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ANALYSIS OF CATECHOLAMINES USING ZIRCONIA-BASED HPLC COLUMNS

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Specialists in High Efficiency, Ultra-Stable Phases for HPLC

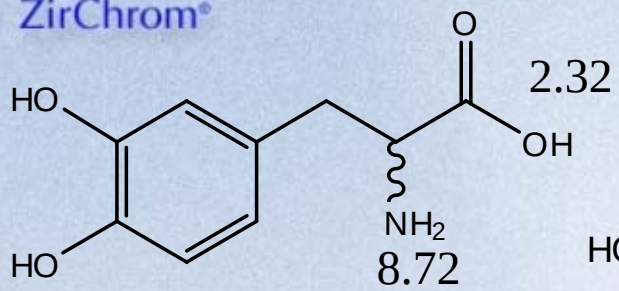
Analysis of Catecholamines

I. Structure & Surface Chemistry

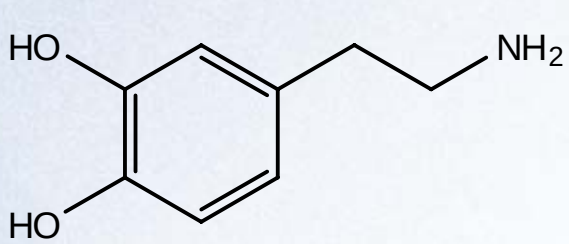
II. Case Study

III. Conclusions

Catecholamine Structure

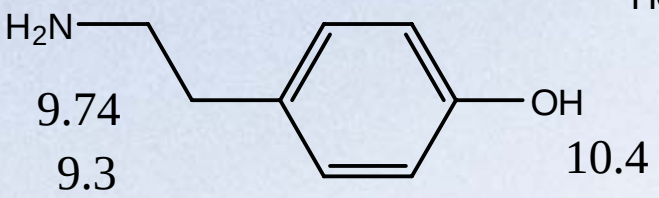


DL-Dopa

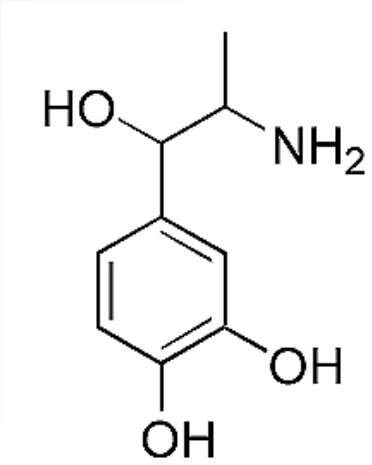


10.10

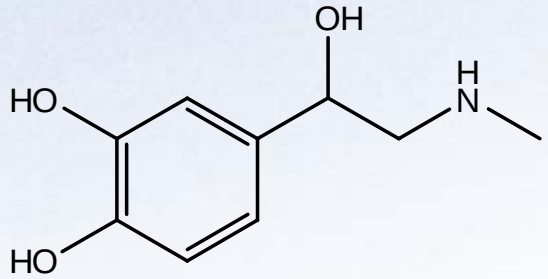
Dopamine



Tyramine



3,4-dihydroxynorephedrine (DHNP)



