ZirChrom®

Part III – Improving Throughput Through the Use of Elevated Column Temperature

- Review potential approaches to improving the speed of HPLC
- Review the concepts of Ultra-Fast High Temperature Liquid Chromatography (UFHTLC)
- Examine current hardware used to provide elevated temperature in HPLC
- Introduce the Metalox^{TM} Model 200-C high temperature column heater

Potential Approaches to Improving Throughput in HPLC

ZirChrom®

Approach	Advantage	Disadvantage
Shorter Columns	Works with most equipment, stationary phases	Low plate count and resolution
Monolithic Columns	Low backpressure	Narrow-bore columns are not available
Ultra-High Pressure LC	High plate counts with small particles	Specialized equipment needed, losses in N at high velocity
High Temperature LC	Low backpressure, high efficiency	Requires adequate heating, stable phases

High temperature LC **is the only approach that allows a significant fraction of the column plate count to be retained as the column linear velocity is increased to values that allow *significantly faster HPLC***

From a Recent Email Advertisement from a Leading Manufacturer…

ZirChrom®

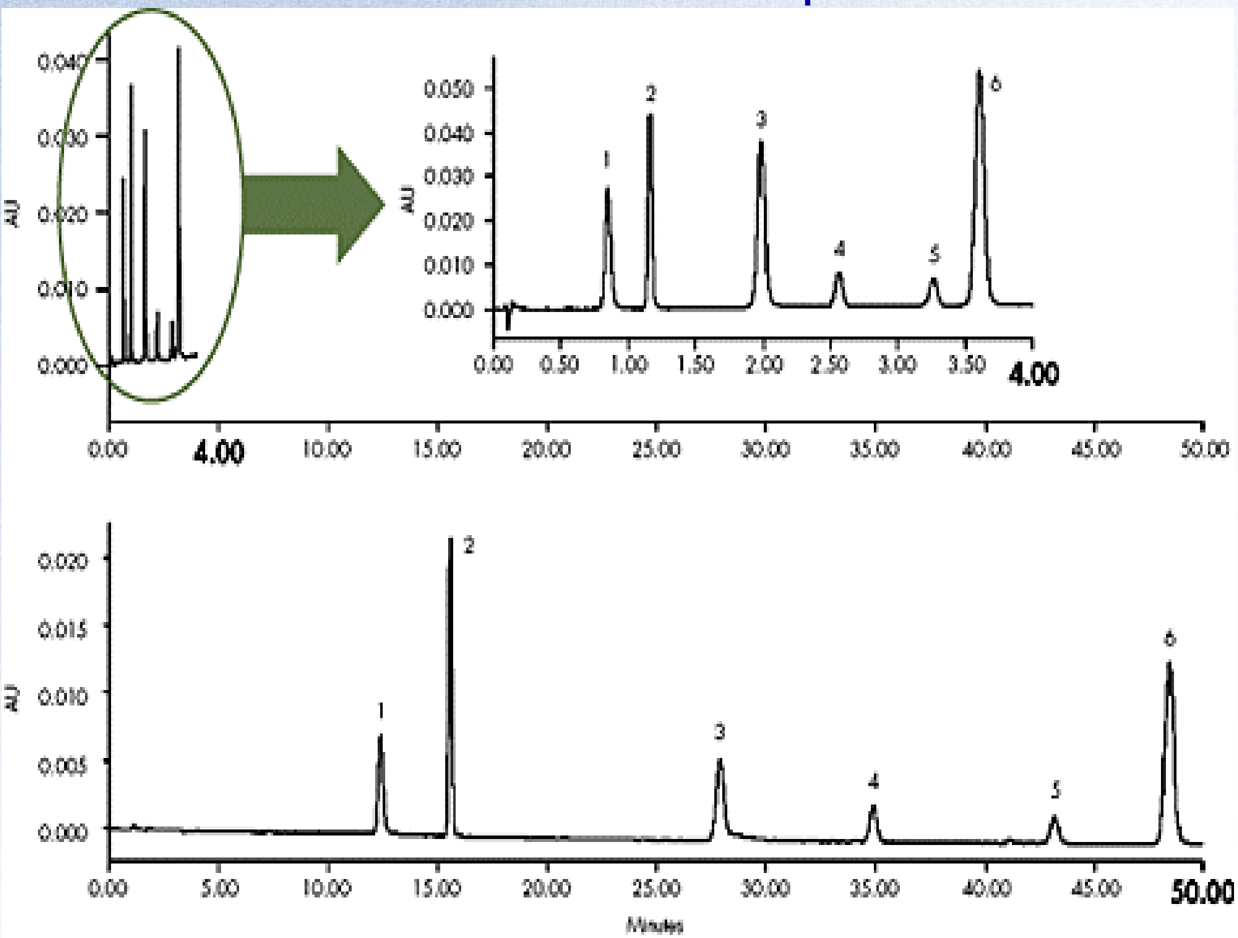
"Forget 60 minute separations…Think short columns and 5 minute separations…"

- Reduce run times by up to 90%
- **No loss in resolution**
- Fast and easy methods transfer
- Shorten development time
- Increase throughput

ZirChrom®

Since N ∝ L, Resolution Must Be Lost When the Column Length is Reduced

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



Conditions

Column: Xterra® MS C_{18} IS

20 mm x 4.6 mm i.d., 3.5 µm

Flow rate: 3.0 ml/min.

Mobile phase: Ternary gradient

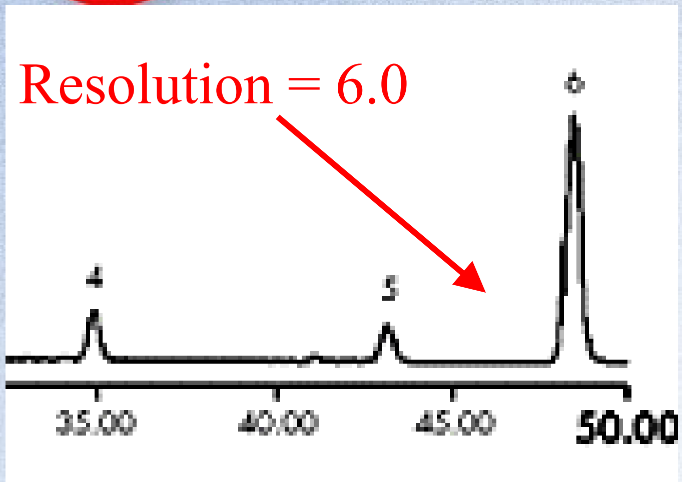
Column: Xterra® MS C_{18} IS

150 mm x 4.6 mm i.d., 5 µm

Flow rate: 1.4 ml/min.

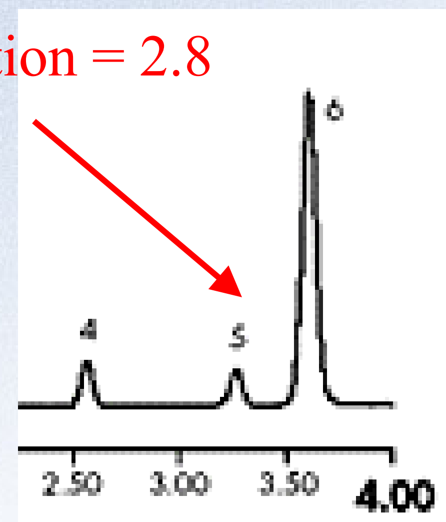
Mobile phase: Ternary gradient

Resolution is NOT Preserved !!



	Peak Number	
	5	**6**
Retention Time (min.)	43.1	48.4
Peak Width ($w_{1/2}$ min.)	0.46	0.46
Retention Factor (k')	33.5	37.7
Selectivity (α)	1.12	
Plate Number (N)	**50,000**	

Resolution = 2.8

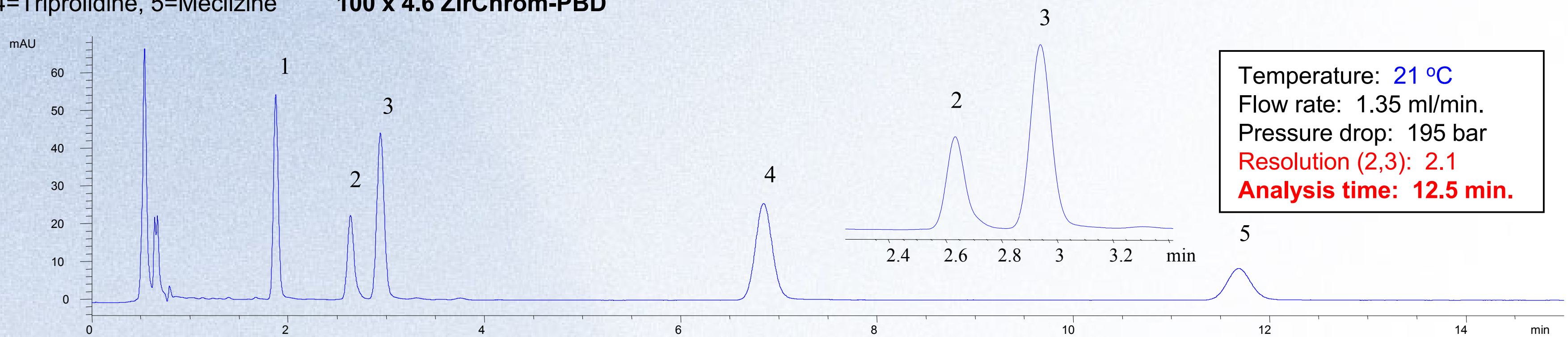


	Peak Number	
	5	**6**
Retention Time (min.)	3.26	3.62
Peak Width ($w_{1/2}$ min.)	0.066	0.094
Retention Factor (k')	31.6	35.2
Selectivity (α)	1.12	
Plate Number (N)	**13,500**	

