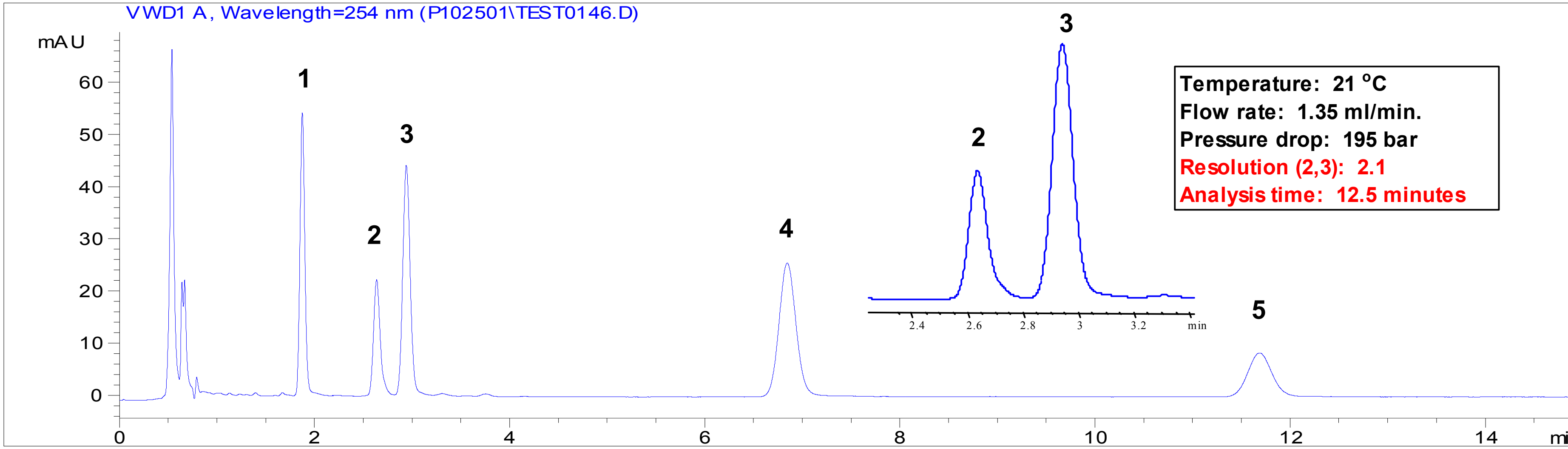
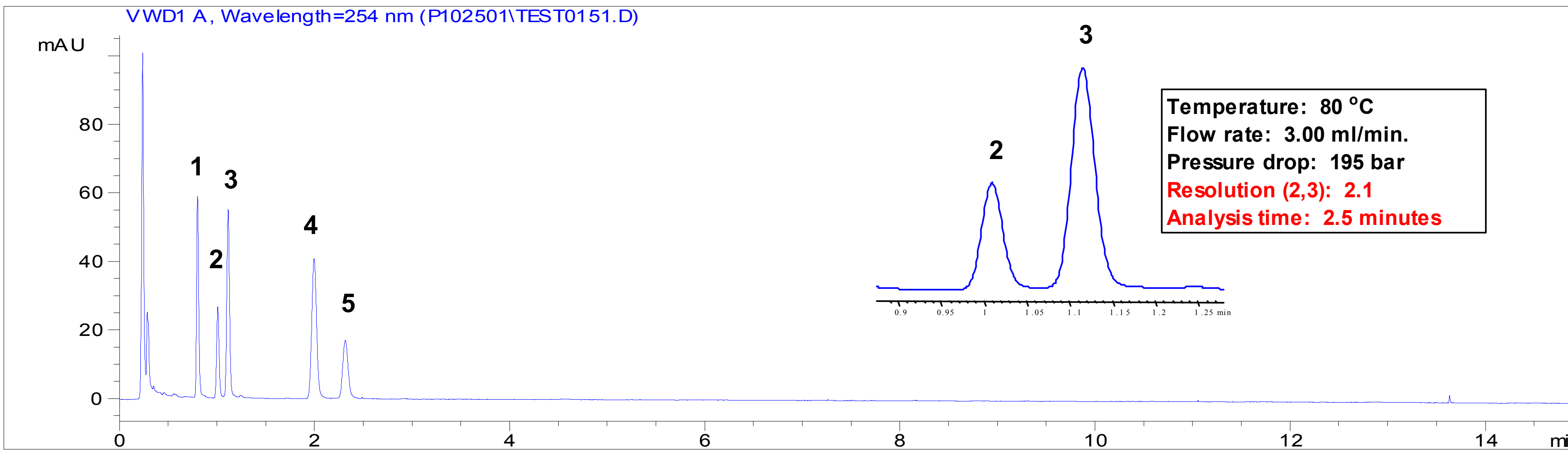
# High Temperature Ultra Fast Liquid Chromatography

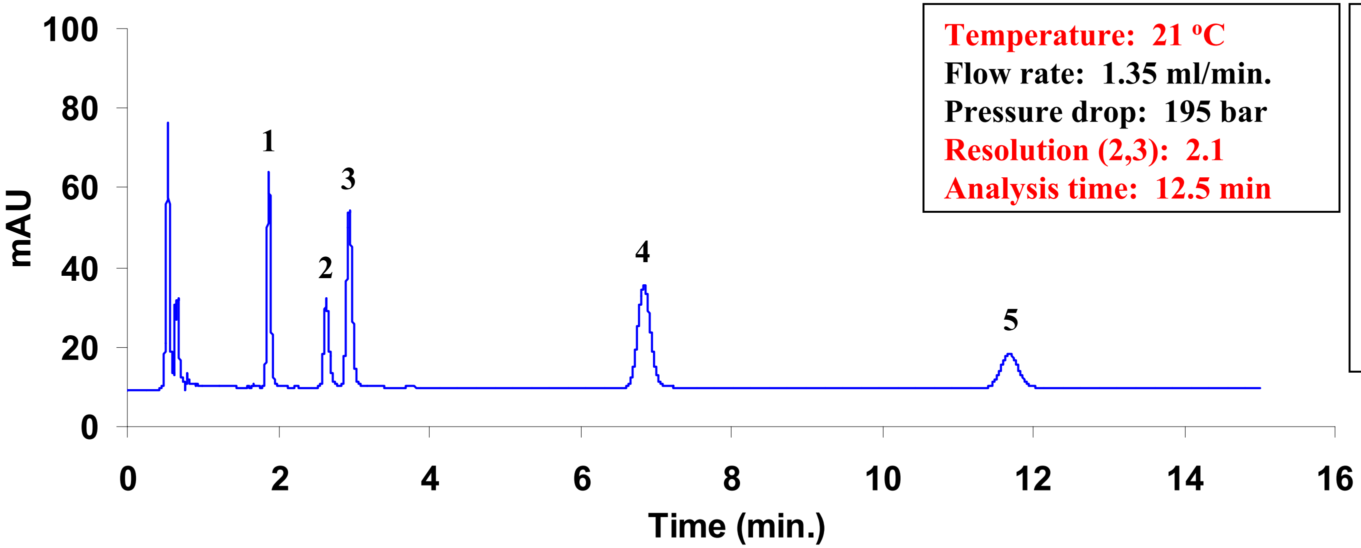
Peter W. Carr, Jon Thompson and
Dwight Stoll

# Fast Separation of Antihistamines



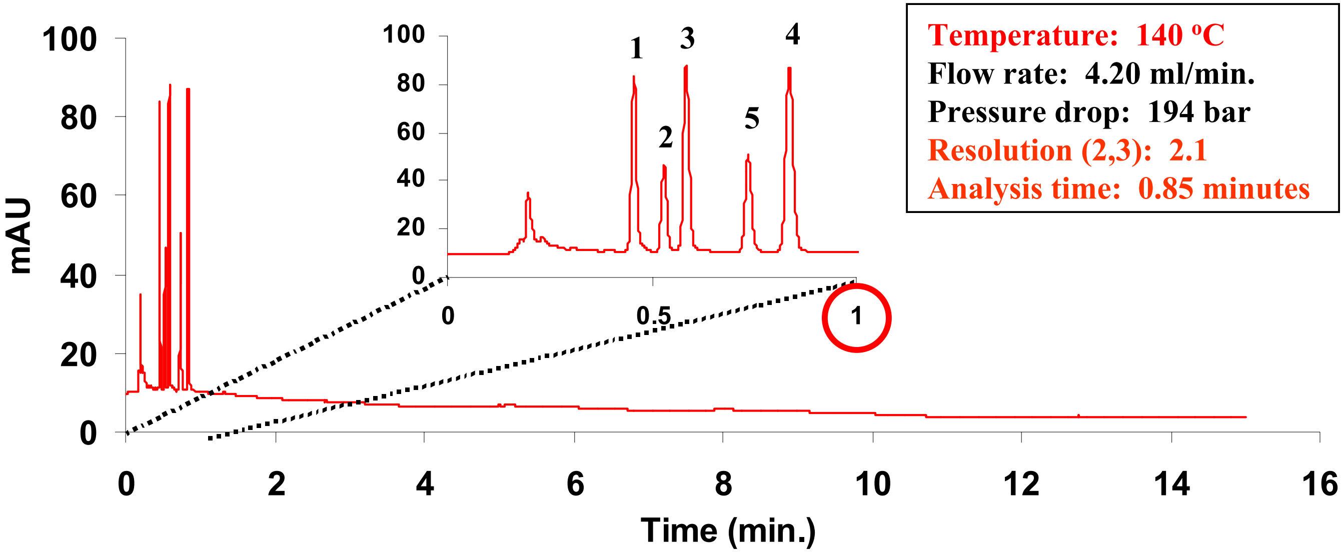




**LC Conditions: (A) Mobile Phase, 29/71 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Flow Rate, 1.35 mL/min.; Injection volume, 0.5 ul; 254 nm detection; Temperature, 21°C; Pressure drop = 195 bar; Solutes: 1=Doxylamine, 2=Methapyrilene, 3=Chlorpheniramine, 4=Triprolidine, 5=Meclizine4=Triprolidine, 5=Meclizine, 100 x 4.6 ZirChrom-PBD (B) same as A, except Mobile Phase, 26.5/73.5 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Flow Rate, 3.00 mL/min.; Column Temperature, 80°C; Pressure drop = 195 bar**




**Temperature: 21 °C**
**Flow rate: 1.35 ml/min.**
**Pressure drop: 195 bar**
**Resolution (2,3): 2.1**
**Analysis time: 12.5 min**

