Advantages of Sub-2 µm Zirconia-PBD Columns for UHPLC at pH and Temperature Extremes

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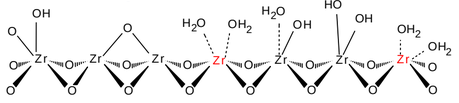
Surface Chemistry of Zirconia



Zirconia chemistry is dominated by Lewis acid-base reactions

Lewis Acid: $Zr(IV): H_2O + RPO_3^{2-} \rightleftharpoons Zr(IV): RPO_3^{2-} + H_2O$

Other Lewis base examples: PO_4^{3-}, RCO_2^- , Catechol

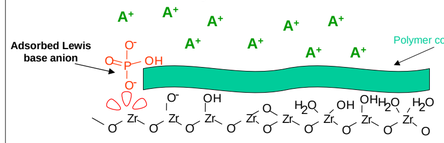
Interaction Strength of Lewis Bases with Zirconia[3]

Interaction Strength	Lewis Base (L)
Strongest	Hydroxide
	Phosphate
	Fluoride
	Citrate
	Sulfate
	Acetate
	Formate
	Nitrate
	Chloride
Weakest	Water

Small Lewis bases with high electron density and low polarizability interact more strongly with Zr atoms.

[3] 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).

Retention of Basic Analytes on ZirChrom-PBD



➢PBD, PS Coating — Reversed-Phase Interaction

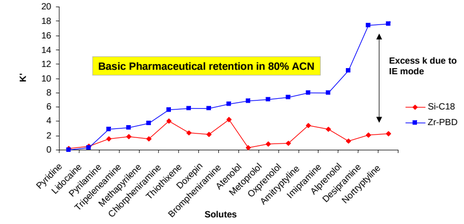
➢Lewis Base Anions — Ion-Exchange (IEX) Sites

$Zr\text{-}L::X^+ + A^+ = Zr\text{-}L::A^+ + X^+$

$Zr\text{-}O::X^+ + A^+ = Zr\text{-}O::A^+ + X^+$

A^+: analyte cation, X^+: counterion, L: adsorbed Lewis base anion.

Retention Comparison: Si-C18 vs Zr-PBD



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 1 µL; Temperature, 35 ºC; Detection at 254 nm; Columns, ZirChrom-PBD, 50 x 4.6 mm I.D., 3 mm (part #: ZR03-0546) Silica-C18 150 x 4.6 mm I.D., 3.5 mm.

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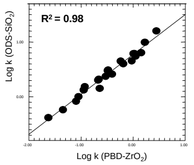
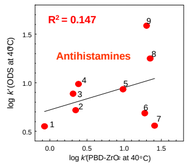
Orthogonality of Zirconia to Silica

Zr-PBD *vs.* Silica-C18



Nonionic solutes: Columns are very similar due to retention by RP mode only, but Zr-PBD is less retentive.



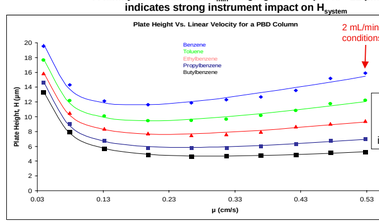
Ionic solutes: Columns are very different due to retention by combined RP and IEC modes; Zr-PBD becomes more retentive.

Introduction

- Coated and uncoated zirconia substrates interact with solutes by very different mechanisms than silica substrates; Lewis acid-base chemistry plays an extremely important role.
- HPLC columns with 3 µm and 5 µm zirconia particles have become widely accepted for making difficult separations and operating at extreme conditions of pH and temperature.
- Sub-2 µm zirconia columns have been demonstrated previously to have the efficiency advantages and very flat van Deemter plots expected for small particles[7].
- Limitations of using a traditional 400 bar HPLC instrument with column heater for applications at higher temperatures and faster speeds are explored in this poster.

