# Ion-Exchange Separations on Zirconia-based Stationary Phases

**Pittcon 2009**

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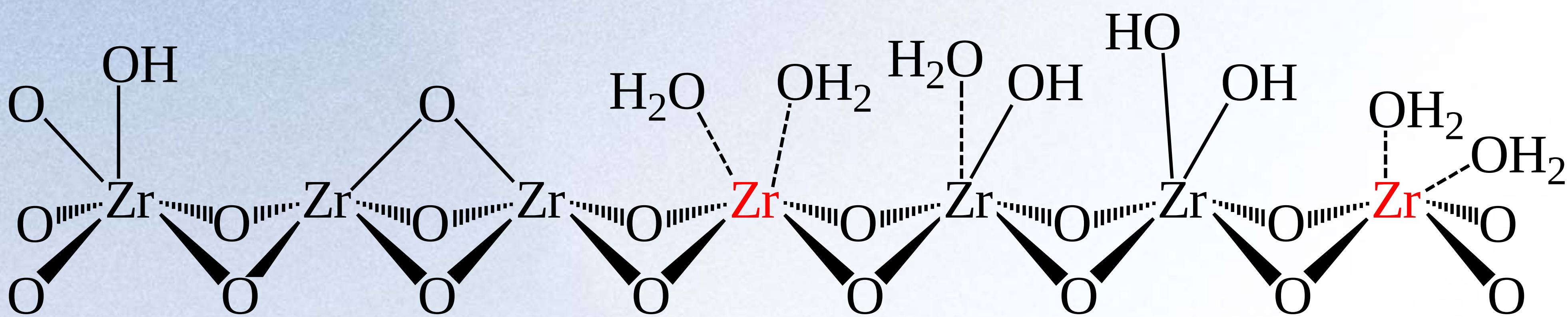
**Specialists in High Efficiency, Ultra-Stable Phases for HPLC**

# 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**

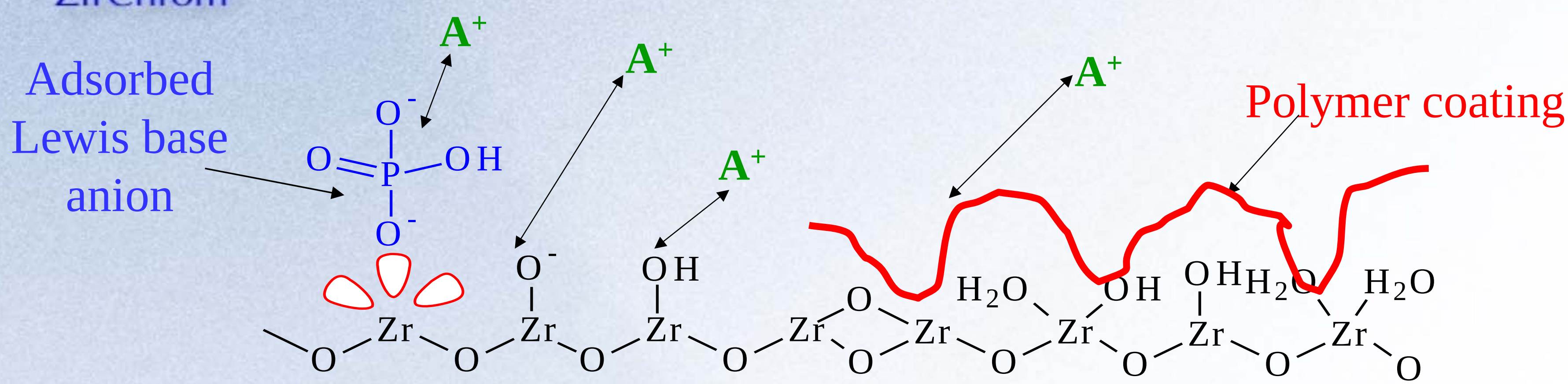
# Interaction Strength of Lewis Bases with Zirconia[3]

| Interaction Strength | Lewis Base (L) |
|---|---|
| Strongest ↓ Weakest | Hydroxide<br>Phosphate<br>Fluoride<br>Citrate<br>Sulfate<br>Acetate<br>Formate<br>Nitrate<br>Chloride<br>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

