Combining High Temperature and Small Particles: The Advantages of Zirconia

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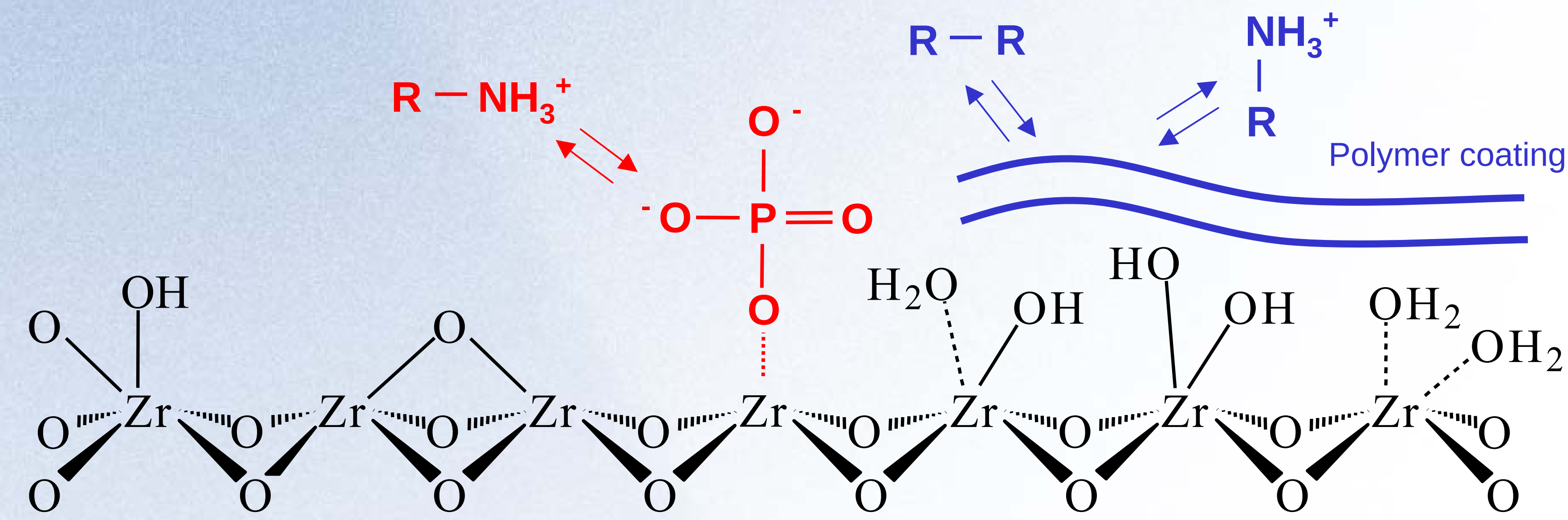
Specialists in High Efficiency, Ultra-Stable Phases for HPLC

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-2µm particles can be observed (at ambient or elevated temperature) for other packings.

Addition of RP Behavior with Coated Zirconia Phases



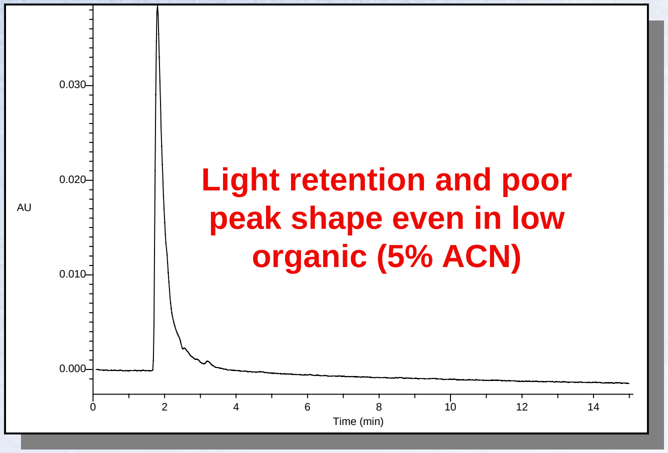
- Ionic solute retention (and selectivity) is modulated by pH, buffer/salt type and concentrations, and temperature.
- RP solute retention is modulated by organic solvent.
- *Five* important mobile phase variables must be controlled.

Difficult Compounds for Silica Often Separate on Zirconia

ZirChrom®

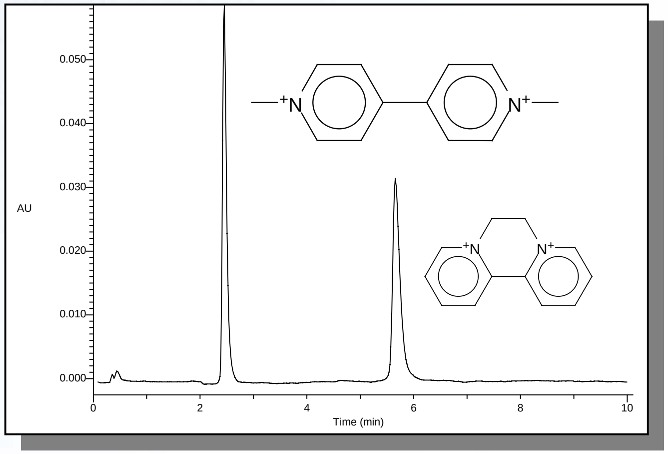
Quaternary amines paraquat and diquat are retained and resolved on Zr-PS (also Zr-PBD or bare ZrO_2) due to the cation exchange mechanism; 50% ACN is useful to suppress or regulate retention by RP mode.