Performance of a Factory HPLC Instrument with Sub-2 µm Zirconia

A family of curves with H_{min} ranging from <5 µm to >12 µm indicates strong instrument impact on H_{system}

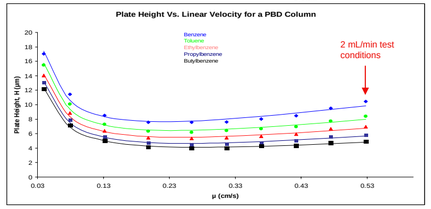


A rise in H at high flow velocity is also indicative of excess instrument dispersion

Plate height vs linear velocity for alkylbenzenes, Temperature 30 ºC, Mobile phase: 50/50 ACN/water, Column: 50 x 4.6 mm, Agilent 1100/UV with Standard Cell and 0.007'' I.D. tubing.

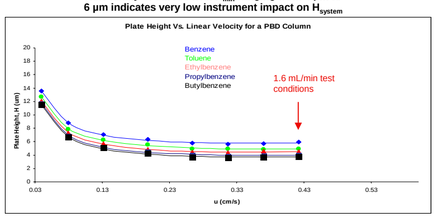
Optimizing Factory Instrument by Adding Micro Flow Cell

New family of curves with H_{min} ranging from <5 µm to 8 µm indicates lower instrument impact on H_{system}



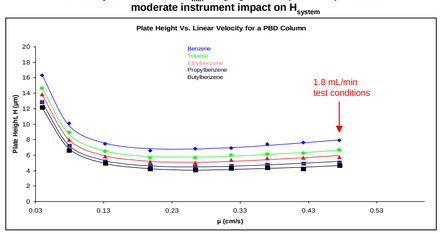
Optimized Factory Instrument with Micro Cell + 0.005" ID Tubing

New family of curves with H_{min} ranging from 4 µm to 6 µm indicates very low instrument impact on H_{system}



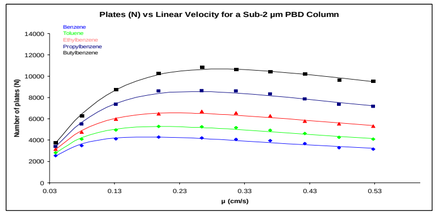
Factory Instrument with Micro Cell + 0.005" ID Tubing + Heat Exchanger

New family of curves with H_{min} ranging from <5 µm to >7 µm indicates moderate instrument impact on H_{system}



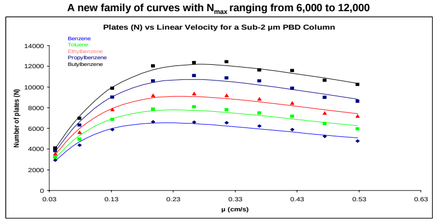
Instrument Optimization Can Be Done with Theoretical Plate Curves

A family of curves with N_{max} ranging from 4,000 to >10,000 on factory Agilent 1100



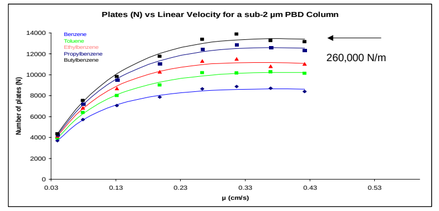
Instrument Optimized with Micro Cell

A new family of curves with N_{max} ranging from 6,000 to 12,000



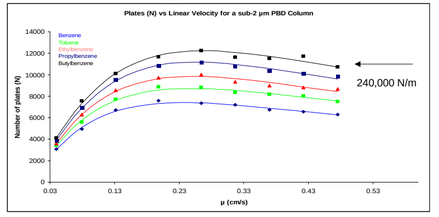
Instrument Optimized with Micro Cell + Tubing

A new family of curves with N_{max} ranging from 8,000 to >13,000 for a 5 cm column.



Instrument Optimized with Micro Cell + Tubing + Heat Exchanger

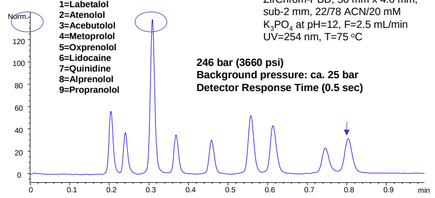
A new family of curves with N_{max} ranging from 6,000 to >12,000 for a 5 cm column.