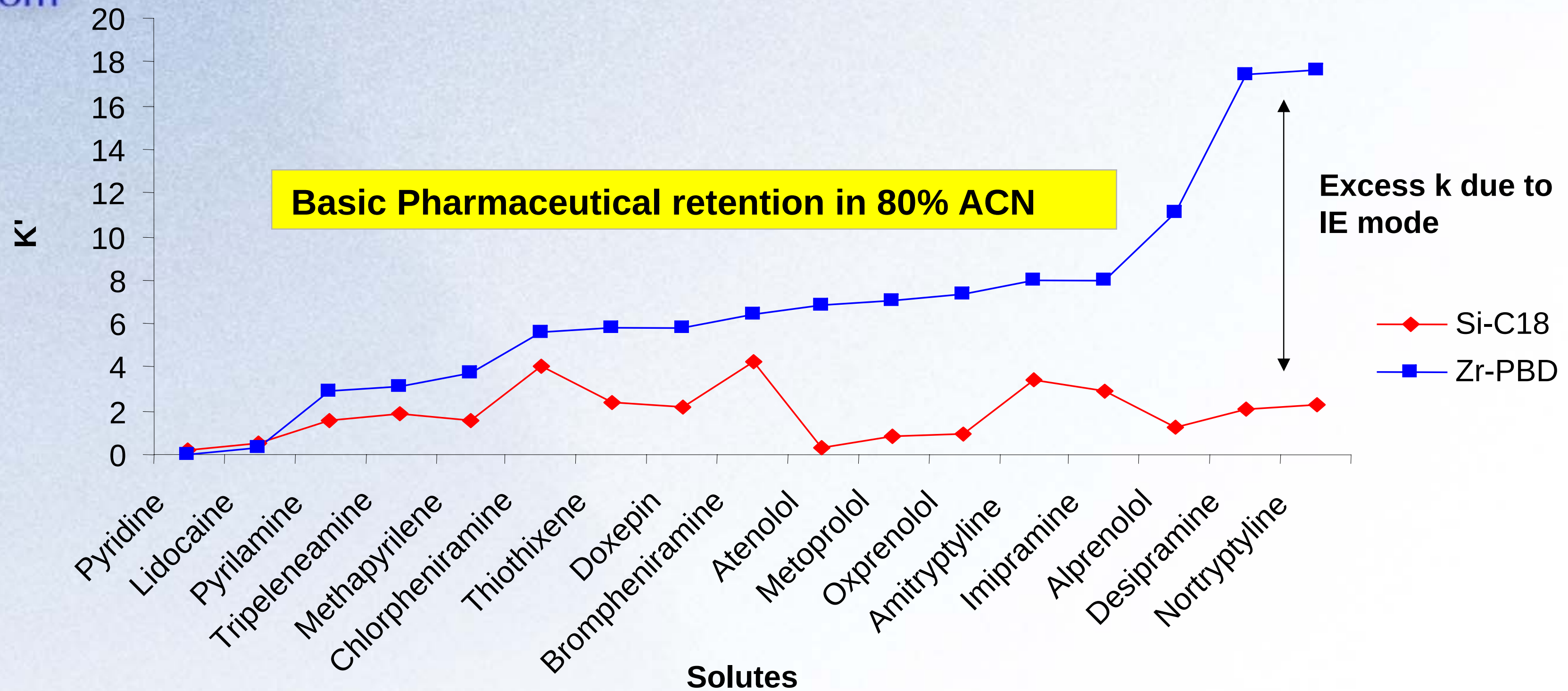
➢Lewis Base Anions — Ion-Exchange (IEX) Sites

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

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

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

# Retention Comparison: Si-C18 vs Zr-PBD

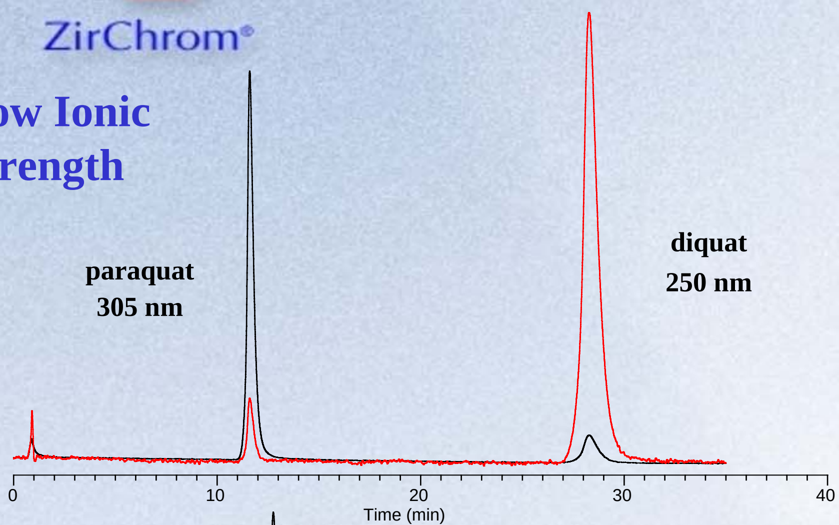



LC Conditions: Machine-mixed 80/20 ACN/10 mM ammonium acetate pH=6.7 without pH adjustment; Flow rate, 1.0 ml/min.; Injection volume 0.1 ul; Temperature, 35 $^{o}$C; Detection at 254 nm; Columns, ZirChrom®-PBD, 50 x 4.6 mm i.d. (3 um particles); Silica-C18 150 x 4.6 mm i.d., (3.5 um particles).

