TIPS AND TRICKS ’09:
GET ON THE RIGHT ROAD.

Tips and Tricks of Reducing Solvent Consumption in Conventional and UHPLC Analyses

Contents

- *What has caused the Acetonitrile Shortage*
- *What can I change? Regulated or Not?*
- *Effect of changing column dimensions*
- *Effect of changing column particle size*
- *Investigating alternative separation techniques*

A SOLVENT DRIES UP

ACETONITRILE is in short supply, and chemists are concerned

A SHORTAGE of acetonitrile is leaving chemists around the U.S. and beyond wondering how long their supplies will last and what their options will be if stocks run dry.

There are good reasons why the situation is making chemists feel vulnerable. Thousands of them use the polar solvent in high-performance liquid chromatography. It is also used in pharmaceutical synthesis and in the extraction of butadiene from streams of C_4 hydrocarbons.

bothers to extract it for sale to the merchant market, which it does at plants in Green Lake, Texas, and Lima, Ohio. Most acrylonitrile producers incinerate the coproduct as fuel.

And it is acetonitrile's status as a minor coproduct that has led to its present scarcity. Amin Dhalla, business director for Ineos Nitriles, says acrylonitrile production has been ebbing. Demand for ABS resins, used in cars, electronic housings, and small appliances, is slumping

The Acetonitrile Shortage

- *Acetonitrile is a by-product of Acrylonitrile production*
- *Due to the global economic slowdown, the production and demand for of acrylonitrile has decreased sharply*
- *China had ceased production to improve air quality for the Olympic games*
- *A major US factory in Texas was damaged during Hurricane Ike.*

The Acetonitrile Shortage

- *In 2008 the price of 2.5L of Acetonitrile was $50*
- *Today the price of 2.5L of Acetonitrile is over $300!*
- *For a 30 minute method at 1ml/min, 50% ACN this means every batch of 100 samples will cost $216 in ACN**
- *An increase of $180 per 100 samples*

** +20% used to wash and re-equilibrate*

What Can I Change?

- ***Regulated Methods***
  - *Follow FDA/USP guidance*
  - *Additional changes will require validation and regulatory approval*
- ***Unregulated Methods***
  - *More freedom but always check method is robust and valid.*

Regulated Methods

- *Review what is a method adjustment and what requires revalidation.*
- *Make method adjustments as soon as possible to save solvent.*
- *Documentation will still be required*
- *Start to validate alternate methods*
  - *Focus on methods with only 1 or 2 analytes (i.e. content uniformity, dissolution) because these will be the easiest*
  - *Focus on methods that are run most often/use the most solvent*
  - *Save acetonitrile solvent for most complex separations*

USP and FDA Method Adjustment Criteria:
LATEST GUIDANCE

Parameter	Maximum Specifications	Comments/Examples
Column Length	± 70%	250mm → 75mm 150mm → 50mm
Column Internal Diameter	±25%* USP – Column ID can be adjusted provided linear velocity is constant**	4.6 mm → 3.0 mm (-35%) 4.6 mm → 2.1 mm (-54%) 3.0 mm → 2.1 mm (-30%)
Flow Rate	±50%	
Injection Volume	Reduce as much as needed – must still meet detection limits and precision	If you change to a smaller/ shorter column make the appropriate change in injection volume
Particle Size	Reduce by up to 50%	You can change column length **and** particle size to keep Rs same

*For the current and official copy, check the Intranet at :http://www.fda.gov/ora/science_ref/lm/pdf/attachments/vol2_5_4_5_attachment_a.pdf

** USP 30 Second Supplement Revisions, PF34(1)

What Can I Change?

- *Review Method Efficiency*
- *Column diameter*
- *Column length and particle size*
- *All of the above*

- *Switch from Acetonitrile to methanol*

Method Efficiency

- *Can you reduce column equilibration/re-equilibration time? Most reversed phase columns will equilibrate in 10 column volumes.*

- *Is your stop time set too long after the last peak elutes and you are using extra solvent?*

- *Are you losing time between runs? Can you set your LC to inject more quickly or be ready to inject faster?*

- *While acetonitrile is a good cleaning/storage solvent can you switch to methanol?*

ORA LABORATORY PROCEDURE Food and Drug Administration	Document No.: ORA-LAB.5.4.5	Version No.: 1.4 Page 10 of 14
Title: ATTACHMENT A – MODIFICATION CRITERIA		Effective Date: 10-01-03 Revised: 02-24-09

If adjustments to operating conditions are needed, each of the following is the maximum specification (USP General Chapter <621> Chromatography) that can be considered. All adjustments falling outside the maximum specifications will be considered as method modifications and will be subject to the method modification protocol.

pH of Mobile Phase (HPLC): The pH of the aqueous buffer used in the preparation of the mobile phase can be adjusted to within ±0.2 pH units of the value or range specified.

Concentration of Salts in Buf
buffer used in the mobile phase ca

Ratio of Components in Mob
the mobile phase (specified at 50%
absolute (i.e., in relation to the tot
exceed ±10% absolute, nor can th
for binary and ternary mixtures ar

Binary Mixtures: Specified Ratio
permitted change of ±10% absolu
the range of 40:60 to 60:40. Speci
adjustments up to ±2% absolute a
of 2:98 – Thirty percent of 2 is 0.6
would reduce the amount of the fi
range of 1.4:98.6 to 2.6:97.4.

Ternary Mixtures: Specified Ratio
which exceeds the maximum permitted change of ±10% absolute in any component. Therefore, the second component may be adjusted only within the range of 25 to 45% absolute. For the third component, thirty percent of 5 is 1.5% absolute. Since ±2% absolute is permitted and provides more flexibility, the third component may be adjusted within the range of 3 to 7% absolute. In all cases, a sufficient quantity of the first component is used to give a total of 100%.

Wavelength of UV-Visible Detector (HPLC): Deviations from the wavelengths specified in the method are not permitted. The procedure specified by the detector manufacturer, or another validated procedure, is to be used to verify that error in the detector wavelength is, at most, ±3 nm.

Column Length (GC, HPLC): May be adjusted by as much as ±70%.
Column Inner Diameter (GC, HPLC): may be adjusted by as much as ±25% for HPLC and ±50% for GC.
Flow Rate (GC, HPLC): May be adjusted by as much as ±50%.
Injection Volume (GC, HPLC): May be reduced as far as is consistent with accepted precision and detection limits. It may be increased to as much as twice the volume specified, provided there are no adverse effects on factors such as baseline, peak shapes, resolution, linearity and retention times.
Particle Size (HPLC): May be reduced by as much as 50%.
Column Temperature (HPLC): May be adjusted by as much as ±10°.
Oven Temperature (GC): May be adjusted by as much as ±10%.
Film Thickness (Capillary GC): May be adjusted by as much as –50 to +100%.

Column Temperature (GC): May be adjusted by as much as ±2%, in terms of absolute temperature.
Oven Temperature Program (GC): Adjustment of temperatures is permitted as stated above. For the times specified for the temperature to be held or for the temperature to be changed from one to another, an adjustment of up to ±20% is permitted.

Column Length (GC, HPLC): May be adjusted by as much as ±70%.
Column Inner Diameter (GC, HPLC): may be adjusted by as much as ±25% for HPLC and ±50% for GC.
Flow Rate (GC, HPLC): May be adjusted by as much as ±50%.
Injection Volume (GC, HPLC): May be reduced as far as is consistent with accepted precision and detection limits. It may be increased to as much as twice the volume specified, provided there are no adverse effects on factors such as baseline, peak shapes, resolution, linearity and retention times.
Particle Size (HPLC): May be reduced by as much as 50%.
Column Temperature (HPLC): May be adjusted by as much as ±10°.

- ***When sponsors make changes in the analytical procedure, drug depends on the nature of the change."***
- ***"FDA intends to provide guidance in the future on post-approval changes in analytical procedures."***
- ***At the moment method verification and documentation should be provided to the FDA – follow your SOP's on this.***

Changing Column Diameter

- *Column diameter will dramatically impact the solvent use because flow rate is proportional to column diameter*
- *As you change ID you want to keep linear velocity the same*
- *By reducing column diameter you can:*
  - *Reduce solvent use and waste*
  - *Maintain analysis time and resolution*
  - *Increase sensitivity (therefore you can reduce injection volume with change in ID)*

Changing Column Diameter

	Standard Analytical	Solvent Saver	Narrow Bore
	●	●	●
Column Internal Diameter	4.6 mm	3.0 mm	2.1 mm
Actual Solvent Used	100 mL	40 mL	20 mL
% Solvent Use Decrease	-	60%	80%

By reducing column ID solvent use is reduced dramatically

Changing Column Diameter

- *Scale flow rate (maintain linear velocity)*

$$F_2 = F_1 \times (d_2)^2/(d_1)^2$$

- *Scale Injection Volume*

$$V_2 = V_1 \times [(d_2^2 \times L_2)/(d_1^2 \times L_1)]$$

d = diameter, L= length, F= flowrate, V= Inj Volume

Changing Column Diameter

4.6 mm → 3 mm

Reduce flow rate and Inj Vol by factor of 0.4

e.g. 1ml/min *0.4ml/min* *60% less ACN*

4.6 mm → 2.1 mm

Reduce flow rate and Inj Vol by factor of 0.2

e.g. 1ml/min *0.2ml/min* *80% less ACN*

N.B. On a typical standard HPLC system 3mm i.d. columns usually give better performance than 2.1mm columns.

