Synthesis of a Novel C18 Polar-Embedded Reversed-Phase Zirconia For HPLC

EAS 2005

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Outline

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- Project Goal – First ***Stable*** Bonded Phase C18 Zirconia Stationary Phase.
- Zirconia Surface Chemistry
- Synthesis of C18 Bonded Phases
- Chromatographic Data
  - ***Selectivity*** Comparison between Silica-C18 and ***Zr-C18***
  - ***Chemical Stability*** Testing
  - ***Example Applications***
- **Conclusion** – The new Zr-C18 column is stable from pH 1 to 9 and has ***very different chromatographic selectivity for basic compounds compared to Silica-C18***. The column also performs well under MS-compatible conditions.

Project Goal

To produce a first of its kind *bonded phase C18* Reversed-Phase Zirconia Stationary Phase that has unique selectivity and *good peak shape for basic drugs*.

Zirconia **-** The difference is the surface chemistry.

Surface Chemistry of Zirconia

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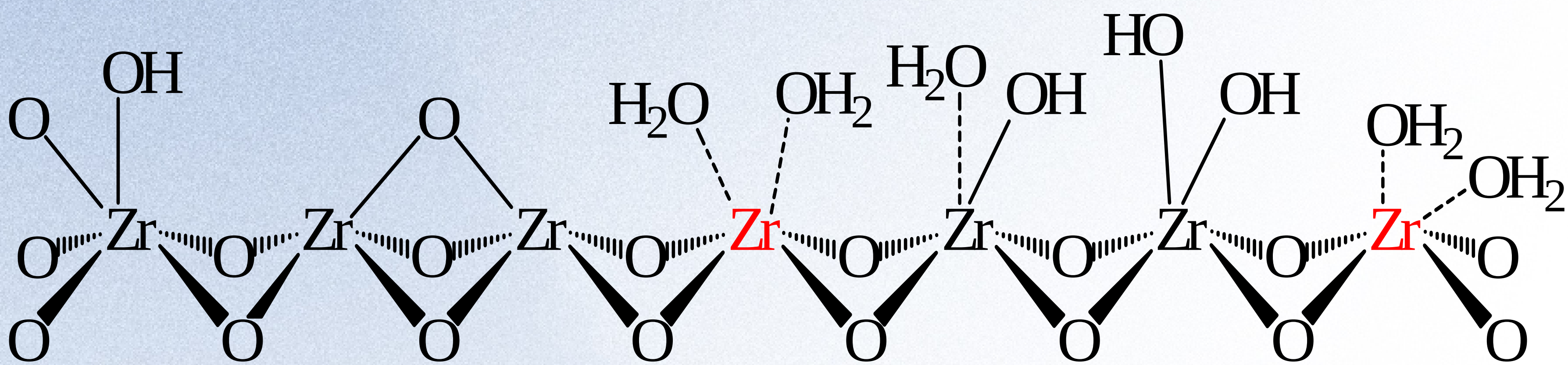


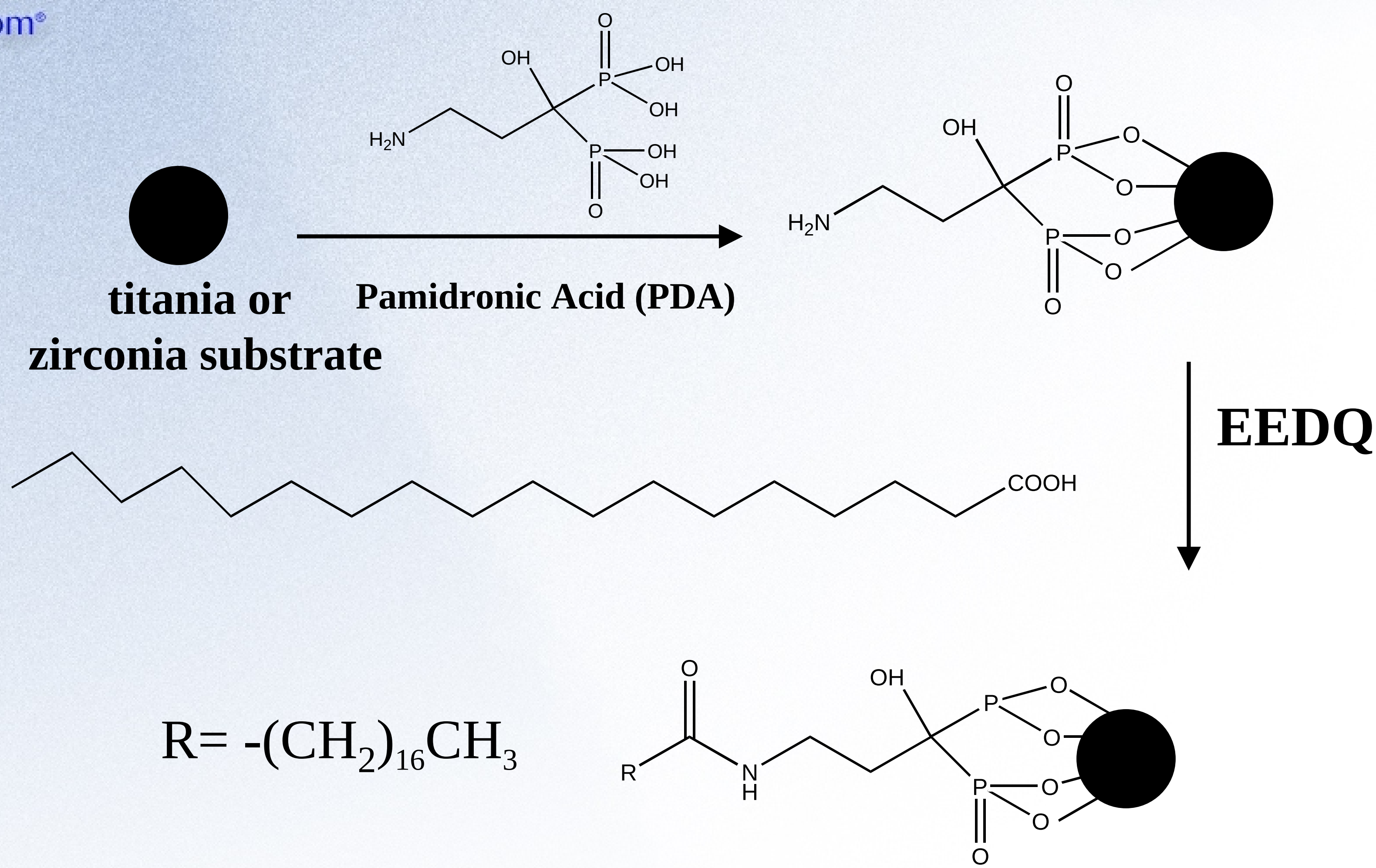
Brönsted Acid: $ZrOH + {}^{-}OH \rightleftharpoons ZrO^{-} + H_2O$