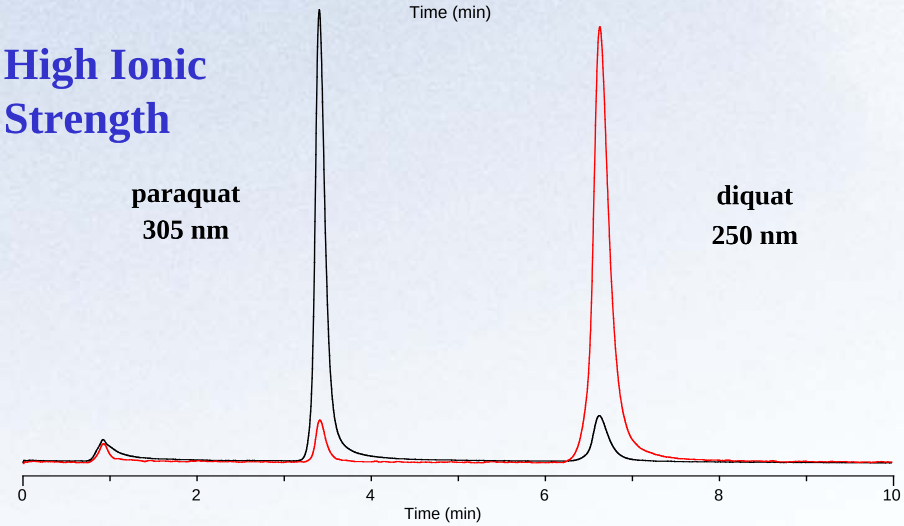
# Effect of Ionic Strength on the Separation of Quaternary Amines[4]

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 $NH_4HCO_3$, pH 7.0 w/ $NH_4OH$) : Acetonitrile