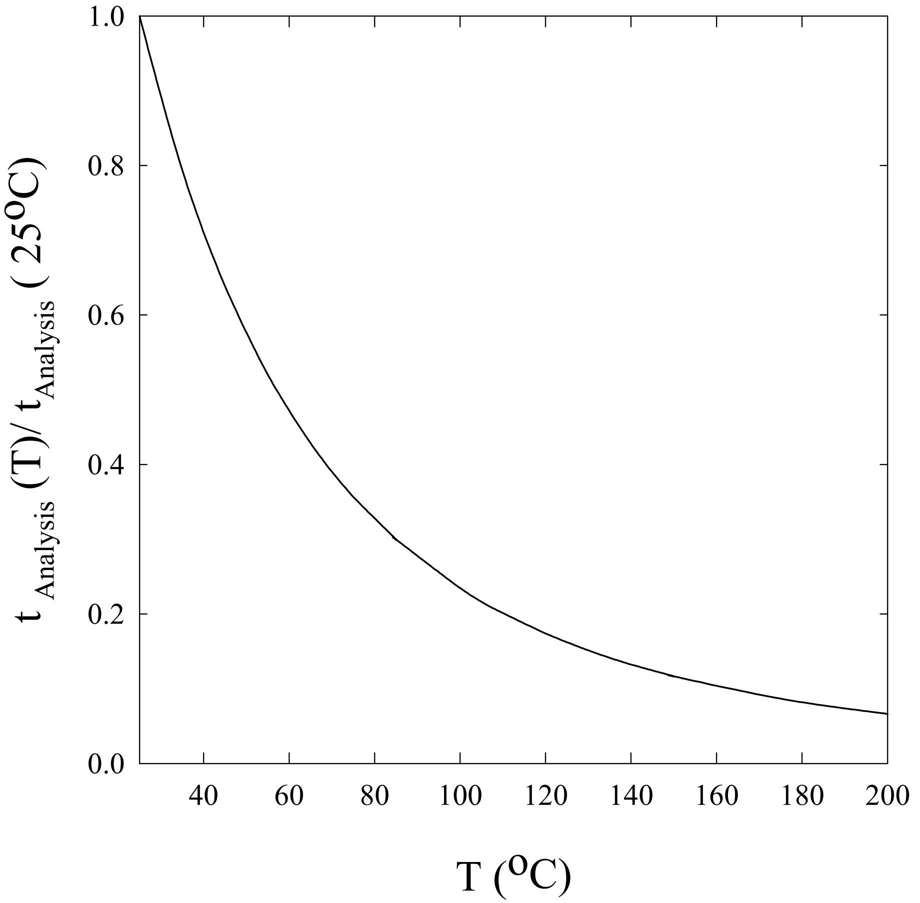
Mobile Phase, 29/71 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Injection volume, 0.5 µl; 254 nm detection; Solutes: 1=Doxylamine, 2=Methapyrilene, 3=Chlorpheniramine, 4=Triprolidine, 5=Meclizine; Column, 100 mm x 4.6 mm i.d. ZirChrom®-PBD




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

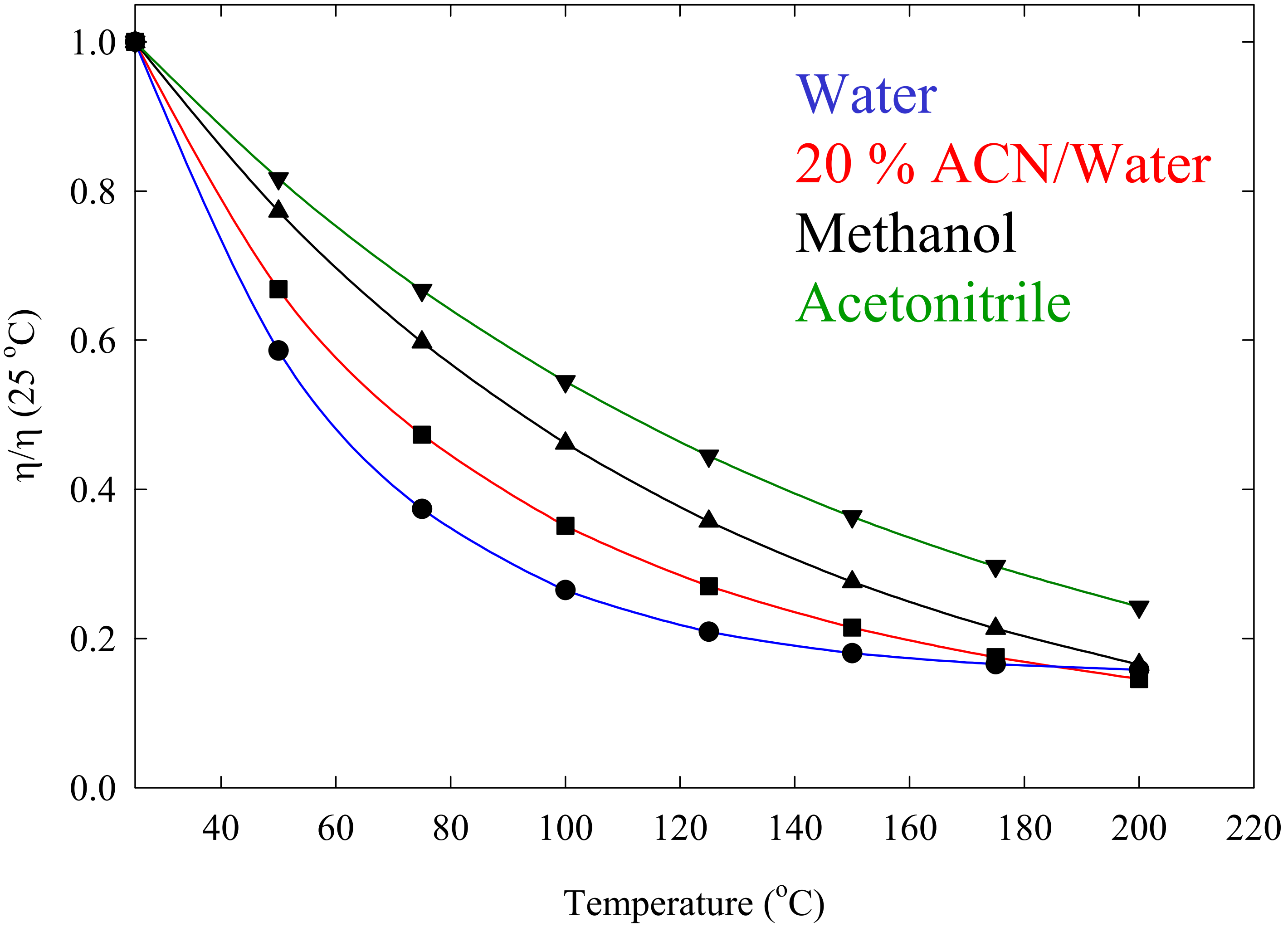
Mobile Phase, 21/79 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Injection volume, 0.5 µl; 254 nm detection; Solutes: 1=Doxylamine, 2=Methapyrilene, 3=Chlorpheniramine, 4=Triprolidine, 5=Meclizine; Column, 100 mm x 4.6 mm i.d. ZirChrom®-PBD

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

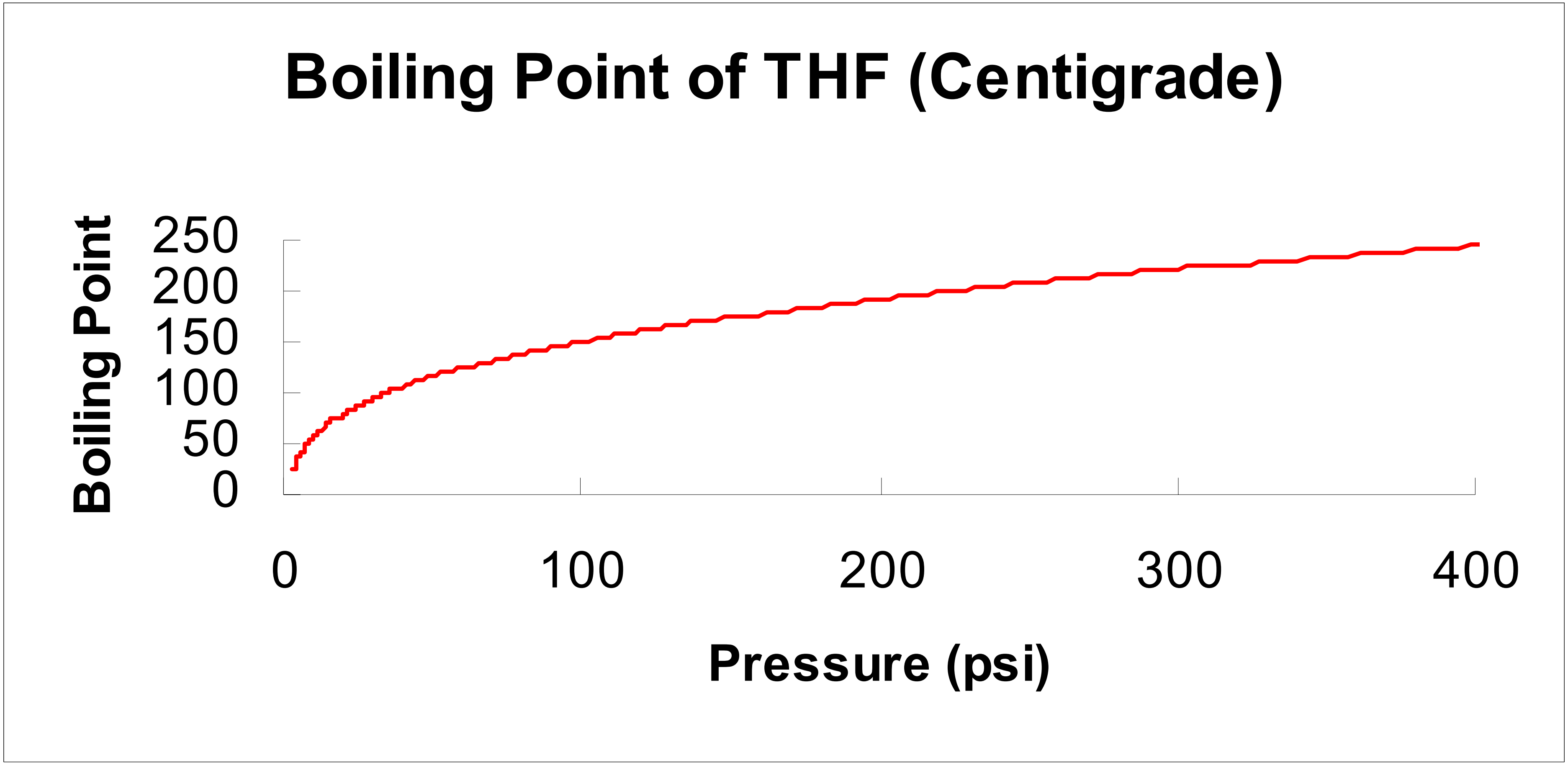



**“High-Performance Liquid Chromatography at Elevated Temperatures: Examination of Condition for the Rapid Separation of Large Molecules,” R. D. Antia and Cs. Horvath, *J. Chromatogr*., 435, 1-15 (1988).**

