

Six Productivity Innovations in Small Molecule Analysis in 30 minutes



Tony Brand and Doug McIntyre

The Field Application Group's 12 Innovations

(in small molecule analysis, not considering a lot of great stuff in macromolecule analysis)

1. **1.8 μ particle columns without excessive back pressure**
2. **A new challenge, columns at higher temperatures.**
3. **True 10,000 amu/sec scanning: Maintaining resolution/sensitivity**
4. **Simultaneous Electrospray and APCI: The Multi-mode Source**
5. **CAN-network and Consequences: 95:95 Prep Performance, for example**
6. **The Agilent 1200 RFID tagging: Beyond columns....**
7. **MS-Chip: Nanoflow LC/MS... Defining a new standard**
8. **2-D GC and GC/MS: The Agilent Implementation of the Dean Switch**
9. **Fast precision gradients at low, low flow rates: The Agilent nano-flow controller**
10. **The Agilent 6000 Series QQQ and QTOF: New Collision Cell Technology**
11. **Dynamic Solvent Specific Compressibility Compression: Higher pressures with minimal baseline variation**
12. **New solutions for Metabolomics: MassHunter with Molecular Feature Extraction and GeneSpring-MS**

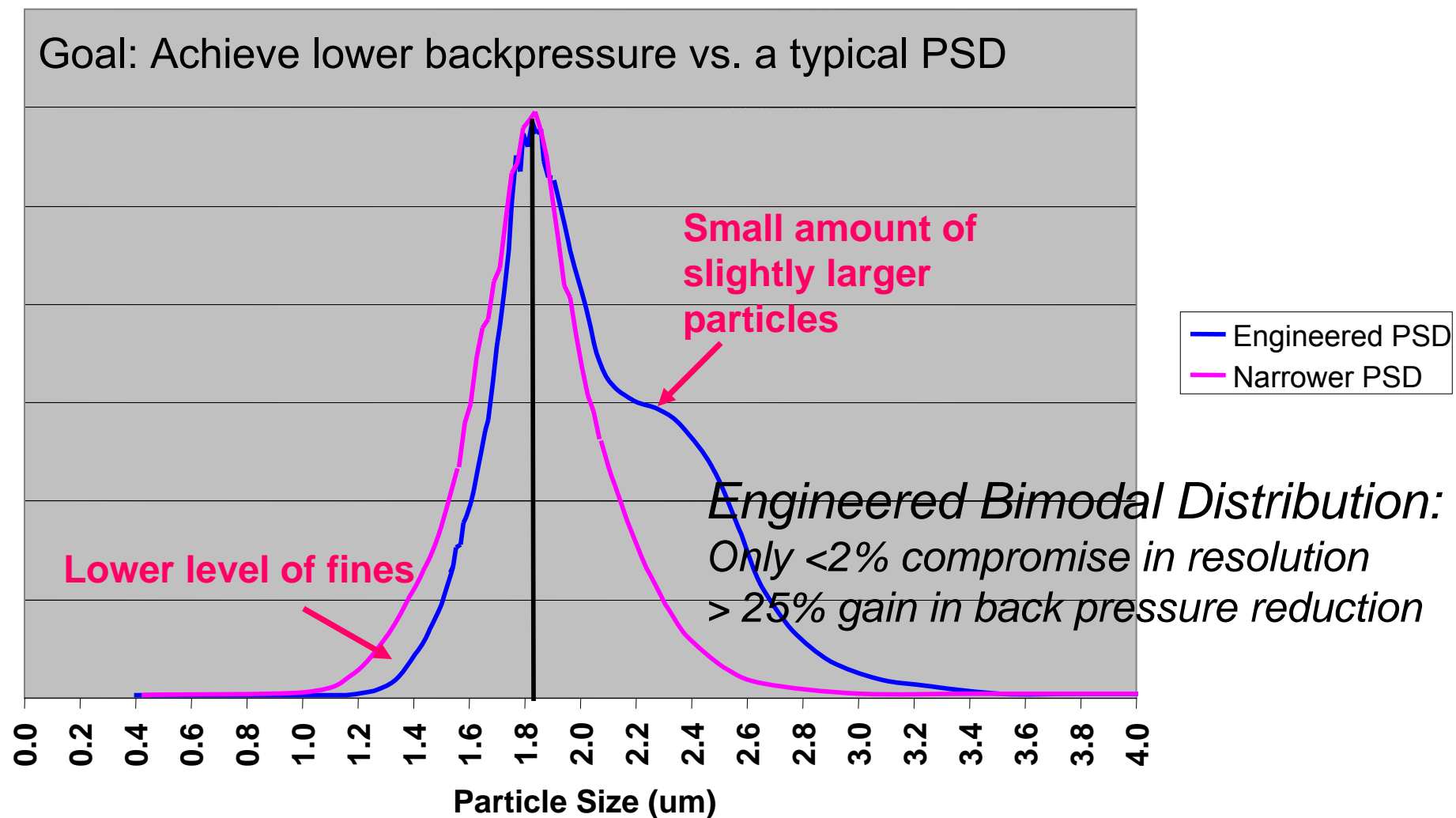
The Field Application Group's Top 6

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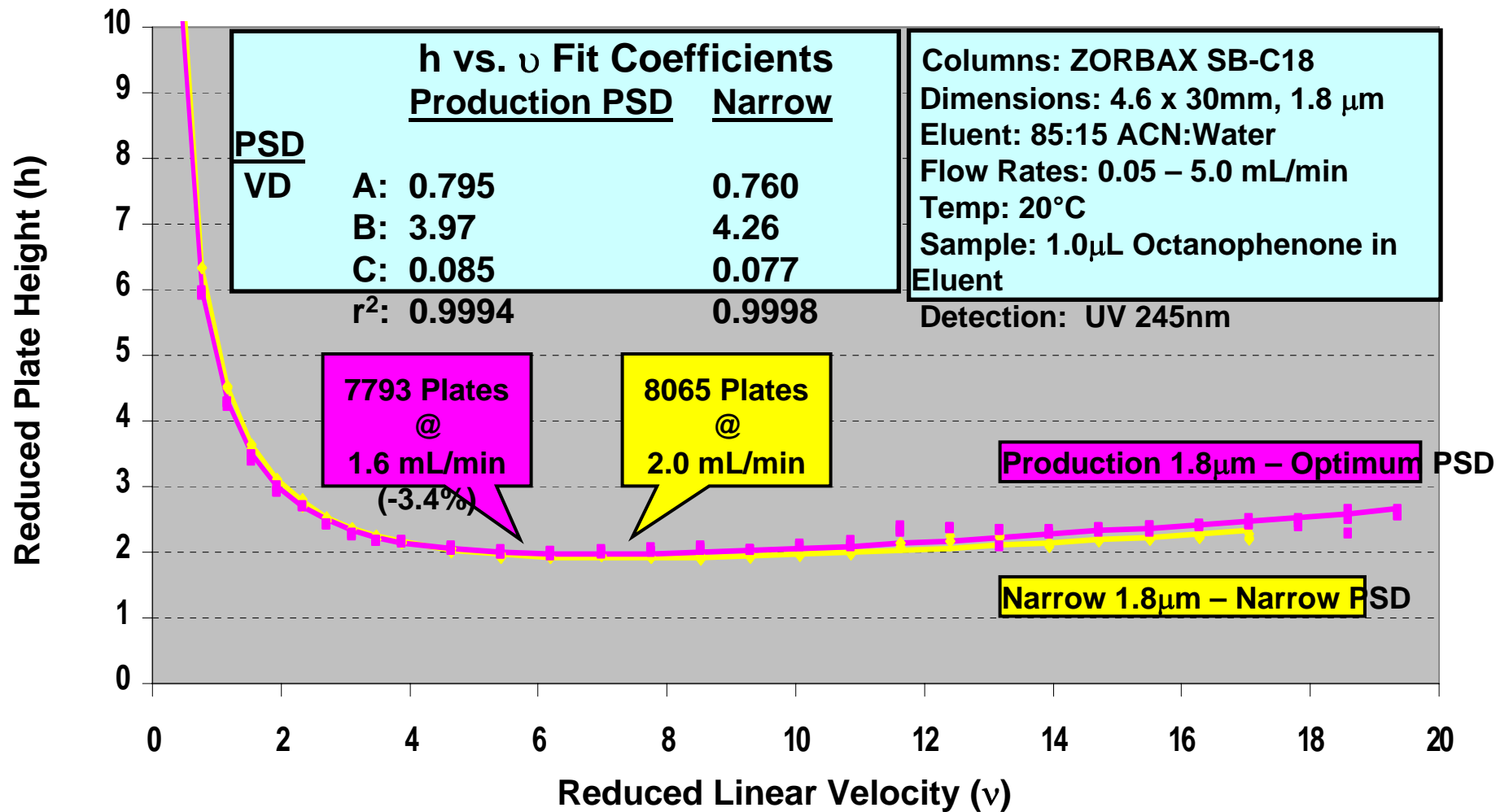
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Zorbax 1.8u RRHT Columns:

Comparison of Typical Particle Size Distribution (PSD) and Engineered PSD

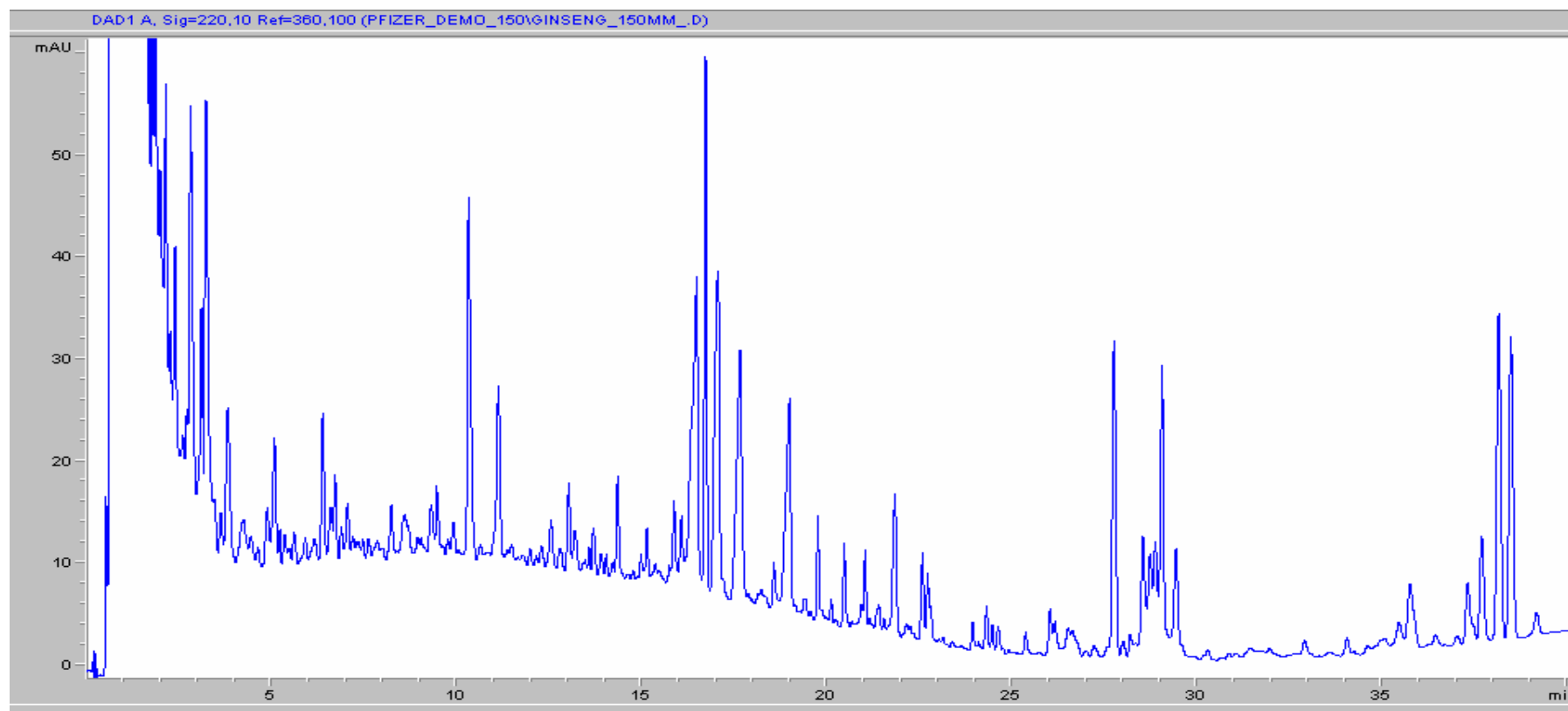


Van Deemter Curves for Narrow vs. Production Particle Size Distribution 1.8 μ m SB-C18



