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Acquity™
Ultra Performance LC

ACQUITY UPLC™ in the Chromatography Lab: Analytical Considerations

- **ACQUITY UPLC™ in the Chromatography Lab: Analytical Considerations**

  Ultra Performance LC™ delivers improved speed, sensitivity and resolution that can benefit many liquid chromatography applications in today's laboratory. We will discuss analytical performance along with considerations and recommendations for successful implementation of UPLC™ in the chromatography laboratory.

Why Advance the Science and Technology of LC?

Challenges in the LC Lab

- Chromatography
  - Resolution
  - Reproducibility
  - Ruggedness
  - Sensitivity
  - Speed
  - Detector optimization
- Business
  - Complex samples
  - Regulatory considerations
  - Increased # samples
  - Time pressures
  - Cost savings

Can you meet today's challenges?

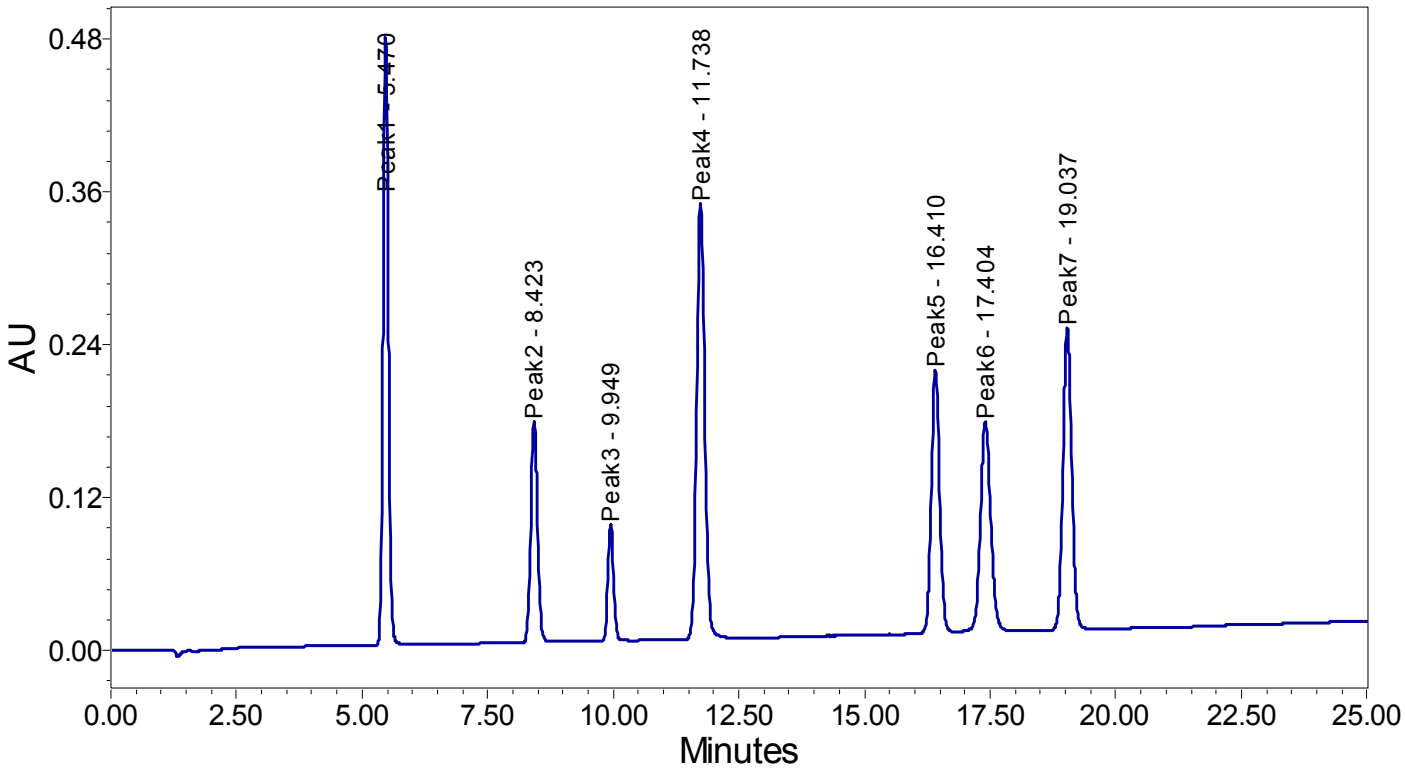
HPLC: 7 Analgesics in 25 minutes

Gradient Table

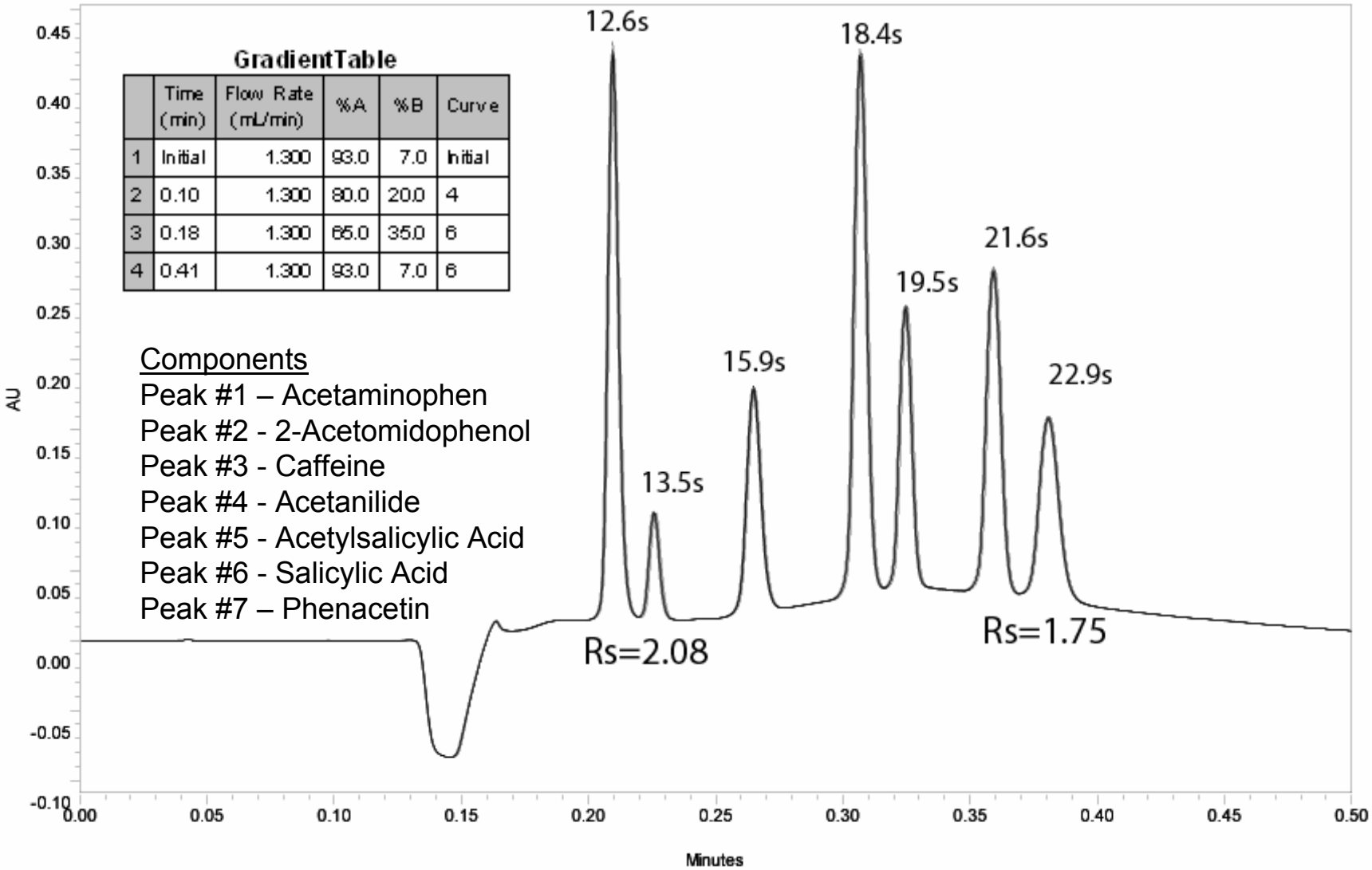
Time	Flow	%A	%B	%C	%D	Curve
	1.00	98.0	2.0	0.0	0.0	
25.00	1.00	75.0	25.0	0.0	0.0	6
25.01	1.00	98.0	2.0	0.0	0.0	6
60.00	0.05	98.0	2.0	0.0	0.0	6

Components

Peak #1 – Acetaminophen
Peak #2 - 2-Acetomidophenol
Peak #3 - Caffeine
Peak #4 - Acetanilide
Peak #5 - Acetylsalicylic Acid
Peak #6 - Salicylic Acid
Peak #7 – Phenacetin



UPLC™:7 analgesics in 25 seconds

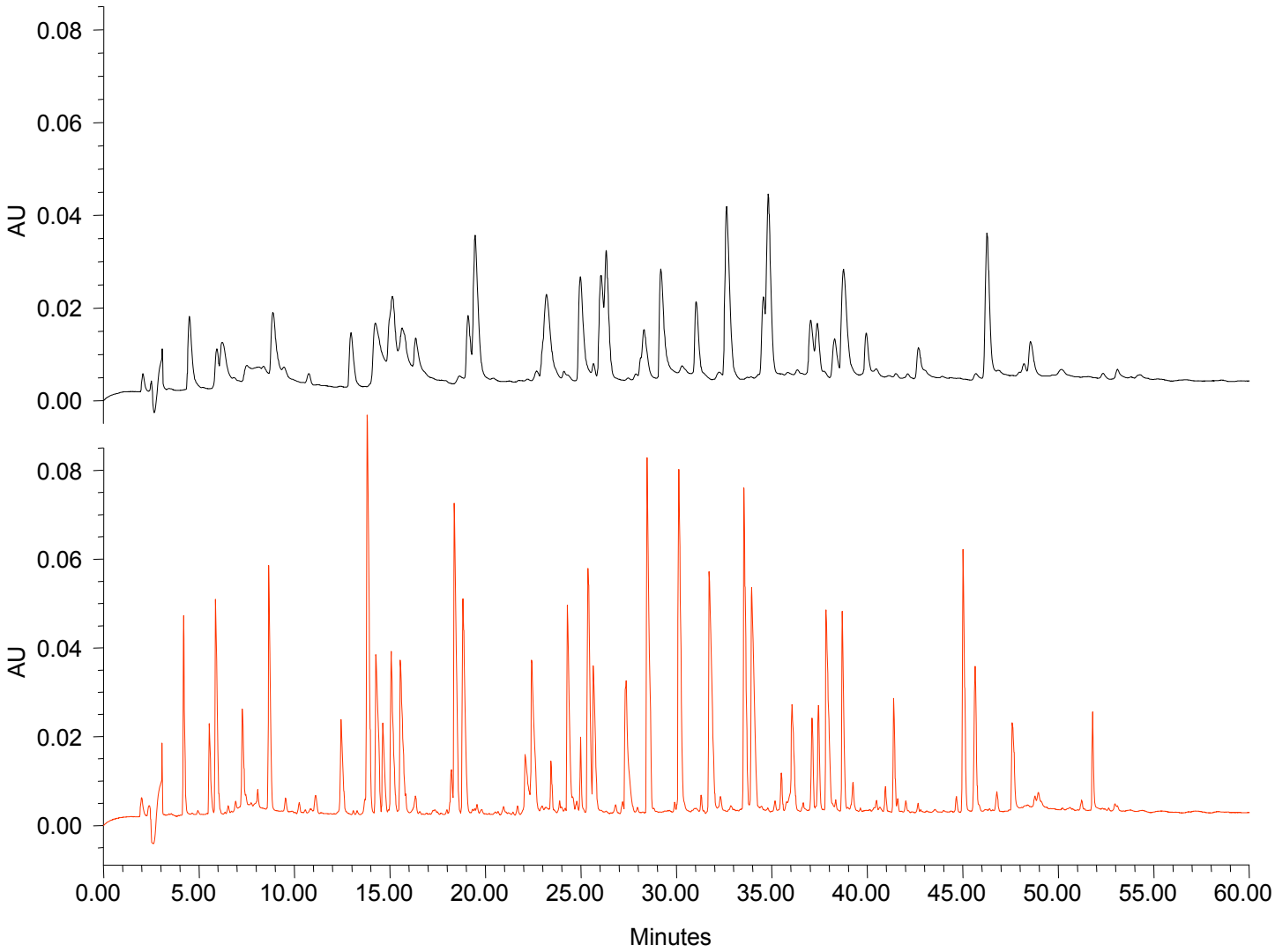


Why Change from HPLC to UPLC™? Time and solvent savings

		Column Volume (X5)	System Volume (x3)	Re-equilibration time (min.)	Total cycle time (min.)	# of injections in 24hours	Solvent consumed per injection
HPLC 4.6 x 100 5µ 25 min. run time	1.0 mL/min.	1.66mL (8.3mL)	620uL (1.86mL)	10.2.	35.2	41	35.2mL
UPLC™ 2.1 x 50 1.7µ 0.41 min run time	1.3 mL/min	0.20mL (1.0mL)	120uL (0.360mL)	1.04	1.45	993	1.9mL

Total Liters of solvent for 1000 injections:
HPLC: 35.2L Vs. UPLC™: 1.9L

Why Change from HPLC to UPLC™? More information about your sample



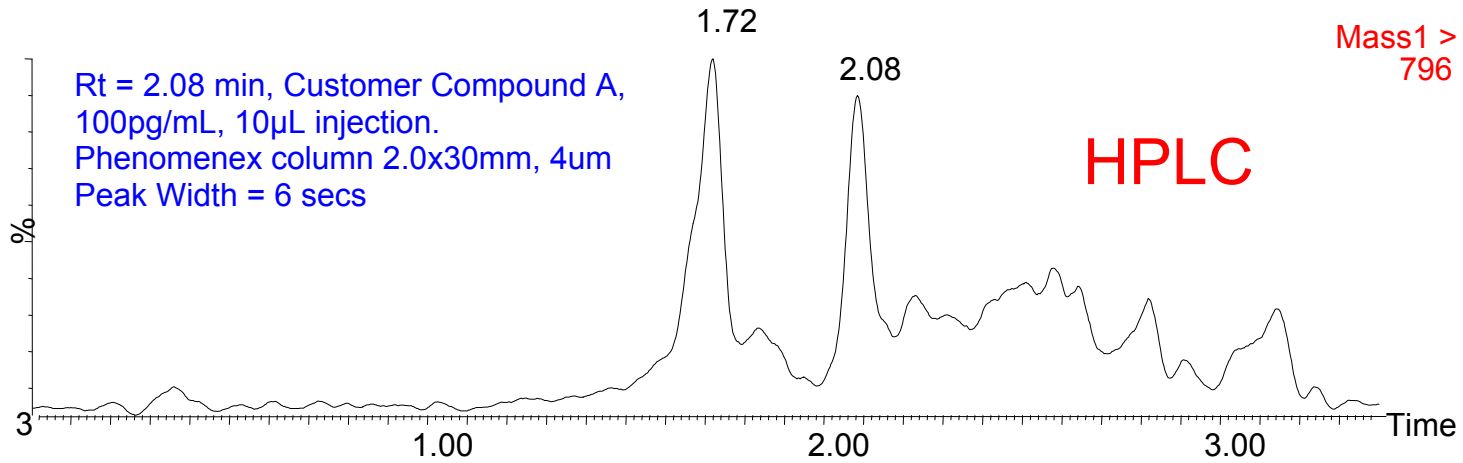
HPLC
4.8 µm
Peaks = 70
P_c = 143

UPLC™
1.7 µm
Peaks = 168
P_c = 360

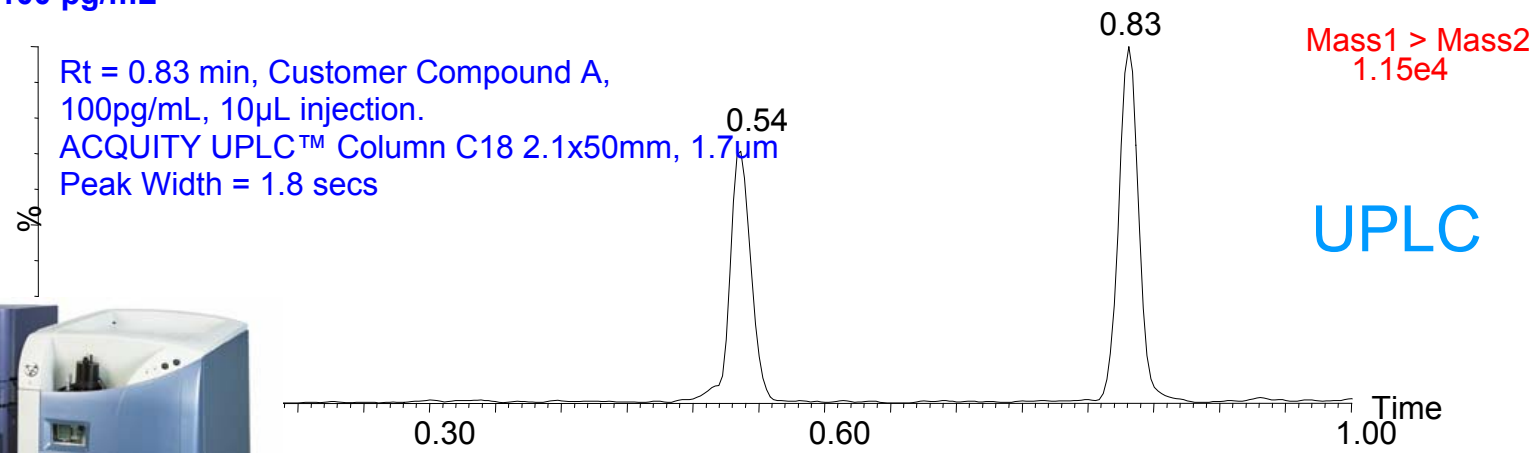
2.5X increase

Why Change from HPLC to UPLC™? Improved sensitivity

100 pg/mL



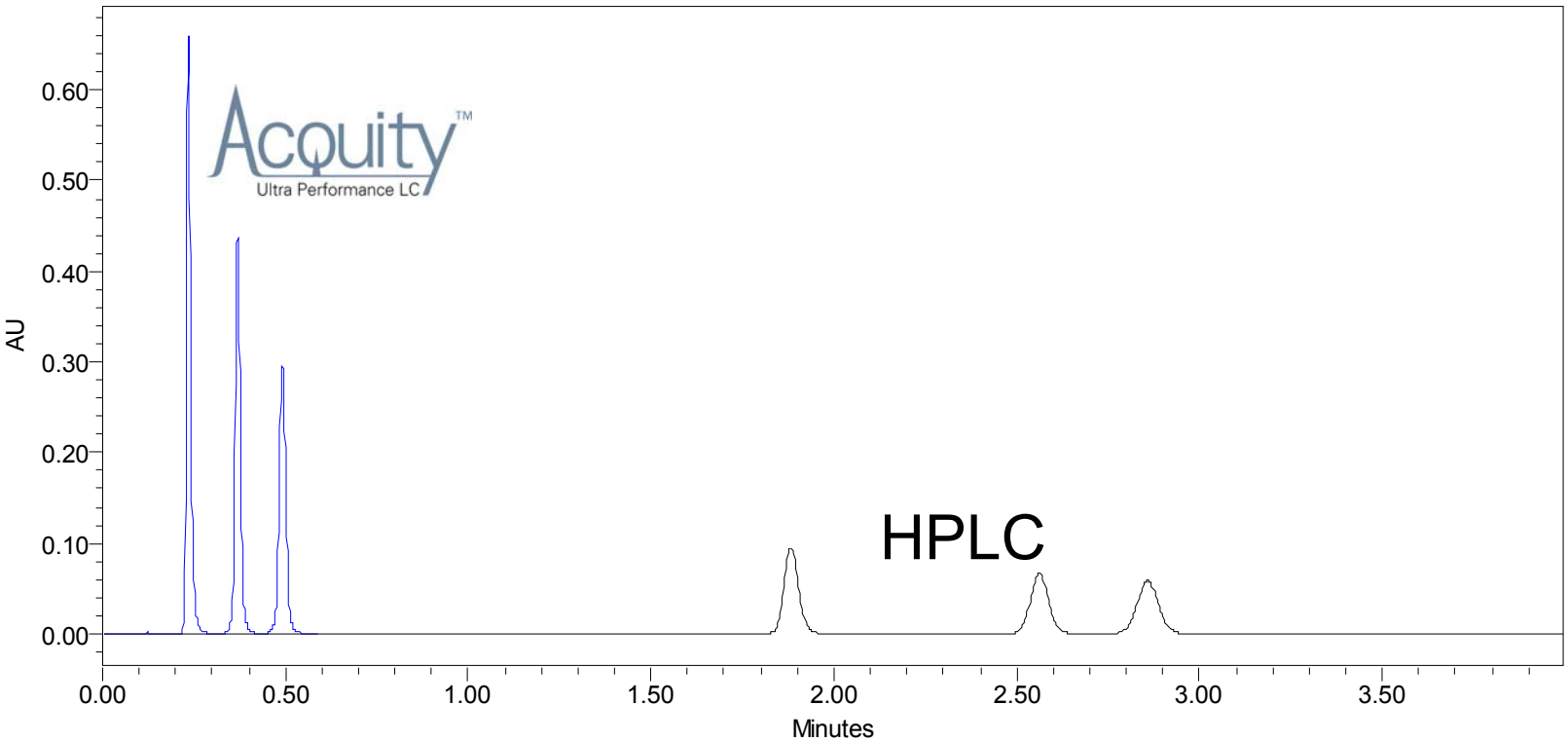
100 pg/mL



Method Comparison 100pg/mL, 10µL injection

Nicholas Ellor

Speed, Sensitivity, Resolution

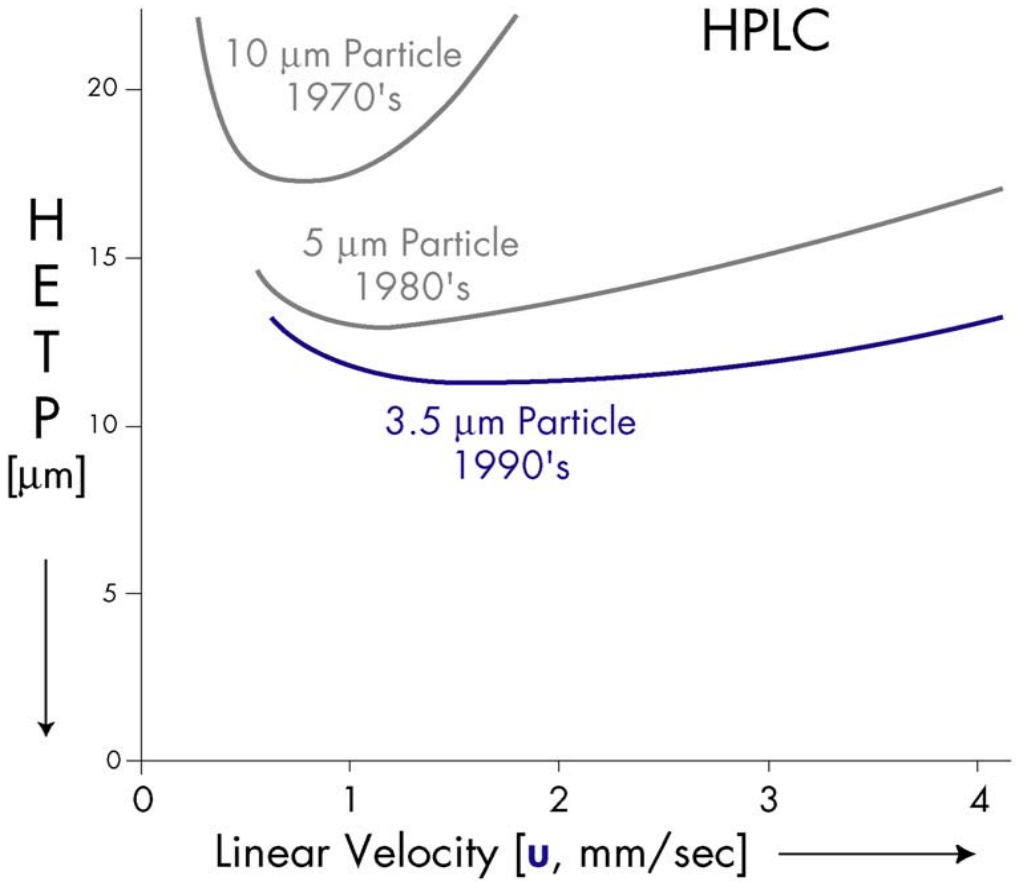


Waters

HPLC Today
The state of the science

- The quality of the separation in HPLC is both driven and limited by column chemistry
- Optimized chemistry can bring speed, sensitivity and resolution benefits to the chromatographic process
- Particle size and selectivity are primary contributors to column performance
- Today's classical HPLC instruments cannot maximize the reality of "state of art" smaller particles

