Recent Developments in LC/MS Analysis of Food Contaminants

(Agilent 1290 Infinity uHPLC, Agilent 6230 TOF, 6460/6490 QQQ and 6540 Q-TOF.)

Dr Peter Stone

Agilent Technologies Inc,

Santa Clara, CA.

Fast Target Screening of Analytes Using high sensitivity 6460 Triple Quadrupole with 1290 uHPLC

- Subfemtogram, on-column sensitivity
- Five Orders of Linearity
- 3,000 m/z mass range
- Fast Pos/Neg switching, 30 millisec
- New Method Development Tool – MH Optimizer
- **"Dynamic Multiple Reaction Monitoring"** for MRMs scheduled by peak retention times instead of time segments

MassHunter “Dynamic MRM”

Many applications require screening of 100 – 1000 compounds in one MRM method !

- Food, environmental and Forensic analysis (e.g. Doping Control)
- Subsequent quantitation of positives in dedicated methods

WITHOUT Dynamic MRM

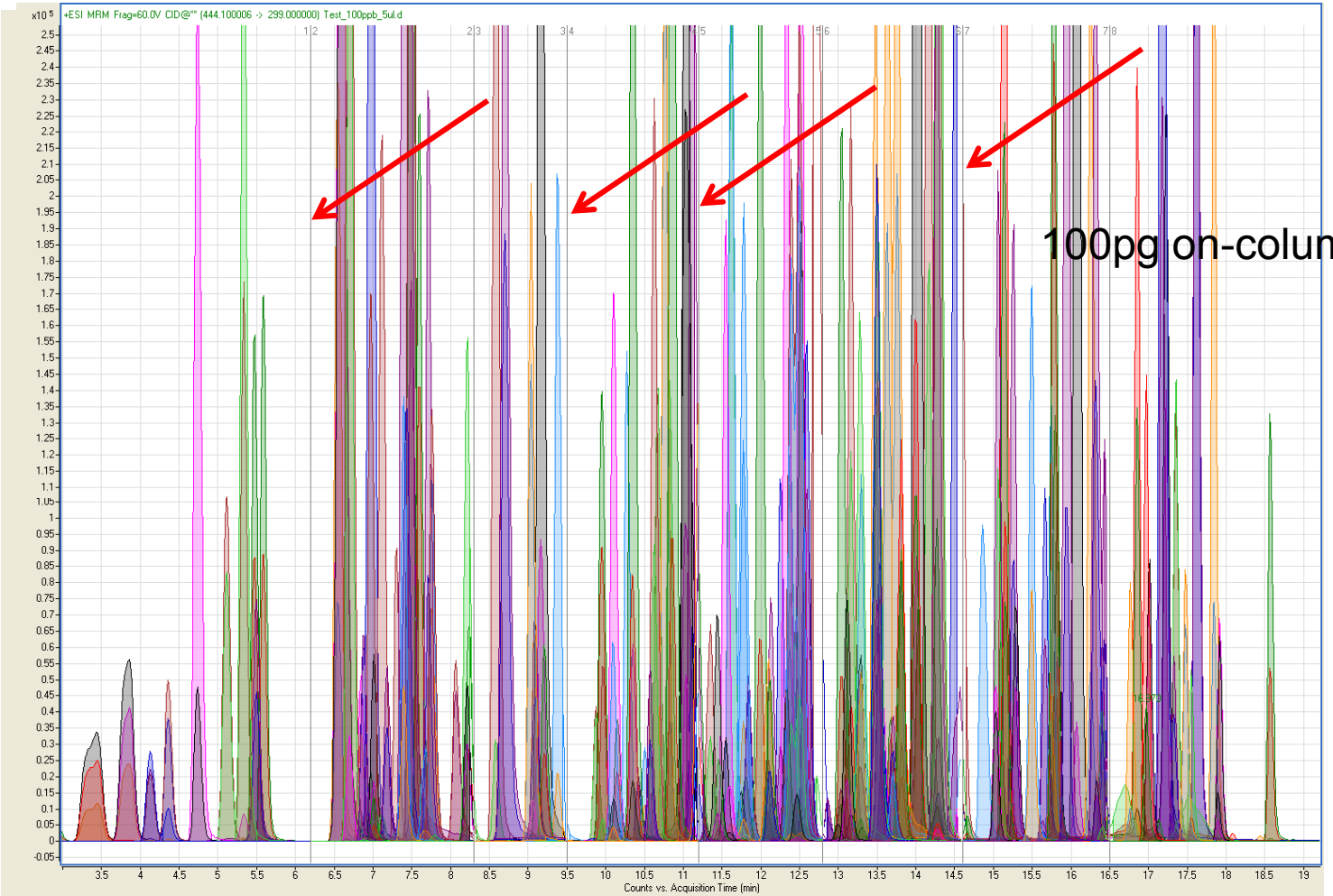
- Need to manually set up multiple time segments to maximize dwell time
- Tedious and fragile for chromatographic time shifts

WITH Dynamic MRM

- Automatic setup of overlapping time segments without user intervention
- Even less MRMs per time results in even longer dwell time / sensitivity
- Unaffected by chromatographic time shifts

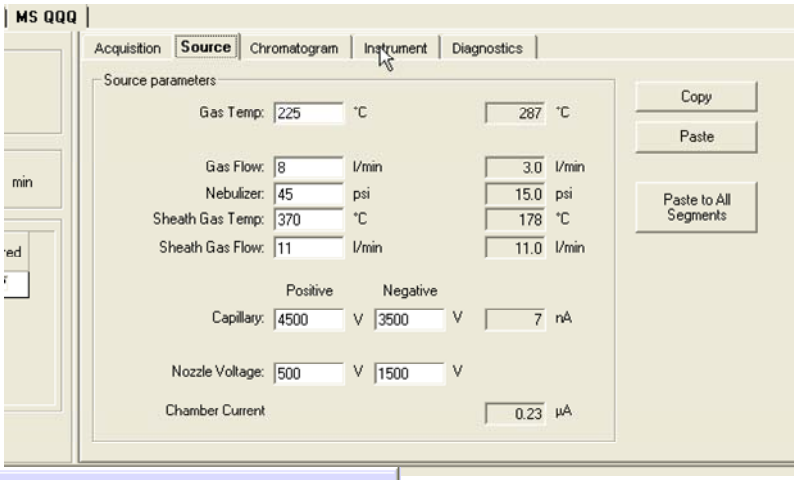
Segmentation with NO Dynamic MRM functionality

300 Component Standard



Agilent Technologies

6460 QqQ Method Conditions



Acquisition | Source | Chromatogram | Instrument | Diagnostics

Scan segments

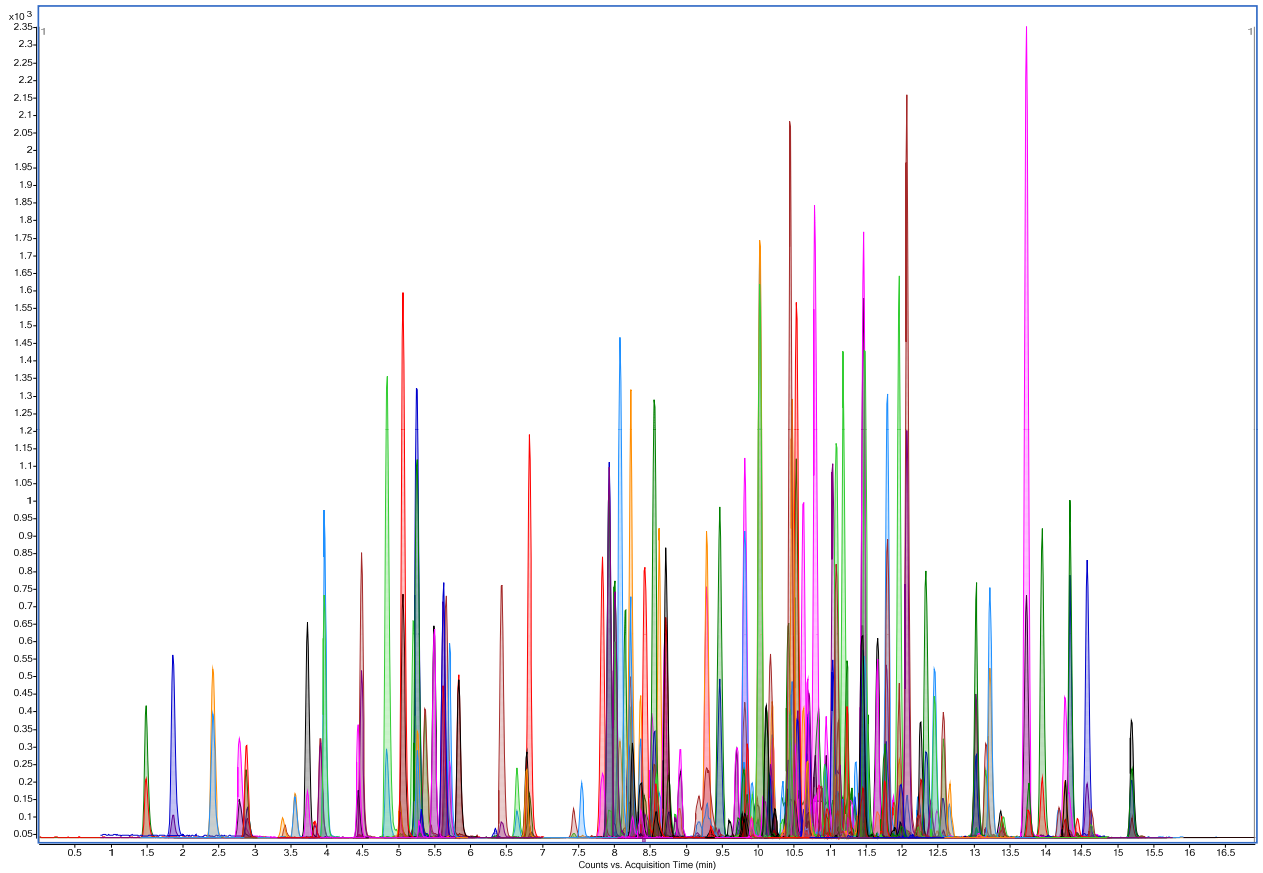
Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Fragmentor	Collision Energy	Ret Time (min)	Delta Ret Time	Polarity
Methamidophos	☐	142	Unit	125	Unit	80	10	0.435	0.2	Positive
Methamidophos	☐	142	Unit	94	Unit	80	10	0.435	0.2	Positive
Acephate	☐	184	Unit	143	Unit	90	5	0.557	0.2	Positive
Acephate	☐	184	Unit	95	Unit	90	20	0.557	0.2	Positive
Formetanate	☐	222.1	Unit	165.1	Unit	100	10	0.724	0.2	Positive
Formetanate	☐	222.1	Unit	120.1	Unit	100	25	0.724	0.2	Positive
Omethoate	☐	214	Unit	183	Unit	90	5	0.735	0.2	Positive
Omethoate	☐	214	Unit	125	Unit	90	20	0.735	0.2	Positive
Propamocarb	☐	189	Unit	101.9	Unit	100	15	0.816	0.2	Positive

Dynamic MRM Parameters

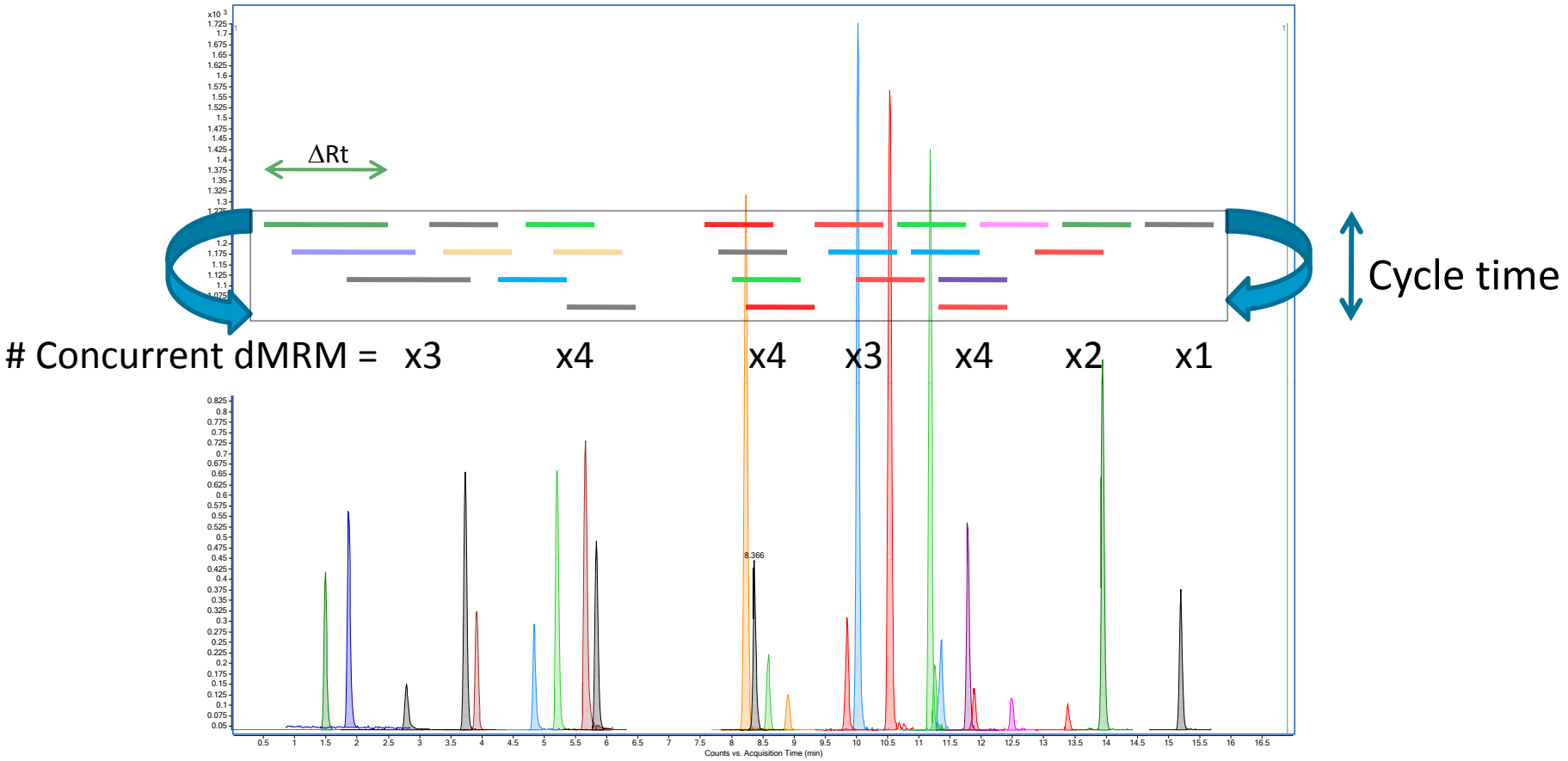
Cycle Time 300 ms

Total MRMs = 438 Max Concurrent MRMs = 52 Min/Max Dwell = 2.27 ms/296.50 ms

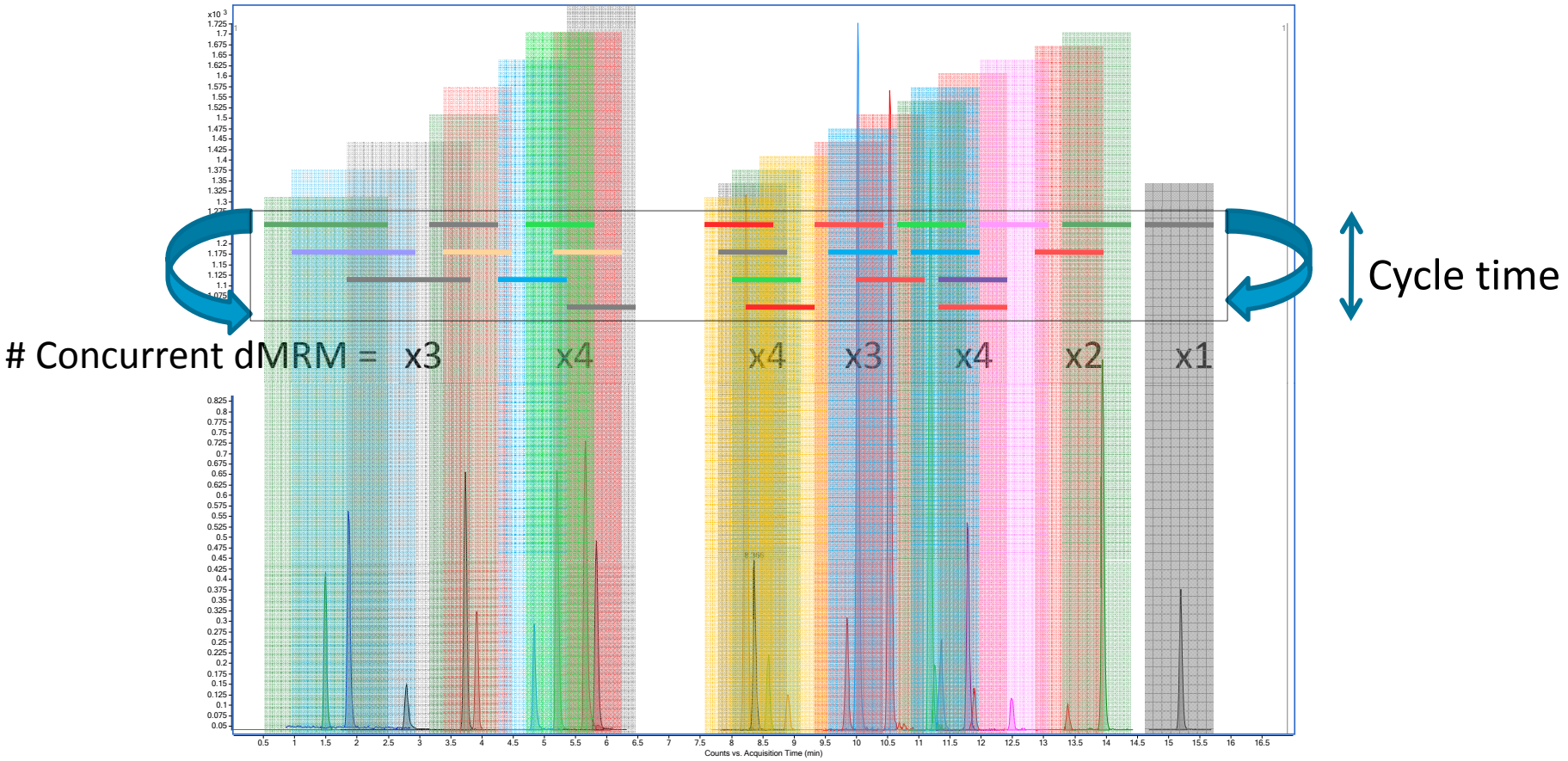
NO Seqmentation with Dynamic MRMs



EIC Overlay of 250 pesticide Mix spiked into Food matrix
(500 total transitions, on-column amount 2.5pg) using dynamic MRMs.

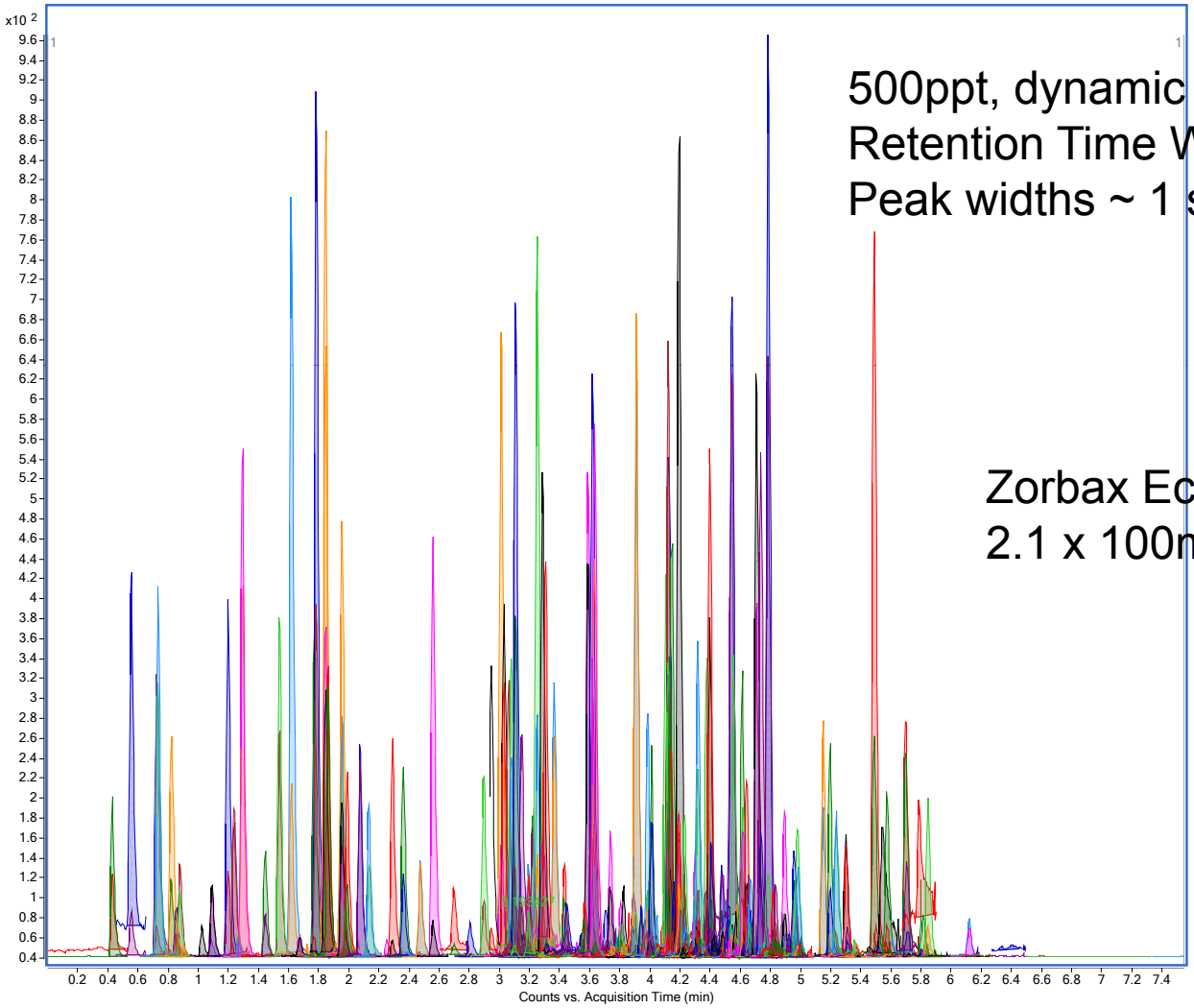


Selection of 24 pesticide transitions from previous slide
To illustrate efficiency of cycle times using dynamic MRMs.



Selection of 24 pesticide transitions from previous slide
To illustrate efficiency of cycle times using dynamic MRMs.

8 Minute Dynamic MRM Analysis 6460 QqQ - 250 Pesticide Screen.



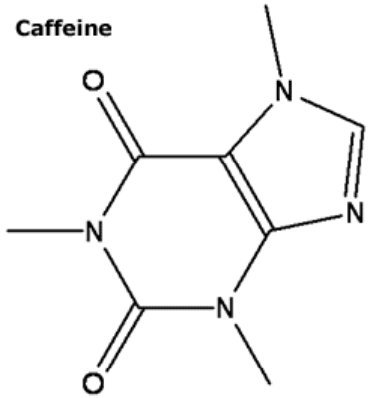
500ppt, dynamic MRM
Retention Time Window = 12 sec
Peak widths ~ 1 second

Zorbax Eclipse Plus C18
2.1 x 100mm (1.8µm)

Target Screening of PPCPs in Aquatic Food Matrices
LC/MS/MS QQQ

Pharmaceuticals and Personal Care Products (PPCPs) refer, in general, to any product used by individuals for personal health or cosmetic reasons or used by agribusiness to enhance growth or health of livestock.

PPCPs comprise a diverse collection of thousands of chemical substances, including prescription and over-the-counter therapeutic drugs, veterinary drugs, fragrances, and cosmetics.