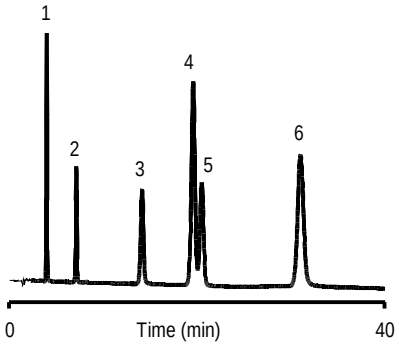
Check HPLC can reliably inject lower injection volumes and handle smaller peaks volumes

Changing Column Diameter

Separation of Antibacterials

Column: ZORBAX SB-C18 Mobile Phase*: 20% ACN: 80% Citrate/phosphate pH 2.6 *200/87/713 ACN/0.2M Na_2HPO_4/0.1M citric acid
Temperature: ambient Sample: Antibacterials 1. Sulfamerazine 2. Furazolidone 3. Oxolinic acid 4. Sulfadimethoxine 5. Sulfaqunioxoline 6. Nalidixic acid

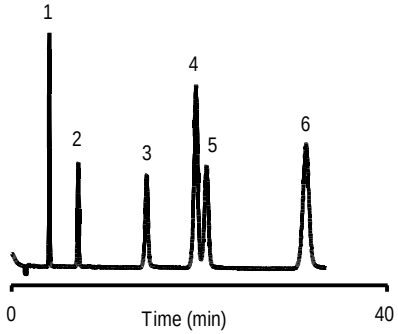
SB-C18
4.6 x 150 mm, 5 um



Solvent Used: 31 mL

Flow Rate: 1.0 mL/min
Injected: 3 uL
Detector Cell Volume: 8 uL

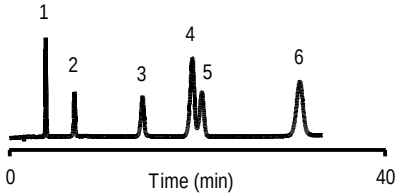
Solvent Saver SB-C18
3.0 x 150 mm, 5 um



Solvent Used: 15 mL
% Solvent Saved = 52%

Flow Rate: 0.5 mL/min
Injected: 2 uL
Detector Cell Volume: 8 uL

SB-C18
2.1 x 150 mm, 5 um



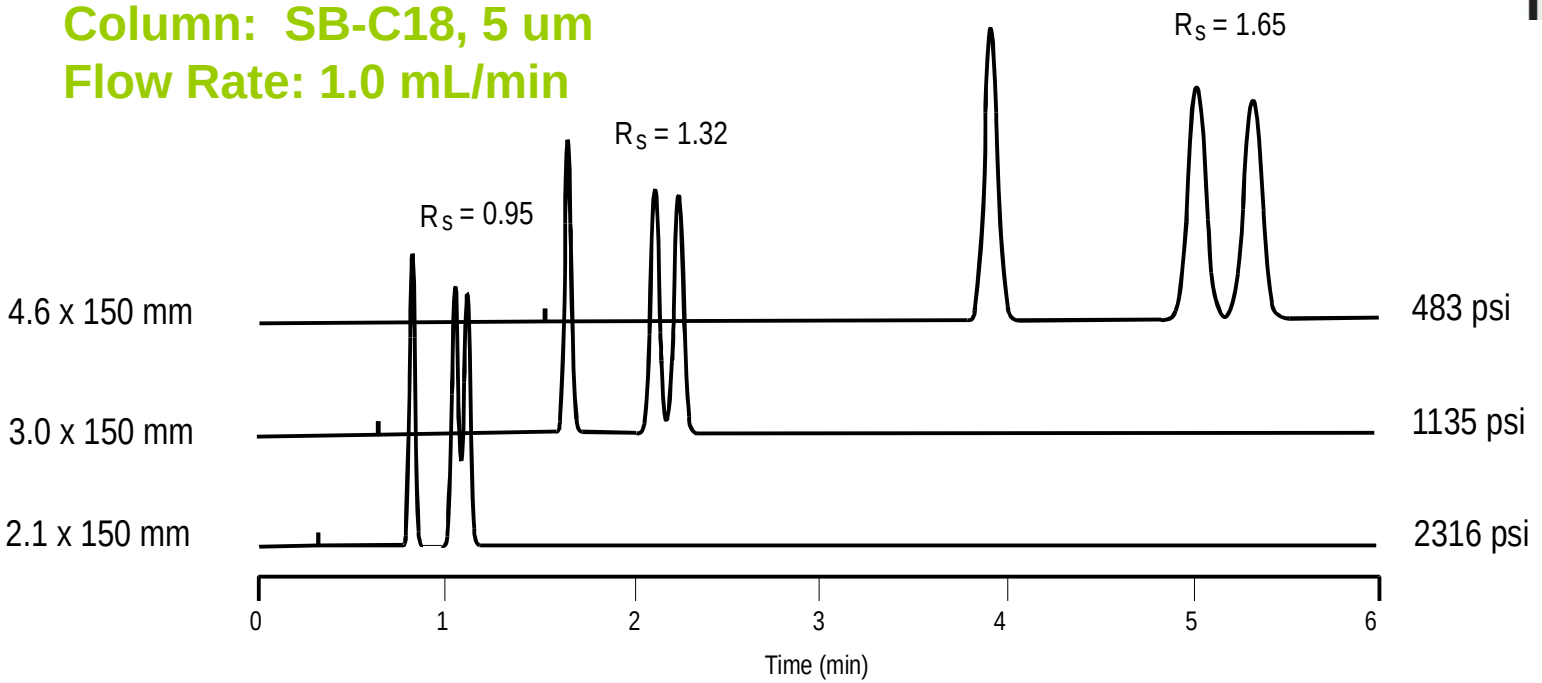
Solvent Used: 8 mL
% Solvent Saved = 74%

Flow Rate: 0.25 mL/min
Injected: 1 uL
Detector Cell Volume: 2 uL

Solvent Saver column reduces solvent use by more than 50% while keeping particle size, bonded phase, column length the same.

Changing Column Diameter

Column: SB-C18, 5 um
Flow Rate: 1.0 mL/min



Separation of Nitrobenzenes on Different Diameters with the Same Flow

- *If flow rate is not changed as column ID changes, resolution and analysis time will change*

- *Therefore you must change flow rate to maintain linear velocity*

Changing Column Diameter

Solvent Saver Columns Can Be Used on most LCs without Modification

		Peak Volume (mL)		
Column Dimension	Void Volume (uL)	k=1	k=3	k=5
Analytical 4.6 x 150 mm 1.0 mL/min	1.50	114	229	343
Solvent Saver 3.0 x 150 mm 0.4 mL/min	0.64	46	92	137
Narrow Bore 2.1 x 150 mm 0.2 mL/min	0.28	23	46	69

$k = (t_r - t_o)/t_o$ **N = 11,000 (constant)**

- *Peak volumes below 60 uL require optimized instrumentation for maximum efficiency.*

Changing Column Diameter

Summary of Benefits and Recommendations

➢First Choice

- ➢*Solvent Saver Columns (3.0 mm id)*
- ➢*Solvent savings up to 60% with almost standard HPLC*

➢Second Choice

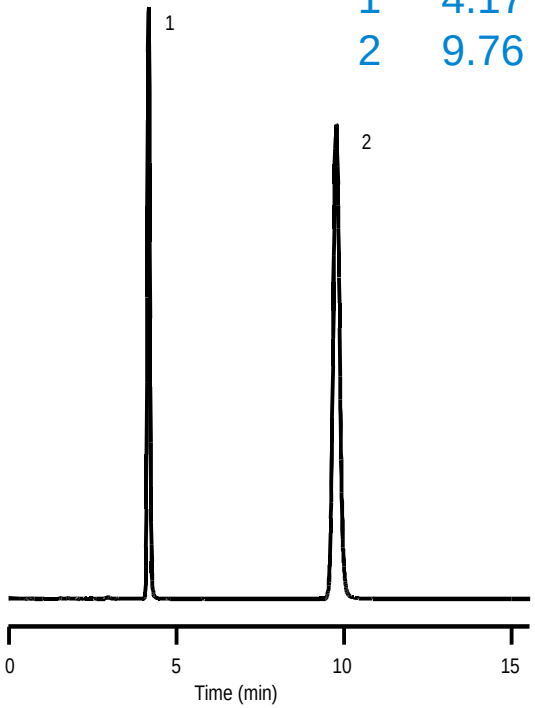
- ➢*Narrow-bore columns (2.1 mm id)*
- ➢*Solvent savings up to 80% with optimized HPLC*
  - ➢ *Detector cell volume of 2 mL or less*
  - ➢ *Reduced injection size*
  - ➢ *Capillary tubing 0.12 mm id*
  - ➢ *Injection volume is 10% of peak volume of the first peak, usually this is < 5 mL*

USP Analysis of Diazepam –
Original Definition of Column 4.6 x 250mm, L1

Column: 4.6 x 250 mm Mobile Phase: 35% Water: 65% MeOH Flow Rate: 1.2 mL/min
Sample: 1. Ethylparaben 2. Diazepam