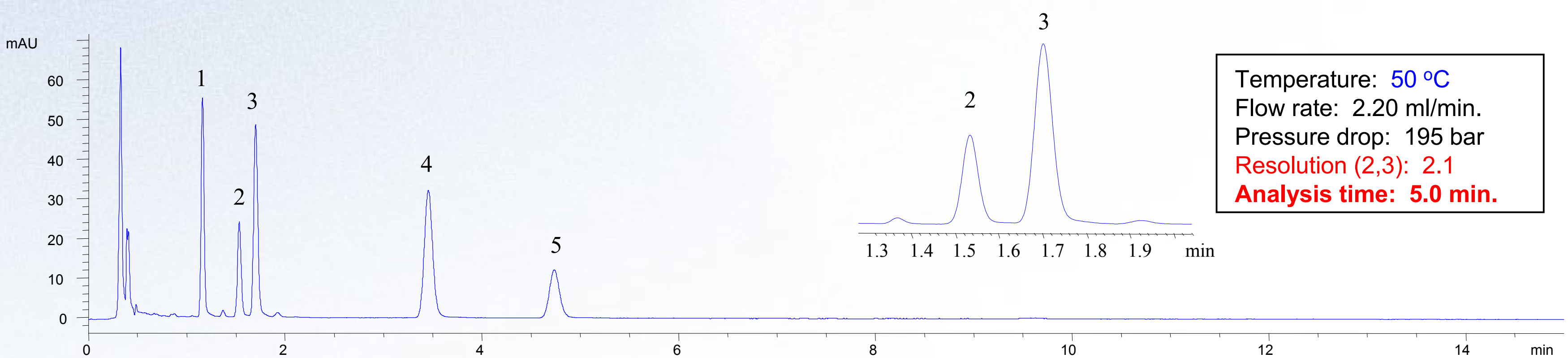
Analysis Time May Be Reduced Without Loss of Resolution Through *Column Heating*

ZirChrom®

LC Conditions: Mobile Phase, 29/71 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Flow Rate, 1.35 mL/min.; Injection volume, 0.5 ul; 254 nm detection; Column Temperature, 21°C; Pressure drop = 195 bar; Solutes: 1=Doxylamine, 2=Methapyrilene, 3=Chlorpheniramine, 4=Triprolidine, 5=Meclizine **100 x 4.6 ZirChrom-PBD**



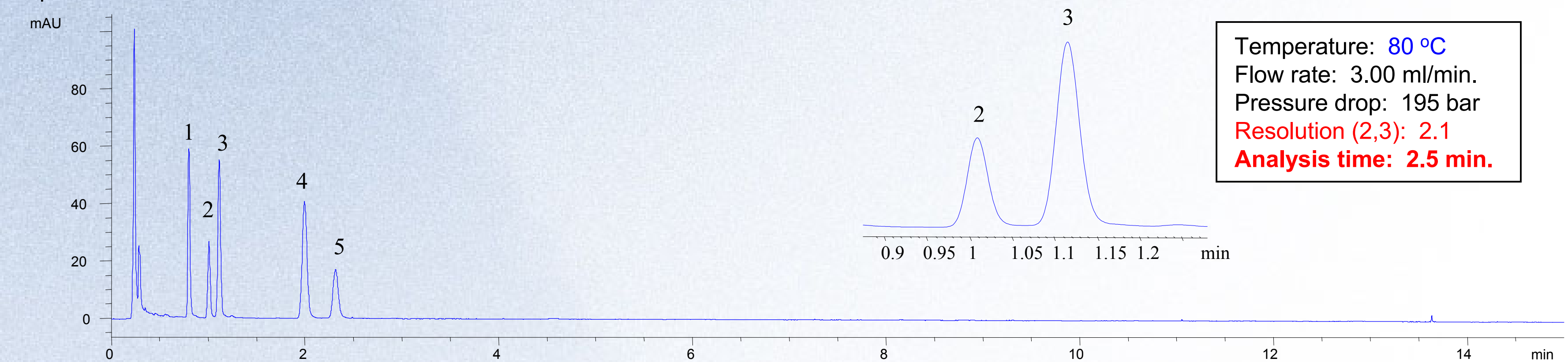
LC Conditions: Mobile Phase, 28/72 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Flow Rate, 2.20 mL/min.; Injection volume, 0.2 ul; 254 nm detection; Column Temperature, 50°C; Pressure drop = 195 bar; Solutes: 1=Doxylamine, 2=Methapyrilene, 3=Chlorpheniramine, 4=Triprolidine, 5=Meclizine **100 x 4.6 ZirChrom-PBD**



Analysis Time May Be Reduced Without Loss of Resolution Through *Column Heating*

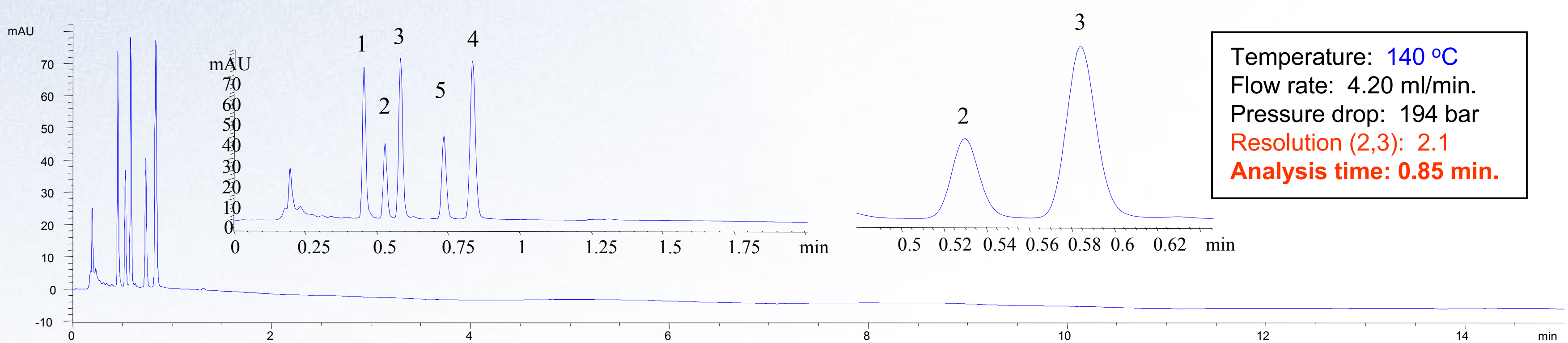
ZirChrom®

LC Conditions: Mobile Phase, 26.5/73.5 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Flow Rate, 3.00 mL/min.; Injection volume, 0.2 ul; 254 nm detection; Column Temperature, 80°C; Pressure drop = 195 bar; Solutes: 1=Doxylamine, 2=Methapyrilene, 3=Chlorpheniramine, 4=Triprolidine, 5=Meclizine **100 x 4.6 ZirChrom-PBD**



Temperature: 80 °C
Flow rate: 3.00 ml/min.
Pressure drop: 195 bar
Resolution (2,3): 2.1
Analysis time: 2.5 min.

LC Conditions: Mobile Phase, 20.5/79.5 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Flow Rate, 4.20 mL/min.; Injection volume, 0.5 ul; 254 nm detection; Column Temperature, 140°C; Pressure drop = 194 bar; Solutes: 1=Doxylamine, 2=Methapyrilene, 3=Chlorpheniramine, 4=Triprolidine, 5=Meclizine 100 x 4.6 ZirChrom-PBD



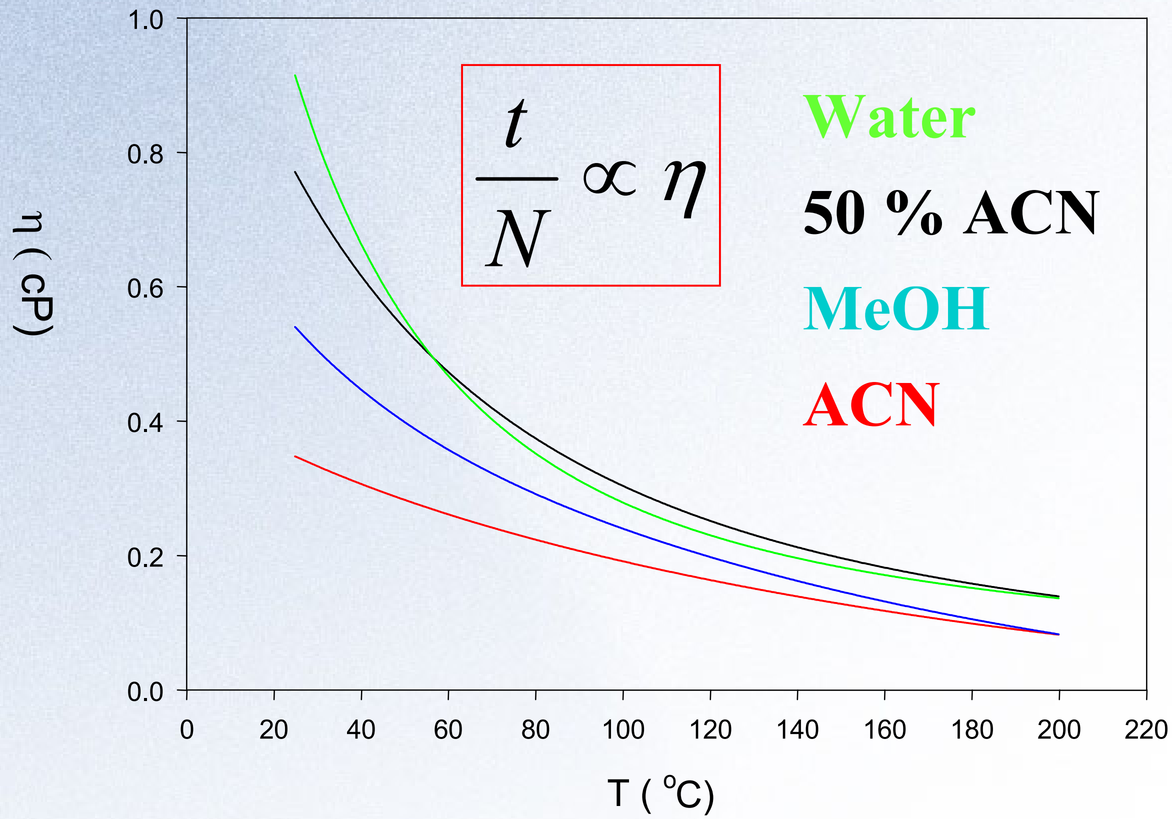
Temperature: 140 °C
Flow rate: 4.20 ml/min.
Pressure drop: 194 bar
Resolution (2,3): 2.1
Analysis time: 0.85 min.

