Double the Throughput of Your UPLC™ Separations

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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

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

- t_O 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

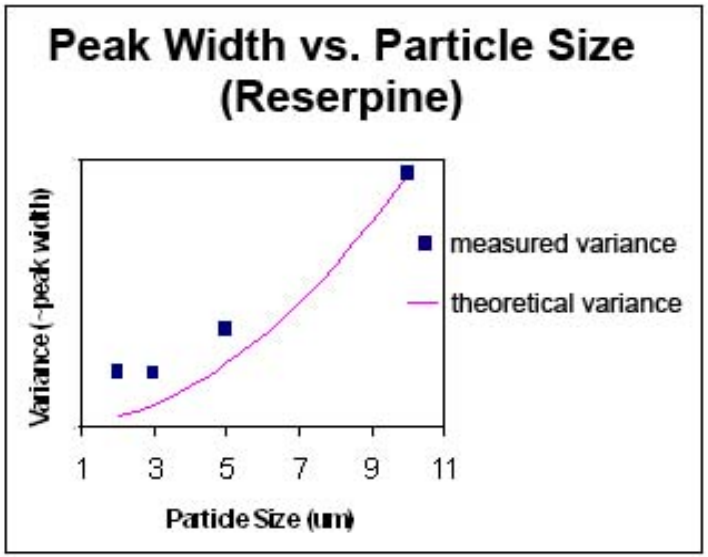
- HPLC systems with pressure limits above 10,000 psi
  - Remove passive preheating units and replace with a short piece of 0.005”ID tubing
- Standard HPLC conditions
  - Optimum flow is 5mL/ min with a 4.6 X 50 mm column using 3 µm particles and 45°C.

Choose the Appropriate Column Dimensions and Particle Size

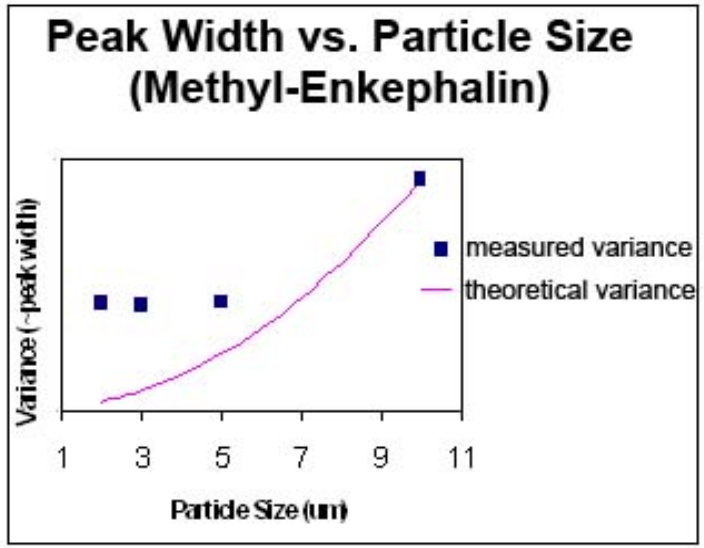
- Method approach will not work without high quality columns
  - Peak width differences of up to 5 fold can be demonstrated between best/worst columns that are commercially available
- Head to head comparisons are the only way to optimize this parameter
- 50 mm lengths with 3µm particles provide a good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

