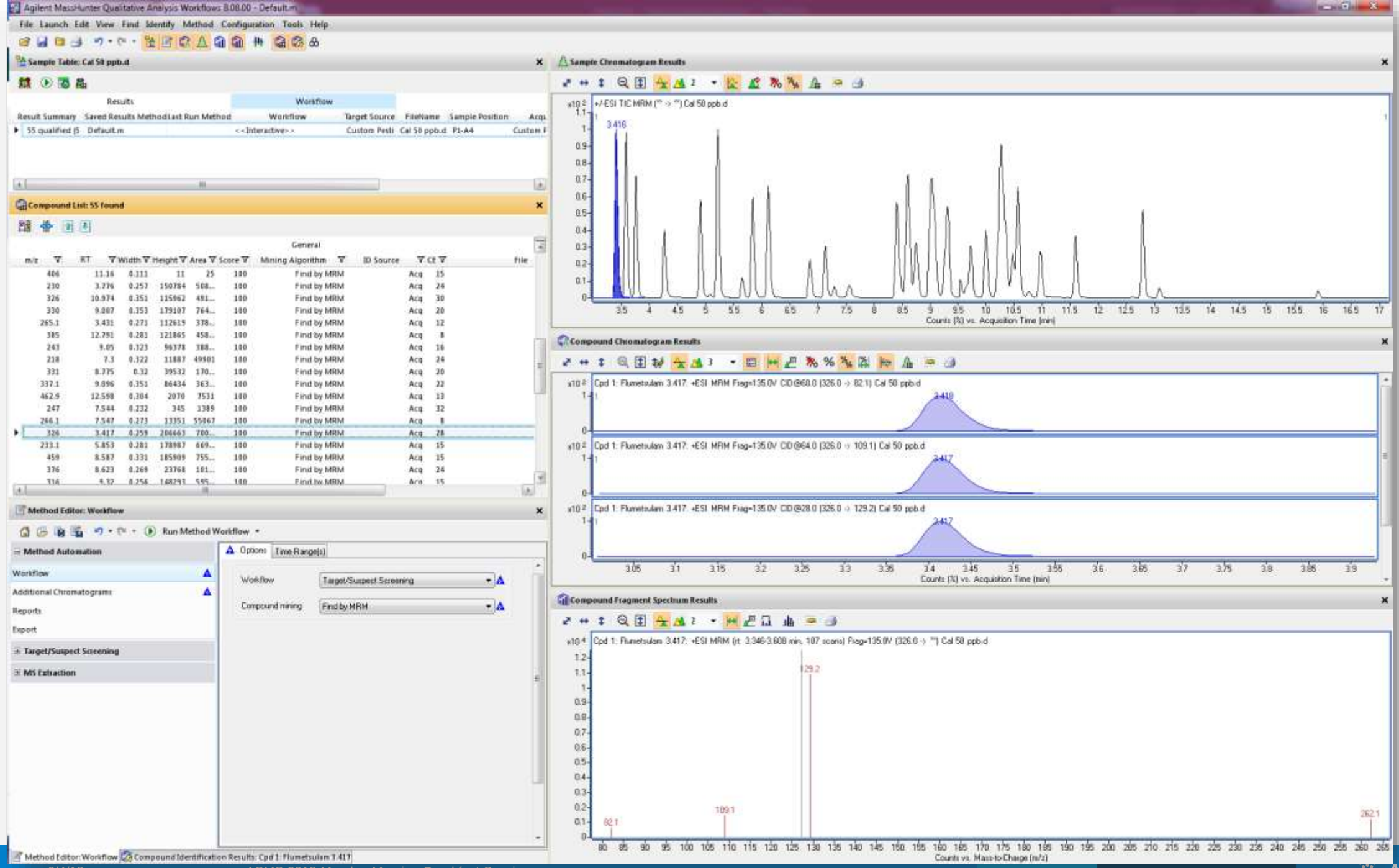
Reliability – Overall (mean time to service), sample throughput, consumable components (TMP, ion injector)

Ease-of-Use - Initial set-up / post-sales support (e.g. method development), training, fit into workflow, software

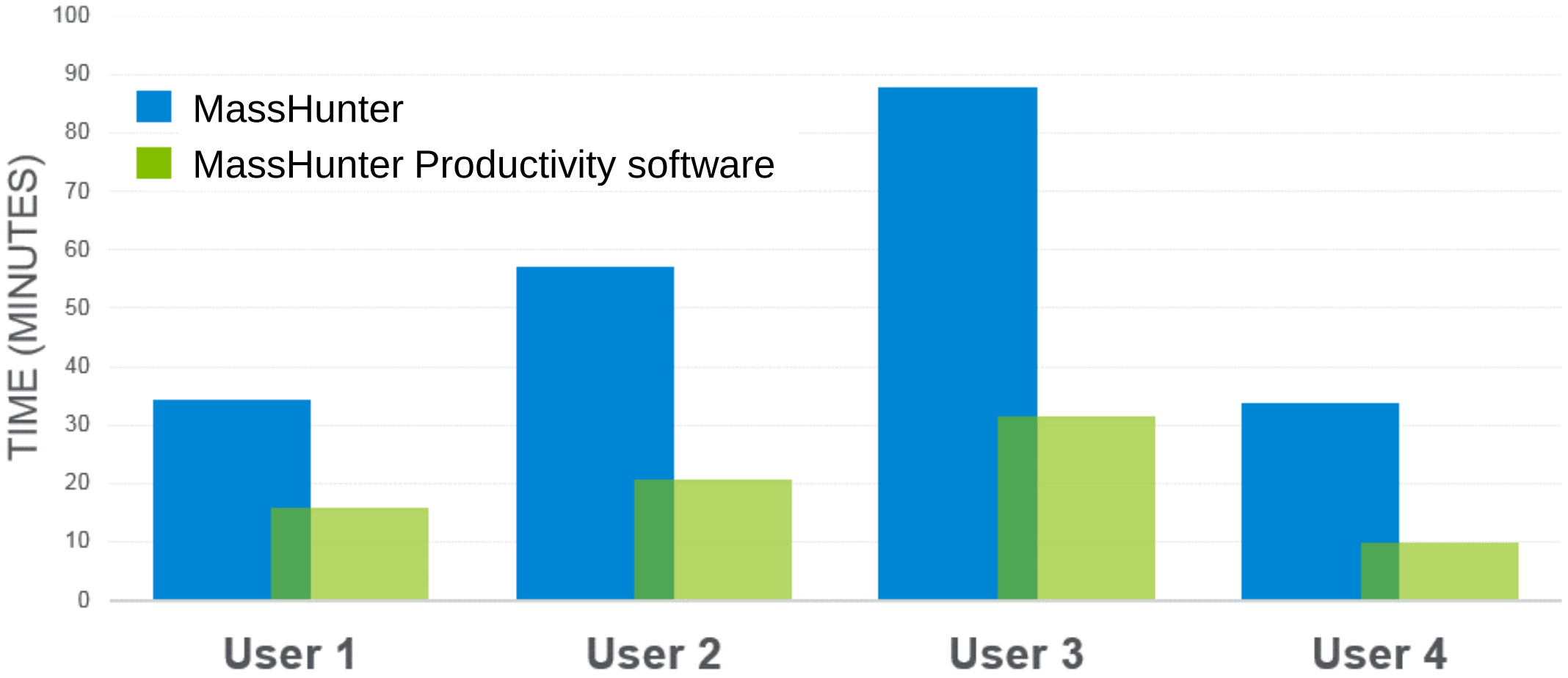
Performance – Sensitivity, selectivity, throughput, positive/ negative switching, mass range / resolution, matrix

Total Cost of Ownership – Initial cost, servicing cost, gas usage, training, noise reduction

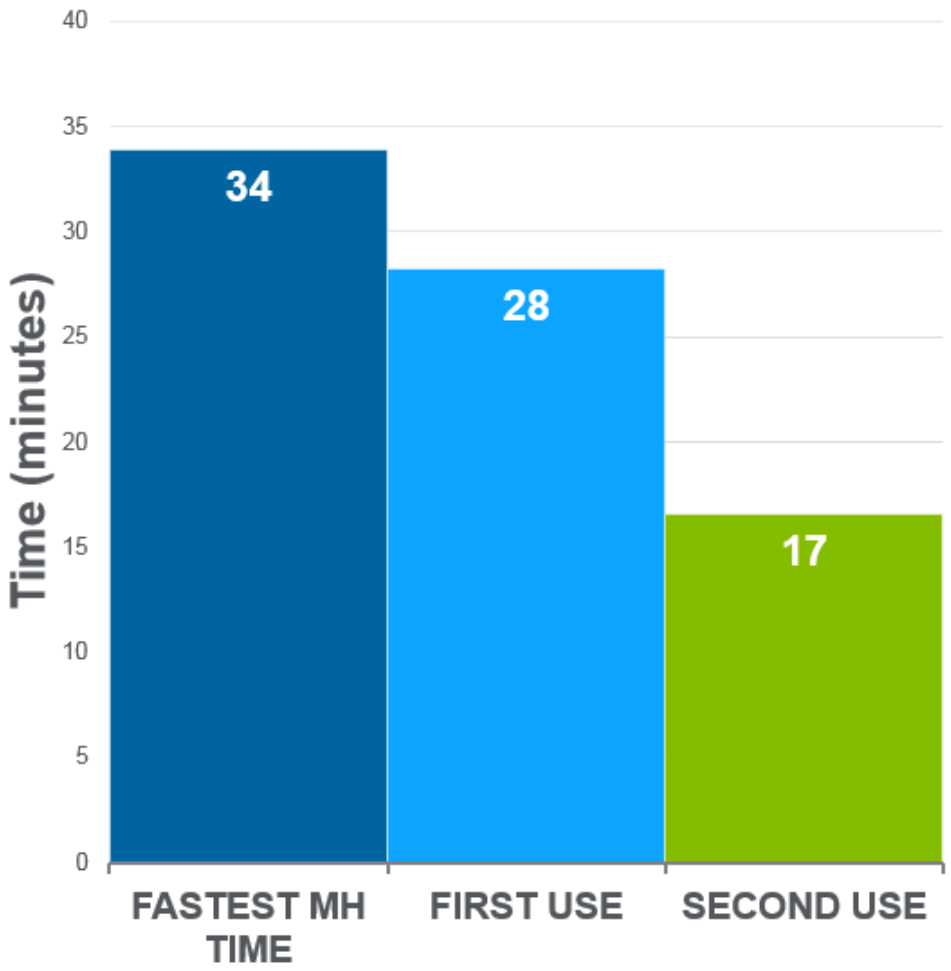
MassHunter is frequently the right tool…


Agilent MassHunter Qualitative Analysis Workflows B.08.00 - Default.m
File Launch Edit View Find Identify Method Configuration Tools Help
Sample Table: Cal 50 ppb.d
Compound List: 55 found
Method Editor: Workflow
Run Method Workflow
Method Automation
Workflow
Additional Chromatograms
Reports
Export
Target/Suspect Screening
MS Extraction
Options
Time Range(s)
Target/Suspect Screening
Find by MRM
Sample Chromatogram Results
+/-ESI TIC MRM (* -> *) Cal 50 ppb.d
3.416
Counts (%) vs. Acquisition Time (min)
Compound Chromatogram Results
Cpd 1: Flumetsulam 3.417: +ESI MRM Frag=135.0V CID@60.0 (326.0 -> 82.1) Cal 50 ppb.d
Cpd 1: Flumetsulam 3.417: +ESI MRM Frag=135.0V CID@64.0 (326.0 -> 109.1) Cal 50 ppb.d
Cpd 1: Flumetsulam 3.417: +ESI MRM Frag=135.0V CID@28.0 (326.0 -> 129.2) Cal 50 ppb.d
Counts (%) vs. Acquisition Time (min)
Compound Fragment Spectrum Results
Cpd 1: Flumetsulam 3.417: +ESI MRM (rt: 3.346-3.608 min, 107 scans) Frag=135.0V (326.0 -> *) Cal 50 ppb.d
129.2
109.1
82.1
262.1
Counts vs. Mass-to-Charge (m/z)

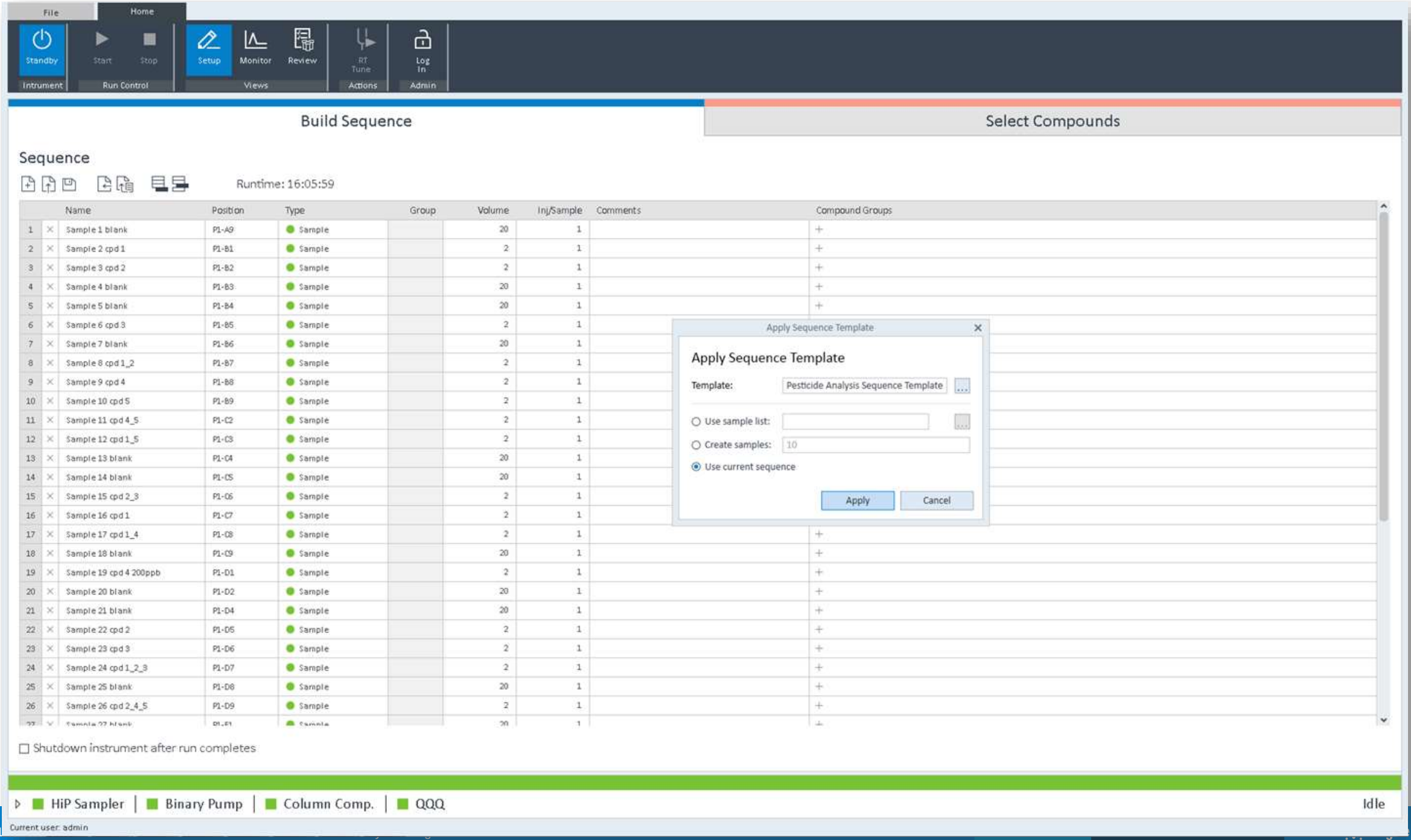
What about production laboratories?