The Importance of Analysis Time Reduction Through *Column Heating*

Temperature (degrees C)	Cost per Analysis*	Throughput per Instrument*
21	$2.66	1 X
50	$1.87	2.1 X
80	$1.50	3.3 X
140	$1.32	5.2 X

*** Based on Quantitative Value Assessment Tool - http://www.zirchrom.com/documents/value.xls**

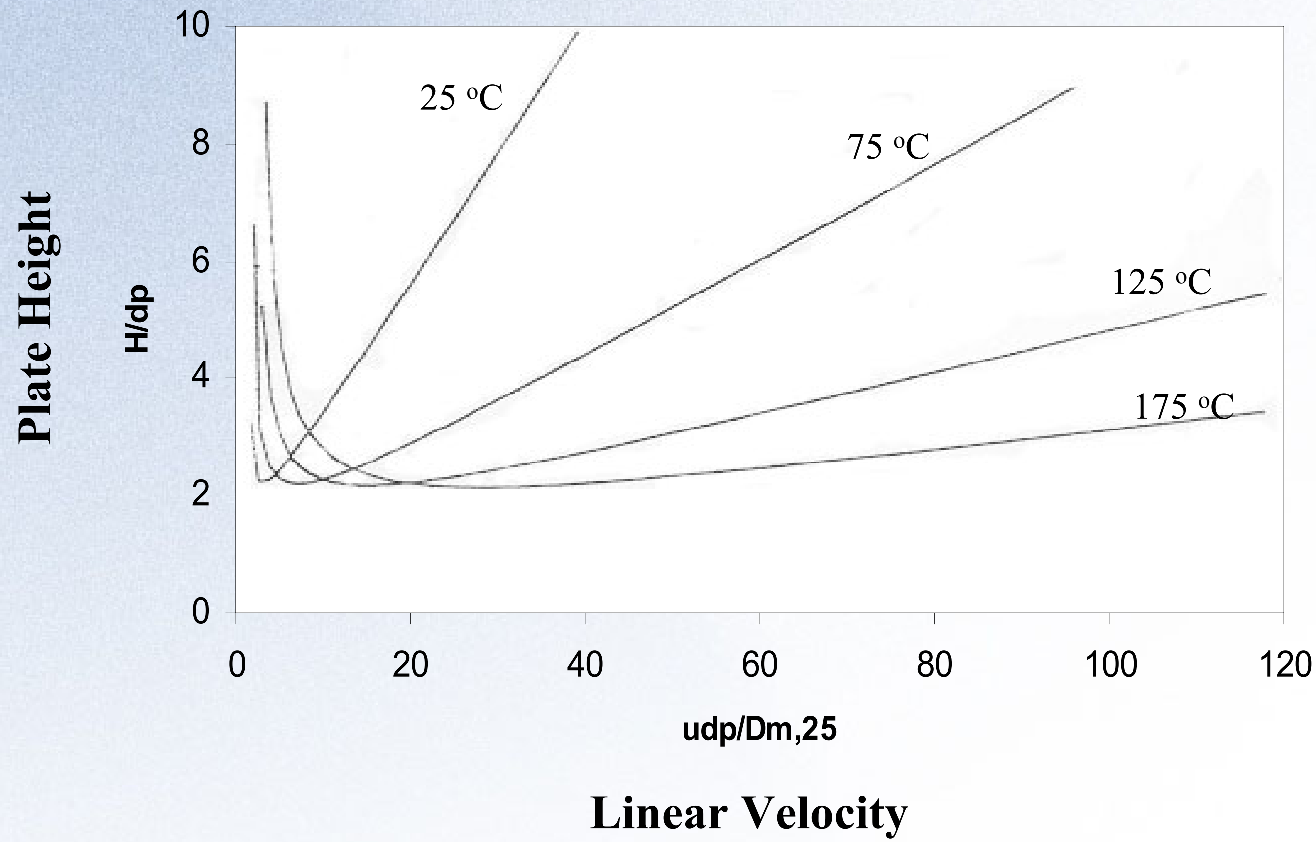
Estimated Effect of Temperature on Viscosity*



$$\frac{t}{N} \propto \eta$$

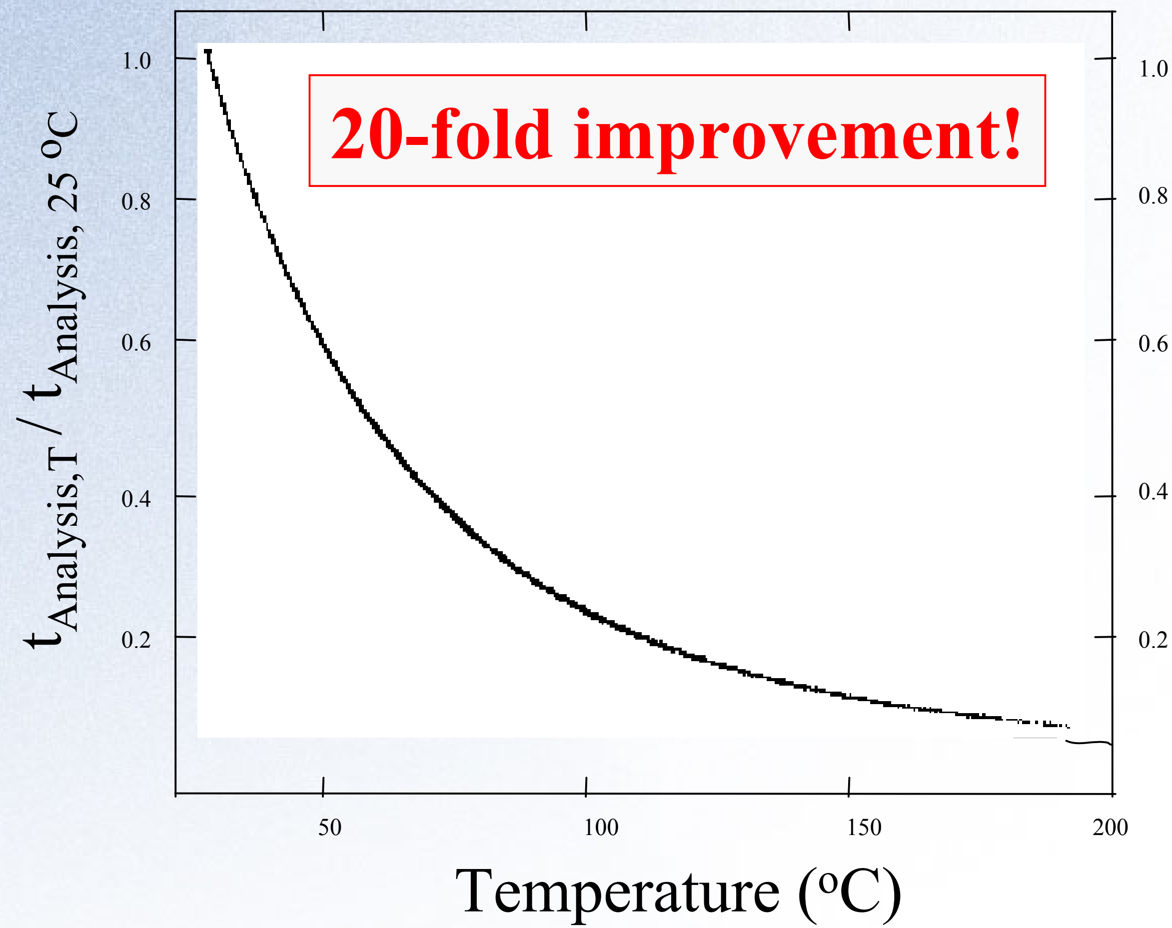
*H. Chen and Cs. Horvath, "Rapid Separation of Proteins by RP-HPLC at Elevated Temperatures," *Anal. Methods Instrum.*, ***1***, 213-222 (1993).

Theoretical Effect of Temperature on Efficiency



F. D. Antia and Cs. Horvath, J. Chromatogr. 1988, 435, 1-15

Theoretical Effect of Temperature on Analysis Time at Constant Pressure, Plate Count and Retention Factor*



*R. D. Antia and Cs. Horvath, *J. Chromatogr.*, **435**, 1-15 (1988).

