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 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 co-product as fuel.

And it is acetonitrile’s status as a minor coproduct that has led to its present scarcity. Amin Dhalia, 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 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

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

*For the current and official copy, check the Intranet at [http://www.fda.gov/ora/science_ref/ln/pdf/attachments/vol2_5_4_5_attachment_a.pdf](http://www.fda.gov/ora/science_ref/ln/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?
When sponsors make changes in the analytical procedure, drug substance, or drug product, the changes may necessitate revalidation of the analytical procedures. The degree of revalidation 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

<table>
<thead>
<tr>
<th></th>
<th>Standard Analytical</th>
<th>Solvent Saver</th>
<th>Narrow Bore</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Internal Diameter</td>
<td>4.6 mm</td>
<td>3.0 mm</td>
<td>2.1 mm</td>
</tr>
<tr>
<td>Actual Solvent Used</td>
<td>100 mL</td>
<td>40 mL</td>
<td>20 mL</td>
</tr>
<tr>
<td>% Solvent Use Decrease</td>
<td>-</td>
<td>60%</td>
<td>80%</td>
</tr>
</tbody>
</table>

*By reducing column ID solvent use is reduced dramatically*
Changing Column Diameter

- **Scale flow rate (maintain linear velocity)**

  \[ F_2 = F_1 \times \frac{(d_2)^2}{(d_1)^2} \]

- **Scale Injection Volume**

  \[ V_2 = V_1 \times \frac{(d_2^2 \times L_2)}{(d_1^2 \times L_1)} \]

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  
*200/87/13 ACN/0.2M Na2HPO4/0.1M citric acid
Temperature: ambient  
Sample: Antibacterials  
1. Sulfamerazine  2. Furazolidone  3. Oxolinic acid  

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

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 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
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**

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

\[ k = \frac{(t_r-t_o)}{t_o} \quad \text{N} = 11,000 \quad \text{(constant)} \]

- 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

<table>
<thead>
<tr>
<th>SB-C18</th>
<th>ODS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak</td>
<td>Time</td>
</tr>
<tr>
<td>1</td>
<td>4.17</td>
</tr>
<tr>
<td>2</td>
<td>9.76</td>
</tr>
</tbody>
</table>

- Many C18 columns provide results in line with the USP method.
**Column Choices to Save Solvent and Time**

Mobile Phase: 35% water: 65% methanol

**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 Used Saved
  - 8 min 4.0 mL 5.6 mL
  - 2.5 min 3.0 mL 6.6 mL

Rs(1,2) = 12.05

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

Disconnect only here!

Standard delay volume
(600-800µl delay)

- Compatible with 1100/1200
- Required for high flow rates
**Changing Column Diameter**

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

- 1200 Series Solvent A
- 1200 Series Solvent B
- Passive Damper 0-300 ul
- Passive Mixer 400 ul
- Very Good Noise ~ 800 µl

- 1290 Infinity Solvent A
- 1290 Infinity Solvent B

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**Active Damping**

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

**Microfluidic Mixing**

- Jet Weaver Mixer (35ul)

**10µl / 45µl DV:**

- 20 - 80x lower
- < 50%

**Lowest Noise**
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

<table>
<thead>
<tr>
<th>Plates</th>
<th>Selectivity</th>
<th>Retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_s = \sqrt{\frac{N}{4}}$</td>
<td>$\frac{\alpha-1}{\alpha}$</td>
<td>$\frac{k'}{k'+1}$</td>
</tr>
</tbody>
</table>

Where:
- $N \propto \frac{L}{d_p}$
- $N \propto \frac{P}{\alpha}$

**To Maintain $R_s$:**
- e.g.: $d_p/2 \rightarrow L/2$

**Reduction in $R_s$:**
- Column Length \(\downarrow\) \(\rightarrow N\)
- Particle Size \(\downarrow\) \(\rightarrow P\)
Changing Column Length and Particle Size

- Reduce column length by factor of 1.5
  - e.g. 150mm → 100m (33% less ACN)

- 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

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

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

<table>
<thead>
<tr>
<th>Dimension</th>
<th>5 um</th>
<th>3.5 um</th>
<th>5 um</th>
<th>3.5 um</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analysis Time</td>
<td>30 min.</td>
<td>40% reduction</td>
<td>18 min.</td>
<td>50% reduction</td>
</tr>
<tr>
<td>Solvent Waste</td>
<td>30 mL</td>
<td>40% reduction</td>
<td>18 mL</td>
<td>50% reduction</td>
</tr>
<tr>
<td>N</td>
<td>20,000</td>
<td>20,000</td>
<td>12,000</td>
<td>10,000</td>
</tr>
</tbody>
</table>

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.
Changing Column Length and Particle Size
1/3 Solvent Use, Plus Increased Sensitivity

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

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

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

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

Tips and Tricks '09:
Get on the Right Road
USP Assay for Ibuprofen Oral Suspension

**USP Requirements:**

- L7 column
- \( R > 1.5 \)
- TF(5%) < 2.0 for each

**Mobile phase:** (63:37) water:acetonitrile + 1.8 ml H3PO4

**Flow:** 2.0 ml/min  
**Temp.: ambient**  
**LC:** Agilent 1100

**Sample:** Childrens ibuprofen oral suspension, with benzophenone as internal std. prepared as described in USP

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**Changing Column Length and Particle Size**