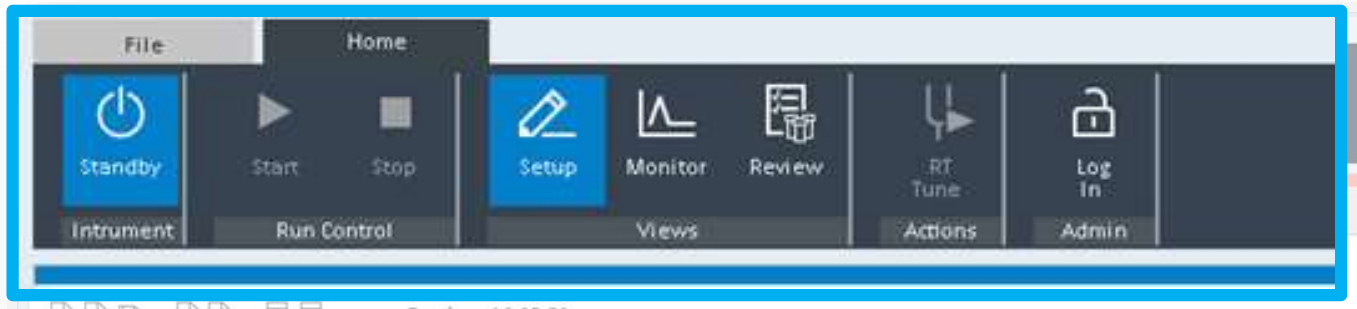


Time to execute standard targeted quant workflow



Increased productivity gains after only two uses


File
Home
Standby
Start
Stop
Setup
Monitor
Review
RT Tune
Log In
Intrument
Run Control
Views
Actions
Admin
Build Sequence
Select Compounds
Sequence
Runtime: 16:05:59
Name
Position
Type
Group
Volume
Inj/Sample
Comments
Compound Groups
Apply Sequence Template
Apply Sequence Template
Template:
Pesticide Analysis Sequence Template
Use sample list:
Create samples:
10
Use current sequence
Apply
Cancel
Shutdown instrument after run completes
HiP Sampler
Binary Pump
Column Comp.
QQQ
Idle
Current user: admin



Select Compounds

Runtime: 16:05:59

		Name	Position	Type	Group	Volume	Inj/Sample	Comments	Compound Groups
1		Sample 1 blank	P1-A9	Sample		20	1		
2		Sample 2 cpd 1	P1-B1	Sample		2	1		
3		Sample 3 cpd 2	P1-B2	Sample		2	1		
4		Sample 4 blank	P1-B3	Sample		20	1		
5		Sample 5 blank	P1-B4	Sample		20	1		
6		Sample 6 cpd 3	P1-B5	Sample		2	1		
7		Sample 7 blank	P1-B6	Sample		20	1		
8		Sample 8 cpd 1_2	P1-B7	Sample		2	1		
9		Sample 9 cpd 4	P1-B8	Sample		2	1		
10		Sample 10 cpd 5	P1-B9	Sample		2	1		
11		Sample 11 cpd 4_5	P1-C2	Sample		2	1		
12		Sample 12 cpd 1_5	P1-C3	Sample		2	1		
13		Sample 13 blank	P1-C4	Sample		20	1		
14		Sample 14 blank	P1-C5	Sample		20	1		
15		Sample 15 cpd 2_3	P1-C6	Sample		2	1		
16		Sample 16 cpd 1	P1-C7	Sample		2	1		
17		Sample 17 cpd 1_4	P1-C8	Sample		2	1		
18		Sample 18 blank	P1-C9	Sample		20	1		
19		Sample 19 cpd 4 200ppb	P1-D1	Sample		2	1		
20		Sample 20 blank	P1-D2	Sample		20	1		
21		Sample 21 blank	P1-D4	Sample		20	1		
22		Sample 22 cpd 2	P1-D5	Sample		2	1		
23		Sample 23 cpd 3	P1-D6	Sample		2	1		
24		Sample 24 cpd 1_2_3	P1-D7	Sample		2	1		
25		Sample 25 blank	P1-D8	Sample		20	1		
26		Sample 26 cpd 2_4_5	P1-D9	Sample		2	1		

Apply Sequence Template

Apply Sequence Template

Template: Pesticide Analysis Sequence Template

Use sample list:

Create samples: 10

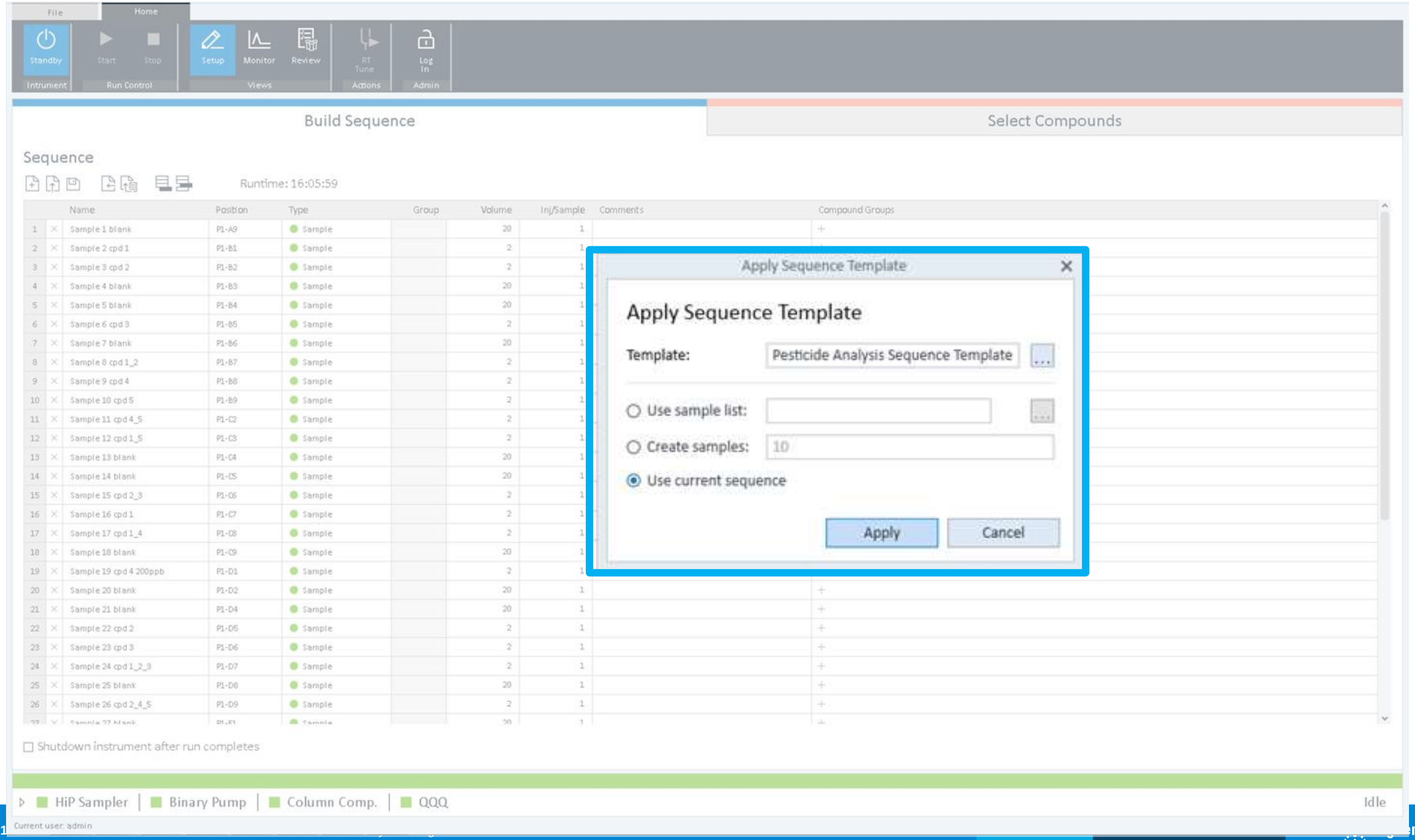
Use current sequence

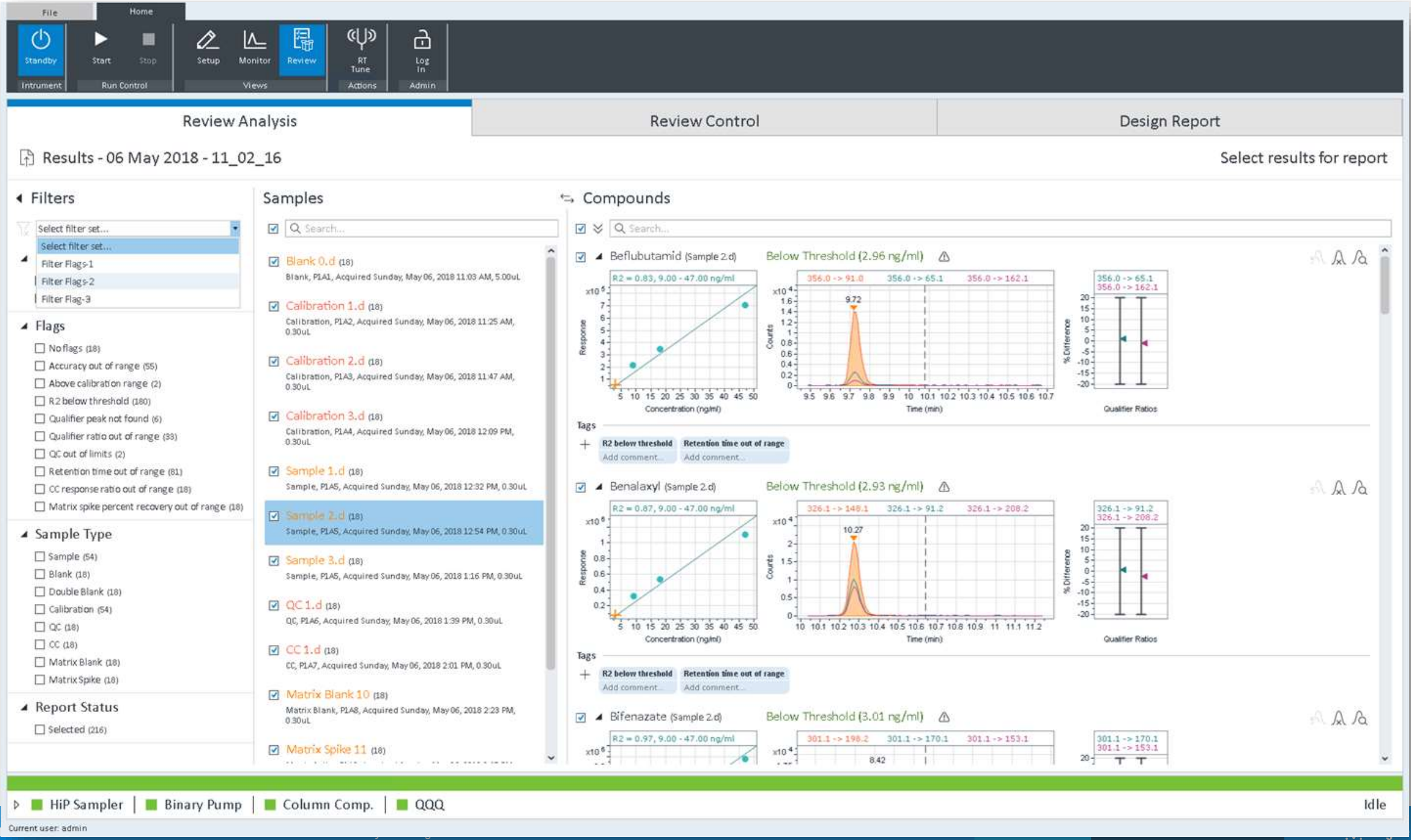
Apply Cancel

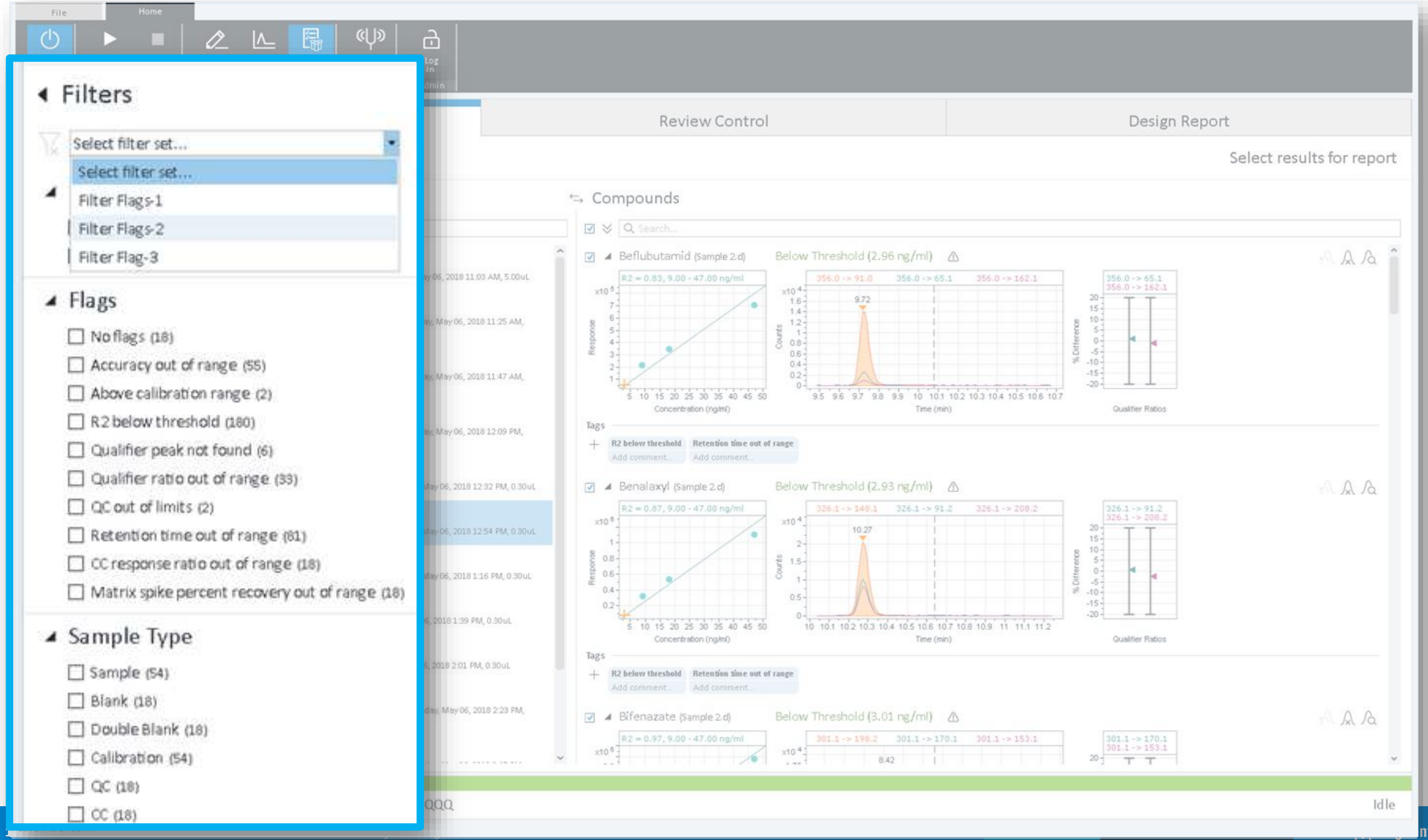
Shutdown instrument after run completes

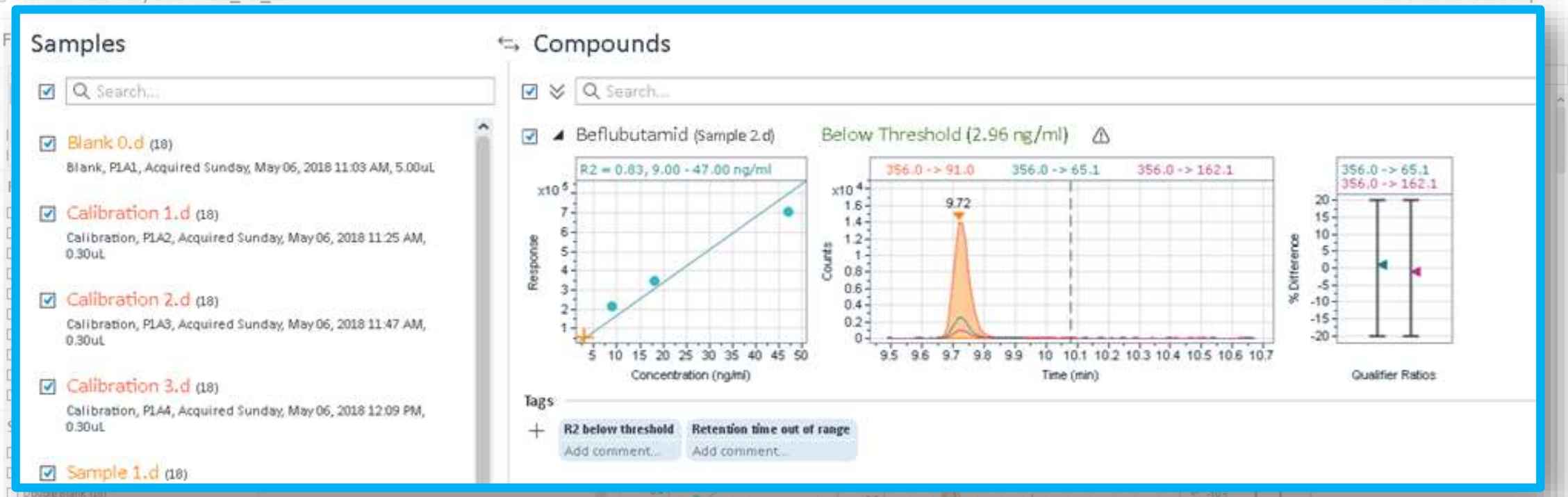
HiP Sampler | Binary Pump | Column Comp. | QQQ Idle

Current user: admin


File
Home
Standby
Start
Stop
Setup
Monitor
Review
RT Tune
Log In
Intrument
Run Control
Views
Actions
Admin
Build Sequence
Select Compounds
Sequence
Runtime: 16:05:59
Name
Position
Type
Group
Volume
Inj/Sample
Comments
Compound Groups
Apply Sequence Template
Apply Sequence Template
Template:
Pesticide Analysis Sequence Template
Use sample list:
Create samples:
10
Use current sequence
Apply
Cancel
Shutdown instrument after run completes
HiP Sampler
Binary Pump
Column Comp.
QQQ
Idle
Current user: admin