Three Obstacles to Routine Use of High Temperature HPLC

ZirChrom®

Traditional Obstacles:		Here and Now:
Silica-based phases: NOT THERMALLY STABLE	→	Zirconia-based Phases are THERMALLY STABLE
Temperature mismatch broadening	→	Temperature Mismatch Broadening Has Been Solved – Metalox 200-C
Questions about thermal stability of small organic molecules?	→	A large majority of small organic molecules are stable on the timescale of ultra-fast separations

Slide courtesy of Dr. Jon Thompson

High Temperature HPLC and the End User

ZirChrom®

Column temperature and the hardware and software that control it are one of the least understood facets of the HPLC technique

And why is that
???

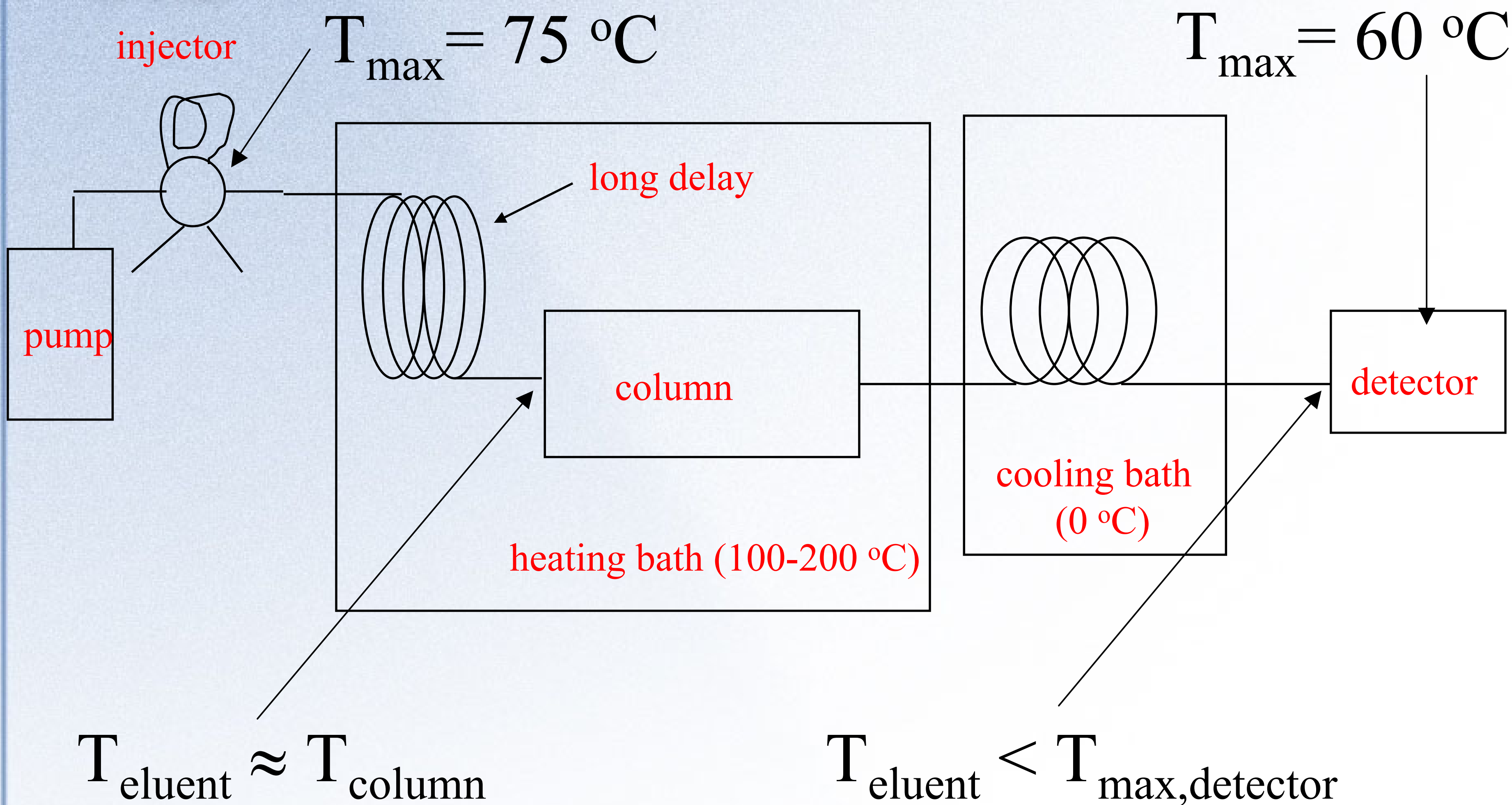
Because utilizing temperature offers little or no advantage to most suppliers of columns and instrumentation.

The benefits of temperature are most appreciated by those interested in controlling the **cost of analysis**!

ZirChrom®

"A poorly engineered column heater can ruin superb chromatography."

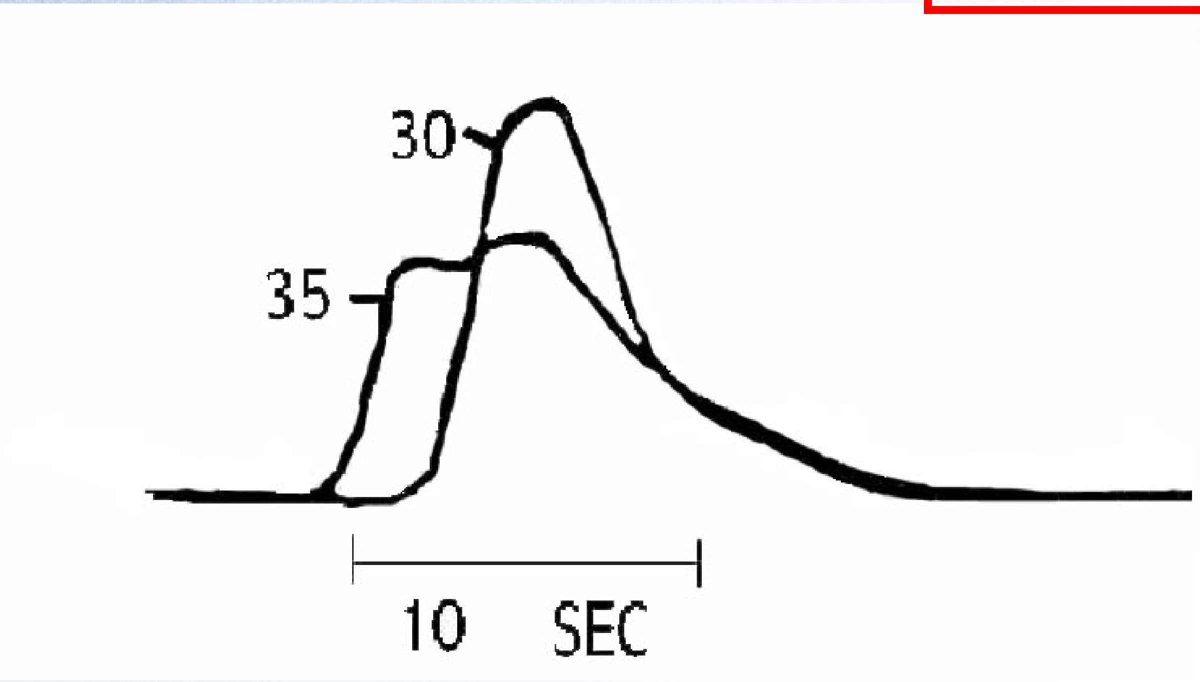
A PRIMITIVE High Temperature Liquid Chromatography System