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Epinephrine

- Zwitterionic
- Metal Chelator
- Lewis Base

Surface Chemistry of Zirconia-Based Supports for HPLC

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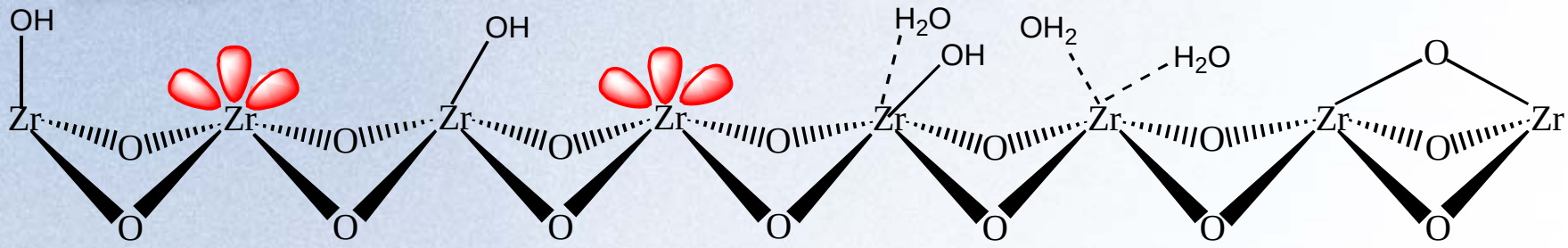
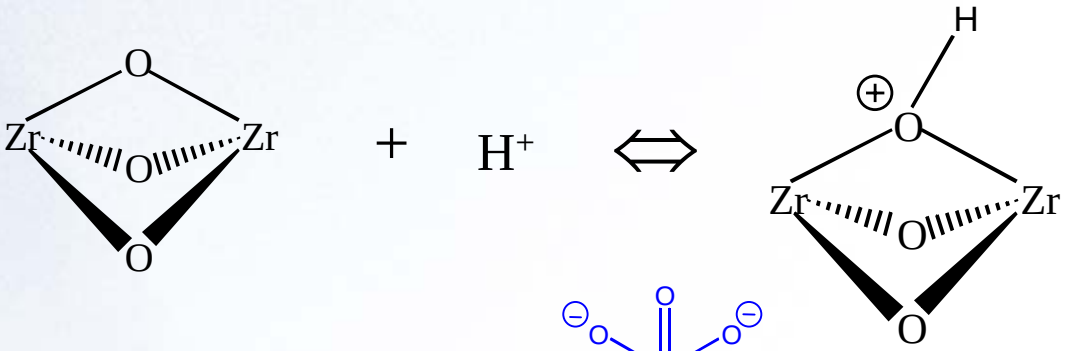
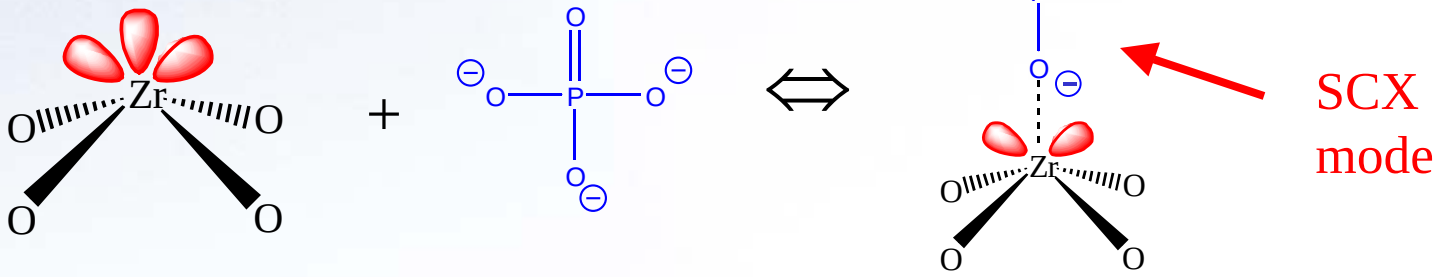


Weak Brönsted Acid: $ZrOH + OH^- \Leftrightarrow ZrO^- + H_2O$

Weak Brönsted Base:



Strong Lewis Acid:



Slide Courtesy of Dr. Dwight Stoll

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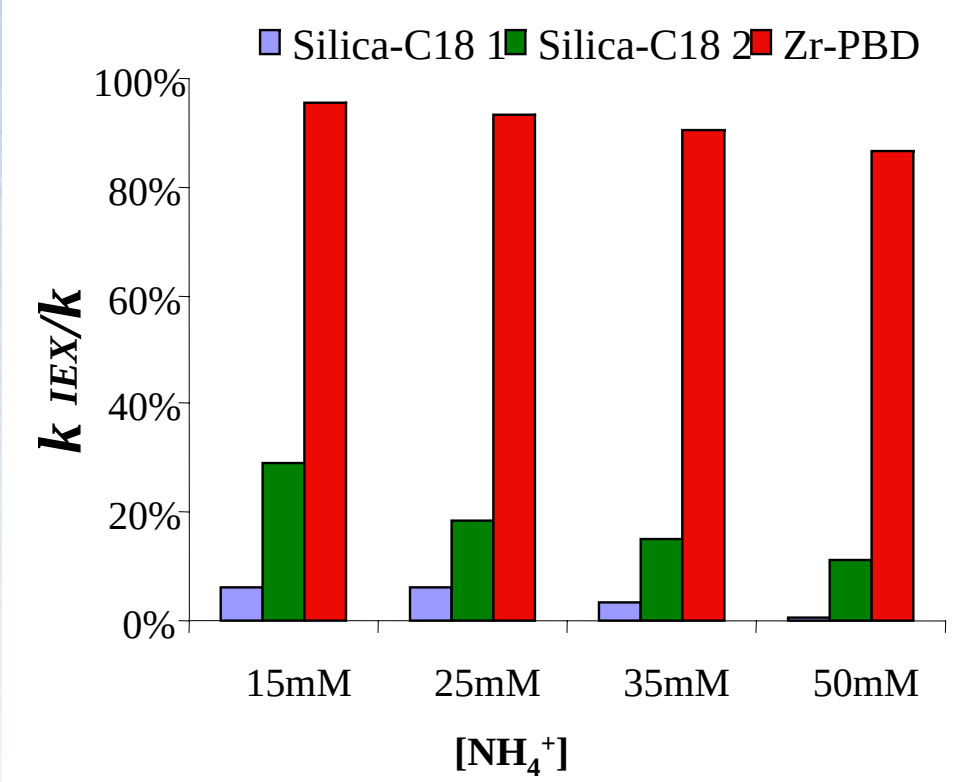
Interaction Strength of Lewis Bases with Lewis Acid Sites on Zirconia

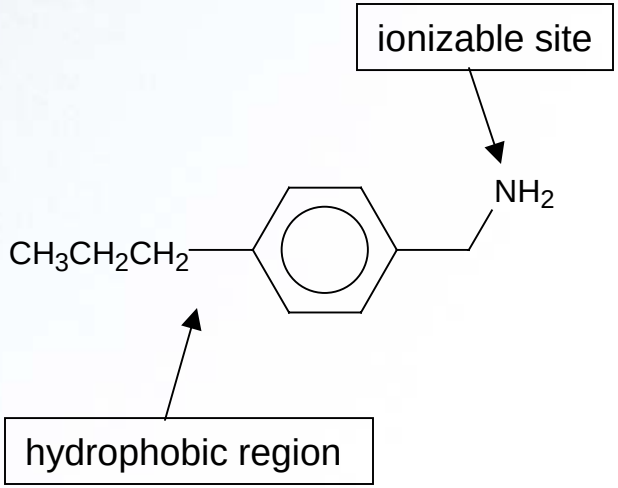
Interaction Strength	Lewis Base Anion (A^-)
Strongest	Hydroxide (cleaning method)
	Phosphate
	Fluoride
	Citric acid
	Sulfate
	Acetic acid
	Formic acid
	Nitrate
Weakest	Chloride

- Lewis bases with higher electron density and lower polarizability interact more strongly with zirconia.

Cation-Exchange Character

Retention of p-propylbenzylamine demonstrates the presence of more ion-exchange sites on ZirChrom®-PBD compared to C18-silicas.