File
Home
Standby
Start
Stop
Setup
Monitor
Review
RT Tune
Log In
Intrument
Run Control
Views
Actions
Admin
Review Analysis
Review Control
Design Report
Results - 06 May 2018 - 11_02_16
Select results for report
Filters
Select filter set...
Select filter set...
Filter Flags-1
Filter Flags-2
Filter Flag-3
Flags
No flags (18)
Accuracy out of range (55)
Above calibration range (2)
R2 below threshold (180)
Qualifier peak not found (6)
Qualifier ratio out of range (33)
QC out of limits (2)
Retention time out of range (81)
CC response ratio out of range (18)
Matrix spike percent recovery out of range (18)
Sample Type
Sample (54)
Blank (18)
Double Blank (18)
Calibration (54)
QC (18)
CC (18)
Matrix Blank (18)
Matrix Spike (18)
Report Status
Selected (216)
Samples
Search...
Blank 0.d (18)
Blank, P1A1, Acquired Sunday, May 06, 2018 11:03 AM, 5.00uL
Calibration 1.d (18)
Calibration, P1A2, Acquired Sunday, May 06, 2018 11:25 AM, 0.30uL
Calibration 2.d (18)
Calibration, P1A3, Acquired Sunday, May 06, 2018 11:47 AM, 0.30uL
Calibration 3.d (18)
Calibration, P1A4, Acquired Sunday, May 06, 2018 12:09 PM, 0.30uL
Sample 1.d (18)
Sample, P1A5, Acquired Sunday, May 06, 2018 12:32 PM, 0.30uL
Sample 2.d (18)
Sample, P1A5, Acquired Sunday, May 06, 2018 12:54 PM, 0.30uL
Sample 3.d (18)
Sample, P1A5, Acquired Sunday, May 06, 2018 1:16 PM, 0.30uL
QC 1.d (18)
QC, P1A6, Acquired Sunday, May 06, 2018 1:39 PM, 0.30uL
CC 1.d (18)
CC, P1A7, Acquired Sunday, May 06, 2018 2:01 PM, 0.30uL
Matrix Blank 10 (18)
Matrix Blank, P1A8, Acquired Sunday, May 06, 2018 2:23 PM, 0.30uL
Matrix Spike 11 (18)
Compounds
Search...
Beflubutamid (Sample 2.d)
Below Threshold (2.96 ng/ml)
R2 = 0.83, 9.00 - 47.00 ng/ml
Response
Concentration (ng/ml)
356.0 -> 91.0
356.0 -> 65.1
356.0 -> 162.1
9.72
Counts
Time (min)
% Difference
Qualifier Ratios
Tags
R2 below threshold
Retention time out of range
Add comment...
Benalaxyl (Sample 2.d)
Below Threshold (2.93 ng/ml)
R2 = 0.87, 9.00 - 47.00 ng/ml
326.1 -> 148.1
326.1 -> 91.2
326.1 -> 208.2
10.27
Bifenazate (Sample 2.d)
Below Threshold (3.01 ng/ml)
R2 = 0.97, 9.00 - 47.00 ng/ml
301.1 -> 198.2
301.1 -> 170.1
301.1 -> 153.1
8.42
HiP Sampler
Binary Pump
Column Comp.
QQQ
Idle
Current user: admin


File
Home
Filters
Select filter set...
Select filter set...
Filter Flags-1
Filter Flags-2
Filter Flag-3
Flags
No flags (18)
Accuracy out of range (55)
Above calibration range (2)
R2 below threshold (180)
Qualifier peak not found (6)
Qualifier ratio out of range (33)
QC out of limits (2)
Retention time out of range (81)
CC response ratio out of range (18)
Matrix spike percent recovery out of range (18)
Sample Type
Sample (54)
Blank (18)
Double Blank (18)
Calibration (54)
QC (18)
CC (18)
Review Control
Design Report
Select results for report
Compounds
Beflubutamid (Sample 2.d)
Below Threshold (2.96 ng/ml)
Benalaxyl (Sample 2.d)
Below Threshold (2.93 ng/ml)
Bifenazate (Sample 2.d)
Below Threshold (3.01 ng/ml)
Tags
R2 below threshold
Retention time out of range
Idle


Samples
Search...
Blank 0.d (18)
Blank, P1A1, Acquired Sunday, May 06, 2018 11:03 AM, 5.00uL
Calibration 1.d (18)
Calibration, P1A2, Acquired Sunday, May 06, 2018 11:25 AM, 0.30uL
Calibration 2.d (18)
Calibration, P1A3, Acquired Sunday, May 06, 2018 11:47 AM, 0.30uL
Calibration 3.d (18)
Calibration, P1A4, Acquired Sunday, May 06, 2018 12:09 PM, 0.30uL
Sample 1.d (18)
Compounds
Search...
Beflubutamid (Sample 2.d)
Below Threshold (2.96 ng/ml)
R2 = 0.83, 9.00 - 47.00 ng/ml
Response
Concentration (ng/ml)
356.0 -> 91.0
356.0 -> 65.1
356.0 -> 162.1
9.72
Counts
Time (min)
% Difference
Qualifier Ratios
Tags
R2 below threshold
Add comment...
Retention time out of range
Add comment...

File | Home

Standby | Start | Stop | Setup | Monitor | Review | Log In

Intrument | Run Control | Views | Admin

Review Analysis | Review Control | Design Report

Report Options

Template

Default Report

Options

☐ Generate chromatogram images

Report Preview

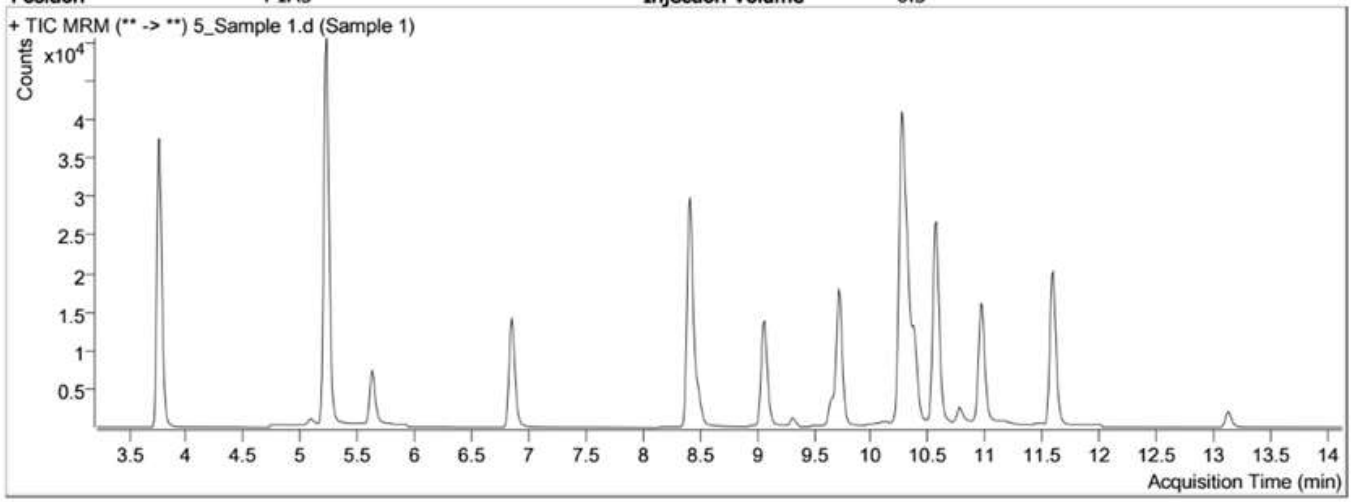
9 /24 | 98 % | Find:

Bookmarks

- Blank 0 (1_Blank 0.d)
- Calibration 1 (2_Calibration 1.d)
- Calibration 2 (3_Calibration 2.d)
- Calibration 3 (4_Calibration 3.d)
- Sample 1 (5_Sample 1.d)
- Sample 2 (6_Sample 2.d)
- Sample 3 (7_Sample 3.d)
- QC1 (8_QC1.d)
- CC1 (9_CC1.d)
- Matrix Blank 10 (10_Matrix Blank 10.d)
- Matrix Spike 11 (11_Matrix Spike 11.d)
- Double Blank 9 (12_Double Blank 9.d)

Analysis Info

Instrument	Instrument 1	**Operator**	
Data File	5_Sample 1.d	**Sample Name**	Sample 1
Sample Type	Sample	**Dilution**	1
Acq. Method	AcquisitionTempMethod.m	**Acq. Date**	5/6/2018 12:32 PM
Position	P1A5	**Injection Volume**	0.3



Compound	RT	Ref RT	Transition(T)	Transition(Q)	T-Resp	Q-Resp	QRatio	Ref QRatio	Final Conc.	Units
Dimethoate	3.756	3.820	230.0 -> 125.0	230.0 -> 199.0	41325	36083	87.3	87.8	3.1821	ng/ml
R2 below threshold										
Dichlorvos	5.095	5.200	221.0 -> 109.0	221.0 -> 79.0	1888	242	12.8	12.2	3.5974	ng/ml
Carbofuran	5.232	5.340	222.1 -> 123.1	222.1 -> 165.1	79339	71692	90.4	90.2	2.9662	ng/ml
R2 below threshold										
Imazalil	5.629	5.700	297.0 -> 159.0	297.0 -> 255.1	9571	4873	50.9	52.6	3.1928	ng/ml
R2 below threshold										

HiP Sampler | Binary Pump | Column Comp. | QQQ | Idle

Current user: admin

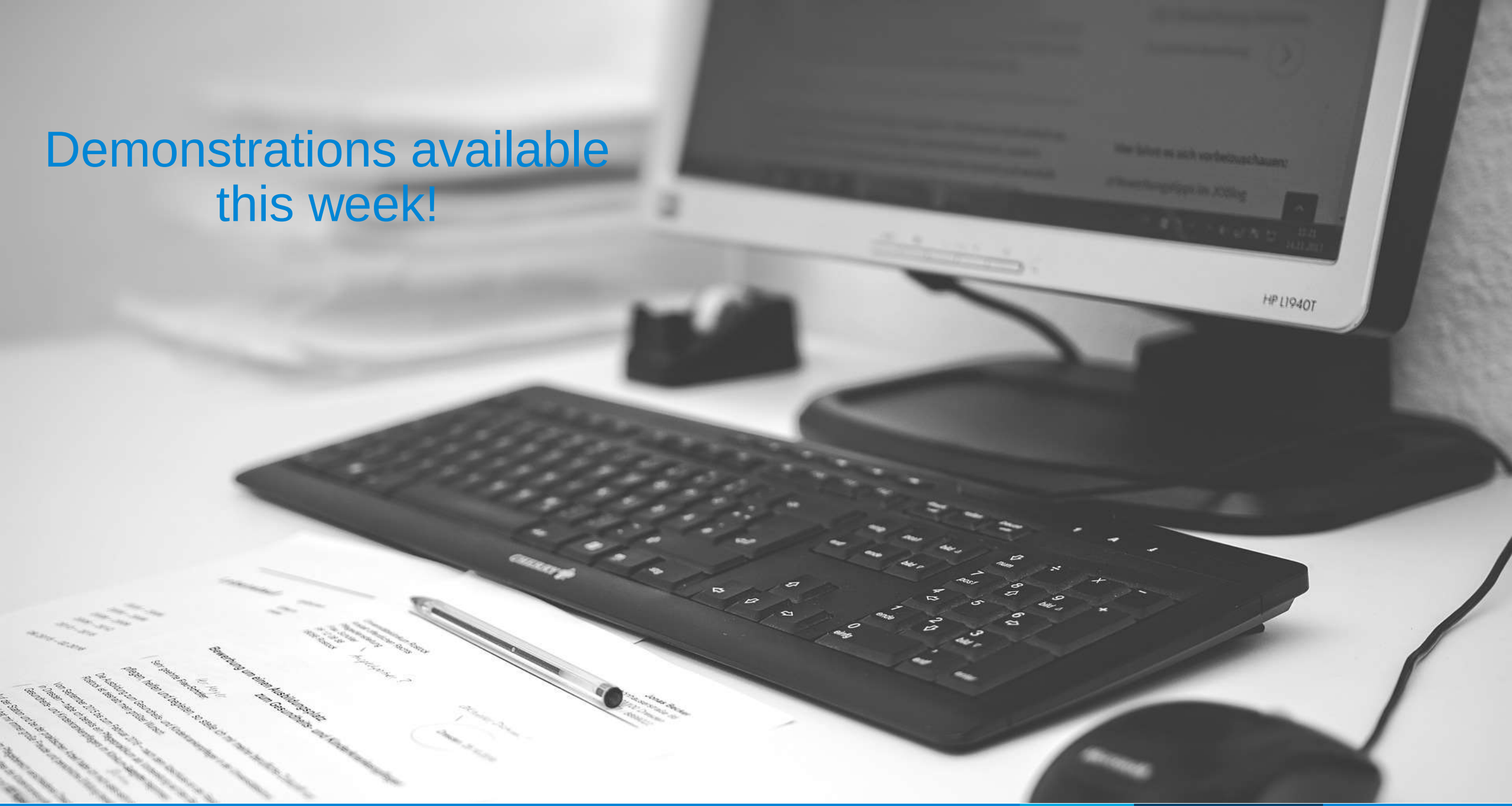
MassHunter Productivity Software Application

Targeted Quant with Pesticides MRM database

Use your methods and MRM database or use ours!

- MassHunter Productivity Software
- Pesticides dMRM DB for LC/TQ
  - >600 commonly used pesticides with RT
  - 2-4 optimized MRM transitions per compound
  - 3-year free updates
- Workflow Guide
- Acquisition and Quant Methods
- Suite of Customizable Report Templates
- Optional Consumables

Demonstrations available this week!



Analytical Determination of Small Molecules Drugs in Serum and Amino Acids in Animal Feed Matrices using Ultivo LC/TQ

Yanan Yang, PhD
LCMS Application Scientist
Agilent Technologies, Inc.
Santa Clara, CA

Agilent
Trusted Answers

Sample Preparation and LCMS Method

Sample Preparation

- 0.1 g homogenized animal feed sample was weighed in a 250 mL Erlenmeyer flask and hydrolyzed using 25 mL 6N HCl with phenol.
- Incubation under nitrogen flow for 23 hours.
- 0.1N HCl was filled to 50 mL and mixed. Aliquots were transferred to sample vials for introduction into the LC/TQ system.

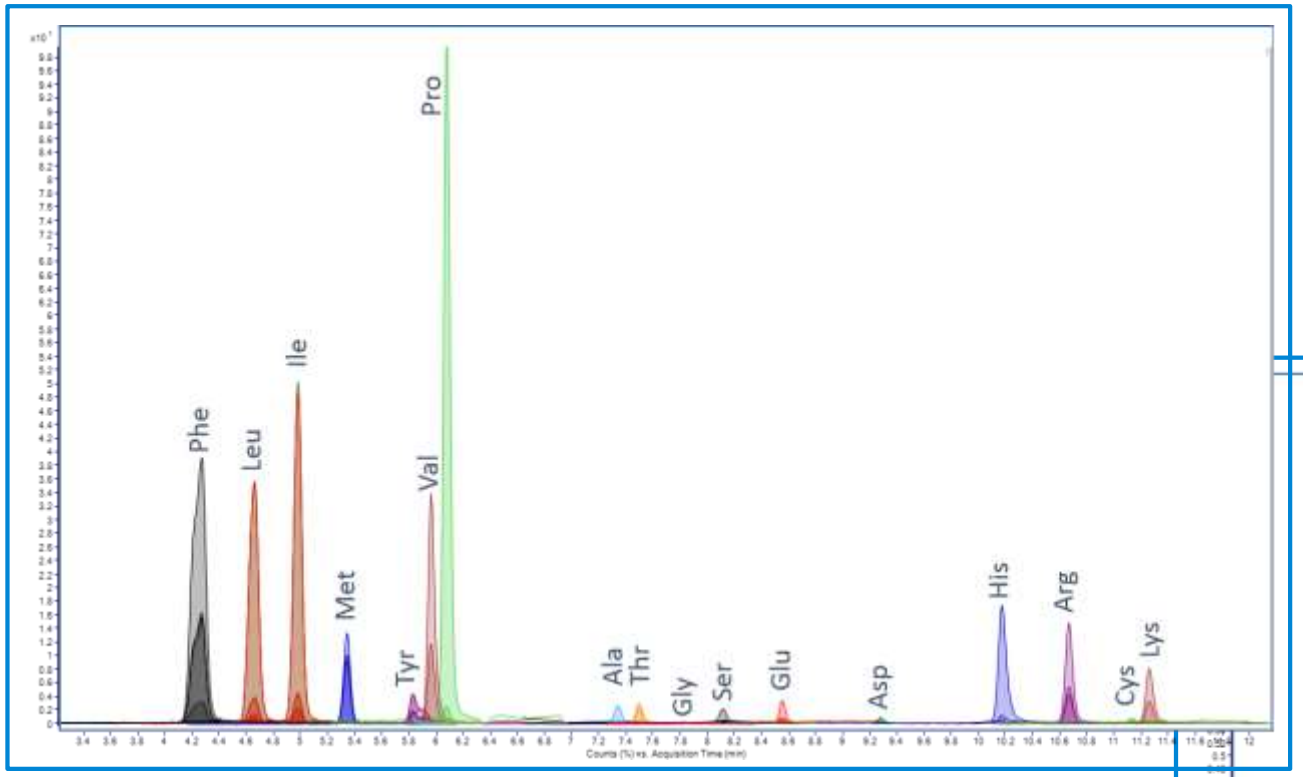
Column	Poroshell 120 HILIC-Z 2.1 x 150 mm, 2.7µm (p/n 6x3775-924)	
Column temp	25°C	
Injection volume	2 µL	
Mobile phase	A: 20 mM ammonium acetate + 0.1% formic acid in water, pH 3 B: 20 mM ammonium acetate in 90% acetonitrile, pH 3	
Flow rate	0.50 mL/min	
Gradient	Time 0 11.5 12.0 15.0 19.0	B% 100 70 100 100 *stop-time* 100 *post time*
Drying gas temp	150 °C	
Drying gas flow	15 L/min	
Sheath gas temp	400 °C	
Sheath gas flow	12 L/min	
Nebulizer pressure	20 psi	
Capillary voltage	2000 V(+)	
Nozzle voltage	0 V(+)	
Cycle Time	500 ms	