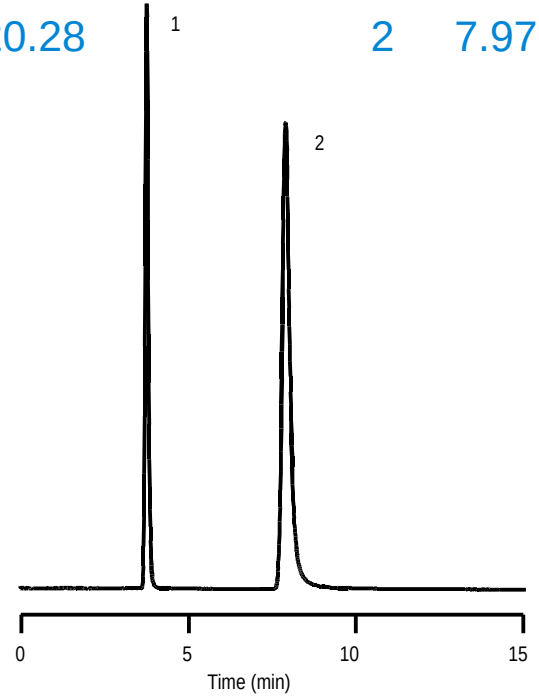
SB-C18

Peak	Retention Time	Plates	Rs
1	4.17	13850	0.00
2	9.76	12154	20.28



ODS

Peak	Retention Time	Plates	Rs
1	3.81	8034	0.00
2	7.97	6020	13.45



- **Many C18 columns provide results in line with the USP method.**

Column Choices to Save Solvent and Time

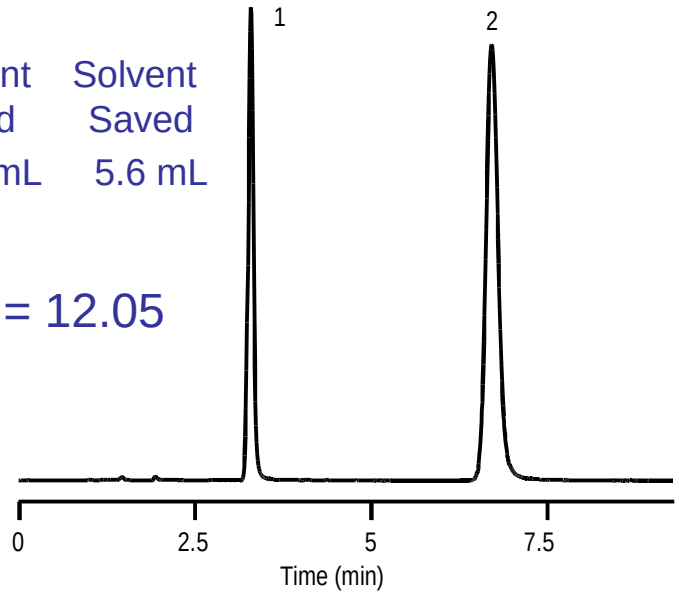
Mobile Phase: 35% water: 65% methanol

Sample: 1. Ethylparaben 2. Diazepam

Solvent Saver, Rx-C18
3.0 x 250 mm, 5 um
Flow Rate: 0.5 mL/min

Analysis Time	Solvent Used	Solvent Saved
8 min	4.0 mL	5.6 mL

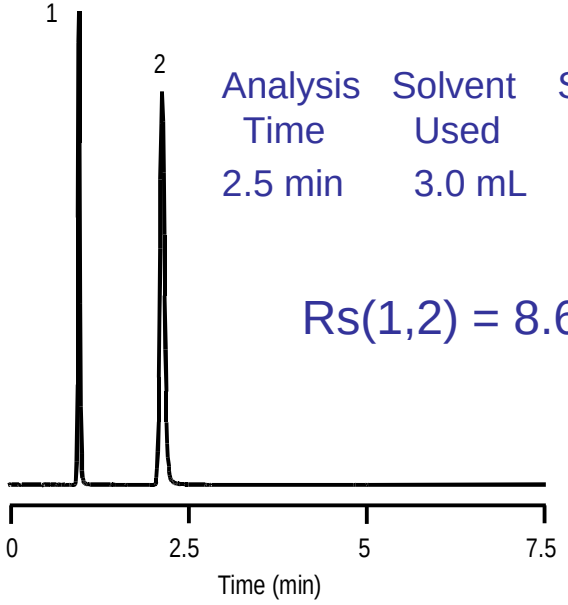
Rs(1,2) = 12.05



Rapid Resolution, Rx-C18
4.6 x 75 mm, 3.5 um
Flow Rate: 1.2 mL/min

Analysis Time	Solvent Used	Solvent Saved
2.5 min	3.0 mL	6.6 mL

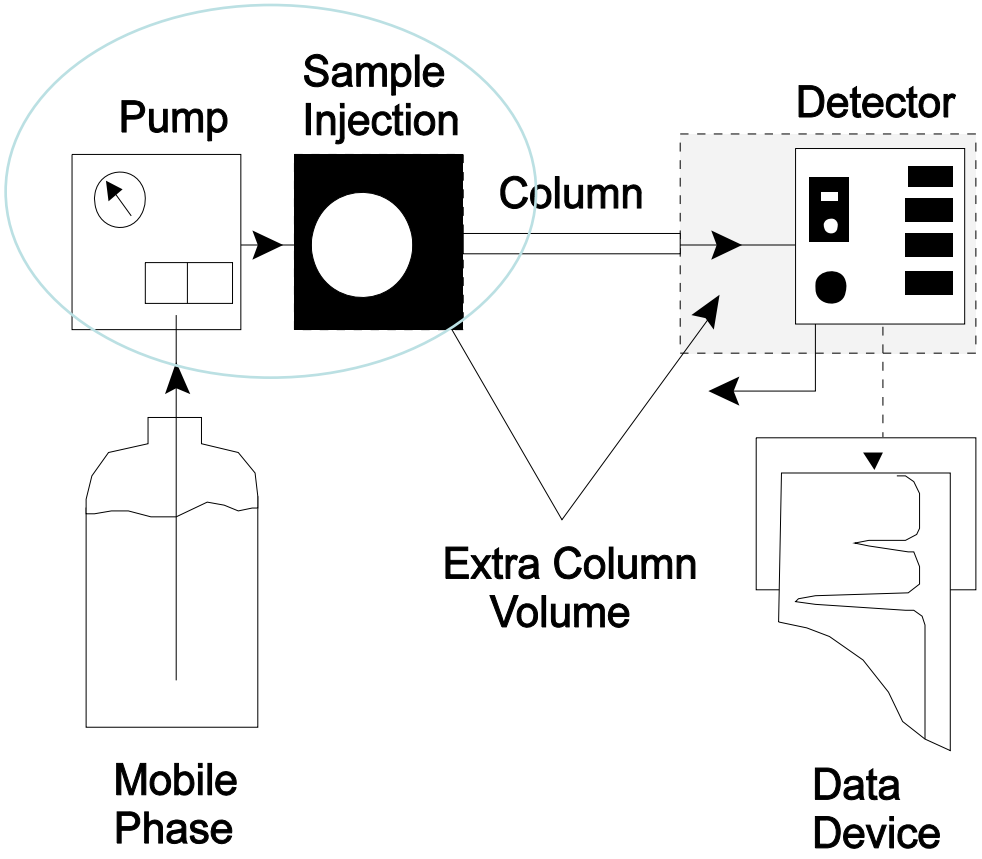
Rs(1,2) = 8.62



- Consider all column configuration options for saving solvent and time.
- If you meet Rs requirements then use shortest column to save time and solvent.

Changing Column Diameter

Let's go beyond the columns….



Maintain Resolution for Low Volume Peaks by Minimizing Extra-Column Volume

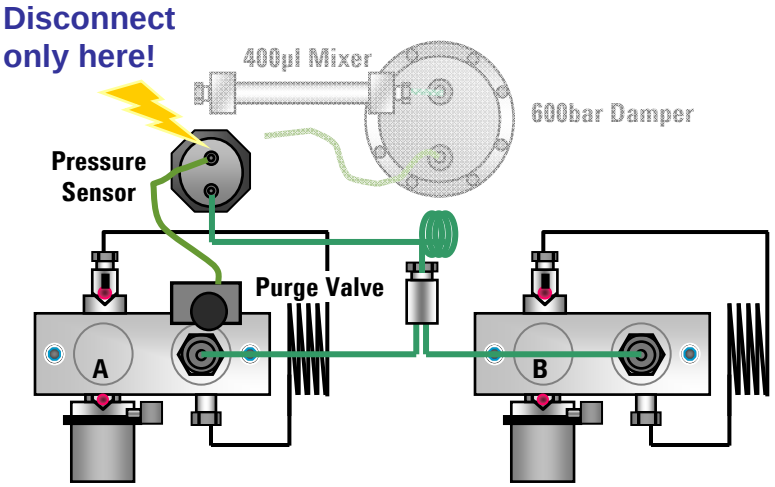
- sample volume
- connecting tube volume
- fitting volume
- detector cell volume

Changing Column Diameter

Agilent 1200 Series Binary Pump SL Configurations

Ultra-Fast Gradient Configuration
Low delay volume - 120µl

- Ideal for sub-1 minute gradients
- Very low mixing noise
- Best for flow rates < 2mL/min

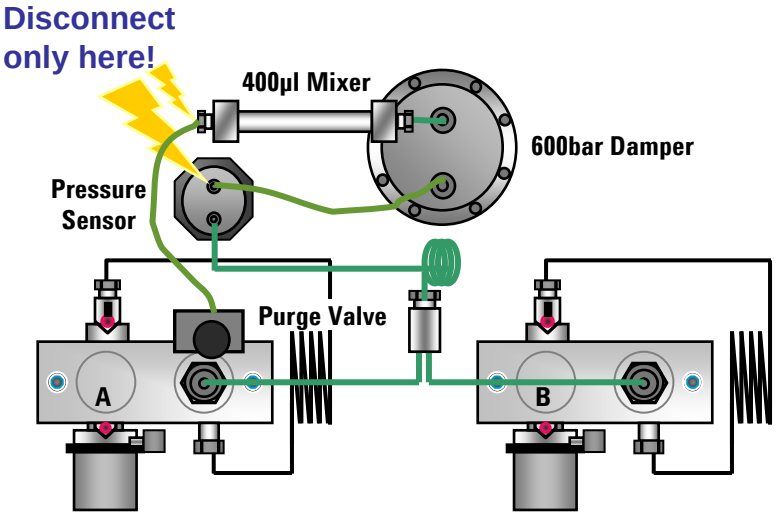


Flow Path

Mixing-T → Pressure-Sensor → Purge Valve

Standard delay volume
(600-800µl delay)

- Compatible with 1100/1200
- Required for high flow rates

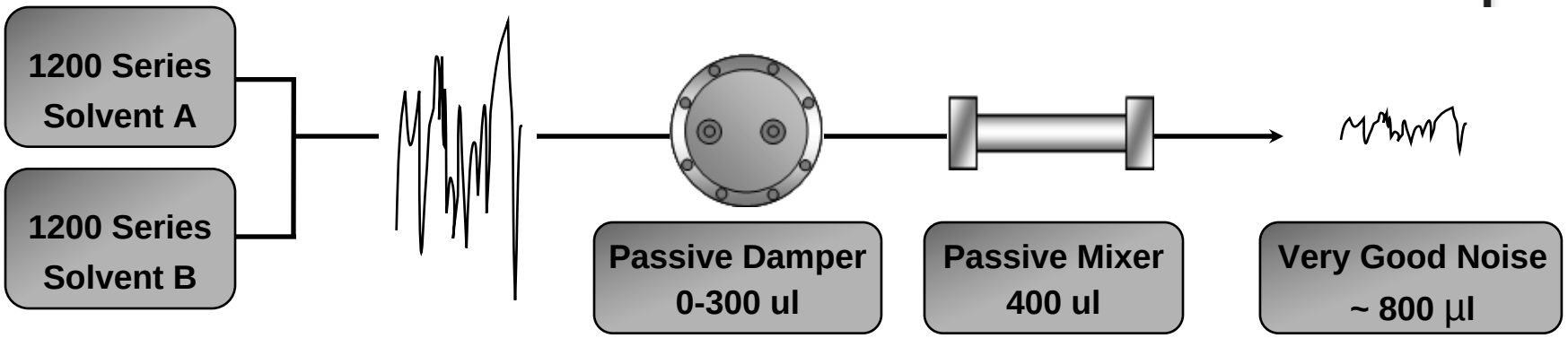


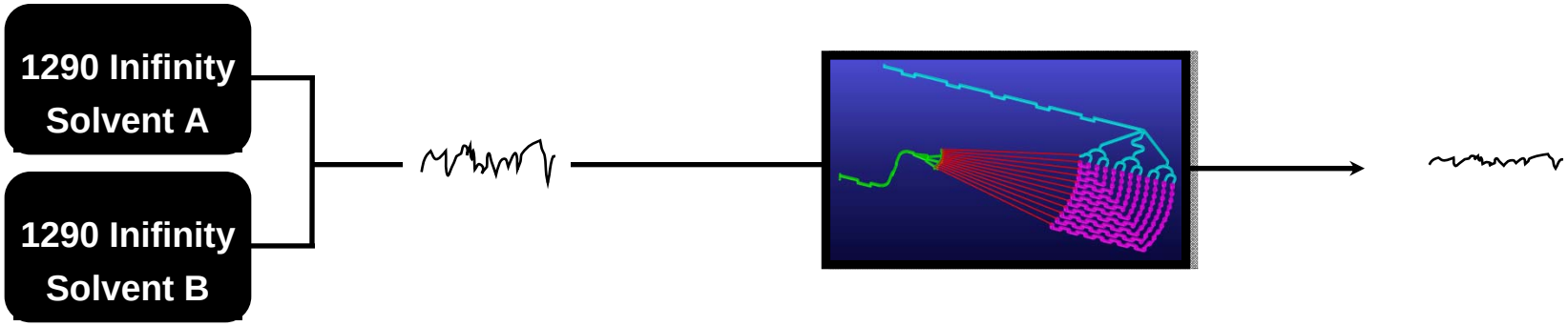
Flow Path

Mixing-T → Pressure-Sensor → Damper → 400µl Mixer → Purge Valve

Changing Column Diameter

1290 Infinty LC: How to achieve lowest delay volume and lowest noise?





Active Damping

Knowledge Intelligence + Dual core Ultra-Res (50x)

