

# Double the Throughput of Your UPLC™ Separations

Jody Clark, Brian A. Jones, Mark Hayward, Qing Ping Han

1. Selerity Technologies USA, Inc., 2484 W. Custer Road  
Salt Lake City, UT 84104
2. Lundbeck Research USA, 215 College Road  
Paramus, NJ 07652



[www.selerity.com](http://www.selerity.com)

# Goal When Developing Fast Methods

- Productivity is proportional to resolution per unit time, ( $R_s/t_r$ ),
- Maintain the resolution as the flow rate is increased, otherwise no improvement in productivity is realized



# Theory

$$R_S = 2(t_{r2} - t_{r1}) / (w_1 + w_2)$$

- Maximizing productivity, while minimizing time requires that peak width ( $w$ ) must be minimized.
- This is done by focusing on:
  - Reducing system volume
  - Increasing flow rate
  - Optimizing temperature
  - Reducing particle size



# Minimizing Time

$$\text{Retention Time} = t_0 + t_{DG} + t_C$$

- $t_0$  is the retention time of the unretained compound
- $t_{DG}$  is the delay time between when a gradient is requested and when it occurs on column
- $t_C$  is the time that an analyte spends in the stationary phase.



# Thinking in Terms of Velocity

$$\text{Retention Time} = (V_O + V_{DG} + V_C)/F$$

- $V$  is the mobile phase volume
- $F$  is the flow rate

key in developing a rugged, reliable procedure for high throughput separations:

- balance the linear velocity with increased pressure and decreased column sizes.



# How to Optimize Your System

- Step 1 - Optimize System Plumbing
- Step 2 - Choose the Appropriate Column Dimensions and Particle Size
- Step 3 - Choose the Appropriate Mobile Phase Composition
- Step 4 - Preheat the Mobile Phase with an Active Preheating Unit
- Step 5 - Increase the Flow Rate



# Optimize System Plumbing

- HPLC systems with pressure limits above 10,000 psi
  - Remove passive preheating units and replace with a short piece of 0.005"ID tubing
- Standard HPLC conditions
  - Optimum flow is 5mL/ min with a 4.6 X 50 mm column using 3  $\mu$ m particles and 45°C.



# Choose the Appropriate Column Dimensions and Particle Size

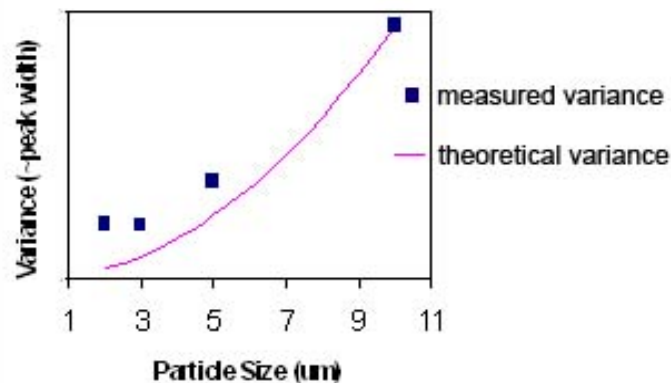
- Method approach will not work without high quality columns
  - Peak width differences of up to 5 fold can be demonstrated between best/worst columns that are commercially available
- Head to head comparisons are the only way to optimize this parameter
- 50 mm lengths with 3 $\mu$ m particles provide a good balance between speed and resolution
- Use the new generation high purity silica, packed consistently & with low backpressure



# 50mm, 3 $\mu$ m provide a good balance between speed and resolution

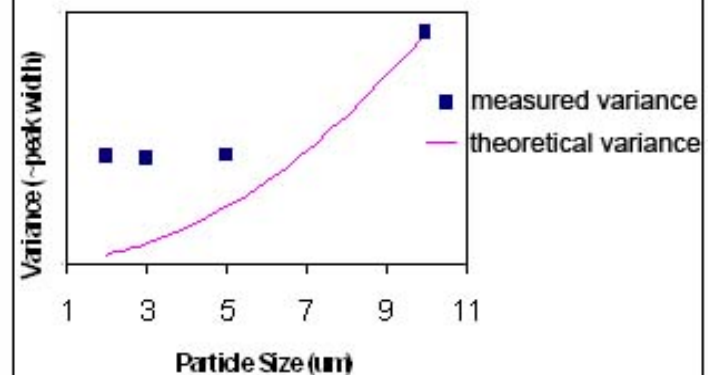
With a Well Chosen Stationary Phase  
Smaller Particles do not Yield Narrower Peaks

