New Applications of Zirconia Based Stationary Phases

EAS 2008

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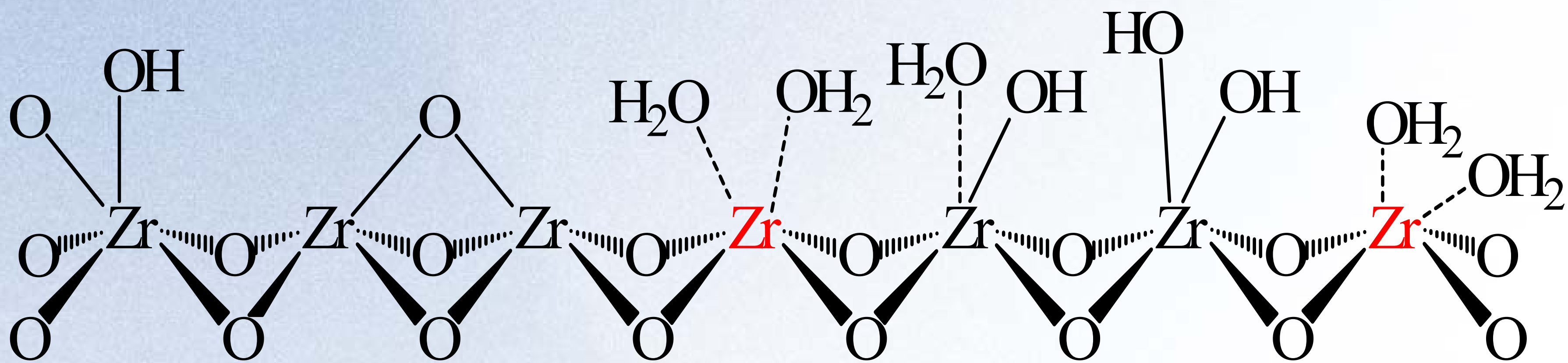
Surface Chemistry of Zirconia



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]

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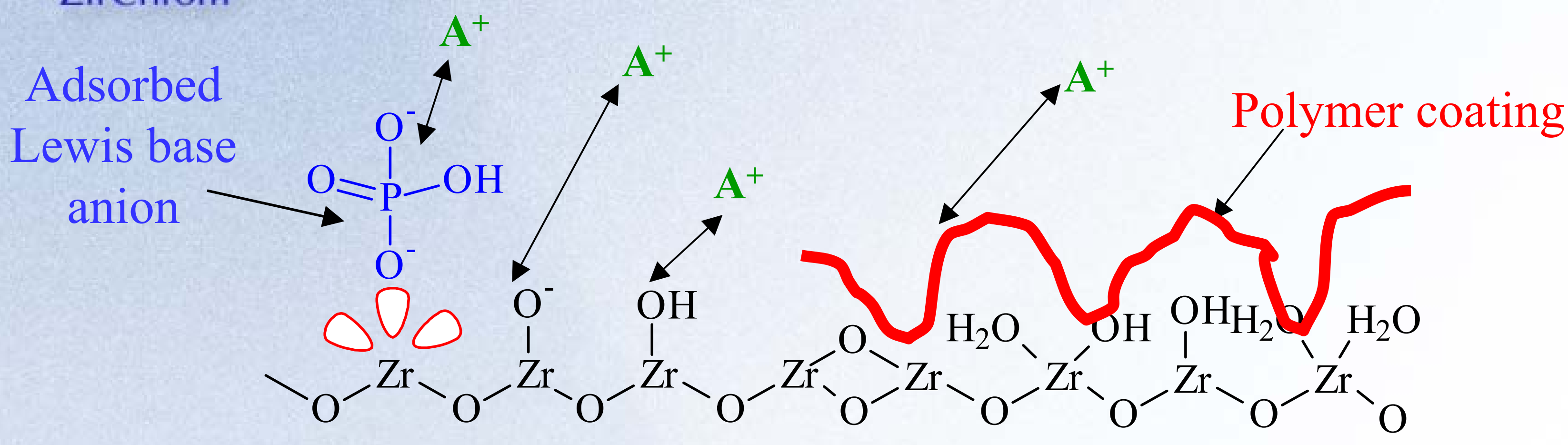
Interaction Strength	Lewis Base (L)
Strongest	Hydroxide
	Phosphate
	Fluoride
	Citrate
	Sulfate
	Acetate
	Formate
	Nitrate
	Chloride
Weakest	Water

