Environmental and Food Safety Analyses by UHPLC and LC/MS Using the New Agilent 1290 Infinity LC System and Agilent Mass Spectrometers

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Topics of Discussion

UHPLC theory and advantages

Challenges in Instrument design

- Pump: flow and pressure range, mixing requirements
- Injector: injection range, principle, carryover
- Column availability, stability, variety
- Column management: temperature control, column dimensions and valve configurations
- UV/VIS detection: balancing dispersion and sensitivity
- MS Considerations: acquisition rate, optimal flow rate

Resolution Equation

Although we may be aiming for faster separations, we normally also need to maintain or increase resolution

$$R_S = \frac{\sqrt{N}}{4} \frac{(\alpha-1)}{\alpha} \frac{k}{(k+1)}$$

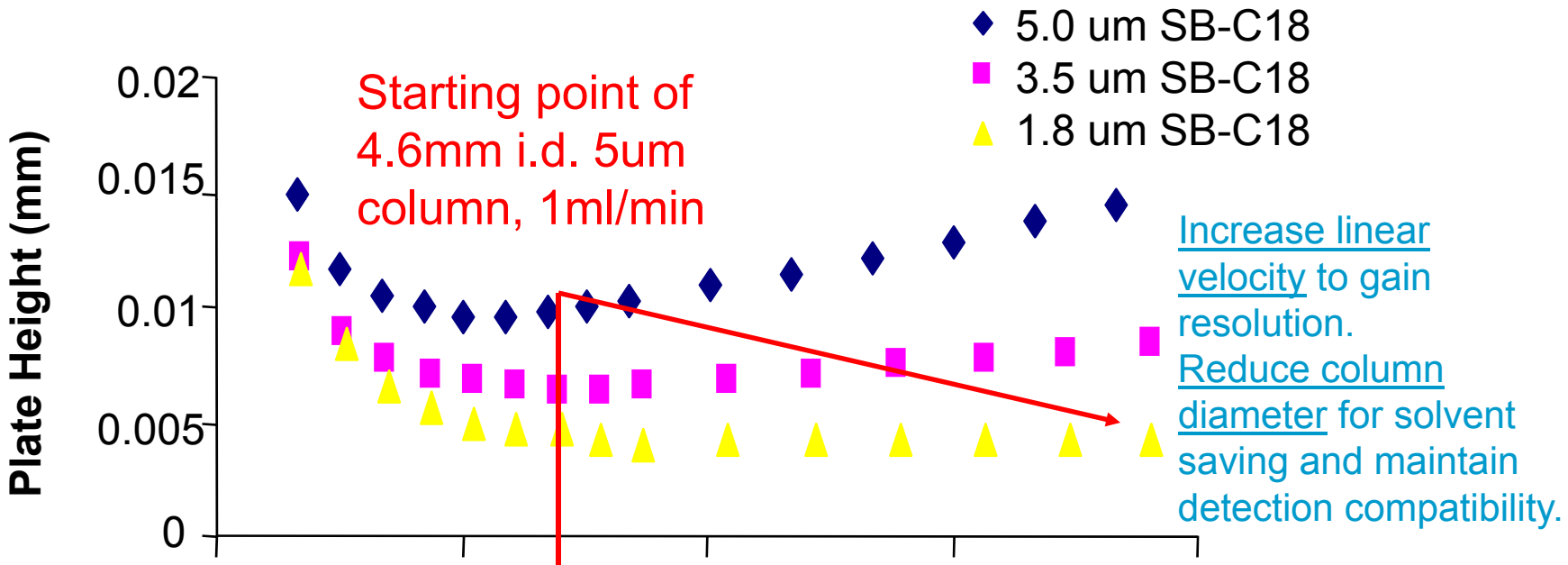
α = **Selectivity**– influenced by mobile and stationary phase

N = Column Efficiency – influenced by length and particle size

k = Capacity Factor (retention) – influenced by stationary and mobile phase, gradient slope and dwell volume (gradients)

Method Transitions to UHPLC sub 2 micron materials

Maintain Acceptable Flow Rates at High Linear Velocity by Progressively Decreasing Column Diameter



Lin. Vel.	mm/sec	1	2	3	4	5
4.6 mm	ml/min	0.7	1.4	2.1	2.8	3.5
3 mm	ml/min	0.3	0.6	0.9	1.2	1.5
2.1 mm	ml/min	0.14	0.29	0.44	0.58	0.73
1 mm	ml/min	0.033	0.066	0.1	0.133	0.166

Increased Pressure Requirements and Enhanced Speed and Performance Associated with Reduced Particle Size

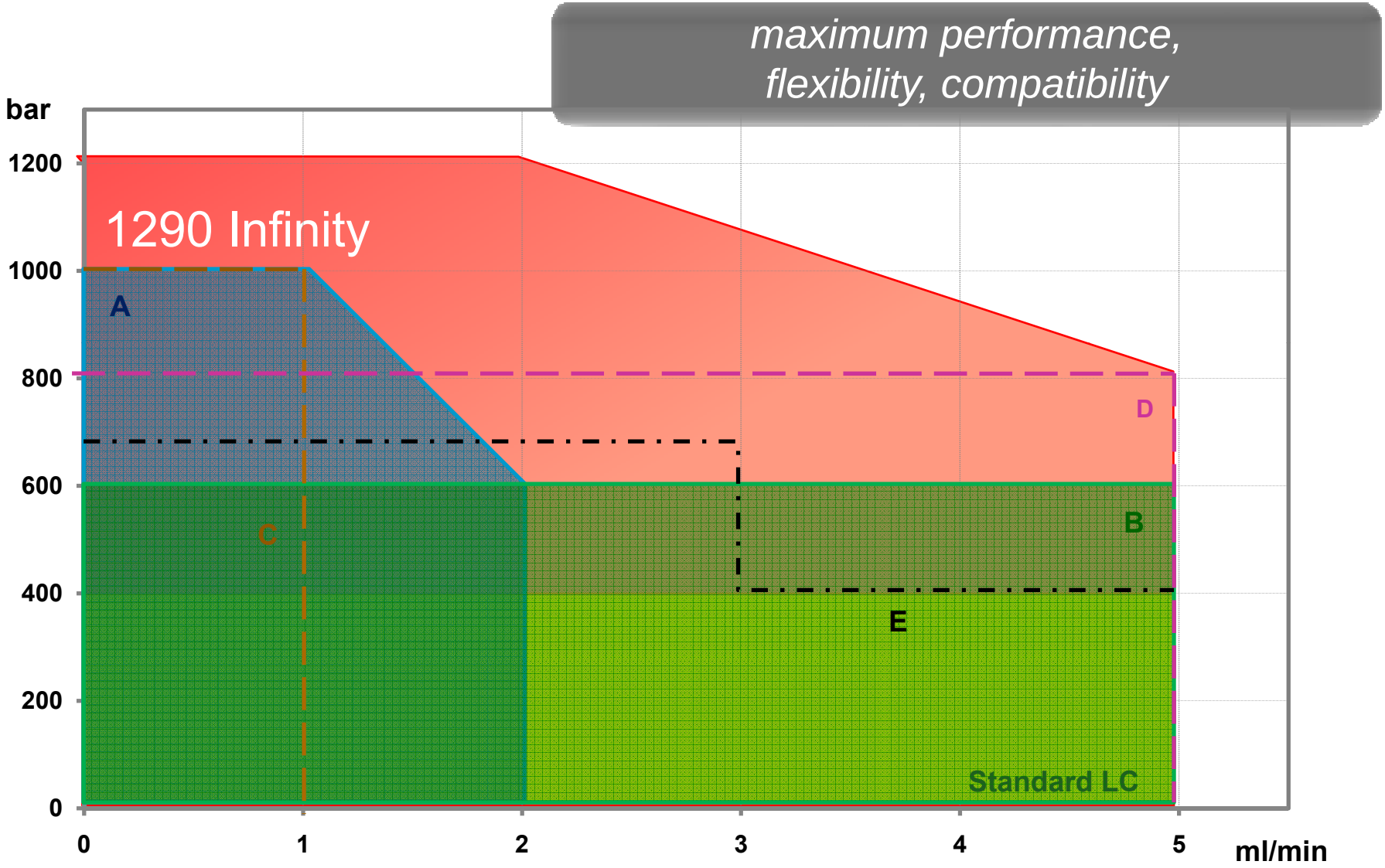
d_p (µm)	ΔP (bar)	t_R (min)	H_{MIN} (µm)	N
5.0	14.5	34.7	10.0	25,000
3.0	66.9	20.8	6.0	41,000
1.5	531	10.4	3.0	83,000
1.0	1800	6.9	2.0	125,000
0.75	4270	5.2	1.5	166,000
0.50	14400	3.5	1.0	250,000

