

METHOD TRANSFER

TIME CONSTRAINTS

REPRODUCIBILITY

Clear your workflow hurdles and make every milliliter of solvent count.

Agilent ZORBAX Solvent Saver and RRHT columns



A global shortage of Acetonitrile (ACN) – the most common organic HPLC solvent – is forcing labs to take strict measures toward controlling the amount of solvent used during each run. But the good news is... Agilent has a simple solution to meet this challenge:

Agilent ZORBAX Solvent Saver (3.0 mm I.D.) and Rapid Resolution High Throughput (RRHT) (1.8 μ m) columns can reduce HPLC solvent waste by 50% or more.

With Agilent Solvent Saver and RRHT columns, you can:

- Dramatically decrease solvent usage while maintaining resolution and decreasing your analysis time.
- Lower your solvent disposal costs.
- Lengthen your periods of unattended operation.

Best of all, you don't have to change your bonded phase or modify your LC configurations. That's because ZORBAX Solvent Saver and RRHT columns are compatible with standard HPLC systems, and are available in all ZORBAX phases.

To learn how every drop of solvent can work harder for you, visit www.agilent.com/chem/solventsaver1

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Agilent Technologies

LC Columns for Reducing Solvent Use

Approaches to Limiting Solvent Use
and Waste

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 C₄ 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

Current Issues in Acetonitrile Solvent Use

- Acetonitrile is one of the most commonly used and versatile organic modifiers for HPLC.
- It is currently in short supply worldwide.
- The supply is getting lower and prices are increasing dramatically, especially for HPLC grade ACN.
- Agilent has a number of LC column solutions for reducing solvent – acetonitrile – use in HPLC separations.
- These are easy to implement and don't require changing bonded phases!

First Determine What Your Method Flexibility Might Be and What You Need Today

•Do you have a regulated method where you want to make only method adjustments?

- 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, cleaning verifications) 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

First Determine What Your Method Flexibility Might Be and What You Need Today

- **Do you have a non-regulated method, but need to take time to modify the method?**

- Find your least complex methods to modify first
- Choose methanol first as your alternate solvent
- Start with same solvent strength (isoeluotropic) and then make adjustments to solvent, using same column
- Change column selectivity (bonded phase) if needed

Options for Method Adjustments

1. Review method adjustment criteria – FDA and USP
 - ICH Guidelines and other pharmacopeia guidelines are often very similar, but always check documents and company SOP's
2. Review approaches to reducing solvent use with method adjustment criteria in mind
3. Review examples on different columns to see potential impact

USP and FDA Method Adjustment Criteria

Method Adjustment Criteria for Column Dimensions

Parameter	Maximum Specifications	Comments/Examples
Column Length	± 70%	250mm → 75mm 150mm → 50mm
Column Internal Diameter	±25% (FDA ORA-LAB 5.4.5*) USP – ± 25% Column ID can be adjusted provided linear velocity is constant** (in process)	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 5um → 3.5um (-30%) 3.5um → 1.8um (-49%)

*Changed on 2/24/09 - For the current and official copy, go to <http://web.ora.fda.gov/dfs/policies/manuals/default.html>

** USP 30 (2nd Supplement) Revisions, PF34(5), in process expected to be final Dec 2009

Several Approaches to Reducing Solvent Use in HPLC

- Reduce Column Diameter – Change to Solvent Saver
 - Complies with FDA guidelines and USP guidelines
- Reduce Column Length – Shorter columns
 - Lots of flexibility, but need to make sure R_s meets needs
- Reduce Multiple Parameters at Once
 - Shorter columns with small particle sizes
 - Rapid Resolution Columns for any LC (3.5 μ m)
 - Rapid Resolution HT Columns – 50mm for any LC and longer for RRLC
 - This can be done within USP and FDA guidelines on official methods




Column Diameter Selection

- Column diameter selection will dramatically impact the solvent used because flow rate depends on column internal diameter!
- As you change ID you want to keep linear velocity the same.

Column Diameter

- 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)

HPLC Columns of Smaller Inner Diameter Reduce Solvent Waste

	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%

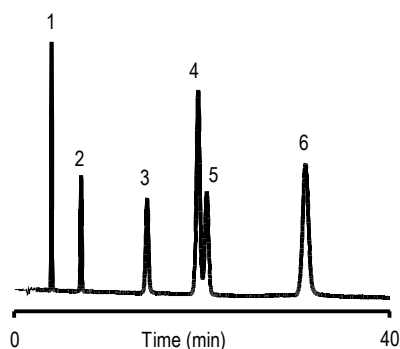
By reducing column ID solvent use is reduced dramatically – 1st step to save solvent!

By Reducing Column
Diameter:
Solvent Savings are Realized
Because Analysis Time is
Unchanged and Flow Rate is
Reduced.

Separation of Antibacterials on Columns Having Different Diameters

Column: ZORBAX SB-C18 Mobile Phase*: 20% ACN: 80% Citrate/phosphate pH 2.6 *200/87/713 ACN/0.2M Na₂HPO₄/0.1M citric acid
Temperature: ambient Sample: Antibacterials 1. Sulfamerazine 2. Furazolidone 3. Oxolinic acid 4. Sulfadimethoxine 5. Sulfaquinoxoline 6. Nalidixic acid

SB-C18
4.6 x 150 mm, 5 um



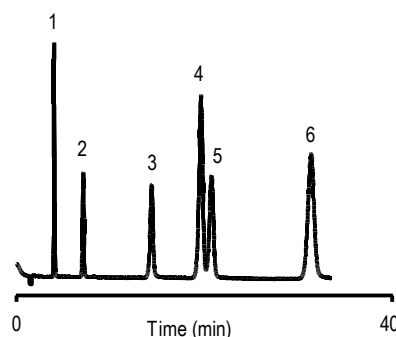
Solvent Used: 31 mL

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



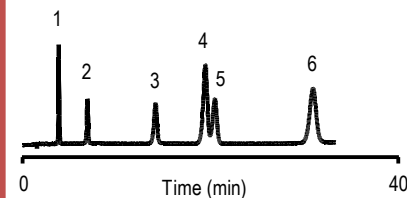
Solvent Used: 15 mL
% Solvent Saved = 52%

