Multi-mode Separations Using Zirconia-based Stationary Phases

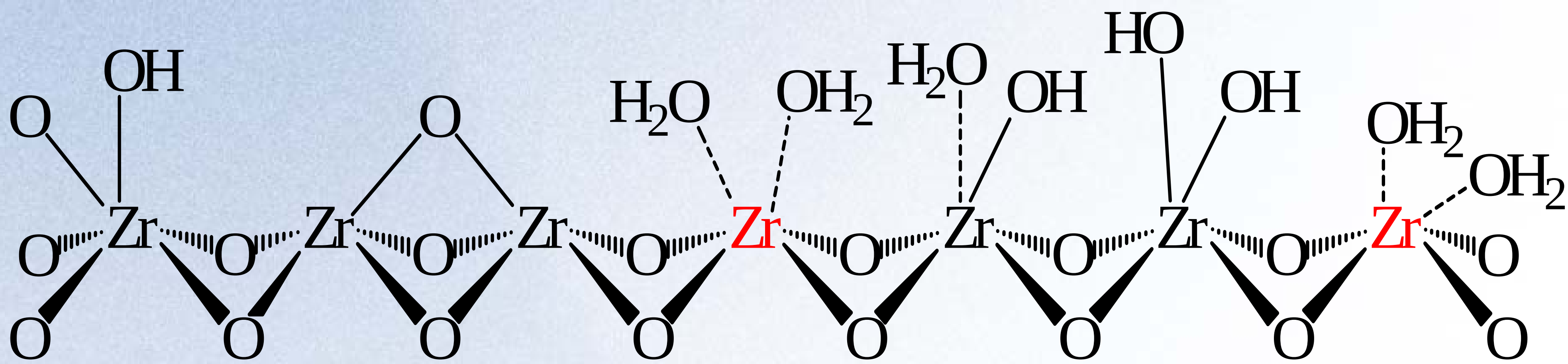
PITTCON 2010

Dan Nowlan[1], Bingwen Yan[1], Clayton V. McNeff[1] , R.A. Henry[2]

[1] ZirChrom Separations, Inc. 617 Pierce St., Anoka, MN 55303
[2] Independent Consultant, 983 Greenbriar Drive, State College, PA 16801

Specialists in High Efficiency, Ultra-Stable Phases for HPLC

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

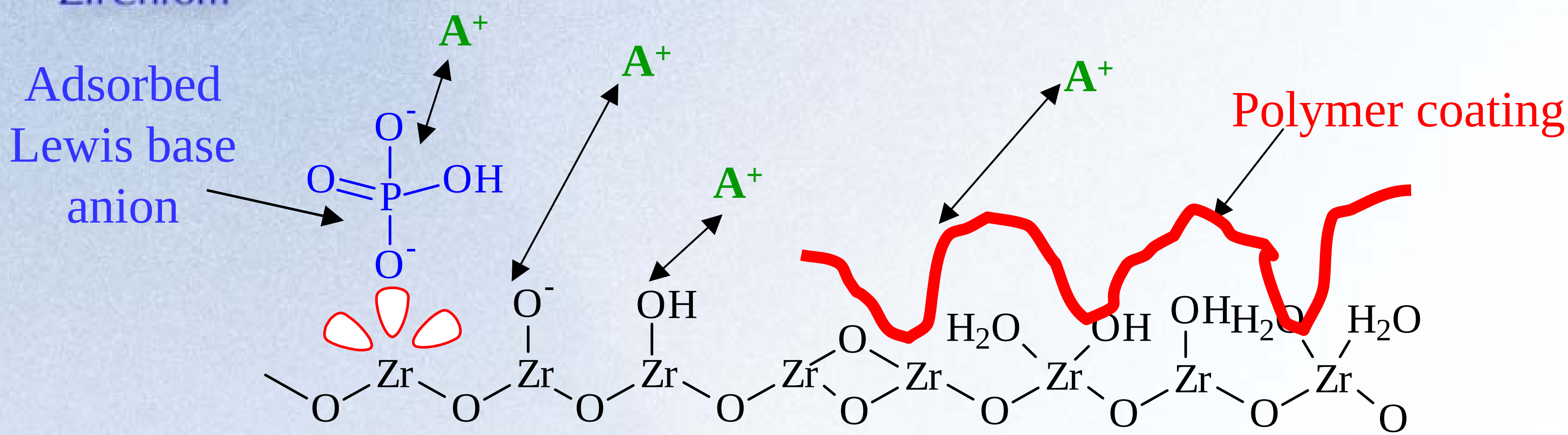
Interaction Strength of Lewis Bases with Zirconia[3]