Peak Shapes Observed for Various Mobile-Phase Feed Temperatures*

ZirChrom®

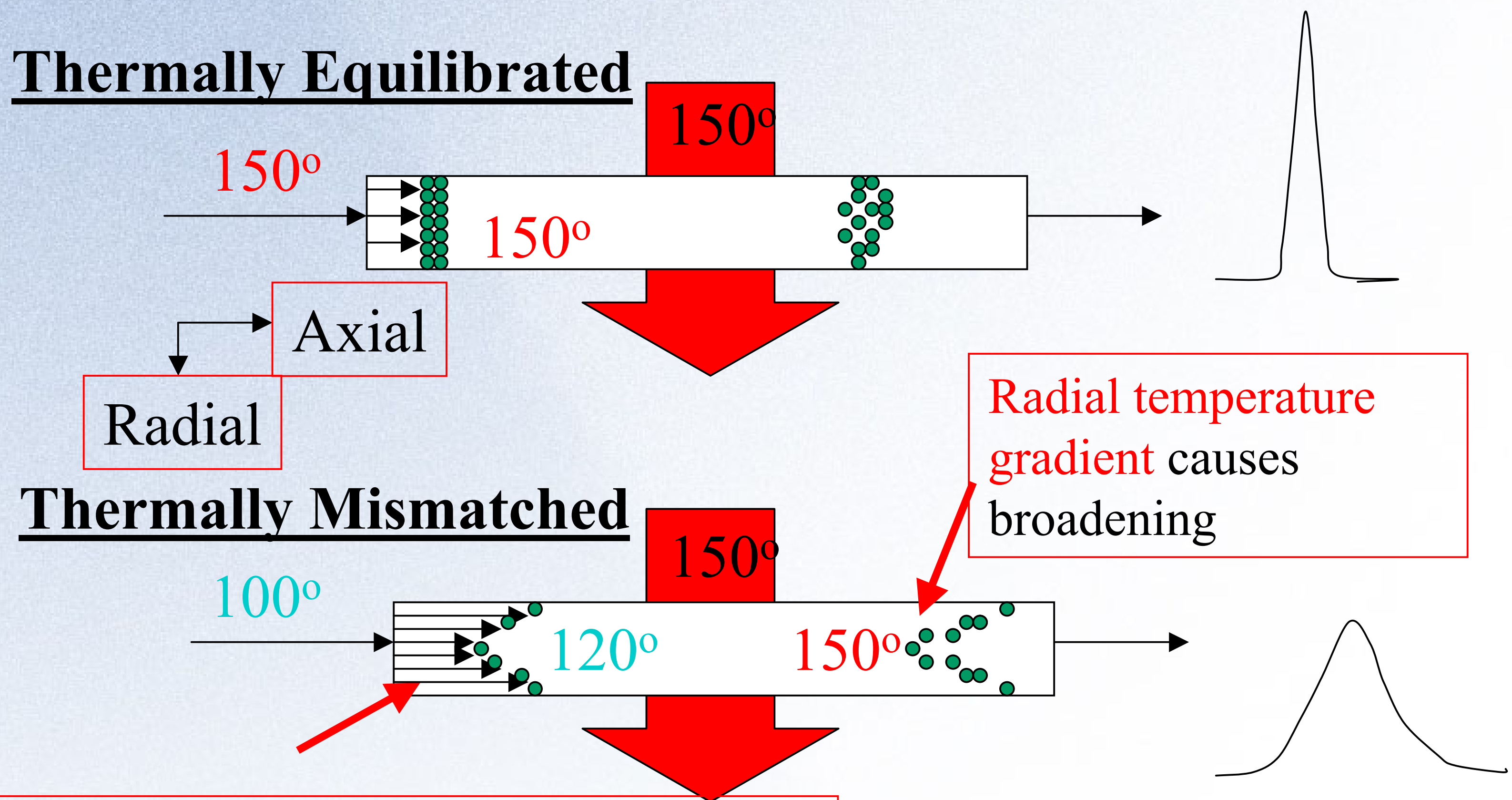
$$\sigma^2_{obs} = \sigma^2_{column} + \sigma^2_{extra-columnn} + \sigma^2_{thermal-mismatch}$$



LC conditions: Mobile phase, 50/50 ACN/water; Flow rate, 5 ml/min.; Temperature, 30 ^{o}C with column water jacket; Column, 80 mm x 6.2 mm i.d. 3 μm Zorbax ODS; Solute, nitrobenzene.

*H. Poppe and J.C. Kraak, *J. Chromatogr*., **282**, 399-412 (1983).

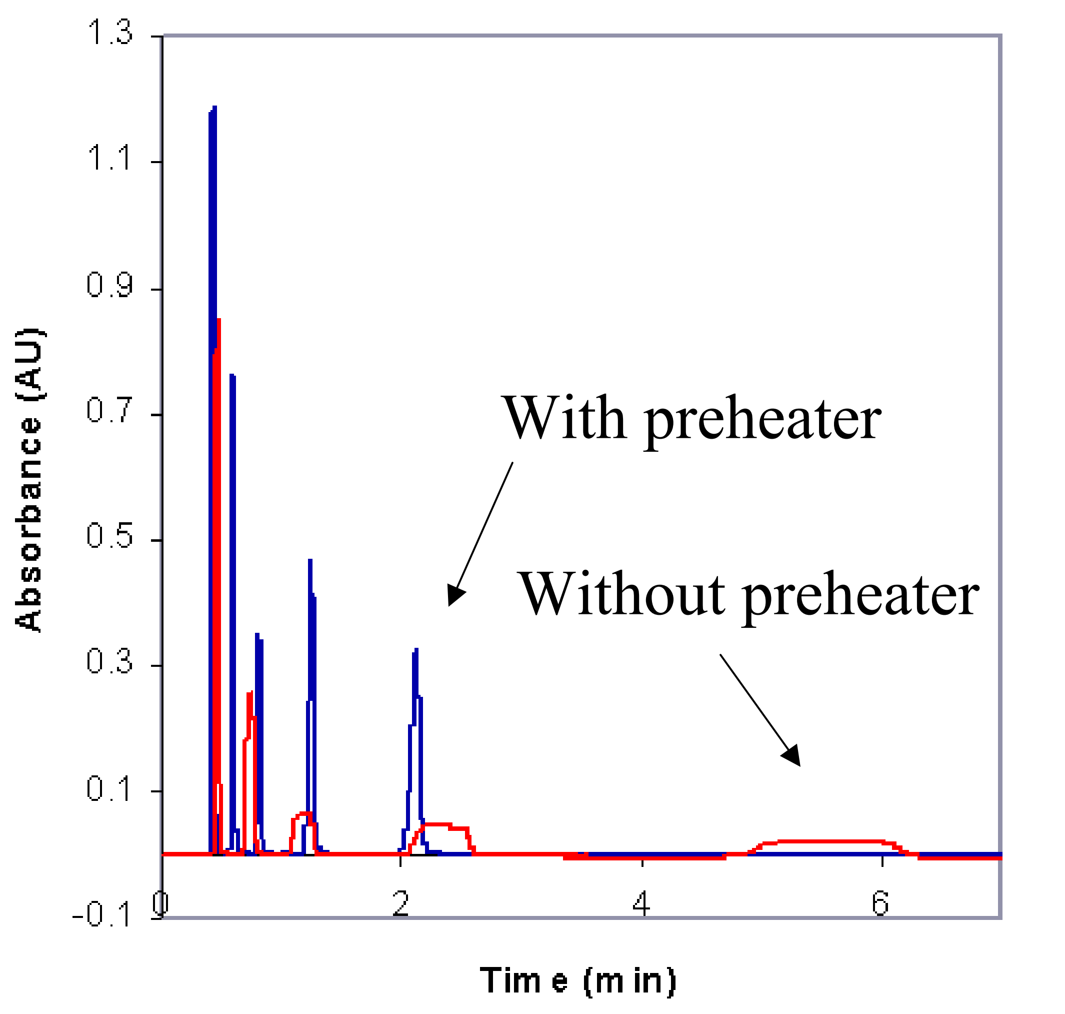
The Effect of Incomplete Thermal Equilibration



Cooler in the center:
Lower viscosity and higher retention

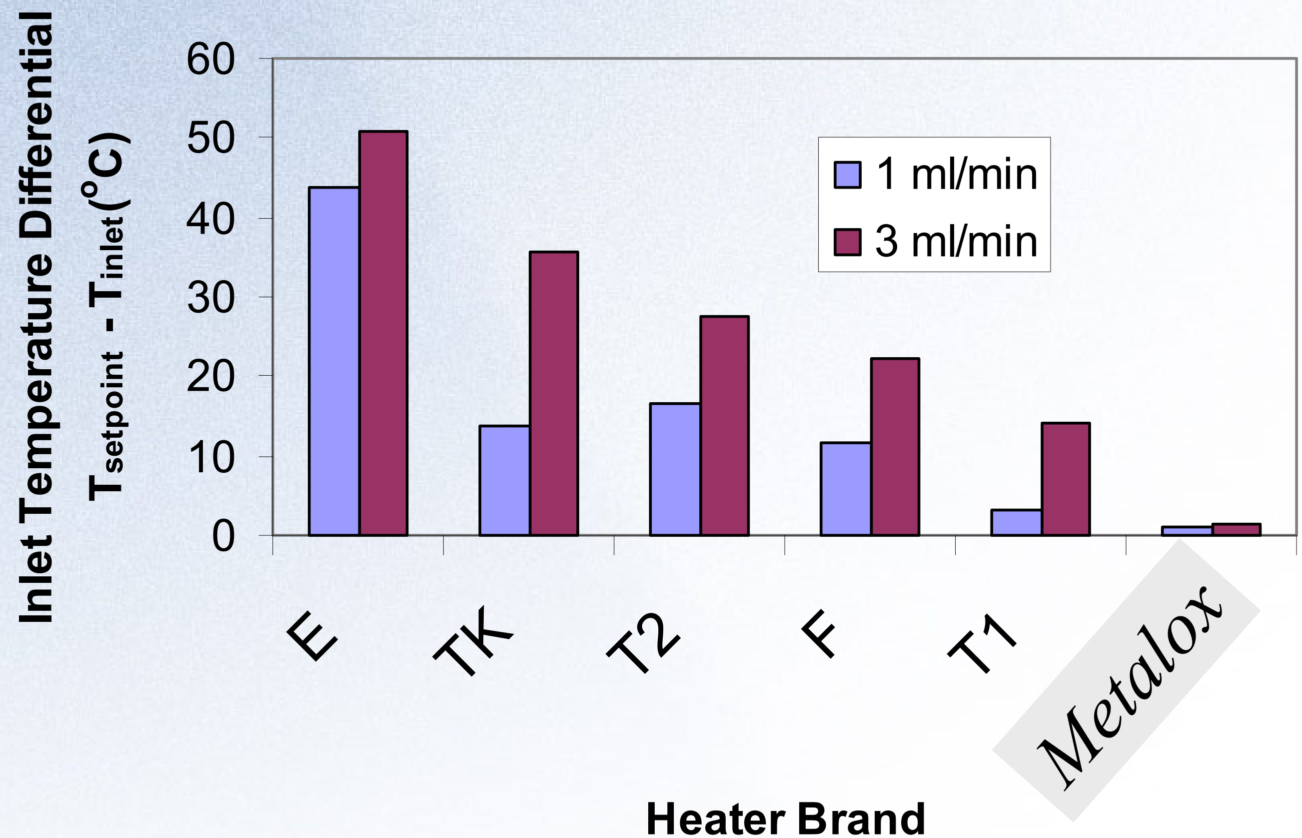
Diagram Courtesy Dr. Jon Thompson, Systec