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

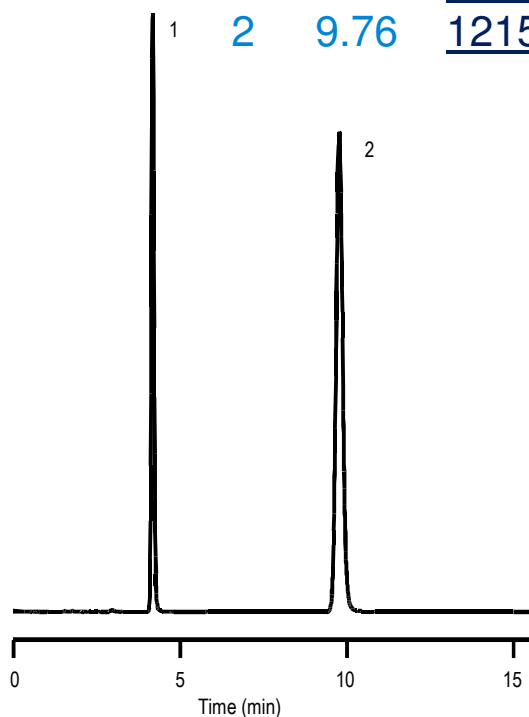
Solvent Saver column reduces solvent use by more than 50% while keeping particle size, bonded phase, column length the same. This is in line with FDA guidelines for a method adjustment

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

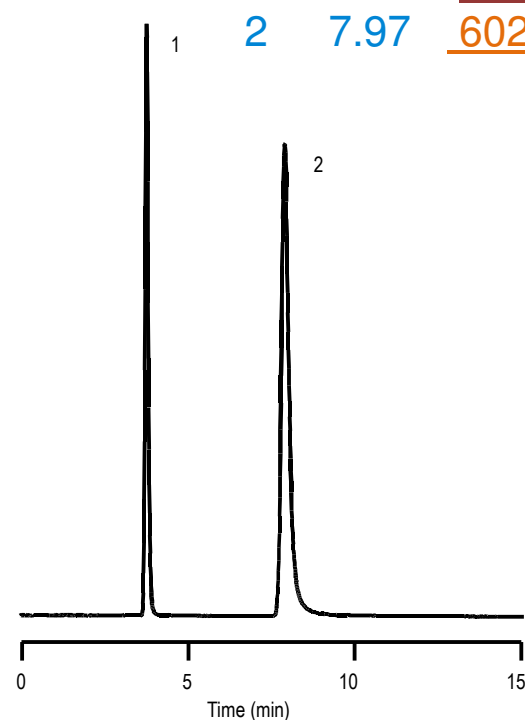
SB-C18

Peak	Retention Time	Plates	R _s
1	4.17	<u>13850</u>	0.00
2	9.76	<u>12154</u>	<u>20.28</u>



ODS

Peak	Retention Time	Plates	R _s
1	3.81	<u>8034</u>	0.00
2	7.97	<u>6020</u>	<u>13.45</u>



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

Column Choices to Save Solvent and Time

Mobile Phase: 35% water: 65% methanol

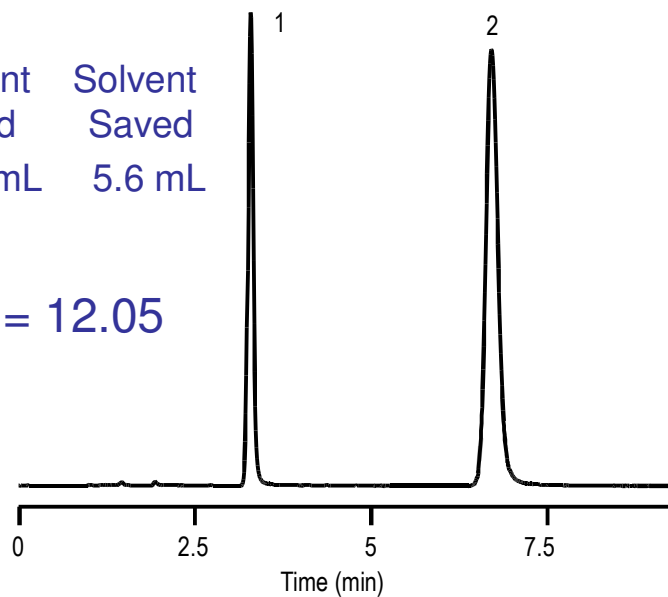
Sample: 1. Ethylparaben 2. Diazepam

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

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

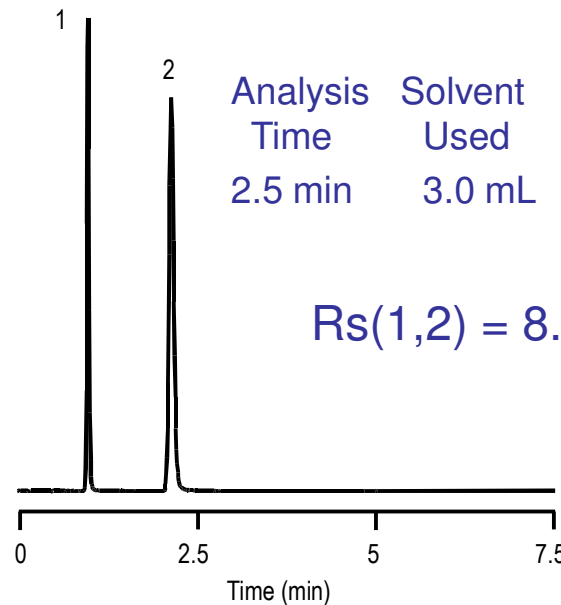
Analysis Time	Solvent Used	Solvent Saved
8 min	4.0 mL	5.6 mL