Smaller Particles
The enabler of productivity



- Smaller particles provide:
  - increased efficiency
  - maintain efficiency over a wider linear velocity
  - ability for both added resolution and increased speed of separation
- Particles are central to the quality of the separation

van Deemter plot for classical HPLC

Fundamental Resolution Equation

At constant column length

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

System Efficiency | Selectivity | Retentivity

- In UPLC™ systems, N (efficiency) is the primary driver
- Assuming the Selectivity and retentivity are the same as in HPLC
- Then, Resolution, Rs, is proportional to the square root of N

$$Rs \propto \sqrt{N}$$

And, Efficiency (N), is inversely proportional to Particle Size , dp

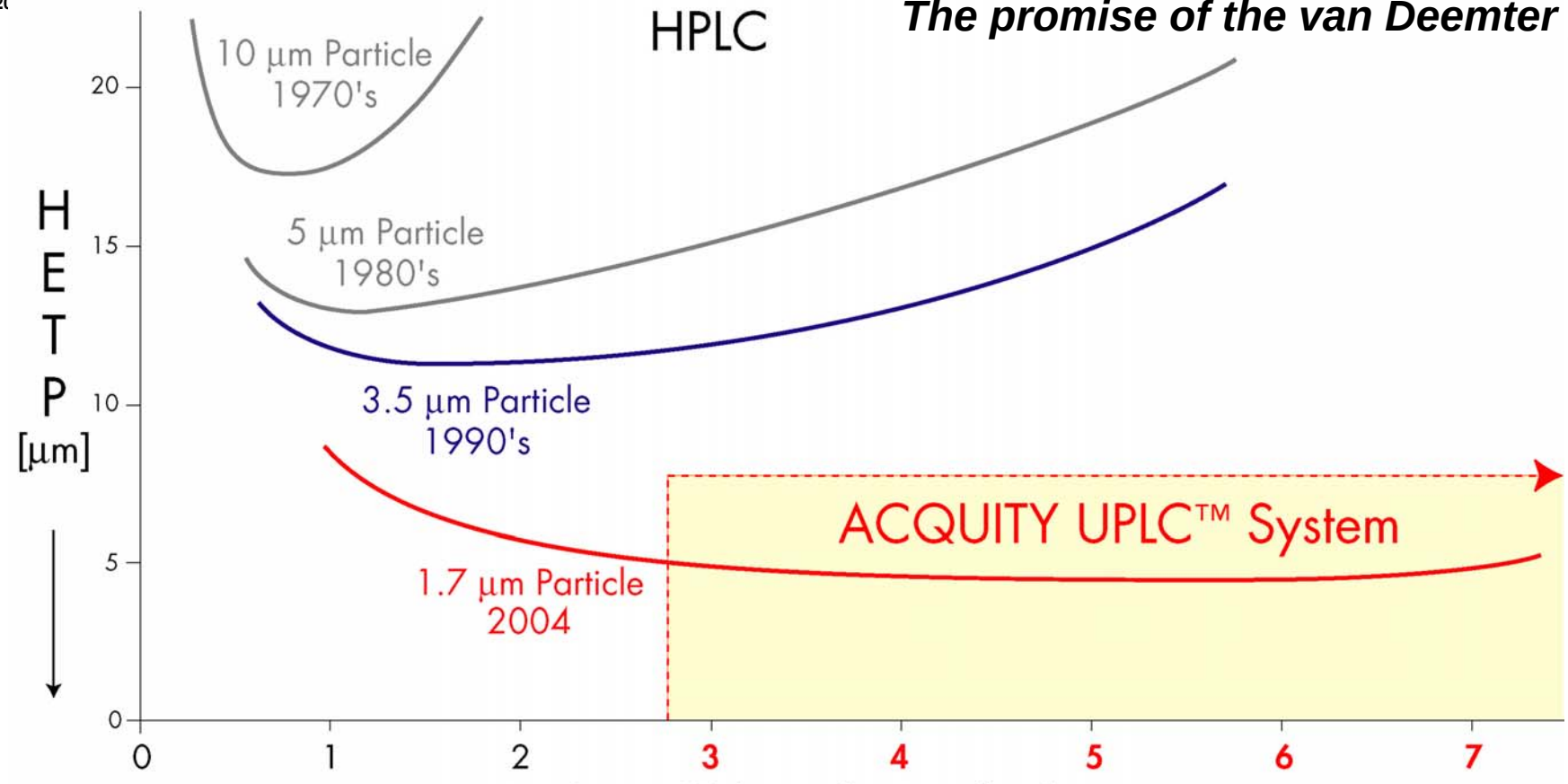
So: $N \propto \frac{1}{dp}$

Therefore if: dp ↓ 3X, Then: N ↑ 3X, Rs ↑ 1.7X

Waters

Smaller Particles
The enabler of productivity

The promise of the van Deemter plot



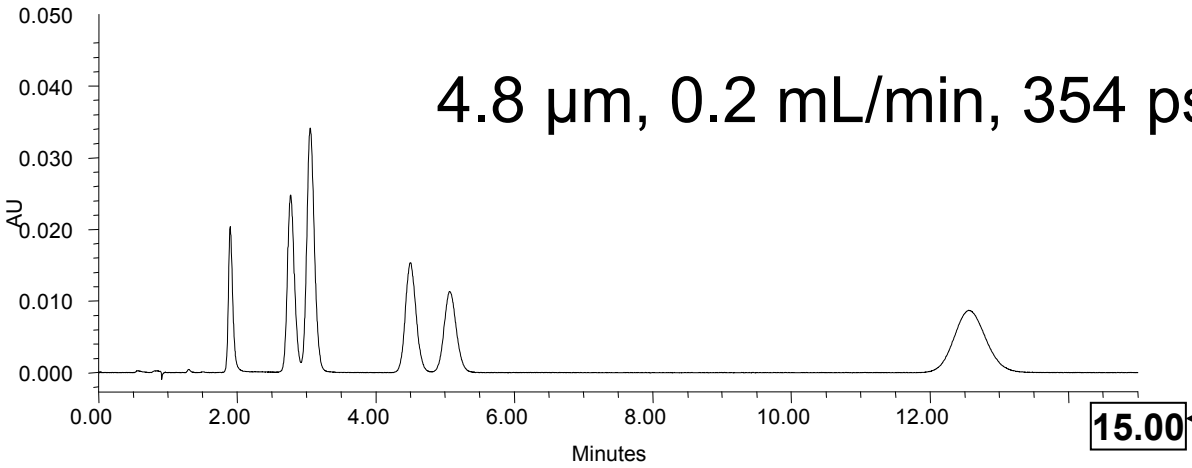
Flow Rate [mL/min]:							
ID = 1.0 mm	0.04	0.07	0.10	0.13	0.17	0.20	0.24
ID = 2.1 mm	0.15	0.3	0.45	0.6	0.75	0.9	1.05
ID = 4.6 mm	*0.7*	*1.4*	*2.1*	*2.8*	*3.5*	*4.2*	*4.9*

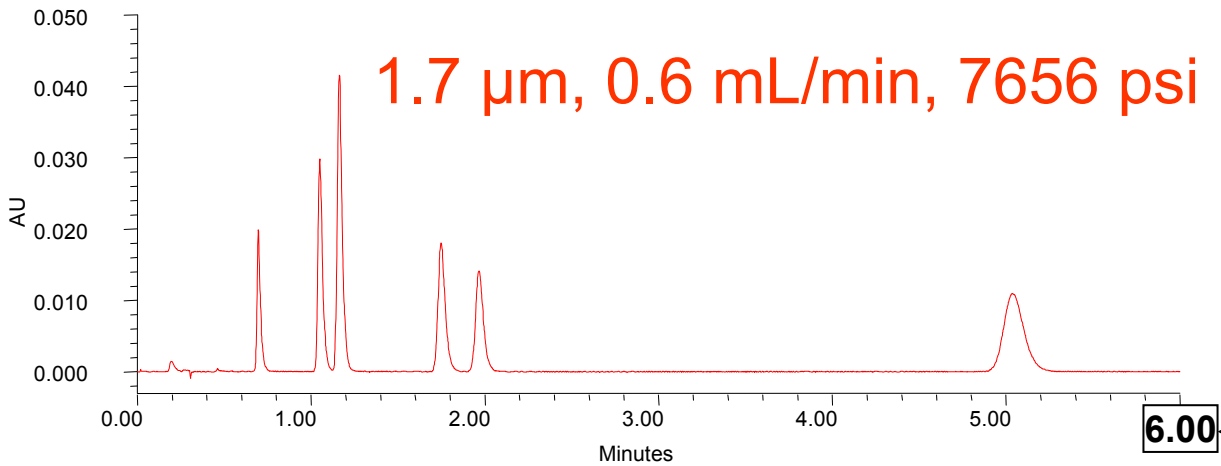
Waters

Summary of Key System Attributes
(at constant column length)

	Resolution Improvement	Speed Improvement	Sensitivity Improvement	Back Pressure
1.7 vs. 5 µm particles	1.7X	3X	1.7X	27X
1.7 vs. 3 µm particles	1.3X	2X	1.3X	6X

Influence of Particle Size on Chromatography





Theory
1.7X Resolution
3X Faster
1.7X Sensitivity
25X Pressure

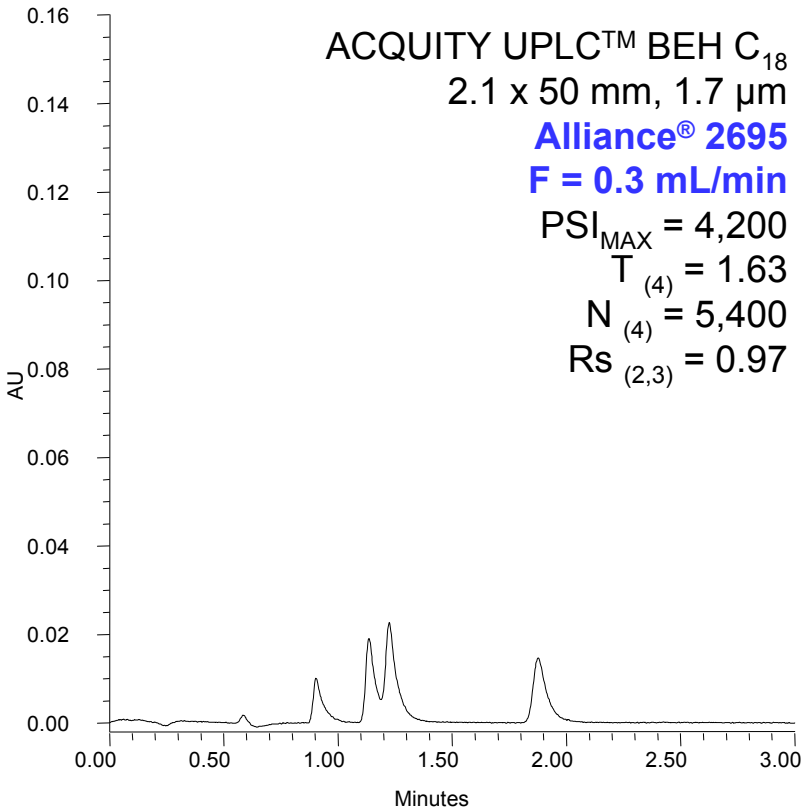
Actual
1.5X Resolution
2.6X Faster
1.4X Sensitivity
22X Pressure

Isocratic methods with 2.1 x 50 mm columns

Optimal Linear Velocity on ACQUITY UPLC™: Isocratic Caffeine Metabolites Separation

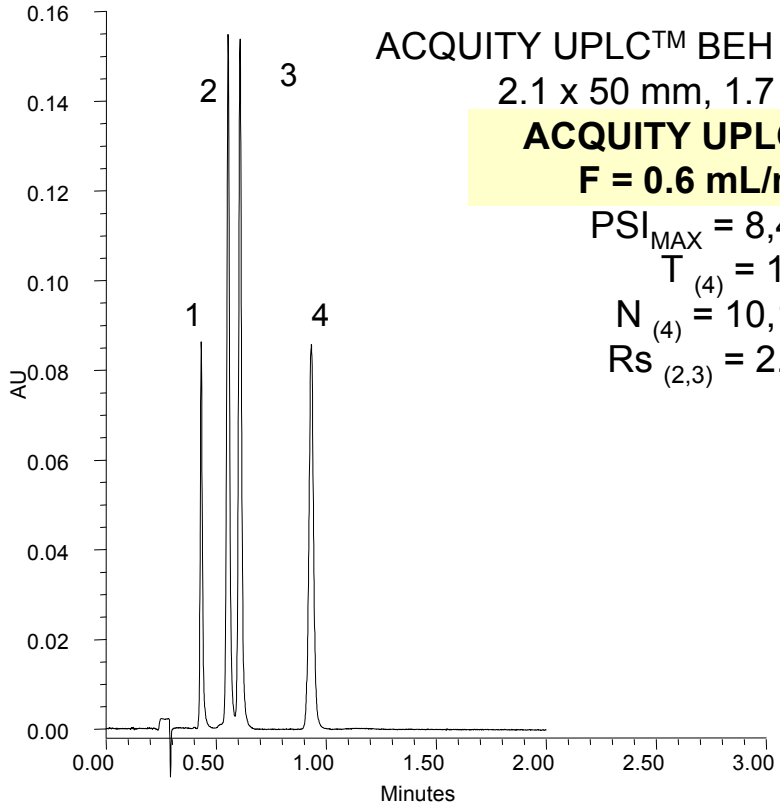
HPLC
Non-Optimal Linear Velocity

ACQUITY UPLC™ BEH C_{18}
2.1 x 50 mm, 1.7 µm
Alliance® 2695
F = 0.3 mL/min
PSI_{MAX} = 4,200
$T_{(4)}$ = 1.63
$N_{(4)}$ = 5,400
$Rs_{(2,3)}$ = 0.97



UPLC™
Optimal Linear Velocity

ACQUITY UPLC™ BEH C_{18}
2.1 x 50 mm, 1.7 µm
ACQUITY UPLC™
F = 0.6 mL/min
PSI_{MAX} = 8,400
$T_{(4)}$ = 1.02
$N_{(4)}$ = 10,100
$Rs_{(2,3)}$ = 2.25



Same ACQUITY UPLC™ Column on HPLC vs UPLC™ instruments

ACQUITY UPLC™ Technology
Ultra performance by design

Acquity™ Ultra Performance LC

Detectors:
Optical and/or Mass Spec
Tunable UV or Photodiode Array
Optimized flow cell for UPLC™
High speed data sampling for

System Considerations:
Small Footprint
Redesigned tubing and fittings
Consolidated waste management
Integrated system diagnostics
Connections Insight™ remote diagnostics

Sample Organizer: (option)
Expands capacity (22/15/8)
Shuttles plate feed
Heated/chilled

Column Manager:
Innovative pivot design
Positions column to detector
E-Cord™ connection

Sample Manager:
Low dispersion XYZZ' Format
Fast cycle times
Low carryover
Plates and/or vials
Optional Sample Organizer

Binary Solvent Manager:
High pressure blending
Binary gradients
Four solvent choices
On-line degassing
Low dispersion design
UPLC pressure capabilities

UPLC™ Technology Challenges

How do we take advantage of smaller particles?

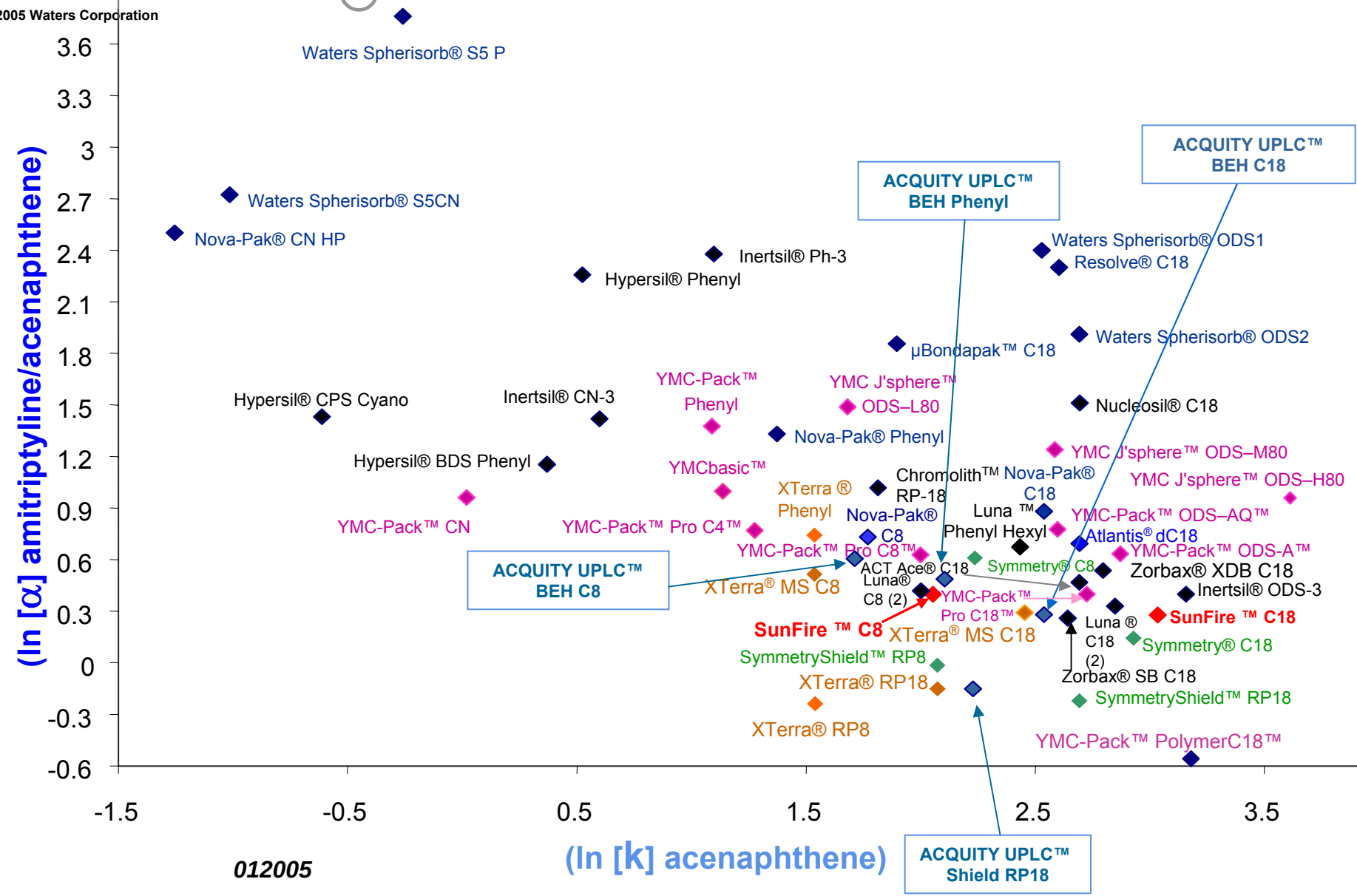
- Pressure tolerant particle/column
- High pressure fluidic modules
- Holistic design, low system volume, optimized flow paths
- Reduced cycle time autosampler with minimum carryover
- High speed detectors; optical and mass
- Controlled and coordinated system interaction
  - Software designed for system integration
  - Comprehensive diagnostic suite

Advancing the Science

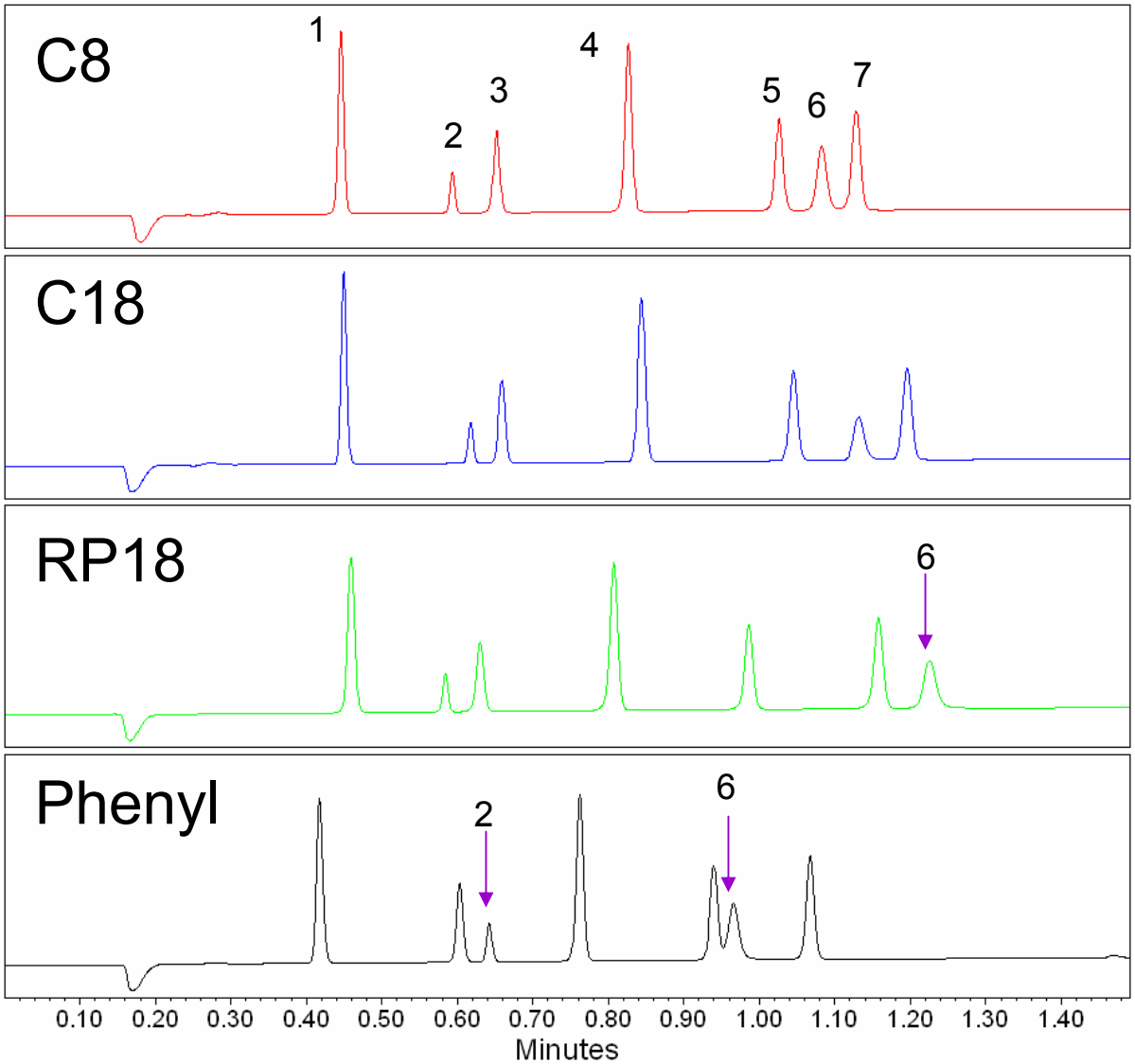
- Requires innovation in every aspect of column:
  - New patented bridged hybrid particle required for pressure tolerance and outstanding chromatographic performance
    - Sub 2µm particles
    - Porous for optimum mass transfer
  - Column hardware
    - New patented frit technology to retain particles
    - New end fittings for high pressure/low dispersion operation
  - Packing technology
    - New column packing processes to optimize stability
  - eCord™
    - New information chip to store column history

Waters

Reversed-Phase Column Selectivity Chart



ACQUITY UPLC™ Column Selectivity Comparison for Analgesics



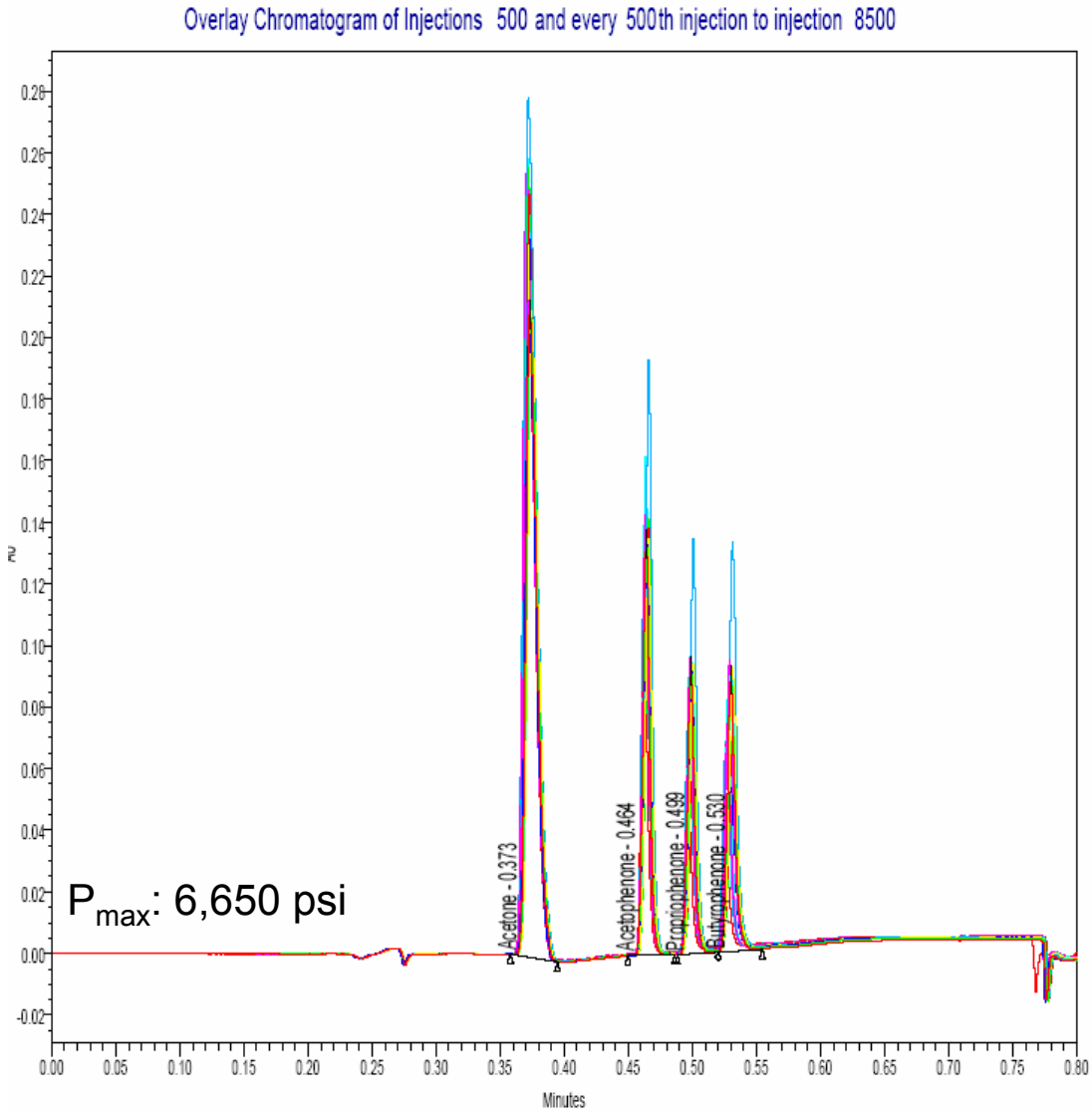
1. Acetaminophen
2. 2-Acetomidophenol
3. Caffeine
4. Acetanilide
5. Acetylsalicylic Acid
6. Salicylic Acid
7. Phenacetin

ACQUITY UPLC™ BEH C18 1.7µm
ACQUITY UPLC™ BEH RP18 1.7µm
ACQUITY UPLC™ BEH C8 1.7µm
ACQUITY UPLC™ BEH Phenyl 1.7µm

Column Lifetime: *>8,500 Injections*

Conditions

Column:	ACQUITY UPLC™ BEH C18 2.1 x 50 mm			
Mobile Phase A:	0.1% HCOOH			
Mobile Phase B:	0.1% HCOOH in ACN			
Flow Rate:	0.65 mL/min			
Gradient:	Time (min)	Profile %A	%B	Curve
	0.0	95	5	Initial
	0.1	95	5	6
	0.4	5	95	3
	0.5	95	5	11
	0.8	95	5	
Injection Volume:	5 µL			
Temperature:	40°C			
Detection:	UV @ 254 nm			



Column Lifetime Customer Example

- Isocratic aging experiment
  - Cycle: low pressure runs/injections (data shown) → 200/400/600 etc. high pressure runs/injections (13,000 psi) → low pressure runs/injections
  - This aging cycle was repeated
- Columns lasted over 4,000 high pressure injections

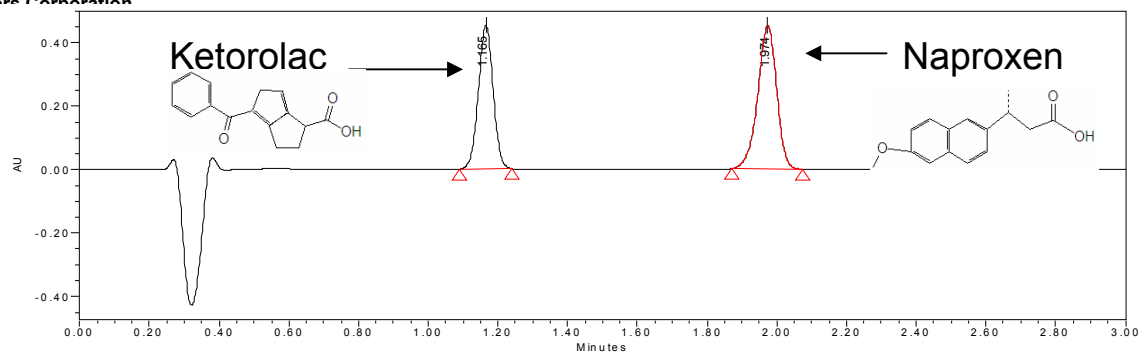
Conditions (Isocratic Slow Flow/Low Pressure)