EPA 1694 - Identification of PPCPs in Water

Antibiotics

Acetaminophen
Albuterol
Ampicillin
Anhydrochlortetracycline (ACTC)
Anhydrotetracycline (ATC)
Azithromycin
Caffeine
Carbadox
Carbamazepine
Cefotaxime
Chlortetracycline (CTC)
Cimetidine
Ciprofloxacin
Clarithromycin
Clinafloxacin
Cloxacillin
Codeine
Cotinine
Dehydronifedipine
Demeclocycline
Digoxigenin
Digoxin
Diltiazem
1,7-Dimethylxanthine
Diphenhydramine
Doxycycline
Enrofloxacin
4-Epianhydrochlortetracycline (EACTC)
4-Epianhydrotetracycline (EATC)
4-Epichlortetracycline (ECTC)
4-Epioxytetracycline (EOTC)
4-Epitetracycline (ETC)
Erythromycin
Erythromycin anhydrate
Flumequine
Fluoxetine
Gemfibrozil

Anti-Depressants

Pain Killers

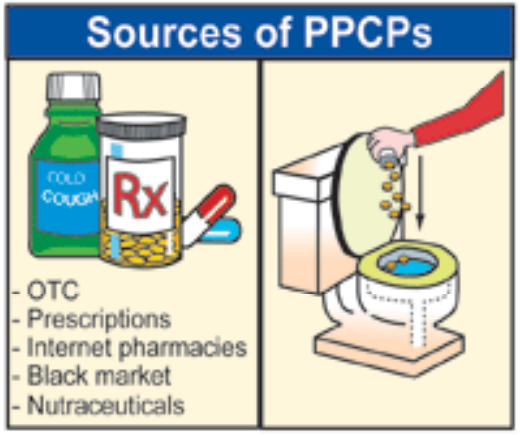
Ibuprofen
Isochlortetracycline (ICTC)
Lincomycin
Lomefloxacin
Metformin
Miconazole
Minocycline
Naproxen
Norfloxacin
Norgestimate
Ofloxacin
Ormetoprim
Oxacillin
Oxolinic acid
Oxytetracycline (OTC)
Penicillin V
Penicillin G
Ranitidine
Roxithromycin
Sarafloxacin
Sulfachloropyridazine
Sulfadiazine
Sulfadimethoxine
Sulfamerazine
Sulfamethazine
Sulfamethizole
Sulfamethoxazole
Sulfanilamide
Sulfathiazole
Tetracycline (TC)
Thiabendazole
Triclocarban
Triclosan
Trimethoprim
Tylosin
Virginiamycin
Warfarin
Other standards

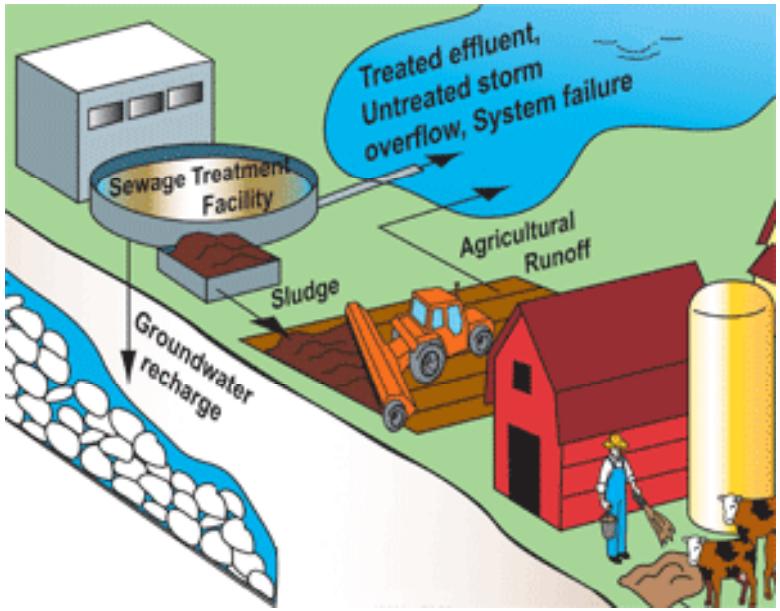
EPA

Birth Control Steroids

Where are PPCPs found in the environment?

PPCPs in the environment are frequently found in aquatic environments because PPCPs dissolve easily and don't evaporate at normal temperature and pressure. Practices such as the use of sewage sludge ("biosolids") and reclaimed water for irrigation bring PPCPs into contact with the soil (landfill also).



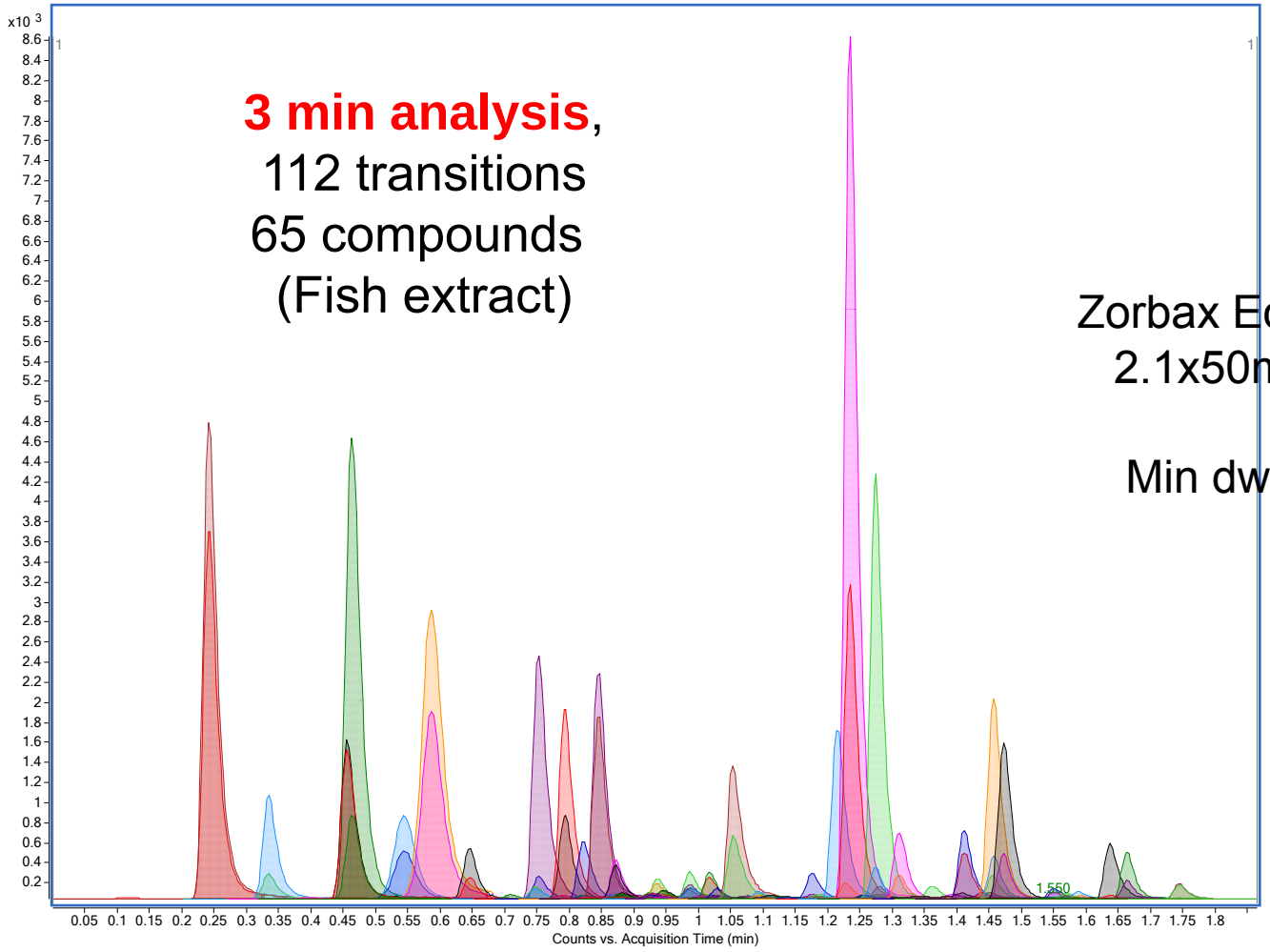


List of PPCP Target Analytes (EPA 1694, Positive Analysis)

Compound Name	Precursor Ion	Product Ion	Fragmentor (V)	Collision Energy (V)	RT	RT Window	Ion Polarity
1,7-Dimethylxanthine	181.0	124.0	90	15	0.658	0.50	Positive
4-Epianhydrotetracycline	427.0	410.0	90	15	3.473	0.50	Positive
4-Epichlortetracycline	479.0	462.0	134	17	2.416	0.50	Positive
4-Epitetracycline	445.0	410.0	110	15	1.777	0.50	Positive
Acetaminophen	152.0	110.0	90	15	0.656	0.50	Positive
Albuterol	240.0	148.0	90	15	0.489	0.50	Positive
Amphetamine	136.1	91.0	70	13	0.880	0.50	Positive
Anhydrotetracycline	427.0	410.0	90	15	3.473	0.50	Positive
Atenolol	267.2	145.0	134	21	0.502	0.50	Positive
Azithromycin	749.5	591.4	130	30	3.254	0.50	Positive
Caffeine	195.0	138.0	110	15	1.072	0.50	Positive
Carbadox	263.0	130.0	80	35	3.097	0.50	Positive
Chlorotetracycline	479.0	462.0	110	15	2.416	0.50	Positive
Cimetidine	253.0	159.0	100	10	0.489	0.50	Positive
Clarithromycin	748.5	158.0	110	25	4.384	0.50	Positive
Clonidine	230.0	44.0	150	25	0.764	0.50	Positive
Cloxacillin degradate	469.0	160.0	70	15	4.625	0.50	Positive
Cloxacillin	436.0	160.0	90	15	4.557	0.50	Positive
Codeine	300.0	215.0	130	25	0.709	0.50	Positive
Cotinine	177.0	80.0	90	25	0.420	0.50	Positive
Dehydronifedipine	345.0	284.0	130	25	4.662	0.50	Positive
Demeclocycline	465.0	448.0	130	15	2.394	0.50	Positive
Dextromethorphan	272.2	170.9	152	41	3.365	0.50	Positive
Diazepam	285.1	193.0	162	33	4.818	0.50	Positive
Digoxigenin	391.0	355.0	90	15	3.036	0.50	Positive
Diltiazem	415.0	178.0	130	25	3.702	0.50	Positive
Diphenhydramine	256.0	167.0	70	15	3.463	0.50	Positive
Doxycycline	445.0	428.0	110	15	3.008	0.50	Positive
Enrofloxacin	360.0	316.0	130	15	2.237	0.50	Positive
Erythromycin Anhydrate	716.5	158.0	90	25	4.201	0.50	Positive
Erythromycin	734.5	158.0	90	35	3.854	0.50	Positive
Erythromycin-Labeled	736.5	578.0	90	15	3.851	0.50	Positive

33/65 PPCP Analytes (Only quantifier ion transitions shown)

uHPLC - PPCPs from EPA 1694 (Positive Polarity)

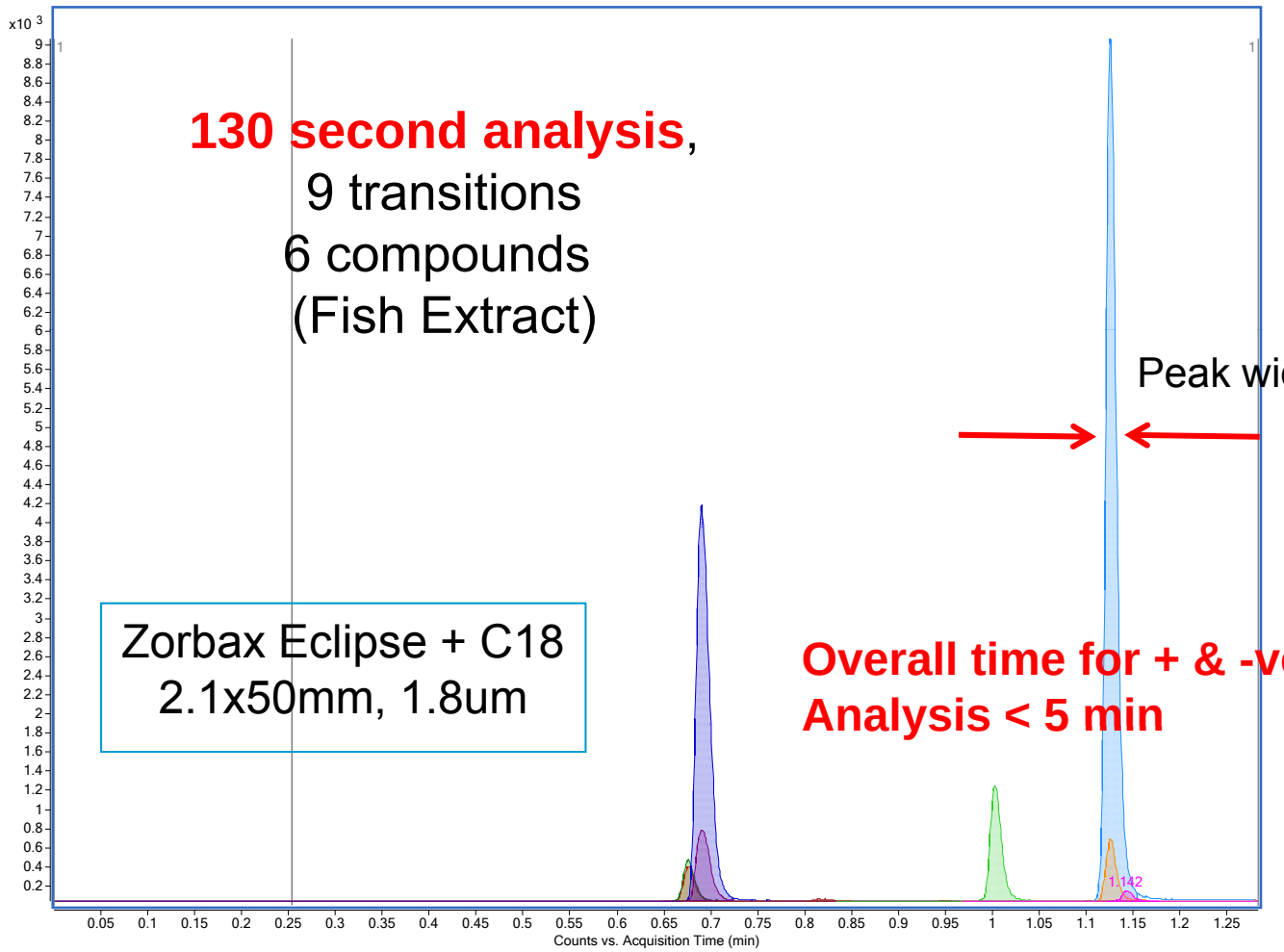


3 min analysis,
112 transitions
65 compounds
(Fish extract)

Zorbax Eclipse + C18
2.1x50mm, 1.8um

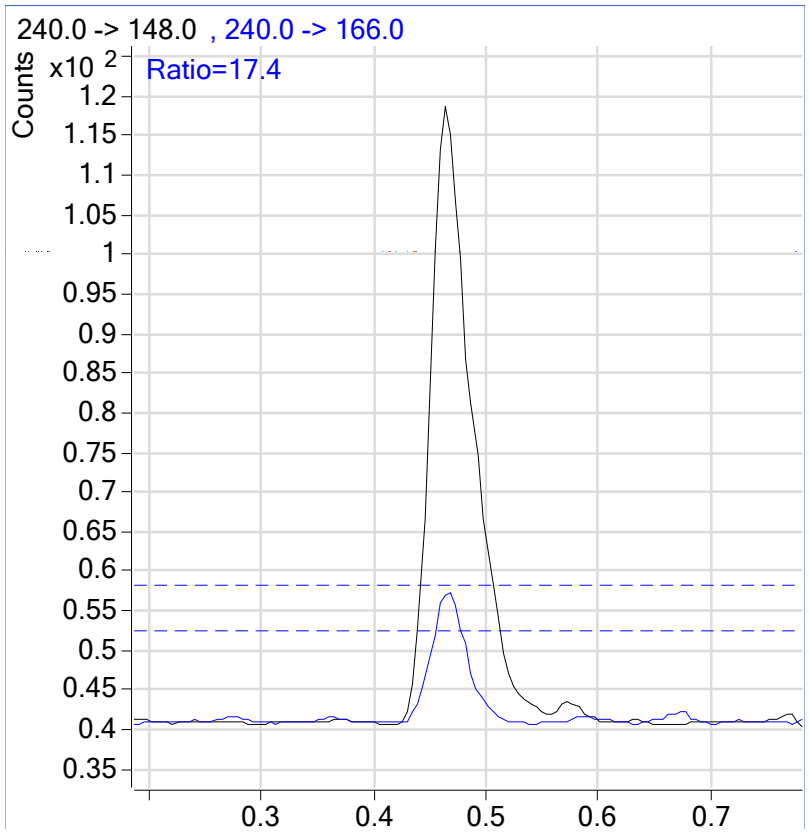
Min dwell=4.3ms

uHPLC - PPCPs from EPA 1694 (Negative Polarity)



Chromatographic Precision (1290/6460, dynamic MRM)

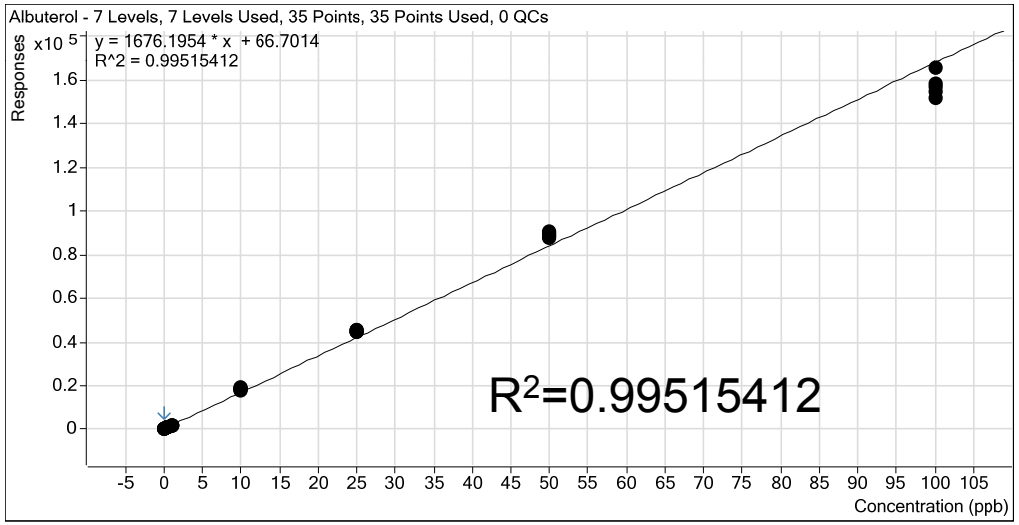
Albuterol, 100fg on-column Fish Extract Spike



Quant & Qual transitions
20% outlier ratio boundary

N= 5 (@100fg), %RSD (area) = 5.436907

N= 35 (all Cal levels), %RSD (RT) = 0.5182964

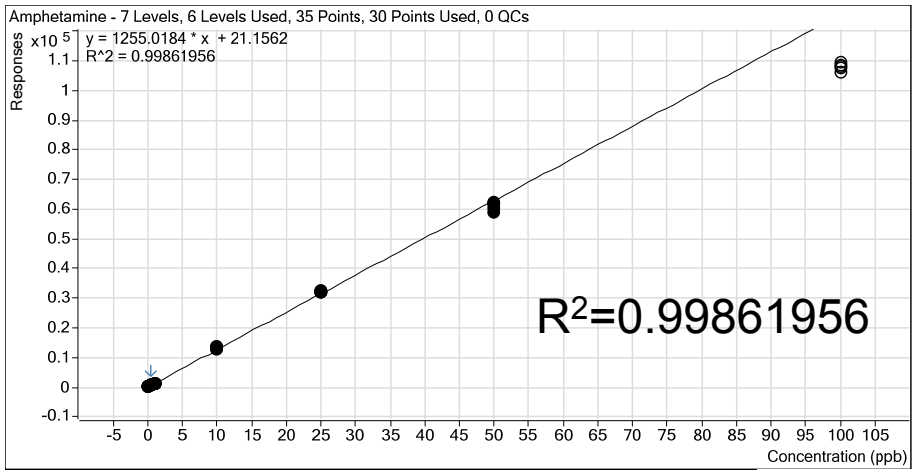


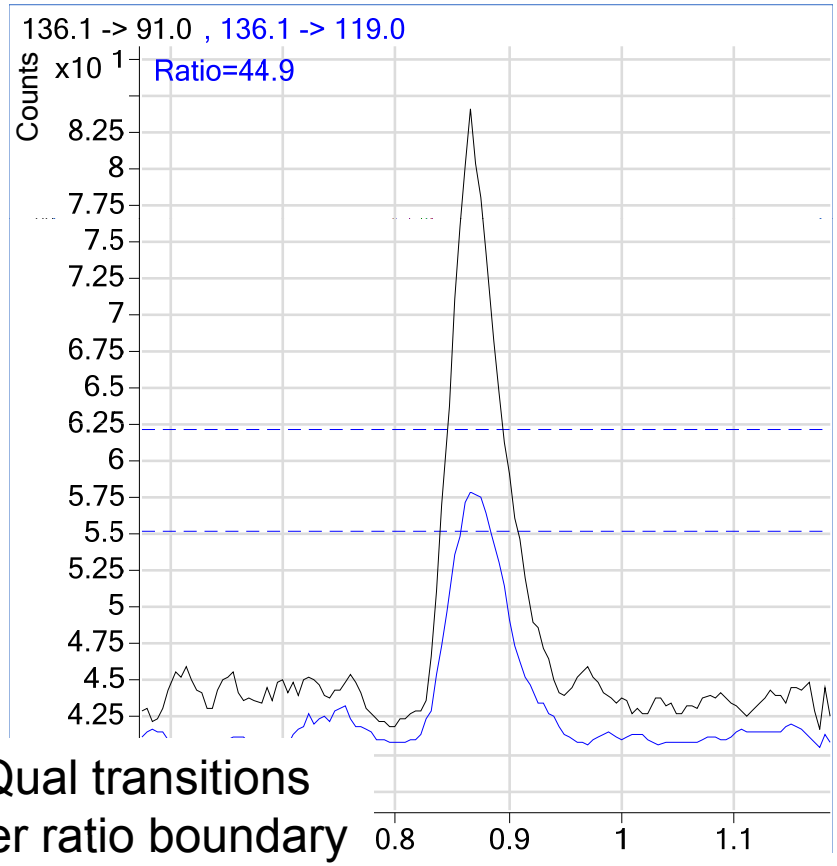
Chromatographic Precision (1290/6460, dynamic MRM)