**Mobile Phase2:** 50:50, (20 mM $H_3PO_4$, 100 mM $NH_4HCO_3$, pH 7.0 w/ $NH_4OH$) : Acetonitrile

**Flow:** 0.2 mL/min

**Temp:** as indicated

**Det:** UV at 250nm & 305nm

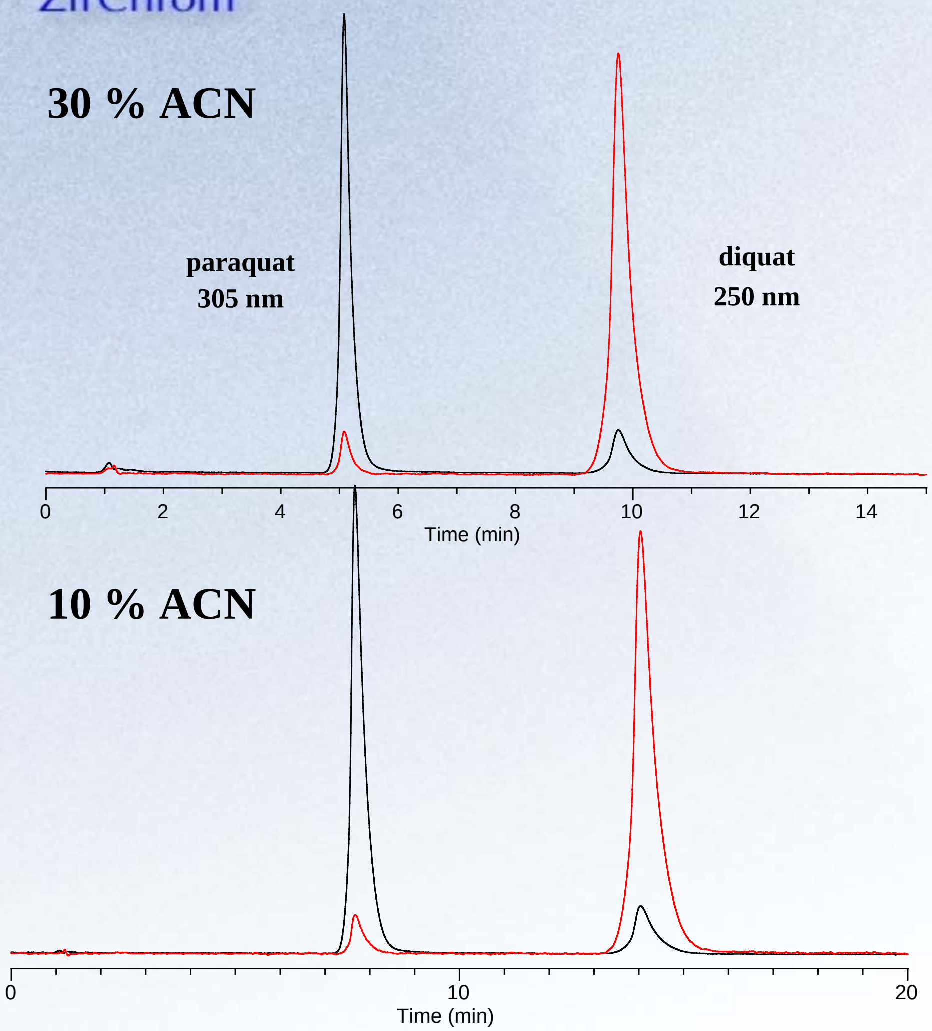
**Inj:** 1 µL

**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

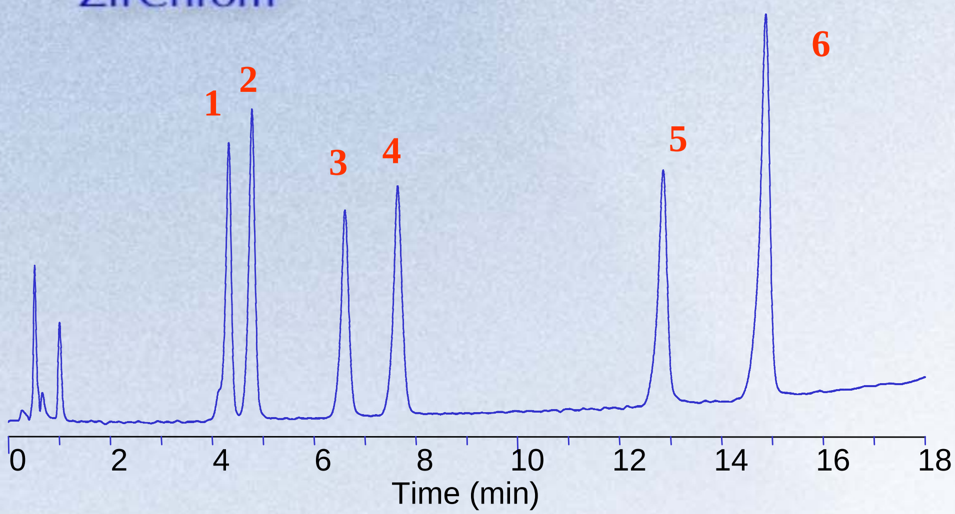
**Inj:** 1 µL

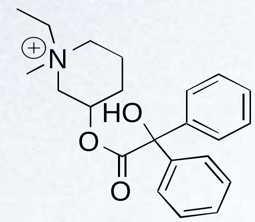
**Sample:** diquat & paraquat in water; 100 mg/L ea.