Rapid Resolution System – Resolution

Increased Peak Capacity for Complex Samples



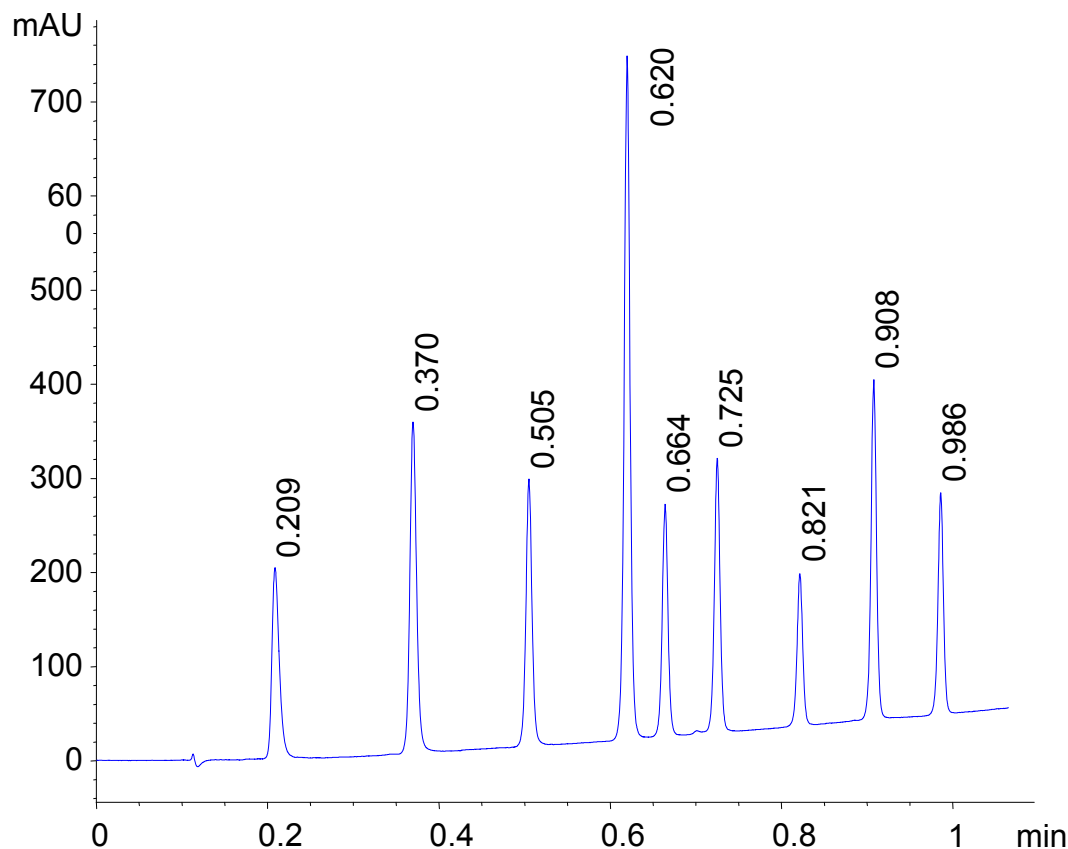
- Sample: Ginseng Extract
- 60 min Gradient
- **2.1 x 150mm, SB-C18 RRHT**

- **Peak Width ~ 6 sec**
- **Peak Capacity: ~ 600**



NEW 1200 Rapid Resolution LC System:

Speed - Better Performance at *Significantly* Lower Pressure

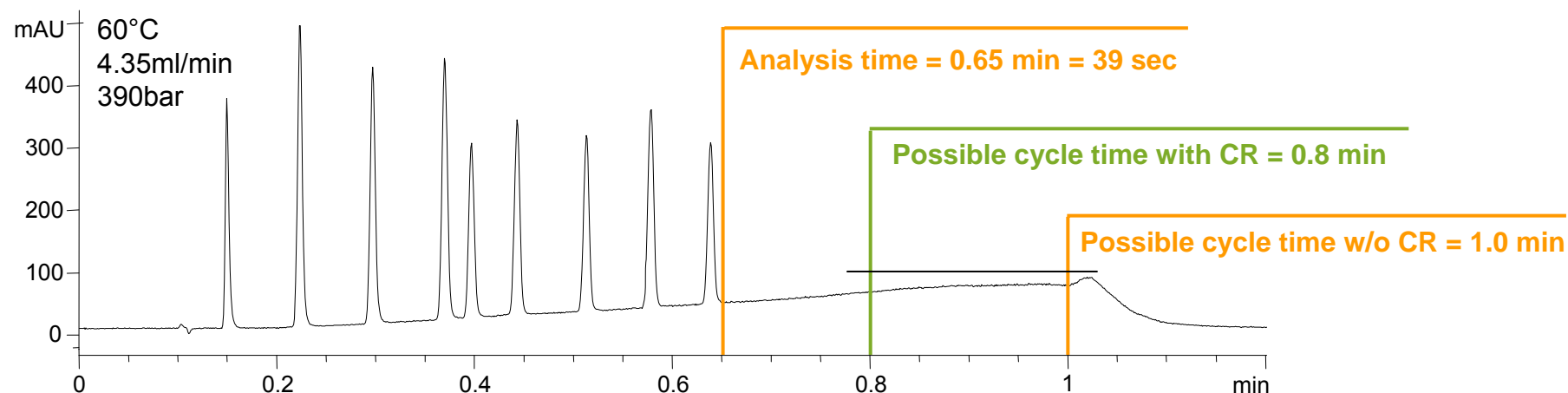


	Rapid Resolution
Anal. Time	0.949 min
Resolution	3.69
Precision	0.025 – 0.094 %RSD
Pressure	440 bar

Sample: Phenones Test Mix
Columns: SB-C18, 2.1x50
Flow Rate: 1ml/min
Gradient: 35-95% ACN in 0.9min
Temperature: 50°C
Injection volume: 1µl
Injection technique*: ADVR, OI, MCO
Wavelength: 245nm
Data rate: 80Hz

Ultra-fast Gradient Analysis of 9 Phenones

Optimizing Cycle Time



Analysis speed 9 Phenones baseline separated in 29 sec

Analysis time 39 sec

Cycle time 0.8 min (with column regeneration and run time = 0.7min)

1.0 min (without column regeneration and run time = 0.7min)

Resolution > 2.65 for all peaks

4 σ peakwidth 0.50 sec (average)

Peak capacity 79 (0.65min gradient)

RT precision < 0.2% RSD without column regeneration

< 1.0% RSD with column regeneration

Carry over < 0.003% (limit of detection)

Robustness 7.2 days or 8000 injections of continuous operation with stable performance

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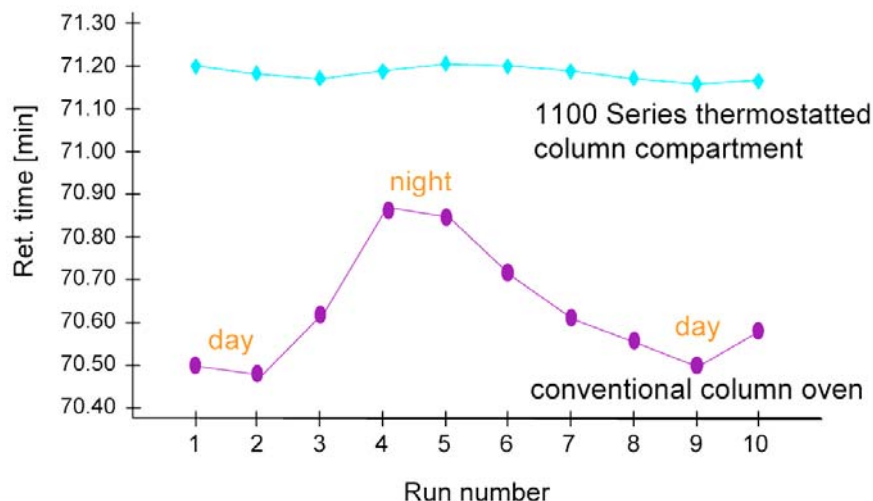
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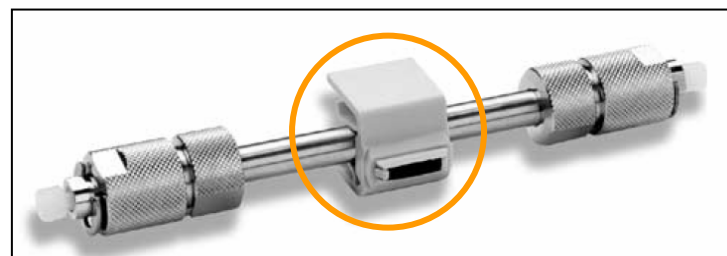
New Agilent 1200 Thermostatted Column Compartment SL Inherits the performance of the 1100 TCC



Peltier-controlled column thermostating

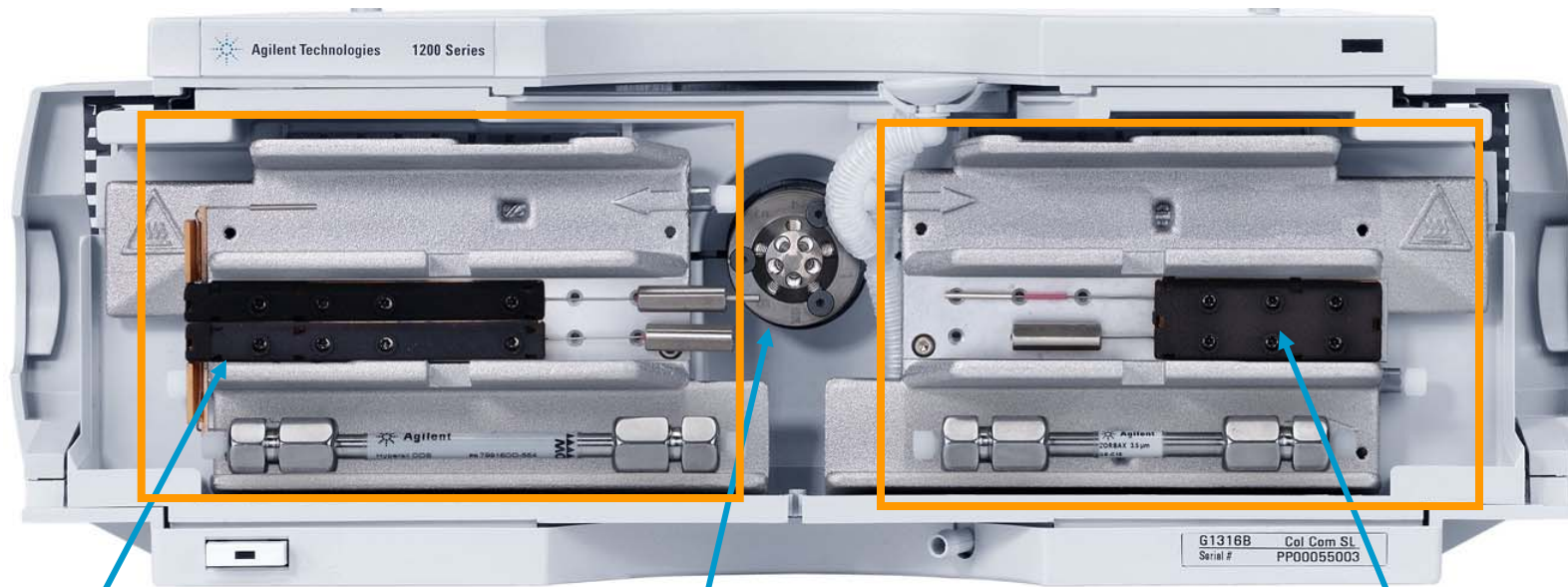


- **Ultra-fast high-temperature analysis up to 100°C**
- **Temperature stability of +/-0.15 °C for robust 24x7 operation**
- **Reliable sub-ambient method development down to RT – 10°C**
- **Integrated column switching or alternating column regeneration valves**
- **Wireless column ID module for tracking of injections**
- **Unmatched capacity in a stacked unit**



Agilent 1200 Thermostatted Column Compartment SL

Takes advantage of the CAN network and modular intelligence



Pre-Column heating

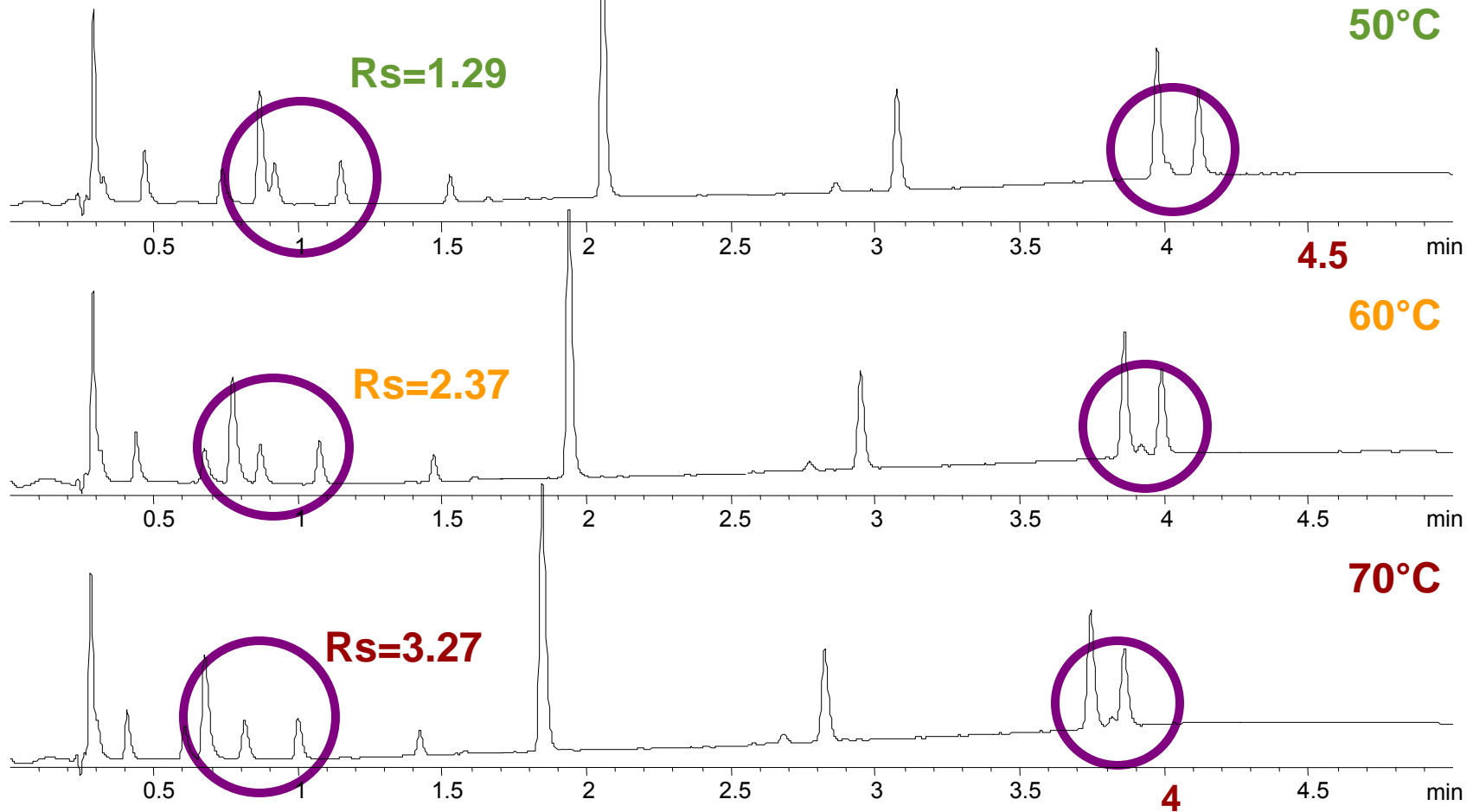
Integrated Column Selection or Column Regeneration Valve