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 and ZirChrom®-PS



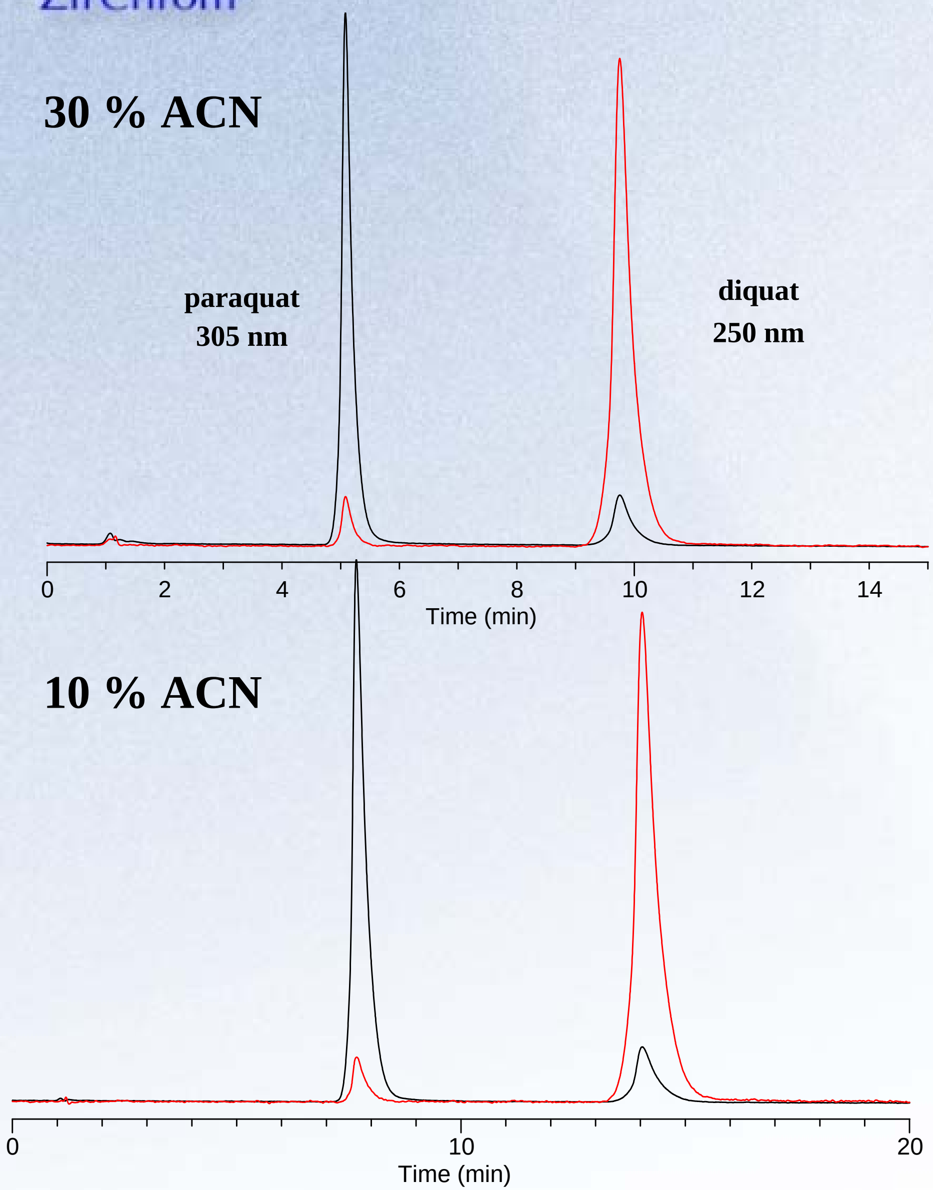
- PBD, PS Coating — Reversed-Phase (RP) Moieties
- Lewis Base Anions — Ion-Exchange (IEX) Sites

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

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

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

Effect of Reversed Phase Character on the Separation of Quaternary Amines[4]



Column: Discovery® Zr-PS, 150cm x 2.1mm ID, 3µ particles
Mobile Phase 1: 50: 20 : 30, (20 mM H_3PO_4, 100 mM NH_4HCO_3, pH 7.0 w/ NH_4OH) : Water : Acetonitrile
Mobile Phase 2: 50: 40 : 10, (20 mM H_3PO_4, 100 mM NH_4HCO_3, pH 7.0 w/ NH_4OH) : Water : Acetonitrile
Flow: 0.3 mL/min
Temp: 50° C
Det: UV at 250nm & 305nm
Inj: 1 µL
Sample: diquat & paraquat in water; 100 mg/L ea.

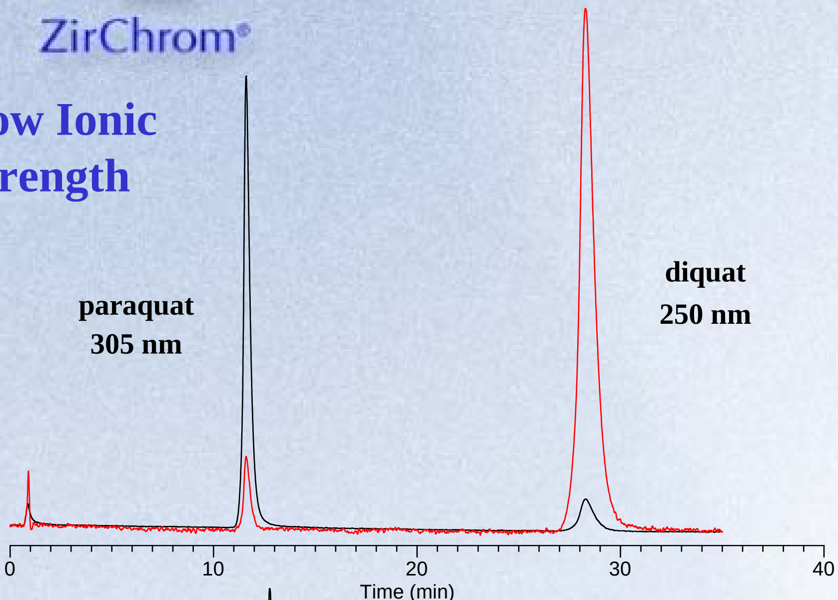
- **At 30% ACN, the polymer coating adds very little to retention or selectivity for these ionic compounds.**
- **When nonionic compounds are present, changes in organic solvent strength will have a greater impact and can be used for optimizing resolution.**

[4] Data used by permission of Supelco

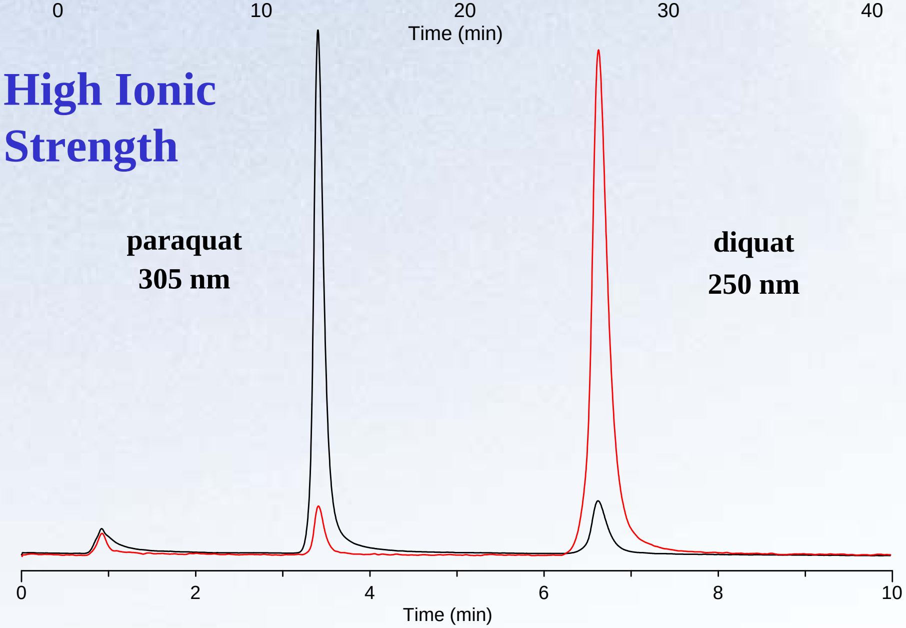
Effect of Ionic Strength on the Separation of Quaternary Amines[4]