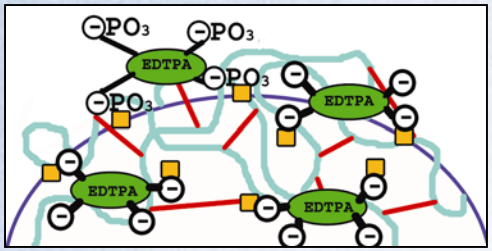




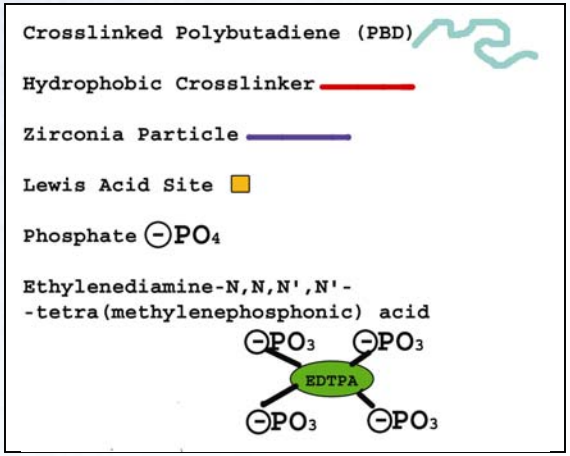
Conditions: 55% CH_3OH in ammonium phosphate (pH, 6.0); 35°C; 1.0 mL/min.; UV 254 nm

Slide Courtesy of Dr. Richard Henry

Phase Selection



ZirChrom®-MS



Ethylenediamine-N,N’-tetra(methylenephosphonic) acid = EDTPA

- EDTPA treatment (reflux particles in EDTPA solution)
  - Strong Lewis base chelate attaches to the surface
  - Probably multidentate attachment- very strongly held
  - Blocks undesirable Lewis acid/base interactions
  - Imparts cation exchange (SCX) properties to zirconia
  - Minimal RP behavior

Mobile Phase Selection

- Acidic pH Required
  - Compound Stability, Very unstable
  - Ensure all compounds carry only one charge

Ion Exchange Contribution

- Acetic acid pH 5.0, concentrations 10 – 100 mM

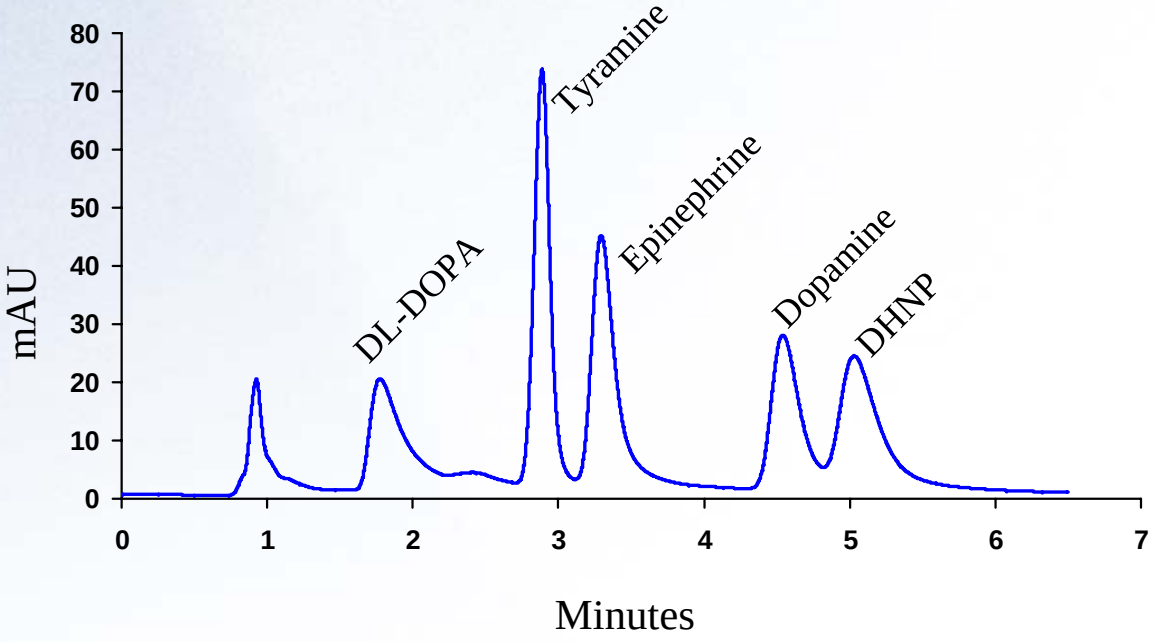
  - Result: No peak elution, elution of tyramine