\+

Microfluidic Mixing

Jet Weaver Mixer (35ul)

=

10µl / 45µl DV:
- **20 - 80x lower Lowest Noise**
- **< 50%**

Changing Column Length and Particle Size

- *Reduce column length and particle size simultaneously to:*
  - *Reduce analysis time*
  - *Reduce solvent use and waste*
  - *Maintain resolution*

Changing Column Length and Particle Size

Plates ***Selectivity*** ***Retention***

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha-1}{\alpha} \cdot \frac{k'}{k'+1}$$

$$N \propto \frac{L}{d_p}$$

To Maintain Rs:

e.g.: $d_p/2$ ➡ $L/2$

↓ *Column Length*	=	↓ N
↓ *Particle Size*	=	↑ N
↓ *Particle Size*	=	↑ P

Changing Column Length and Particle Size

5um → 3.5um

Reduce column length by factor of 1.5

e.g. 150mm *100m* *33% less ACN*

5um → 1.8um

Reduce column length by factor of 3

e.g. 150mm *50mm* *66% less ACN*

Changing Column Length and Particle Size

Maintain Rs and reduce Solvent Usage Dramatically

Column Length (mm)	Resolving Power N(5 µm)	Resolving Power N(3.5 µm)	Resolving Power N(1.8 µm)	Typical Pressure Bar (1.8 µm)
150	12,500	21,000	32,500	580
100	8,500	14,000	24,000	410
75	6000	10,500	17,000	320
50	4,200	7,000	12,000	210
30	N.A.	4,200	6,500	126
15	N.A.	2,100	2,500	55

Analysis Time	-33%
	-50%
Peak Volume	-67%
	-80%
Solvent Usage	-90%

Based on your current starting point you can quickly pick the column that will give you the same resolution in less time.

- ***pressure determined with 60:40 MeOH/water, 1ml/min, 4.6mm ID***

Changing Column Length and Particle Size

- *Often an older method has more Rs than needed*
- *Larger length reductions are possible*
- *Changing from a 250mm to a 75mm length column maximizes the allowed change in column length by FDA/ USP*

	5 um		3.5 um	5 um		3.5 um
						
Dimension	**4.6 x 250 mm**		**4.6 x 150 mm**	**4.6 x 150 mm**		**4.6 x 75 mm**
Analysis Time (min)	**30 min.**	40% reduction	**18 min.**	**18 min.**	50% reduction	**9 min.**
Solvent Waste (mL)	**30 mL**	40% reduction	**18 mL**	**18 mL**	50% reduction	**9 mL**
N	**20,000**		**20,000**	**12,000**		**10,000**

Changing Column Length and Particle Size

1/3 Solvent Use, Plus Increased Sensitivity

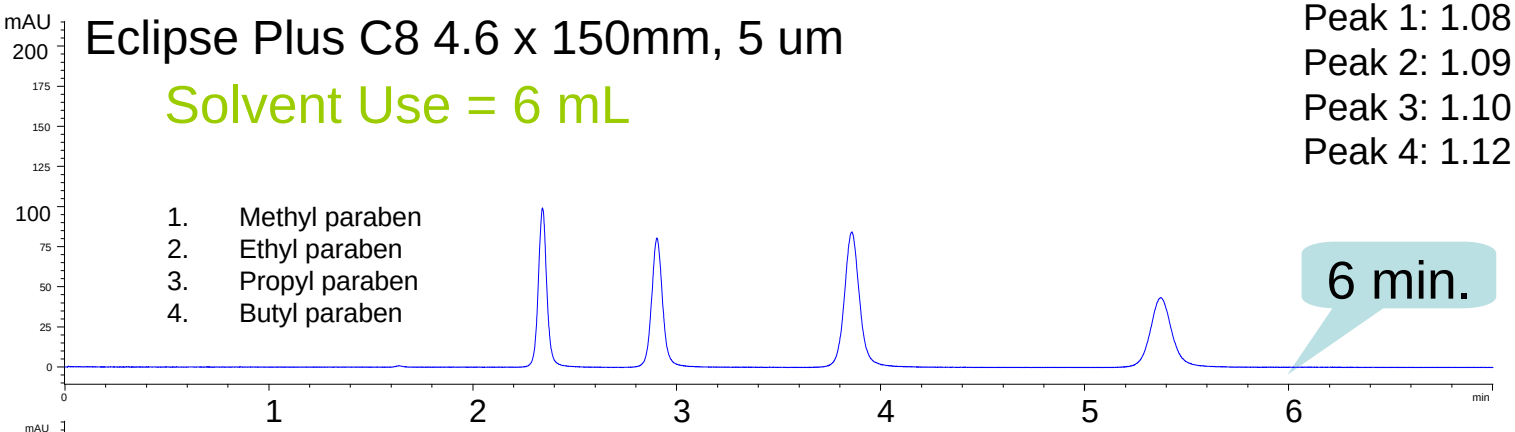
Mobile Phase: 50% ACN:50% Water, Flow Rate: 1 mL/min

Tailing factors:
Peak 1: 1.08
Peak 2: 1.09
Peak 3: 1.10
Peak 4: 1.12

Eclipse Plus C8 4.6 x 150mm, 5 um

Solvent Use = 6 mL

1. Methyl paraben
2. Ethyl paraben
3. Propyl paraben
4. Butyl paraben

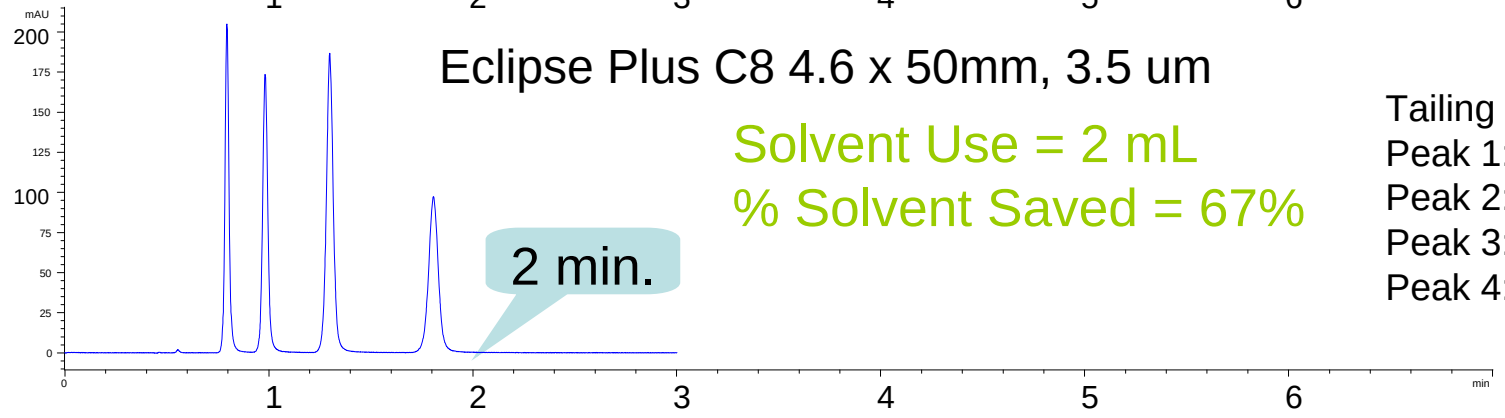


Eclipse Plus C8 4.6 x 50mm, 3.5 um

Solvent Use = 2 mL

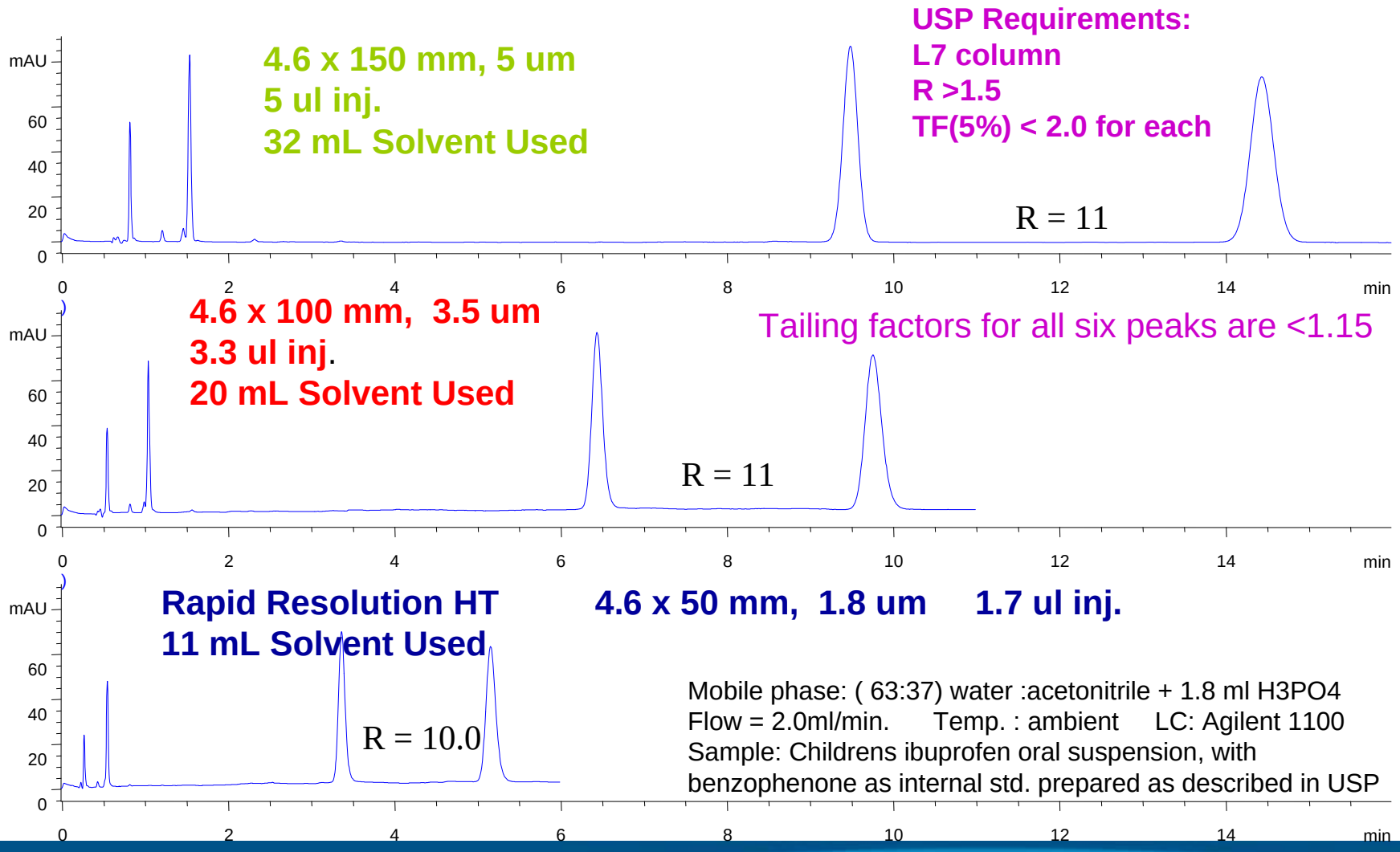
% Solvent Saved = 67%

Tailing factors:
Peak 1: 1.11
Peak 2: 1.07
Peak 3: 1.04
Peak 4: 1.01



Changing Column Length and Particle Size

USP Assay for Ibuprofen Oral Suspension



Mobile phase: (63:37) water :acetonitrile + 1.8 ml H3PO4
Flow = 2.0ml/min. Temp. : ambient LC: Agilent 1100
Sample: Childrens ibuprofen oral suspension, with benzophenone as internal std. prepared as described in USP