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

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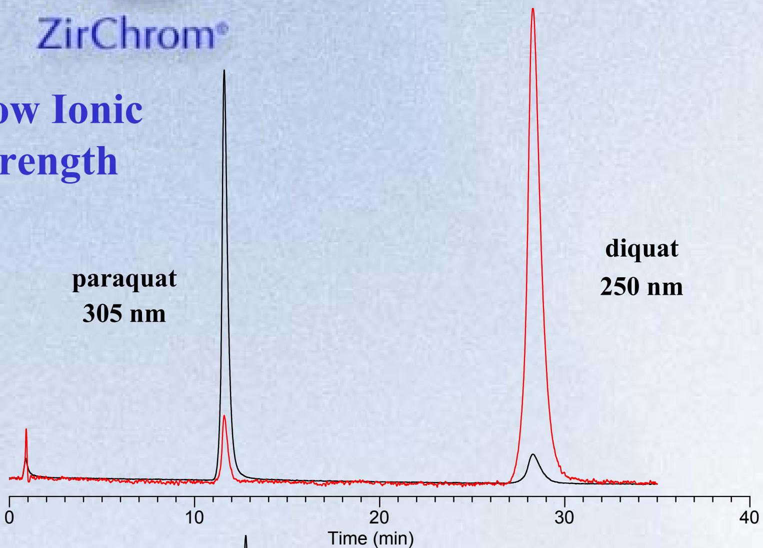
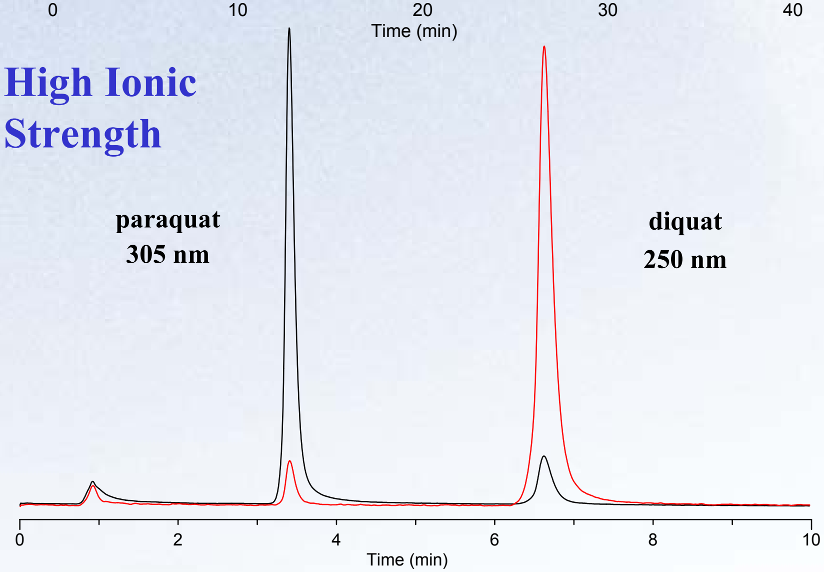
Low Ionic Strength