Column:	ACQUITY UPLC™ BEH C18 (2.1 x 50 mm, 1.7 μm) ACQUITY UPLC™ BEH Shield RP18 BEH (2.1 x 50 mm, 1.7 μm)
Part Number:	186002350
Mobile Phase A:	0.1% HCOOH in Waters
Mobile Phase B:	0.1% HCOOH in ACN
Flow Rate:	0.5 mL/min (~6,000 psi)
Condition:	67% A / 33% B
Injection Volume:	5.0 µL
Sample Concentration:	208 μg/mL ketorolac tromethamine and 13 μg/mL naproxen in MeOH
Temperature:	65°C
Detection: UV	225 nm (Sample Rate: 10 Hz, Filter: Normal)

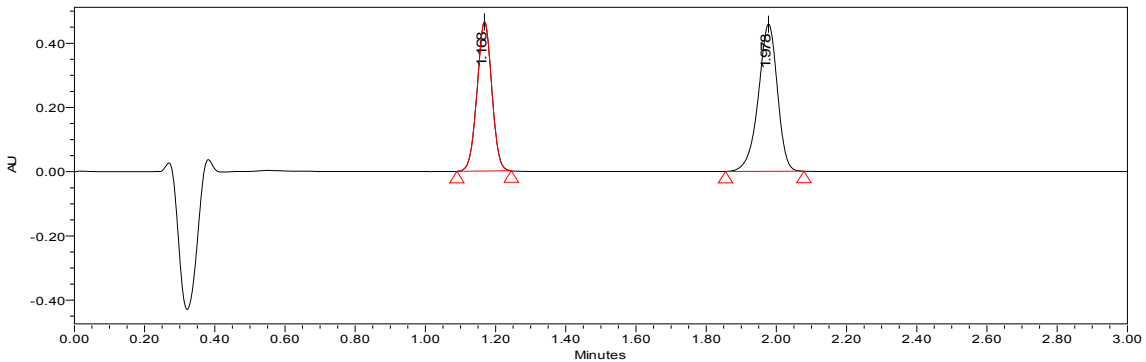
Conditions (Isocratic Fast Flow/High Pressure)

Columns:	ACQUITY UPLC™ BEH C18 (2.1 x 50 mm, 1.7 μm) ACQUITY UPLC™ BEH Shield RP18 (2.1 x 50 mm, 1.7 μm)
Mobile Phase A:	0.1% HCOOH in Water
Mobile Phase B:	0.1% HCOOH in ACN
Flow Rate:	1.250 mL/min (Pressure ~13,000 psi)
Condition:	62% A / 38% B
Injection Volume:	5.0 µL
Sample Concentration:	208 μg/mL ketorolac tromethamine and 13 μg/mL naproxen in MeOH
Temperature:	65°C
Detection: UV	225 nm (Sample Rate: 10 Hz, Filter: Normal)

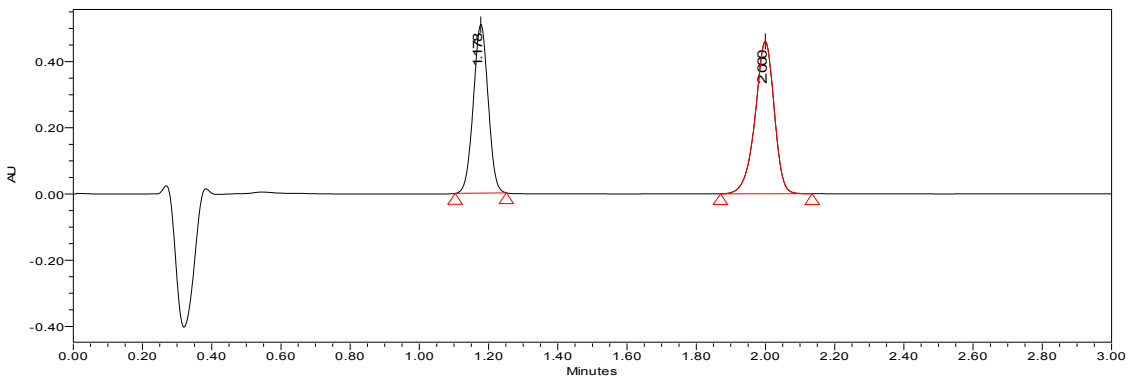
Column Lifetime Customer Example



First Injection on Column
No high pressure runs
N = 3483 for Peak 1
N = 6352 for Peak 2



Injection after
1600 high pressure runs
N = 3659 for Peak 1
N = 6489 for Peak 2



Injection after
4000 high pressure runs
N = 3573 for Peak 1
N = 6466 for Peak 2

Chromatograms obtained under low (~6000 psi) isocratic conditions

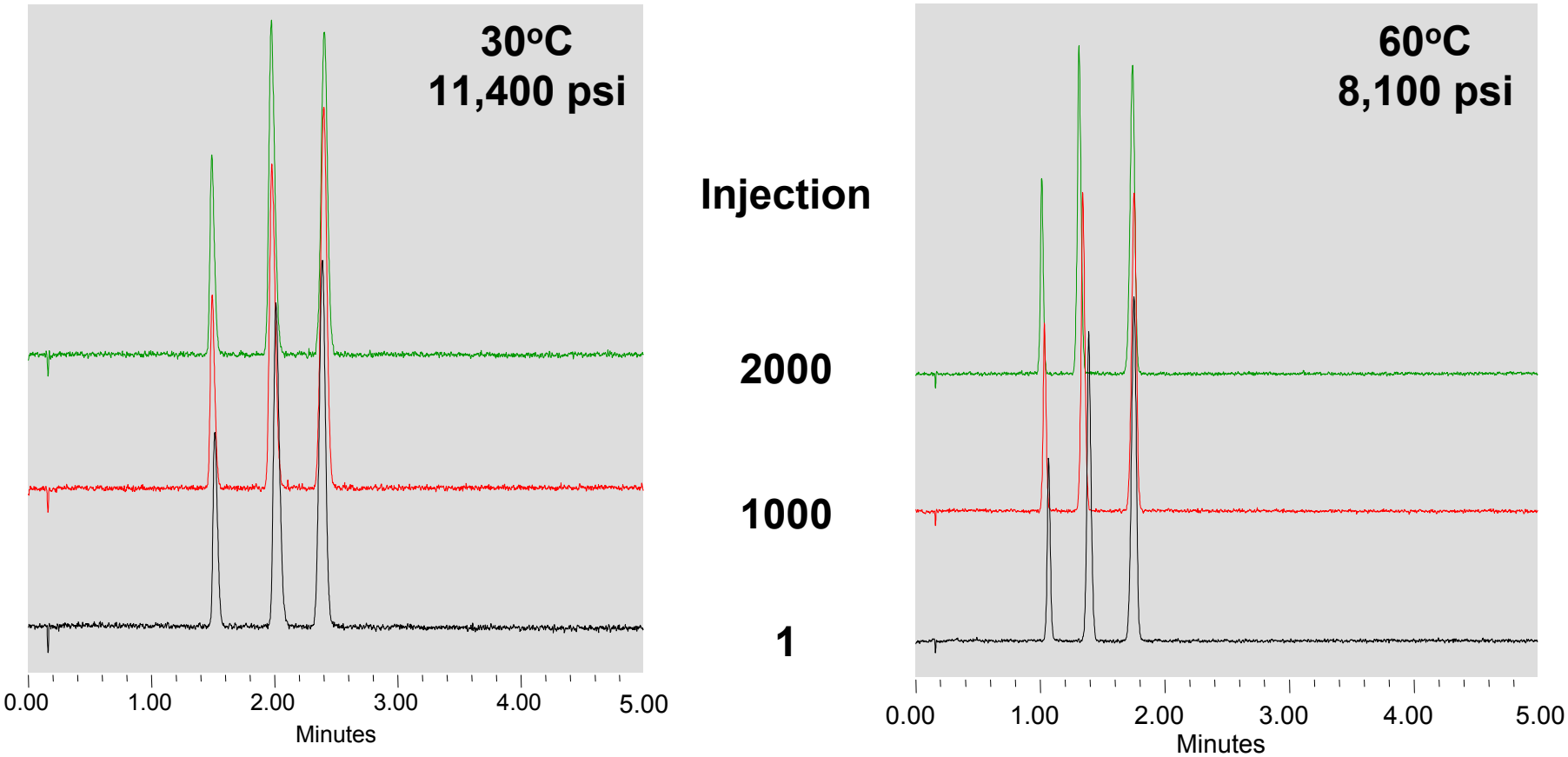
pH Stability At The Extremes

- What about chemical stability of the column at the extremes of pH, temperature and pressure?
- A 2 x 2 experimental design (at UPLC™ pressures, of course):

	30°C	60°C
pH 2.0		
pH 11.3		

Column Stability at pH 2.0

Direct Overlays

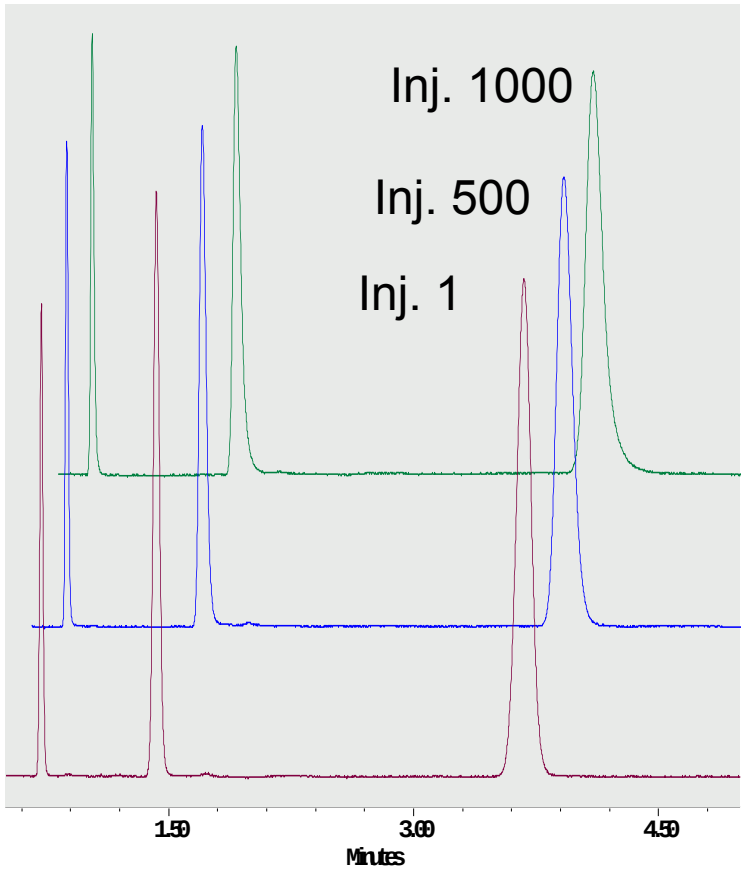


ACQUITY UPLC™ BEH C18, 2.1 x 50 mm
30% ACN (v/v) with 0.1% TFA (pH 2.0) at 0.9 mL/min
Analytes: protriptyline, amitriptyline, butyrophenone

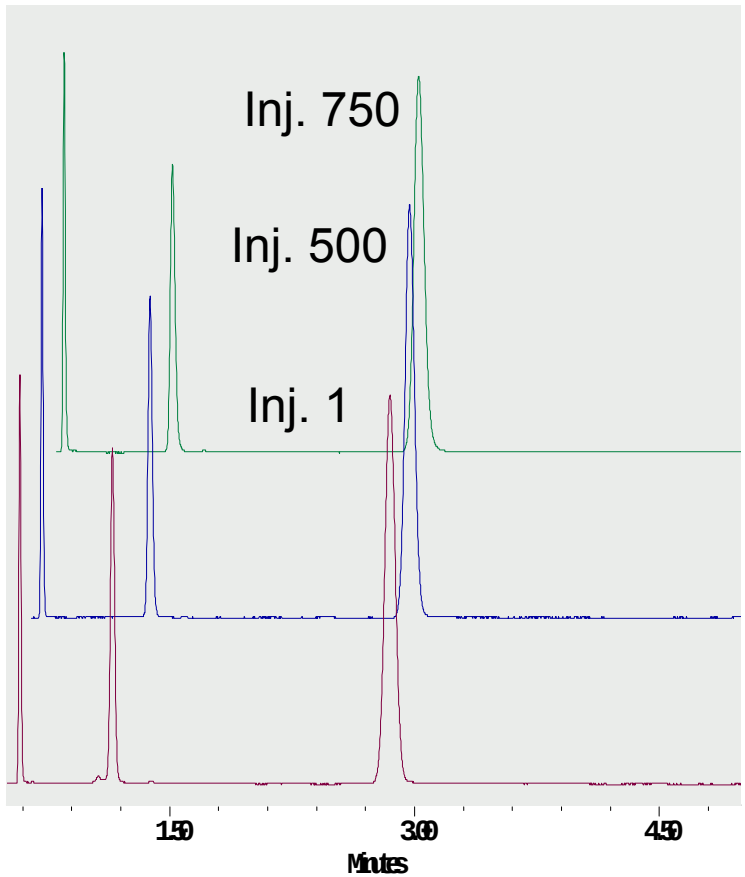
Column Stability at pH=11.3

Offset Overlays

30°C, 11,500 psi



60°C, 7,700 psi



Acetonitrile-Pyrrolidine Buffer (pH=11.3) 45:55 (v/v) at 0.9 mL/min flow rate
Analytes: butyrophenone, protriptyline and amitriptyline

UPLC™ Technology Challenges

How do we take advantage of smaller particles?

- Pressure tolerant particle/column
- High pressure fluidic modules
- Holistic design, low system volume, optimized flow paths
- Reduced cycle time autosampler with minimum carryover
- High speed detectors; optical and mass
- Controlled and coordinated system interaction
  - Software designed for system integration
  - Comprehensive diagnostic suite

ACQUITY UPLC™ Technology
Ultra performance by design

Acquity™ Ultra Performance LC

Detectors:
- Optical and/or Mass Spec
- Tunable UV or Photodiode Array
- Optimized flow cell for UPLC™
- High speed data sampling for

System Considerations:
- Small Footprint
- Redesigned tubing and fittings
- Consolidated waste management
- Integrated system diagnostics
- Connections Insight™ remote diagnostics

Sample Organizer: (option)
- Expands capacity (22/15/8)
- Shuttles plate feed
- Heated/chilled

Column Manager:
- Innovative pivot design
- Positions column to detector
- E-Cord™ connection

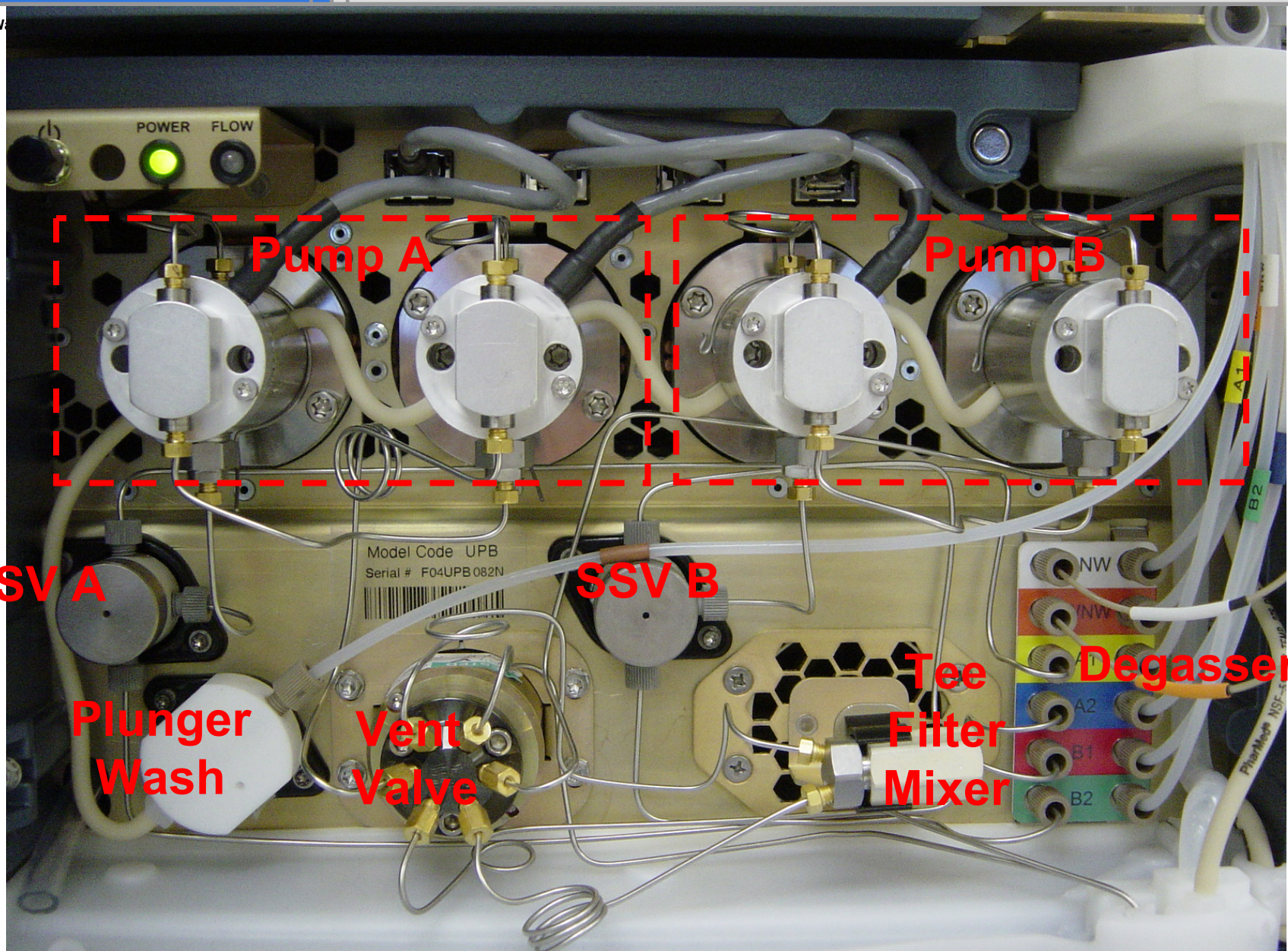
Sample Manager:
- Low dispersion XYZZ' Format
- Fast cycle times
- Low carryover
- Plates and/or vials
- Optional Sample Organizer

Binary Solvent Manager:
- High pressure blending
- Binary gradients
- Four solvent choices
- On-line degassing
- Low dispersion design
- UPLC pressure capabilities

- Binary gradient
  - Independent style piston drives
  - Serial flow for each solvent, parallel gradient formation
  - **High Pressure Gradient**
    - Solvent select, 4 solvent choices
  - On board solvent degasser
  - 50µl mixer, ~120µL total system volume
  - Ability to deliver non-linear gradients
  - UPLC pressure capabilities
    - 0-1mL/min to 15K psi,
    - 1 -2mL/min to 9K psi
  - Dedicated transducer for each head. Enhances performance, provides diagnostic information

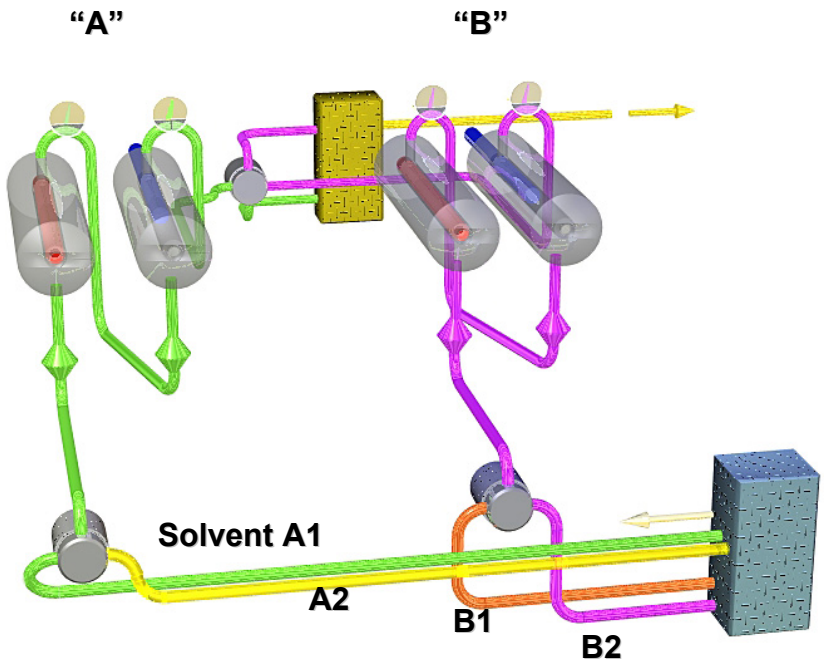
Waters

BSM Components



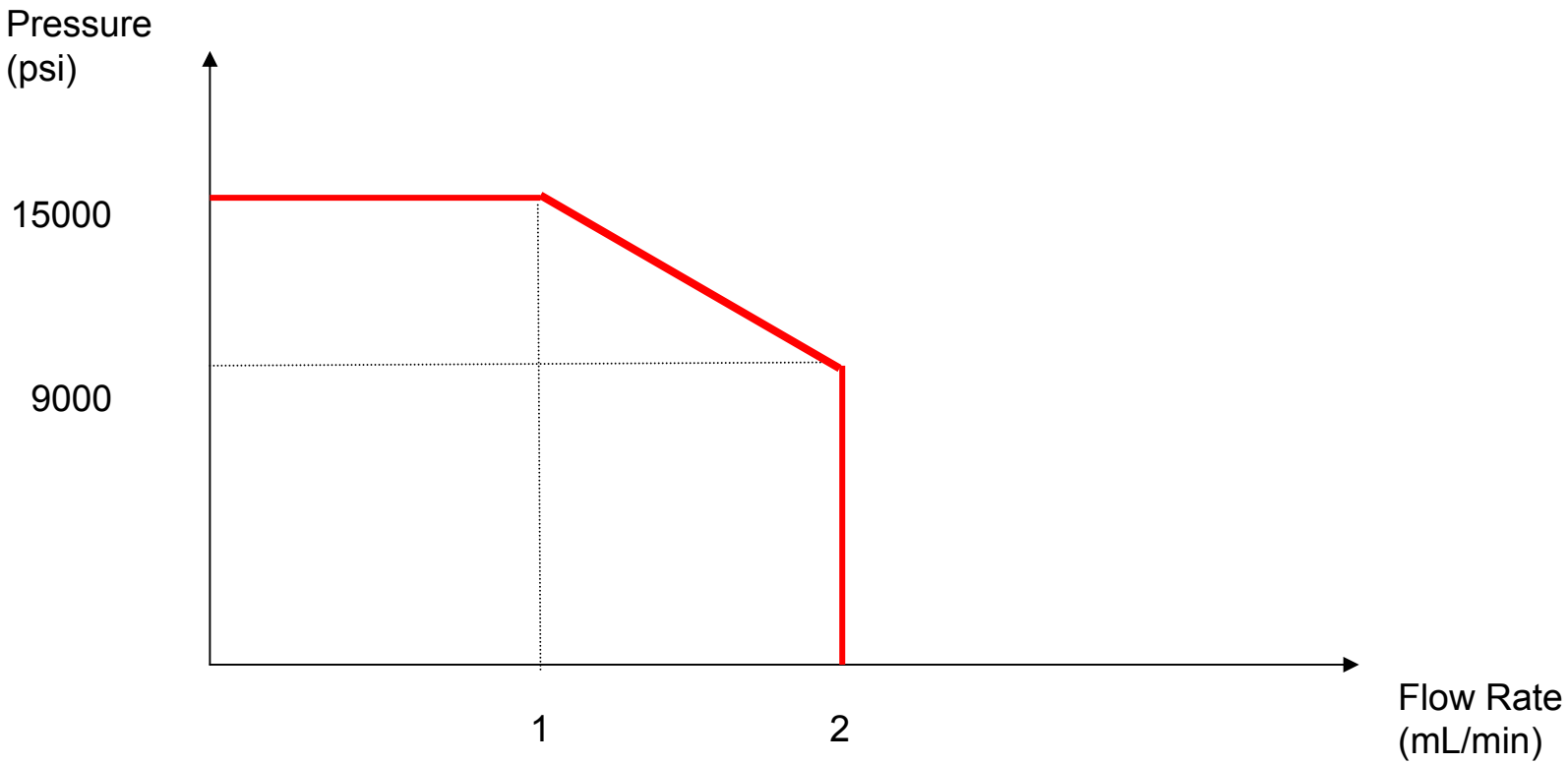
From Serial to Parallel Flow

- Two independently driven primary and accumulator piston drives
  - Separate drive motors
  - Two transducers
  - Two inlet check valves
- Four Solvents Capability
  - A1 or A2 and B1 or B2
  - Two solvents per gradient
- Six Channel Degasser
  - 4 chromatographic eluents
  - 2 solvents for Sample Manager
    - Strong & weak wash



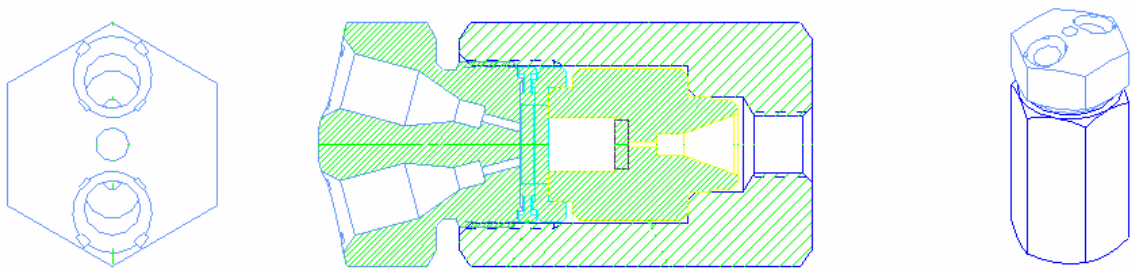
Pressure Envelope

- Fluidic components optimized to support the pressure demands of UPLC™ separations
  - Seals, head, bolts, check valves, fittings, tubing, mixer, pressure transducers
- UPLC™ compatible pressure with flow rate range



Tee/ Filter/ Mixer

- Glass Bead Mixing Technology
  - Tortured Mixing
  - Durable and will not disintegrate or affect delicate compounds
  - Highly resistant to attack by most liquids and/or vapors.
- Glass Bead Mixer
  - Standard 4 x 5, approximate volume 50 µL (P/N 700002631)
  - Optional Mixer 6 x 6, approximate volume 100 µL (P/N 205000252)
- Filter
  - Similar to Alliance, 2 micron filter (P/N 700002674)