Silica-C18:
reversed-phase



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

Anticholinergics on Zr-PBD

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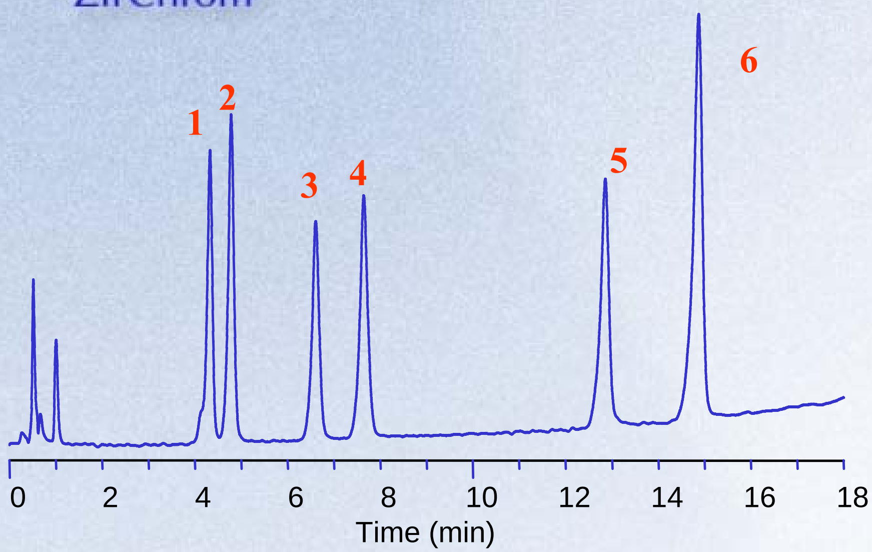
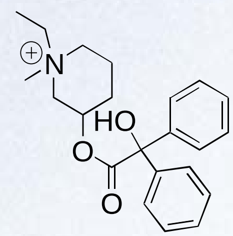


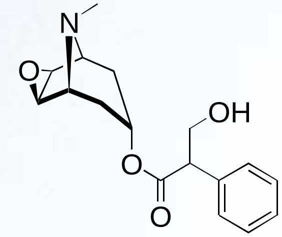
<u>LC Conditions</u>

Discovery® Zr-PBD, 100mm x 2.1mm i.d., 3 µm
Mobile Phase A: 10 mM NH_4PO_4, pH 7.0
Mobile Phase B: 80/20 20 mM NH_4PO_4, pH 7.0/ACN
Gradient: 10-100% B over 18 minutes
Temp: 80 °C,
Flow: 0.3 mL/min
Inj vol: 2 µL in 60% MeOH
Detector: UV@225 nm

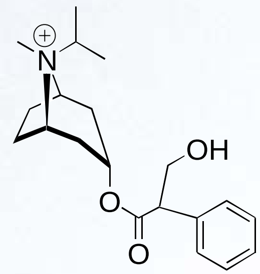
Quaternary amines and related compounds



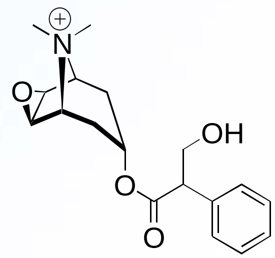
1, Pipenzolate (20 mg/L)



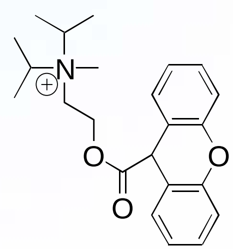
2, Scopolamine (100 mg/L)



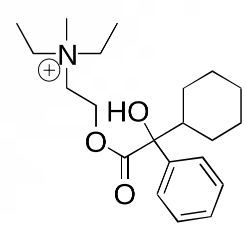
3, Ipratropium (100 mg/L)



4, Methscopolamine (100 mg/L)



5, Propantheline (20 mg/L)

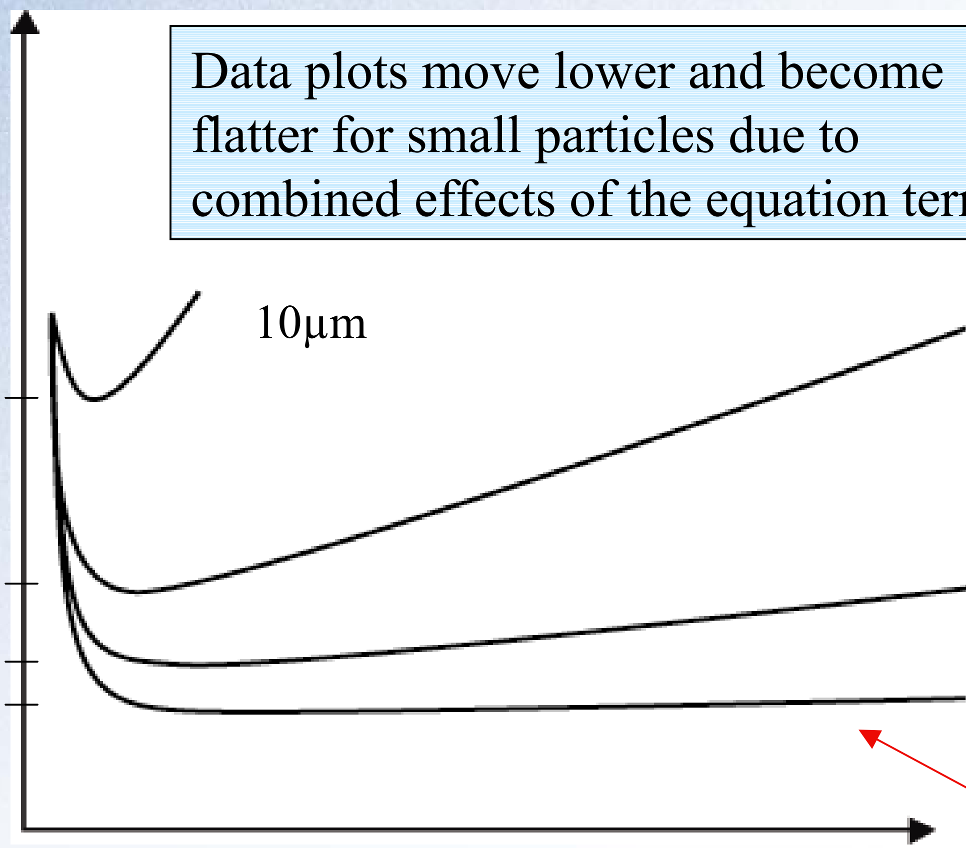


6, Oxyphenonium (100 mg/L)