50mm, 3µm provide a good balance between speed and resolution

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

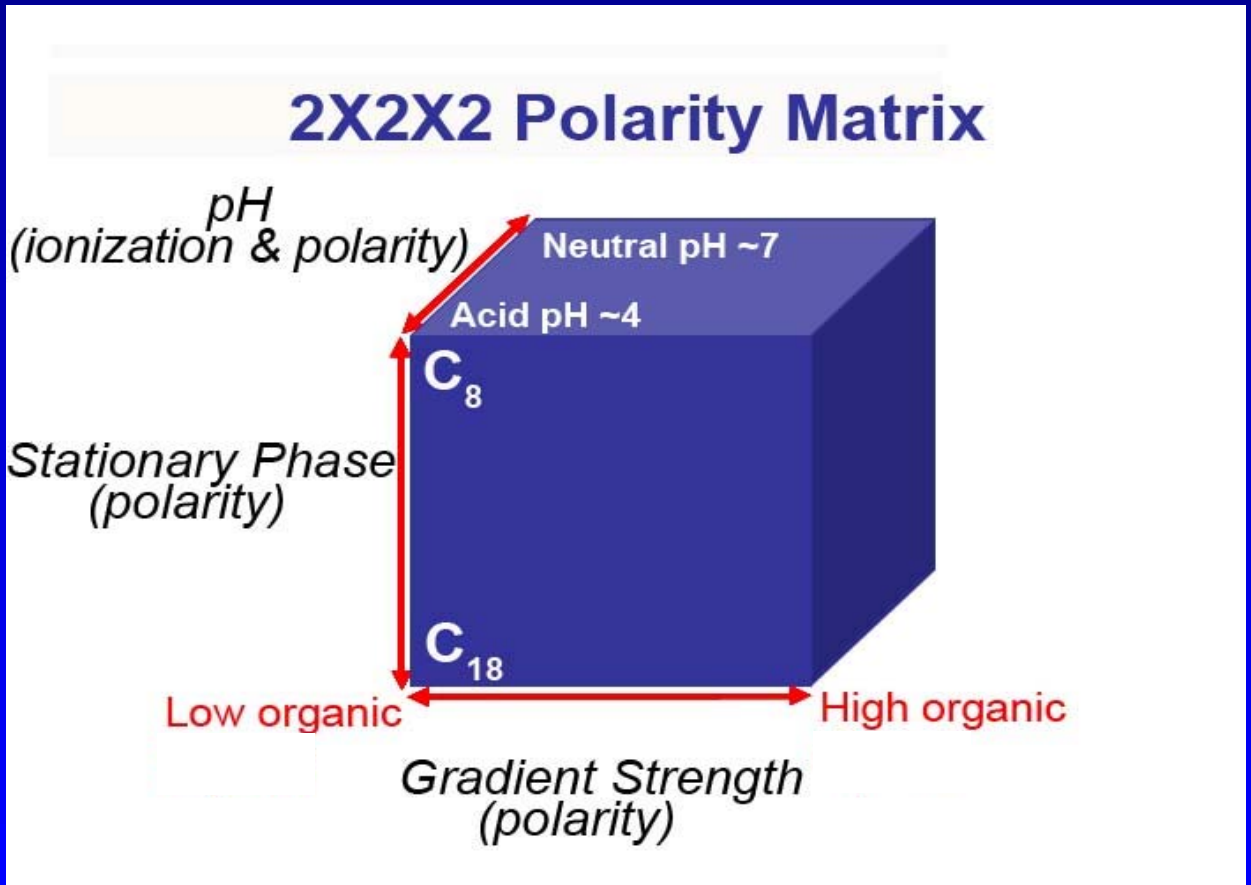


Acetonitrile soluble compounds:
Peak widths level out at 3µm



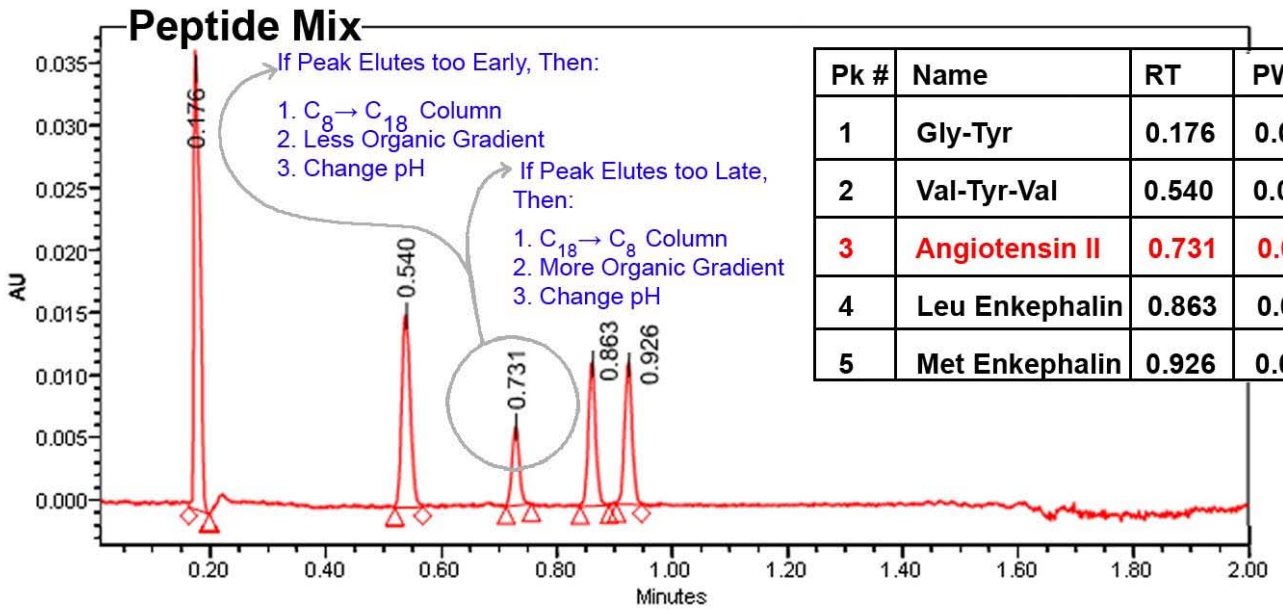
Water soluble compounds:
Peak widths level out at 5µm

Choose the Appropriate Mobile Phase Composition/Phase



How to Make Adjustments