Post-Column heating/cooling with DAD temperature tracking

- ✓ New low dispersion heat exchangers for precolumn heating and post-column cooling
- ✓ Temperature control up to 100° C
 - to reduce pressure in rapid separations
 - to support high temp. applications (certain sugars, polymer separ. with viscous eluents)
- ✓ Door w/ improved insulation
- ✓ 600 bar support for built-in micro valves: 2 pos/6 port for column switching, 2 pos/10 port for automated column regeneration for RR HTPost-column cooling) and improved peltier temperature control ($< \pm 0.05^{\circ}\text{C}$) for minimized UV noise under most demanding conditions ($< 50\mu\text{AU}$ at high flow rates and temperatures)
- ✓ Improved peltier temperature control ($< \pm 0.05^{\circ}\text{C}$) for min. UV noise under most demanding conditions ($< 50\mu\text{AU}$ at high flow rates and temperatures)
- ✓ Post-column cooling

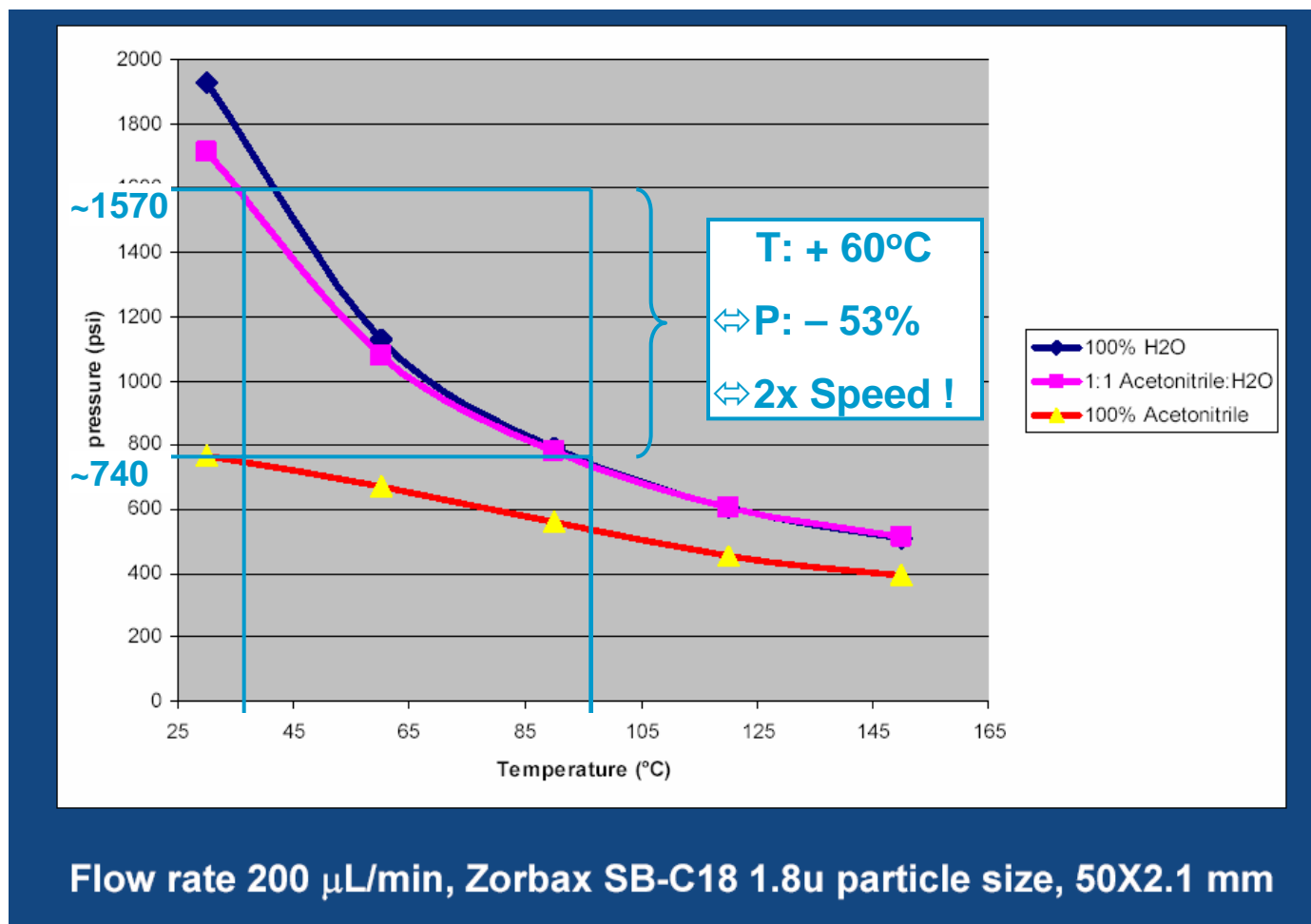
Zorbax Stablebond C18 1.8u particle RRHT Column: High Temperature Optimizes Resolution and Selectivity

Gradient of Ten Cardiac Drugs on SB-C18 RRHT, at low pH



RRLC using Temperature

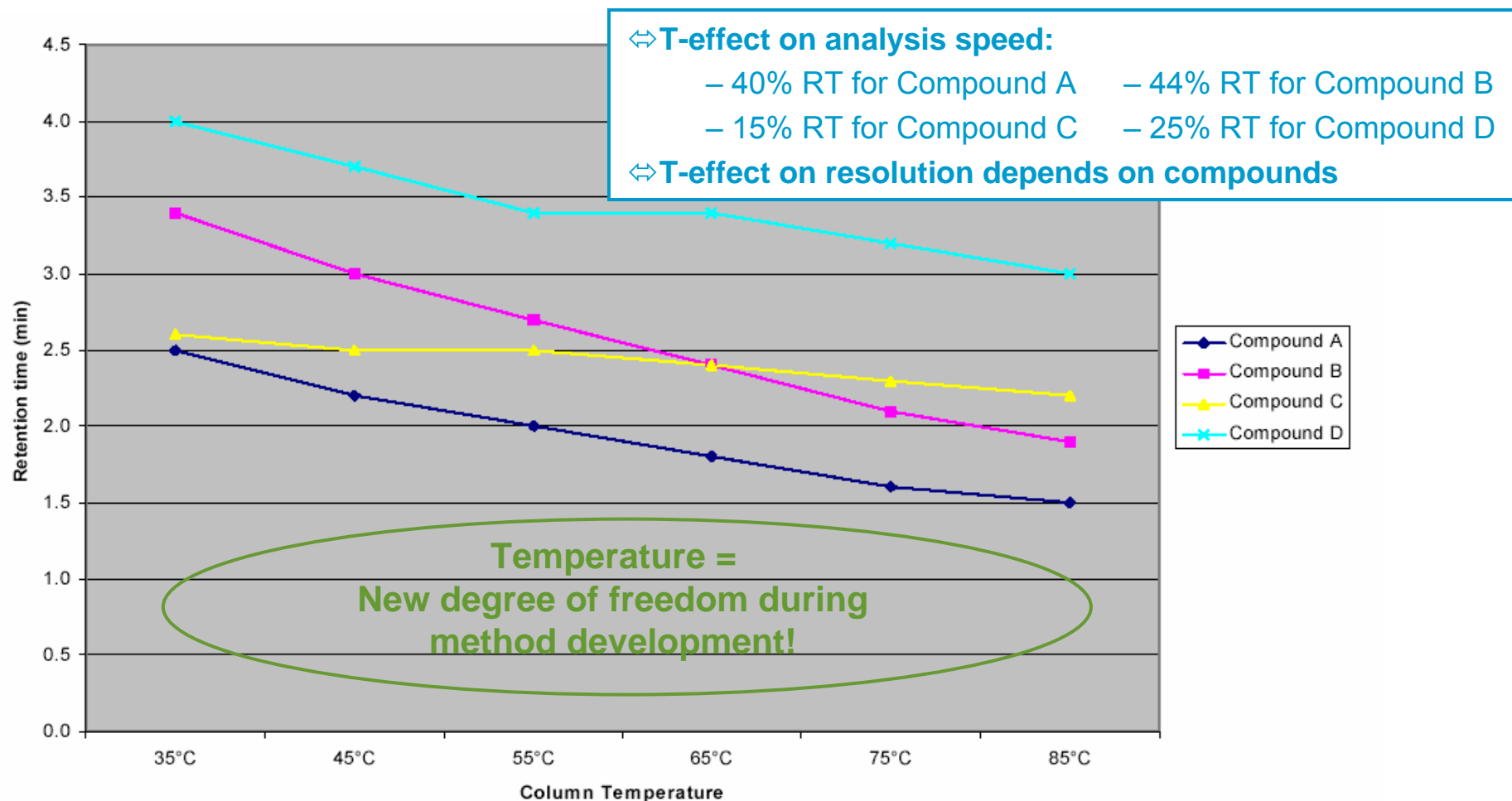
Effect of Temperature on Column Pressure



High Temperature LC-MS/MS (HTLC-MS/MS) for High Throughput Bioanalysis
Daniel Tang, PhD, PDM, Michigan Laboratories, PGRD, Pfizer Pharmaceuticals

RRLC using temperature

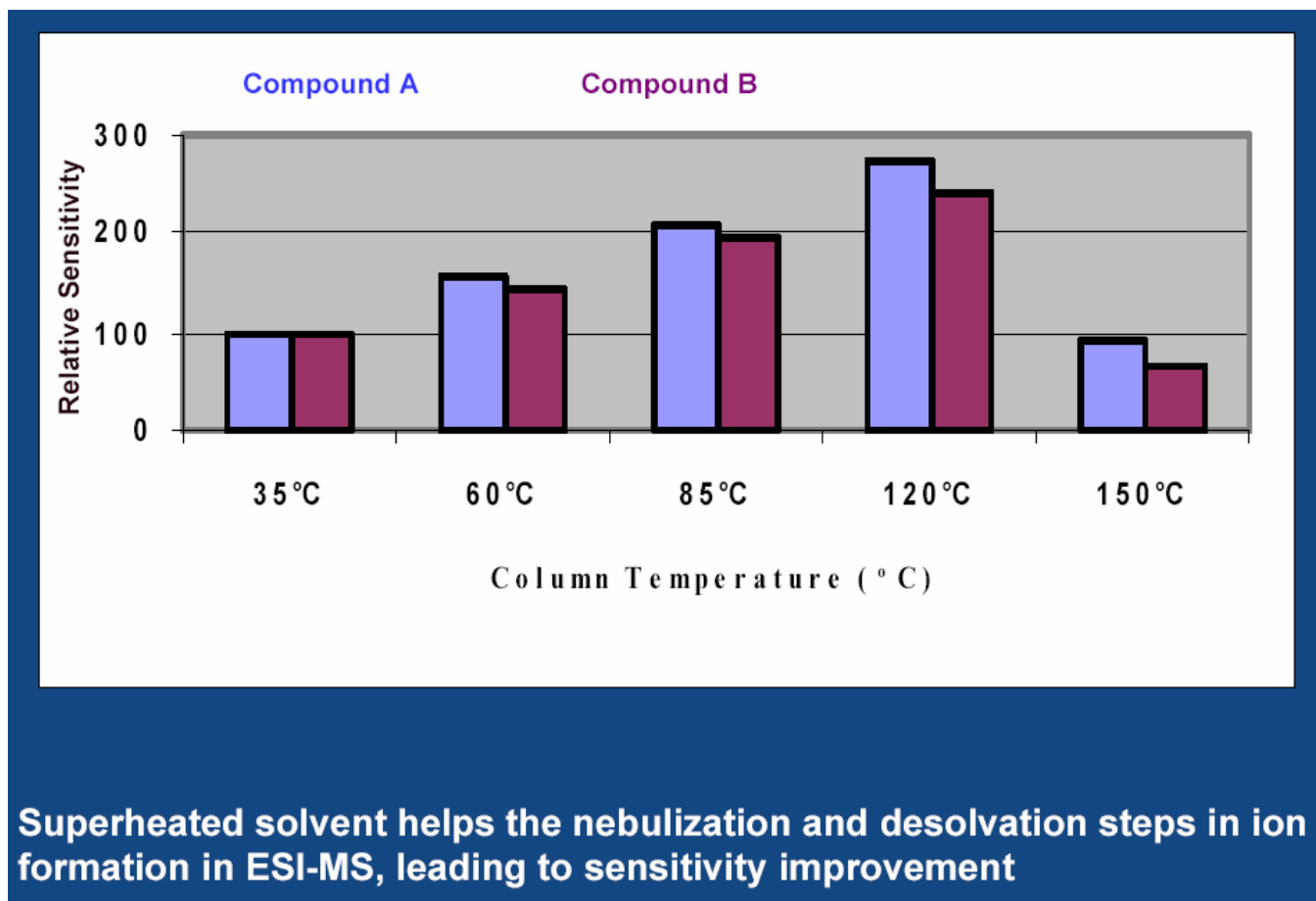
Temperature Effect on RT and Resolution



High Temperature LC-MS/MS (HTLC-MS/MS) for High Throughput Bioanalysis
Daniel Tang, PhD, PDM, Michigan Laboratories, PGRD, Pfizer Pharmaceuticals