# Relative Viscosity vs. Temperature

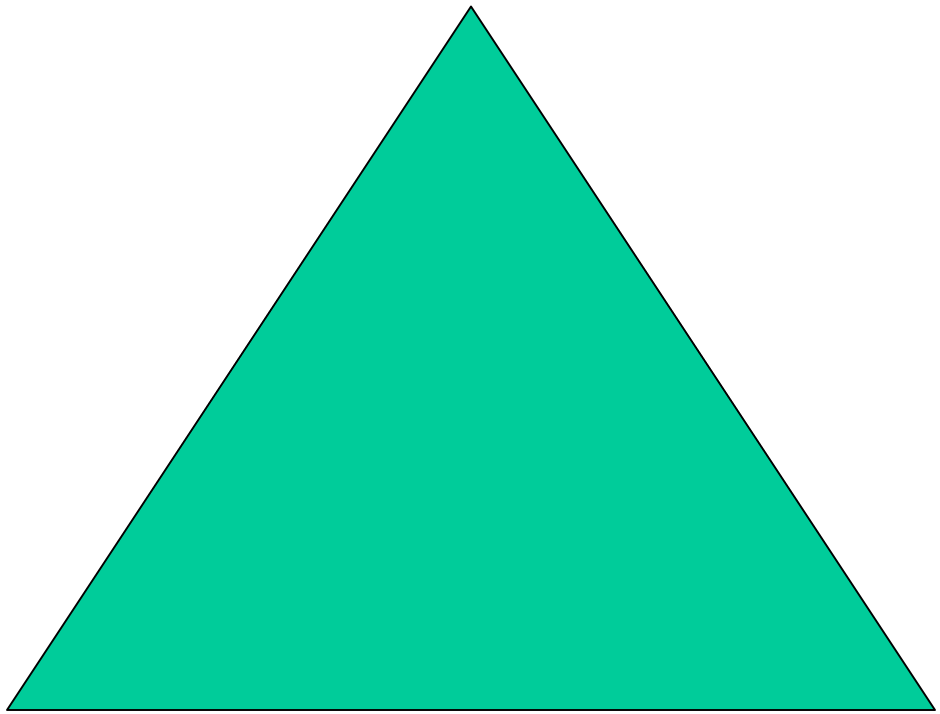


# How to Prevent Boiling



# Requirements for High Temperature LC

Stationary Phase Stability

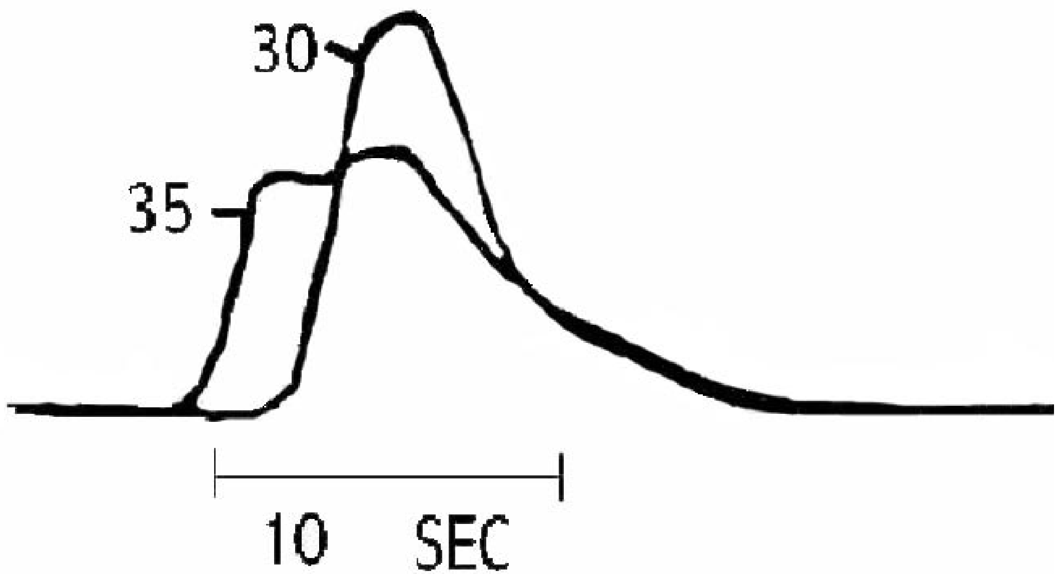


Thermal Mismatch Broadening

On-Column Analyte Instability

# Peakshapes Observed for Various Mobile-Phase Feed Temperatures*

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

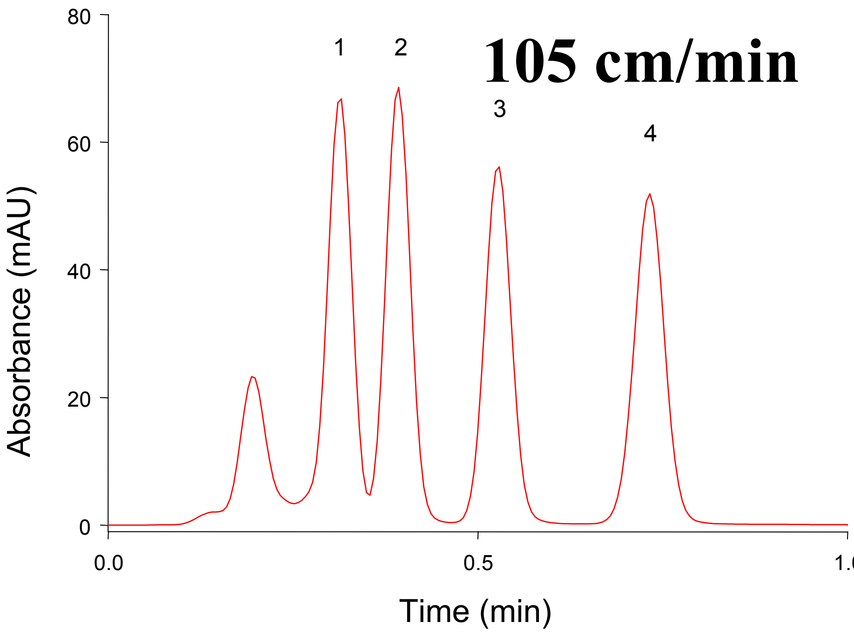



LC conditions: Column at 30 °C; 6.2 mm IDx8cm; 3μ Zorbax ODS; at 5 mL/min; 50/50 (v/v) ACN,$H_2O$; nitrobenzene

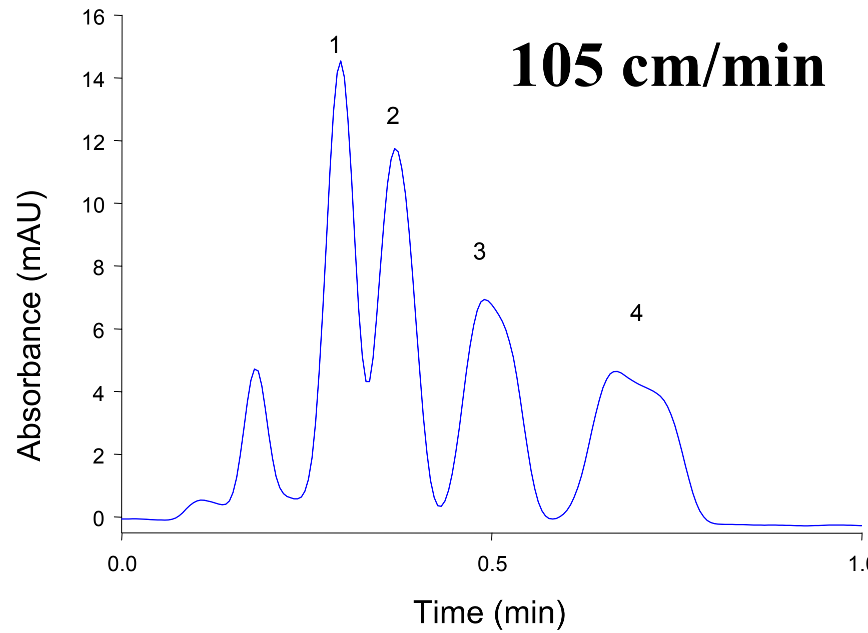
*H. Poppe and J.C. Kraak, *J. Chromatogr.*, **282**, 399-412 (1983).

# Comparison of the Effect of Incomplete Thermal Equilibration on Column Performance

2.1 mm ID



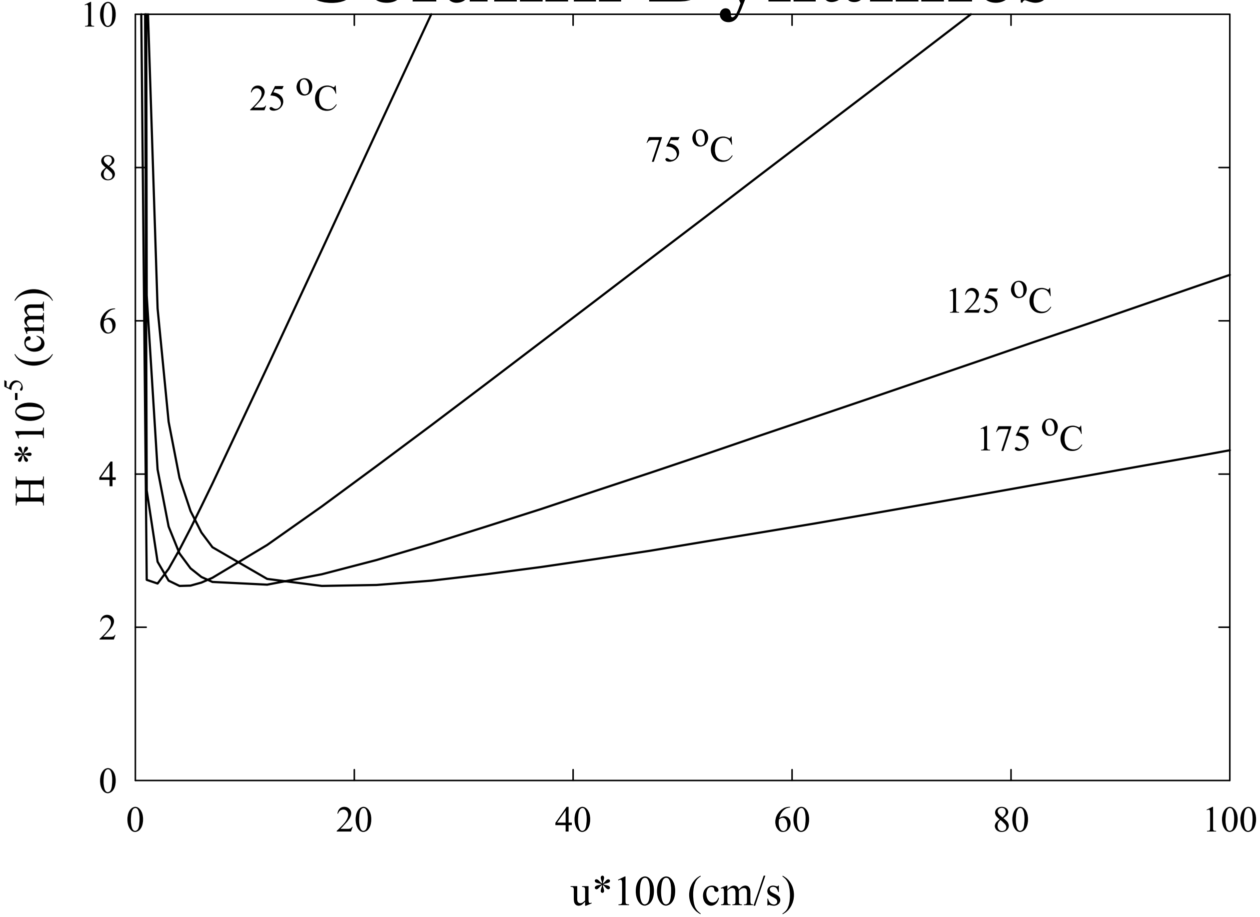

4.6 mm ID




<u>LC conditions</u>: 2.1 x 5 cm, C-18 INERT, 55 % ACN, 5 cm preheater, 60 $^{o}$C
4.6 x 5 cm, C-18 INERT, 60% ACN, 5 cm preheater, 60 $^{o}$C.