Thermal Mismatch Broadens Peaks

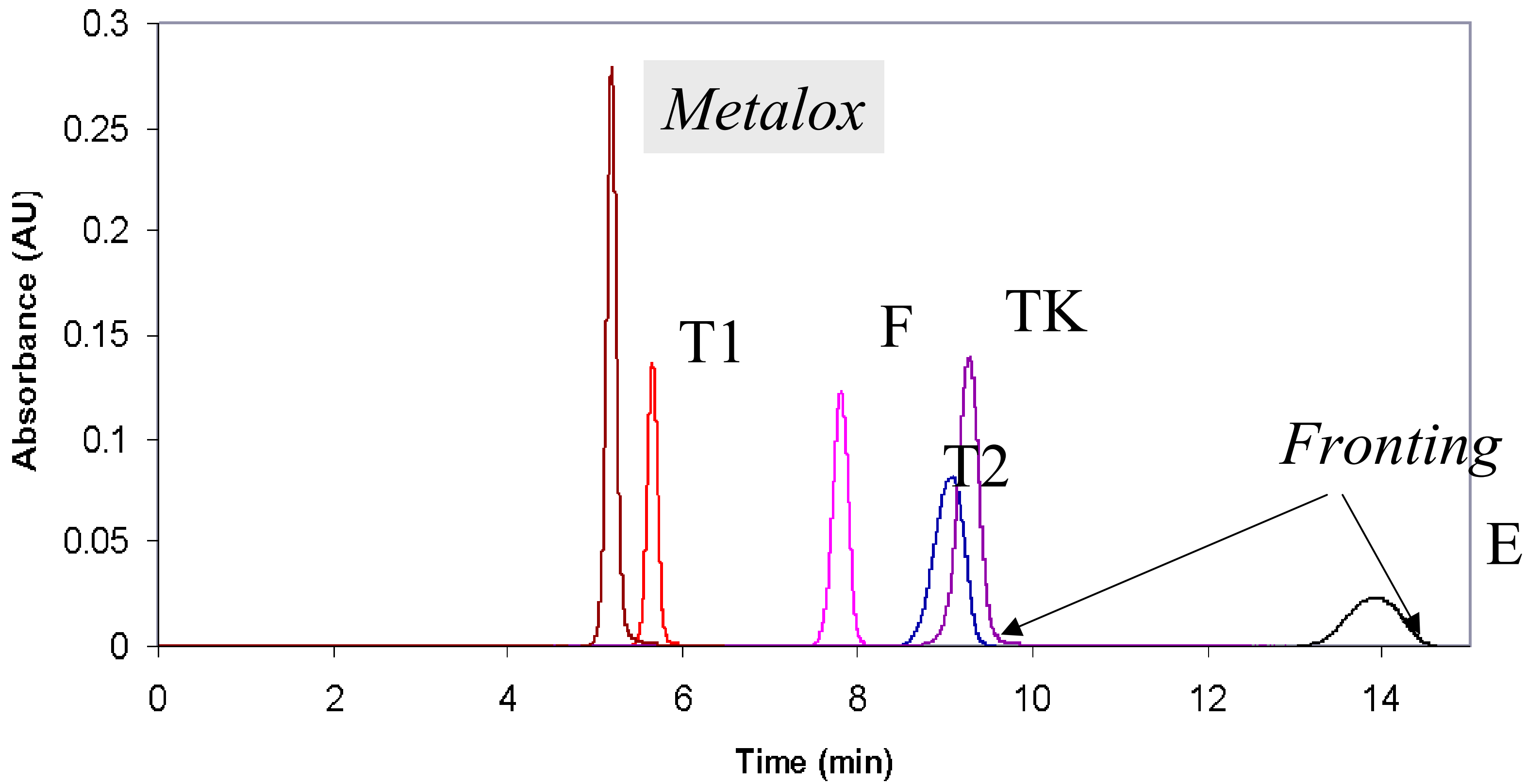


Conditions:_ Mobile phase, 50/50 ACN/water; Setpoint temperature, 165 °C, Flow rate, 3 ml/min.; Heater, F with 1.68 m preheater tubing; 100 mm x 4.6 mm i.d. ZirChrom®-PBD; Solutes, alkylphenones.

Comparison of Inlet Temperature Differential for Various Heater Brands



Effect of Heater Manufacturer on Retention and Peak Shape

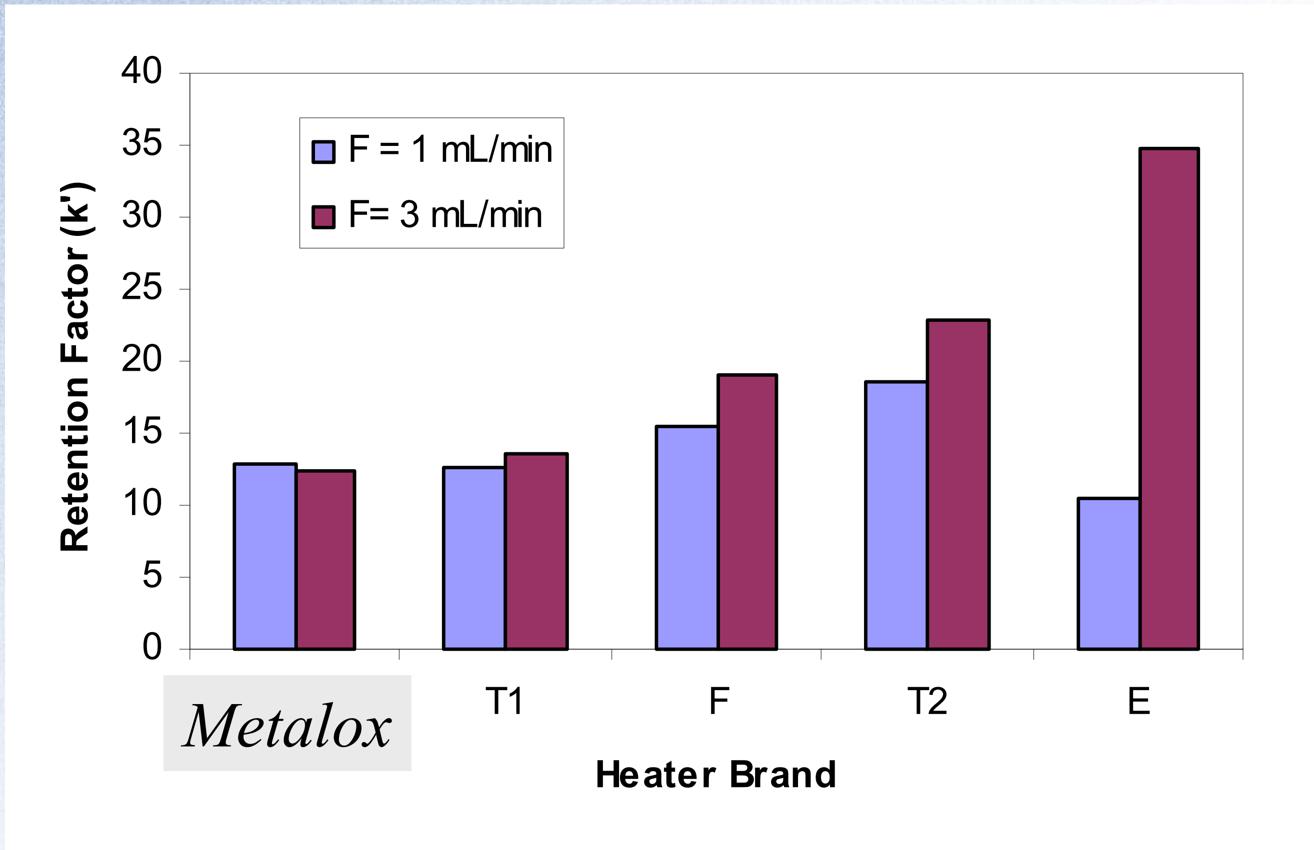


Conditions: Mobile phase, 50/50 ACN/water; Setpoint temperature, 85 °C; Flow rate, 3.0 ml/min.; Column 100 mm x 4.6 mm I.d. ZirChrom®-PBD; Solute, tetradecaphenone.

Retention Factor is Independent of Flow Rate, Right?