Post-Column Heating

Temperature Effect on LC/MS Sensitivity



High Temperature LC-MS/MS (HTLC-MS/MS) for High Throughput Bioanalysis
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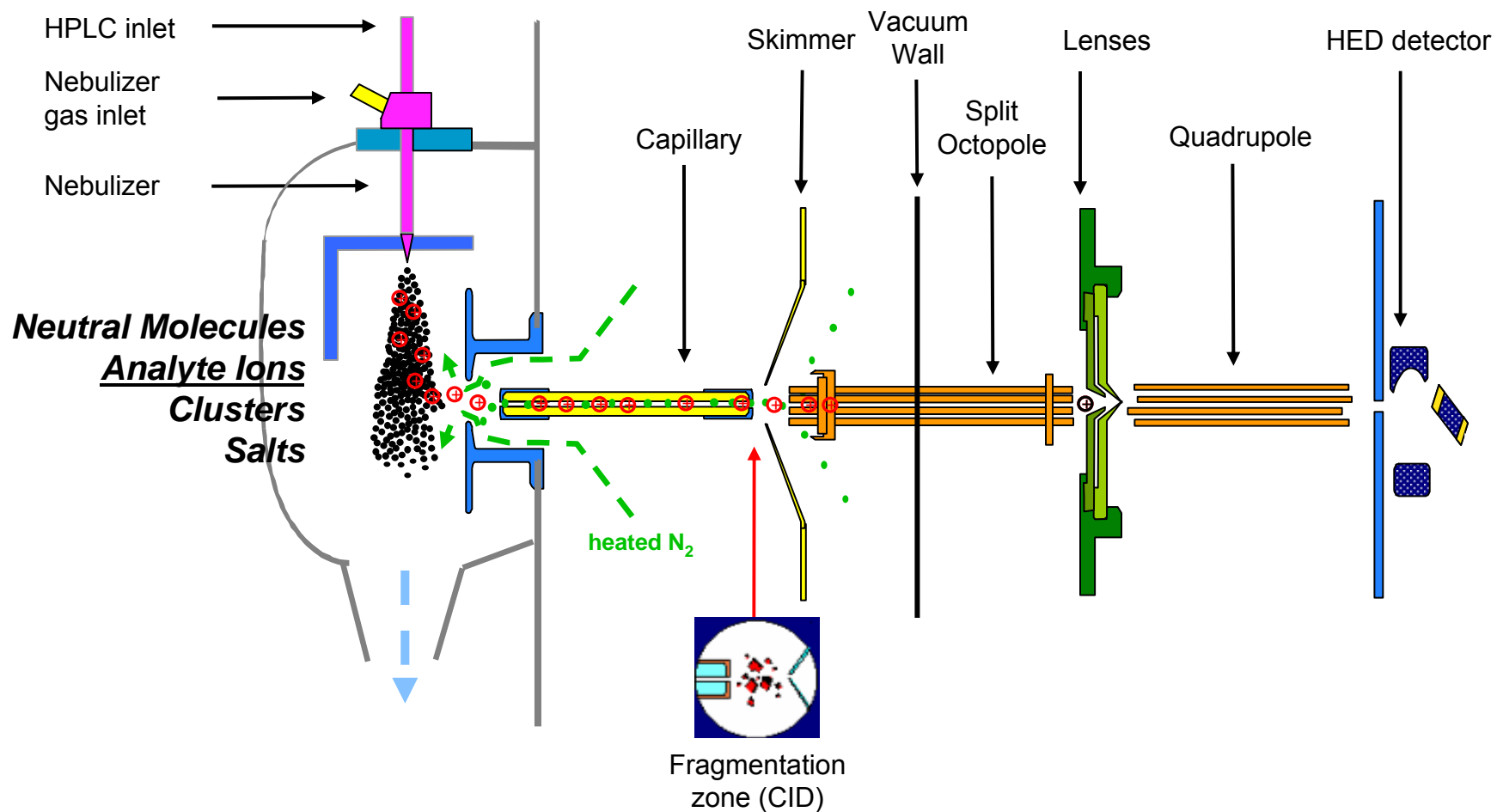
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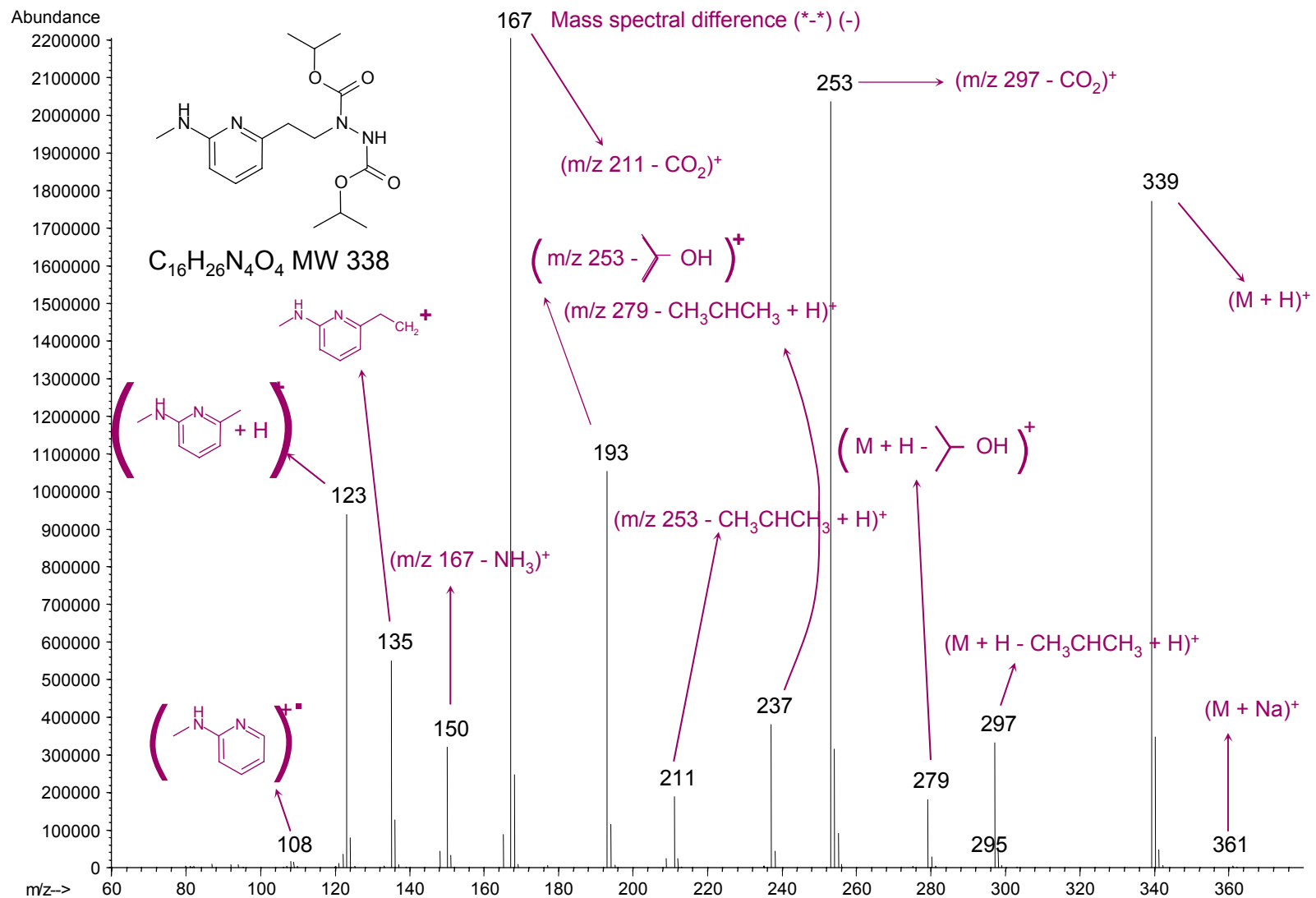
Agilent 6100 Series: Core Technology

AP Electrospray



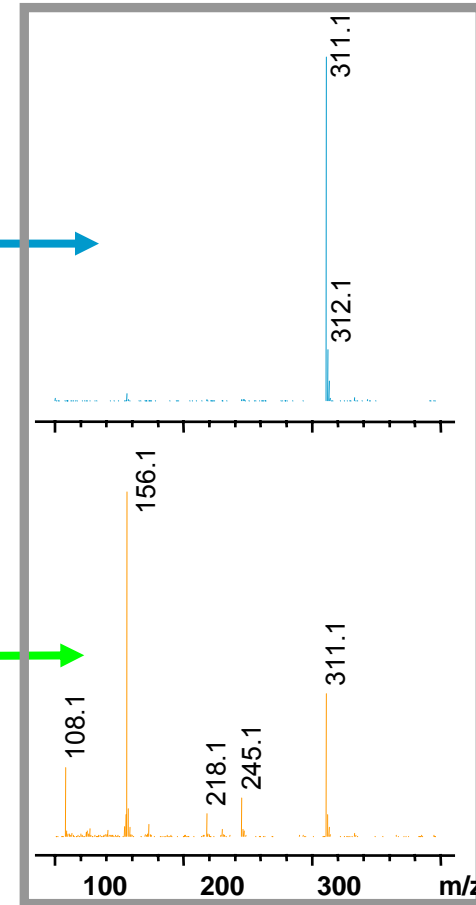
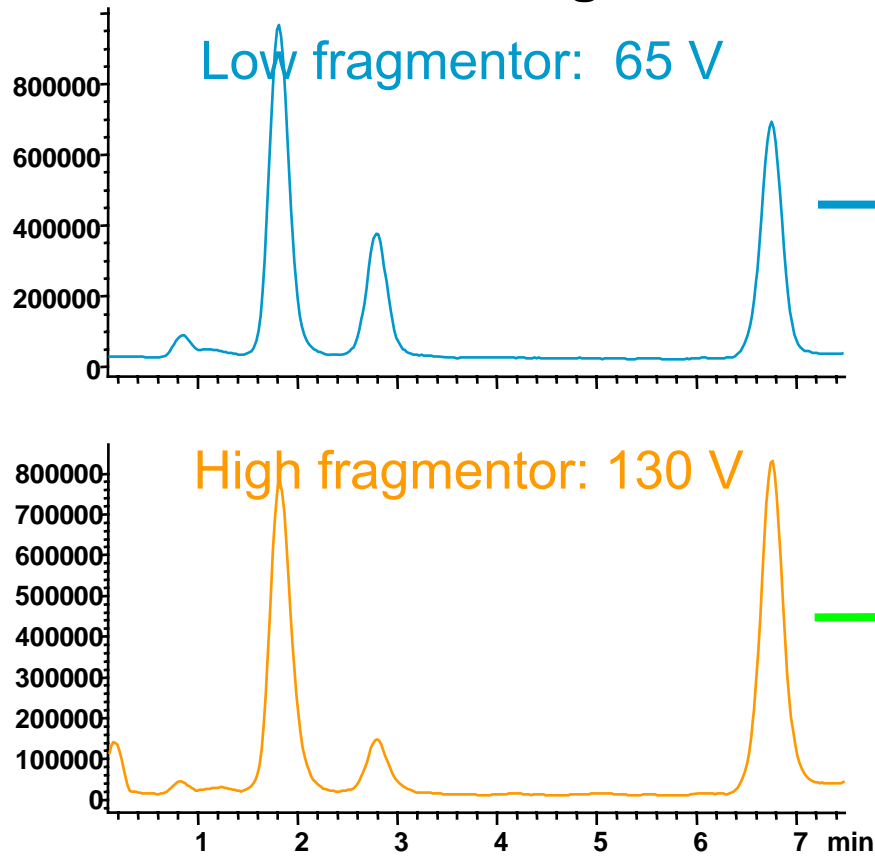
LC/MSD LC/MS Spectrum

Courtesy: Paul Cummings, GSK, 1998



Acquire Multiple MS Signals: High/Low Fragmentor

Sulfa drugs



Scanning Modes for the 6100: Ion Energy



All models use a log amp collecting data every 20 μsec (50000 samples/second)

Scan Type	Step (amu)	Samples	Ion Energy (eV)	Quad Freq. (MHz)	Scan Speed
Normal (All Models)	0.1	2	5	1.0	2500u/sec
Fast Scan (6130)	0.1	1	10	1.0	5200u/sec (using data interpolation)
Ultra Fast Scan (6140)	0.2	1	15	1.4	10,000u/sec

Standard mode uses 5eV ion energy

65 μsec for ion at **m/z 100** to pass through quadrupole

200 μsec for ion at **m/z 1000** to pass through quadrupole

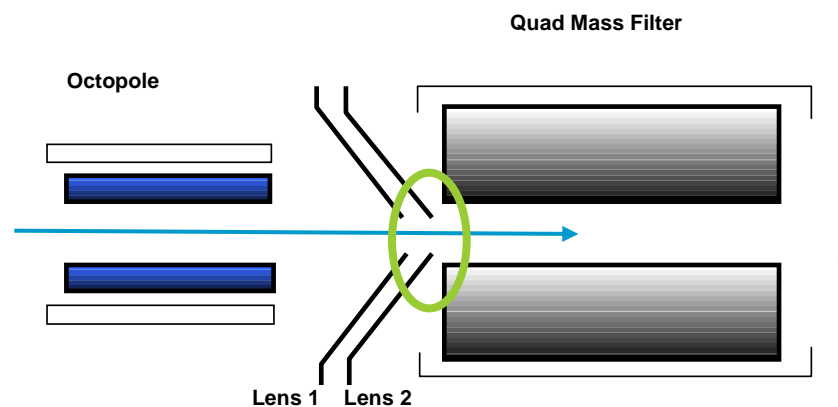
At *faster* scan speeds, quadrupole has moved to different optimum mass as ion passes through (1 amu for m/z 1000) \rightarrow *reduced transmission (lower sensitivity)*

Pushing faster (e.g. 10 eV) results in fewer quadrupole cycles \rightarrow *less filtering, poorer resolution*

Lens2RF – A Patented Agilent Solution



Issue: As m/z value goes up, amplitude of quadrupole RF increases, more difficult to inject ions into the quadrupole and ion transmission drops.