With a 5.0 to 1.5 µm particle size reduction

- t_R decreases >3-fold
- N increases >3-fold

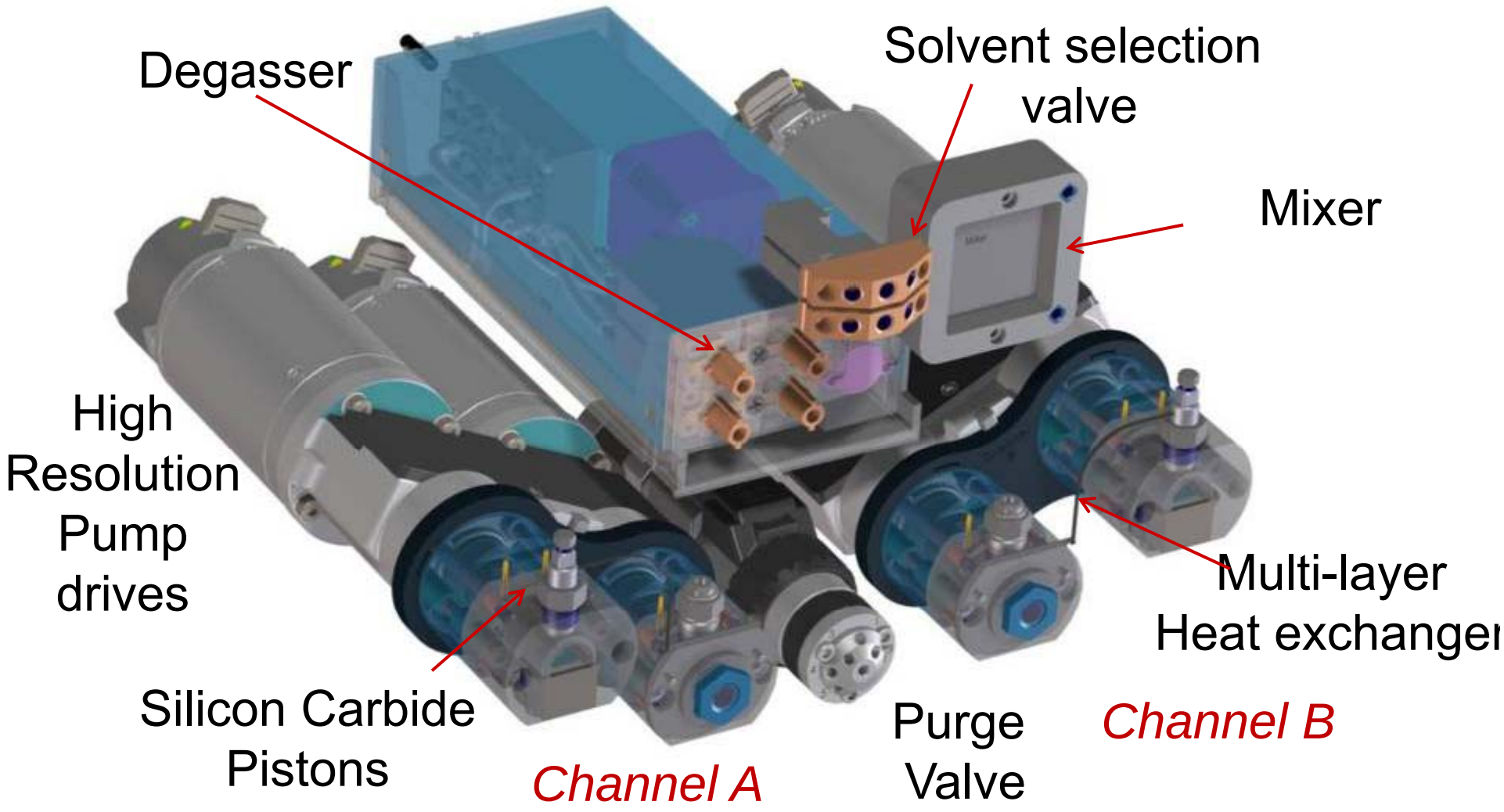
But . . . ΔP increases >35-fold

Maximum Power Range



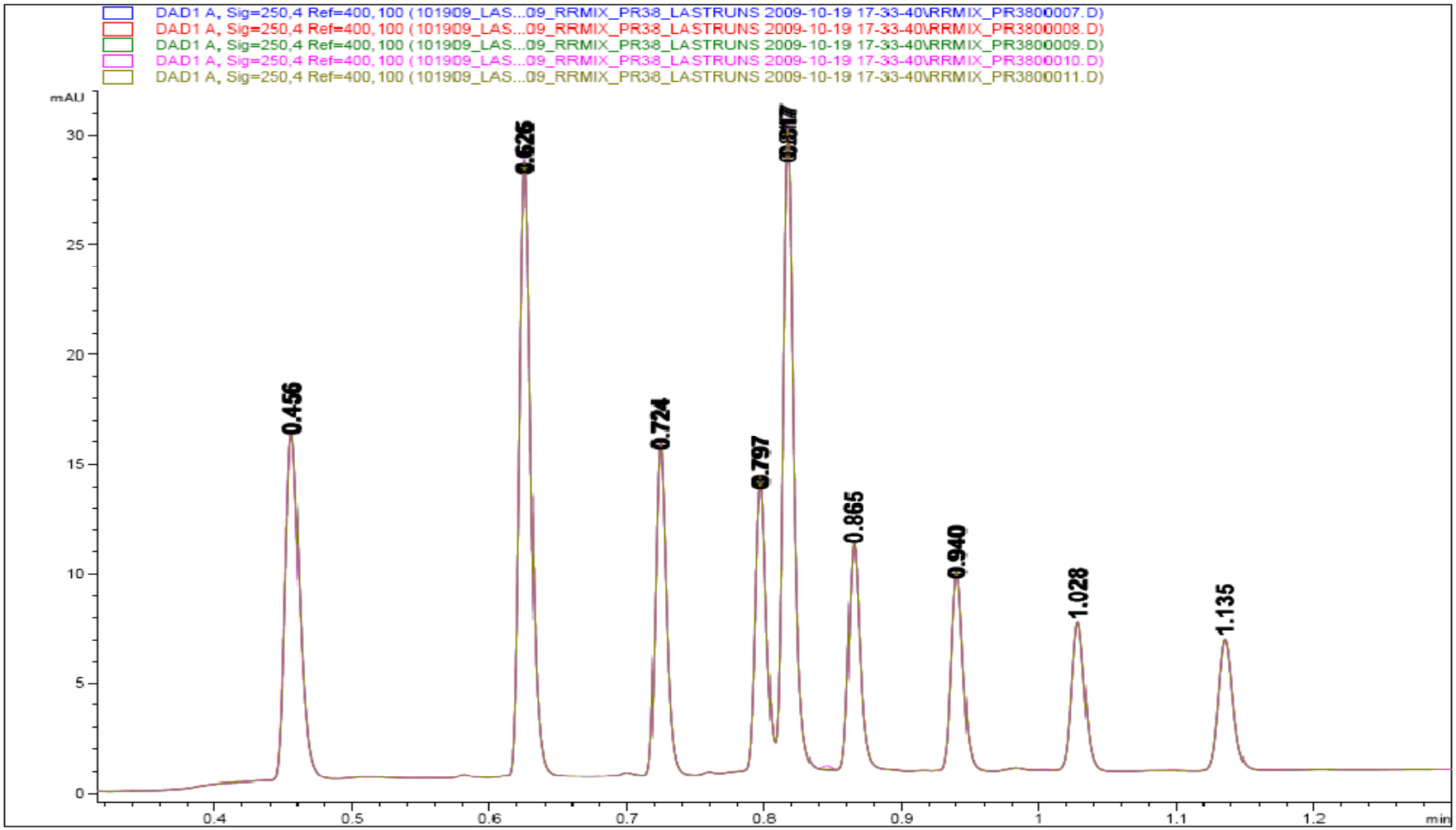
Advanced UHPLC Binary Pump

- Flow path and integration of new materials



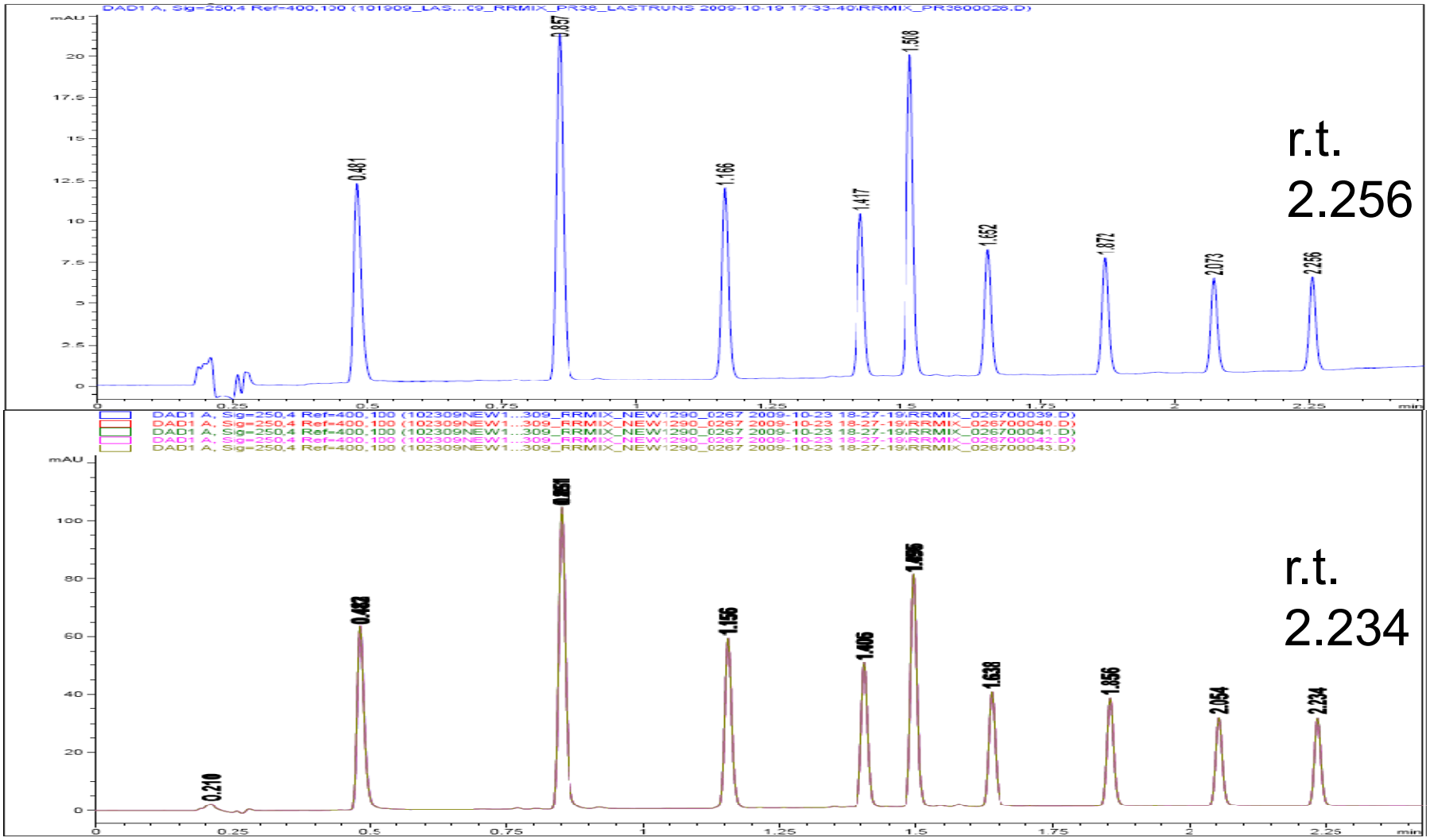
What to Look for in Gradient Performance

- Steep 0.5ml (25%/cv) Gradient, alkylphenones, system 38



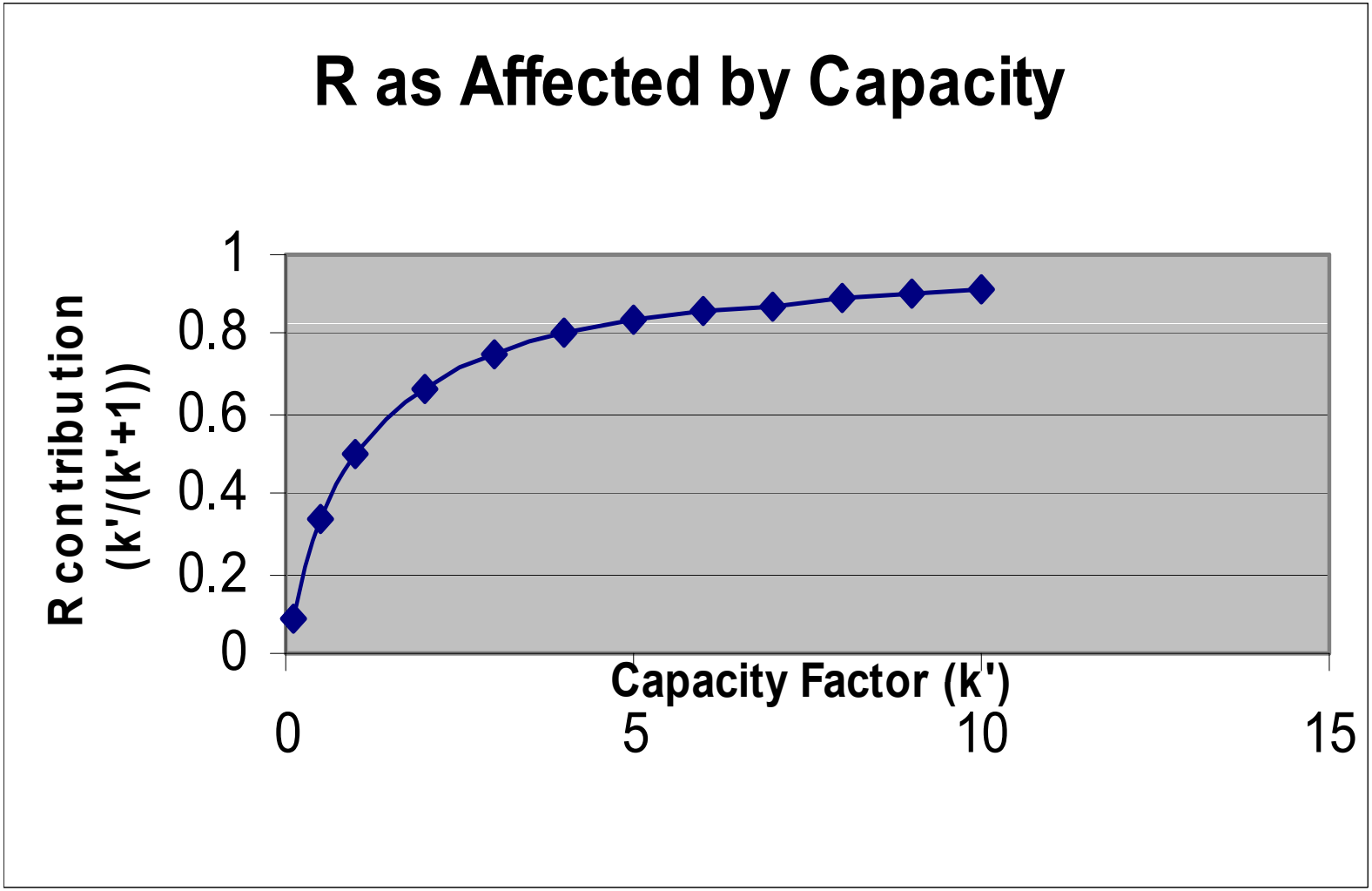
System to System Performance

- systems 38 and 0x67, same column installed, 2ml gradient

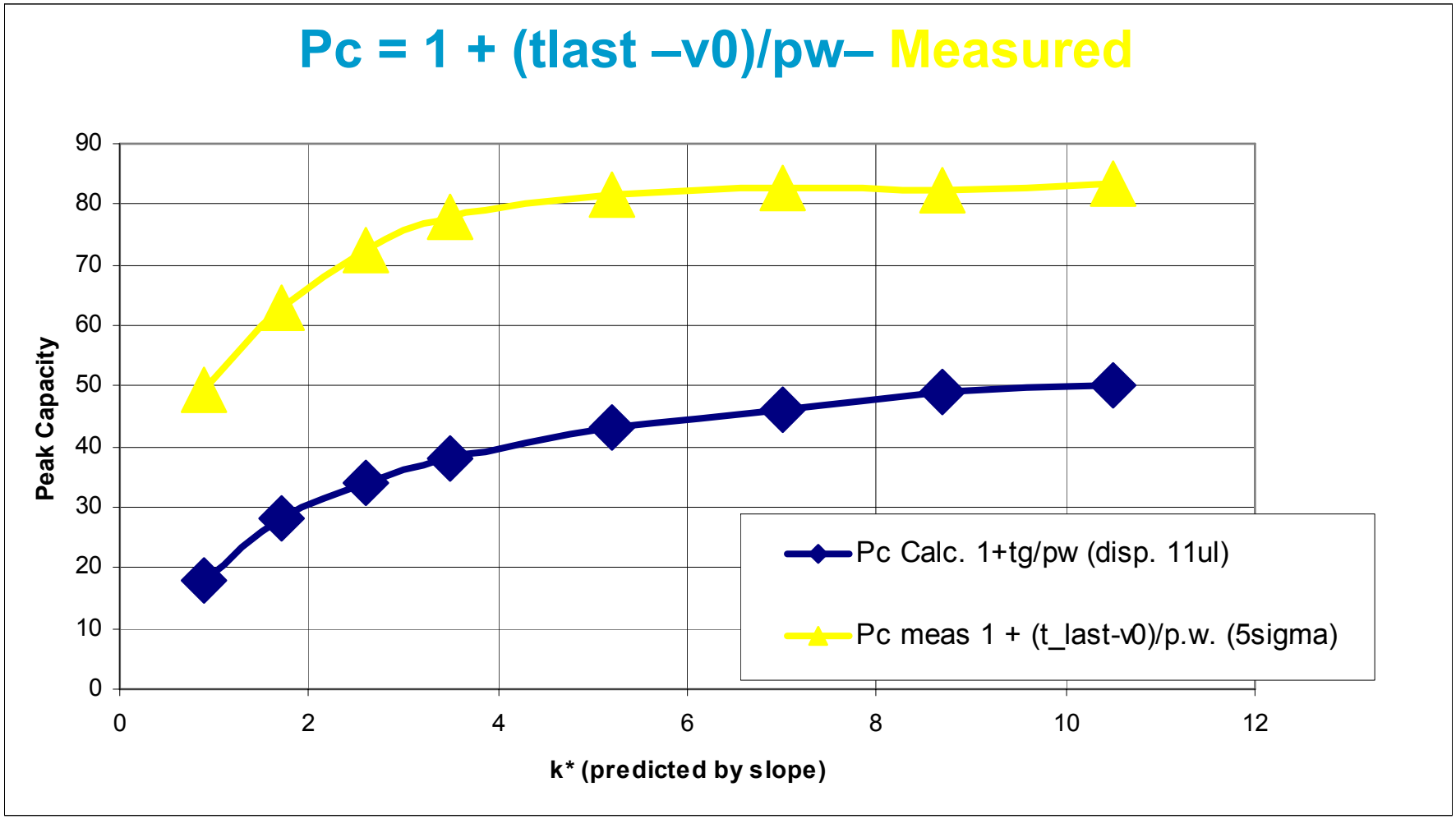


Agilent Technologies

Resolution – effect of increasing capacity factor k' in isocratic Resolution Equation



Resolution – effect of increasing capacity factor k* in gradient peak capacity equations



for a 1ml/min gradient, 30-90% ACN, 3x50mm 1.8um, 11ul disp., avg. analyte mc.wt. 162g/m

My Lab Environment?

1290 Infinity LC

Fluorescence

Diode Array

Outboard 12 position solvent select valves

Column Compartment with 2 position 6 port valve

Autosampler

Binary pump with integral vacuum degasser and solvent select valves

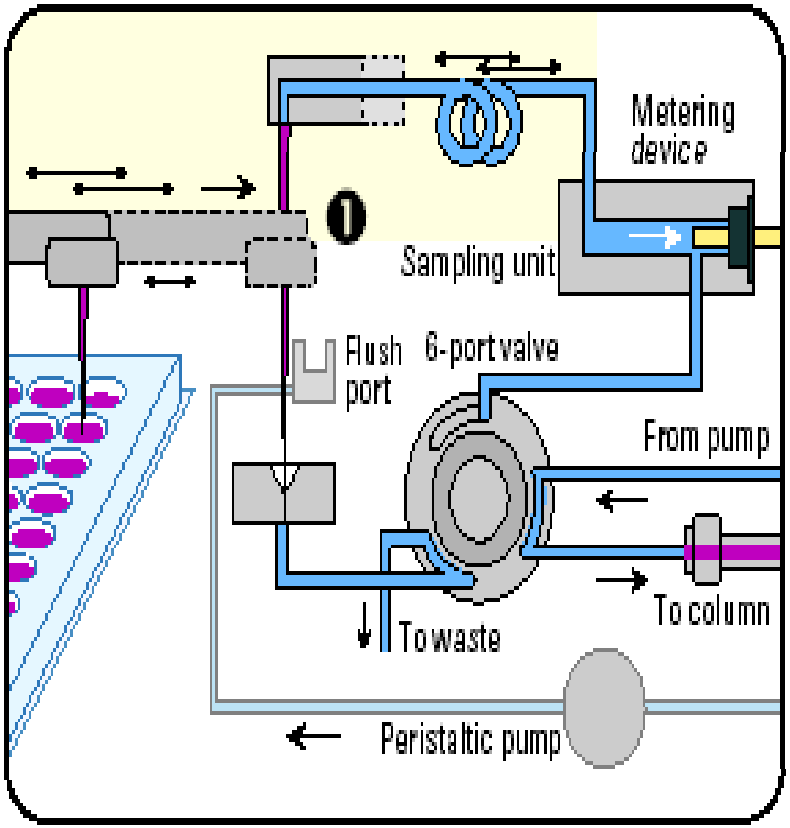
What Kind of Lab Environment is Needed?