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



Pk #	Name	RT	PW(60%)
1	Gly-Tyr	0.176	0.011716
2	Val-Tyr-Val	0.540	0.013900
3	Angiotensin II	0.731	0.011213
4	Leu Enkephalin	0.863	0.011412
5	Met Enkephalin	0.926	0.011619

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

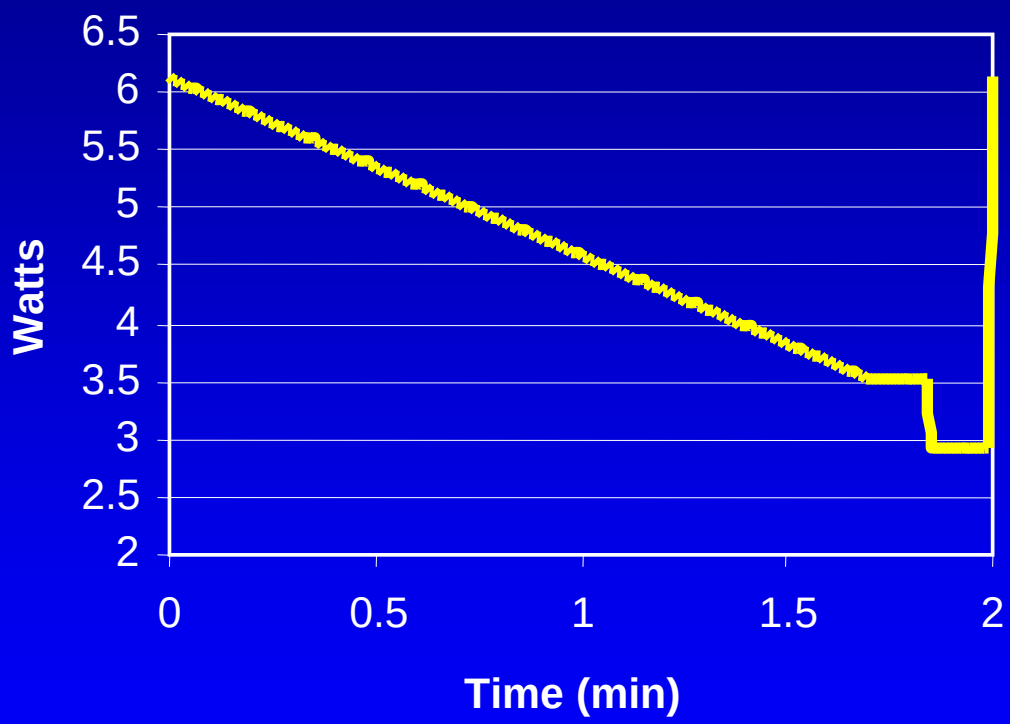
Mobile Phase Energy Demand During a Gradient Run at 45°C

