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

Pittcon 2004

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ZirChrom Separations, Inc.

Outline

- The Goal
- Chromatographic Data
  - ***Selectivity*** Comparison between Silica C18 and the ***new ZirChrom®-MS***
  - ***Stability*** Testing
  - ***Applications and MS testing***
- 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***. The column also performs well under MS-compatible conditions.

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The Goal

To produce a new MS compatible Reversed-Phase Zirconia Stationary Phase that has unique selectivity for basic pharmaceuticals.

Selectivity: The Key to Success

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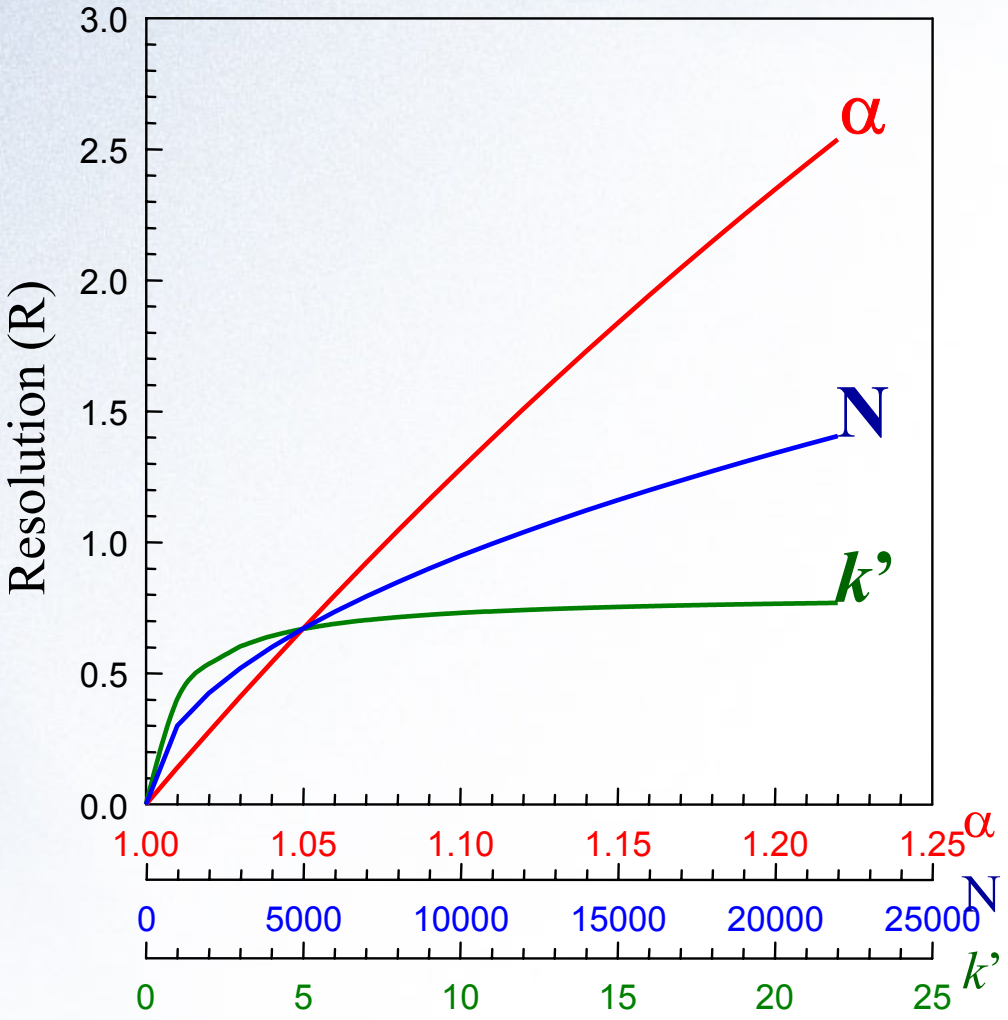
Efficiency	Retention	Selectivity
↘	↓	↙
$R = \frac{\sqrt{N}}{4}$	$\frac{k'}{k'+1}$	$\frac{\alpha-1}{\alpha}$

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



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

Selectivity Comparison Solutes

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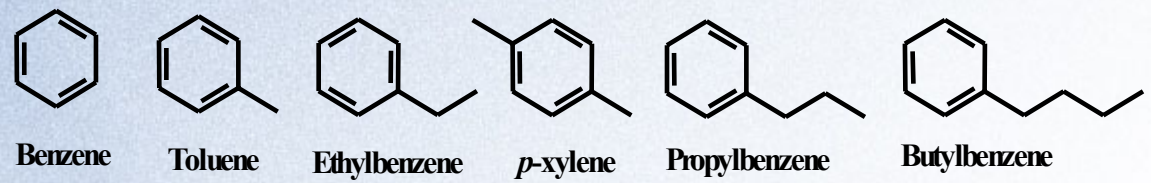
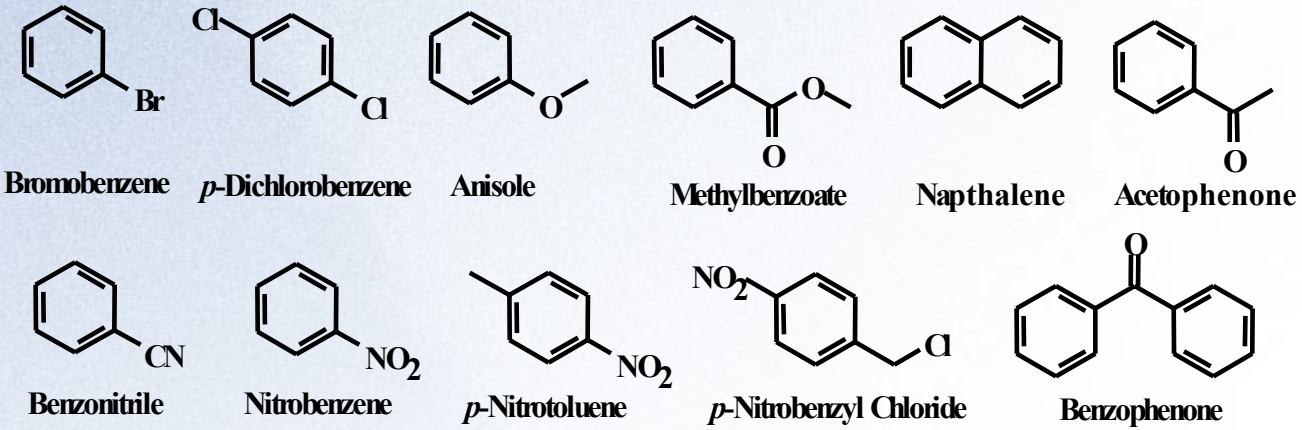
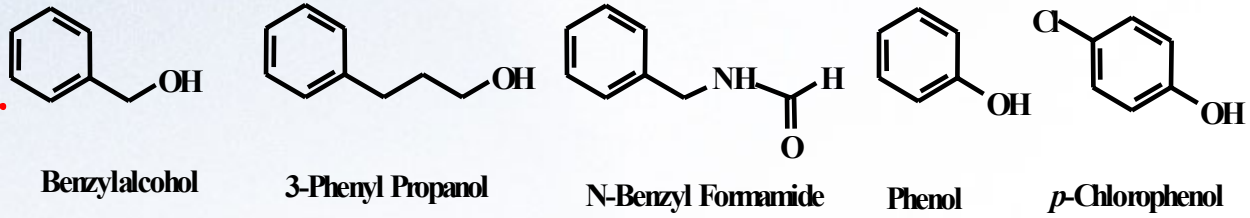
Nonpolar