Changing Column Length and Particle Size

50% Less Acetonitrile in the Analysis of Propranolol

SB-C18

4.6 x 150 mm, 5 mm

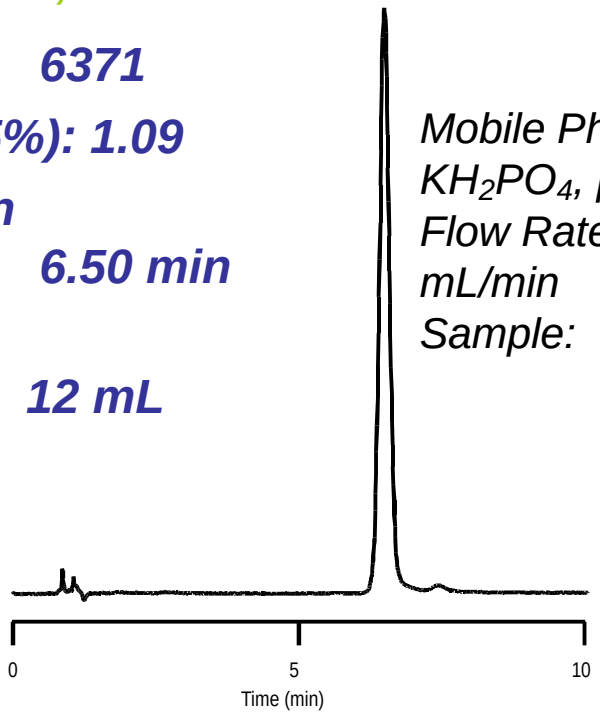
Plates: 6371

USP T_f (5%): 1.09

Retention Time: 6.50 min

Solvent Used: 12 mL

Mobile Phase: 75% 50 mM KH_2PO_4, pH 4.4: 25% ACN
Flow Rate: 1.5 mL/min
Sample: 1. Propranolol



SB-C18 Rapid Resolution

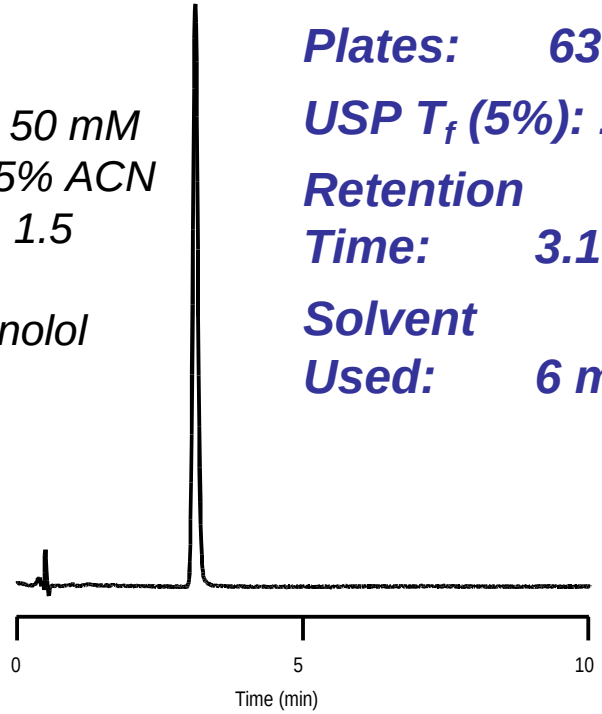
4.6 x 75 mm, 3.5 mm

Plates: 6370

USP T_f (5%): 1.14

Retention Time: 3.11 min

Solvent Used: 6 mL



Solvent Saved: 50%

Changing Column Length and Particle Size

Comparison of Results with Process LC Method on Columns with Different Particle Sizes

	5µm	**3.5µm**	1.8µm
Resolution	4.1	1.4	1.6
Selectivity	1.08	1.06	1.05
Theoretical Plates*	56108	14314	23190
k'*	6.362	4.485	4.12
Run Time (inc. re-equil)	**25 min**	**6.5 min**	**2 min**
Solvent Usage	**37.5ml**	**14.25ml**	**3ml**

Solvent Savings 92%

Changing Length, Diameter & Particle Size

e.g. 150mm x 4.6mm *100m x 4.6mm* *100 x 3.0mm*

Reduce column length by factor of 0.66

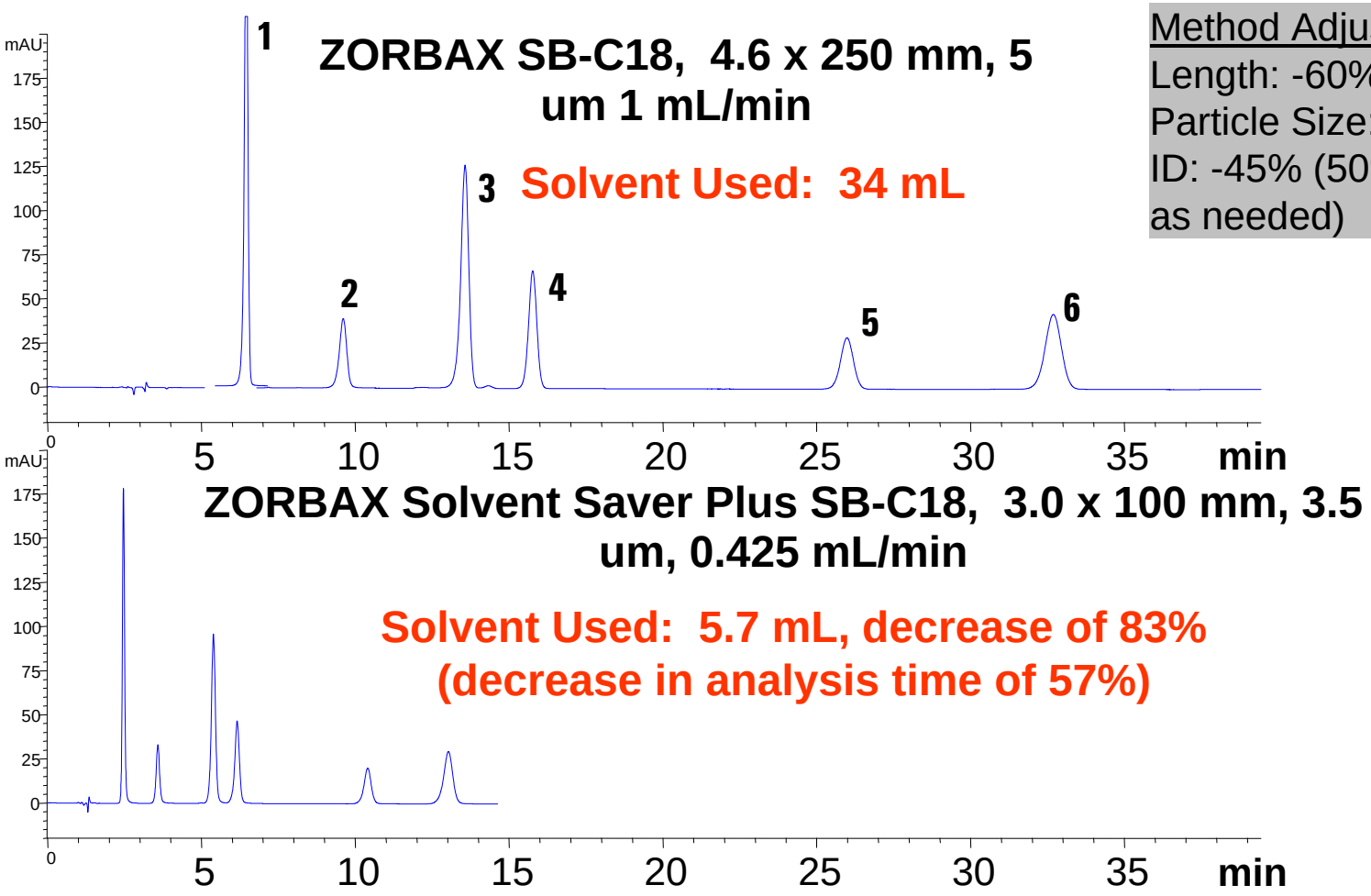
Reduce flow by factor of 0.4

73% less ACN

Changing Length, Diameter & Particle Size

Change from a 4.6 x 250 mm (5 um) to a 3.0 x 100 mm (3.5 um) Column

Mobile Phase: 25% methanol in 0.4% Formic Acid



ZORBAX SB-C18, 4.6 x 250 mm, 5 um 1 mL/min

Solvent Used: 34 mL

ZORBAX Solvent Saver Plus SB-C18, 3.0 x 100 mm, 3.5 um, 0.425 mL/min

Solvent Used: 5.7 mL, decrease of 83%
(decrease in analysis time of 57%)

Method Adjustment:
Length: -60% (70%)
Particle Size:-30% (50%)
ID: -45% (50% or as much as needed)

Changing to Methanol

- Considerations
  - *May (most likely) require revalidating the method*
  - *May require substantial redevelopment of the method for changes in selectivity*
  - *Reversed-phase separations with methanol often have longer analysis times than with acetonitrile*

Changing to Methanol

- *Start by calculating the appropriate percentage of methanol to have the same solvent strength as acetonitrile.*

- *Run the sample (same column)*

- *Evaluate retention and resolution of method*
  - *Expect longer analysis times if your method contains basic compounds*
  - *Neutral compounds may not show as much change*
  - *Therefore changes in selectivity and resolution can occur*