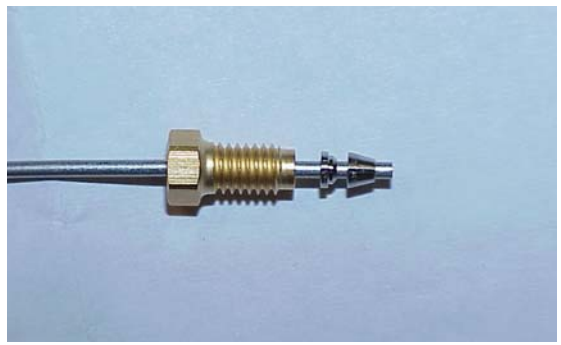
New Tubing & Fittings

- HPLC Fittings
  - Traditional drawn tubing

  - Traditional Waters Fittings

- UPLC™ Fittings
  - Center-less ground tubing

  - Gold Compression Screw
  - 2 Piece Swagelock Ferrules



ACQUITY 9/1000 SS316 Tubing

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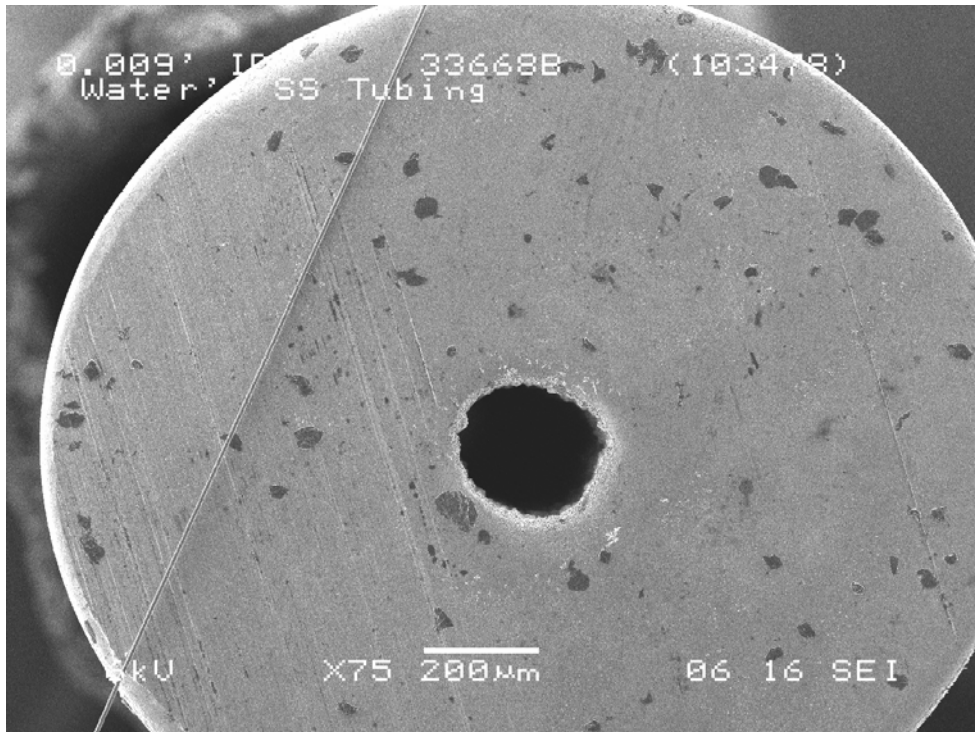

Standard 5/1000 inch 316 SS Tuning

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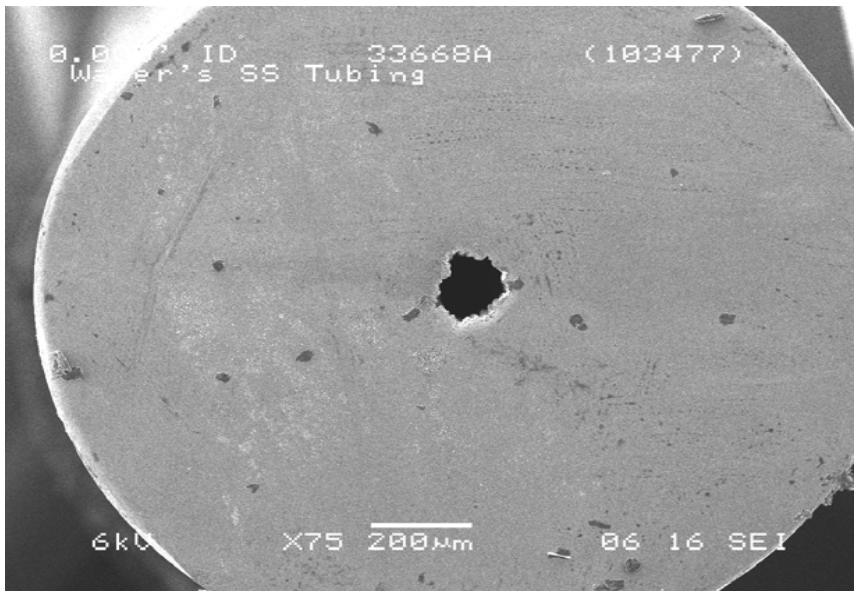

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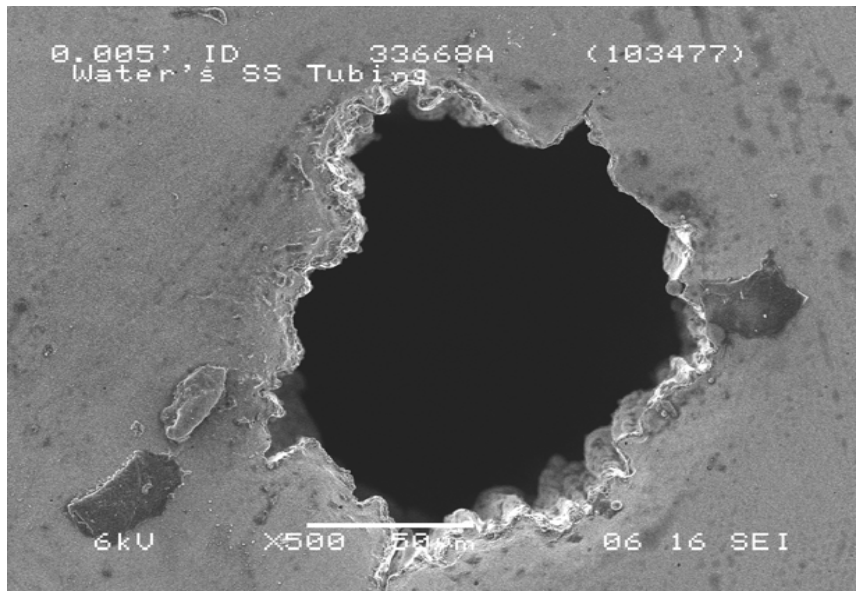
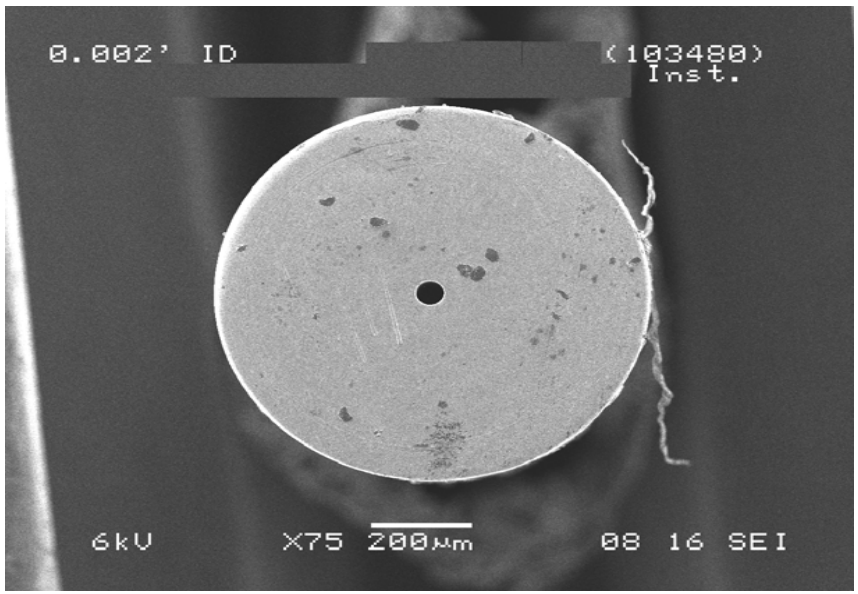
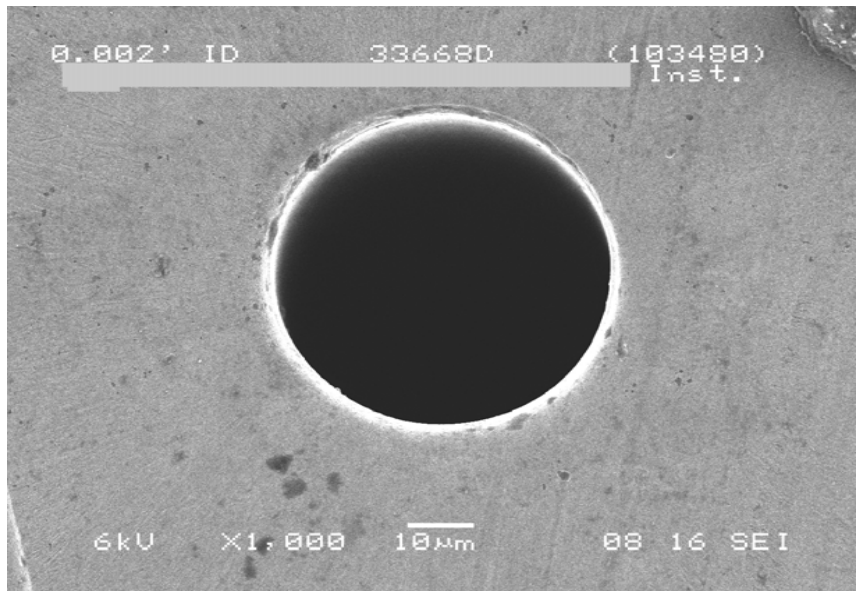


New ACQUITY 2/1000 316 SS Tubing

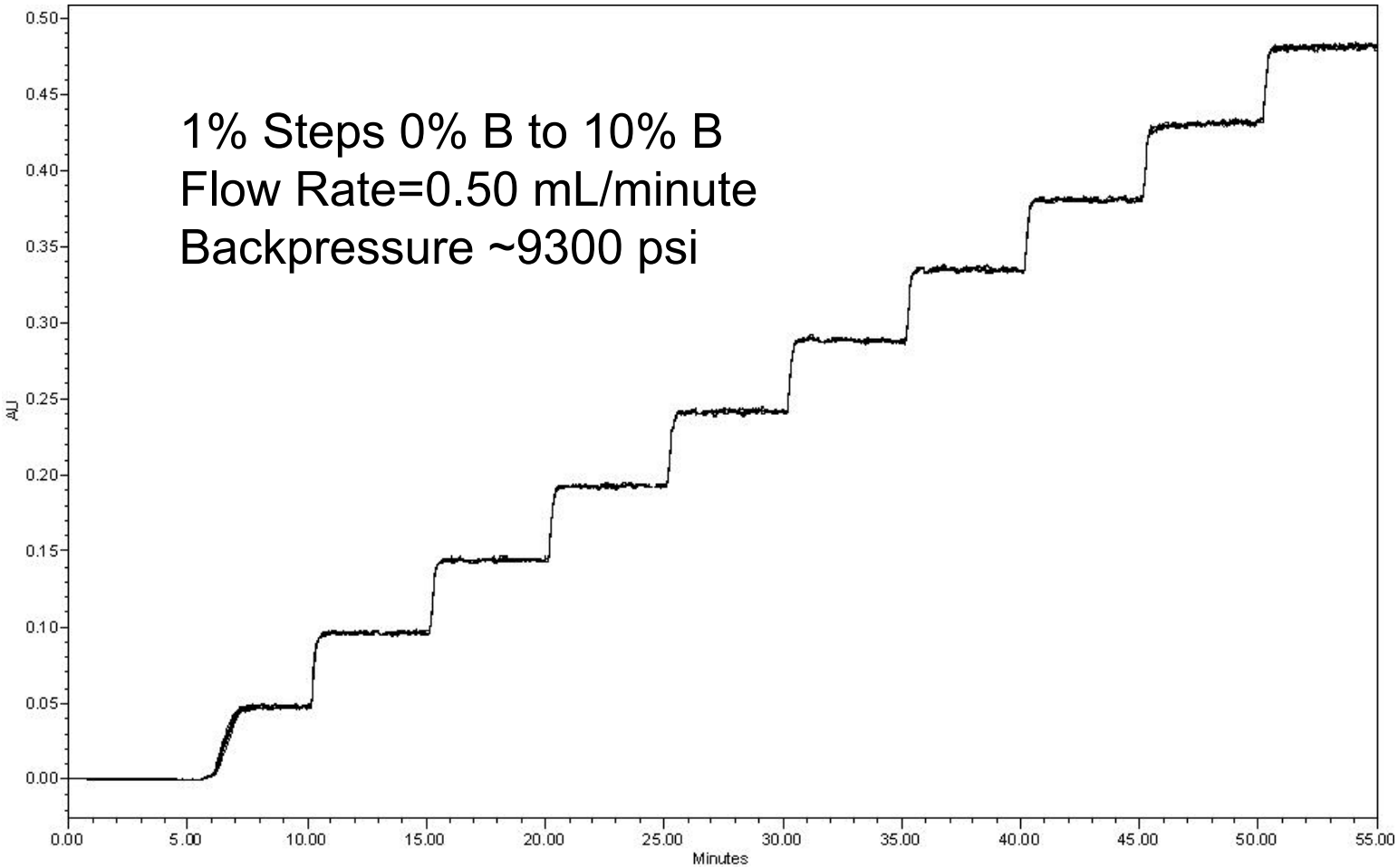
@75 Times

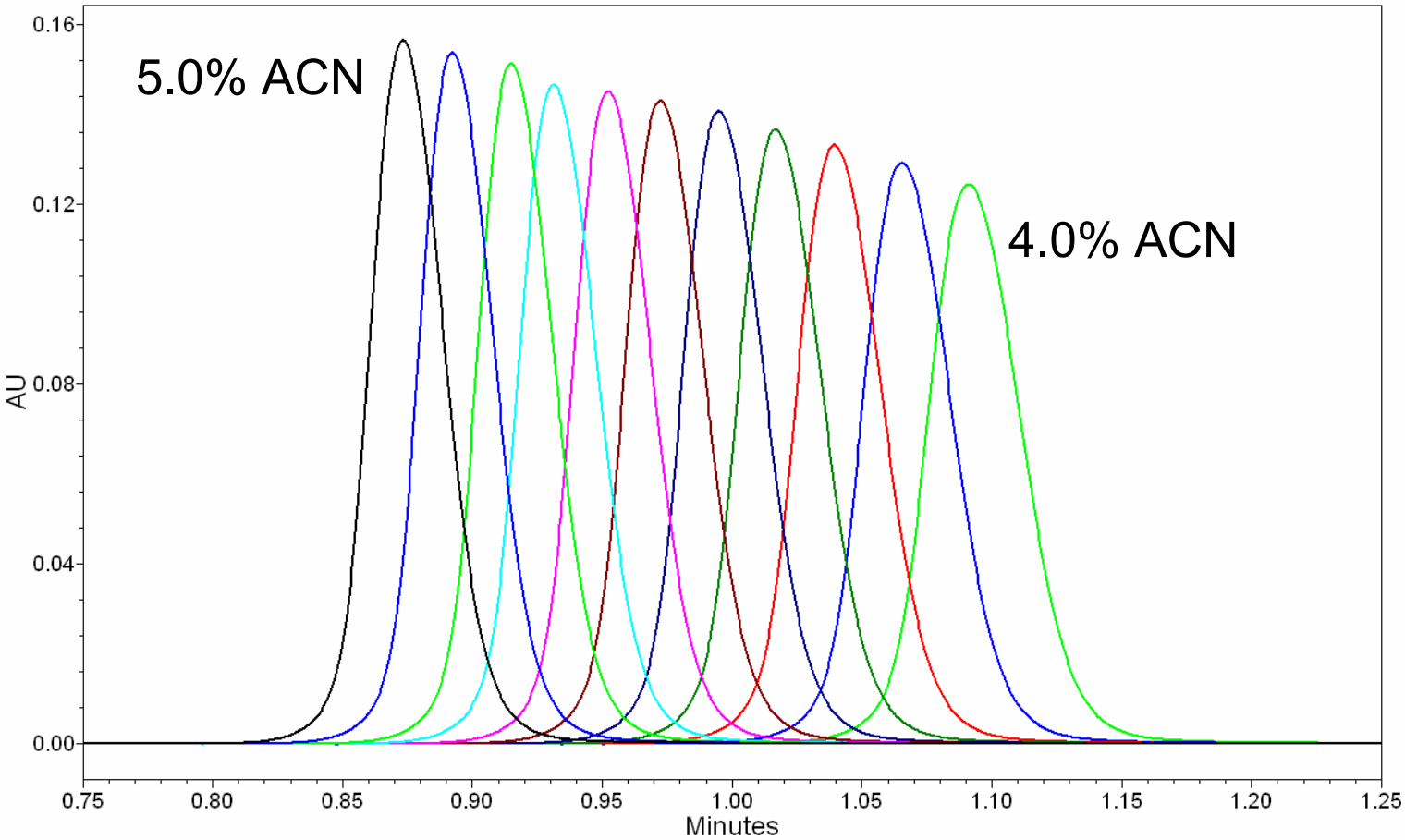


@1000 Times



N = 6





Injections of Caffeine with Dial-a-Mix at 1.0mL/min
4.0% to 5.0% ACN in 0.1% Increments

Original Assay Method from the USP for: Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Solution

- *Mobile phase*— Prepare a mixture of methanol, water, and 0.25 *M* sodium 1-heptanesulfonate (500:500:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621).

- *Chromatographic system* (see *Chromatography* [621])—The liquid chromatograph is equipped with a 313-nm detector, and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute.

- *Standard preparation*— Transfer about 140 mg of *USP Benzocaine RS*, accurately weighed, to a 100-mL volumetric flask with the aid of 25 mL of methanol, and swirl. Transfer about 140 *J* mg of *USP Butamben RS*, accurately weighed, to the same volumetric flask with the aid of 25 mL of water, *J* being the ratio of the labeled amount, in percent, of butamben to the labeled amount, in percent, of benzocaine in the Topical Solution. Transfer about 140 *J¢* mg of *USP Tetracaine Hydrochloride RS*, accurately weighed, to the same volumetric flask with the aid of 25 mL of water, *J¢* being the ratio of the labeled amount, in percent, of tetracaine hydrochloride to the labeled amount, in percent, of benzocaine in the Topical Solution. Sonicate for about 1 minute, dilute with *Diluent* to volume, and mix.

- *Procedure*— Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas for the major peaks.

- Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.3 for benzocaine, 0.8 for butamben, and 1.0 for tetracaine; the resolution, ***R,*** **between the benzocaine peak and the butamben peak and between the butamben peak and the tetracaine peak is not less than 2; and the relative standard deviation for replicate injections is not more than 2.0% for each of the three analyte peaks.**

Acquity

Component Summary Area RT Report

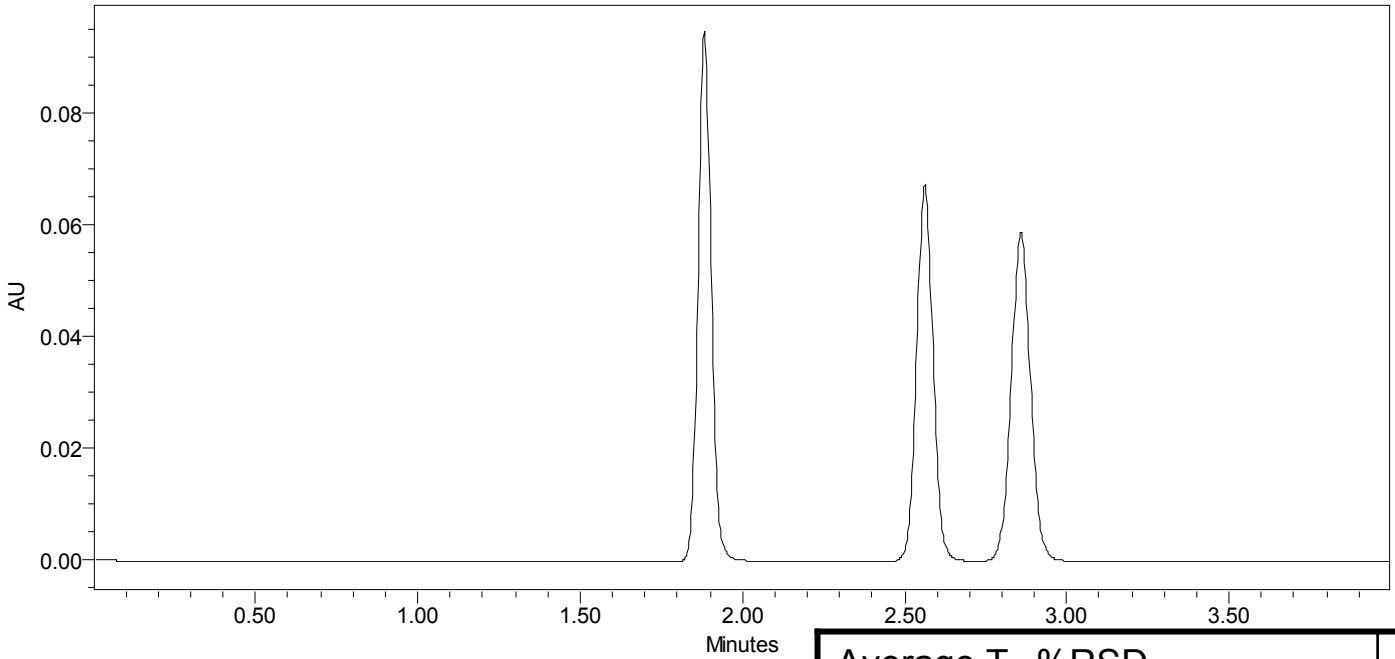
Reported by User: Andrew Aubin (Aubin) Project Name: BBT

Component Summary For Retention Time

	SampleName	Inj	Vial	Benzocaine	Butamben	Tetracaine
1	Ben Tetra Butam	10	2:F,1	0.234	0.366	0.487
2	Ben Tetra Butam	11	2:F,1	0.236	0.371	0.493
3	Ben Tetra Butam	12	2:F,1	0.235	0.370	0.492
4	Ben Tetra Butam	13	2:F,1	0.235	0.369	0.492
5	Ben Tetra Butam	14	2:F,1	0.235	0.369	0.492
6	Ben Tetra Butam	15	2:F,1	0.235	0.370	0.492
7	Ben Tetra Butam	16	2:F,1	0.234	0.368	0.491
8	Ben Tetra Butam	17	2:F,1	0.234	0.366	0.487
9	Ben Tetra Butam	18	2:F,1	0.236	0.371	0.494
10	Ben Tetra Butam	19	2:F,1	0.235	0.369	0.492
11	Ben Tetra Butam	20	2:F,1	0.235	0.369	0.492
Mean				0.235	0.369	0.491
Std. Dev.				0.001	0.002	0.002
% RSD				0.250	0.426	0.426

Standard Deviations of 35, 94, 125 milliseconds

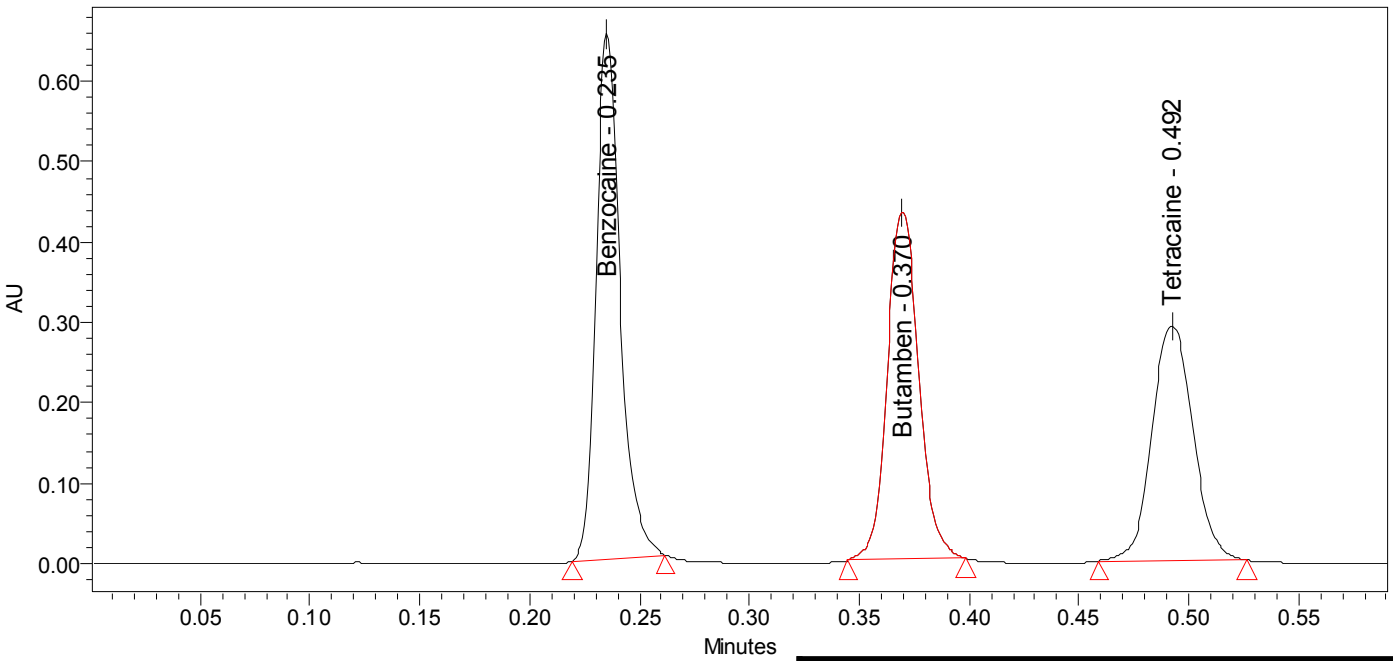
Separation of Benzocaine, Butamben, and Tetracaine



Column – XTerra RP C18, 5 um, 4.6 X 250 mm
Solvent A – 10 mMol Ammonium Bicarbonate pH=10
Solvent B – Acetonitrile
Flow Rate - 2.0 mL/min
Column Temperature – 45 °C
Isocratic 40% A + 60% B
Detection - PDA in 2D mode, 301 nm at 4.8 nm bandwidth
Data rate: 5 pps filtering constant 0.5
Injection Volume - 2 uL of 0.07 mg/mL (5/100 dilution of USP Standard)

Average T_R %RSD	0.133%
Average Peak Area %RSD	0.467%
Average Peak Width @ 4.4%	7.2 sec
Average $w_{½}$ Peak Width	3.2 sec.
Minimum Resolution	2.9
Total Cycle Time	351 sec
Solvent Used	11.7 mL