$Rs(1,2) = 12.05$



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

$Rs(1,2) = 8.62$

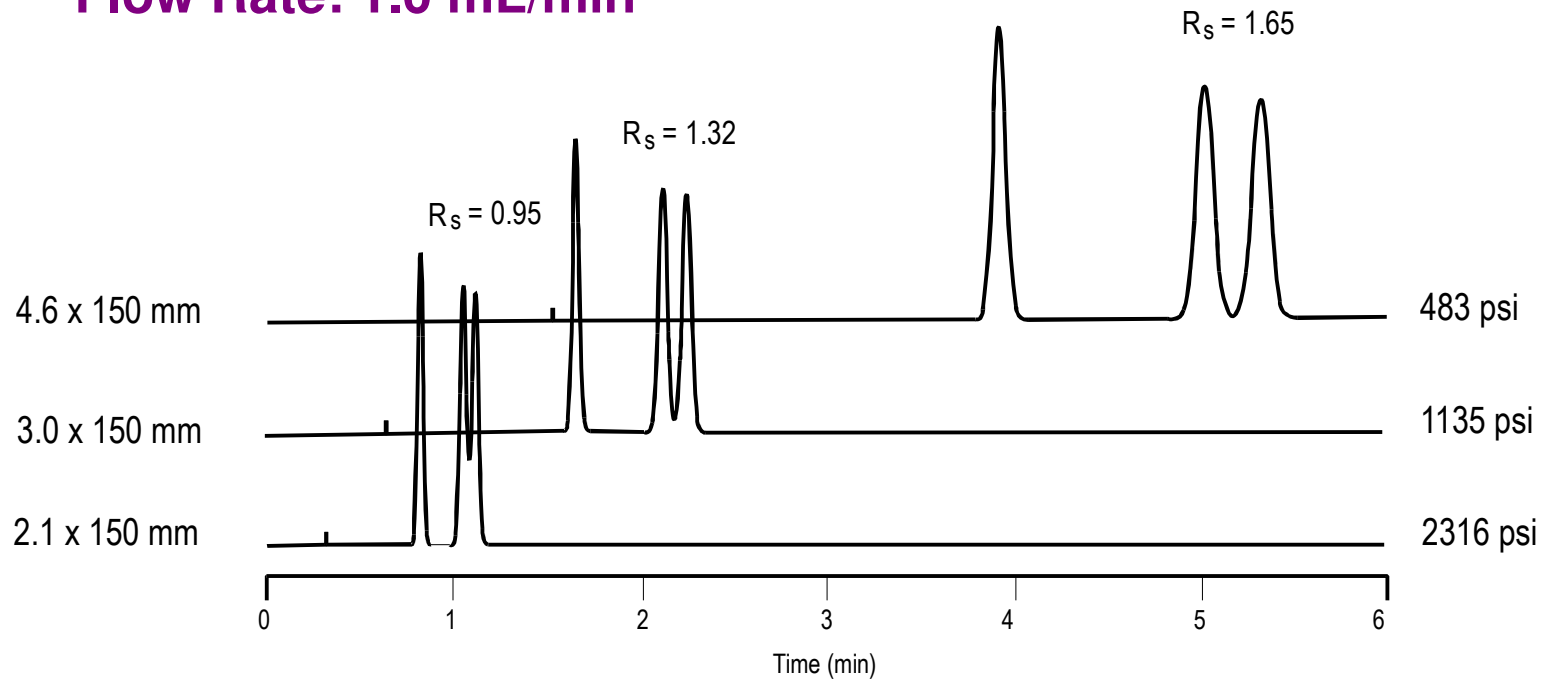


- Consider all column configuration options for saving solvent and time.
- If you meet R_s requirements then use shortest column to save time and solvent.

Reducing Column Diameter
Requires Reducing the Flow Rate
to
Maintain Resolution

Separation of Nitrobenzenes on Columns of Different Diameters with the Same Flow Rate

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



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!

Equations for Calculating Flow Rates and Injection Volumes When You Change Column ID

- Scale flow rate (maintain linear velocity) to maintain results:

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

- Example 1: $F_2 = 1.0 \text{ mL/min} \times (3.0\text{mm})^2 / (4.6 \text{ mm})^2$

- $F_2 = 0.43 \text{ mL/min}$

- Example 2: $F_2 = 1.0 \text{ mL/min} \times (2.1\text{mm})^2 / (4.6 \text{ mm})^2$