Data provided by Sigma-Supelco

van Deemter Plots Reveal Column Performance

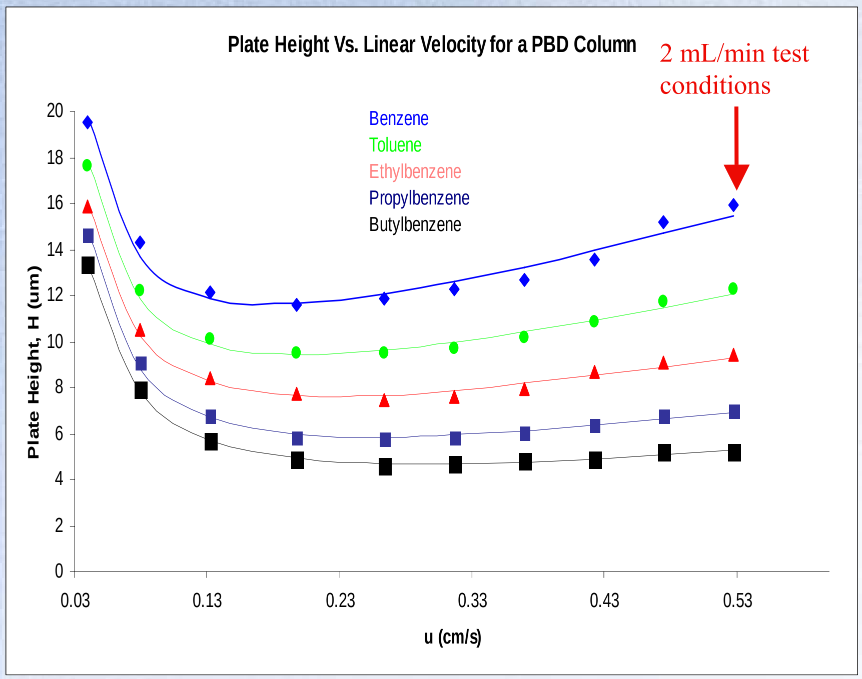
H = A + B/ν + Cν (shown below for a single solute)



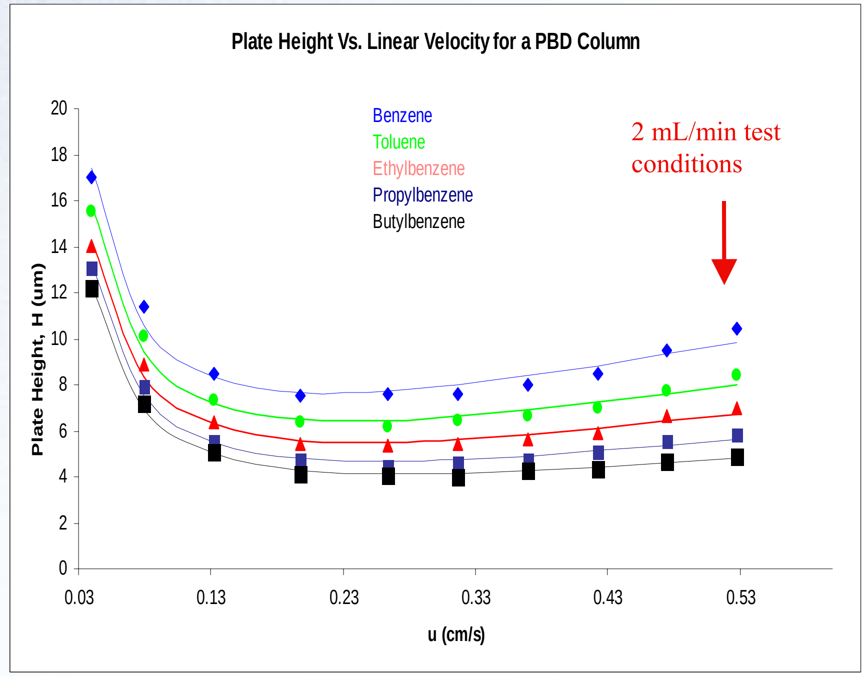
Idealized plot provided by Sigma-Supelco