Peak Width vs. Particle Size  
(Reserpine)



Acetonitrile soluble compounds:  
Peak widths level out at 3 $\mu\text{m}$

Peak Width vs. Particle Size  
(Methyl-Enkephalin)

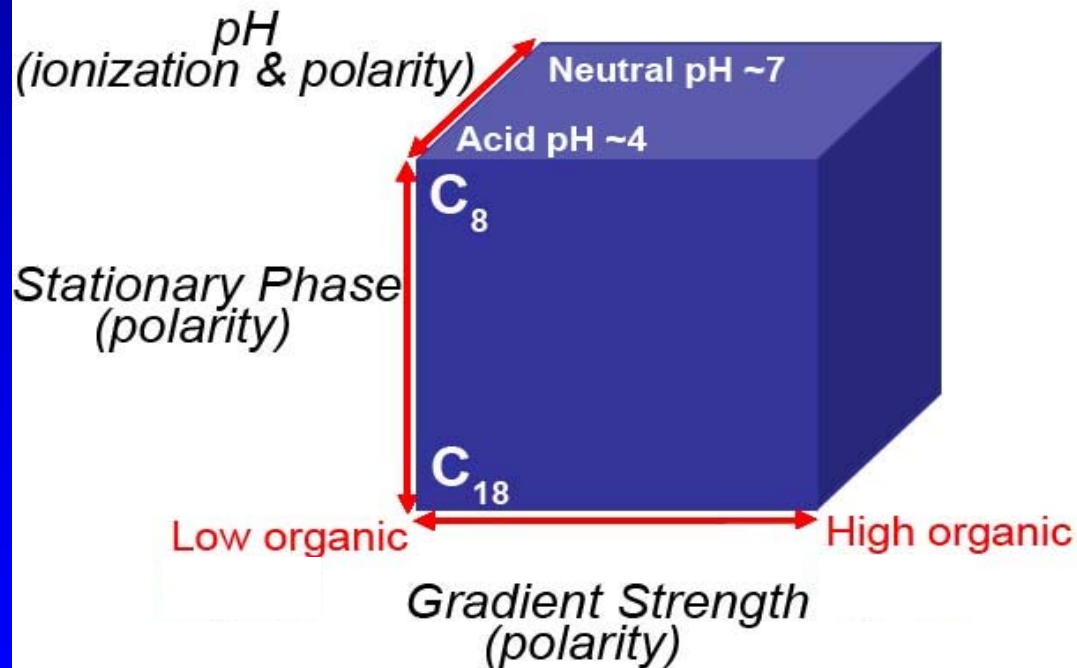


Water soluble compounds:  
Peak widths level out at 5 $\mu\text{m}$



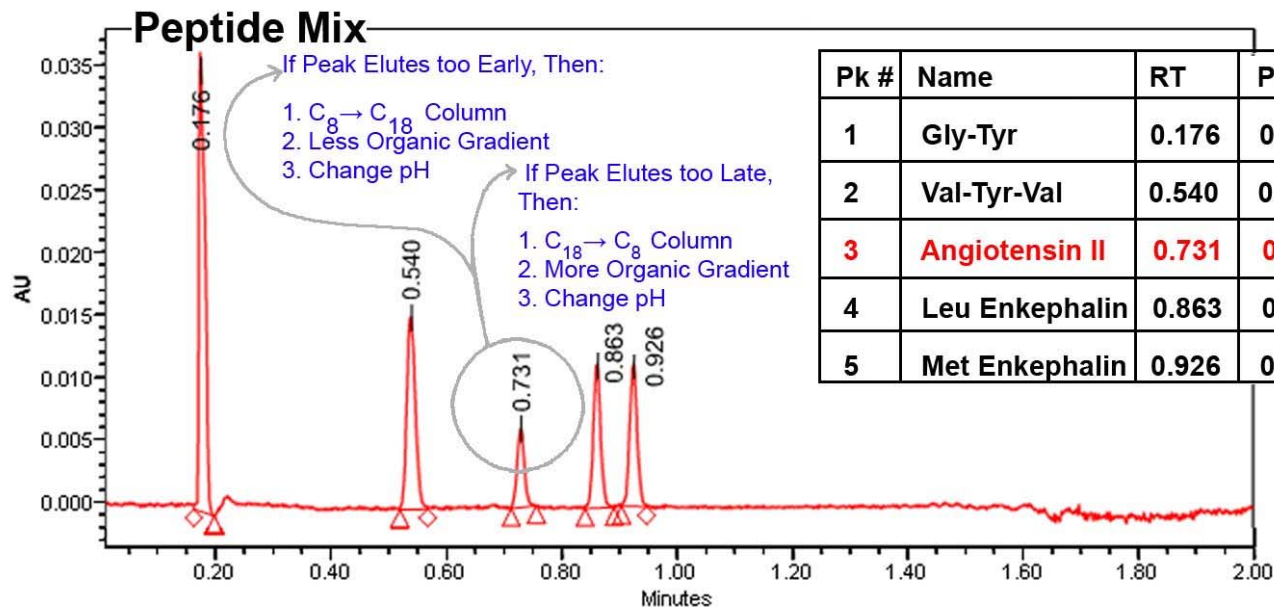
# Choose the Appropriate Mobile Phase Composition/Phase

## 2X2X2 Polarity Matrix



# How to Make Adjustments

**Goal for Mobile Phase:**  
Smallest Peak Widths Should be the Peaks Eluting in the Middle of the Chromatogram



# Temperature Parameter Deviates from Common Practice

- Control the temperature of the mobile phase before it enters the column
- Important to use an active mobile phase
  - Mobile phase properties, i.e., viscosity, change during a solvent gradient
  - Different amounts of energy input are needed to maintain the temperature during a gradient
- Passive preheaters cannot respond fast enough during fast gradient programming



