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Synthesis of a New Thermally and Chemically Stable Lewis-Acid Deactivated Reversed-Phase Zirconia Stationary Phase for HPLC

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... For Peak Performance

Outline

- The Problem and the Goal
- Stationary Phase Synthesis
- Chromatographic Data
  - Reversed-phase characteristics
  - Selectivity Comparison between Silica C18 and the ***new ZirChrom-MS***
  - Stability Testing
  - Applications
- Conclusion – The new ZirChrom-MS column is thermally and pH stable over a wide range and has very different chromatographic selectivity for basic compounds compared to silica C18.

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The Problem -

Zirconia surface interacts with Lewis bases and in order to "block" this interaction, it is necessary to use non-volatile inorganic buffers such as phosphates to achieve good peak shapes for some compounds.

The Goal -

To produce a new RP Zirconia Stationary Phase that does not require the use of nonvolatile buffers and therefore is HPLC-MS Compatible.

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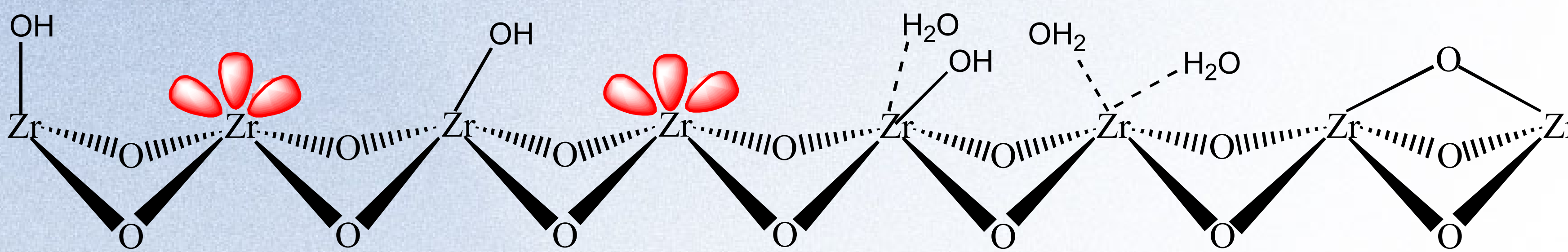
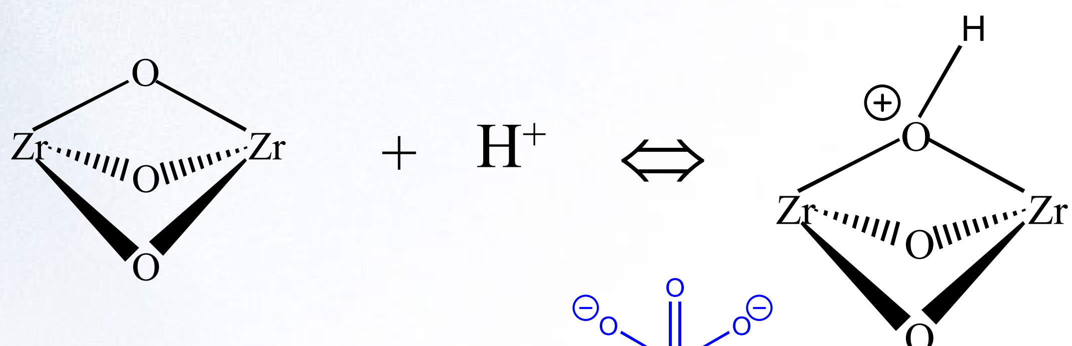
Surface Chemistry of Zirconia-Based Supports for HPLC

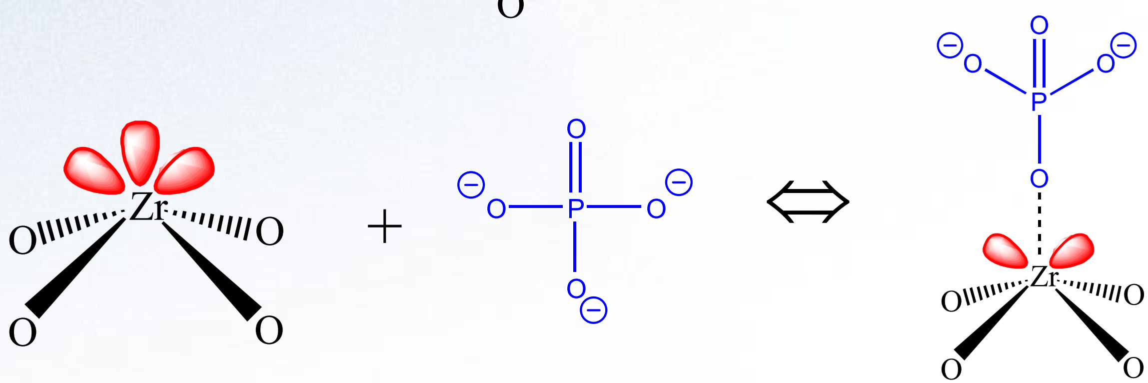


Brönsted Acid: $ZrOH + OH^- \Leftrightarrow ZrO^- + H_2O$

Brönsted Base:



Lewis Acid:



A Difficult Separation: Alkoxy Benzoic Acids.

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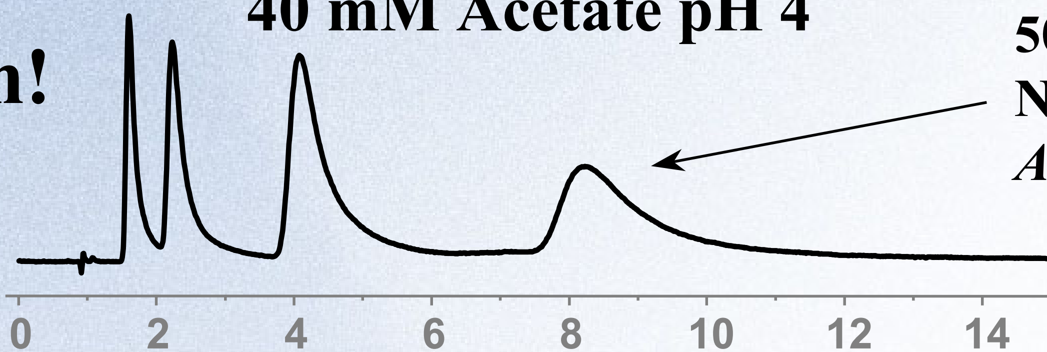
40 mM Acetate pH 4