- *Adjust methanol composition to change retention as needed*

- *Change bonded phase as a last option to adjust selectivity needs where current column choice is not working*

Changing to Methanol

Isoelutropic Strength Table

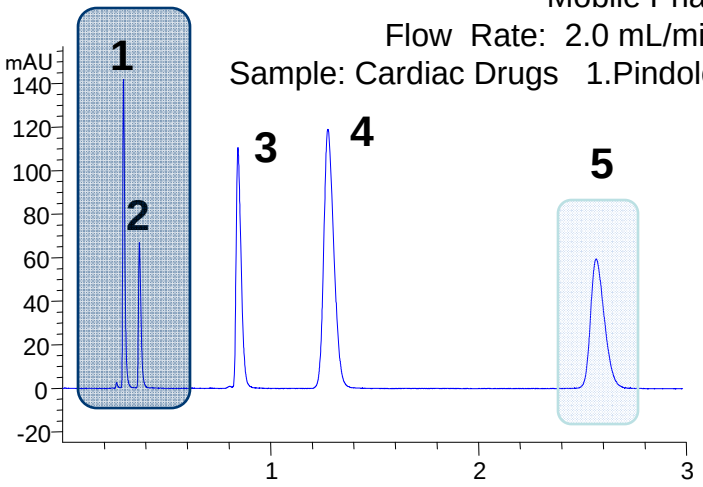
% MeOH in H2O	% ACN in H2O	Relative k'
0	0	100
10	6	40
20	14	16
30	22	6
40	32	2.5
50	40	1
60	50	0.4
70	60	0.2
80	73	0.06
90	86	0.03
100	100	0.01

http://www.sanderkok.com/techniques/hplc/eluotropic.html

Changing to Methanol

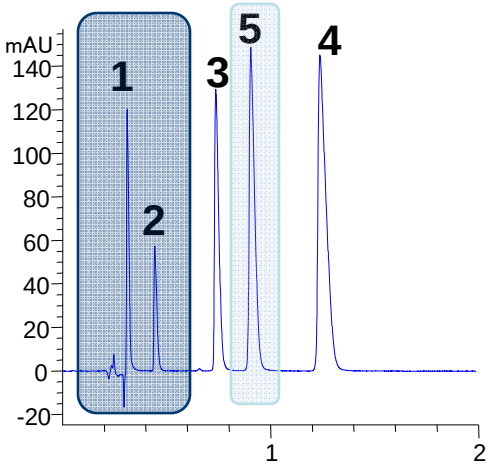
Comparison of Acetonitrile and MeOH Separations

Column: ZORBAX RRHT Eclipse Plus C18, 4.6 x 50 mm, 1.8 µm
Mobile Phase: A: 25 mM NaH_2PO_4 , pH 3.0 B: organic
Flow Rate: 2.0 mL/min Temperature: 30°C Detection: UV 240 nm
Sample: Cardiac Drugs 1.Pindolol 2. Disopyridamide 3.Propranolol 4.Diltiazem 5. Dipyridamole



50% MeOH

Resolution of pairs (2,3), (3,4), (4,5) is better in MeOH. Peak 5 selectivity shift



30% ACN

Resolution of critical pair (1,2), is better in ACN

Changing to Methanol

How can Switching to Methanol be made easier?

- *Automated Method Development System*

- *An instrument capable of automatically switching between columns and solvents with an appropriate software to set up experiments.*

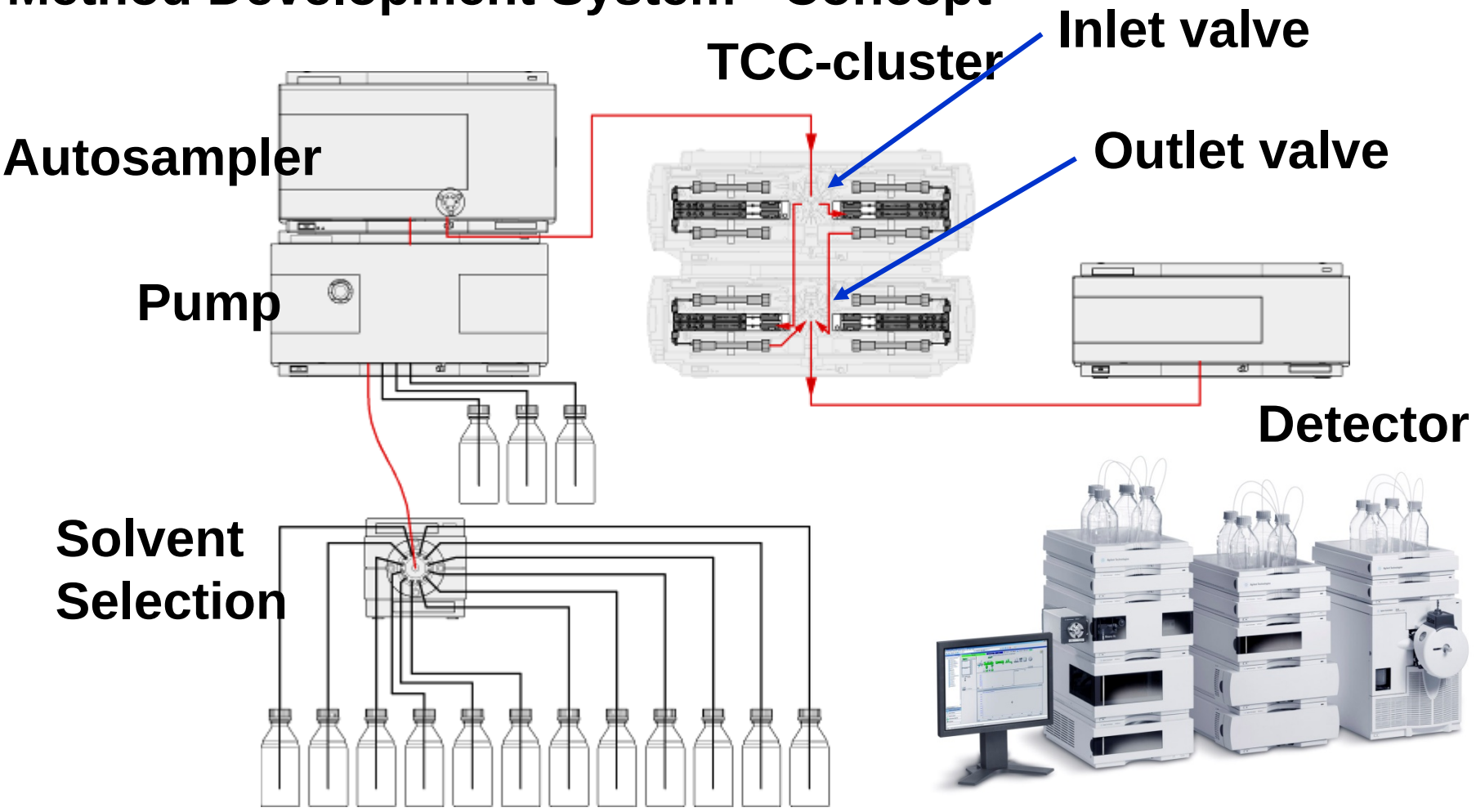
Changing to Methanol

Agilent 1200 Series Method Development Solution

- *New and clustered thermostatted column compartments (TCC) with integrated 400 or 600 bar column selection valves*
  - *8 columns (bypass and/or waste)*
  - *2.1 – 4.6 mm ID*
  - *30 – 300mm length*
  - *same thermal behavior as standard Agilent TCC*
  - *independent temperature zones*
  - *simple one-click column selection*
- *Pump clustered with external solvent selection valve*
  - *12+3 solvents to select*
  - *simple one-click solvent selection*