Brönsted Base: $Zr{-}O{-}Zr + H^{+} \rightleftharpoons Zr{-}O(H^{+}){-}Zr$

RPO_3^{2-} or Catechol

Lewis Acid: **$Zr^{4+}{:}\ H_2O + R\text{-}COO^{-} \rightleftharpoons Zr^{4+}{:}{}^{-}OOC\text{-}R + H_2O$**

Zr-C18 Synthesis



Selectivity: The Key to Success

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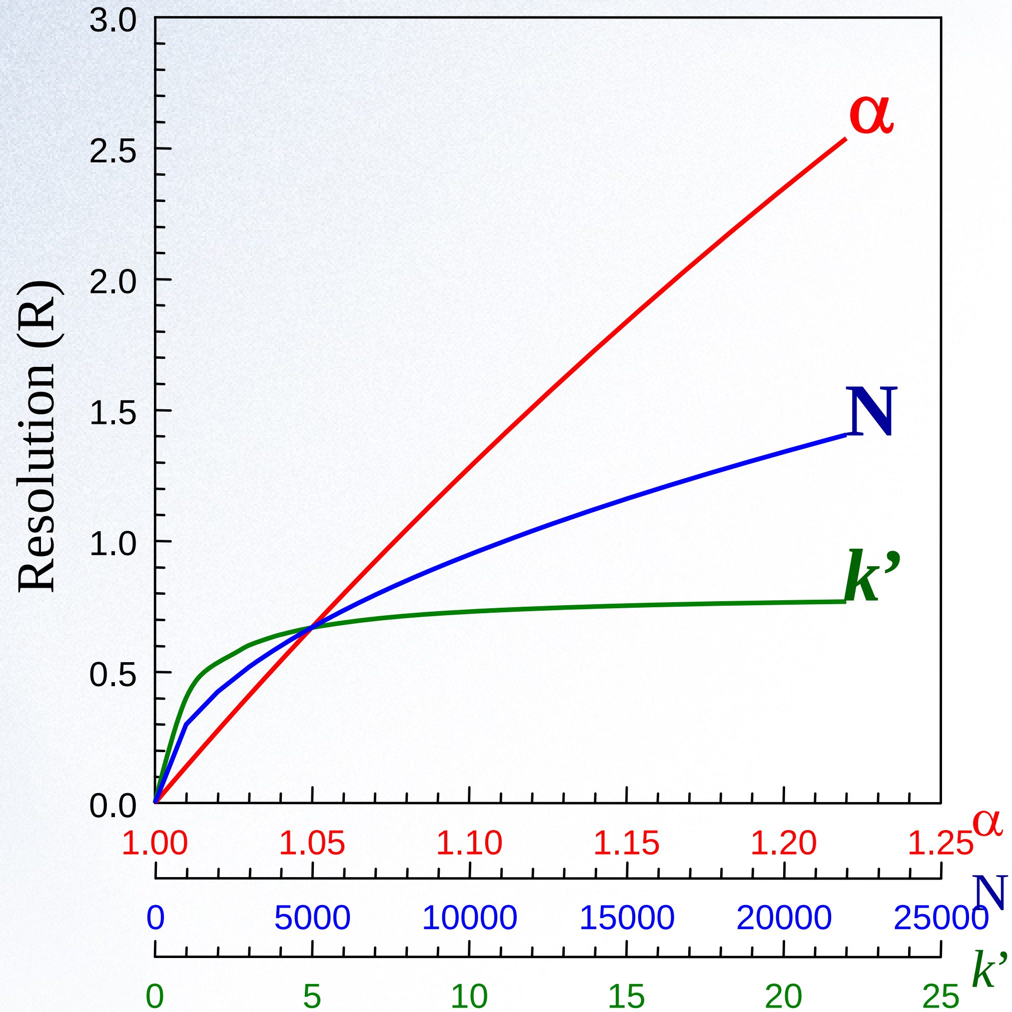
Efficiency	Retention	Selectivity

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

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

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



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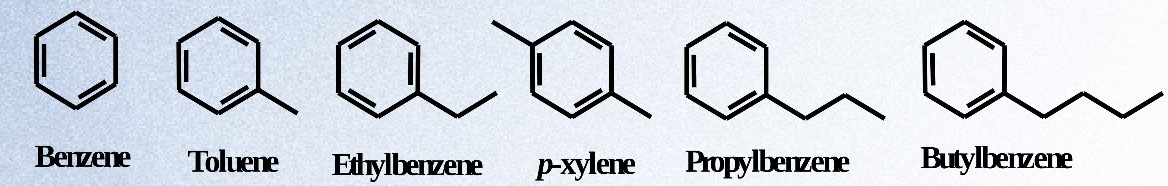
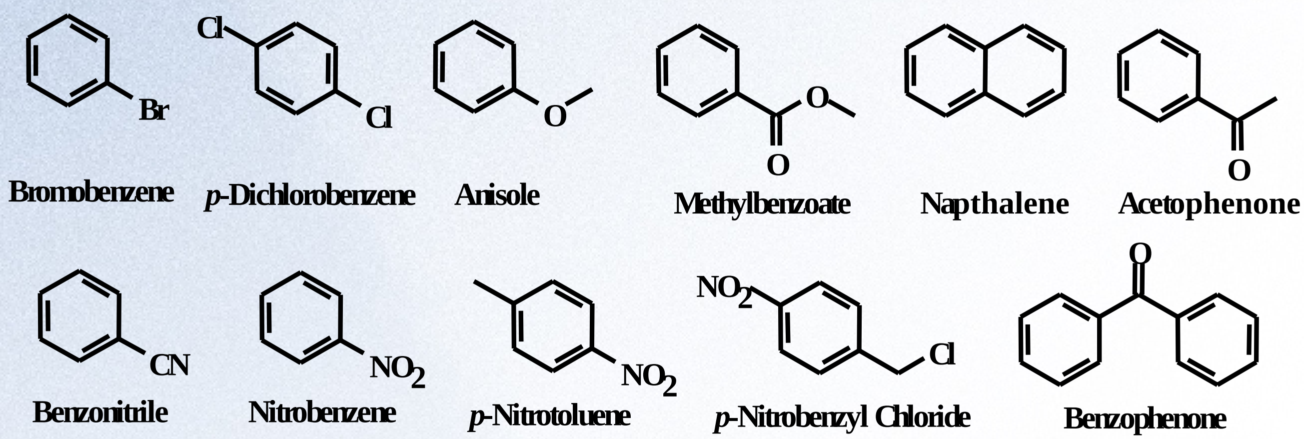
Selectivity Comparison Solutes