High Ionic Strength

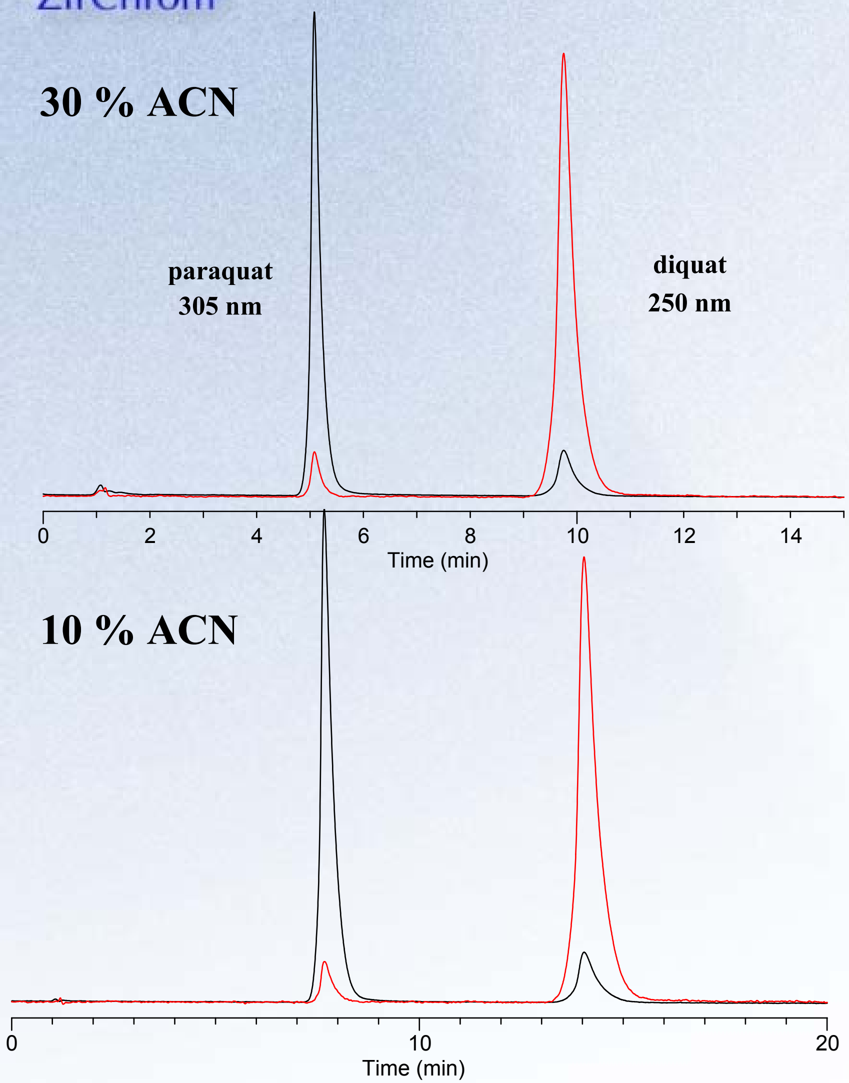


Column: Discovery® Zr-PS, 7.5cm x 2.1mm ID, 3µ particles
Mobile Phase1: 50:50, (20 mM H_3PO_4, 40 mM NH_4HCO_2, pH 7.0 w/ NH_4OH) : Acetonitrile
Mobile Phase2: 50:50, (20 mM H_3PO_4, 100 mM NH_4HCO_2, pH 7.0 w/ NH_4OH) : Acetonitrile
Flow: 0.2 mL/min
Temp: as indicated
Det: UV at 250nm & 305nm
Inj: 1 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

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

Anticholinergics on Zr-PBD[4]

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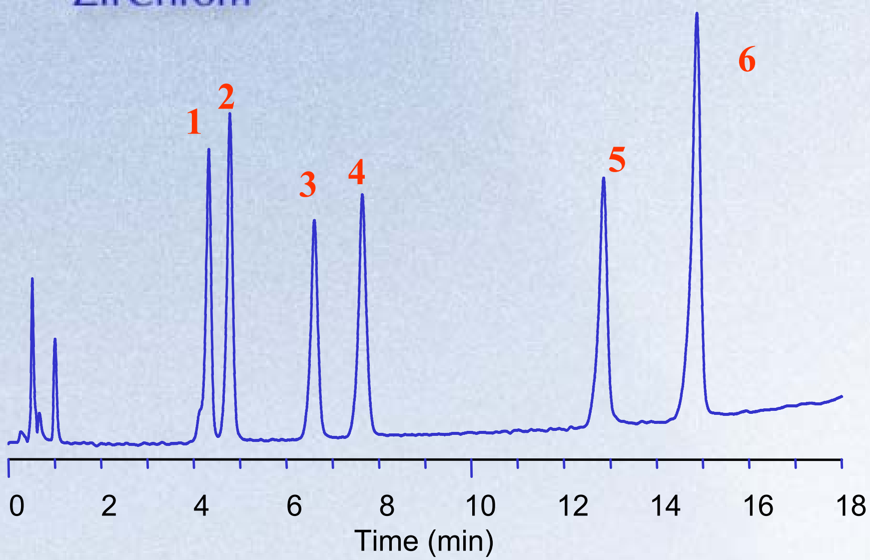
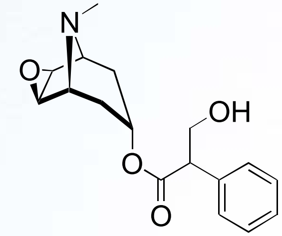
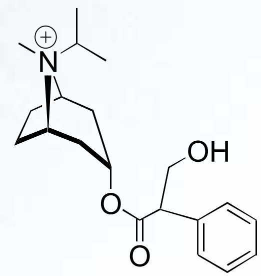


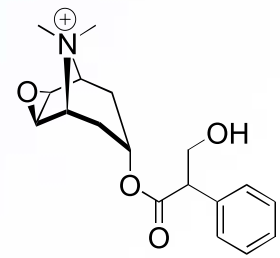
1, Pipenzolate (20 mg/L)



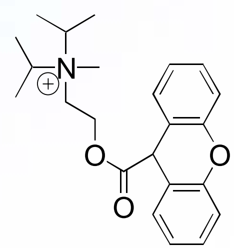
2, Scopolamine (100 mg/L)



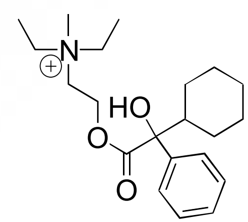
3, Ipratropium (100 mg/L)



4, Methscopolamine (100 mg/L)



5, Propantheline (20 mg/L)



6, Oxyphenonium (100 mg/L)