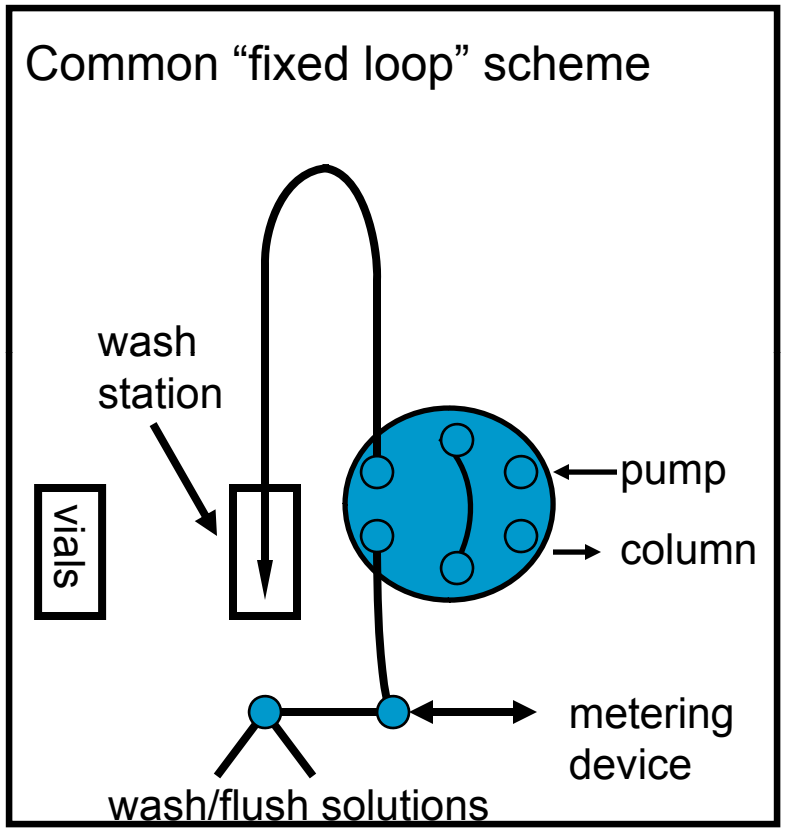
Design Considerations for UHPLC Sample Introduction

- Injection principle (variable loop, fixed loop designs)
- Usable injection volume range
- Speed of injection cycle
- Minimal dispersion
- Minimal carryover
- Minimal sample waste

Design Considerations for UHPLC Samplers

- Injection principle (variable loop, fixed loop designs)





Fixed loop designs leave flush solution in loop, which can distort peak shape or alter selectivity in scouting separations

Tips for minimizing dispersion in LC systems

- Keep injection volumes small, ensure weak diluent when possible
- Ensure autosampler flush solvent is matched with starting conditions

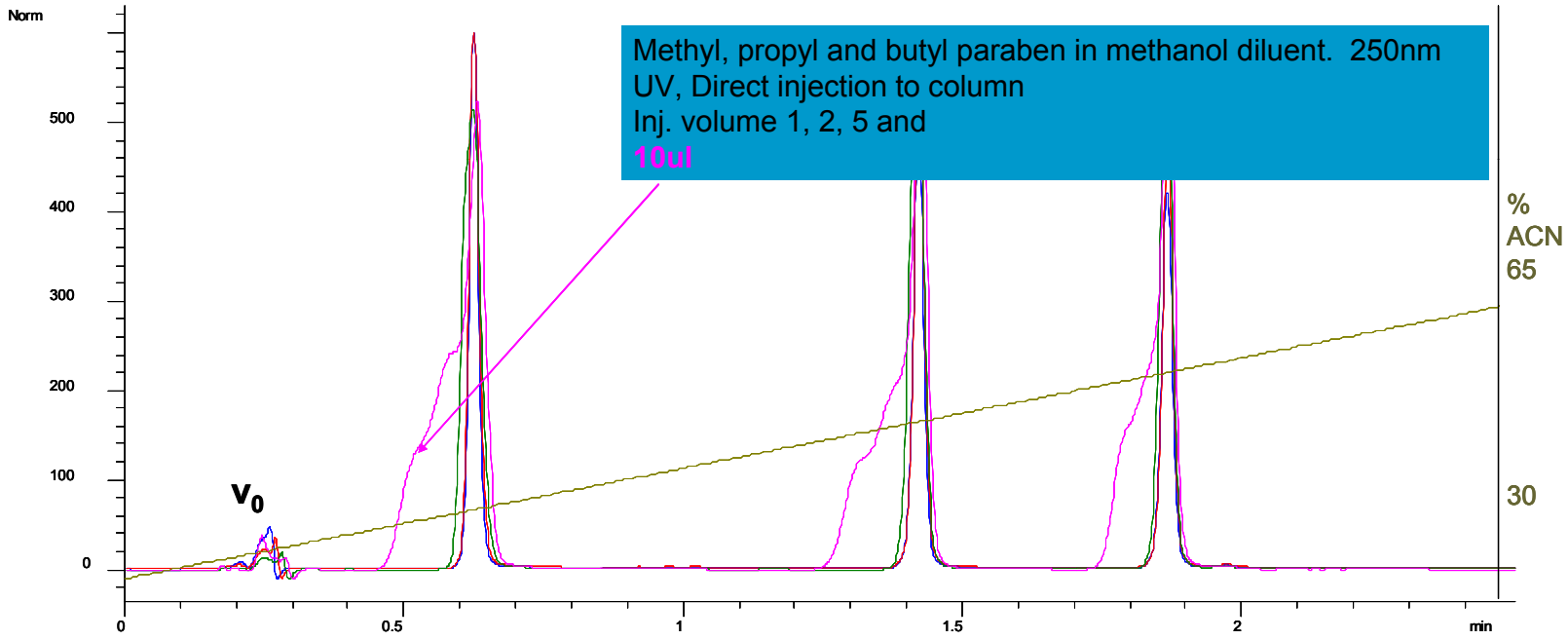
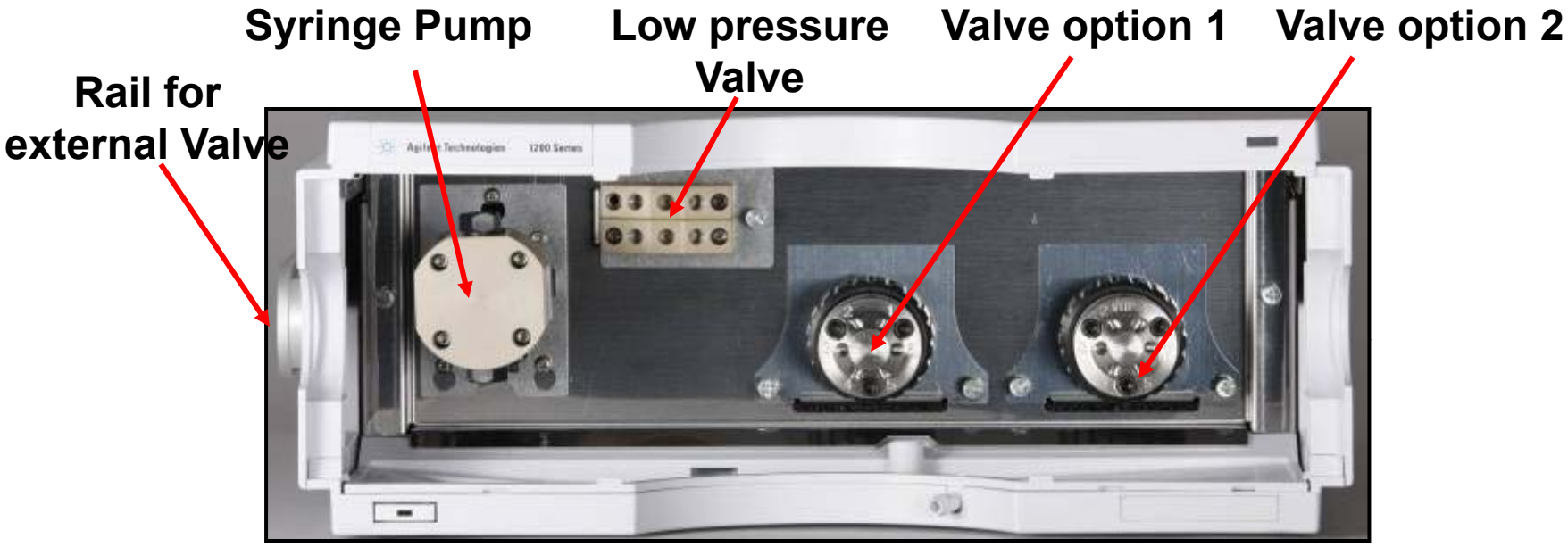


Figure 2. Effect of Strong Diluent Injections – Bandbroadening

Methanol diluted sample, gradient from 30% ACN initial condition, 3x50mm 1.8um column

Flexibility in injection options – more freedom of choice *- extending the capabilities*



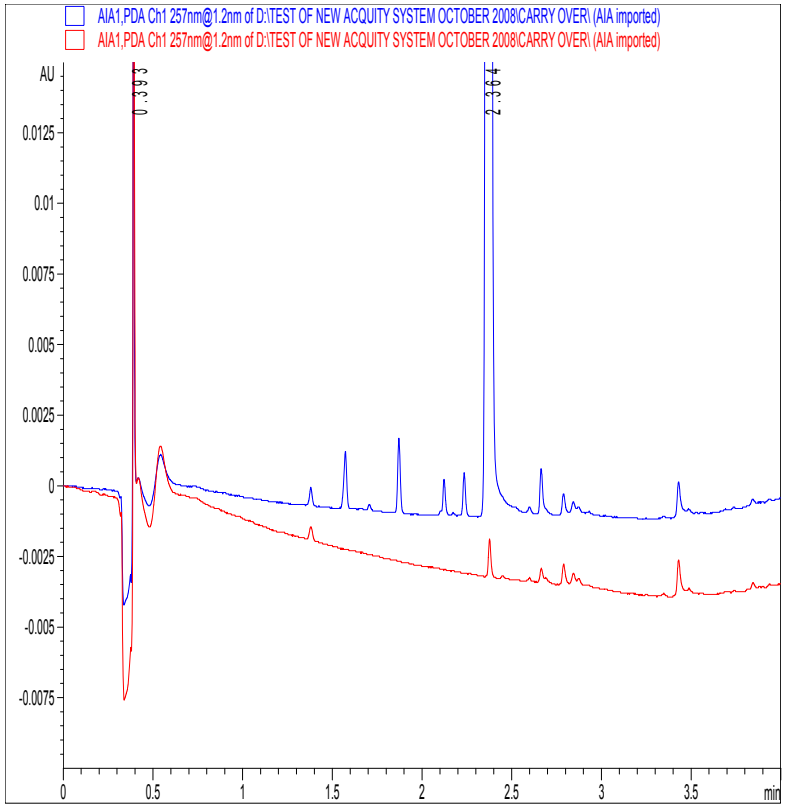
Applications:

a)Automated needle seat back-flushing for lower carry over
b)Fixed loop injection mode for fastest cycle times
c)By-pass metering device for lower delay volume
d)...and more .....

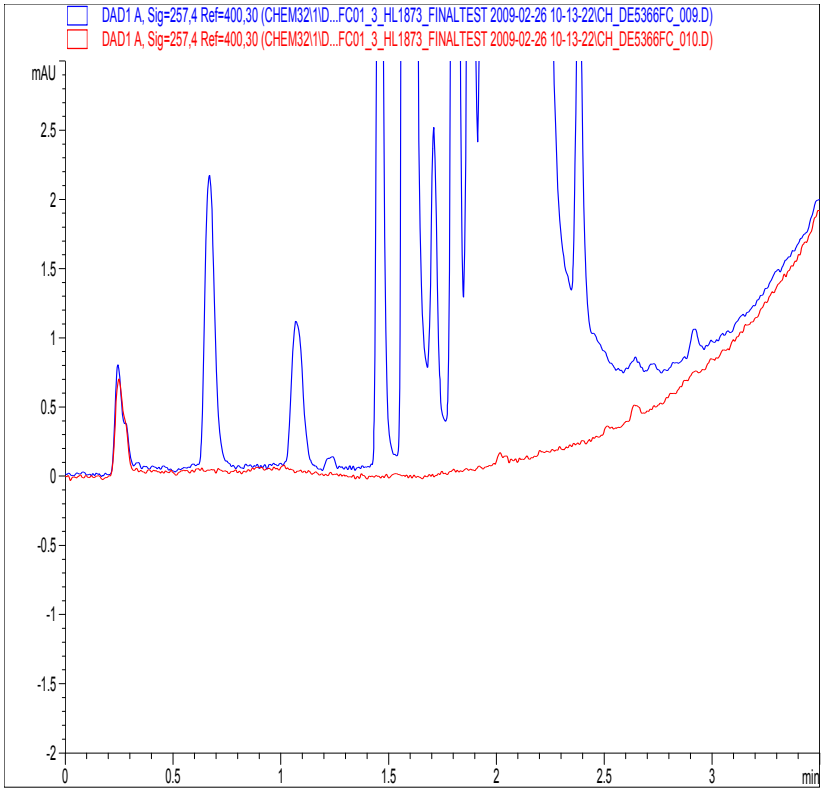
Carry-over comparison

- two system designs

0.021% on popular UHPLC system (PLUNO mode)



0.002% to 0.004% on another UHPLC without needle seat backflushing



Column: 2.1x50mm BEH C 18

Design Considerations for Column Compartments

- Column management: use any analytical column size or hardware
- Use fully porous, superficially porous or monolithic materials
- Temperature control: sub-ambient to 90C for all application types with independent temperature zones for each column compartment
- Valve configurations: 6-port, 10-port, two position valves for 2 column selection, including alternating column regeneration (10 port only)
- With 2 column compartment modules, 9-port eight position column selection valves, you may expand column selection to 4 columns with independent thermal zones, or up to 8 columns total
- Change valve type in minutes using universal drive valves and RFID identified liquid ends

UHPLC Therm. Column Compartment

- look for flexibility:

RFID-tag, stores type, pressure range, valve switches

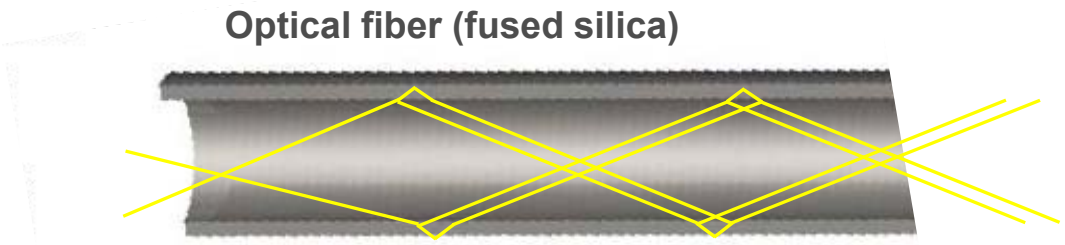
New 2PS/6PT, 2PT/10PT and 9PT/8PS high pressure valves

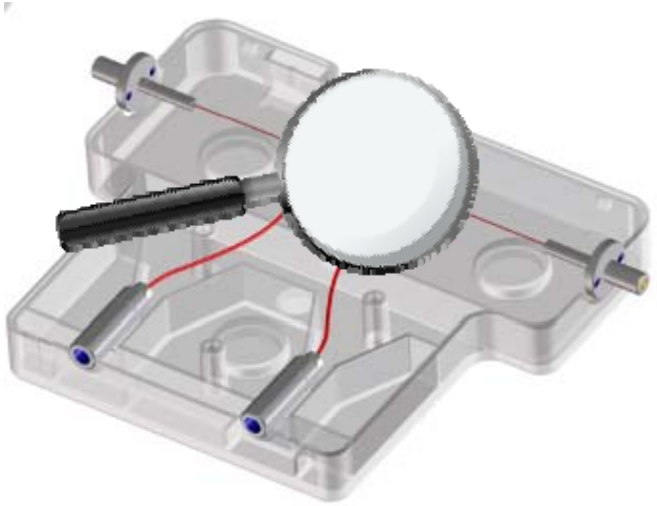
Design Considerations for UHPLC Optical Detection

- Maximizing sensitivity without excessive cell volume
- Flow rate and gradient compositional stability
- Refractive index correction approaches
- - direct correction through optical design,
- - acquisition with reference wavelength,
- - baseline subtraction from reference chromatogram

Long Path Cartridge Cell

- Optofluidic waveguides





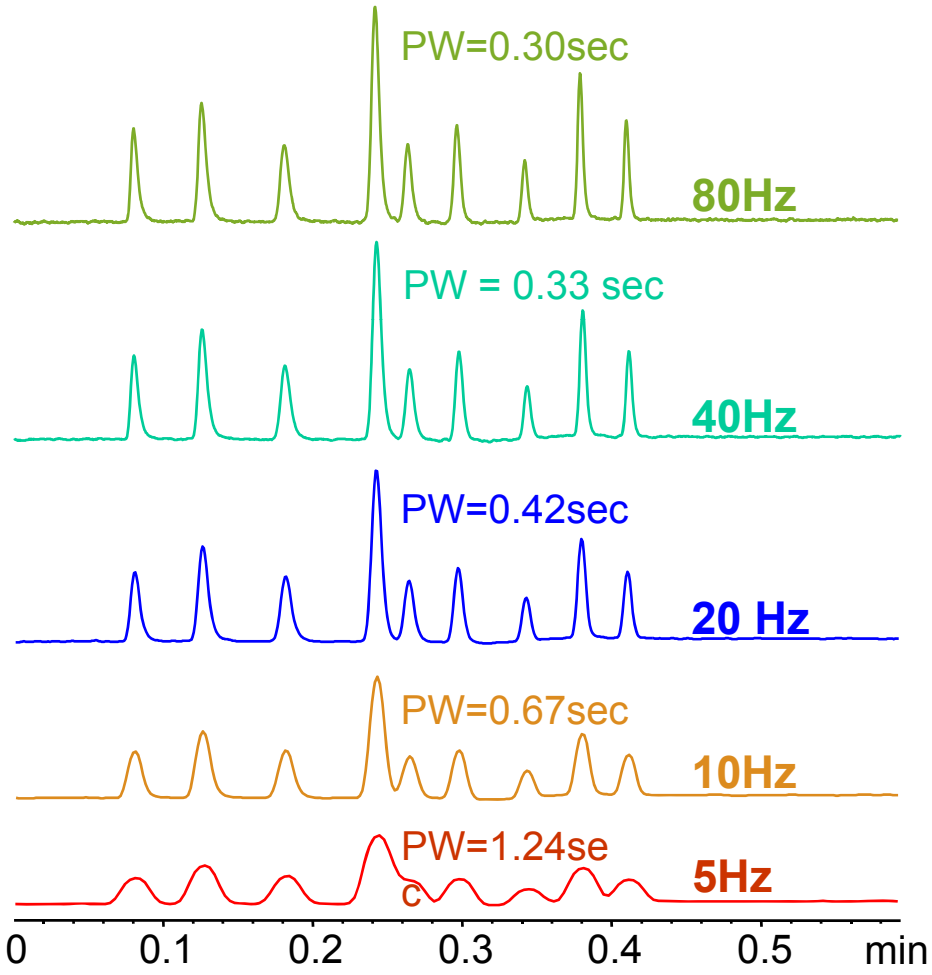
High Light Transmission due to Total-Internal Reflection (TIR) principle (~ 100 % Light efficiency) supports longer path length cell designs

Benefits:

- *High sensitivity (S/N) with small cell volumes (dispersion effects)*
- *Coating-free fused silica (no special care instructions or smiling baseline effects)*
- *Easy cell selection (one cell for all major applications)*
- *Cartridge design for ease of use*

Effect of Data Acquisition Rate (time constant)

Peak Width, Resolution and Peak Capacity in Ultra-Fast LC



80Hz **20Hz**

– 30% Peak Width

+ 30% Resolution

+ 40% Peak Capacity

+ 70% Apparent Column Efficiency

80Hz **10Hz**

– 55% Peak Width

+ 90% Resolution

+ 120% Peak Capacity

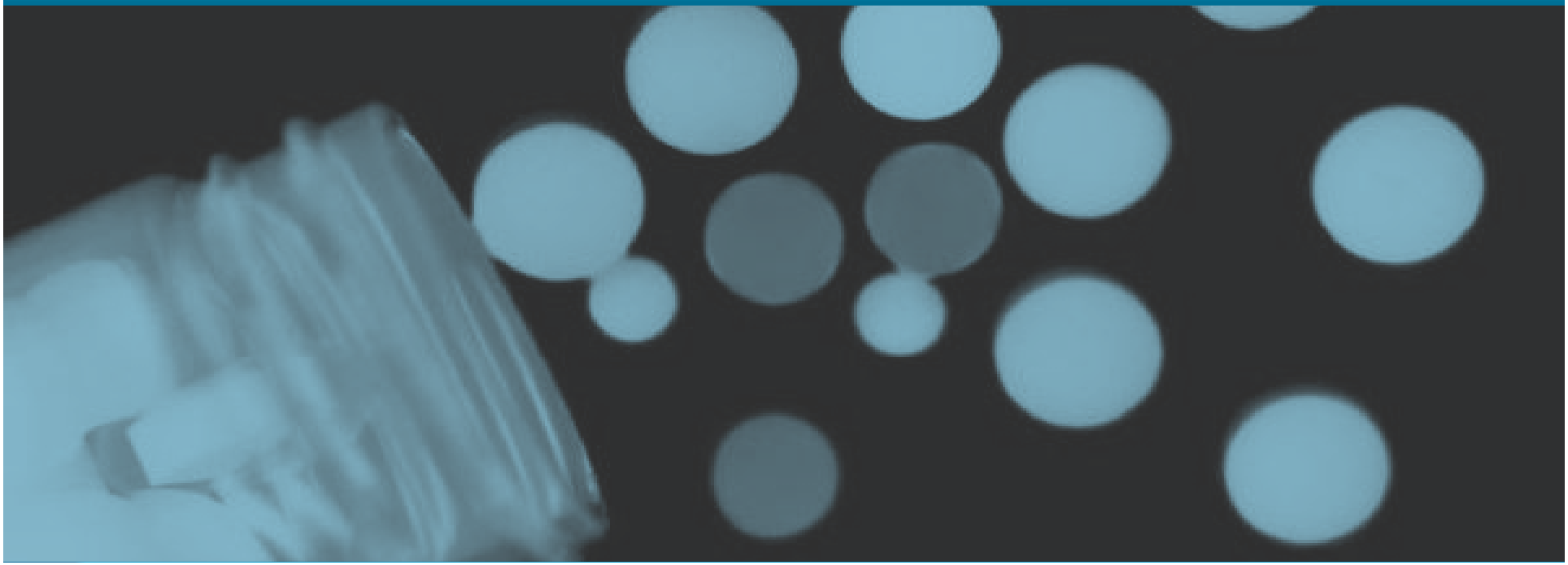
+ 260% Apparent Column Efficiency

Conclusion –UHPLC Design Requirements

- Look for best overall performance and feature set according to your application requirements (routine vs. method development, etc.)
- Choose flexible injection designs that allow variable or fixed loop capability, should usage priorities change
- Versatile column compartments support a wide range of applications on one common platform
- Understand available column technologies to ensure a good mix of performance and chemical selectivity

Pharmaceuticals in Water

- Rising concern about pharmaceuticals in surface water
- Wide range of analytes at low levels
- High speed MS sampling and mass resolution needed



Choose the Appropriate MS Technology

MS/MS

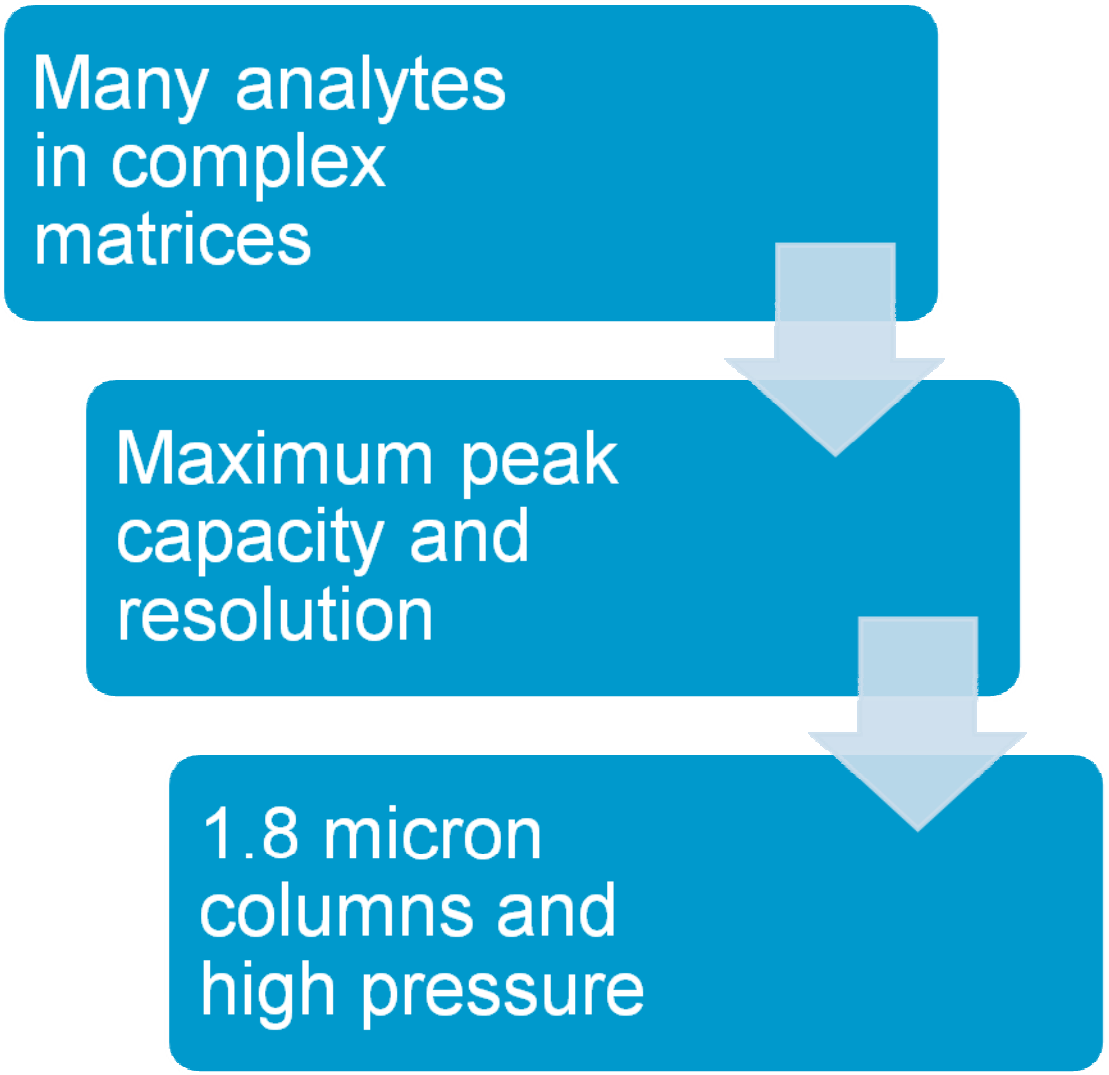
- Target compound analysis
- MRM
- Quantitation in complex matrices

TOF

- Identification of unknowns
- High mass accuracy
- Fast acquisition
- Full scan

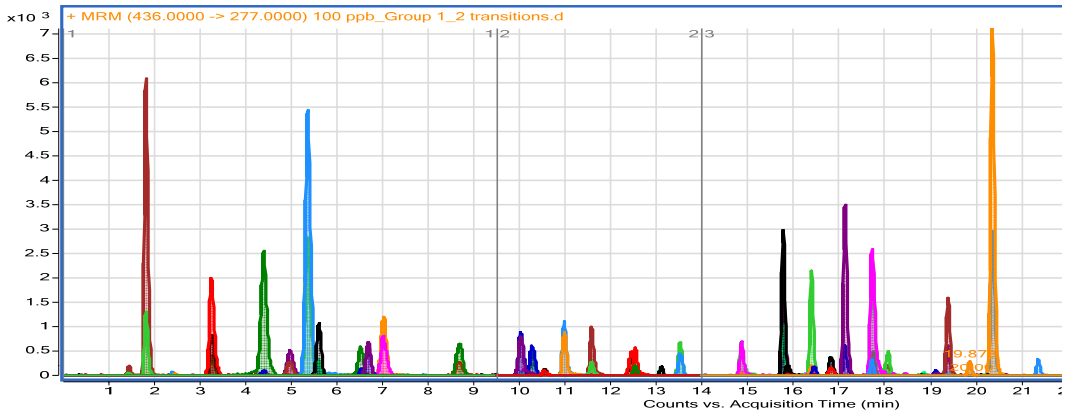
Note: MRM with two transitions is normally required