Solution: ramping the DC and RF amplitudes with mass on lens 2 and offsetting the phase relative to the quadrupole, ion transmission into the quadrupole increases.

Result: Ion transmission increased by ~3X at m/z 600 and up to 10X at m/z 3000

Implemented on the 6130 and 6140 models

TIC Chromatogram: Customer Sample #48

Chromatographic parameters:

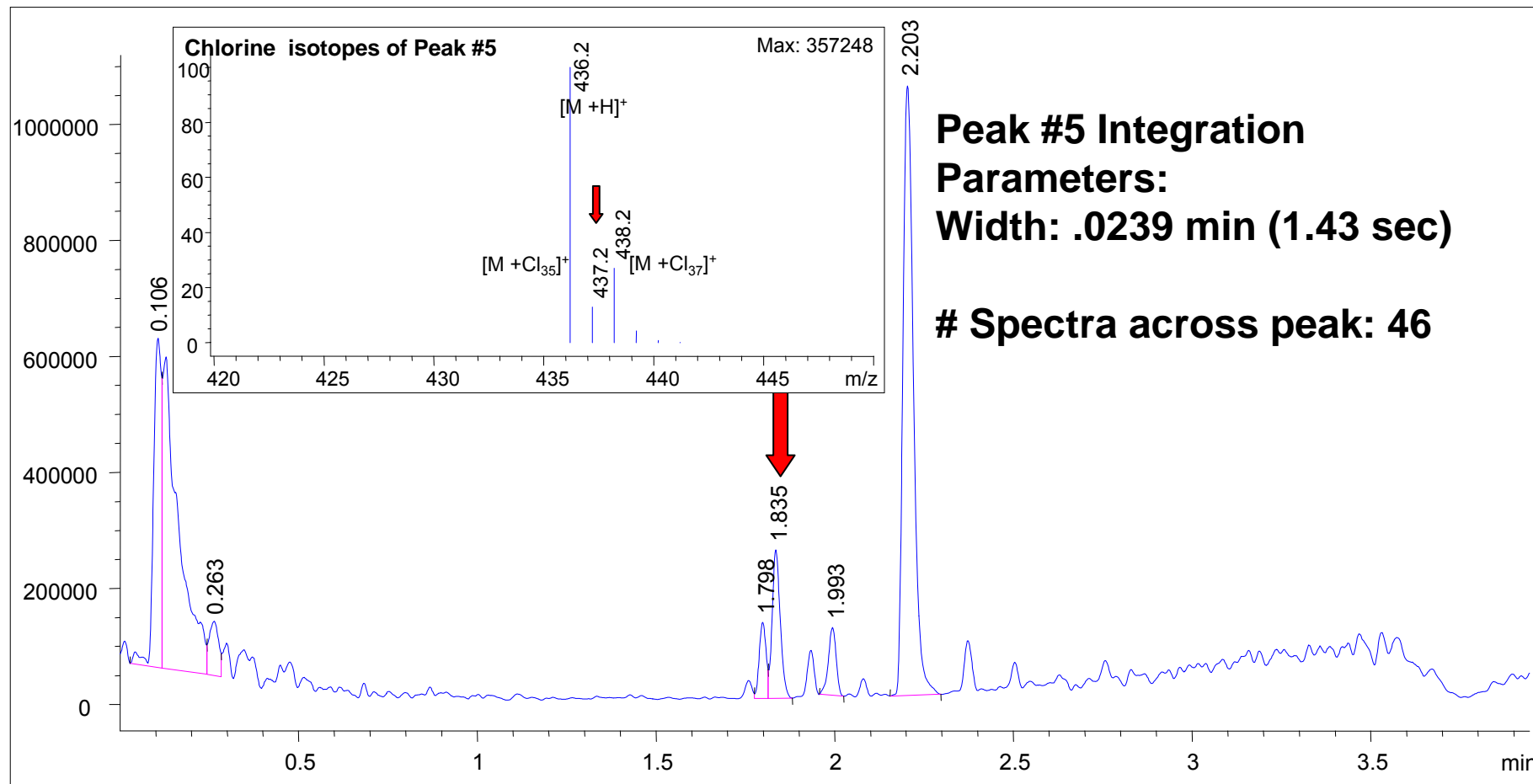
Column: Zorbax SB C-18 RR 2.1x50mm

Gradient: 5% ACN to 95% over 3 min at 1.0 ml/min.

MS Acquisition parameters:

Scan range: m/z 100 to 900

Cycle time: 0.085 sec. (~9400 u/sec)

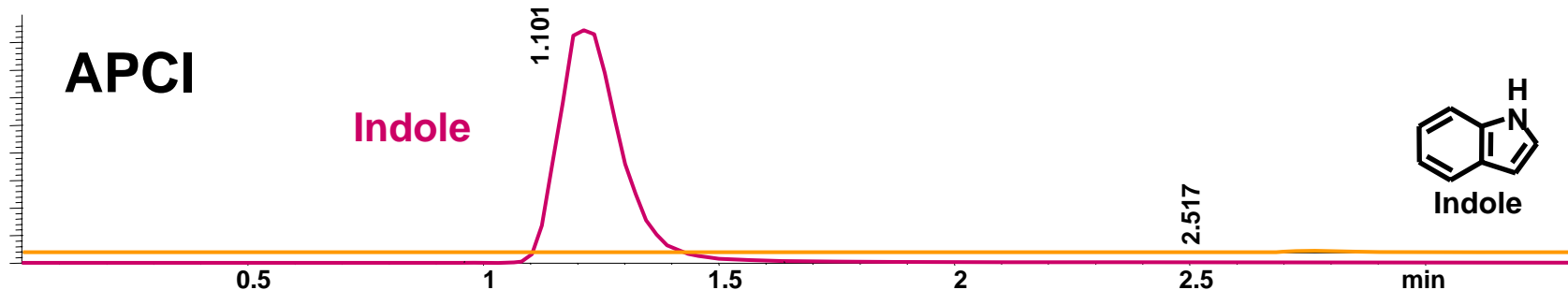
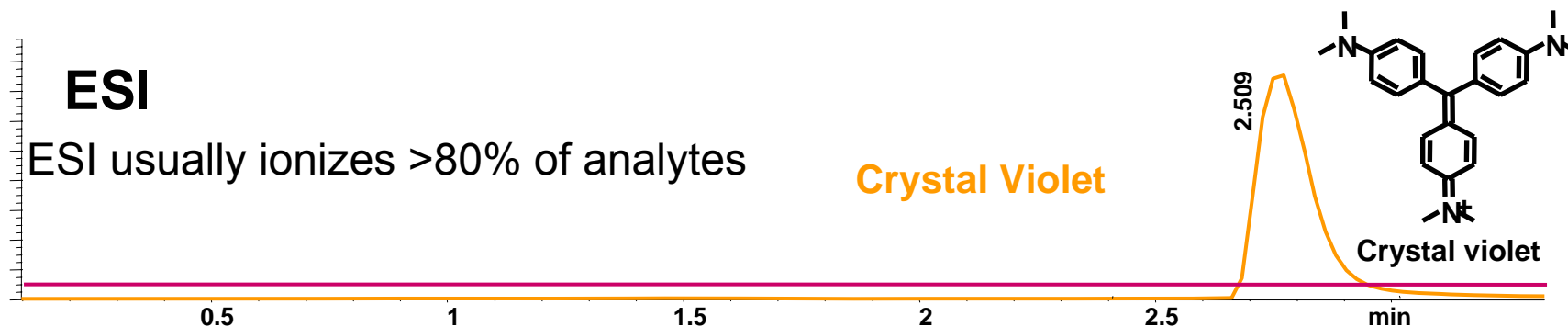


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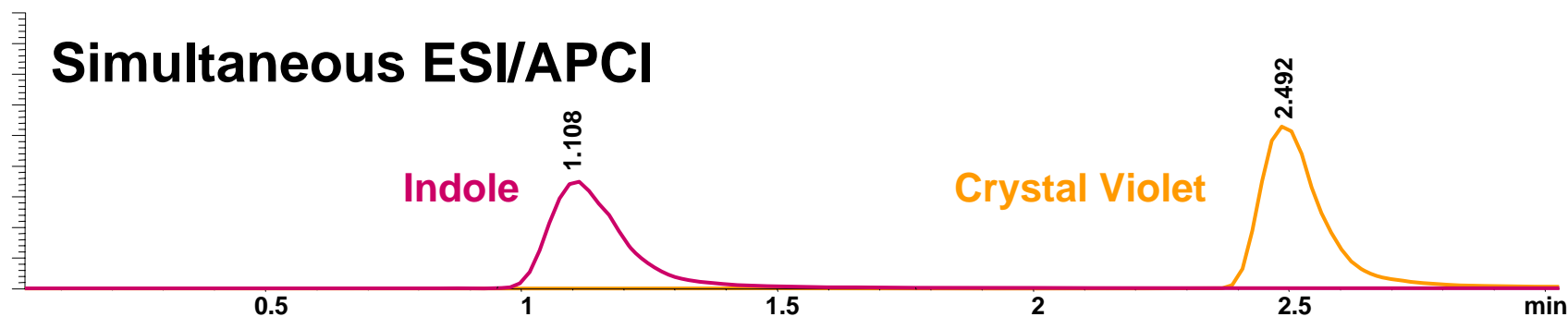
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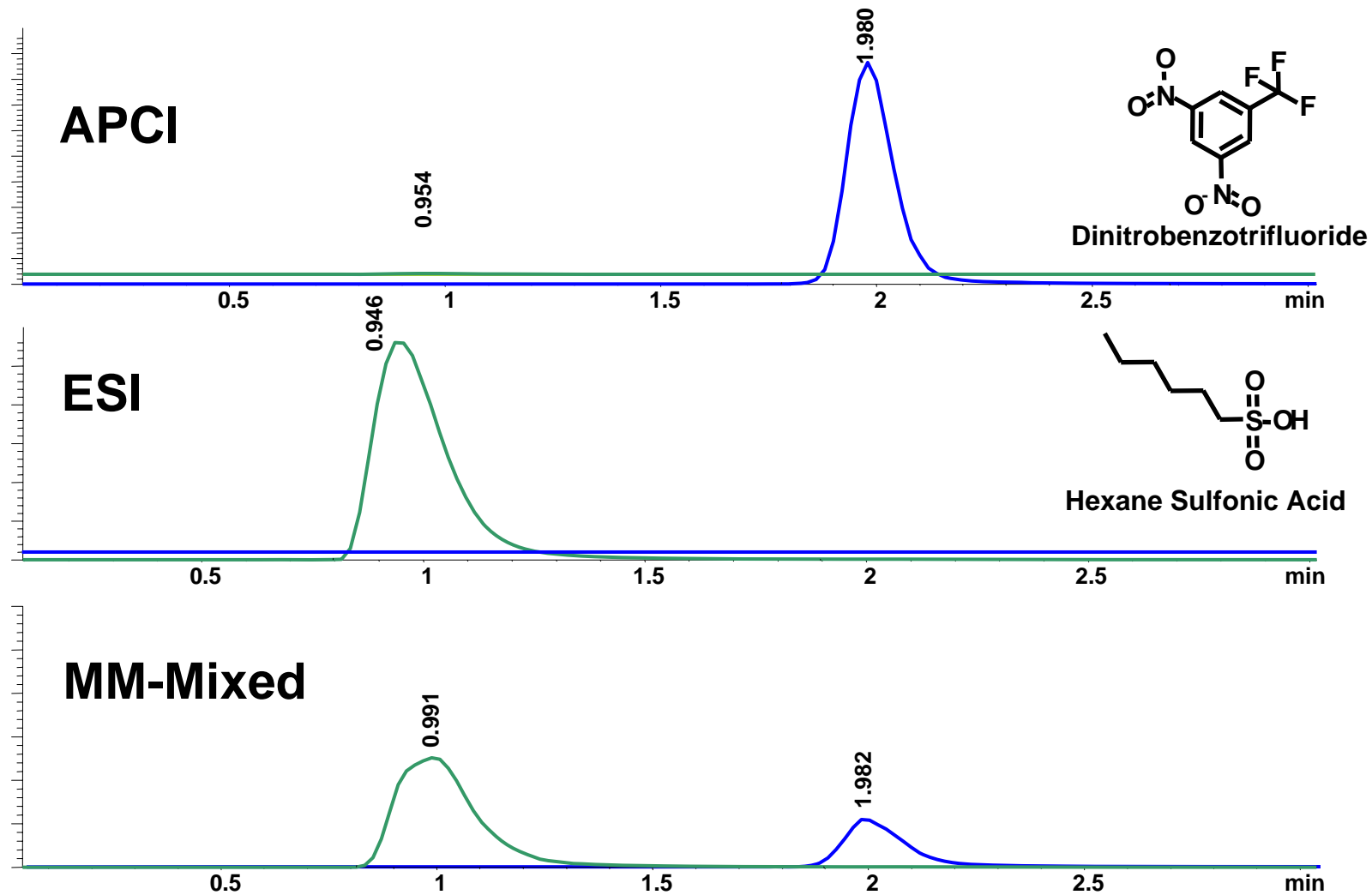
Studying mixtures and unknowns: ESI or APCI?