MRM Transition List

Compound Name	Precursor (m/z)	Product (m/z)	RT (min)	RT Window (min)	Fragmentor (V)	CE (V)
Alanine	90.1	62.1	6.83	2	75	0
Alanine	90.1	44.1	6.83	2	75	0
Arginine	175.1	116.1	10.43	2	105	2
Arginine	175.1	70.1	10.43	2	105	8
Arginine	175.1	60.1	10.43	2	105	4
Aspartic Acid	134.1	88.0	9.03	2	75	0
Aspartic Acid	134.1	74.0	9.03	2	75	4
Aspartic Acid	134.1	70.0	9.03	2	75	6
Cystine	241.0	241.0	11.16	2	105	0
Cystine	241.0	152.0	11.16	2	105	0
Cystine	241.0	120.0	11.16	2	105	0
Cystine	241.0	74.1	11.16	2	105	25
Glutamic Acid	148.1	148.1	8.27	2	85	0
Glutamic Acid	148.1	84.0	8.27	2	85	6
Glutamic Acid	148.1	56.1	8.27	2	85	22
Glutamic Acid	148.1	41.0	8.27	2	85	18
Glycine	76.0	48.0	7.36	2	65	0
Glycine	76.0	30.0	7.36	2	65	0
Histidine	156.1	110.1	9.81	2	95	4
Histidine	156.1	95.1	9.81	2	95	6
Isoleucine	132.1	86.1	4.90	2	85	0
Isoleucine	132.1	44.1	4.90	2	85	16
Isoleucine	132.1	41.0	4.90	2	85	18
Isoleucine	132.1	30.0	4.90	2	85	6
Leucine	132.1	86.1	4.62	2	85	0

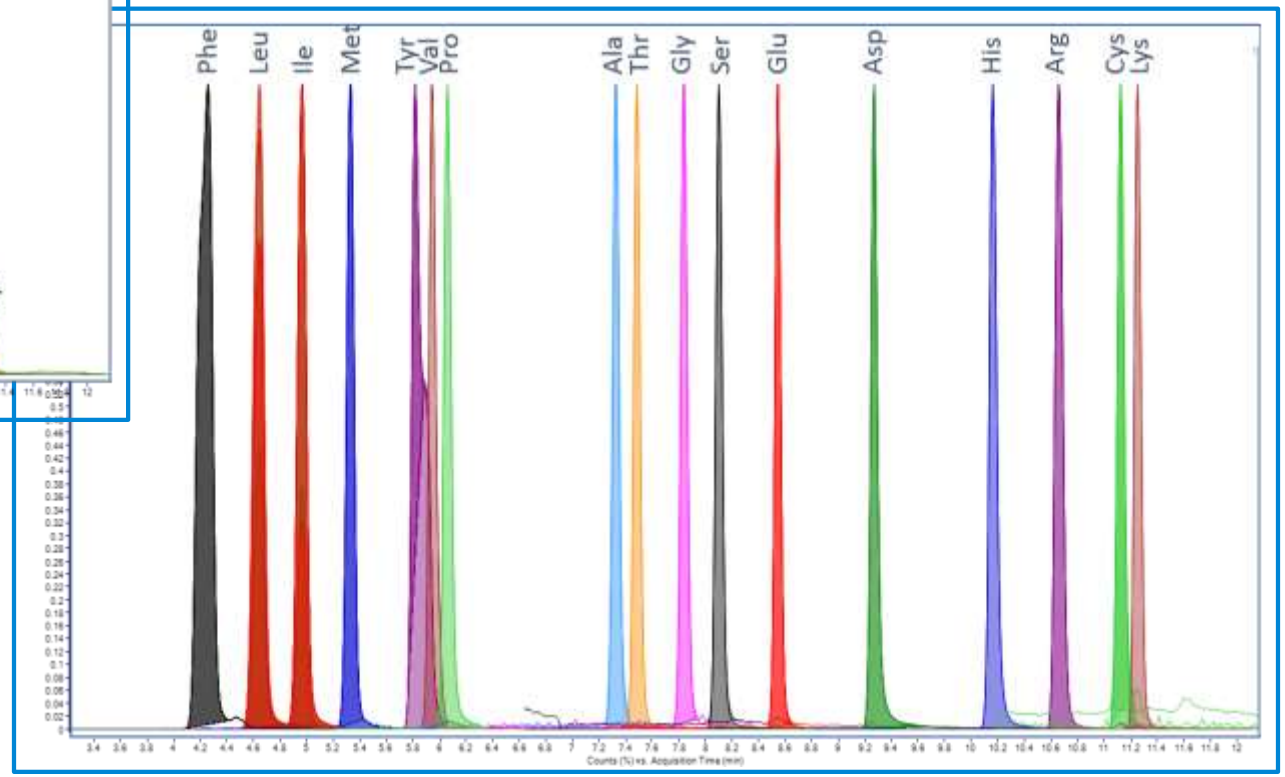
Compound Name	Precursor (m/z)	Product (m/z)	RT (min)	RT Window (min)	Fragmentor (V)	CE (V)
Leucine	132.1	44.1	4.62	2	85	14
Leucine	132.1	41.0	4.62	2	85	25
Leucine	132.1	30.0	4.62	2	85	4
Lysine	147.1	130.1	11.12	2	85	0
Lysine	147.1	84.1	11.12	2	85	6
Methionine	150.1	104.1	5.16	2	75	0
Methionine	150.1	61.0	5.16	2	75	14
Methionine	150.1	56.1	5.16	2	75	6
Methionine	150.1	28.0	5.16	2	75	26
Phenylalanine	166.1	120.1	4.23	2	85	4
Phenylalanine	166.1	103.1	4.23	2	85	22
Phenylalanine	166.1	91.1	4.23	2	85	32
Phenylalanine	166.1	77.0	4.23	2	85	36
Proline	116.1	70.1	6.01	2	85	6
Proline	116.1	43.1	6.01	2	85	25
Serine	103.1	88.1	7.63	2	65	0
Serine	103.1	60.1	7.63	2	65	0
Threonine	120.0	74.1	6.98	2	75	0
Threonine	120.0	56.1	6.98	2	75	6
Tyrosine	182.1	136.1	5.53	2	95	0
Tyrosine	182.1	119.1	5.53	2	95	10
Tyrosine	182.1	91.1	5.53	2	95	22
Tyrosine	182.1	77.0	5.53	2	95	34
Valine	118.1	72.1	5.84	2	75	0
Valine	118.1	55.1	5.84	2	75	14

Composite dMRM Chromatogram of Amino Acids

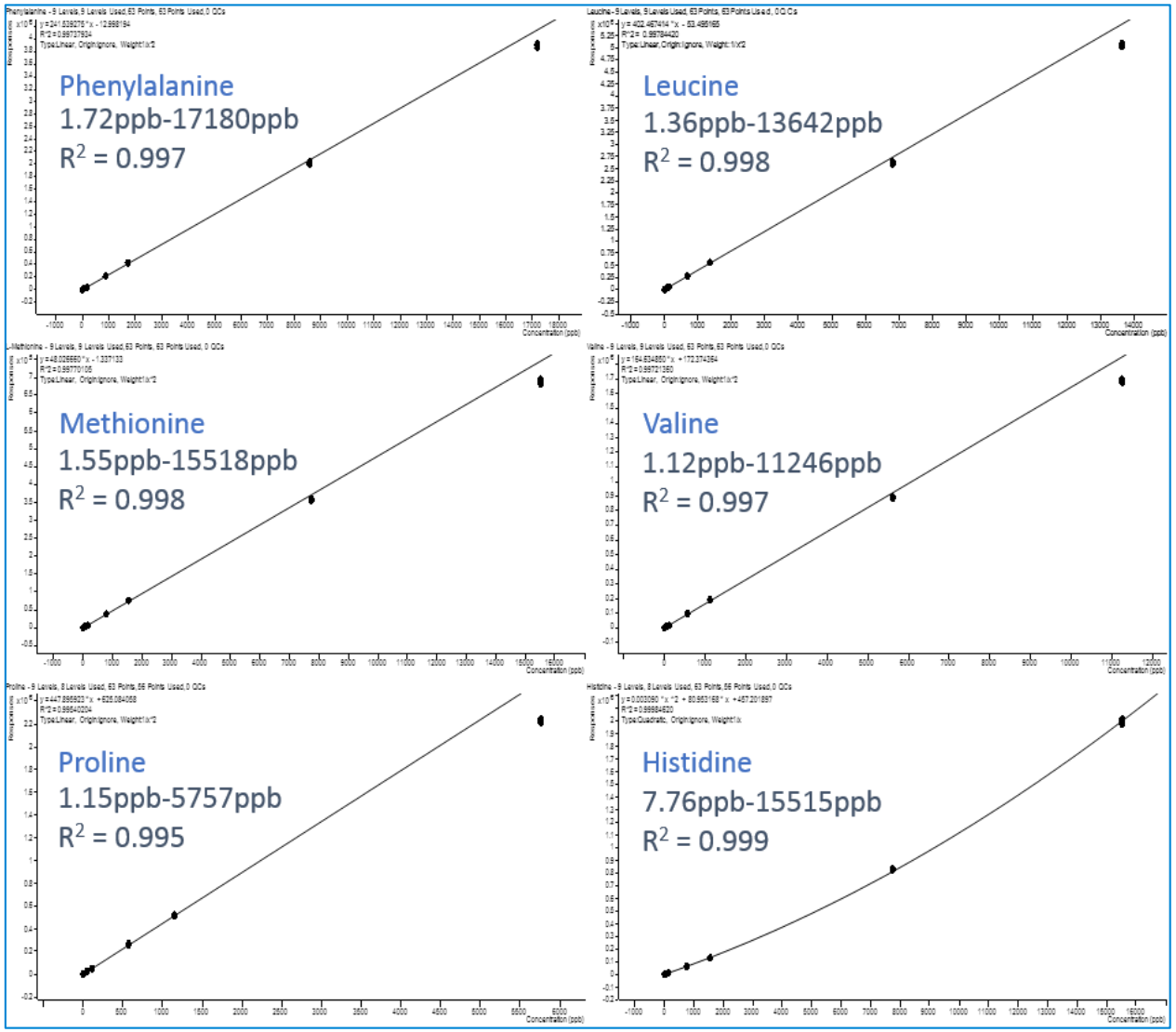


Overlaid dMRM chromatograms of amino acids

Peak height normalized

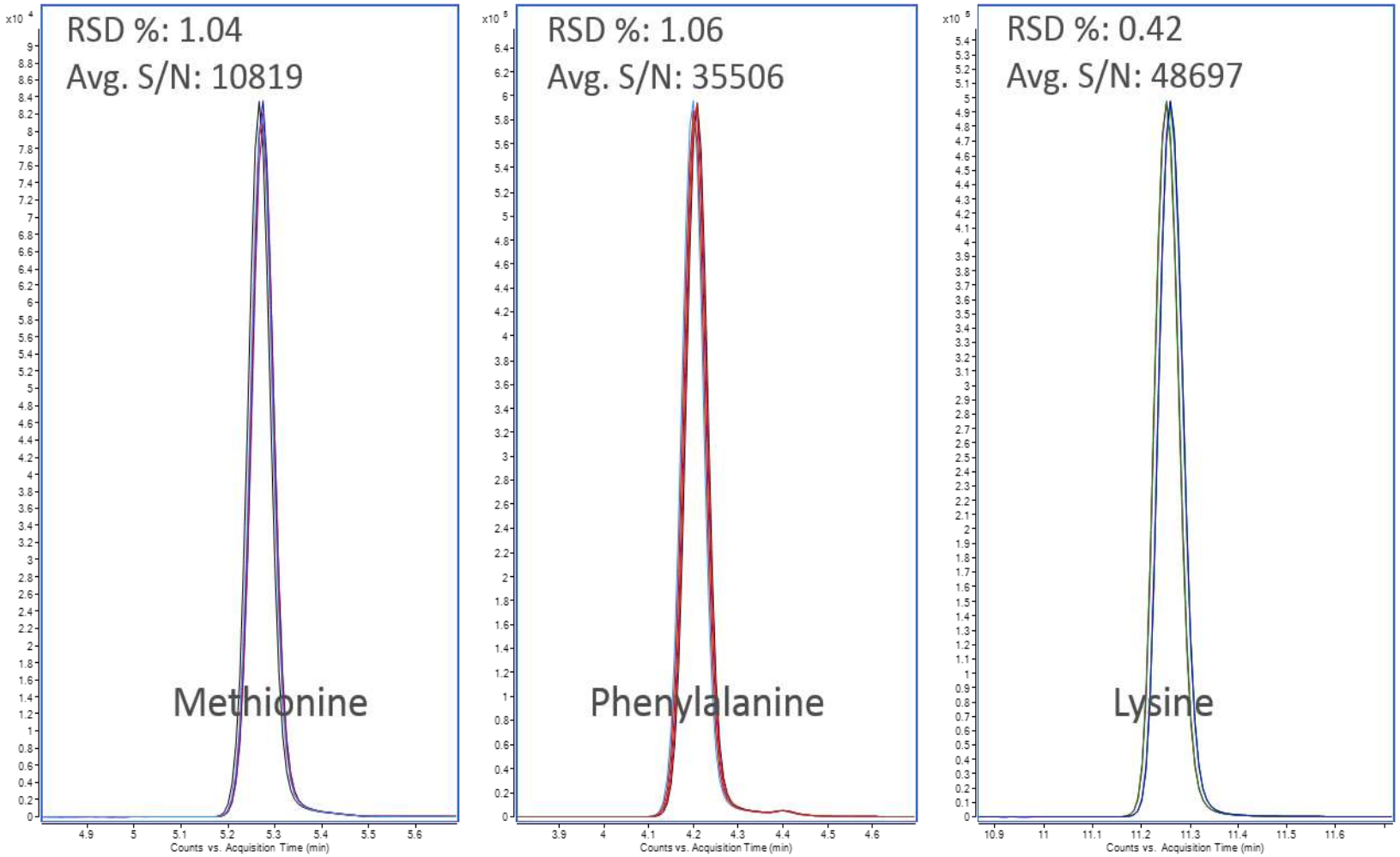


Calibration Curves and LOQs

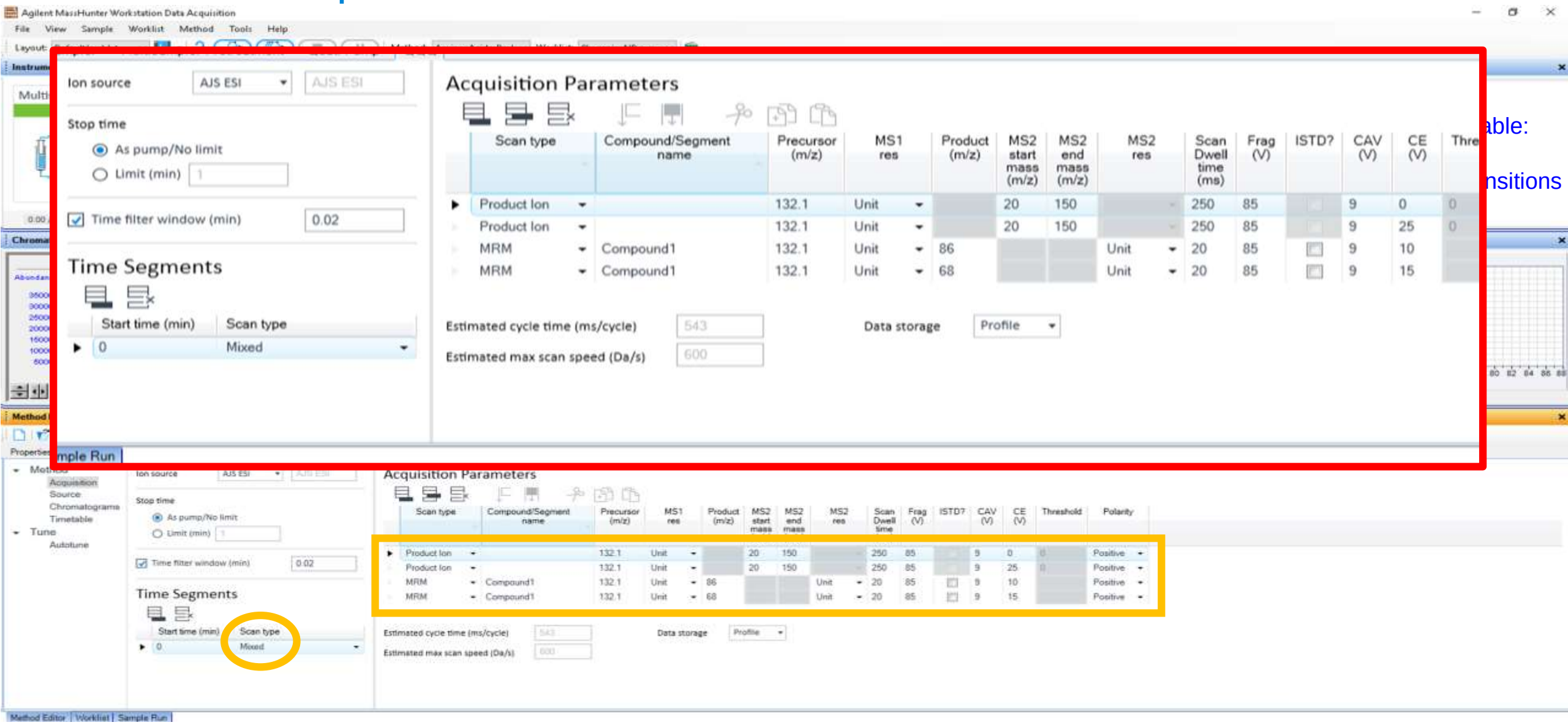


Compound Name	Curve Fit	R^2	LOQ (ppb)	S/N at LOQ
Alanine	Linear	0.9997	46.33	4.35
Arginine	Quadratic	0.9920	8.71	10.75
Aspartic Acid	Linear	0.9971	13.31	5.76
Cystine	Linear	0.9969	61.04	13.51
Glutamic Acid	Quadratic	0.9998	7.65	5.37
Glycine	Linear	0.9986	75.07	3.51
Histidine	Quadratic	0.9998	7.76	15.92
Isoleucine	Linear	0.9961	1.36	5.88
Leucine	Linear	0.9978	1.36	4.03
Lysine	Quadratic	0.9934	7.60	18.94
Methionine	Linear	0.9977	1.55	14.57
Phenylalanine	Linear	0.9974	1.72	4.82
Proline	Linear	0.9954	1.15	6.03
Serine	Quadratic	0.9987	10.51	5.43
Threonine	Linear	0.9995	11.91	4.35
Tyrosine	Linear	0.9943	1.88	3.86
Valine	Linear	0.9972	1.12	6.19