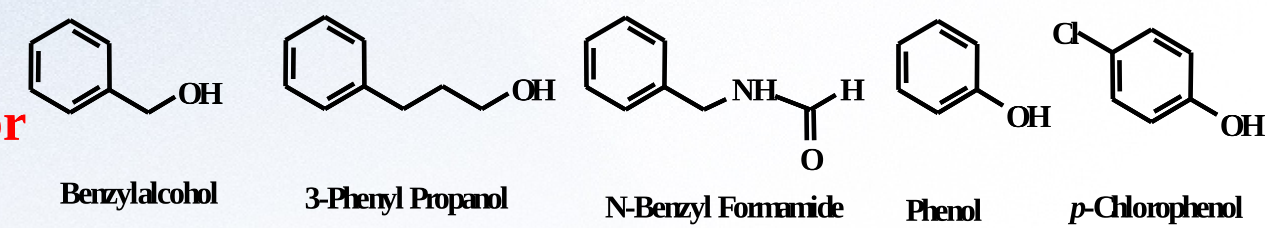
Nonpolar



Polar

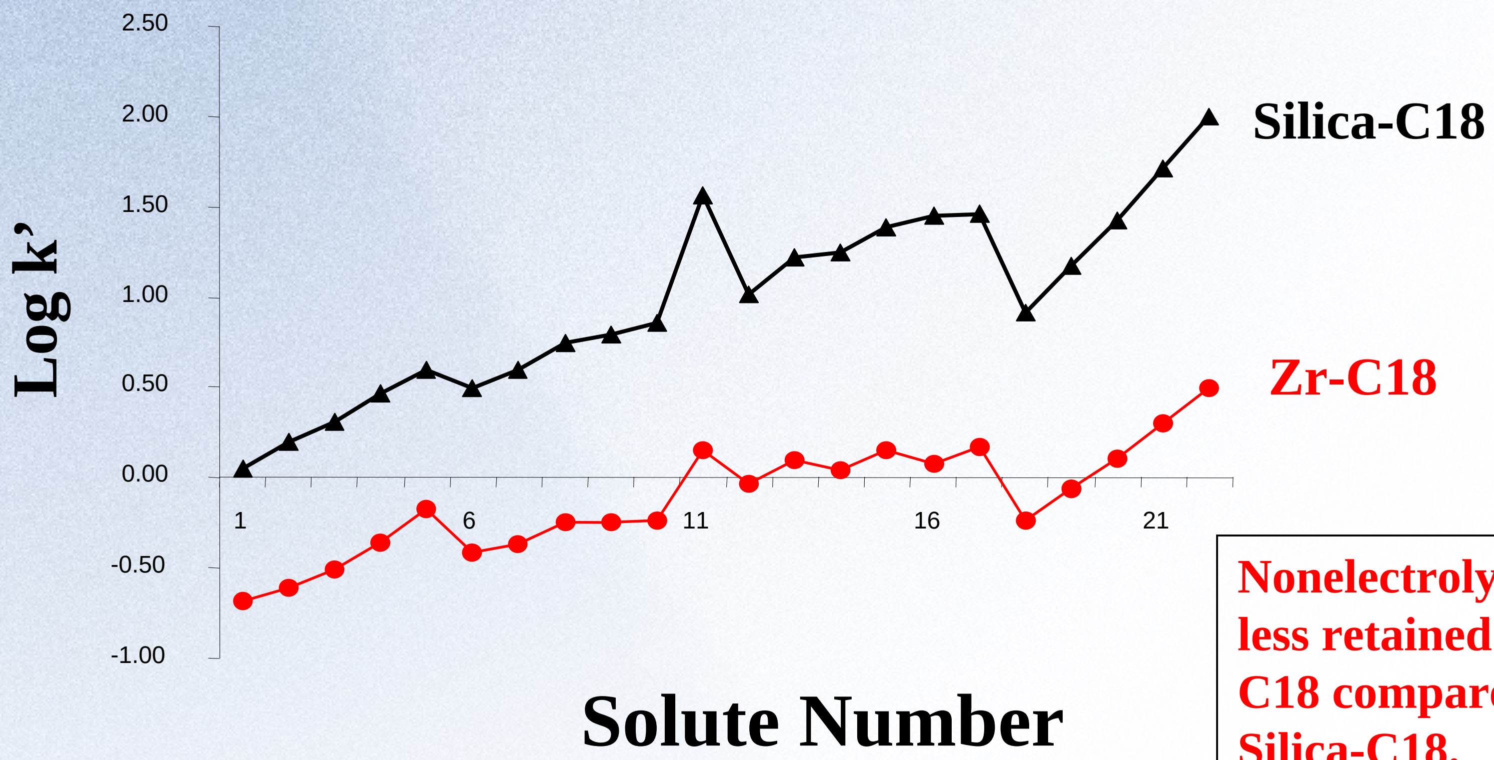


HB Donor



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

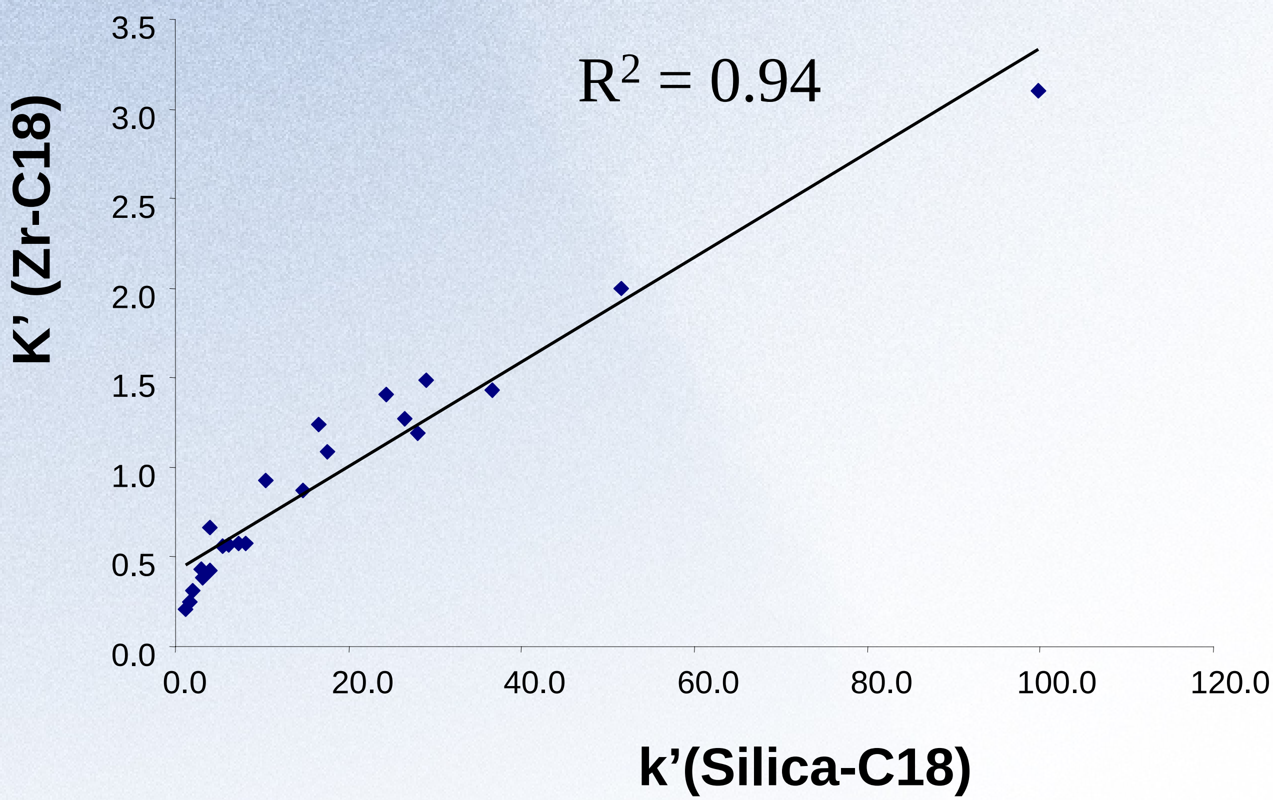
Comparison of Selectivity for Zr-C18 and Silica-C18 for Neutral Solutes



Nonelectrolytes are less retained on Zr-C18 compared to Silica-C18.

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

K–K Plot for 22 Solutes on Zr-C18 and Silica-C18



Zr-C18 has similar selectivity to Silica-C18 for nonelectrolytes.