Amphetamine, 100fg on-column Fish Extract spike

N= 5 (@100fg), %RSD (area) = 6.902997

N= 35 (all Cal levels), %RSD (RT) = 0.50386361

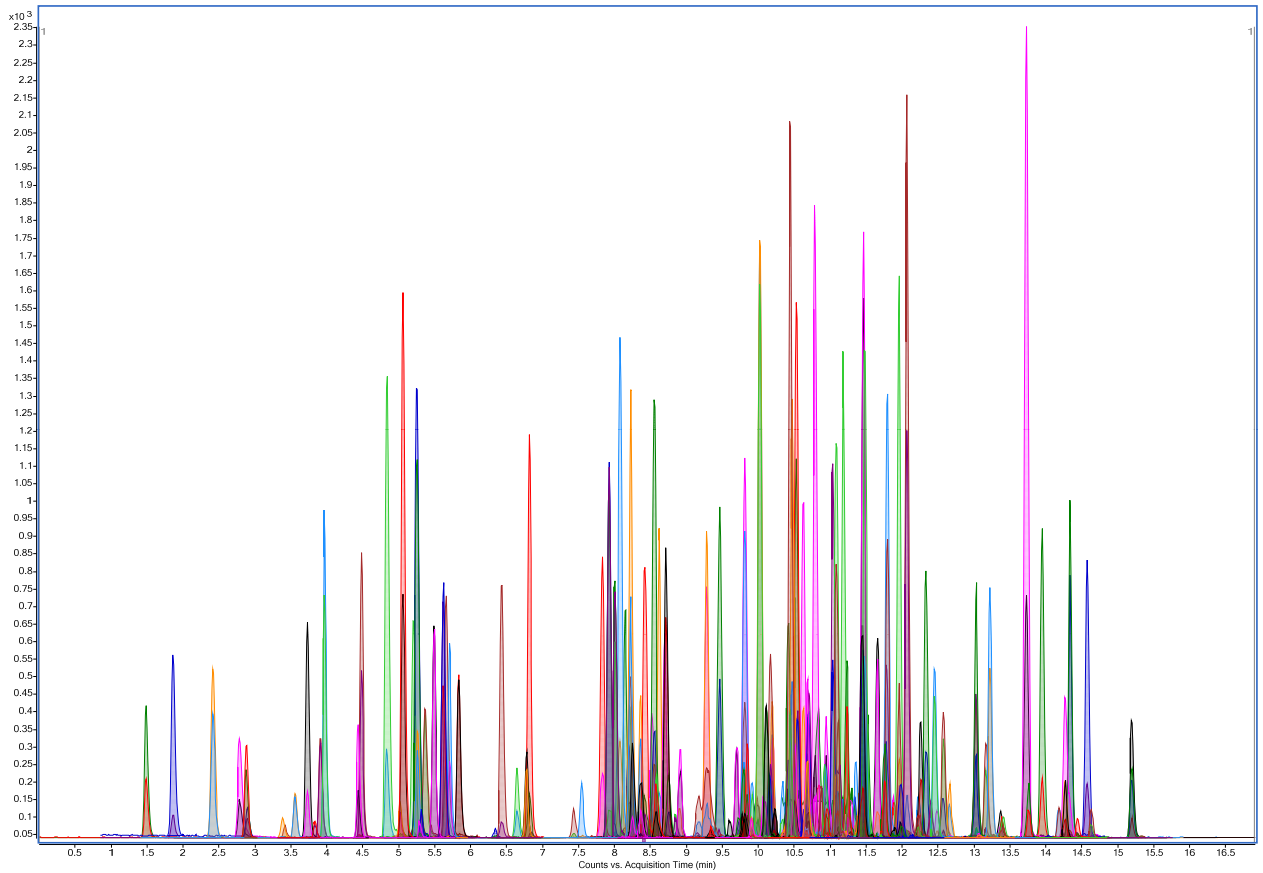




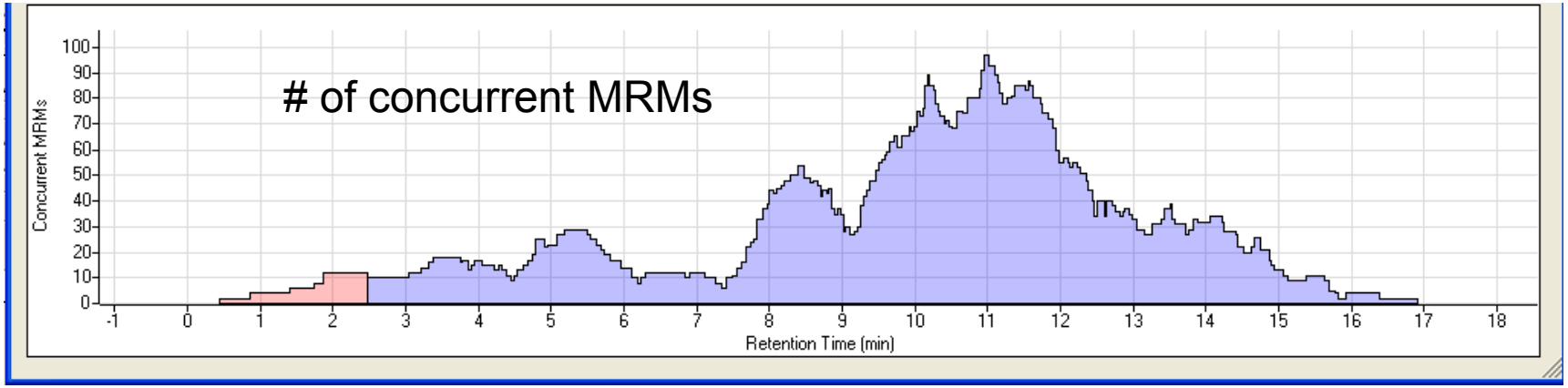
Quant & Qual transitions

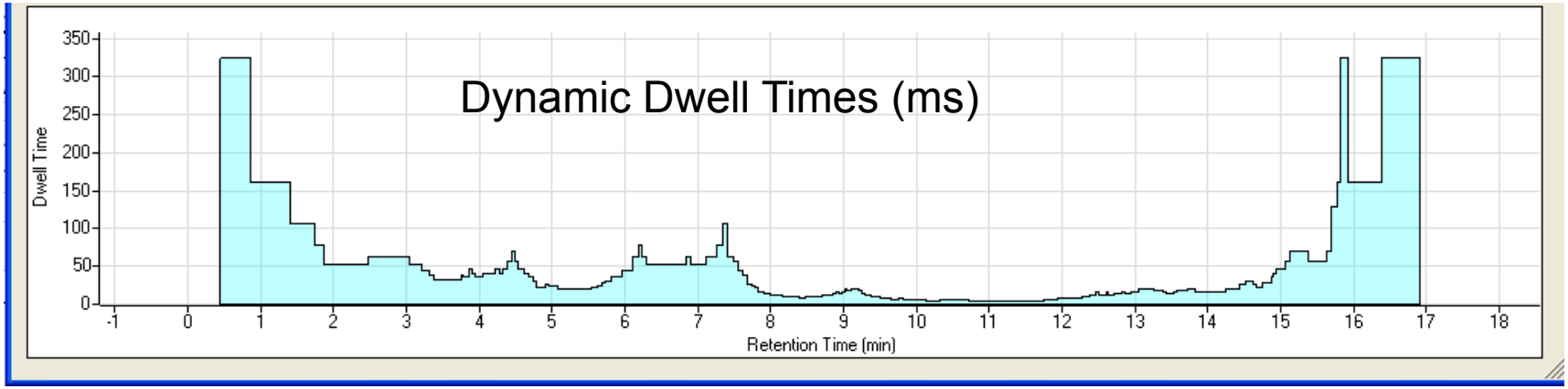
20% outlier ratio boundary

NO Seqmentation with Dynamic MRMs

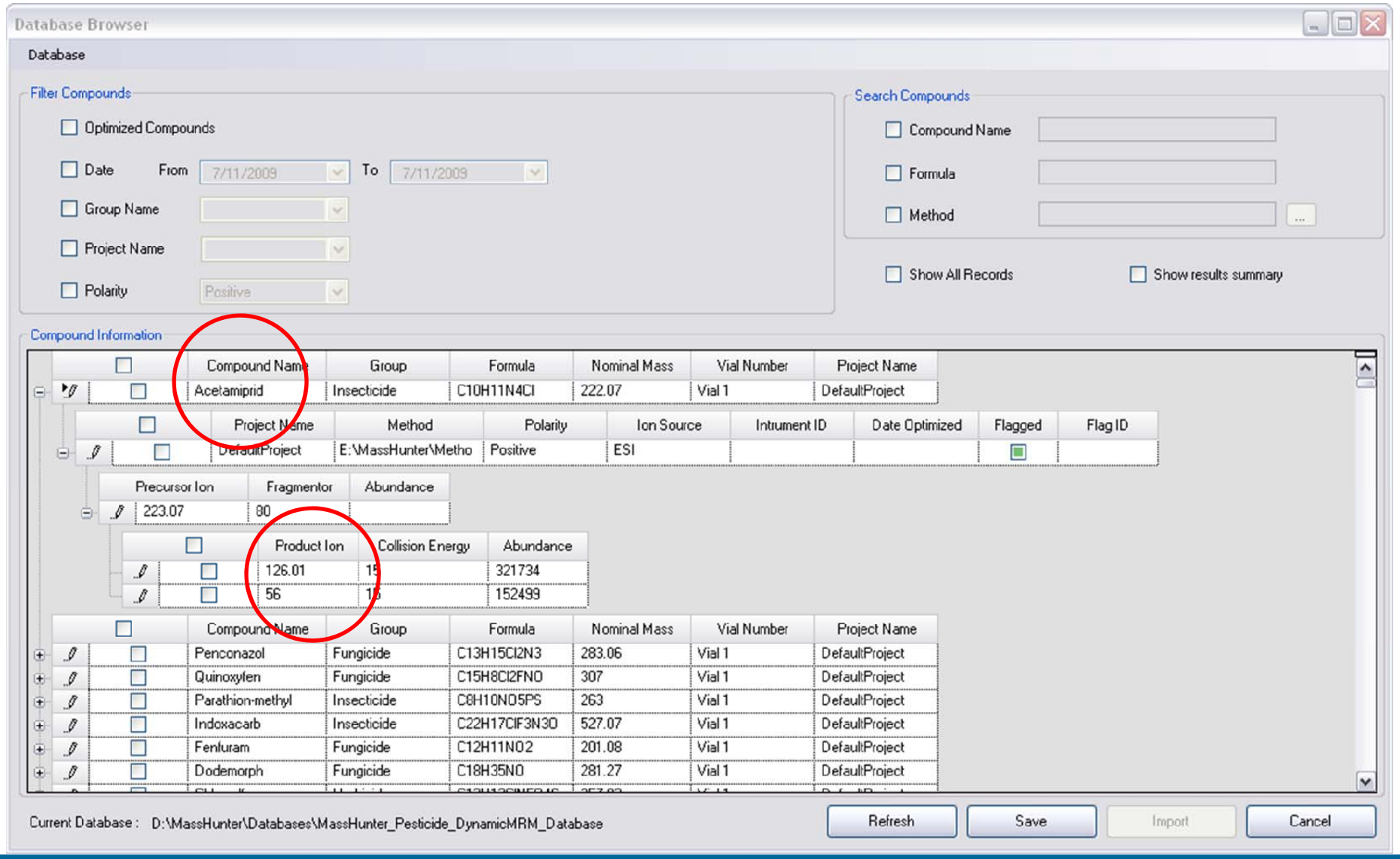


EIC Overlay of 250 pesticide Mix spiked into Food matrix
(500 total transitions, on-column amount 2.5pg) using dynamic MRMs.


# of concurrent MRMs
Concurrent MRMs
Retention Time (min)


Dynamic Dwell Times (ms)
Dwell Time
Retention Time (min)

Agilent >600 Compound Dynamic MRM Database



Analyses of PFCs with Agilent Jetstream ESI 6460 QQQ

Peter JW Stone
Agilent Technologies Inc.
Santa Clara, California

Compounds Analyzed in the Study

Target compound	Precursor ion (m/z)	Product ion (m/z)
perfluoro-1-butanesulfonate (PFBS)	298.8	80.3
perfluoro-n-hexanoic acid (PFHxA)	312.8	268.9
perfluoro-n-heptanoic acid (PFHpA)	362.9	318.9
perfluoro-1-hexanesulfonate (PFHxS)	398.8	79.9
perfluoro-n-octanoic acid (PFOA)	412.8	368.9
perfluoro-n-nonanoic acid (PFNA)	462.8	418.9
perfluoro-1-octanesulfonate (PFOS)	498.8	79.9
perfluoro-n-decanoic acid (PFDA)	512.8	468.9
perfluoro-n-undecanoic acid (PFUA)	562.8	518.9
perfluoro-1-decanesulfonate (PFDS)	598.8	80
perfluoro-n-dodecanoic acid (PFDoA)	612.9	568.9
perfluoro-n-tridecanoic acid (PFTriA)	662.9	619
perfluoro-n-tetradecanoic acid (PFTA)	712.8	669

Rapid Resolution HPLC Parameters

RRLC Configuration: Agilent 1200 Series Binary Pump SL (G1312B) , High Performance Well Plate Sampler SL Plus (G1367D) with Thermostat (G1330B), Thermostated Column Compartment SL with 10Port2Pos (G1316B)

Column:	Zorbax Eclipse Plus C18, 2.1 x 50mm (1.8um)
Column temperature:	55°C
Injection volume:	1 uL (test 1A- LOD); 0.2-1ul (test 1B - MDL)
Autosampler temp:	4°C
Needle wash:	flushport (100% Methanol), 5 seconds
Mobile phase:	A = 2mM NH4-Acetate in Water B = 2mM NH4-Acetate in MeOH
Gradient flow rate:	0.5 mL/min (no split)
Gradient:	Time (min) %B 0 6 0.5 6 6 95 8 95

Agilent Jetstream QQQ MS Parameters

MS: Agilent 6460A Triple Quadrupole LC/MS/MS

QQQ MS Conditions

Ion Mode	ESI+Agilent Jet Stream, Negative

Source conditions:

Capillary Voltage	2500 V
Charging electrode	0 V
Drying gas (nitrogen)	4 L/min
Drying gas temperature	350 °C
Nebulizer gas (nitrogen)	60 psi
Sheath Gas temperature	350 °C
Sheath Gas flow	12 L/min

MRM acquisition:

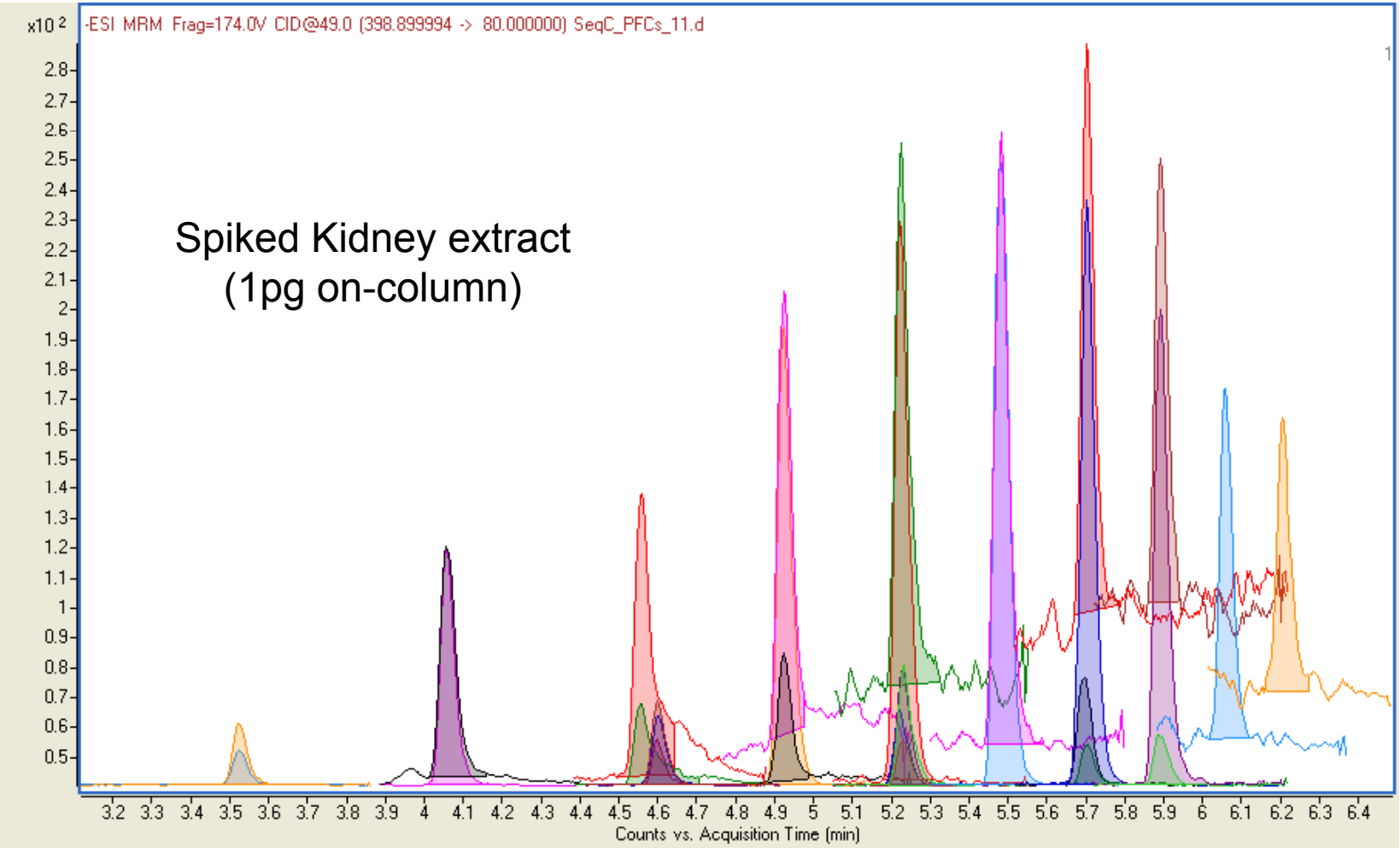
Q1 and Q2 Resolution	0.7 amu [autotune]
Delta EMV	0V

Dynamic MRM PFC Transitions

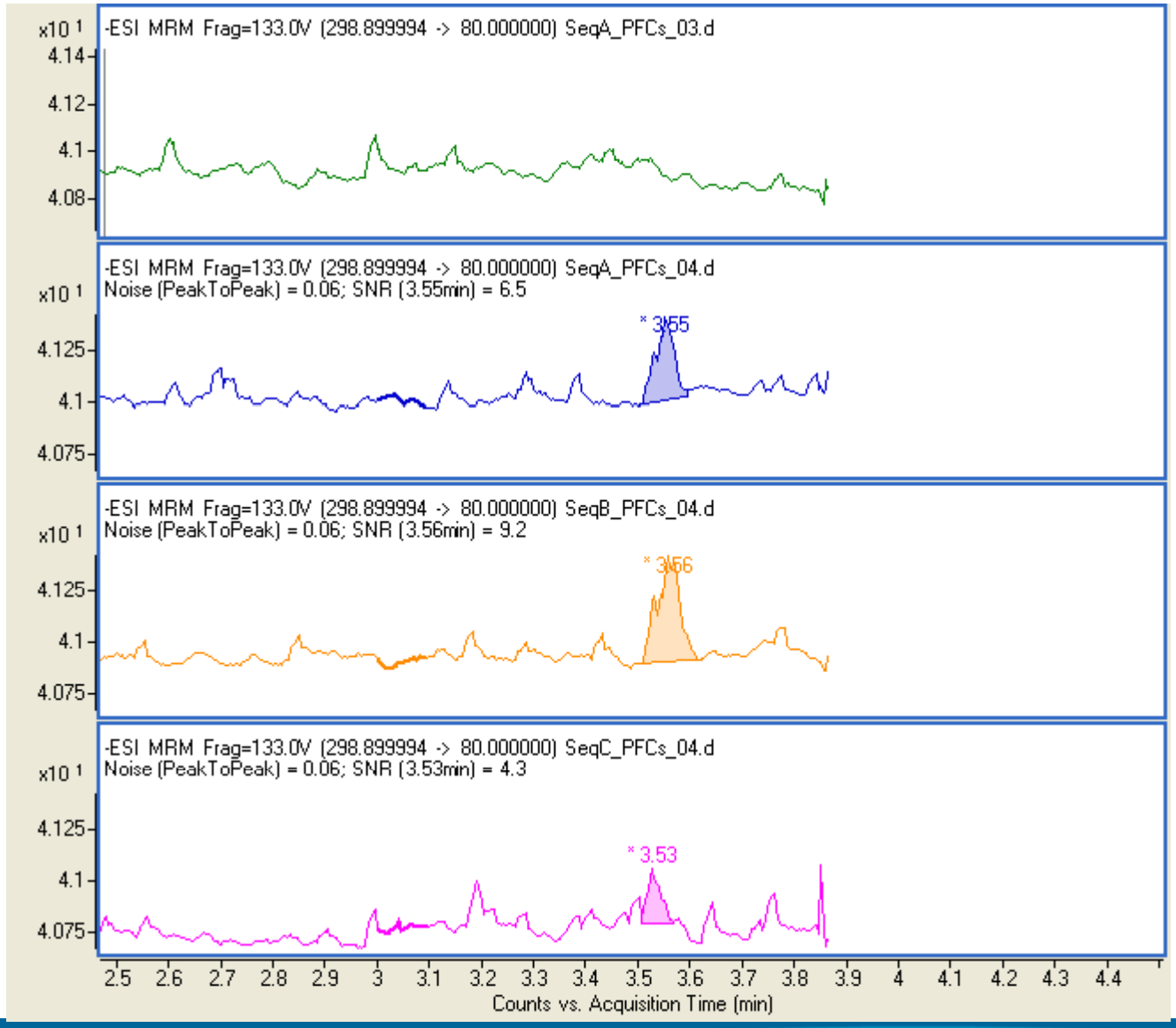
Quant ion Name	Precursor	Q1-Res	Product	Q2-Res	Frag Voltage	CE	RT	RT Delta	IonPolarity
PFBS	298.9	unit	80.0	unit	133	45	3.623	1	Negative
PFDA (C13)2	514.9	unit	469.9	unit	102	5	5.542	1	Negative
PFDA	512.9	unit	469.0	unit	102	5	5.543	1	Negative
PFDoA (C13)2	614.9	unit	570.0	unit	97	5	5.961	1	Negative
PFDoA	612.9	unit	569.0	unit	97	5	5.961	1	Negative
PFDS	598.9	unit	80.0	unit	205	94	5.752	1	Negative
PFHpA	362.9	unit	319.0	unit	66	5	4.626	1	Negative
PFHxA (C13)2	314.9	unit	269.9	unit	66	5	4.141	1	Negative
PFHxA	312.9	unit	268.9	unit	66	5	4.143	1	Negative
PFHxS (O18)2	402.9	unit	83.9	unit	174	49	4.671	1	Negative
PFHxS	398.9	unit	80.0	unit	174	49	4.671	1	Negative
PFNA (C13)5	467.9	unit	423.0	unit	66	5	5.296	1	Negative
PFNA	462.9	unit	418.9	unit	66	5	5.296	1	Negative
PFOA (C13)4	416.9	unit	371.9	unit	86	5	5.001	1	Negative
PFOA	412.9	unit	368.9	unit	86	5	5.003	1	Negative
PFOS (C13)4	502.9	unit	80.0	unit	210	50	5.301	1	Negative
PFOS	498.9	unit	80.0	unit	210	50	5.302	1	Negative
PFTA	712.9	unit	669.0	unit	112	9	6.255	1	Negative
PFTriA	662.9	unit	619.0	unit	102	9	6.117	1	Negative
PFUA (C13)2	564.9	unit	519.9	unit	92	5	5.764	1	Negative
PFUA	562.9	unit	519.0	unit	92	5	5.762	1	Negative

Qual ion Name	Precursor	Q1-Res	Product	Q2-Res	Frag	CE	RT	RT Delta	IonPolarity
PFBS	298.9	unit	98.9	unit	133	29	3.623	1	Negative
PFDoA	612.9	unit	169.0	unit	97	25	5.961	1	Negative
PFHpA	362.9	unit	169.0	unit	66	13	4.626	1	Negative
PFHxS	398.9	unit	99.0	unit	174	45	4.671	1	Negative
PFNA	462.9	unit	169.0	unit	66	17	5.296	1	Negative
PFOA	412.9	unit	169.0	unit	86	13	5.003	1	Negative
PFOS	498.9	unit	99.0	unit	210	50	5.302	1	Negative
PFUA	562.9	unit	169.0	unit	92	21	5.762	1	Negative

PFCs - Overlay of all transitions – Dynamic MRM



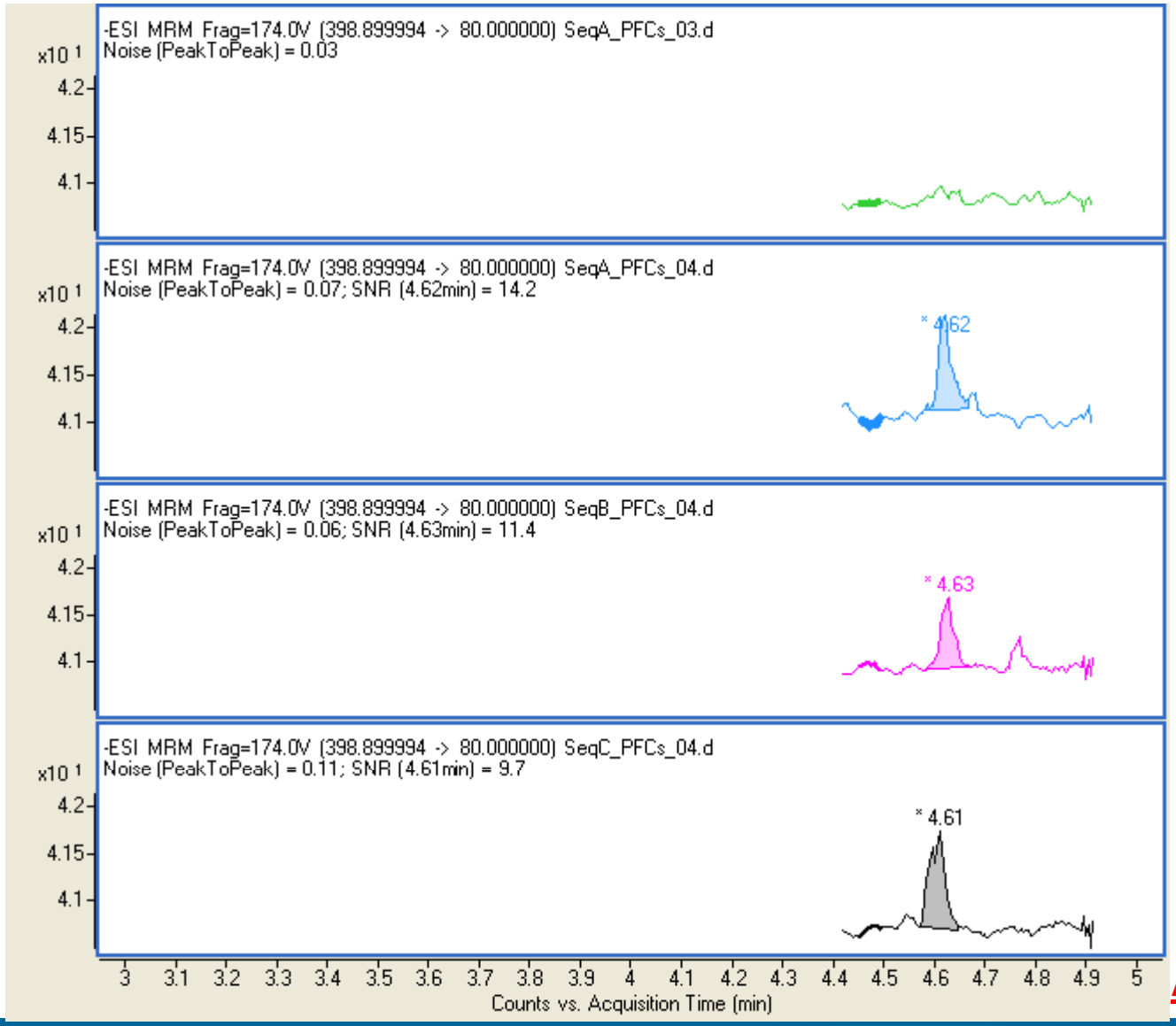
LOD – PFBS – less than 10 fg on-column (5 fg on-column)



LOD (fg on column) SNR 3:1
4.6
3.3
7

Ave (n=3): 5

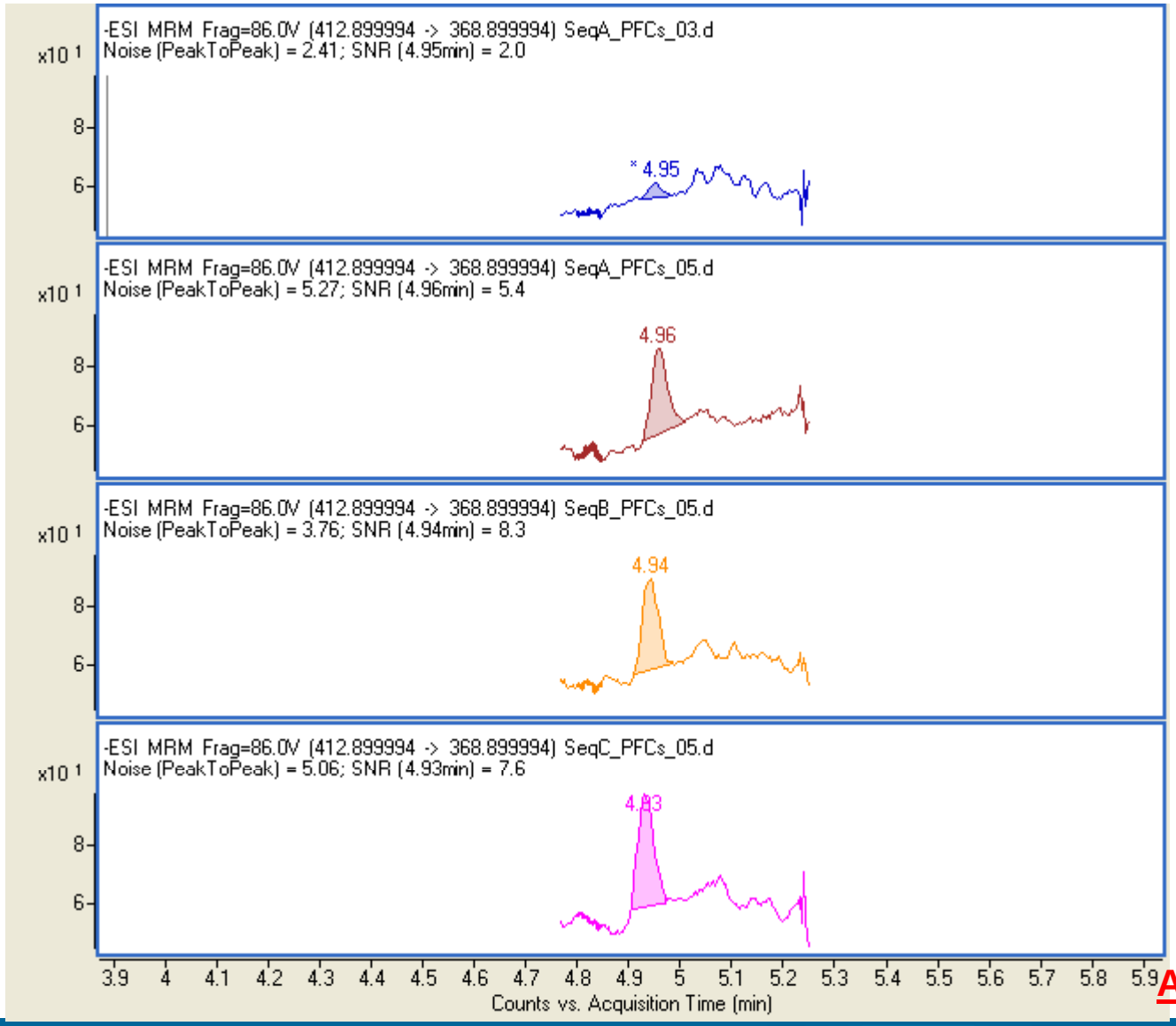
LOD – PFHxS – < 10 fg on-column (2.6 fg on-column)