# Mobile Phase Energy Demand During a Gradient Run at 45°C

Gradient: Time(min)	%A	%B
0.00	80	20
1.70	15	85
1.84	15	85
1.85	0	100
2.00	80	20

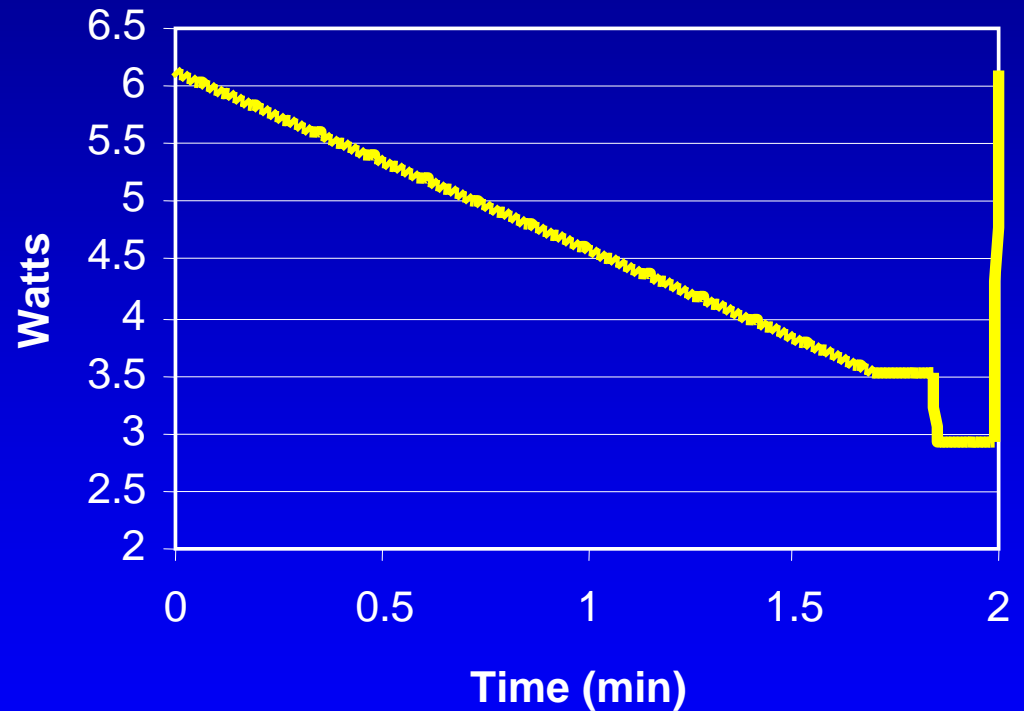
A=1% ACN

B=99.8%ACN

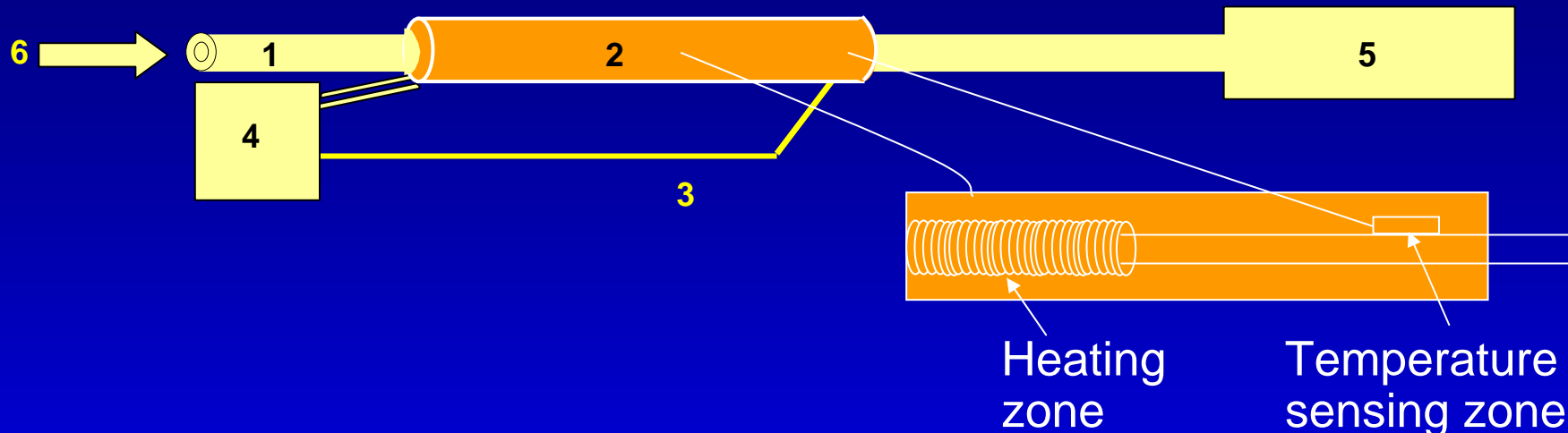
Flow Rate: 5 ml/min

Solvents Initially at 25°C

(Neglecting Endothermic Mixing)



# Caloratherm Preheater Design



(1) stainless steel tubing, (2) heater, (3) thermocouple sensor, (4) temperature controller, (5) column, (6) from pump



Patent pending Selerity Technologies, Inc.