Reproducibility: Six Replicates in Sample Matrix



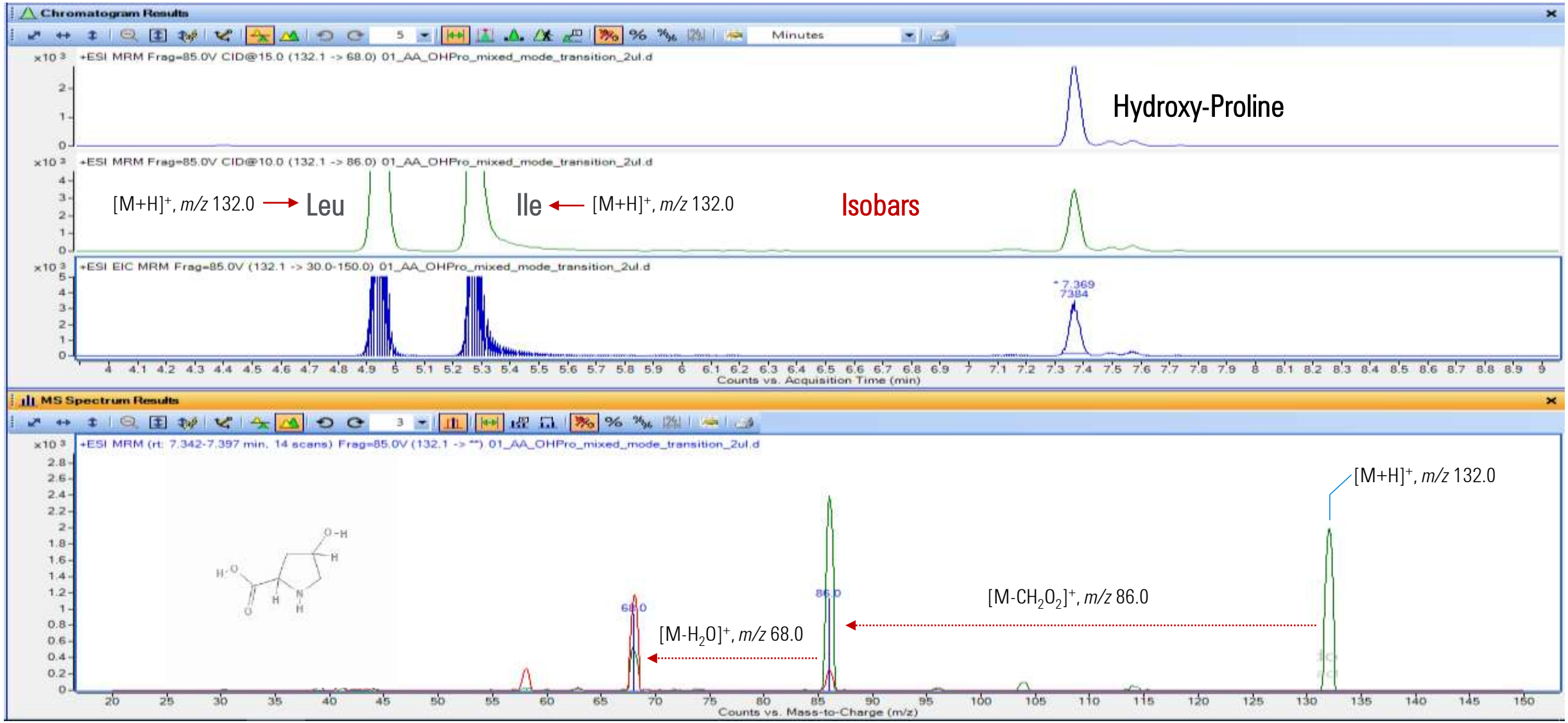
New Ultivo Acquisition Feature: Mixed Mode



able:

nsitions

Mixed-Mode Acquisition: Hydroxyproline MRM and Product Ion Scan



Eight Small Molecule Drugs in Serum

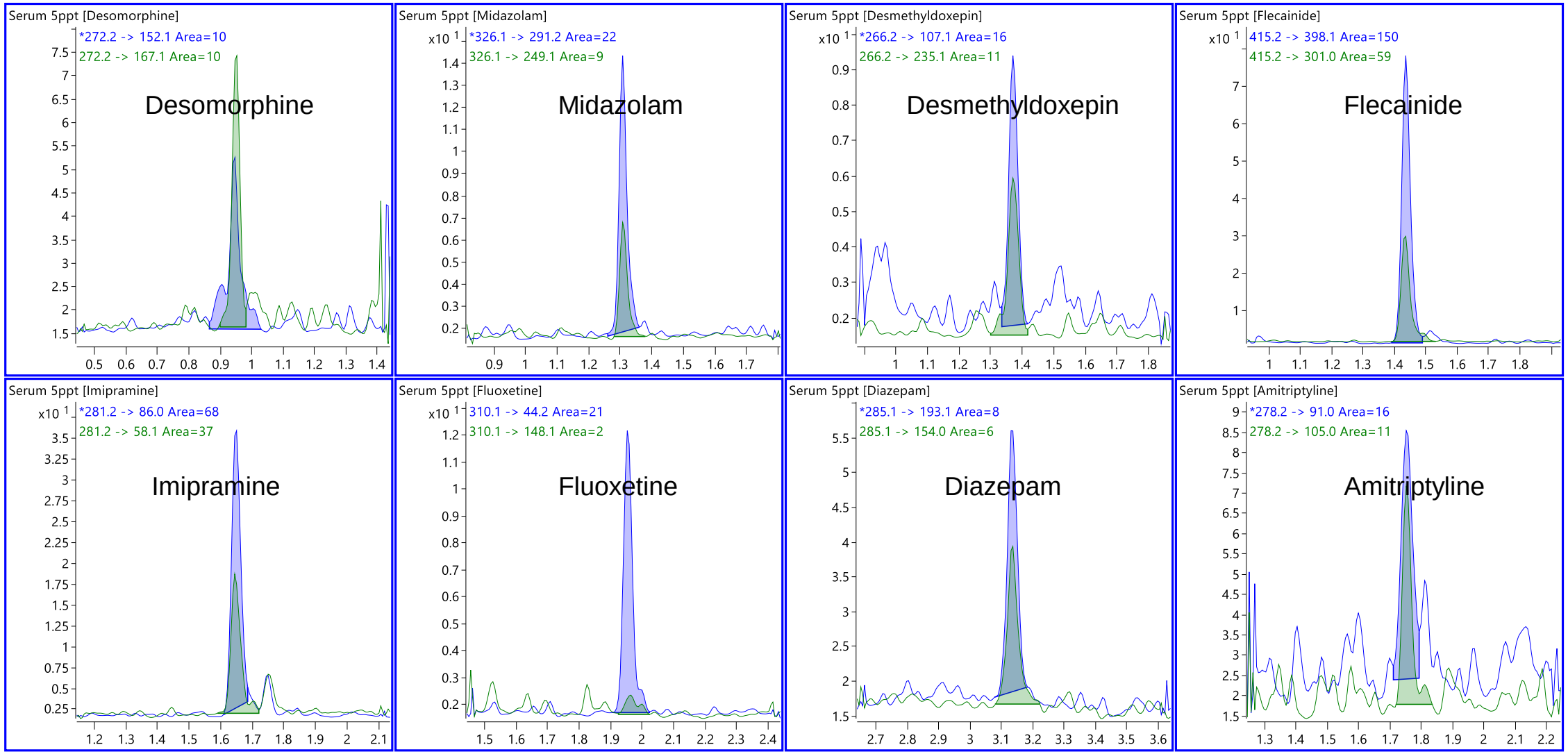
Sample Preparation

- 250 uL human serum was crashed with 500 uL acetonitrile, vortexed for 1 min and centrifuged for 4 min at 10,000 rpm.
- 500 uL supernatant was transferred and diluted with 500 uL of water.
- The 11-point calibration ranges of all drugs was from 0.001 to 100 ng/mL with 25 ng/mL sufficient internal standard across all sample types injected.

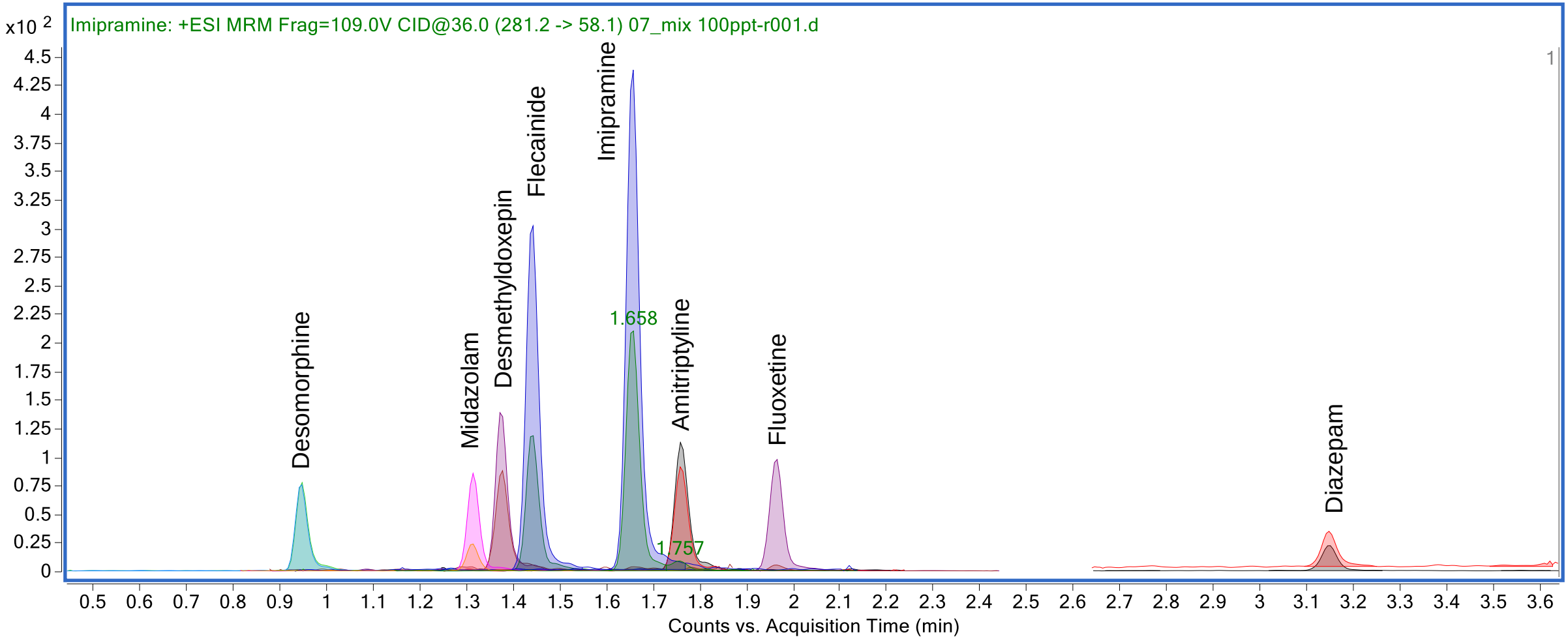
Column	Zorbax Eclipse Plus C8, 2.1 x 100 mm, 1.8 µm (p/n 959758-906)	
Column temp	40°C	
Injection volume	2 µL	
Mobile phase	A: Water B: Acetonitrile	
Flow rate	0.6 mL/min	
Gradient	Time 0 0.5 2 3 3.5 3.51 4 5	B% 5 35 40 50 75 95 95 (stop time) (post time)

Drying gas temp	200 °C
Drying gas flow	11 L/min
Sheath gas temp	400 °C
Sheath gas flow	12 L/min
Nebulizer pressure	35 psi
Capillary voltage	2750 V(+)
Nozzle voltage	0 V(+)
Cycle Time	350 ms

LODs: 5 pg/mL in dilute and shoot serum



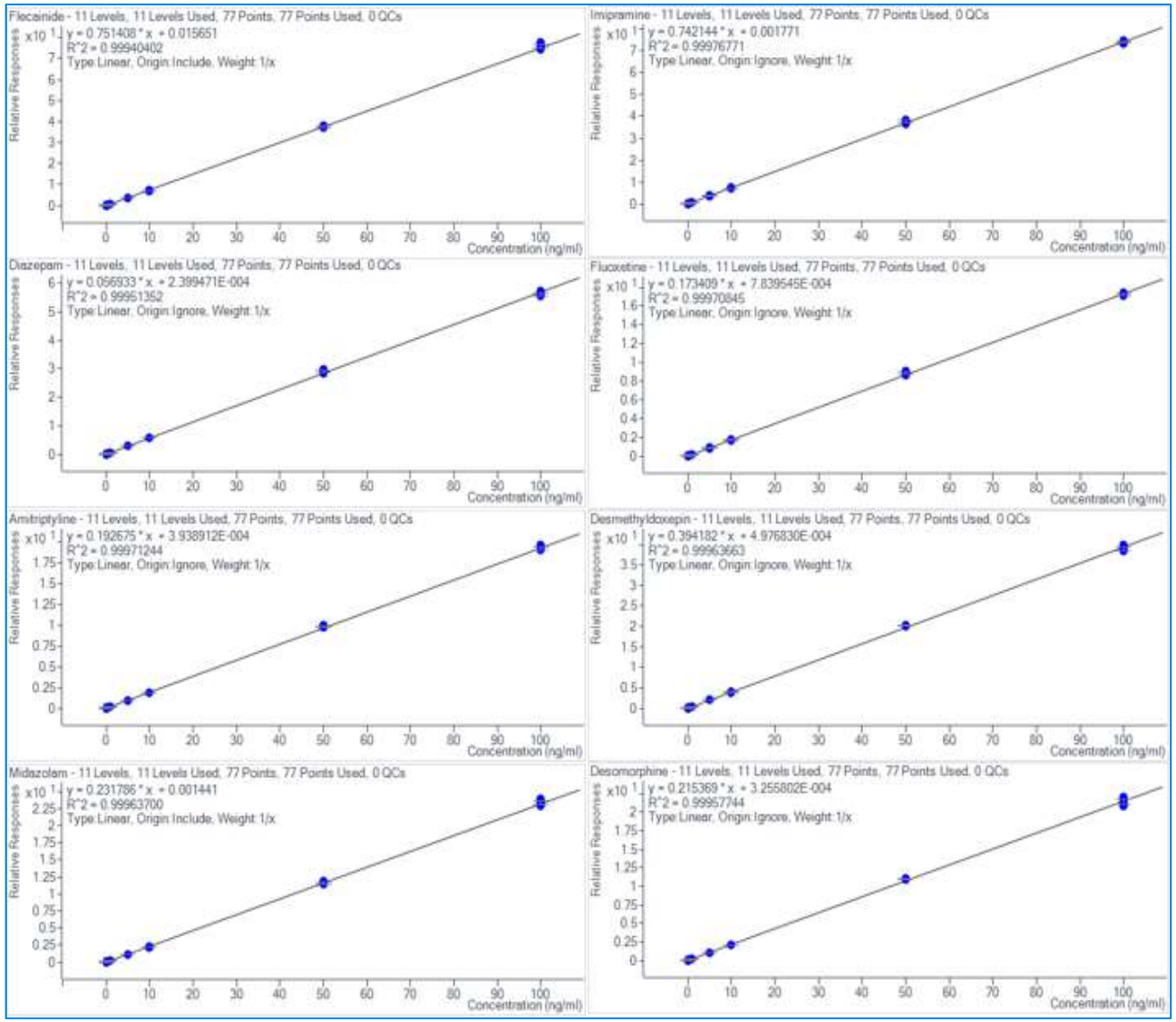
Analytical Analysis of Drugs in Human Serum: 0.1 ng/mL



Accuracy and Reproducibility (n=7)

Level	Desomorphine		Midazolam		Desmethyldoxepin		Flecainide		Imipramine		Amitriptyline		Fluoxetine		Diazepam	
ng/mL	Accuracy	%RSD	Accuracy	%RSD	Accuracy	%RSD	Accuracy	%RSD	Accuracy	%RSD	Accuracy	%RSD	Accuracy	%RSD	Accuracy	%RSD
0.005	101.0	9.31	102.9	44.66	87.6	14.97	85.6	6.33	83.0	3.55	78.3	7.65	100.1	8.39	129.1	9.91
0.01	105.1	6.96	156.1	10.78	103.9	8.44	96.1	11.29	99.0	5.90	97.6	4.53	110.4	8.46	147.3	6.19
0.05	94.4	3.66	91.6	2.69	99.5	6.12	88.0	4.17	96.7	2.52	101.9	3.03	101.1	2.21	101.5	4.59
0.1	96.7	3.26	93.1	3.55	99.9	3.70	93.4	3.63	98.5	1.11	98.1	4.41	99.2	2.43	103.3	2.92
0.5	97.1	1.90	99.0	1.49	102.7	2.06	96.2	2.08	101.0	1.34	108.2	1.47	103.1	1.98	106.6	2.79
1	96.8	1.49	97.5	2.28	99.5	2.25	93.8	1.99	96.6	0.95	96.0	1.69	100.4	1.47	103.0	1.23
5	97.2	0.79	97.3	1.85	101.6	1.86	94.7	0.86	98.6	1.33	98.3	1.07	100.0	1.54	102.8	1.40
10	96.6	1.18	95.3	0.82	99.3	0.65	93.4	1.03	96.7	1.20	96.7	0.95	97.6	0.85	100.1	0.68
50	102.2	0.83	100.2	1.22	102.2	0.44	99.8	0.94	101.3	1.63	101.6	1.03	102.0	1.59	102.5	1.25
100	99.5	1.57	100.5	1.28	98.9	1.41	101.1	1.27	99.8	0.58	99.6	1.01	99.2	0.57	98.5	0.92

Calibration Curves and LOQs



Compound name	Limits of Quantification	
	Ultivo (fg on column)	Ultivo (pg/mL)
Desomorphine	17.3	1.73
Desmethyldoxepin	27.8	2.78
Flecainide	11.7	1.17
Midazolam	40.0	4.0
Imipramine	6.57	0.66
Amitriptyline	14.2	1.42
Fluoxetine	15.6	1.56
Diazepam	18.4	1.84

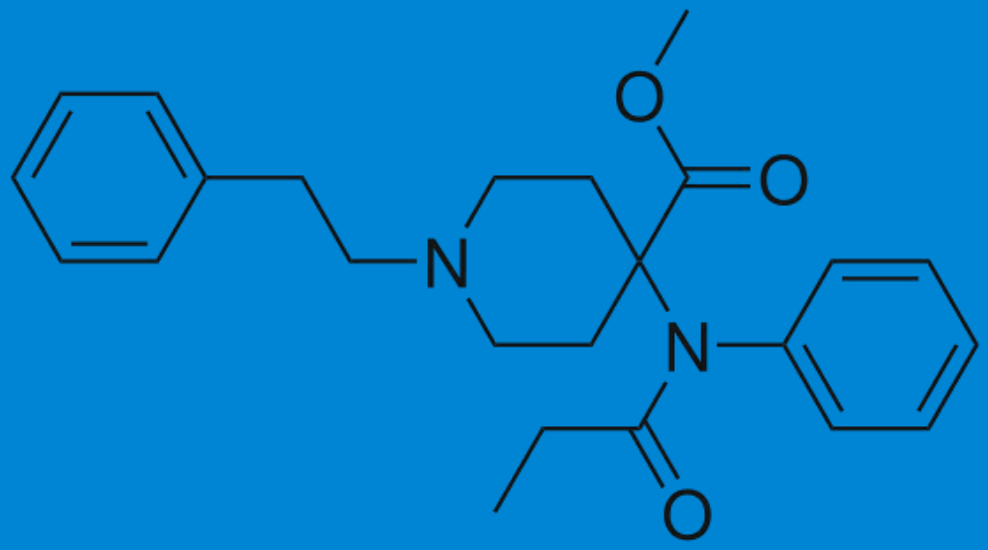
Summary

- Agilent Ultivo LC/TQ system demonstrated the accurate and sensitive detection of commonly small molecule monitored amino acids in a pet food matrix, while the use of the Poroshell 120 HILIC-Z column enabled underivatized analytes to be monitored, resulting in a simplified workflow.