ZirChrom®

Low Ionic Strength



High Ionic Strength



Column: Discovery® Zr-PS, 7.5cm x 2.1mm ID, 3µ particles

Mobile Phase1: 50:50, (20 mM H_3PO_4, 40 mM NH_4HCO_2, pH 7.0 w/ NH_4OH) : Acetonitrile

Mobile Phase2: 50:50, (20 mM H_3PO_4, 100 mM NH_4HCO_2, pH 7.0 w/ NH_4OH) : Acetonitrile

Flow: 0.2 mL/min

Temp: as indicated

Det: UV at 250nm & 305nm

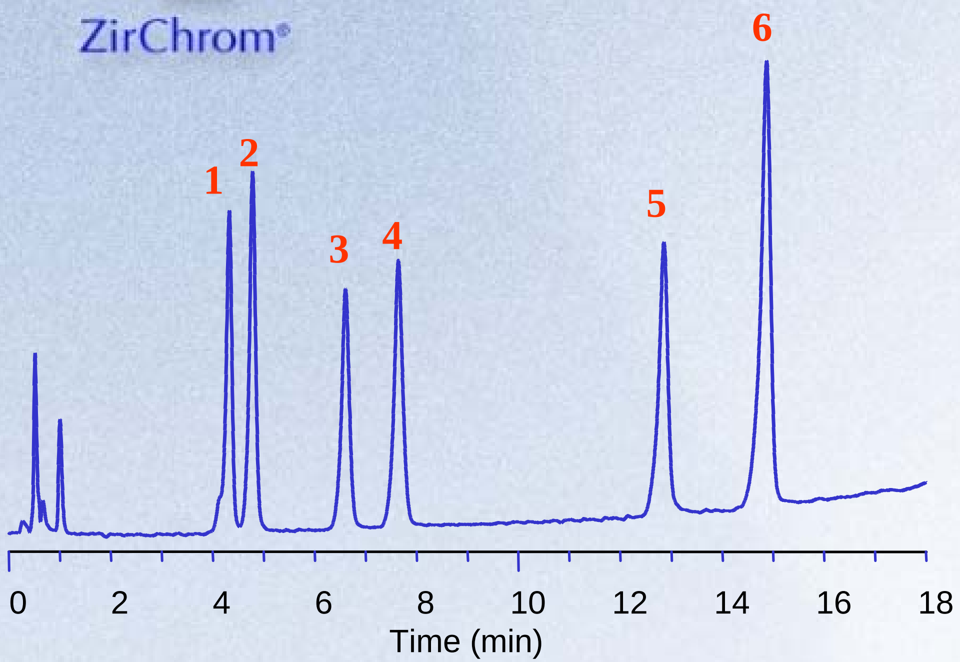
Inj: 1 µL

Sample: diquat and paraquat in water; 50 mg/L ea.

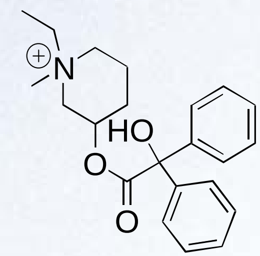
- **k values for diquat are 25-30 at low ionic strength in 50% ACN.**
- **k values for diquat decrease to about 5 at high ionic strength without changing %ACN.**
- **The classic method for reducing k in IE mode is to increase ionic strength, confirming IE mode.**

[4] Data used by permission of Supelco

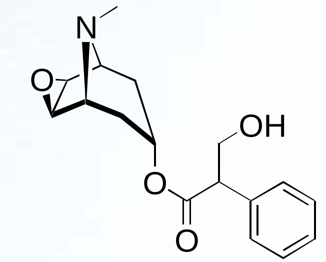
Anticholinergics on Zr-PBD[4]



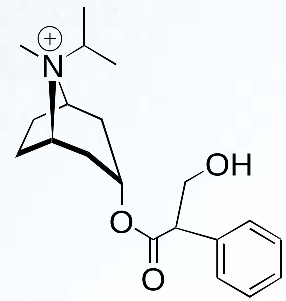
Quaternary amines and related compounds



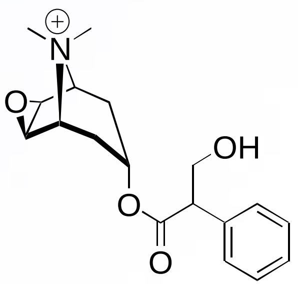
1, Pipenzolate (20 mg/L)



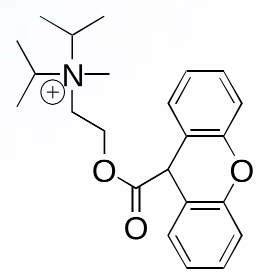
2, Scopolamine (100 mg/L)



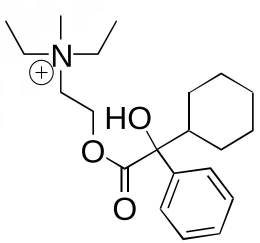
3, Ipratropium (100 mg/L)



4, Methscopolamine (100 mg/L)



5, Propantheline (20 mg/L)