<u>Peaks</u>: 1. toluene, 2. ethylbenzene, 3. propylbenzene,4. butylbenzene

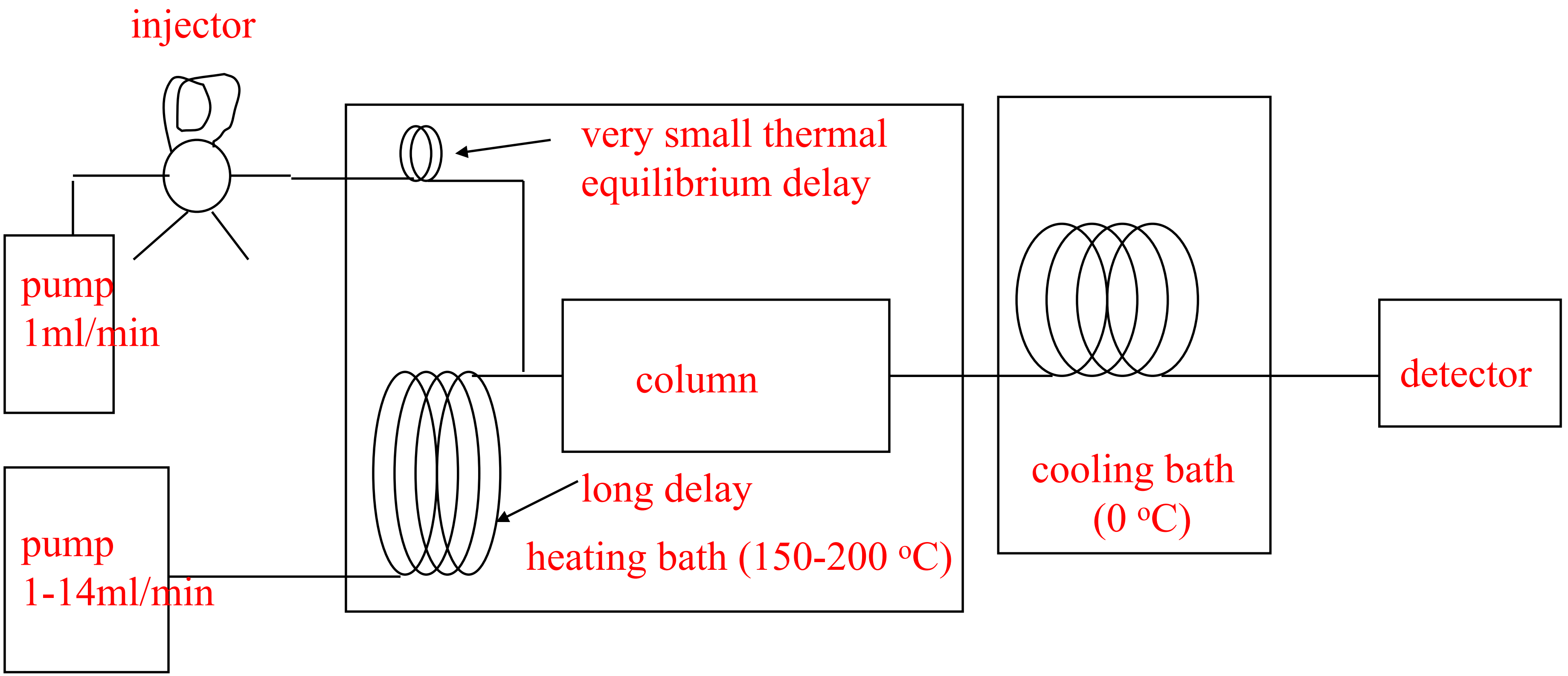
# Theoretical Effect of Temperature on Column Dynamics



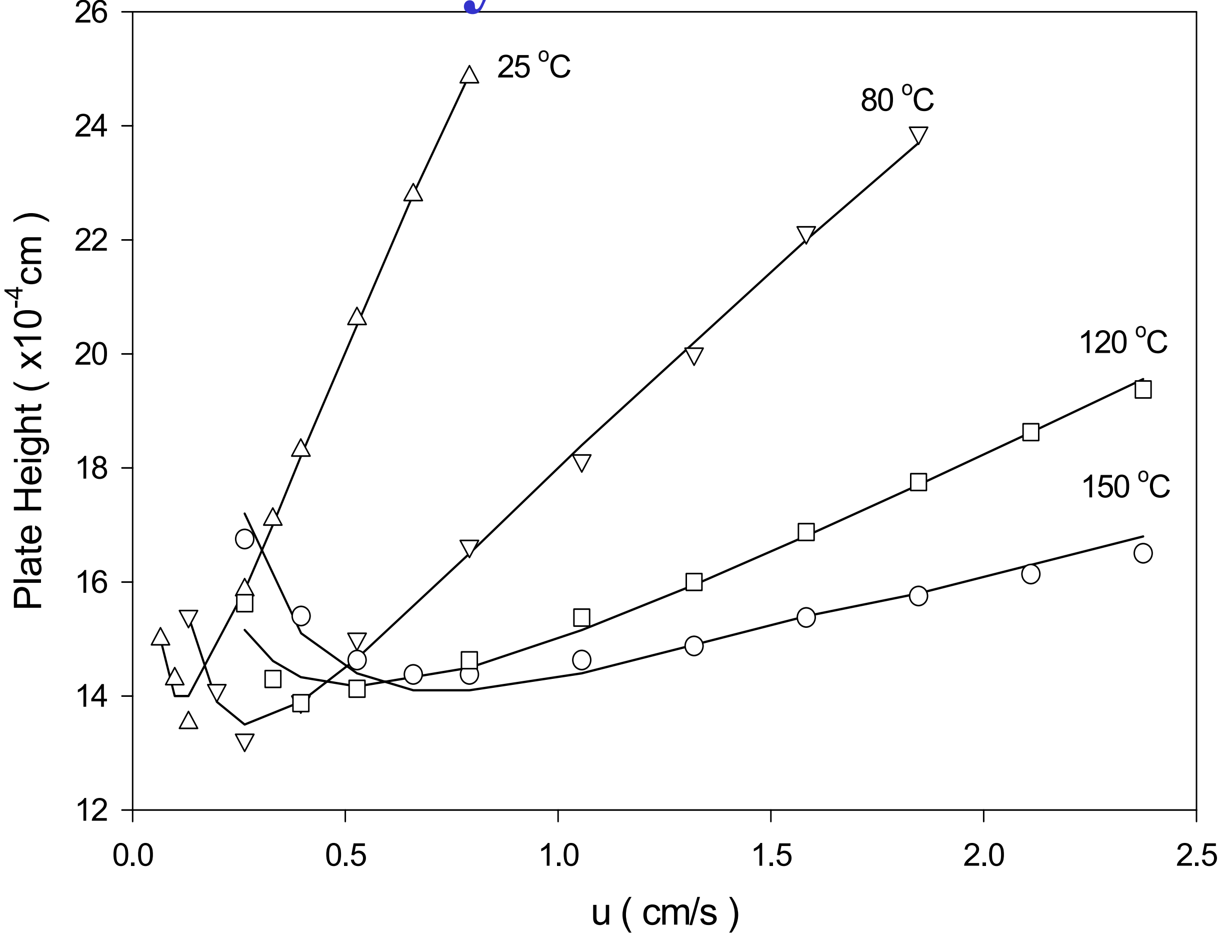

**Conditions**: The particle diameter is 3 μm and the reduced linear velocity does not change with temperature (($D_{m,25\,^{o}C} = 6*10^{-7}$ cm$^2$/s). The linear velocity (u) is increased and the reduced plate height is calculated from a modified Knox equation (A = 1.5, B = 0.8, C = 0.3, D = 0.04) at each velocity and temperature. Fast desorption kinetics are assumed ($E_a$ = 20 kJ/mol, $k_o$ = 1*10$^{13}$s).

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

# A Novel High Temperature Ultrafast Liquid Chromatography (HTUFLC) System



# Effect of Temperature on Column Efficiency in HTUFLC




**Conclusion**: **Resistance to mass transfer is greatly reduced at elevated column temperature**. Δ , 25 °C (decanophenone, k'=12.23), ∇, 80 °C (dodecanophenone, k'=7.39), □ , 120 °C (tetradecanophenone, k'=12.32).

# Effect of Temperature on Column Dynamics

| Experimental Conditions[a] | | | van Deemter Equation Coefficients | | | |
|---|---|---|---|---|---|---|
| T(°C) | Mobile Phase (% ACN (v/v)) | $D_m$ x10$^4$ (cm$^2$/s)[b] | A x 10$^3$ cm | B x 10$^4$ (cm$^2$/s) | C x 10$^3$ (s) | $u_{opt}$ (cm/s) |
| 25 | 40 | 0.08 | 1.1±0.04 | 0.18±0.03 | 1.4±0.06 | **0.1** |
| 80 | 40 | 0.15 | 0.90±0.05 | 0.6±0.09 | 0.80±0.03 | **0.3** |
| 120 | 30 | 0.25 | 0.91±0.03 | 1.2±0.08 | 0.44±0.01 | **0.6** |
| 150 | 25 | 0.36 | 1.0±0.05 | 1.3±0.08 | 0.31±0.03 | **0.7** |

[a] Solutes: alkylphenones

[b] Estmated solute diffusion coefficient in the indicated mobile phase at temperature of the calculation based on modified Wilke-Chang equation.

# References

B. Yan, J. Zhao, J.S. Brown, J. Blackwell, and P.W. Carr, "High Temperature Ultrafast Liquid Chromatography," *Anal. Chem.* **72,** 1253-62 (2000).