- A fast, sensitive, accurate method was presented for the analytical determination of eight drugs in human serum on the new Ultivo TQ LC/MS.

- Ultivo is an exceptionally innovative new mass spectrometer, which can minimize laboratory workspace needs, as well as reduce maintenance challenges, creating a productive work environment for high-throughput laboratories.

Analytical Determination of New Synthetic Fentanyls and Pesticides in Cannabis Using Ultivo LC/TQ



Jennifer Hitchcock, PhD
LCMS Application Scientist
Agilent Technologies, Inc.
Santa Clara, CA

Agilent
Trusted Answers

OR the Pete Stone Showcase

Presented by Jennifer Hitchcock

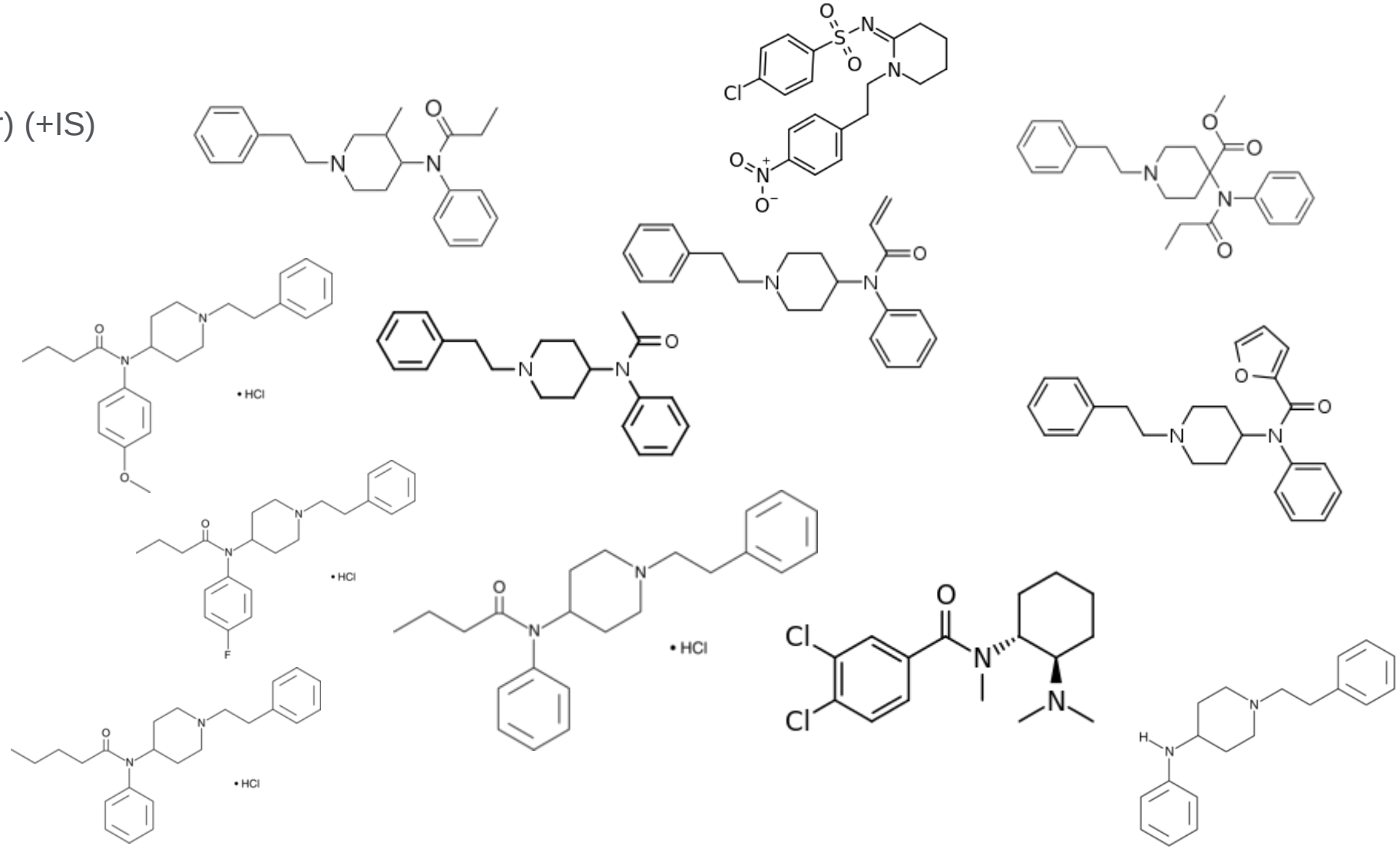
Agilent
Trusted Answers

Introduction

- Opioid misuse is severe, with approximately 100 reported overdose fatalities per day across the US
- It’s thought that historically abused opiates like heroin are being “cut” with many new synthetic, more potent opioids like fentanyl
- Keeping up with new synthetic illicits and analyzing for them is difficult
- Research method tested on Ultivo for a list of 12 new synthetic fentanyl opioids and 4-ANNP, the DEA-scheduled precursor

Synthetic fentanyls used in research study

- 4-ANNP (synthetic precursor) (+IS)
- 3-methylfentanyl
- Acetylfentanyl (+IS)
- Acetylnorfentanyl (+IS)
- Acrylfentanyl (+IS)
- Butyrylfentanyl (+IS)
- Carfentanyl (+IS)
- Furanylfentanyl
- N-desmethyl U-47700
- Norcarfentanyl
- Para-fluorobutyrylfentanyl
- Valerylfentanyl (+IS)
- U-47700 (+IS)
- W-18 RM



Sample preparation

Standards and ISTDs from Cerilliant and Cayman Chemicals
Serum and negative urine from Golden West Biologicals

Urine Matrix:

1. Negative urine diluted 10x using DI water
2. Diluted negative urine centrifuged at 10,000 rpm through nanosep 3K filters, then supernatant combined as stock solution
3. ISTDs spiked into diluted negative urine stock at concentration of 25 ng/mL
4. Serial dilutions made for calibrators from 500 ng/mL to 1 pg/mL using ISTD-spiked filtered stock (equivalence dilution 1/10)

Serum Matrix:

1. Gold serum (10 mL) mixed with 20 mL cold acetonitrile
2. Serum/ACN mix vortexed for 2 min, then centrifuged at 5000 rpm for 15 min
3. Supernatant (20 mL) spiked with ISTD at concentration of 50 ng/mL
4. Supernatant and ISTDs diluted with 20 mL DI water (ISTD conc. 25 ng/mL)
5. Serial dilutions made for calibrators from 500 ng/mL to 1 pg/mL (equivalence dilution 1/6)

Synthetic fentanyls in serum and urine

Column	Poroshell 120 EC-C18, 2.7 µm, 2.1x50 mm (pn 699775-902)	
Column temp	55°C	
Injection volume	5 µL	
Mobile phase	A: 5mM ammonium formate + 0.01% formic acid in water B: 0.01% formic acid in methanol	
Flow rate	0.5 mL/min	
Gradient	Time 0.00 0.50 3.50 5.50 6.00 7.00	B% 10 15 50 95 95 (stop time) 10 (post time)

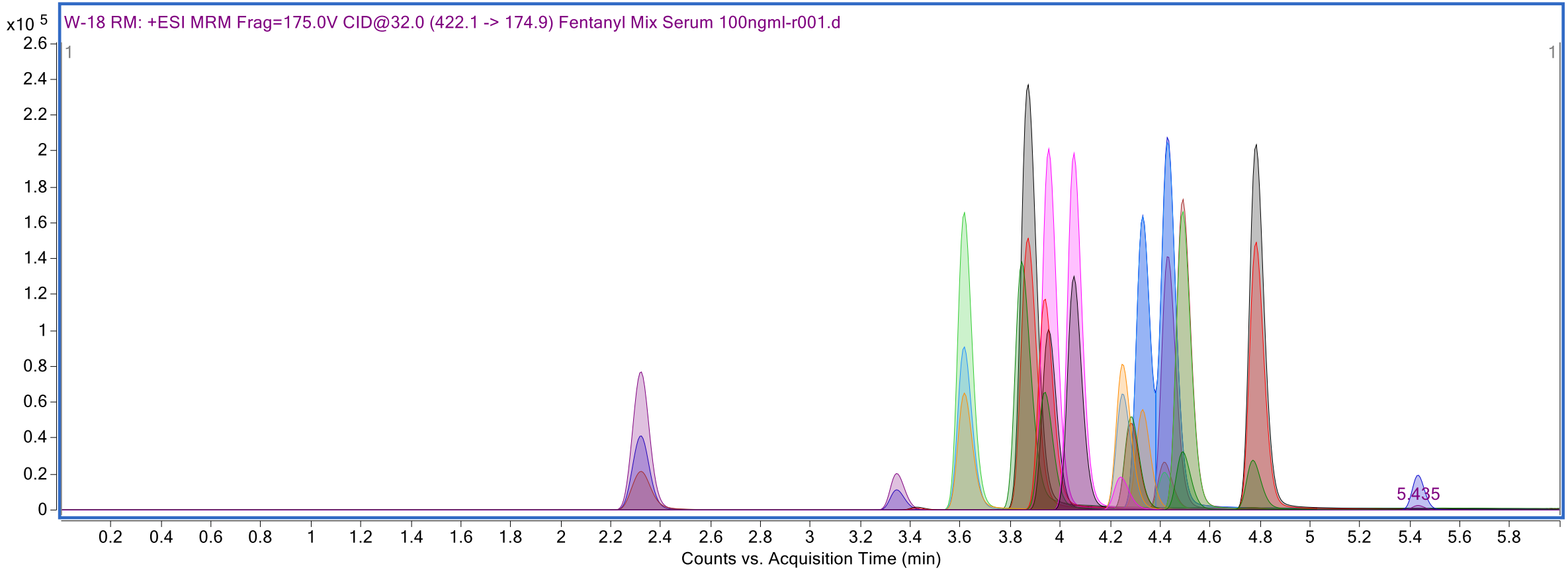
Drying gas temp	325 °C
Drying gas flow	9 L/min
Sheath gas temp	350 °C
Sheath gas flow	11 L/min
Nebulizer pressure	35 psi
Capillary voltage	3500 V(+)
Nozzle voltage	0 V(+)
Cycle Time	400 ms

MRM transition list

Compound Name	Precursor (*m/z*)	Product (*m/z*)	Dwell Time (ms)	Frag (V)	CE (V)
W-18 RM	422.1	174.9	10	175	32
W-18 RM	422.1	110.9	10	175	56
Furanyl fentanyl	375.2	188.1	10	170	24
Furanyl fentanyl	375.2	105	10	170	48
Valeryl fentanyl-D5	370.3	105	10	180	48
p-fluorobutyrylfentanyl	369.2	188.1	10	180	24
p-fluorobutyrylfentanyl	369.2	105	10	180	52
Valeryl fentanyl	365.3	188.1	10	180	24
Valeryl fentanyl	365.3	105	10	180	48
Butyrylfentanyl-D5	356.3	188.1	10	180	24
Butyrylfentanyl-D5	356.3	105.1	10	180	48
Cis-3-methyl fentanyl	351.2	202.1	10	180	24
Butyrylfentanyl	351.2	188.1	10	180	24
Butyrylfentanyl	351.2	105	10	180	48
Cis-3-methyl fentanyl	351.2	105	10	180	48
Acrylfentanyl-D5	340.2	188.1	10	165	24
Acrylfentanyl-D5	340.2	105	10	165	44
U-47700-D6	335.2	284	10	120	16
Acrylfentanyl	335.2	188.1	10	165	24
Acrylfentanyl	335.2	105	10	165	44

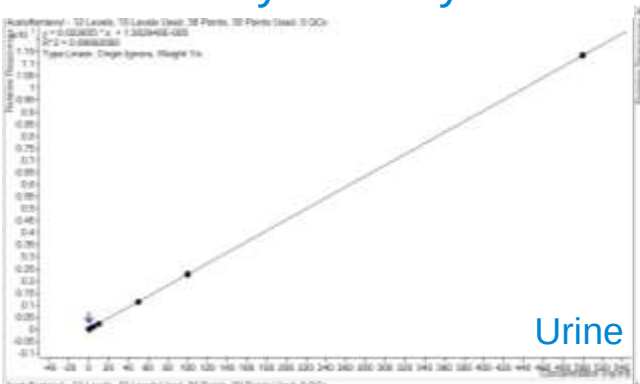
Compound Name	Precursor (*m/z*)	Product (*m/z*)	Dwell Time (ms)	Frag (V)	CE (V)
Acetylfentanyl-13C6	329.2	105	10	170	44
U-47700	329.1	284	10	120	16
U-47700	329.1	172.9	10	120	36
Acetyl fentanyl	323.2	188.1	10	170	24
Acetyl fentanyl	323.2	105.1	10	170	44
N-desmethyl U-47700	315.1	284	10	120	16
N-desmethyl U-47700	315.1	172.9	10	120	36
Norcarfentanyl	291.2	231.1	10	95	12
Norcarfentanyl	291.2	142	10	95	16
4-ANNP-D5	286.2	105.1	10	140	36
4-ANNP	281.2	188.1	10	140	16
4-ANNP	281.2	105	10	140	36
Acetyl norfentanyl-13C6	225.2	84.1	10	120	20
Acetyl norfentanyl	219.1	84.1	10	120	20
Acetyl norfentanyl	219.1	55.2	10	120	44
Carfentanyl-D5	400.3	113	10	130	36
Carfentanyl	395.2	335.2	10	135	16
Carfentanyl	395.2	113	10	135	36

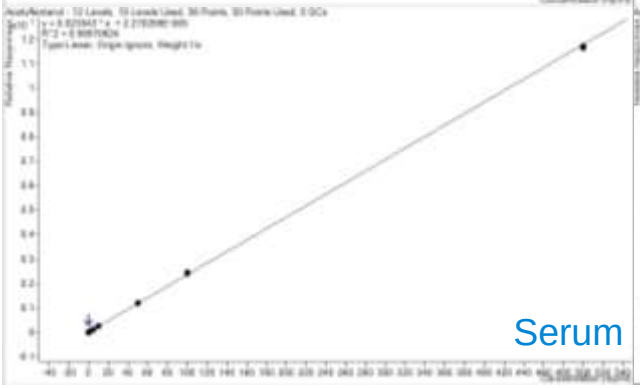
Composite EIC of each fentanyl opioid transition
(Serum mix calibrant 100 ng/mL)



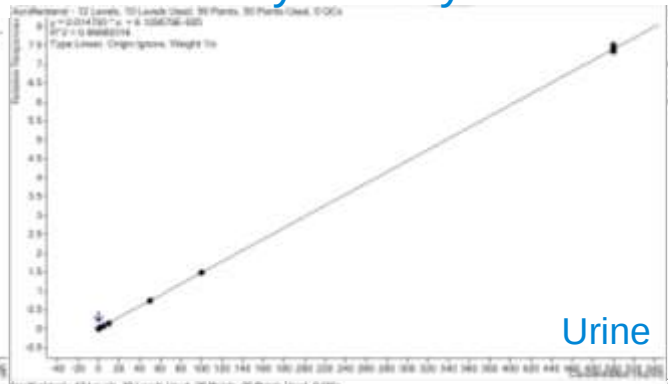
Selected calibration curves
1pg/mL→500ng/mL