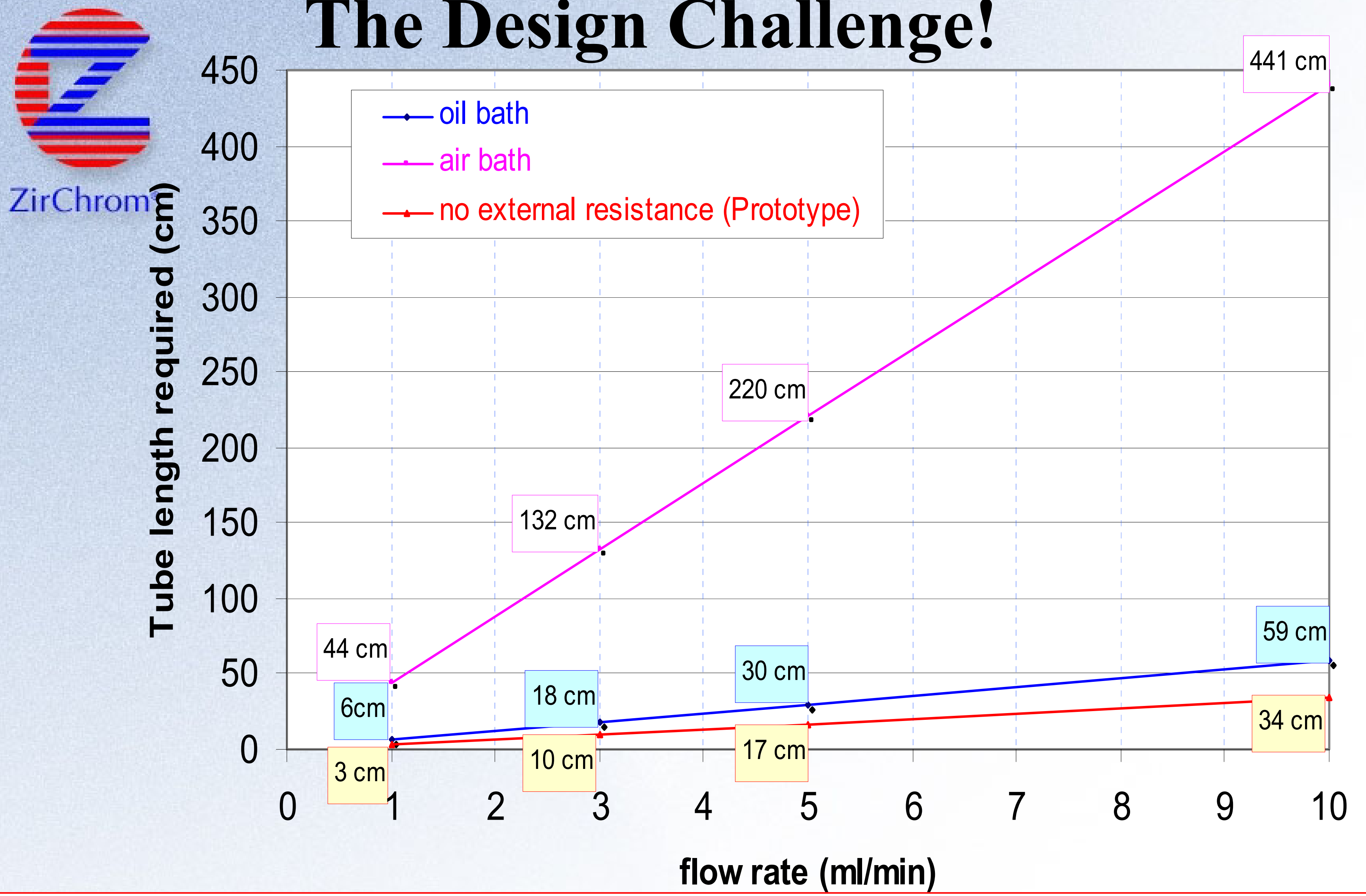
$$k' = \frac{t_R - t_m}{t_m}$$

Wrong…if your heater does not work properly



Conditions: Mobile phase, 50/50 ACN/water; Setpoint temperature, 85 ^{o}C; Flow rate, 3.0 ml/min.; Column 100 mm x 4.6 mm I.d. ZirChrom®-PBD; Solute, tetradecaphenone.

The Design Challenge!



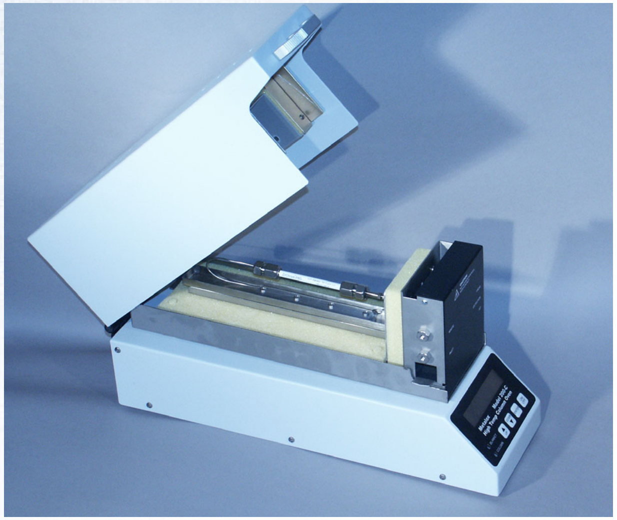
How do we get the eluent and column to high temperature and high velocity without intolerable extra-column band broadening?

B Yan, et al. ***Anal. Chem*****. 72, 1253-62 (2000)**.

The Metalox™ 200-C System

ZirChrom®

- Mobile phase pre-heating heat exchanger
- Direct heating of mobile phase and pre-detector heat exchange cooling
- Active shield quasi-adiabatic column jacket
- Adjustable back pressure regulator
- A method development kit including three of the ultra-stable RPLC ZirChrom® phases (introductory offer)

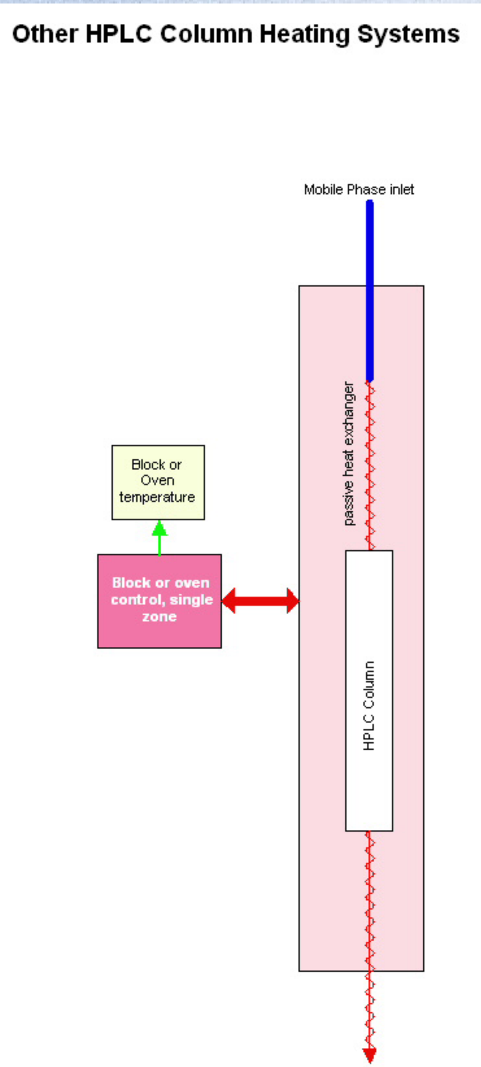


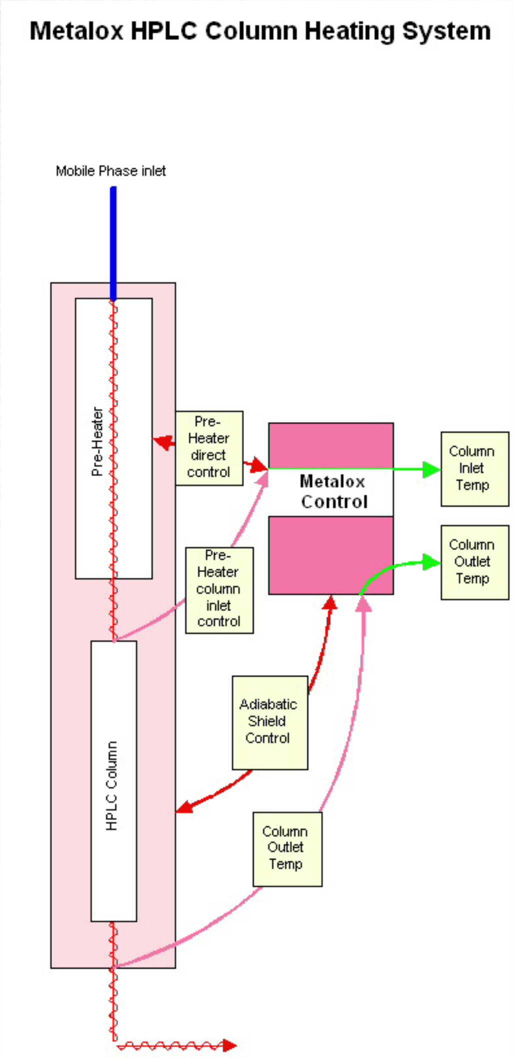
Design Goals for the Metalox™ Model 200-C