J.D. Thompson, J.S. Brown, and P.W. Carr, "Dependence of **Thermal Mismatch Broadening** on Column Diameter in High-Speed Liquid Chromatography at Elevated Temperatures," *Anal. Chem.***73,** 3340-7 (2001).

J.D. Thompson and P.W. Carr, "A Study of the Critical Criteria for **Analyte Stability** in High-Temperature Liquid Chromatography," *Anal. Chem.* **74,** 1017-23 (2002).

J.D. Thompson and P.W. Carr, "High-Speed Liquid Chromatography by Simultaneous Optimization of Temperature and Eluent Composition," *Anal. Chem.* **74,** 4150-9 (2002).

# Theory of High Speed HPLC

# Rearrangement to Obtain the Guiochon Equation

Fundamental Equation # 1

$$L = NH = Nhd_p$$

Fundamental Equation # 2

$$u = \frac{\nu D_m}{d_p}$$

Fundamental Equation # 3

$$t_R = \frac{L}{u}(1 + k')$$

Guiochon Equation

$$\frac{t_R}{N} = \frac{(1 + k')}{D_m} \frac{h}{\nu} d_p^2$$

Knox Equation

$$h = A\nu^{1/3} + \frac{B}{\nu} + C\nu$$

G. Guiochon, Anal. Chem., **1980,** 52, 2002-2008

# Limit 1: the "C term"

**Knox, Saleem, Guiochon Equation**

$$\frac{t_R}{N} = \frac{(1+k')}{D_m}\frac{h}{\nu}d_p^2$$

**Theoretical Limit**

**h→Cν as ν→∞**

$$h \cong C\nu$$

**Theoretical Limit for $t_R$/N**

$$\left.\frac{t_R}{N}\right|_{\nu\to\infty} \cong \frac{C(1+k')}{D_m}d_p^2$$

**Stokes-Einstein**

$$D_m = \frac{RT}{6n\pi r\eta}$$

**Result**

$$\left.\frac{t_R}{N}\right|_{\nu\to\infty} \propto \frac{\eta C(1+k')}{T}d_p^2$$

G. Guiochon, Anal. Chem., **1980,** 52, 2002-2008

# Limit 2: the "A term"

**Practical Limit for h**

$$h \cong A\nu^{1/3}$$

**$t_R$/N f(A)**

$$\frac{t_R}{N} = \frac{A d_p^{4/3}(1+k')}{D_m^{1/3} u^{2/3}}$$

**Kozeny-Carman Permeability and Darcy's Law**

$$\Delta P = \frac{\Phi}{d_p^2} u L \eta$$

**Maximum Linear Velocity**

$$u_{max} = \frac{d_p^2 \Delta P_{max}}{L \phi \eta}$$

**Practical Limit Temperature Dependence ($A\nu^{1/3} > C\nu$)**

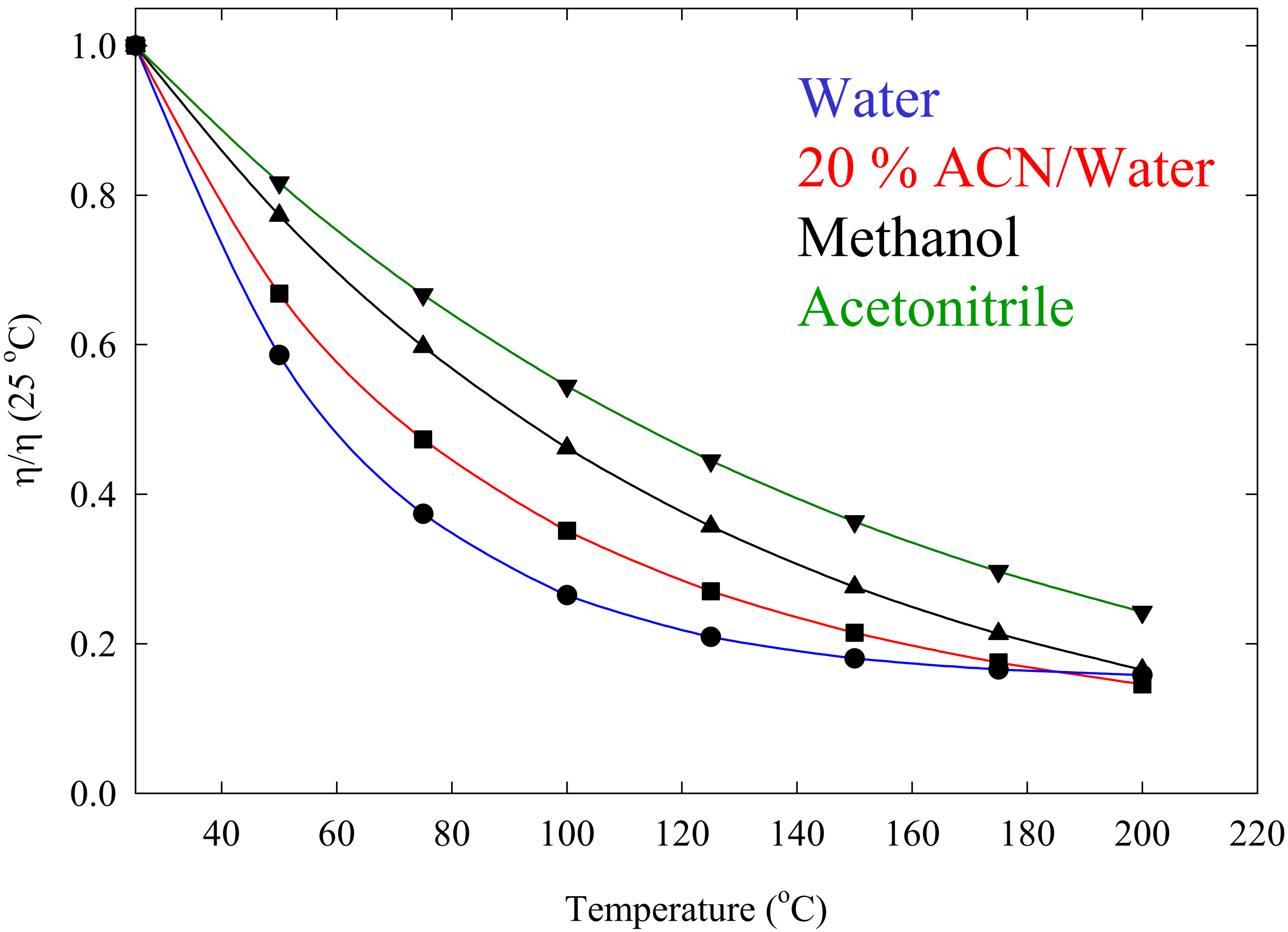
$$\left.\frac{t_R}{N}\right|_{\frac{h}{\nu} \to A\nu^{-2/3}} \propto (1+k') \frac{L^{2/3}}{\Delta P_{max}^{2/3}} \frac{\eta}{T^{1/3}}$$

# Dependence of t/N on Velocity in the Limit of Exponent of $\nu^x$

## Critical Exponents

| $\nu^x$ | $d_p^x$ | $L^x$ | $\Delta P^x$ | $\eta^x$ | $T^x$ |
|---|---|---|---|---|---|
| **0** | -1 | 1 | -1 | **1** | **0** |
| **½** | ½ | ½ | -0.5 | **1** | **-0.5** |
| **1/3** | 0 | 2/3 | - 2/3 | **1** | **- 1/3** |
| **1** | 2 | 0 | 0 | **1** | **-1** |

# Relative Viscosity vs. Temperature



# Limit 3: the Resolution

**Rearrangement of Darcy's Law**

$$\nu = \frac{d_p^2 \Delta P}{h N \Phi \eta D_m}$$

**Knox-Saleem Equation**

$$\frac{t_R}{N} = \frac{(1+k')}{D_m} \frac{h}{\nu} d_p^2$$

**Retention Time**

$$t_R = \frac{(1+k')N^2 h^2 \eta \Phi}{\Delta P}$$

**General Resolution Equation**

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

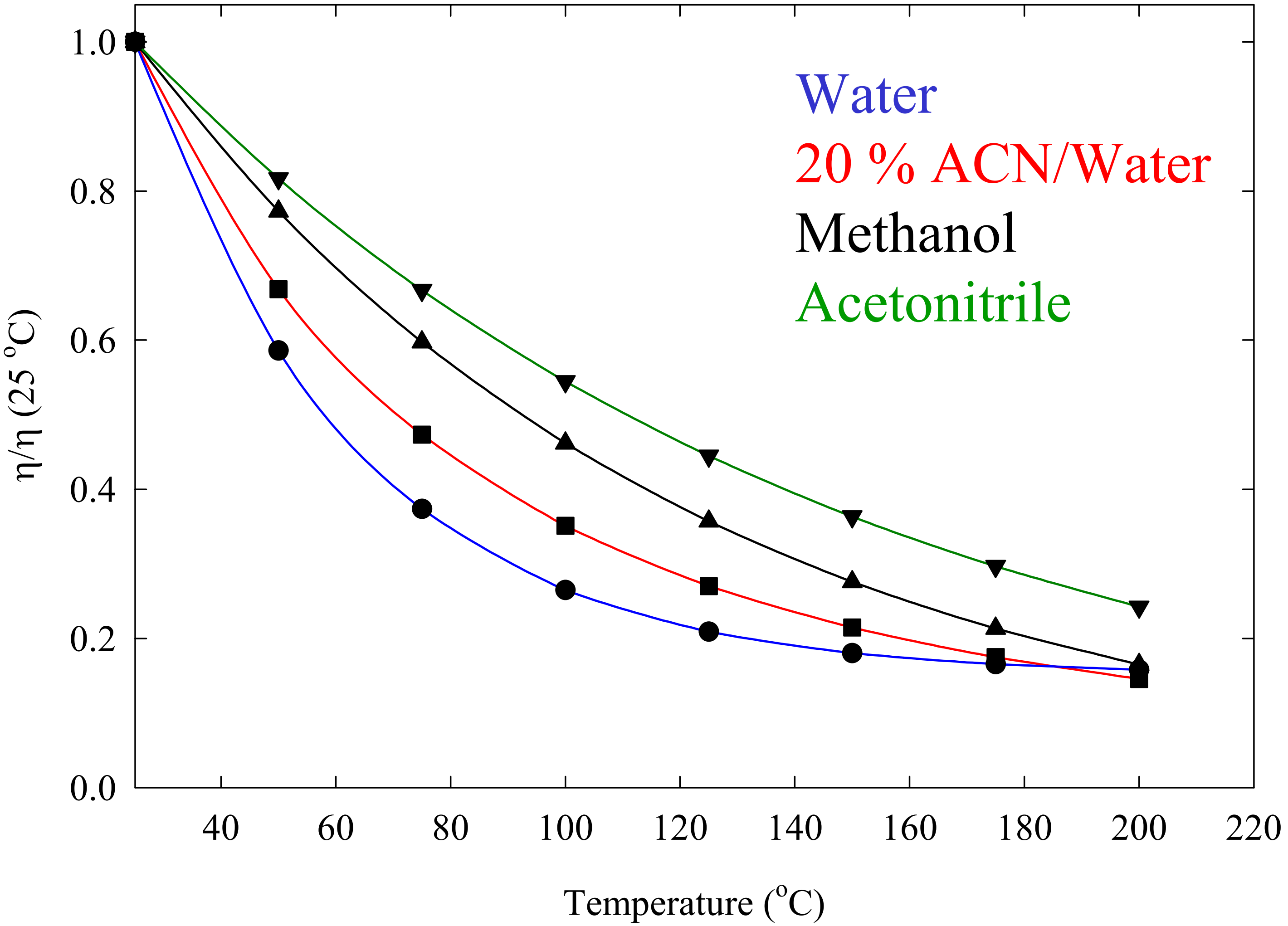
**Result**

$$t_R = \frac{256 R^4 h^2 \eta \Phi}{\Delta P} \left( \frac{\alpha}{\alpha - 1} \right)^4 \frac{(1+k')^6}{k'^4}$$