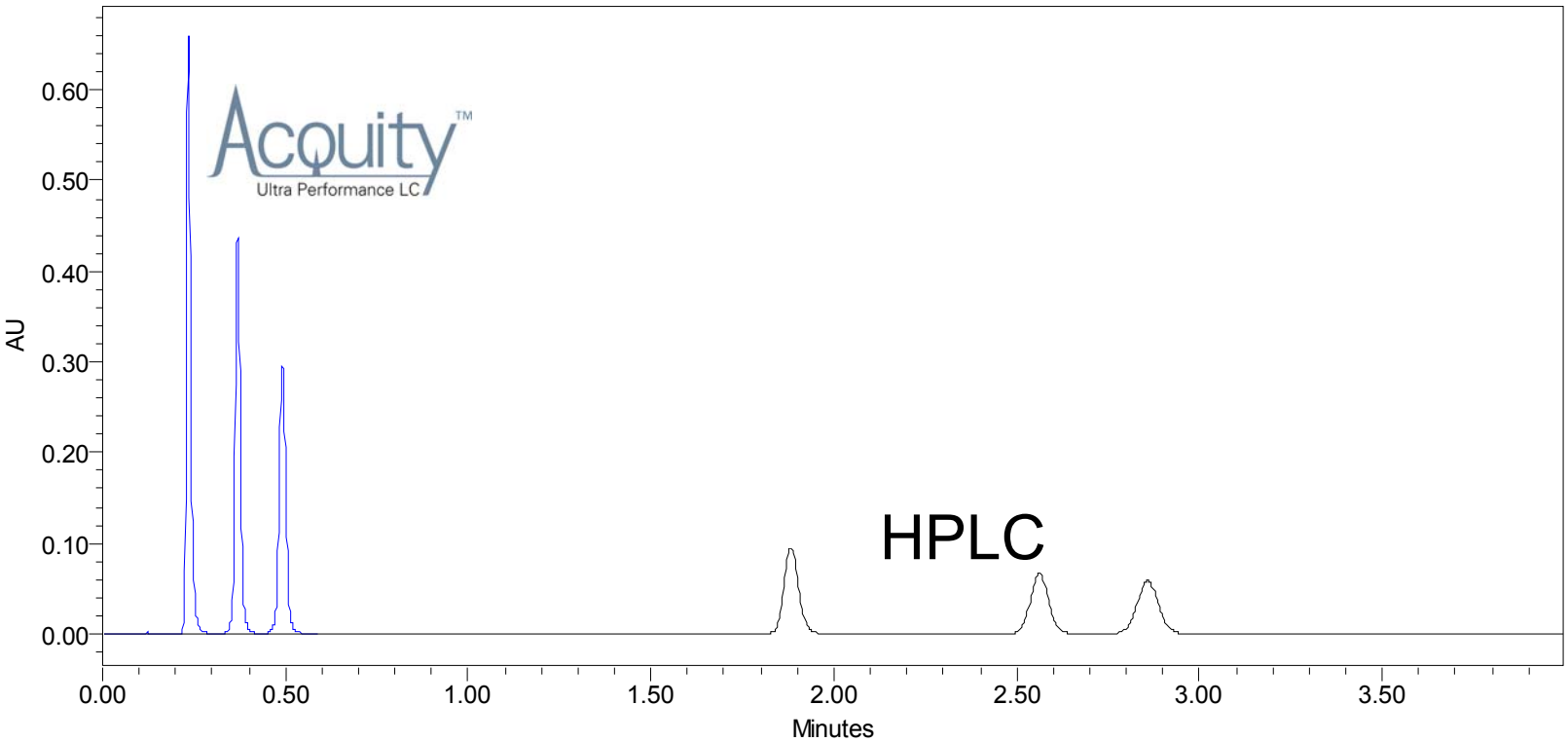
USP Separation of Benzocaine, Butamben, and Tetracaine



Column – ACQUITY UPLCTM BEH C18 2.1 X 50 mm, 1.7 µm
Solvent A – 10 mMol Ammonium Bicarbonate pH=10
Solvent B – Acetonitrile
Flow Rate - 1.0 mL/min
Column Temperature – 45 °C
Isocratic 50% A + 50% B
Detection - PDA in 2D mode, 301 nm at 4.8 nm bandwidth
Data rate: 20 pps Filter constant: 0.1
Injection Volume 2 uL of 0.07 mg/mL (5/100 dilution of USP Standard)

Average T_R %RSD	0.370%
Average Peak Area %RSD	0.467%
Average 4 Sigma Peak Width	2.1 sec
Average $w_{½}$ Peak Width	0.93 sec.
Minimum Resolution	4.2
Total Cycle Time	65 sec
Solvent Used	1.1 mL

Speed, Sensitivity, Resolution



Changes to Original Assay Method from the USP for: Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Solution

- *Mobile phase*— Prepare a mixture of acetonitrile, water containing 10 mMol ammonium bicarbonate at a pH of 10.0 (1:1) for UPLC, 4:6 for HPLC. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621).

- *Chromatographic system* (see *Chromatography* [621])—The liquid chromatograph is equipped with a 301-nm detector, and a 4.6-mm × 25-cm column that contains packing L1 (XTerra RP18, 5 um) for HPLC and a 2.1 X 50 mm 1.7 um for UPLC. The flow rate is about 1 mL per minute for UPLC and 2 mL per minute for HPLC.

- *Standard preparation*— Transfer about 140 mg of *USP Benzocaine RS*, accurately weighed, to a 100-mL volumetric flask with the aid of 25 mL of methanol, and swirl. Transfer about 140 *J* mg of *USP Butamben RS*, accurately weighed, to the same volumetric flask with the aid of 25 mL of water, *J* being the ratio of the labeled amount, in percent, of butamben to the labeled amount, in percent, of benzocaine in the Topical Solution. Transfer about 140 *J¢* mg of *USP Tetracaine Hydrochloride RS*, accurately weighed, to the same volumetric flask with the aid of 25 mL of water, *J¢* being the ratio of the labeled amount, in percent, of tetracaine hydrochloride to the labeled amount, in percent, of benzocaine in the Topical Solution. Sonicate for about 1 minute, dilute with *Diluent* to volume, and mix. Further dilute this stock 5 in100 for UPLC use.

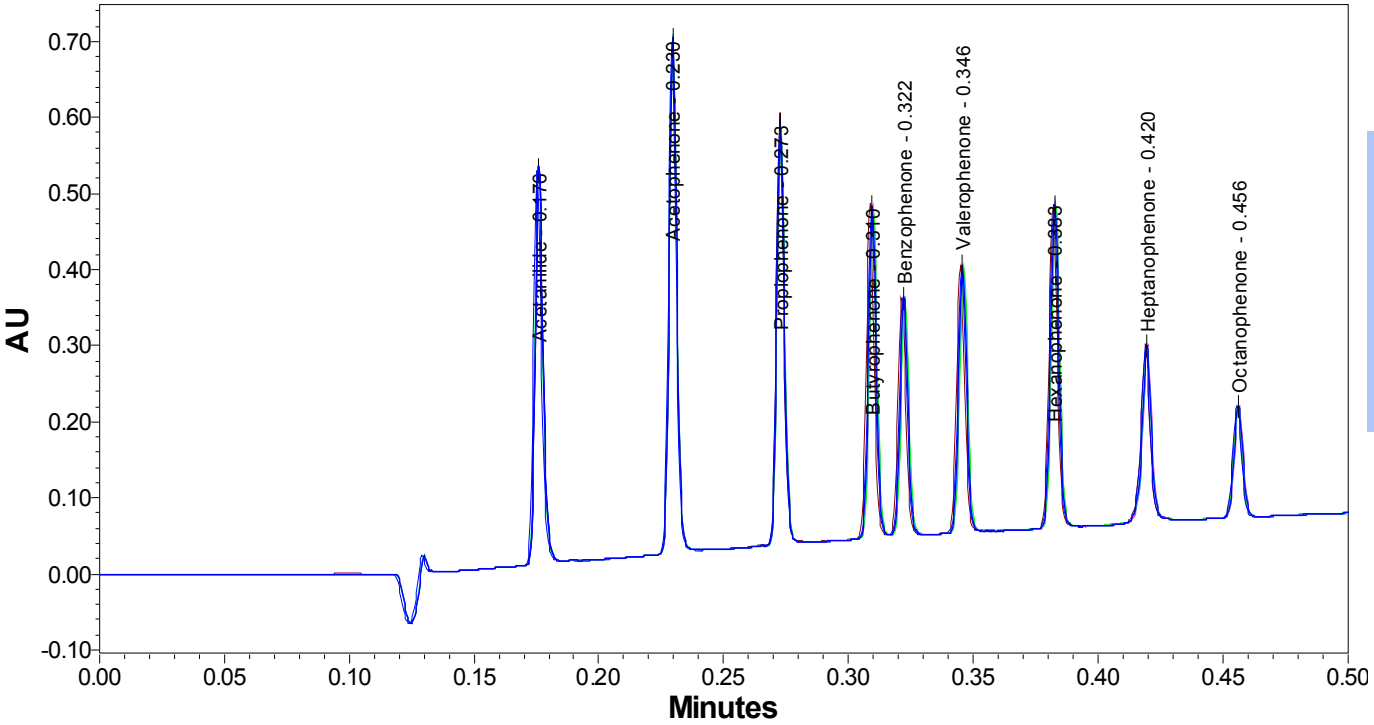
- *Procedure*— Separately inject equal volumes (about 2 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas for the major peaks.

- Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.3 for benzocaine, 0.8 for butamben, and 1.0 for tetracaine; the resolution, *R,* between the benzocaine peak and the butamben peak and between the butamben peak and the tetracaine peak is not less than 2; and the relative standard deviation for replicate injections is not more than 2.0% for each of the three analyte peaks.

Waters

ACQUITY UPLC™

Analytical Performance with Gradient conditions



6 injections
9 phenones
(3µL, 0.1 mg/mL)
30 sec. gradient

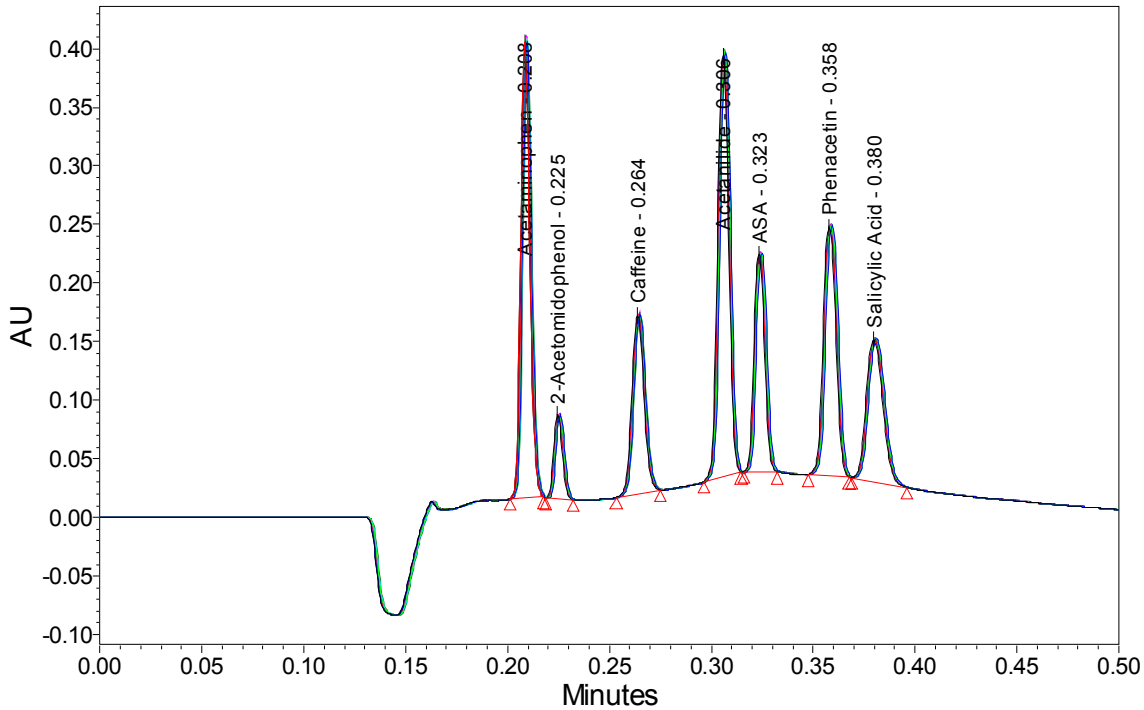
Column:	2.1 x 50 mm @ 65°C
Mobile Phase A:	0.1% Formic Acid in Water
Mobile Phase B:	0.1% Formic Acid in ACN
Flow Rate:	1.3 mL/min
Gradient:	30% - 90% B over 0.5minutes, Curve 5
Wash Solvents:	Strong – 50/50 Water/ACN (200µL); Weak – 95/5 Water/ACN 600µL)
Wavelength:	240nm
Data Rate:	40 pts/s, Filter Constant: 0.0

Average T_R %RSD	0.109%
Average Peak Area %RSD	0.368%
Average 4 Sigma Peak Width	0.369 sec
Average $w_{½}$ Peak Width	0.210 sec
Minimum Resolution	2.15
Total Cycle Time	56 sec
Maximum Pressure	13,500 psi

Waters

ACQUITY UPLC™

Analytical Performance with Gradient conditions



Ave RT %RSD	0.115%
Ave Area %RSD	0.133%
Minimum Resolution	1.70
Total Cycle Time	56 sec
Maximum Pressure	11,000 psi

10 Replicate Injections of a 7 component Analgesic Mix

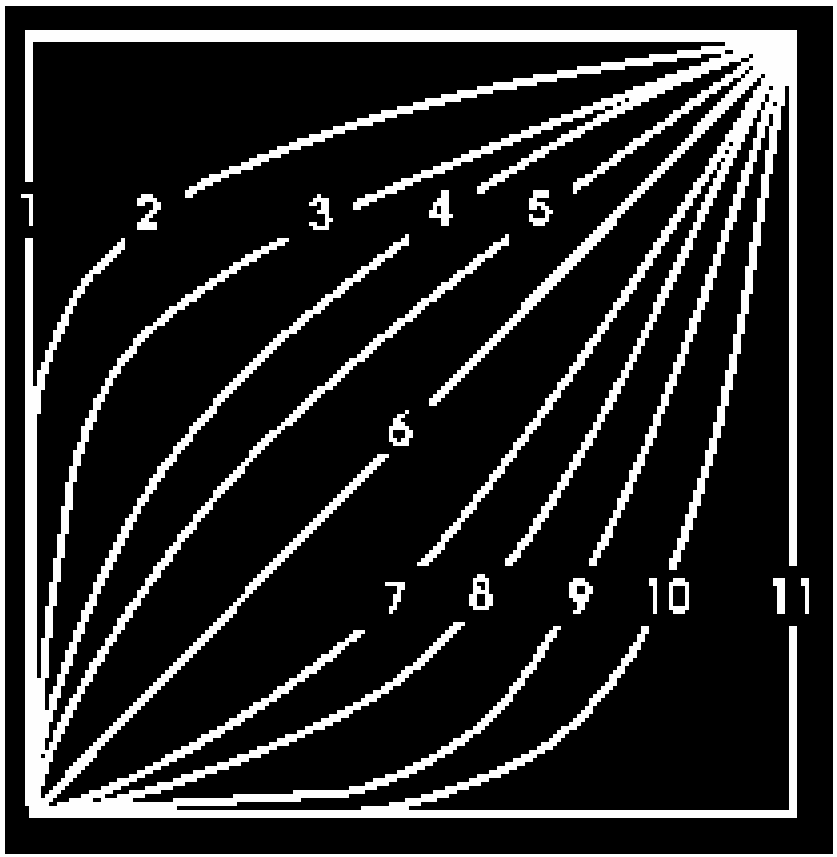
(5µL, 0.1 – 0.2 mg/mL in water)

Column: 2.1x50mm @ 65°C
Mobile Phase A: 0.1% Formic Acid in Water
Mobile Phase B: 0.1% Formic Acid in ACN
Flow Rate: 1.3 mL/min
Gradient:

Time	%A	%B	Curve
Initial	93	7	-
0.10	80	20	4
0.18	65	35	6
0.41	93	7	6

Wash Solvents: Strong – 50/50 Water/ACN, 100µL
Weak – 98/2 Water/ACN, 500µL
Wavelength: 240nm
Data Rate: 40 pts/s, Filter Constant: 0.0

Gradient Profiles on ACUITY UPLC™



Curve #	Effect
1	Immediatly to specified condition
2 to 5	Convex
6	Linear
6 to 10	Concave
11	Maintain start conditions

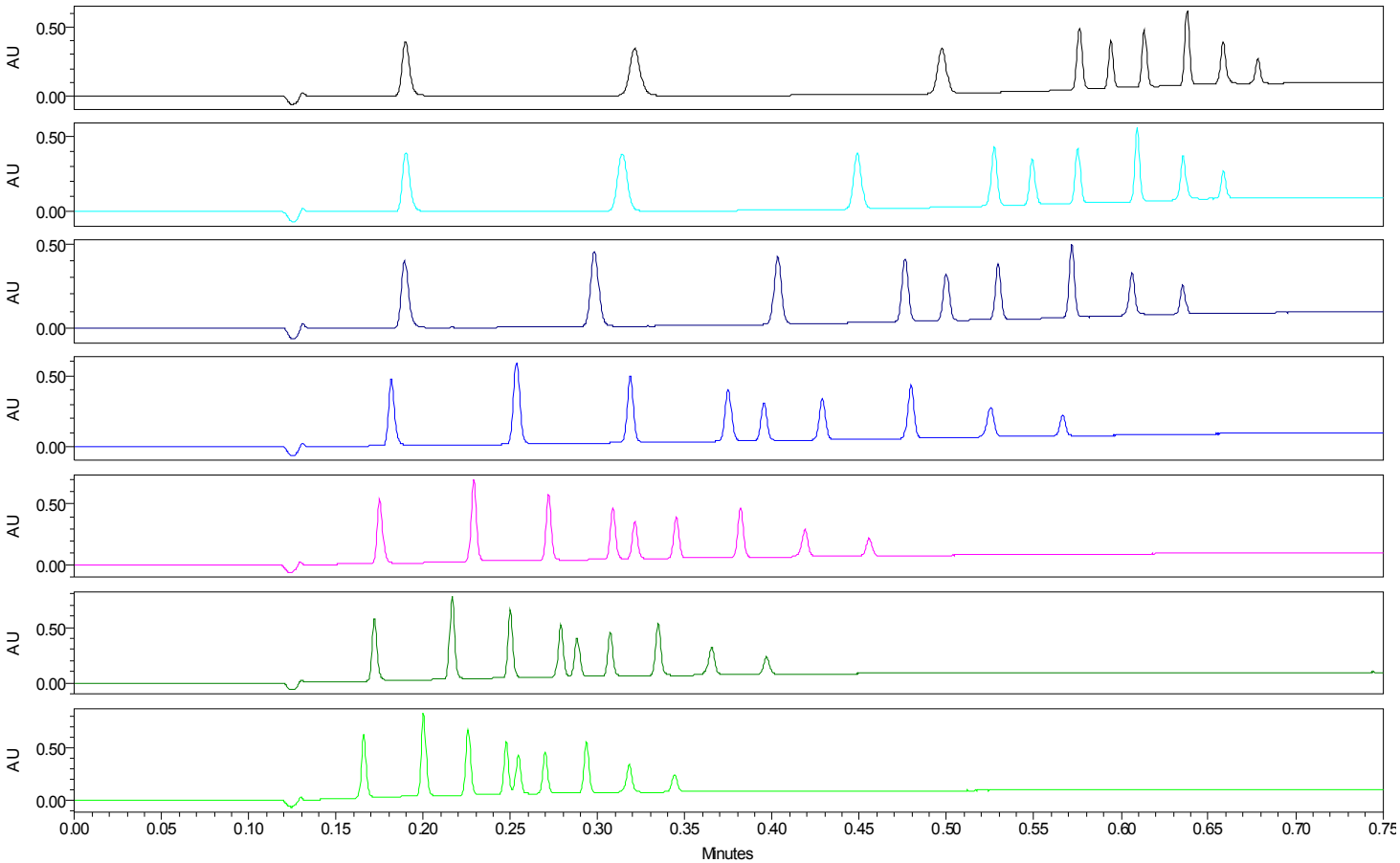
Utility of Non-linear gradient capability

Method Development Challenge:

Gradient elution of 9 component mix

- To go as fast as possible
- To meet a minimum resolution requirement of 2.0
- Start with generic method 30s gradient with 15s hold at final conditions. (5% to 95% ACN)
- Run gradient using curves 3-9

Chromatograms of Curves 3-9



Curve 9

Curve 8

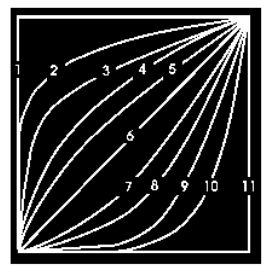
Curve 7

Curve 6

Curve 5

Curve 4

Curve 3



Run Time Results and Minimum Resolution

Curve	3	4	5	6	7	8	9
Resolution	1.32	1.76	2.17	3.01	3.59	3.61	3.36
Elution Time of Last Peak	0.34	0.40	0.46	0.57	0.64	0.66	0.68

Shortest Run Time with required resolution is using Curve 5

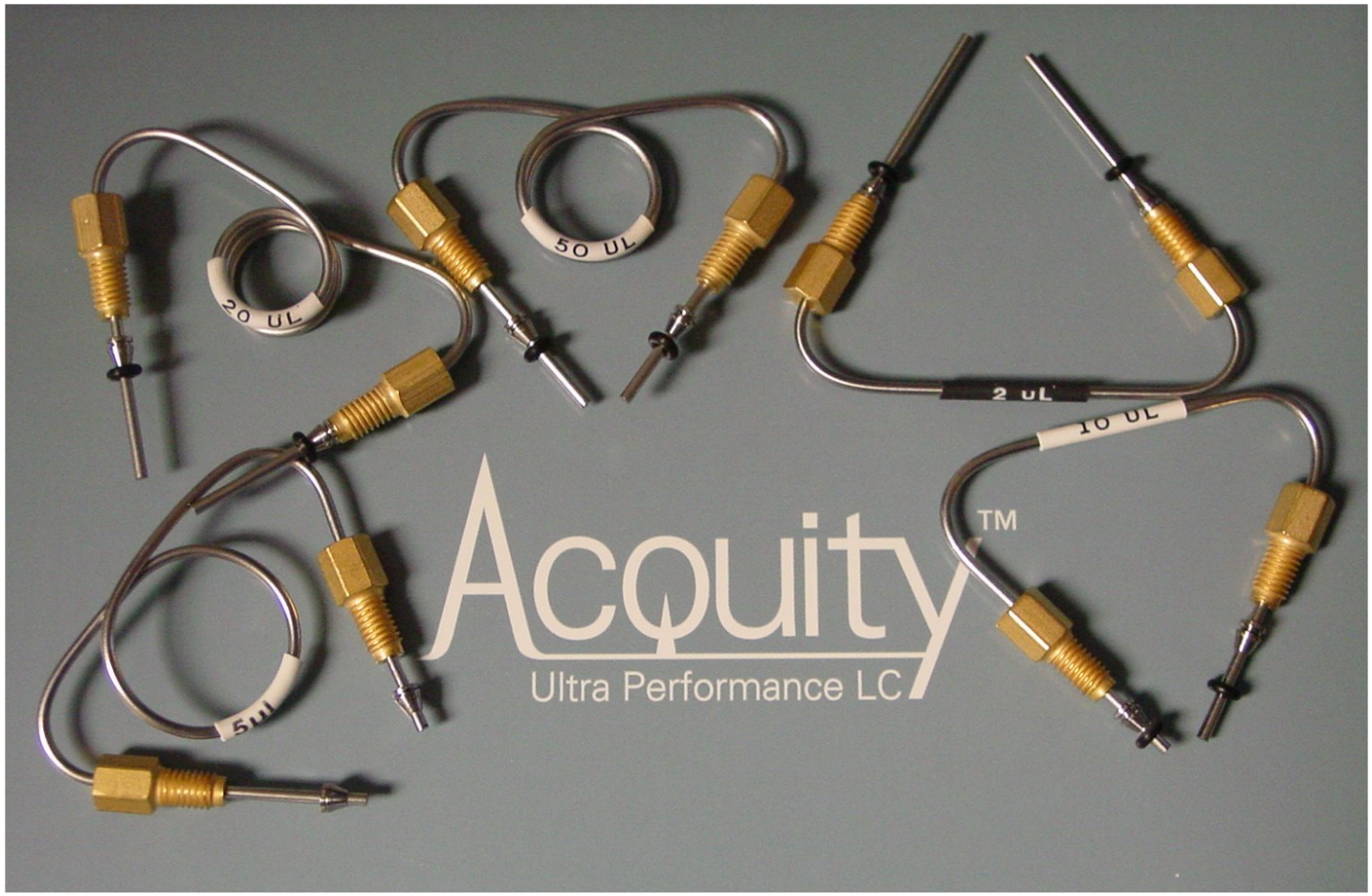
New Technologies - *all-new* injection process

- Pressure tolerant, low volume flow-through isolation & injection valves
- Fast cycle times, low carry over
- 2 Plate capacity, multiple formats
  - Well plates, microtube plates, vials
- Thermal control (4 - 40°C)
- Low dispersion column management
- Sample Organizer (optional)