- **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.**

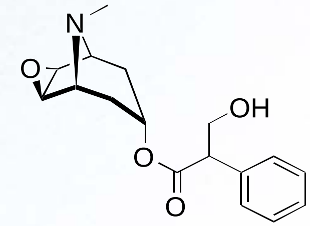
[4] Data used by permission of Supelco

# Anticholinergics on Zr-PBD[4]

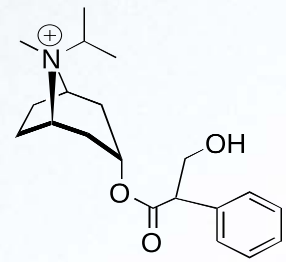


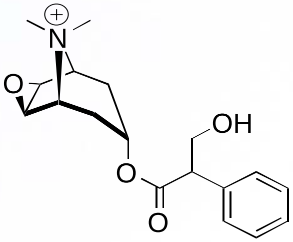

**1**, Pipenzolate (20 mg/L)



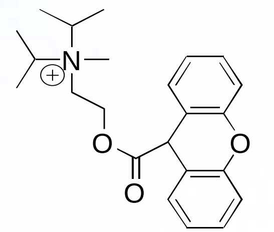

**2**, Scopolamine (100 mg/L)



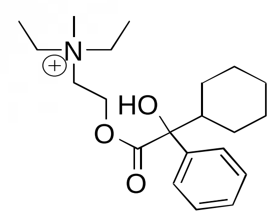

**3**, Ipratropium (100 mg/L)




**4**, Methscopolamine (100 mg/L)




**5**, Propantheline (20 mg/L)



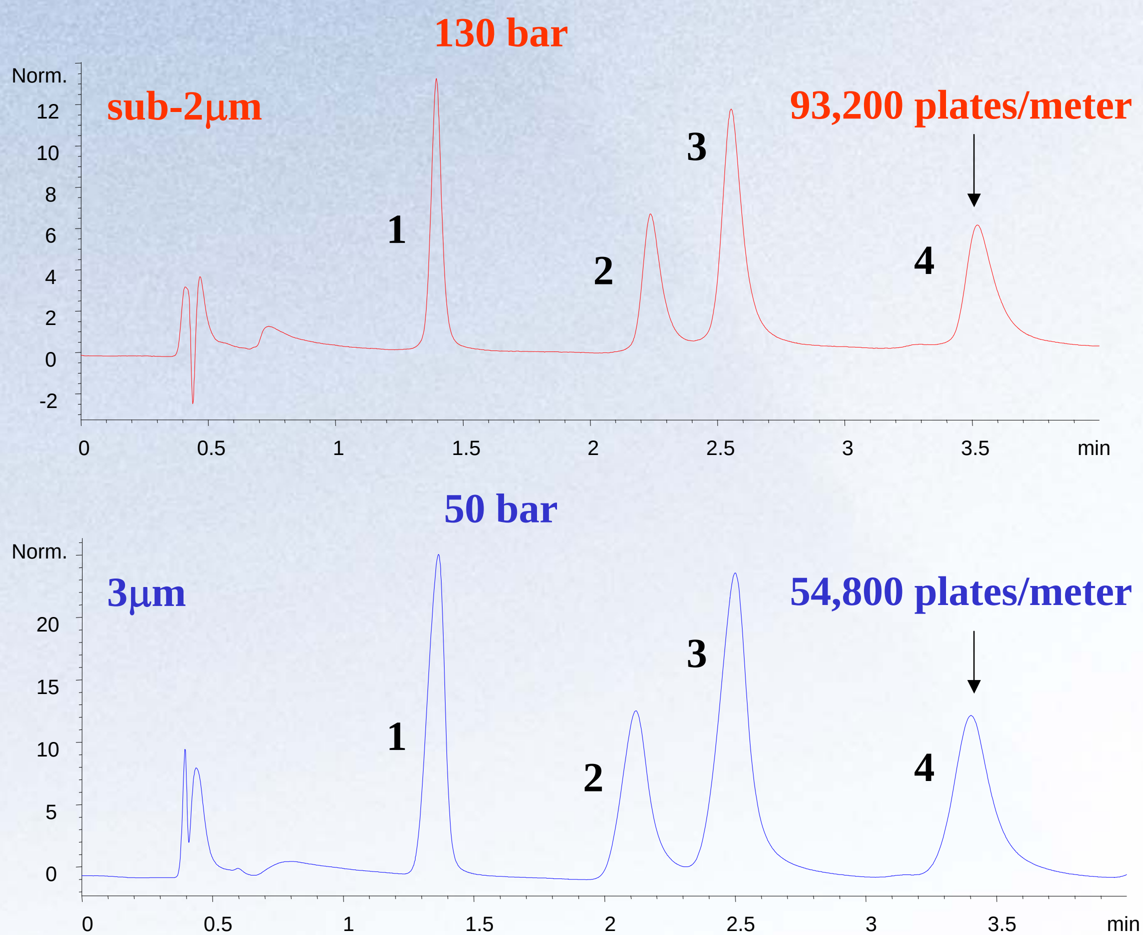

**6**, Oxyphenonium (100 mg/L)

## 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

# ZirChrom-PBD Separation of Catecholamines: sub-2µm vs 3µm, T = 30 $^{0}$C



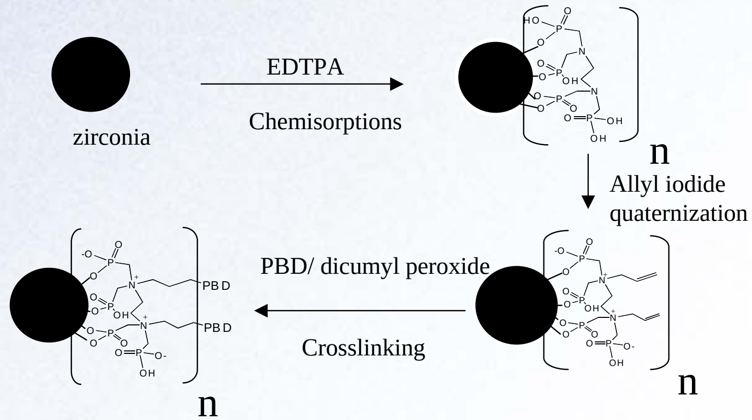

**<u>Conditions</u>**

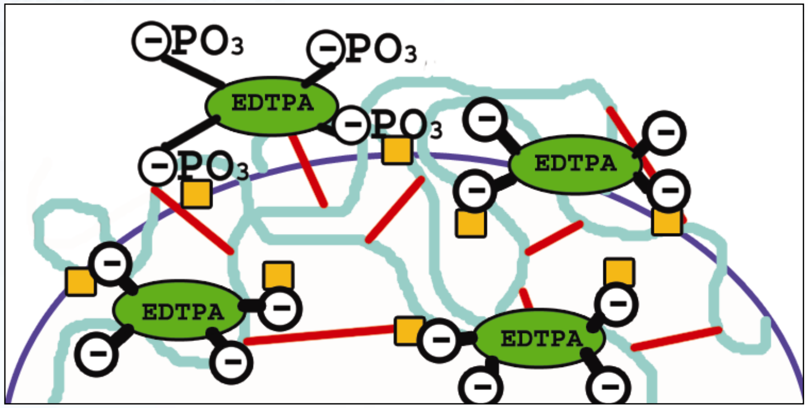
Column: ZirChrom-PBD 50mm x 4.6mm
Mobile Phase: 85/15 ACN/30mM $NH_4OAc$, 10mM $NH_4H_2PO_4$ adjusted to pH=3.4 w/ HCl
Flow rate = *1.5 mL/min*
Temperature = 30$^{o}$C
Inj Vol = 5 µL
Elution order: **1=**Tyramine, **2=**Epinephrine
**3**=Dopamine,
**4**=3,4-dihydroxynorephedrine

- **Smaller particle gives increased efficiency**
- **Good reproducibility**
- **Fast separation times**