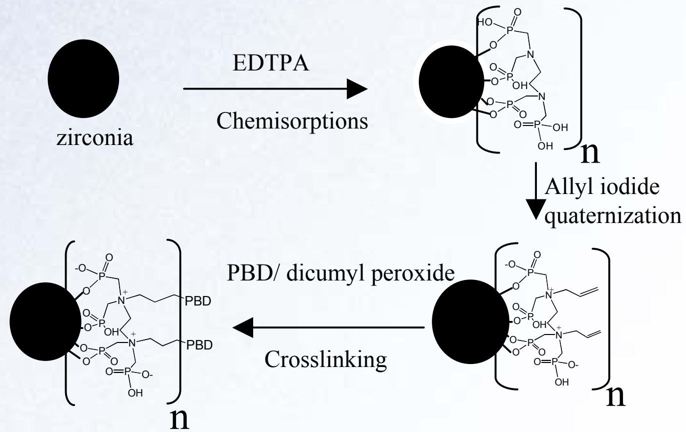
LC Conditions

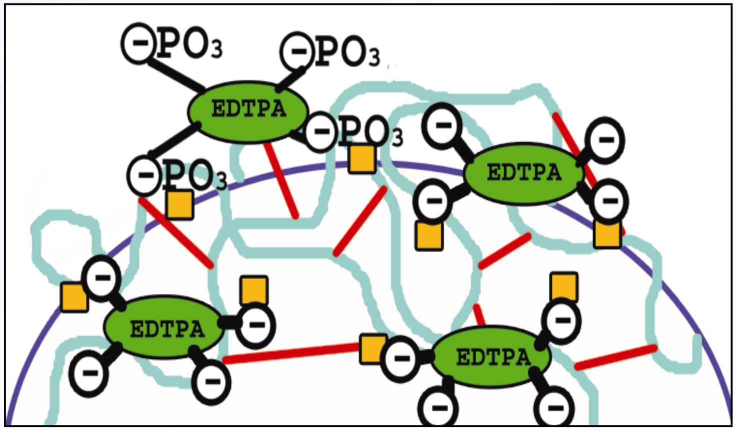
Discovery® Zr-PBD 100mm x 2.1mm i.d., 3 µm
Mobile Phase A: 50:50 [20 mM H_3PO_4, pH 7.0 w/ NH_4OH]:water
Mobile Phase B: 50:30:20 [20 mM H_3PO_4, pH 7.0 w/ NH_4OH]:water:ACN
Gradient 90:10 to 0:100 A:B over 18 minutes
Temp = 80 °C, Flow = 0.3 mL/min, Inj vol = 2 µL,
UV 225 nm, sample in ~60:40 Mobile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

[4] Data used by permission of Supelco

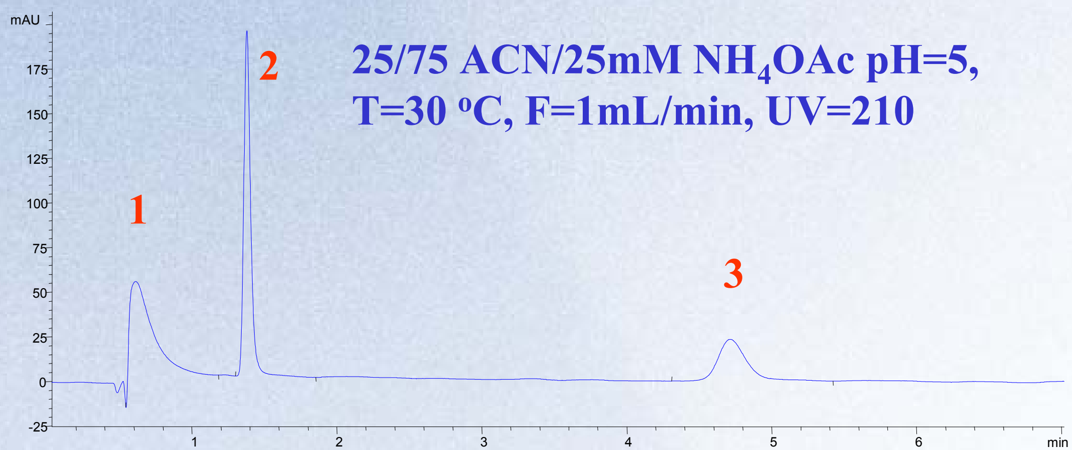
MS Stationary Phase Strategy

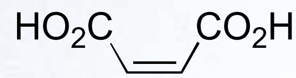
1. Chemisorb Ethylenediamine N,N,N',N'-tetra(methylenephosphonic)acid (EDTPA) to the zirconia surface.
2. Quaternize amines on the zirconia surface with allyl iodide.
3. Coat polybutadiene (PBD) on the chelator-modified zirconia surface and crosslink PBD with allyl group and PBD itself using dicumyl peroxide as initiator.