**goal:
ca. 250,000 N/m
Instrument optimization very important!**

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



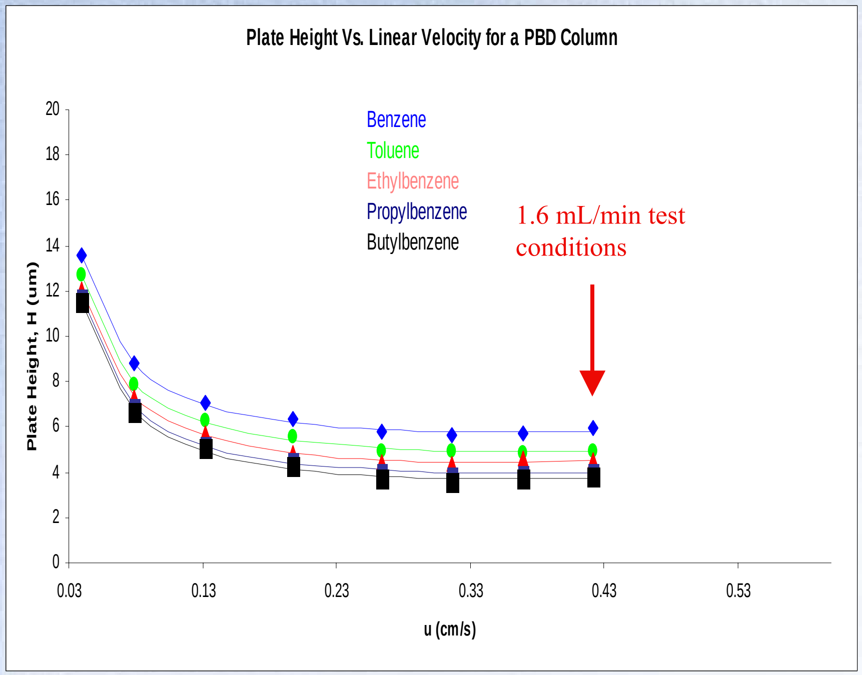
Factory Instrument



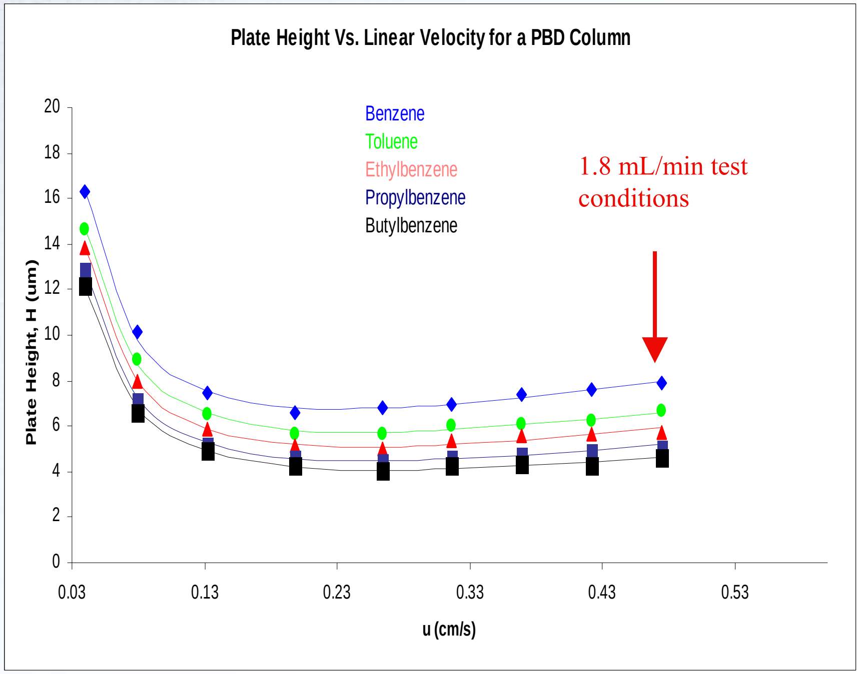
Micro Cell Only

Plate height based on van Deemter Equation vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 ºC, Mobile phase: 50/50 ACN/water ZirChrom®-PBD column: 50 x 4.6mm sub-2µm (part #: ZR03-0546-1.9), Agilent 1100/UV

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



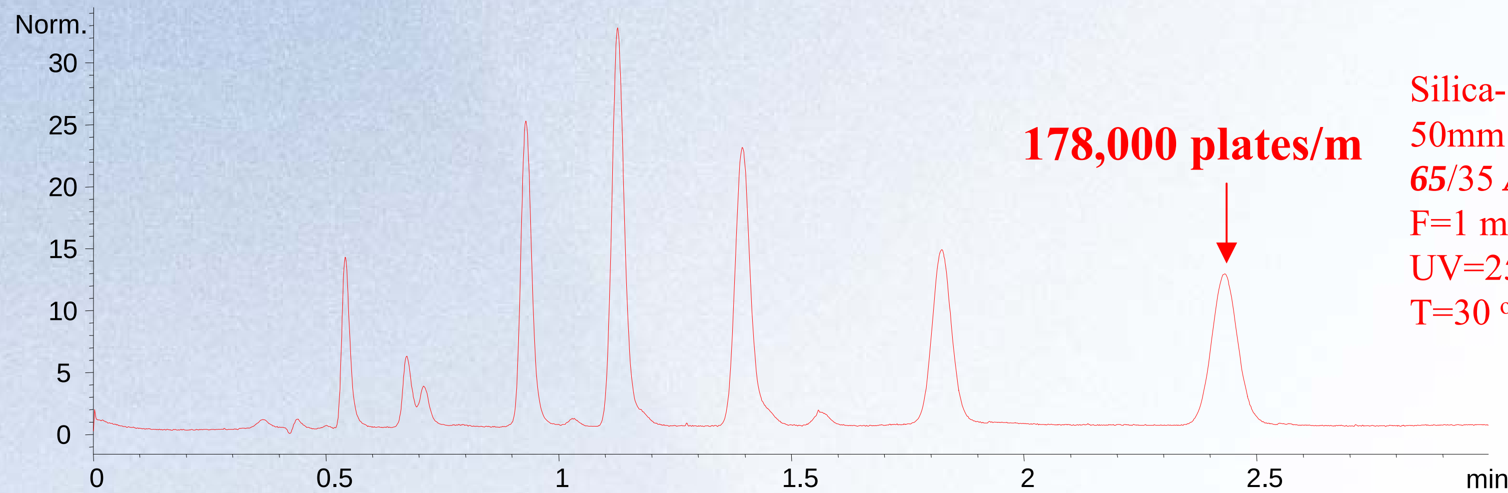
Micro cell + Optimized tubing (bypass instrument heat exchanger)



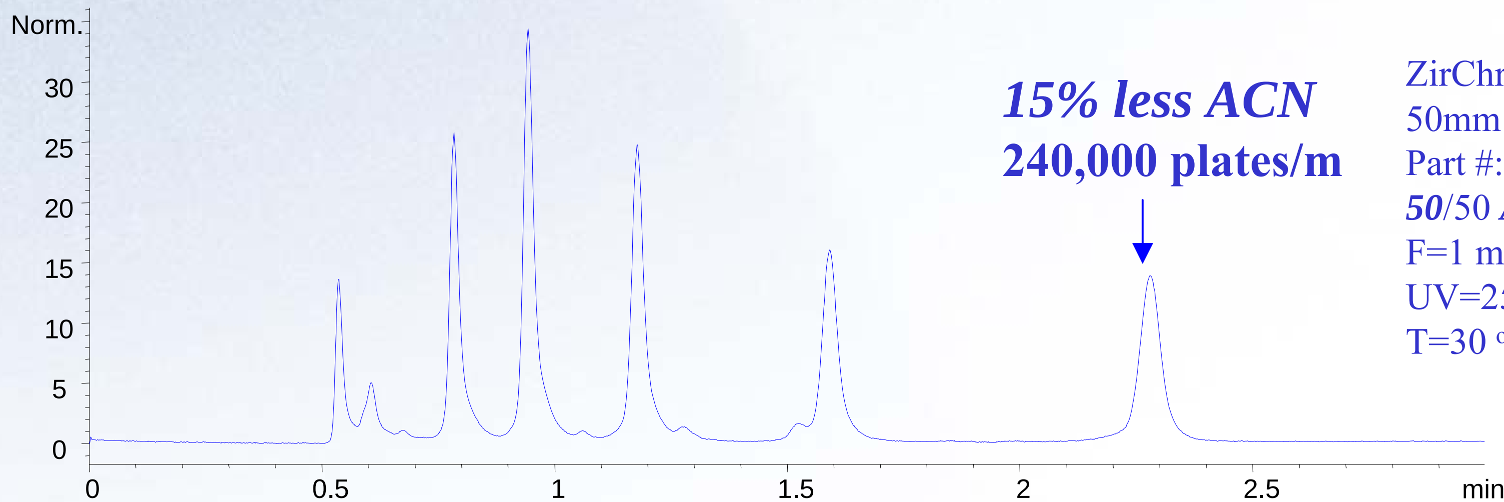
Micro Cell + High pressure fitting + Heat exchanger