- Lower Ion Exchange by Lower pH

  -Less net negative charge at lower pH

pH 3.5

Conditions: ZirChrom® -MS 50 mm x 4.6 mm, 70/30 ACN/50 mM Ammonium dihydrogenphosphate, pH=3.5, Flow=0.6 ml/min , Temperature =35° C, Detection UV= 254nm, 5 ul injection. Solutes: Impurity (DHNP & Ddg, of Epinephrine), DL-DOPA, Tyramine, Epinephrine, Dopamine, DHNP.



Effect of Organic Type

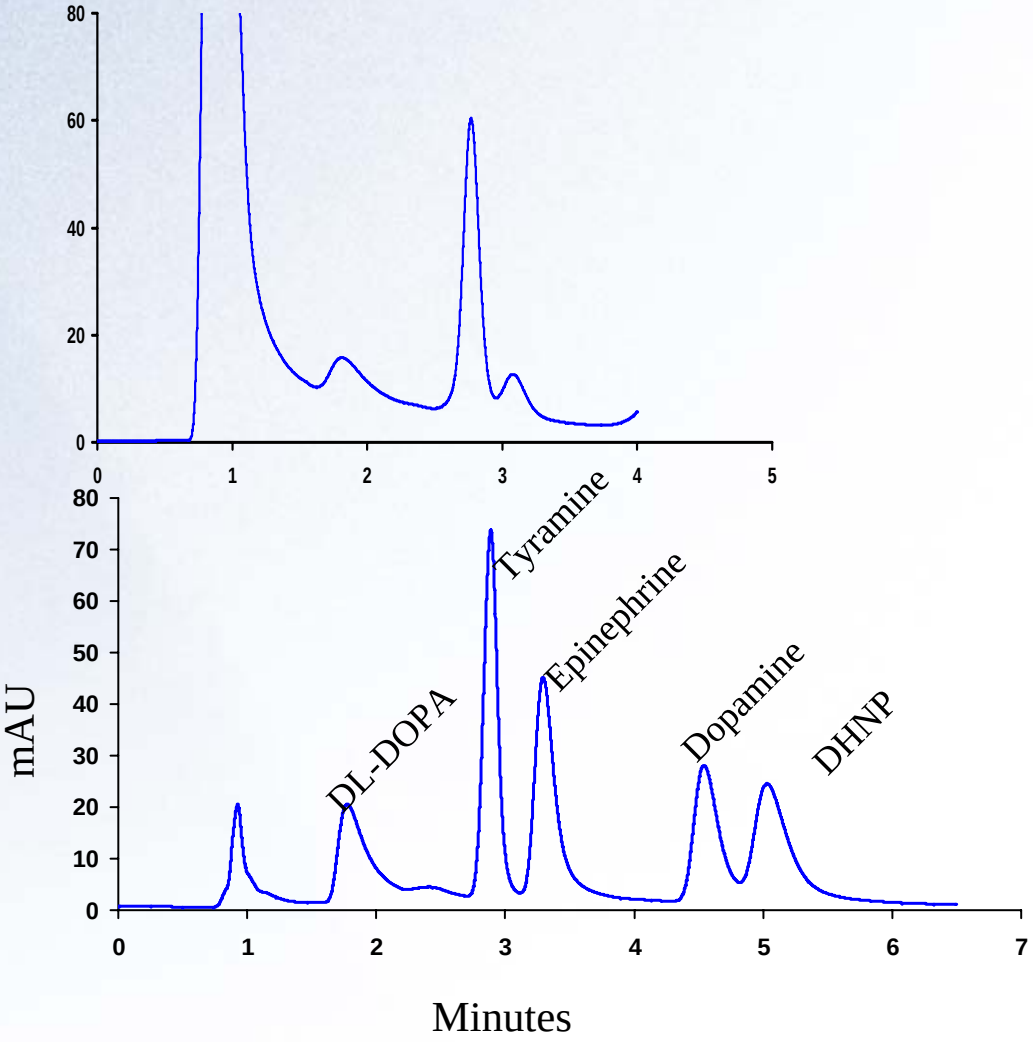
ACN/Methanol /Buffer

65/5/30

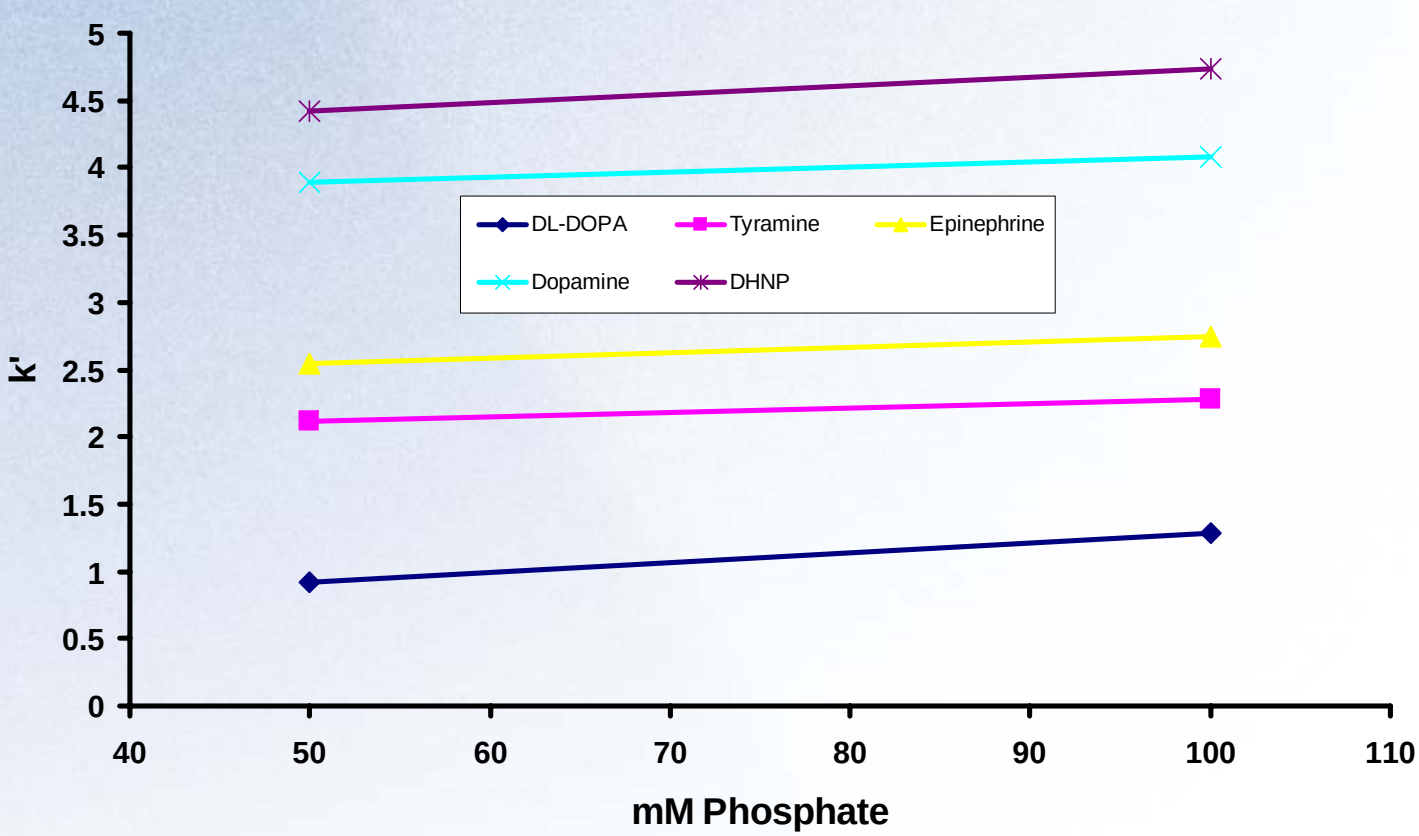
ACN/Buffer

70/30

Conditions: same as previous slides except where noted



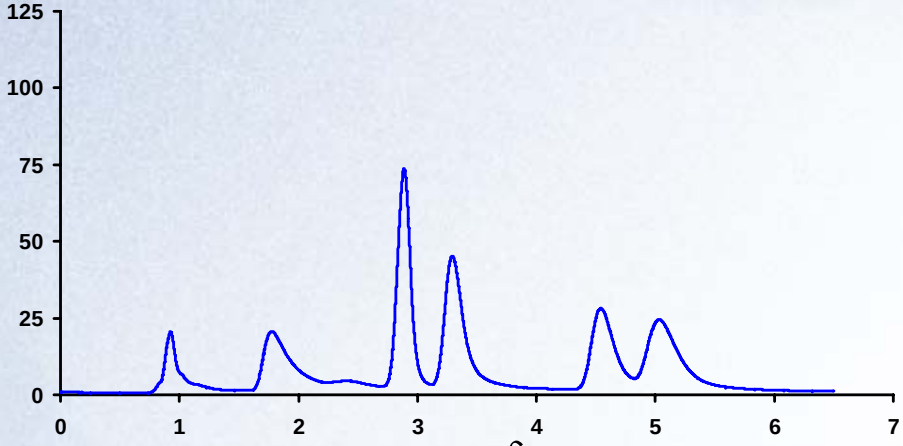