- $F_2 = 0.21 \text{ mL/min}$

- Scale Injection Volume

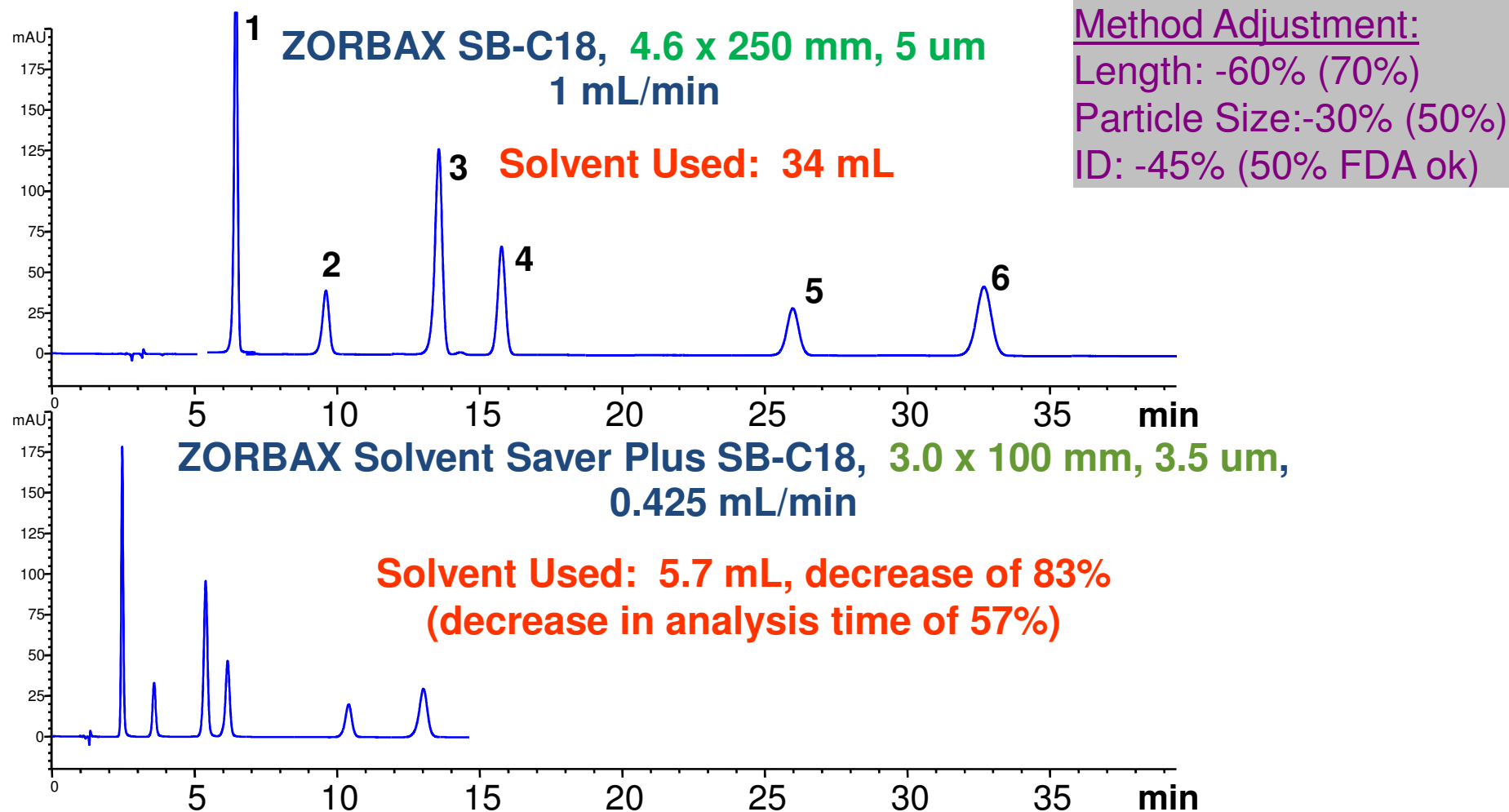
- $V_2 = V_1 \times [(r_2^2 \times L_2) / (r_1^2 \times L_1)]$

- $r = \text{radius of column, } L = \text{length}$

Changing Column Length, Diameter & Particle Size

Change from a 4.6 x 250 mm (5 μ m) to a 3.0 x 100 mm (3.5 μ m) Column.

Mobile Phase: 25% methanol in 0.4% Formic Acid



Reduce Column Diameter - Benefits and Recommendations

First Choice

- Solvent Saver Columns (3.0 mm id)
- Solvent savings up to 60% with almost standard HPLC
 - Capillary tubing 0.12 mm id (red)

Second Choice

- Narrow-bore columns (2.1 mm id)
- Solvent savings up to 80% with optimized HPLC
 - Detector cell volume of 2 μL 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 $< 5 \mu\text{L}$

Column Length and Particle-Size Selection

Column Length and Particle Size

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

Short Column/Smaller Particle Maintains Rs Reduce Solvent Usage Dramatically

* Reduction in analysis time compared to 150 mm column

Column Length (mm)	Resolving Power N(5 μ m)	Resolving Power N(3.5 μ m)	Resolving Power N(1.8 μ m)	Typical Pressure Bar (1.8 μ m)	Analysis Time*
150	12,500	21,000	32,500	580	
100	8,500	14,000	24,000	410	-33%
75	6000	10,500	17,000	320	-50%
50	4,200	7,000	12,000	210	-67%
30	N.A.	4,200	6,500	126	-80%
15	N.A.	2,100	2,500	55	-90%

Analysis Time
Peak Volume
Solvent Usage

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

Rapid Resolution HPLC Columns Reduce Analysis Time and Solvent Waste

Going from a 250mm to a 75mm length column maximizes the allowed change in column length.

Method Adjustment: Length: -70% (70%) Particle Size:-30% (50%)

ID: **No Change**

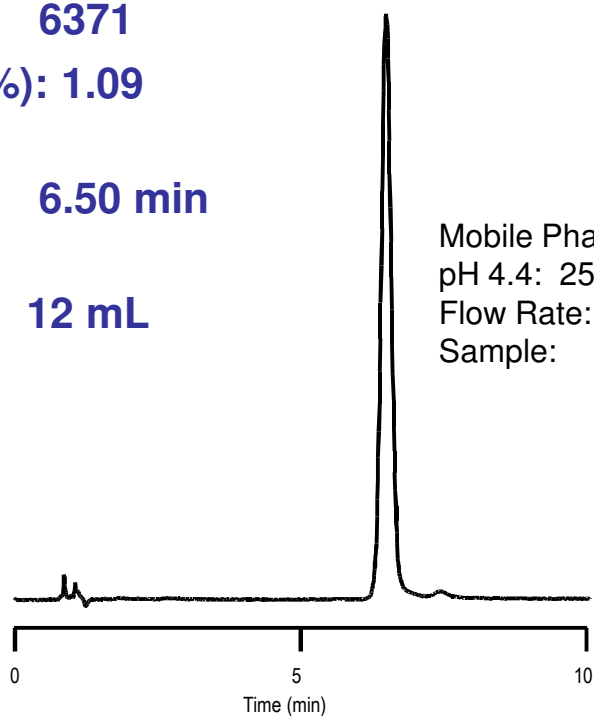
	5 um	3.5 um	5 um	3.5 um
				
Dimension	4.6 x 250 mm	4.6 x 150 mm	4.6 x 150 mm	4.6 x 75 mm
Analysis Time (min)	30 min.	18 min.	18 min.	9 min.
		40% reduction		50% reduction
Solvent Waste (mL)	30 mL	18 mL	18 mL	9 mL
		40% reduction		50% reduction
N	20,000	20,000	12,000	10,000