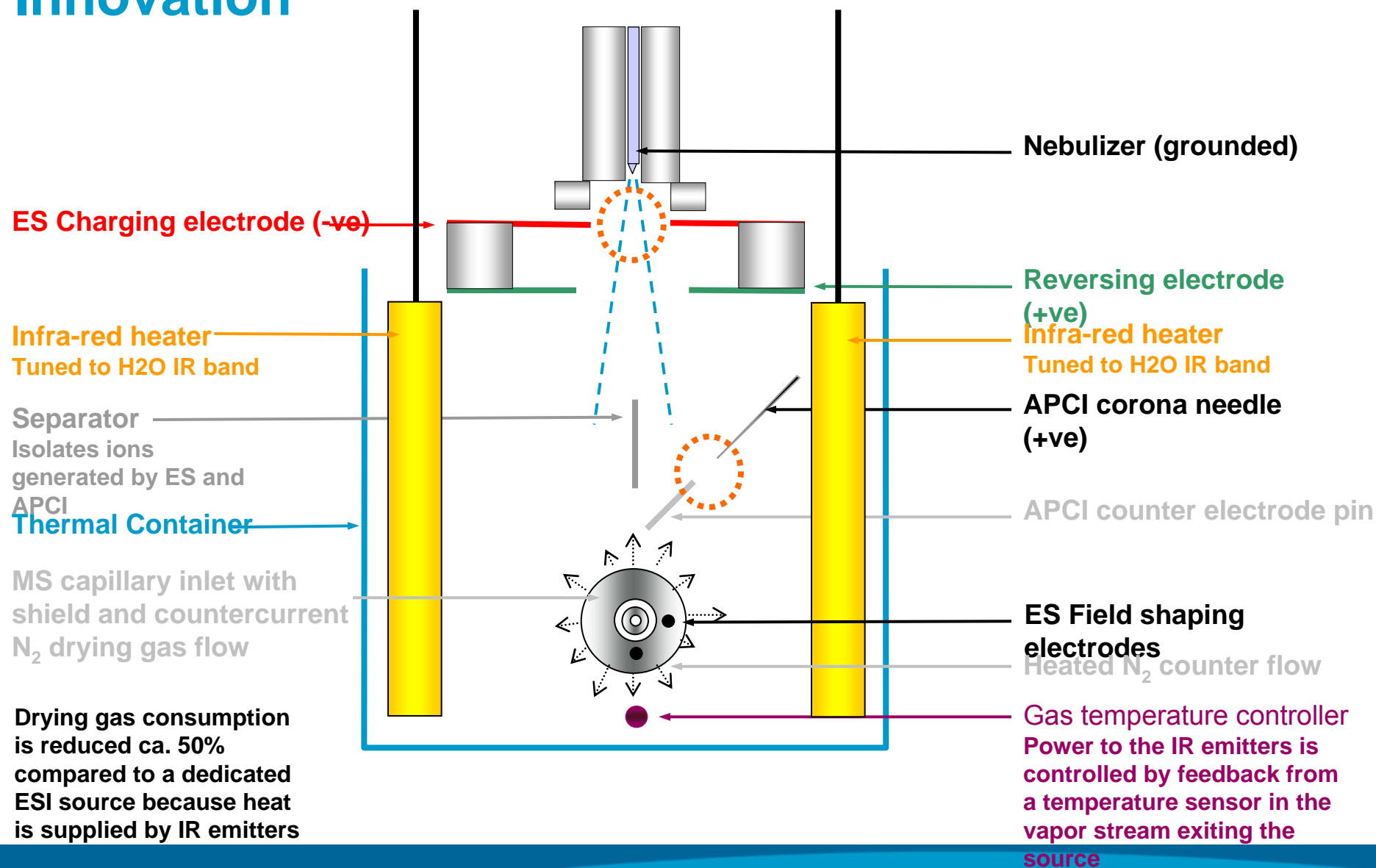
Almost all compounds ionize by ESI or APCI



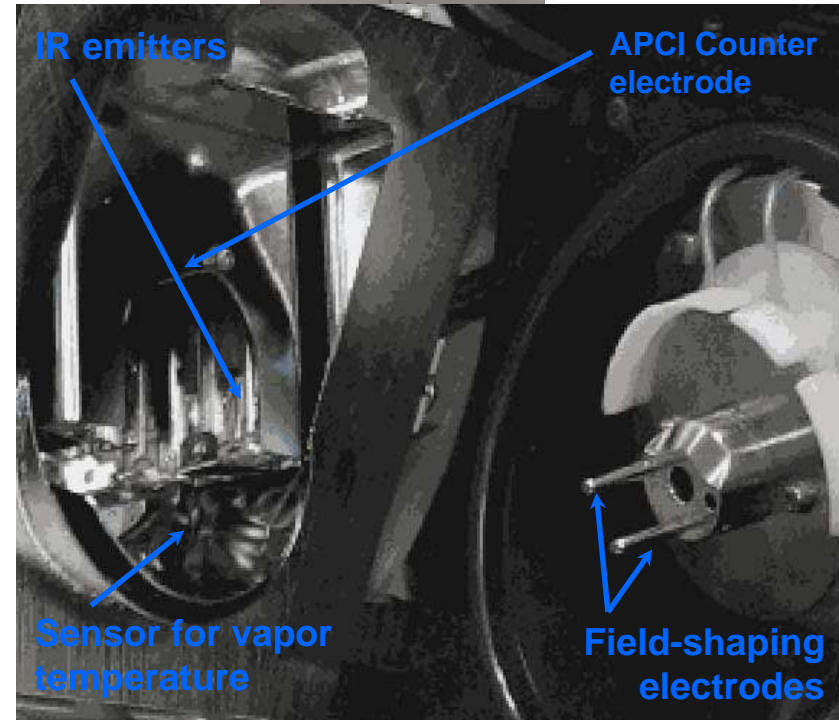
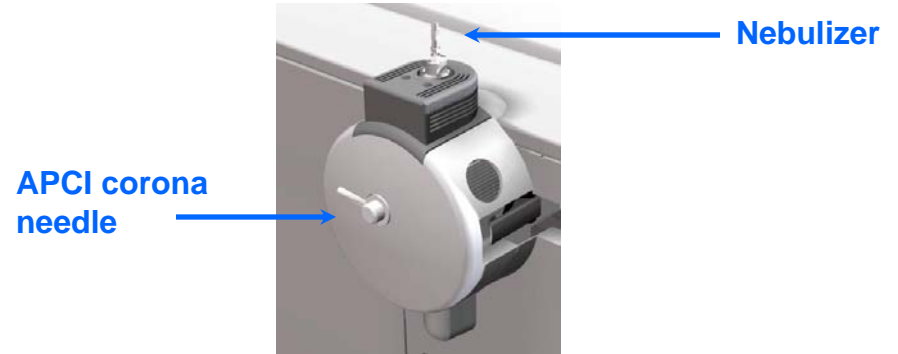
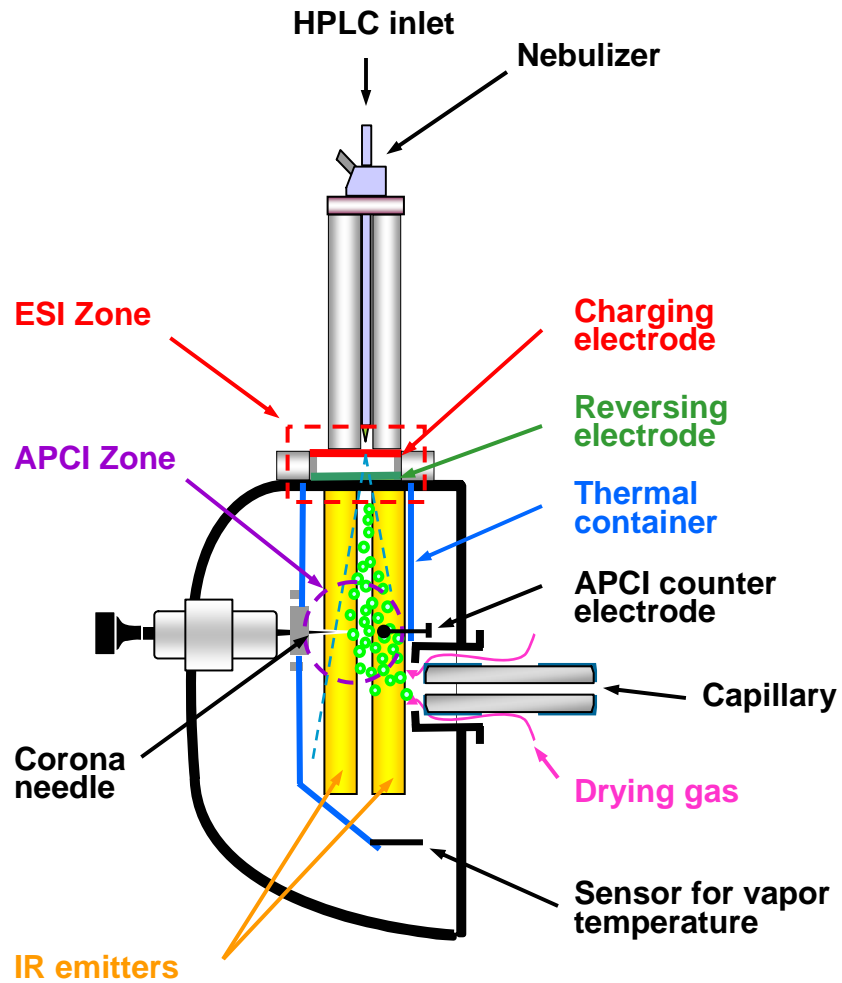
Source Comparison - Negative Ion Mode



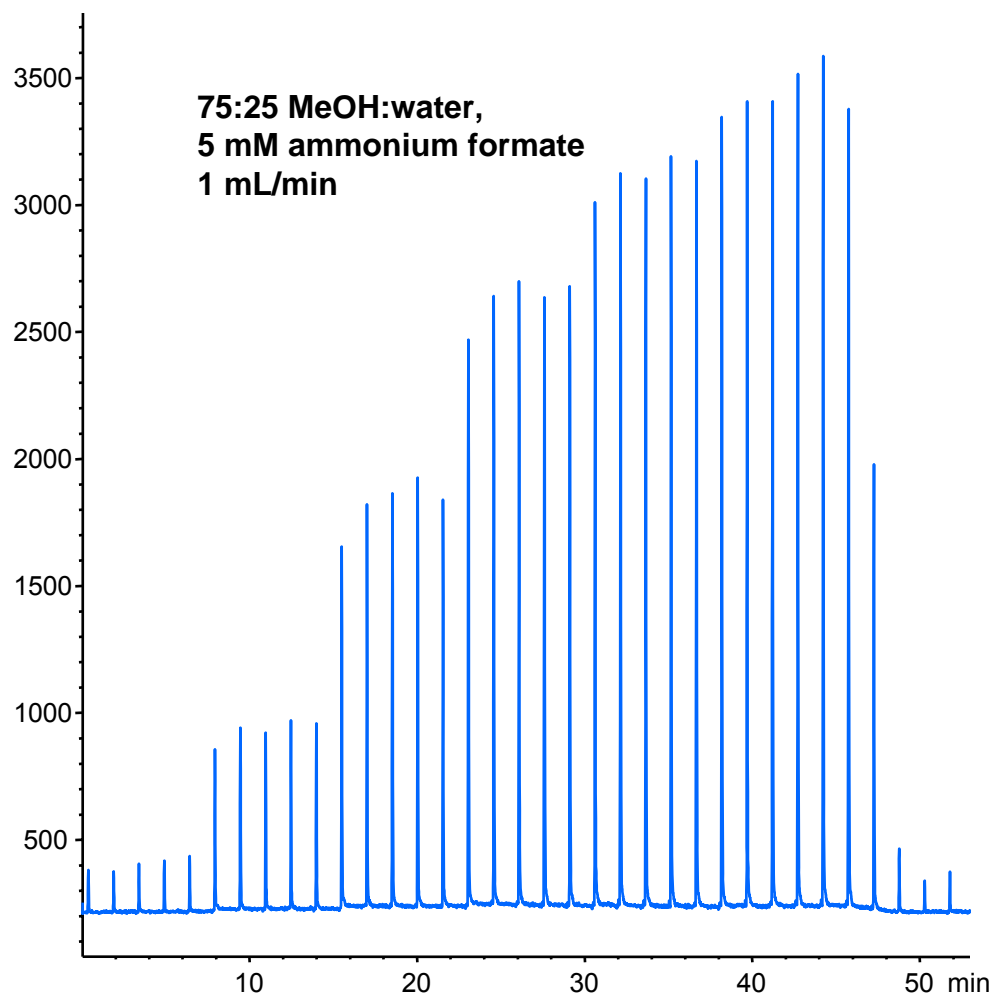
The Agilent Multi-Mode Source Design: Innovation