Plate height based on van Deemter Equation vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 ºC, Mobile phase: 50/50 ACN/water ZirChrom®-PBD column: 50 x 4.6mm sub-2μm (part #: ZR03-0546-1.9), Agilent 1100/UV

Alkylbenzenes on ZirChrom®-PBD sub-2µm and Silica-C8



Silica-C8
50mm x 4.6mm, sub-3µm
65/35 ***ACN***/H_2O
F=1 mL/min
UV=254nm
T=30 °C

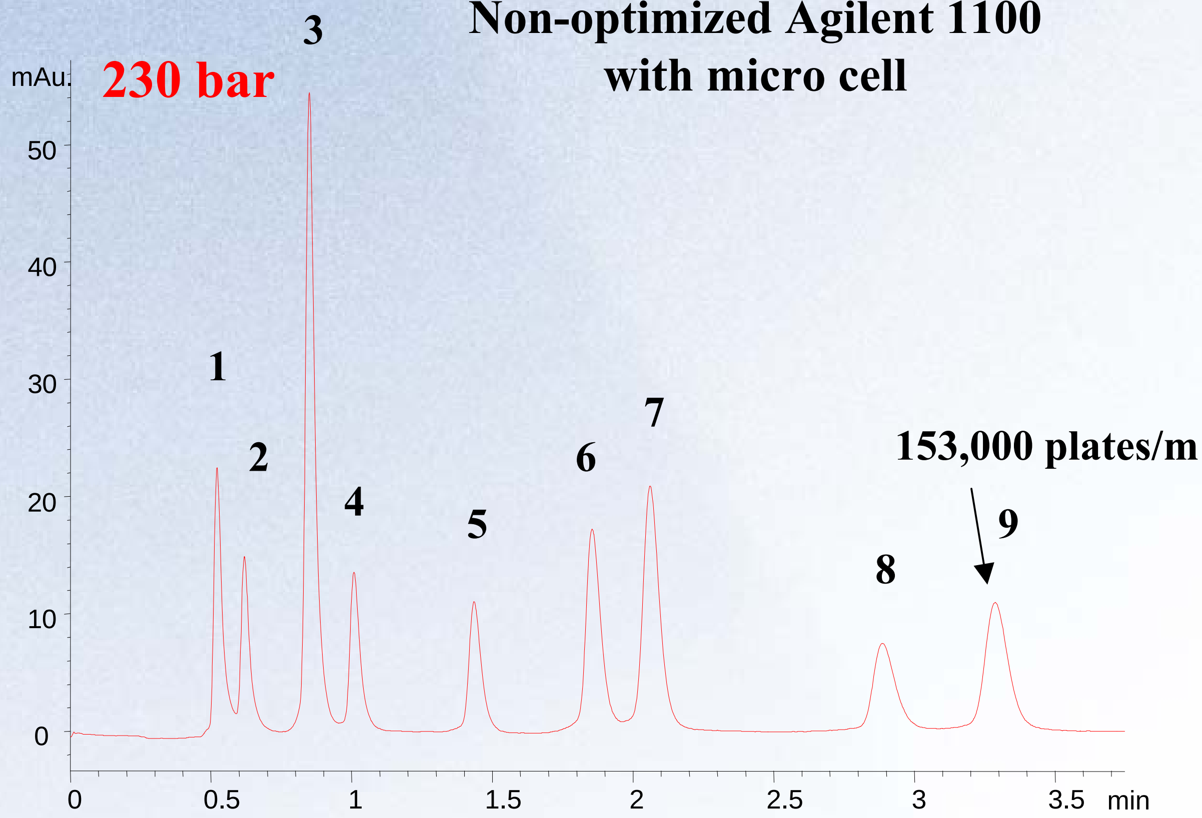


ZirChrom®-PBD
50mm x 4.6mm, sub-2µm
Part #: ZR03-0546-1.9
50/50 ***ACN***/H_2O
F=1 mL/min
UV=254nm
T=30 °C

Drug Mix* Separation on Zr-PBD
sub-2µm – Ambient

ZirChrom®

Non-optimized Agilent 1100
with micro cell



Analytes

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

Column: ZirChrom®-PBD, 50 x 4.6 mm i.d., sub-2µm; Part #: ZR03-0546-1.9 **Mobile phase**: 24/76 ACN/20 mM K_3PO_4 at pH=12; **Flow rate**: 1.0 mL/min; **Temp.**: 30 °C; **Injection vol.**: 2.0 µL; **Detection**: UV at 254 nm