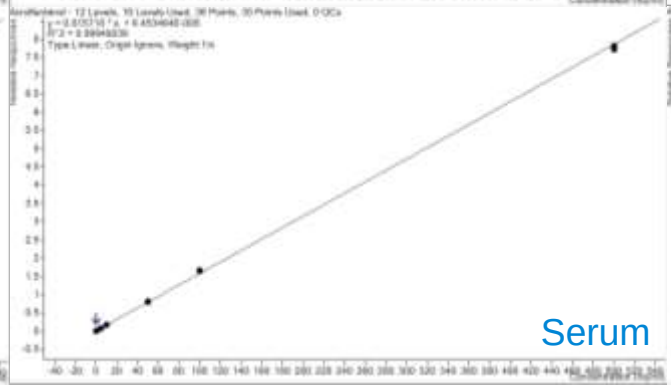
Acetyl fentanyl



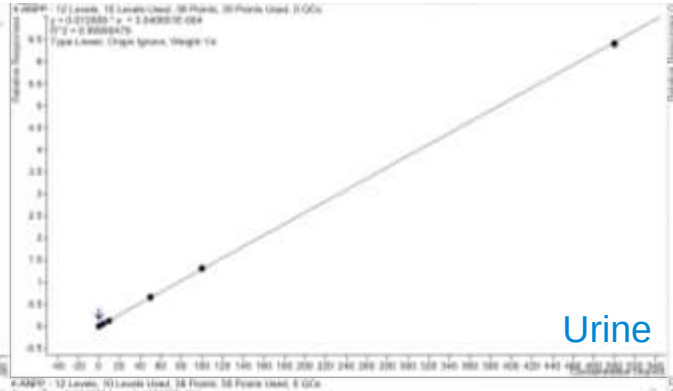


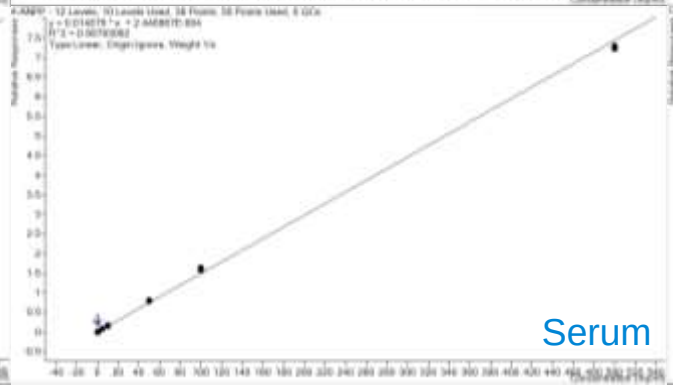
Acrylfentanyl



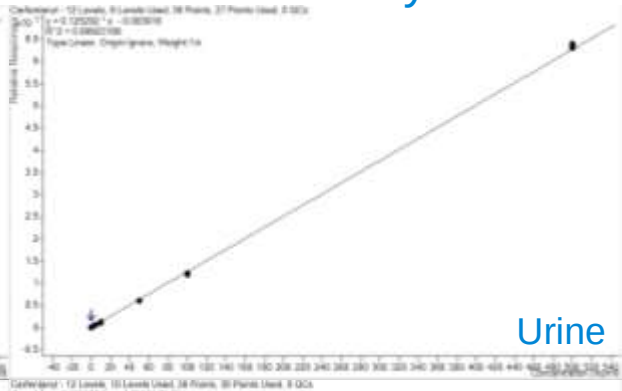


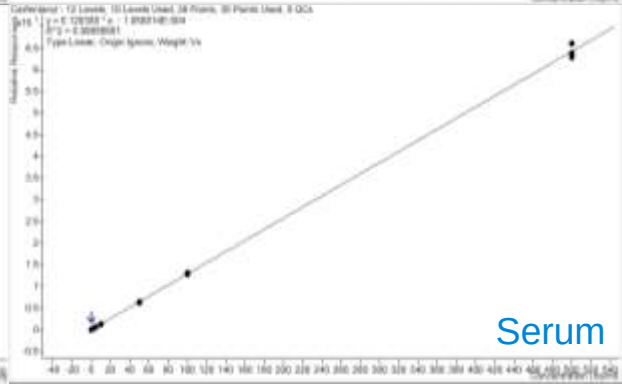
4-ANNP





Carfentanyl

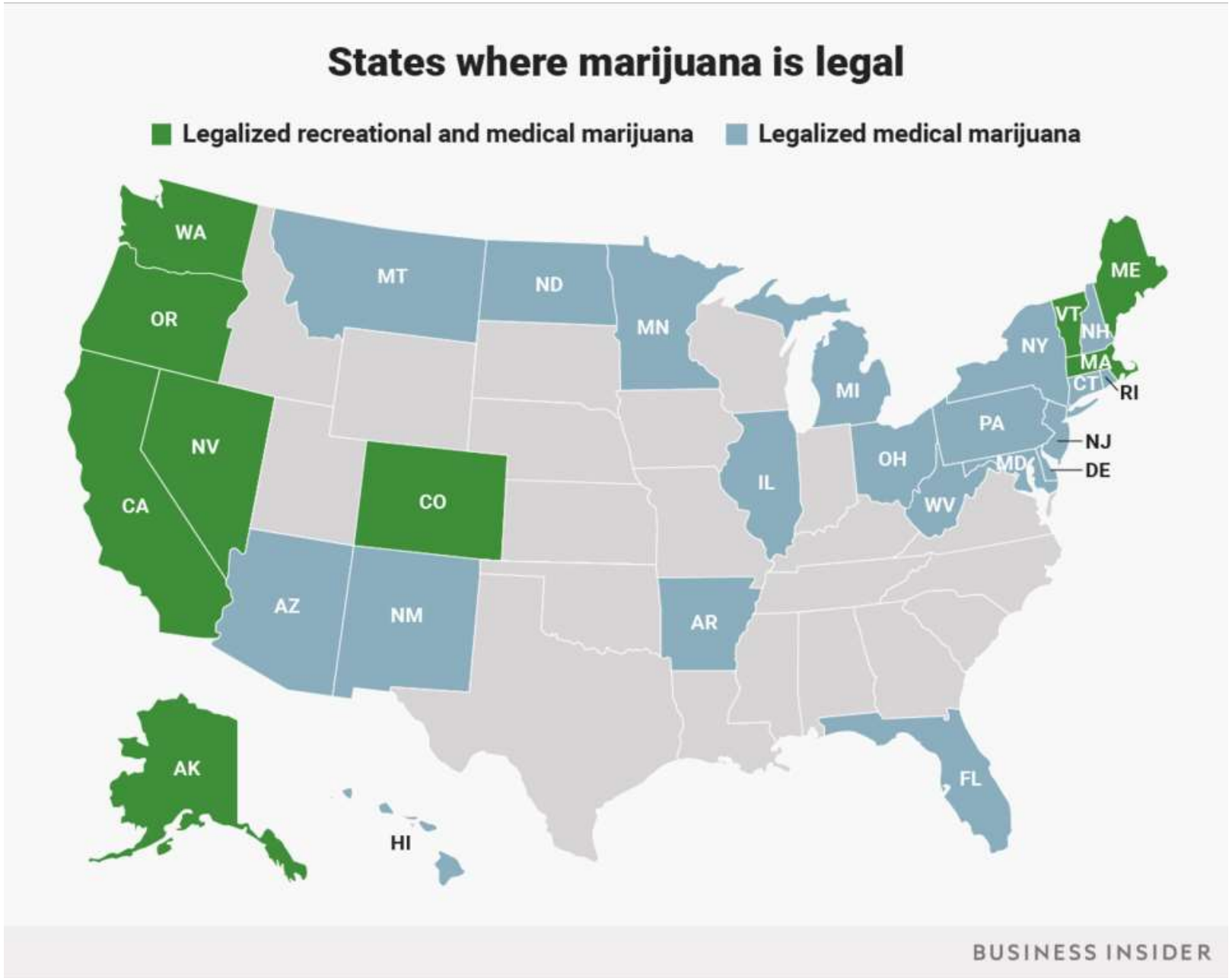




LLOQ results table for synthetic fentanyls

Analyte	Actual LLOQ (Urine) pg/ml	Actual LLOQ (Serum) pg/ml	Equivalence LLOQ (Urine) pg/ml (1/10 dilution)	Equivalence LLOQ (Serum) pg/ml (1/6 dilution)
4-ANPP	100	10	1000	60
3-methylfentanyl	100	50	1000	300
Acetylfentanyl	50	10	500	60
Acetylnorfentanyl	100	50	1000	300
Acrylfentanyl	50	10	500	60
Butyrylfentanyl	100	50	1000	300
Carfentanyl	50	10	500	60
Furanylfentanyl	50	50	500	300
Para-fluorobutyrylfentanyl	50	50	500	300
Norcarfentanyl	100	50	1000	300
N-desmethyl U-47700	100	50	1000	300
Valerylfentanyl	100	50	1000	300
U-47700	100	50	1000	300
W-18 RM	50	50	500	300

*Equivalence refers to the actual concentration of the urine or serum prior to dilution


States where marijuana is legal
Legalized recreational and medical marijuana
Legalized medical marijuana
WA
OR
CA
NV
MT
ND
MN
CO
AZ
NM
AR
IL
MI
OH
WV
PA
NY
VT
NH
ME
MA
CT
RI
NJ
DE
MD
FL
AK
HI
BUSINESS INSIDER

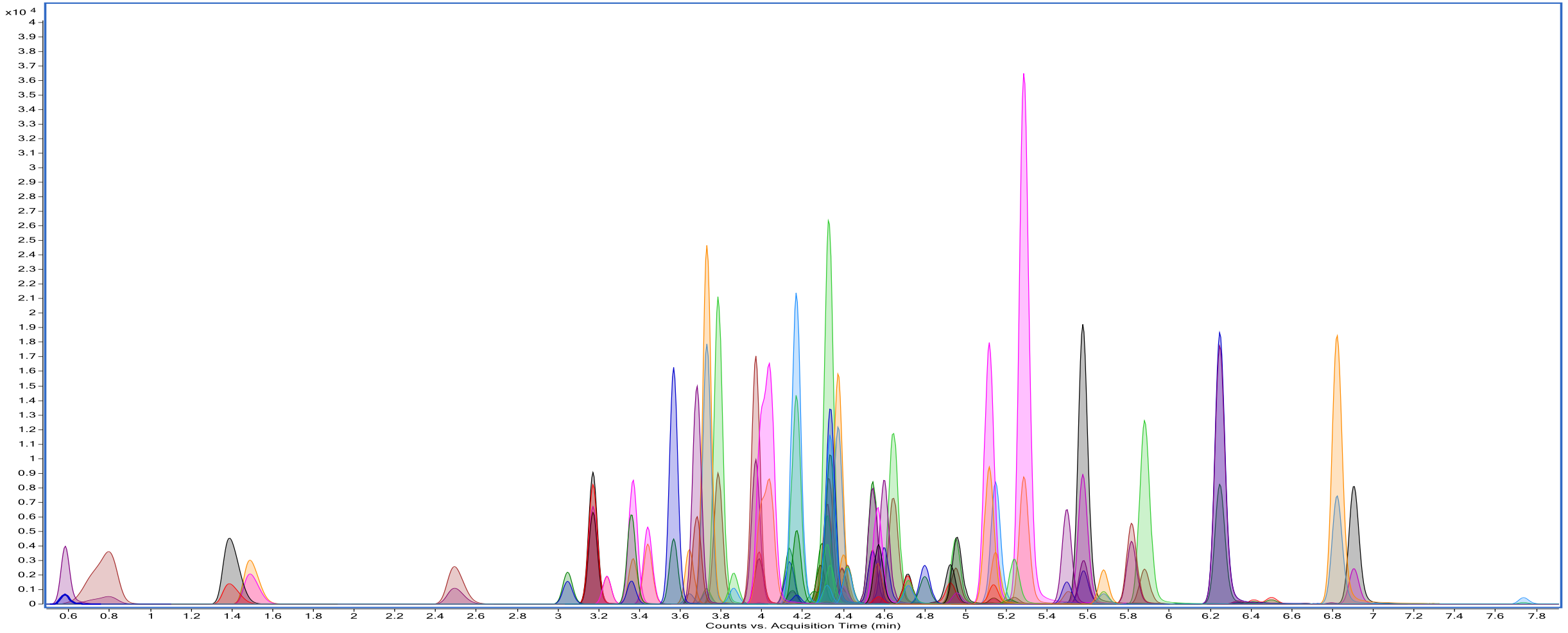
Cannabis pesticide testing

- Cannabis is a complex botanical with many endogenous compounds that can make analytical detection of trace amounts of pesticides difficult
- Each state determines which pesticides to analyze and what appropriate action limits are
- Primary focus has been on California list as most comprehensive in US for cannabis pesticide testing
  - 66 total pesticides
  - Category I (detected or not) has 21
  - Category II (specific action levels for different matrices) has 45
- Canada has proposed regulations containing an additional 33 compounds when compared to the California panel
- Levels are best guesses at this point and may change as more information is acquired

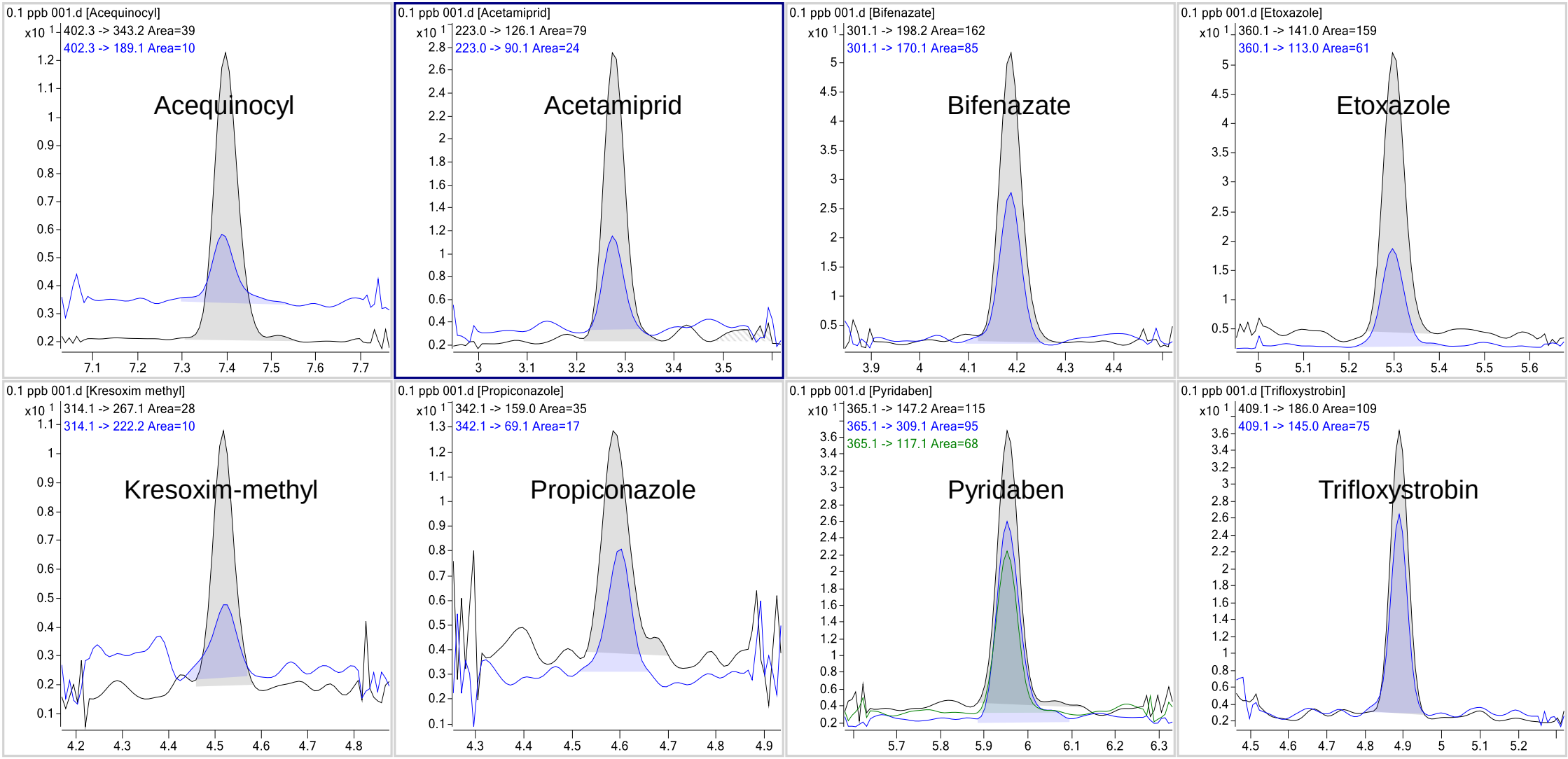
Pesticides in cannabis

Column	Poroshell 120 Phenyl-Hexyl 2.7 µm, 3.0x100 mm (pn 695975-312)	
Column temp	55°C	
Injection volume	2 µL	
Mobile phase	A: 5 mM ammonium formate + 0.1% formic acid in water B: 0.1% formic acid in methanol	
Flow rate	0.50 mL/min	
Gradient	Time 0.00 1.00 2.00 8.00 9.00 9.50 9.51 11.00	B% 20 20 75 96 100 100 20 20 *stop time*
Drying gas temp	200 °C	
Drying gas flow	13 L/min	
Sheath gas temp	200 °C	
Sheath gas flow	10 L/min	
Nebulizer pressure	55 psi	
Capillary voltage	5500 V (+) / 3500 V (-)	
Nozzle voltage	2000 V (+) / 500 V (-)	
Cycle Time	500 ms	