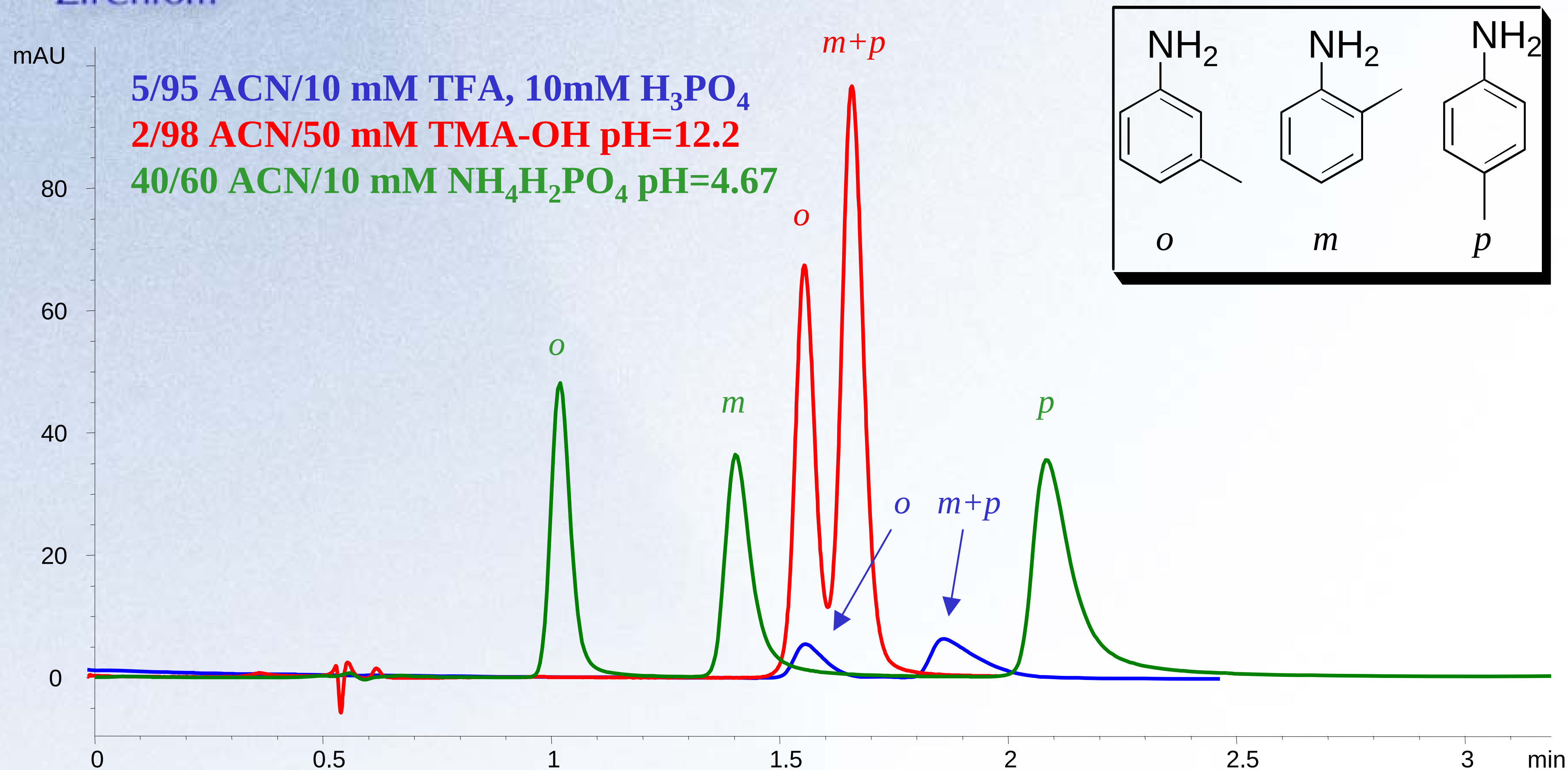
6, Oxyphenonium (100 mg/L)

LC Conditions

Discovery® Zr-PBD 100mm x 2.1mm i.d., 3 µm
Mobile Phase A: 50:50 [20 mM H_3PO_4, pH 7.0 w/ NH_4OH]:water
Mobile Phase B: 50:30:20 [20 mM H_3PO_4, pH 7.0 w/ NH_4OH]:water:ACN
Gradient 90:10 to 0:100 A:B over 18 minutes
Temp = 80 °C, Flow = 0.3 mL/min, Inj vol = 2 µL,
UV 225 nm, sample in ~60:40 Mobile phase A:MeOH