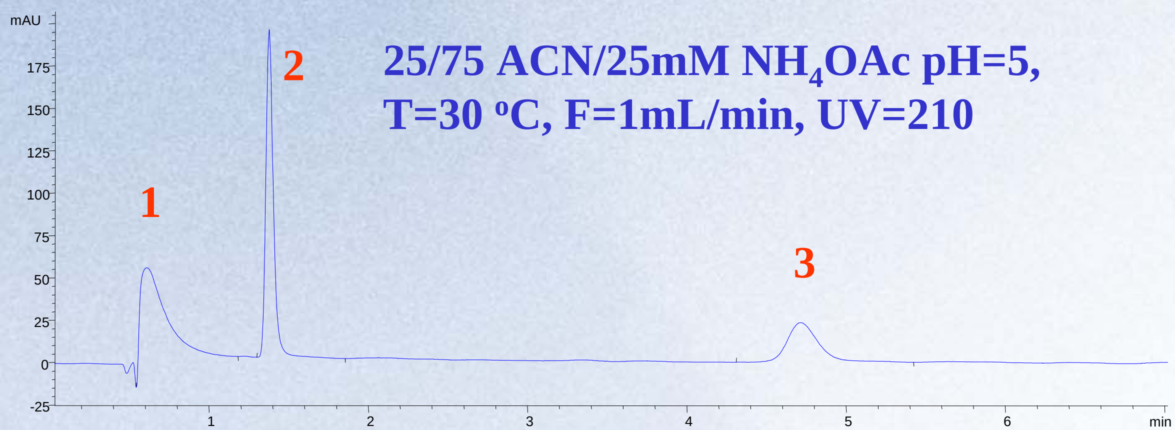
# MS Stationary Phase Strategy

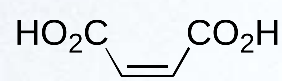
1. **Chemisorb Ethylenediamine N,N,N',N'-tetra(methylenephosphonic) acid (EDTPA) to the zirconia surface.**
2. **Quaternize amines on the zirconia surface with allyl iodide.**
3. **Coat polybutadiene (PBD) on the chelator-modified zirconia surface and crosslink PBD with allyl group and PBD itself using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