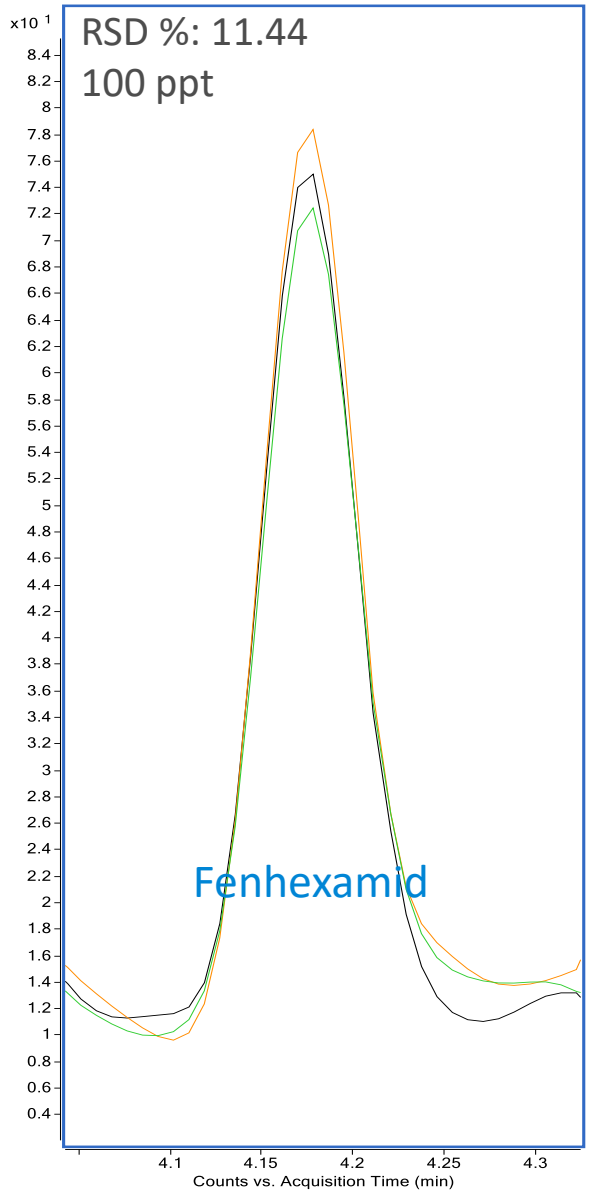
Composite EIC of pesticides and aflatoxins: 10 ppb

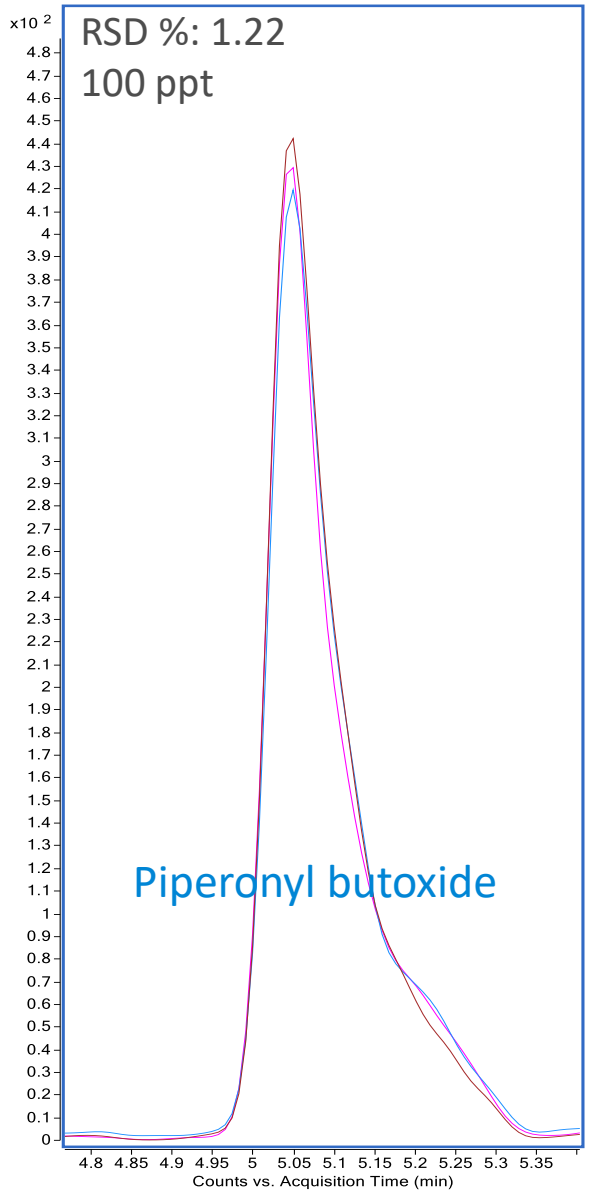


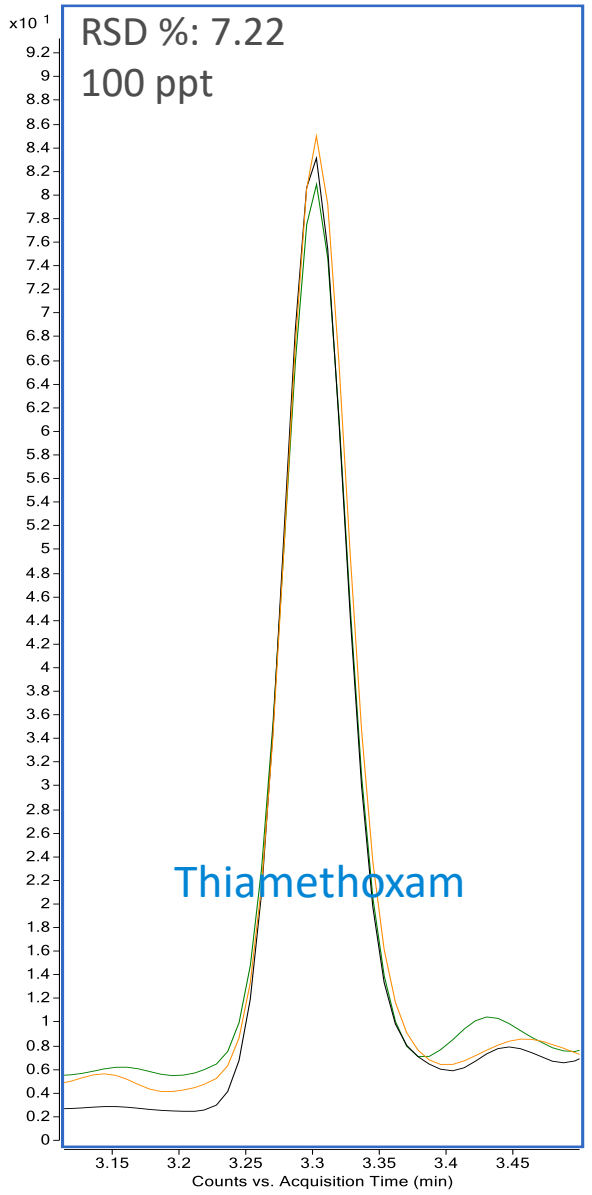
Example chromatograms at 0.1 ppb



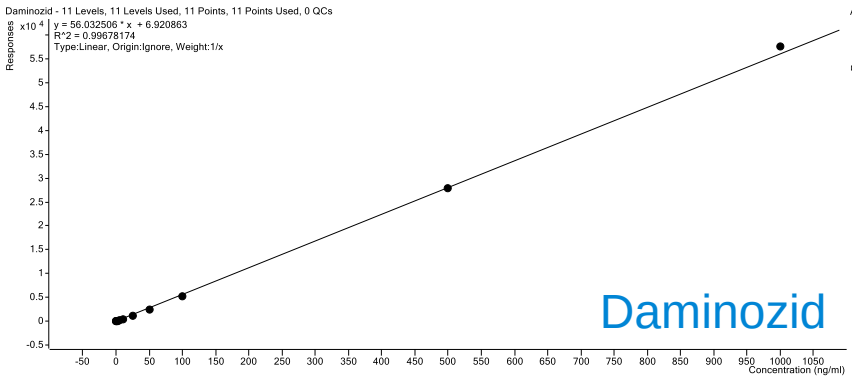
Reproducibility in matrix (n=3)

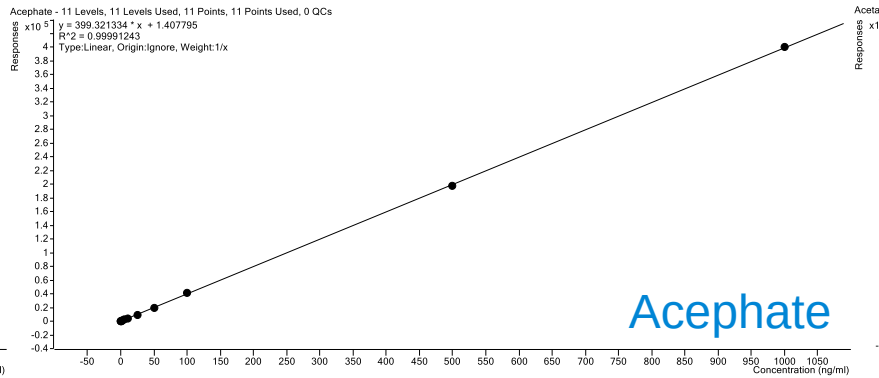


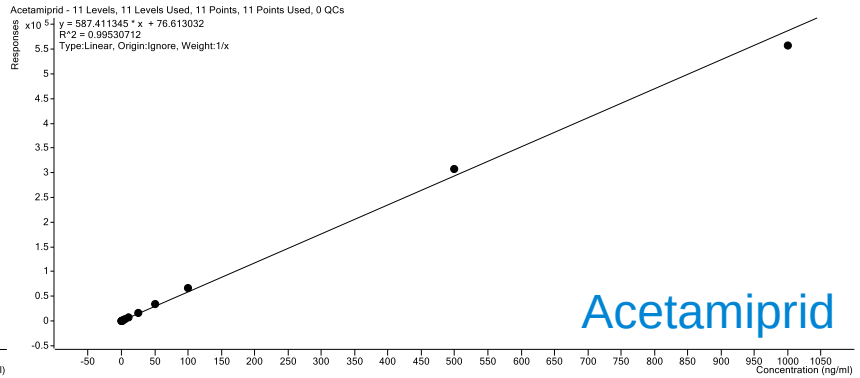


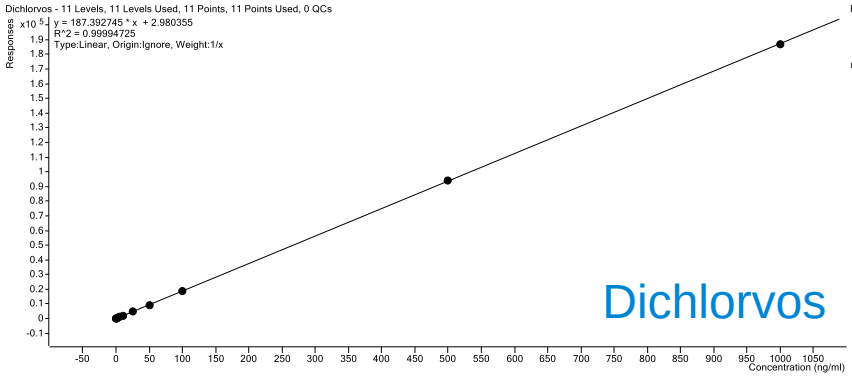


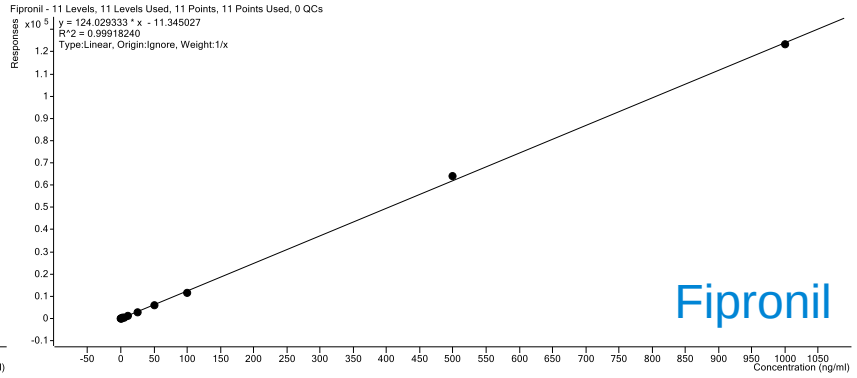
Representative calibration curves

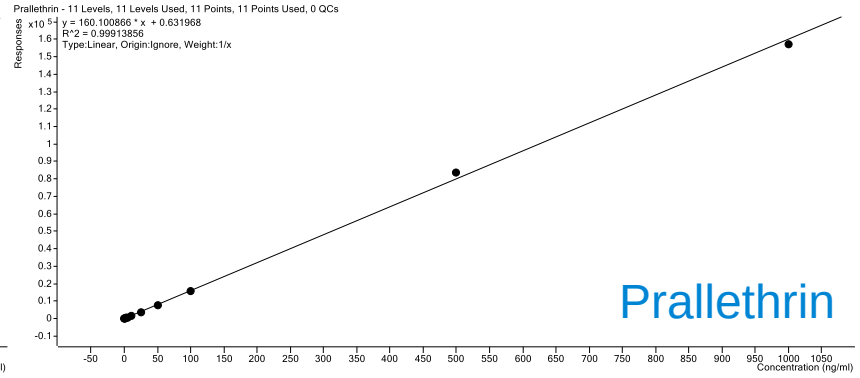


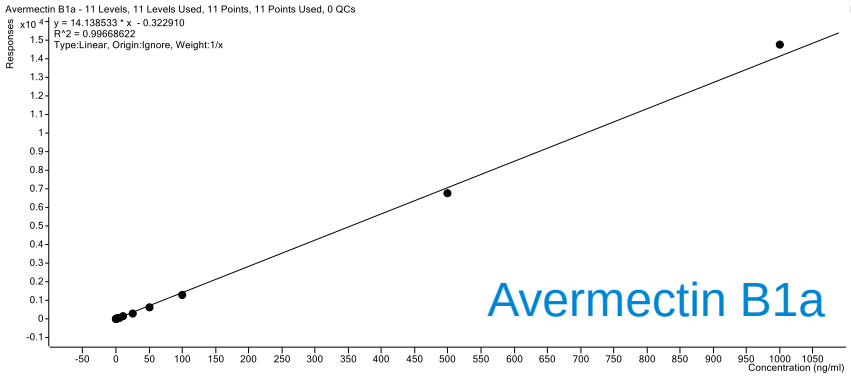


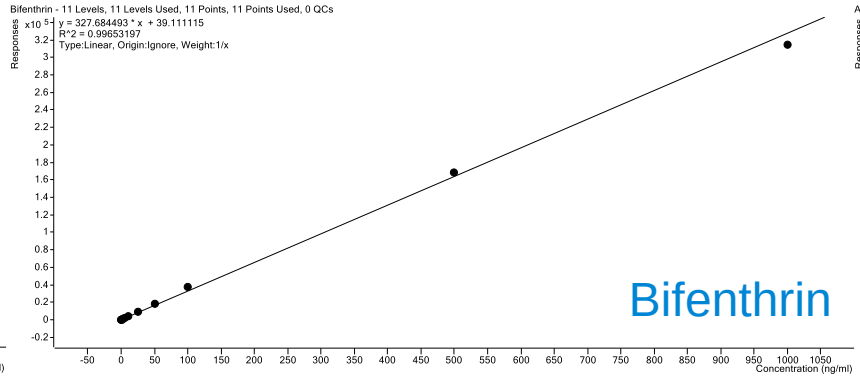


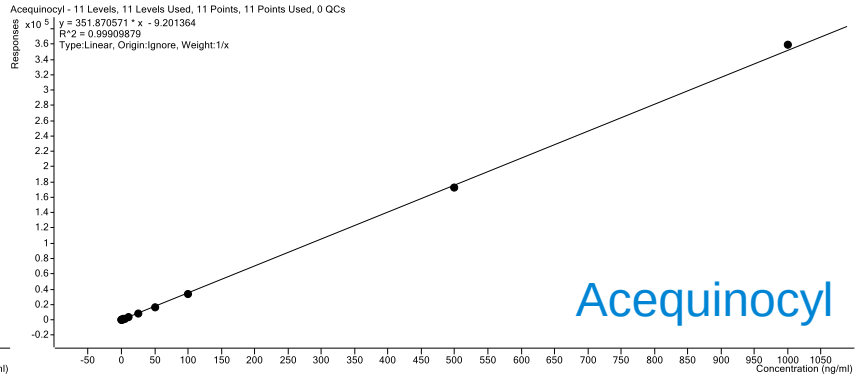








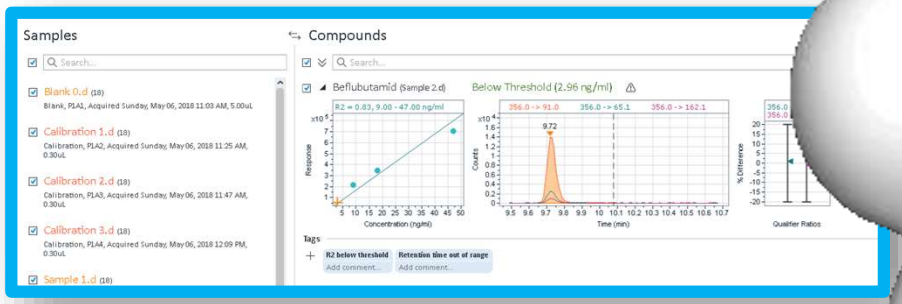




Summary

- Excellent sensitivity has been demonstrated in a variety of matrices and applications, including synthetic fentanyls in biological fluids and pesticides in cannabis
- In these applications, results on Ultivo are similar to those determined on the 6470
- Ultivo innovates the mass spec lab by minimizing bench space requirements and reducing maintenance challenges

Agilent – Your Partner!



Your success

Is our success

Stay ahead of the Status-Quo!

Ruben Garnica, Ph.D.
Yanan Yang, Ph.D.
Jennifer Hitchcock, Ph.D.
Patrick Jeanville, Ph.D.