	LOD (fg on column) SNR 3:1
BLANK	
PFC-STD #1	2.1
PFC-STD #2	2.6
PFC-STD #3	3.1

Ave (n=3): 2.6

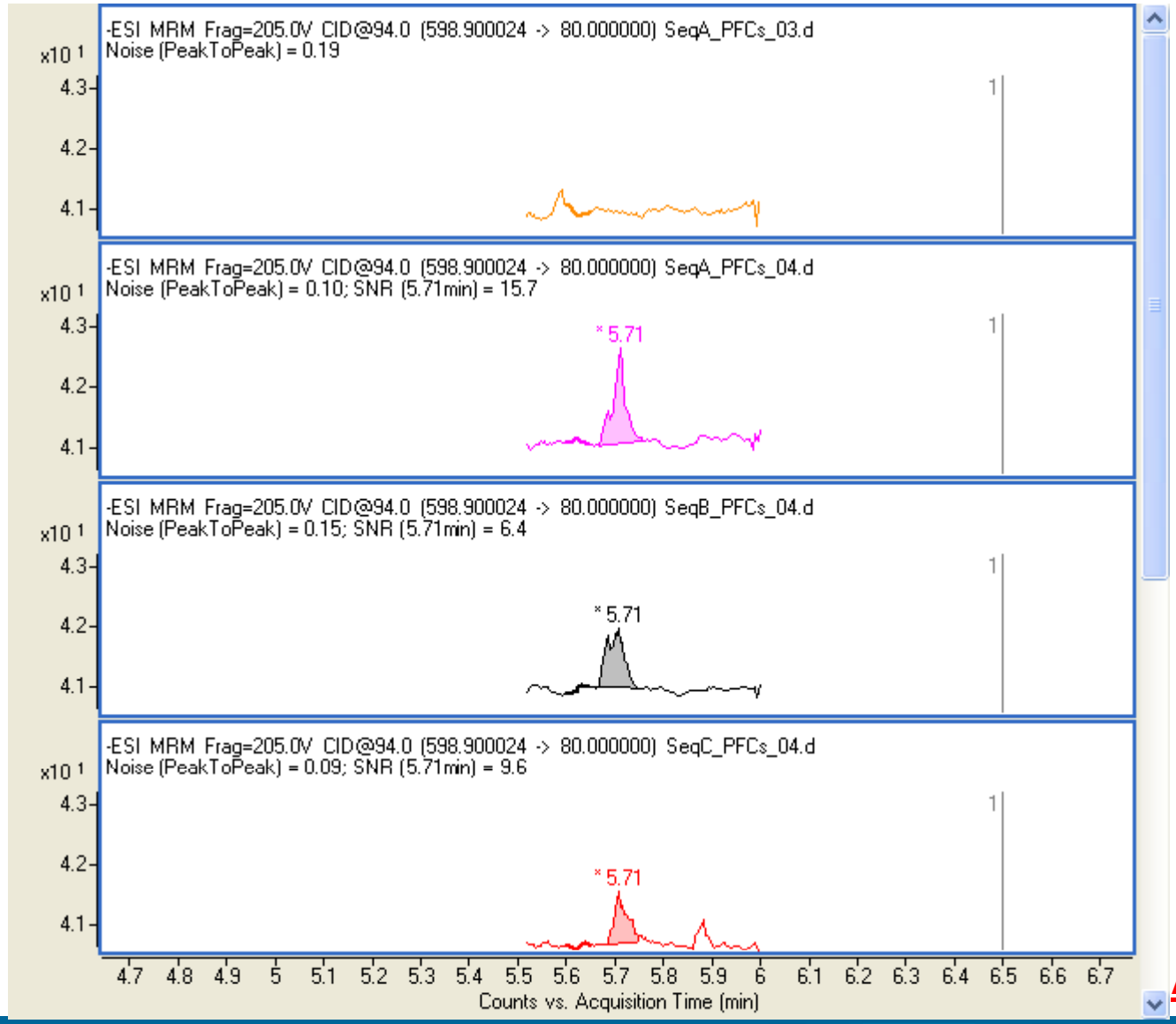
LOD – PFOA – < 10 fg on-column (43.7 fg on-column)



	LOD (fg on column) SNR 3:1 estimated
BLANK	
PFC-STD #1	55.6
PFC-STD #2	36.1
PFC-STD #3	39.5

Ave (n=3): 43.7

LOD – PFDS – less than 10 fg on-column (3.2 fg on-column)



	LOD (fg on column) SNR 3:1 estimated
BLANK	
PFD-ST6 #1	1.9
PFD-ST6 #2	4.7
PFD-ST6 #3	3.1

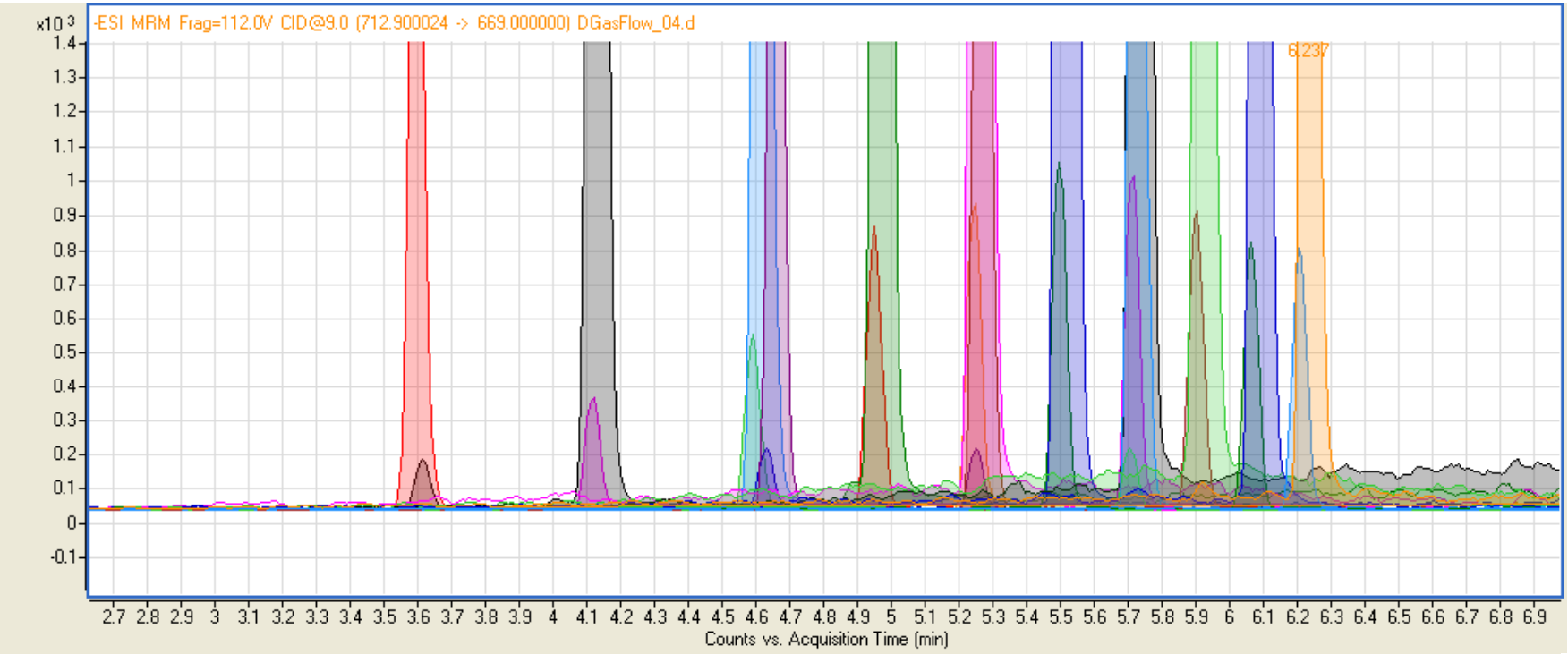
Ave (n=3): 3.2

PFC Suite LODs – Summary

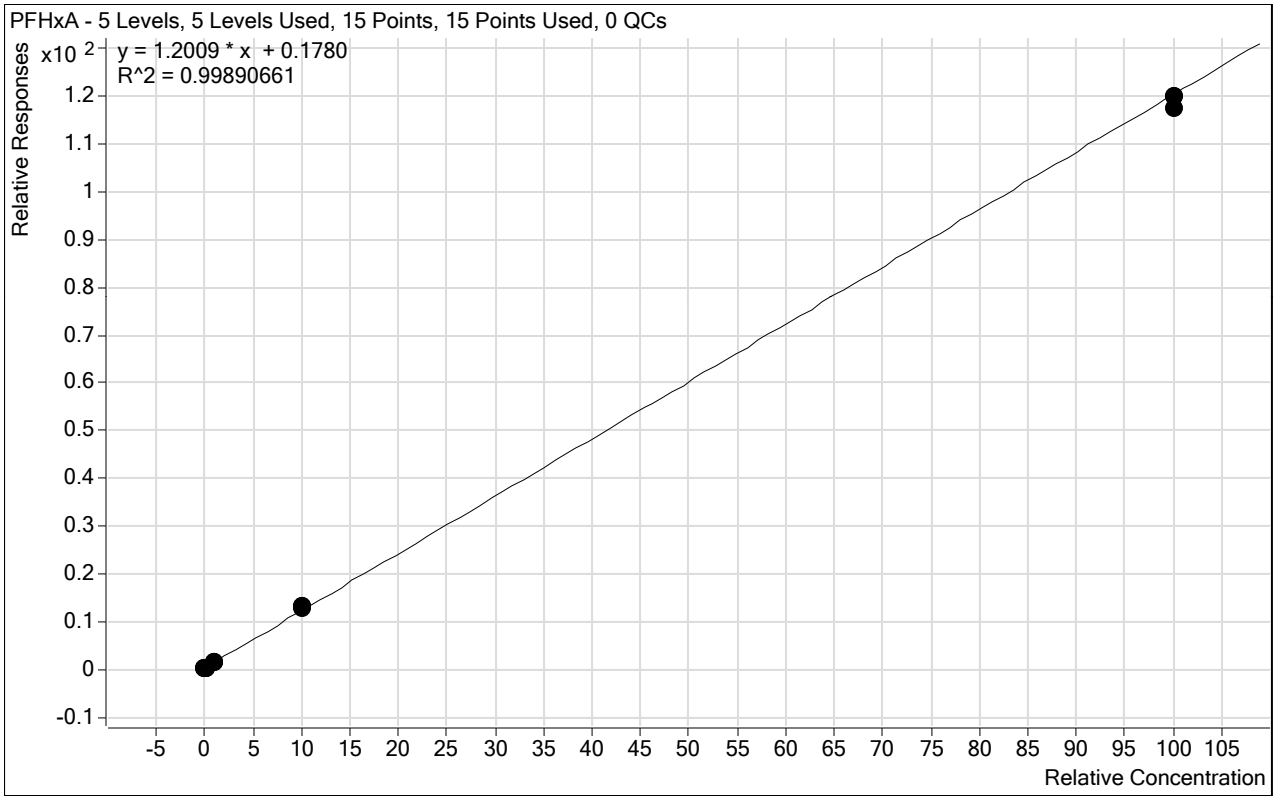
Compounds	LODs (fg on column) SNR 3:1
PFBS	5.0
PFHxA	8.4
PFHpA	12.2
PFHxS	2.6
PFOA	43.7
PFNA	75.0
PFOS	5.7
PFDA	36.3
PFUA	44.0
PFDS	3.2
PFDoA	55.9
PFTriA	74.2
PFTA	21.7

The lowest concentration that all 13 compounds can be detected is at level of less than 75fg on column.

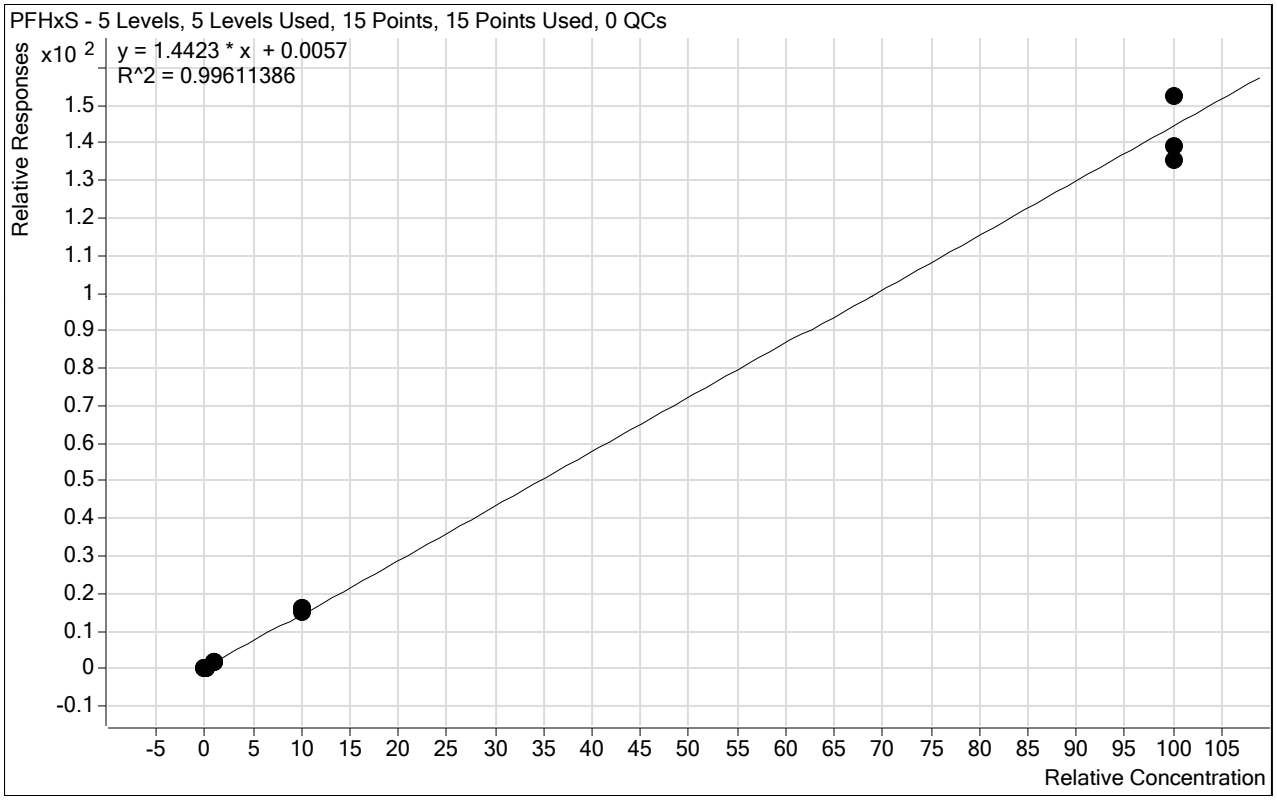
Overlay Chromatograms of Non-Optimised versus AJS-Optimized Responses



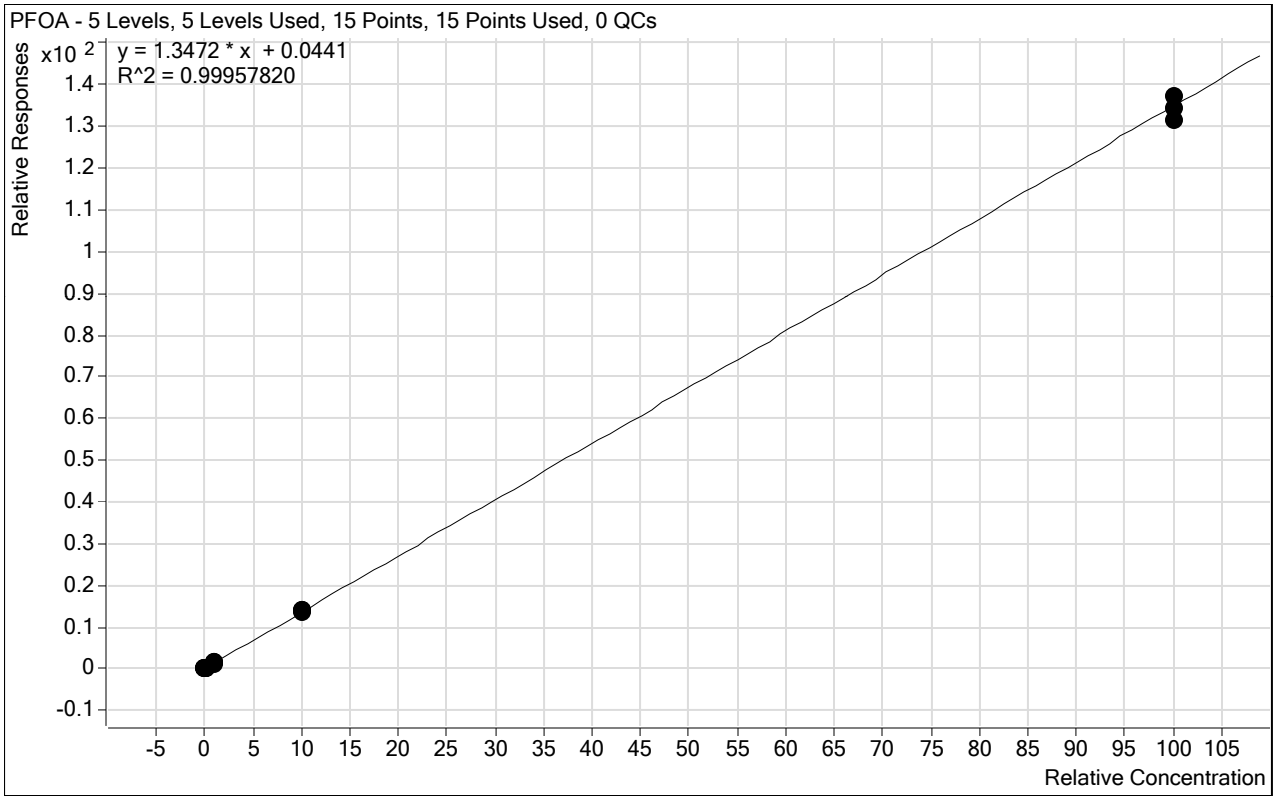
Calibration Curve for PFHxA
Linear Correlation = 0.99890661



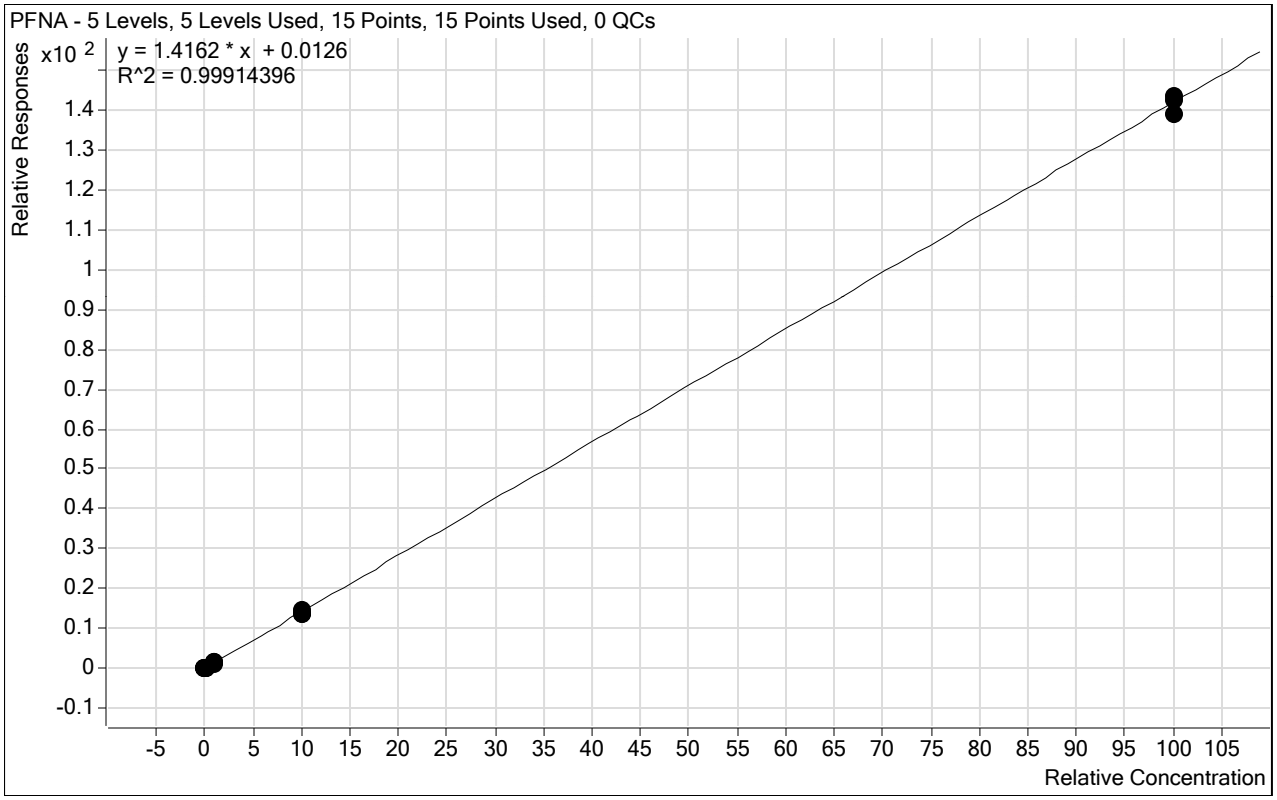
Calibration Curve for PFHxS
Linear Correlation = 0.99611386



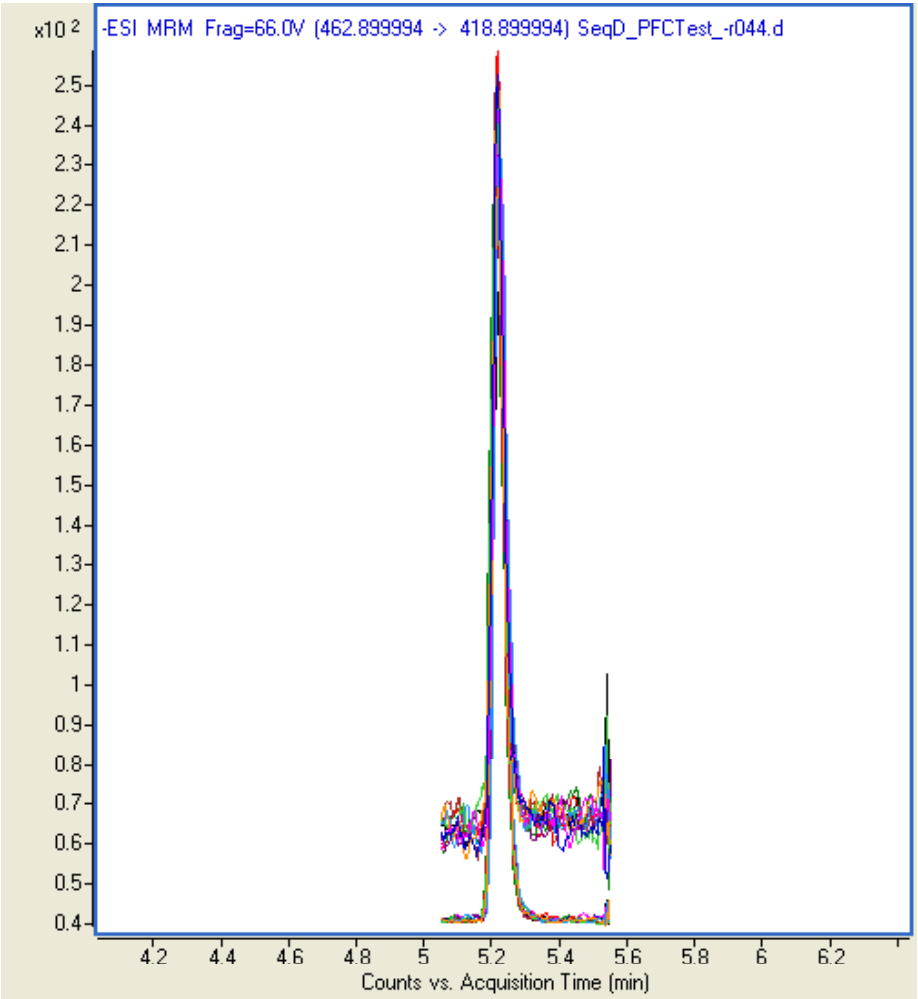
Calibration Curve for PFOA
Linear Correlation = 0.99957820



Calibration Curve for PFNA
Linear Correlation = 0.99914396



Overlaid chromatograms N=10 replicate injections of PFNA with ISTD @ MDL in Matrix



ISTD Response Ratio
1.002
1.010
1.007
1.009
1.015
0.892
0.894
0.970
1.016
0.982
% RSD = 4.9