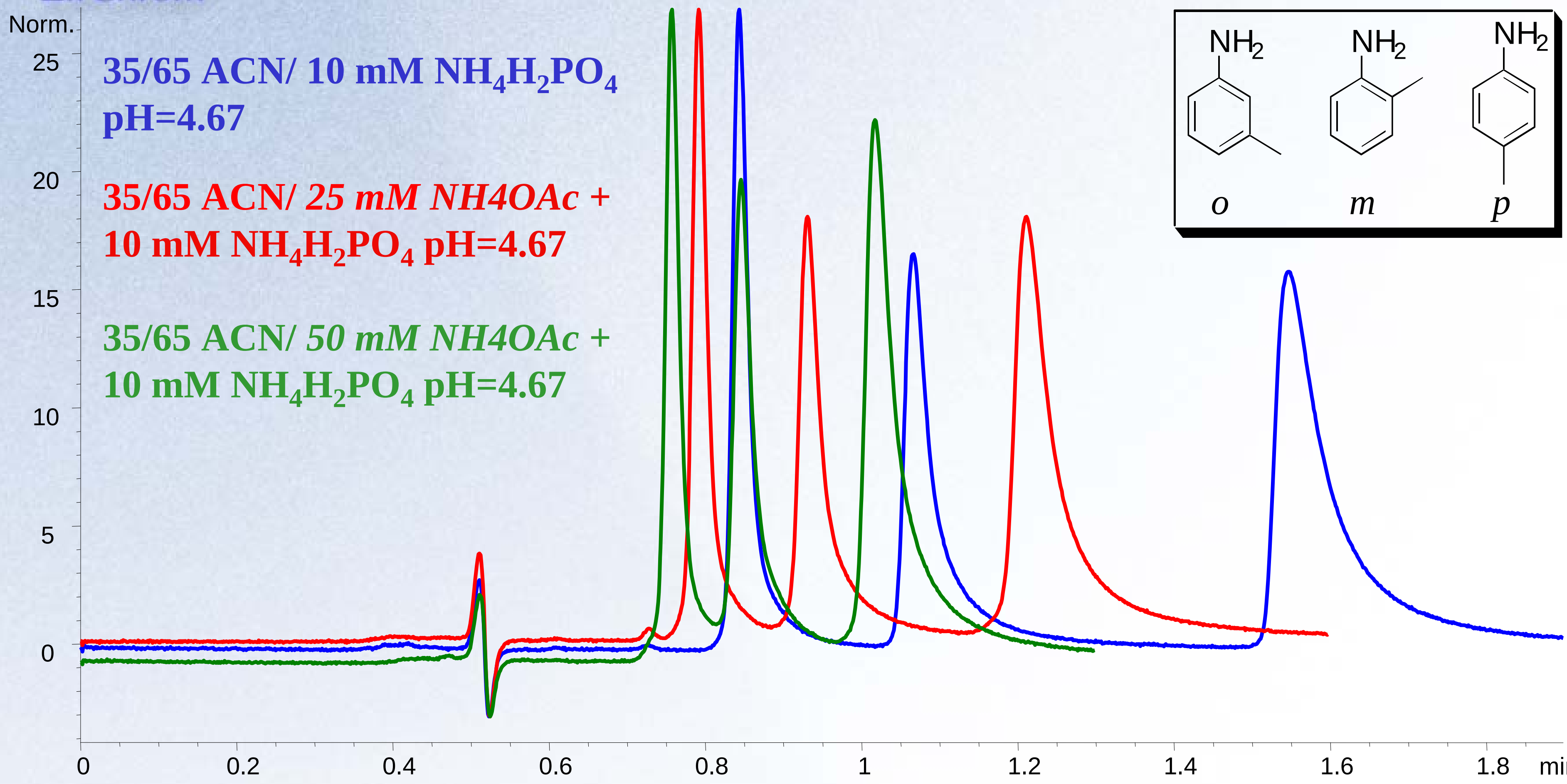
[4] Data used by permission of Supelco

Toluidines Separation on 3μm Zr-PBD



LC Conditions: Column: ZirChrom®-PBD, 50 x 4.6 mm i.d., 3μm (part #: ZR03-0546); Flow rate: 1.0 mL/min; Temp: 25 ºC; Injection Vol.: 2.0 μL; Detection: UV at 254 nm

Toluidines Separation on sub-2μm PBD: Ionic Strength

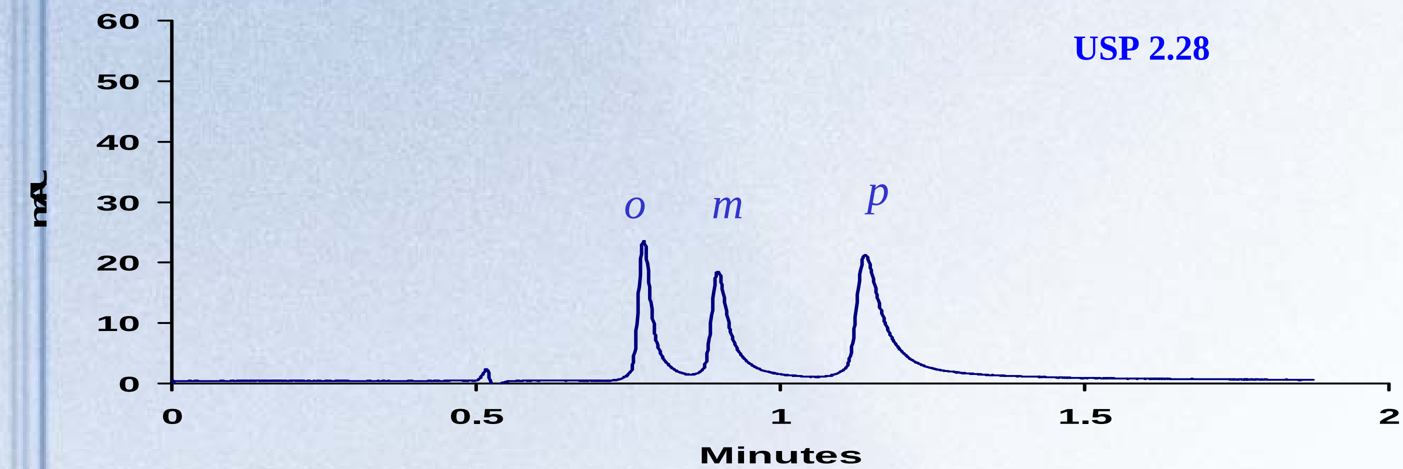


LC Conditions: Column: ZirChrom®-PBD, 50 x 4.6 mm i.d., sub-2μm (part #: ZR03-0546-1.9); Flow rate: 1.0 mL/min; Temp: 25 °C; Injection Vol.: 2.0 μL; Detection: UV at 254 nm