Changing to Methanol

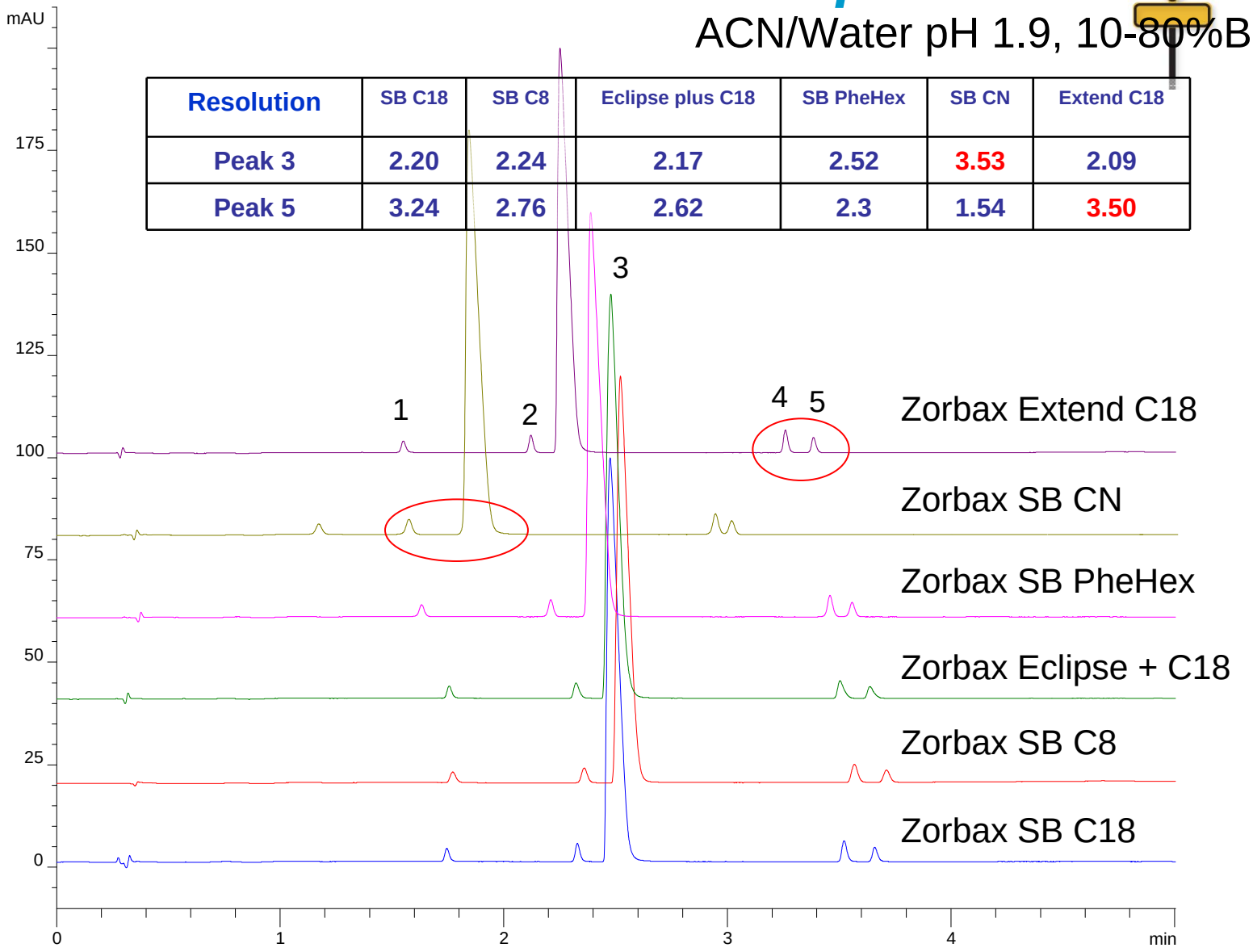
Method Development System - Concept



Agilent 1200 Series Method Development Solution

ACN/Water pH 1.9, 10-80%B

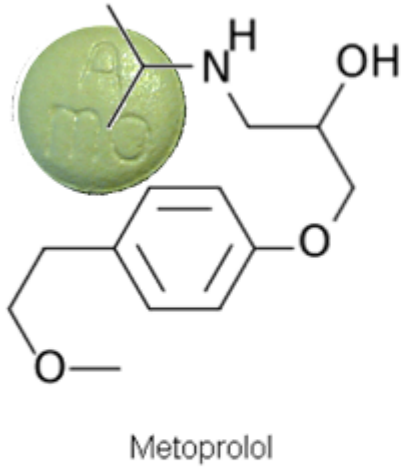
Resolution	SB C18	SB C8	Eclipse plus C18	SB PheHex	SB CN	Extend C18
Peak 3	2.20	2.24	2.17	2.52	3.53	2.09
Peak 5	3.24	2.76	2.62	2.3	1.54	3.50



Agilent 1200 Series Method Development Solution

Metoprolol + decomposition products from Metoprolol tablets

Columns: **2.1x50mm** columns packed with **1.8µm** particles
Mobile phase: water +0.2% TFA / ACN+0.16%TFA Flow rate: 0.5ml/min
Gradient: 5 to 50% in **5min,**
Column temperature: all 30° C
Detector DAD: 210, 230,254, 280/10nm



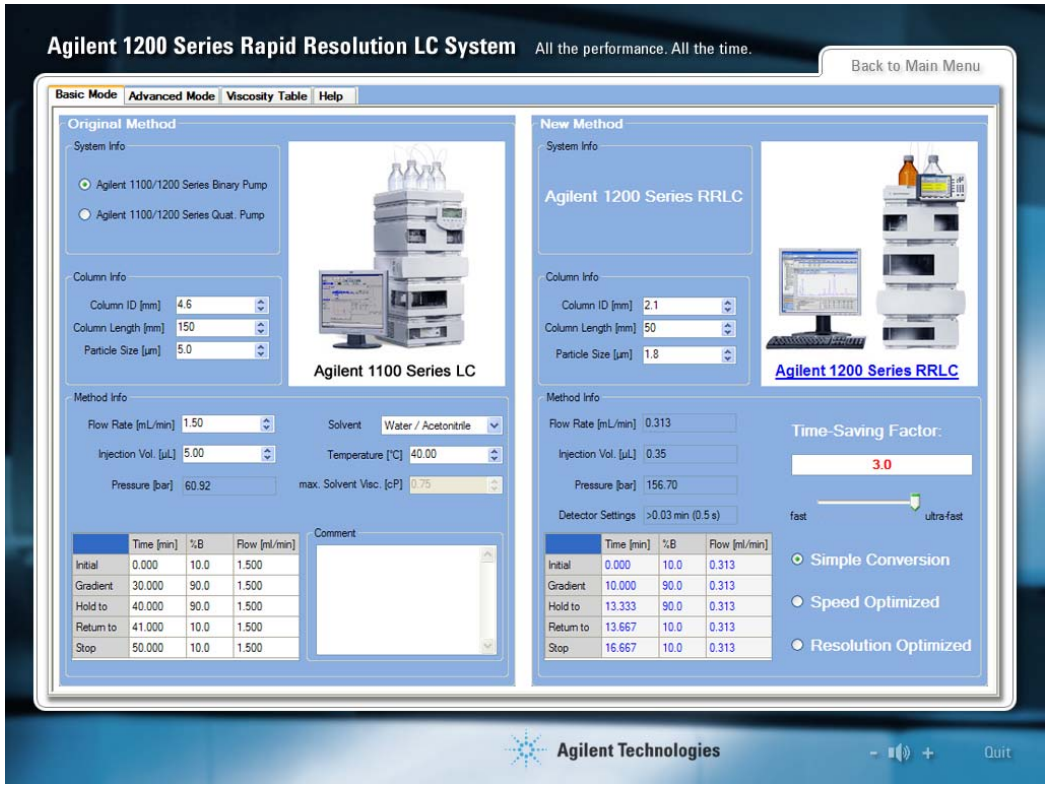
Metoprolol

Other ideas

- Solvent recycling with or without peak detection
  - *Only usable with isocratic methods with premixed solvents*
  - *Risks contamination of solvent*
  - *Potential unexplained noise and peaks in the chromatogram, unacceptable to trace level and regulated analyses*

Tools

Mini-Demo Method Translator



Alternative Separation Techniques

Super Critical Fluid Chromatography

Aurora SFC Fusion A5 module: From HPLC to SFC….in Minutes

- ✓ One module takes LC to SFC… and back again
- ✓ Aurora SFC Fusion A5 is an add-on module to Agilent LC's
- ✓ Re-defines cost and performance standards for Analytical SFC
  - ✓ Re-defines noise performance, making SFC applicable to impurity analysis
  - ✓ Uses standard LC components and software
- ✓ Green with lower costs, uses no ACN, with food-grade CO_2 (does not require expensive SFC-grade CO_2)
- ✓ More information: www.aurorasfc.com

Aurora SFC™ Systems

Alternative Separation Techniques

Super Critical Fluid Chromatography

- Does not use ANY Acetonitrile
- Uses inexpensive CO_2 as primary mobile phase
- SFC offers all the speed associated with HPLC at significantly lower pressures
- SFC mobile phases have 1/10th the viscosity of normal liquids
- Solutes diffuse much faster in SFC mobile phases compared to normal liquids
- SFC can be performed using any column or particle size used in HPLC but produces peaks 3 to 5 times narrower.

Aurora SFC™ Systems

Alternative Separation Techniques

Super Critical Fluid Chromatography

Conventional LC Method:
3mL/min. 24x7. 50/50 Solvent/Fluid gradient
HPLC: 788 L /year Fluid & Solvent
Fluid cost (Water) $40/L. Solvent cost (ACN) $100/L

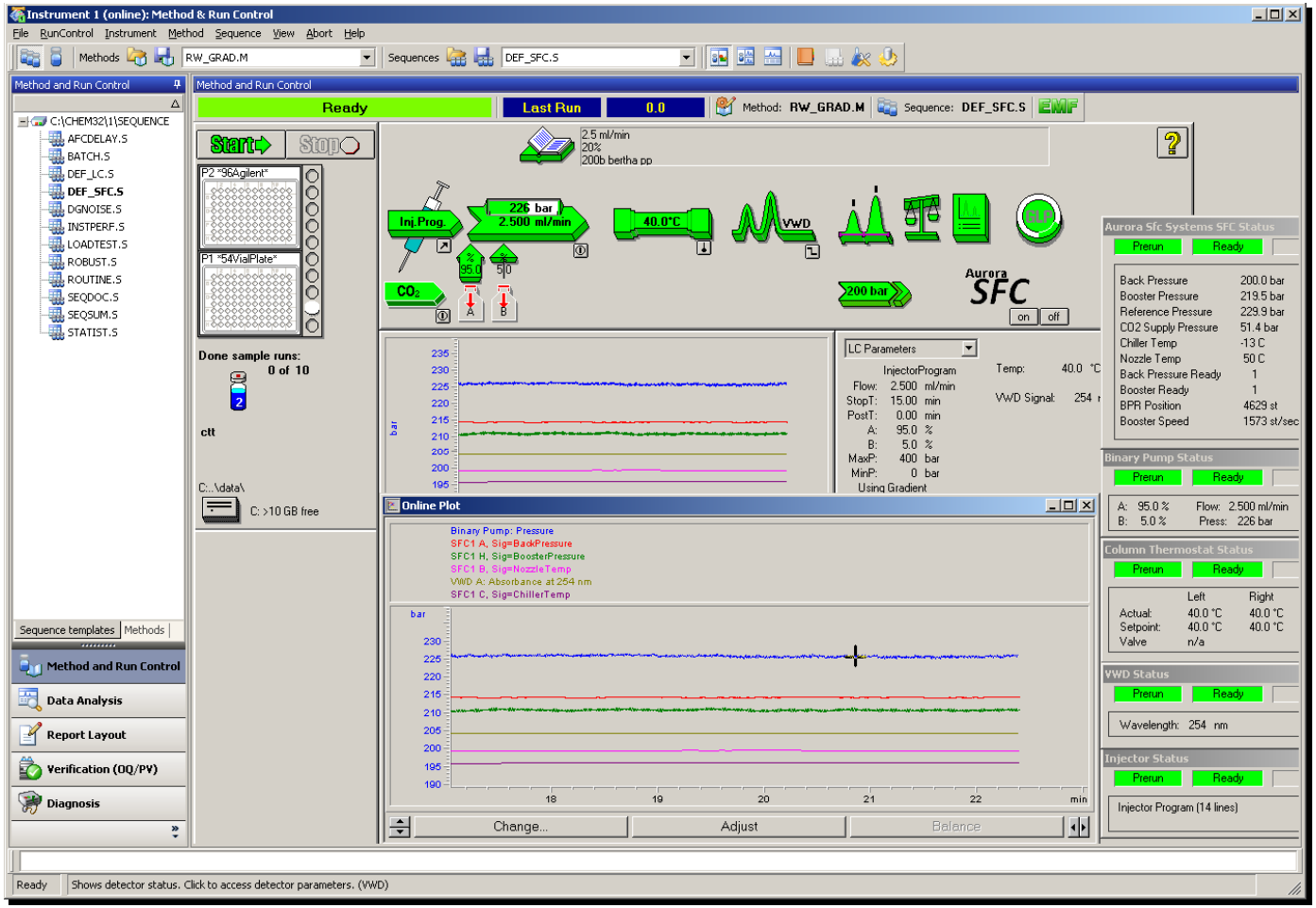
SFC Method:
3mL/min. 24x7. 20/80% Solvent/Fluid gradient
sp-LC: 1418L /year Fluid. 157 L/year Solvent
Fluid cost (CO2) $1/L. Solvent cost (Methanol) $36/L