50% Less Acetonitrile (Solvent) Used in the Analysis of Propranolol with a Rapid Resolution Column

SB-C18
4.6 x 150 mm, 5 μ m

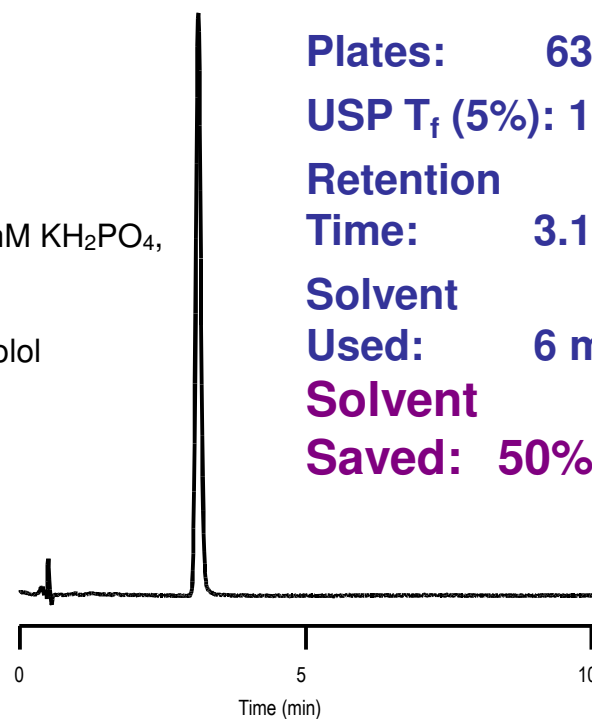
SB-C18 Rapid Resolution
4.6 x 75 mm, 3.5 μ m

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

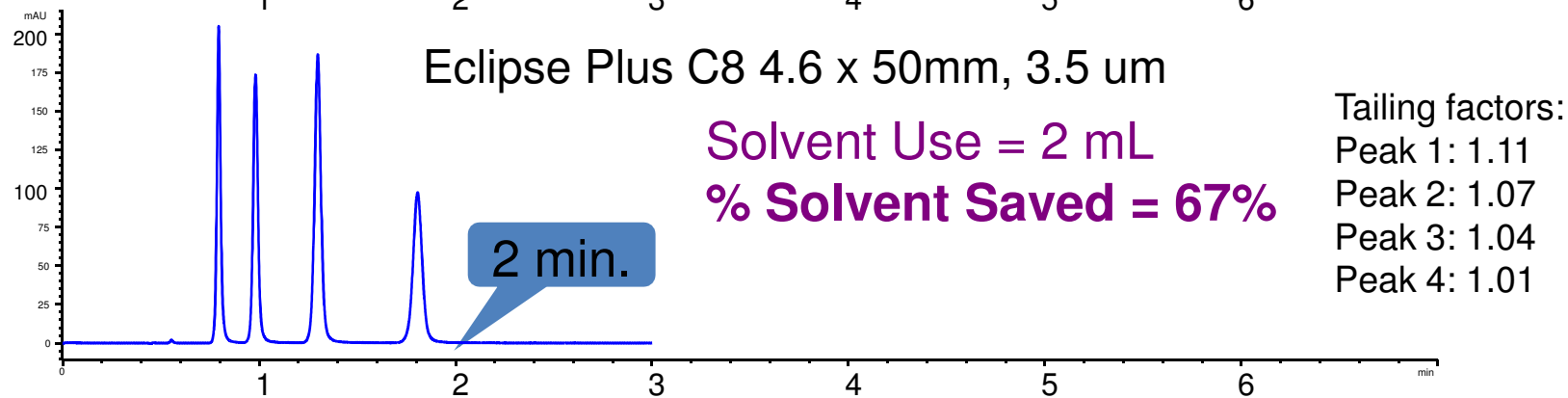
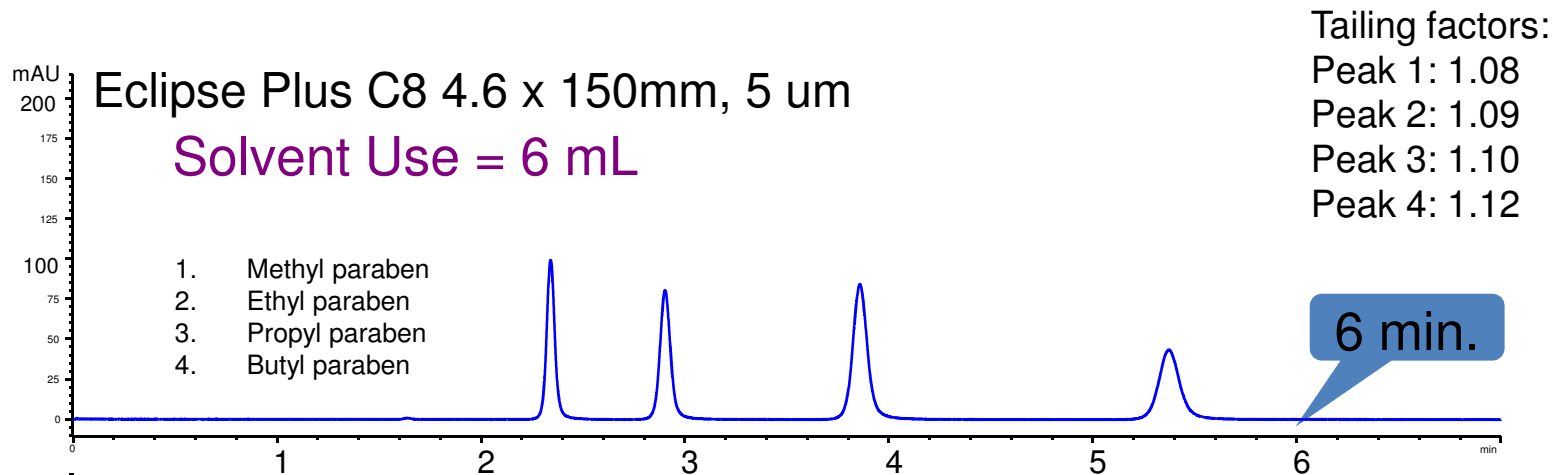
Plates: 6370
USP T_f (5%): 1.14
Retention Time: 3.11 min
Solvent Used: 6 mL
Solvent Saved: 50%