1. **4.6 x 150 mm, 5 um**
   - 5 ul inj.
   - 32 mL Solvent Used
   - \( R = 11 \)
   - Tailing factors for all six peaks are <1.15

2. **4.6 x 100 mm, 3.5 um**
   - 3.3 ul inj.
   - 20 mL Solvent Used
   - \( R = 11 \)
   - Tailing factors for all six peaks are <1.15

3. **Rapid Resolution HT**
   - **4.6 x 50 mm, 1.8 um**
   - 1.7 ul inj.
   - 11 mL Solvent Used
   - \( R = 10.0 \)
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$_2$PO$_4$, pH 4.4: 25% ACN
Flow Rate: 1.5 mL/min
Sample: 1. Propranolol

Solvent Saved: 50%
### Changing Column Length and Particle Size

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

<table>
<thead>
<tr>
<th></th>
<th>5µm</th>
<th>3.5µm</th>
<th>1.8µm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resolution</strong></td>
<td>4.1</td>
<td>1.4</td>
<td>1.6</td>
</tr>
<tr>
<td><strong>Selectivity</strong></td>
<td>1.08</td>
<td>1.06</td>
<td>1.05</td>
</tr>
<tr>
<td><strong>Theoretical Plates</strong></td>
<td>56108</td>
<td>14314</td>
<td>23190</td>
</tr>
<tr>
<td><strong>k'</strong></td>
<td>6.362</td>
<td>4.485</td>
<td>4.12</td>
</tr>
<tr>
<td><strong>Run Time (inc. re-equil)</strong></td>
<td>25 min</td>
<td>6.5 min</td>
<td>2 min</td>
</tr>
<tr>
<td><strong>Solvent Usage</strong></td>
<td>37.5ml</td>
<td>14.25ml</td>
<td>3ml</td>
</tr>
</tbody>
</table>

**Solvent Savings 92%**
Changing Length, Diameter & Particle Size

5um → 3.5um → 3.5um

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

### Isoeluotropic Strength Table

<table>
<thead>
<tr>
<th>% MeOH in H2O</th>
<th>% ACN in H2O</th>
<th>Relative k’</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>40</td>
</tr>
<tr>
<td>20</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>30</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td>40</td>
<td>32</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>50</strong></td>
<td><strong>40</strong></td>
<td><strong>1</strong></td>
</tr>
<tr>
<td>60</td>
<td>50</td>
<td>0.4</td>
</tr>
<tr>
<td>70</td>
<td>60</td>
<td>0.2</td>
</tr>
<tr>
<td>80</td>
<td>73</td>
<td>0.06</td>
</tr>
<tr>
<td>90</td>
<td>86</td>
<td>0.03</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Comparing Acetonitrile and MeOH Separations

Column: ZORBAX RRHT Eclipse Plus C18, 4.6 x 50 mm, 1.8 μm
Mobile Phase: A: 25 mM NaH₂PO₄, pH 3.0  B: organic
Flow Rate: 2.0 mL/min  Temperature: 30°C  Detection: UV 240 nm

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

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

Changing to Methanol
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

- TCC-cluster
- Inlet valve
- Outlet valve
- Detector

Autosampler

Pump

Solvent Selection

Changing to Methanol
Agilent 1200 Series Method Development Solution

ACN/Water pH 1.9, 10-80% B

<table>
<thead>
<tr>
<th>Resolution</th>
<th>SB C18</th>
<th>SB C8</th>
<th>Eclipse plus C18</th>
<th>SB PheHex</th>
<th>SB CN</th>
<th>Extend C18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak 3</td>
<td>2.20</td>
<td>2.24</td>
<td>2.17</td>
<td>2.52</td>
<td>3.53</td>
<td>2.09</td>
</tr>
<tr>
<td>Peak 5</td>
<td>3.24</td>
<td>2.76</td>
<td>2.62</td>
<td>2.3</td>
<td>1.54</td>
<td>3.50</td>
</tr>
</tbody>
</table>

Tips and Tricks '09: Get on the Right Road
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

Detected Peaks:
- Metoprolol
- Zorbax SB C18, 38 peaks
- Zorbax Eclipse + C18, 31 peaks
- Zorbax SB C8, 28 peaks
- Zorbax Phenyl, 35 peaks
- Zorbax Extend, 38 peaks
- Zorbax CN, 29 peaks
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 CO₂ (does not require expensive SFC-grade CO₂)
- More information: www.aurorasfc.com
Alternative Separation Techniques

Super Critical Fluid Chromatography

- Does not use ANY Acetonitrile
- Uses inexpensive CO₂ 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.
Alternative Separation Techniques

Super Critical Fluid Chromatography

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

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
Alternative Separation Techniques

Super Critical Fluid Chromatography

➢ 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).
Alternative Separation Techniques
Super Critical Fluid Chromatography

Lowest Noise SFC

Berger Instruments
SFC

Aurora Fusion A5 + Agilent 1200SL

Chiral Separation of .1% of original Warfarin

Tips and Tricks '09: Get on the Right Road
## 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

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

**Fusion A5: Reproducible and Linear**

Tips and Tricks '09:
Get on the Right Road
Alternative Separation Techniques
Super Critical Fluid Chromatography ~ SFC-MS

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

22 component mix

Princeton Chromatography
22 Component Mix

APCI positive ion

Fusion A5 w/ Agilent 1200 SL-Single Quad

Tips and Tricks '09:
Get on the Right Road
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:

Tackle the acetonitrile shortage!
Today, tomorrow and in the future.