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

Selectivity Study: Acidic Solutes

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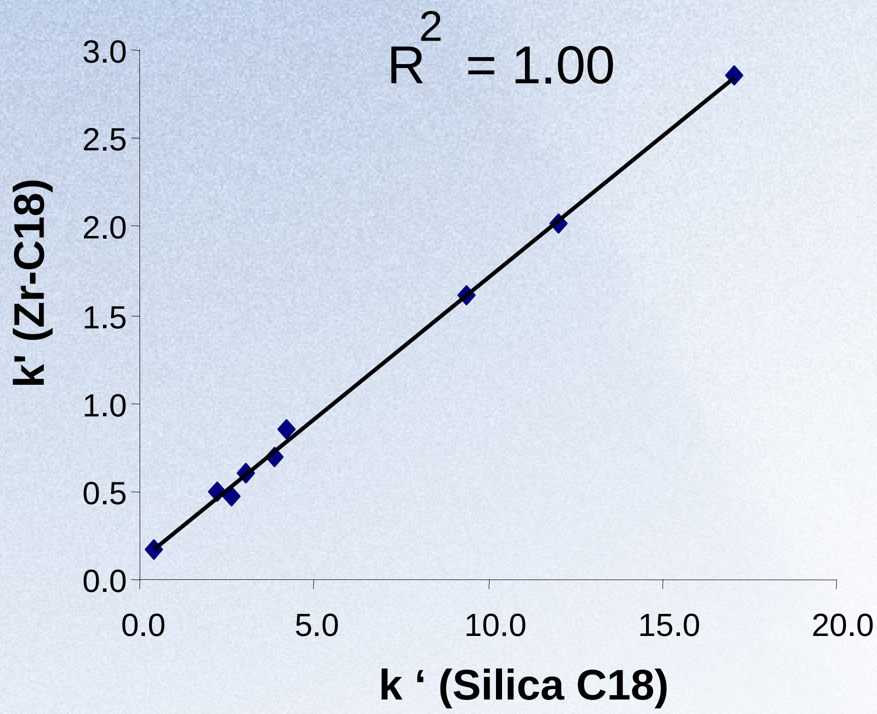
1. Benzoic acid
2. 4-hydroxybenzoic acid
3. 4-cyanobenzoic aicd
4. 4-methoxybenzoic acid
5. 4-fluorobenzoic acid
6. 4-nitrobenzoic aicd
7. 4-chlorobenzoic acid
8. 4-bromobenzoic acid
9. 4-iodobenzoic aicd

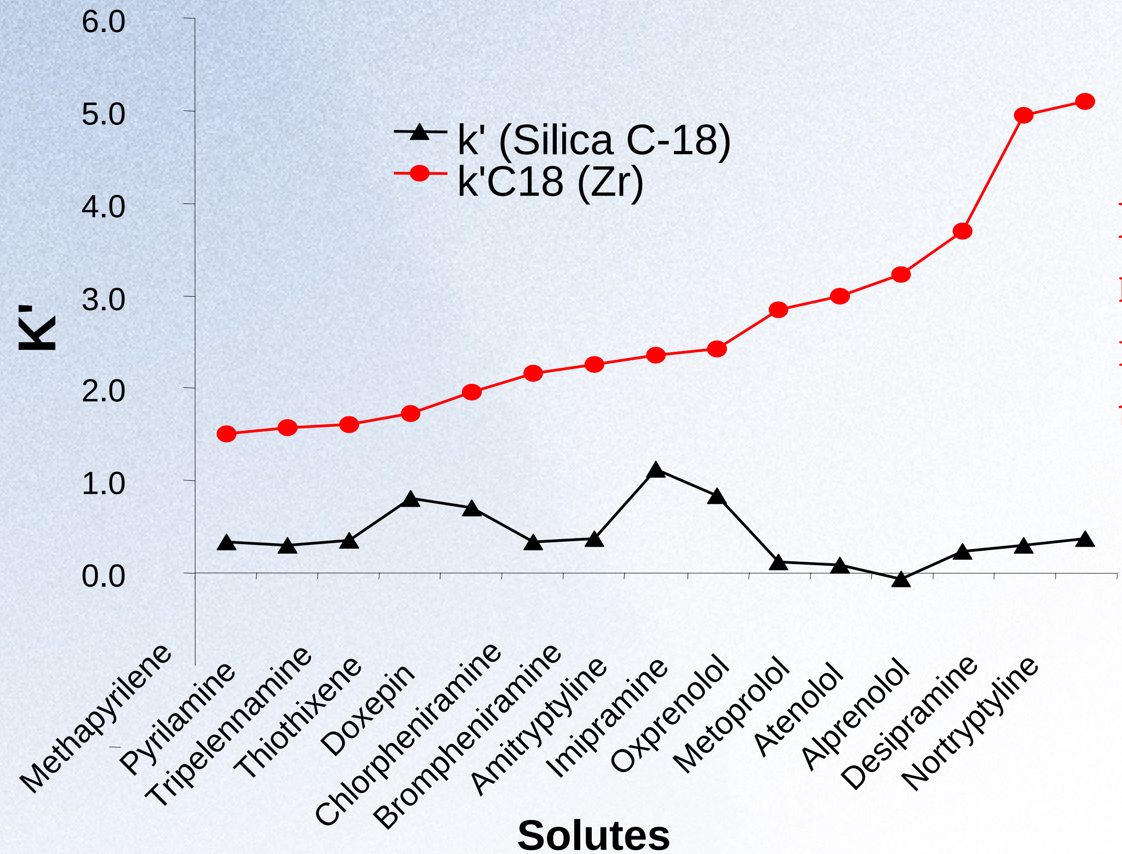
Comparison Between Zr-C18 and Silica-C18