- Excellent peak shape on SB-C18 columns, easily meets requirements with very low tailing.
- Rapid Resolution SB-C18 reduced analysis time with equivalent results.

Rapid Resolution Analysis – 1/3 Analysis Time and Solvent Use, Plus Increased Sensitivity

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



Benefits of Using the ZORBAX Rapid Resolution Approach

- Reduce analysis time and solvent waste up to 50%
- Maintain resolution
- Pressure typically <3000 psi for 4.6 x 150 mm, 4.6 x 100 mm and 4.6 x 75 mm columns
- Use standard HPLC equipment
- Minimal loss in efficiency (N) at higher flow rates
- No instrument modifications needed
- Long column life due to strong silica
- Susceptibility to column plugging is not an issue
 - Narrow silica-particle distribution allows use of 2 um frit

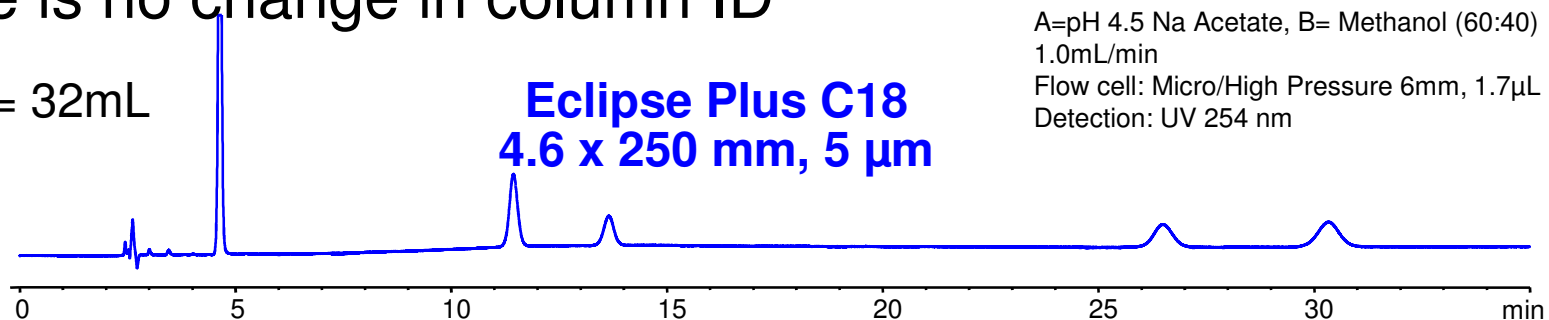
The Next Step – Rapid Resolution HT Columns

- Rapid Resolution HT columns are those with 1.8um particles
- This step down in particle size means even shorter columns with the same resolution.
- Shorter columns means shorter run times and more substantial solvent savings!

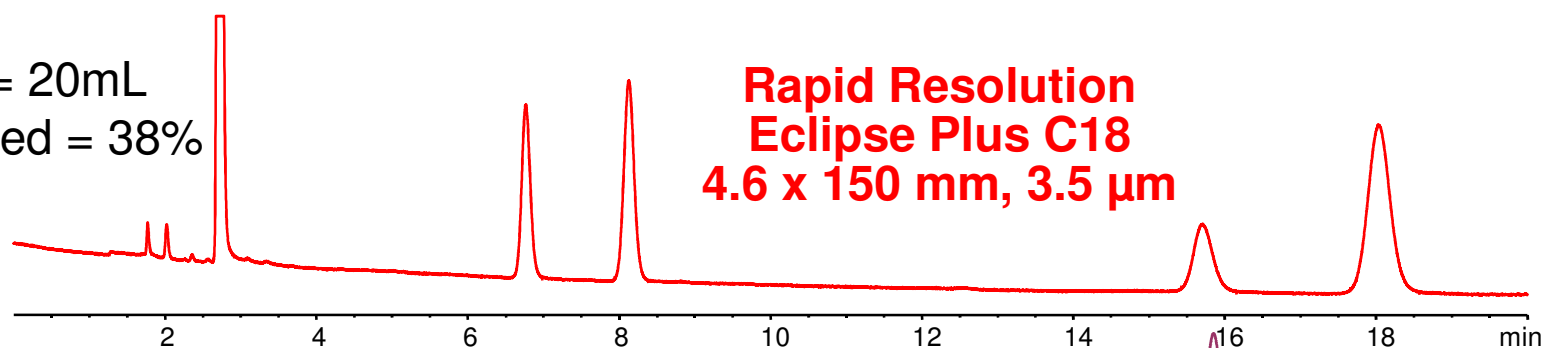
Reduce Column Length, Particle Size for High Resolution with Solvent Savings