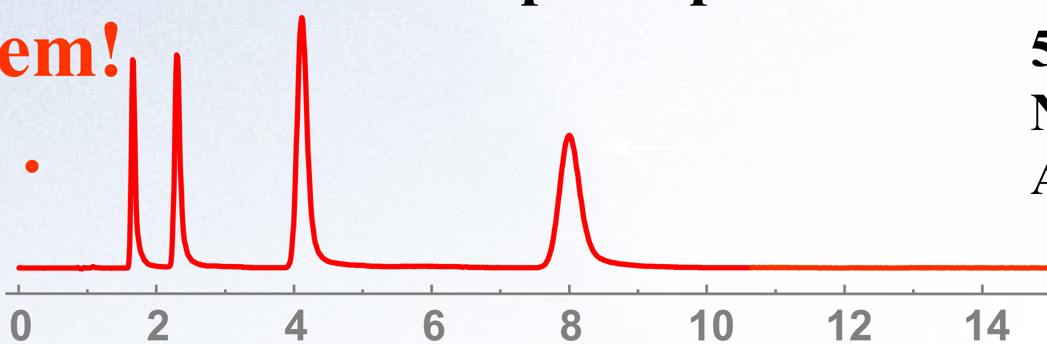
Problem!



50 x 4.6 mm
N = 260
A_s = 3.35

40 mM Phosphate pH 2.15

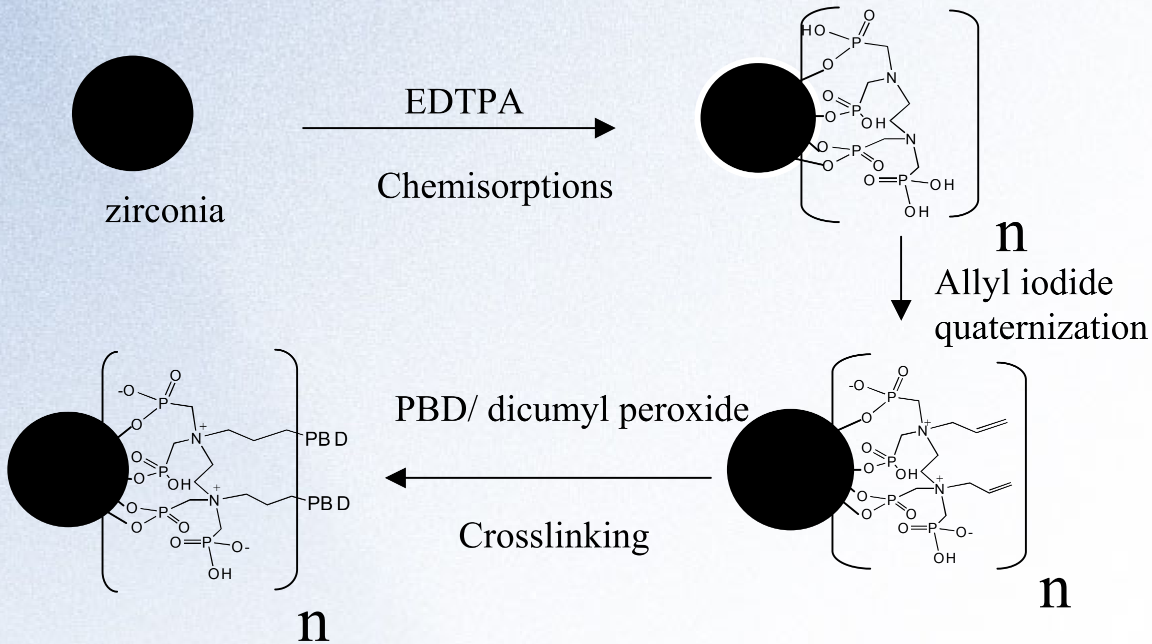
No Problem!
Except . . .
for MS.



50 x 4.6 mm
N = 3250
As = 1.07

25% ACN, 40 mM above additive, 5 mM NH_4F; 0.6 mL/min; 30 °C; 254 nm.

