High Temperature Ultra Fast Liquid Chromatography

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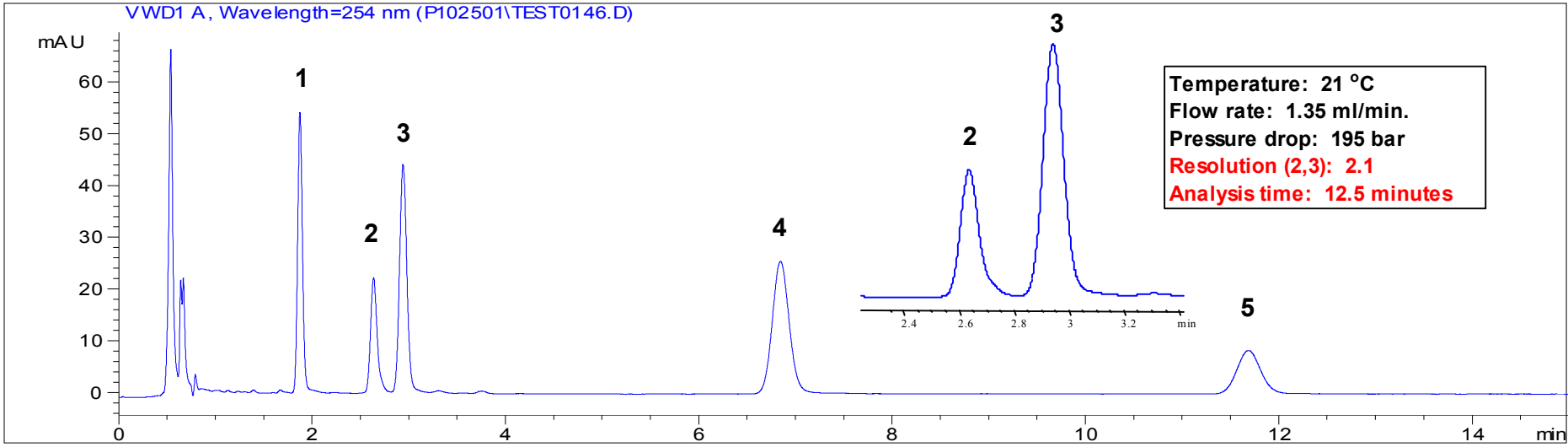
Dwight Stoll, and Adam Schallinger

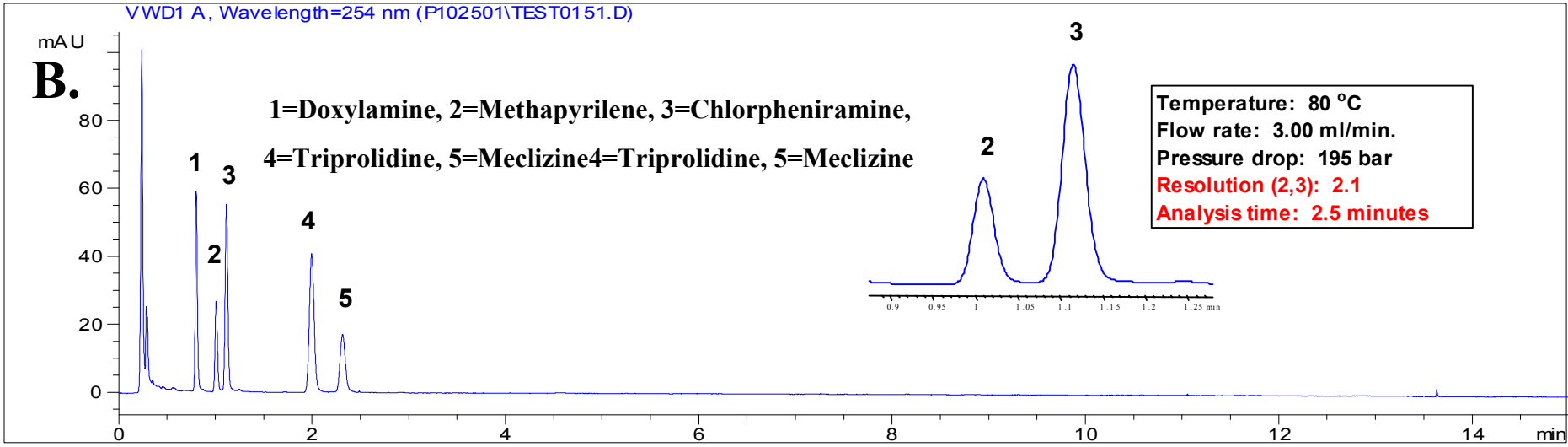
Conclusions

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

2. Use a highly retentive column so that you can work at lowest possible viscosity!

A. Fast Separation of Antihistamines at 80 °C

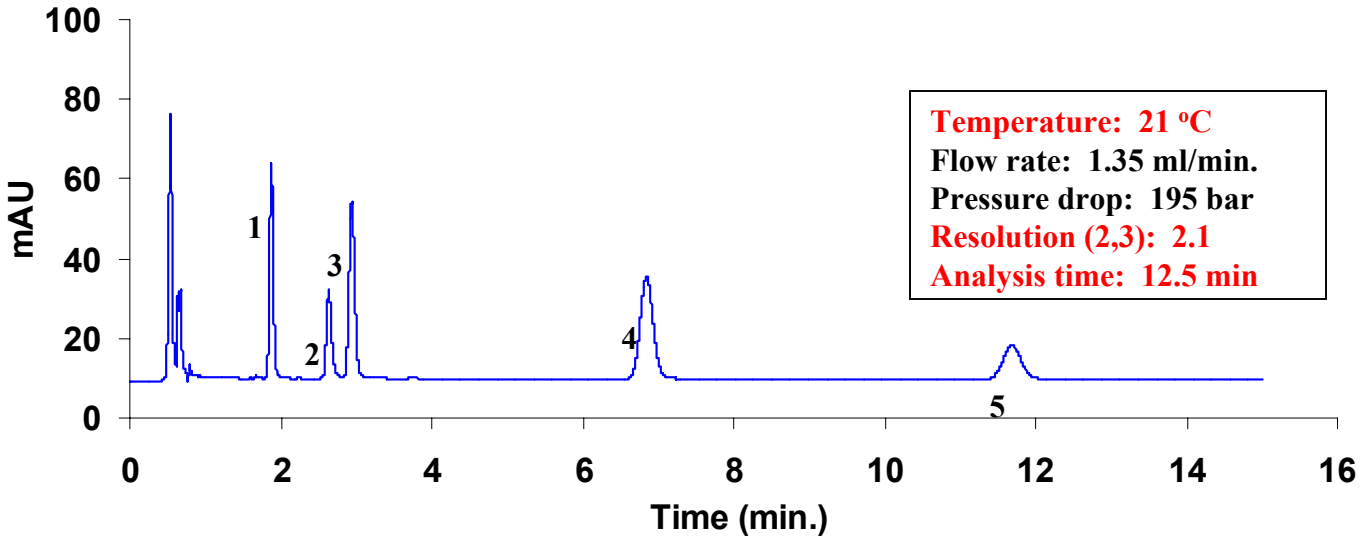


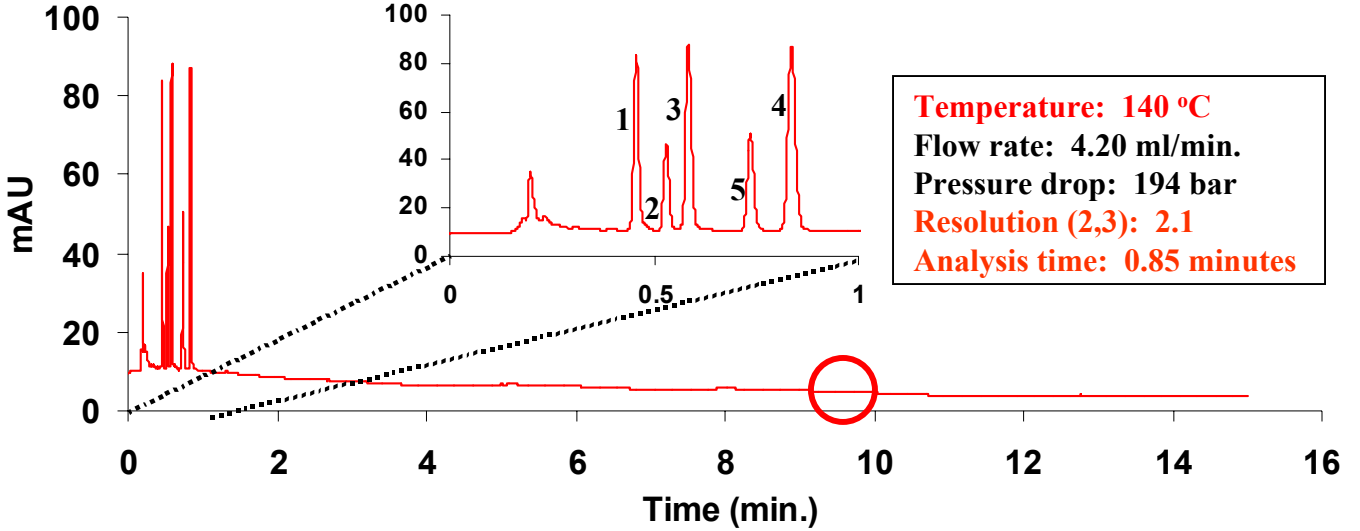


LC Conditions: (A) Mobile Phase, 29/71 ACN/50mM Tetramethylammonium hydroxide; Injection volume, 0.5 ul;

254 nm detection; 100 x 4.6 ZirChrom-PBD (B) same as A, except Mobile Phase, 26.5/73.5 ACN/50mM Tetramethylammonium hydroxide, pH 12.2

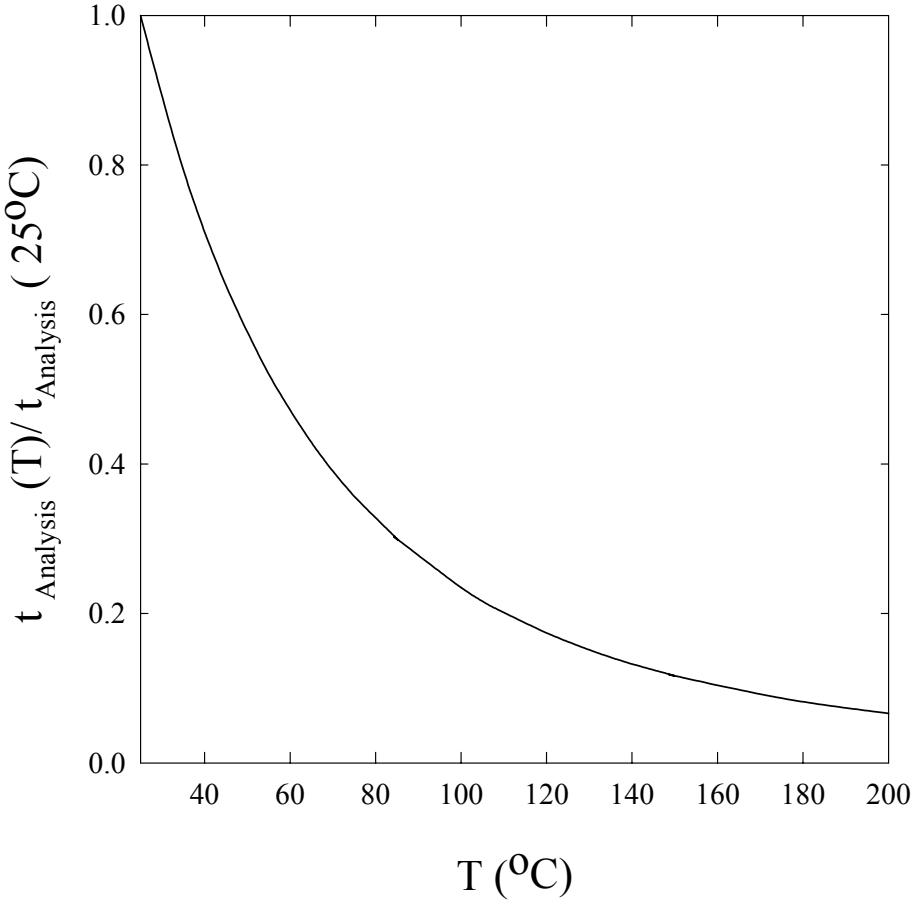
Faster Separation at 140 °C





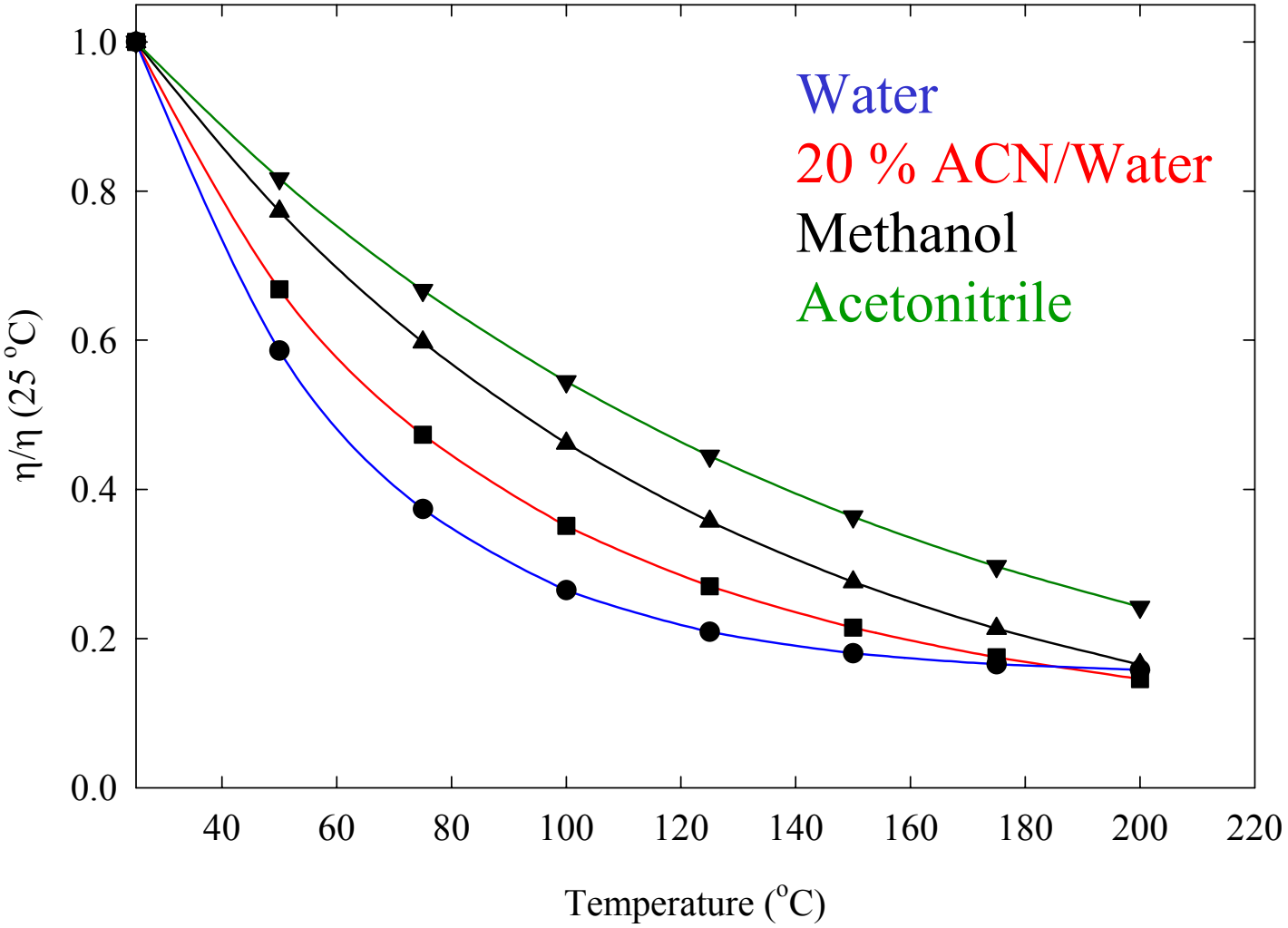
Mobile Phase, 29/71 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Injection volume, 0.5 µl; 254 nm detection; Solutes: 1=Doxylamine, 2=Methapyrilene, 3=Chlorpheniramine, 4=Triprolidine, 5=Meclizine; Column, 100 mm x 4.6 mm i.d. ZirChrom®-PBD

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

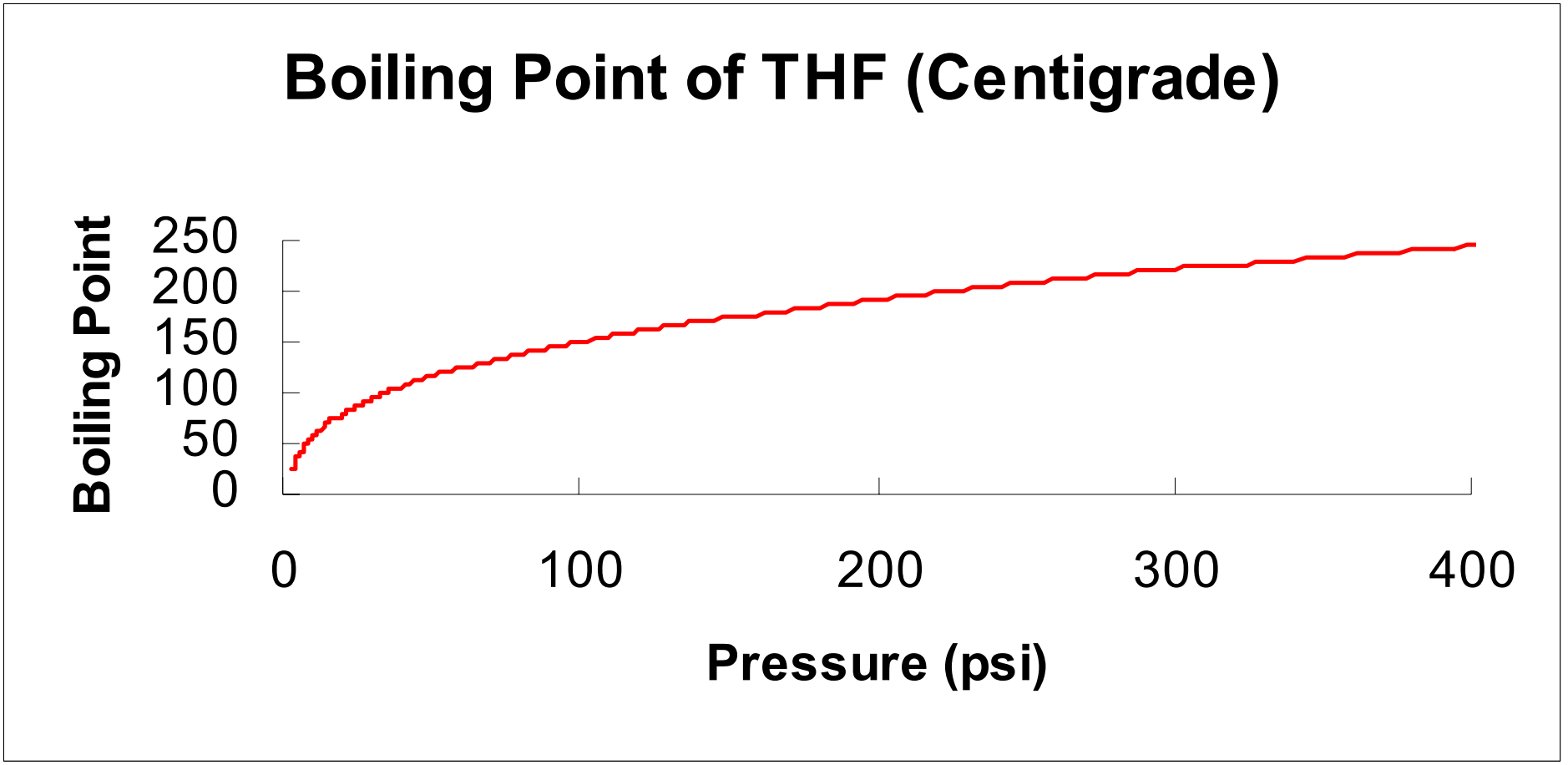


"High-Performance Liquid Chromatography at Elevated Temperatures: Examination of Condition for the Rapid Separation of Large Molecules," R. D. Antia and Cs. Horvath, *J. Chromatogr*., 435, 1-15 (1988).

Relative Viscosity vs. Temperature

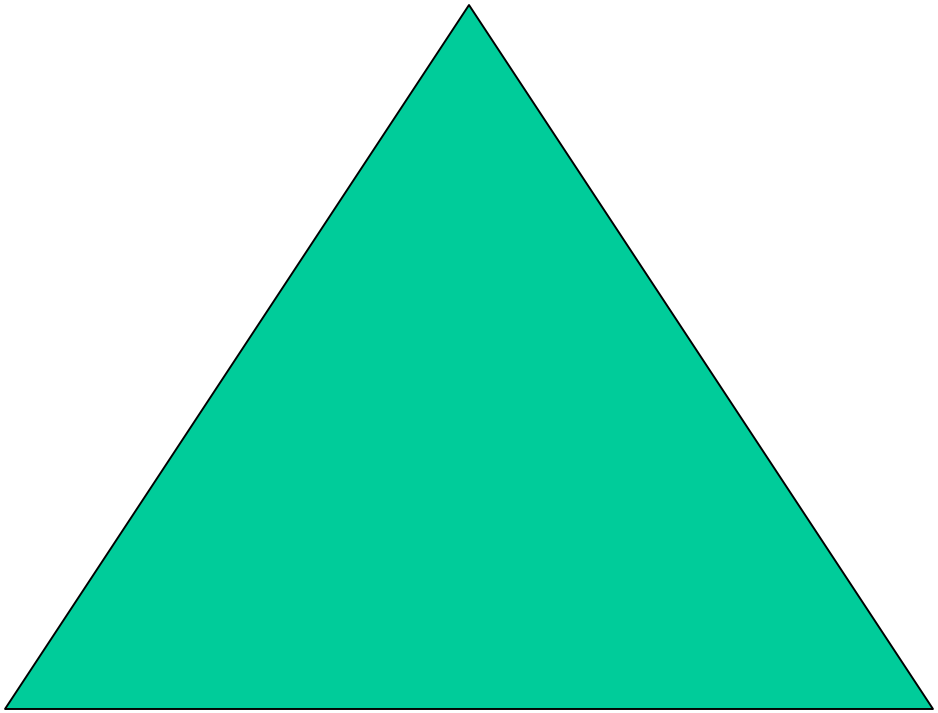


How to Prevent Boiling



Requirements for High Temperature LC

Stationary Phase Stability

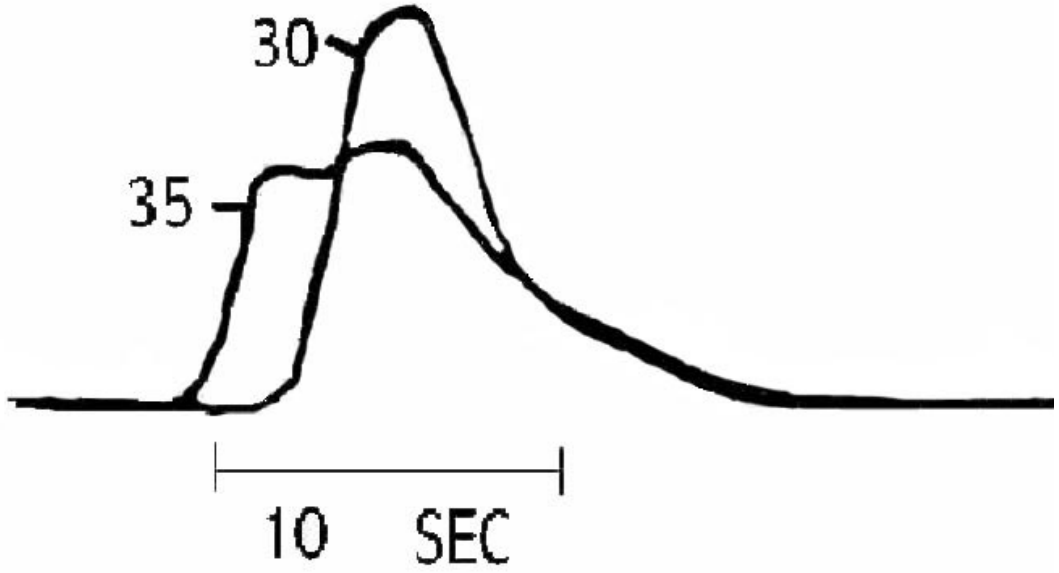


Thermal Mismatch Broadening

On-Column Analyte Instability

Peak Shapes Observed for Various Mobile-Phase Feed Temperatures*

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

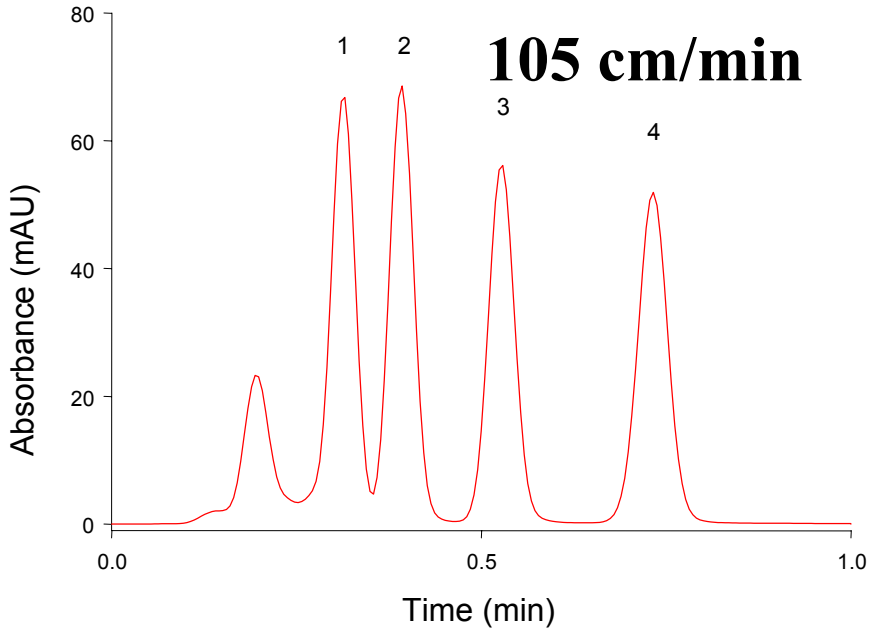


LC conditions: Column at 30 °C; 6.2 mm IDx8cm;
3μ Zorbax ODS; at 5 mL/min; 50/50 (v/v) ACN,H_2O;
nitrobenzene

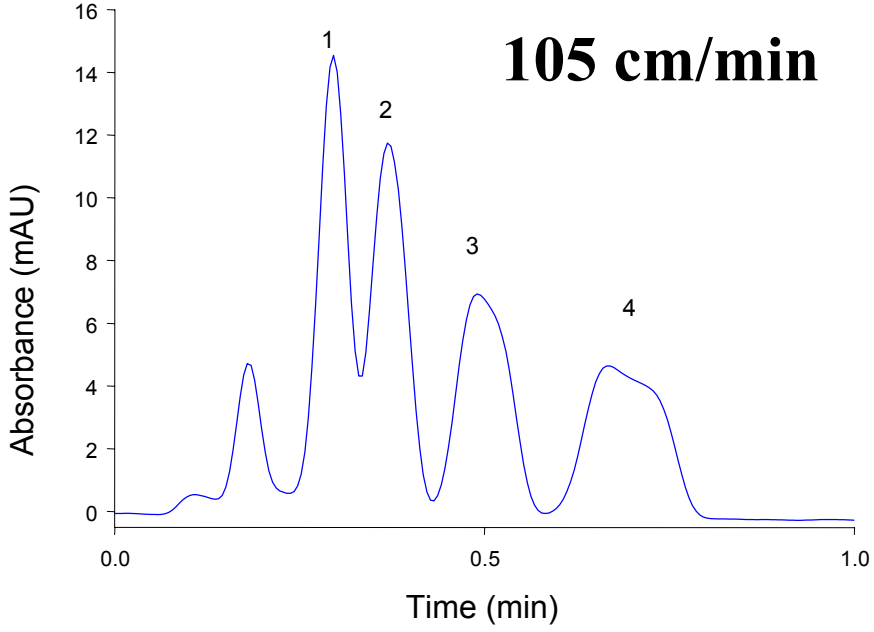
*H. Poppe and J.C. Kraak, *J. Chromatogr.*, **282**, 399-412 (1983).

Comparison of the Effect of Incomplete Thermal Equilibration on Column Performance

2.1 mm ID



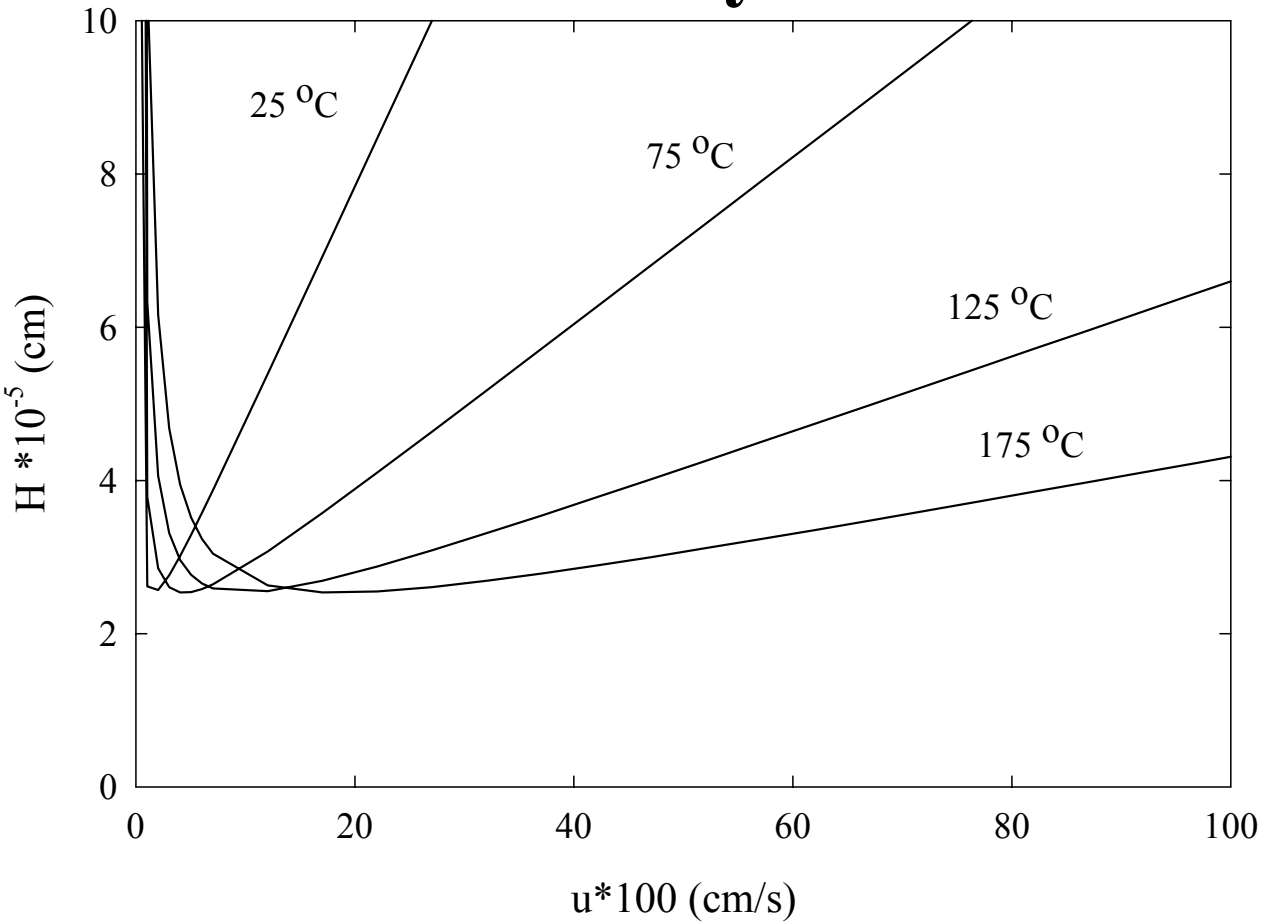
4.6 mm ID



LC conditions: 2.1 x 5 cm, C-18 INERT, 55 % ACN, 5 cm preheater, 60 °C
4.6 x 5 cm, C-18 INERT, 60% ACN, 5 cm preheater, 60 °C.

Peaks: 1. toluene, 2. ethylbenzene, 3. propylbenzene,4. butylbenzene

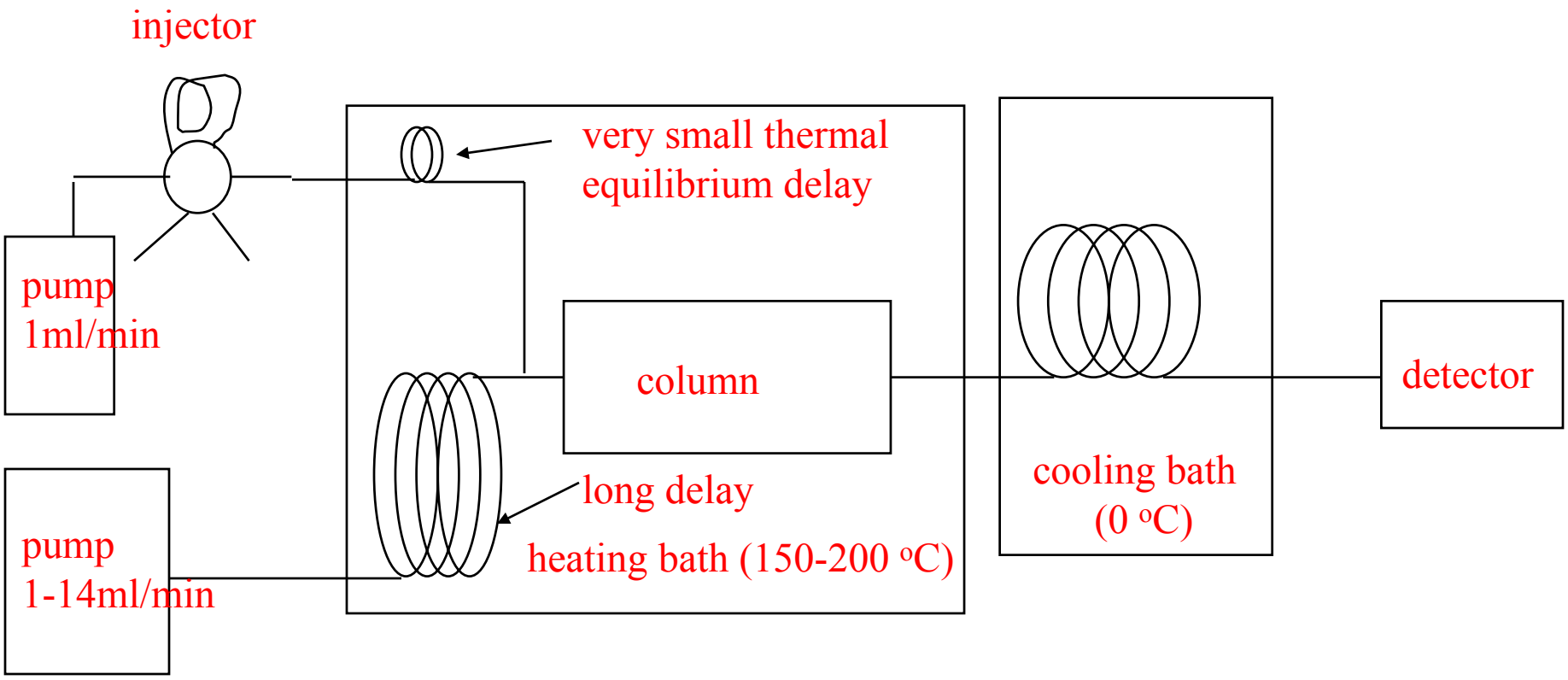
Theoretical Effect of Temperature on Column Dynamics



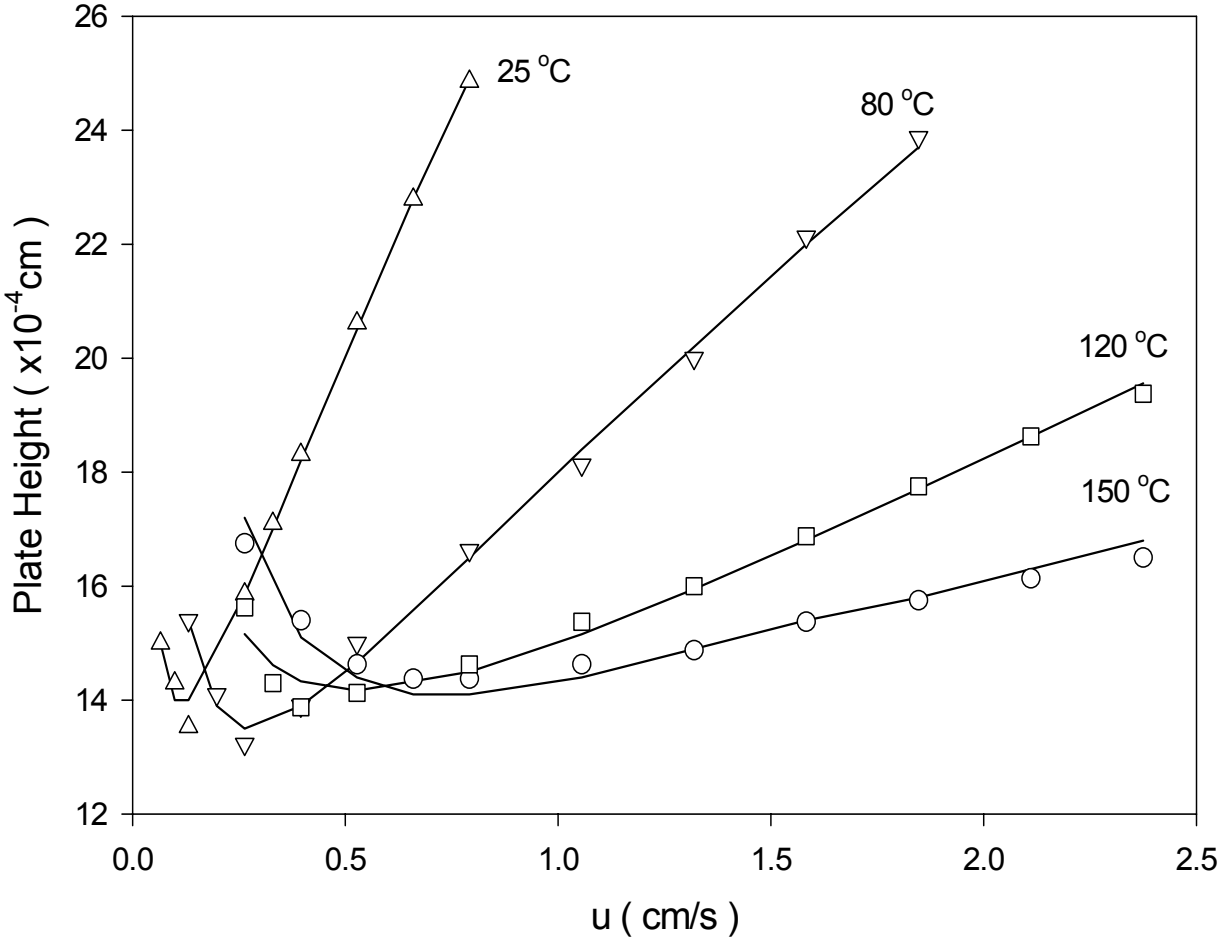
Conditions: The particle diameter is 3 μm and the reduced linear velocity does not change with temperature ($(D_{m,25\ ^oC} = 6*10^{-7}\ cm^2/s$). The linear velocity (u) is increased and the reduced plate height is calculated from a modified Knox equation (A = 1.5, B = 0.8, C = 0.3, D = 0.04) at each 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 Totally Impractical 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).

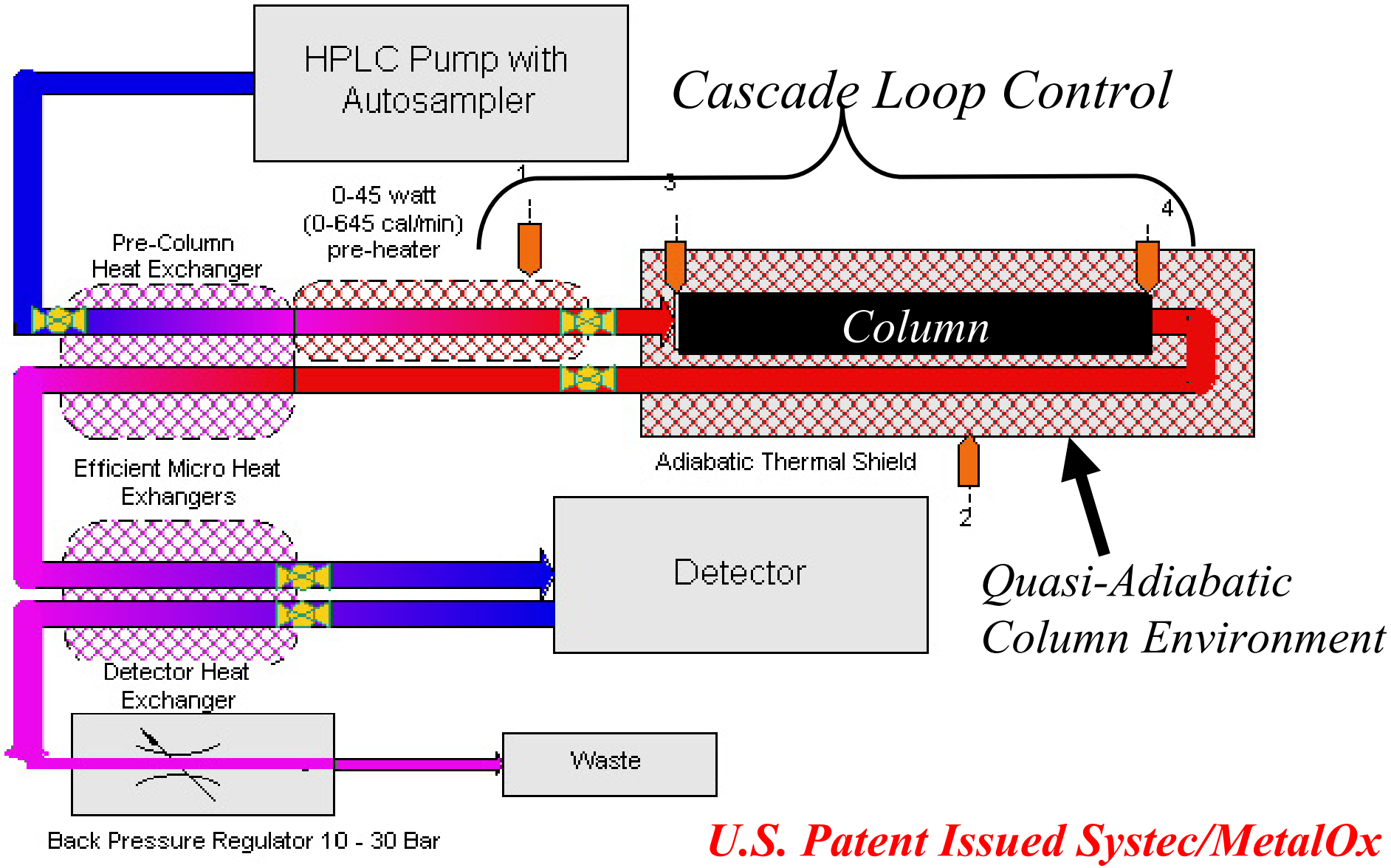
Effect of Temperature on Column Dynamics

Experimental Conditions[a]			van Deemter Equation Coefficients			
T(^{o}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.

A Totally Practical Heating System



U.S. Patent Issued Systec/MetalOx

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: "C term"

Knox, Saleem, Guiochon Equation

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

Theoretical Limit
$h \rightarrow C\nu$ as $\nu \rightarrow \infty$

$$h \cong C\nu$$

Theoretical Limit for t_R/N

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

Stokes-Einstein

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

Result

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

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

Limit 2: "A term"

Practical Limit for h

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

t_R/N f(A)

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

Kozeny-Carman Permeability and Darcy's Law

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

Maximum Linear Velocity

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

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

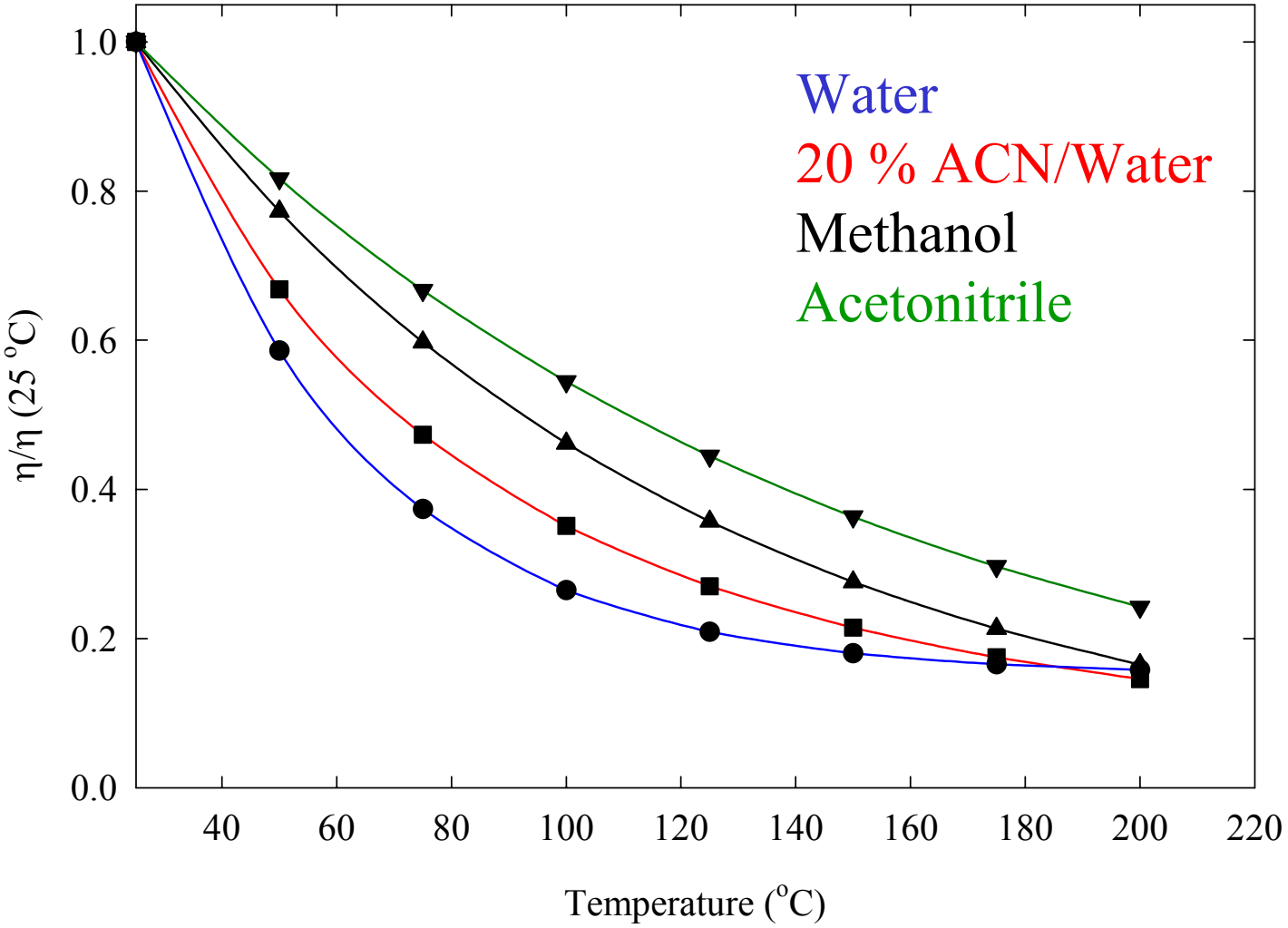
$$\left.\frac{t_R}{N}\right|_{\frac{h}{\nu} \to A\nu^{-2/3}} \propto (1+k')\frac{L^{2/3}}{\Delta P_{\max}^{2/3}}\frac{\eta}{T^{1/3}}$$

Dependence of t/N on Velocity in the Limit of Exponent of ν^x

Critical Exponents

ν^x	d_p^x	L^x	ΔP^x	η^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: Resolution

Rearrangement of Darcy's Law

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

Knox-Saleem Equation

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

Retention Time

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

General Resolution Equation

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

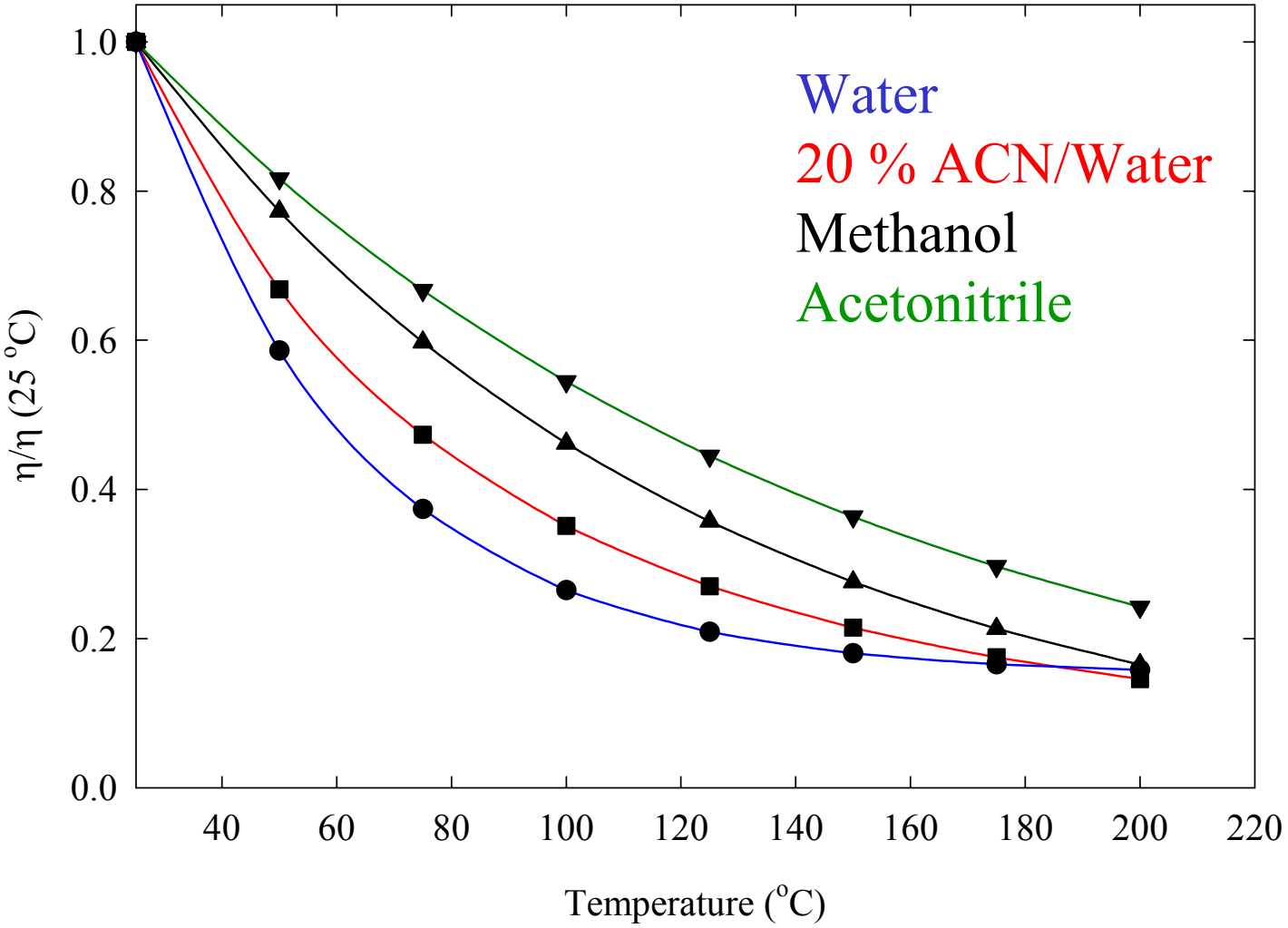
Result

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

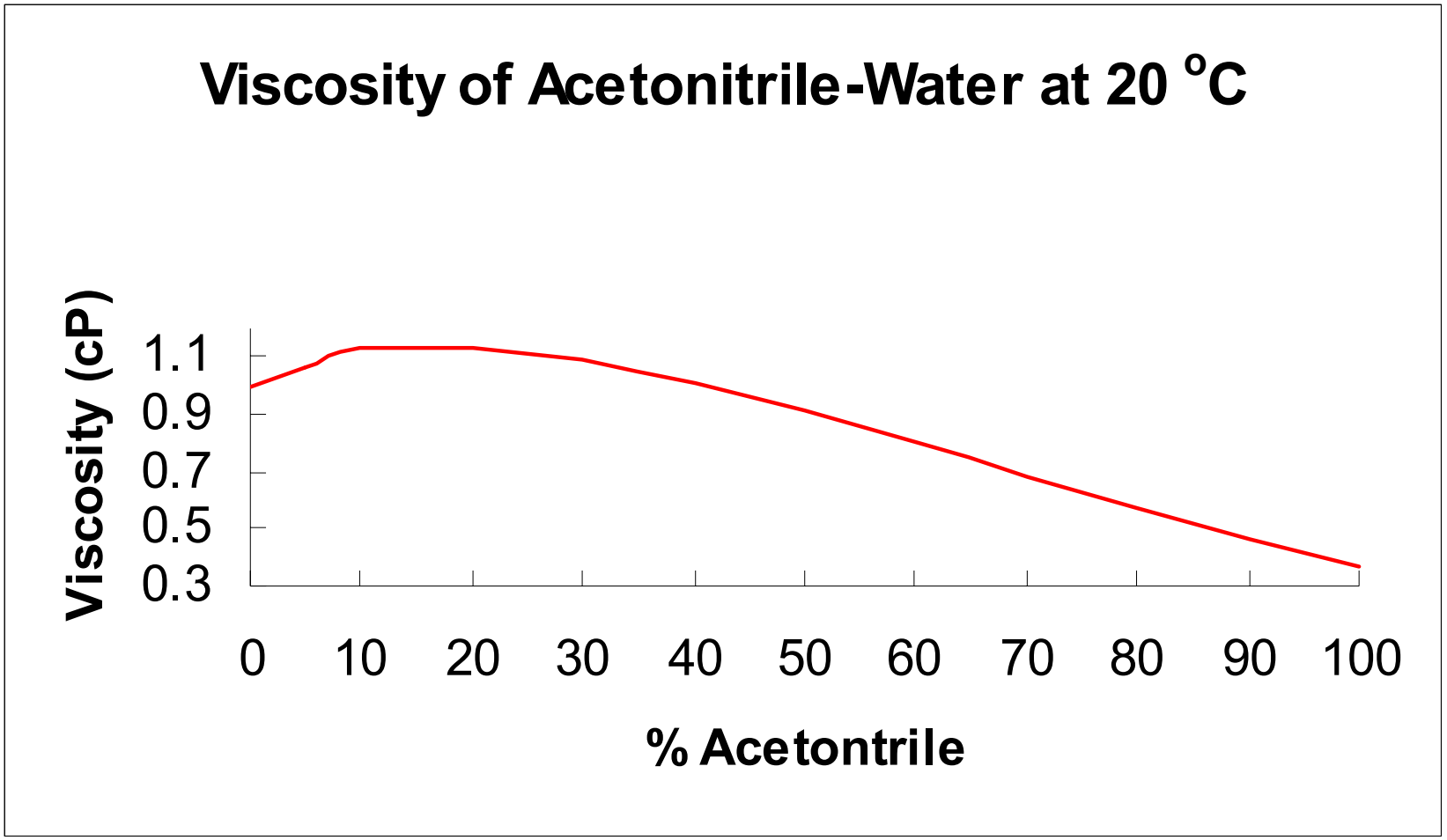
Dependence of t/N on Optimization Parameters

	d_p^x	L^x	ΔP^x	η^x
C Limit	2	0	0	1
A limit ($\nu^{1/3}$)	0	2/3	- 2/3	1
Resolution Limit	0	0	-1	1

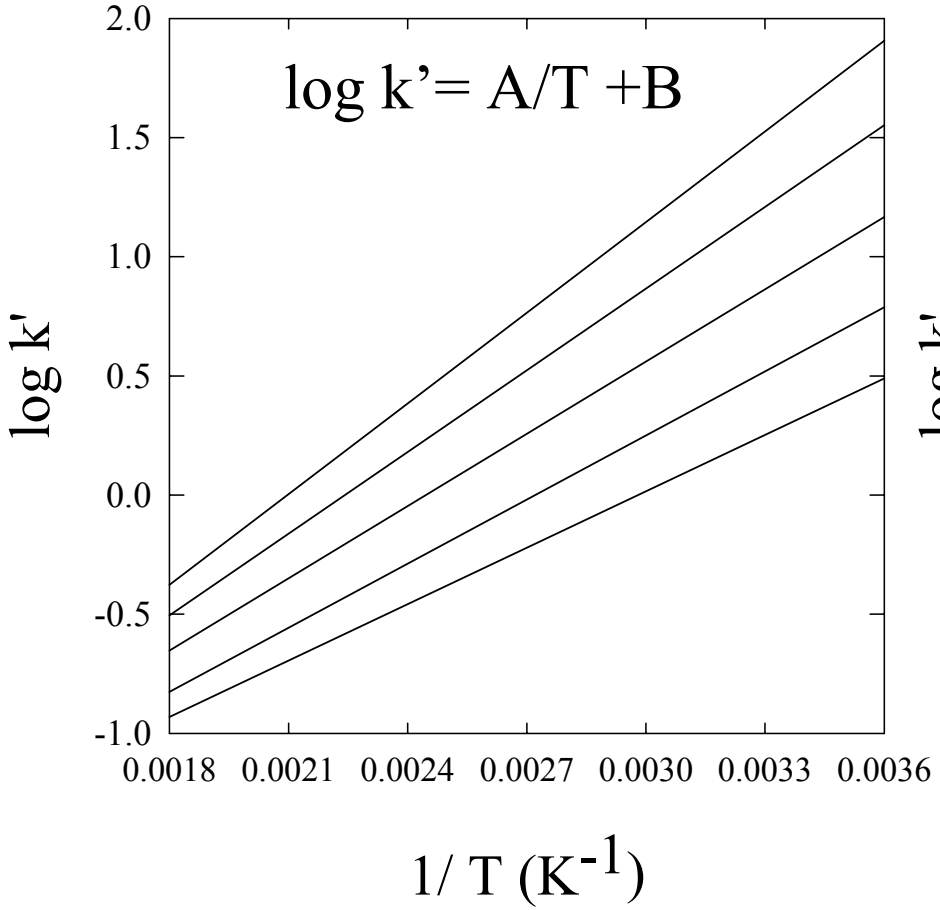
Relative Viscosity vs. Temperature

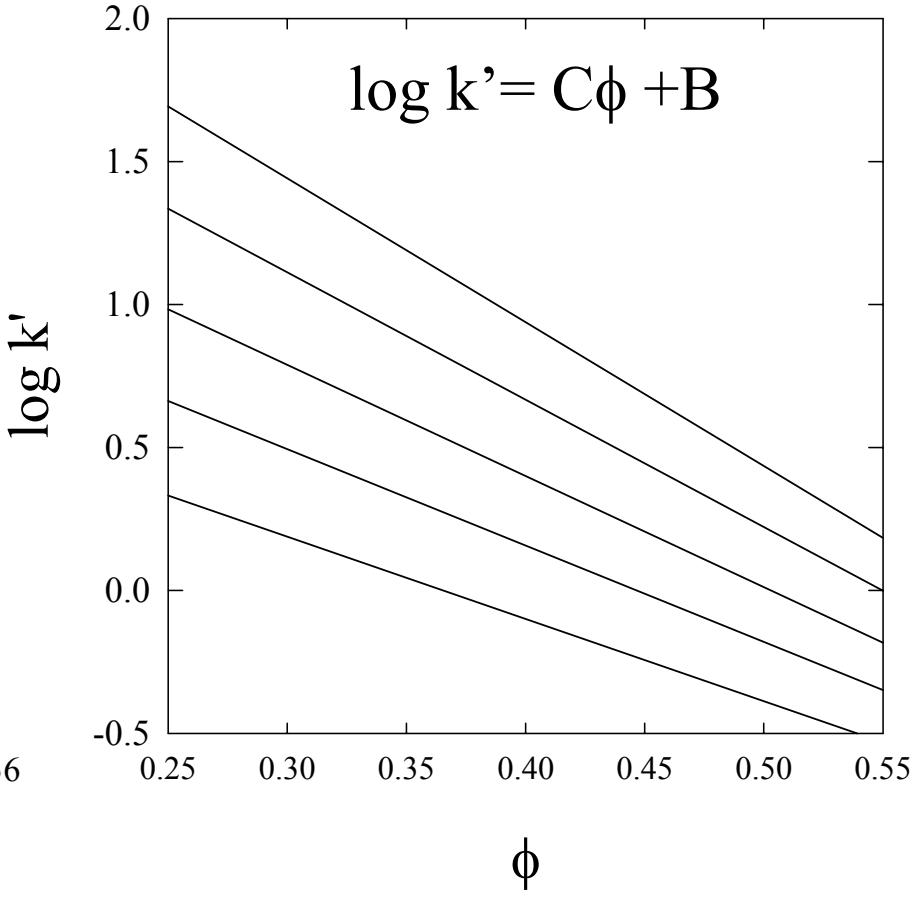


Effect of Composition on Viscosity



Effect of ϕ, & T on k'





How Should the Separation Be Done?

The same k' can be achieved by use of :

a. low temperature and organic rich eluent.

OR

b. high temperature and organic poor eluent.

Which allows the faster separation?

Is High T Optimal 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 ϕ and T (at k' = 5) on η

k' (ϕ,T)	% ACN (v/v)	T (^{o}C)	η(cP) (ϕ,T)	η (T)/η (25 ^{o}C)
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' based on butyl benzene on a C_{18} Zorbax column.

Conclusions

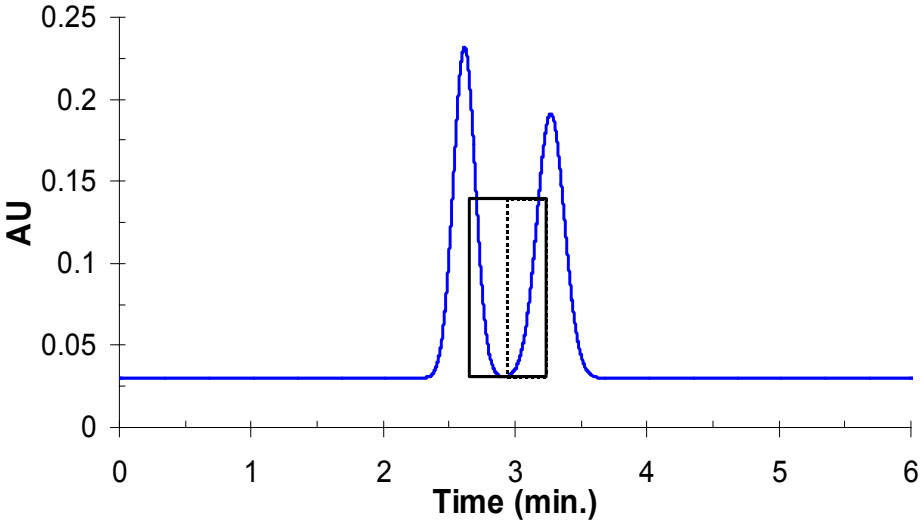
1. To do **fast** LC, use a **WEAK** eluent and a **HOT** column.

2. Use a highly retentive column so that you can work at lowest possible viscosity!

The Importance of Speed in Comprehensive Two-Dimensional HPLC

For comprehensive 2DLC, the speed of the **second dimension separation** is the **rate limiting step** in completing the entire 2D chromatogram.

Each first dimension peak must be chromatographed **3-4 times** by the second dimension column.



$$t_{rtotal} = \frac{\sqrt{N_1} L_{c2} \left(k'_{\max 1} + 1\right)\left(k'_{\max 2} + 1\right)}{\upsilon_2}$$

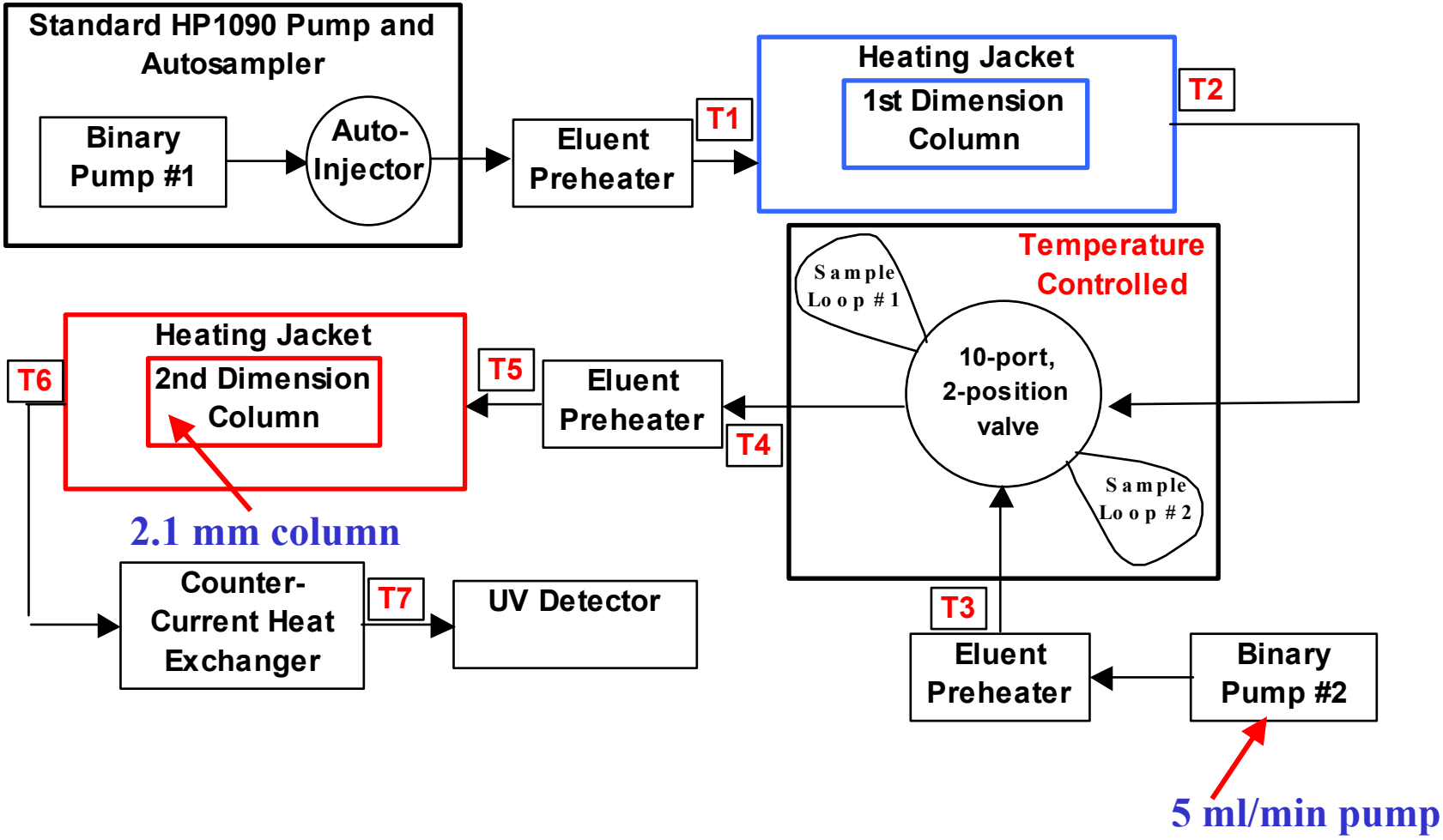
	Typical	Fast
1st Dim. k'_{max}	10	10
2nd Dim. k'_{max}	5	5
N_1 (Plates/column)	10000	10000
$L_{c,2}$ (cm)	3.3	3.3
u_2 (cm/s)	0.5	5.0
Total Analysis Time (Hrs)	12	1

Potential Approaches to Improving the Speed of HPLC

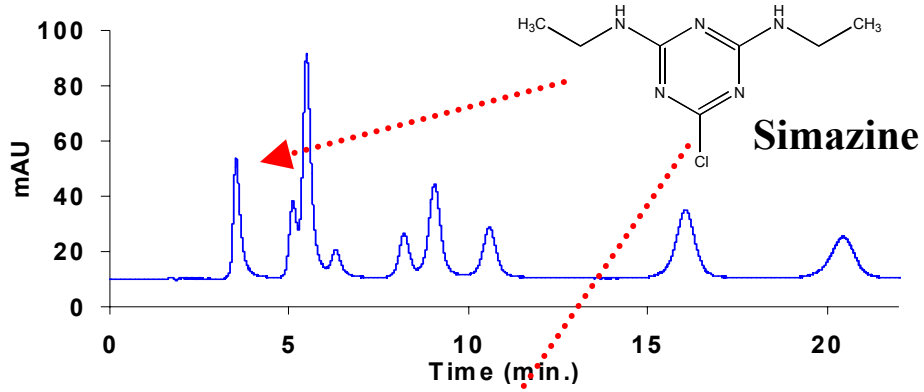
Approach	Advantage	Disadvantage
Shorter Columns	Works with most equipment, stationary phases	Low plate count and resolution
Monolithic Columns	Low backpressure	Narrow-bore columns are not available, high sovent useage
Ultra-High Pressure LC	High plate counts with small particles	Specialized equipment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

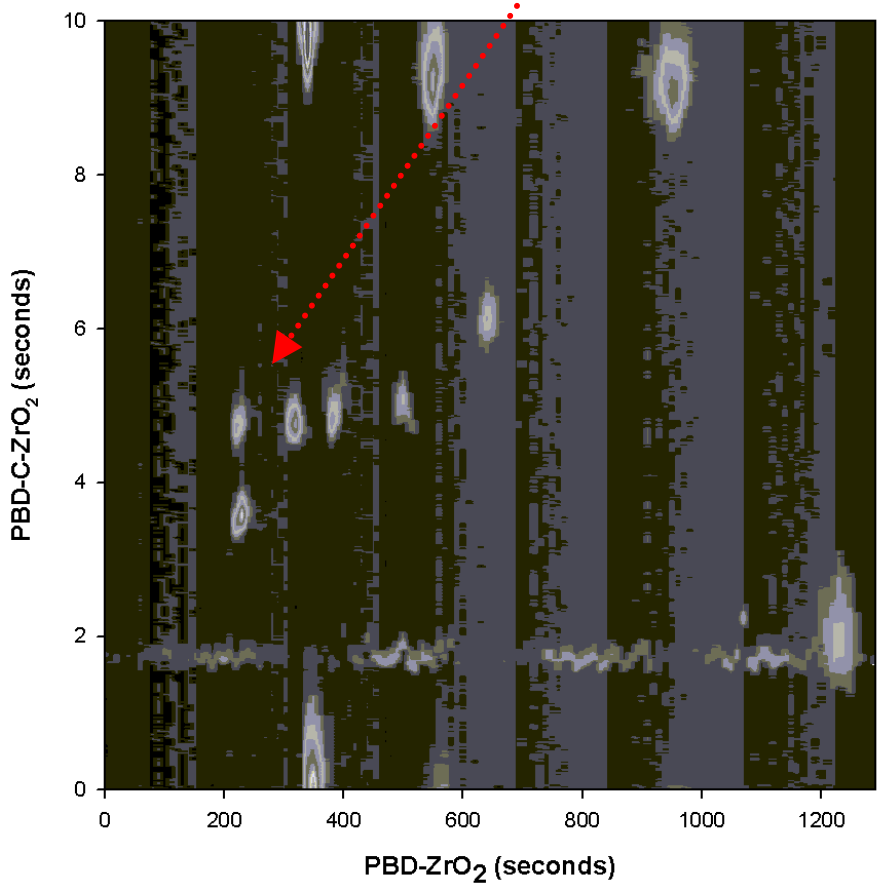
High temperature LC **is the only approach that allows a significant fraction of the column plate count to be retained as the column linear velocity is increased to values that allow** ***much faster HPLC***

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 °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 °C; 1st dimension sampling frequency, 0.1 Hz

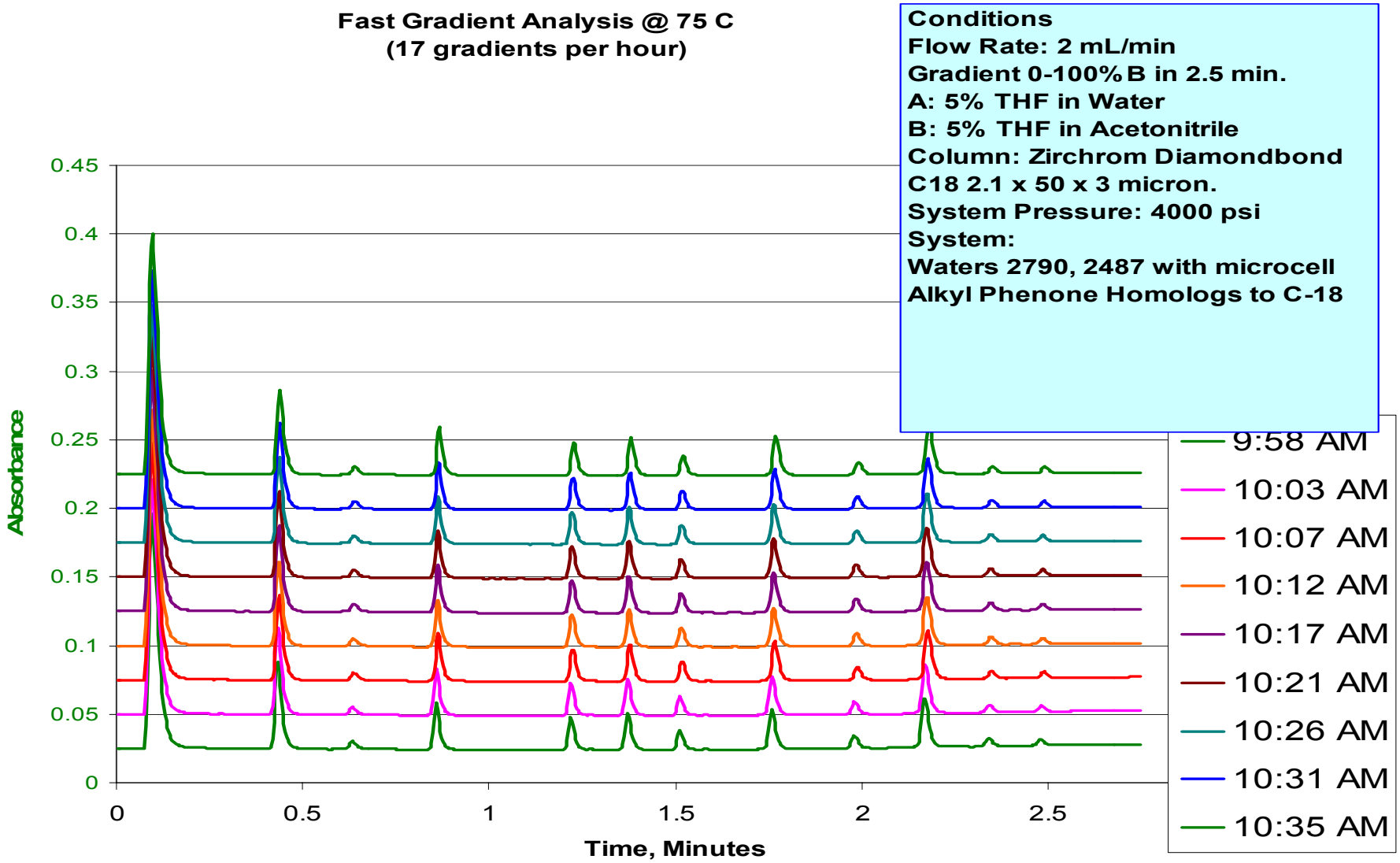
Total LC × UFHTLC peak capacity = 185

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

Thanks!

- Ben Yan (ZirChrom).
- NIH.
- Carl Sims and Systec, Inc.
- ZirChrom Separations, Inc.

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.

Second Dimension Chromatograms