UHPLC is Important for Environmental Chemistry

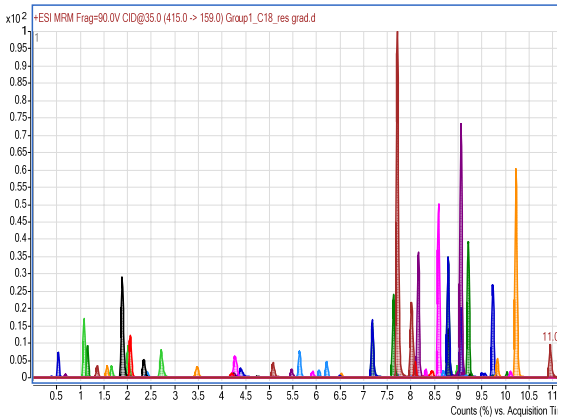


Agilent ZORBAX
RR-HT columns – 600 bar
RR-HD columns – 1200 bar

Improve Group 1 Analysis with 1.8 Micron



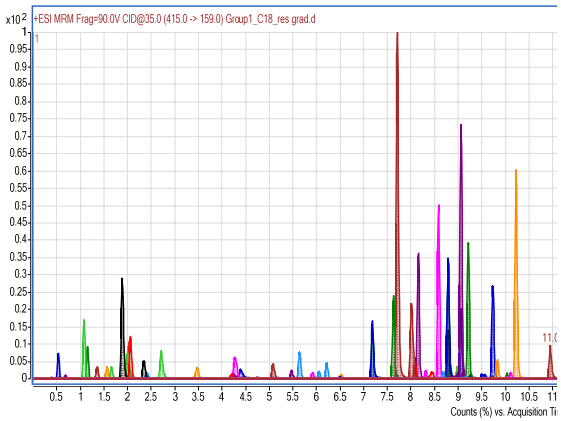
EPA method
2.1 x 100 mm
3.5 micron
22 minutes



Modified method
2.1 x 100 mm
1.8 micron
Eclipse Plus C-18
750 bar
11 minutes

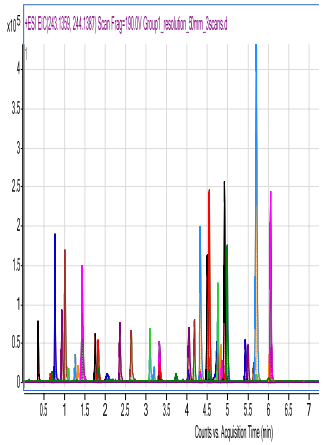
Higher pressure with reduced particle size – more power

Improve Further with Gradient Change



2.1 x 100 mm
1.8 micron
Eclipse Plus C-18
750 bar
11 minutes

Time	Composition
0.0	10% ACN
3.4	10% ACN
20.0	100% ACN
20.3	100% ACN

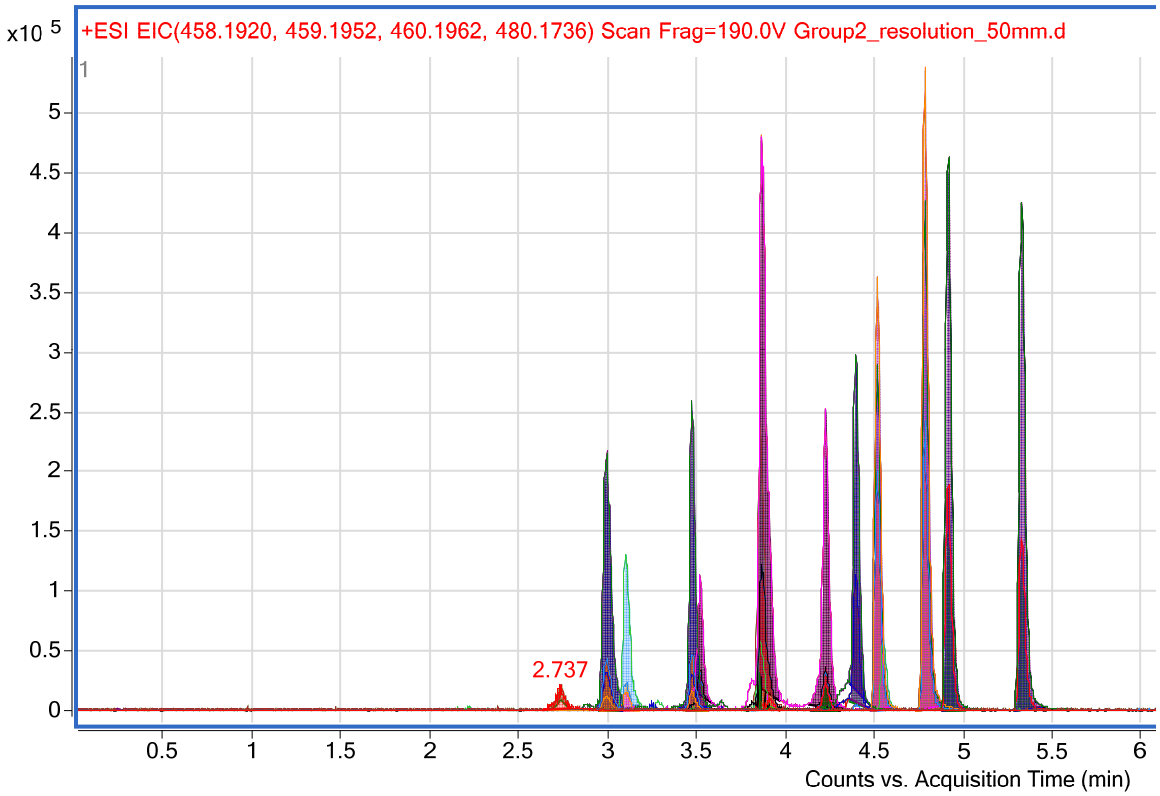


2.1x 50 mm
1.8 micron
Eclipse Plus C-18
375 bar
6 minutes

Time	Composition
0.0	10% ACN
1.7	10% ACN
10.0	100% ACN
10.3	100% ACN

shorter column further increases throughput

Fast Resolution of 12 Tetracyclines

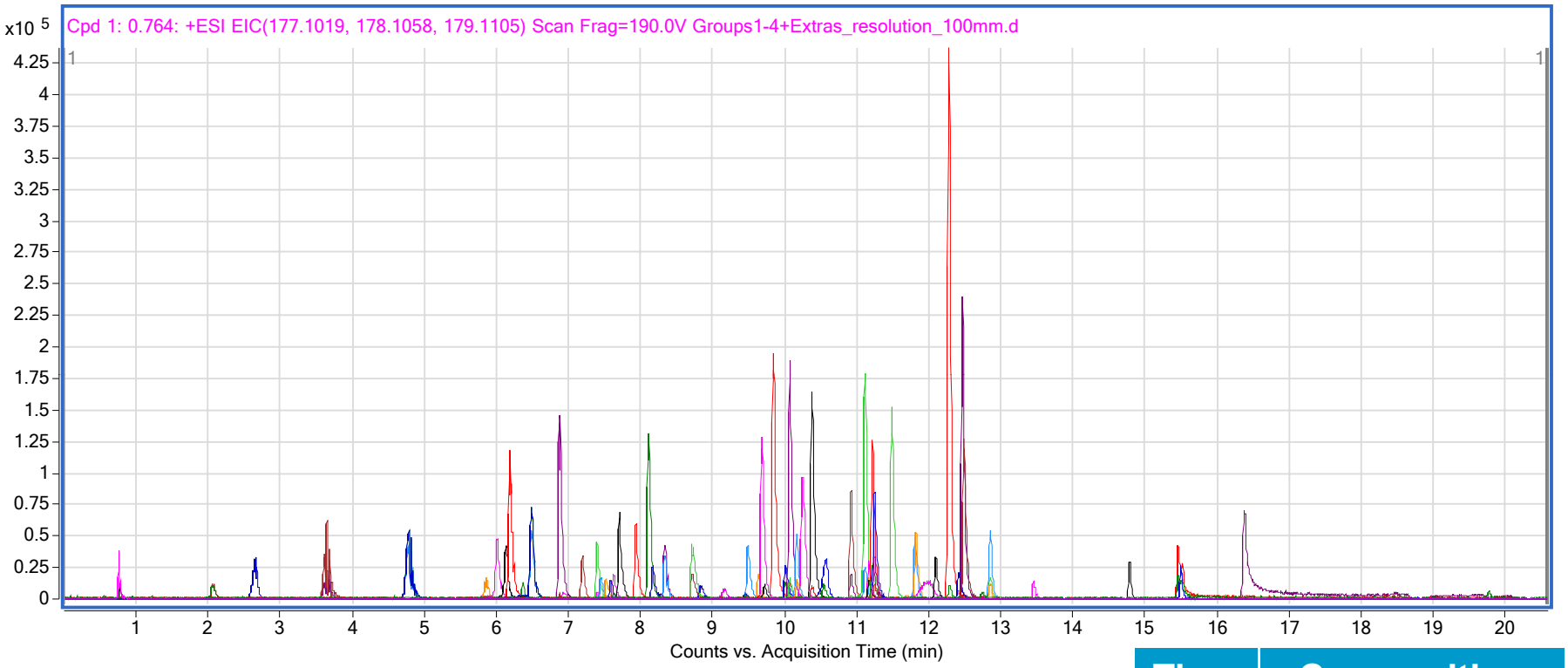


2.1x 50 mm
1.8 micron
Eclipse Plus C-18
375 bar
6 min

Time	Composition
0.0	10% ACN
1.7	10% ACN
10.0	100% ACN
10.3	100% ACN

Good resolution with shallow gradient on short column

Peak Capacity for 90 Pharmaceuticals in 20 min

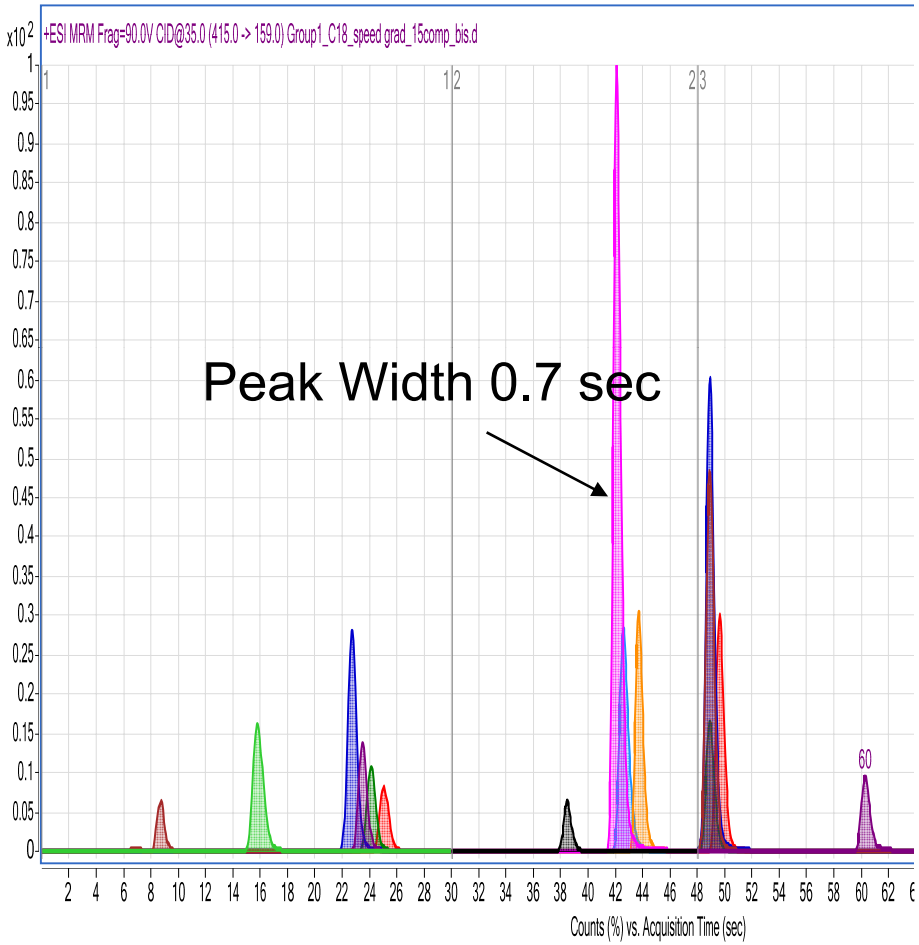


2.1 x 50 mm, 1.8 micron Eclipse Plus C-18,
750 bar observed operating pressure at 0.6 ml/min
1290 Infinity LC

Time	Composition
0.0	10% ACN
3.4	10% ACN
20.0	100% ACN
20.3	100% ACN

"Infinity" resolution for complex mixtures

Ultrafast Chromatography for 15 Analyte Subset

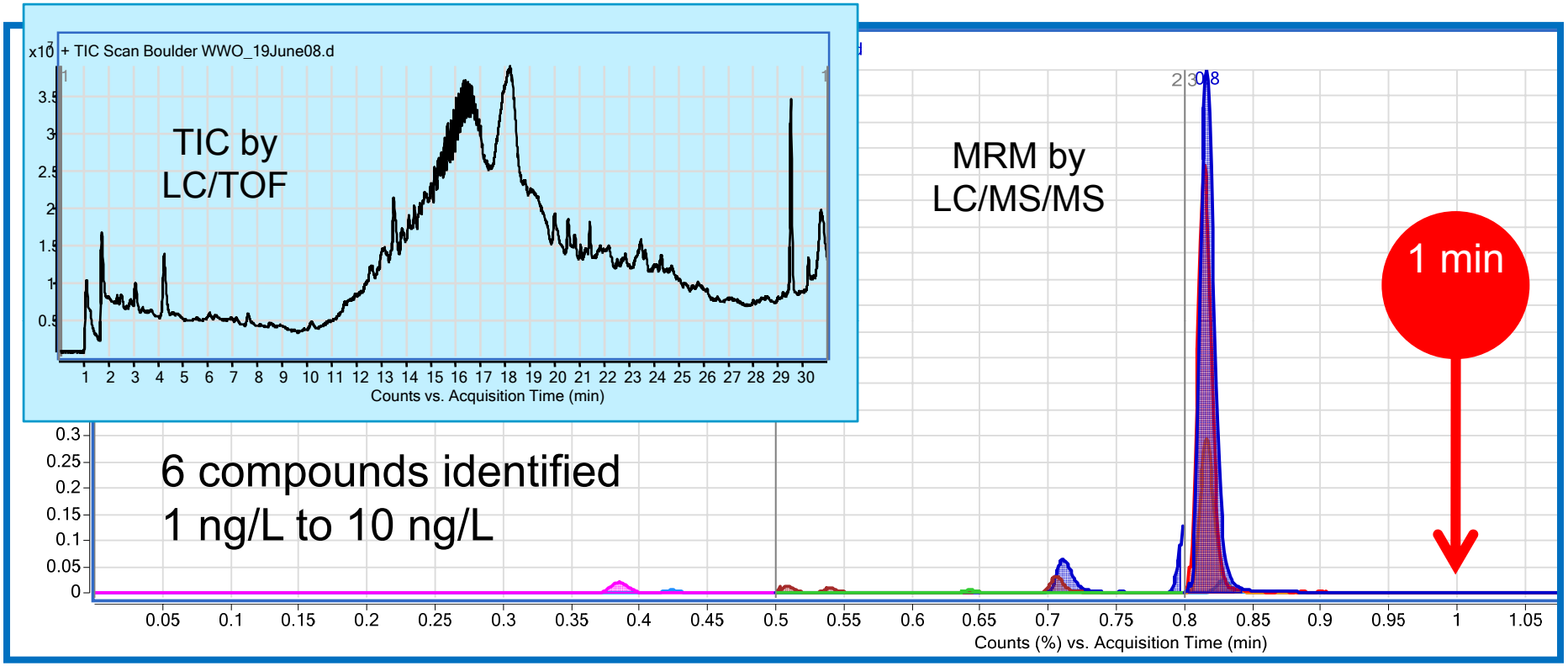


2.1x 50 mm
1.8 micron
Eclipse Plus C-18
750 bar
1 minute

Time	Composition
0.0	10% ACN
1.5	100% ACN

Ultimate speed on a short column with ballistic gradient

Rapid Analysis of Boulder Wastewater Effluent



2.1x 50 mm, 1.8 micron Eclipse Plus C-18, running at 750 bar

Time	Composition
0.0	10% ACN
1.5	100% ACN

It works on real samples, too!!