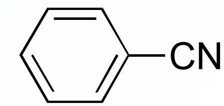


ZirChrom®-MS Multi-Mode Character

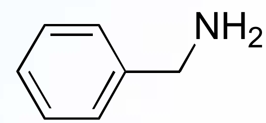




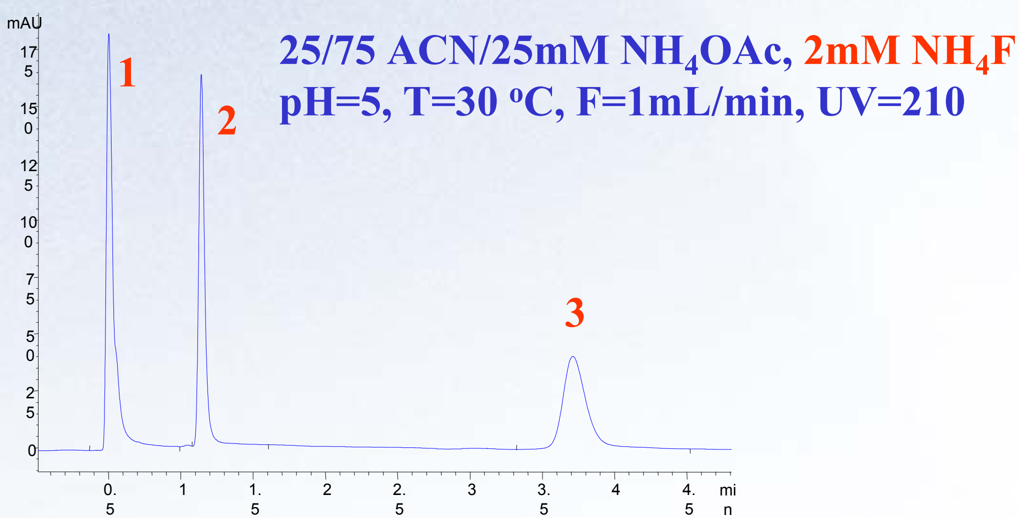
1, Maleic acid



2, Benzonitrile

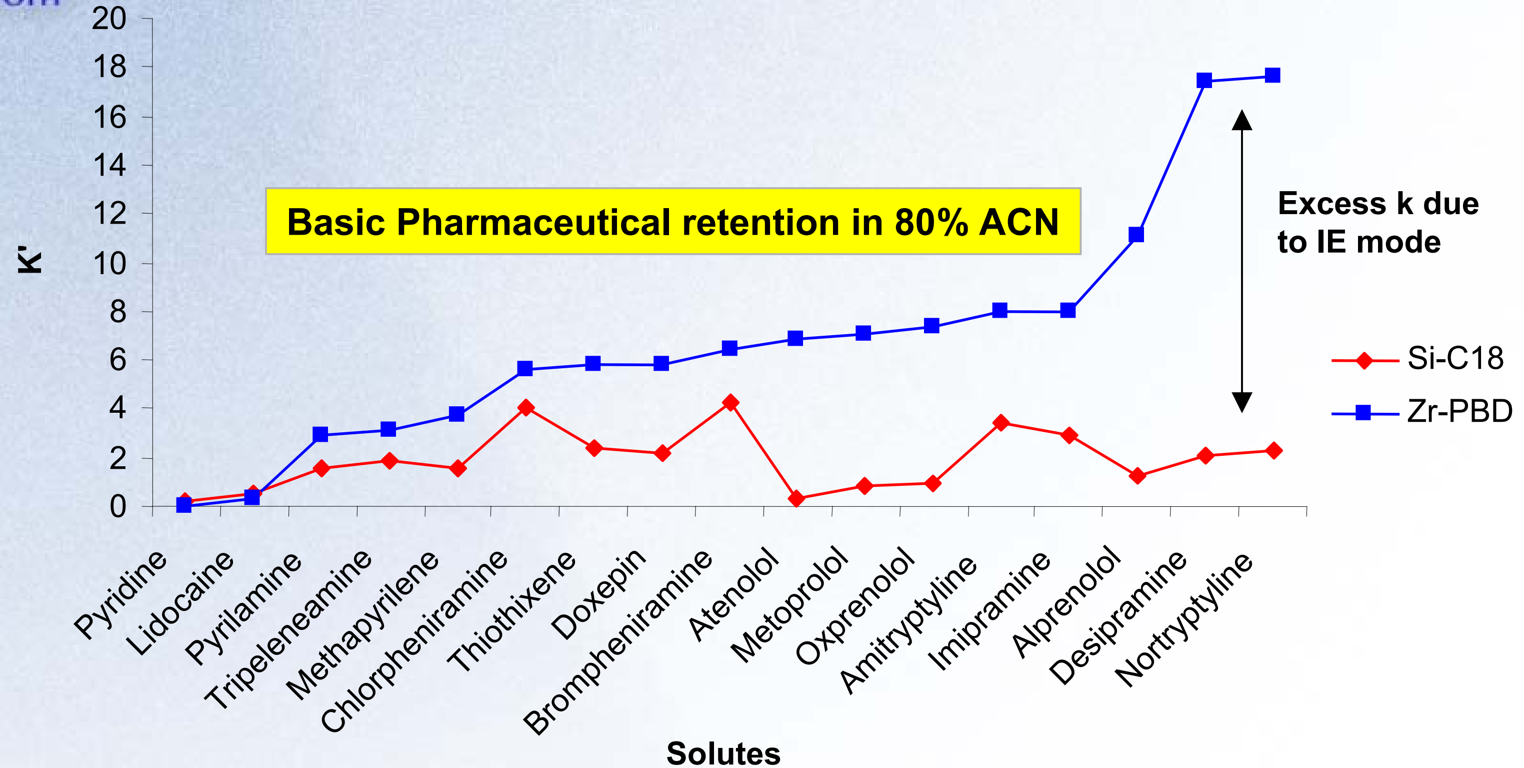


3, Benzyl amine



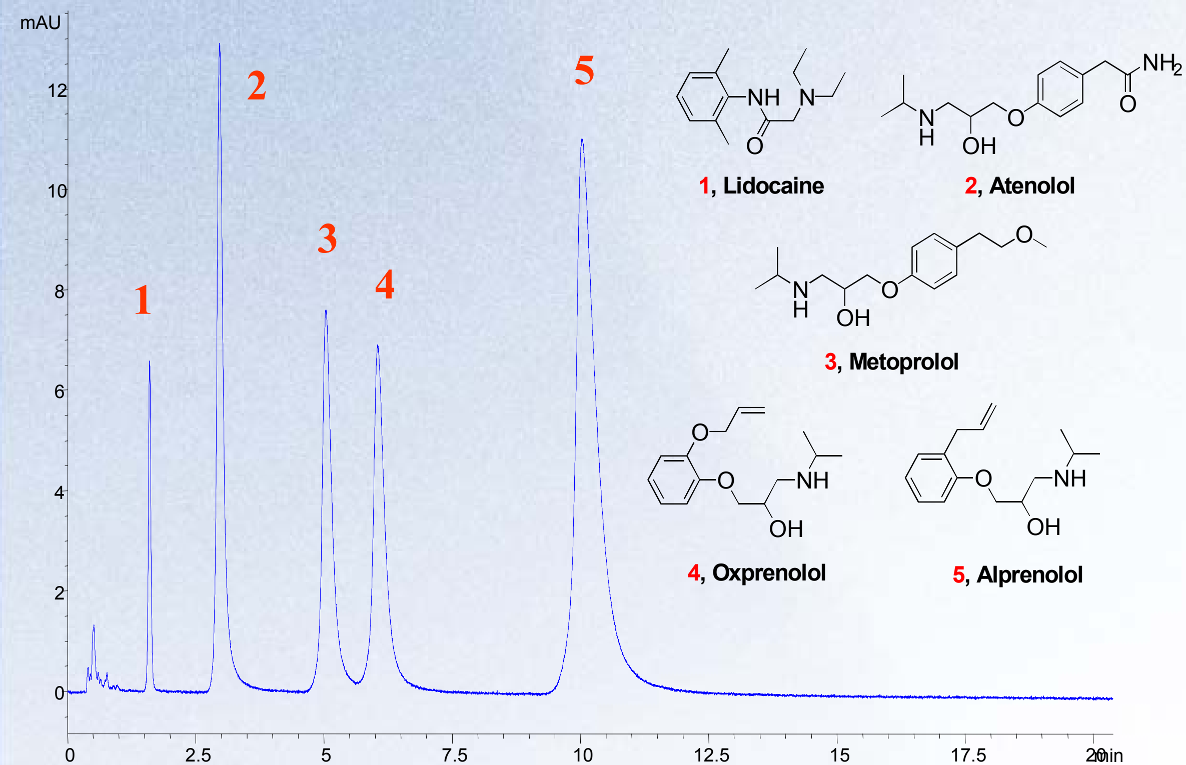
- **Multi-mode retention on ZirChrom®-MS (MS01-0546)**
- **Addition of fluoride blocks Lewis acid sites, decreasing the ligand exchange between zirconia and maleic acid.**

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 °C; Detection at 254 nm; Columns, ZirChrom®-PBD, 50 x 4.6 mm i.d. (3 um particles); Silica-C18 150 x 4.6 mm i.d., (3.5 um particles).