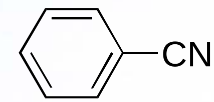



# ZirChrom®-MS Multi-Mode Character

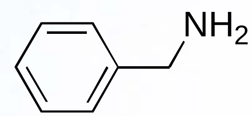


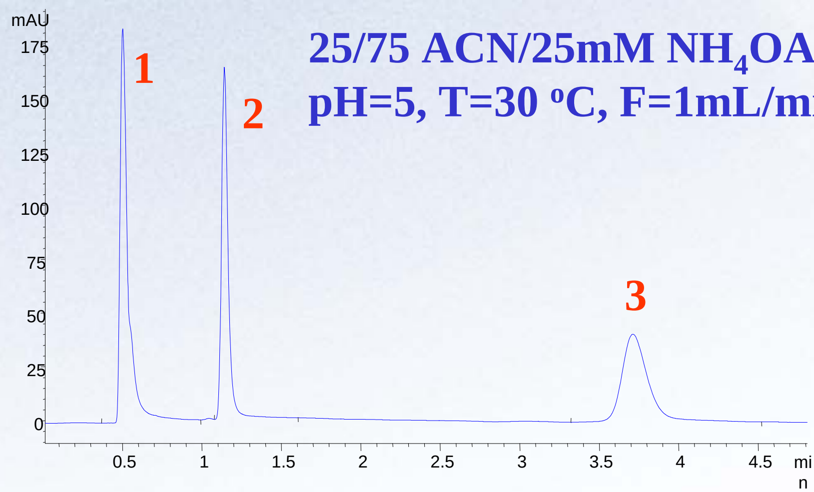

1, Maleic acid




2, Benzonitrile

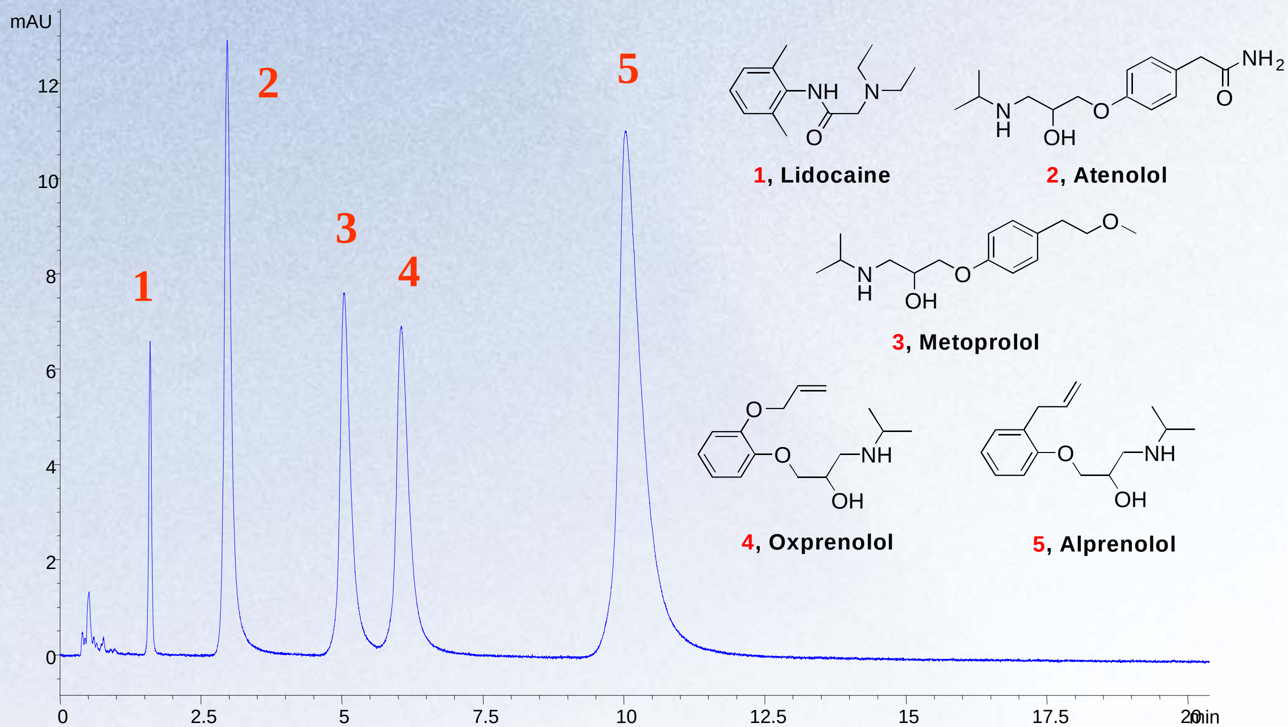



3, Benzyl amine




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

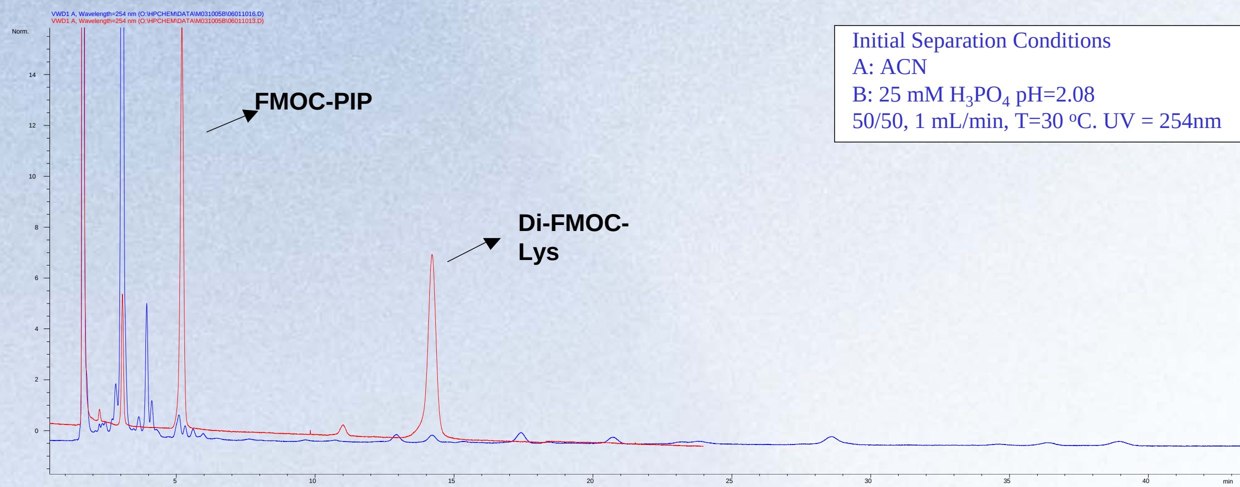
# Beta Blockers on ZirChrom®-MS



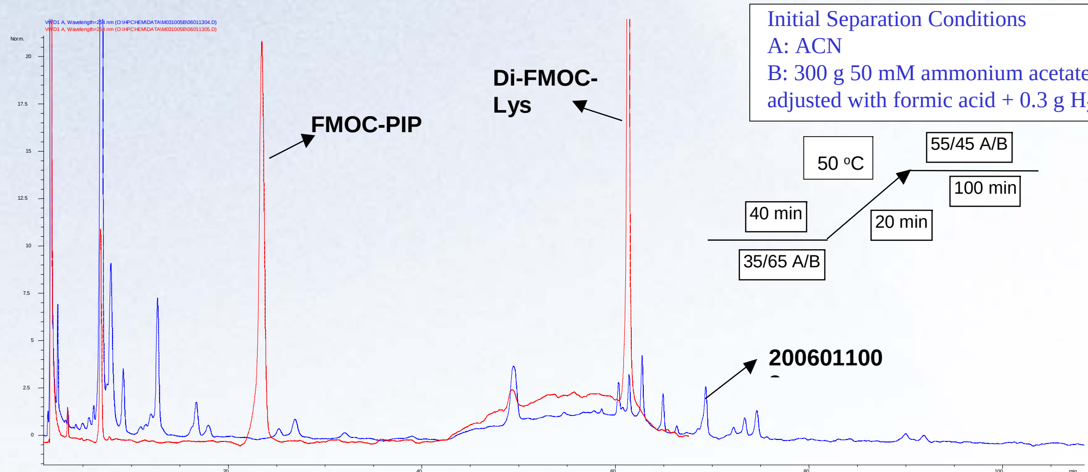

**<u>LC Conditions</u>**: 65/35 ACN/10 mM ammonium acetate pH=5.0; Flow rate, 1.0 mL/min.; Injection volume 5.0 µL; Temperature, 35 °C; Detection at 254 nm; Columns, ZirChrom®-MS, 50 x 4.6 mm i.d. (3 um particles)