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

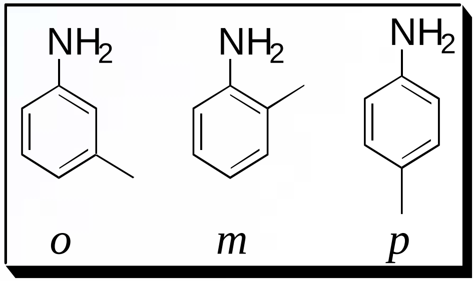
ZirChrom®

T=25 ºC, 221 bar

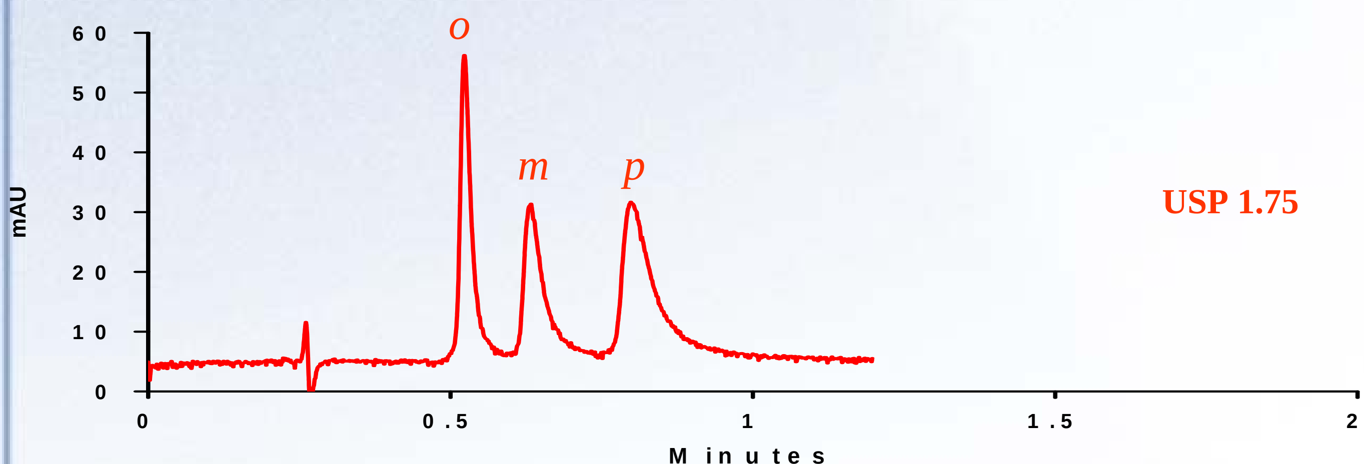


LC Conditions:

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



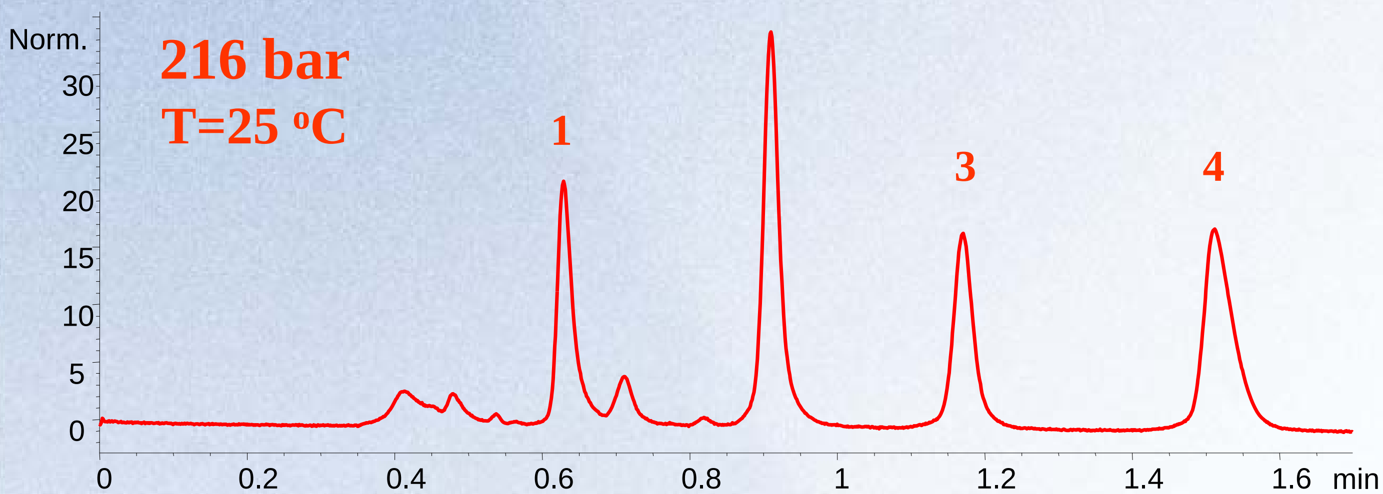
T=80 ºC, 208 bar



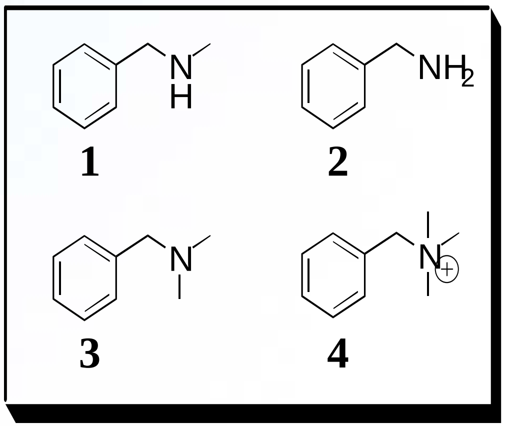
LC Conditions:

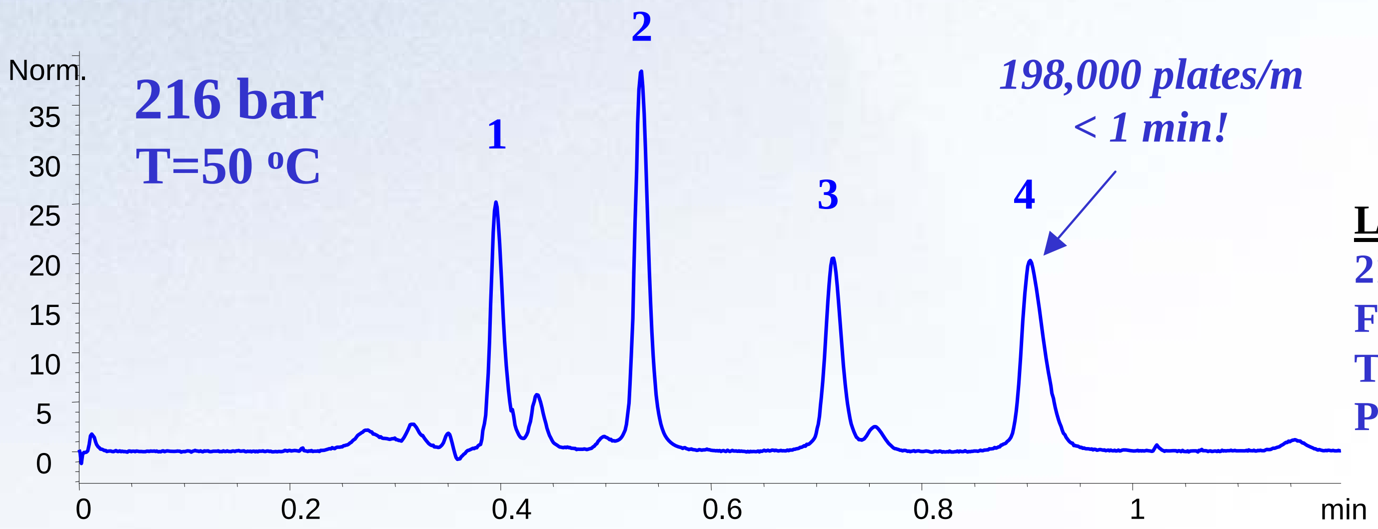
10/90 ACN/ 25 mM NH4OAc +
10 mM $NH_4H_2PO_4$ pH=4.67
F=2 mL/min, UV=254nm, T=80 ºC
50x4.6mm, 1.9 μm, 7 μL inj
Part #: ZR03-0546-1.9