Polar



HB Donor



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

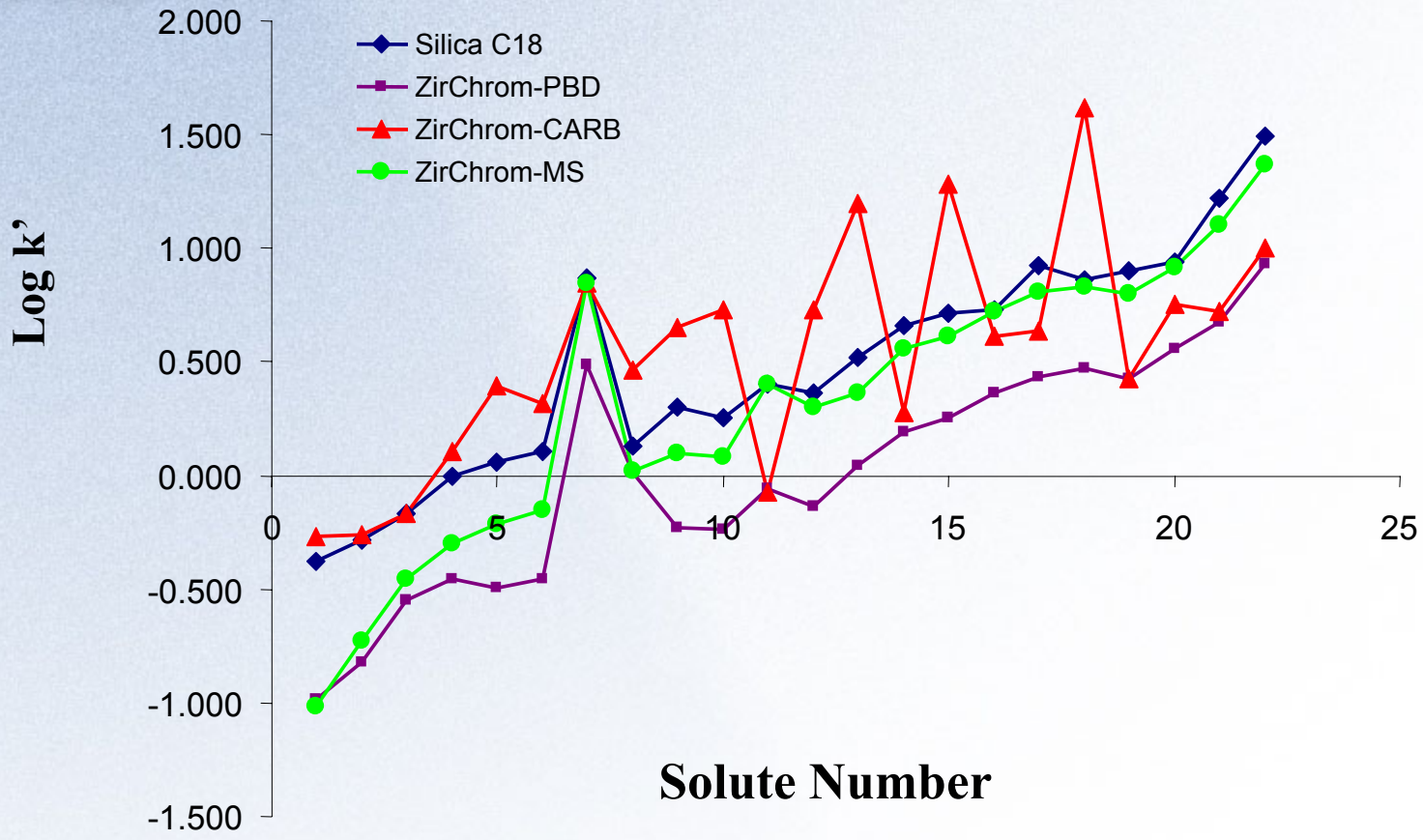
Selectivity Matrix for Nonelectrolytes

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

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

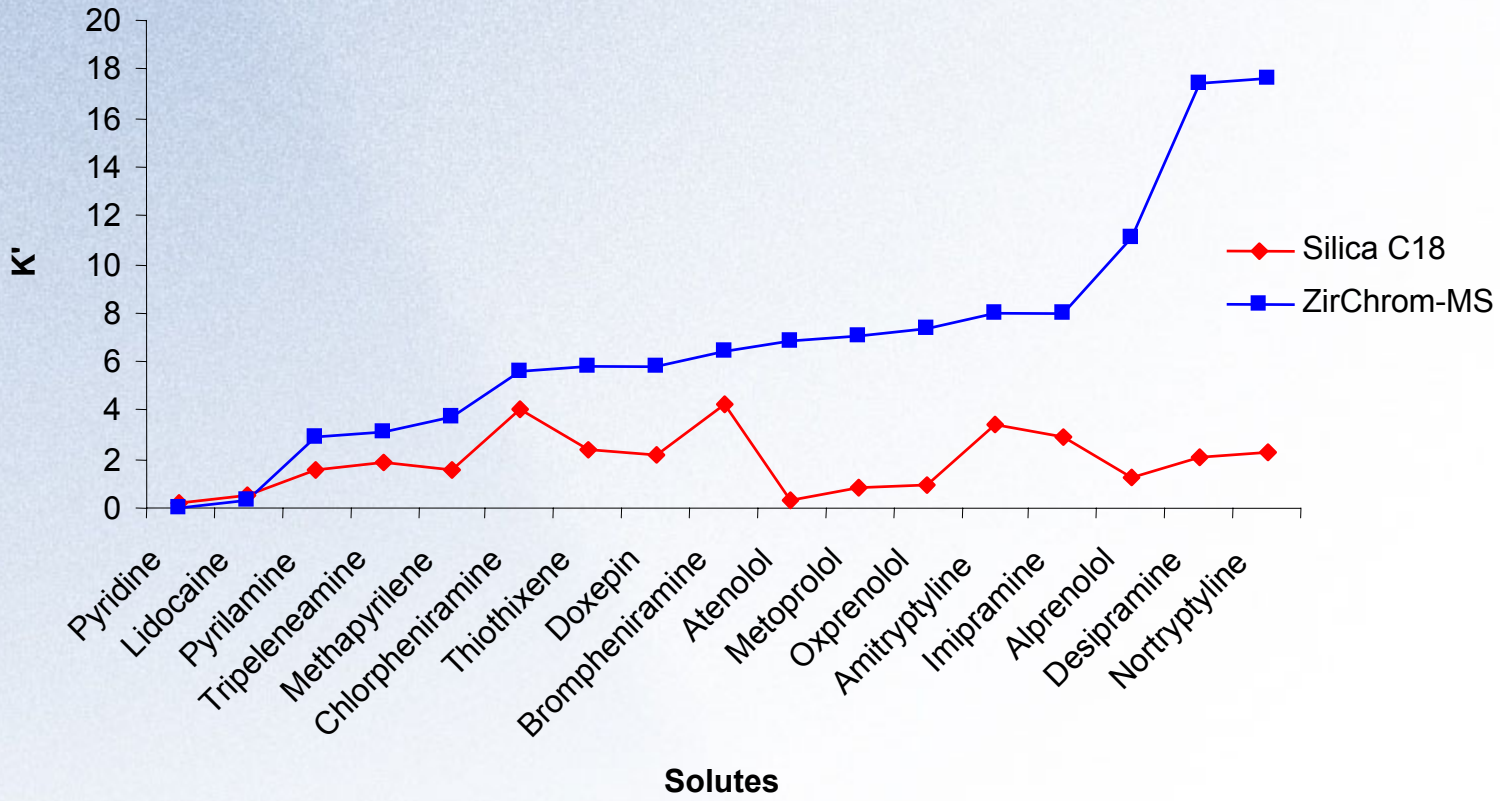
LC Conditions: Mobile phase, 40/60 ACN/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 5 µl; Detection at 254 nm.