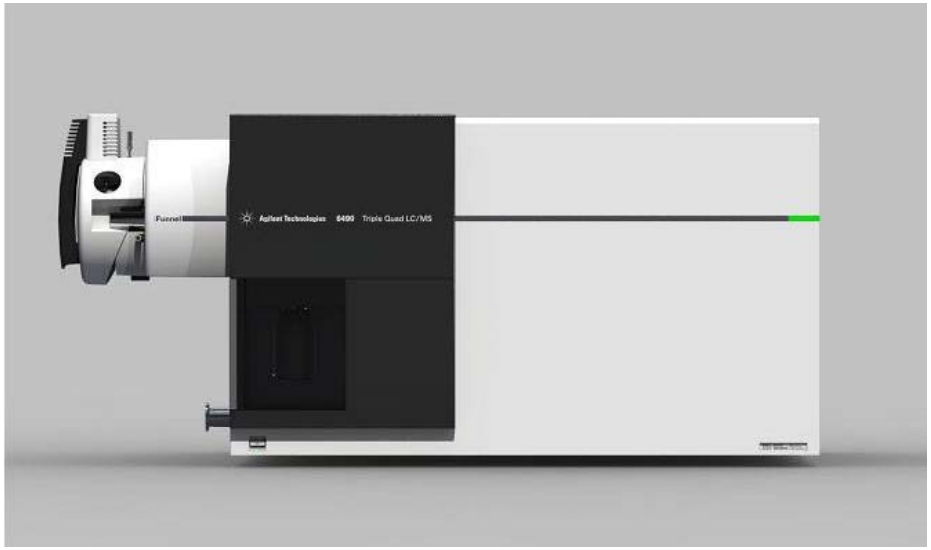
MDLs from PFC_Test – Summary (n=10) (Liver Spike)

Compounds	MDLs (injection volume ul)	% RSD	MDLs (fg on column)	LODs (fg on column) SNR 3:1	3 x LODs	MDLs (fg on column) SNR 10:1
PFBS	0.4	7.4	400	5.0	14.9	97.7
PFHxA	0.4	4.3	400	8.4	25.3	110.5
PFHpA	0.8	11.3	800	12.2	36.7	249.0
PFHxS	0.2	20.2	200	2.6	7.8	44.62
PFOA	0.6	5.9	600	43.7	131.2	291.5
PFNA	1.0	4.9	1000	75.0	225.1	421.3
PFOS	0.2	23.9	200	5.7	17.0	58.3
PFDA	0.6	11.0	600	36.3	109.0	275.3
PFUA	0.8	5.7	800	44.0	132.0	303.9
PFDS	0.2	18.6	200	3.2	9.7	54.9
PFDoA	0.8	6.2	800	55.9	167.7	594.5
PFTriA	0.8	5.9	800	74.2	222.5	494.5
PFTA	0.8	10.8	800	21.7	65.1	503.2

New 6490 - World's Most Sensitive LC/QQQ

iFunnel Technology

- Agilent Jet Stream
- Hexabore capillary
- Dual-stage ion funnel



Collision Cell – hexapole

- Tapered structure for increased ion acceptance
- Reduction of background noise

Improved Quad Drive Electronics

- Higher RF power capability
- Quad drive frequency increased to 1.4 MHz

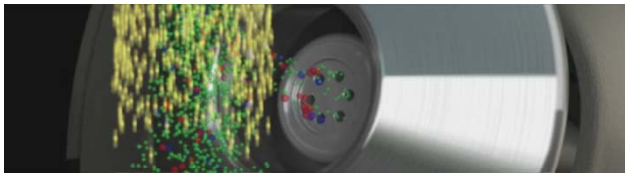
iFunnel Technology captures 10X more ions

Agilent Jet Stream

- Thermal confinement of ESI plume
- Efficient desolvation to create gas phase ions
- Creates an ion rich zone

Hexabore Capillary

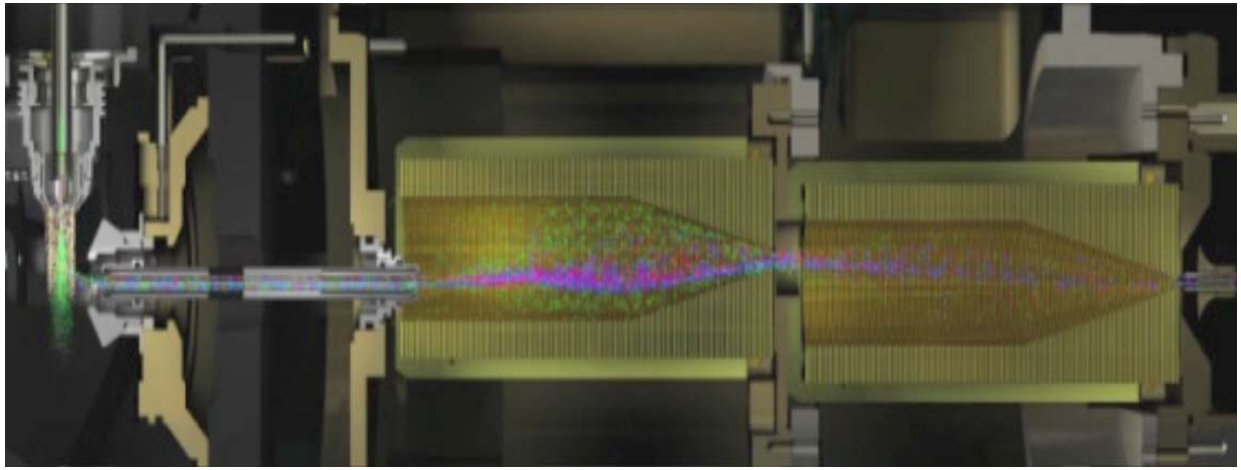
- 6 capillary inlets
- Samples 6X more ion rich gas from the source
- Captures the majority of the gas from the source region



Dual Ion Funnel

- Removes the gas but captures the ions
- Removes neutral noise
- Extends turbo pump life

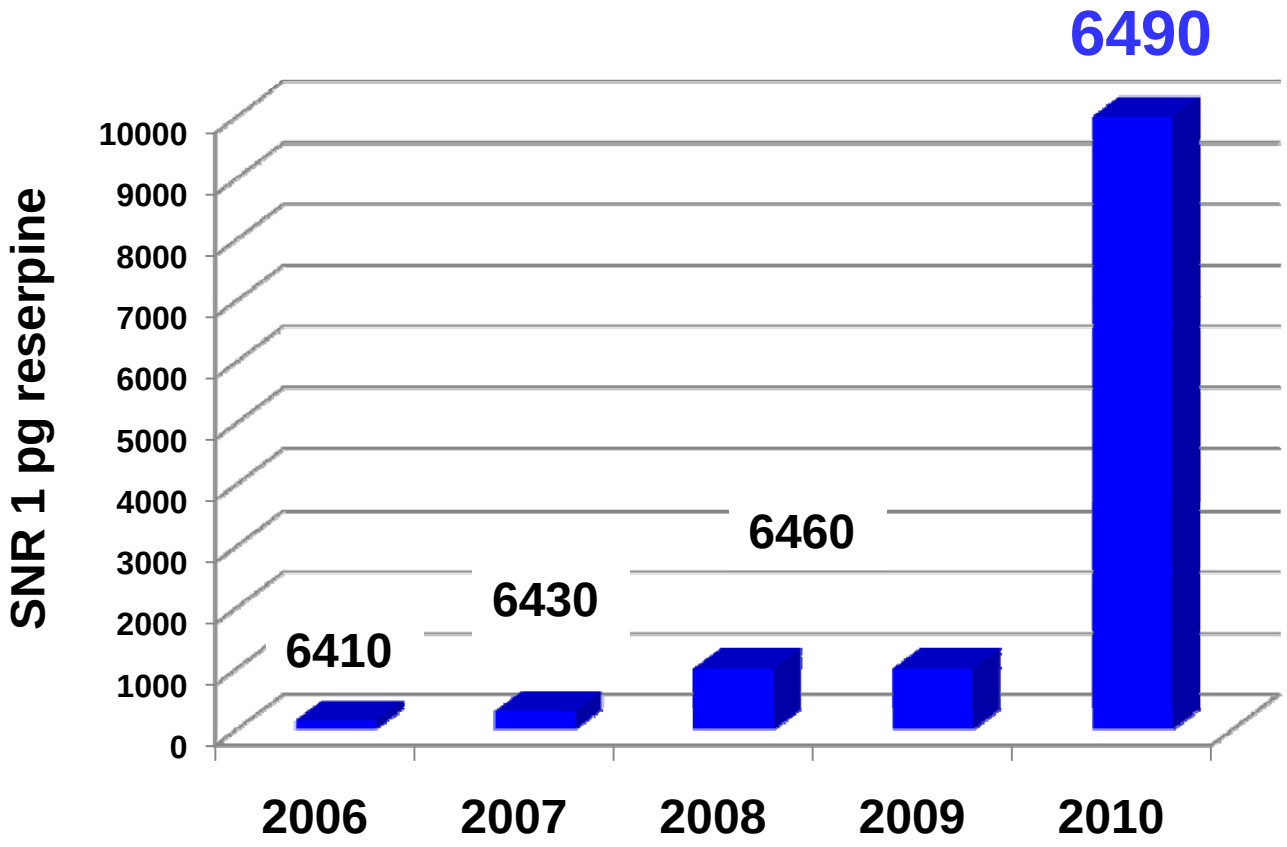
New iFunnel Technology Revolutionizes Atmospheric Sampling



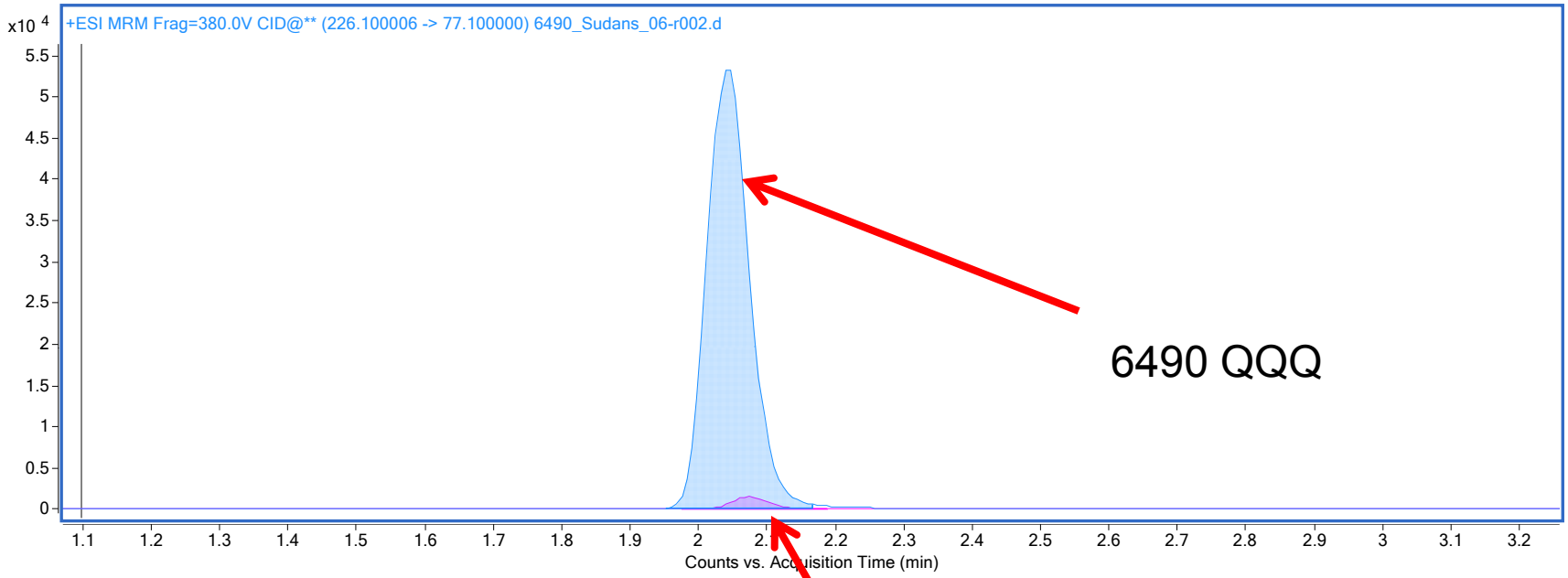
The combined result of all three elements of iFunnel Technology is a dramatic gain in sensitivity.

Agilent Triple Quads: 2006 – 2010

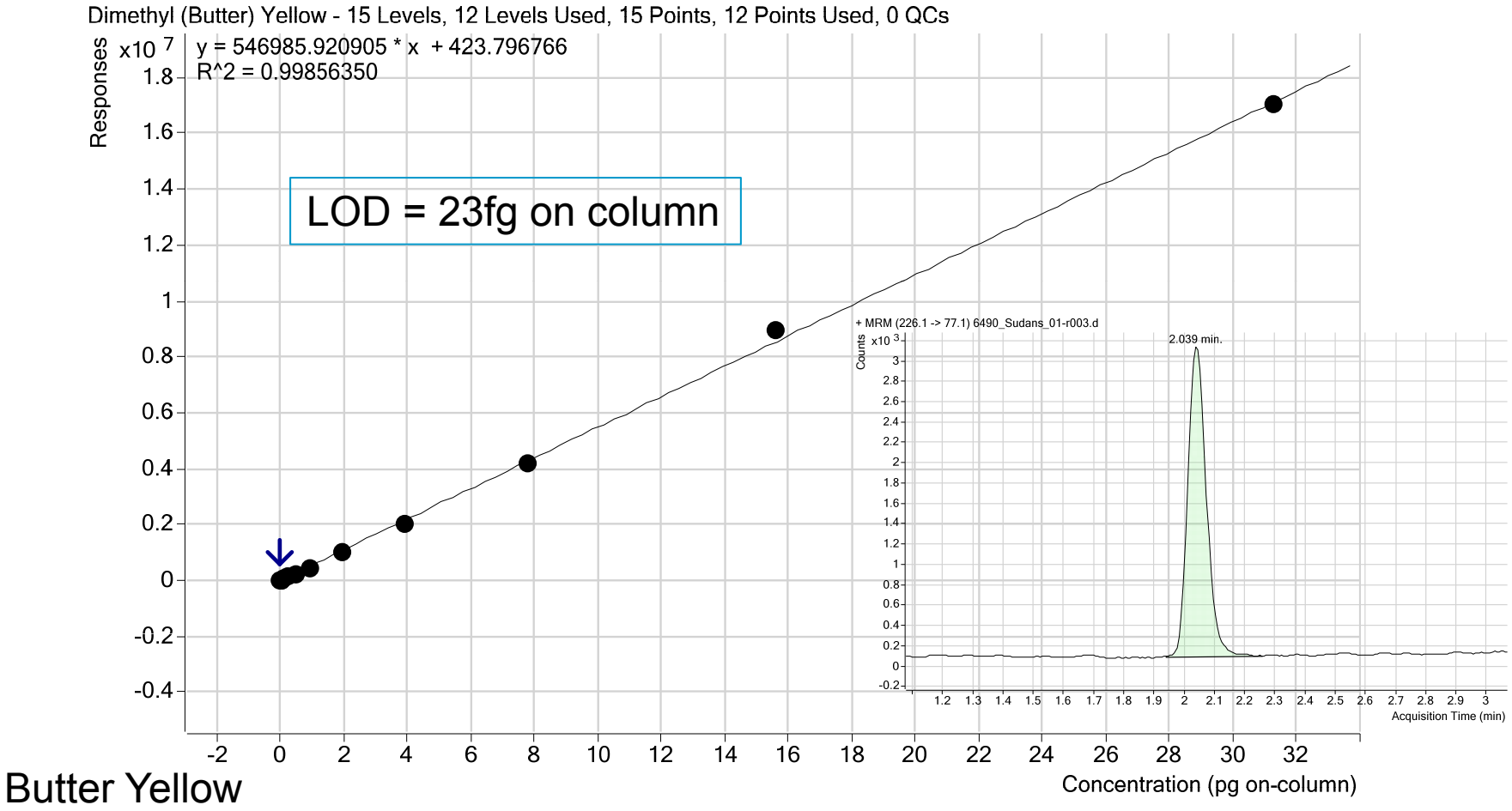
Relentless increase in signal-to-noise specs



Sudan Red Dyes
Baby formula, extracted via Quechers



Sudan Red Dyes
Baby formula, extracted via Quechers

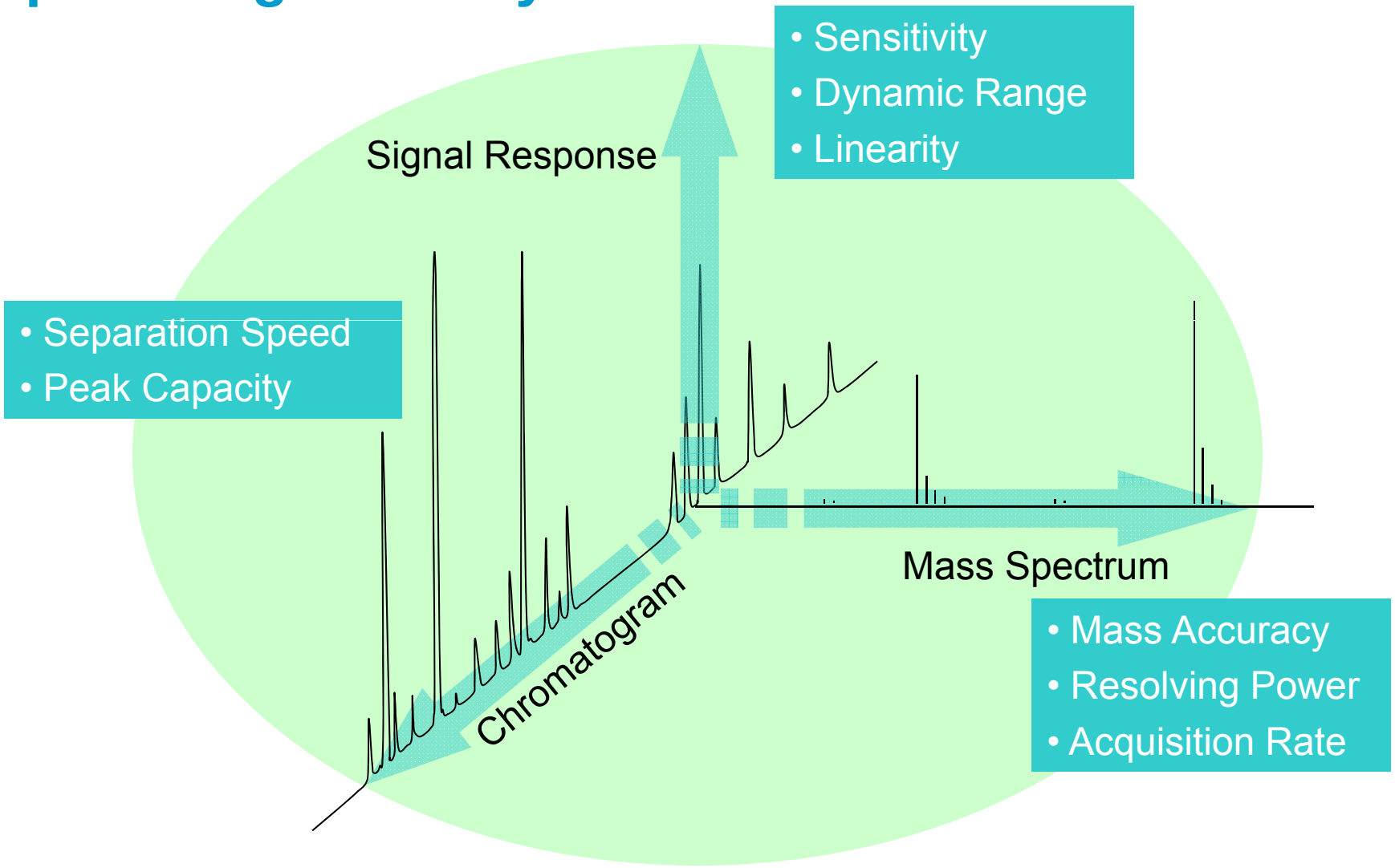


Recent Advances in Ultra High Definition Mass Spectrometry with the 1290 Infinity uHPLC System

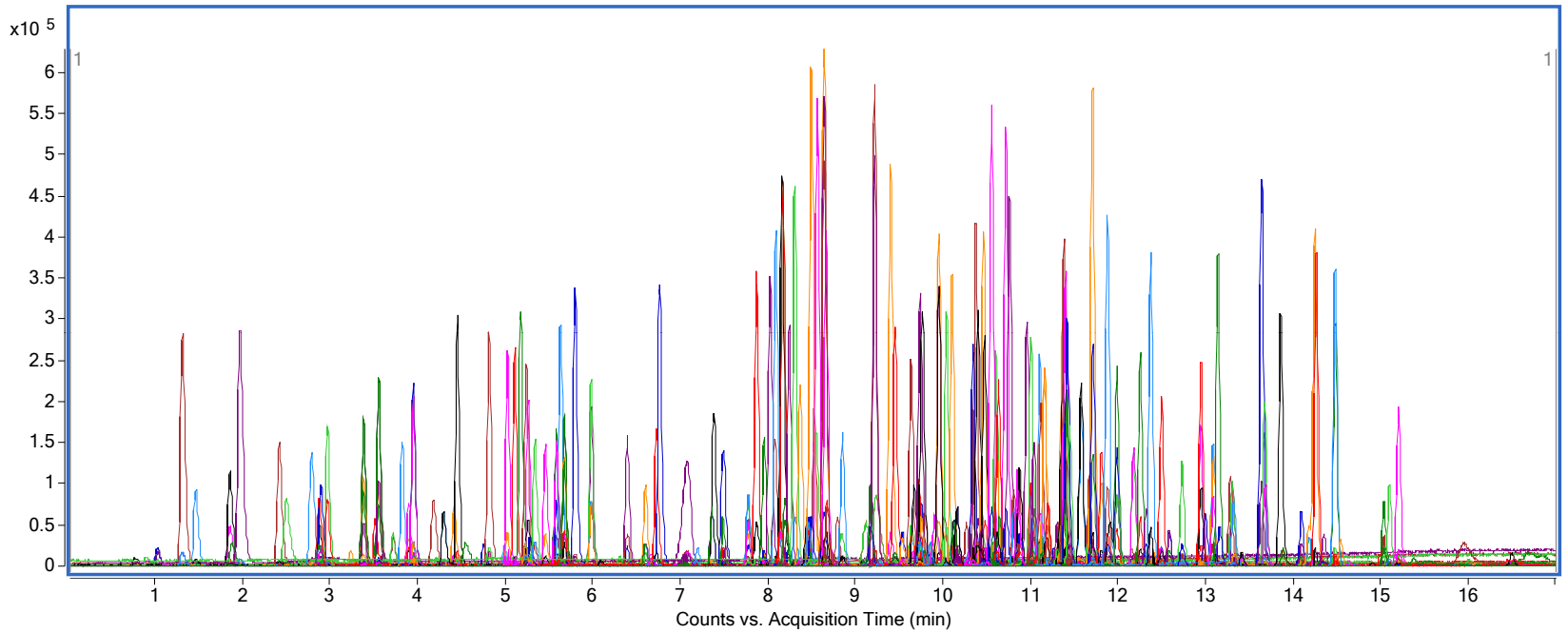
(Agilent 1290 Infinity uHPLC, Agilent 6230 TOF and 6540 Q-TOF.)

Peter Stone
Agilent Tecnologies Inc,
Santa Clara, CA.