“Pod” Style Injector

- Modular design (removable “Pod”) permits easy access

ACQUITY UPLC™ Loops available



2 µL
5 µL
10 µL
20 µL
50 µL

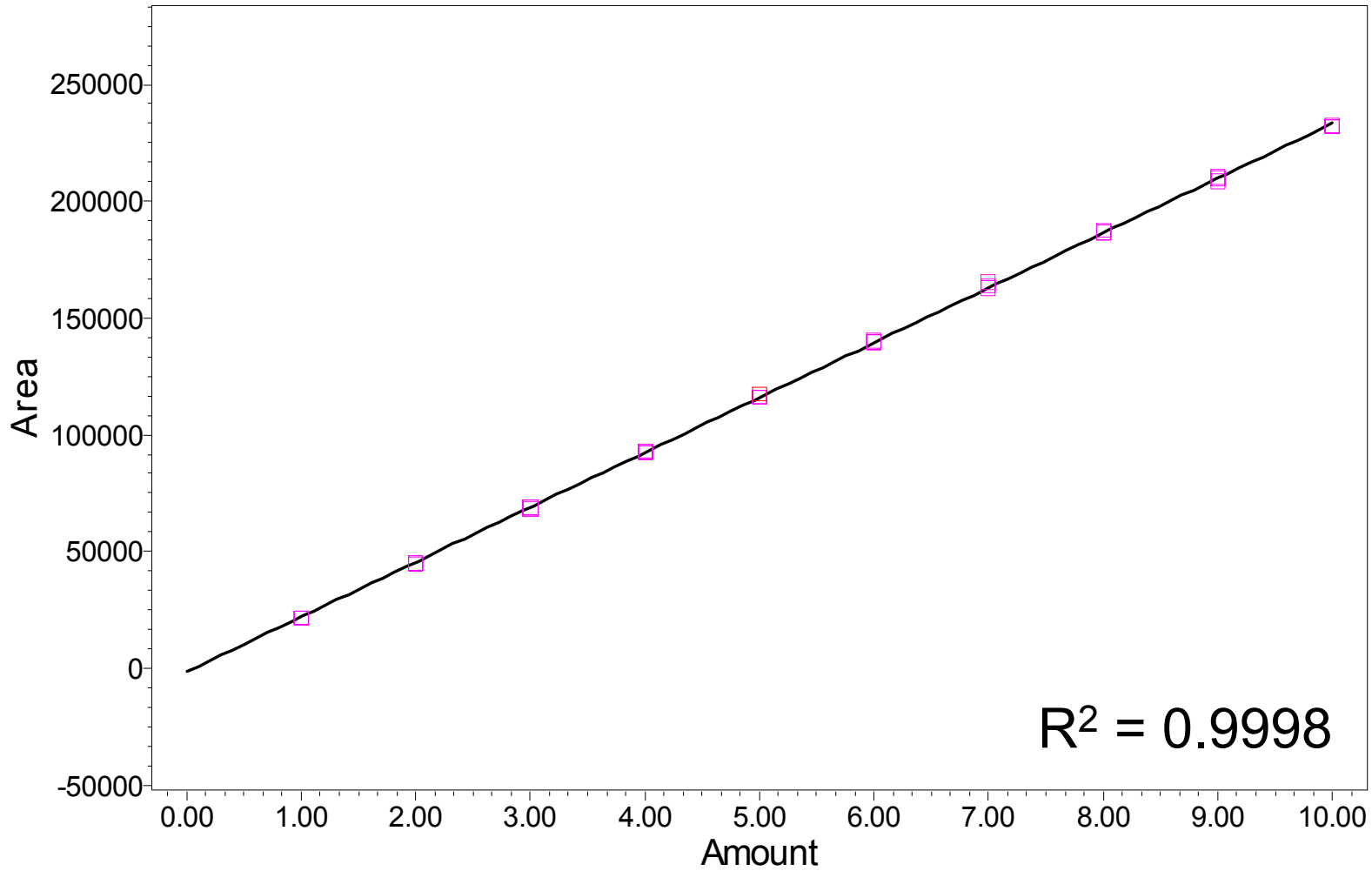
Acquity Ultra Performance LC

Component Summary Area RT Report

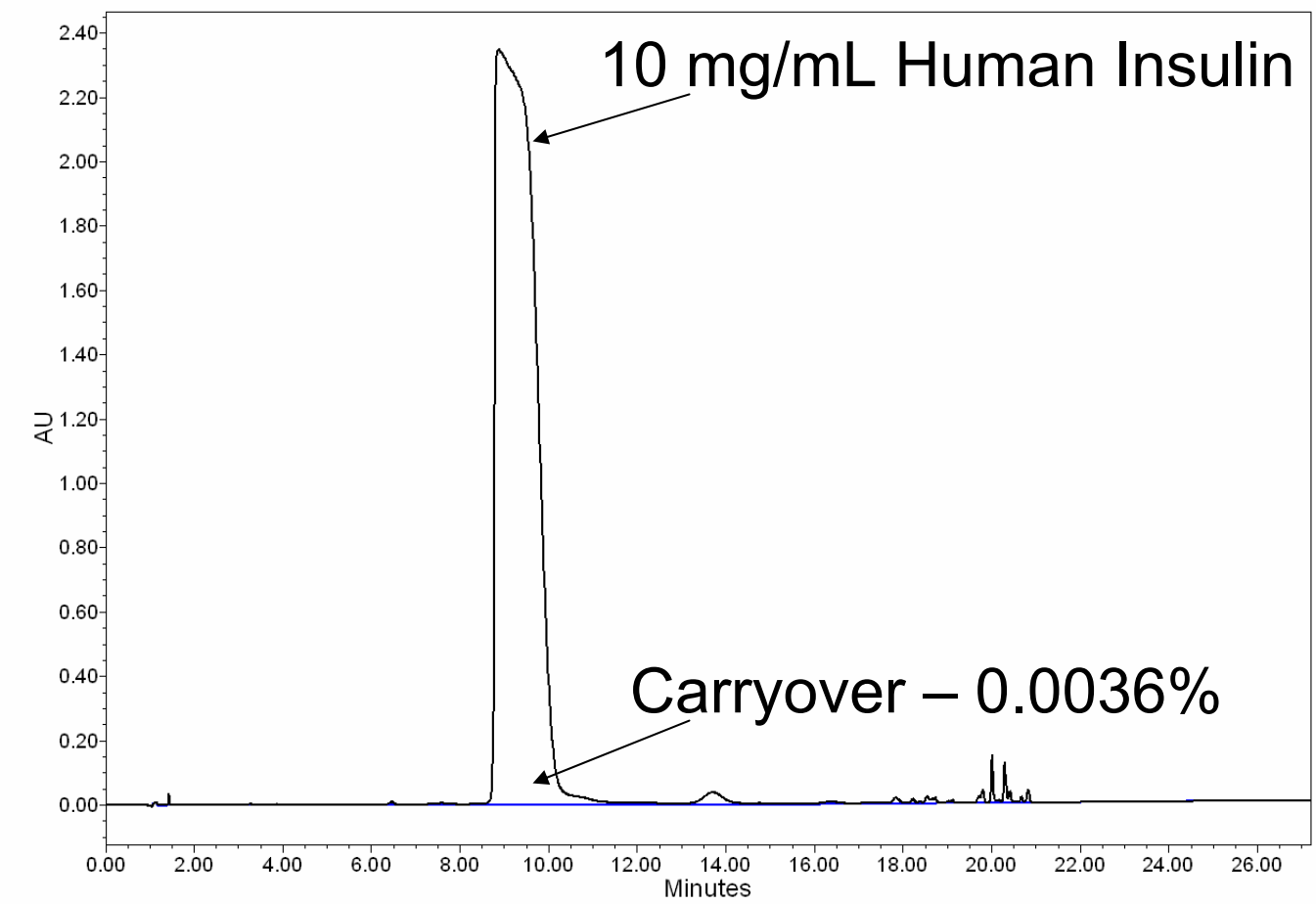
Reported by User: Andrew Aubin (Aubin) Project Name: PITTCON_2005

Component Summary For Area

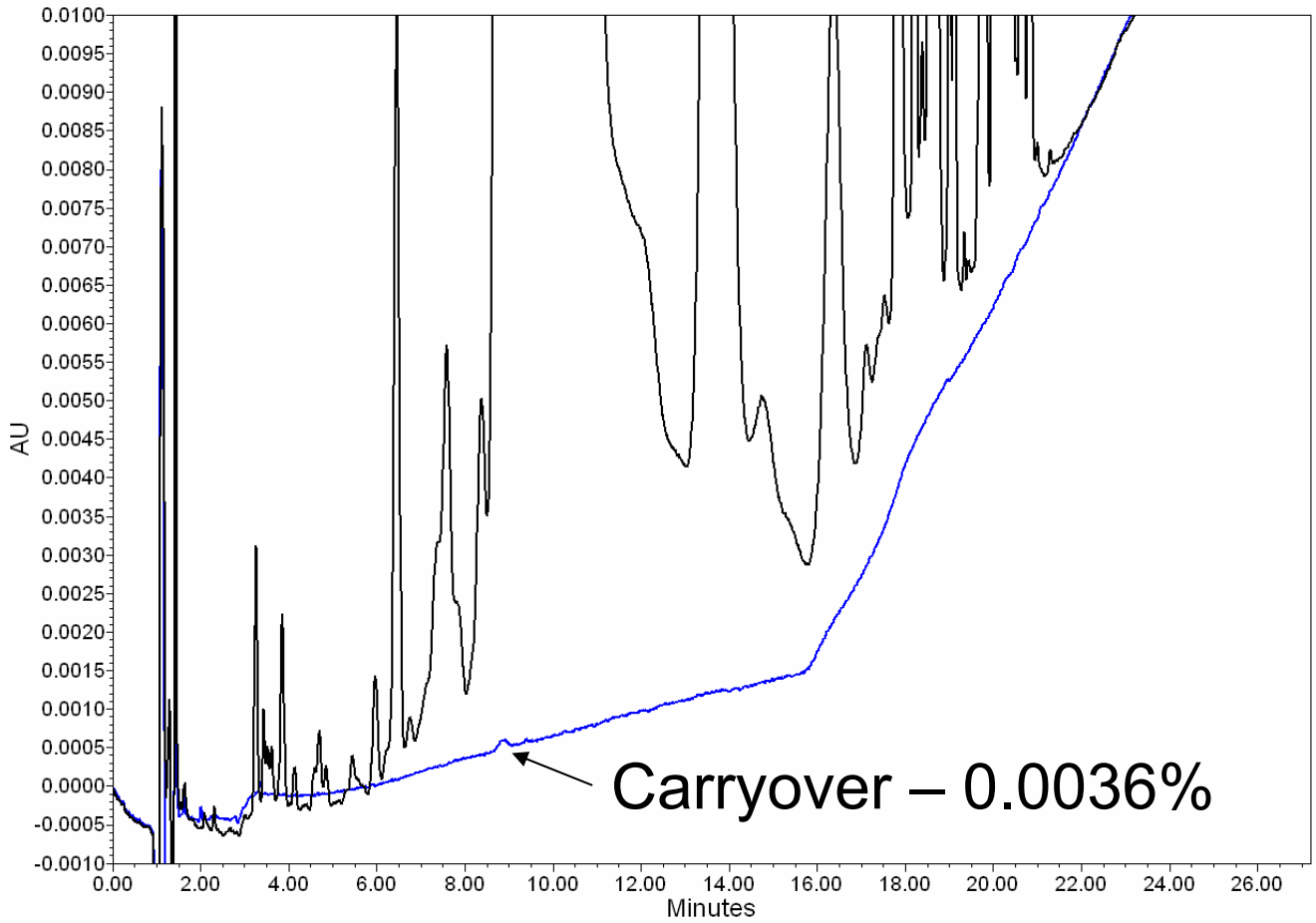
	SampleName	Date Acquired	Inj Vol (ul)	Inj	Vial	3,4-MDA	2,3-MDA	3,4-MDMA	2,3-MDMA
1	2,3+3,4 MDA MDMA	2/14/2005 10:28:57 AM	5.00	1	2:D,1	252746	169926	235323	164552
2	2,3+3,4 MDA MDMA	2/14/2005 10:33:42 AM	5.00	1	2:D,1	251676	169802	235335	163520
3	2,3+3,4 MDA MDMA	2/14/2005 10:36:38 AM	5.00	1	2:D,1	252557	169395	235366	163371
4	2,3+3,4 MDA MDMA	2/14/2005 10:39:35 AM	5.00	1	2:D,1	251382	169090	232696	162642
5	2,3+3,4 MDA MDMA	2/14/2005 10:42:31 AM	5.00	1	2:D,1	251354	168659	234721	163114
6	2,3+3,4 MDA MDMA	2/14/2005 10:45:27 AM	5.00	1	2:D,1	250666	169286	234564	163830
7	2,3+3,4 MDA MDMA	2/14/2005 10:48:24 AM	5.00	1	2:D,1	252694	168439	235468	163120
8	2,3+3,4 MDA MDMA	2/14/2005 10:51:20 AM	5.00	1	2:D,1	252331	169251	235365	163195
9	2,3+3,4 MDA MDMA	2/14/2005 10:54:14 AM	5.00	1	2:D,1	252360	168626	234133	164507
10	2,3+3,4 MDA MDMA	2/14/2005 10:57:11 AM	5.00	1	2:D,1	252659	168037	234904	162978
Mean						252043	169051	234787	163483
Std. Dev.						722	604	856	636
% RSD						0.29	0.36	0.36	0.39



0.1 mg/mL Acetaminophen with 20µL loop in Pressure-Assist Mode



Wash Conditions:
200µL Strong (6:3:1 / 0.1% phosphoric in Water:ACN:IPA) 600µL Weak (0.01M HCl)



Wash Conditions:
200µL Strong (6:3:1 / 0.1% phosphoric in Water:ACN:IPA) 600µL Weak (0.01M HCl)

ACQUITY UPLC™ Minimized Carryover

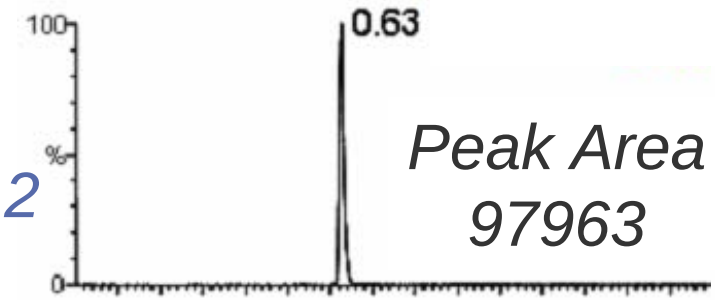
Alprazolam in Plasma

MRM transition

1000 ng/mL

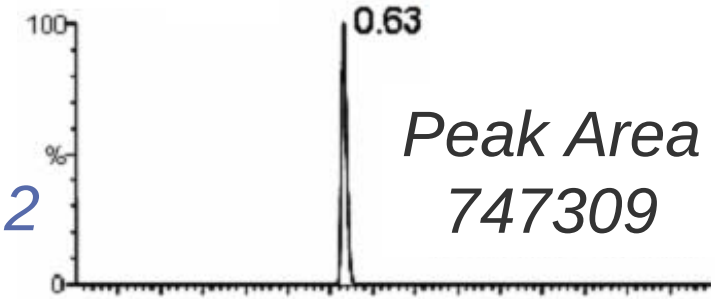
Plasma Blank

314.1>210.2

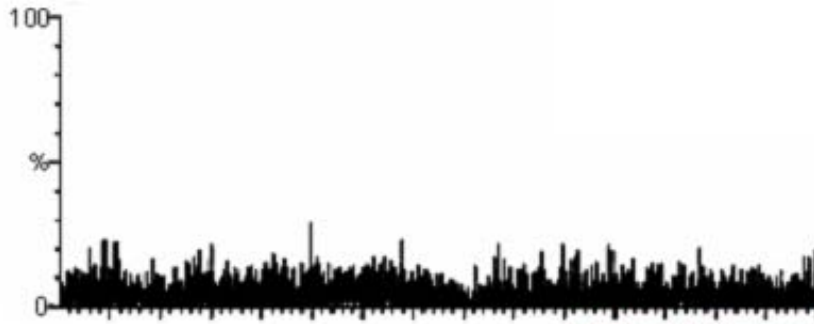


Peak Area 97963

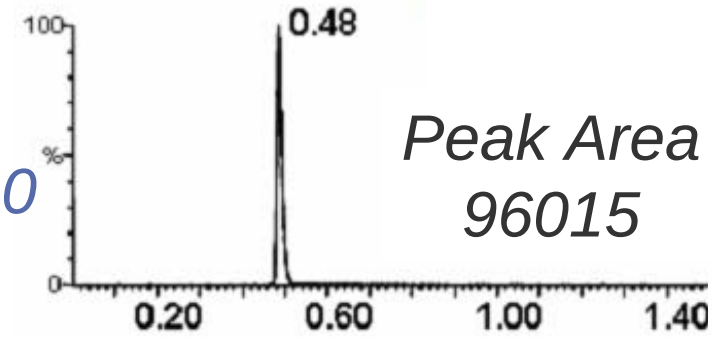
309.0>205.2



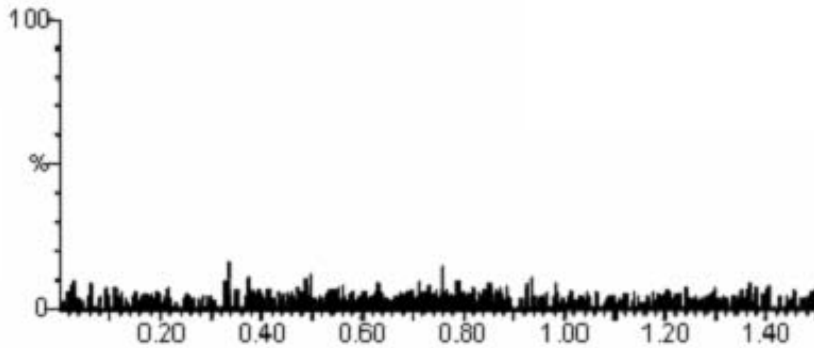
Peak Area 747309



260.2>116.0



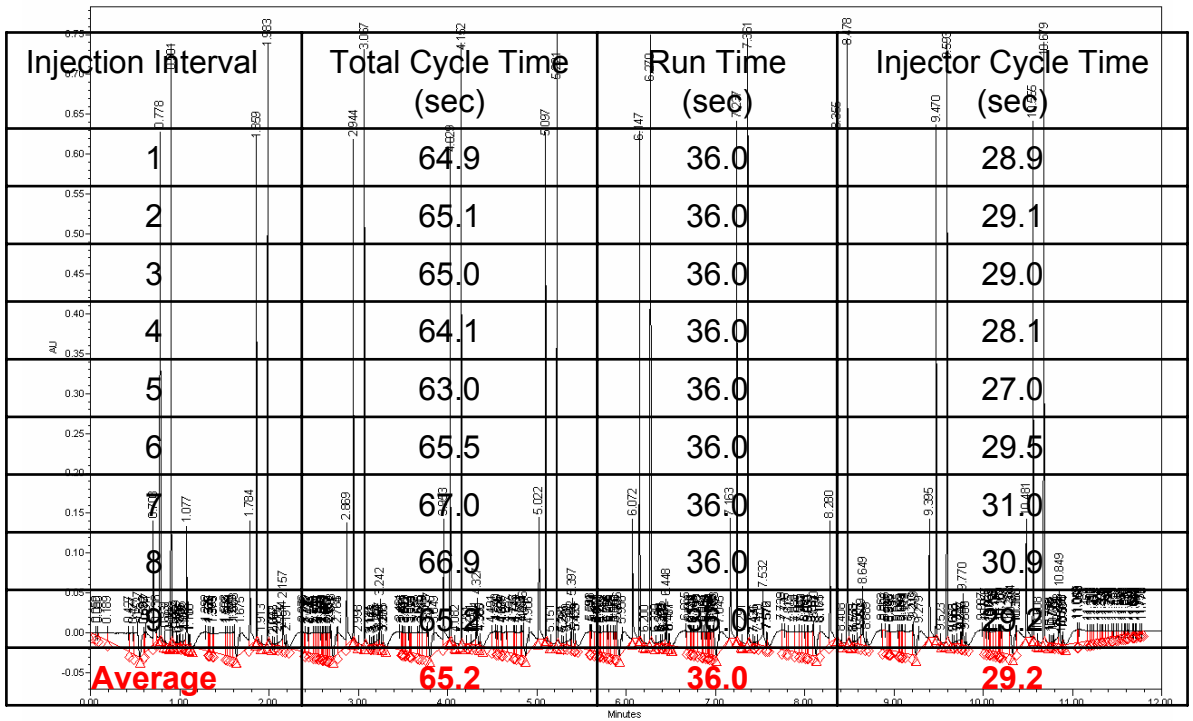
Peak Area 96015



Plumb

ACQUITY UPLC™ Injection-to-Injection Cycle Times

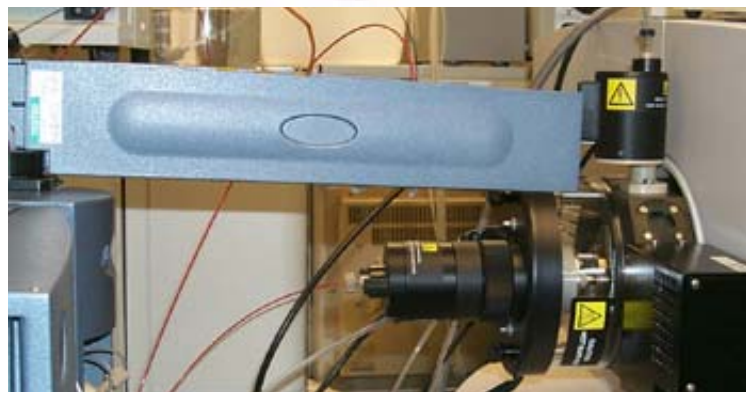
Injection-to-injection cycle times are calculated as the time it takes for the injector to perform all of the tasks during the injection cycle from the point of initiation by the data acquisition software until the valve turns and the injection is made. To determine this value a series of injections are monitored by a detector that is continually collecting data, even between the run times. The difference in time between corresponding peaks of each injection gives the total cycle time. Subtracting the run time from the total cycle time will yield the injection-to-injection cycle time.



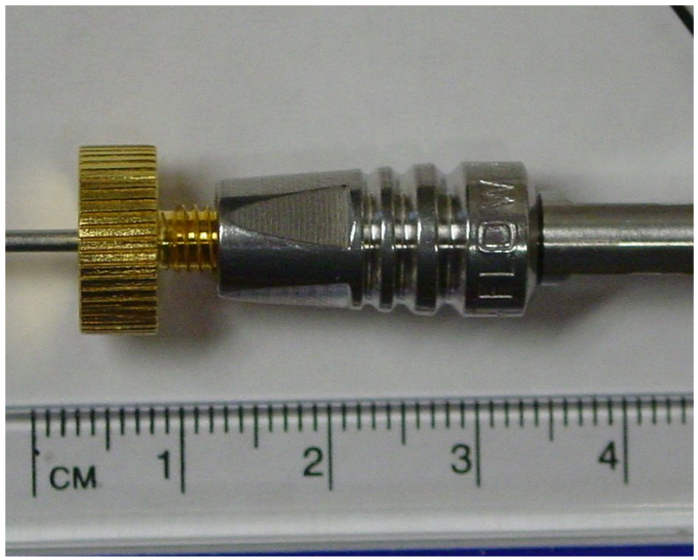
Injection Interval	Total Cycle Time (sec)	Run Time (sec)	Injector Cycle Time (sec)
1	64.9	36.0	28.9
2	65.1	36.0	29.1
3	65.0	36.0	29.0
4	64.1	36.0	28.1
5	63.0	36.0	27.0
6	65.5	36.0	29.5
7	67.0	36.0	31.0
8	66.9	36.0	30.9
Average	65.2	36.0	29.2

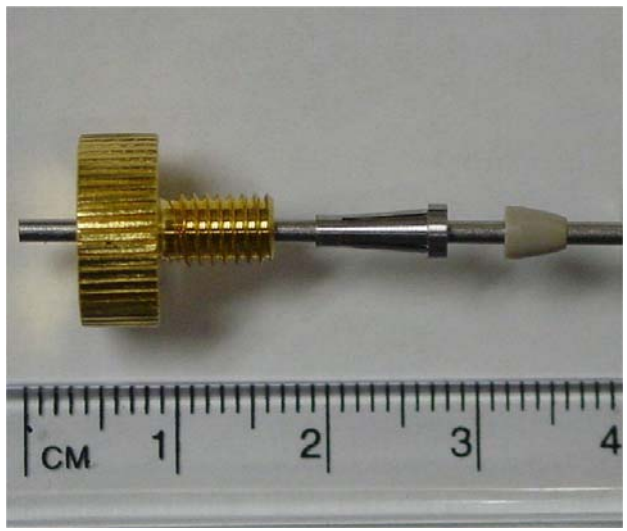
Column Manager

- Symbiotic with Sample Manager
  - Flow path distance optimized
  - 65°C upper limit
  - Up to 150 mm column
- Pivot positioning
  - *Stacked* mode
    - w/ optical detector
  - *Swung out* mode
    - w/ MS detector only
  - Column may be accessed from either side

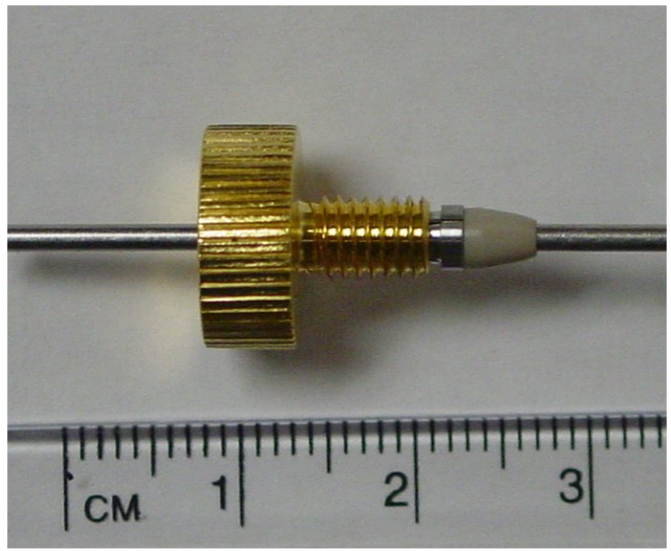


Attaching The Column to the System



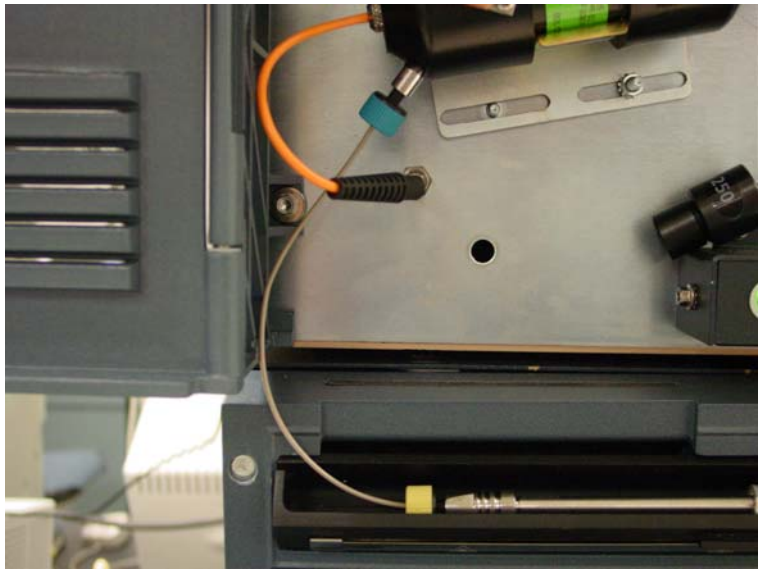


High Pressure Finger Tight Fittings
Holds >15000 psi



Patent Pending

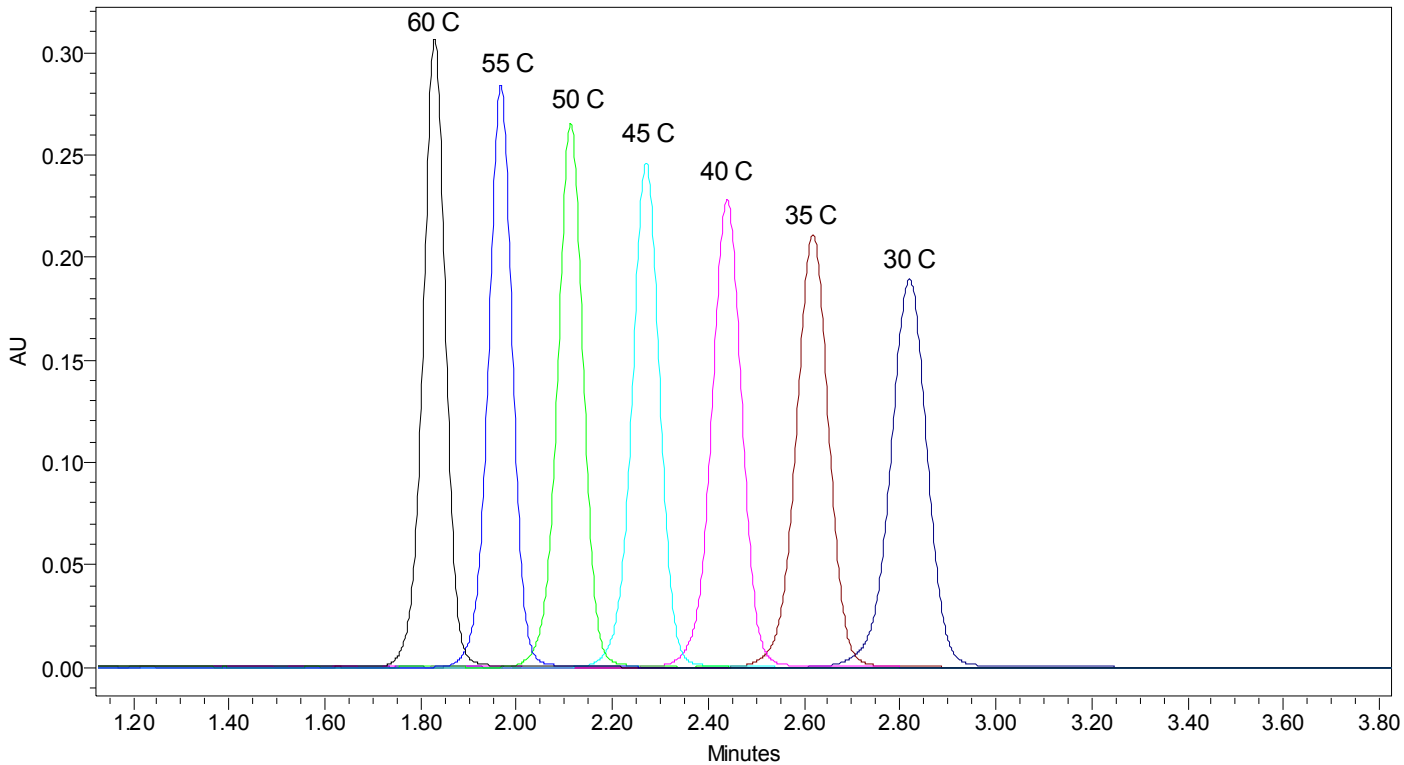
Column Outlet



- One piece finger tight fittings work well
- Cut 0.0025” Peek to length
- Keep as short as possible to minimize dispersion