*** Mainly beta-blockers**

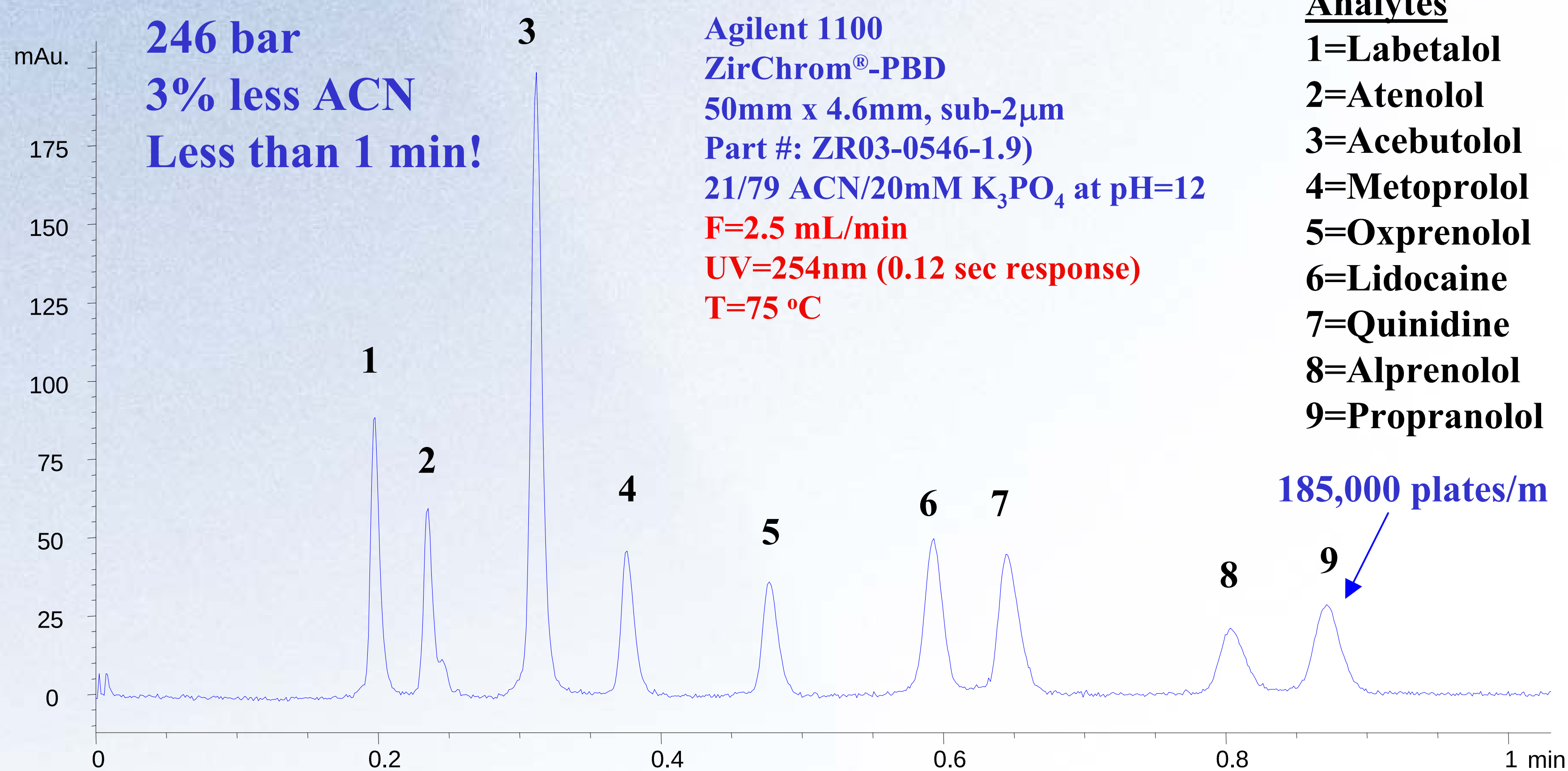
Drug Mix on ZirChrom®-PBD sub-2µm, 75 °C

ZirChrom®

246 bar
3% less ACN
Less than 1 min!

Agilent 1100
ZirChrom®-PBD
50mm x 4.6mm, sub-2µm
Part #: ZR03-0546-1.9)
21/79 ACN/20mM K_3PO_4 at pH=12
F=2.5 mL/min
UV=254nm (0.12 sec response)
T=75 °C

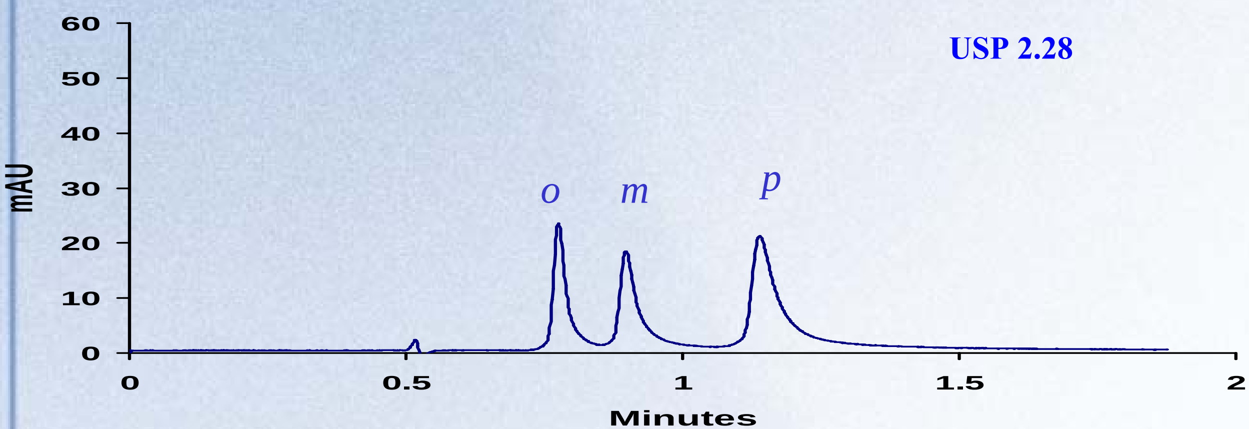
Analytes
1=Labetalol
2=Atenolol
3=Acebutolol
4=Metoprolol
5=Oxprenolol
6=Lidocaine
7=Quinidine
8=Alprenolol
9=Propranolol



Toluidines Separation on sub-2µm Zr-PBD: Temperature

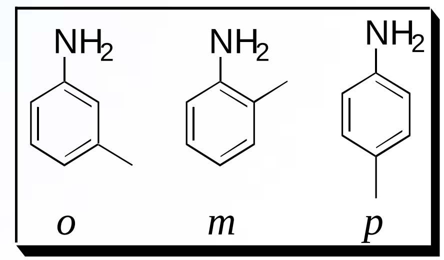
ZirChrom®

T=25 °C, 221 bar

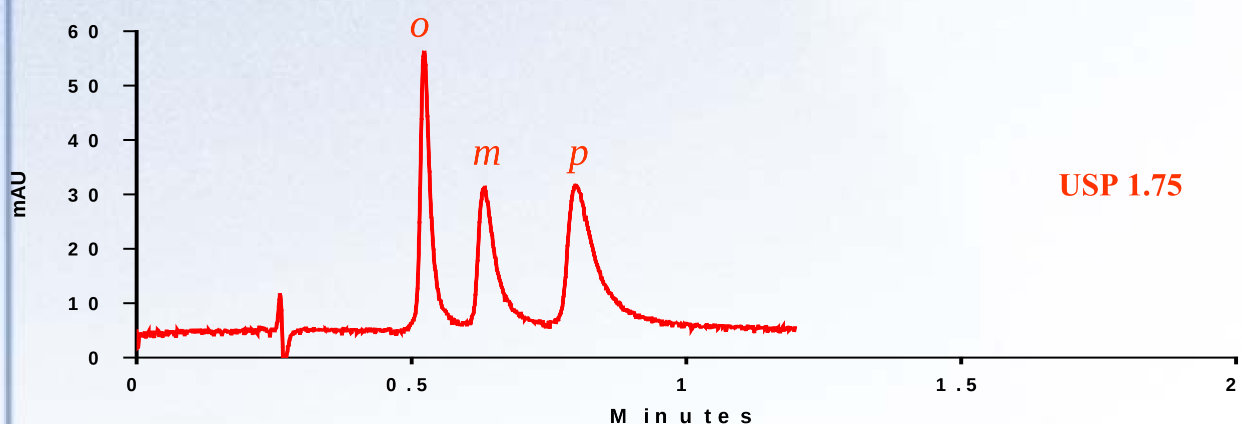


LC Conditions:

35/65 ACN/ 25 mM NH4OAc +
10 mM $NH_4H_2PO_4$ pH=4.67
F=1 mL/min, UV=254nm, T=25 °C
50x4.6mm, 1.9 µm, 2 µL inj
Part #: ZR03-0546-1.9



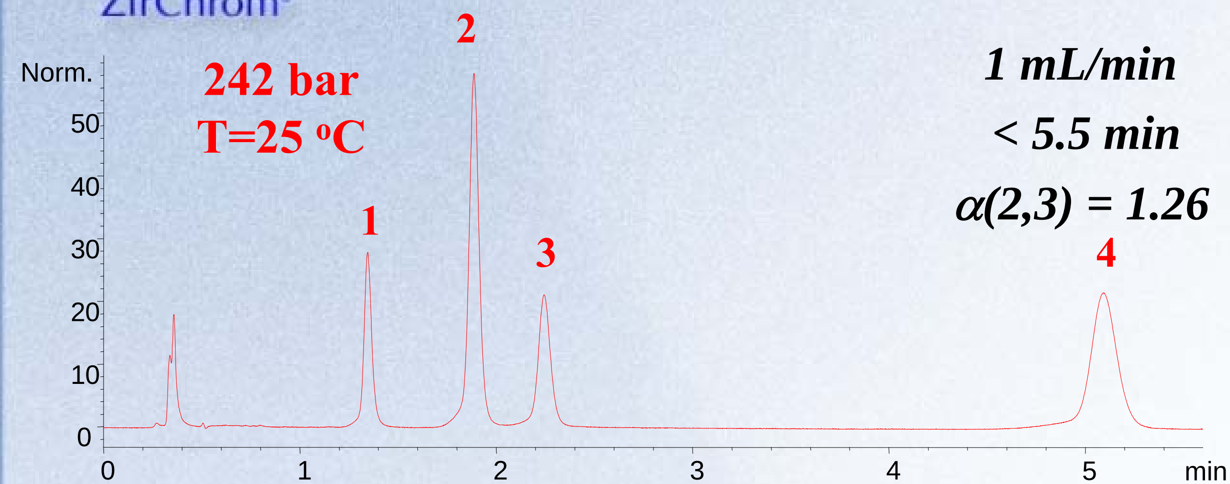
T=80 °C, 208 bar

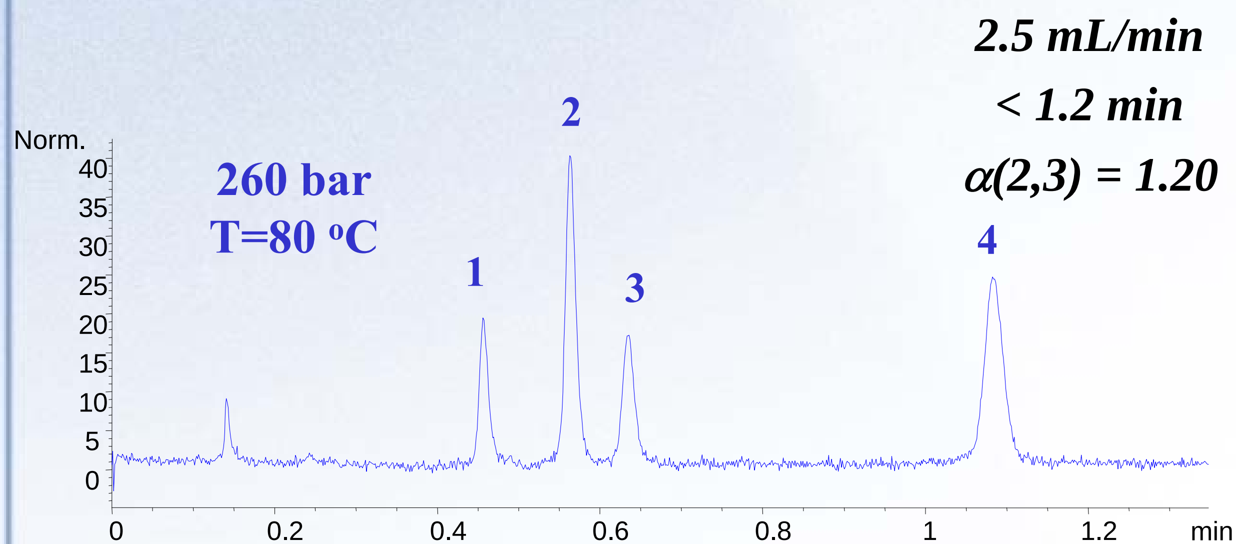


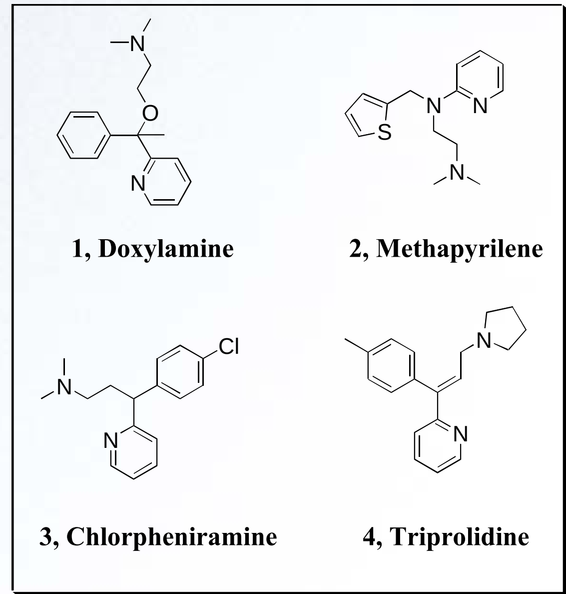
LC Conditions:

10/90 ACN/ 25 mM NH4OAc +
10 mM $NH_4H_2PO_4$ pH=4.67
F=2 mL/min, UV=254nm, T=80 °C
50x4.6mm, 1.9 µm, 7 µL inj
Part #: ZR03-0546-1.9