(1) Benzoic acids and phenols on Zr-C18 have much less retention than on silica.
(2) There is no selectivity difference between Zr-C18 and Silica-C18 for acidic compounds.

LC Conditions: Machine-mixed 74//26 ACN/25 mM phosphoric acid (H_3PO_4) pH=2.08 without pH adjustment; Flow rate, 1.0 ml/min.; Injection volume 1 μl; Temperature, 35 ^{o}C; Detection at 254 nm; Columns, ZR-C18, 50 x 4.6 mm i.d. (3um particles); Silica-C18 50 x 4.6 mm i.d.

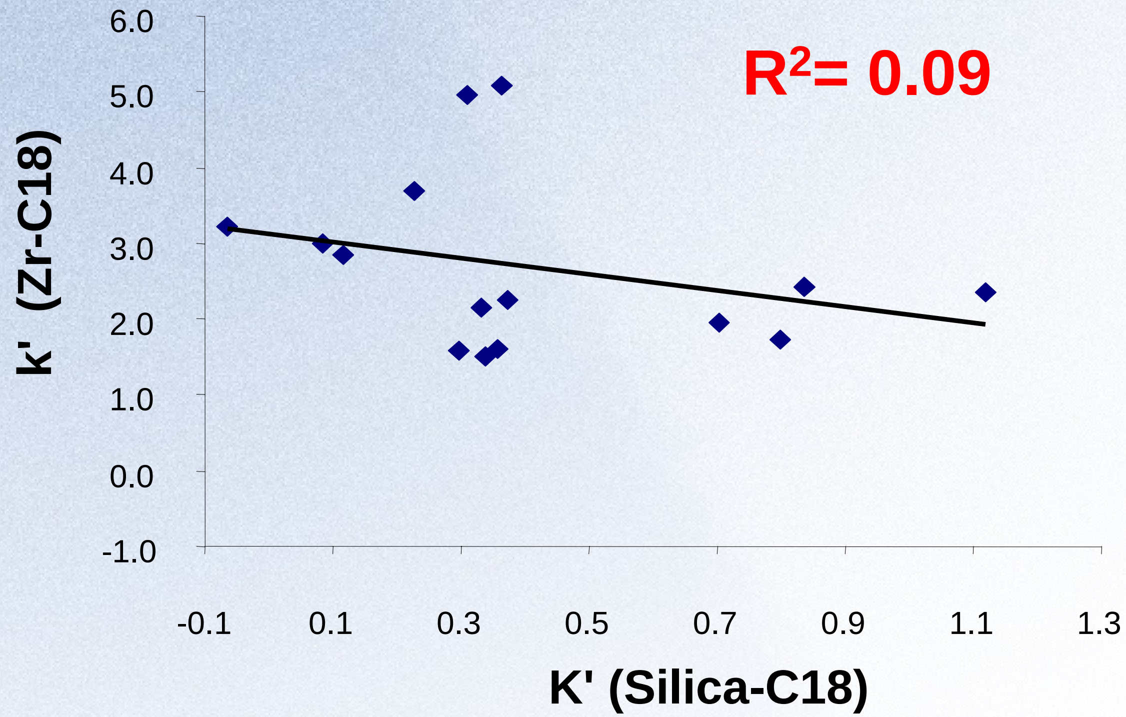
Comparison of Retention of Basic Pharmaceuticals for Zr-C18 and Silica-C18



Basic drugs have much higher retained on Zr-C18 than on Silica-C18.

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, Zr-C18, 50 x 4.6 mm i.d. (3um particles); Silica-C18 150 x 4.6 mm i.d., (3 um particles).

K−K Plot for Basic Pharmaceuticals on Zr-C18 and Silica-C18

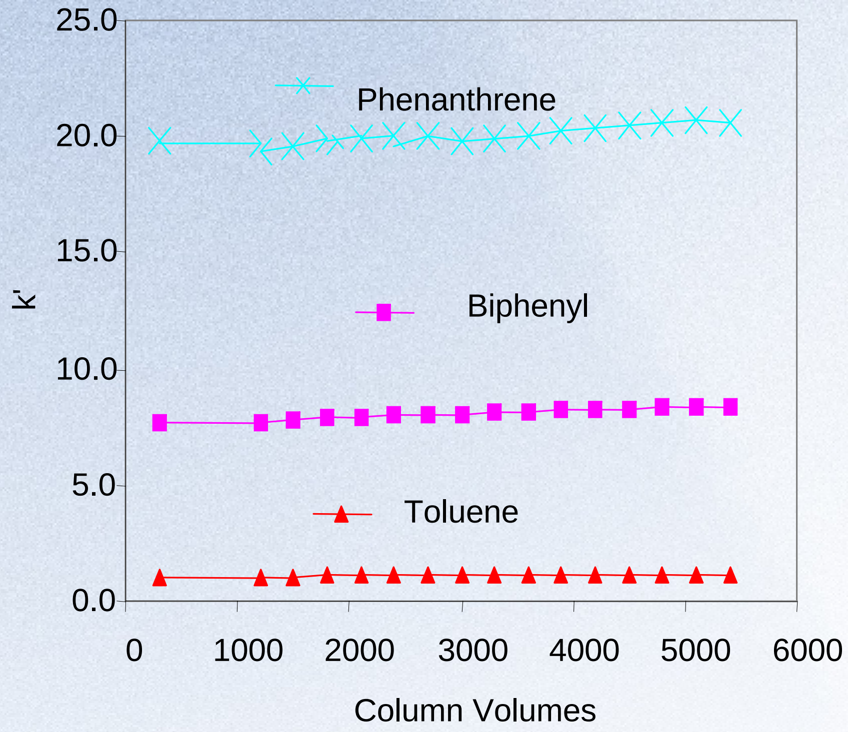


Zr-C18 has *very different chromatographic selectivity for basic drugs* compared to Silica-C18.