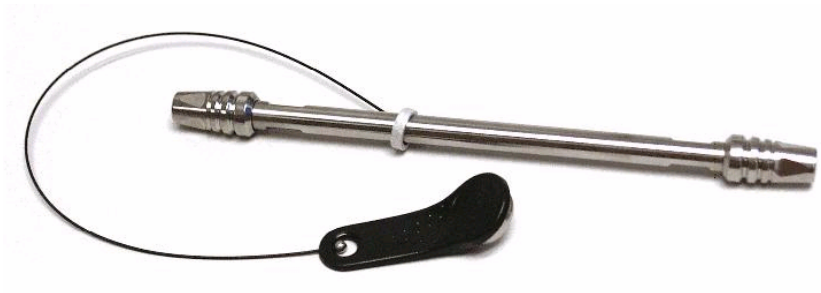
Column Heating

- Column heating considerations
  - Consistent temperature for RT stability
  - Solvent viscosity is lower at higher temperatures
  - Lower backpressures at higher temperatures



eCord™ Technology

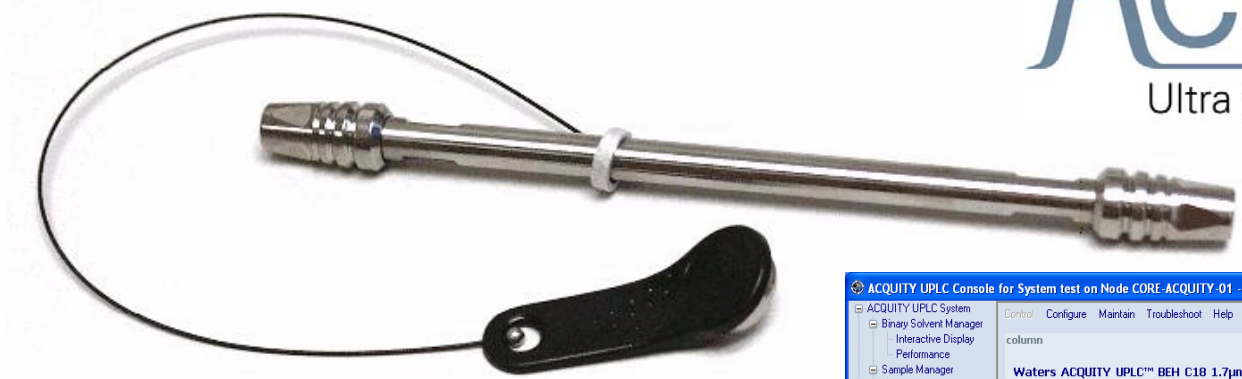
- Column Information
  - Certificate of Analysis
- Column Usage History
  - Total number of samples
  - Injections
  - Pressure/Temperature history
  - And much more…



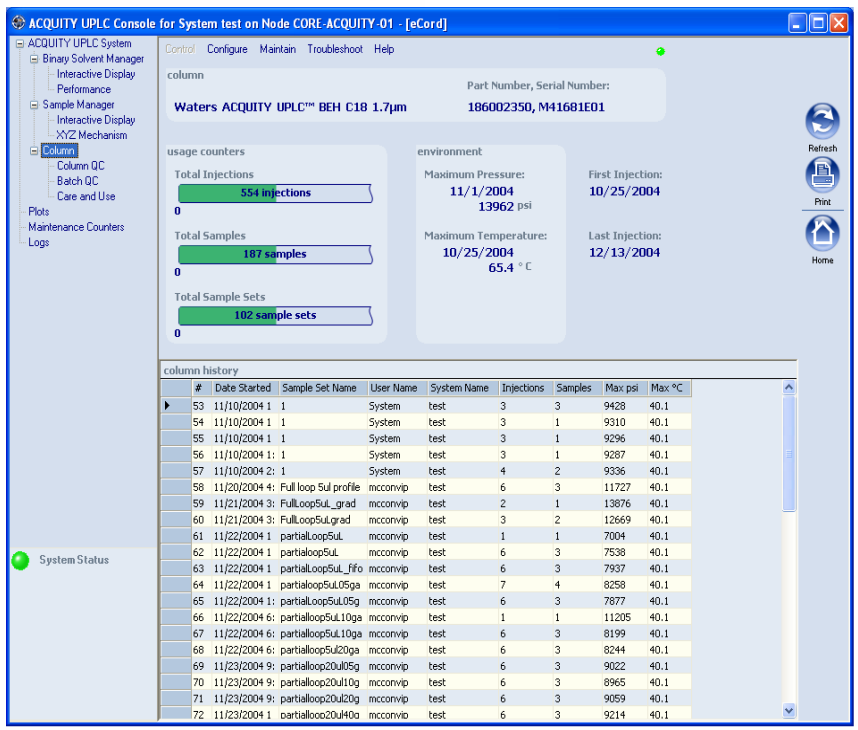
eCord™

ACQUITY UPLC™ *eCord™*

Acquity™ Ultra Performance LC Columns



eCord™



#	Date Started	Sample Set Name	User Name	System Name	Injections	Samples	Max psi	Max °C
53	11/10/2004 1	1	System	test	3	3	9428	40.1
54	11/10/2004 1	1	System	test	3	1	9310	40.1
55	11/10/2004 1	1	System	test	3	1	9296	40.1
56	11/10/2004 1:	1	System	test	3	1	9287	40.1
57	11/10/2004 2:	1	System	test	4	2	9336	40.1
58	11/20/2004 4:	Full loop 5ul profile	mcconvip	test	6	3	11727	40.1
59	11/21/2004 3:	FullLoop5uL_grad	mcconvip	test	2	1	13876	40.1
60	11/21/2004 3:	FullLoop5uLgrad	mcconvip	test	3	2	12669	40.1
61	11/22/2004 1	partialLoop5uL	mcconvip	test	1	1	7004	40.1
62	11/22/2004 1	partialoop5uL	mcconvip	test	6	3	7538	40.1
63	11/22/2004 1	partialLoop5uL_fifo	mcconvip	test	6	3	7937	40.1
64	11/22/2004 1	partialoop5uL05ga	mcconvip	test	7	4	8258	40.1
65	11/22/2004 1:	partialLoop5uL05g	mcconvip	test	6	3	7877	40.1
66	11/22/2004 6:	partialloop5uL10ga	mcconvip	test	1	1	11205	40.1
67	11/22/2004 6:	partialloop5uL10ga	mcconvip	test	6	3	8199	40.1
68	11/22/2004 6:	partialloop5ul20ga	mcconvip	test	6	3	8244	40.1
69	11/23/2004 9:	partialloop20ul05g	mcconvip	test	6	3	9022	40.1
70	11/23/2004 9:	partialloop20ul10g	mcconvip	test	6	3	8965	40.1
71	11/23/2004 9:	partialloop20ul20g	mcconvip	test	6	3	9059	40.1
72	11/23/2004 1	partialloop20ul40g	mcconvip	test	6	3	9214	40.1

Waters

eCord™ Column QC

ACQUITY UPLC Console for System test on Node CORE-ACQUITY-01 - [Column QC]

- ACQUITY UPLC System
  - Binary Solvent Manager
    - Interactive Display
    - Performance
  - Sample Manager
    - Interactive Display
    - XYZ Mechanism
  - Column
    - Column QC
    - Batch QC
    - Care and Use
  - Plots
  - Maintenance Counters
  - Logs

System Status

Control Configure Maintain Troubleshoot Help

Waters ACQUITY UPLC™ BEH C18 1.7µm P/N: 186002350 S/N: M41681E01

Print

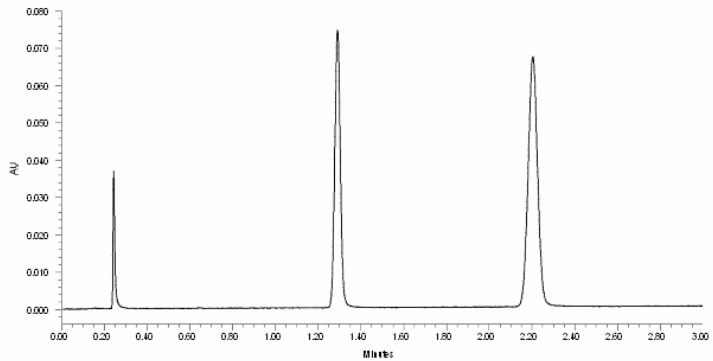
Home

Acquity Ultra Performance LC™

Part Number: 186002350
Column Dimension: 2.1mm x 50mm
Name: Waters ACQUITY UPLC™ BEH C18 1.7µm

Column Serial No: M41681E01 Batch Number: 105

Test Method
Eluent: Acetonitrile-Water 55:45 (v/v)
Sample:
1. Thiourea 0.01mg/mL
2. Naphthalene 0.05mg/mL
3. Acenaphthene 0.2mg/mL
Flow Rate: 0.43 mL/min
Detection: 254 nm
Temperature: Ambient
Injection Volume: 1.2 µl



Test Results For: Acenaphthene Retention Time (min.): 2.20

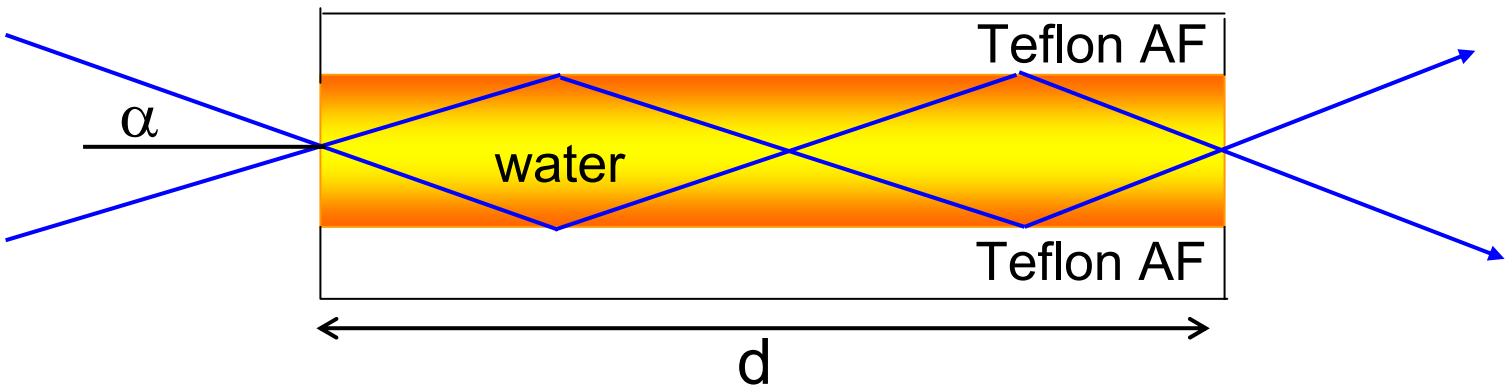
USP Tangent Efficiency: 11363 Retention Factor: 8.07

USP Tailing: 1.03 Accepted By: SJS

Pressure (psig): 5011 Date: 6/16/2004 7:35:57 AM

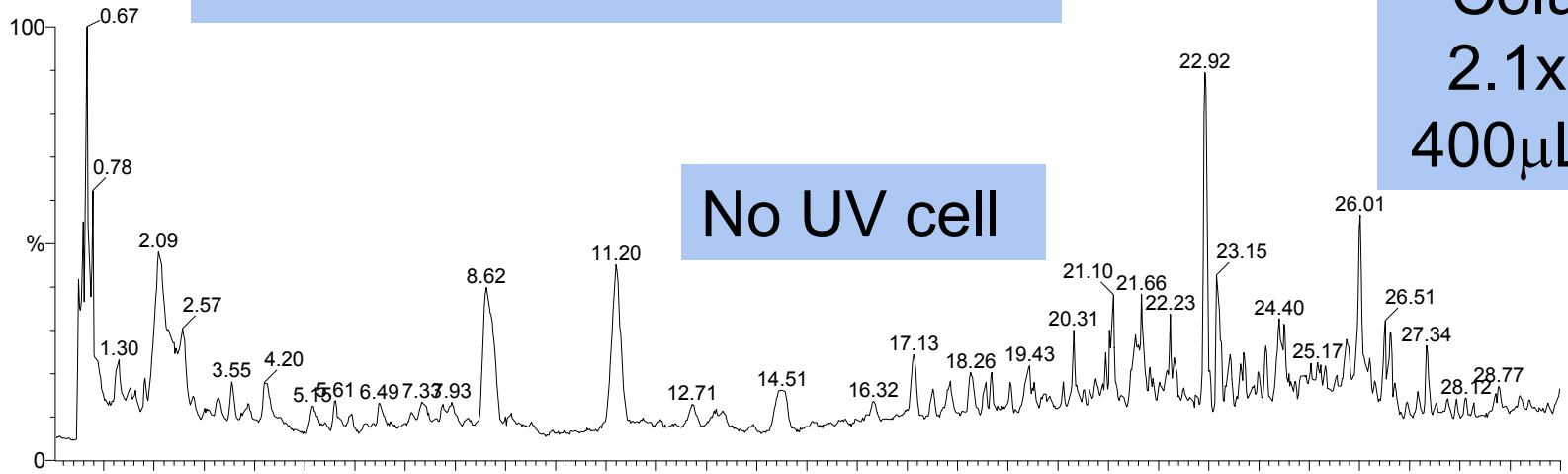
- Faster eluting peaks require higher data rates
  - High light throughput/transmission
  - Faster digital filter time constants
- UPLC™ separations require low dispersion flow cells
  - Maintain chromatographic fidelity
- Tunable UV, PDA and ELSD Options
- MS Options

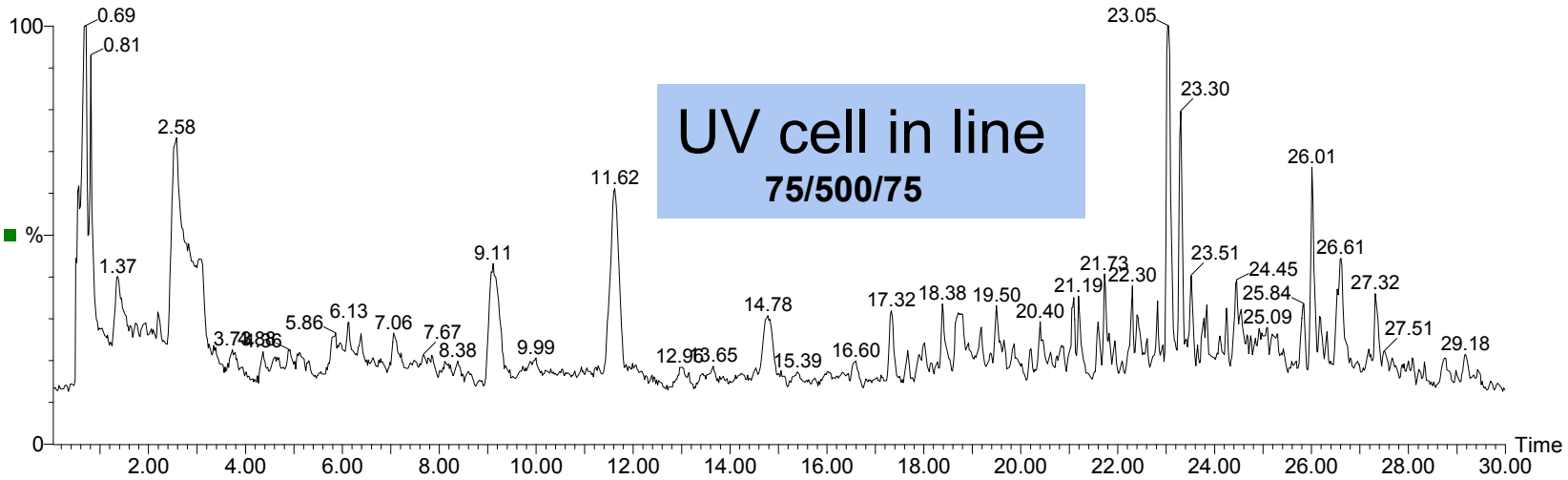
- Light – Guided UPLC™ flow cells
  - 10 mm pathlength, 500 nL volume
  - Flow cell channel is the inside of a low-index Teflon AF tube
  - Total internal reflection at walls, like optical fiber cladding