# Features of the Caloratherm

**Selerity Caloratherm  
Pre-Heater: active heat  
sensor, non-invasive and  
easy to use**



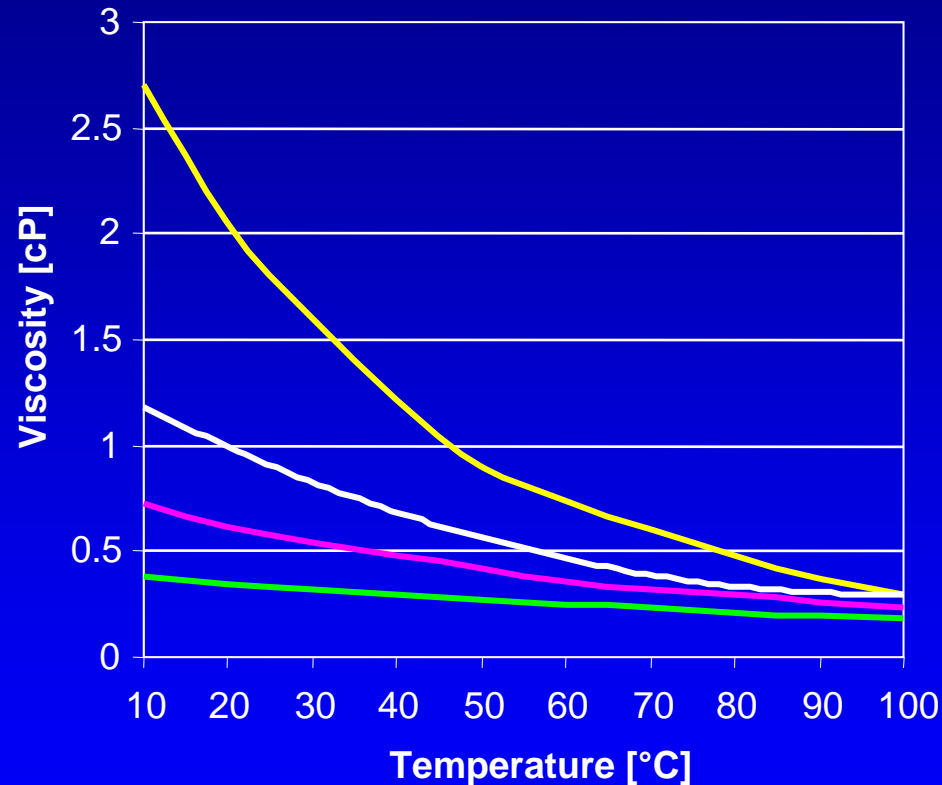
- Capable of efficiently preheating the mobile phase, by continuously sensing the temperature and inputting the energy required to maintain the temperature