Pesticides

- Number of Compounds limited with Fast Target Analyses
- Rapid screening of many pesticides needed
- Complex matrices make identification difficult

Find These Compounds in a Sample

Methamidophos
1-Naphthol,
Methomyl
Fenuron
Acephate
Fuberidazole
Propamocarb
Tricyclazole
Butocarboxim
Carbendazim
Isoprocarb
Cymoxanil
Cycluron
Pyrimethanil
Diamidafos
Thiabendazole
Carbaryl
Dinotefuran
Aldicarb-sulfoxide
Isoproturon
Promecarb
Aminocarb
Propoxur
Chlortoluron
Omethoate
Simetryn
Monolinuron
Metribuzin
Pymetrozine
Pyracarbolid
Thiofanox
Oxamyl
Thidiazuron
Methabenzthiazuron

Carbofuran
Formetanate
Acetamiprid
Aldicarb-sulfone
Butoxycarboxim
Mexacarbate
Monocrotophos
Bendiocarb
Dioxacarb
Mepanipyrim
Mevinphos
Ethiofencarb
Methiocarb
Cyprodinil
Prometon
Secbumeton
Terbumeton
Ametryn
Tebuthiuron
Dimethoate
Flonicamid
Diuron
Fluometuron
Siduron
Carbetamide
Dicrotophos
Carbofuran,
-3 hydroxy
Pirimicarb
Prometryn
-Terbutryn
-Ethoprop
-Cyanophos
-Fonophos

Forchlorfenuron
-Linuron
-Fludioxonil
-Clothianidin
-Thiacloprid
-Imidacloprid
Thiobencarb
Metobromuron
-Demeton-S Phorate
Diethofencarb
-Mepronil
-Nitenpyram
Methoprotryne
-Neburon
-Fenthion
-Oxadixyl
-Metalaxyl
-Propetamphos
Penconazole
Ethofumesate
Vamidothion
-Terbufos
- Iprobenfos
-Myclobutanil
Chloroxuron
Thiamethoxam
-Parathion
Cyproconazole
Uniconazole
-Amitraz
-Triadimenol
- Imazalil
-Spiroxamine
-Quinalphos

-Mefenacet
-Ditalimfos
Desmedipham
Phenmedipham
Bifenazate
-Fenhexamid
-Flutriafol
-Fenoxycarb
-Furalaxyl
-Methidathion
-Clofentezin
-Fenamiphos
Fenpropimorph
-Diazinon
-Pirimifos-methyl
-Buprofezin
-Fenazaquin
-Quinoxyfen
-Tebuconazole
Fensulfothion
-Ediphenphos
-Diflubenzuron
-Fenamidone
Triazophos
Hexaconazole
Kresoxim methyl
Nuarimol
Tribufos
Flusilazol
Bupirimate
Azinphos-methyl
Triticonazole
Tebupirimfos
Metconazole

Phenthoate
Iprovalicarb
Pyriproxyfen
Sulfotep
Sulprofos
EPN
Flutolanil
Cyazofamid
Famphur
Diniconazole (E)
Diniconazole (Z)
Benalaxyl
Dimoxystrobin
Etaconazole
Diclobutrazol
Fenarimol
Malathion
Pirimifos-ethyl
Ipconazole
Tebufenpyrad
Zoxamide
Fenbuconazole
Bitertanol
Piperonyl butoxide
Pyridafenthion
Propiconazole
Thiophanate-methyl
Prothioconazole
Azinphos-ethyl
Triflumizol
Isofenphos
Propargite
Hexythiazax
Tebufenoxide

Triflumuron
Isoxaflutole
Clethodim
toxazole
Flufenacet
Benzoximate
Topramezone
Pyridaben
Picoxystrobin
Methoxyfenozide
Spiromesifen
Tetraconazol
Pyrazophos
Famoxadon
Bromuconazole
Fluquinconazole
Prochloraz
Furathiocarb
Ethion
Sulfentrazone
Pyraclostrobin
Dimethomorph(E)
Dimethomorph(Z)
Rotenone Ethiprole
Flucarbazone
Alanycarb
Pinoxaden
Azoxystrobin
Difenconazole
Trifloxystrobin
Benfuracarb
Carfentrazone-ethyl
Mandipropamid
Fenpyroximate(E)

Fenpyroximate(Z)
Dioxathion
Fluoxastrobin
Fluazinam
Temephos
Butafenacil
Hydramethylnon
Lufenuron
Indoxacarb
Moxidectin
Spinosyn A
Spinosyn D
Avermectin B1b
Emamectin B1b
Avermectin B1a
Ivermectin B1a
Emamectin B1a
Doramectin
Eprinomectin B1a

How Did We Do It?

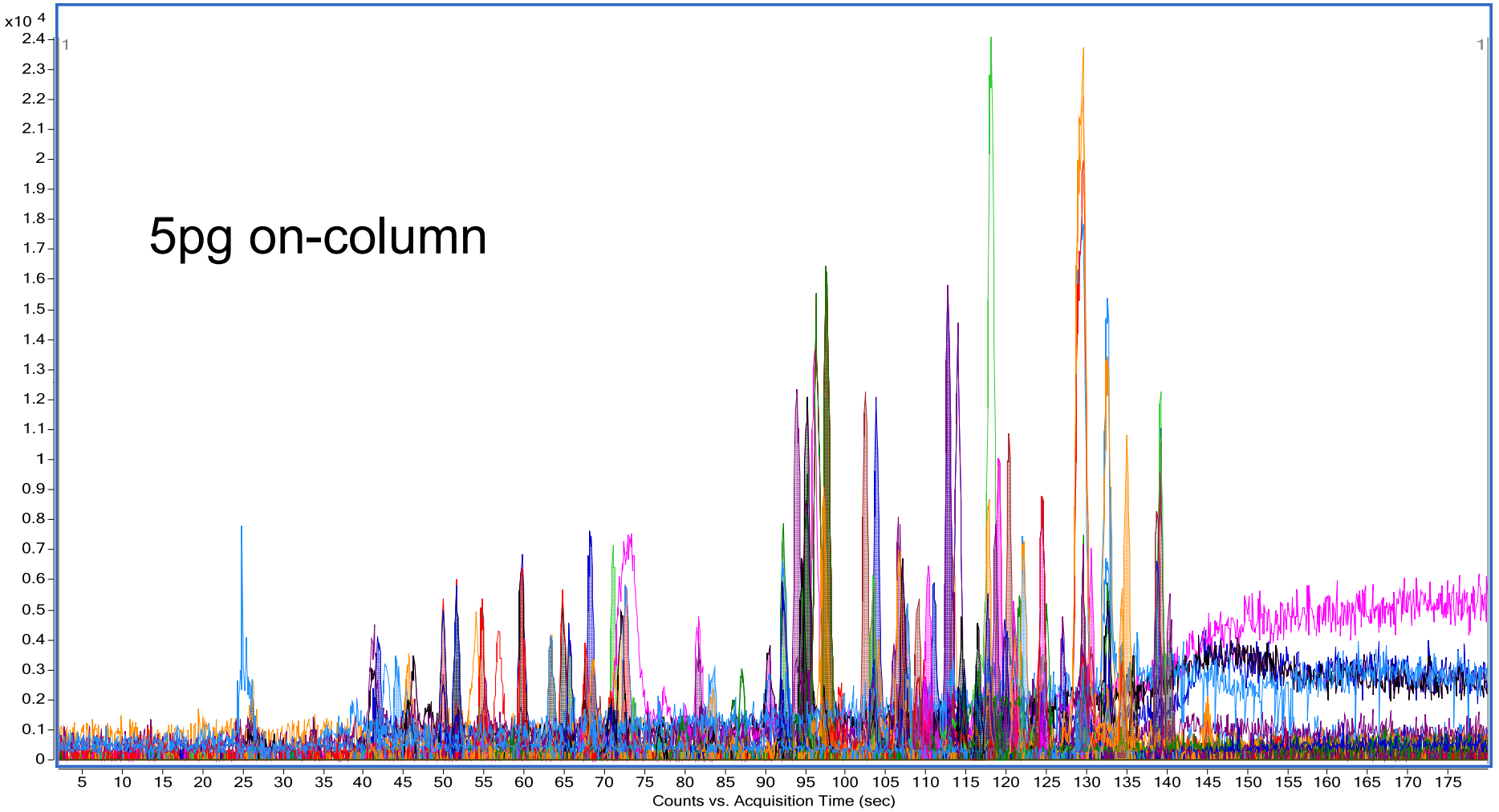
1290 Infinity LC — High pressure

Zorbax Eclipse Plus C18 column — High resolution

6230 TOF with JetStream — High sensitivity

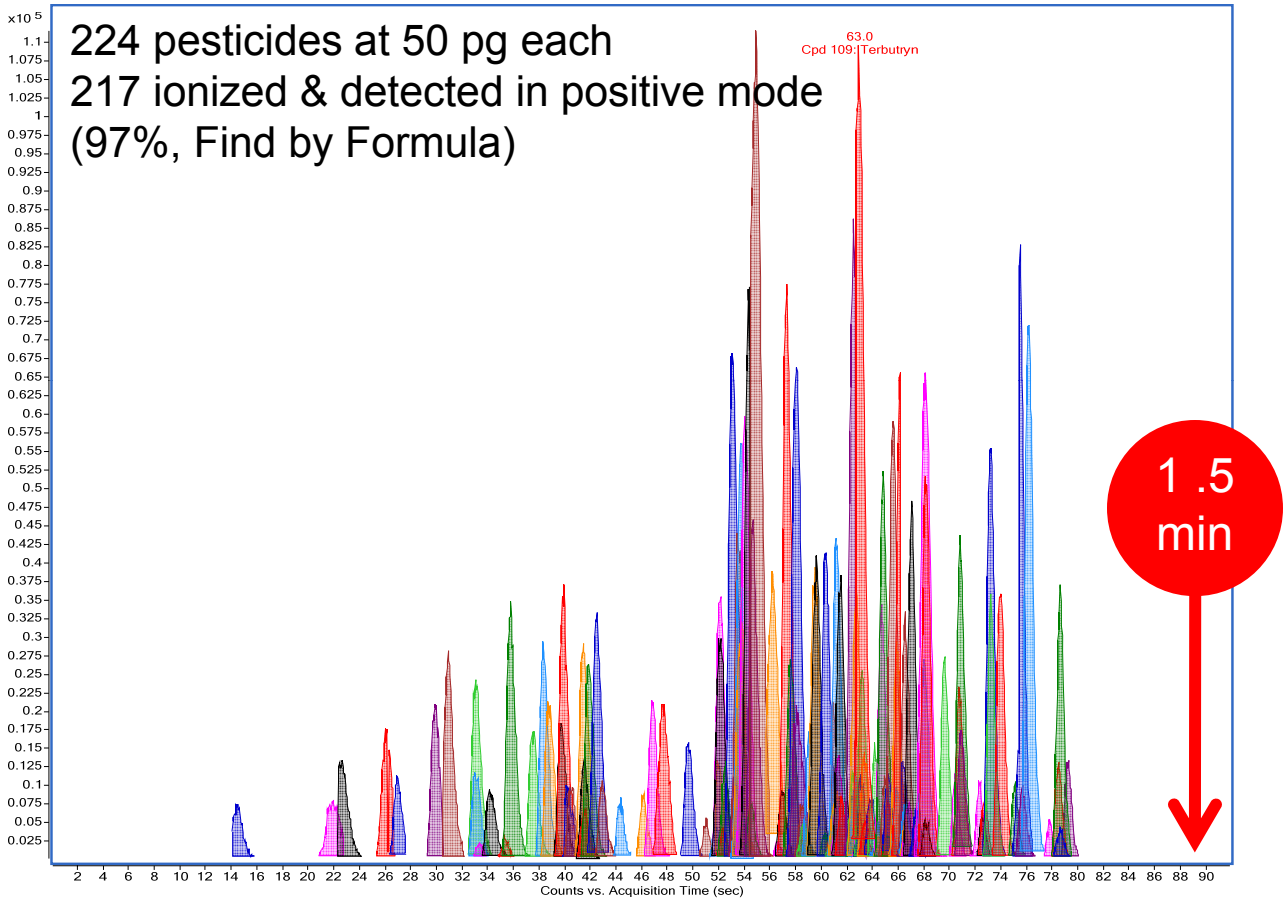
Pesticide database — Screening

Is 3 Minutes Fast Enough?