Ultra High Definition
Optimizing all Analytical Dimensions

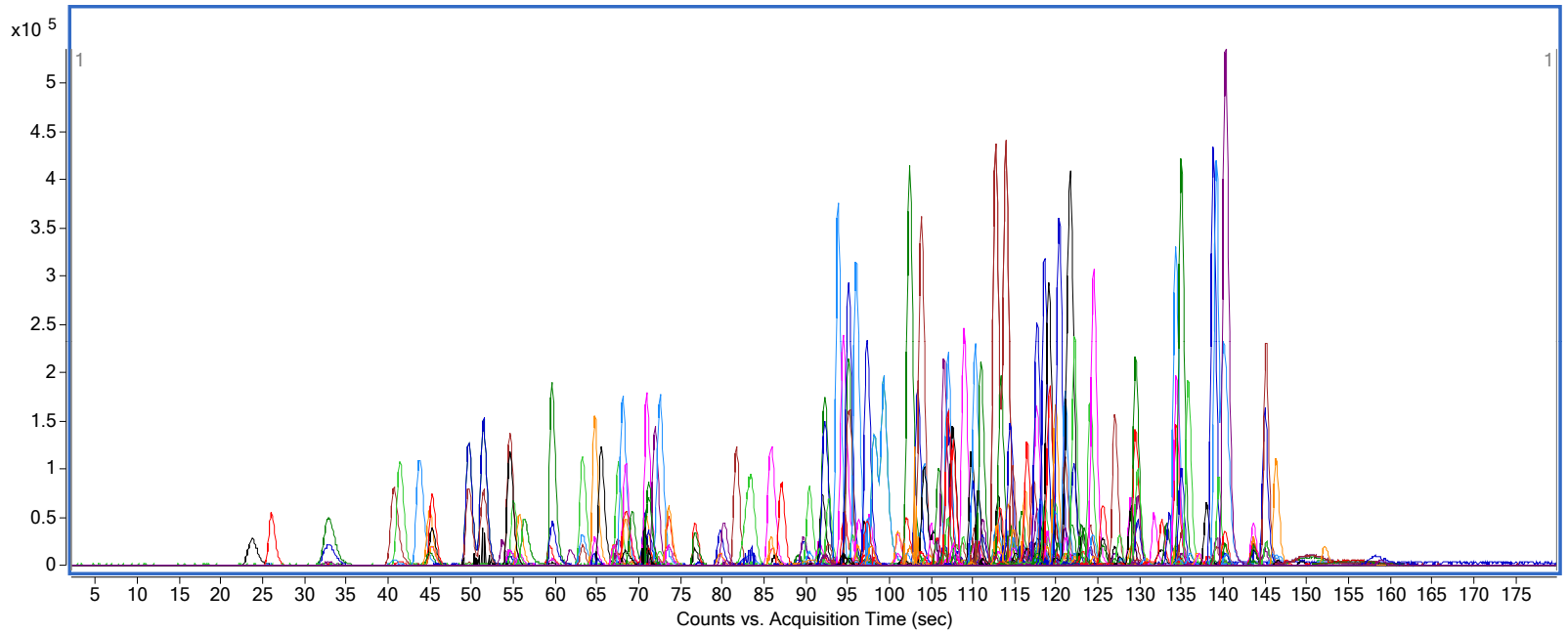


17 minute analysis – 224 Pesticides
6230 TOF/1290 Infinity uHPLC



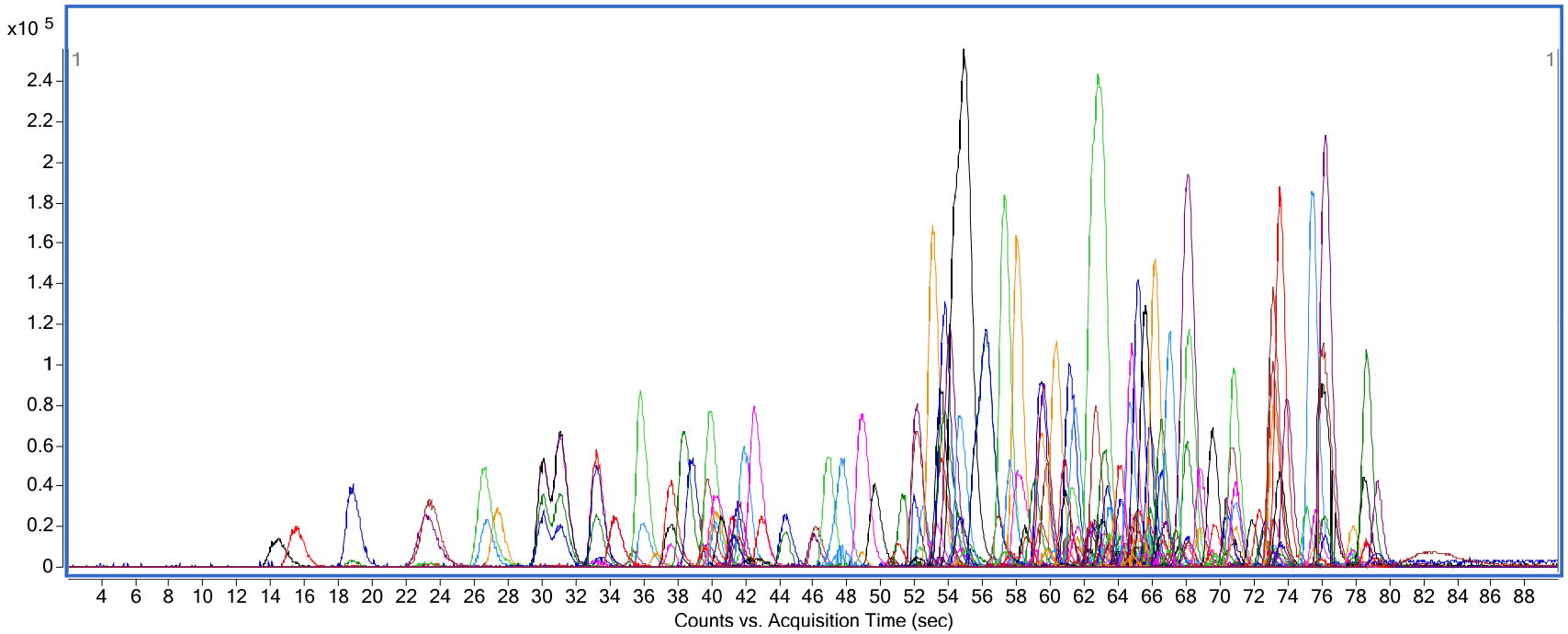
3 Hz Data Acquisition Speed
50pg on-column

3 minute analysis – 224 Pesticides
6230 TOF/1290 Infinity uHPLC



10 Hz Data Acquisition Speed
50pg on-column

90 second analysis – 224 Pesticides
6230 TOF/1290 Infinity uHPLC

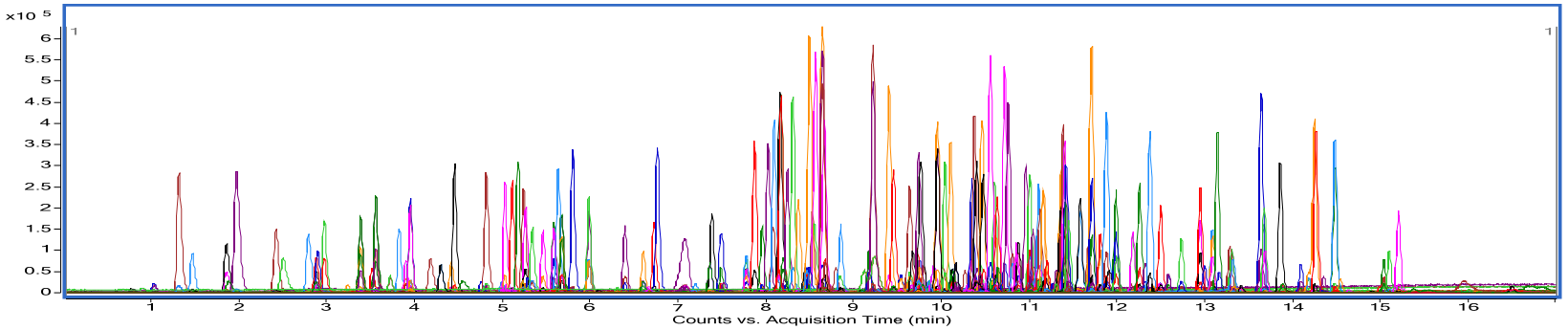


20 Hz Data Acquisition Speed
50pg on-column

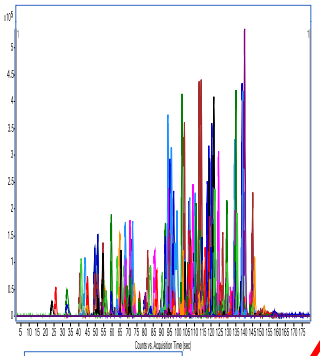
Agilent 6230 TOF Pesticide Screening

224 pesticides, 50 pg on column

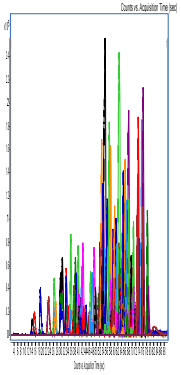
17 min.

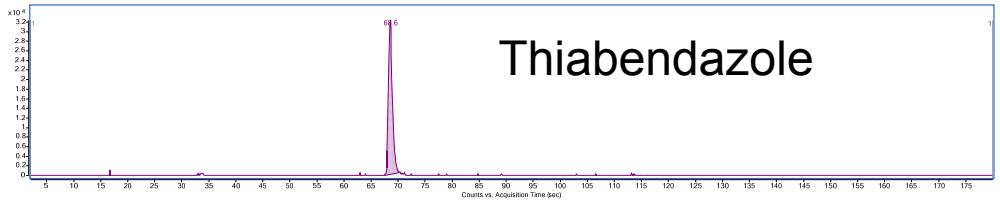


3 min.



1.5 min.

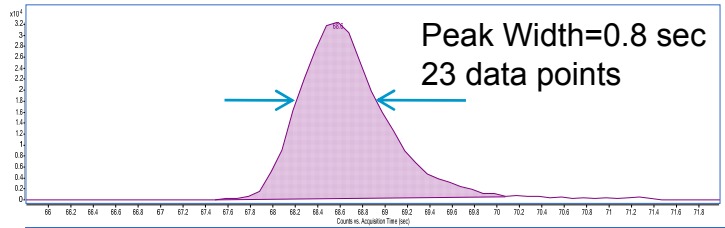


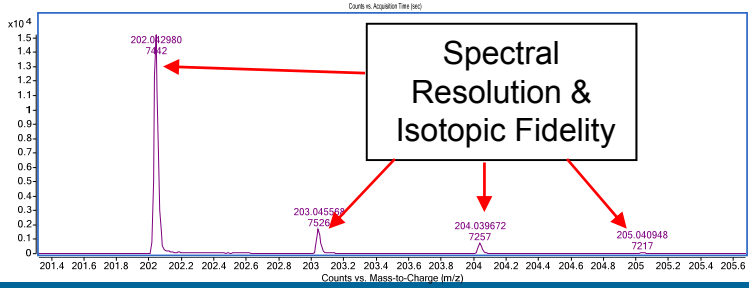


CompoundList

Cpd	Name	RT	Mass	Diff (Tgt, ppm)	Diff (Tgt, mDa)
191	Tebufenpyrad	123	333.1613	1.43	0.48
174	Tebupirimfos	138	318.1172	1.65	0.52
145	Tebupirimfos oxone	107.1	302.1397	0.36	0.11
77	Tebuthiuron	81.7	228.1044	-0.21	-0.05
115	Terbufos	120	288.0437	-1.59	-0.46
74	Terbumeton	97.7	225.1589	-0.05	-0.01
28	Terbuthylazine-desethyl	54.7	201.0798	3.46	0.7
91	Terbutryn	112.8	241.1362	0.5	0.12
215	Tetraconazol	110.8	371.0216	0.24	0.09
25	Thiabendazole	68.6	201.0357	-1.81	-0.36
94	Thiacloprid	77	252.0233	-1.38	-0.35
118					0.08
127					-11.66
167					-0.16
169	Tribufos	146.3	314.0964	0.76	0.24
15	Triciclazole	72	189.0359	-0.97	-0.18

-1.8 ppm





Focused AMRT Databases
(Personal Compound Databases (PCD)

Pesticides, Part # (G6854AA):

- 1600 analyte content (formulae & accurate mass)
- Structures
- CAS Numbers & Links to external website (pubchem)

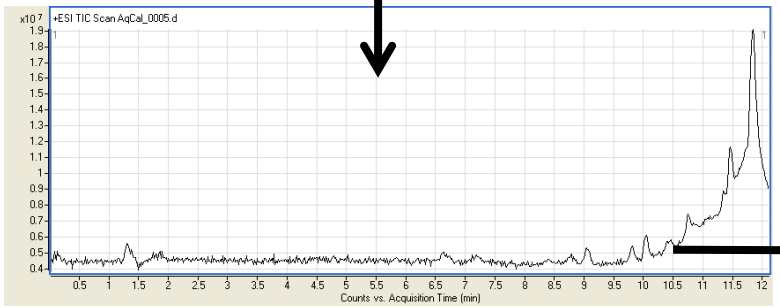
Forensic & Toxicology, Part # (G6855AA):

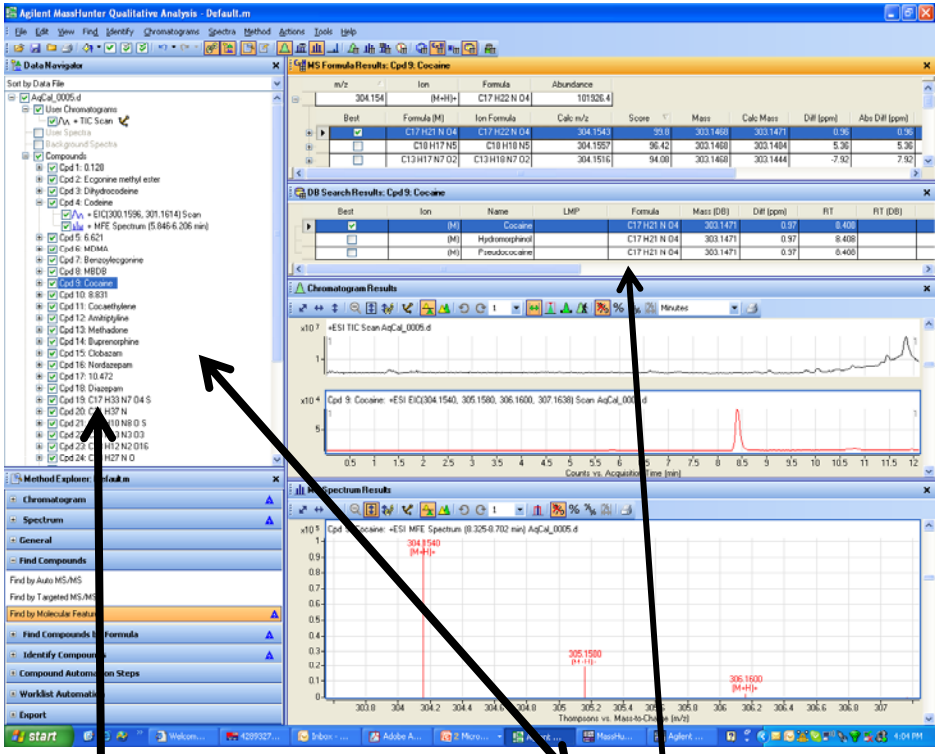
- ~7000 analytes content (formulae & accurate mass)
- Structures
- CAS Numbers & Links to external website (pubchem)

Agilent TOF Screening Solution (PCD)

Agilent 6230 TOF

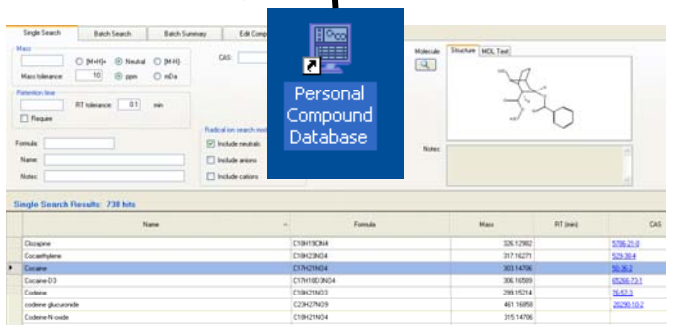
- **20K resolution**
- **5 decades in-scan**
- Low Pg Sensitivity





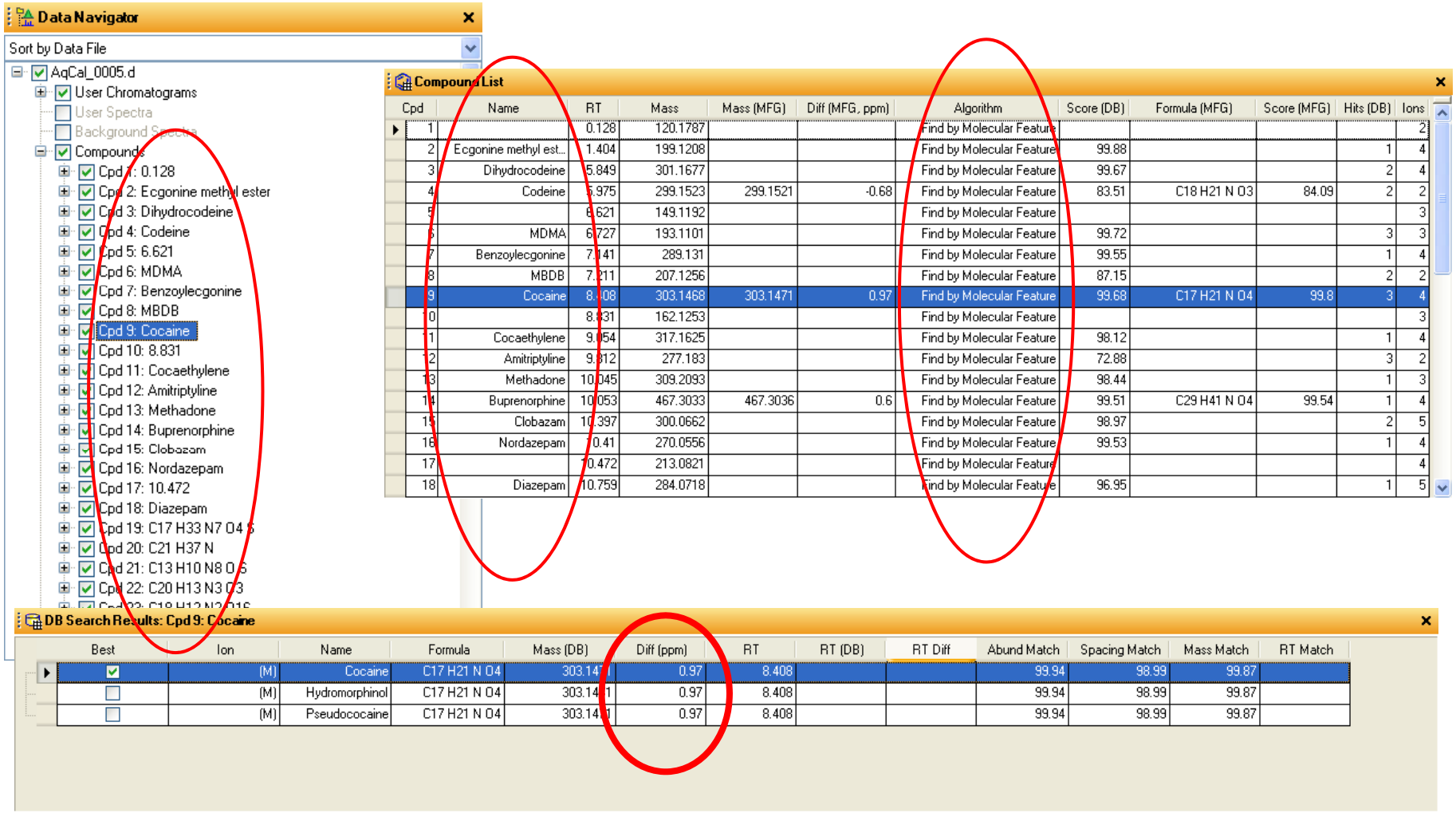
Molecular Feature Extractor

Find by Formula



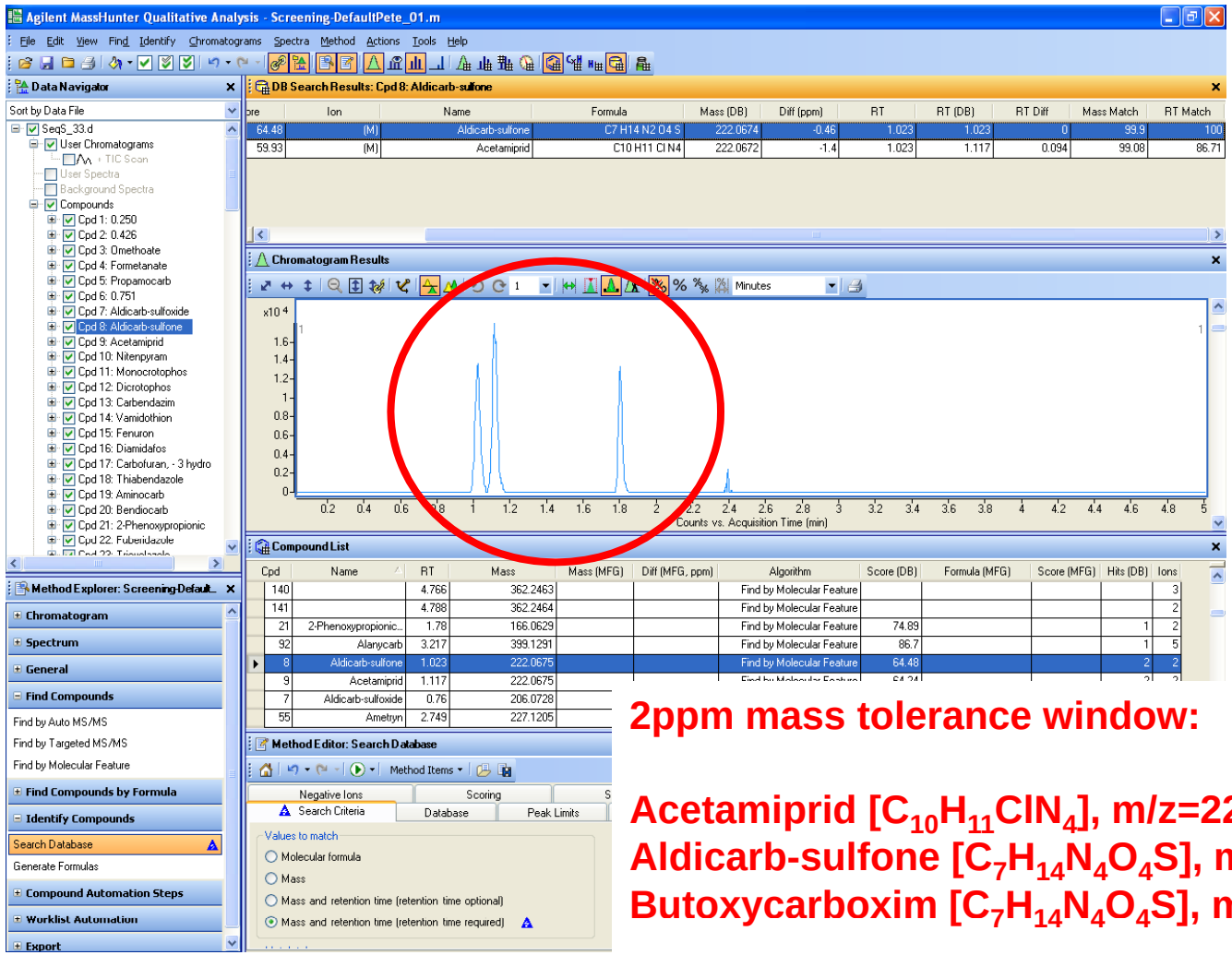
Agilent Technologies

PCD Search Results For 50pg Forensic Spiked Blood – PCD Search Confirmed compound 9 as Cocaine



Agilent Technologies

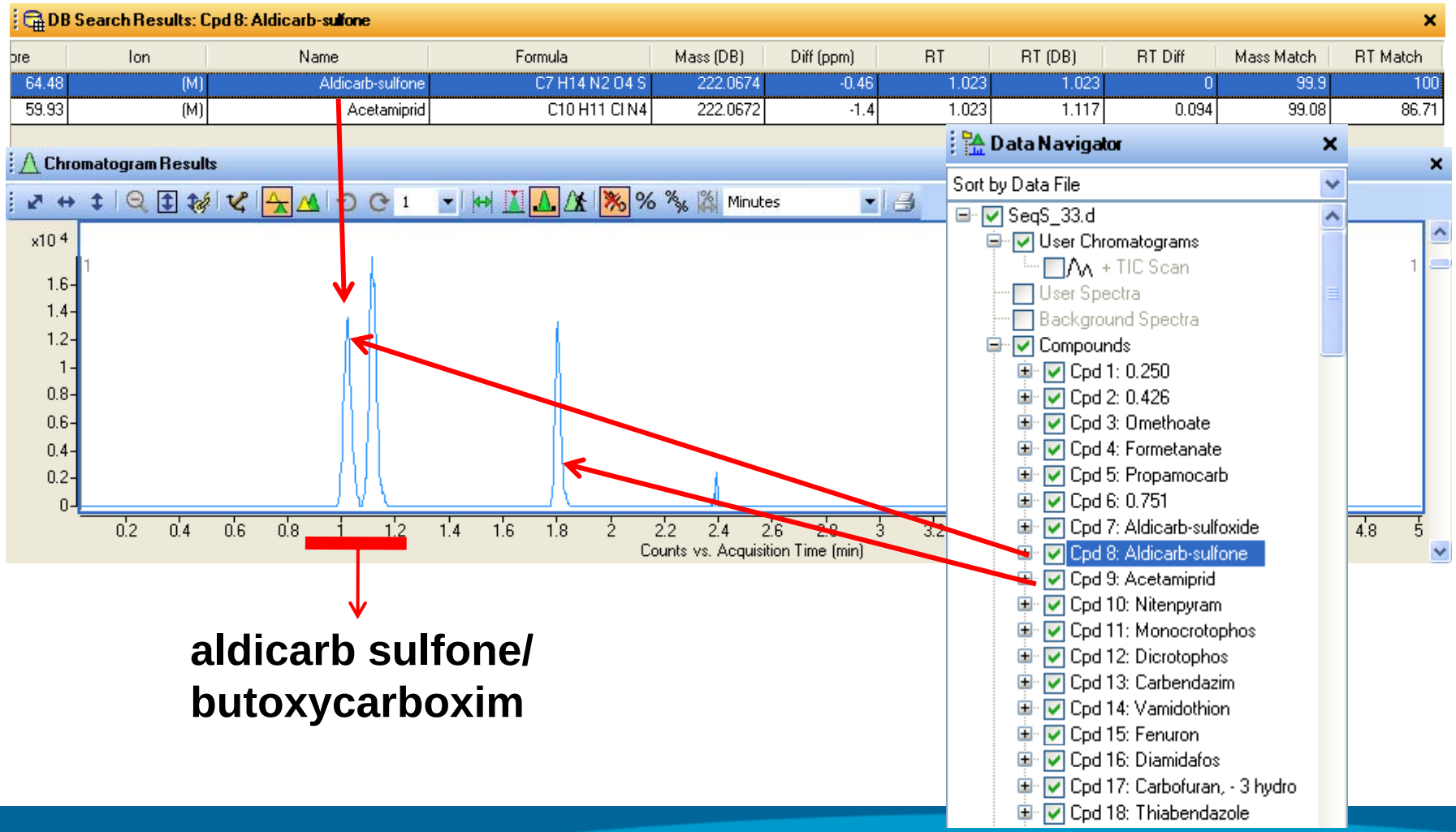
Retention Time Scoring
(example – aldicarb sulfone/acetamiprid/butoxycarboxim)



2ppm mass tolerance window:

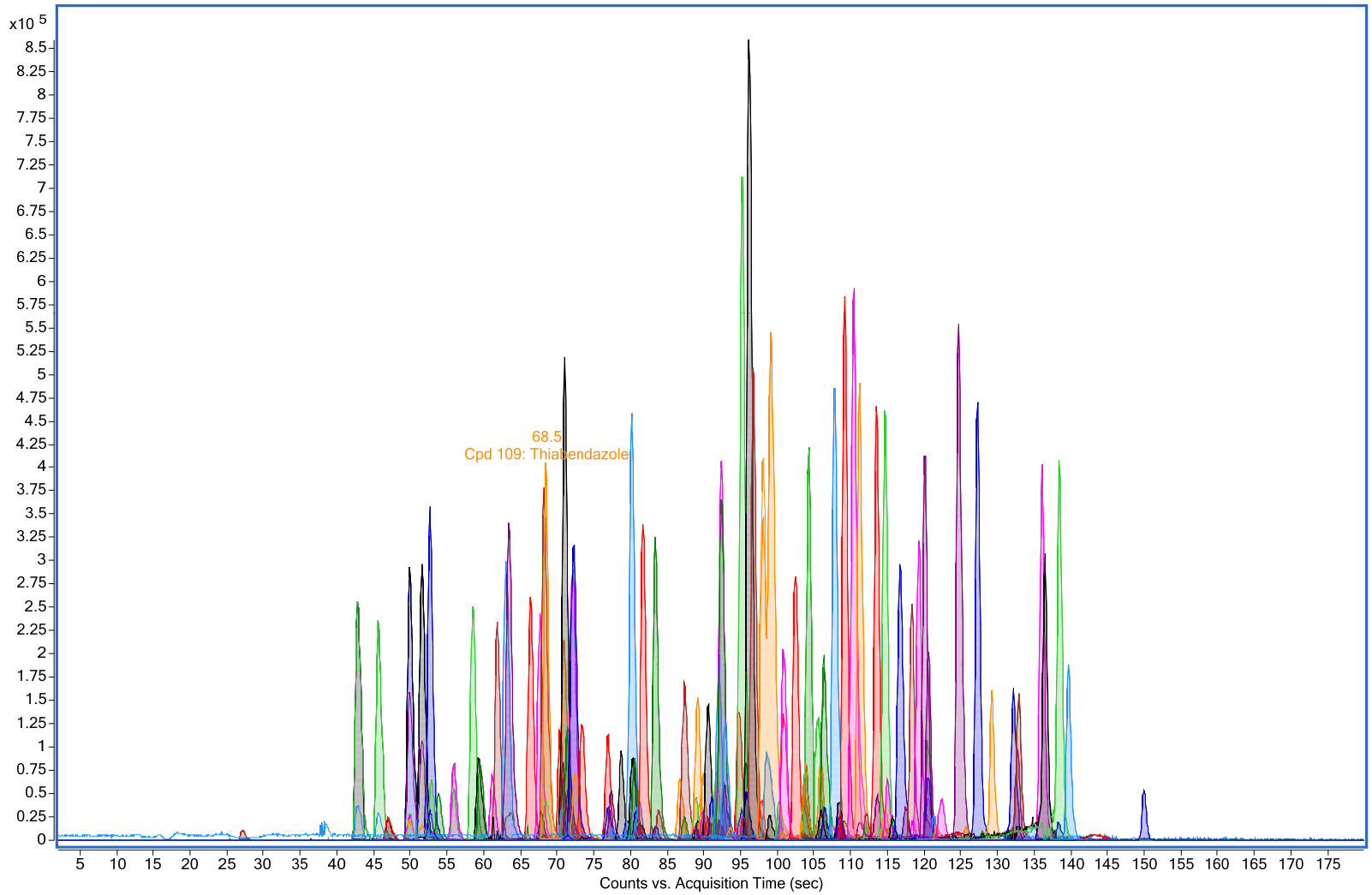
Acetamiprid [$C_{10}H_{11}ClN_4$], m/z=222.06722
Aldicarb-sulfone [$C_7H_{14}N_4O_4S$], m/z=222.06743
Butoxycarboxim [$C_7H_{14}N_4O_4S$], m/z=222.06743

Retention Time Scoring
(example – aldicarb sulfone/acetamiprid/butoxycarboxim)

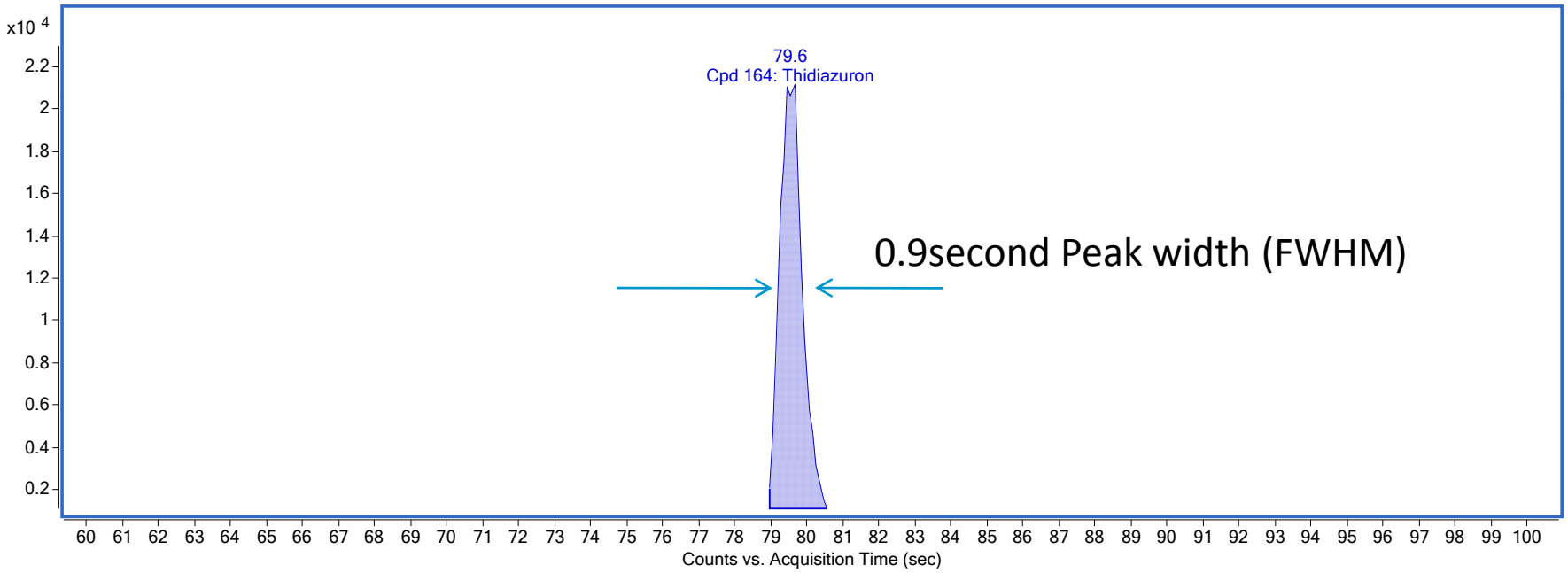


aldicarb sulfone/
butoxycarboxim

PESTICIDE Non-target Screen, 6230 TOF/1290 uHPLC
3 minute analysis, Zorbax Eclipse + C18 2.1x100mm, 1.8um
100pg on-column, 10 Hz Acquisition.



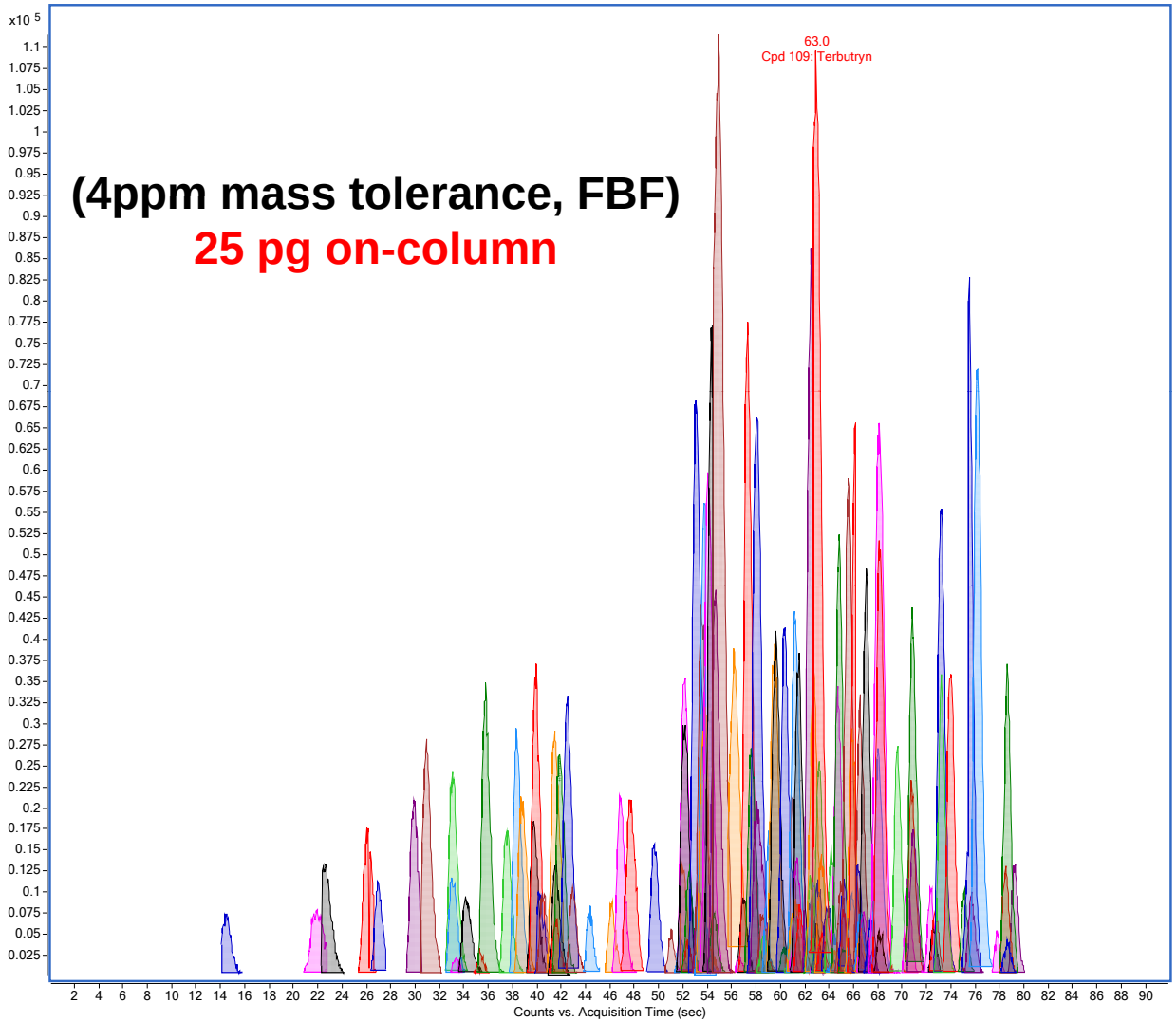
3 minute analysis, Zorbax Eclipse + C18 2.1x100mm, 1.8um 6230 TOF/1290uHPLC, 100pg on-column, 10 Hz Acquisition.



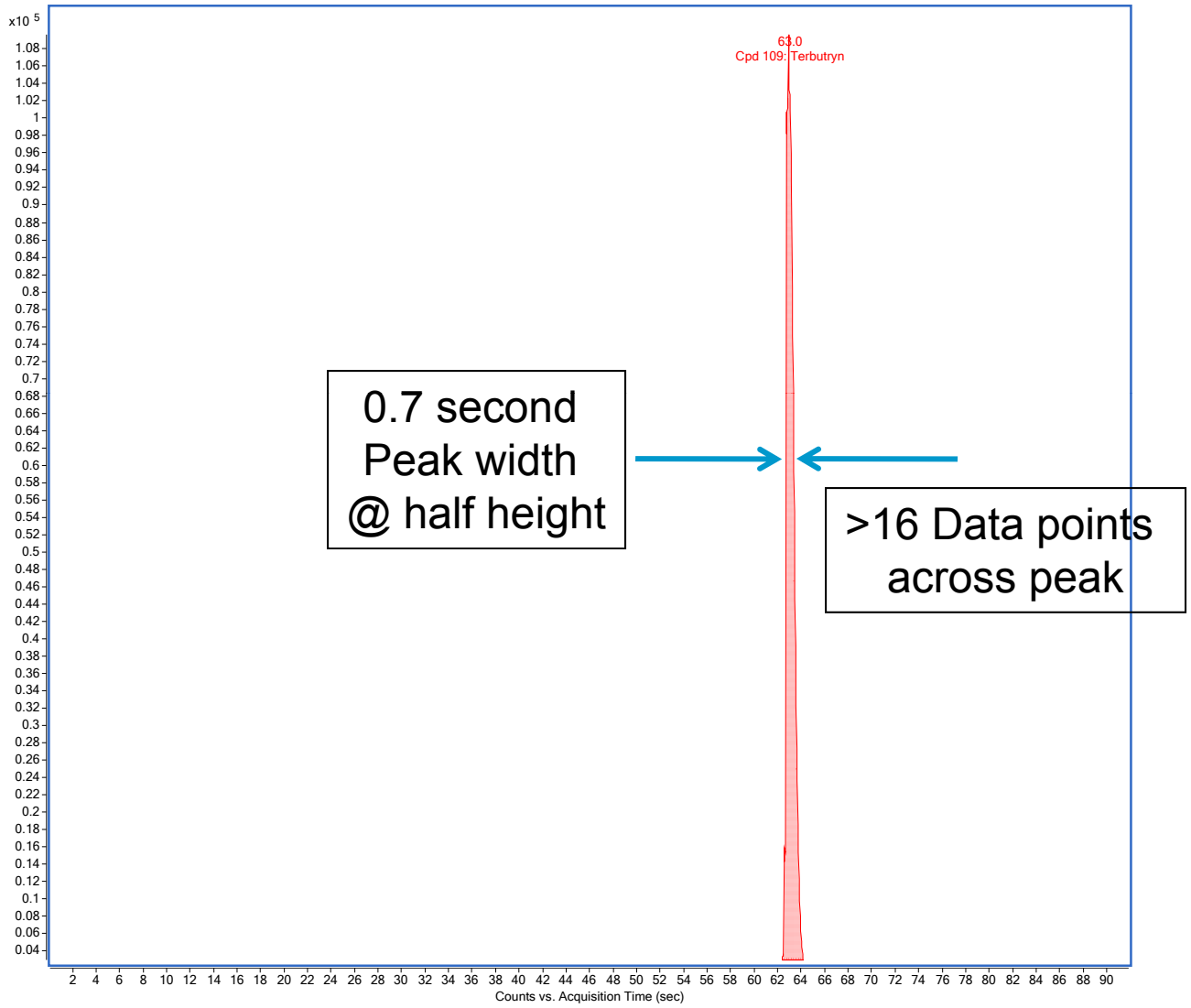
1290 Infinity/6230 TOF
Find By Formula PCD Search

3-minute 1290/6230 TOF Screening (224 +ve Pesticide suite)		
Analyte amount (on-column)	**# of compounds identified (%)**	**Average Mass Accuracy (ppm)**
500fg	45 (20%)	0.24
1.25pg	96 (43%)	0.51
2.5pg	124 (55%)	0.49
5pg	163 (73%)	0.12
25pg	202 (90%)	0.70
50pg	217 (97%)	0.06
125pg	224 (100%)	0.60

Non-target Screen (6230 TOF/1290 uHPLC), 202/224 (90%) Pesticides Identified
90 second Analysis time 20Hz (2GHz mode)



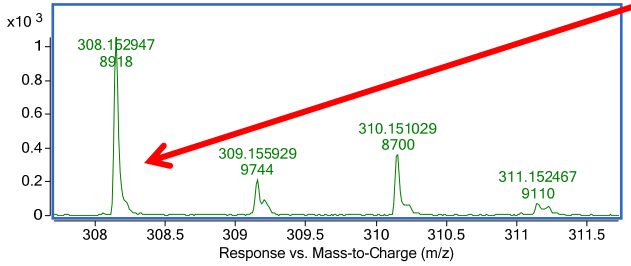
Terbutryn 90 second Analysis time 20Hz (2GHz mode)
Zorbax Eclipse Plus C18 (2.1x50mm x 1.8um) 6230TOF/1290 UHPLC



6230 TOF Speed, Mass Resolution & Dynamic Range – Example: Tebuconazole (2GHz mode)

Tebuconazole (on-column amount)	3 min analysis 10Hz acquisition		1.5 min analysis 20Hz acquisition	
	Mass Error (ppm)	Mass Resolution	Mass Error (ppm)	Mass Resolution
500pg on-column	-0.9	8542	1.46	8546
250pg on-column	-1.79	8626	-1.02	8617
125pg on-column	-0.8	8711	1.82	8803
50pg on-column	-1.77	8822	-0.06	8922
25pg on-column	-2.03	8749	1.51	8918
Peak Width FWHM (sec)	0.8		0.5	

$[M+H]^+$=308.152947



6230 TOF Speed, Mass Resolution & Dynamic Range – Example: Tebuconazole (4GHz mode)

Tebuconazole (on-column amount)	3 min analysis 10Hz acquisition		1.5 min analysis 3Hz acquisition	
	Mass Error (ppm)	Mass Resolution	Mass Error (ppm)	Mass Resolution
250pg on-column	-0.26	17765	-0.2	17949
125pg on-column	0.4	17314	0.1	17432
50pg on-column	-0.31	16959	0.2	17226
25pg on-column	-0.35	17122	0.03	17119
5pg on-column	-1.42	16177	0.03	16499
2.5pg on-column	ND	ND	-1.13	16262
Peak Width FWHM (sec)	0.8		0.5	

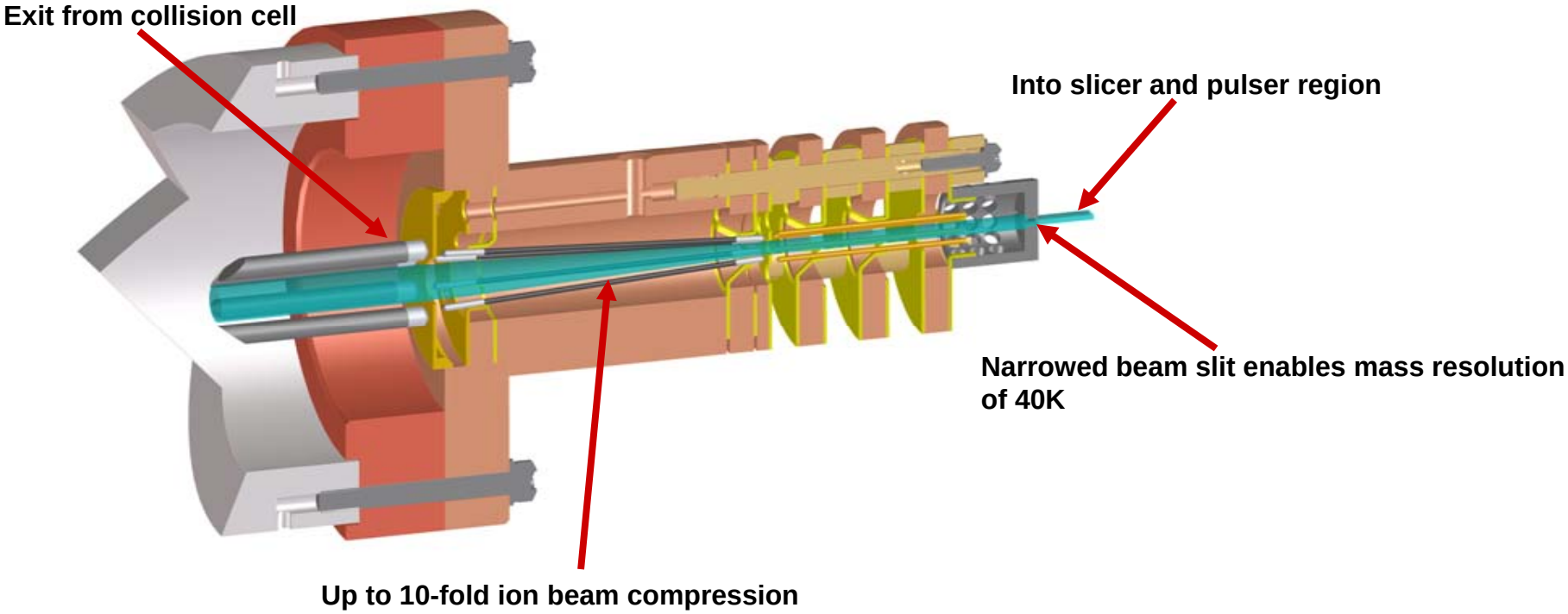
Agilent's New 6540 Ultra High Definition Accurate Mass Q-TOF

Exceptional accurate mass, sensitivity, dynamic range and resolution … perfect match for 1290 Infinity UHPLC

- **500 ppb mass accuracy**
- **femtogram sensitivity**
- **5 decades dynamic range**
- **40,000 resolving power**
- **20 Spectra/sec**
- **Excellent Linearity and Isotopic Fidelity**
- **Supports Agilent Jet Stream and HPLC-Chip**

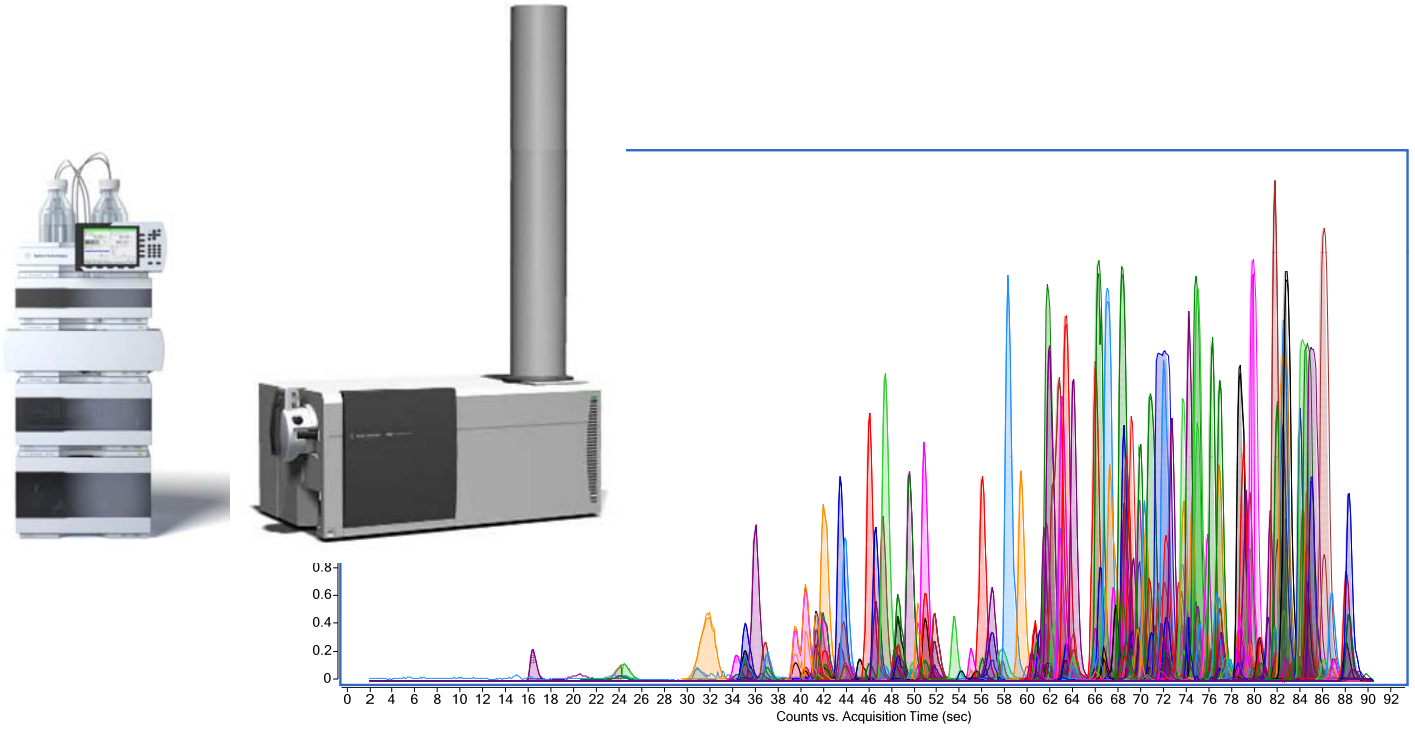
Ion Beam Compression (IBC)* Technology Drives Higher Resolution

Compressed and cooled ion beam ensures the best sensitivity performance in high resolution mode