Beta Blockers on ZirChrom®-MS



- **Multi-mode retention creates high k' for bases even in high organic.**
- **Bases are charged at pH 5 and can interact strongly with negative charges on phosphonate groups of EDTPA.**
- **High organic mobile phase with ammonium acetate is ideal for LC-MS.**

<u>LC Conditions</u>: 65/35 ACN/10 mM ammonium acetate pH=5.0; Flow rate, 1.0 mL/min.; Injection volume 5.0 µL; Temperature, 35 ^{o}C; Detection at 254 nm; Columns, ZirChrom®-MS, 50 x 4.6 mm i.d. (3 um particles)

HPLC Analysis of Cattle Plasma Using ZirChrom®-MS[5]

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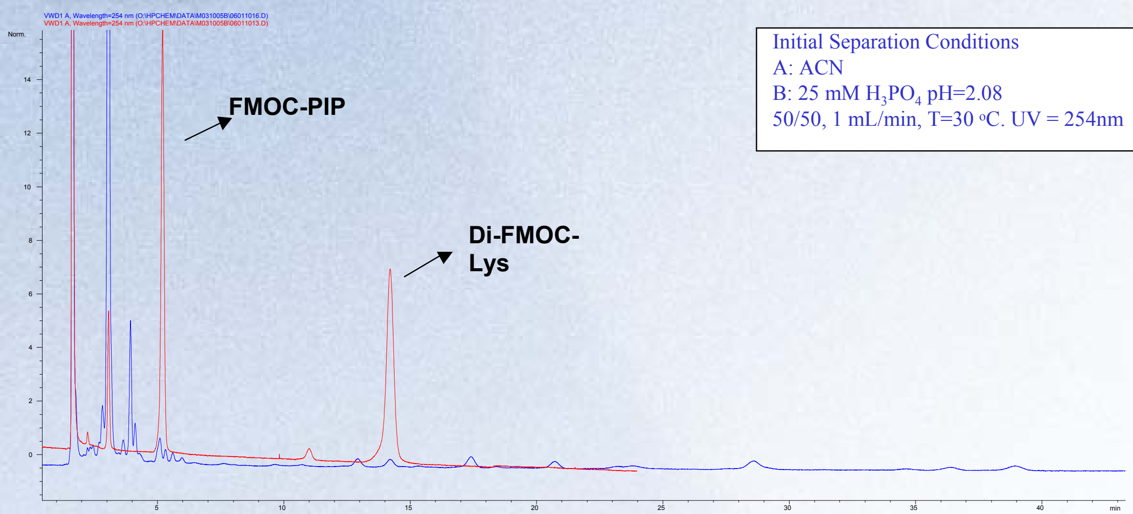
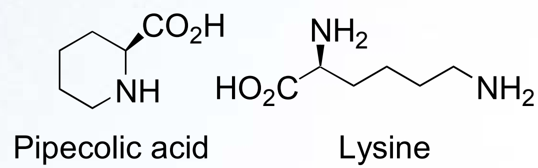
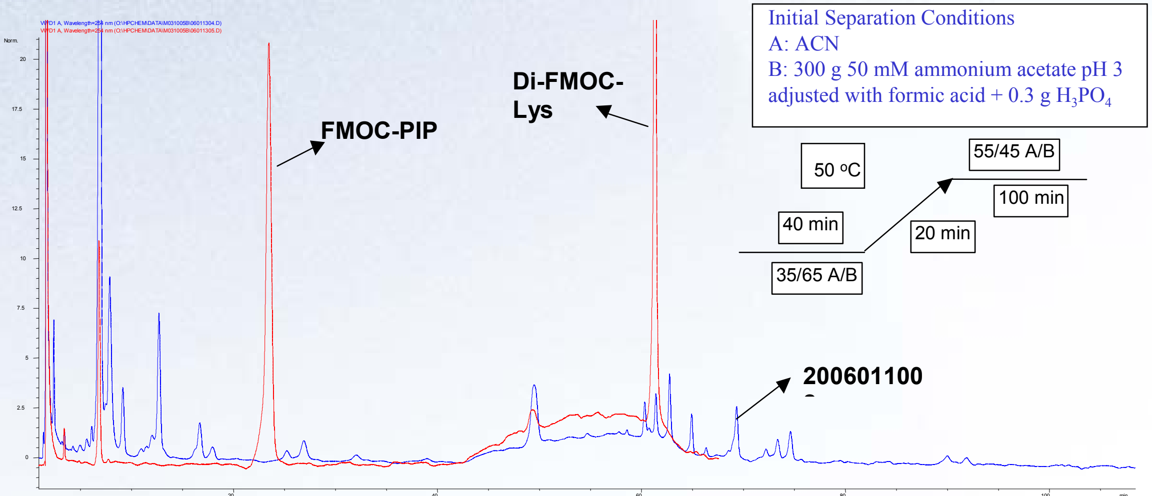