Remarkable specificity, speed and sensitivity

TOF Can Identify More Compounds in Less Time



2.1 x 50 mm x 1.8 micron
Eclipse Plus C-18
900 bar
1.5 mL/min
1290 Infinity

Time	Composition
0.0	10% ACN
1.5	100% ACN

TOF fast acquisition rates ensure maximum throughput

High Accuracy Confirmed by Database

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

More than 70% confirmed with only 5pg on column

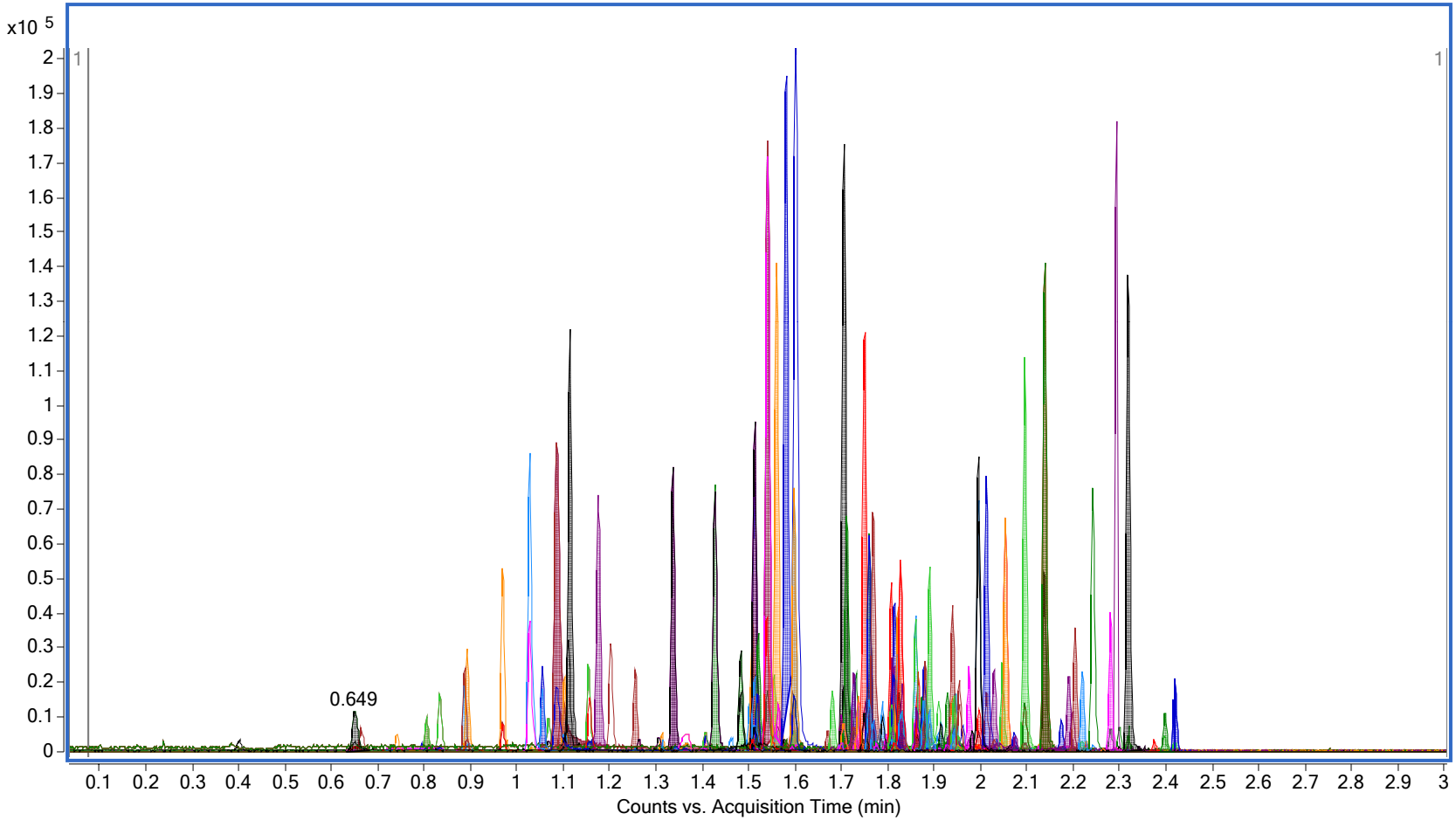
TOF/Q-TOF

Capabilities

- Full-Spectral Acquisition
- Accurate mass
- Fast acquisitions
- High Spectral Resolution

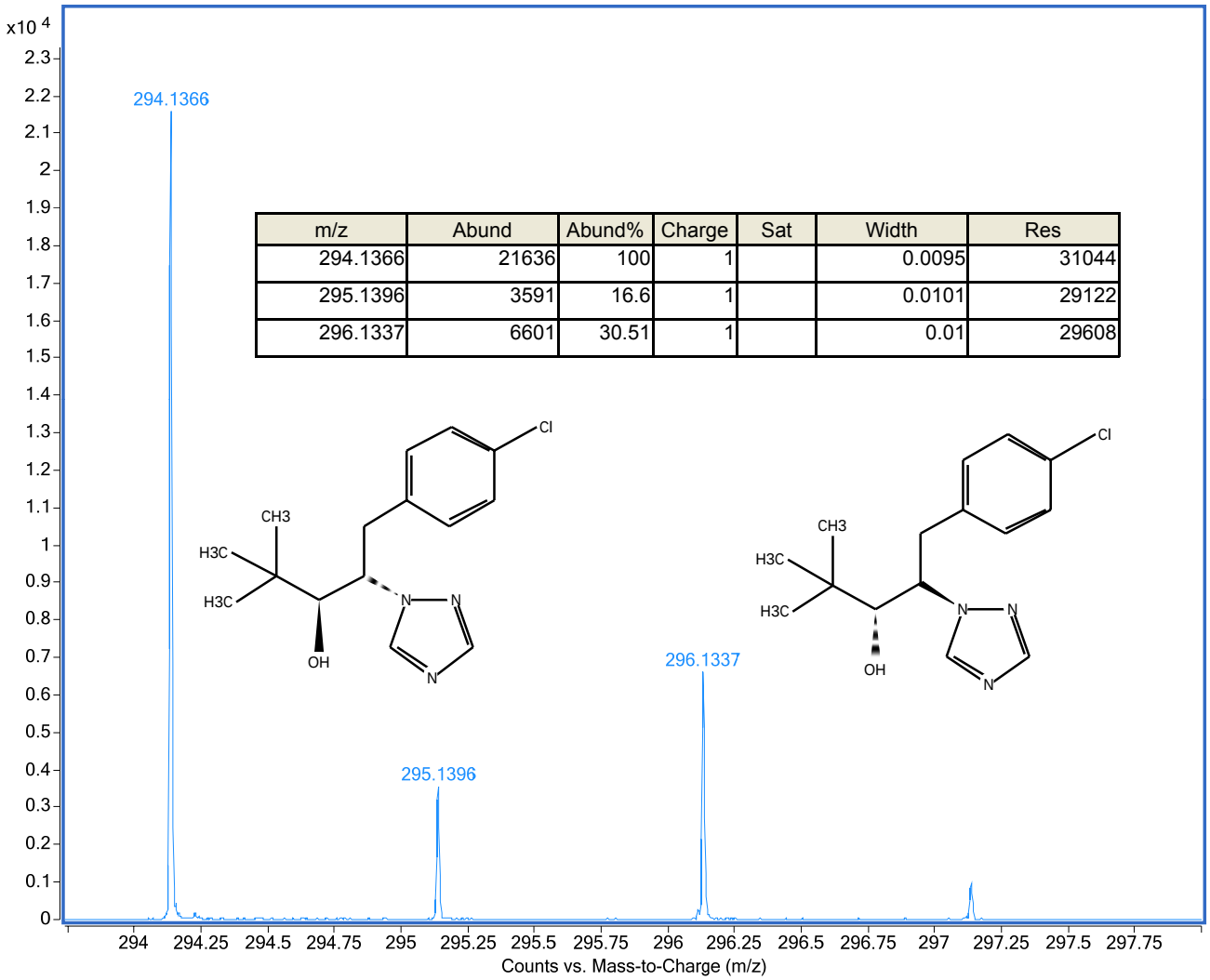
New 1290 Infinity LC with 6540 QTOF LC/MS

ECC of 100 Pesticides in 3 min using new *Agilent 1290 Infinity* LC with the new *Agilent 6540 QTOF*



With FULL SPECTRA Benefit from FAST RUN TIMES

Example mass spectrum from data on 3 min run with 1290 Infinity LC and 6540 QTOF.



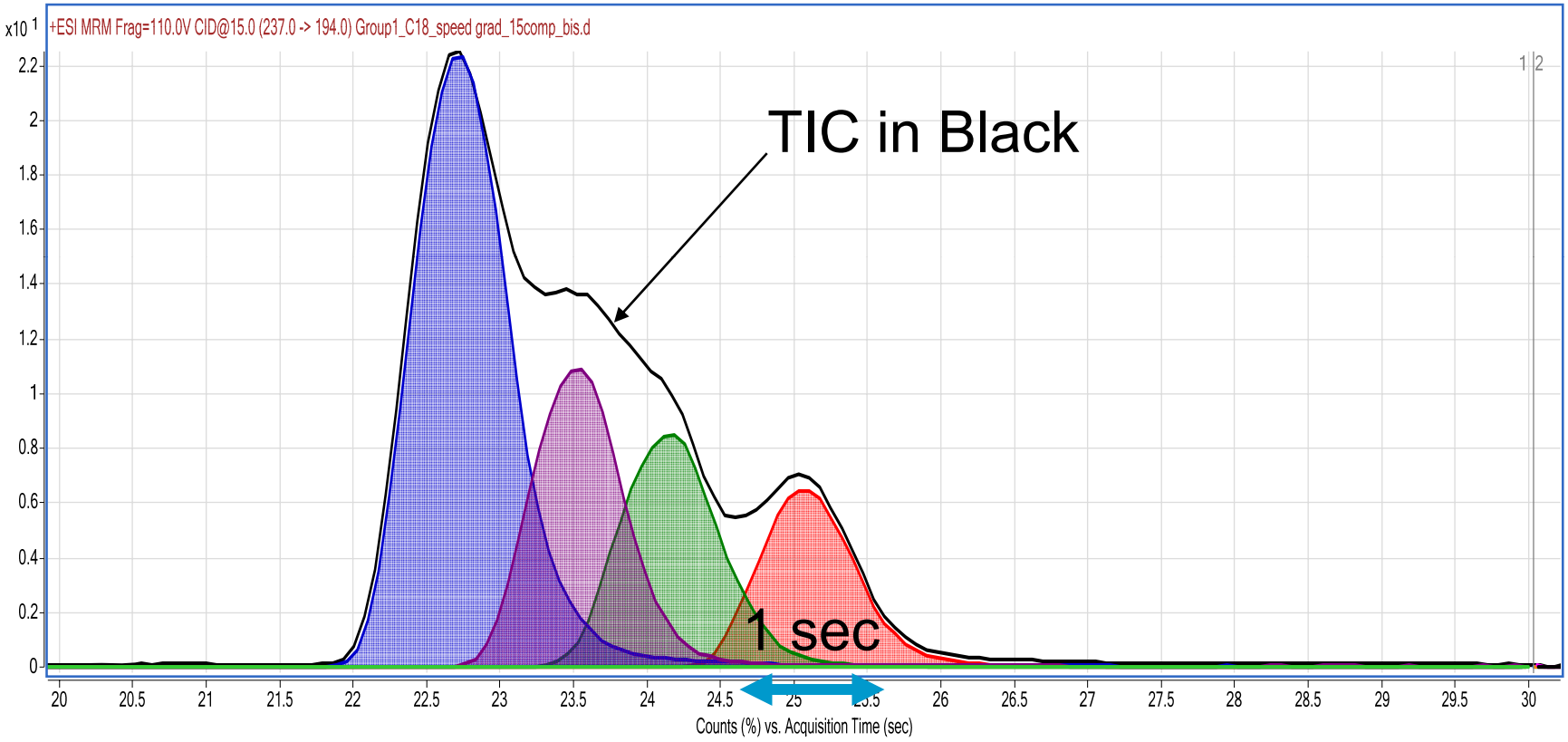
m/z	Abund	Abund%	Charge	Sat	Width	Res
294.1366	21636	100	1		0.0095	31044
295.1396	3591	16.6	1		0.0101	29122
296.1337	6601	30.51	1		0.01	29608

Note the mass resolution at 10 spectra per second

How Do We Do It with a Triple Quad

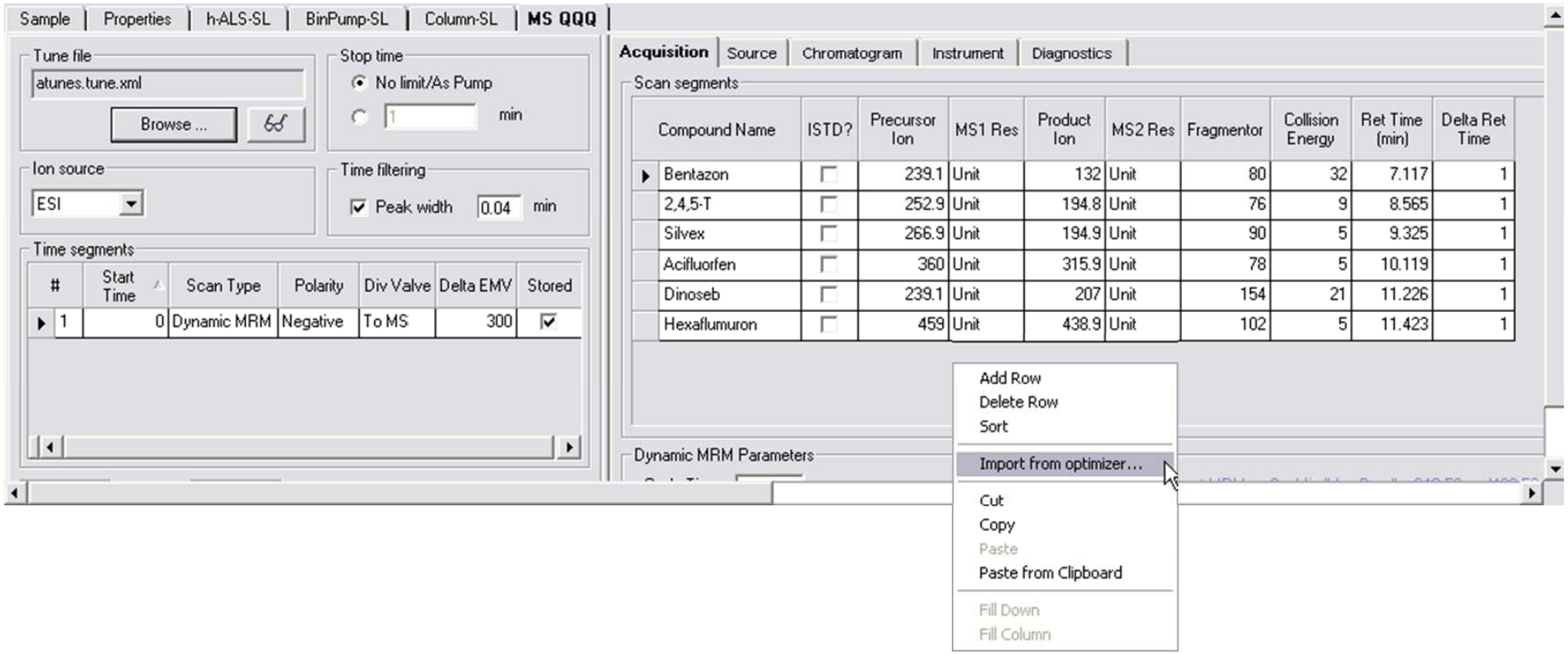
Dynamic MRM Required for Overlapping Peaks