New 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.**

Selectivity: The Key to Success in HPLC

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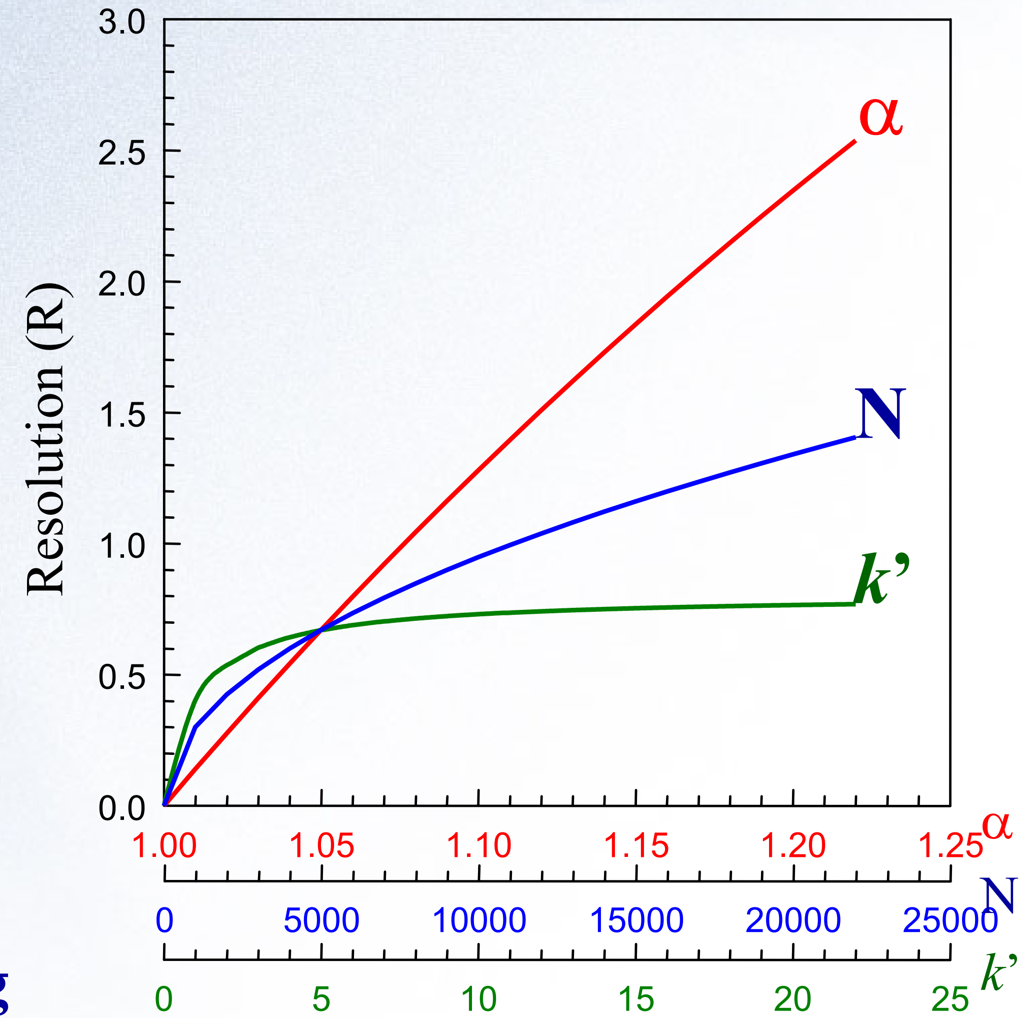
Efficiency	Retention	Selectivity

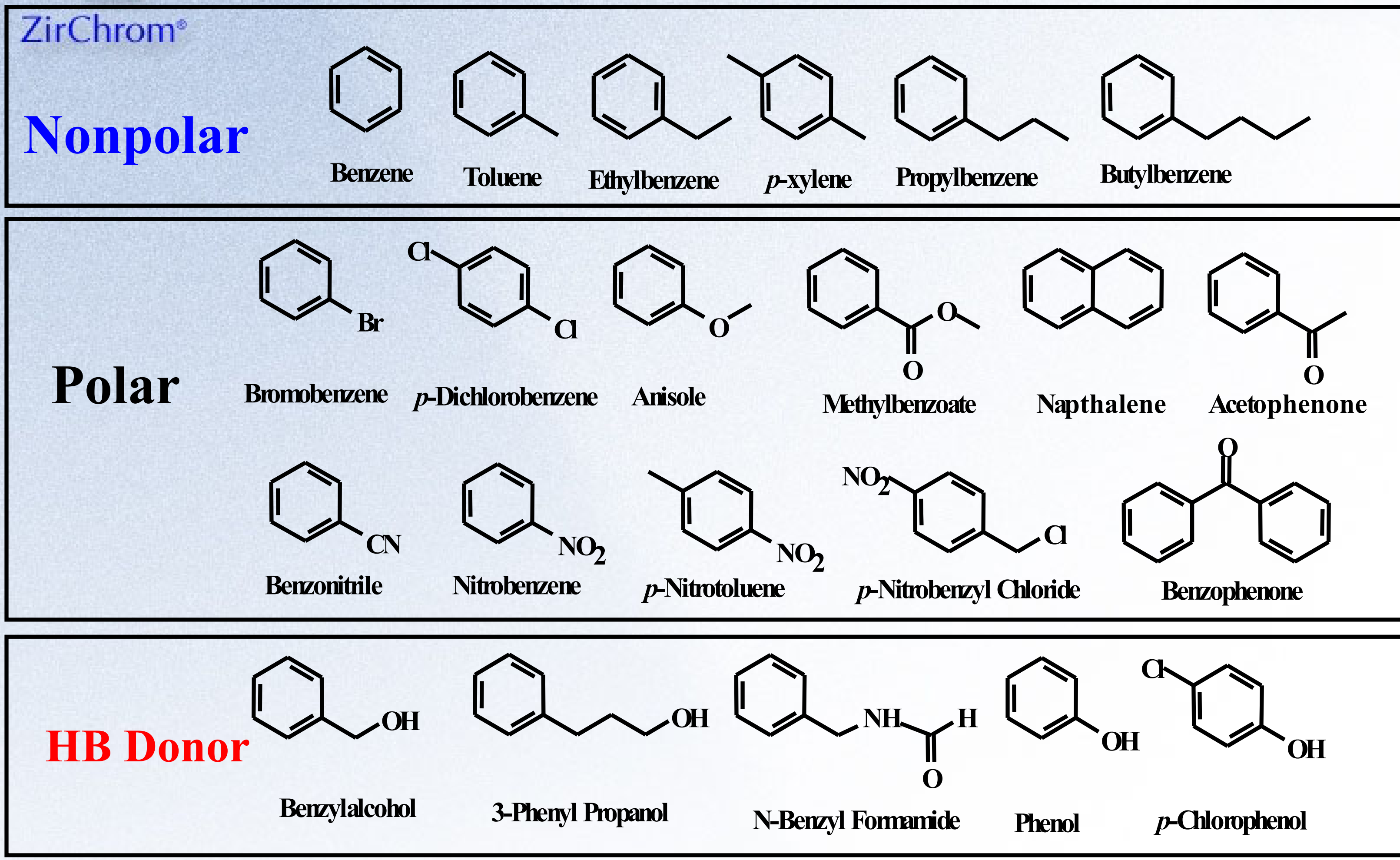
$$R = \frac{\sqrt{N}}{4}\;\frac{k'}{k'+1}\;\frac{\alpha - 1}{\alpha}$$

$$\alpha = \frac{k_j'}{k_i'}$$

➢ **Selectivity (α) has the greatest impact on improving resolution.**

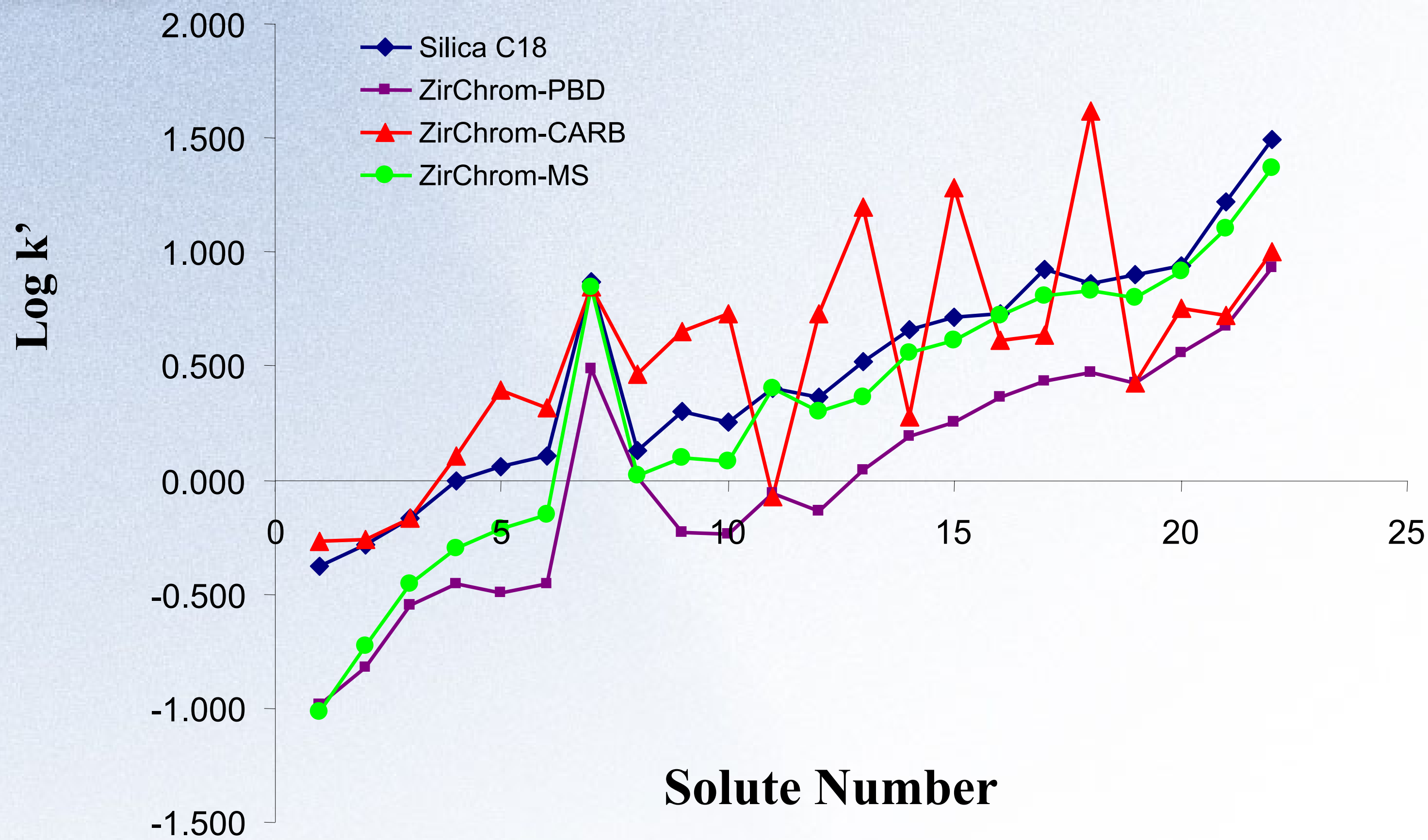


Selectivity Comparison Solutes



Mobile phase, 40/60 Acetonitrile/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Detection at 254nm; 5µl Injection volume.

Comparison of Selectivity of ODS, ZirChrom®-PBD, -CARB and -MS



1.) benzyl formamide 2.) benzyl alcohol 3.) phenol 4.) 3-phenyl propanol 5.) p-chlorophenol 6.) acetophenone 7.) benzonitrile 8.) nitrobenzene 9.) methylbenzoate 10.) anisole 11.) benzene 12.) p-chlorotoluene 13.) p-nitrobenzyl chloride 14.) toluene 15.) benzophenone 16.) bromobenzene 17.) naphthalene 18.) ethyl benzene 19.) p-xylene 20.) p-dichlorobenzene 21.) propyl benzene 22.) butyl benzene

Selectivity Matrix for Nonelectrolytes

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Correlation Coefficient	Waters Xterra (RP18)	Luna	PLRP	Gammabond	ZirChrom-PBD	ZirChrom-CARB	DB-C18	Hypercarb	Discovery BIO Wide Pore C18	ZirChrom-EZ	ZirChrom-MS
Waters Xterra (RP18)	1	0.99	0.96	0.98	0.95	0.71	0.94	0.77	0.96	0.96	0.96
Luna		1	0.98	0.99	0.95	0.70	0.94	0.77	0.96	0.96	0.97
PLRP			1	0.98	0.97	0.70	0.95	0.76	0.98	0.98	0.98
Gammabond				1	0.97	0.70	0.95	0.76	0.98	0.98	0.98
ZirChrom-PBD					1	0.69	0.97	0.77	0.98	0.99	0.99
ZirChrom-CARB						1	0.84	0.97	0.68	0.70	0.70
DB-C18							1	0.90	0.95	0.97	0.97
Hypercarb								1	0.76	0.78	0.77
BIO Wide Pore C18									1	0.99	0.99
ZirChrom-EZ										1	0.998
ZirChrom-MS											1

Summary: All CARBON-BASED Columns have different selectivity for nonelectrolytes. All other column retention is very highly correlated.

LC Conditions: Mobile phase, 40/60 ACN/Water; Flow rate, 1.0 ml/min.; Temperature, 30 ^{o}C; Injection volume, 5 µl; Detection at 254 nm.

Reversed-Phase Characteristics

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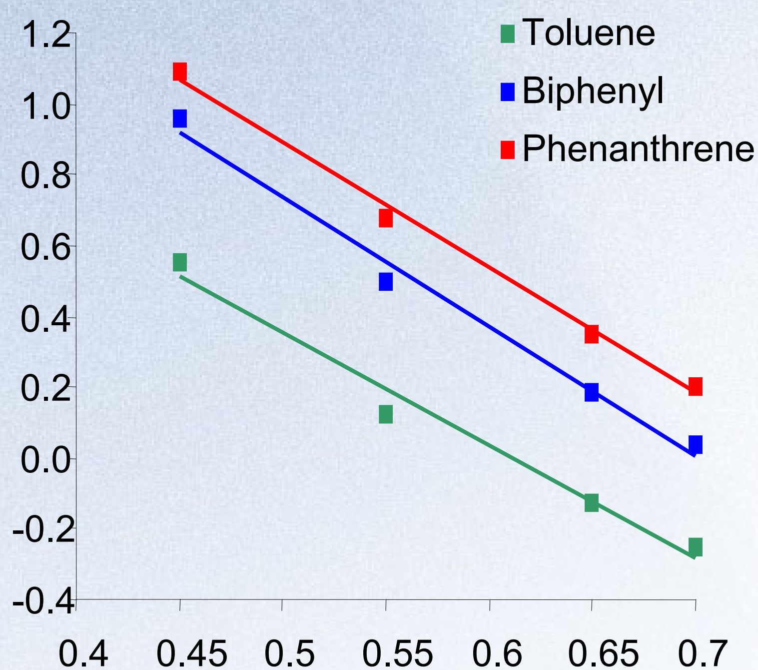
$$\log k'_{RP} = \log k_w - S\phi$$



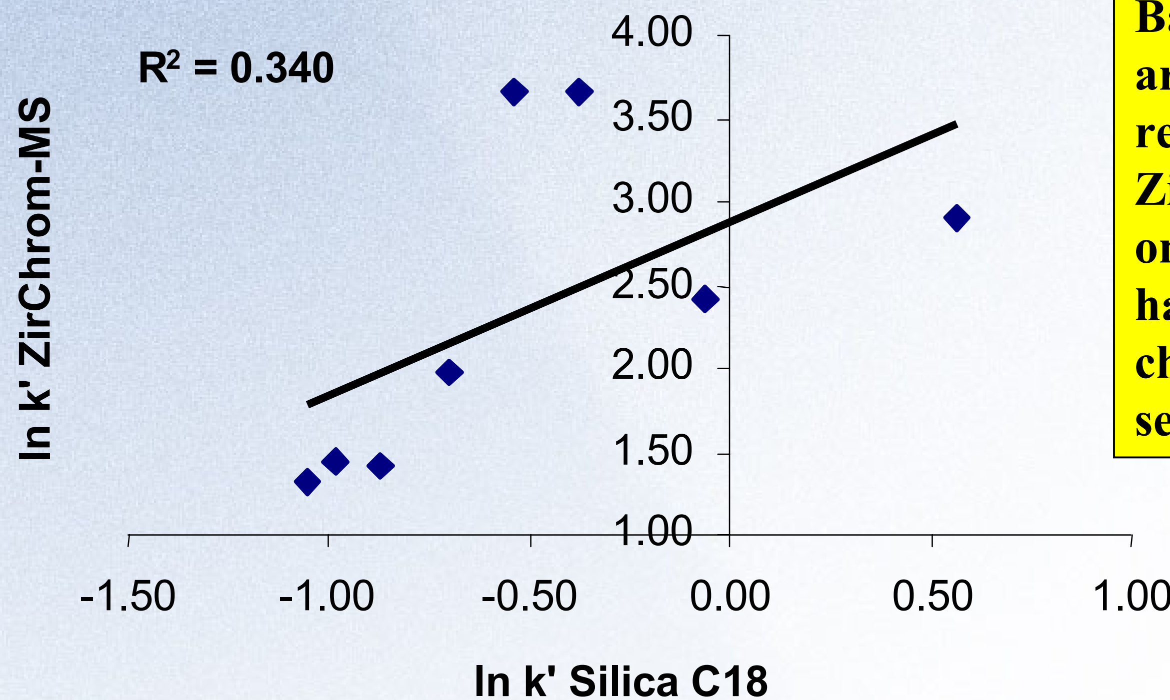
	Toluene	Biphenyl	Phenanthrene
logkw	2.06	2.67	2.75
S*	3.41	3.86	3.71
R^2	0.980	0.990	0.990

* Typical value for S for butylbenzene on silica C18 is 3.4 and intercept of 3.0. (Jianhong Zhao and Peter W. Carr, Anal Chem. Vol. 71 (1999) 5217-5224.)

ZirChrom-MS has very similar RP behavior to Silica C18.

LC Conditions: Mobile phase, indicated composition of ACN/Water; Flow rate, 2.0 ml/min.; Temperature, 35 °C; Injection volume, 5 µl; Detection at 254 nm; Column, 50 mm x 4.6 mm i.d. ZirChrom®-MS.

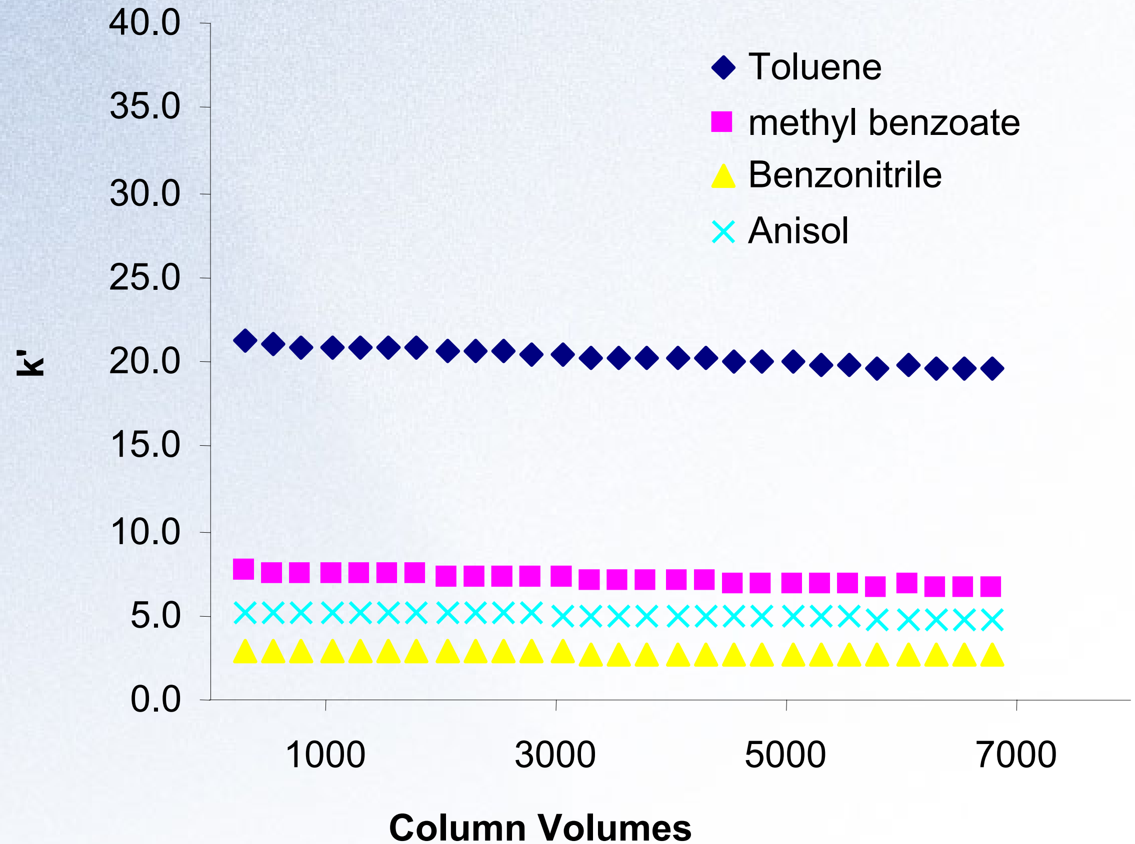
Selectivity Comparison for Basic Pharmaceuticals



Basic Compounds are much more retained on ZirChrom-MS than on Silica C18 and have very different chromatographic selectivity.

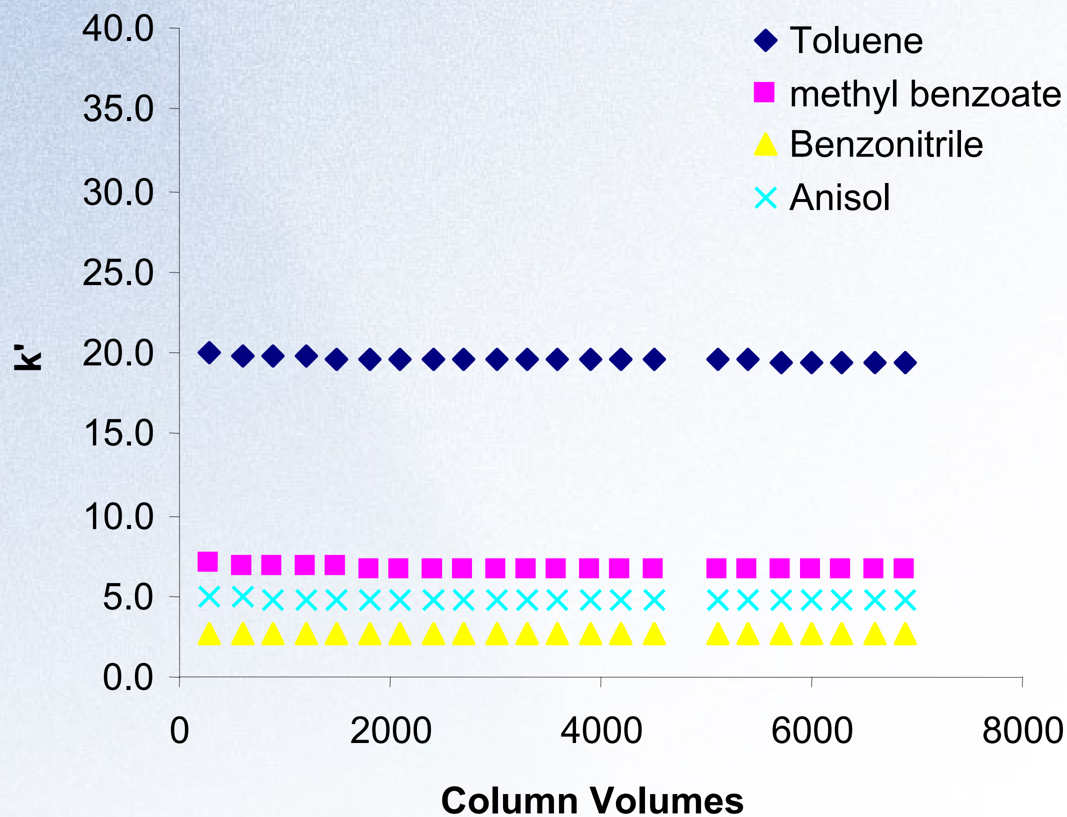
Selectivity comparison for several basic pharmaceuticals - Leading C18 silica column versus the new ZirChrom®-MS column. **LC Conditions**: Mobile Phase, 72/28 MeOH/25mM Ammonium phosphate, pH 6.0; Flow Rate, 1.0 ml/min.; Temperature, 35 ^{o}C; Injection Volume, 5 µl; Detection by UV at 254 nm; Solutes from left to right: Methapyrilene, Pyrilamine, Tripelennamine, Brompheniramine, Desipramine, Nortryptyline, Doxepin, and Amitryptyline.

pH 1 Stability Testing



Column ID: MS0082903X; Mobile phase, 15/85 ACN/pH=1 nitric acid, Temperature: 30 ^{o}C; Injection volume: 5 µl; UV, 254 nm; Solutes (see figure).

pH 10 Stability Testing



Column ID: MS0082903X; Mobile phase, 15/85 ACN/pH=10 with tetramethylammonia hydroxide, Temperature: 30 ^{o}C; Injection volume: 5 µl; UV, 254 nm; Solutes (see figure).

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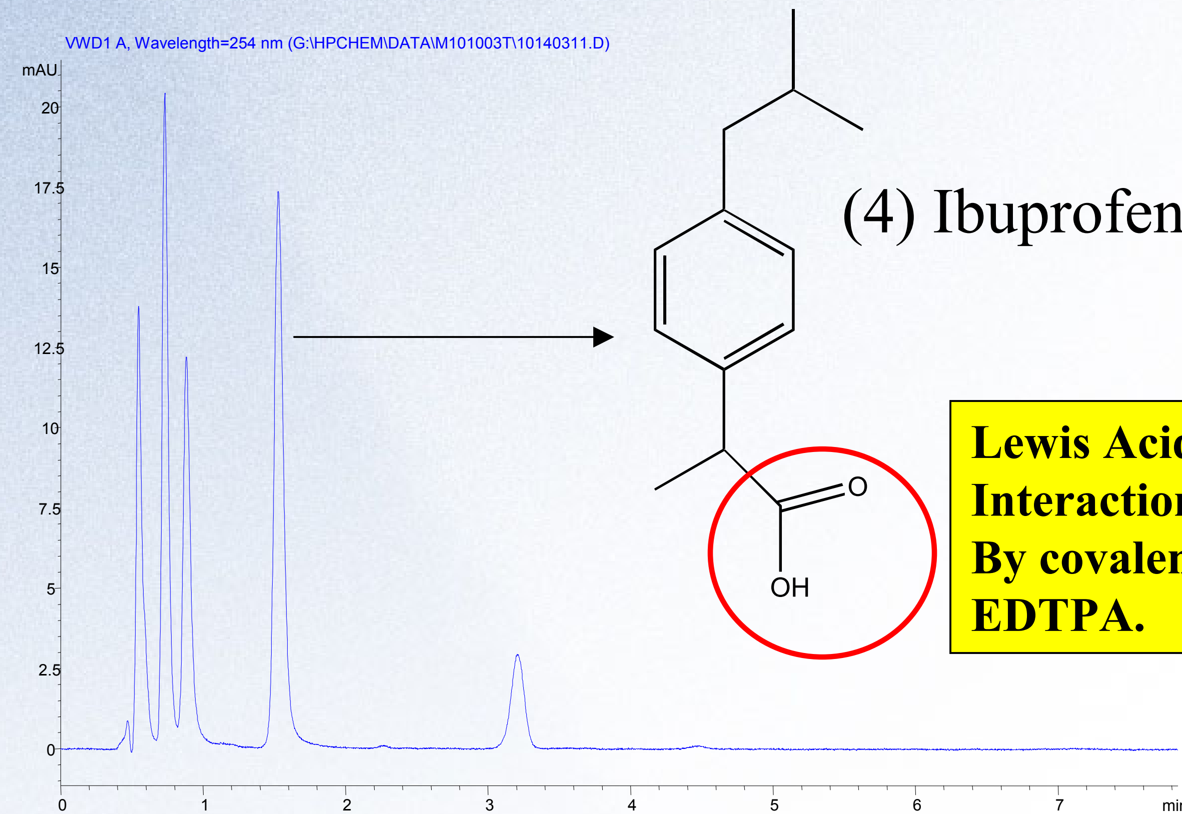
Separation of Acidic Pharmaceuticals

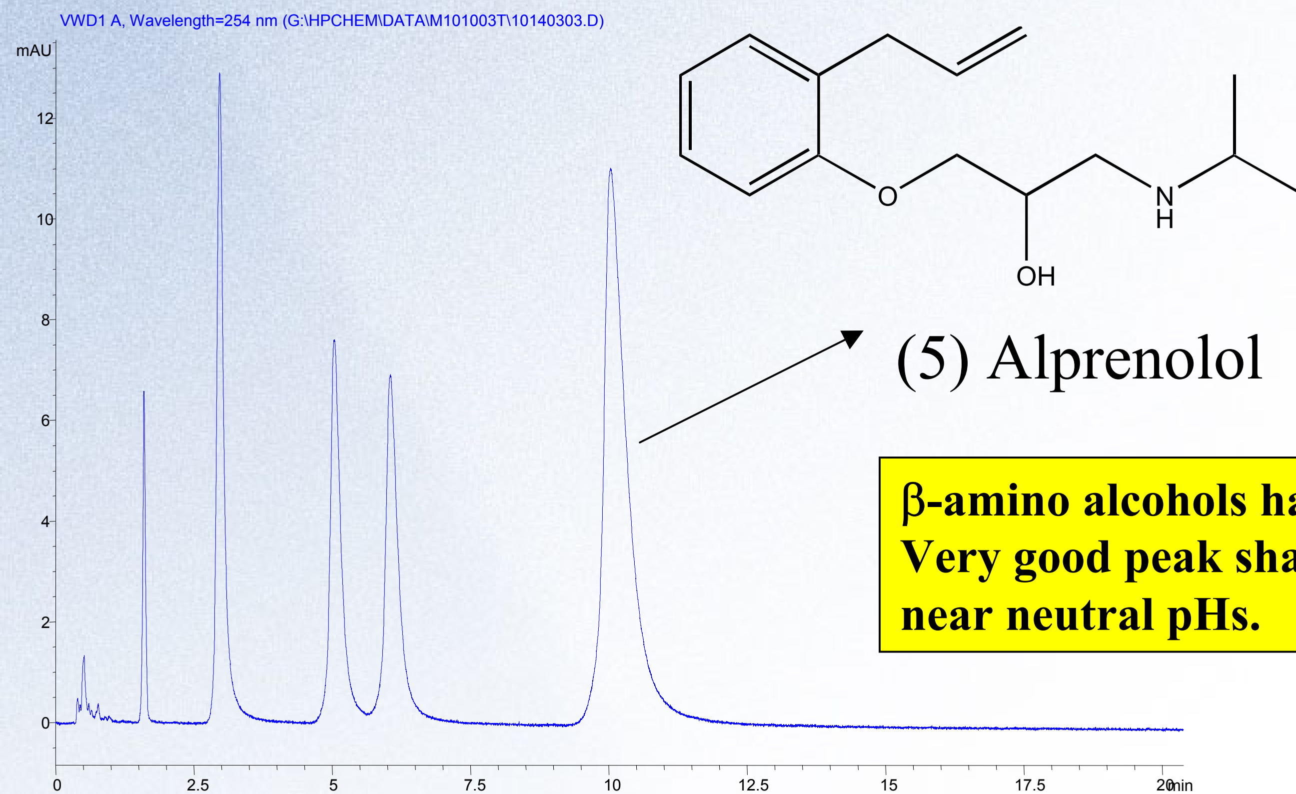


(4) Ibuprofen

Lewis Acid-Base Interaction "blocked" By covalently attached EDTPA.

Chromatographic Conditions: Column Dimension: 50X4.6 MS101003T; Mobile phase, Machine-mixed 40/60 ACN/10 mM ammonium acetate pH=5. Flow rate:1 ml/min, Temperature, 35° C; Injection volume: 5 µl; Solutes eluted in order, (1) Acetaminophen, (2) Ketoprofen, (3) Naproxen, (4) Ibuprofen, (5) Impurity; Detection, 254 nm. Pressure drop, 68 bar.

Separation of β-Blockers



(5) Alprenolol

β-amino alcohols have Very good peak shape at near neutral pHs.

Chromatographic Conditions: Column Dimension: 50X4.6 MS101003T; Mobile phase: Machine-mixed 65/35 ACN/10 mM ammonium acetate pH=5; Flow rate:1 ml/min; Temperature, 35^{o} C; Injection volume: 5 µl. Solutes eluted in order: (1) Lidocaine, (2) Atenolol, (3) Metoprolol, (4) Oxprenolol, (5) Alprenolol
Detection: 254 nm; Pressure drop, 59 bar.

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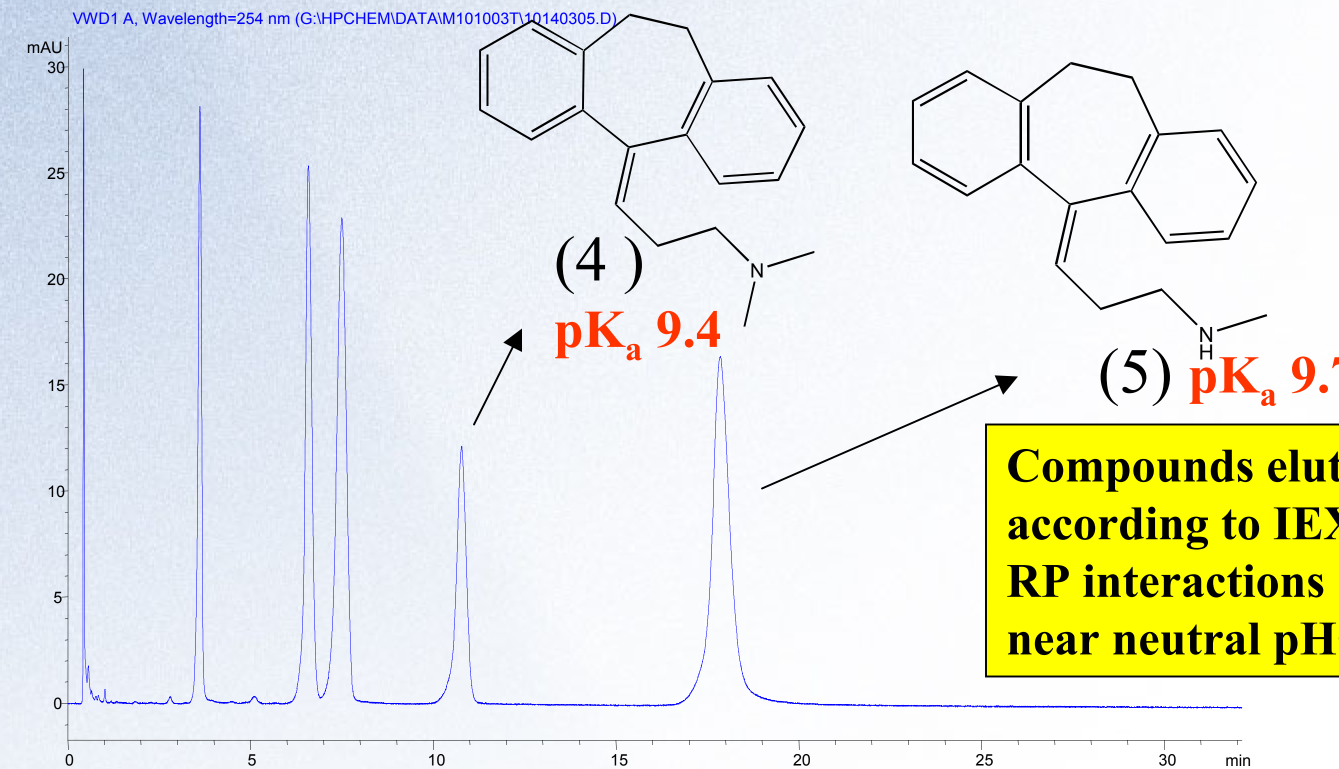
Separation of Basic Pharmaceuticals



Compounds elute according to IEX, not RP interactions at near neutral pHs.

Chromatographic Conditions: Column Dimension: 50X4.6 MS101003T; Mobile phase, Machine-mixed 65/35; ACN/10 mM ammonium acetate pH=5; Flow rate, 1 ml/min; Temperature, 35° C; Inject volume, 1 µl; Solutes eluted in order: (1) Methapyrilene, (2) Bromphenriamine, (3) Doxpin, (4) Amtriptyline, (5) Nortryptyline Detection, 254 nm; Pressure drop, 59 bar.

Conclusions

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The new ZirChrom- MS phase meets our project goals:

- ***Lewis acid site deactivated***.
- ***Similar selectivity*** and RP behavior to silica C18 ***for neutral compounds***.
- ***Chemically stable*** from pH 1-10.
- ***Different selectivity*** than Silica C18 ***for ionizeable*** analyte compounds.
- ***MS detection compatible***.