



# **Evaluation of Sub-2 $\mu$ m Zirconia-PBD Particles for Multi-Modal UHPLC**

**Dan Nowlan, B. Yan, C. V. McNeff and R. A. Henry**

**ZirChrom Separations, Inc., 617 Pierce St., Anoka, MN  
55303**



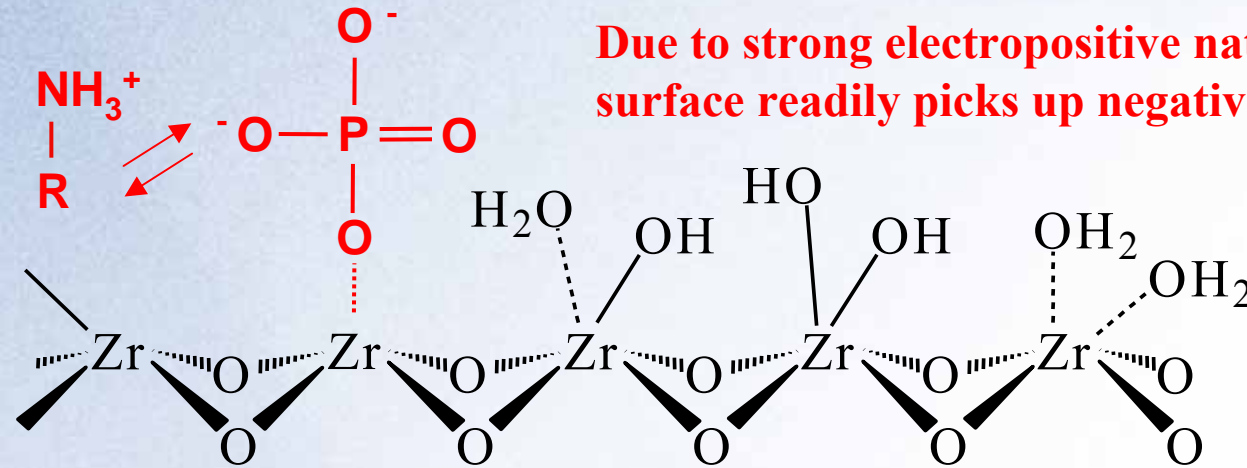
ZirChrom®

# Introduction

- Lately, efficiency has received most of the attention in HPLC. As we study and debate optimum particle geometry and instrument design, higher efficiency columns are being adopted by analysts to improve *resolution, peak capacity, speed, sensitivity and solvent economy*.
- Most of the progress with small particles has been made with silica RP columns so it is important to investigate whether the high efficiency observed with ultra-small silica RP particles can be translated to other substrates and phases, which may retain and separate by other selective modes.
- Zirconia phases often separate by a multi-modal mechanism so they are good candidates to see if the performance advantages of sub-2 $\mu$ m particles can be observed (at ambient or elevated temperature) for other packings.



# Brief Review of Multi-Modal Zirconia Behavior

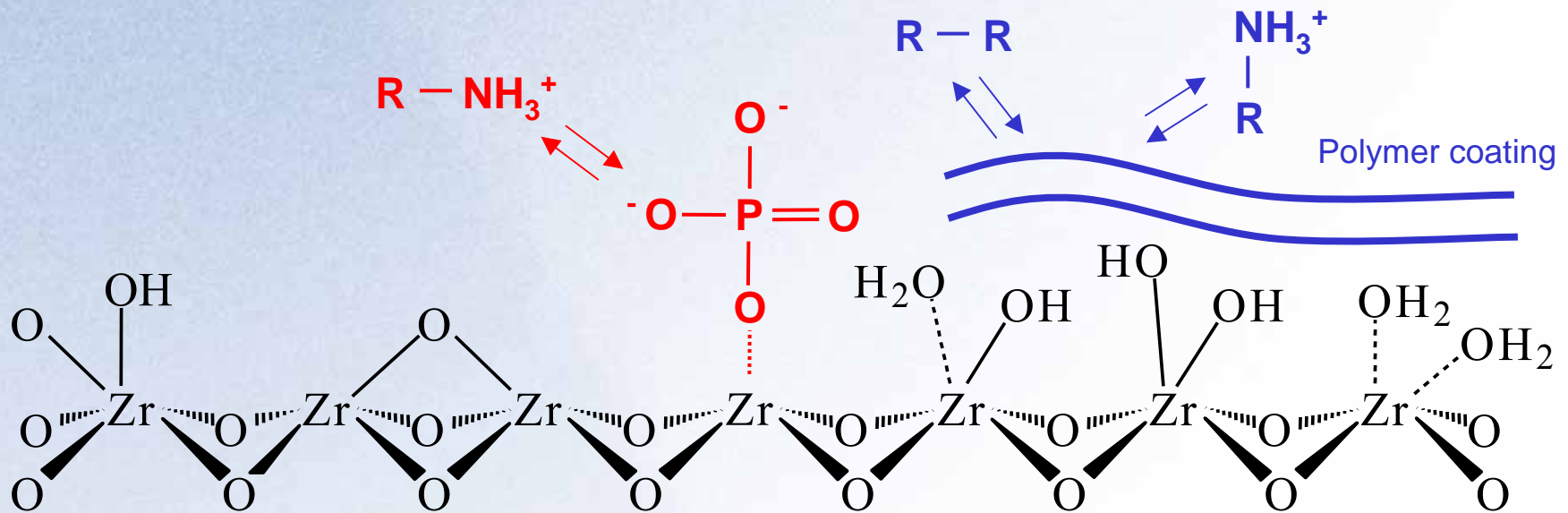


Due to strong electropositive nature, Zr surface readily picks up negative charge

- Zirconia substrate exhibits polar and ionic solute interaction: mainly **cation-exchange**.
- With stable organic coatings, **reversed-phase** interaction creates Multi-Modal behavior.
- Extreme resistance to temperature, pH and mechanical stress are potential advantages.



# Addition of RP Behavior with Coated Zirconia Phases

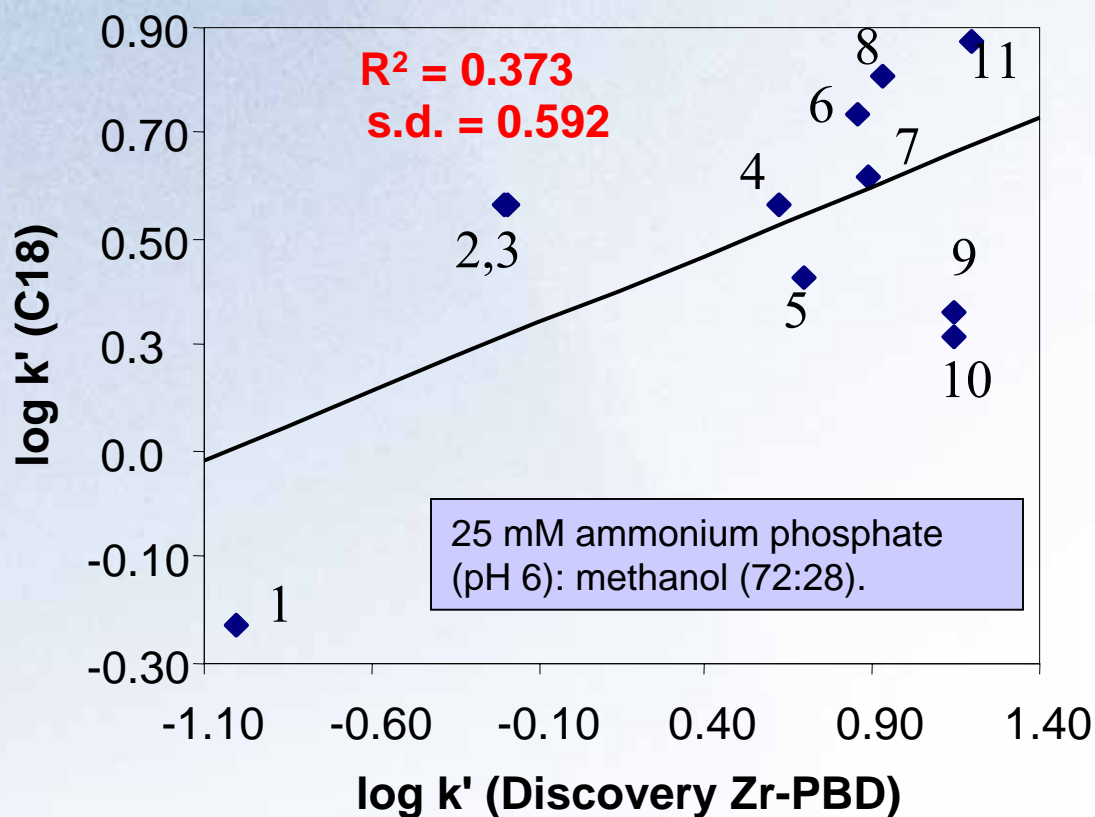


- Ionic solute retention (and selectivity) is modulated by pH, buffer/salt type and concentrations, and temperature.
- RP solute retention is modulated by organic solvent.
- *Five* important mobile phase variables must be controlled.



# Zr-PBD and Si-C18 are Orthogonal for Basic Drugs<sup>2</sup>

C18 (RP) columns separate mainly by hydrophobic forces and Zr-PBD columns separate by a combination of ionic and hydrophobic forces



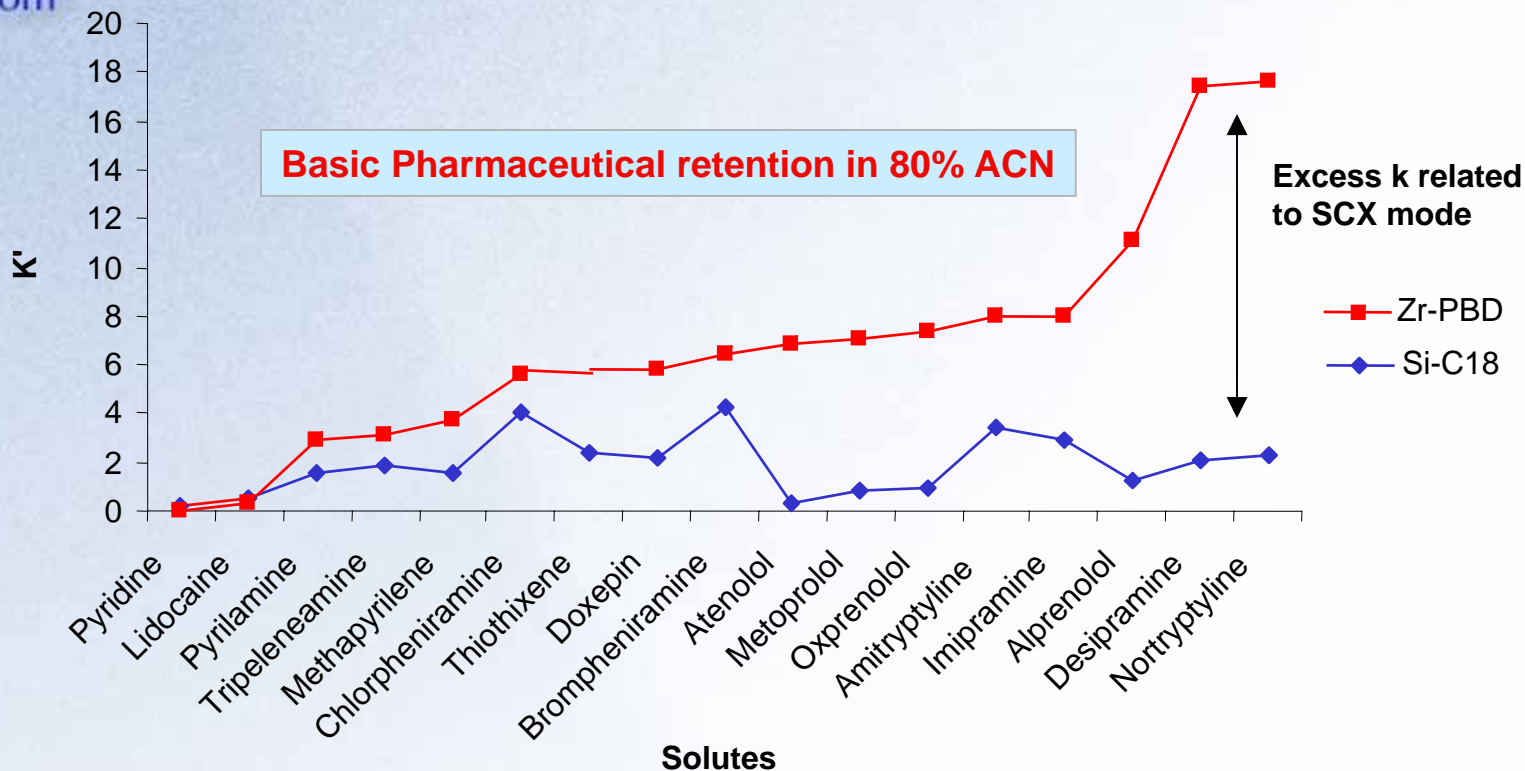
#### Solutes

1. Chlordiazepoxide
2. Hydroxyzine
3. Buclizine
4. Thiothixene
5. Doxepin
6. Amitriptyline
7. Imipramine
8. Perphenazine
9. Nortriptyline
10. Desipramine
11. Thioridazine

Data provided by Sigma-Supelco  
Plot technique by Horvath, Carr, others



# SCX Mode Can Create Zr-PBD Retention Even in High Organic



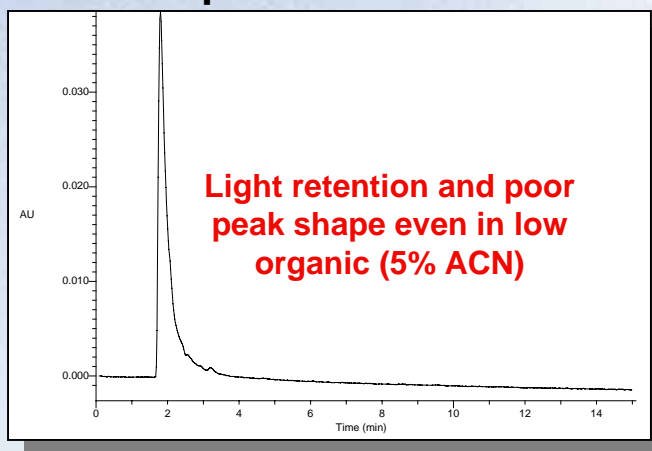
LC Conditions: Machine-mixed 80/20 ACN/10 mM ammonium acetate pH=6.7 without pH adjustment; Flow rate, 1.0 mL/min.; Injection volume 0.1  $\mu$ L; Temperature, 35  $^{\circ}$ C; Detection at 254 nm; Columns, Zr-PBD, 50 x 4.6 mm i.d. (3  $\mu$ m particles); Silica-C18 150 x 4.6 mm i.d., (3.5  $\mu$ m particles).



# Difficult Compounds for Silica Often Separate on Zirconia

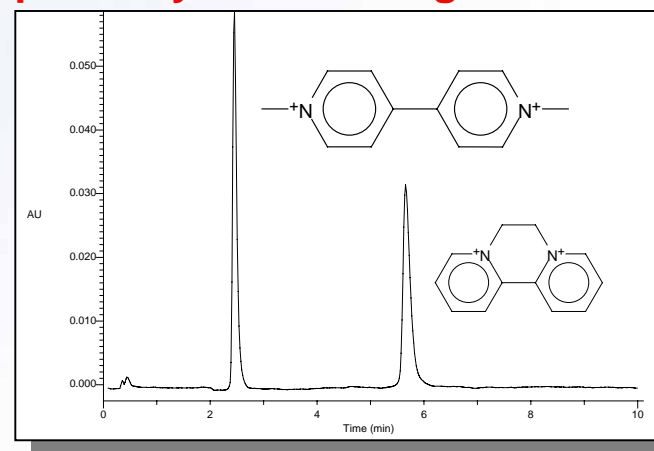
**Quaternary amines** paraquat and diquat are retained and resolved on Zr-PS (also Zr-PBD or bare  $ZrO_2$ ) due to the **cation exchange** mechanism; 50% ACN is useful to suppress or regulate retention by RP mode.

**Silica-C18:**  
reversed-phase



column: Discovery C18, 15 cm x 4.6 mm I.D., 3 $\mu$ m  
mobile phase: 5% acetonitrile in 25 mM phosphate, pH 7  
flow rate: 1 mL/min.  
temp.: 35 °C  
det.: UV 290 nm

**Zirconia-PS:**  
primarily ion-exchange



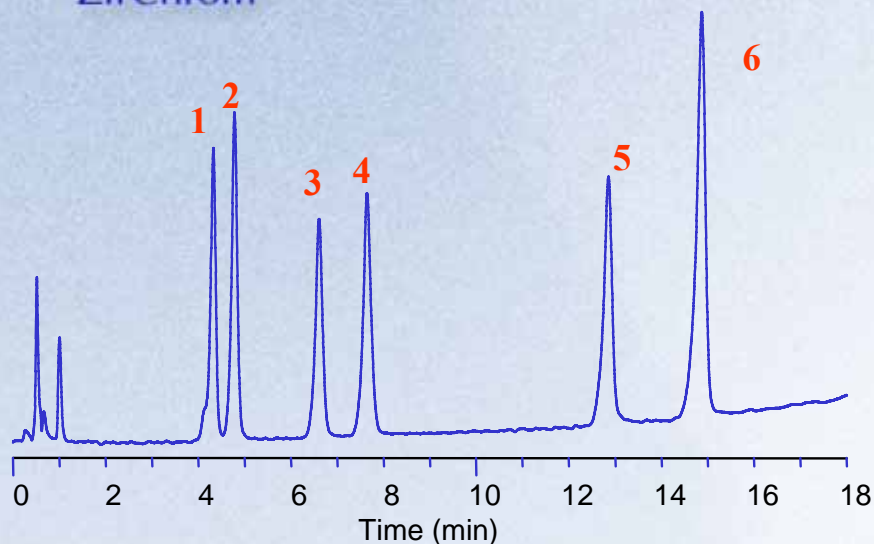
column: Discovery Zr-PS, 7.5 cm x 4.6 mm, 3 $\mu$ m  
mobile phase: 50% acetonitrile in 25 mM phosphate, pH 7  
flow rate: 3 mL/min.  
temp.: 65 °C  
det.: UV 290 nm

Data provided by Sigma-Supelco



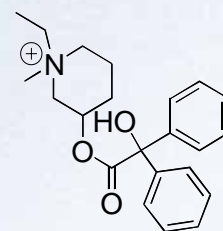
# Anticholinergics on Zr-PBD

## Quaternary amines (except 2)

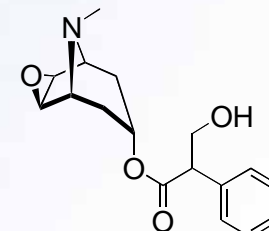


### LC Conditions

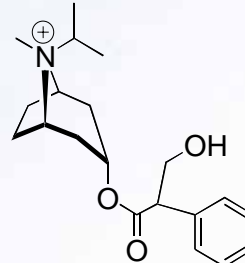
Discovery Zr-PBD, 100mm x 2.1mm i.d., 3  $\mu$ m  
Mobile Phase A: 10 mM  $\text{NH}_4\text{PO}_4$ , pH 7.0  
Mobile Phase B: 80/20 20 mM  $\text{NH}_4\text{PO}_4$ , pH 7.0/ACN  
Gradient: 10-100% B over 18 minutes  
**Temp: 80 °C,**  
Flow: 0.3 mL/min  
Inj vol: 2  $\mu$ L in 60% MeOH  
Detector: UV@225 nm



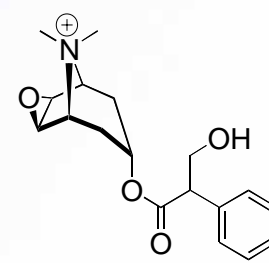
1, Pipenzolate (20 mg/L)



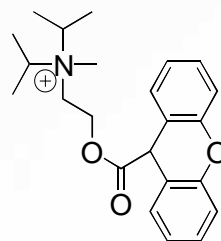
2, Scopolamine (100 mg/L)



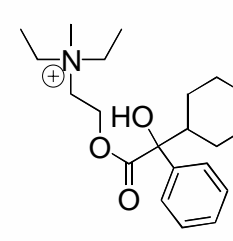
3, Ipratropium (100 mg/L)



4, Methscopolamine (100 mg/L)



5, Propantheline (20 mg/L)



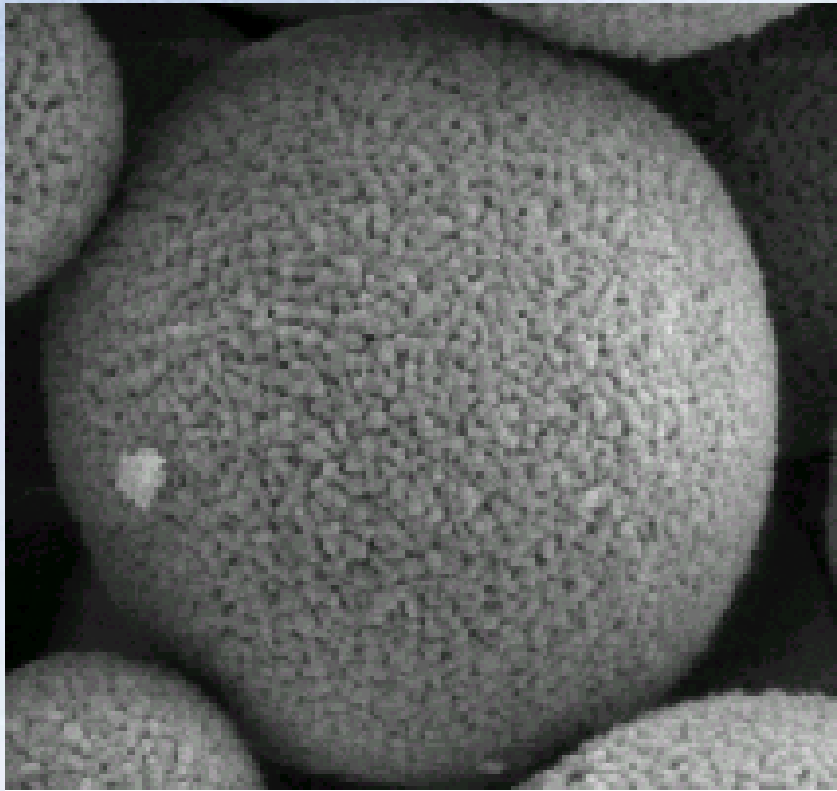
6, Oxyphenonium (100 mg/L)

Data provided by Sigma-Supelco





# Evaluation of Smaller Diameter Porous Zirconia Particles



1 μm 25000X

## Specifications:

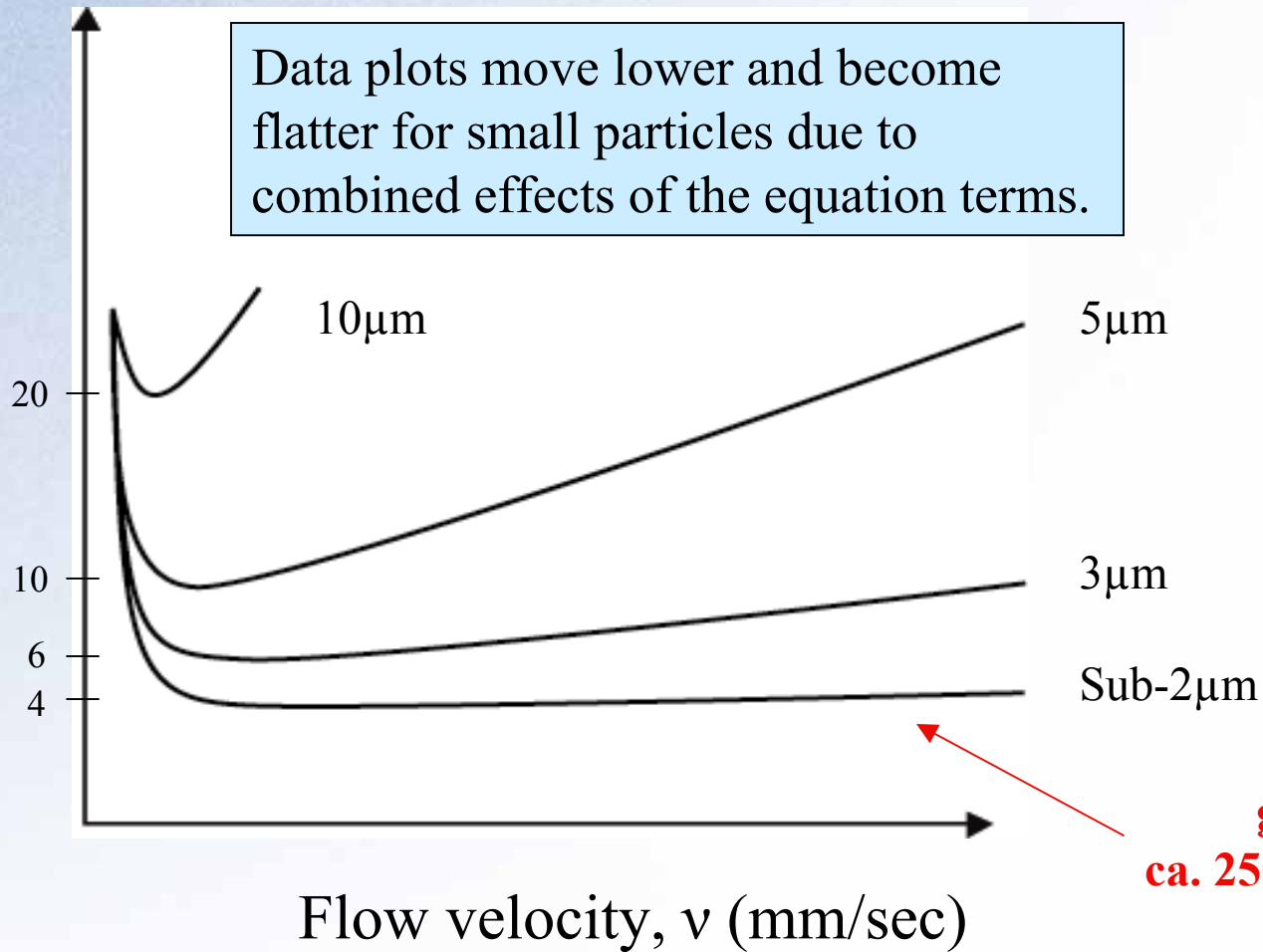
- Particles produced by a sol-gel process with 1000Å sol
- Pore diameter 250-300Å
- Density: 2.6 g/cc (2.5X silica)
- Surface area: 25 m<sup>2</sup>/g
- Particle diameters: 3 μm and **sub-2 μm**
- Totally porous (porosity: 0.45)



ZirChrom®

# van Deemter Plots Reveal Column Performance

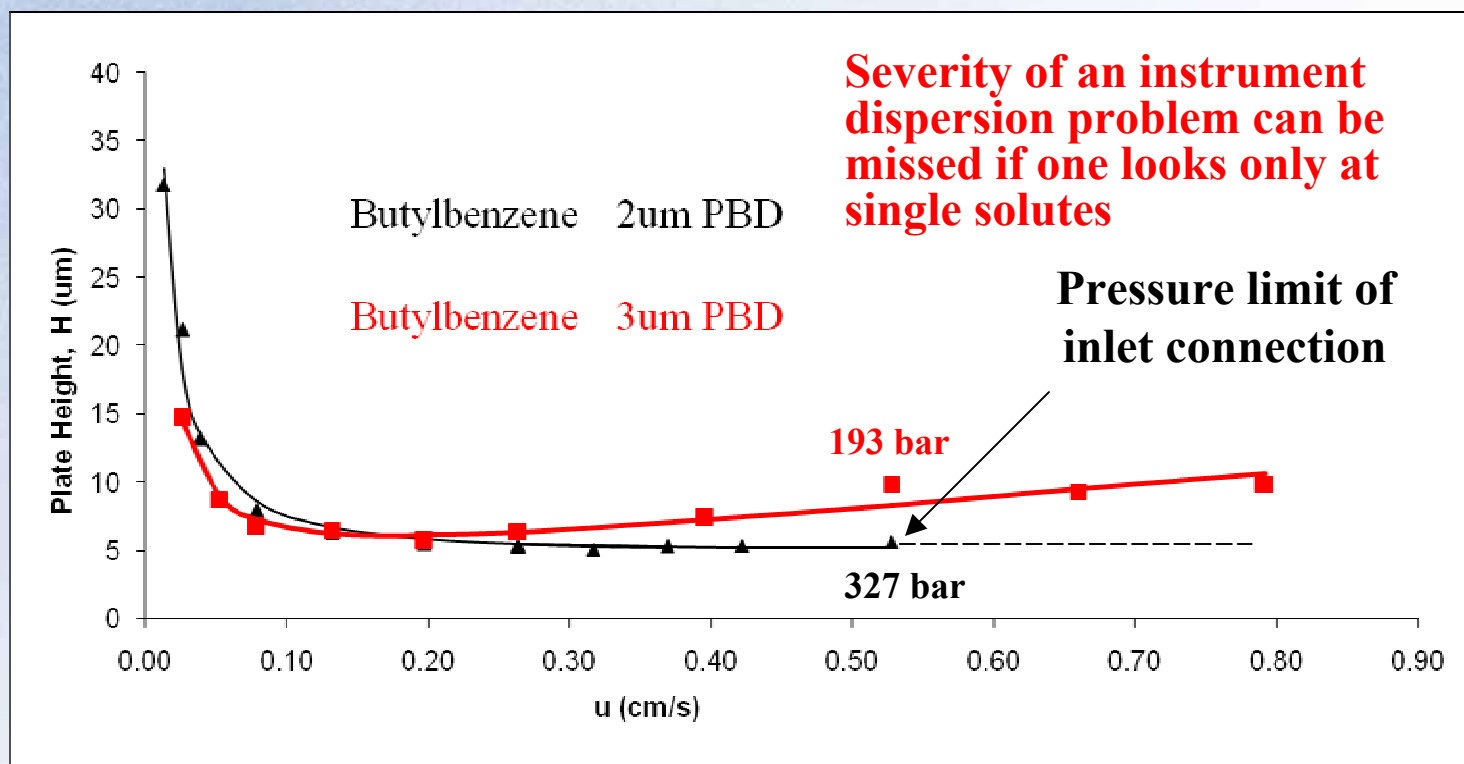
$$H = A + B/v + Cv \quad (\text{shown below for a single solute})$$



Idealized plot provided by Sigma-Supelco



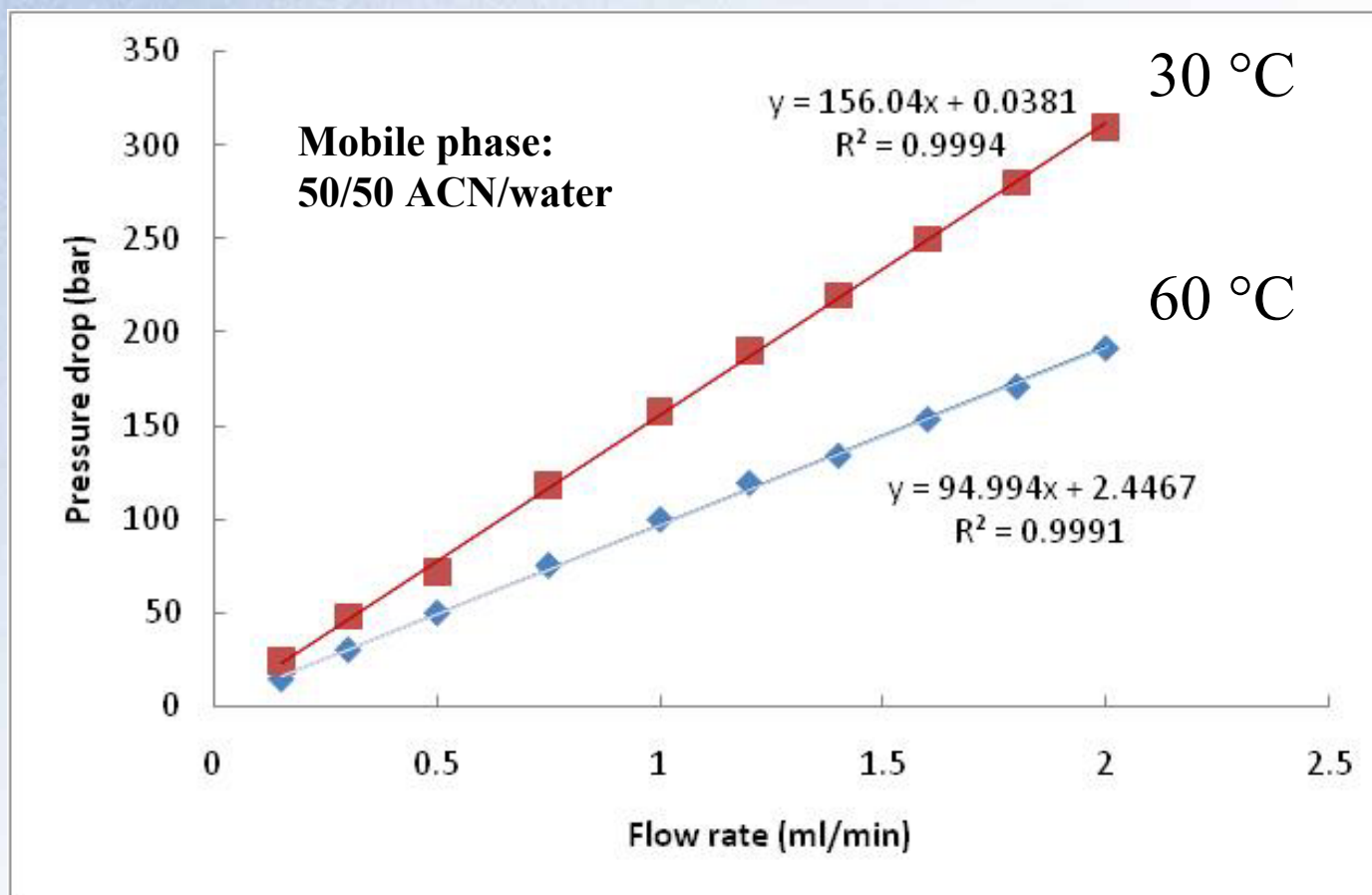
# Comparison Between Sub-2 $\mu\text{m}$ and 3 $\mu\text{m}$ Zr-PBD



Efficiency for the larger particle is about right, but the smaller particle should be better (lower H). Could be the phase coating or the column, but instrument dispersion cannot be ruled out with this data.



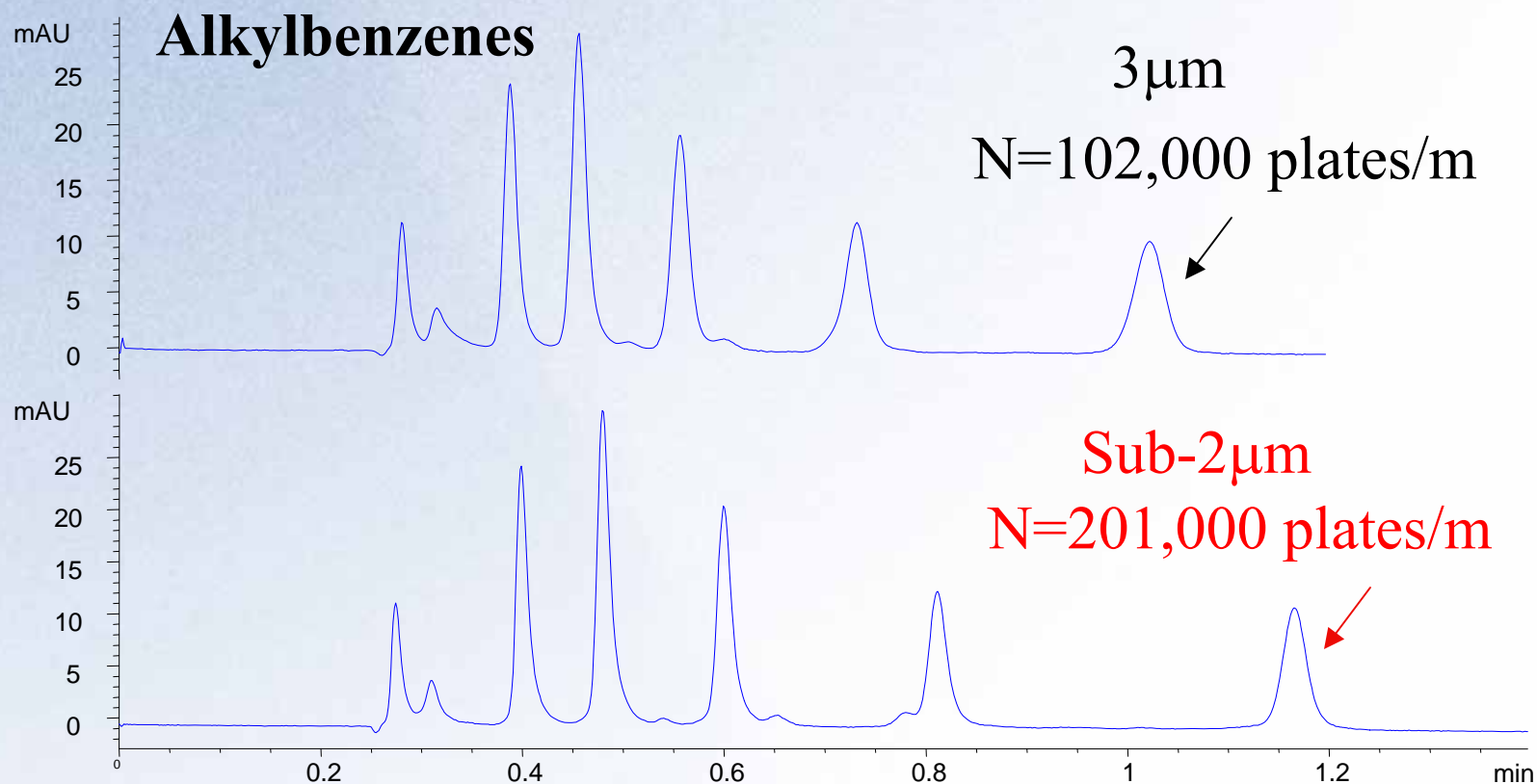
# Sub-2 $\mu\text{m}$ Pressure Drop at Different Temperatures\*



\* 3 $\mu\text{m}$  particles (not shown) have about half the pressure drop



# Initial Comparison Between Sub- $2\mu\text{m}$ and $3\mu\text{m}$ Zr-PBD Particles



Columns: ZirChrom PBD, 50 x 4.6mm; Mobile phase: 50/50 ACN/water; Flow 2.0 mL/min (0.53 cm/sec); Temp.: 30 °C; UV@254nm; Agilent 1100.



# Flow Studies on 3 $\mu$ m Zr-PBD: Alkylbenzenes

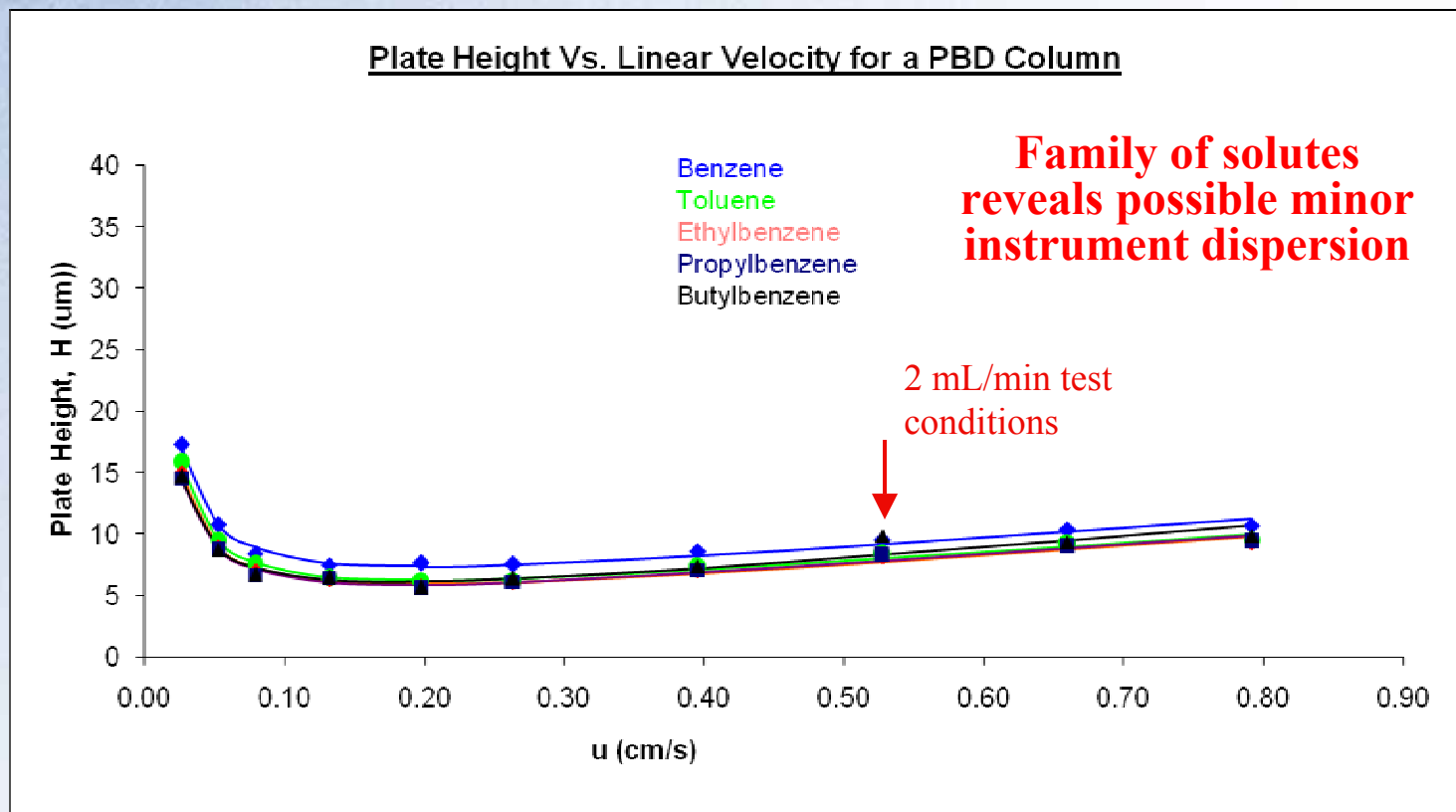


Plate height based on van Deemter Equation vs linear velocity at various temperatures for retained solutes: Alkylbenzenes, Temperature: 30 °C, Mobile phase: 50/50 ACN/water, Column: ZirChrom PBD, 50 x 4.6mm, Agilent 1100/UV micro cell (0.007" i.d. tubing).



# Flow Studies on Sub- $2\mu\text{m}$ Zr-PBD: Alkylbenzenes

“..but we used a micro flow cell!”

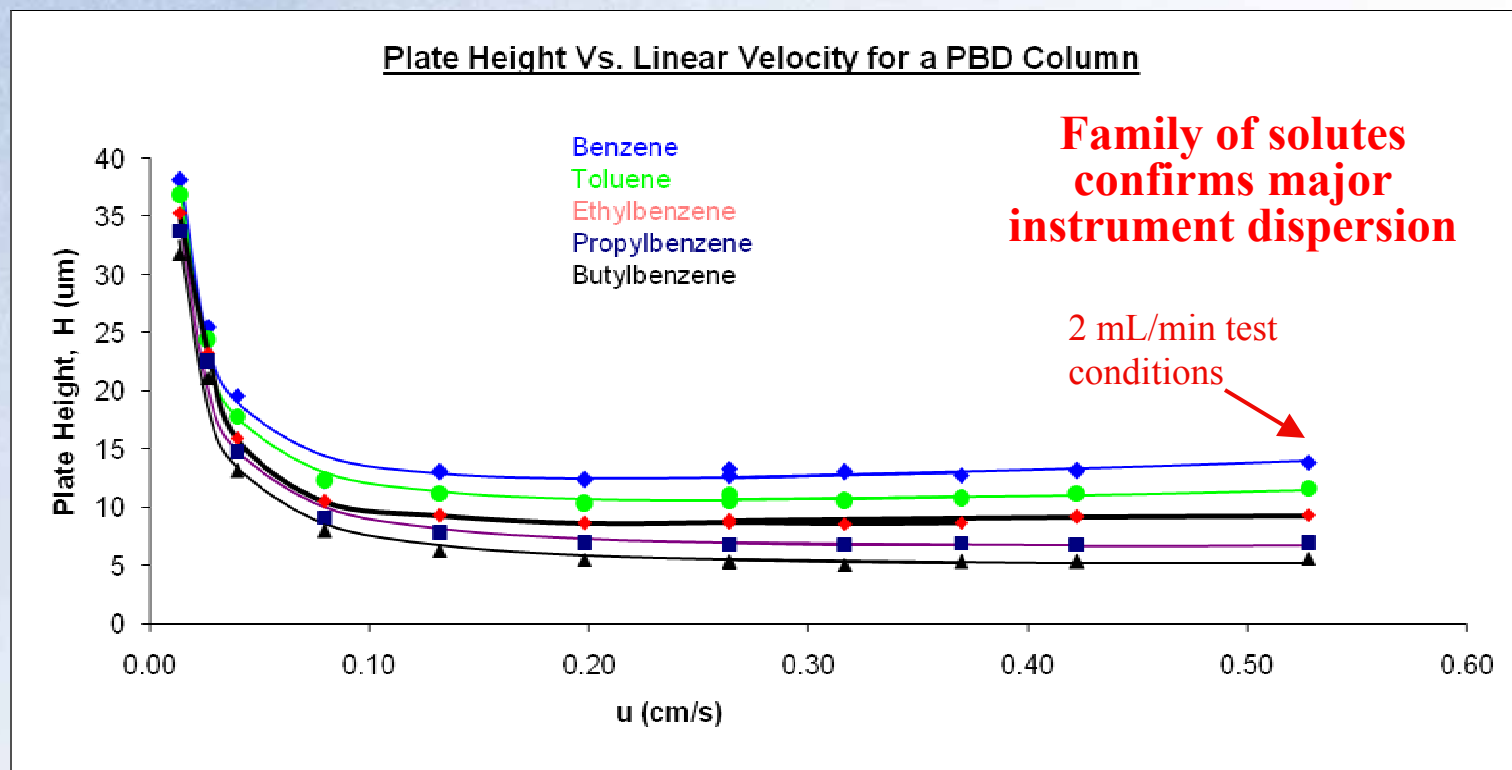


Plate height based on van Deemter equation vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 °C, Mobile phase: **50/50 ACN/water** (keep k in the same range as  $3\mu\text{m}$  particles), Column: 50 x 4.6mm, Agilent 1100/UV micro cell (0.007" i.d. tubing).

**Conclusion: systematic investigation of instrument dispersion needed.**



# Flow Studies on Sub-2 $\mu\text{m}$ Zr-PBD: Factory Instrument at Ambient

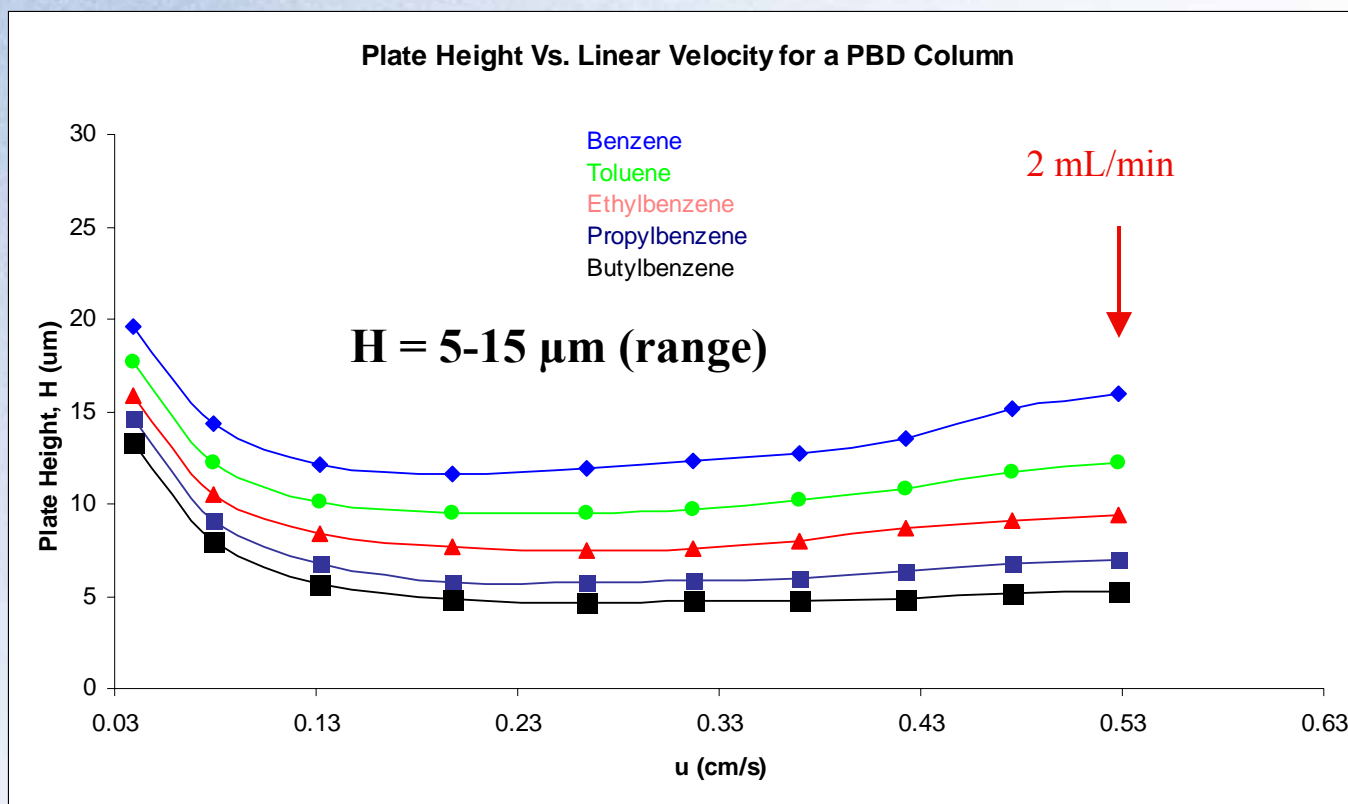


Plate height vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 °C, Mobile phase: 50/50 ACN/water (keep  $k'$  in the same range as 3 $\mu\text{m}$  particles), Column: 50 x 4.6mm, Agilent 1100/UV with Standard Cell and 0.007" i.d. tubing.





# Flow Studies on Sub-2 $\mu\text{m}$ Zr-PBD: Add Micro Cell

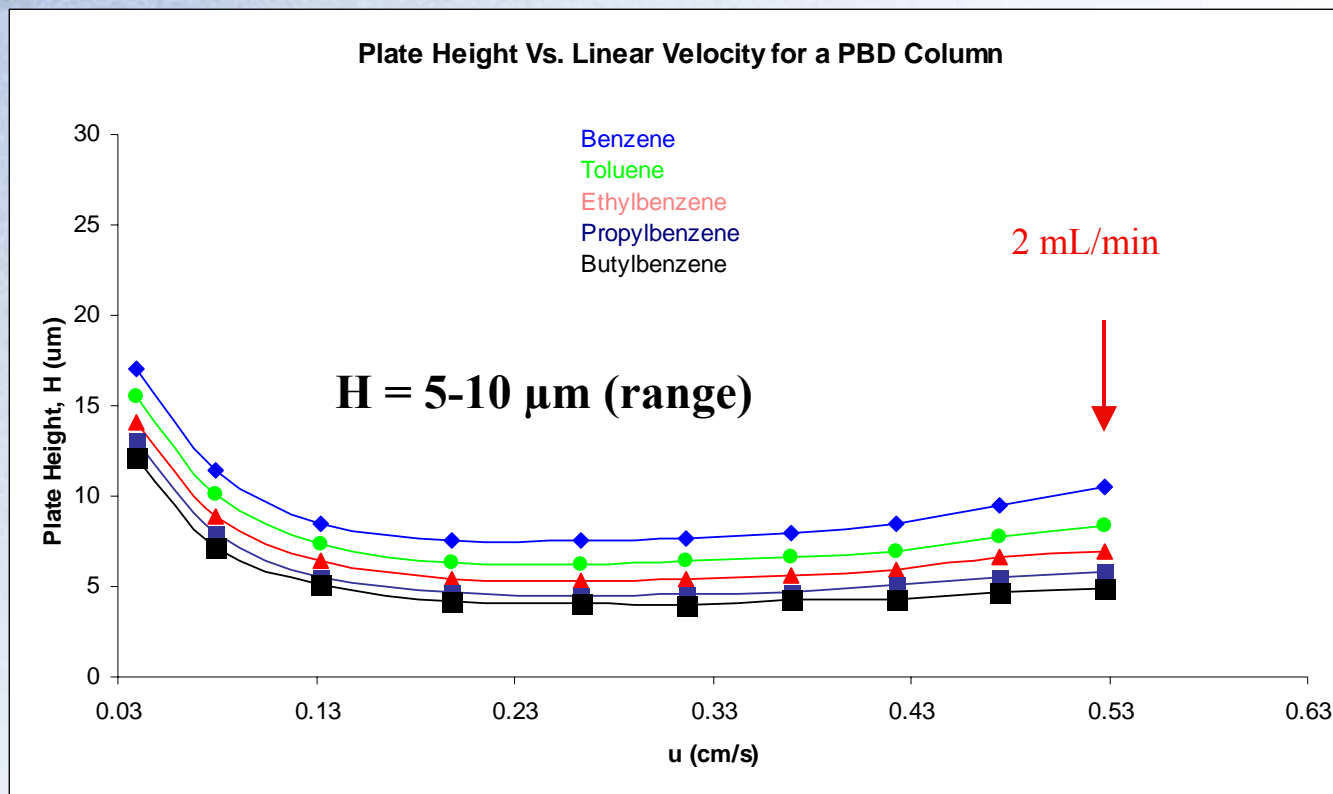


Plate height vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 °C, Mobile phase: **50/50 ACN/water** (keep  $k'$  in the same range as 3 $\mu\text{m}$  particles), Column: 50 x 4.6mm, Agilent 1100/UV with Micro Cell and 0.007" i.d. tubing.



# Flow Studies on Sub-2 $\mu\text{m}$ Zr-PBD: Micro Cell + Optimized Tubing

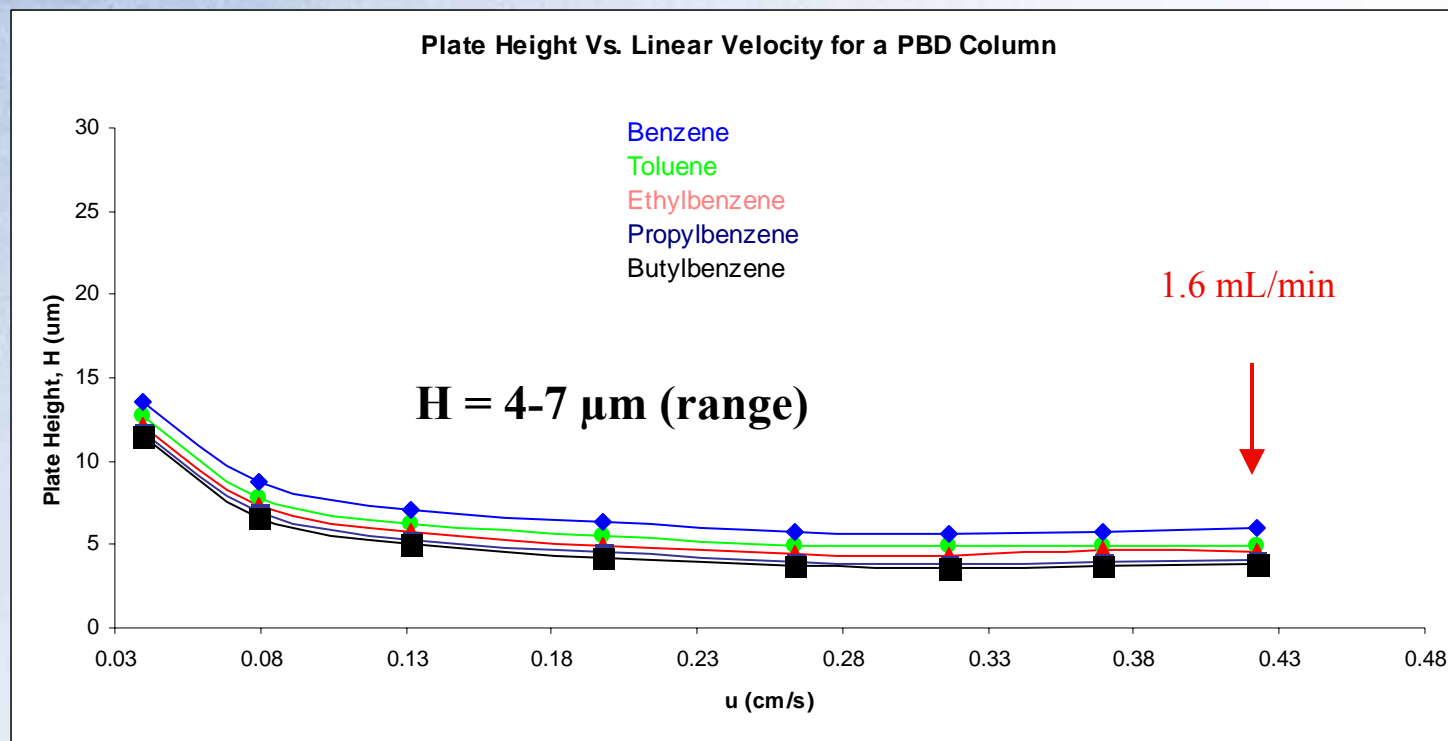


Plate height vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 °C, Mobile phase: **50/50 ACN/water** (keep  $k'$  in the same range as 3 $\mu\text{m}$  particles), Column: 50 x 4.6mm, Agilent 1100/UV with Micro Cell and optimized 0.005'' i.d. tubing.



# Flow Studies on Sub-2 $\mu\text{m}$ Zr-PBD: Add Heat Exchanger and Fitting

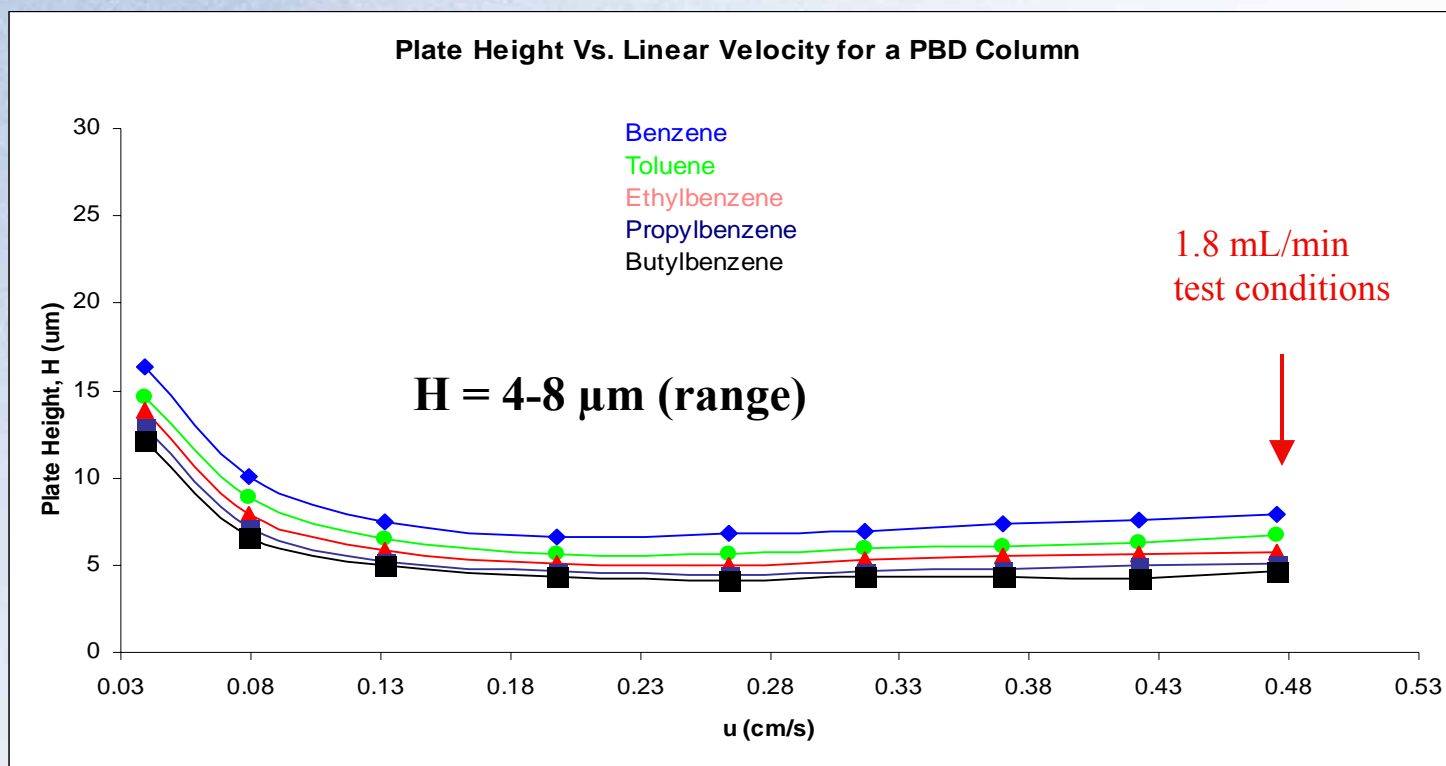


Plate height vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 °C, Mobile phase: **50/50 ACN/water** (keep  $k'$  in the same range as 3 $\mu\text{m}$  particles), Column: 50 x 4.6mm, Agilent 1100/UV with Micro Cell, **high pressure fitting and heat exchanger.**



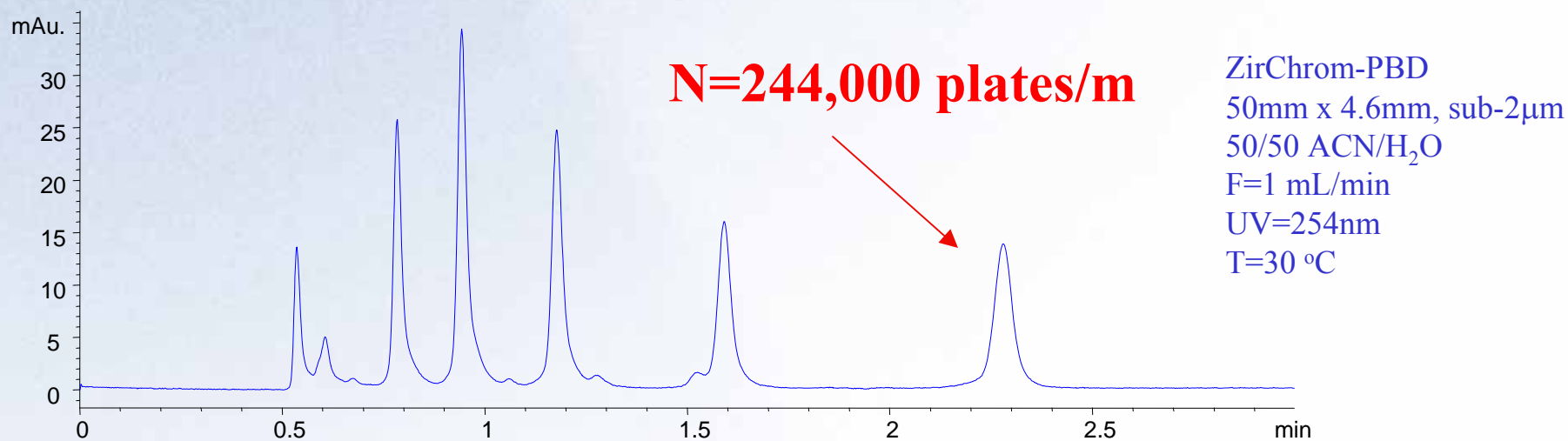
# Optimization and Configuration for Elevated Temperature Operation with High-Pressure Column Fitting





# ZirChrom-PBD sub-2 $\mu$ m; Reduced Instrument Volume

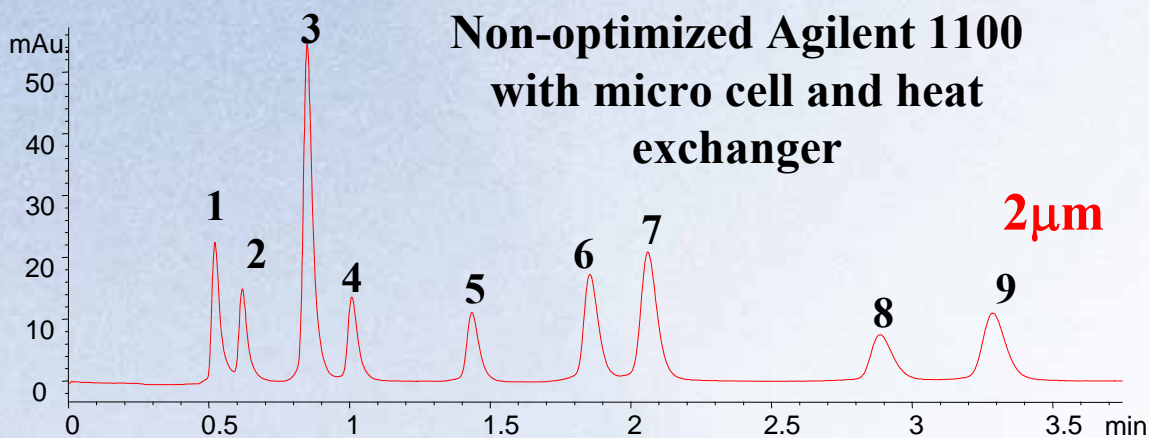
## Alkylbenzenes





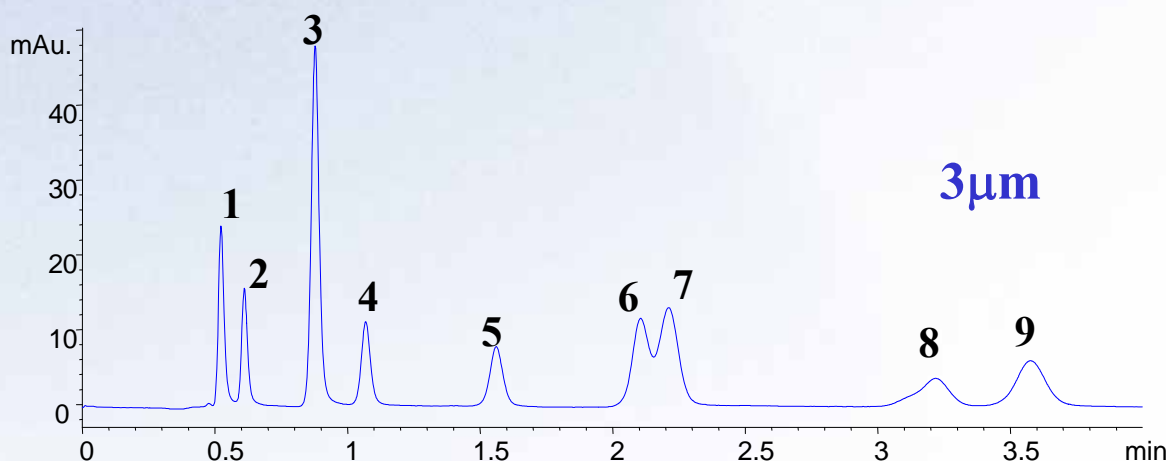
# Drug Mix\* Separation on PBD

## sub-2 $\mu$ m vs 3 $\mu$ m – Ambient



### Analytes

- 1=Labetalol
- 2=Atenolol
- 3=Acebutolol
- 4=Metoprolol
- 5=Oxprenolol
- 6=Lidocaine
- 7=Quinidine
- 8=Alprenolol
- 9=Propranolol



Column: ZirChrom<sup>®</sup>-PBD,  
50 x 4.6 mm i.d., sub-2 $\mu$ m;  
Mobile phase: 24/76 ACN/20  
mM K<sub>3</sub>PO<sub>4</sub> at pH=12; **Flow  
rate: 1.0 mL/min; Temp.: 30  
°C; Injection vol.: 2.0  $\mu$ L;**  
**Detection: UV at 254 nm**

\* Mainly beta-blockers

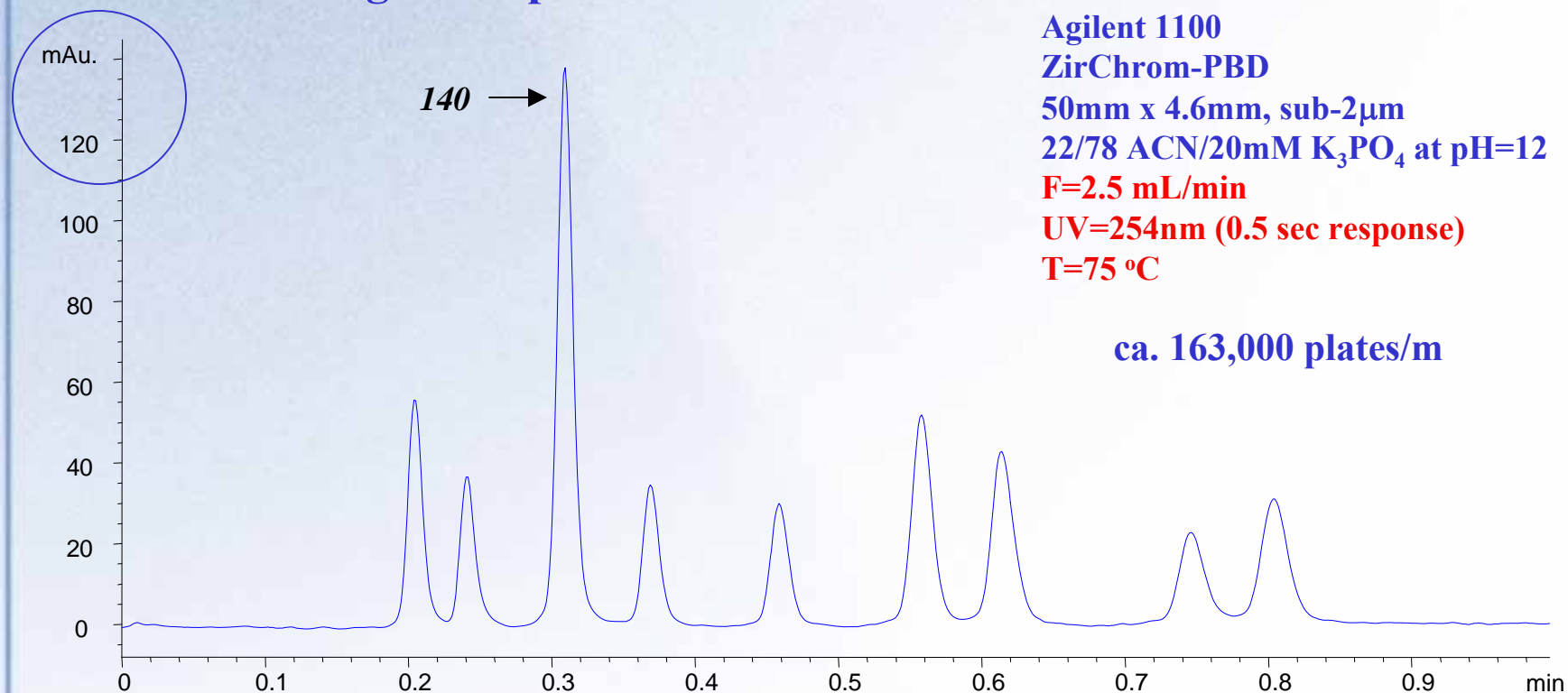


# Drug Mix on ZirChrom-PBD sub-2 $\mu$ m, **High Temperature**

246 bar (3660 psi)  
Background pressure: ca. 25 bar

Agilent 1100  
ZirChrom-PBD  
50mm x 4.6mm, sub-2 $\mu$ m  
22/78 ACN/20mM K<sub>3</sub>PO<sub>4</sub> at pH=12  
**F=2.5 mL/min**  
**UV=254nm (0.5 sec response)**  
**T=75 °C**

ca. 163,000 plates/m

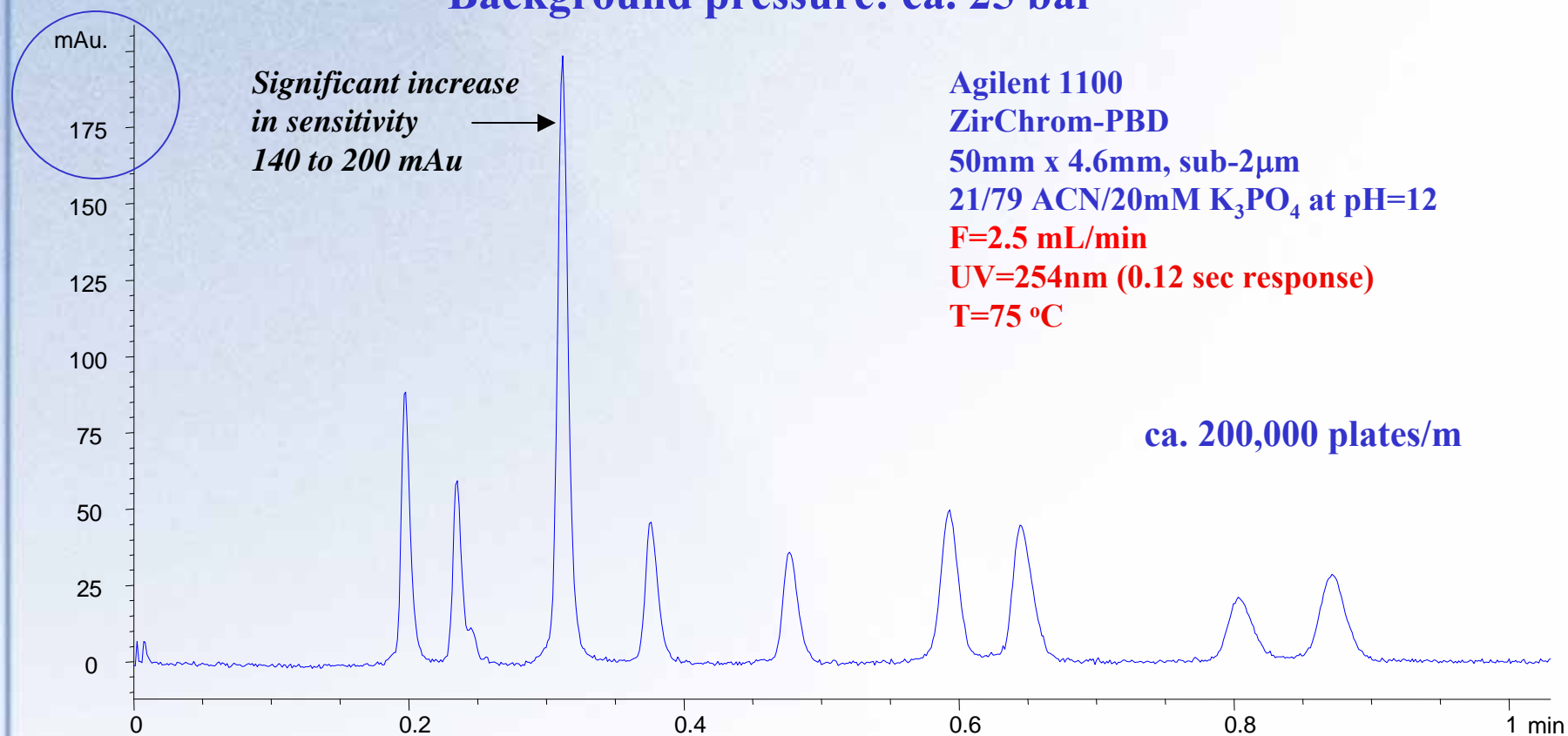




# Drug Mix on ZirChrom-PBD sub-2 $\mu$ m, High Temp, *Faster Detector Response*

246 bar (3660 psi)

Background pressure: ca. 25 bar







## Calibrating Background Pressure Drop for Optimized Agilent 1100

100% H <sub>2</sub> O at 30 °C		100% H <sub>2</sub> O at 75 °C	
Flow (mL/min)	BP (bar)	Flow (mL/min)	BP (bar)
1	26	1	21
2	50	2	39
3	77	3	60

\* Reference point: Waters Acquity (0.005" ID inlet / 0.0025" ID outlet), 60/40 ACN/water, 0.5 mL/min, **background pressure = 1700 psi (113 bar).**



## Conclusions and Plans for Further HPLC Development with Zirconia

- Performance results with a sub-2 $\mu$ m Zr-PBD column in an Agilent 1100 are encouraging.
- The study of ultra-high speed applications using sub-2 $\mu$ m Zr-PBD, especially at higher pH and temperature (“extreme conditions for silica”) will be continued; generic conditions for LC-MS will be investigated.
- Additional advantages of optimizing the IBW of an Agilent Model 1100 HPLC instrument using a high performance (Model 1200) heat exchanger will be studied.
- Other sub-2 $\mu$ m Zr phases (such as CARB) will be prepared and compared to Zr-PBD under ambient and extreme conditions.



## References and Acknowledgements

1. J. A. Blackwell and P. W. Carr, "Development of an Eluotropic Series for the Chromatography of Lewis Bases on Zirconium Oxide," *Anal. Chem.* **64**, 863-73 (1992).
2. R. A. Henry, H. K. Brandes, D. S. Bell and C. T. Santasania, 30<sup>th</sup> Annual HPLC Meeting, Multi-Mode Oral Presentation, 2006, San Francisco, CA.
3. R. A. Henry and H. K. Brandes, Eastern Analytical Symposium, Multi-Mode Oral Presentation, 2006, Somerset, NJ.
4. B. Yan. C. V. McNeff. R. A. Henry and D. Nowlan, Eastern Analytical Symposium, Multi-Mode Poster, 2008, Somerset, NJ.
5. B. Yan. C. V. McNeff. R. A. Henry and D. Nowlan, Pittsburgh Analytical Conf., Evaluation of Small Particle Zirconia, Oral Presentation, 2009, Chicago, IL.

**The assistance of Supelco Division of Sigma-Aldrich is gratefully appreciated, including the use of a high-pressure column fitting.**