Note there is no change in column ID

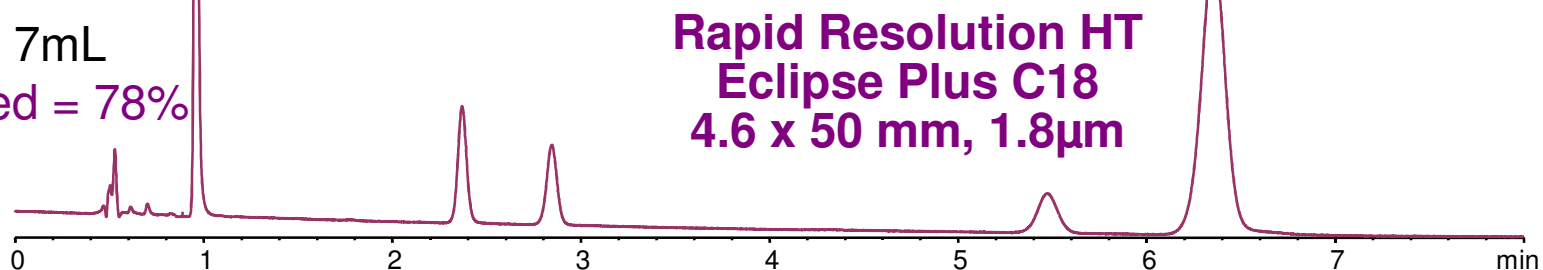
Solvent Used = 32mL



Solvent Used = 20mL
% Solvent Saved = 38%



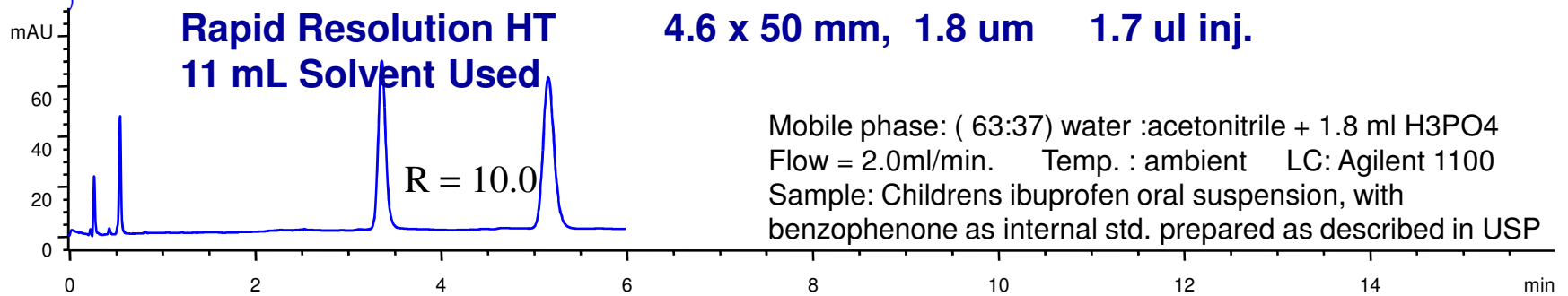
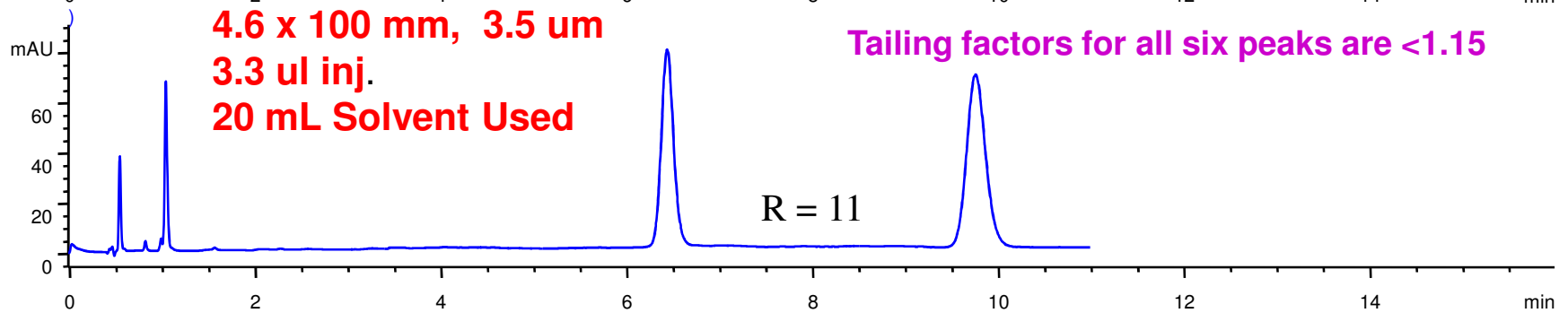
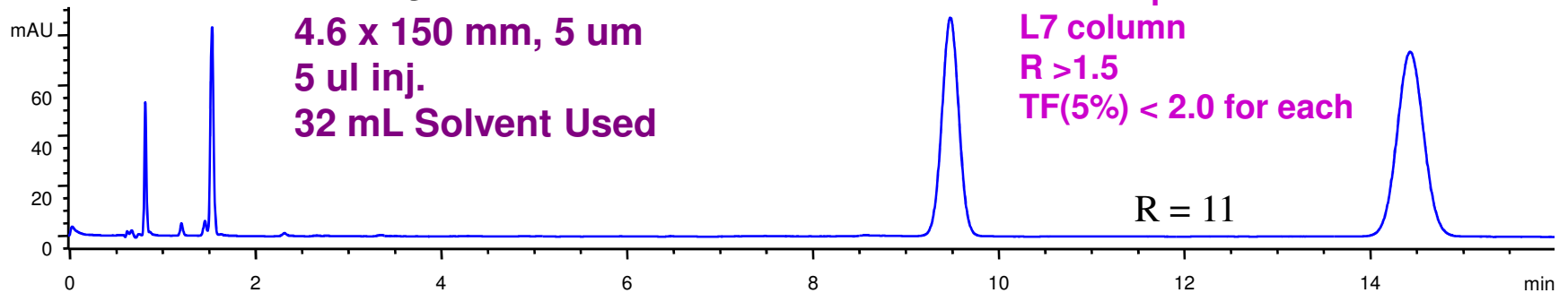
Solvent Used = 7mL
% Solvent Saved = 78%