Necessary to have at least 20 points across the peak



once again, true UHPLC demands fast data acquisition

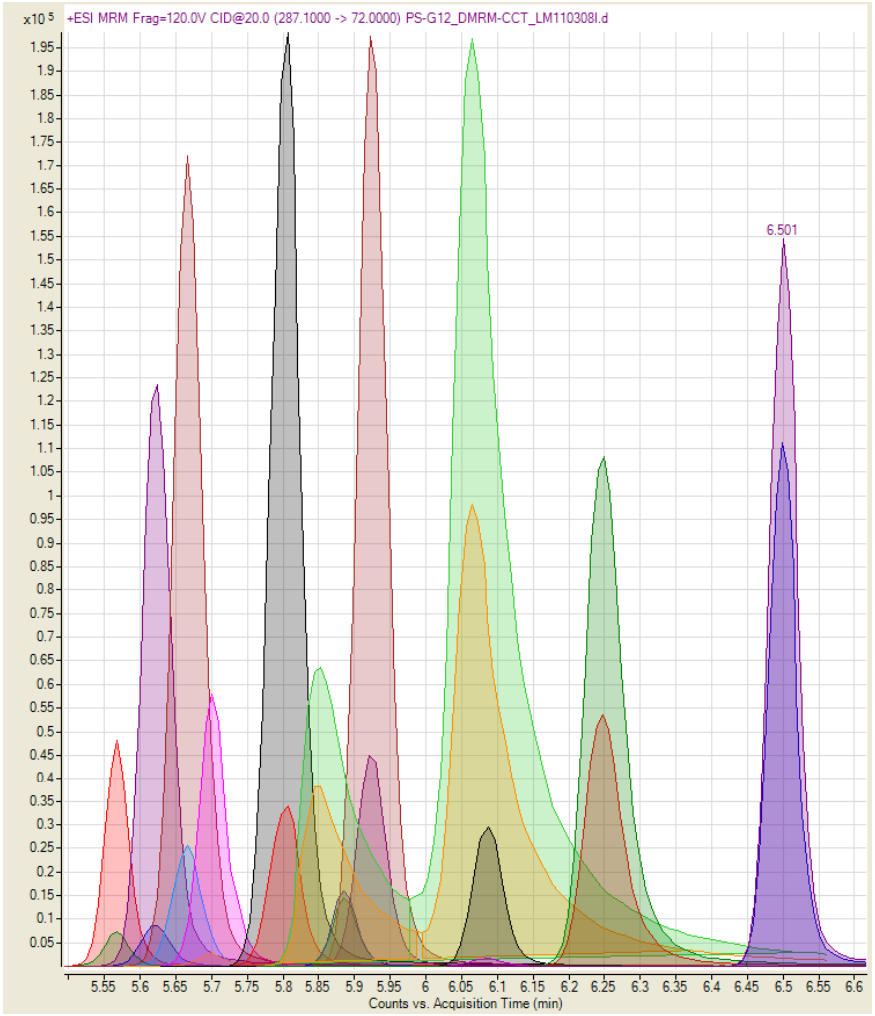
Dynamic MRM Acquisition Parameters – *Customized* Methods from Database



**** Note that, with Dynamic MRM, the user sets the cycle time and dwell times are automatically managed.***

Dynamic MRM 11 Compounds (22 MRMs) eluting in about 1 min window

Compound Name	Precursor Ion	Product Ion	Retention Time
Cinosulfuron	414.1	183	5.579
Cinosulfuron (Q)	414.1	157	5.579
Chlorotoluron	213.1	72	5.642
Chlorotoluron (Q)	213.1	140	5.642
Atrazine	216.1	174	5.682
Atrazine (Q)	216.1	132	5.682
Carbaryl	202.1	145	5.736
Carbaryl (Q)	202.1	117	5.734
Carboxin	236.1	143	5.836
Carboxin (Q)	236.1	87	5.836
Chlorsulfuron	358.0	167	5.896
Chlorsulfuron (Q)	358.0	141	5.896
Ethiofencarb	226.1	107	5.937
Ethiofencarb (Q)	226.1	164	5.936
Dodemorph	282.3	116	6.073
Dodemorph (Q)	282.3	98	6.074
Diuron	233.0	72	6.101
Diuron (Q)	233.0	160	6.101
Cyprodinil	226.1	108	6.245
Cyprodinil (Q)	226.1	93	6.246
Difenoxurone	287.1	123	6.509
Difenoxurone (Q)	287.1	72	6.509

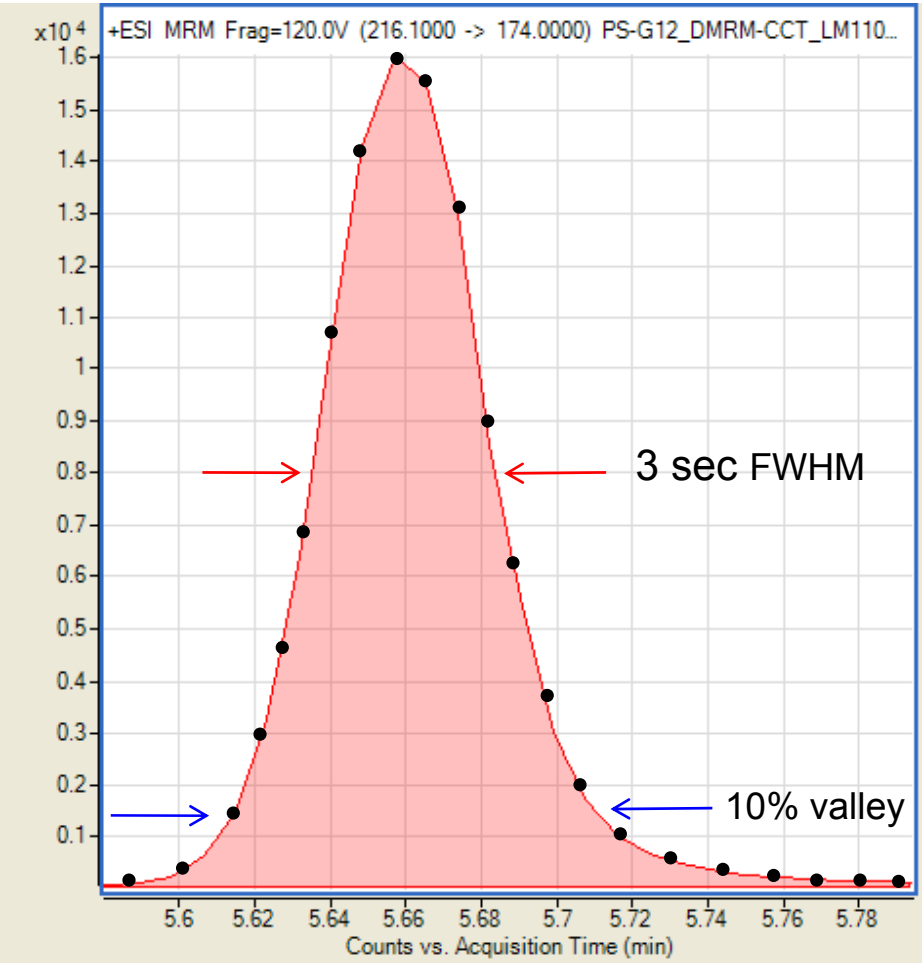


Agilent Technologies

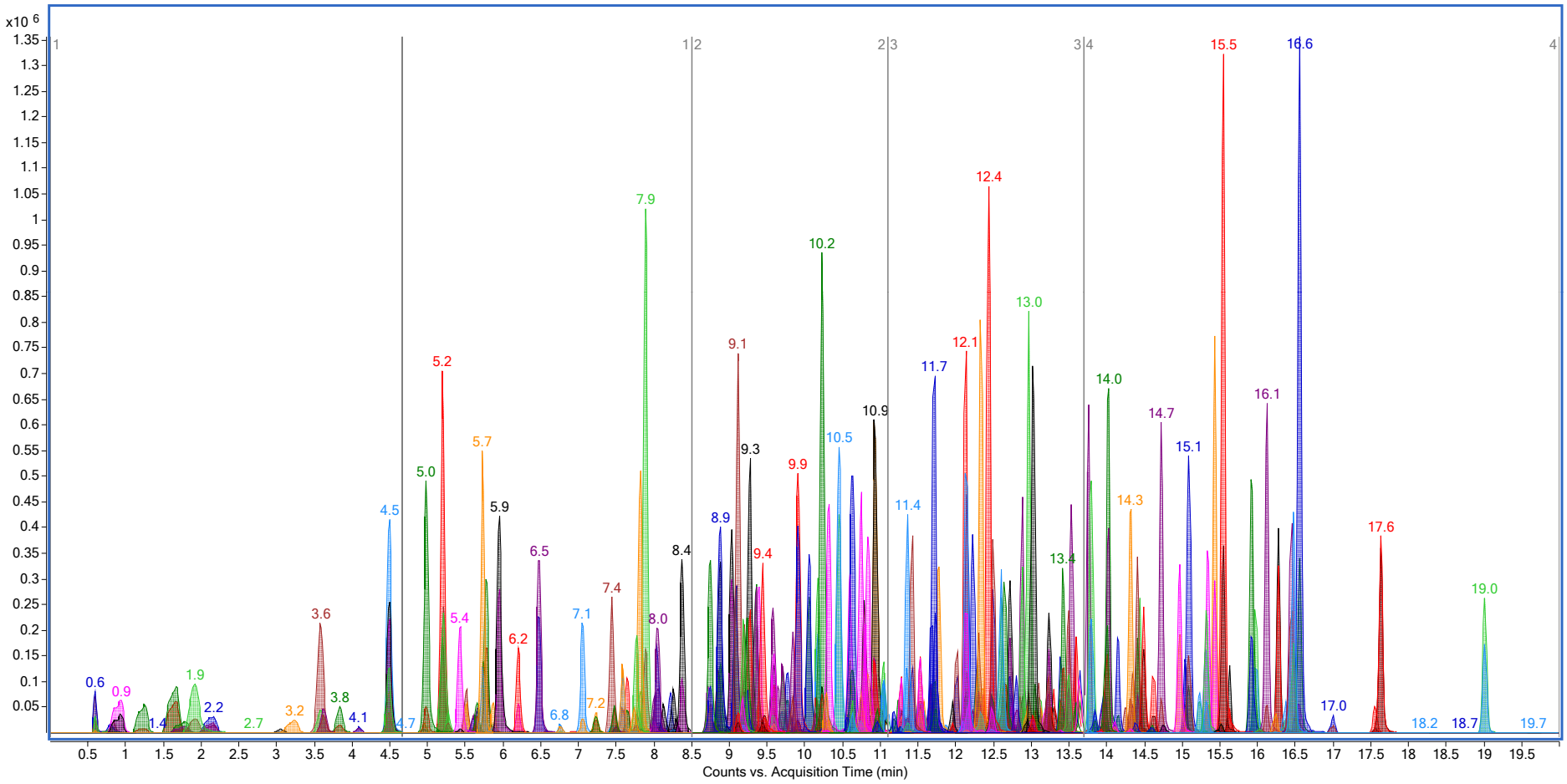
Typical results: 10 pg Atrazine on-column using Dynamic MRM 6460A QQQ with Jet Stream Technology

- *Avg. Signal Height: 15,650*
- *Avg. Signal Area: 50,966*
- *RSD: 3.2 %*
- *Estimated LOQ: 100 fg or less*

- *6-7 data points above FWHM*
- *3 sec FWHM, 6 sec @10% valley*
- *20 data points baseline-to-baseline*

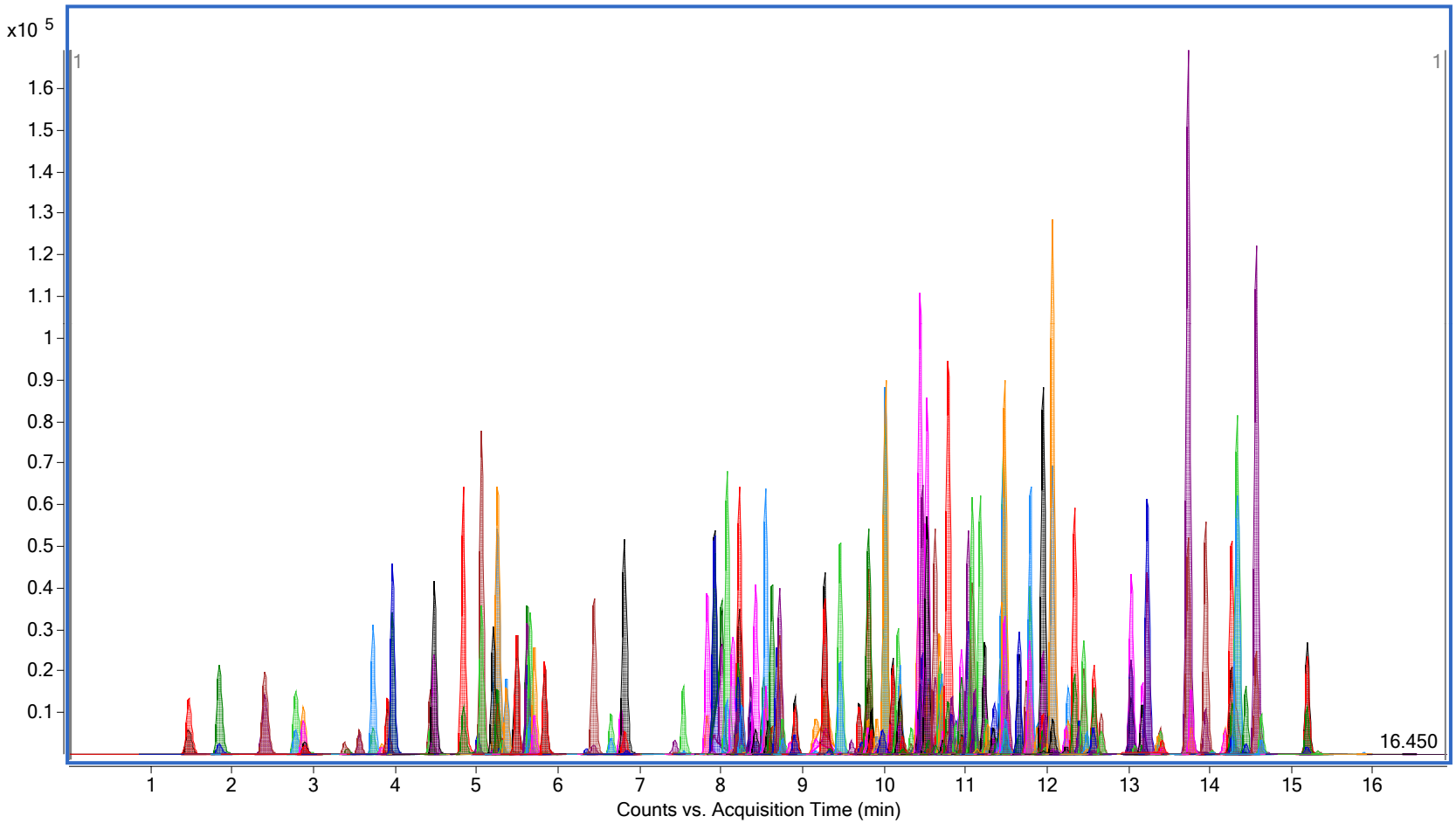


Dynamic MRM of 300 Pesticides with Two Transitions Each



Run with 1200-SL LC 2.1 x 100 C18 Eclipse PLUS 1.8 u

Pesticide Mix run with Agilent 1290 Infinity LC : Benefit of *HIGHER PEAK CAPACITY*



LC 2.1 x 150 C18 Eclipse PLUS HD 1.8 u, 0.6 mL/min, 800 bar

Conclusions

- **For maximum peak capacity, choose high pressure rated columns and optimize effective gradient slope (k*) vs. available system pressure and preferred analysis time. Use the Agilent Method Translator for modeling**

- **Use MS conditions that allow 20 cycles/second for sharp peaks of 1-3 seconds, either**
  LC/MS/MS by triple quadrupole with fast dwell times of 5 msec*, or
  LC/TOF-MS or Q/TOF-MS and 20 cycles per second (i.e.~500 transients per second)

LC Systems and Method Translator: http://www.chem.agilent.com/en-US/Products/Instruments/lc/systems/1290infinitylcsystem/Pages/default.aspx

MS systems:
http://www.chem.agilent.com/en-US/products/instruments/ms/Pages/default.aspx