Alkylbenzylamine Separation on sub-2μm Zr-PBD: 25 and 50 °C



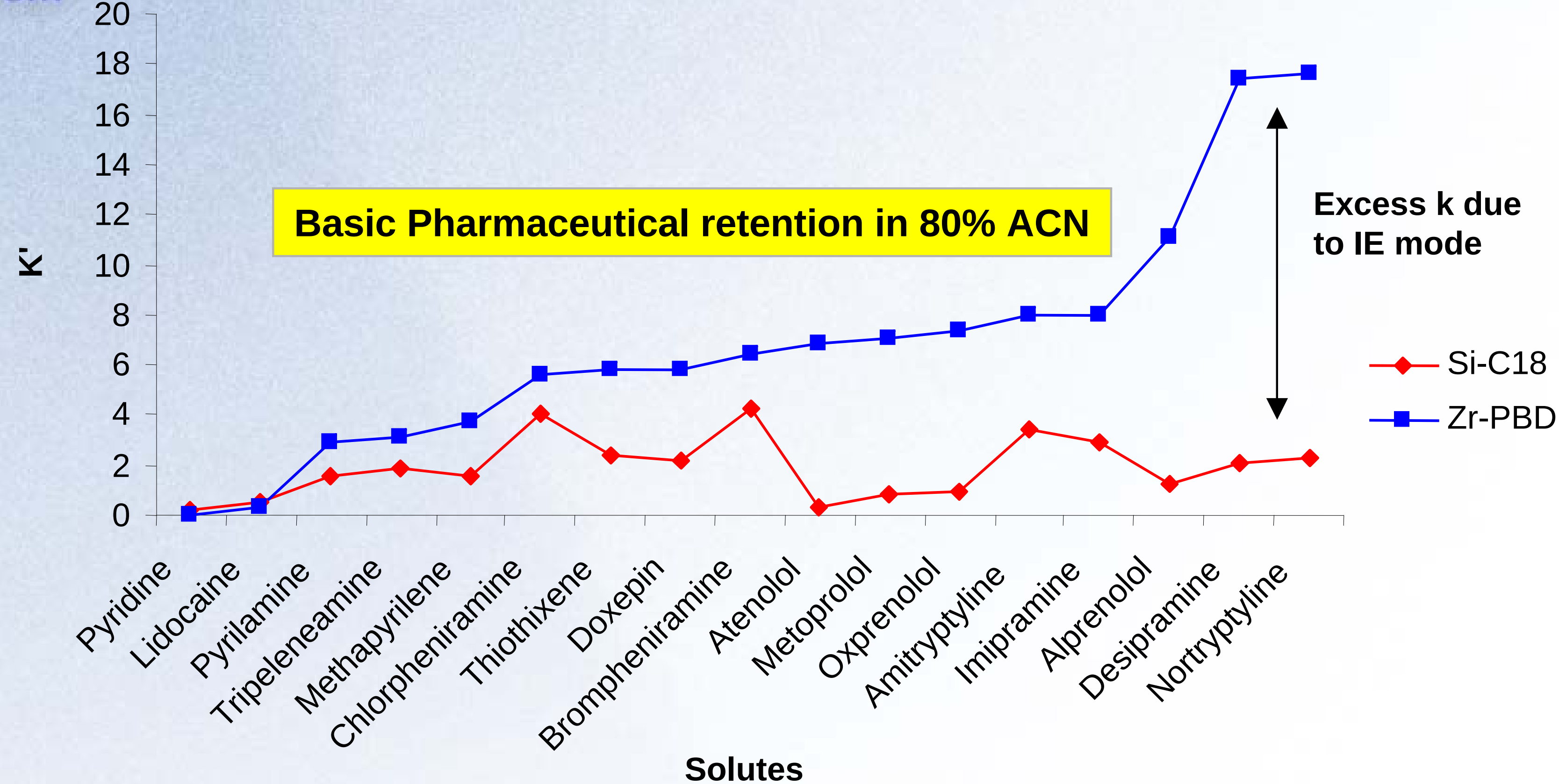
LC Conditions:
21/79 ACN/ 20 mM K_3PO_4 pH=12
F=1.0 mL/min, UV=254nm,
T=25 °C, 50x4.6mm, 1.9 μm, 3 μL inj
Part #: ZR03-0546-1.9