- Use on any HPLC system
- Slide onto standard 1/16 " tubing
- 2.25" in length



# Example: Caloratherm Placement



# Temperature Effects on Mobile Phase Viscosity

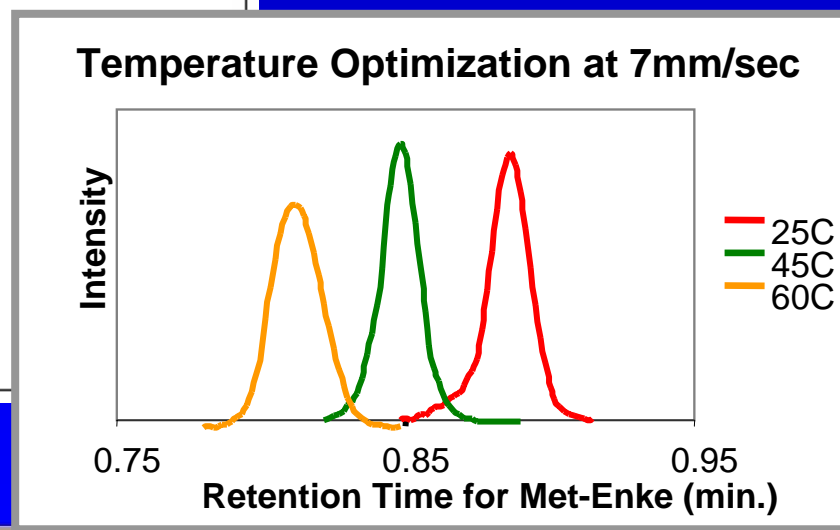
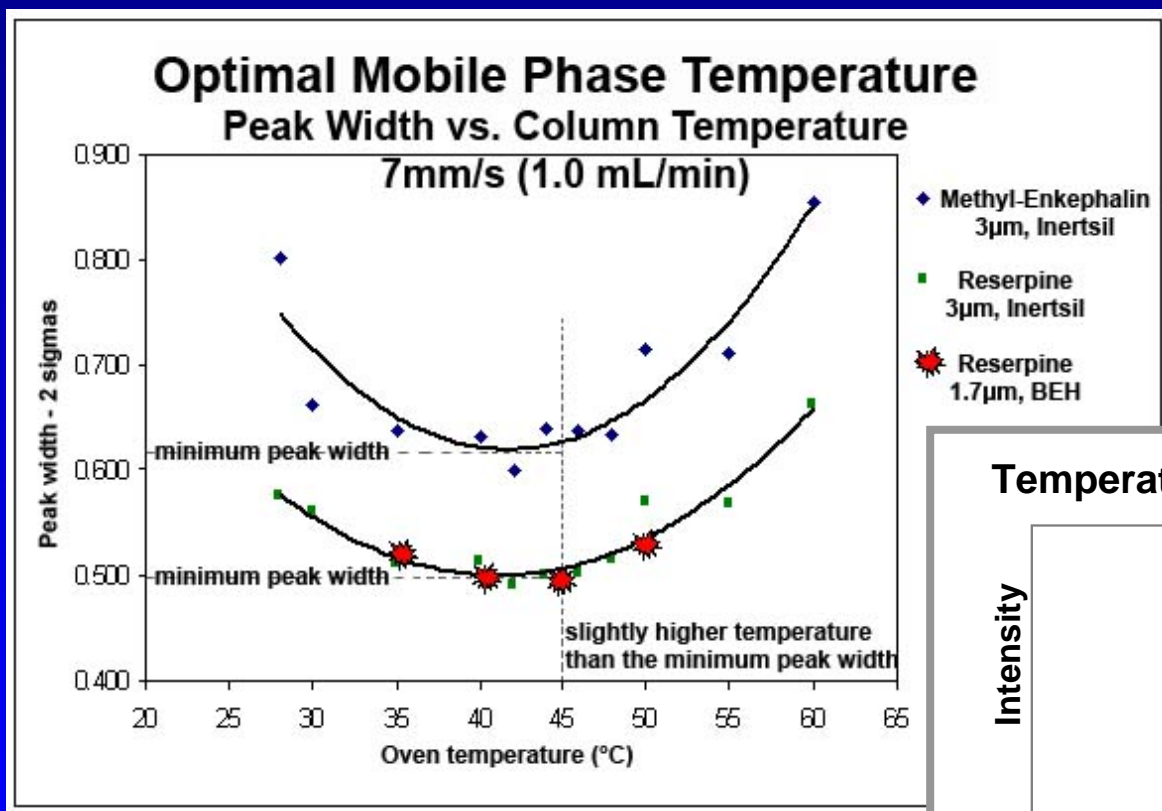