Method Adjustments can be Used to Transfer USP

Assay for Ibuprofen Oral Suspension to RRHT

Note there is no change in column ID



Increase in Sample Throughput and Cost Savings using Fast LC Methods

Cycle time	20 min cycle time	5min cycle time
Runs/year*	26,208	104,832
Approx. costs/analysis	\$ 11.35	\$ 2.85
Approx. cost/1000runs**	\$ 11,350	\$ 2,850
Cost savings/1000 runs	-	\$ 8,500
Increase in throughput	-	4 times

* 24hour/day, 7days/week, 52 weeks/year

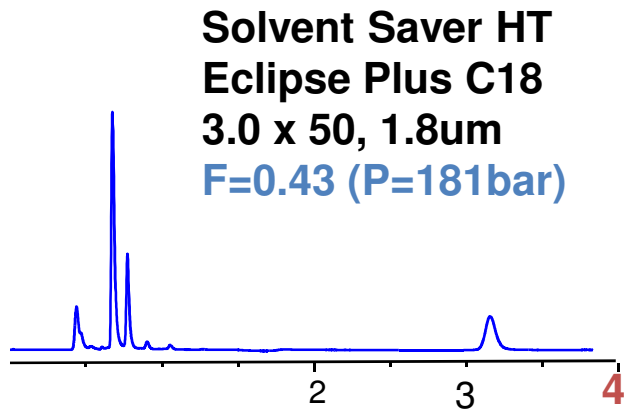
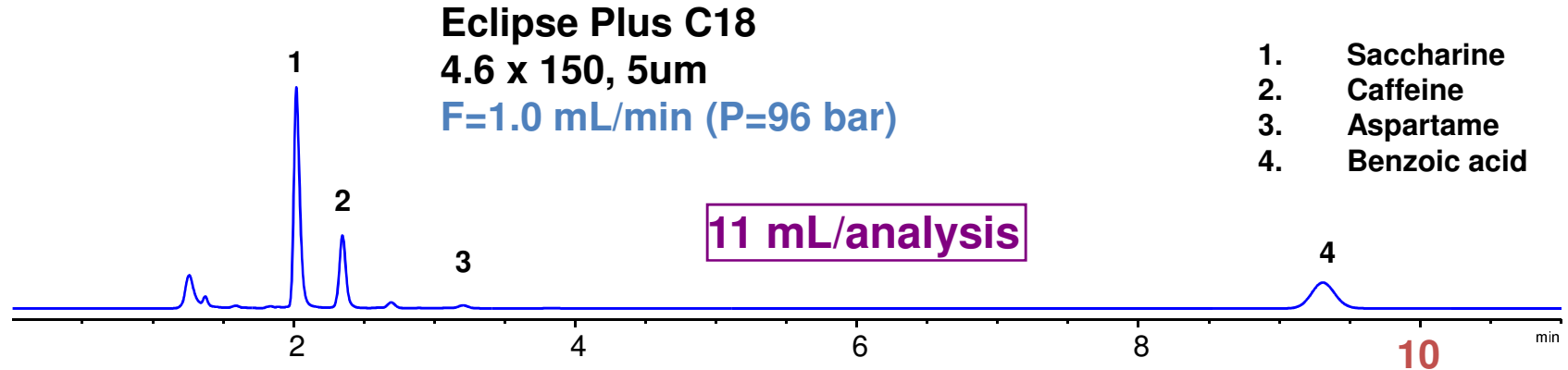
** solvents and disposal = \$31 per liter (old prices), labor = \$30 per hour

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

	5 μ m	3.5 μ m	1.8 μ m
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%

Combine RRHT and Solvent Saver for Maximum Solvent Reduction – Diet Soda Sample



85% less solvent

Mobile Phase.: A: 20 mM Phosphate B: acetonitrile A:B (79:21) Temp: 25 C Detection: UV 220nm,16, ref=420,20
Flow cell: 3mm, 2 μ L Data rate: 1 s Inj. Vol 3 μ L for 4.6, 1.5 for 3.0 Instrument Bin 1200SL
Sample: 0.2 μ m filtered diet soda

Solvent Savings are Easy to Achieve by Simple Column Modifications

- Changing column dimensions – to a Solvent Saver column – can dramatically reduce solvent, especially acetonitrile usage.
- The second option is to change column length and particle size for faster analyses using less solvent.
- Either approach yields substantial solvent savings.

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
- This is critical during this time of very limited ACN availability.
- Many changes can be made within guidelines for method adjustments.
- Documentation will be required, but not complete method revalidation
- Move to revalidate easy methods frequently used methods and methods with high Acetonitrile use to avoid problems.

See Pub No. 5989-7282EN for Complete Poster

ZORBAX Eclipse Plus LC columns are the first choice for HPLC method development.

These columns were engineered to deliver superior performance by:

- Combining excellent peak shape with the efficiency of a silica-based column.
- Providing enhanced reliability from 1.5µm to 5µm to reliably transfer methods without revalidation.
- Giving you the choice of Eclipse Plus Rapid Resolution High Throughput columns for ultra-fast separations.
- Continuing the ZORBAX tradition of long life and reproducibility.