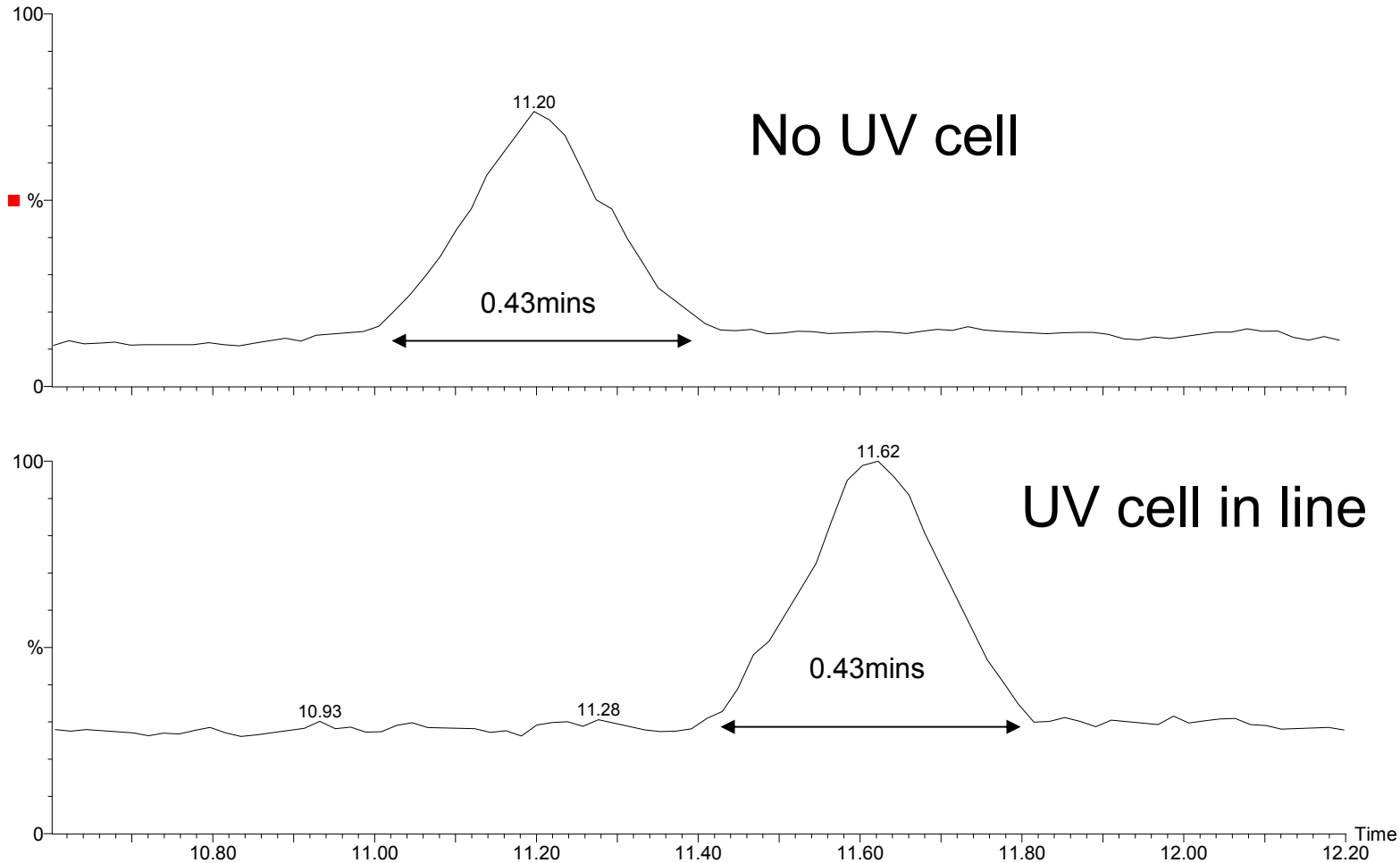
Effect of UV Cell on MS Data

Column
2.1x100
400µL/min



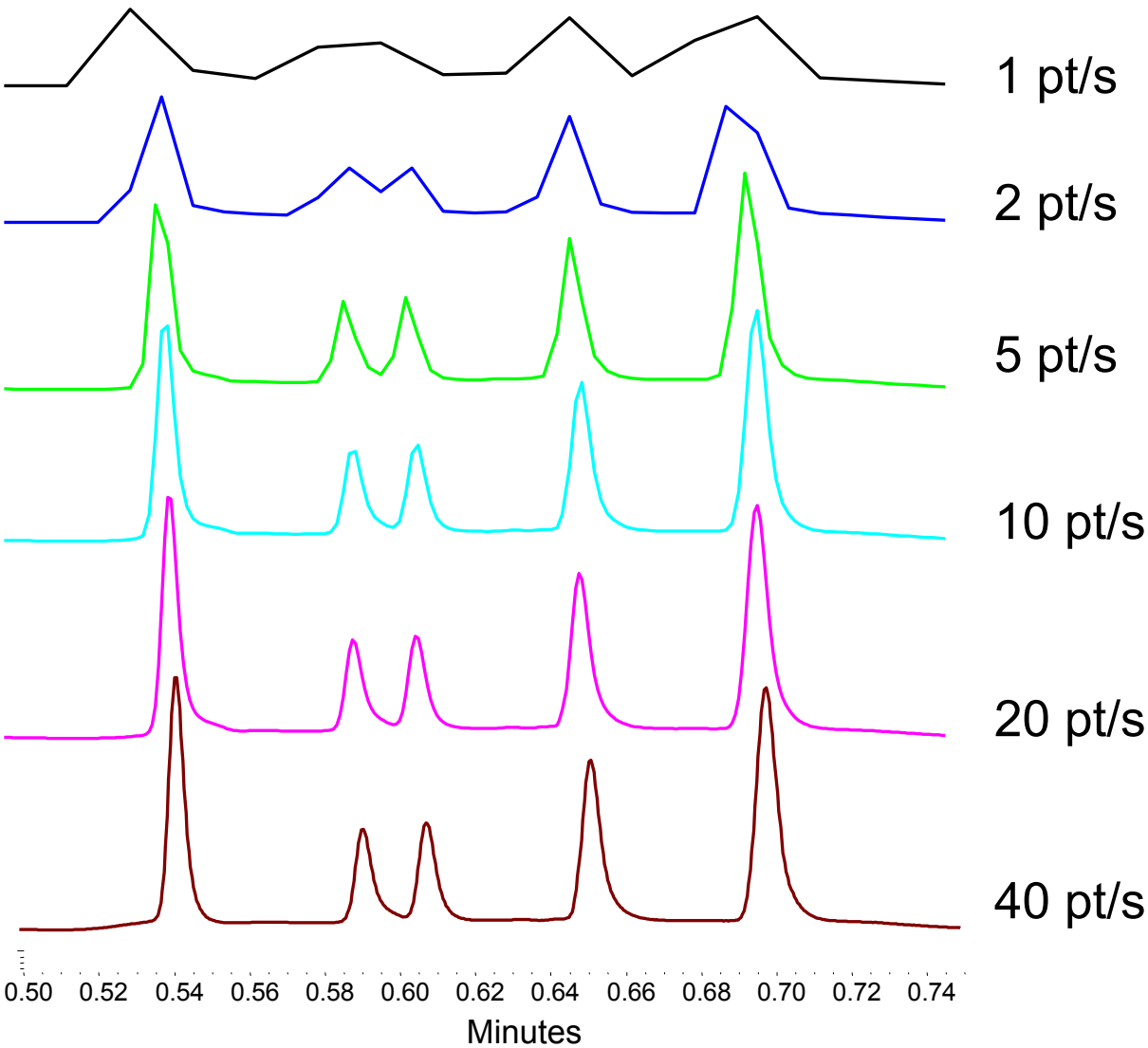


Low dispersion UPLC™ optics
Expanded Region

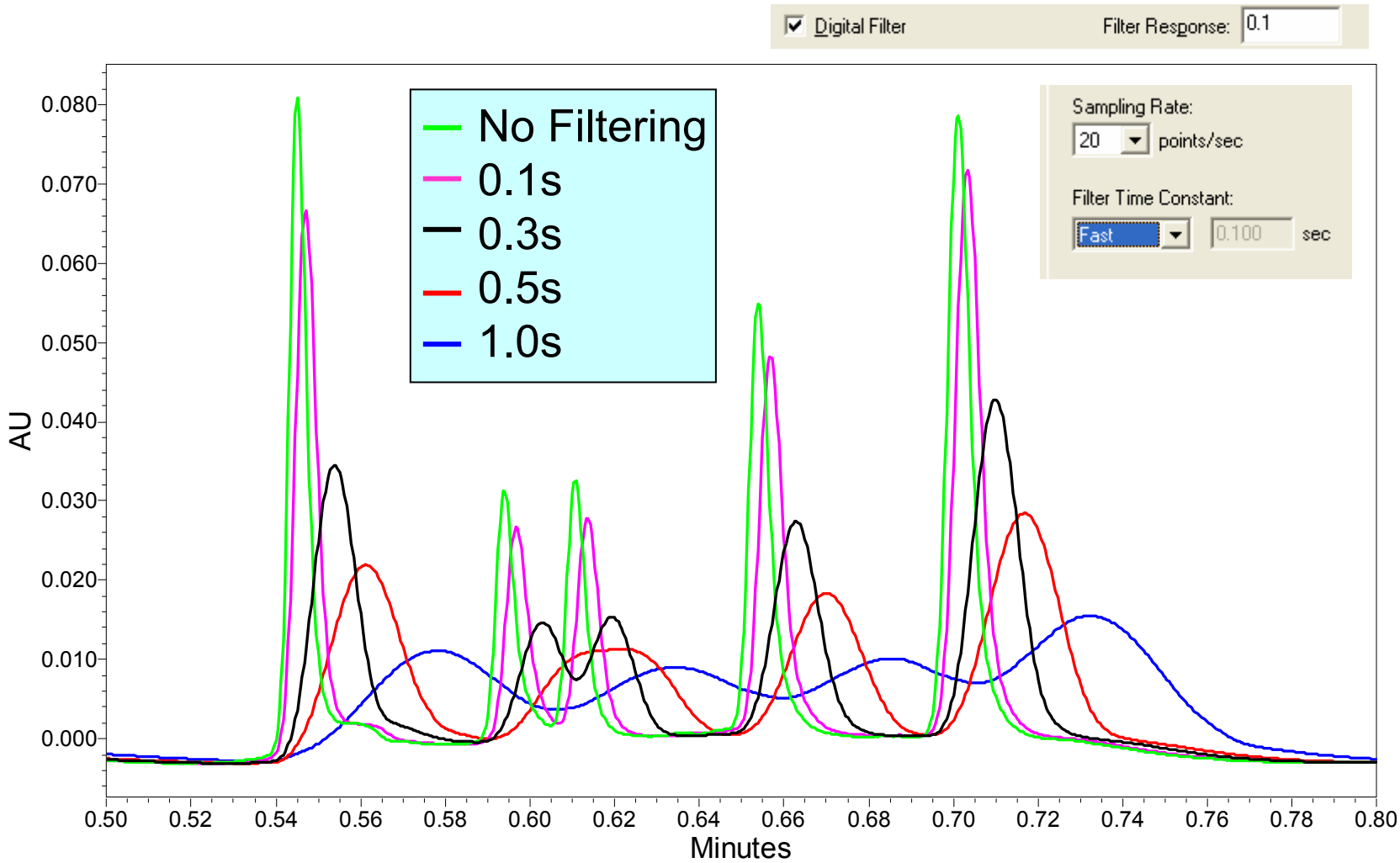


Data Acquisition Rates

Impact on UV chromatography data

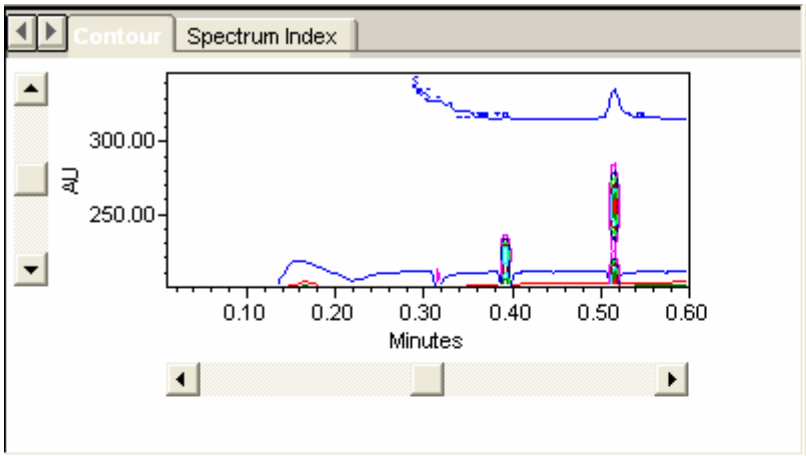


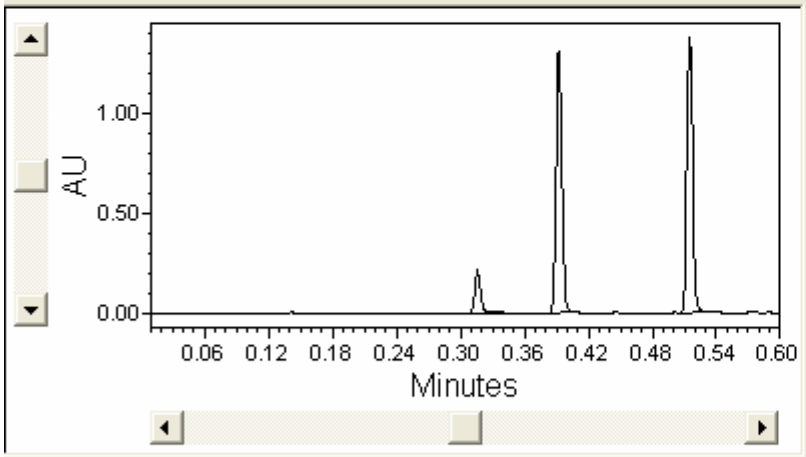
Effect of Filtering Constant on Chromatography

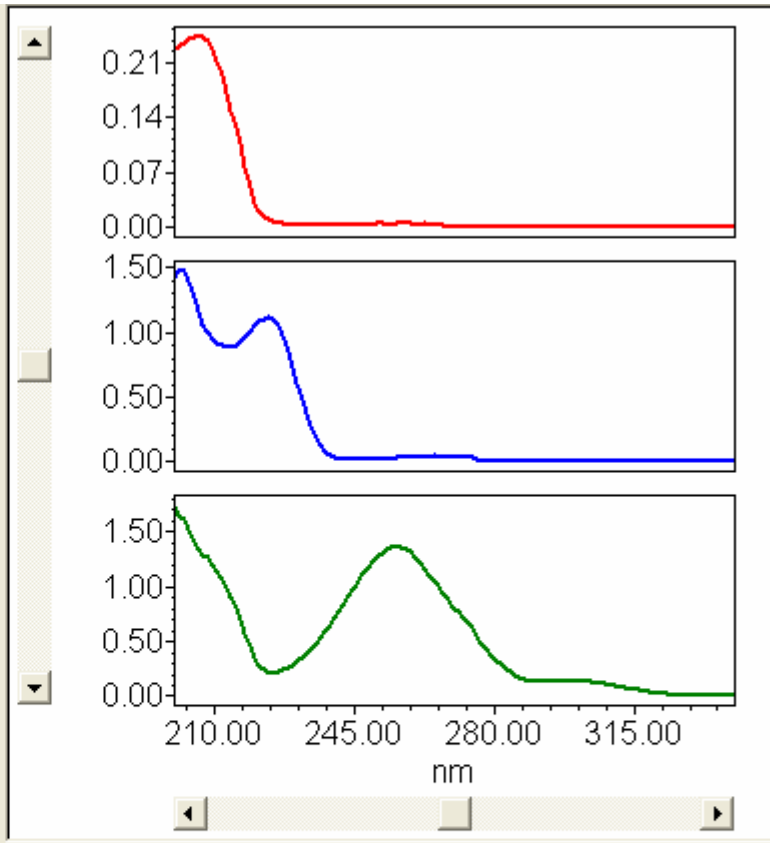


5 component Analgesic Mix on 2.1x50mm ACQUITY UPLC™ Column, 30s gradient

UV Spectra on ACQUITY PDA



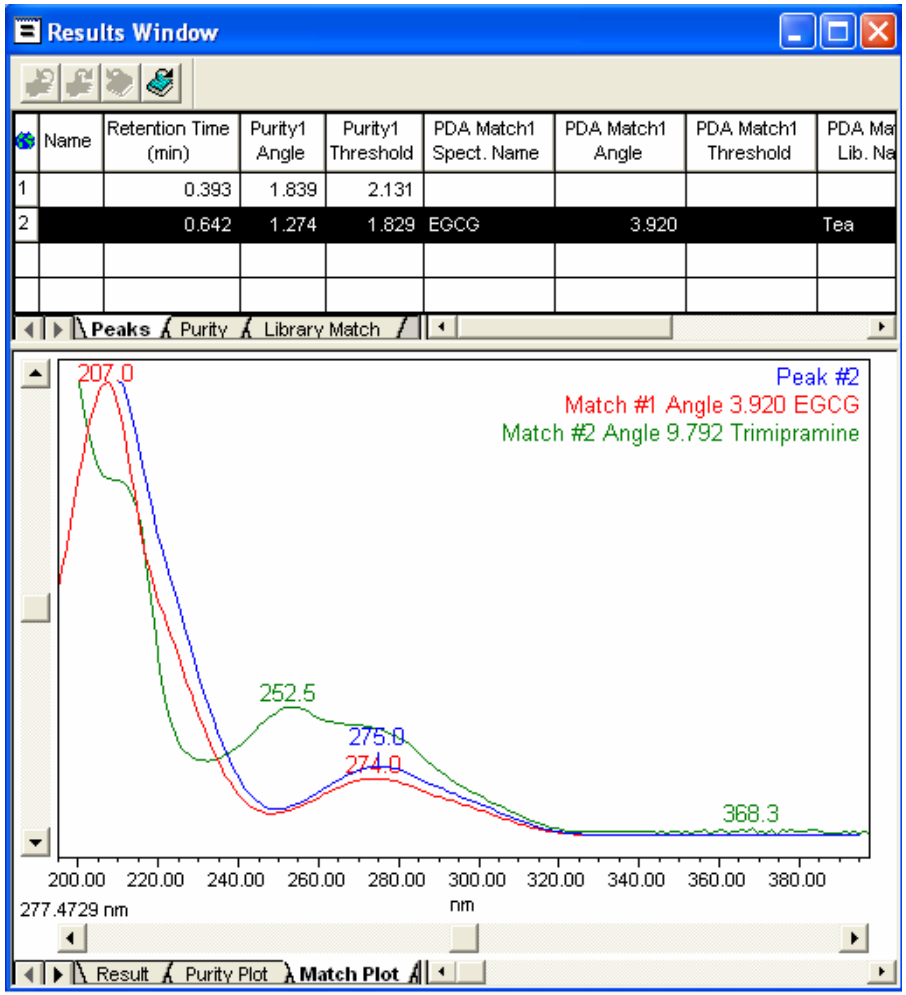


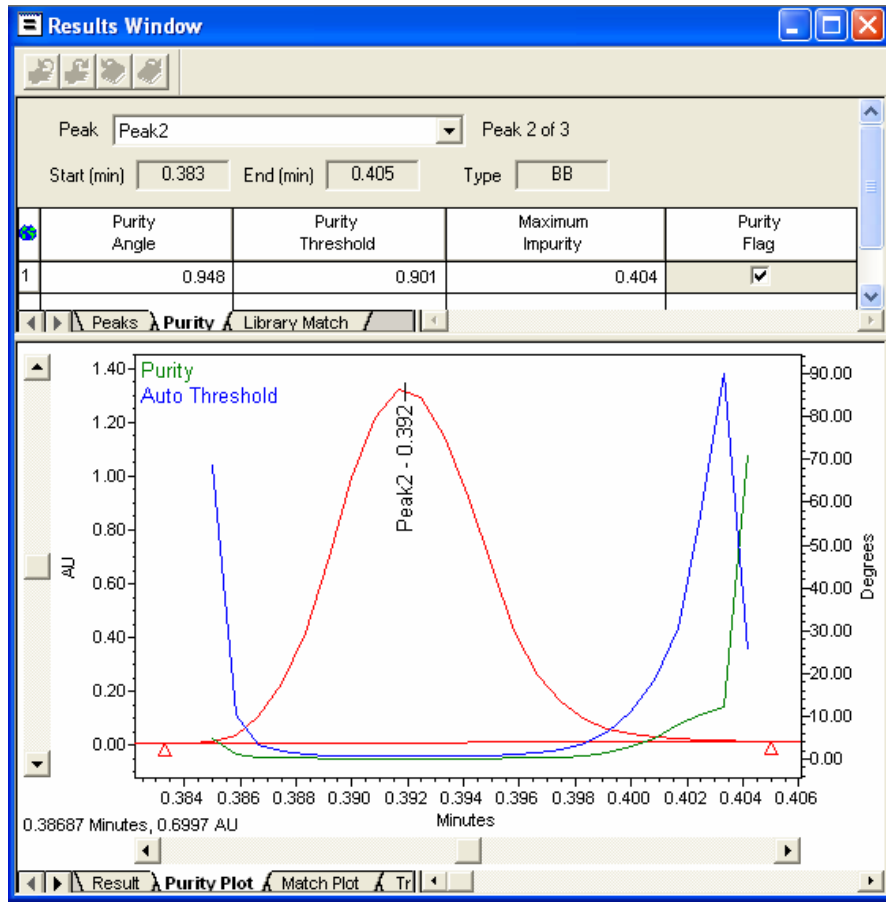


	Name	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)	Int Type	Peak Type
1	PSE	0.316	79514	7.31	218207	BG	Found
2	IBU	0.392	495747	45.59	1307478	BB	Found
3	INSTD	0.515	512260	47.10	1362548	BB	Found

	Image	Select	Retention Time (min)	Info	Max. Absorbance (au)	Baseline Correction	Smoothin Level
1		☑	0.316	PSE	0.24334	☑	None
2		☑	0.393	IBU	1.47971	☑	None
3		☑	0.515	INSTD	1.74742	☑	None

Peak Purity and Library Match Capabilities



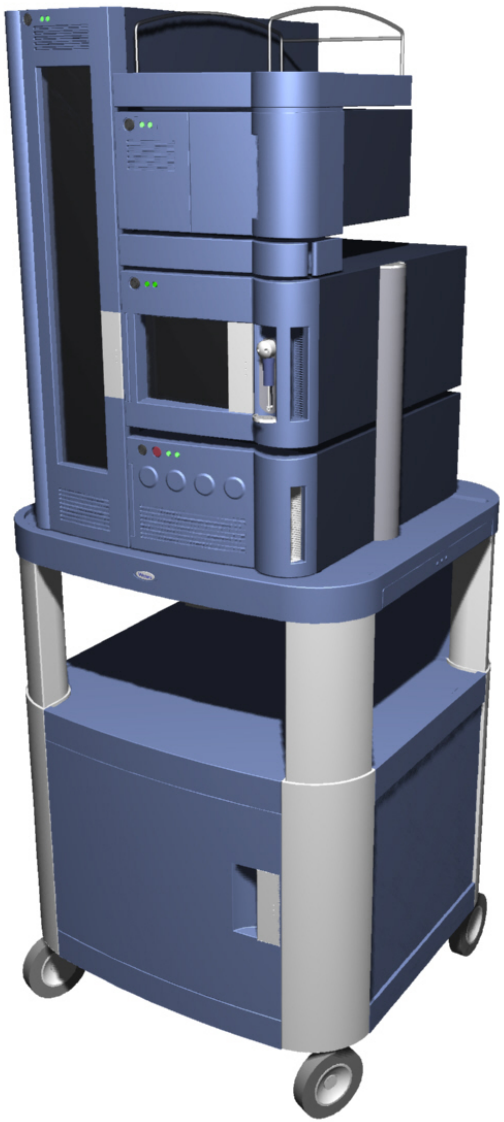


Supports high throughput/high capacity

- Expands capacity-mix or match
  - 22 thin (microtitre) plates
    - 8,448 samples max
  - 15 medium height plates
  - 8 full height (vial) plates
    - 384 x 2mL vials
- Temperature controlled
- Programmable Access to any sample location
- Supports Walk-up Operation

Waters

ACQUITY UPLC™
Optional FlexCart



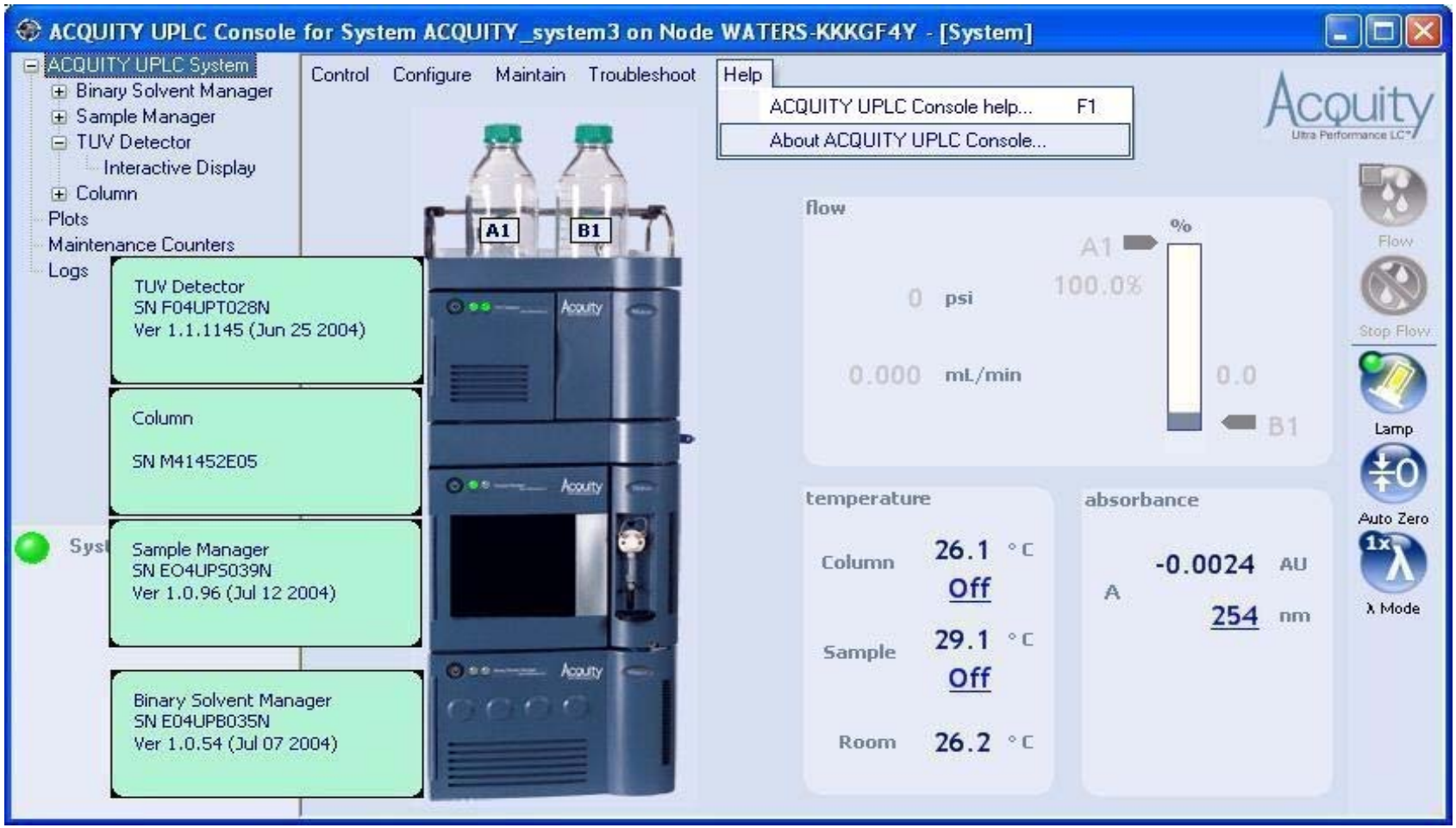
Supports inlet mobility

UPLC™ Technology Challenges

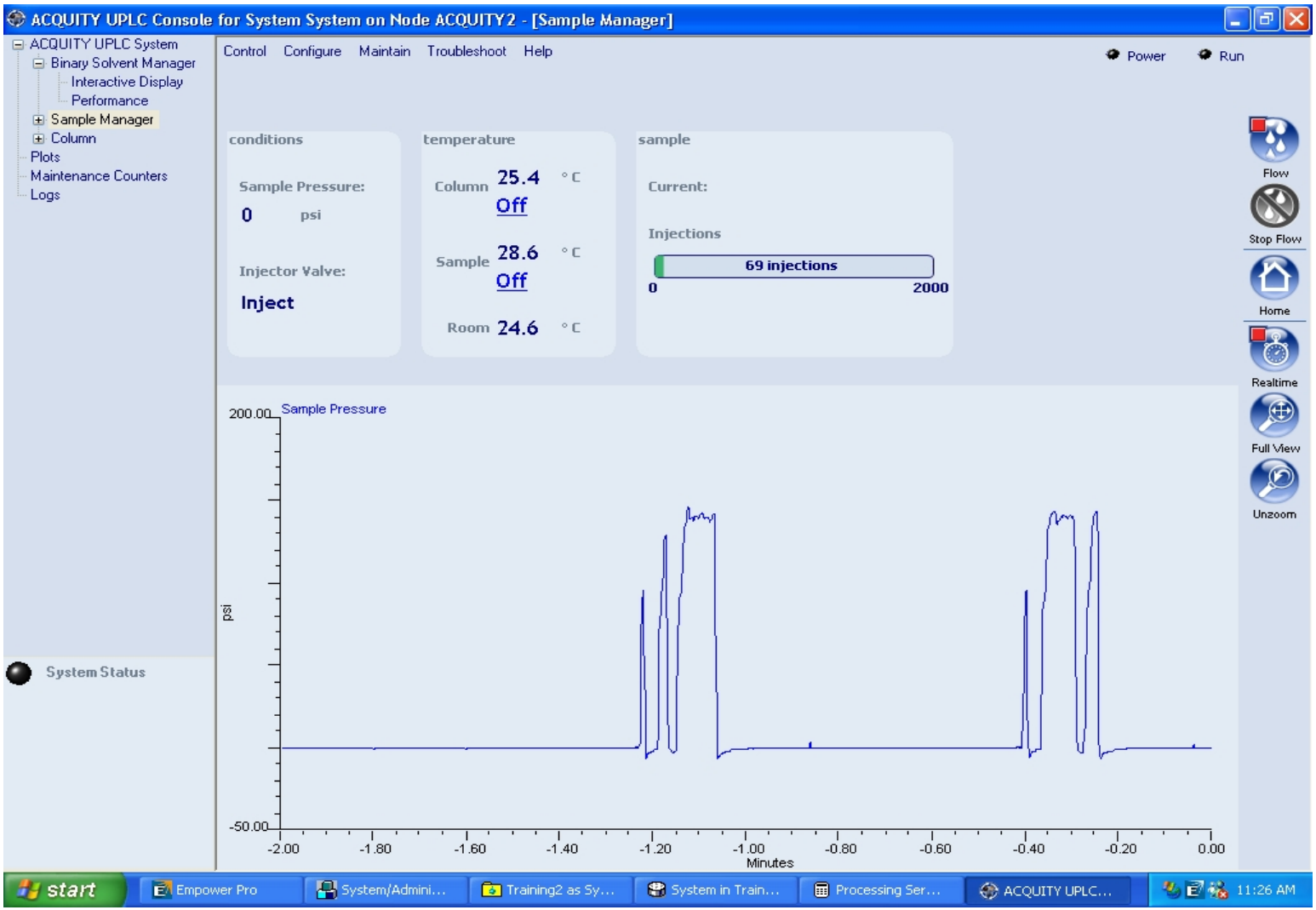
How do we take advantage of smaller particles?

- Pressure tolerant particle/column
- High pressure fluidic modules
- Holistic design, low system volume, optimized flow paths
- Reduced cycle time autosampler with minimum carryover
- High speed detectors; optical and mass
- Controlled and coordinated system interaction
  - Software designed for system integration
  - Comprehensive diagnostic suite

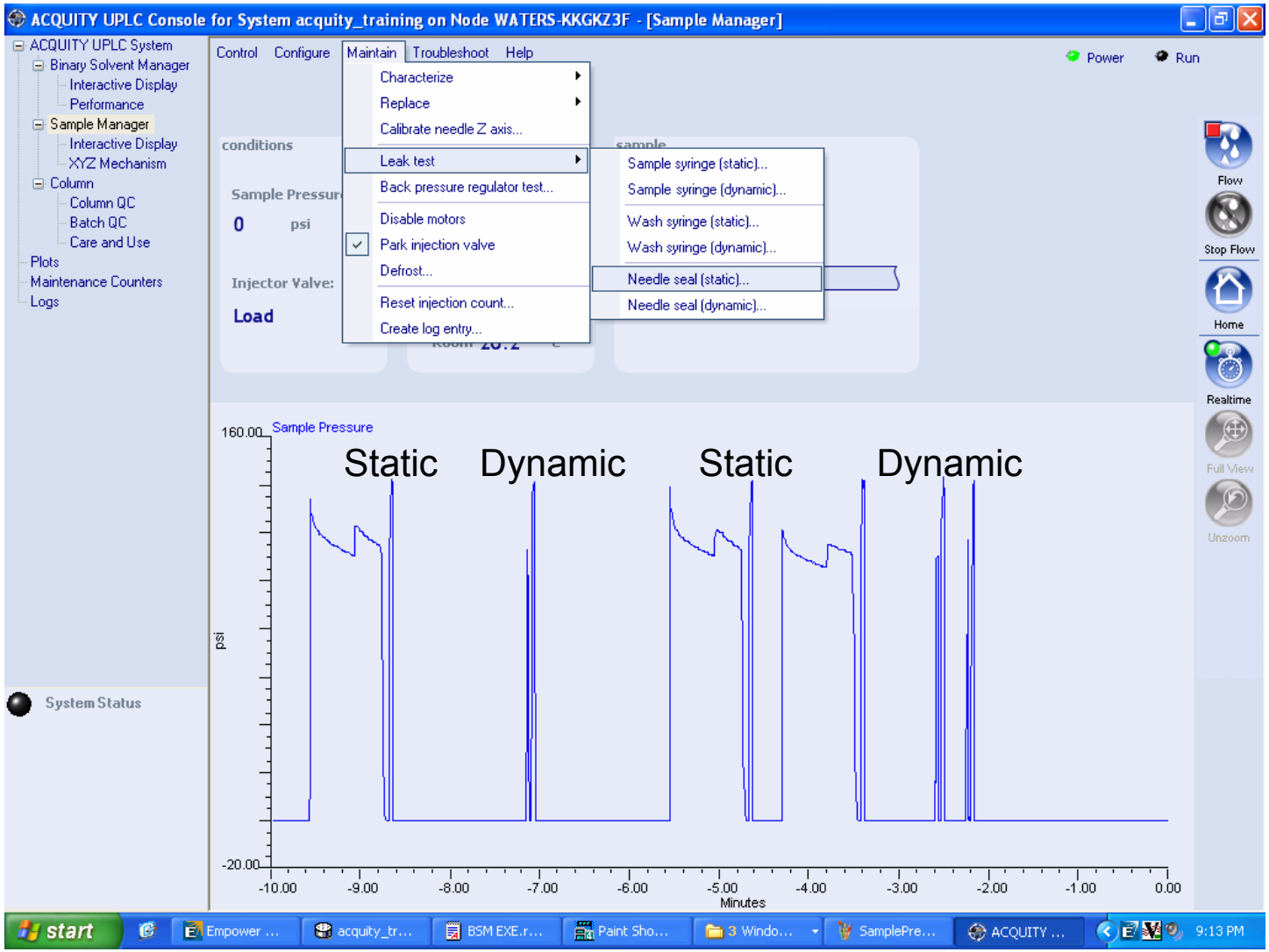
System Information



Sample Syringe Diagnostic



Needle Seal - Static vs Dynamic TEST



UPLC™ enabled, laboratory rugged

- Performance and Reliability
  - UPLC pressure capabilities
  - Low dispersion fluidics
  - Rapid, clean injections
  - Fast detection
  - Rugged chemistry
  - Analytical performance
  - Extensive onboard diagnostics
  - Connections Insight™
- Convenience
  - AQT Qualification
  - Small footprint
  - Integrated waste management
  - eCord™ Technology
  - Intuitive Control Console
  - Multiple plate formats
  - High capacity sample organizer
  - FlexCart

UPLC™ = a higher quality of (more) information faster

Waters

QUESTIONS?

Acquity™

Ultra Performance LC

Waters

Acknowledgements

- John Morawski
- Tanya Jenkins
- Eric Grumbach
- Robert Plumb
- Jeffrey Mazzeo