— ACN — Water — MeOH — 40% MeOH



# Optimized Mobile Phase Temperature

## Set temperature and measure peak width



**UPLC BEH 1.7  $\mu$ m at 7 mm/s yields same results**

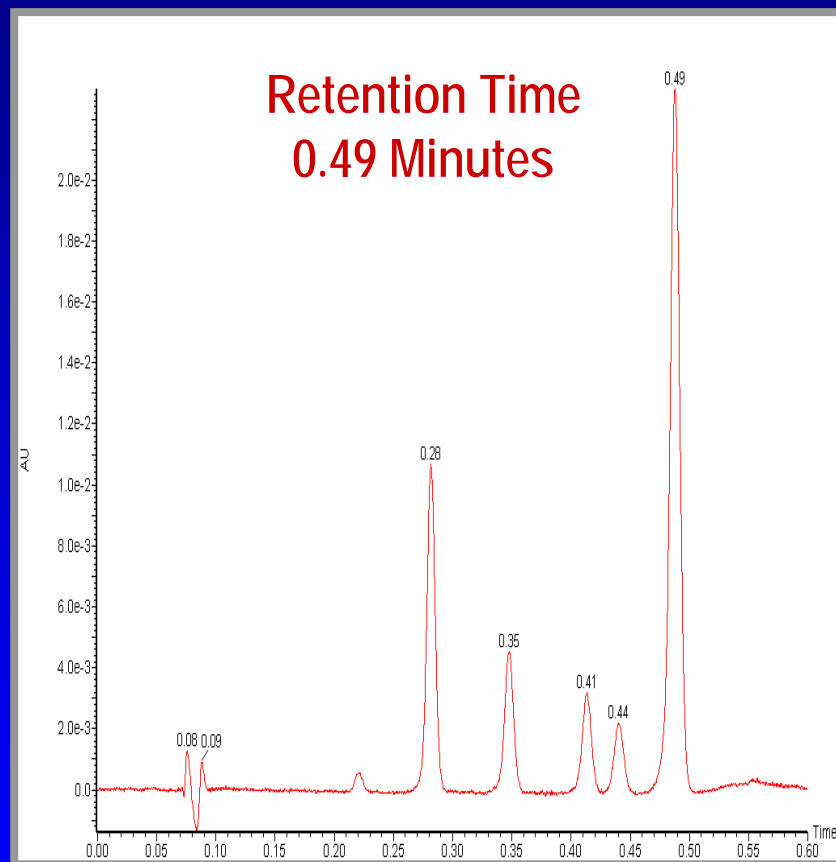
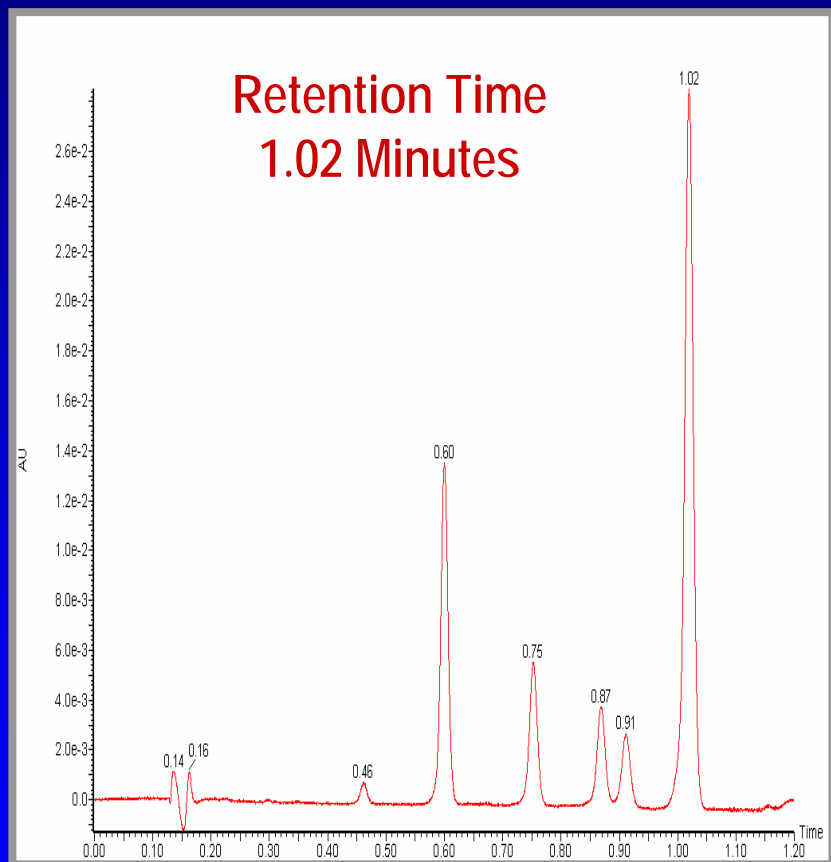
# Increase the Flow Rate

- Choose a flow rate based on pump pressure.
  - The preferred pressure is approximately 50-60% of the maximum operating pressure.
  - Do not operate above 75%.
- Standard HPLC conditions
  - Optimum flow is 5mL/ min with a 4.6 X 50 mm column using 3  $\mu\text{m}$  particles and 45°C.
- High pressure HPLC conditions:
  - Optimum flow is 2mL/ min with a 2.1 X 50 mm column and with 3-3.5  $\mu\text{m}$  particles and 60°C.



# Random synthetic reaction mixture

## 2 times the velocity = 0.5 the time



**Waters Aquity™ UPLC™**  
**3 μm, 2.1 X 50mm**  
**7mm/s (1 mL/min) 45°C**

**Waters Aquity™ UPLC™**  
**3 μm, 2.1 X 50mm**  
**14mm/s (2 mL/min) 60°C**



# Summary Comments

- 3-3.5  $\mu\text{m}$  particles provide the fastest resolution per unit time by allowing higher velocities to be achieved
- Preheating the incoming mobile phase with an active preheater benefits the separation two-fold:
  - Allows the incoming solvent to match that of the column and oven temperature, even with rapidly changing mobile phase heat capacities, reducing radial thermal gradients and peak widths
  - Allows existing column heaters to work more efficiently by decreasing the demand in energy needed to maintain the temperature of the column
  - Enables the use of elevated temperatures for viscosity reduction and thus higher practical mobile phase flow rates



# Acknowledgements

Mark J. Hayward, Qing P. Han, Chi  
Zhang:



Lundbeck Research, Paramus, NJ.