References

1. R. Majors, *Are you Getting the Most Out of Your HPLC Column?*, LCGC NA, Vol. 21, No. 12, 1124-1133 (December 2003).
2. R. A. Henry and D. S. Bell, *Important Guidelines for Optimizing Speed and Sensitivity in Small Molecule LC-UV and LC-MS*, LCGC NA, Vol. 23, No. 5, 2-7 (May 2005).
3. T. Chester, *Sub-2µm Performance with a Conventional Instrument Using 2.7µm Fused-CoreTM Particles,* American Lab, Volume 41, No. 4, 11-15 (2009).
4. S. Fekete, et. al., *Shell and Small Particles: Evaluation of New Technology,* J. of Pharmaceutical and Biomedical Analysis, 49, 64-71 (2009).
5. F. Gritti, G. Guiochon, et. al., *Achieving Full Performance of Columns by Optimizing HPLC Instruments*, J. of Chromatogr. A, 1217, 3000-3012 (2010)
6. H. Brandes, unpublished data, Sigma-Supelco Applications Lab Reports and Notebooks (2008-2009).
7. R. A. Henry and D. Nowlan, *Use of Sub-2µm Zirconia-PBD at Elevated pH and Temperature*, Oral Paper, EAS 2009, Somerset, NJ.

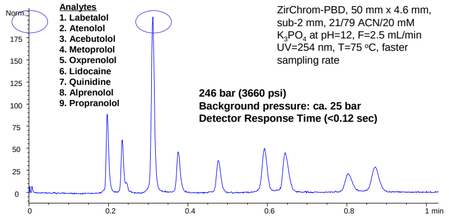
Beta-Blockers on ZirChrom-PBD Sub-2 µm at 75 ºC



ZirChrom-PBD, 50 mm x 4.6 mm, sub-2 mm, 22/78 ACN/20 mM K_2PO_4 at pH=12, F=2.5 mL/min UV=254 nm, T=75 ºC

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

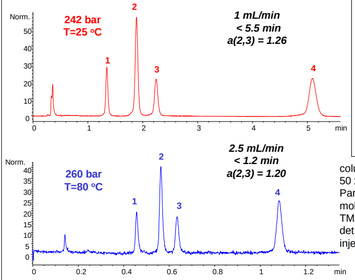
Beta-Blockers Optimized with Faster Detector Response

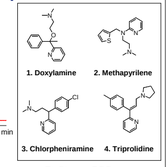


ZirChrom-PBD, 50 mm x 4.6 mm, sub-2 mm, 21/79 ACN/20 mM K_2PO_4 at pH=12, F=2.5 mL/min UV=254 nm, T=75 ºC, faster sampling rate

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

Antihistamines Optimized at Elevated Temperature





column: ZirChrom-PBD, 50 x 4.6 mm I.D., sub-2 mm, Part #: ZR03-0546-1.9
mobile phase: 28/72 ACN/50 mM TMA-OH at pH=12
det.: UV at 254 nm
injection; 2.0 µL

Conclusions

- Zirconia sub-2 µm UHPLC columns can show significant advantages over silica for operating at high pH and elevated temperatures.
- Presence of a family of H-µ curves has been shown to be a good diagnostic for the presence of significant instrument bandspreading; without instrument contribution, curves should superimpose at theoretical values of H and N.
- A rise in the slope of H-µ curves at high flow velocity is also indicative of excessive instrument contribution to system dispersion with sub-2 µm UHPLC columns.
- Instrument contribution to system peak width can be systematically reduced to acceptable levels by changing to smaller volume flow cell and connectors.
- With modern UHPLC columns, current column heater designs may limit system performance.
- Sub-2 µm zirconia can be used with optimized 400 bar HPLC instruments and column heaters with only minor performance loss.