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

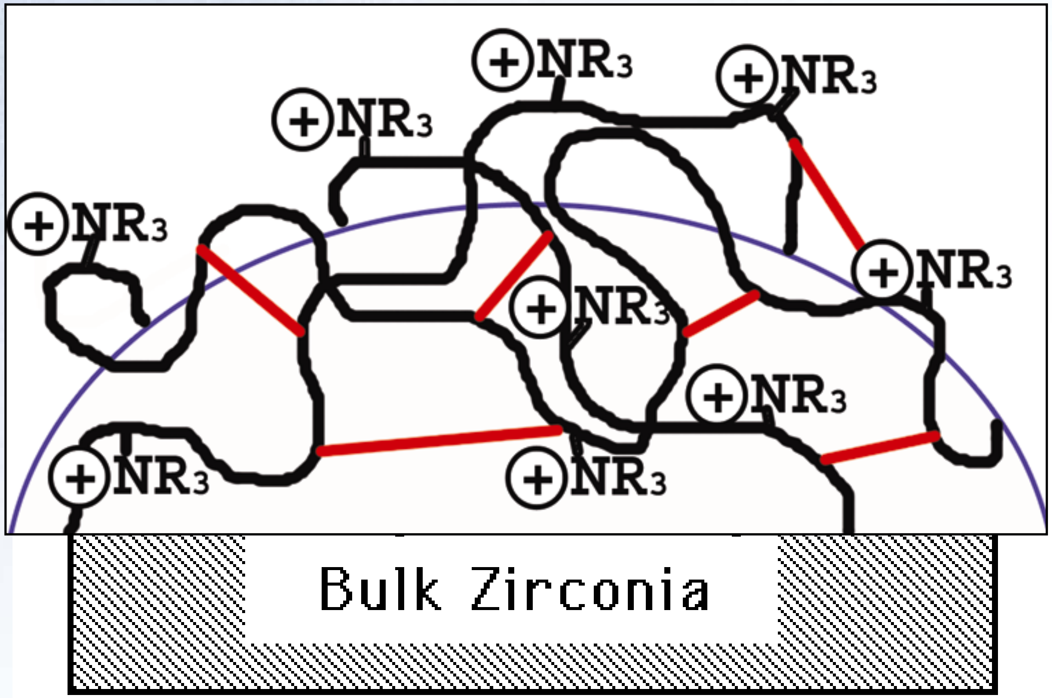
Retention Comparison: Si-C18 vs Zr-PBD



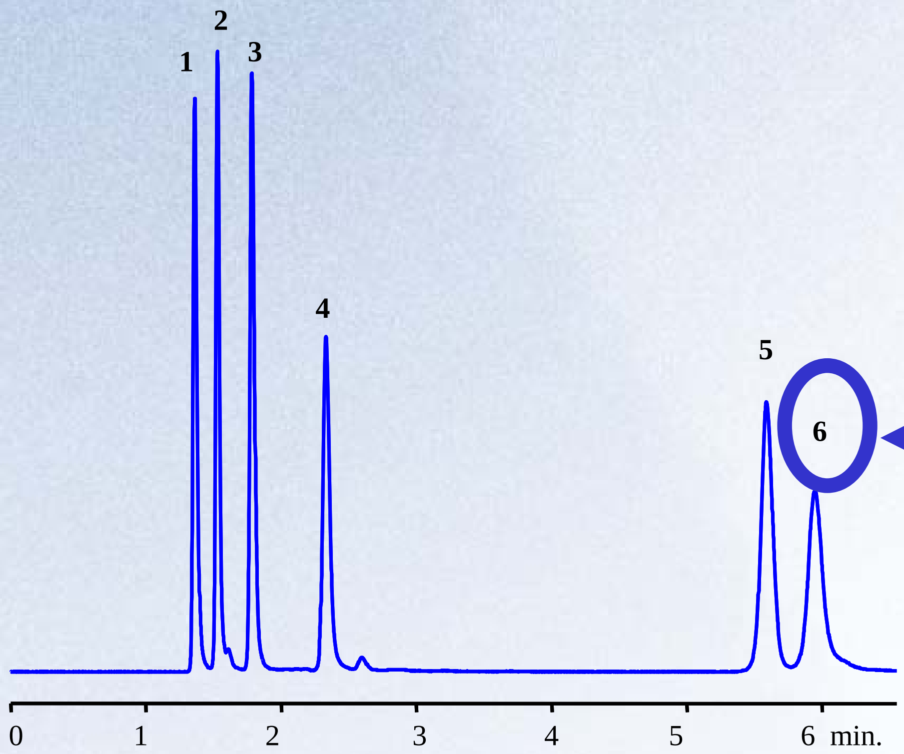
LC Conditions: Machine-mixed 80/20 ACN/10 mM ammonium acetate pH=6.7 without pH adjustment; Flow rate, 1.0 ml/min.; Injection volume 0.1 ul; Temperature, 35 ^{o}C; Detection at 254 nm; Columns, ZirChrom®-PBD, 50 x 4.6 mm i.d., 3µm (part #: ZR03-0546) Silica-C18 150 x 4.6 mm i.d., 3.5 µm.

Surface Chemistry and Retention Mechanisms of QPEI-Zirconia

- **Anion-exchange**
- **Hydrophobic interactions**
- **Lewis acid-base interactions**



Water-Soluble Vitamin Analysis on ZirChrom®-SAX



1 - Thiamine (Vit. B_1)
2 - Pyridoxine (Vit. B_6)
3 - Nicotinamide (form of Vit. B_3)
4 - Riboflavin (Vit. B_2)
5 - Nicotinic acid (form of Vit. B_3)
6 - Ascorbic acid (Vit. C)

Vitamin C is strongly retained on ZirChrom®-SAX

LC Conditions: Column: ZirChrom®-SAX, 150 x 4.6 mm i.d. (part number: ZR06-1546), Mobile Phase: 50 mM Ammonium dihydrogenphosphate, pH 4.5, Flow rate: 1.0 mL/min. Temperature: 30 ^oC, Injection Vol.: 5.0 μL, Detection: UV at 254 nm

Summary and Conclusions

- Mixed-mode applications have become popular for difficult applications where compounds vary widely in chemical nature.
- Several ZirChrom® phases, including Zr-PBD, Zr-PS, Zr-MS and Zr-SAX, are ideal for mixed-mode applications and show unique selectivity.
- ZirChrom® phases are stable and reproducible over a wider range of pH and temperature than silica-based phases.

Acknowledgements

The authors wish to thank Supelco for permitting the use of data on quaternary amine compound.

Trademarks used include: ZirChrom®, Discovery®

For more information contact ZirChrom support at www.zirchrom.com or stop by **Booth 1173**.