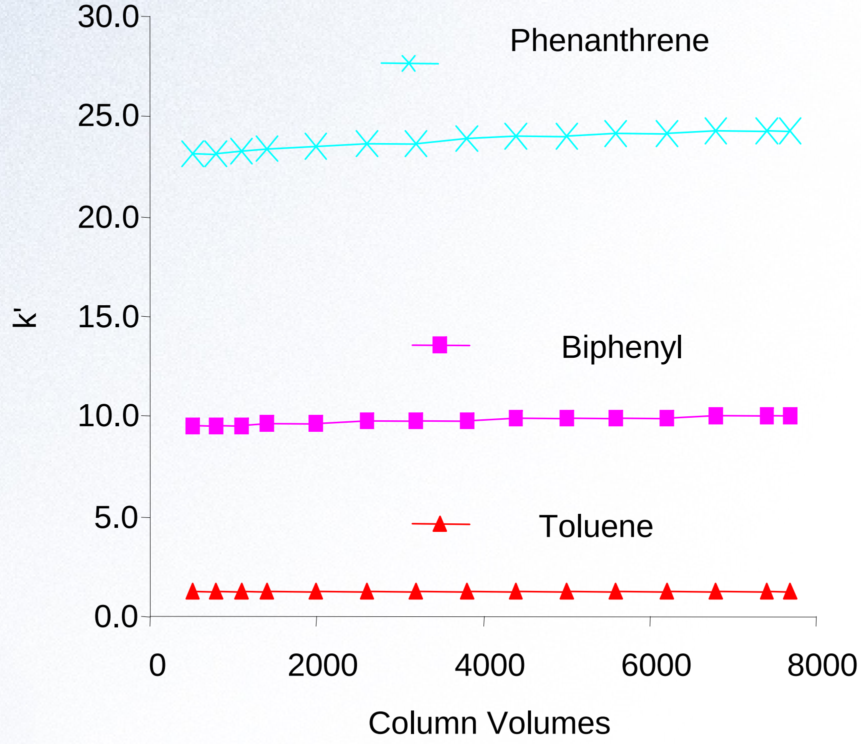
LC Conditions: Machine-mixed 80/20 ACN/10 mM ammonium acetate pH=6.7 without pH adjustment; Flow rate, 1.0 ml/min.; Injection volume 0.1 µl; Temperature, 35 ^{o}C; Detection at 254 nm; Columns, Zr-C18, 50 x 4.6 mm i.d. (3um particles); Silica-C18 150 x 4.6 mm i.d., (3 um particles).