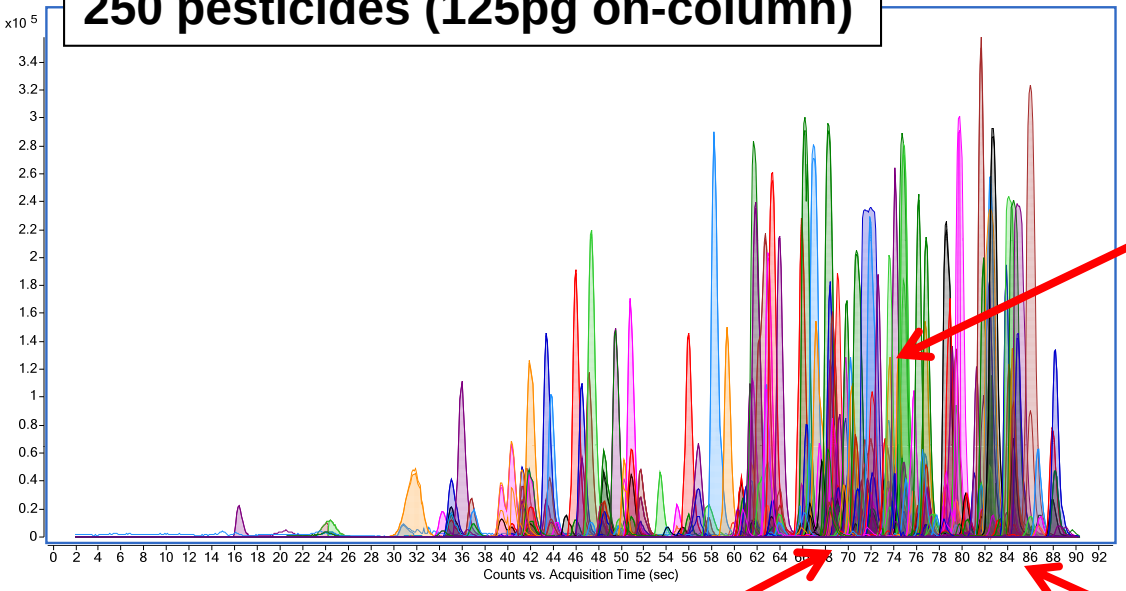


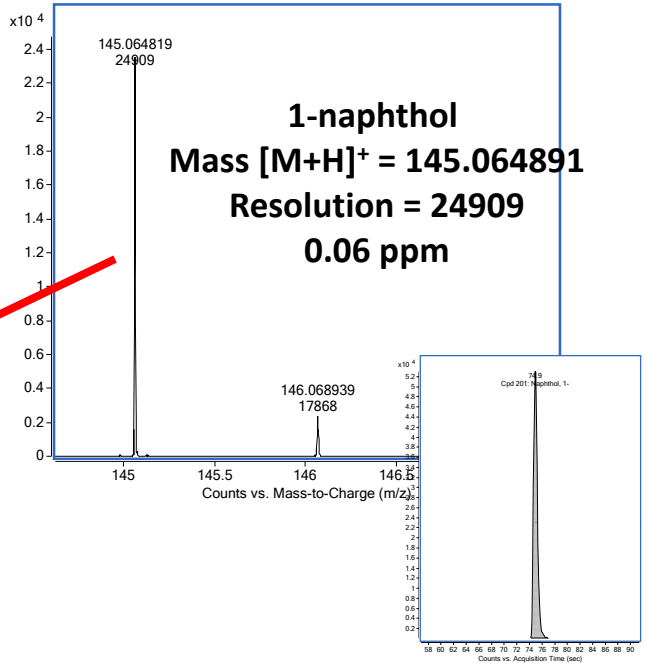
*** Patent pending**

Pesticide Screen 1290 Infinity/6540 Q-TOF

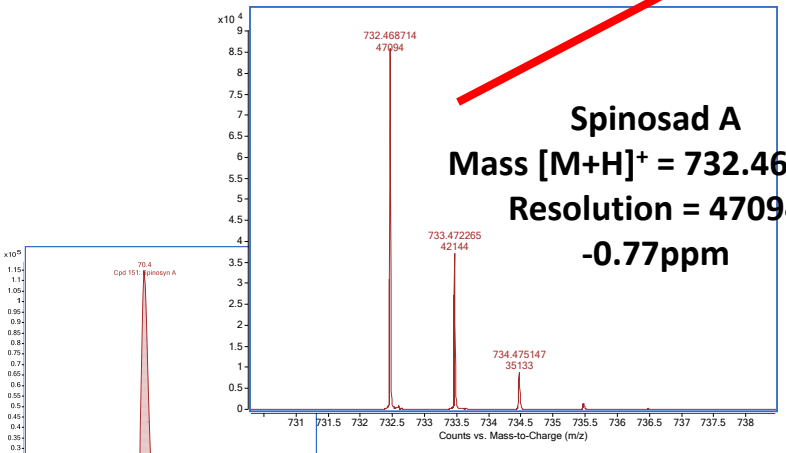


1290/6540 90sec analysis
250 pesticides (125pg on-column)

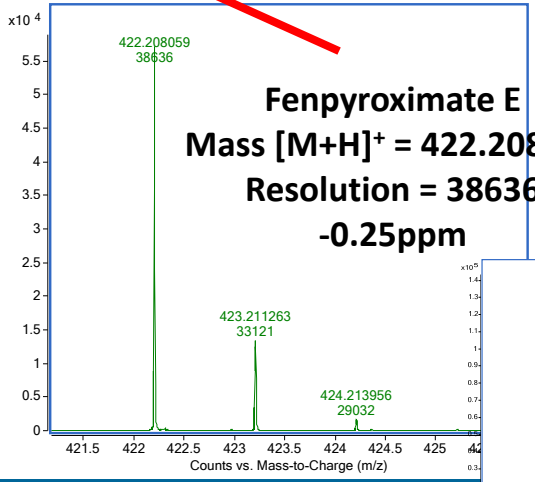




1-naphthol
Mass [M+H]⁺ = 145.064891
Resolution = 24909
0.06 ppm

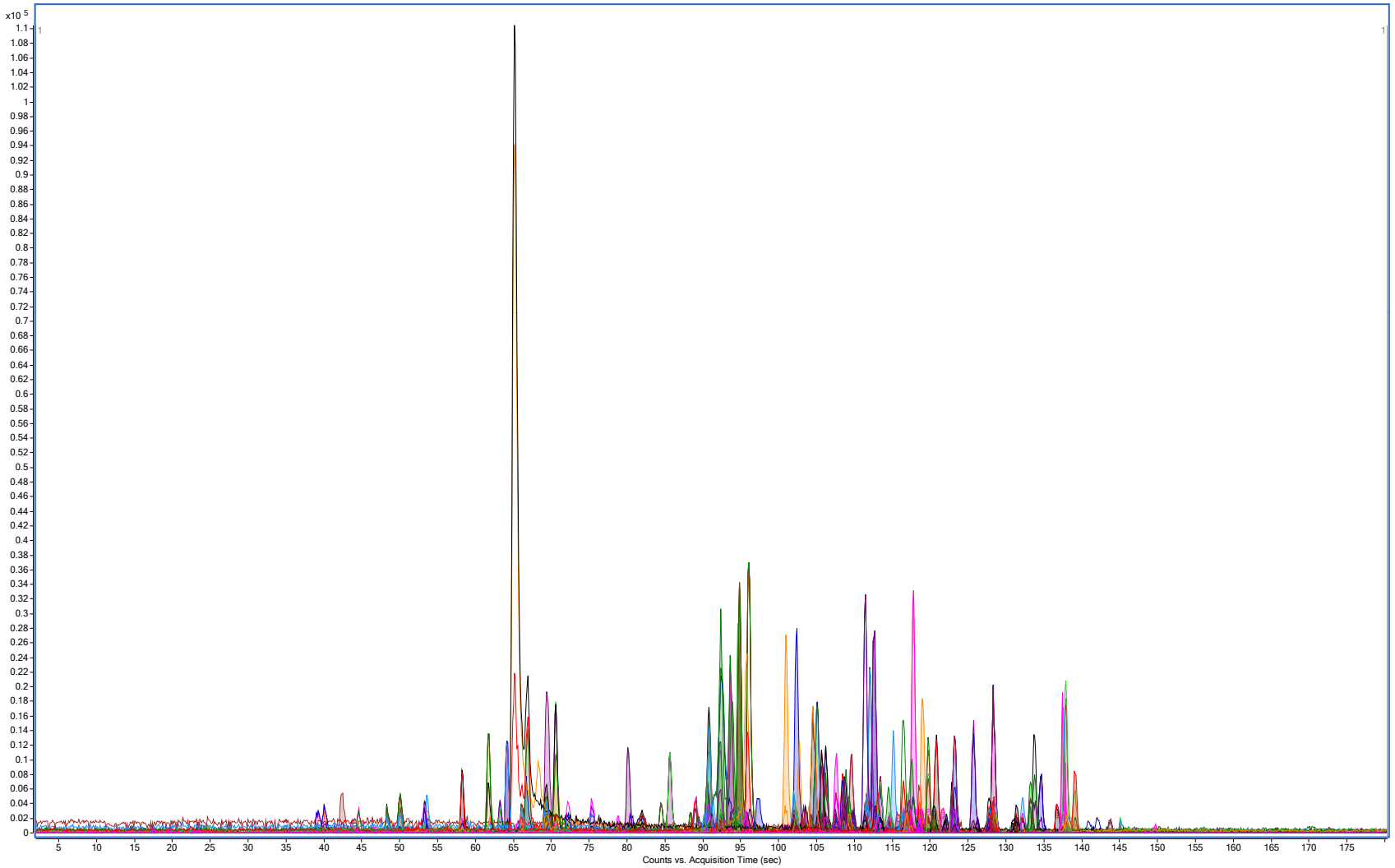


Spinosad A
Mass [M+H]⁺ = 732.468714
Resolution = 47094
-0.77ppm



Fenpyroximate E
Mass [M+H]⁺ = 422.208059
Resolution = 38636
-0.25ppm

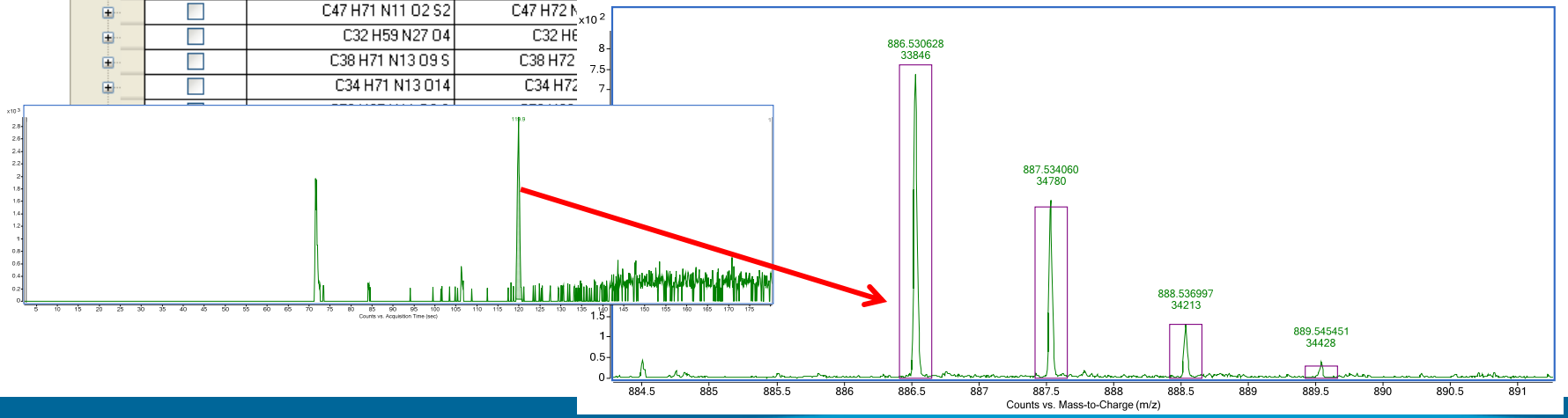
25pg on-column 1290 Infinity/6540 Q-TOF
3min 92% identified pesticides



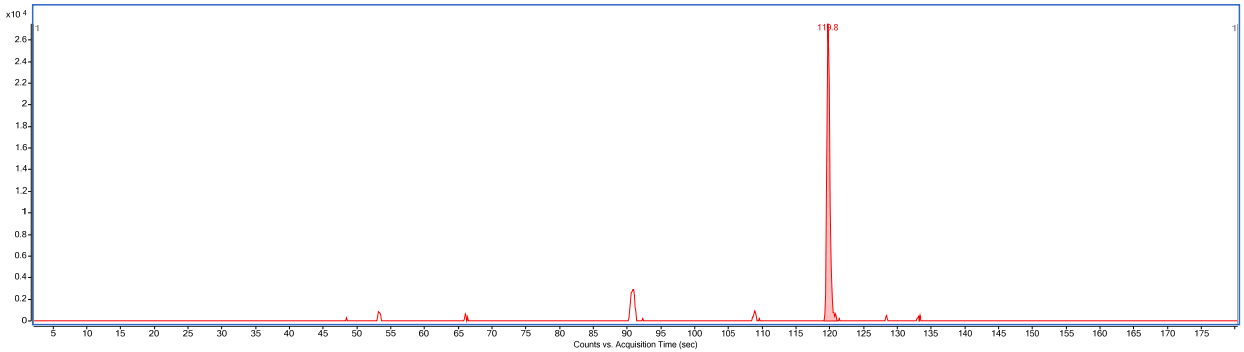
1290 Infinity/6540 Q-TOF
- Emamectin B1a, 50pg on-column 3min

m/z	Ion	Formula	Abundance
886.530628	(M+H)+	C49 H76 N O13	737.4

Best	Formula (M)	Ion Formula	Score	Calc m/z	Diff (ppm)	Mass Match	Abund Match	Spacing Match
☐	C47 H63 N15 O3	C47 H64 N15 O3	98.97	886.531107	0.41	99.72	97.9	98.77
☑	C49 H75 N O13	C49 H76 N O13	98.59	886.531118	0.54	99.53	95.87	99.99
☐	C46 H67 N11 O7	C46 H68 N11 O7	97.89	886.52977	-1.07	98.18	96.24	99.28
☐	C50 H71 N5 O9	C50 H72 N5 O9	96.52	886.532455	2.02	93.63	99.54	99.88
☐	C54 H71 N5 O4 S	C54 H72 N5 O4 S	94.76	886.529953	-0.83	98.9	86.14	96.8
☐	C55 H67 N9 S	C55 H68 N9 S	94.63	886.53129	0.66	99.3	85.44	96.31
☐	C40 H63 N21 O S	C40 H64 N21 O S	93.07	886.531793	1.1	98.06	88.22	88.9
☐	C45 H71 N7 O11	C45 H72 N7 O11	92.9	886.528433	-2.55	90.05	92.01	99.66
☐	C43 H59 N21 O	C43 H60 N21 O	92.56	886.528422	-2.67	89.08	94.48	97.2
☐	C39 H67 N17 O5 S	C39 H68 N17 O5 S	91.85	886.530456	-0.38	99.77	80.29	89.91
☐	C53 H75 N O8 S	C53 H76 N O8 S	91.13	886.528616	-2.31	91.75	85.03	97.19
☐	C51 H67 N9 O5	C51 H68 N9 O5	91	886.533793	3.5	82.05	98.73	99.64
☐	C42 H75 N7 O11 S	C42 H76 N7 O11 S	90.28	886.531803	1.23	97.57	75.25	93.73
☐	C58 H63 N9	C58 H64 N9	89.72	886.52792	-3.13	85.37	88.62	99.75
☐	C41 H79 N3 O15 S	C41 H80 N3 O15 S	88.73	886.530466	-0.24	99.91	65.48	94.28
☐	C43 H71 N11 O7 S	C43 H72 N11 O7 S	88.31	886.533141	2.71	88.79	83.55	93.05
☐	C47 H71 N11 O2 S2	C47 H72 N						
☐	C32 H59 N27 O4	C32 H6						
☐	C38 H71 N13 O9 S	C38 H72						
☐	C34 H71 N13 O14	C34 H72						

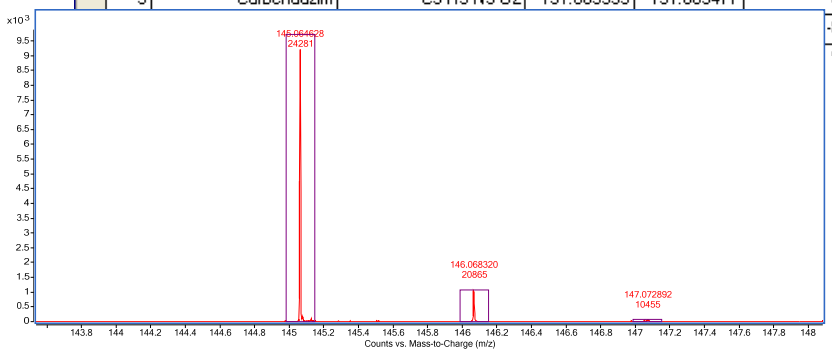


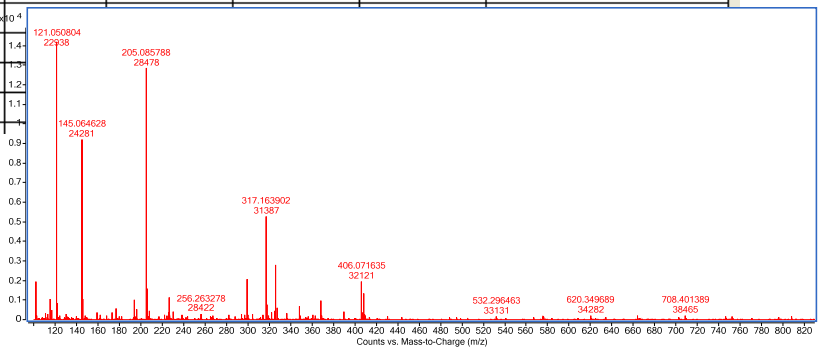
1290 Infinity/6540 Q-TOF
- 1-naphthol, 50pg on-column 3min



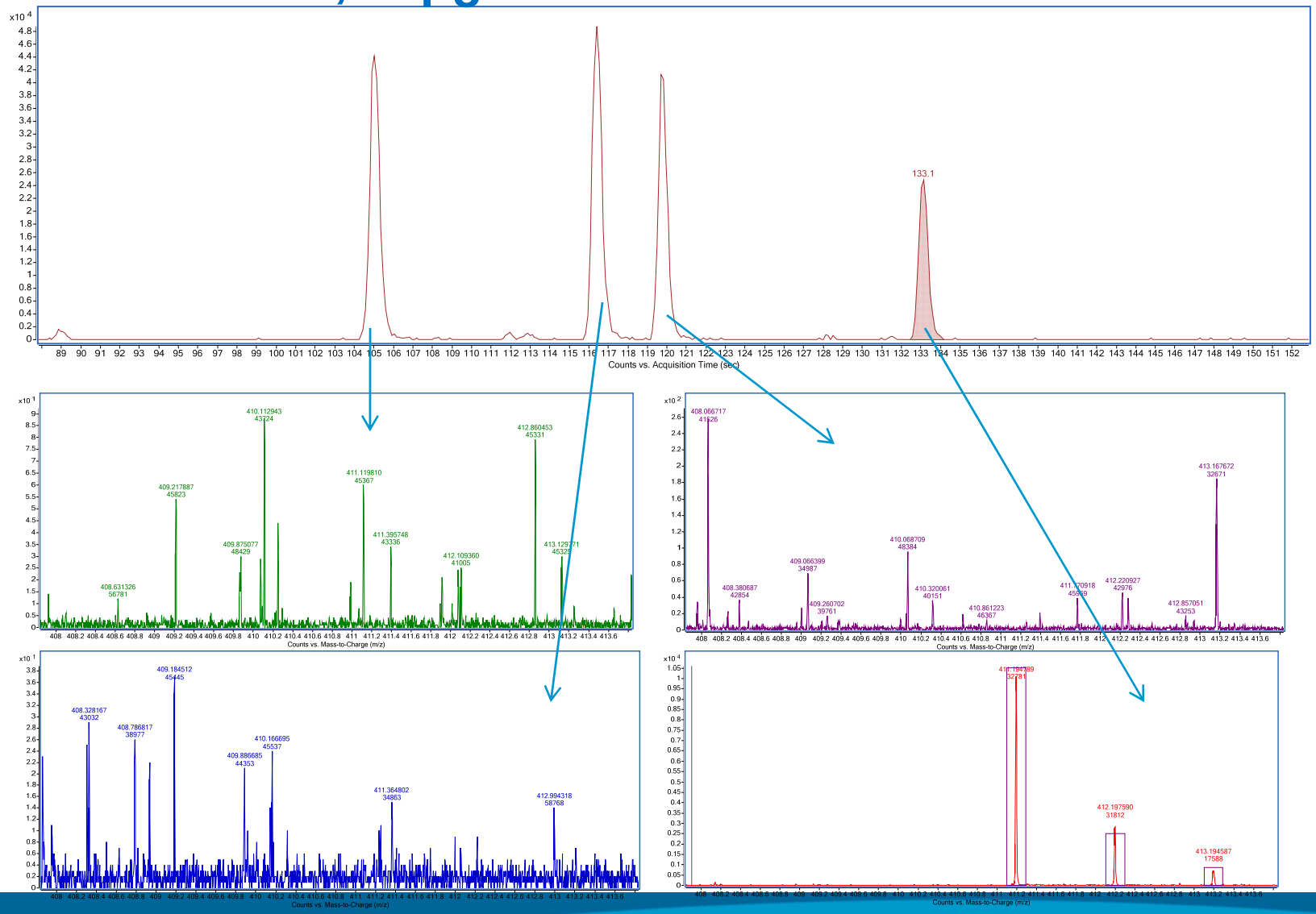
Compound List

Cpd	Name	Formula (Tgt)	Mass	Mass (Tgt)	Diff (Tgt, ppm)	RT	Score (Tgt)	Height	Area	Abund	Algorithm
1	Methamidophos	C2 H8 N O2 P S	141.001262	141.001336	-0.52	24.6	47.59	1590	639	823	Find By Formula
2	Naphthol, 1-	C10 H8 O	144.057502	144.057515	-0.09	119.8	99.16	27504	14953	15022	Find By Formula
3	Methomyl	C5 H10 N2 O2 S	162.04672	162.046298	2.6	82.9	46.69	559	506	296	Find By Formula
4	Fenuron	C9 H12 N2 O	164.094596	164.094963	-2.24	66.8	99.58	16508	11151	7436	Find By Formula
5	Fuberidazole	C11 H8 N2 O	184.063654	184.063663	-0.05	69.5	99.9	53636	31874	31498	Find By Formula
6	Propamocarb	C9 H20 N2 O2	188.15212	188.152478	-1.9	42.3	99.27	14920	10513	7714	Find By Formula
7	Tricyclazole	C9 H7 N3 S	189.035792	189.036068	-1.46	70.6					
8	Butocarboxim	C7 H14 N2 O2 S	190.077627	190.077598	0.15	78.4					
9	Carbendazim	C9 H9 N3 O2	191.069335	191.069477	-0.74	61.7					
							-8.87	85.7			

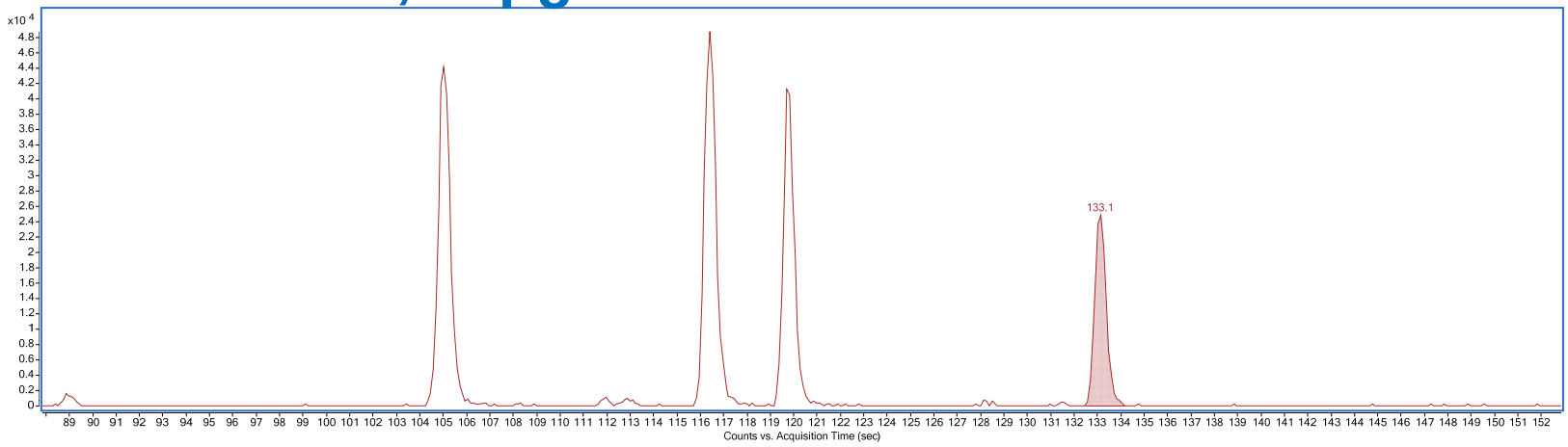




1290 Infinity/6540 Q-TOF
- Benfuracarb, 50pg on-column 3min



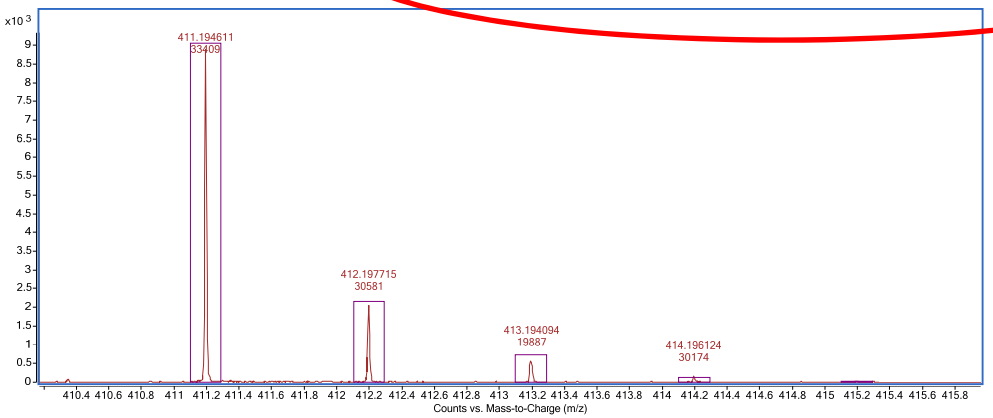
1290 Infinity/6540 Q-TOF
- Benfuracarb, 50pg on-column 3min



MS Formula Results: + Scan (132.9-133.4 sec)

m/z	Ion	Formula	Abundance
411.194611	(M+H)+	C20 H31 N2 O5 S	9225

Best	Formula (M)	Ion Formula	Score	Calc m/z	Diff (ppm)	Mass Match	Abund Match	Spacing Match
✓	C20 H30 N2 O5 S	C20 H31 N2 O5 S	98.62	411.194819	0.51	99.77	95.92	99.56



Non-Targeted Screen Summary (QTOF/TOF)

- Agilent TOF/Q-TOF for ‘unknown’ screening (low ppm mass accuracy.)
- Agilent MassHunter PCD utilizes the accurate mass data to provide reliable ‘positives’ for qualitative screens.
  - Extra Confirmation with Isotope abundance (pattern), spacing and mass matching.
  - 1600 Pesticide analytes
  - Fully Automated integration with MassHunter Qualitative Analysis SW.
- Reliable Data mining Algorithms (MFE or FBF.)
- Low pico-gram on-column Sensitivity.
- Fully Automated.
- Faster data acquisition with 1290 Infinity UHPLC.

Questions?

Thank You for Listening.