Technology: Overview of the Multimode Source



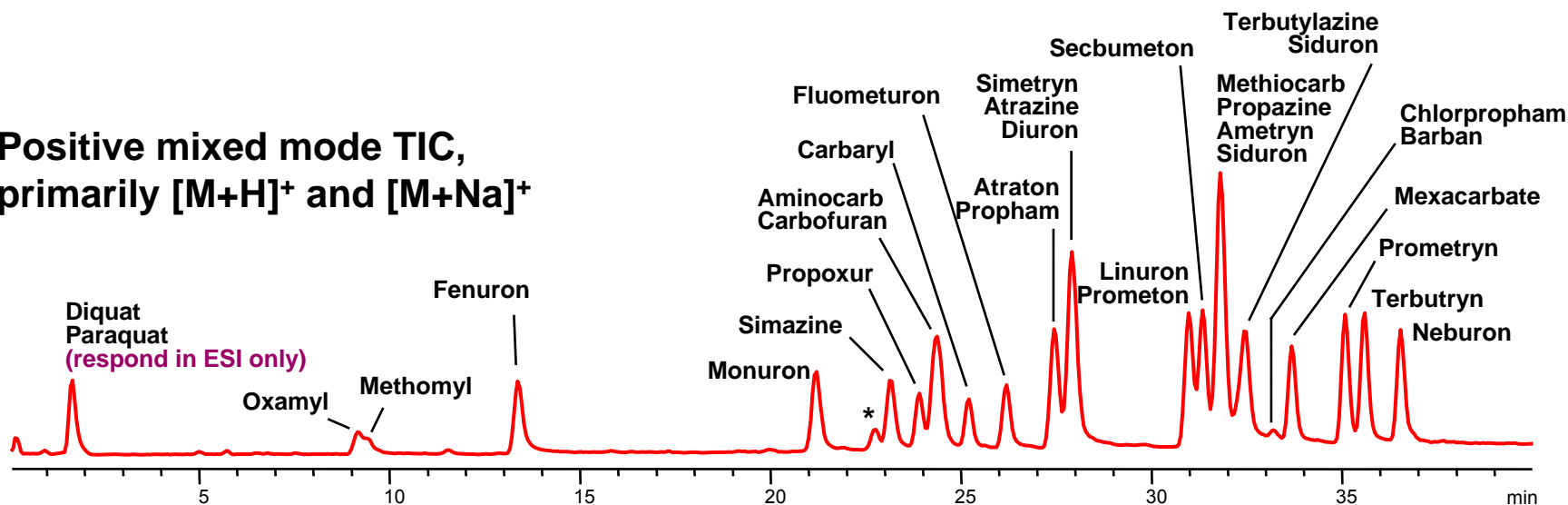
Vaporization Using Infrared Emitters: Optimal APCI Conditions for Reserpine (10 pg)



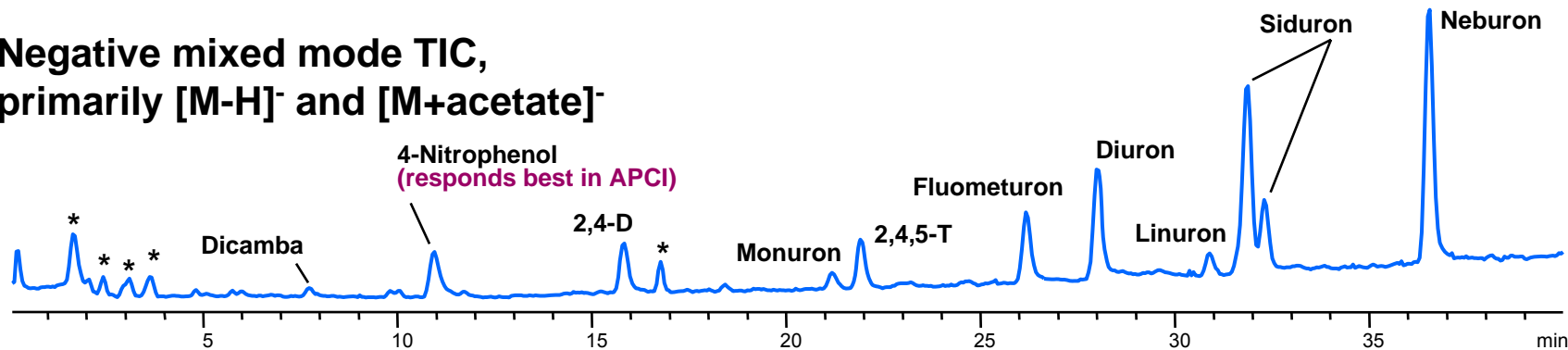
Vaporizer temperature	Relative peak area	Rel. std dev. 4 replicates
250 °C	16.8	2.0%
225 °C	15.1	1.7%
200 °C	12.7	2.1%
175 °C	8.3	2.9%
150 °C	3.7	1.5%
125 °C	1	13.4%

Environmental Compounds, Alternating Positive/Negative Mixed Mode Ionization

Positive mixed mode TIC,
primarily $[M+H]^+$ and $[M+Na]^+$



Negative mixed mode TIC,
primarily $[M-H]^-$ and $[M+acetate]^-$



5 ng each analyte; 2.1 x 150 mm Zorbax XDB-C18, 3.5 μ , 60 $^{\circ}$ C, water/methanol gradient (3-90% methanol) with 1mM ammonium acetate, scan mode m/z 130-330; sample dissolved in 80:20 water/methanol containing 1% acetic acid. * denotes impurity

Compound Detection Test—Results

Compound Ion Polarity	Dedicated APCI		Dedicated ESI		Simultaneous	
	(+)	(-)	(+)	(-)	(+)	(-)
Acetazolamide	U	+	U	+	U	+
Butyl 4-aminobenzoate	+	U	+	U	+	U
Cortisone	+	+	+	U	+	+
Gemfibrozil	U	+	U	+	U	+
Hexahydro-...-dione	+	U	+	U	+	U
Hydroflumethiazide	U	+	U	+	U	+
Indole	+	U	U	U	+	U
Iodipamide	U	U	U	+	+	+
Labetalol	+	+	+	+	+	+
Lidocaine	+	U	+	U	+	U
Morin	+	+	+	+	+	+
Paclitaxel	U	U	+	U	+	U
Phenylbutazone	+	+	+	+	+	+
Procainamide	+	U	+	U	+	U
Progesterone	+	U	+	U	+	U
Sulfamethoxazole	+	+	+	+	+	+
Tolazamide	+	+	+	+	+	+
Uracil	U	+	U	U	U	+
Detected by Polarity	67%	56%	67%	50%	78%	61%
Detected by Source	89%		89%		100%	

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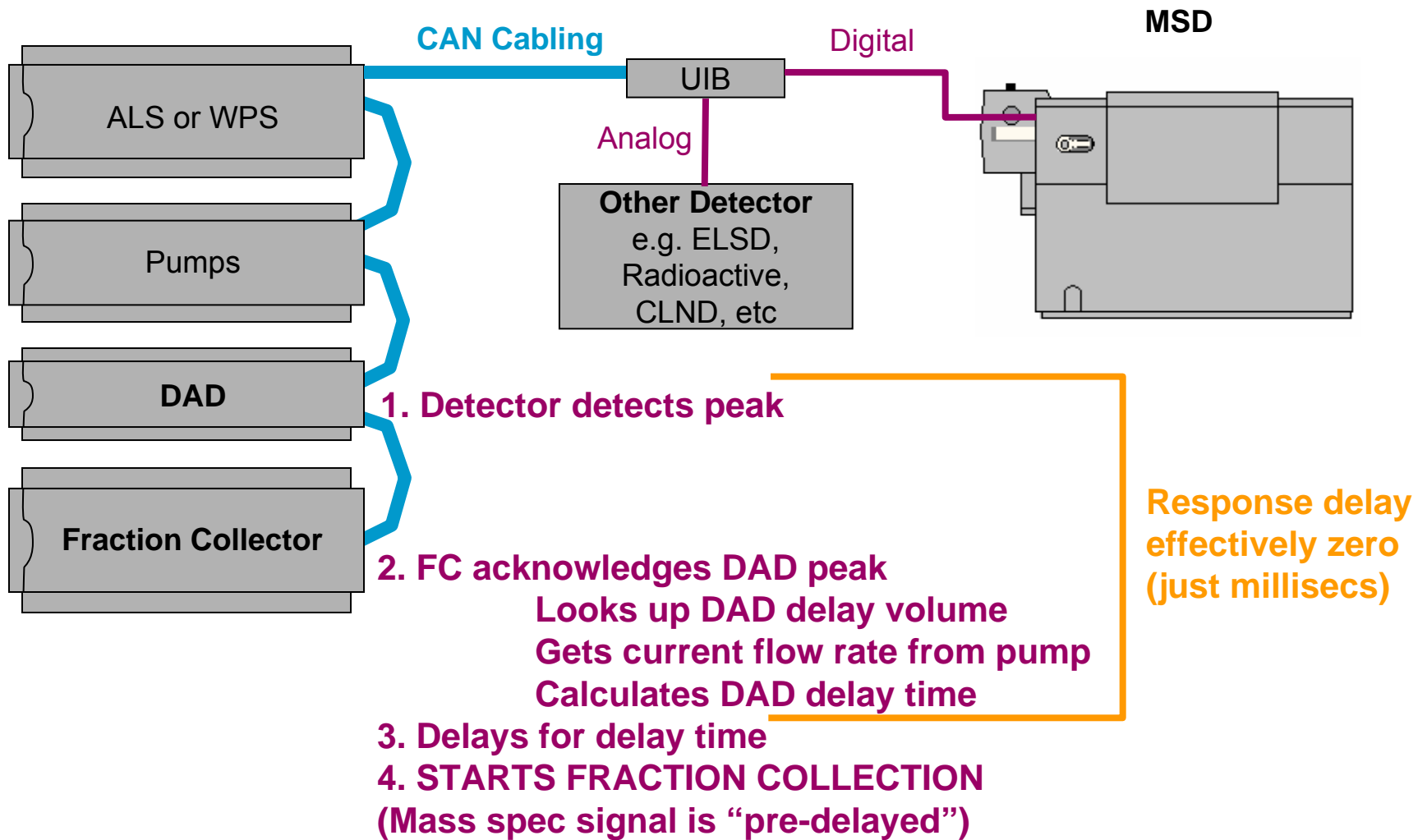
The Agilent Central Automation Network (CAN)

Modular intelligence via the CAN network

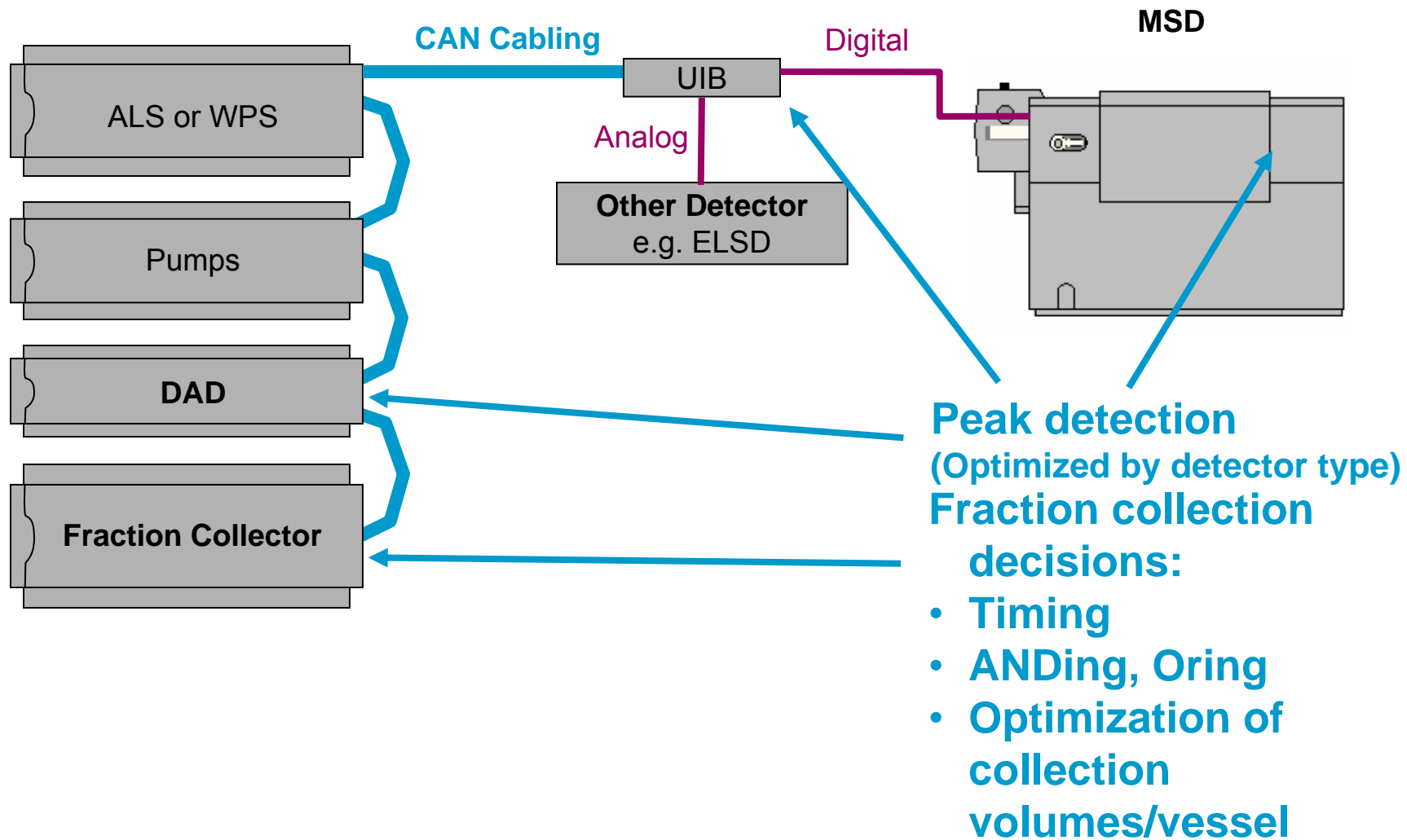
- Acq. Software Independent Diagnostics
- EMF, Early Maintenance Feedback
- Shutdown on Leak Detection
- Shutdown on Instrument error
- High precision prep
- Temperature tracking across modules