- **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.**

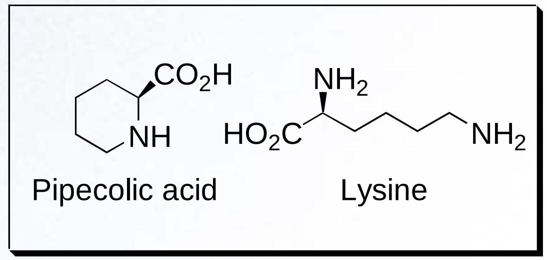
# HPLC Analysis of Cattle Plasma Using ZirChrom®-MS[5]




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 $H_3PO_4$

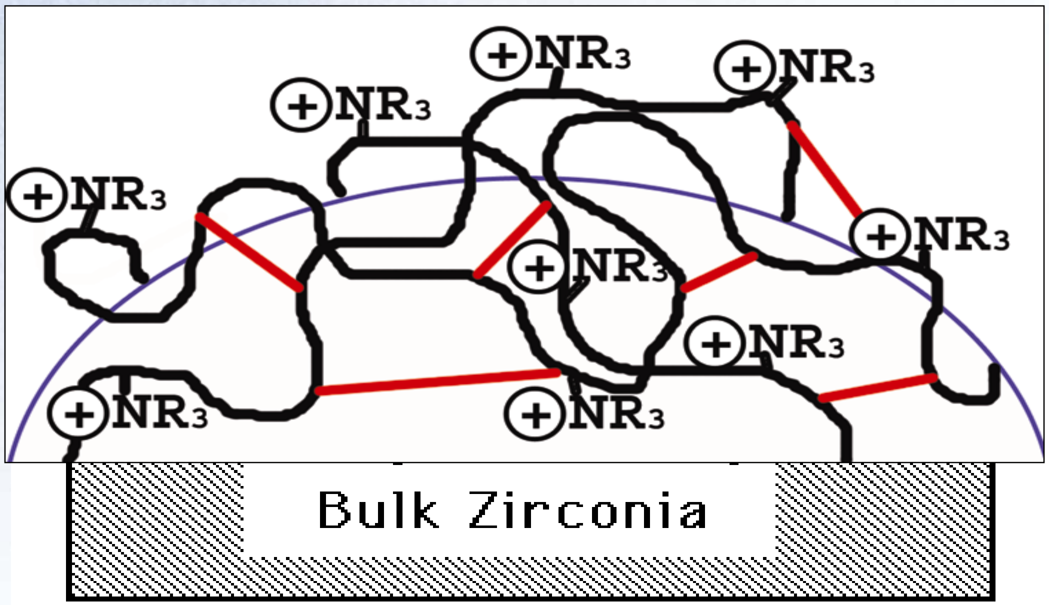



- **Analysis of pipecolic acid and lysine levels in cattle plasma ZirChrom®-MS (MS01-1546).**
- **IEX and RP modes contribute to performing this difficult separation.**
- **Organic solvent, pH and ionic strength are all important variables with ZirChrom-MS.**

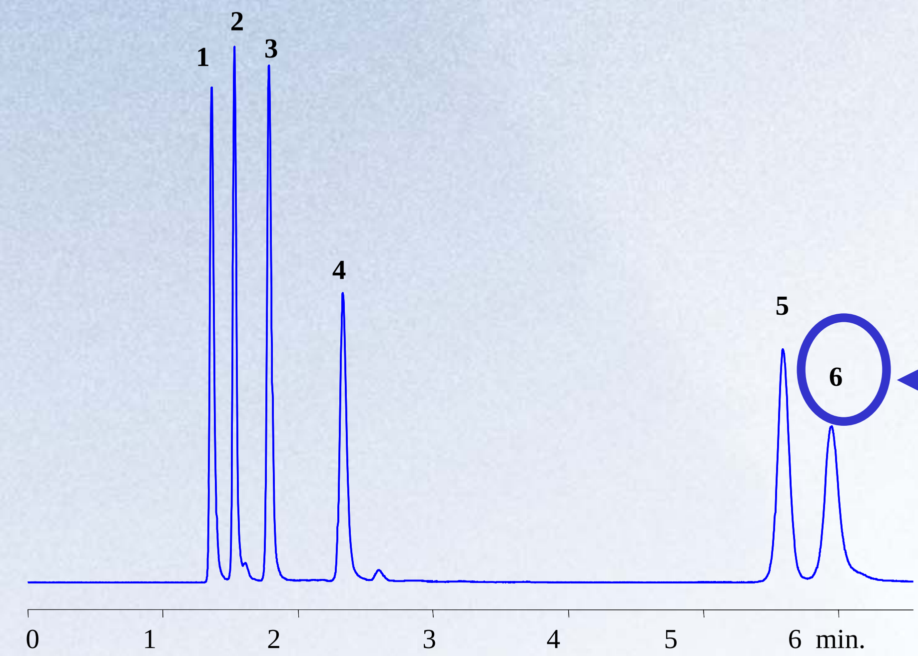
[5] Data used by permission of SarTec Corporation (Anoka, MN)

# 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, Conclusions, Acknowledgements*

- **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.**

For more information contact ZirChrom support
at www.zirchrom.com or stop by **Booth 746**.

*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. Trademarks used include: ZirChrom®, Discovery®