ZirChrom®

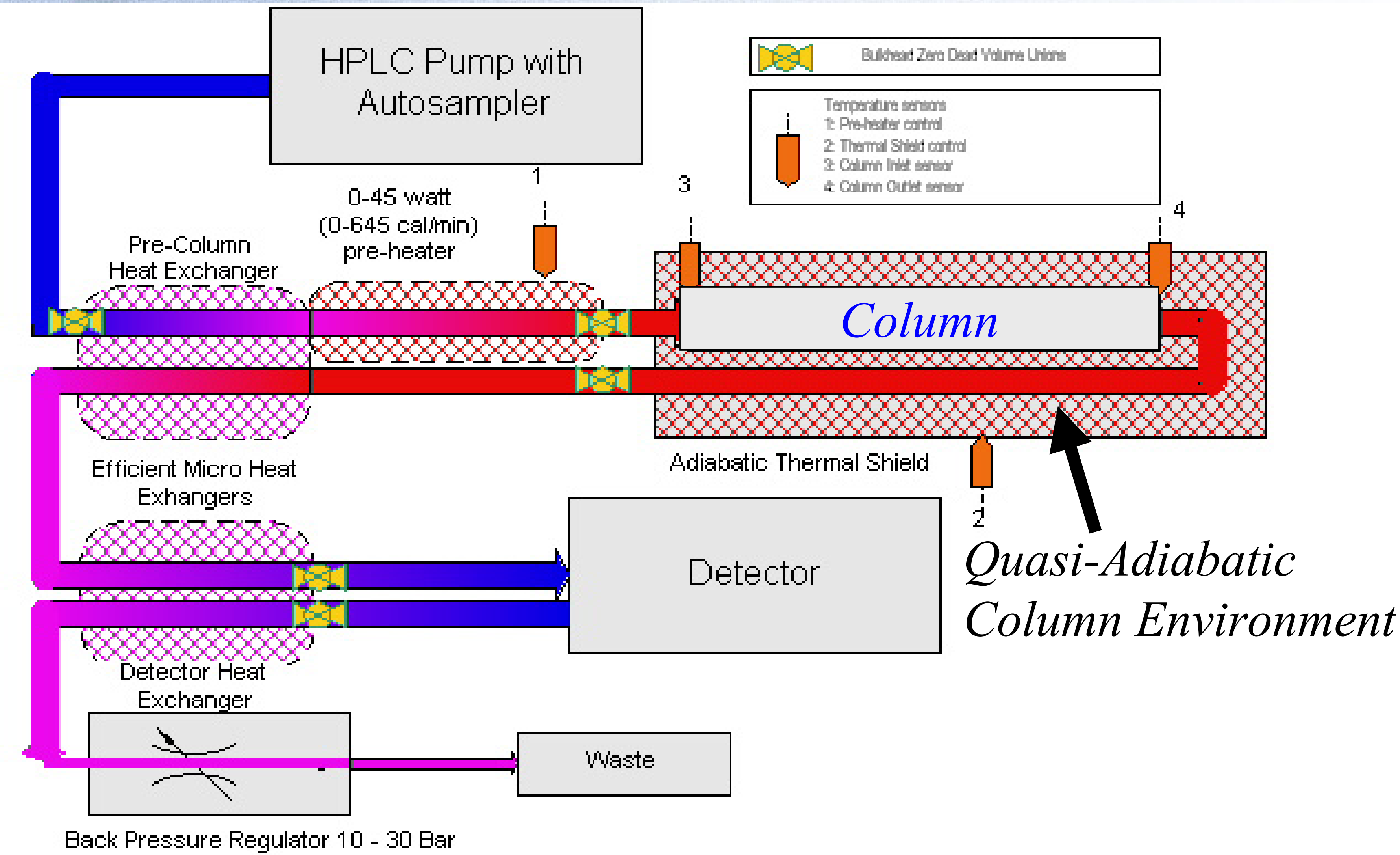
- Small footprint
- Stand alone operation
- Multiple operating positions
- Highly accurate column temperature
- No overheating of incoming mobile phase
- One preheater tube for all specified operating conditions
- Intended as a productivity tool for HPLC columns 15cm or less
- Easy access to parameters necessary for method or instrument validation

Schematic Comparison of Conventional Column Heater vs. Metalox^{TM} 200-C

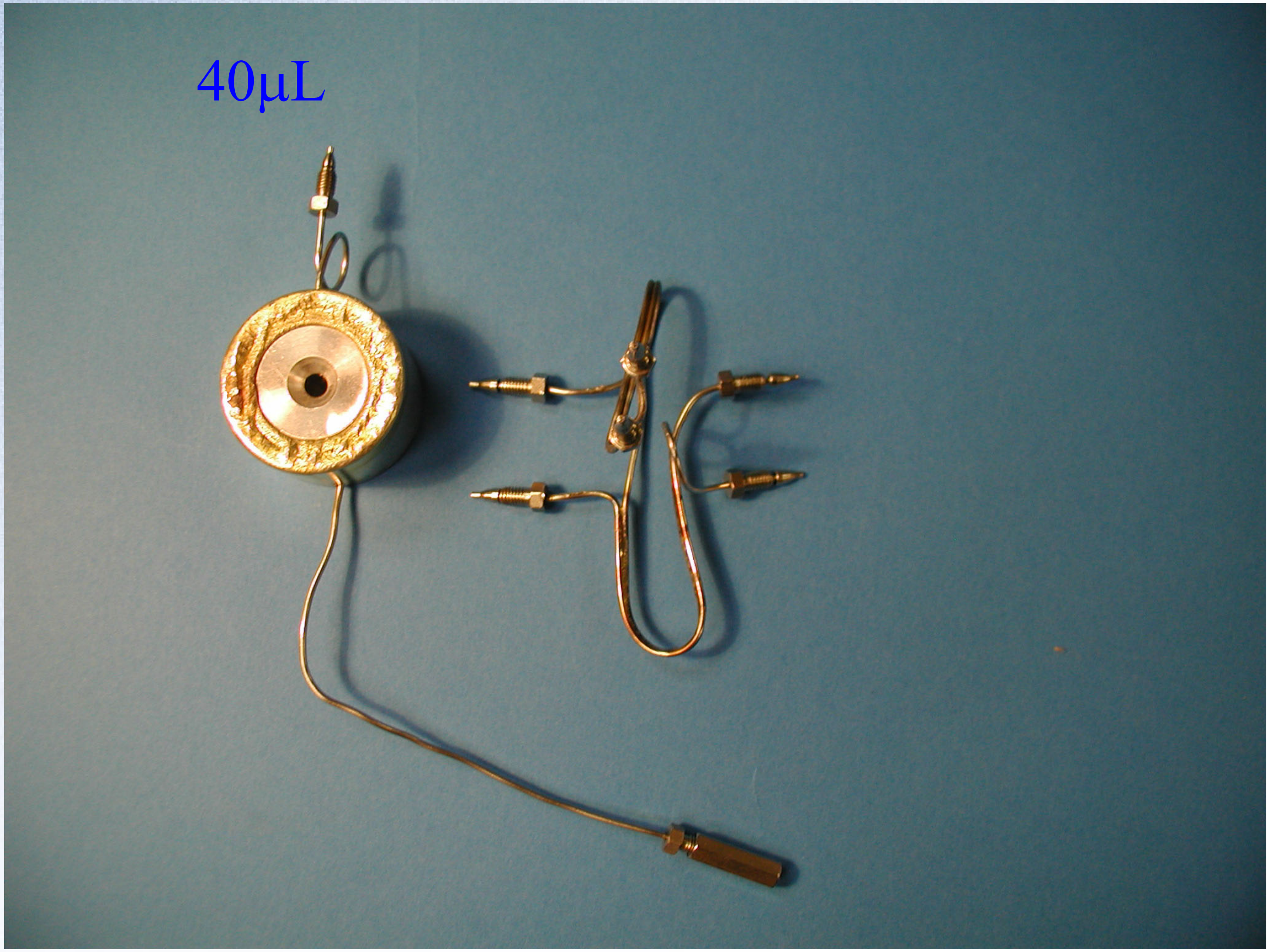




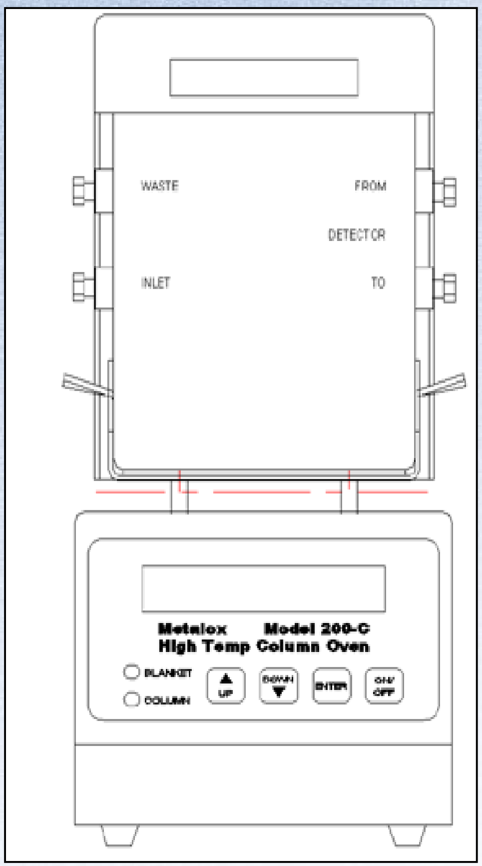
Patented Heating System
Metalox™ Model 200-C



Comparison of Heat Exchangers



Metalox™ 200-C Specifications



- Operational Capabilities
  - Max. Column Operating Temp: 200° C (6 ml/min with water)
  - Min. Temperature: 7° C above ambient
  - Max. Flow Rate: 6ml/min (200° C, water mobile phase)
  - Max. Cal./s: 17.9
  - Temperature Reproducibility: ± 0.5° C
  - Accuracy of Temp. Reading: ± 1%
  - Display Resolution: 1° C
- Physical Specifications
  - Weight: 15lbs
  - Footprint: 6”x10”x16”
  - Power Requirements: 115/230V 47-440 Hz
  - Internal Transfer Volumes:
    - Pre-Column: 10.5 uL
    - Post-Column 4.0 uL

Note: Metalox 200-C design and specifications are patented

ZirChrom®

Summary

- High temperature HPLC is a practical means of achieving significant improvements in HPLC throughput
- ZirChrom offers four unique phases for high tempeature RPLC that are thermally stable up to 150 ^{o}C (200 ^{o}C for carbon phases)
- The Metalox^{TM} 200-C column heater is a state-of-the-art unit that allows monitoring of the actual HPLC column inlet and outlet temperatures up to 200 ^{o}C to ensure the most high performance and reproducible separations