Chemical Stability

pH 1.0

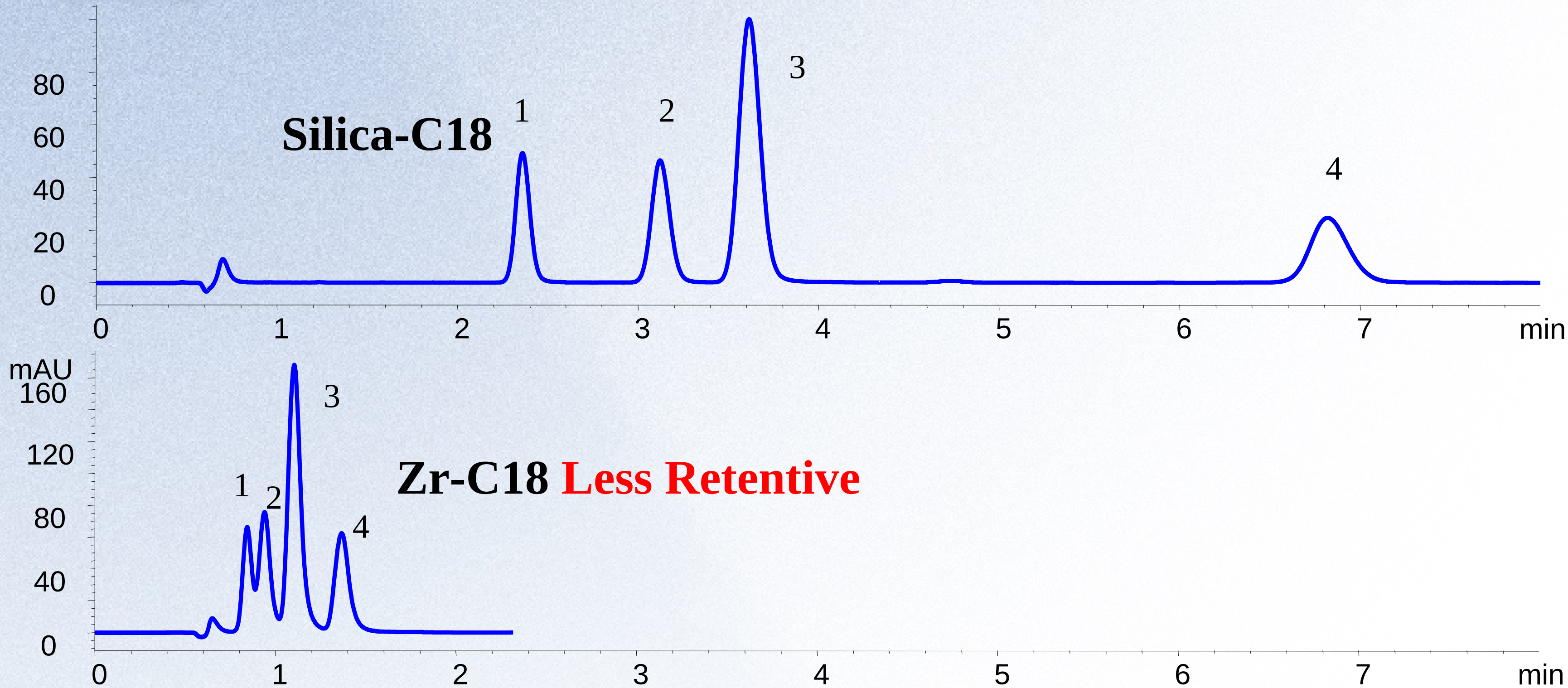


pH 9.0



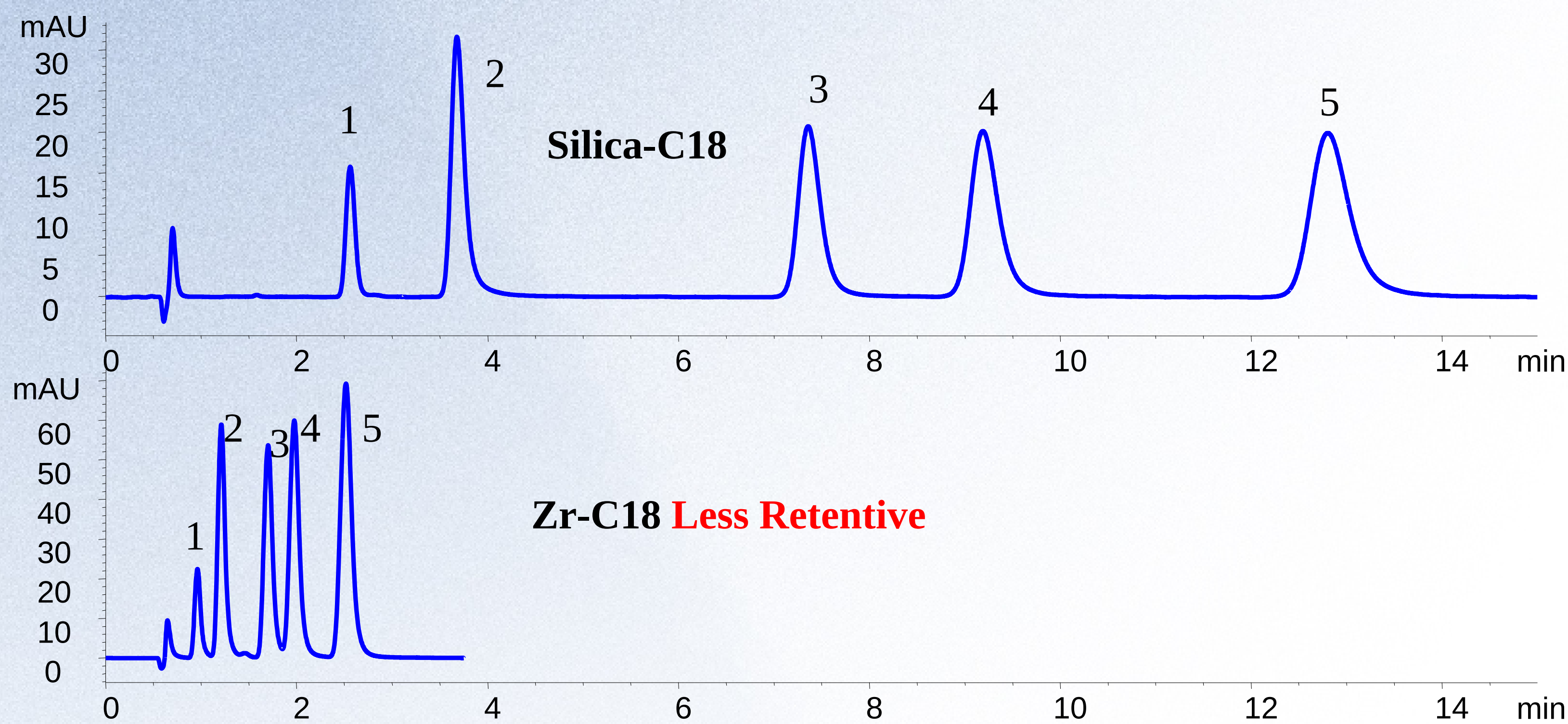
Exposure and Evaluation Conditions: Mobile phase, 15/85 ACN/0.1M Nitric acid, pH 1.0, or 20 mM Ammonium hydrogen carbonate, pH 9.0; Flow rate, 1.0 ml/min.; Temperature, 30 ºC; Injection volume, 5 µl; Detection at 254 nm; Column, 50 mm x 4.6 mm i.d. Zr-C18.

Phenols Separation on Zr-C18 and Silica-C18



LC Conditions: Mechine-mixed 26/74 ACN/25 mM H_3PO_4 pH=2.08 without pH adjustment; Flow rate, 1.0 ml/min.; Injection volume 0.1 ul; Temperature, 35 ^oC ; Detection at 254 nm; Solutes: (1) phenol, (2) 4-fluorophenol, (3) 4-nitrophenol, (4) 4-chlorophenol

Benzoic Acids Separation on Zr-C18 and Silica-C18



LC Conditions: Mechine-mixed 26/74 ACN/25 mM H_3PO_4 pH=2.08 without pH adjustment; Flow rate, 1.0 ml/min.; Injection volume 0.1 ul; Temperature, 35 ^oC ; Detection at 254 nm; Solutes: (1) benzoic acid, (2) 4-nitrobenzoic acid, (3) 4-chlorobenzoic acid, (4) 4-bromobenzoic acid, (5) 4-iodobenzoic acid

Separation Comparison of Basic Pharmaceuticals on Zr-C18 and Silica C18

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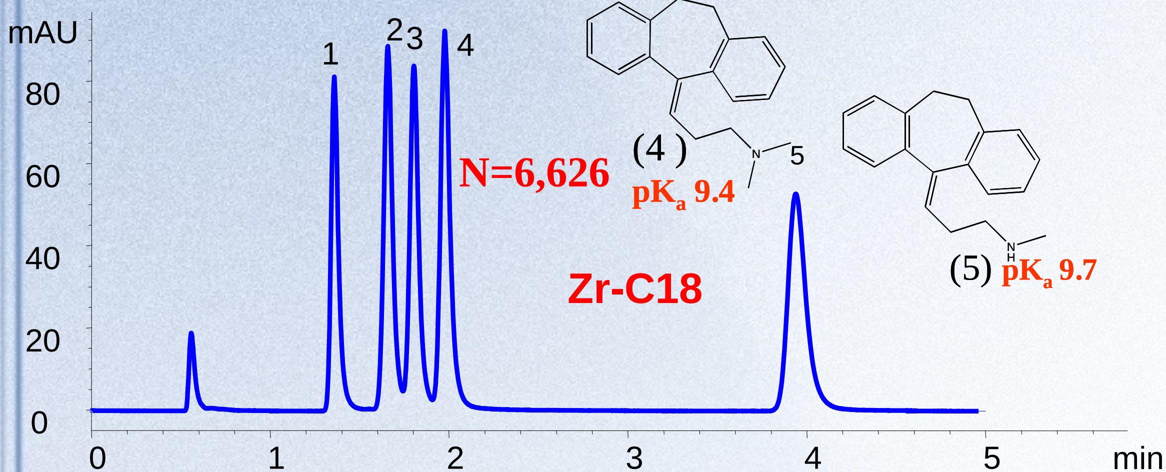

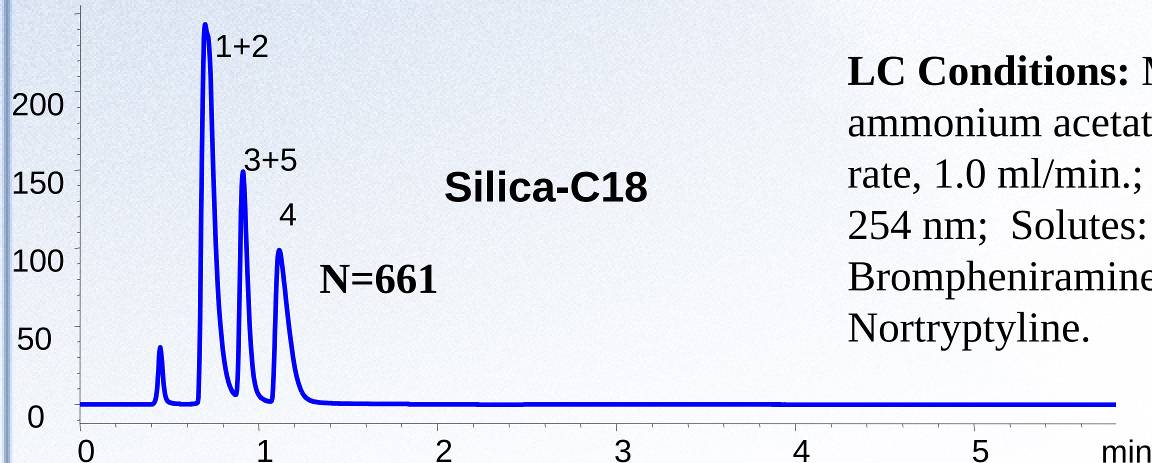

Compounds elute according to IEX, not RP interactions at near neutral pHs.

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 ^{o}C; UV @ 254 nm; Solutes: (1) Methapyrilene, (2) Brompheniramine, (3) Doxepin, (4) Amitriptyline, (5) Nortryptyline.

LC-MS of UV Degraded Nifedipine on Zr-C18 and Silica C18

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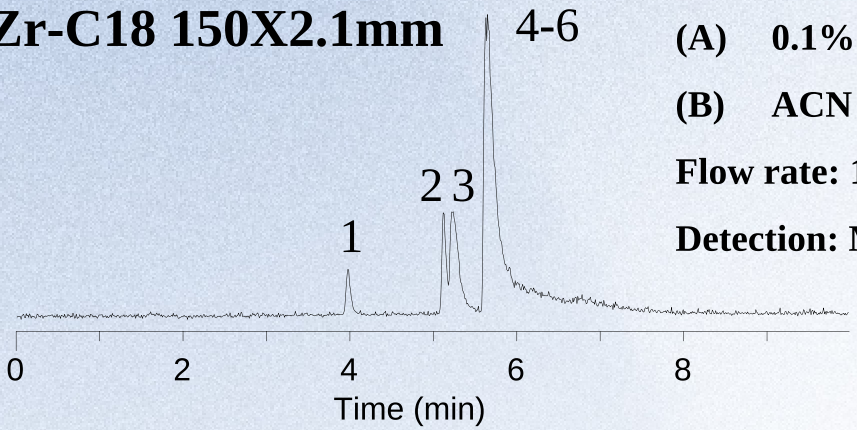
Zr-C18 150X2.1mm



Mobile Phase:

(A) 0.1% ammonium acetate

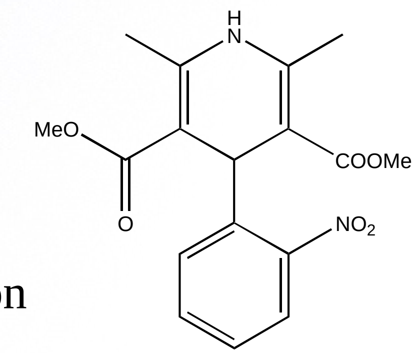
(B) ACN

Flow rate: 1ml/min

Detection: MS, ESI (+)

Gradient program

Time	A%	B%
0	95	5
10	0	100
12	0	100
14	95	5
20	95	5



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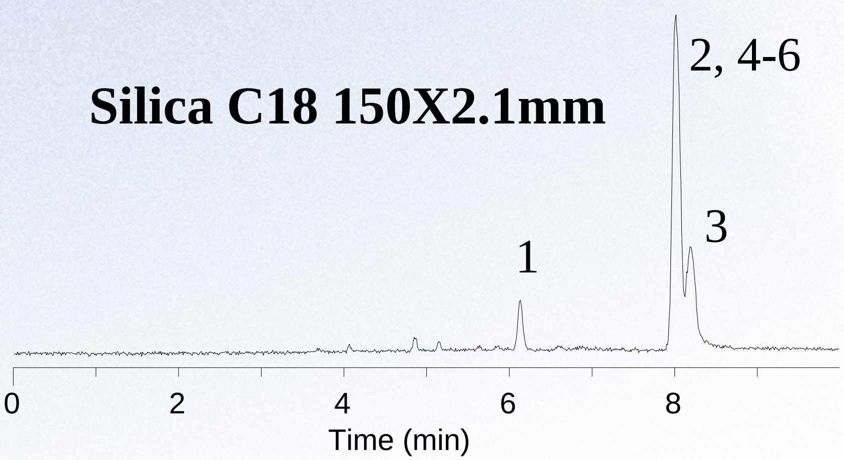
Silica C18 150X2.1mm



Peak Identification

1. m/z 329
2. m/z 345
3. m/z 329
4. Nifedepine m/z 347
5. m/z 315
6. m/z 410

Conclusions

- The Zr-C18 phase is ***a first of its kind*** stable bonded phase ***compatible with MS detection***.
- The Zr-C18 phase is ***Lewis acid site deactivated***.
- The Zr-C18 phase has ***similar selectivity*** and RP behavior to Silica-C18 ***for neutral and acidic compounds***.
- Zr-C18 ***has very different selectivity*** than Silica-C18 ***for basic compounds***.
- Zr-C18 ***is chemically stable from pH 1-9***.

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