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

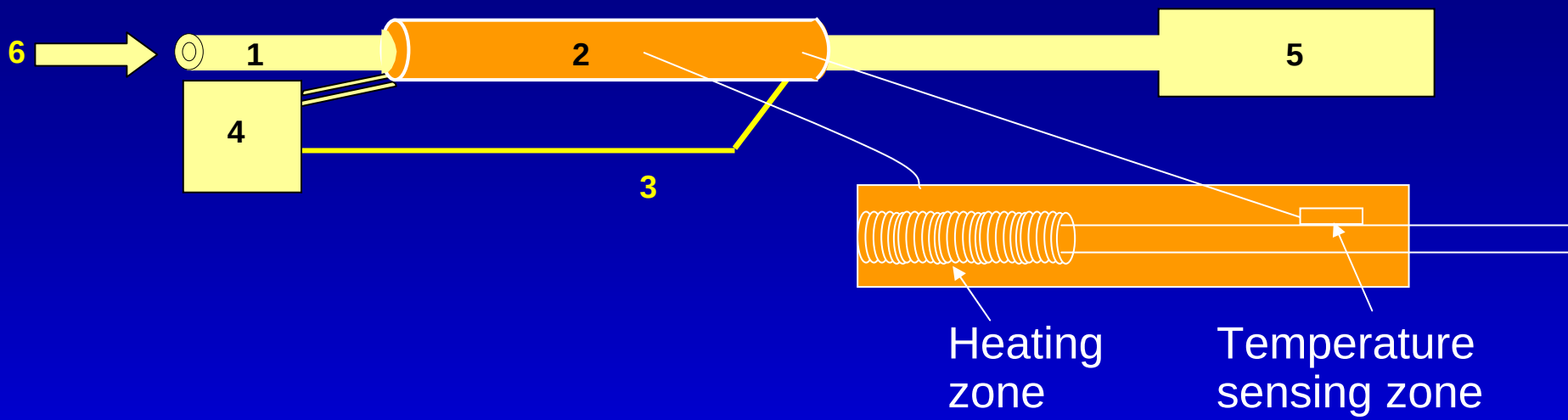
A=1% ACN

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

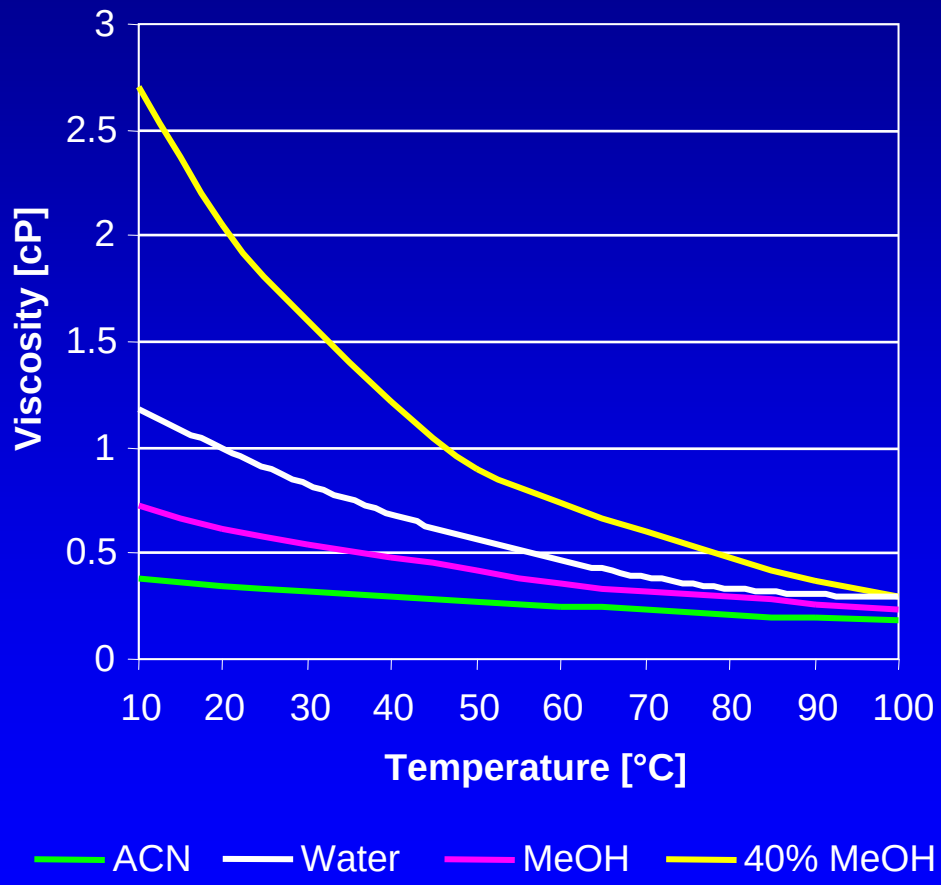