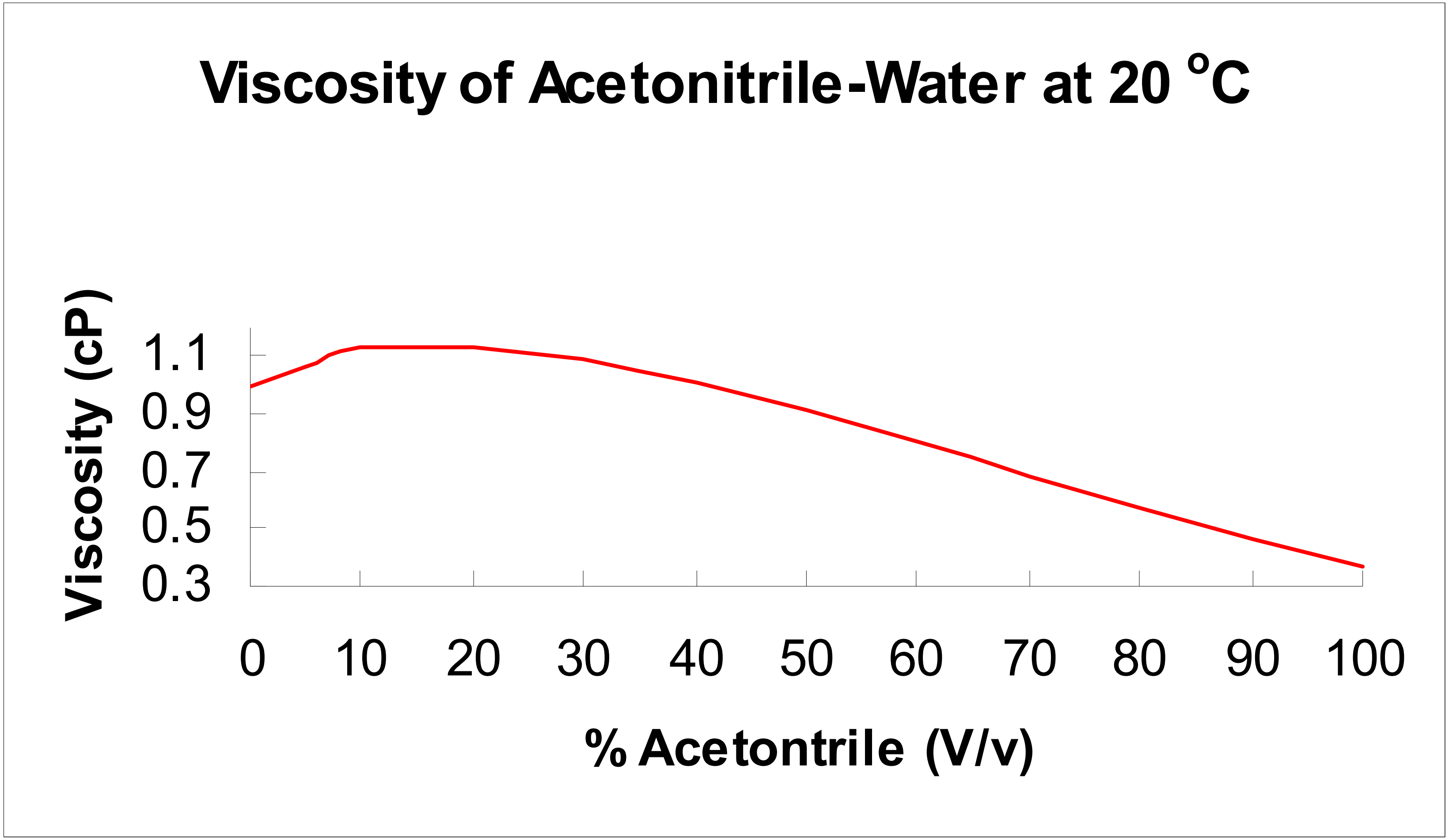
# Dependence of t/N on Optimization Parameters

| | $d_p^x$ | $L^x$ | $\Delta P^x$ | $\eta^x$ |
|---|---|---|---|---|
| C Limit | 2 | 0 | 0 | 1 |
| A limit ($\nu^{1/3}$) | 0 | 2/3 | - 2/3 | 1 |
| Resolution Limit | 0 | 0 | -1 | 1 |

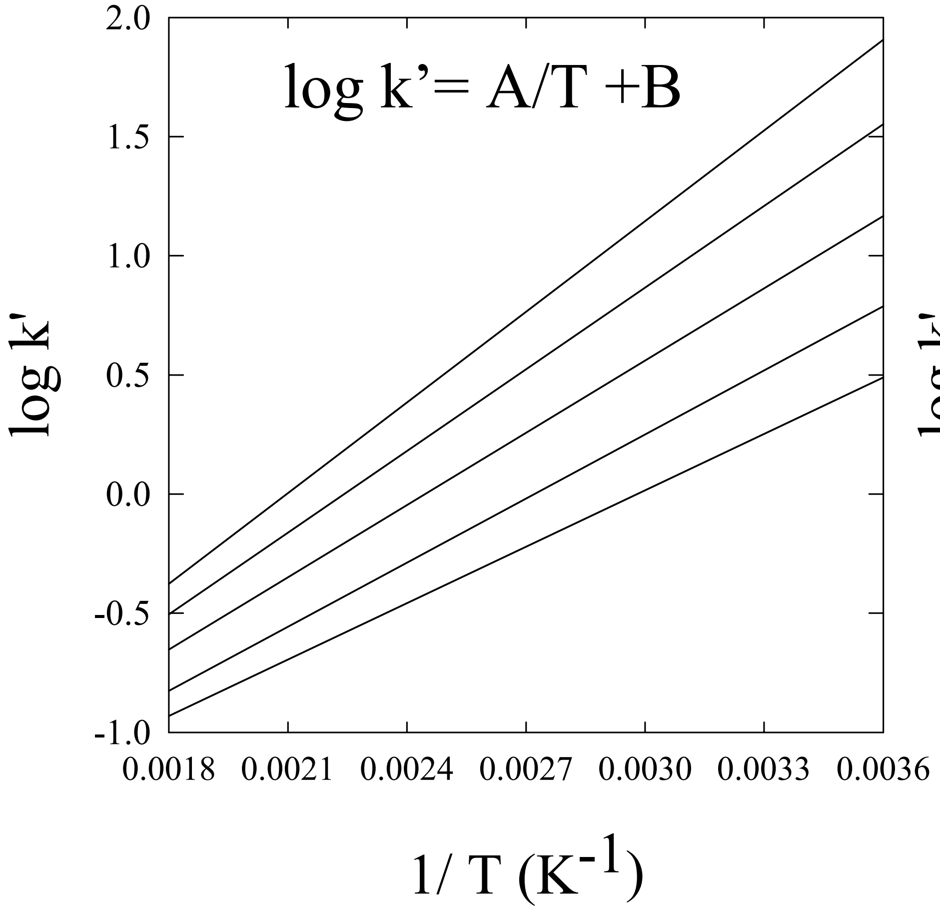
# Relative Viscosity vs. Temperature

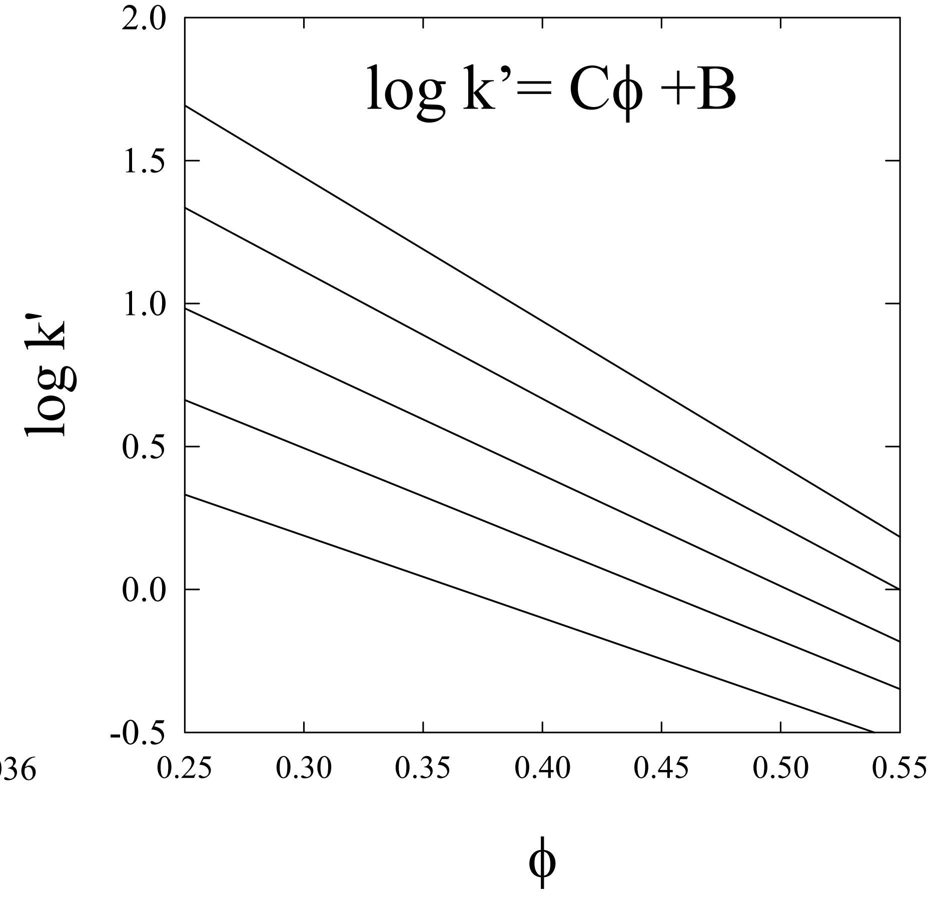


# Effect of Composition on Viscosity



# Effect of ϕ, T on k'



# Is High T the Optimal Conditions for High Speed in LC?

***k'* too high**

| | ϕ (low) | ϕ (high) |
|---|---|---|
| T (low) | low,low | low, high |
| T (high) | high, low | high, high |

Regions of same k' and low viscosity.
Where should we work?

***k'* too low**

# Effect of $\phi$ and T at Const. *k'* on $\eta$*

| k' ($\phi$,T) | % ACN (v/v) | T (ºC) | $\eta$(cP) ($\phi$,T) | $\eta$ (T)/$\eta$ (25 ºC) |
|---|---|---|---|---|
| **5** | 69 | 25 | 0.55 | 1 |
| **5** | 59 | 100 | 0.29 | 0.53 |
| **5** | 52 | 125 | 0.20 | 0.36 |
| **5** | 45 | 200 | 0.14 | 0.25 |

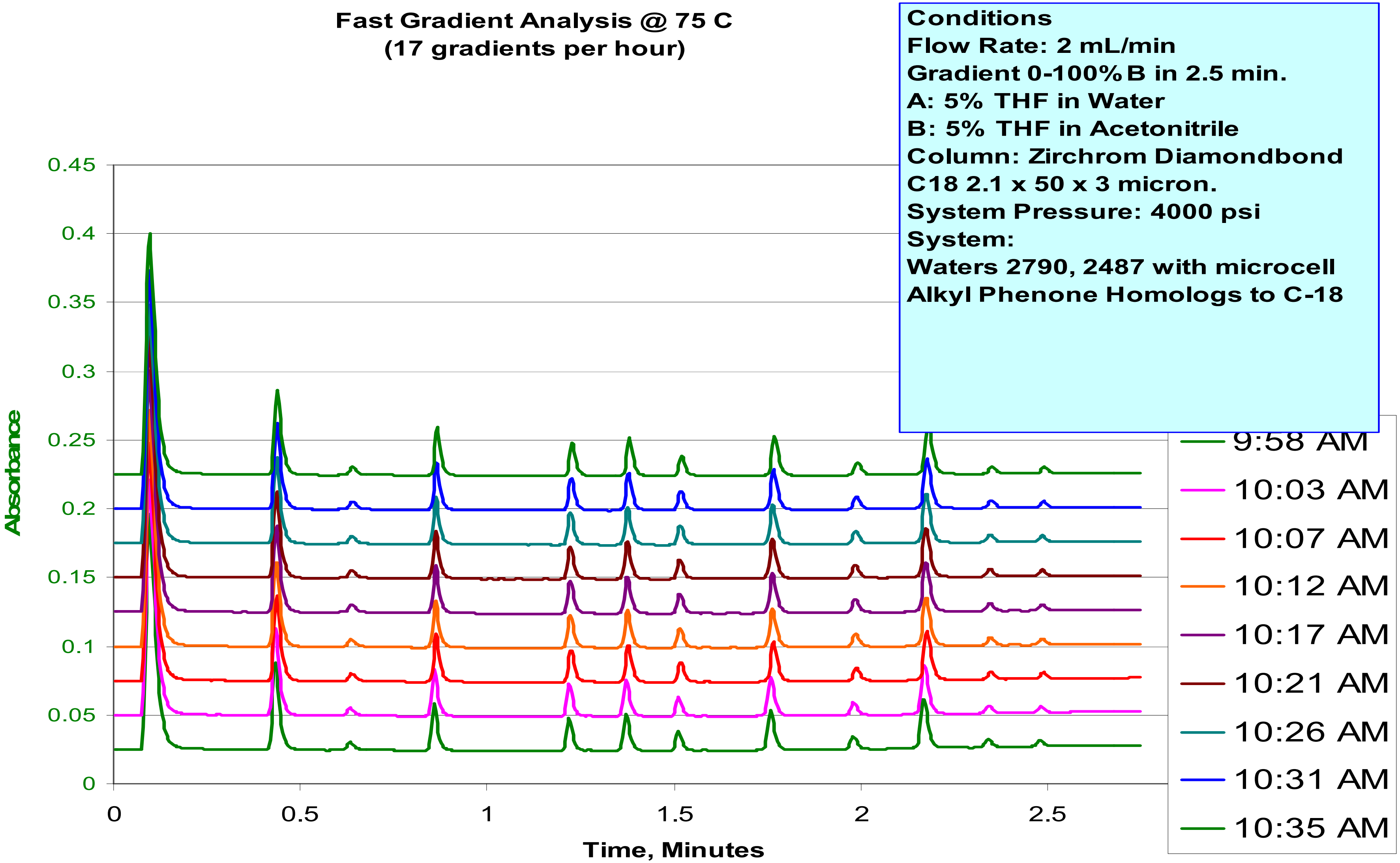
Conditions: k' ($\phi$,T) is based on the retention of butyl benzene at different temperatures (T) at different fraction organic in the eluent ($\phi$) on a C18 Zorbax column.

# Conclusions

1. To do **fast** LC, use a **WEAK** solvent and a **HOT** column.

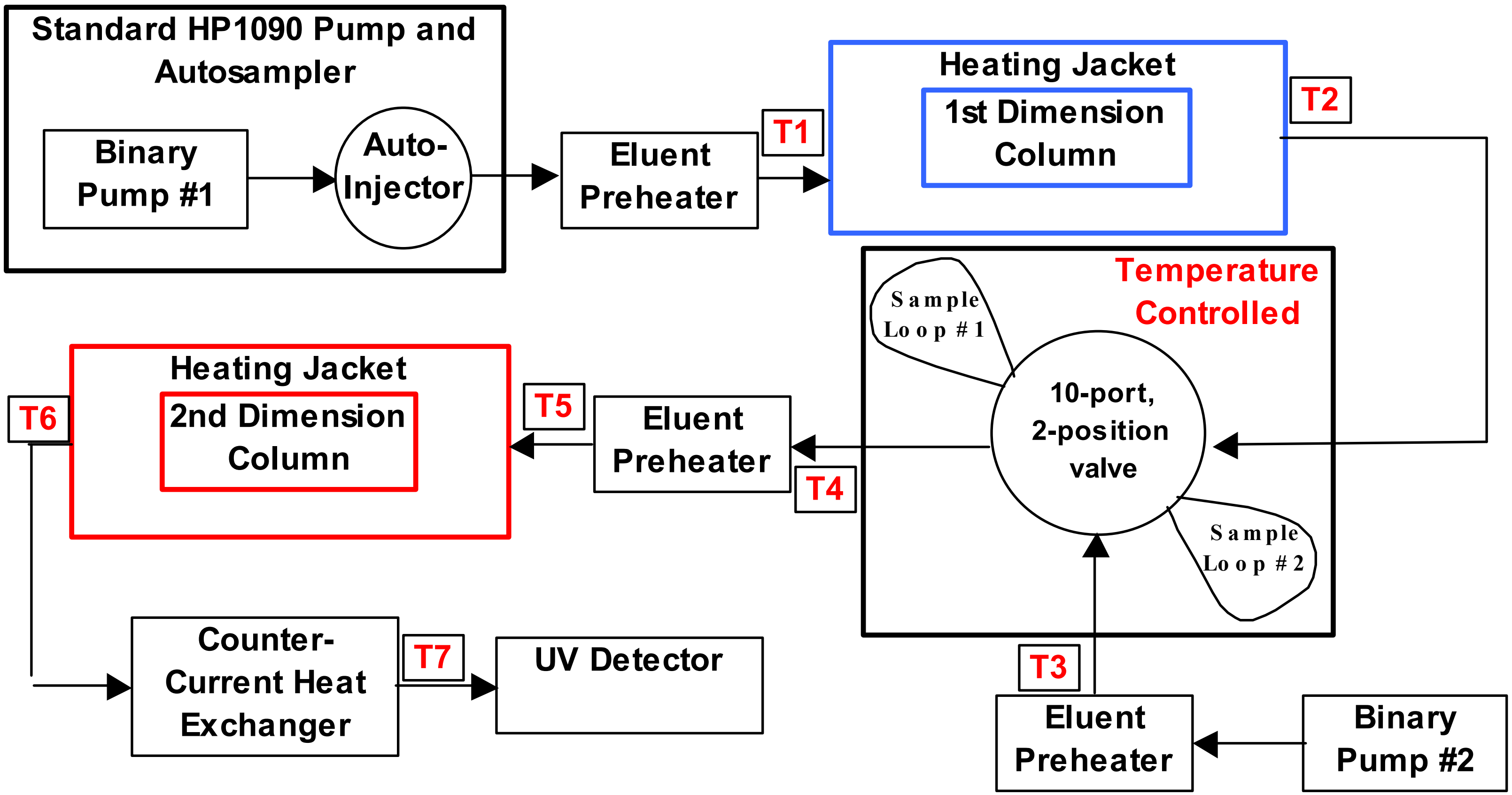
2. Use a highly retentive column!

# High Throughput Gradient Elution

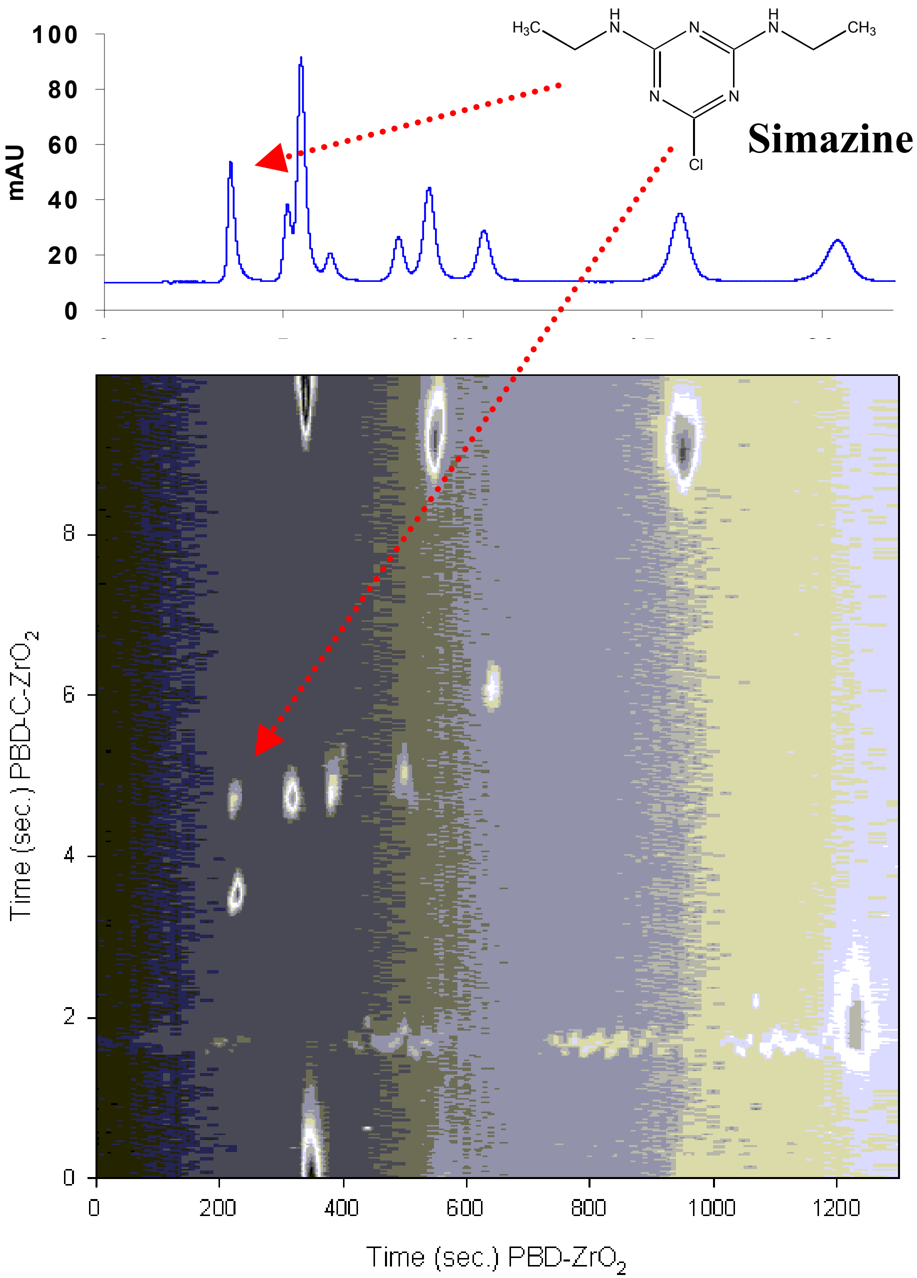



**17 Gradients/Hour**. **Peak capacity is 70!** This speed cannot be done at ambient within the gradient space! Carl Sims—Systec.

# Schematic of a Complete LC × UFHTLC System



# LC × UFHTLC Separation of Ten Triazine Herbicides



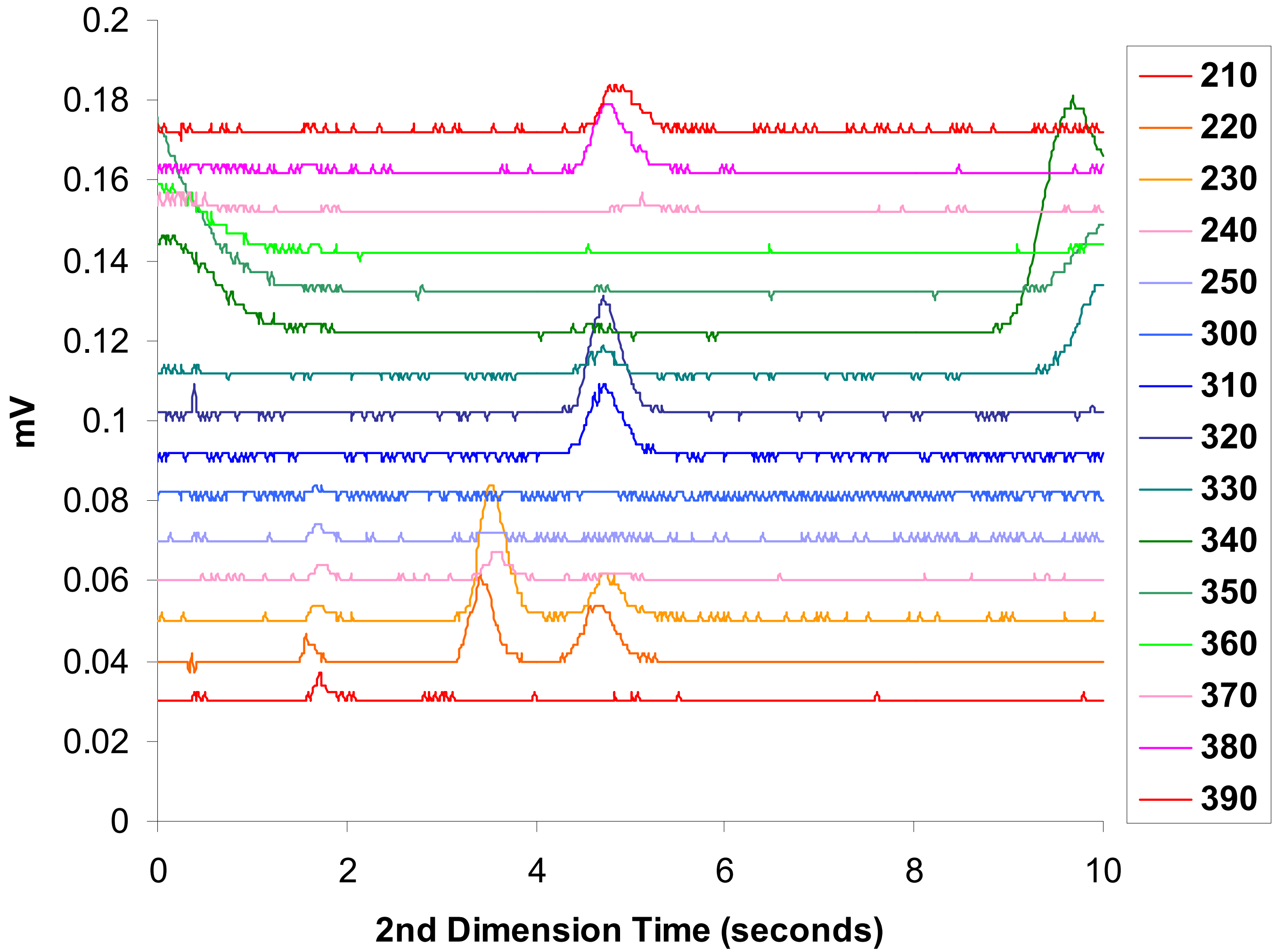

**1st Dimension Conditions**: Column, 50 mm x 2.1 mm i.d. PBD-$ZrO_2$; Mobile phase, 20/80 ACN/Water; Flow rate, 0.08 ml/min.; Injection volume, 20 µl; Temperature, 40 $^{o}$C

**2nd Dimension Conditions**: Column, 50 mm x 2.1 mm i.d. PBD-C-$ZrO_2$; Mobile phase, 20/80 ACN/Water; Flow rate, 7.0 ml/min.; Injection volume, 15 µl; Temperature, 150 $^{o}$C; 1st dimension sampling frequency, 0.1 Hz

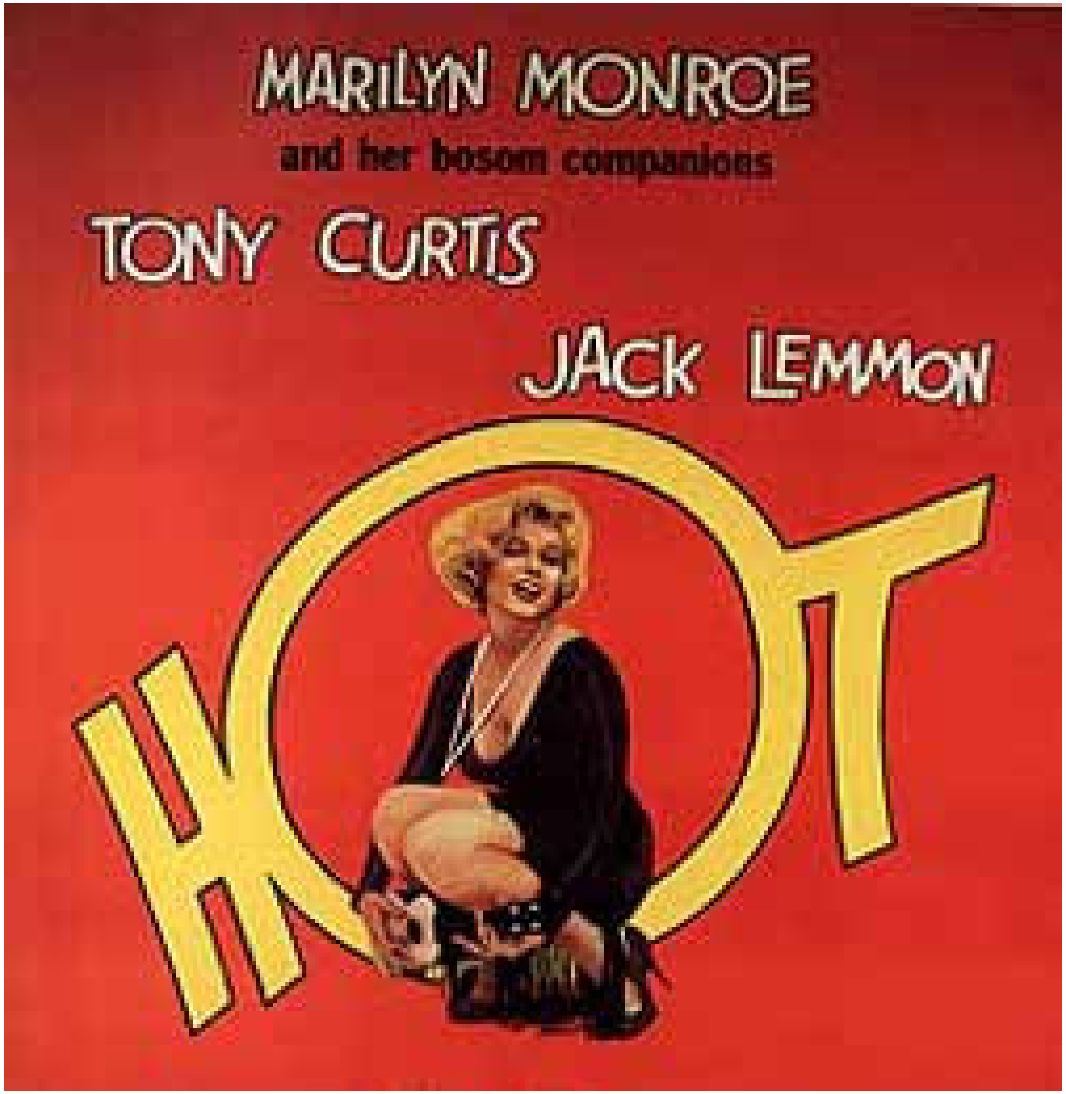
Total LC × UFHTLC peak capacity = **185**

Using a single column, it would take a **2.5 meter** long column and **44 hours** to generate the same peak capacity

# Second Dimension Chromatograms



# High Temperature HPLC?




# Some Like It Hot!

# Thanks!

- Ben Yan,  Dwight Stoll.
- NIH.
- Systec, Inc.
- ZirChrom Separations, Inc.