	HPLC	SFC	% Cost Reduction
Fluid: *(Water/CO2)*	$31,520	$1,418	
Solvent: *(ACN/Methanol)*	$141,840	$5,674	
Liquids Disposal *@ $50/L*	$39,400	$7,880	
	$212,760	$14,972	**93%**

Aurora SFC™ Systems

Alternative Separation Techniques

Super Critical Fluid Chromatography



Seamless Integration Into Agilent Chemstation

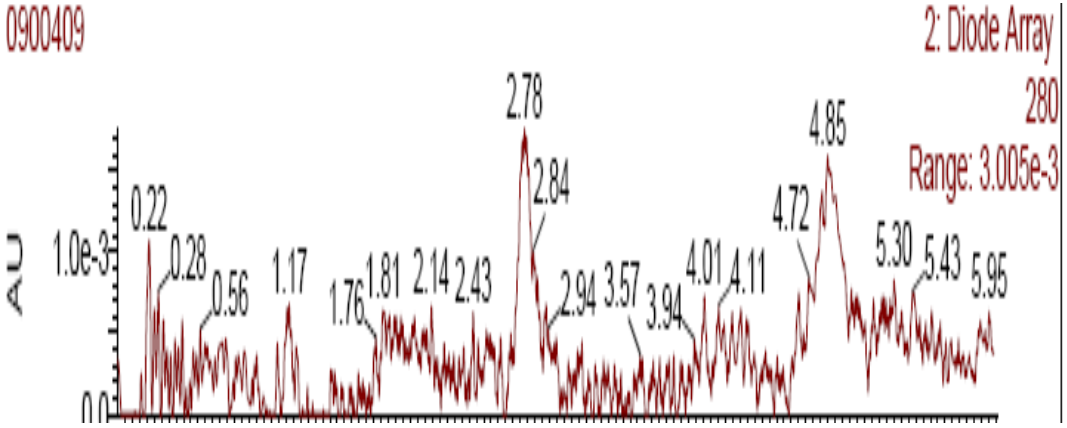
- Beyond standard interfacing, Fusion displays two icons in the system diagram. These icons provide instant feedback of the system state and conditions.
- Pop-up menus provide direct method editing (Settings).

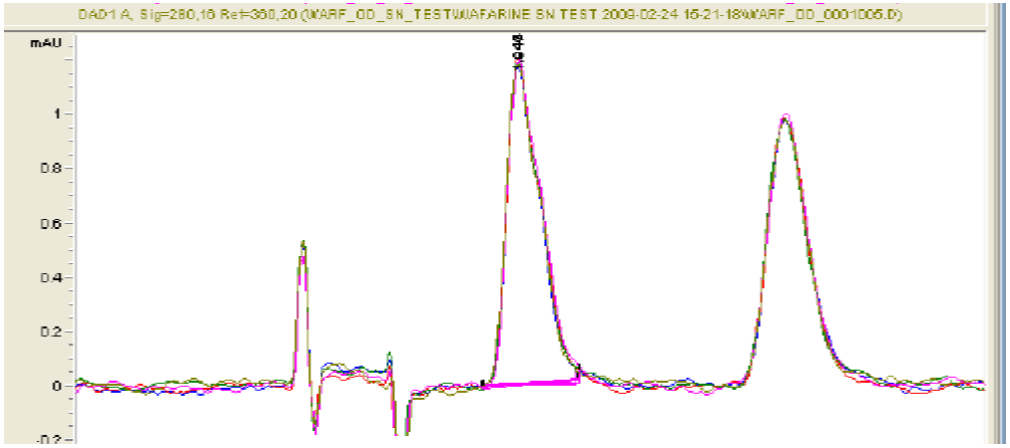
Aurora SFC™ Systems

Alternative Separation Techniques

Super Critical Fluid Chromatography

Lowest Noise SFC





Chiral Separation of .1% of original Warfarin

Berger Instruments SFC

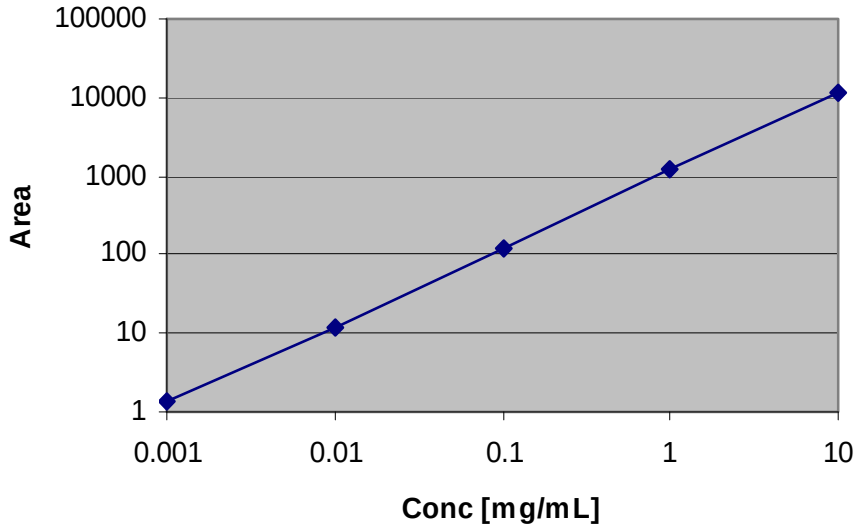
- Same sample (same vial)
- Same injection volume
- Same condition set-points
- Similar detector (1100 vs 1200)
- Same wavelengths and bandwidths
- Same peak width & height
- Same scale
- Same column

Aurora Fusion A5 + Agilent 1200SL

Aurora SFC™ Systems

Alternative Separation Techniques

Super Critical Fluid Chromatography ~ Validation



CORRELATION
COEFFICIENT > 0.99999
Over 5 orders of magnitude

Statistics for 1st peak

~+/- 0.5% RSD on retention time
~+/- 0.35% RSD on peak area IF S/N >100

Conc. mg/mL	Ret. Time, min	Area Counts	Height	S/N .
10.00	1.966=/-0.46%	11,772+/-0.27%	1033.09+/-2.85%	>16K-67K
1.00	1.960+/-0.56%	1232.5+/-0.46%	110.40+/-0.99%	4846
0.10	1.948+/-0.70%	116.97+/-0.31%	11.16+/-1.11%	538
0.010	1.944+/-0.15%	12.30+/-2.07%	1.191+/-1.13%	>57.6
0.001	1.950+/-0.27%	1.332+/-14.22%	0.136+/-8.34%	7.9

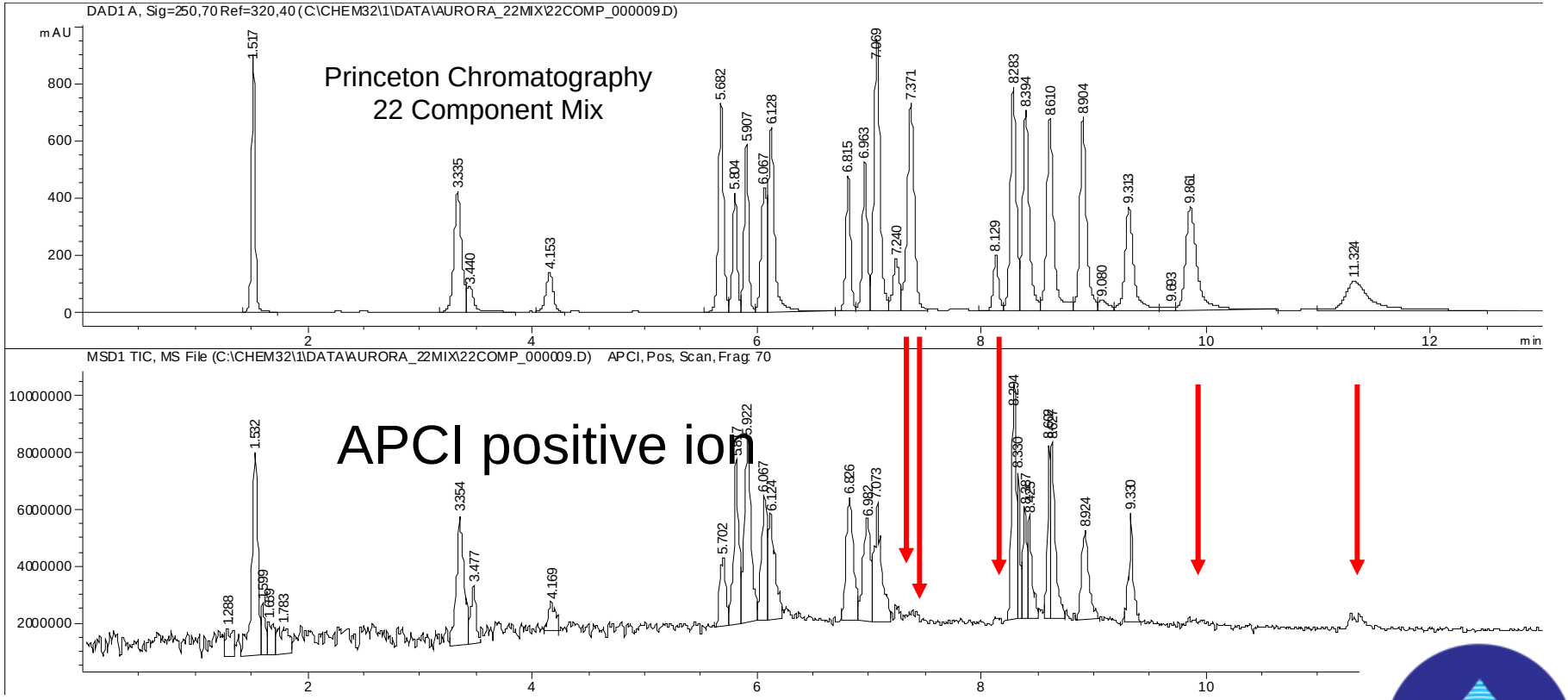
Aurora SFC™ Systems

Alternative Separation Techniques

Super Critical Fluid Chromatography ~ SFC-MS

HA dypyridyl 4.6 x 250, 6µ
3.5ml/min, doubling gradient , APCI

22 component mix



Fusion A5 w/ Agilent 1200 SL-Single Quad

Aurora SFC™ Systems

Conclusions

- *Easy column changes to control solvent use are effective at reducing acetonitrile usage*
  - *Solvent Saver and Narrow Bore*
  - *Rapid Resolution – with reduced column length*
  - *Rapid Resolution HT – with reduced column length*
- *Many changes can be made within guidelines for method adjustments.*
- *Move to revalidate easy methods frequently used methods and methods with high Acetonitrile use to avoid problems.*
- *Optimize instrumentation and overall method can support savings*
- *Switching to methanol – more complex; method development necessary*
- *HPLC to SFC in minutes using new Aurora Fusion A5 system*

Thank You !!

Learn more at: