Applications of Sub-2µm Zirconia-PBD Columns at Elevated pH and Temperature

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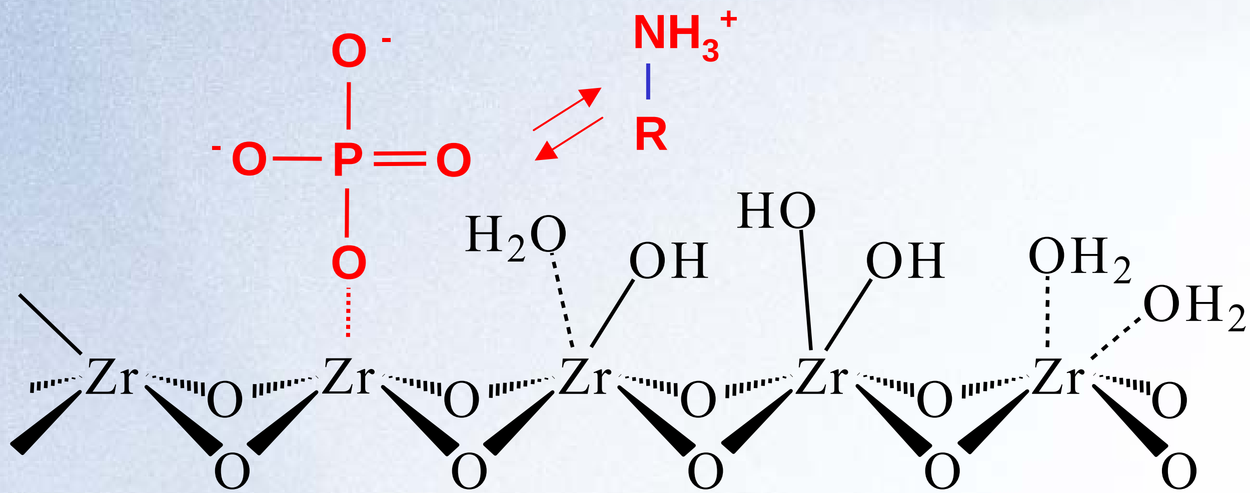
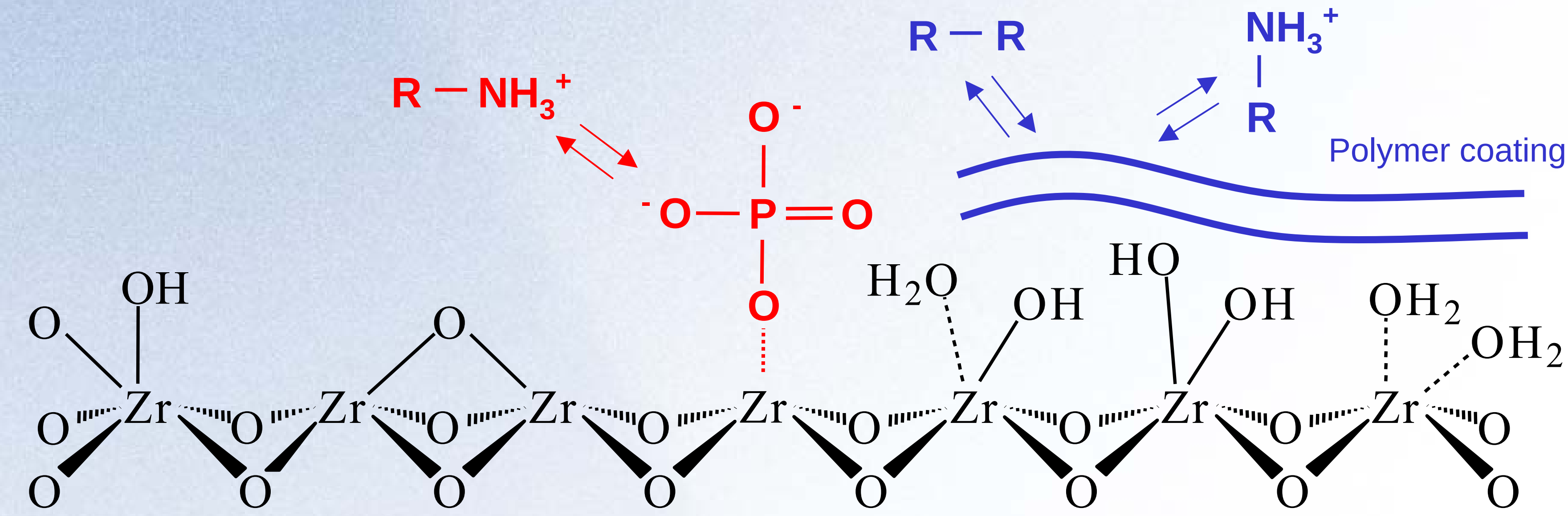
Multi-Mode Behavior of Zirconia



- Zirconia substrate exhibits polar and ionic solute interaction: especially cation-exchange.
- With stable organic coatings, reproducible reversed-phase behavior can be added.
- Extreme resistance to temperature, pH and mechanical stress are unique advantages.

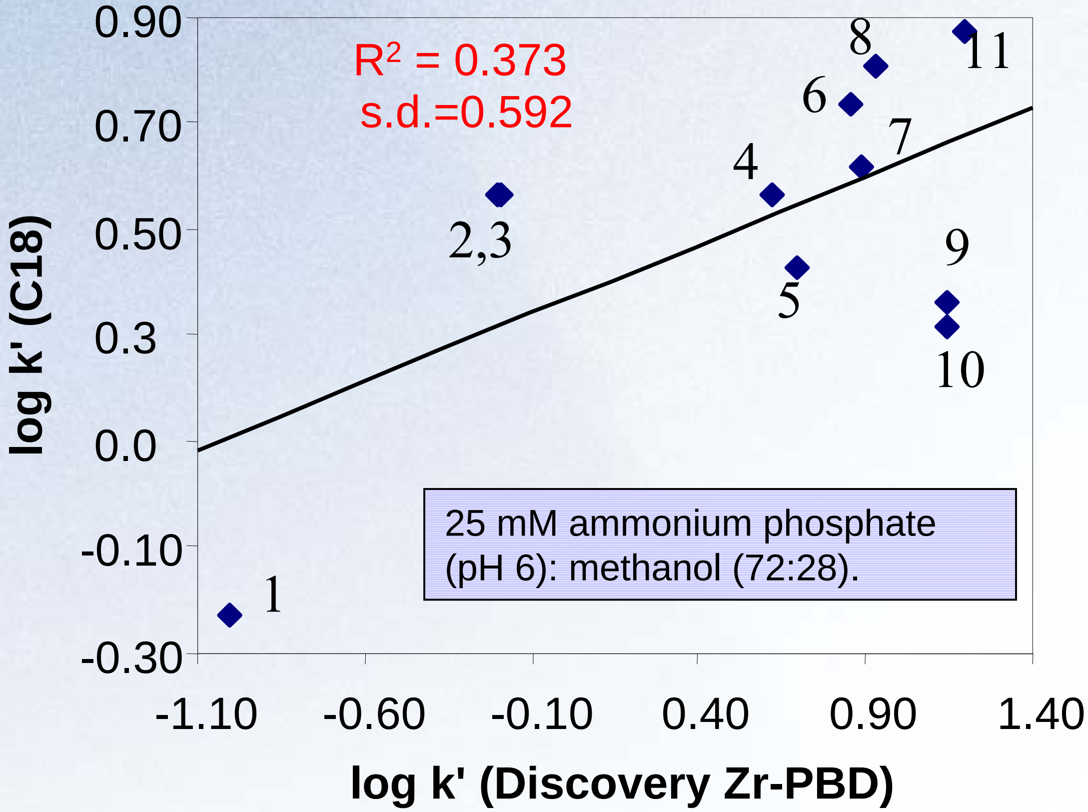
Addition of RP Behavior with Coated Zirconia Phases



- Retention (and selectivity) of ionic analytes modulated by pH, buffer/salt type and concentrations, and temperature.
- Retention of neutral solutes modulated by organic solvent.

C18 and Zr-PBD are Orthogonal for Basic Drugs[2]

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



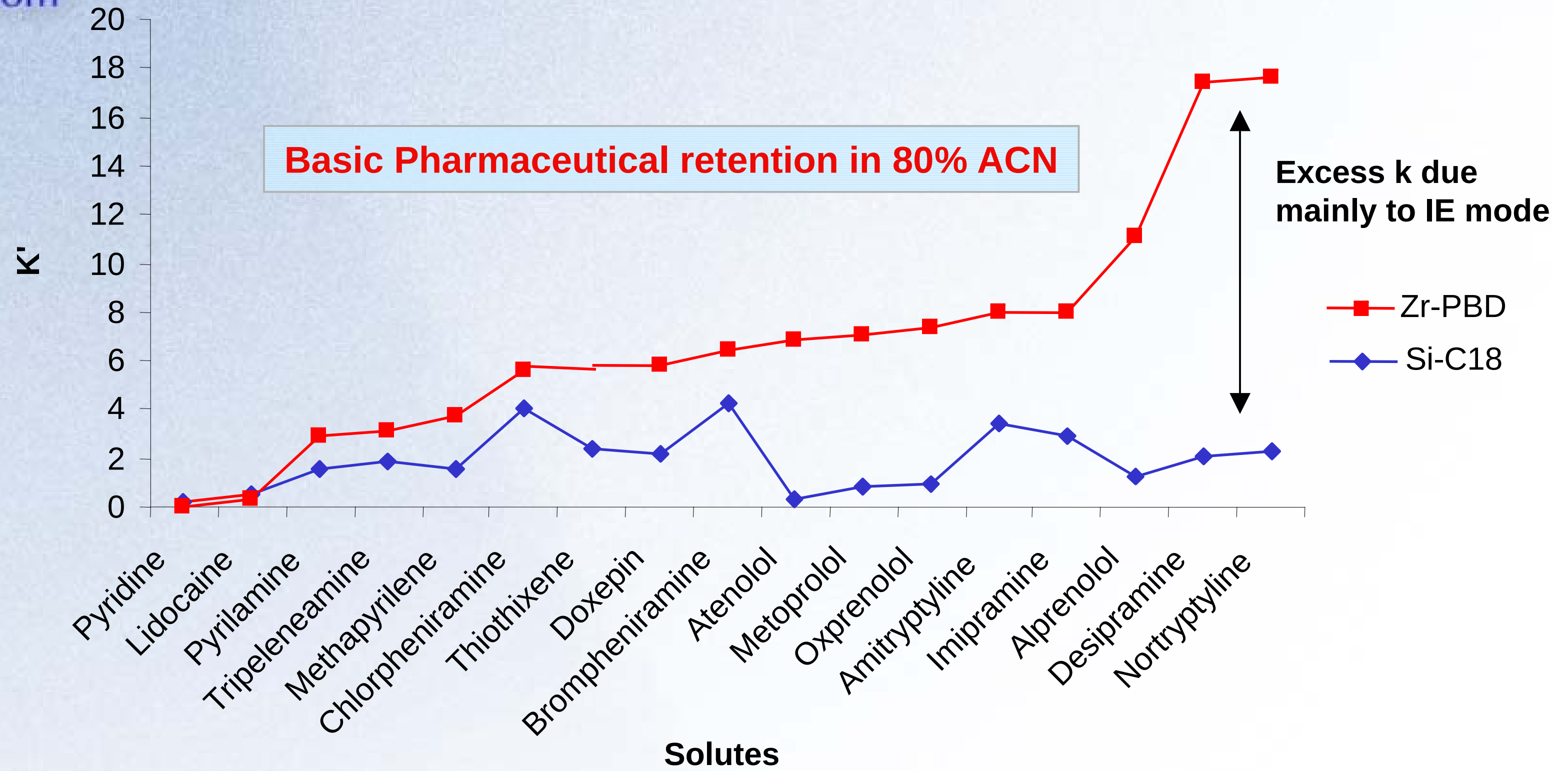
Zr-PBD and Si-C18 have very different selectivity for ionic drugs (especially in phosphate) due to the SCX ZrO_2 component.

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

Cation Retention Observed for Zr-PBD Even in High Organic



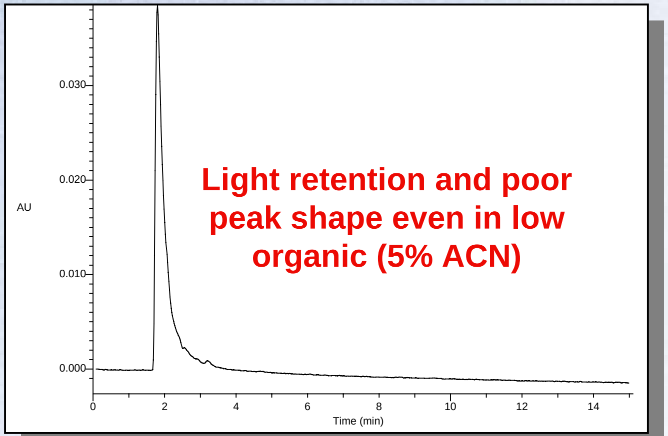
LC Conditions: Machine-mixed 80/20 ACN/10 mM ammonium acetate pH=6.7 without pH adjustment; Flow rate, 1.0 mL/min.; Injection volume 0.1 µL; Temperature, 35 ^{o}C; Detection at 254 nm; Columns, Zr-PBD, 50 x 4.6 mm i.d. (3 µm particles); Silica-C18 150 x 4.6 mm i.d., (3.5 µm particles).

Difficult Compounds for Silica Often Separate on Zirconia

ZirChrom®

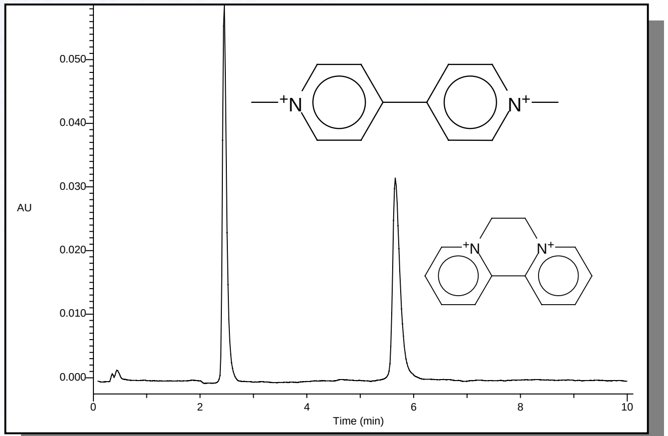
Quaternary amines paraquat and diquat are retained and resolved on Zr-PS (also Zr-PBD or bare ZrO_2) due to the cation exchange mechanism; 50% ACN is useful to suppress or regulate retention by RP mode.

Silica-C18:
reversed-phase (silanol tailing)



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

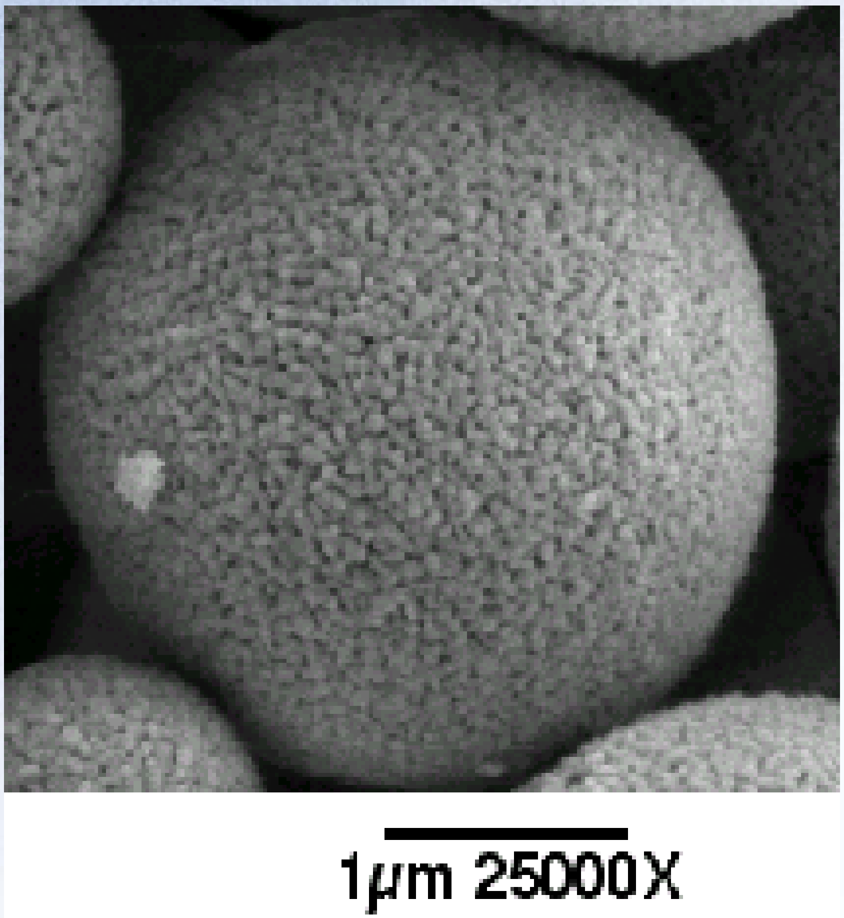
Zirconia-PS:
primarily ion-exchange



column: Discovery® Zr-PS, 7.5 cm x 4.6 mm, 3µm
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

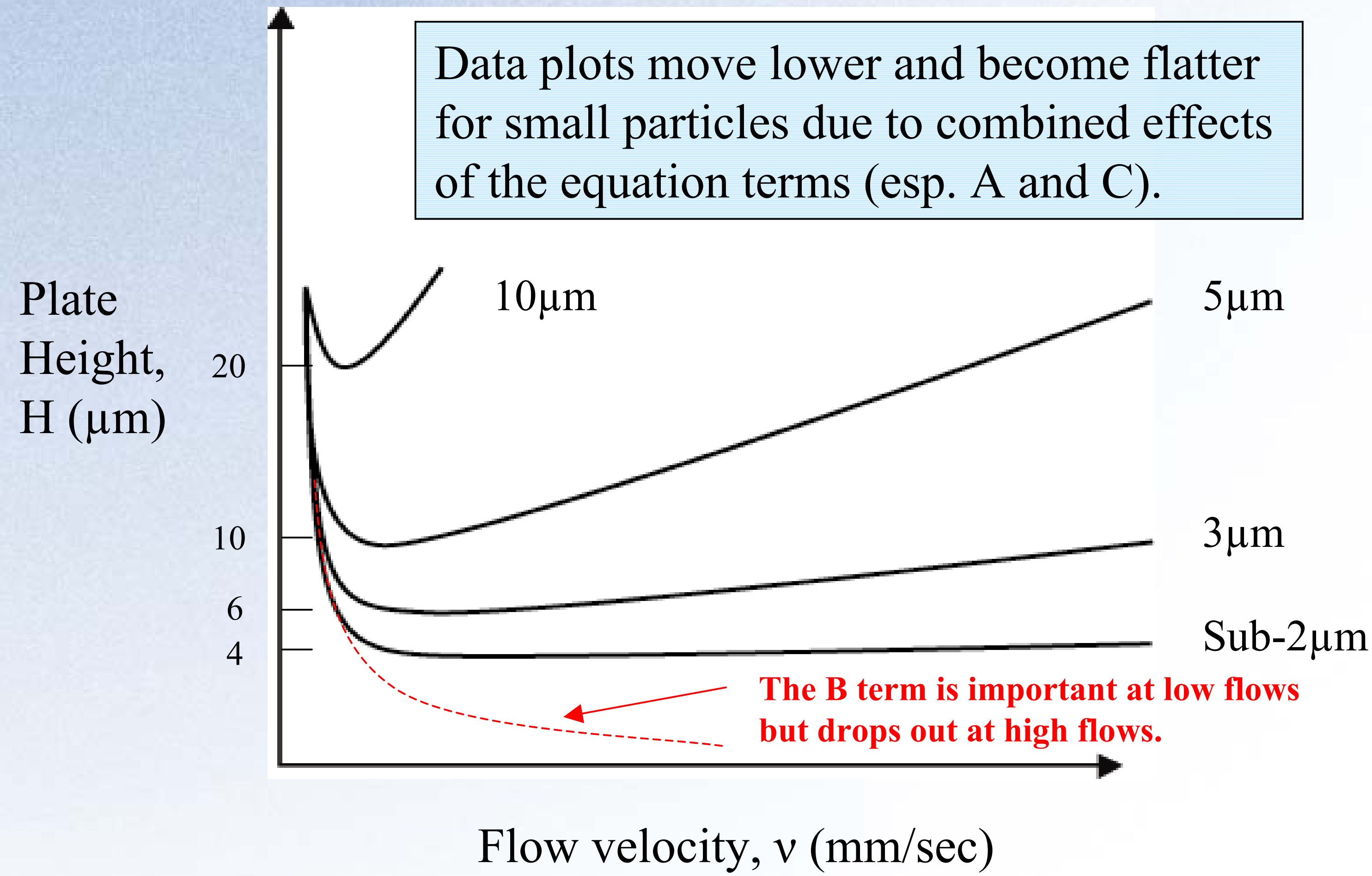
Analytical Diameter Porous Zirconia Particles



- 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^2/g
- Particle diameters: 3μm and sub-2μm
- Totally porous (porosity: 0.45)

Idealized van Deemter Plots

$$H = A + B/v + Cv$$



Elements of the drawing provided by Sigma-Supelco

Flow Studies on 3µm Zr-PBD: Alkylbenzenes

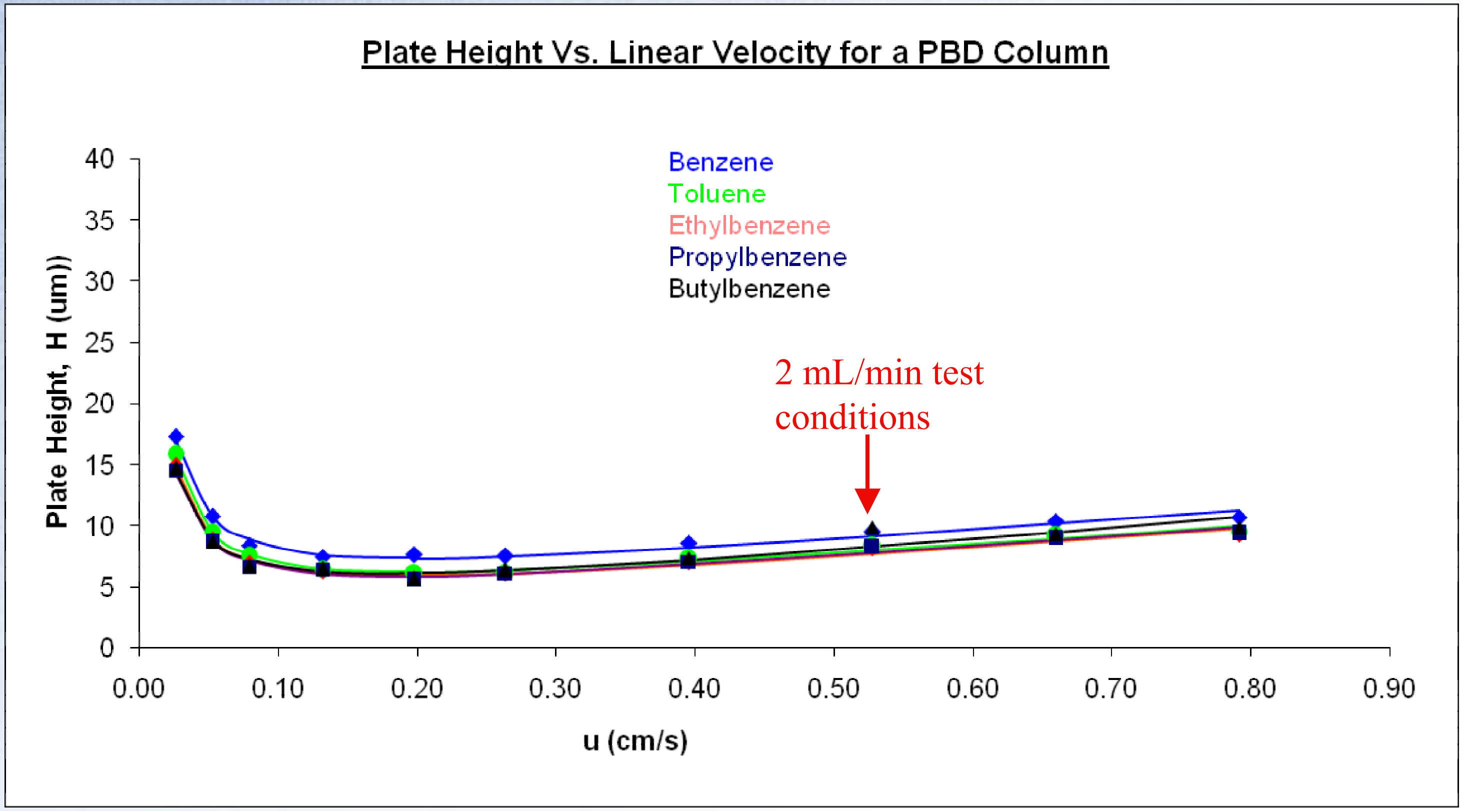


Plate height based on van Deemter Equation vs linear velocity at various temperatures for retained solutes: Alkylbenzenes, Temperature: 30 ºC, Mobile phase: 55/45 ACN/water, Column: ZirChrom®-PBD, 50 x 4.6mm, Agilent 1100/UV with micro cell (0.007''i.d. tubing).

Flow Studies on Sub-2µm Zr-PBD: Alkylbenzenes

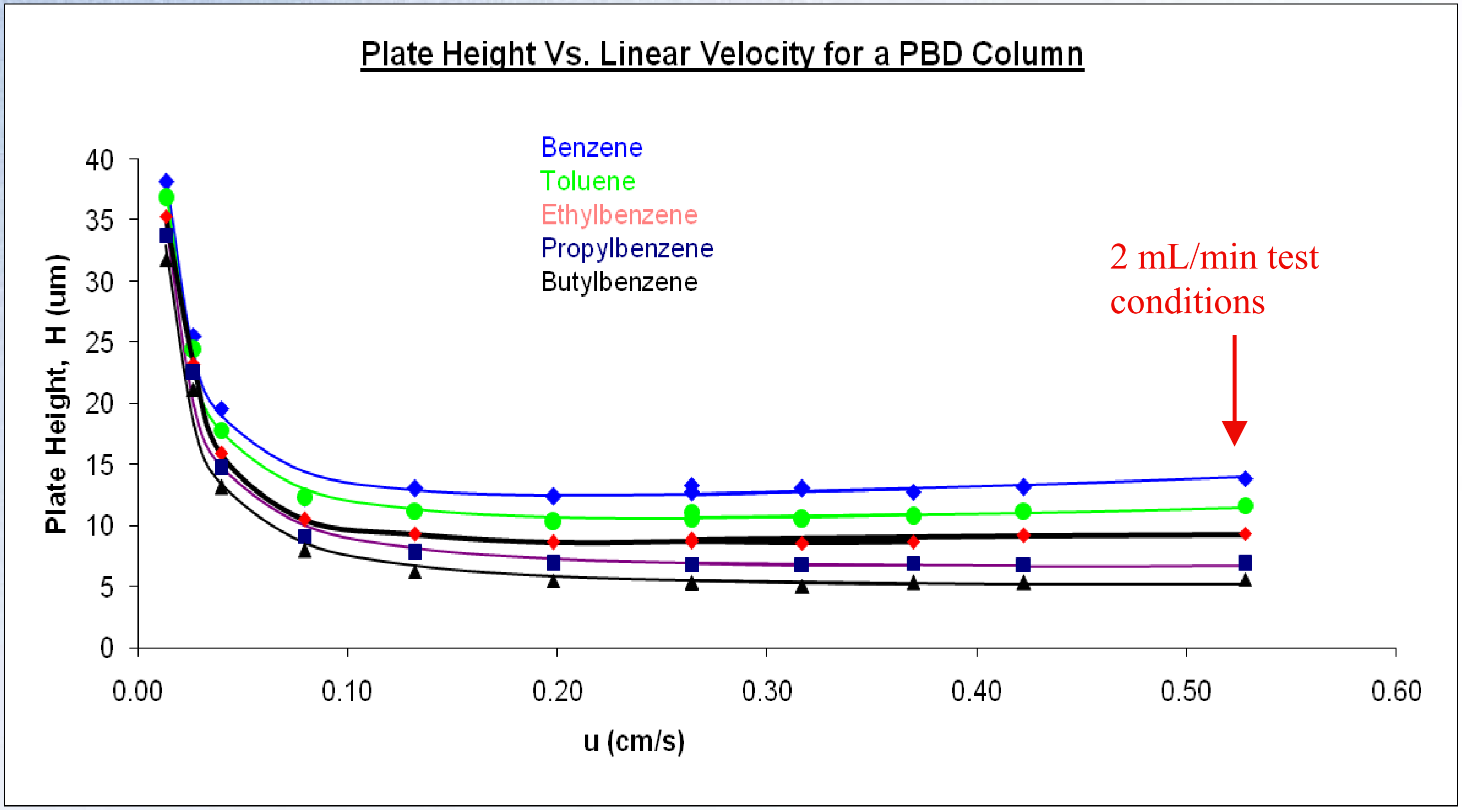
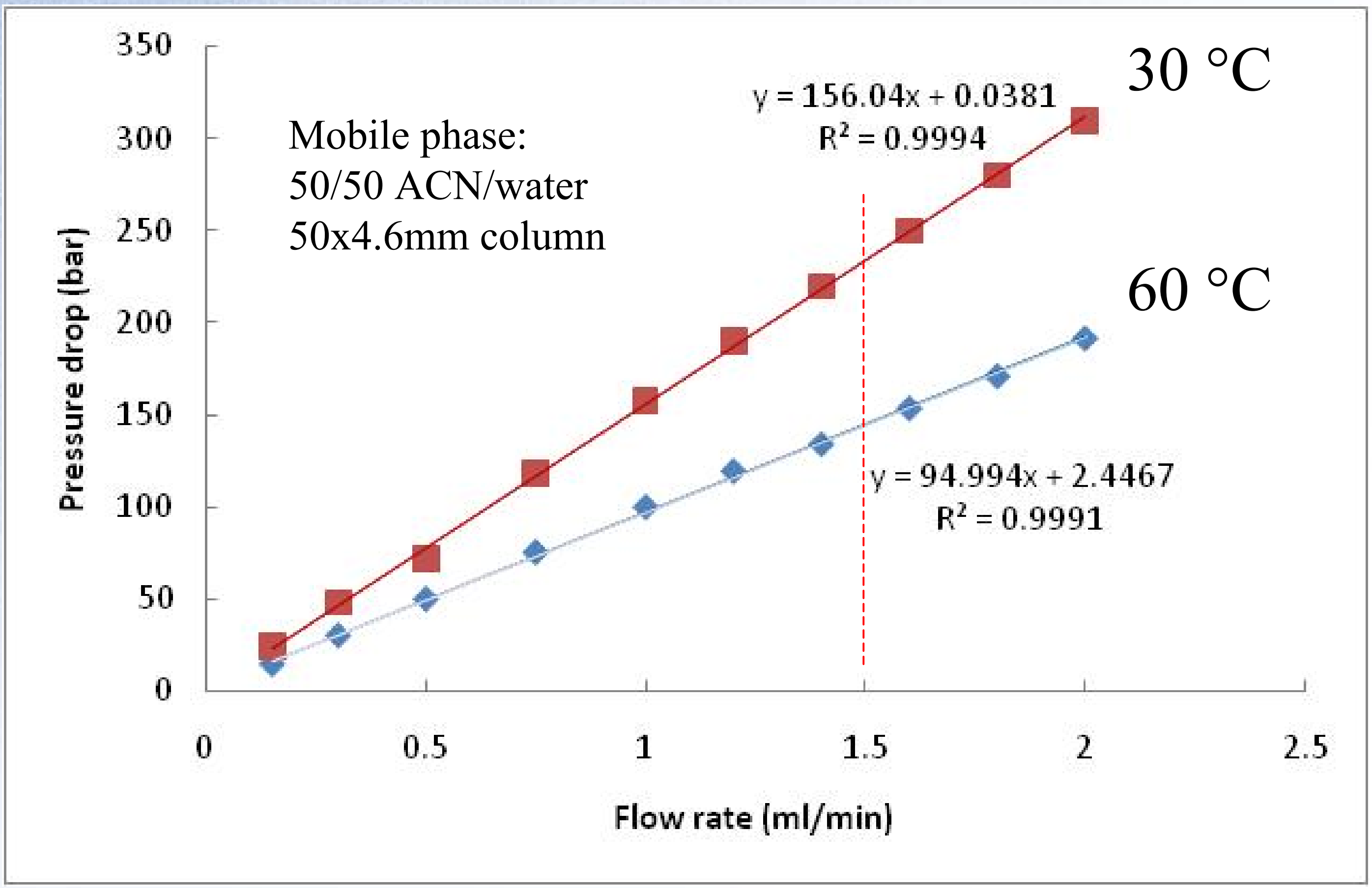


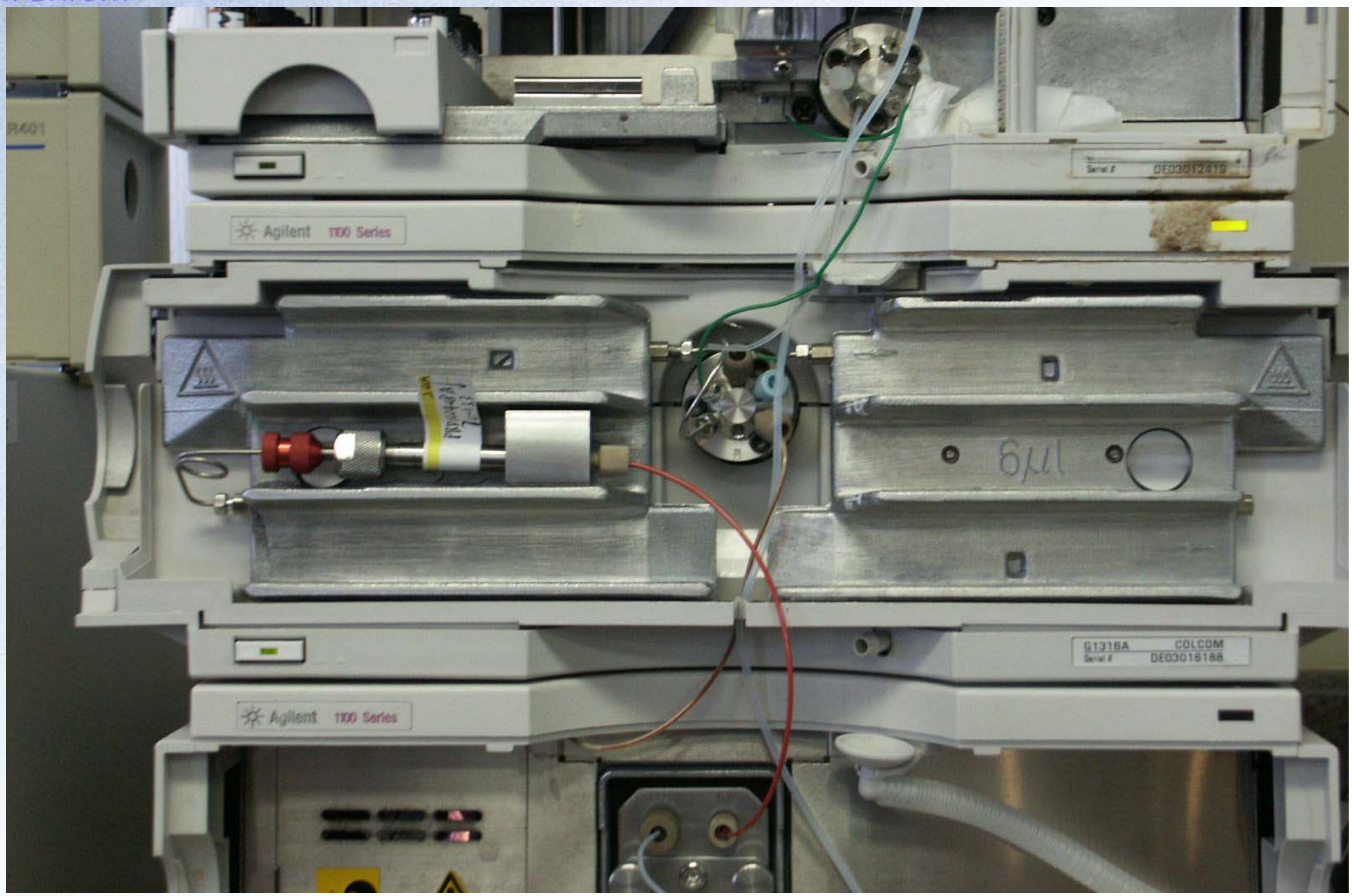
Plate height based on van Deemter Equation vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 ºC, Mobile phase: 50/50 ACN/water (keep k in the same range as 3µm particles), Column: 50 x 4.6mm, Agilent 1100/UV Micro Cell/0.007'' i.d. tubing.

Sub-2µm Pressure Drop at Different Temperatures*



*** 3µm particles show about half the pressure drop**

Optimization and Configuration for Elevated Temperature Operation



Background Pressure Drop Across Agilent 1100 at High Flow Rate

100% H_2O at 30 °C		100% H_2O 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).

Flow Studies on Sub-2μm Zr-PBD: Factory Instrument

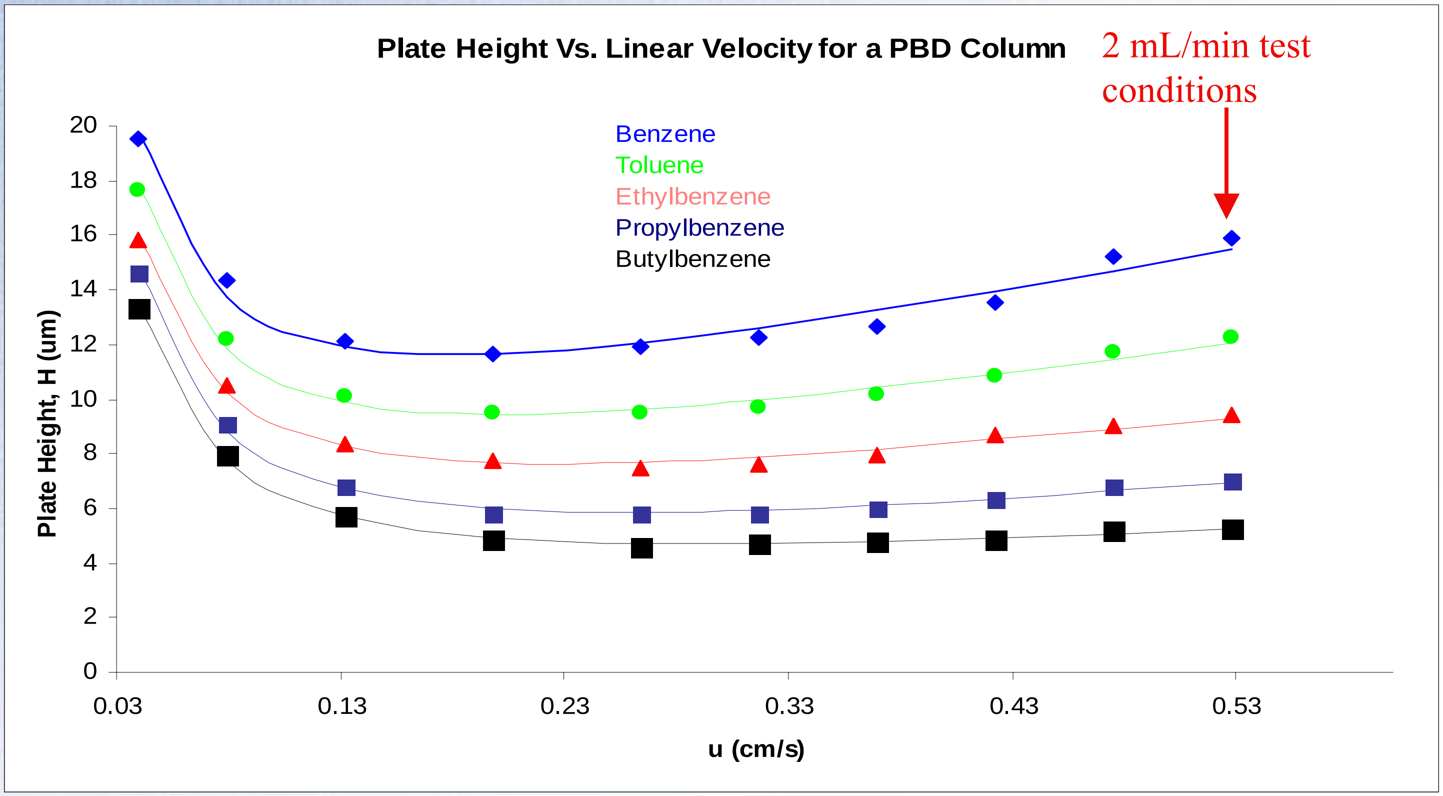


Plate height based on van Deemter Equation vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 ºC, Mobile phase: 50/50 ACN/water (keep k' in the same range as 3μm particles), Column: 50 x 4.6mm, Agilent 1100/UV with Standard Cell and 0.007'' i.d. tubing.

Flow Studies on Sub-2μm Zr-PBD: Factory + Micro Cell Only

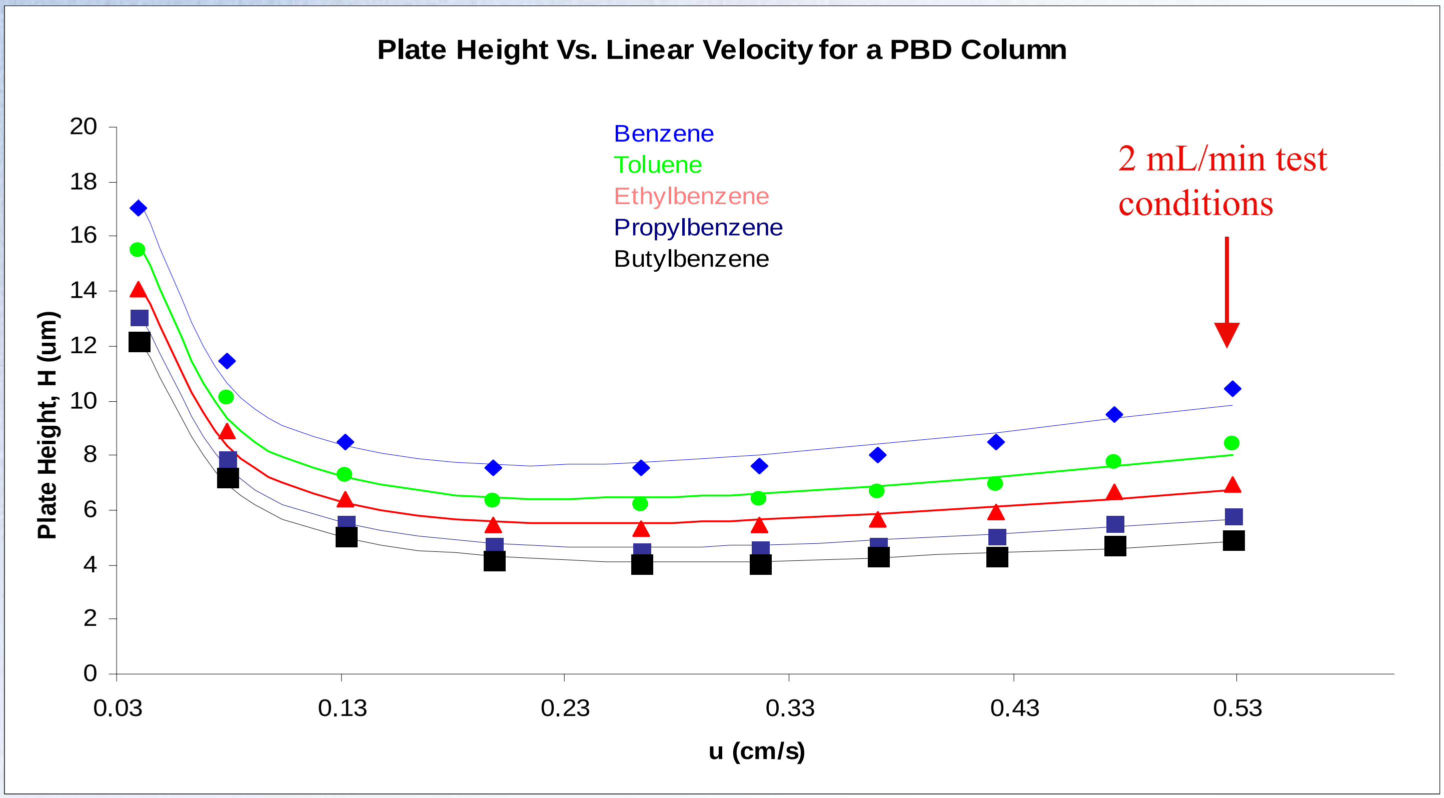


Plate height based on van Deemter Equation vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 ºC, Mobile phase: 50/50 ACN/water (keep k' in the same range as 3μm particles), Column: 50 x 4.6mm, Agilent 1100/UV with Micro Cell and 0.007'' i.d. tubing.

Flow Studies on Sub-2μm Zr-PBD: Micro Cell + Optimized Tubing

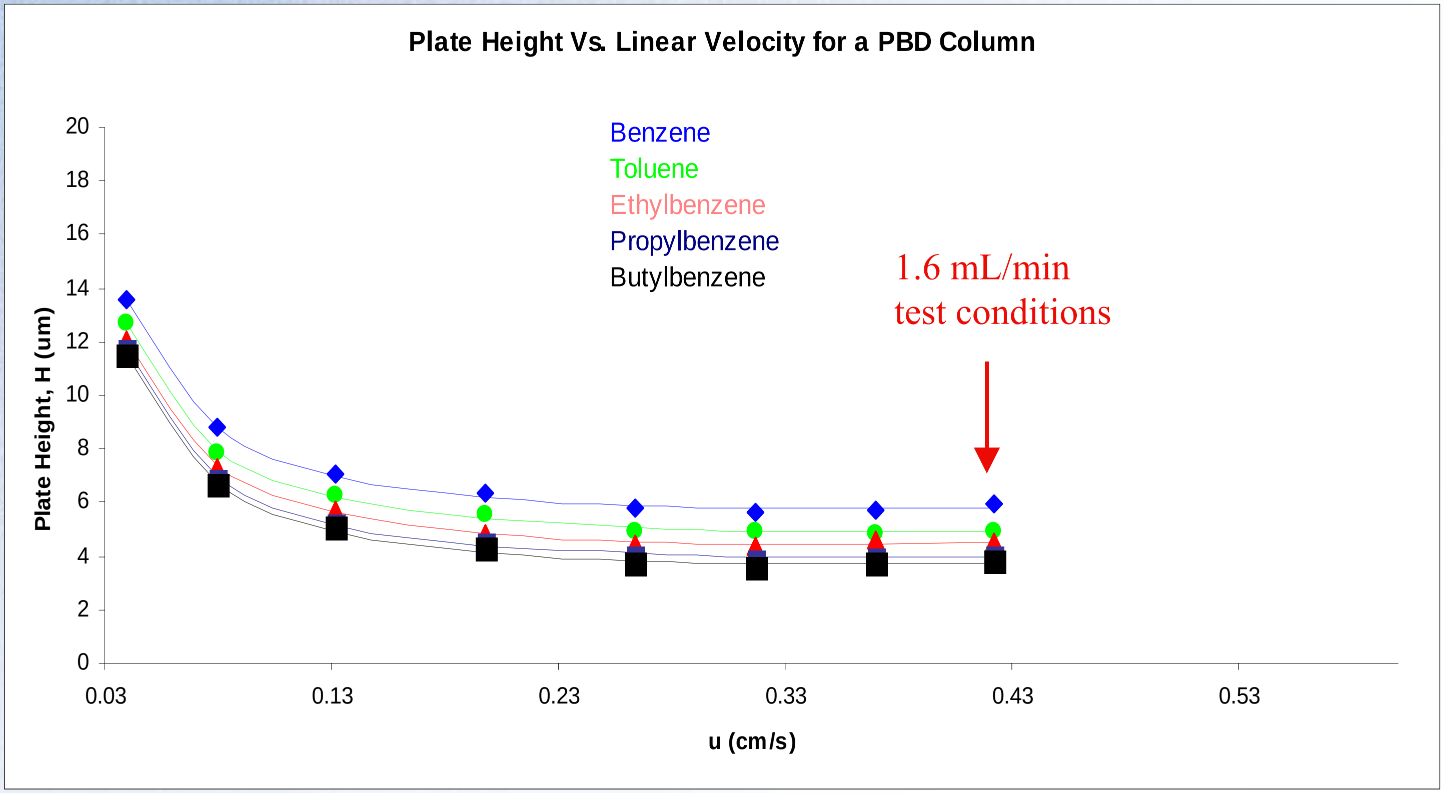


Plate height based on van Deemter Equation vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 ºC, Mobile phase: 50/50 ACN/water (keep k' in the same range as 3μm particles), Column: 50 x 4.6mm, Agilent 1100/UV with Micro Cell and optimized 0.005'' i.d. tubing.

Flow Studies on Sub-2μm Zr-PBD: Heat Exchanger + Fitting + μCell

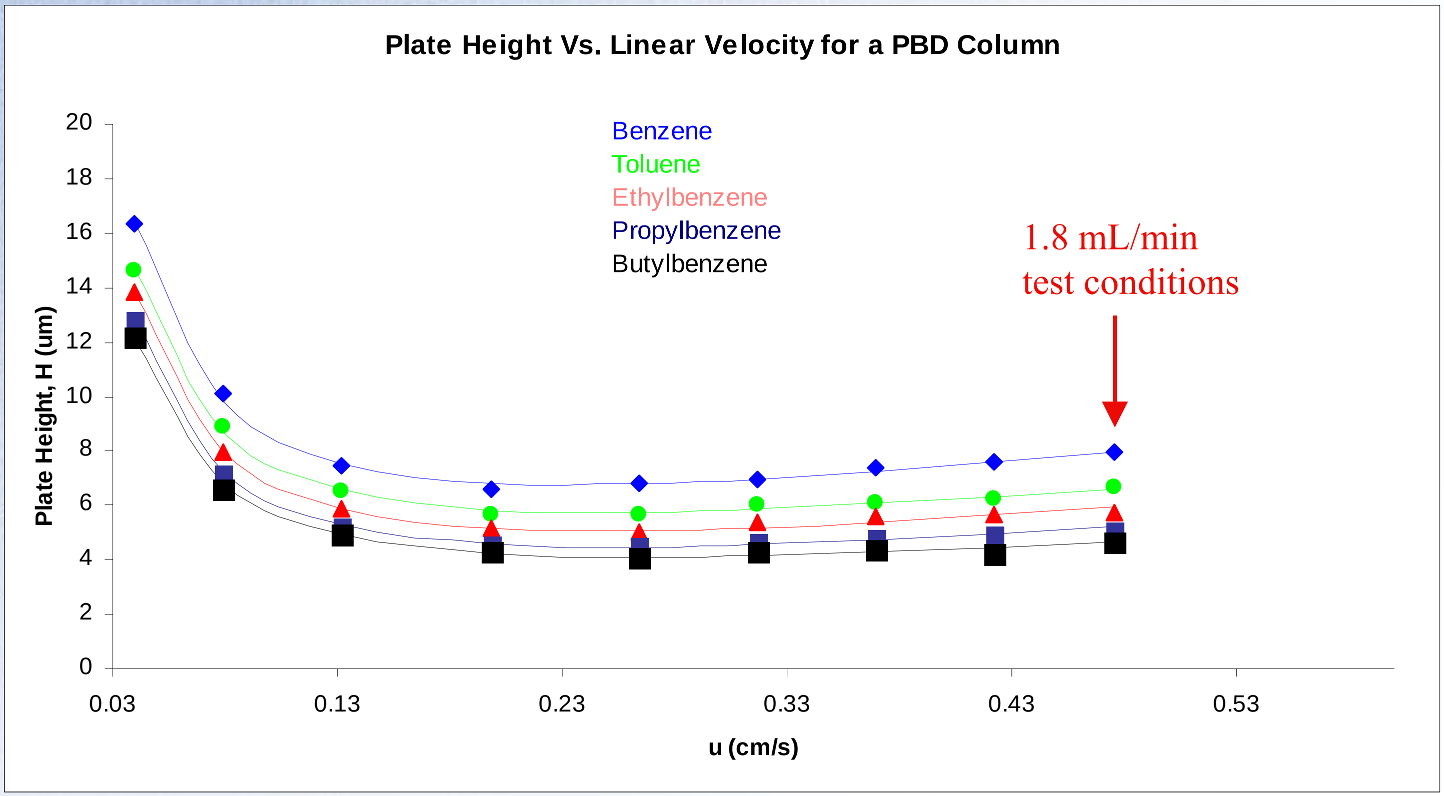


Plate height based on van Deemter Equation vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 ºC, Mobile phase: 50/50 ACN/water (keep k' in the same range as 3μm particles), Column: 50 x 4.6mm, Agilent 1100/UV with Micro Cell, high pressure fitting and passing through heat exchanger.

Flow Studies on Sub-2µm Zr-PBD: Factory Instrument at Ambient

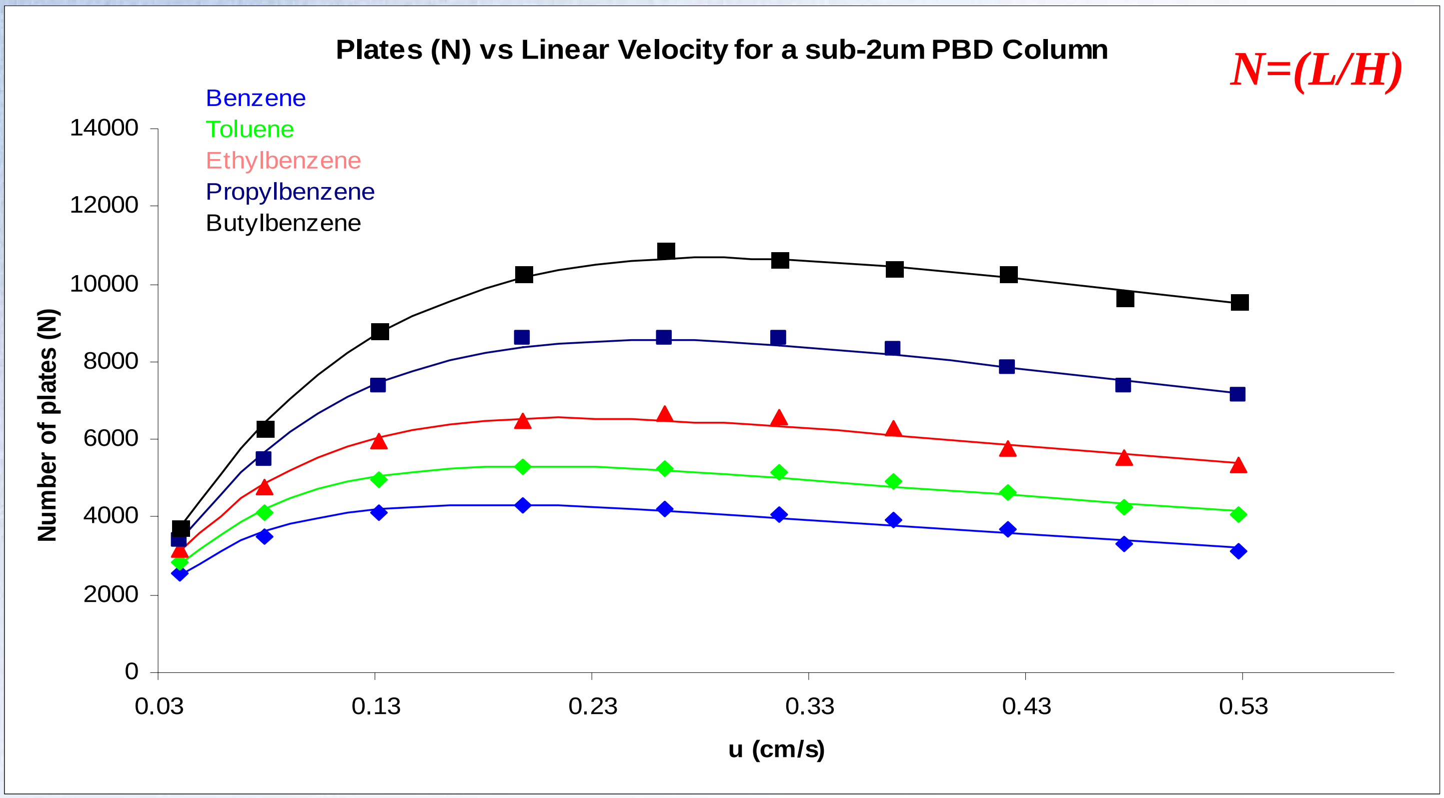


Plate height vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 ºC, Mobile phase: 50/50 ACN/water (keep k' in the same range as 3µm particles), Column: 50 x 4.6mm, Agilent 1100/UV with Standard Cell and 0.007'' i.d. tubing.

Flow Studies on Sub-2µm Zr-PBD: Micro Cell

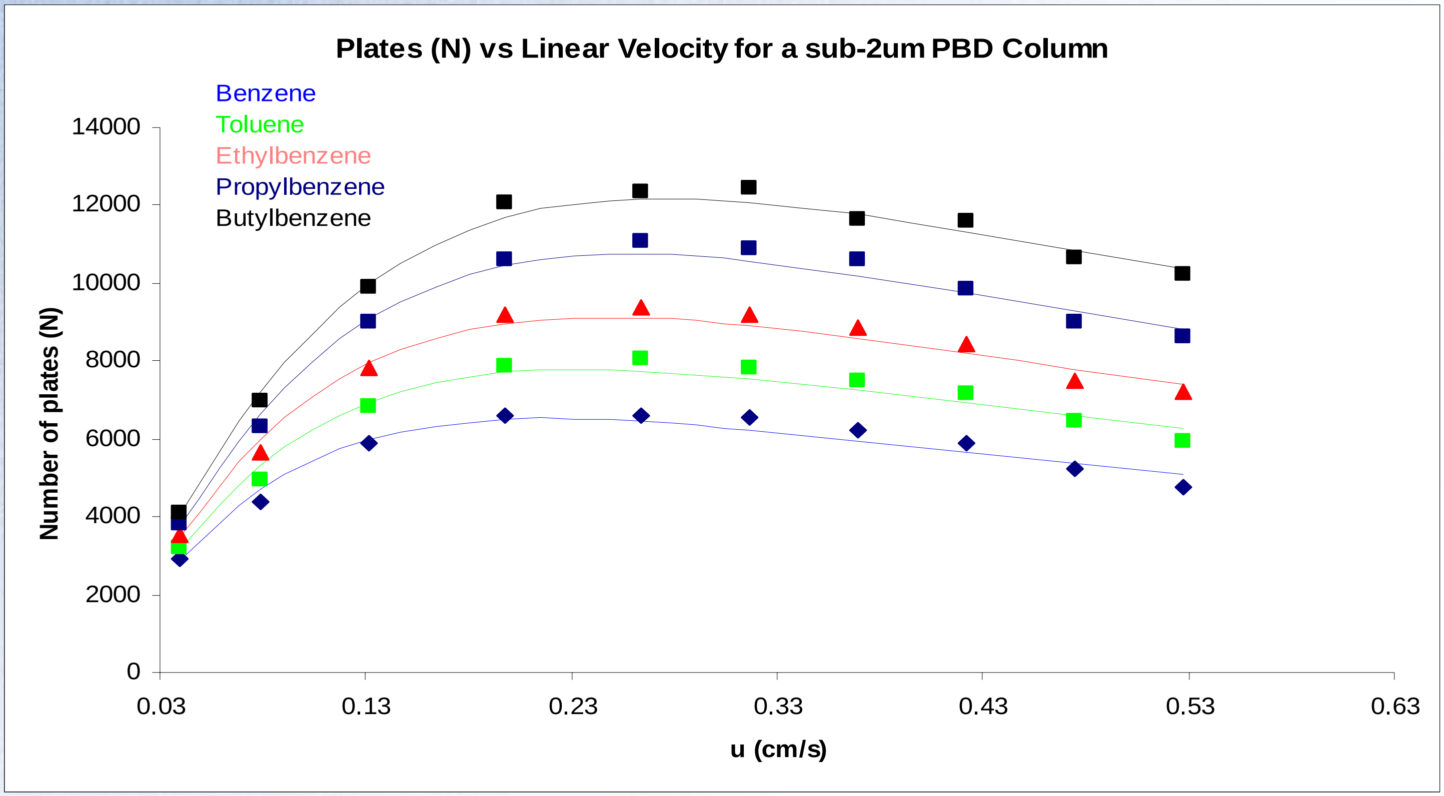


Plate height vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 ºC, Mobile phase: 50/50 ACN/water (keep k' in the same range as 3µm particles), Column: 50 x 4.6mm, Agilent 1100/UV with Micro Cell and 0.007'' i.d. tubing.

Flow Studies on Sub-2μm Zr-PBD: Micro Cell + Tubing

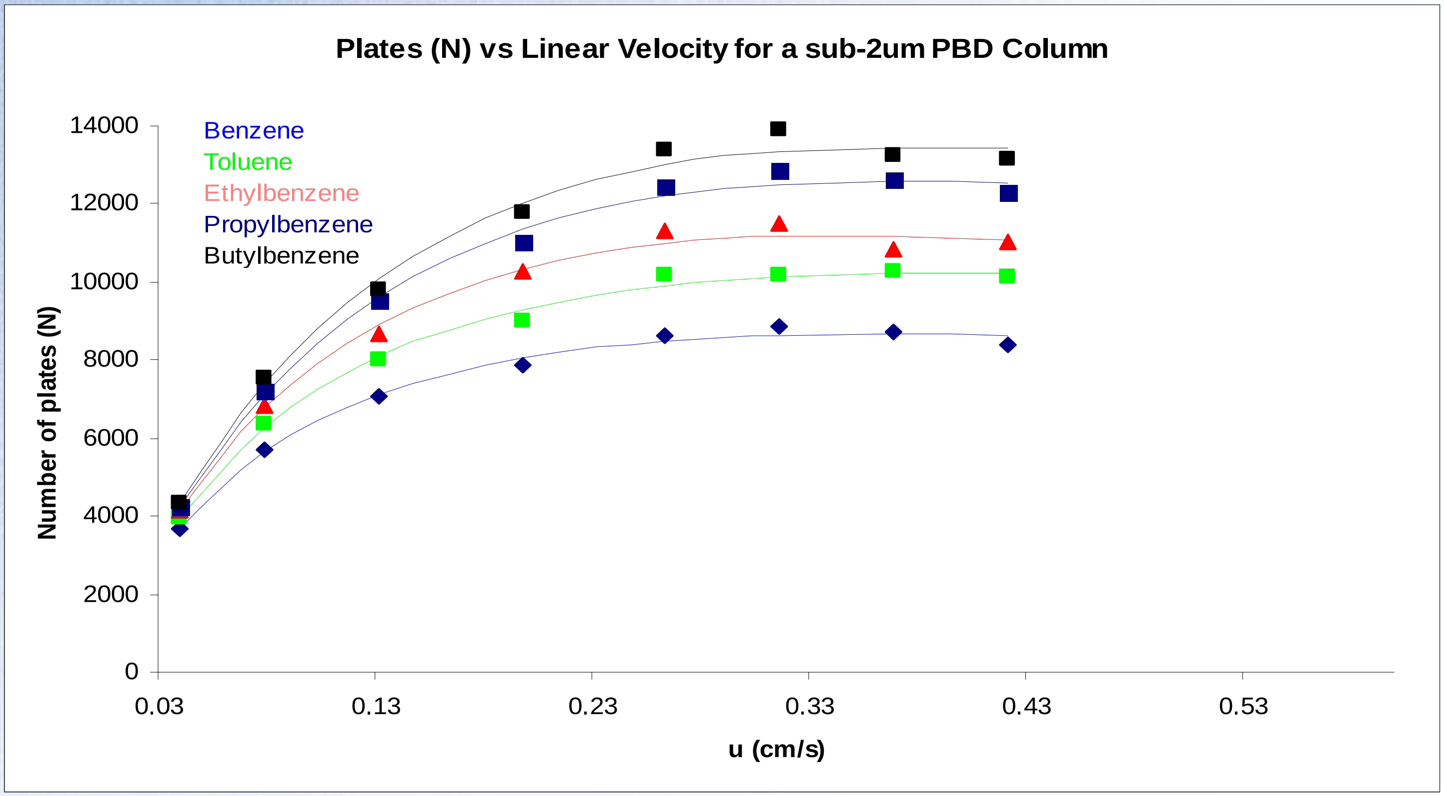


Plate height vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 ºC, Mobile phase: 50/50 ACN/water (keep k' in the same range as 3μm particles), Column: 50 x 4.6mm, Agilent 1100/UV with Micro Cell and 0.005'' i.d. tubing.

Flow Studies on Sub-2μm Zr-PBD: Heat Exchanger + Fitting + μCell

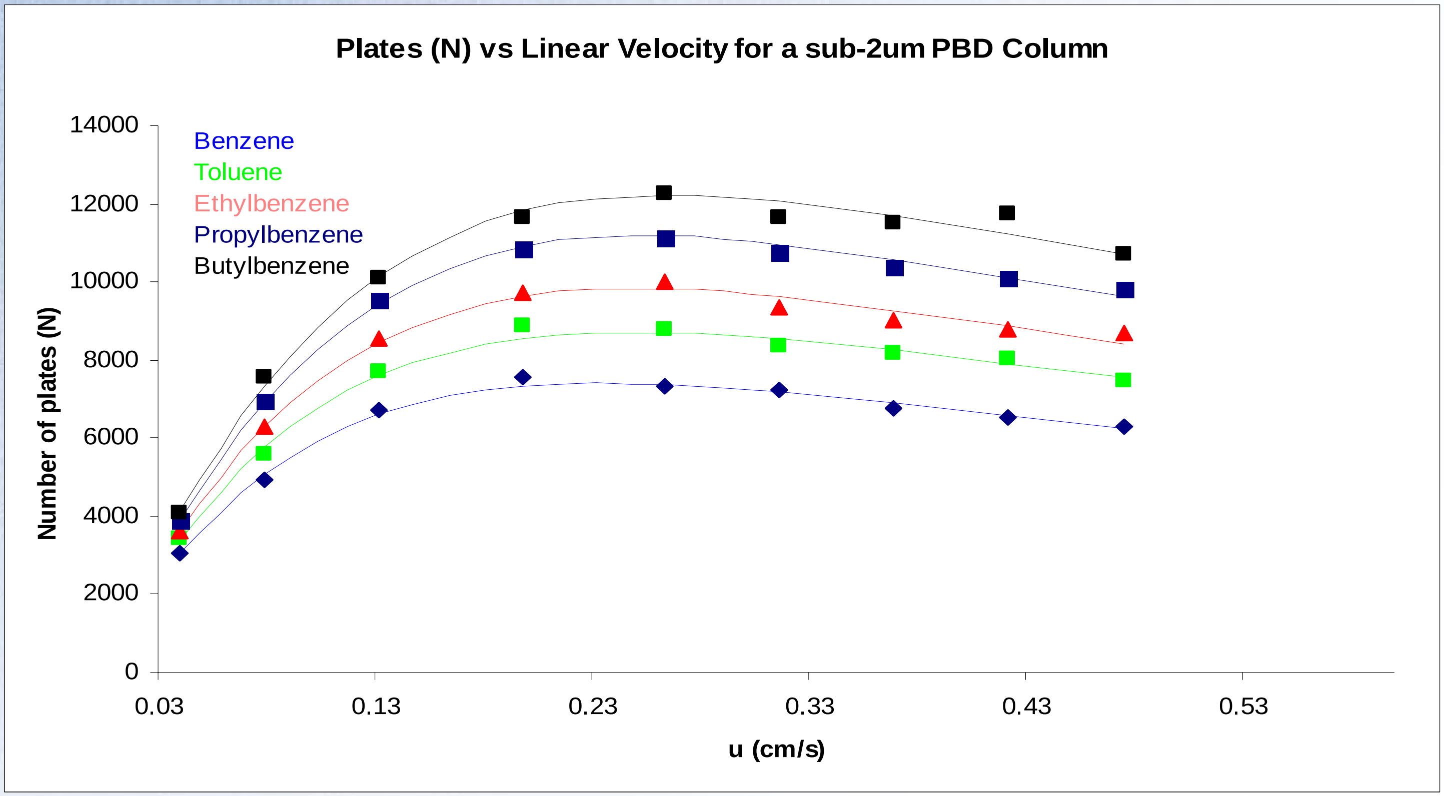


Plate height vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 °C, Mobile phase: 50/50 ACN/water (keep k' in the same range as 3μm particles), Column: 50 x 4.6mm, Agilent 1100/UV with Micro Cell, high pressure fitting and passing through heat exchanger.

Flow Studies on 3μm Zr-PBD: Factory Instrument

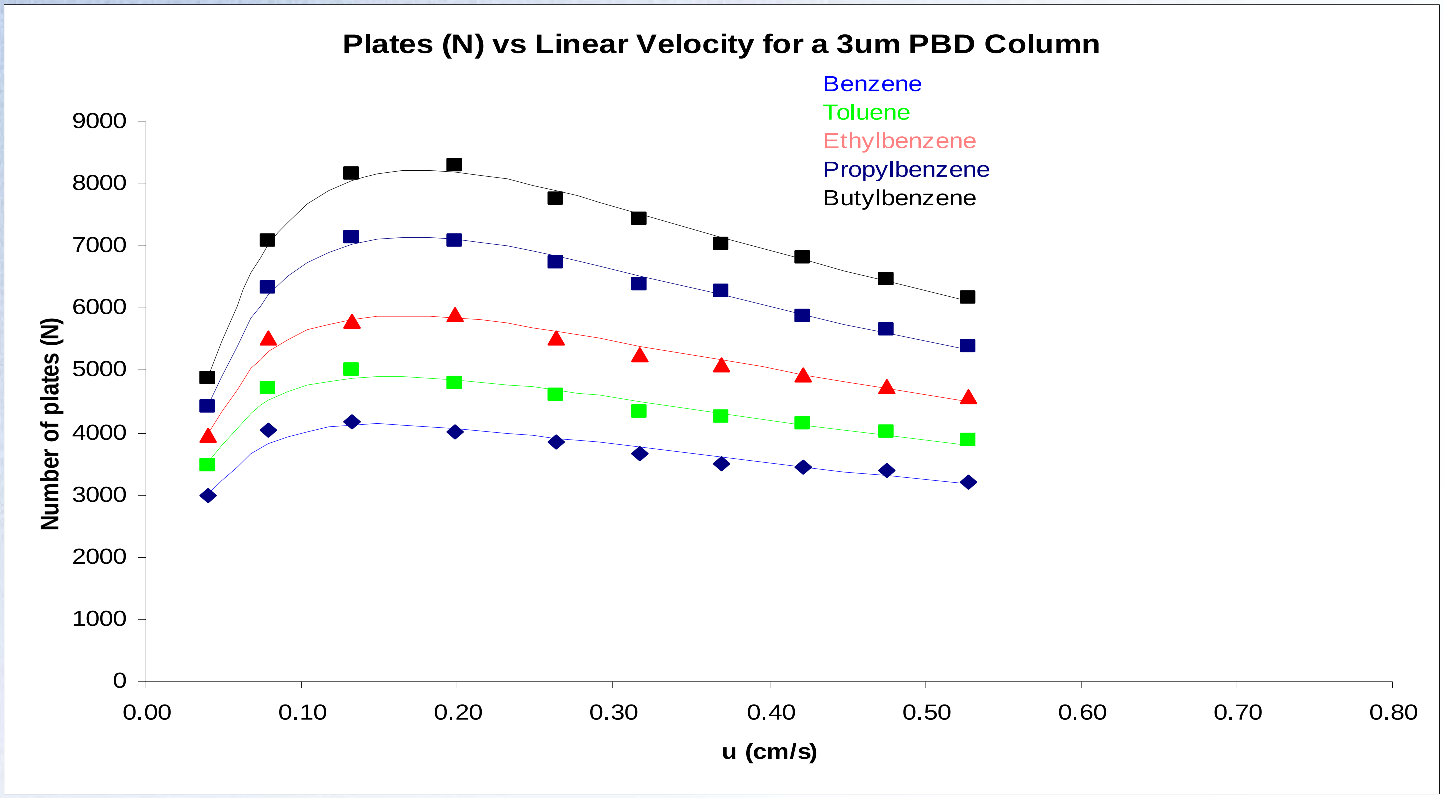


Plate height based on van Deemter Equation vs linear velocity at various temperatures for retained solutes: Alkylbenzenes, Temperature: 30 ºC, Mobile phase: 50/50 ACN/water, Column: ZirChrom®-PBD, 50 x 4.6mm, Agilent 1100/UV standard cell (0.007'' i.d. tubing).

Flow Studies on 3μm Zr-PBD: Factory + Micro Cell

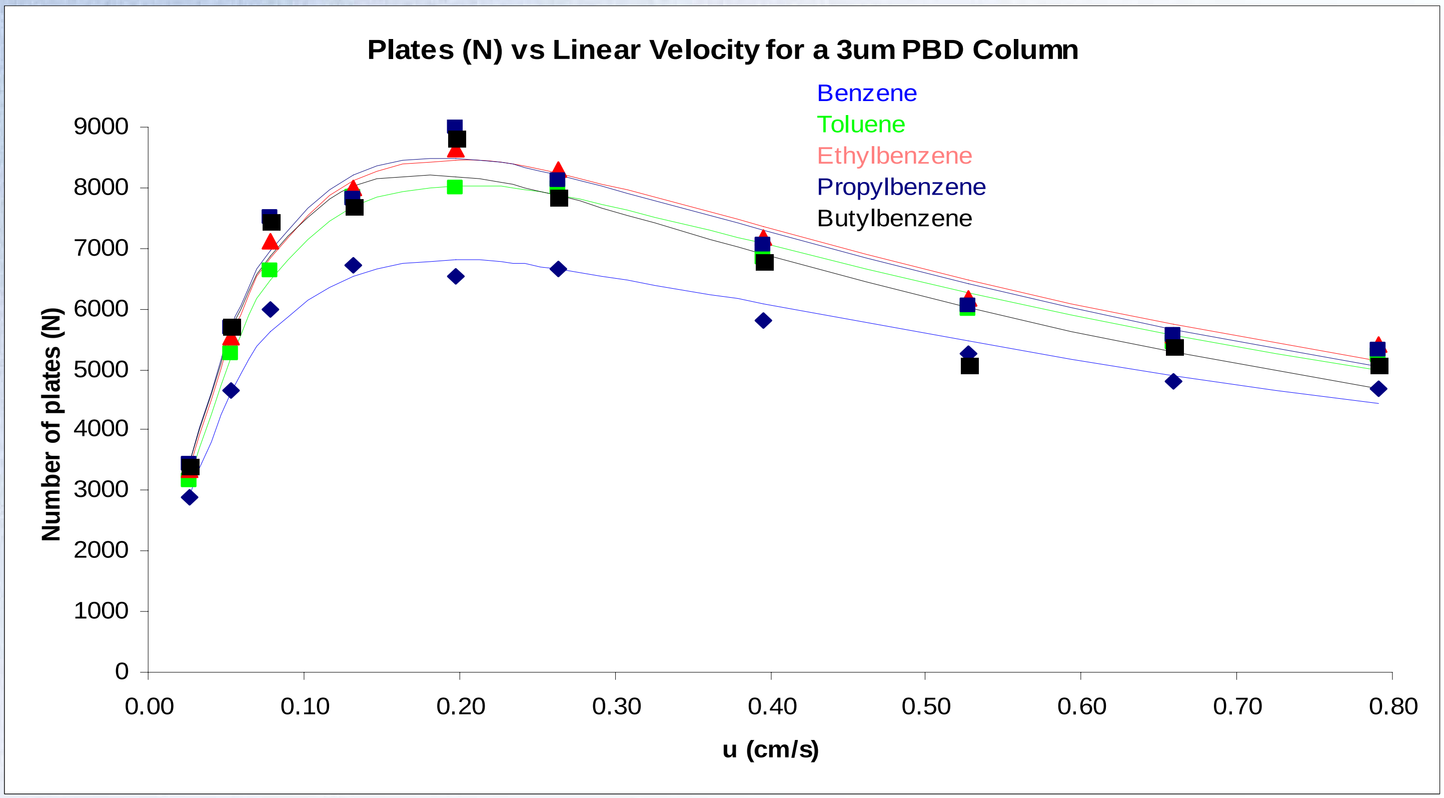
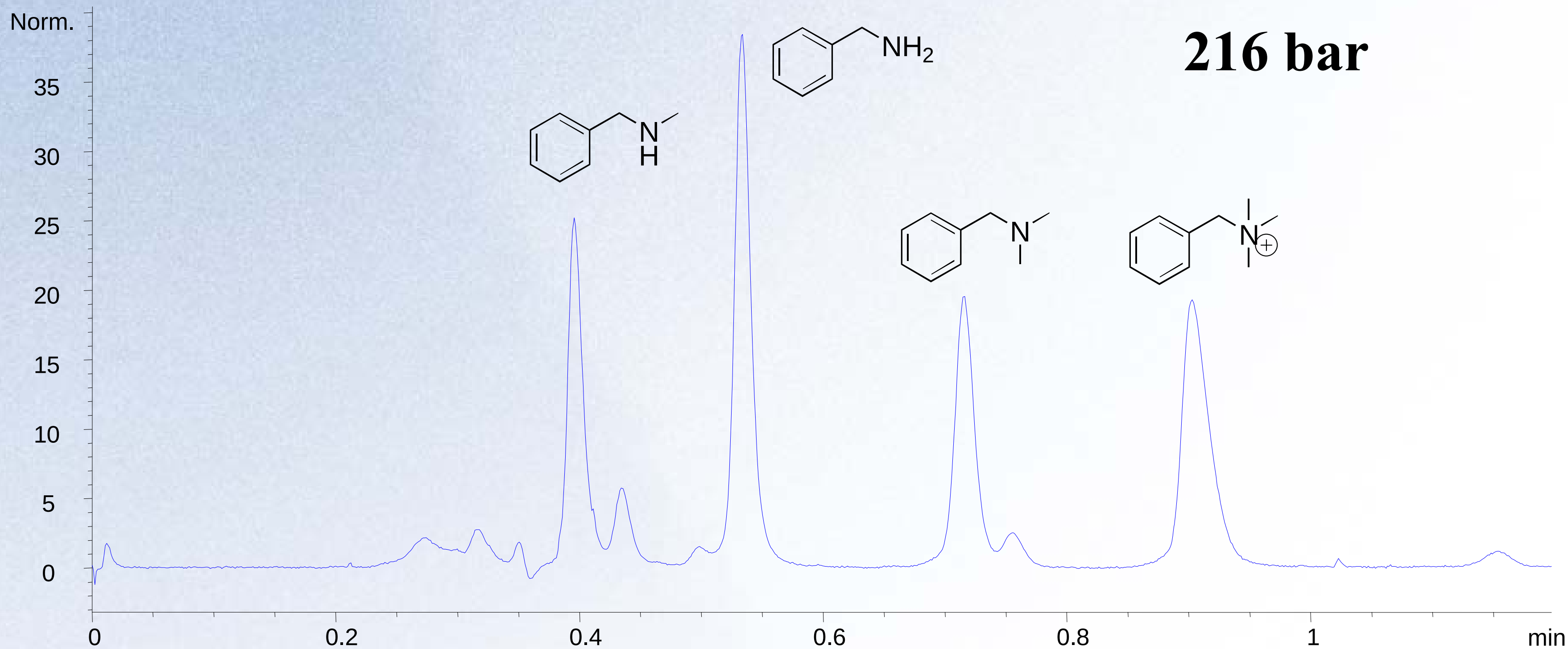


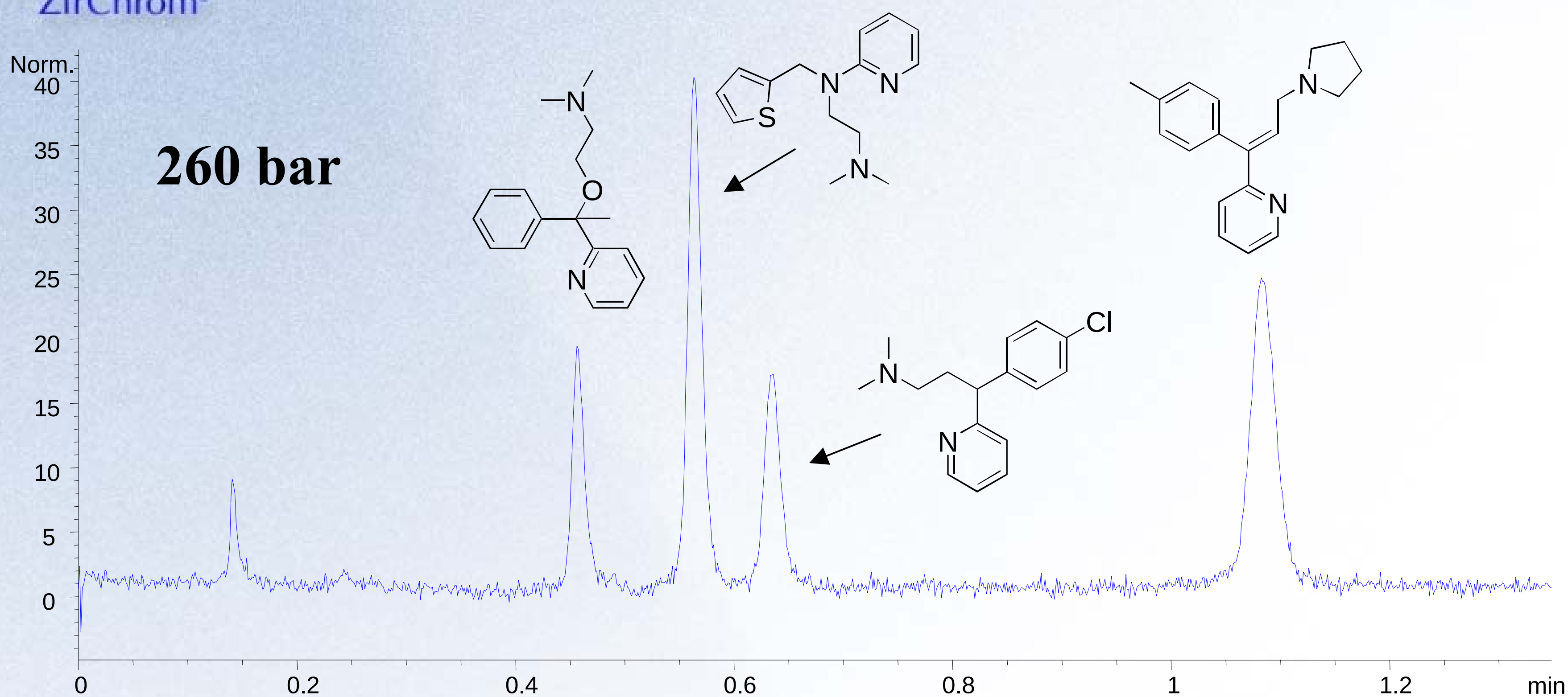
Plate height based on van Deemter Equation vs linear velocity at various temperatures for retained solutes: Alkylbenzenes, Temperature: 30 °C, Mobile phase: 50/50 ACN/water, Column: ZirChrom®-PBD, 50 x 4.6mm, Agilent 1100/UV micro cell (0.007'' i.d. tubing).

Alkylbenzylamine Separation on sub-2µm Zr-PBD, 50 °C



LC Conditions: Column: ZirChrom®-PBD, 50 x 4.6 mm i.d., sub-2µm Mobile Phase: 21/79 ACN/20 mM K_3PO_4 at pH=12; Flow rate: 1.5 mL/min; Temperature: 50 °C; Injection Vol.: 3.0 µL; Detection: UV at 254 nm

Antihistamine Separation on sub-2μm Zr-PBD, 80 °C



LC Conditions: Column: ZirChrom®-PBD, 50 x 4.6 mm i.d., sub-2μm Mobile Phase: 28/72 ACN/50 mM TMA-OH at pH=12.2; Flow rate: 2.5 mL/min; Temperature: 80 °C; Injection Vol.: 2.0 μL; Detection: UV at 254 nm

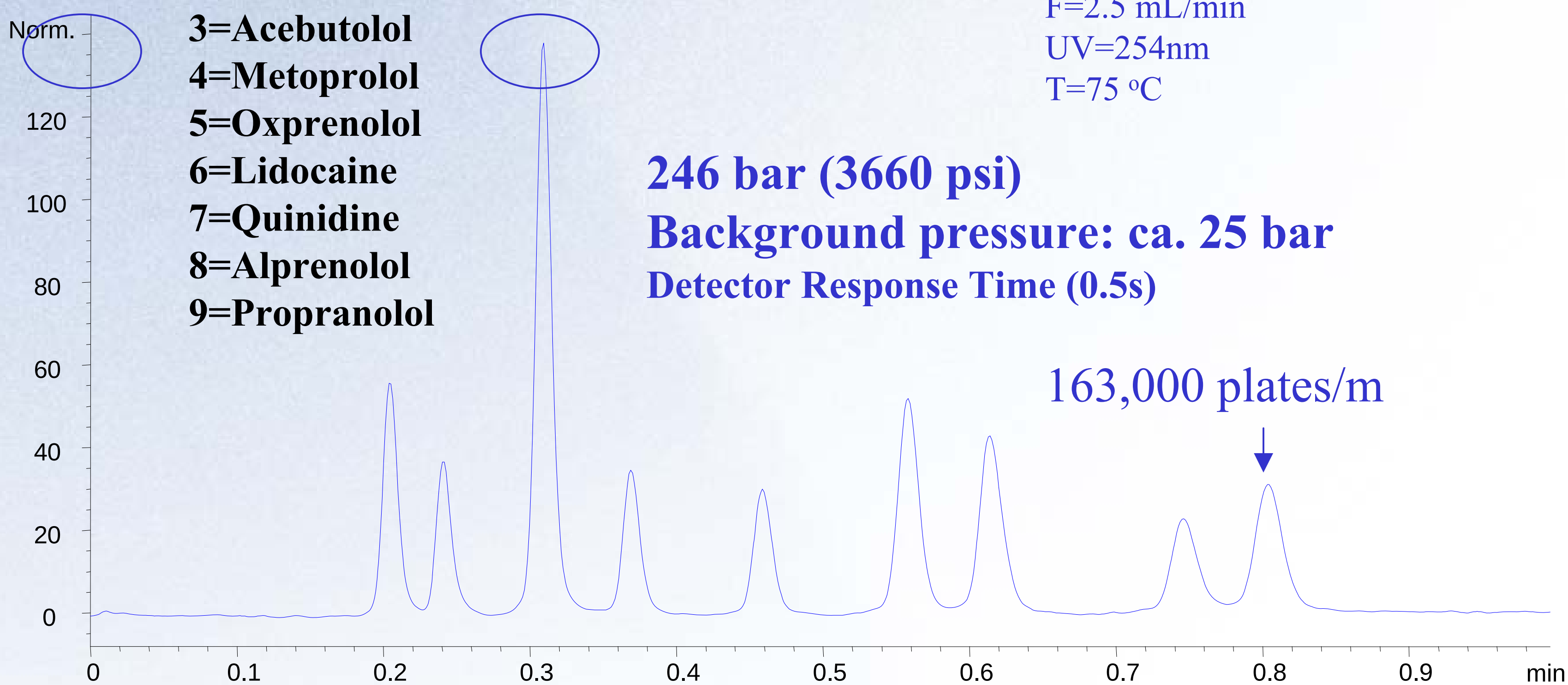
β-blockers on ZirChrom®-PBD sub-2μm, High Temp

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

ZirChrom®-PBD
50mm x 4.6mm, sub-2μm
22/78 ACN/20mM K_3PO_4 at pH=12
F=2.5 mL/min
UV=254nm
T=75 °C

246 bar (3660 psi)
Background pressure: ca. 25 bar
Detector Response Time (0.5s)

163,000 plates/m



β-blockers on ZirChrom®-PBD sub-2μm, High Temp, Faster sampling

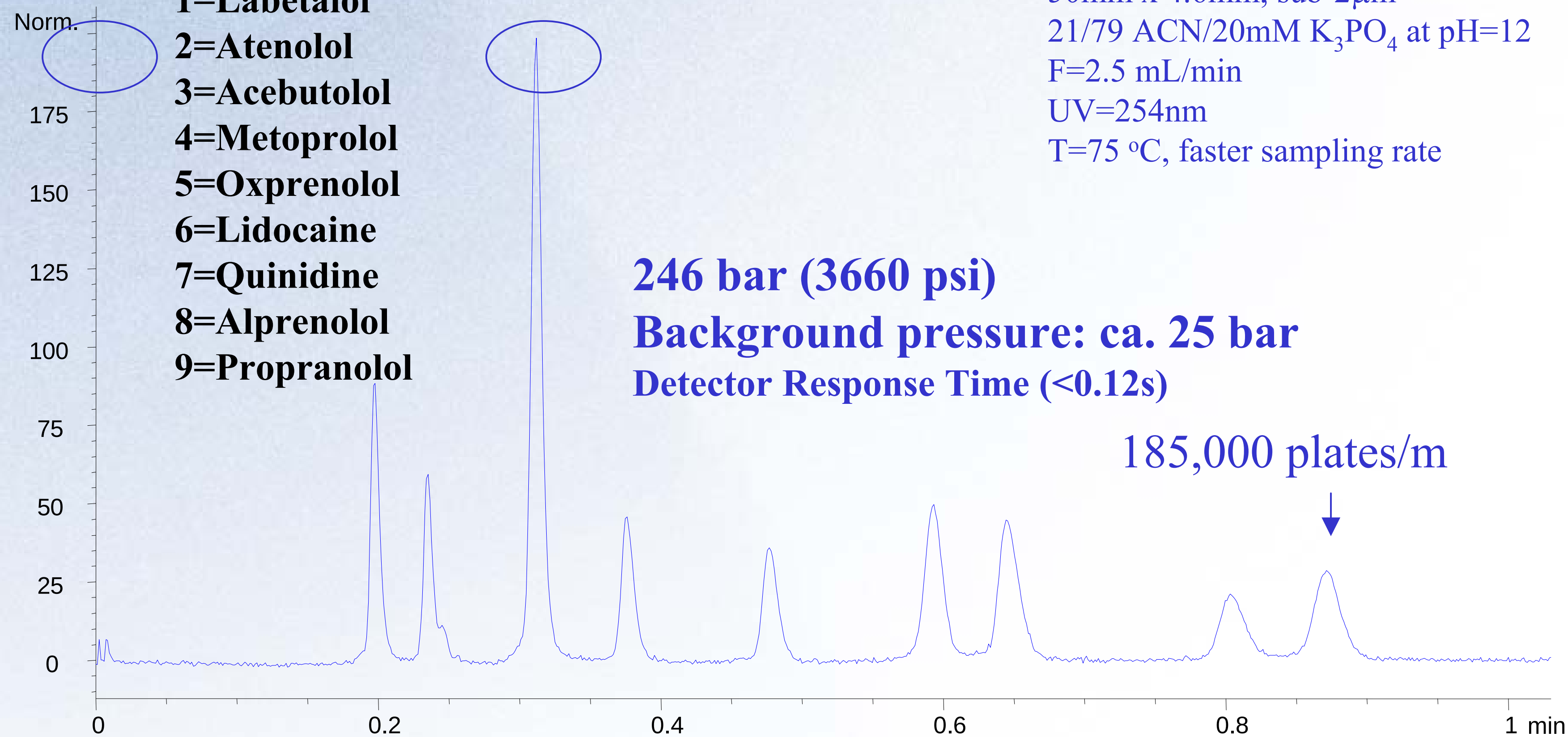
ZirChrom®

<u>Analytes</u>
1=Labetalol
2=Atenolol
3=Acebutolol
4=Metoprolol
5=Oxprenolol
6=Lidocaine
7=Quinidine
8=Alprenolol
9=Propranolol

ZirChrom®-PBD
50mm x 4.6mm, sub-2μm
21/79 ACN/20mM K_3PO_4 at pH=12
F=2.5 mL/min
UV=254nm
T=75 °C, faster sampling rate

246 bar (3660 psi)
Background pressure: ca. 25 bar
Detector Response Time (<0.12s)

185,000 plates/m



ZirChrom®

Plans for Further Development

- Extend the range of ultra-high speed applications using sub-2µm Zr-PBD, especially at high pH and temperature ("extreme conditions for silica"); develop generic conditions for LC-MS.
- Develop sub-2µm Zr-CARB and compare performance to Zr-PBD under ambient and extreme temperature conditions.
- Study additional advantages of optimizing the IBW of an Agilent Model 1100 HPLC instrument using a high performance (Model 1200) heat exchanger.

Conclusions

- Multi-mode HPLC columns have become popular for difficult applications where compounds have ionic character and vary widely in chemical nature. Several ZirChrom® phases are ideal and popular for multi-mode applications and are stable over a much wider range of pH and temperature than any silica-based phase.
- Zirconia 3μm HPLC columns are currently available in a wide range of stable coatings and produce efficiencies in excess of 100,000 N/M.
- New sub-2μm zirconia UHPLC columns with very high efficiency in excess of 200,000 N/M with a PBD polymer-coated phase permit higher speed separations with shorter residence time at elevated temperatures.

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, 30th Annual HPLC Meeting, Oral Presentation, 2006, San Francisco, CA.
3. R. A. Henry and H. K. Brandes, Eastern Analytical Symposium, Oral Presentation, 2006, Somerset, NJ.
4. B. Yan. C. V. McNeff. R. A. Henry and D. Nowlan, Eastern Analytical Symposium, Poster, 2008, Somerset, NJ.
5. B. Yan. C. V. McNeff. R. A. Henry and D. Nowlan, Pittsburgh Analytical Conference, 2009, Oral Paper, Chicago, IL..
6. B. Yan. C. V. McNeff. R. A. Henry and D. Nowlan, Horvath Award Symposium, 2009, Oral Paper, Hartford, CT.

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