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



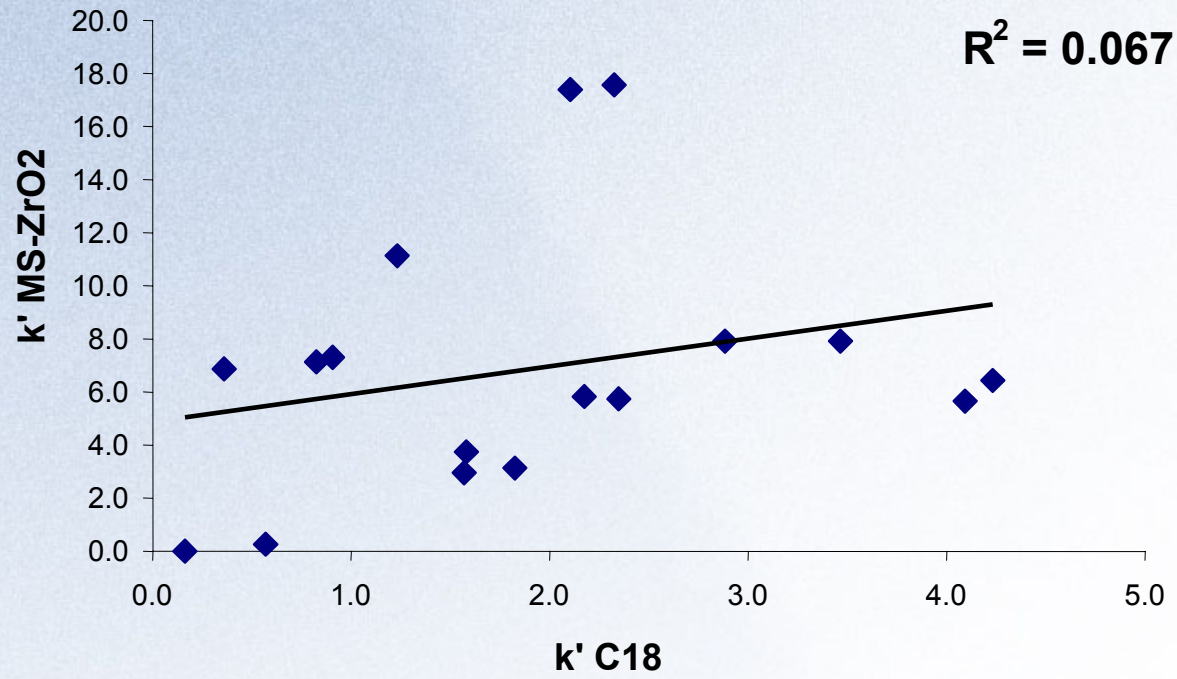
1.) benzyl formamide 2.) benzyl alcohol 3.) phenol 4.) 3-phenyl propanol 5.) p-chlorophenol 6.) acetophenone 7.) benzonitrile 8.) nitrobenzene 9.) methylbenzoate 10.) anisole 11.) benzene 12.) p-chlorotoluene 13.) p-nitrobenzyl chloride 14.) toluene 15.) benzophenone 16.) bromobenzene 17.) naphthalene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

Comparison of Retention of Basic Pharmaceuticals for ODS and ZirChrom®-MS



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®-MS, 50 x 4.6 mm i.d. (3um particles), S/N:MS020204T; Silica-C18 150 x 4.6 mm i.d., (3.5 um particles).

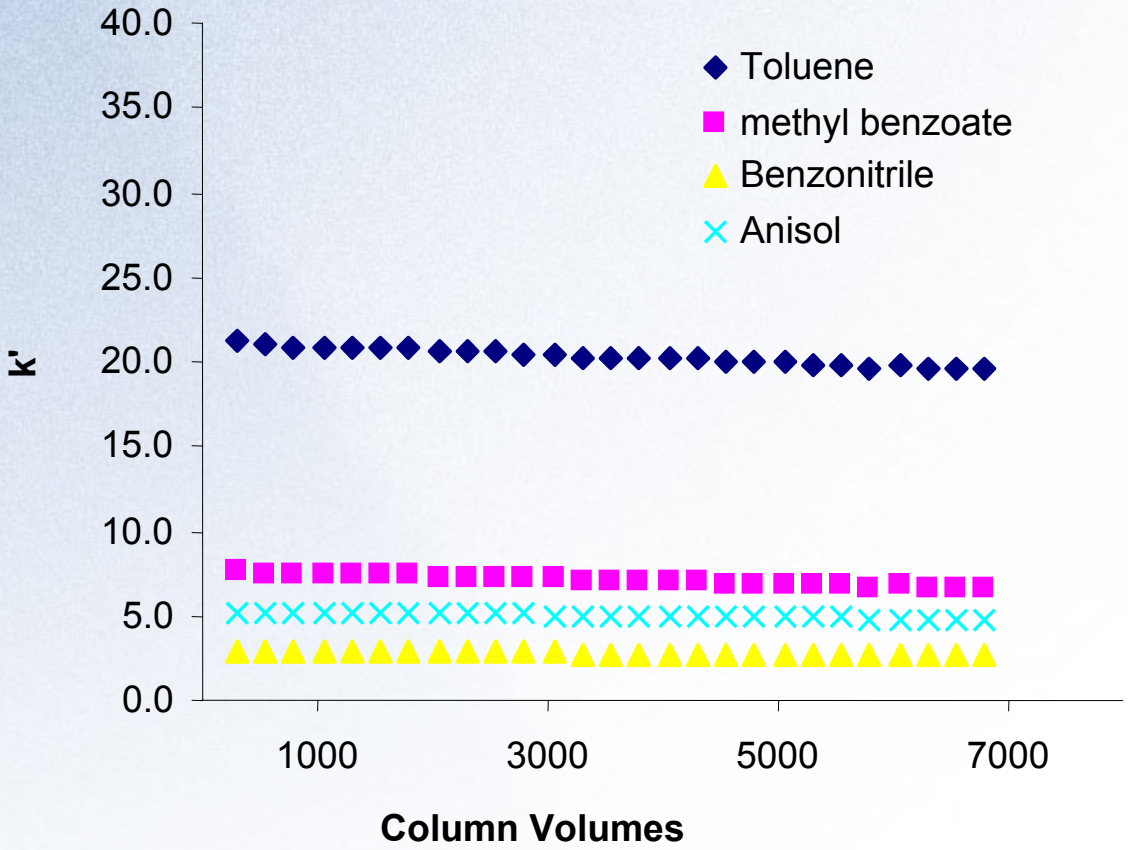
K–K Plot for Basic Pharmaceuticals on ZirChrom®-MS and ODS



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

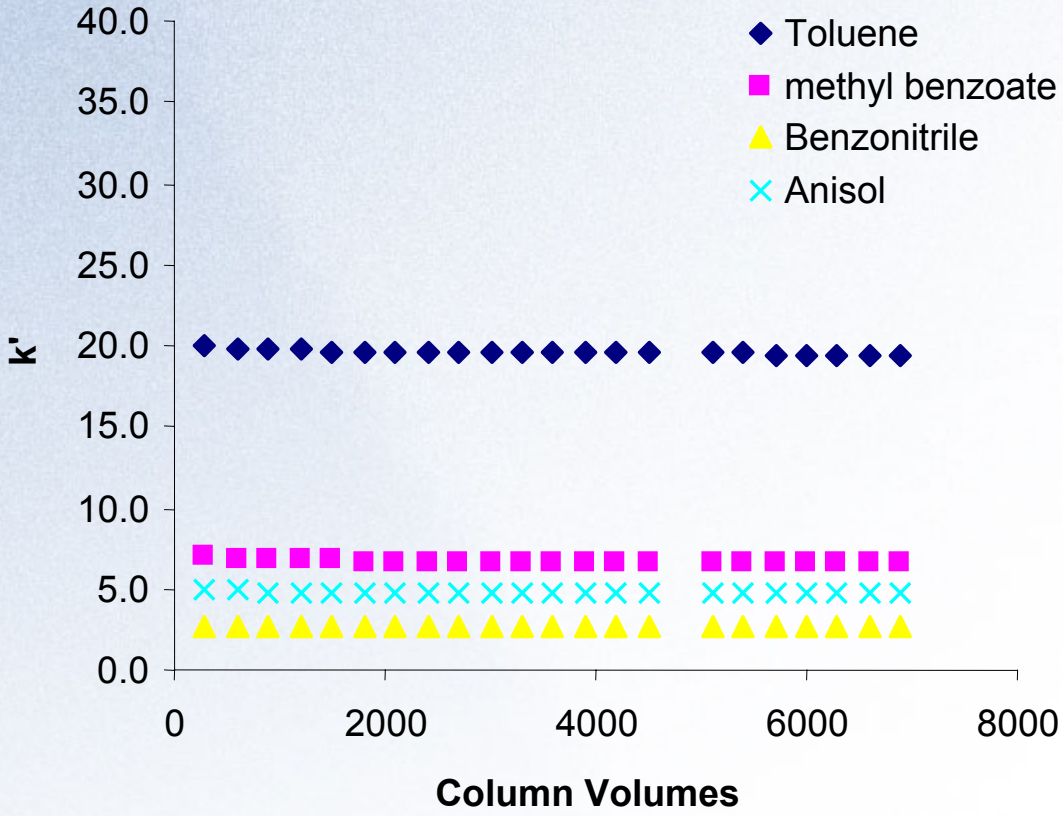
LC Conditions: Machine-mixed 80/20 ACN/10 mM ammonium acetate pH=6.7 without pH adjustment; Flow rate, 1.0 ml/min.; Injection volume 0.1 µl; Temperature, 35 °C; Detection at 254 nm; Columns, ZirChrom®-MS, 50 x 4.6 mm i.d. (3um particles), S/N:MS020204T; Silica-C18 150 x 4.6 mm i.d., (3.5 um particles).

pH 1 Stability Testing



ZirChrom®-MS, S/N: MS0082903X; Mobile phase, 15/85 ACN/pH=1 nitric acid, Temperature: 30 °C; Injection volume: 5 µl; UV, 254 nm; Solutes (see figure).

pH 10 Stability Testing



ZirChrom®-MS, S/N: MS0082903X; Mobile phase, 15/85 ACN/pH=10 with tetramethylammonia hydroxide, Temperature: 30 °C; Injection volume: 5 µl; UV, 254 nm; Solutes (see figure).

HPLC-MS of Basic Pharmaceuticals

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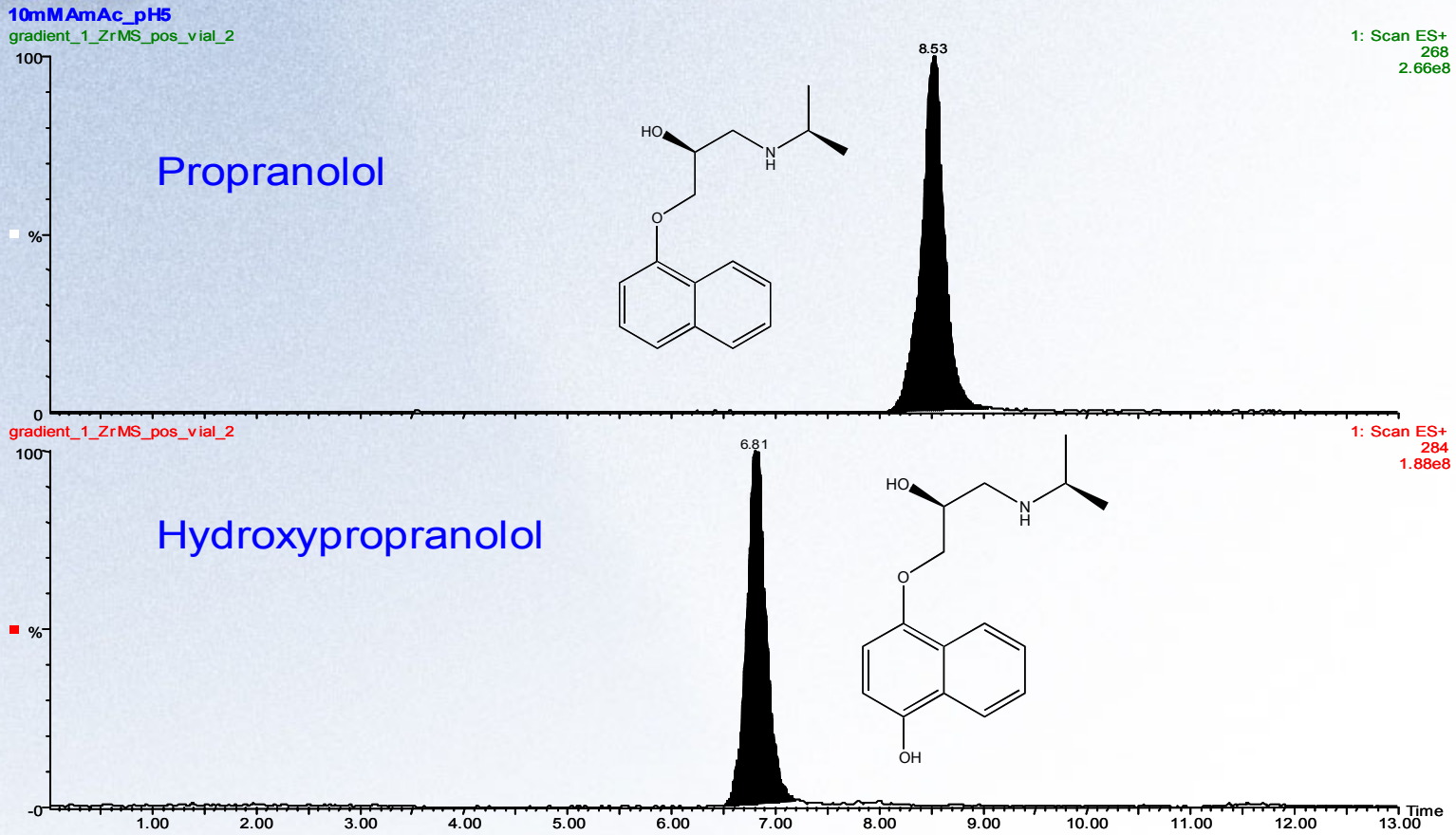

LC Conditions: Column, ZirChrom®-MS, 5 x 2.1 mm i.d. (3 micron particles). Waters Alliance 2795 LC, Flow rate, 0.2mL/min, Mobile phases channel C=10mM ammonium acetate at pH 5, channel D=10mM ammonium acetate at pH 5:acetonitrile (10:90, v/v) , Linear gradient 5% D to 100% D in 6 minutes, hold 100% 6-7.4 min, 100 to 5% D 7.4-8.1min, hold 5% D 8.1-13.0 min. Temperature, 35°C. Waters/Micromass ZQ single quadrupole interfaced with the LC using an electrospray ionization (ESI) interface. Positive ion mode (XIC) from full scan acquisitions from m/z 120-700. Solute concentrations = 10µg/mL, 2µL injections.

HPLC-MS of Beta-Blockers

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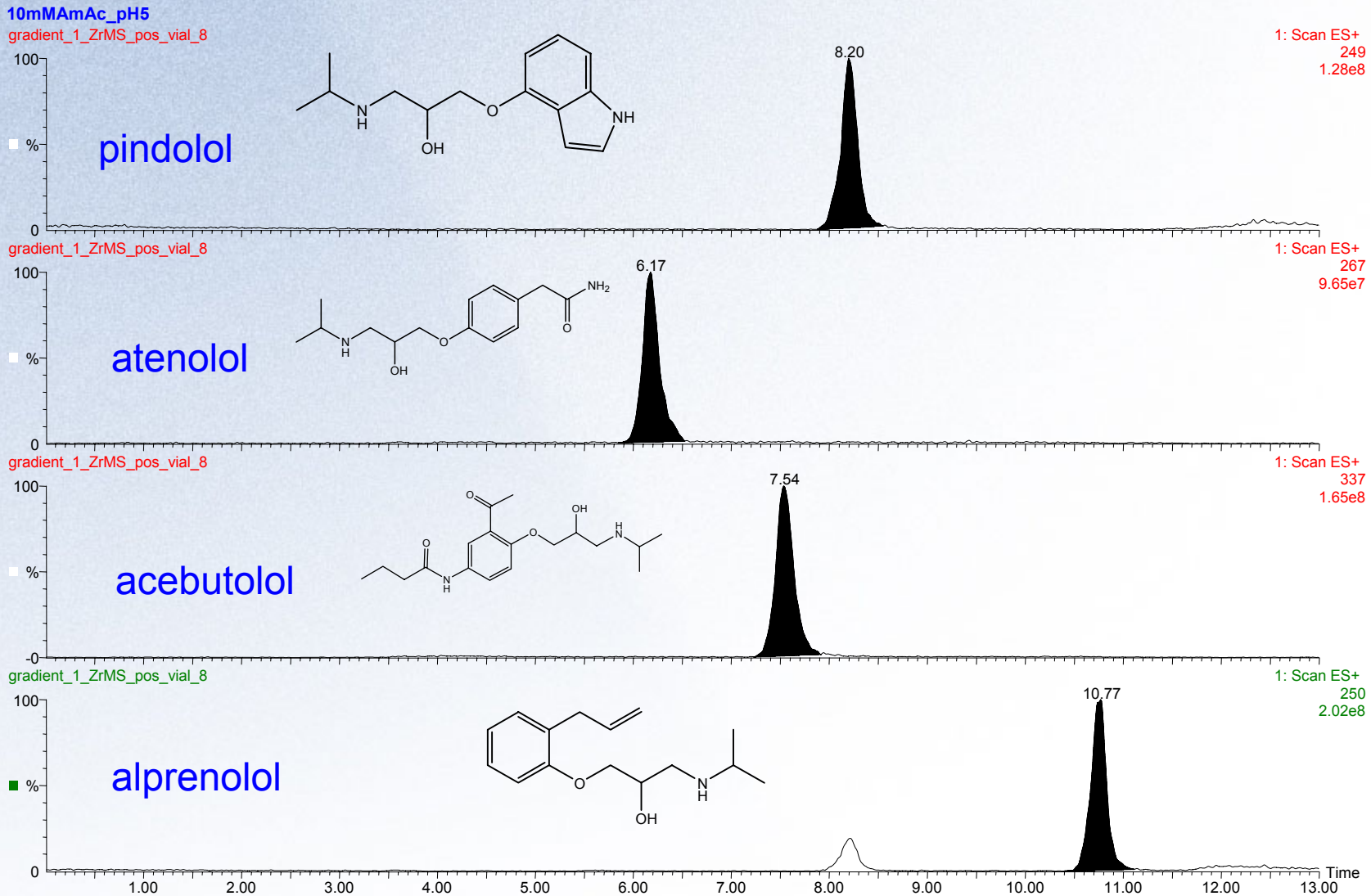
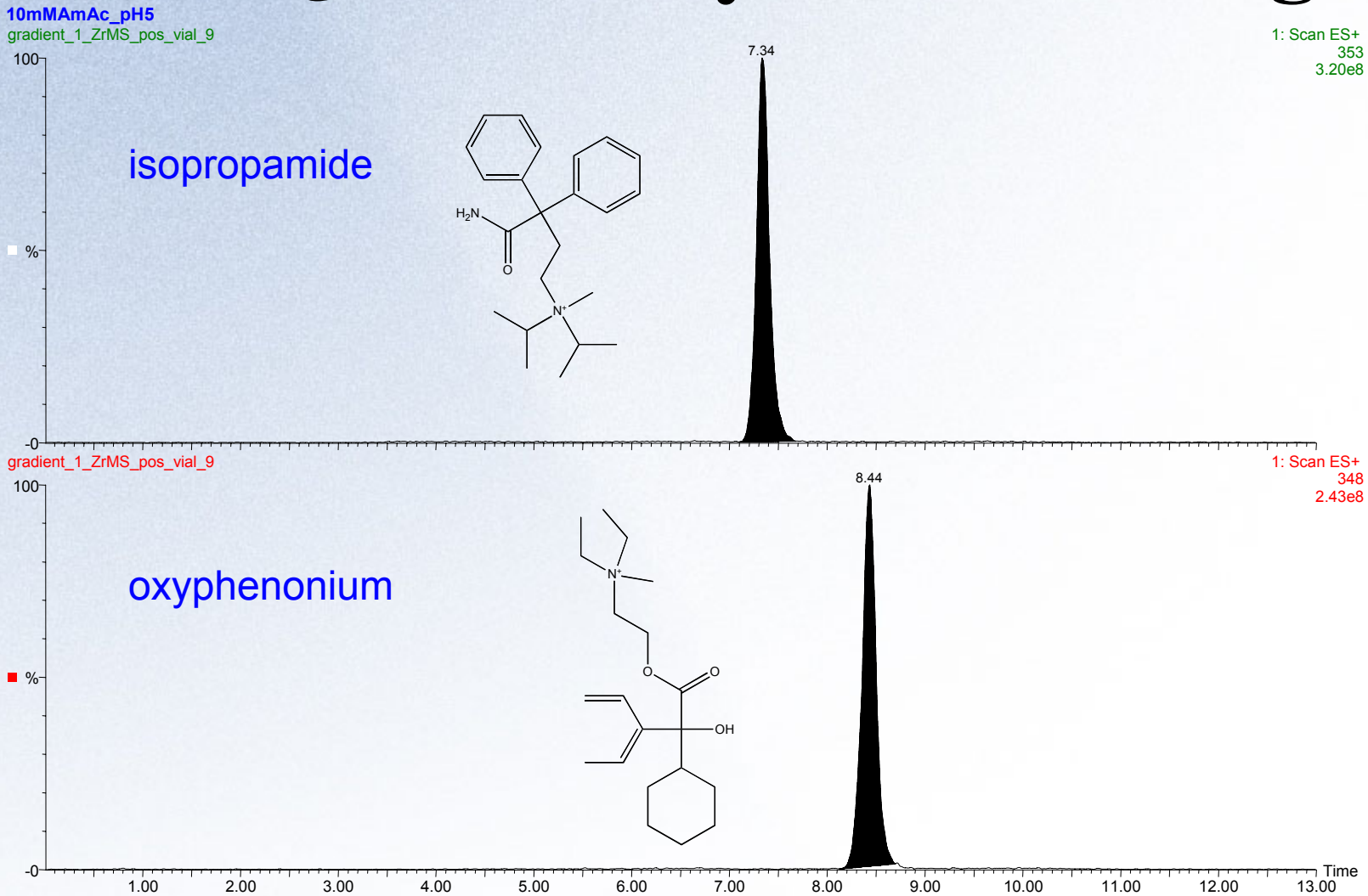
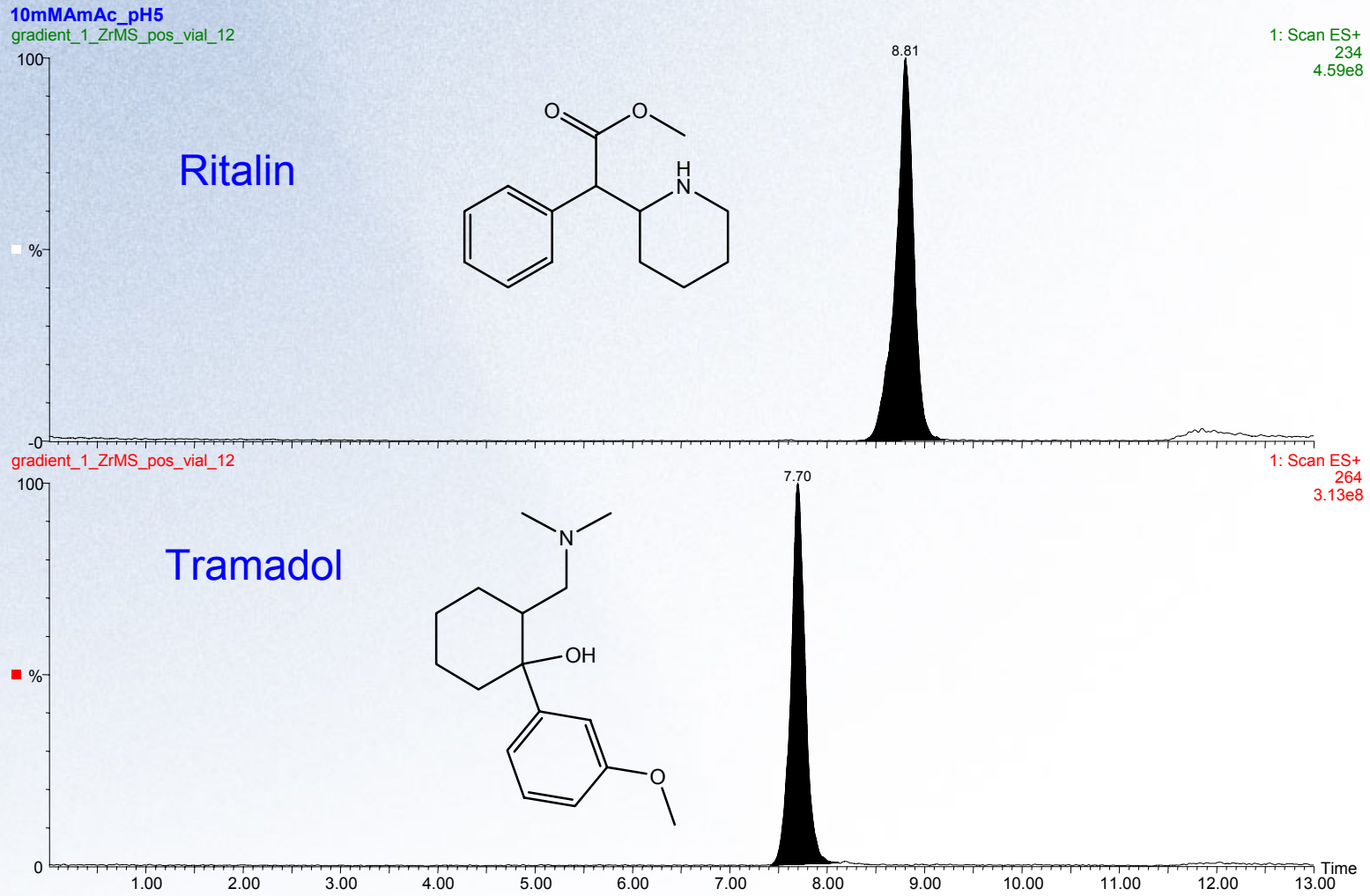


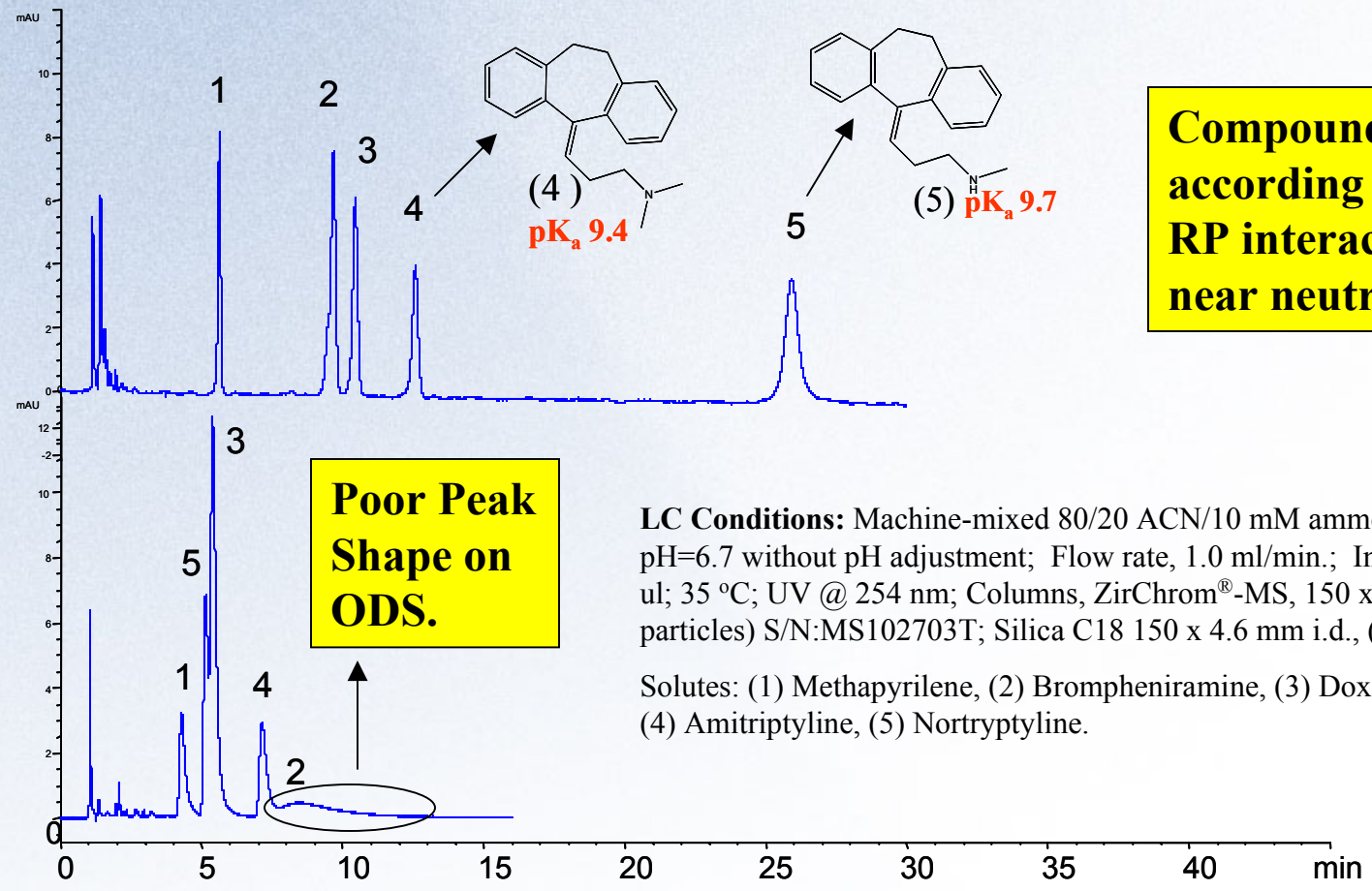
HPLC-MS of Quaternary Amine Drugs



HPLC-MS of Quaternary Amine Drugs



Separation Comparison of Basic Pharmaceuticals on ZirChrom®-MS and ODS



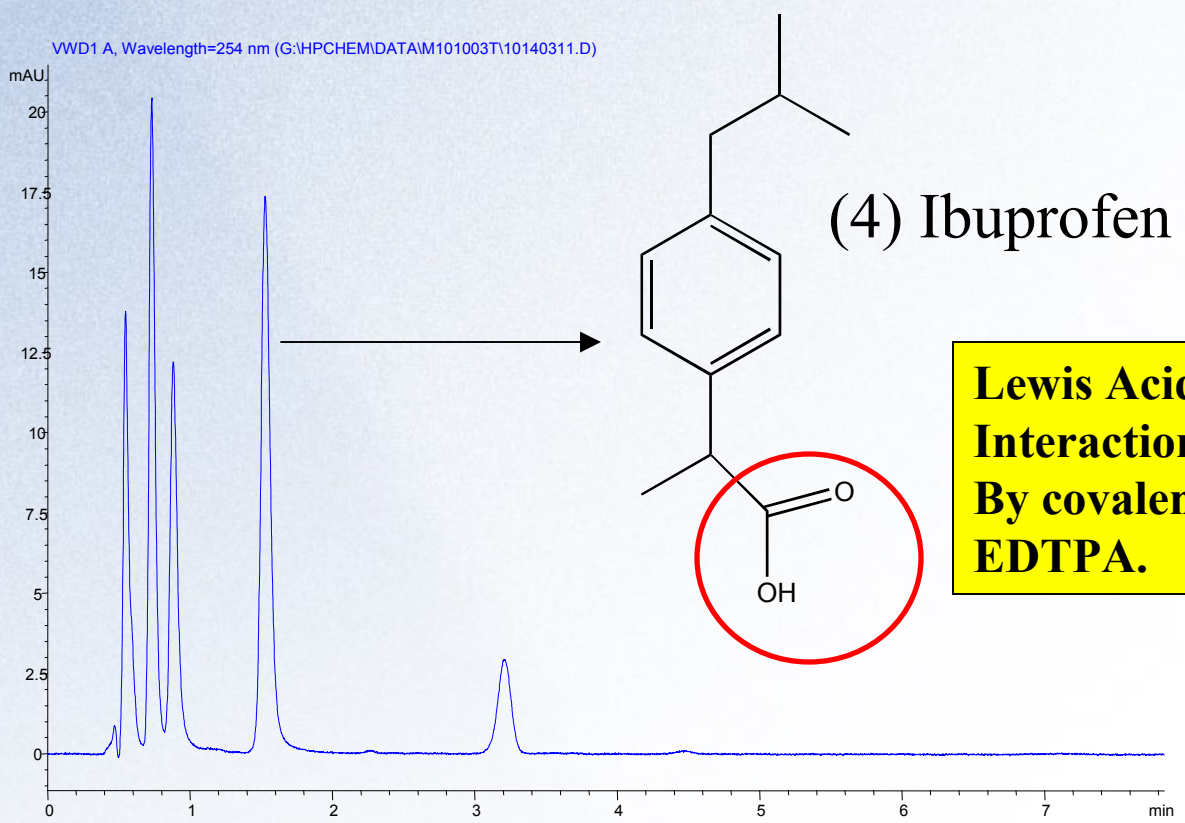
Compounds elute according to IEX, not RP interactions at near neutral pHs.

Poor Peak Shape on ODS.

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, 5 ul; 35 ºC; UV @ 254 nm; Columns, ZirChrom®-MS, 150 x 4.6 mm i.d. (3 um particles) S/N:MS102703T; Silica C18 150 x 4.6 mm i.d., (3.5 um particles).

Solutes: (1) Methapyrilene, (2) Brompheniramine, (3) Doxepin, (4) Amitriptyline, (5) Nortryptyline.

Separation of Acidic Pharmaceuticals



(4) Ibuprofen

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

LC Conditions: Column, ZirChrom®-MS, 50 x 4.6 mm i.d. (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.

Conclusions

- The ZirChrom®-MS phase is a novel zirconia-based RP column ***designed for use with MS***.
- The ZirChrom®-MS phase is ***Lewis acid site deactivated***.
- The ZirChrom®-MS phase has ***similar selectivity*** and RP behavior to silica C18 ***for neutral compounds***.
- ZirChrom®-MS ***is chemically stable*** from pH 1-10.
- ZirChrom®-MS ***has very different selectivity*** than silica C18 ***for basic compounds***.

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Supplemental Slides

Surface Chemistry of Zirconia-Based Supports for HPLC

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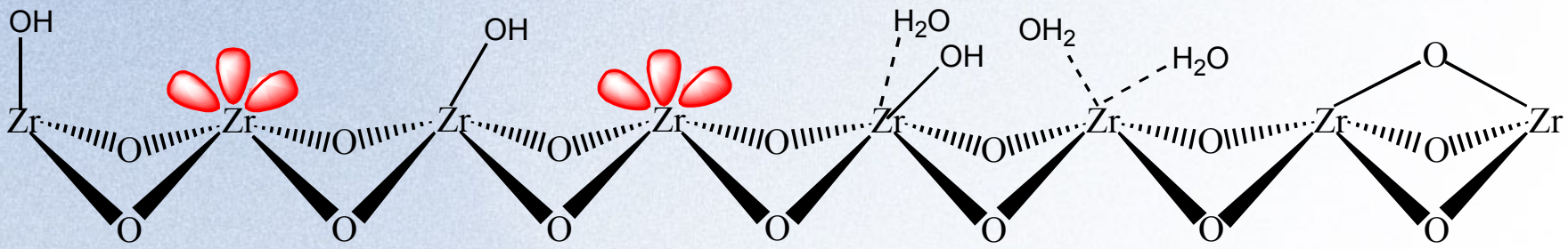
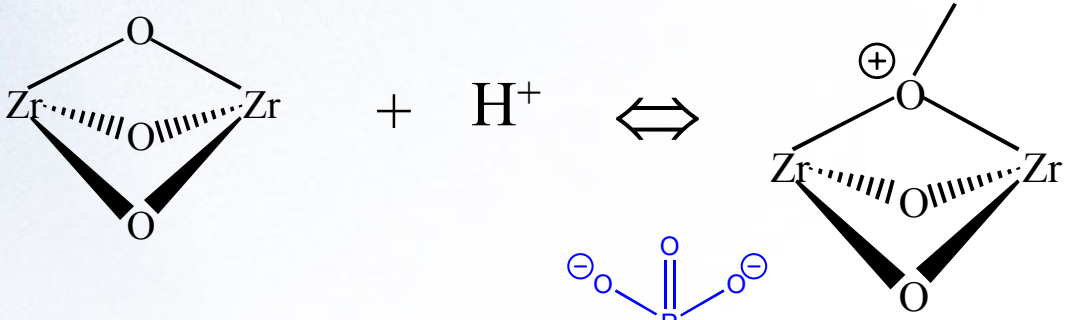
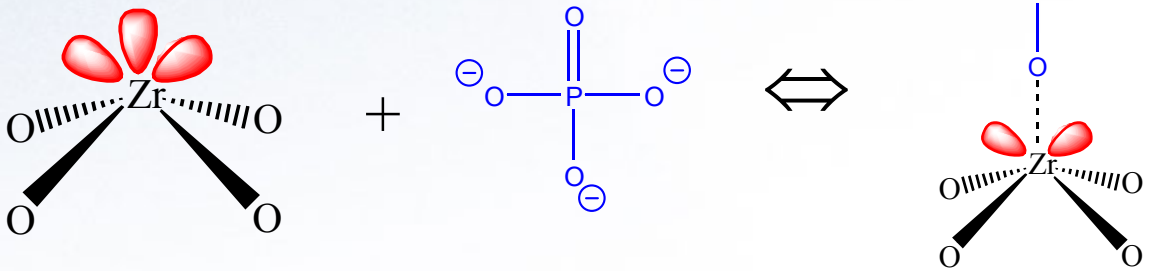


Brönsted Acid: $ZrOH + OH^- \Leftrightarrow ZrO^- + H_2O$

Brönsted Base:



Lewis Acid:



A Difficult Separation: Alkoxy Benzoic Acids.

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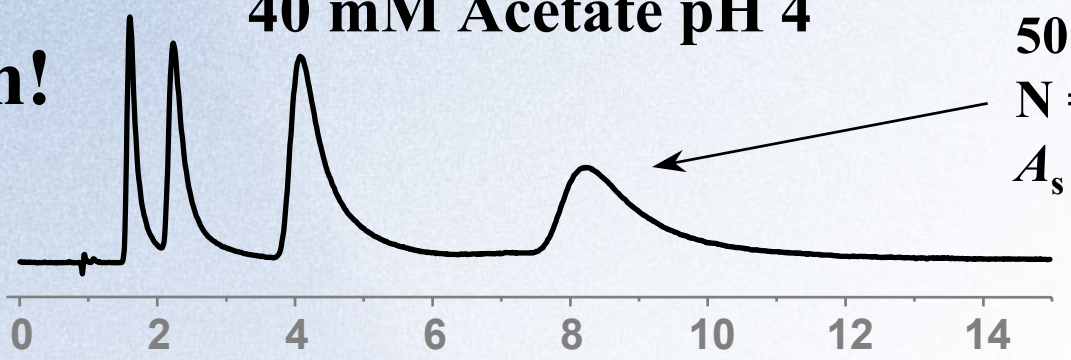
Problem!

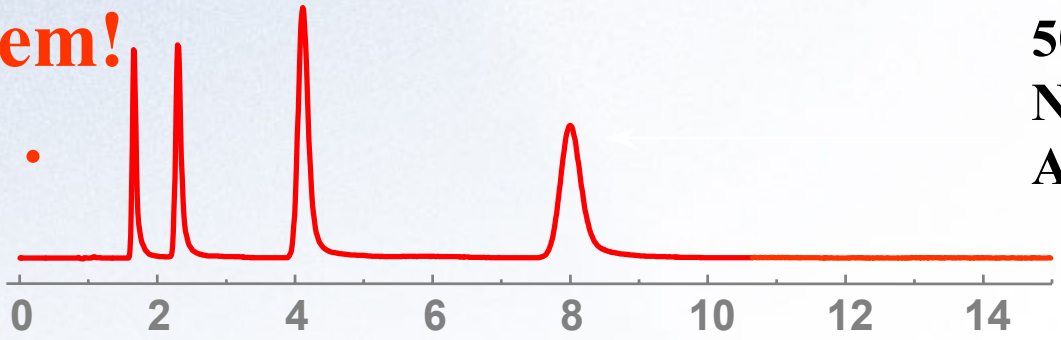
40 mM Acetate pH 4



50 x 4.6 mm
N = 260
A_s = 3.35

40 mM Phosphate pH 2.15

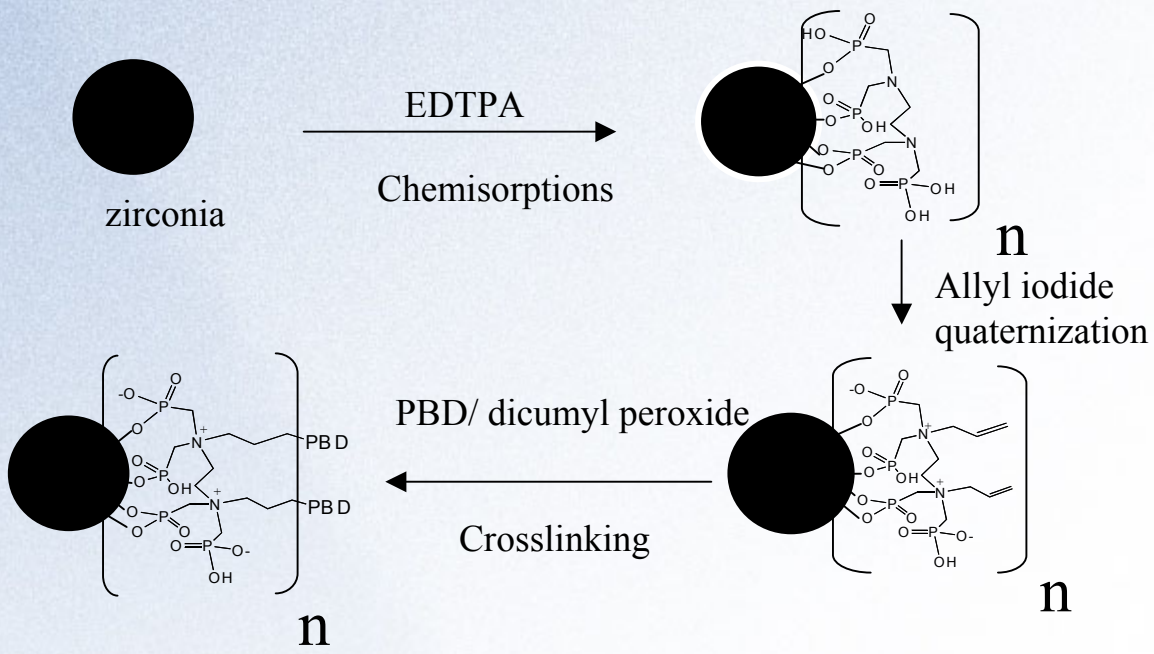
No Problem!
Except . . .
for MS.



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.

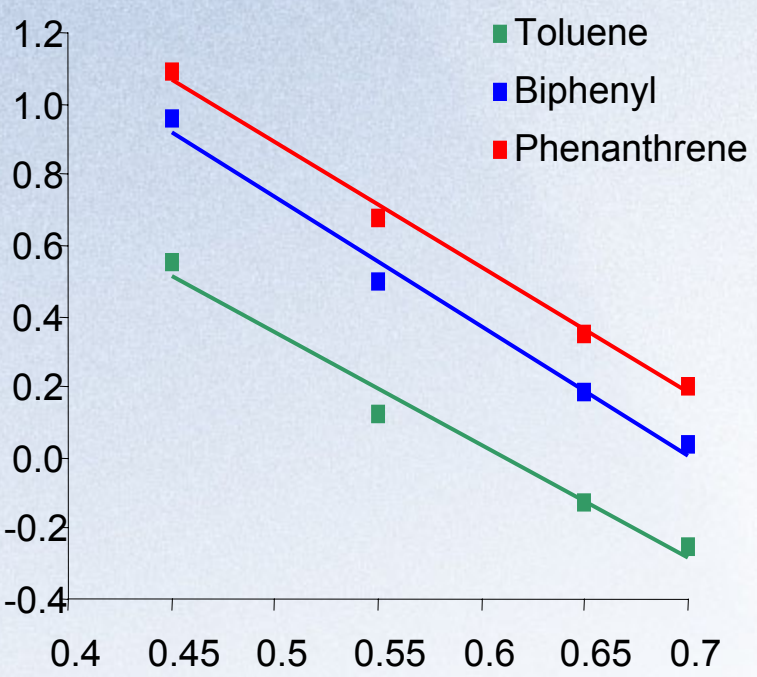
New Stationary Phase Strategy



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.**

Reversed-Phase Characteristics

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



	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.