Features of the Caloratherm

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

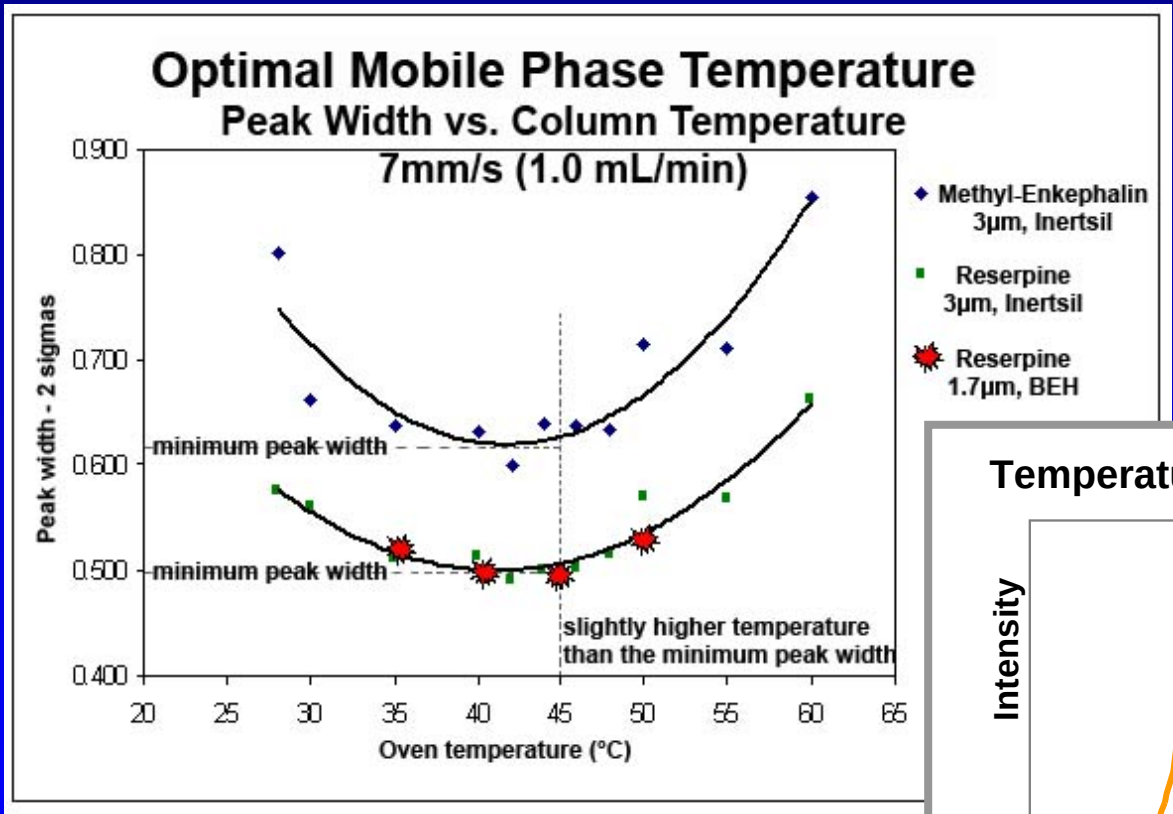
Temperature Effects on Mobile Phase Viscosity