Antihistamine Separation on sub-2μm Zr-PBD

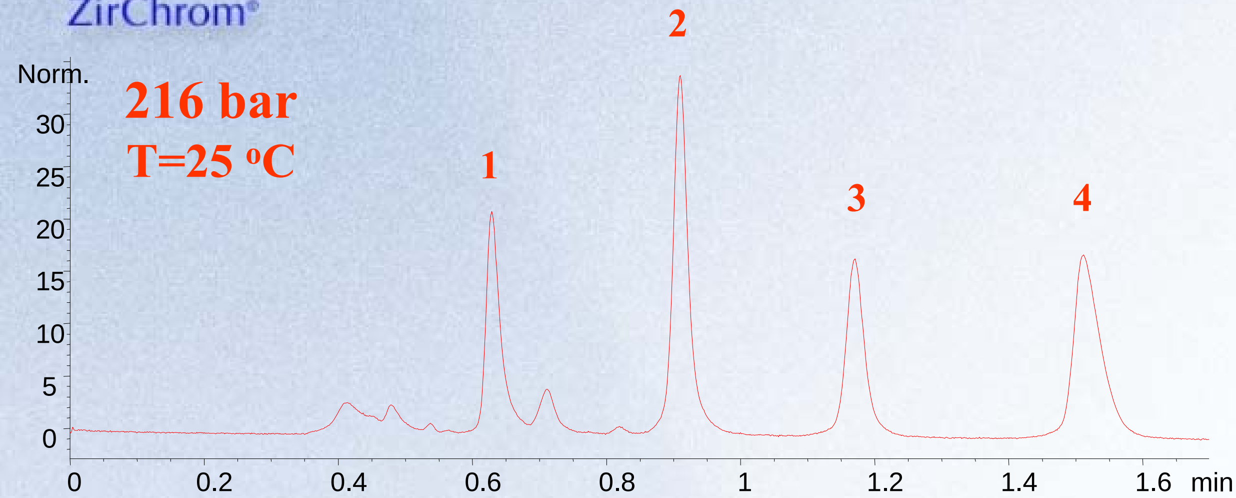




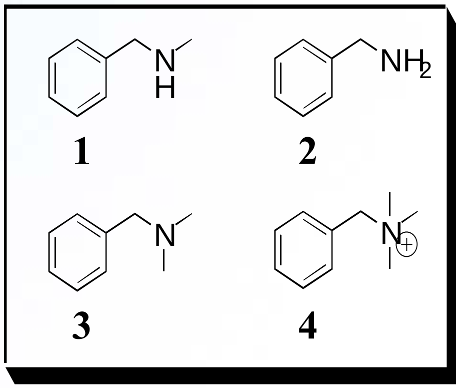


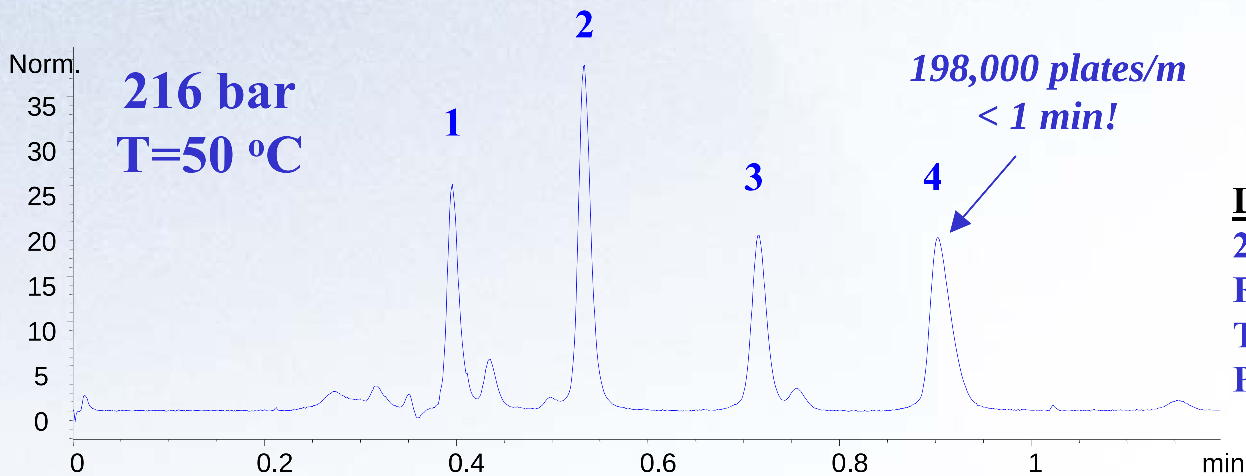
Column: ZirChrom®-PBD, 50 x 4.6 mm i.d., sub-2μm; Part #: ZR03-0546-1.9
Mobile phase: 28/72 ACN/50 mM TMA-OH at pH=12.2;
Injection vol.: 2.0 μL;
Detection: UV at 254 nm

Alkylbenzylamine Separation on sub-2μm Zr-PBD: 25 and 50 ^{o}C



LC Conditions:
21/79 ACN/ 20 mM K_3PO_4 pH=12
F=1.0 mL/min, UV=254nm,
T=25 ^{o}C, 50x4.6mm, 1.9 μm, 3 μL inj
Part #: ZR03-0546-1.9





LC Conditions:
21/79 ACN/ 20 mM K_3PO_4 pH=12
F=1.5 mL/min, UV=254nm,
T=50 ^{o}C, 50x4.6mm, 1.9 μm, 3 μL inj
Part #: ZR03-0546-1.9

Conclusions and Plans for Further HPLC Development with Zirconia

- Performance results with sub-2µm Zr-PBD show comparable efficiency gains for the zirconia based sub-2µm particles. The multi-modal separation mechanisms and high temperature stability of zirconia based sub-2µm particles provide added resolution and lower back pressures. This improved performance enables the use of the particles on an optimized standard HPLC.
- The study of ultra-high speed applications using sub-2µm Zr-PBD, especially at higher pH and temperature ("extreme conditions for silica") will be continued; generic conditions for LC-MS will be investigated.
- Other sub-2µm Zr phases (such as CARB) will be prepared and compared to Zr-PBD under ambient and extreme conditions.

Acknowledgements

For more information contact ZirChrom support at www.zirchrom.com or stop by **Booth 222**.

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