Initial Separation Conditions
A: ACN
B: 25 mM H_3PO_4 pH=2.08
50/50, 1 mL/min, T=30 °C. UV = 254nm





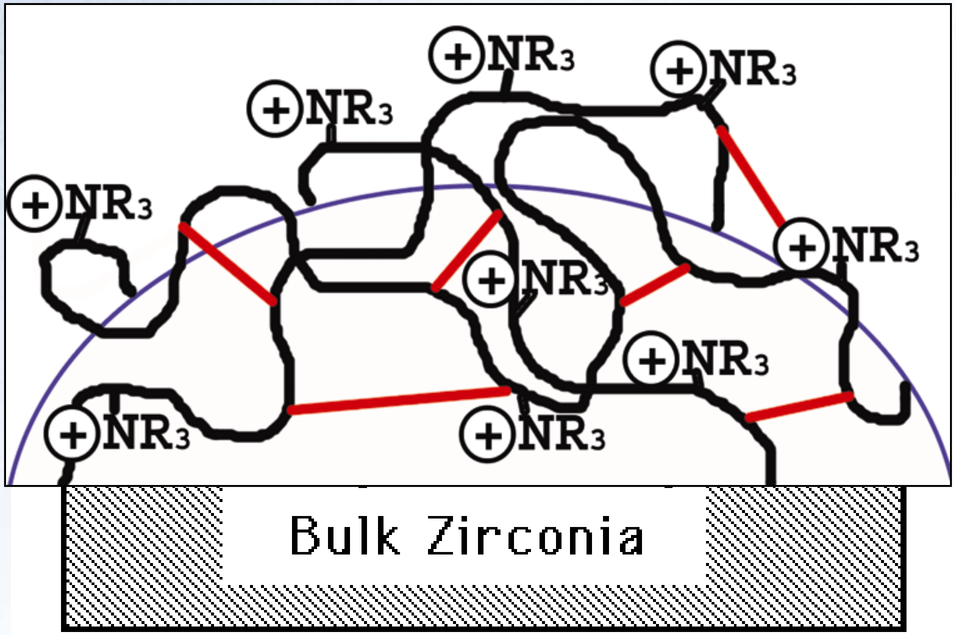
Initial Separation Conditions
A: ACN
B: 300 g 50 mM ammonium acetate pH 3 adjusted with formic acid + 0.3 g H_3PO_4

- **Analysis of pipecolic acid and lysine levels in cattle plasma ZirChrom®-MS (MS01-1546).**
- **IEX and RP modes contribute to making a difficult separation.**
- **Organic solvent, pH and ionic strength are all important variables with ZirChrom-MS.**

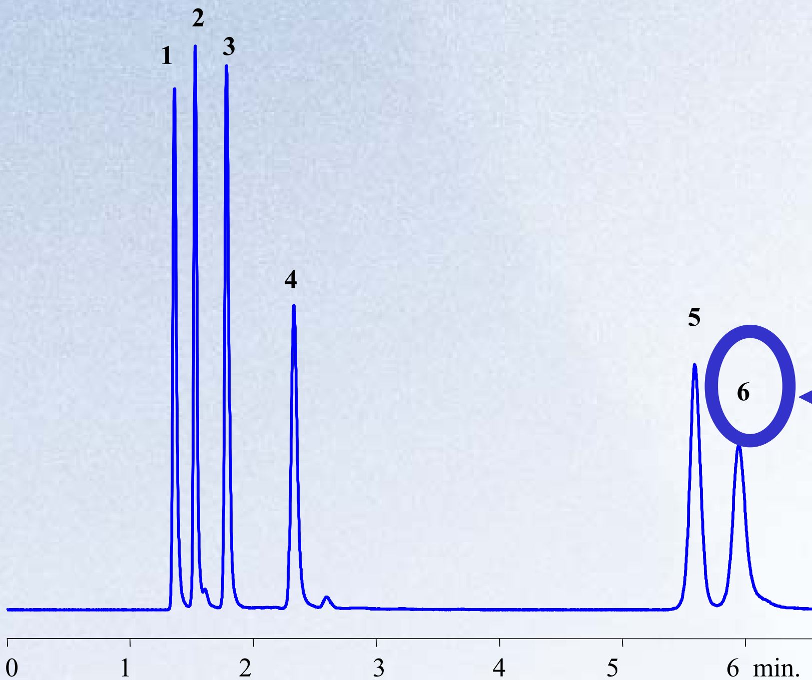
[5] Data used by permission of SarTec Corporation (Anoka, MN)

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 ^{o}C, 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 compounds and SarTec for use of data on cattle plasma compounds.

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