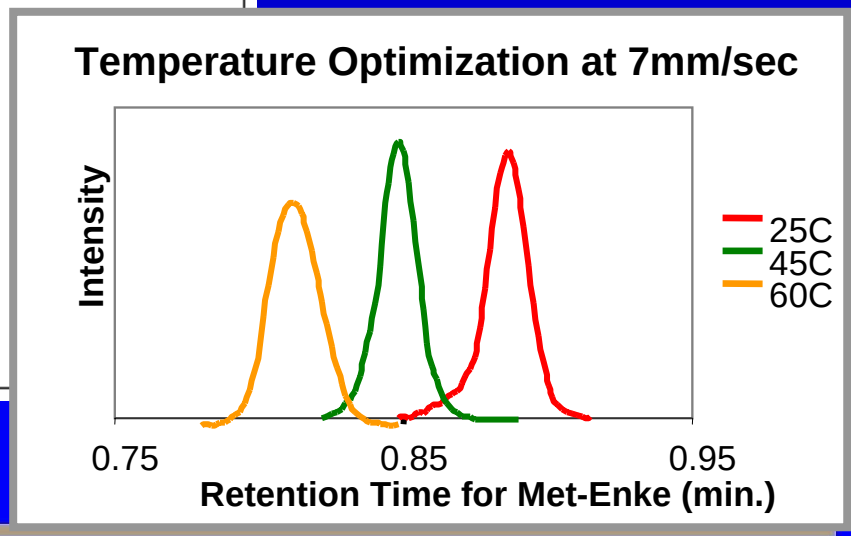


R. Gilmont, www.cepmagazine.org, October 2002, 36-41

Optimized Mobile Phase Temperature

Set temperature and measure peak width



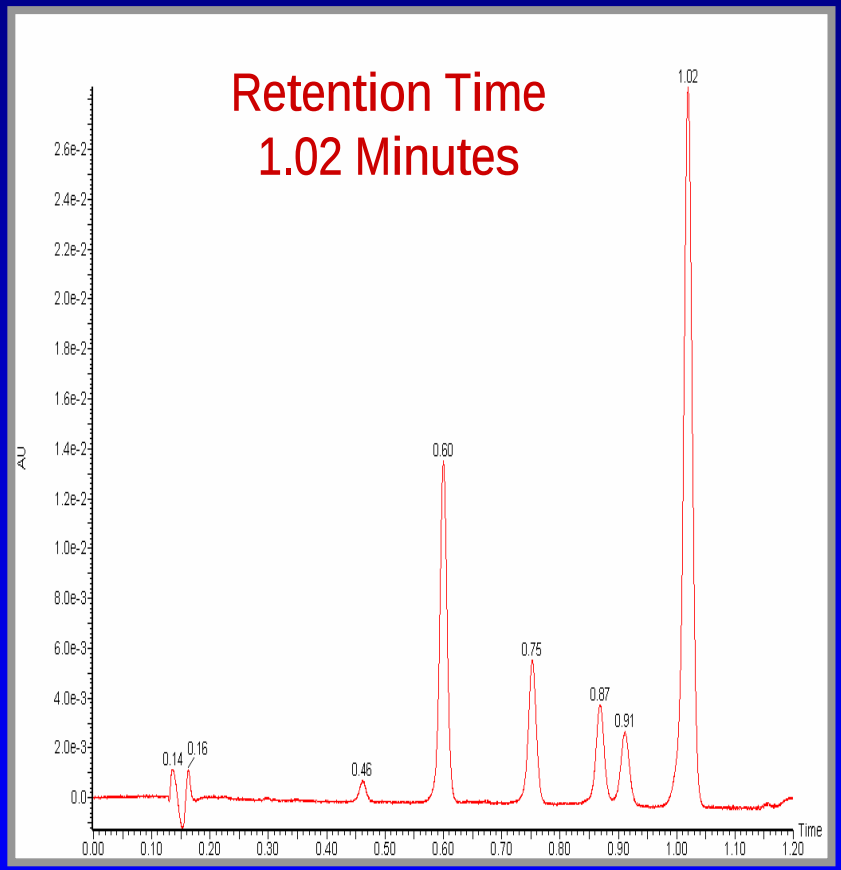


UPLC BEH 1.7 µm at 7 mm/s yields same results

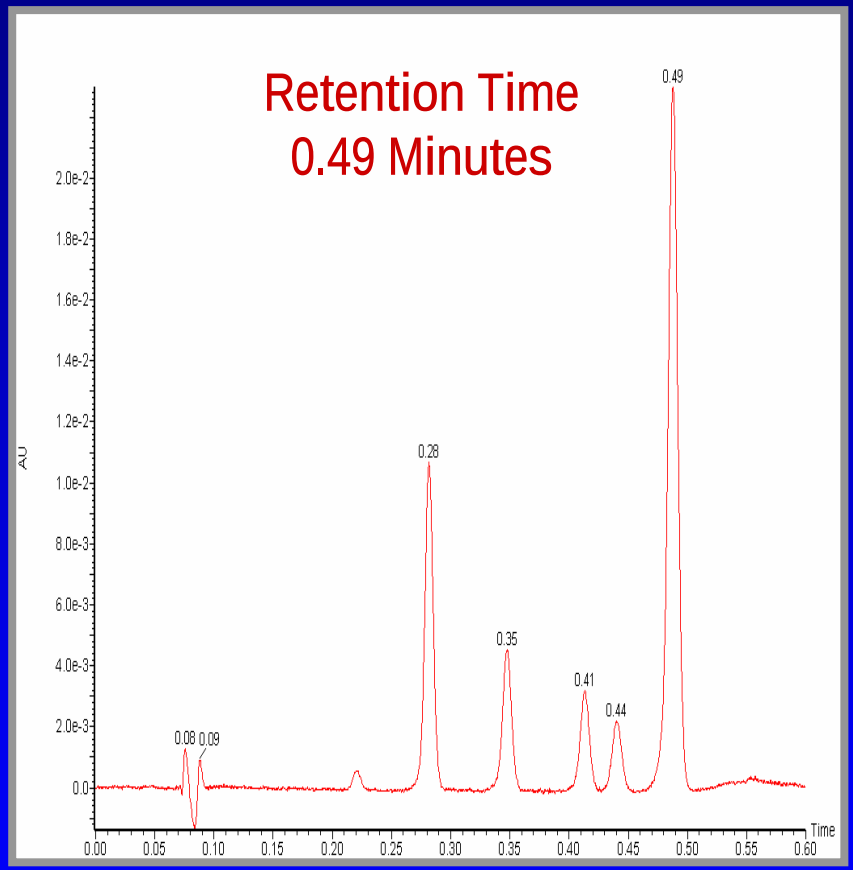
Increase the Flow Rate

- 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 µm 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 µm 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 µm 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

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