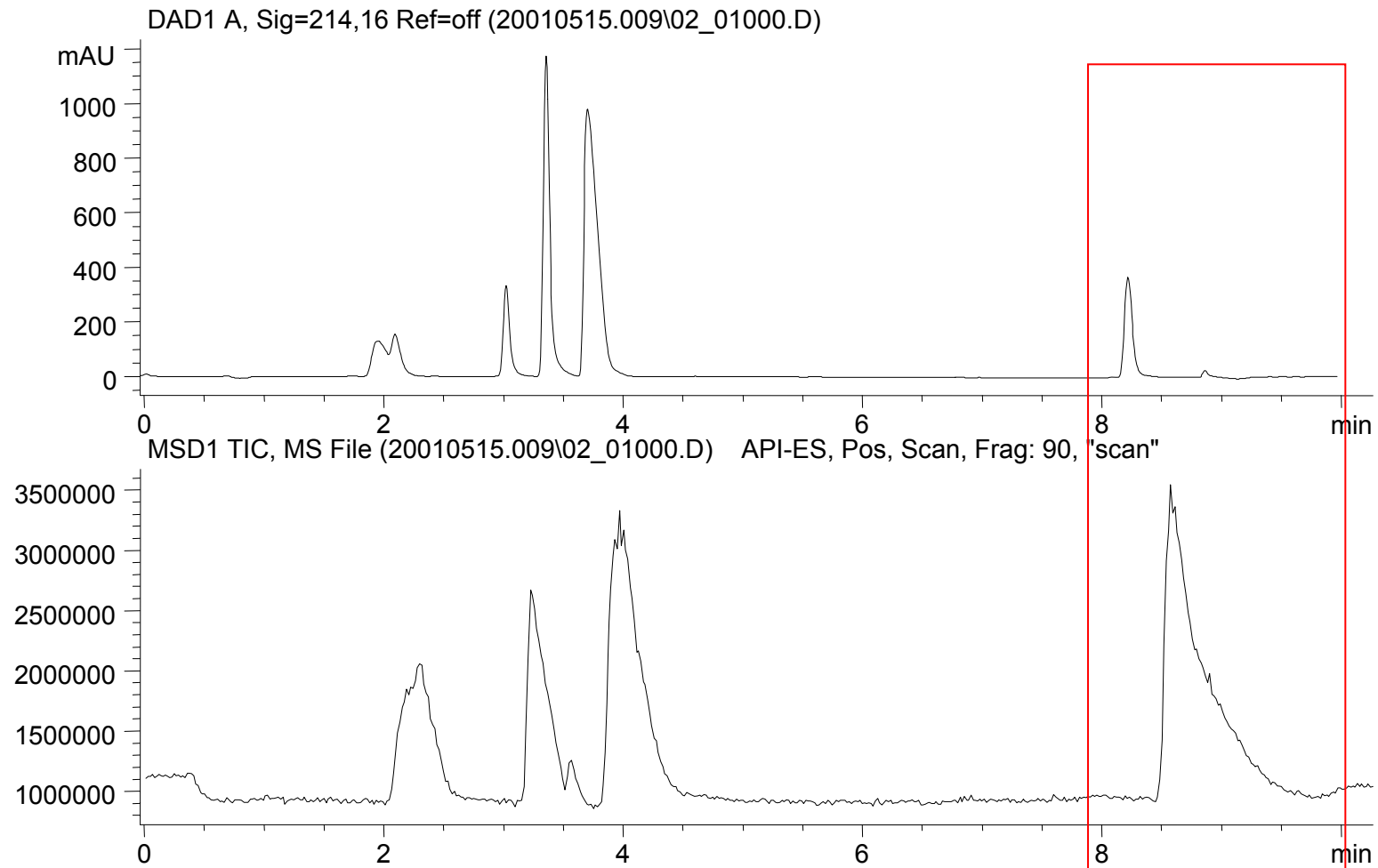
The 1200 CAN Network: Integrated Intelligence



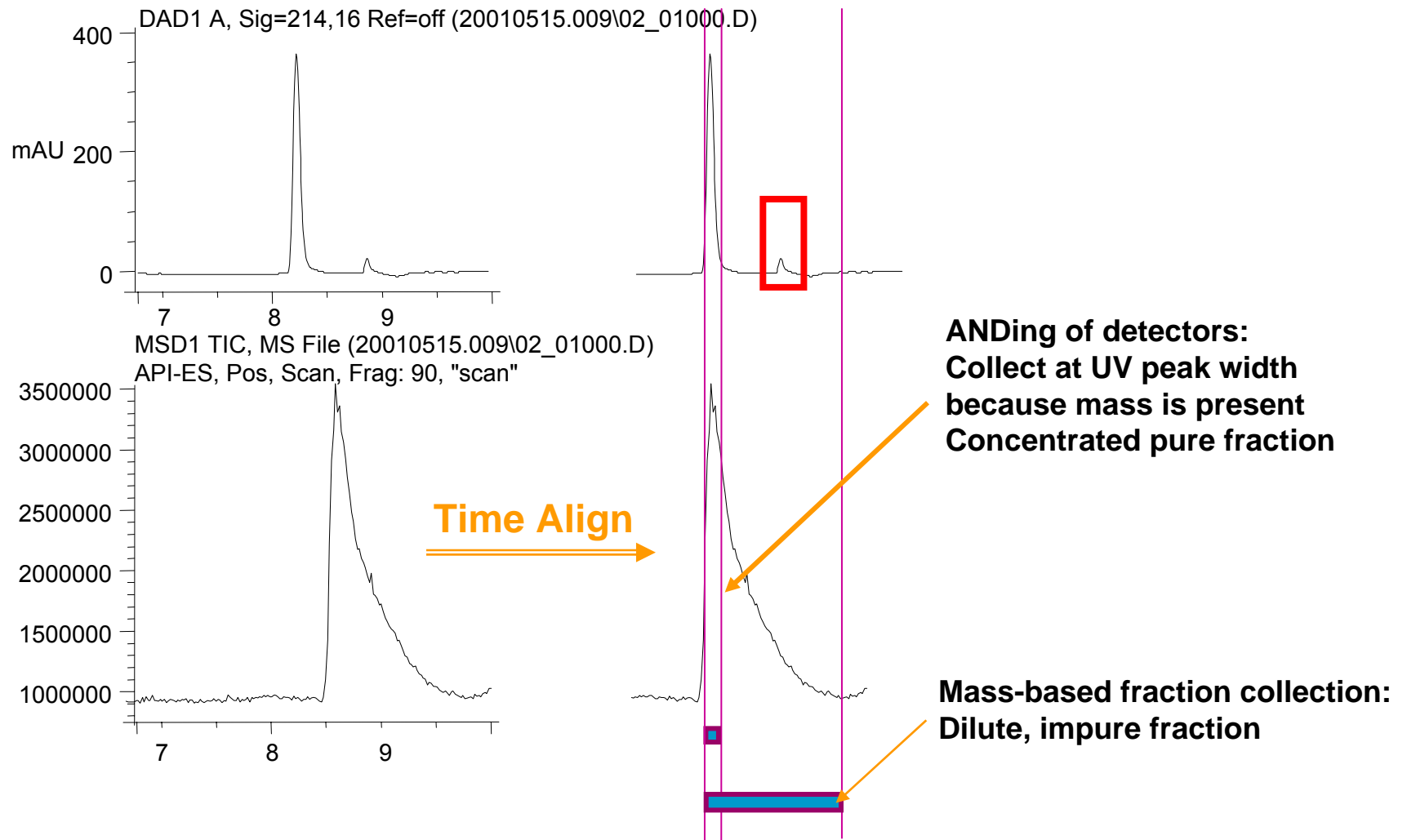
The 1200 CAN Network: Integrated Intelligence



Agilent 1200 Prep System Overloaded Column and Detectors



ANDing signals: Tighter Fractions Lower volumes, Higher Purity



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The Next Level of Data Traceability

RFID Technology for Flow Cells and UV Lamp



Analysis method: C:\CHEM32\1\METHODS\DEF_DEF.M

Sample information for Location:

Sample Name:		Multiplier:	1.00
Injection#:	0	Dilution:	1.00
Injection volume:	Unknown		

Acquisition information:

Operator:			
Date/Time:	19.04.2005 16:41:32		
Data file name:	C:\CHEM32\1\DATA\TAGINFO.D		
Method file name:	D:\CHEM32\1\METHODS\TEST1.M		
Flow:	- ml/min		
Pressure at start:	- bar	Pressure at end:	- bar
Left Temp. at start:	- °C	Left Temp. at end:	- °C
Right Temp. at start:	- °C	Right Temp. at end:	- °C

```

Cell Tag
Product Number: G1315-60022
Serial Number: Proto CR20
Production Date: 08.10.2004 06:51:24
Path Length: 10 mm
Volume: 1000nl
Max. Pressure: 120 bar
Last Cell Test: 14.04.2005 10:36:18
    
```

```

UV Lamp Tag
Product Number: 2140-0820
Serial Number: 214042
Production Date: 08.10.2004 06:51:24
Accum. UV On Time: 873 h
Number of Ignitions: 24
Last Intensity Test: 14.04.2005 12:36:17
    
```

Flashcard
Compact Flashcard: 128 MB

Signal description: DAD1 A, Sig=254,4 Ref=360,100

```

Cell Tag
Product Number: G1315-60022
Serial Number: Proto CR20
Production Date: 08.10.2004 06:51:24
Path Length: 10 mm
Volume: 1000nl
Max. Pressure: 120 bar
Last Cell Test: 14.04.2005 10:36:18
    
```

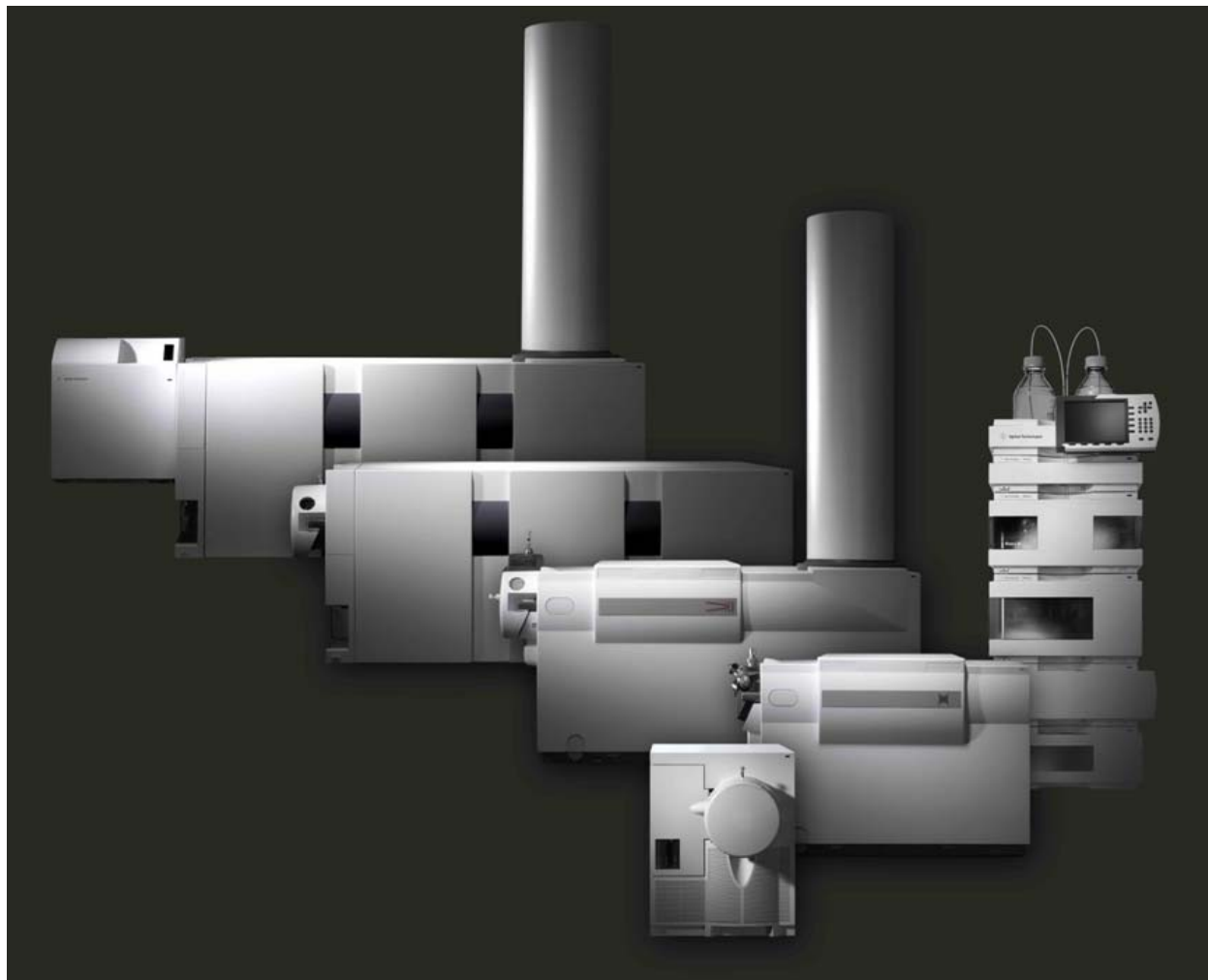
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```

ChemStation Report

RFID-tag info documents run conditions.

The Agilent 1200 series LC and 6000 series MS



Thanks for listening!