Effect of Ionic Strength



Conditions: 70/30 ACN/Buffer, pH 3.5, 50mm x 4.6 mm ZirChrom® -MS, Temperature 35° C.

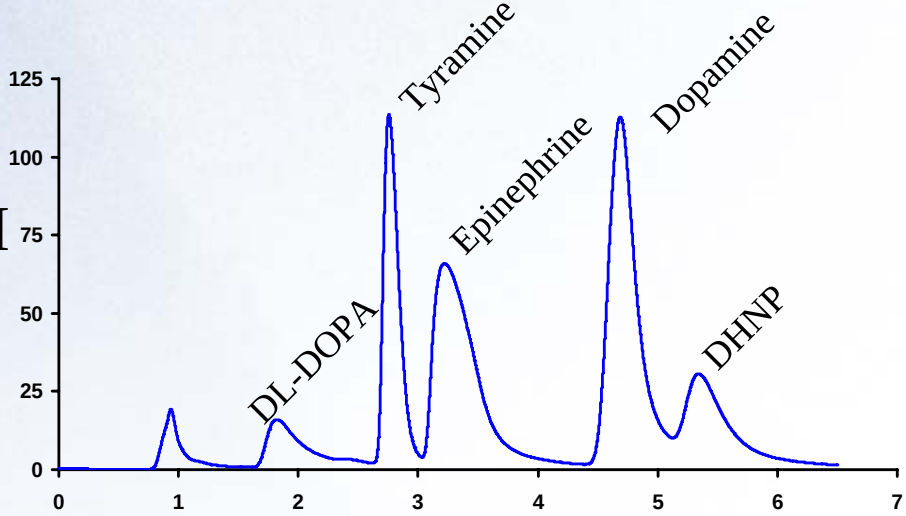
Effect of Buffer Type

50mM Phosphate



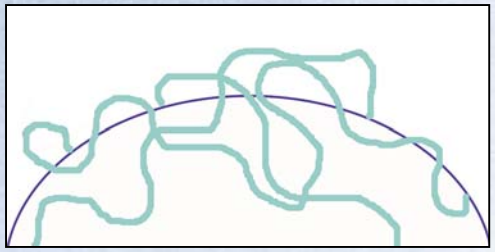
25mM Phosphate/25mM Acetic Acid

(larger injection)



Conditions: same as previous slides except where noted

Phase Change

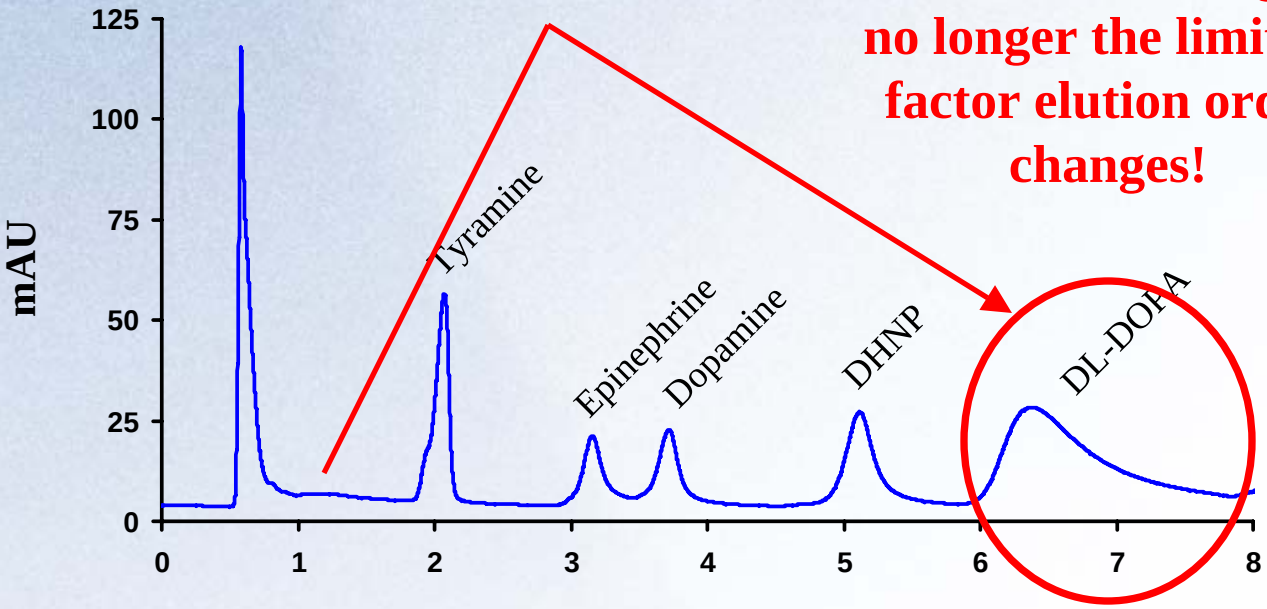


ZirChrom®-PBD

- Less Net Negative Charge
- Less Reversed Phase Retention

Final Separation

When ion exchange is no longer the limiting factor elution order changes!



Conditions: ZirChrom® -PBD 50 mm x 4.6 mm, 85/15 ACN/10 mM Ammonium dihydrogen phosphate, 30 mM Ammonium Acetate pH=3.4, Flow=1.5 ml/min , Temperature =35° C, Detection UV= 254nm, 5 ul injection. Solute : Tyramine, Epinephrine, Dopamine, DHNP, DL-DOPA.

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Conclusions

- Ion exchange contribution to the retention of these molecules is large.

  -Reduction of charge by pH, buffer and phase change required for elution and satisfactory peak shape.

- Methanol is not an effective solvent for these molecules on zirconia based phases

- Once the ion exchange contribution is overcome the metal chelation contribution becomes the controlling factor in retention and peak shape of DL-DOPA.

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Thanks *very much* for listening!

www.zirchrom.com for more information
and web access to the free Buffer Wizard