Zirconia as a Versatile Substrate for Chiral Stationary Phases

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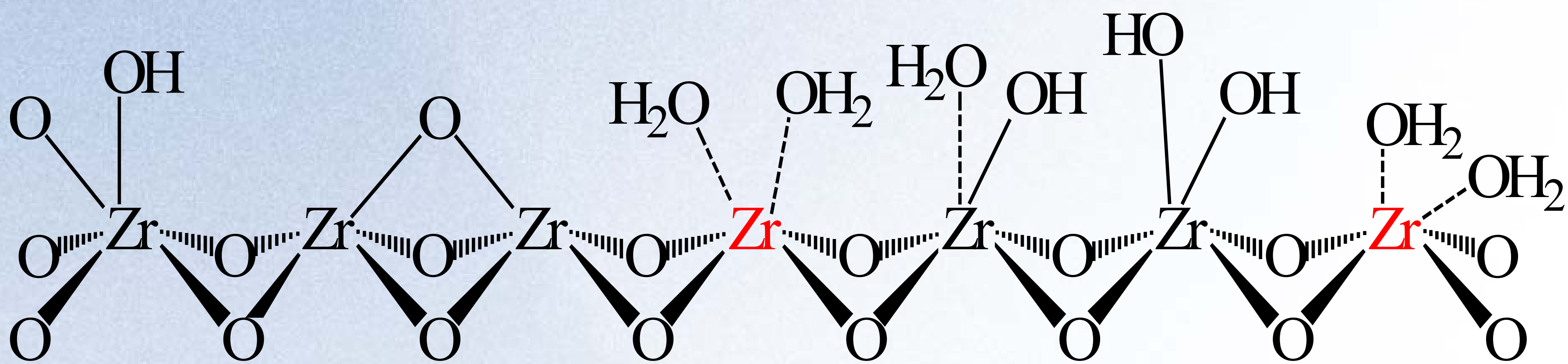
Surface Chemistry of Zirconia

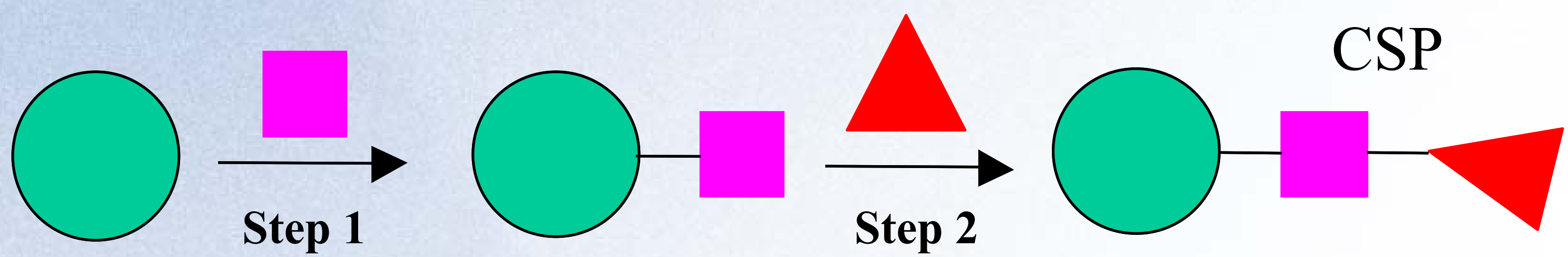


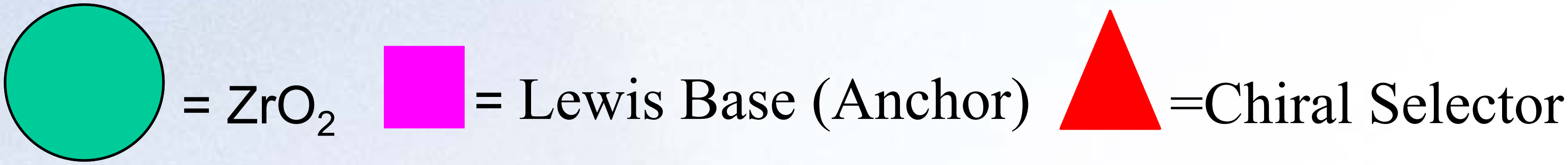
Zirconia chemistry is dominated by Lewis acid-base reactions

Lewis Acid: Zr^{4+}: $H_2O + RPO_3^{2-} \rightleftharpoons Zr^{4+}: RPO_3^{2-} + H_2O$

Other Lewis base examples: PO_4^{3-}, RCO_2^{-}, Catechol

A Novel Approach to Attaching Chiral Selectors[1] to Zirconia[2]





1. William H. Pirkle, et. al., J. Chromatogr., 316 (1984) 585.
2. 2. Phase I SBIR Grant (NIH).

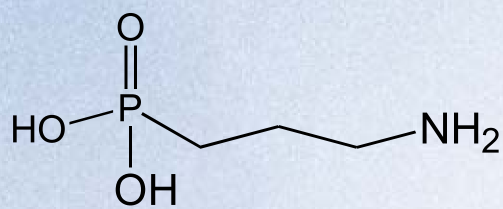
Interaction Strength of Lewis Bases with Zirconia[1]

Interaction Strength	Lewis Base (L)
Strongest	Hydroxide
	Phosphate
	Fluoride
	Citrate
	Sulfate
	Acetate
	Formate
	Nitrate
	Chloride
Weakest	Water

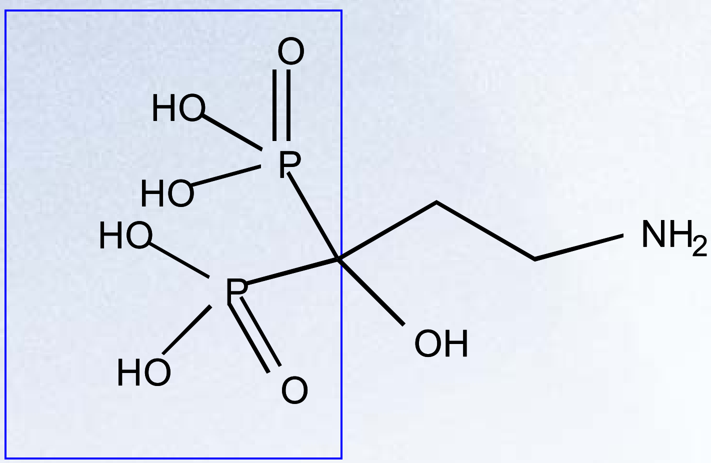
Small Lewis bases with high electron density and low polarizability interact more strongly with Zr atoms.

1. 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).

A Bidentate Phosphonate Anchor– the Key to Improved Stability[1]



Aminopropylphosphonic acid (APPA)



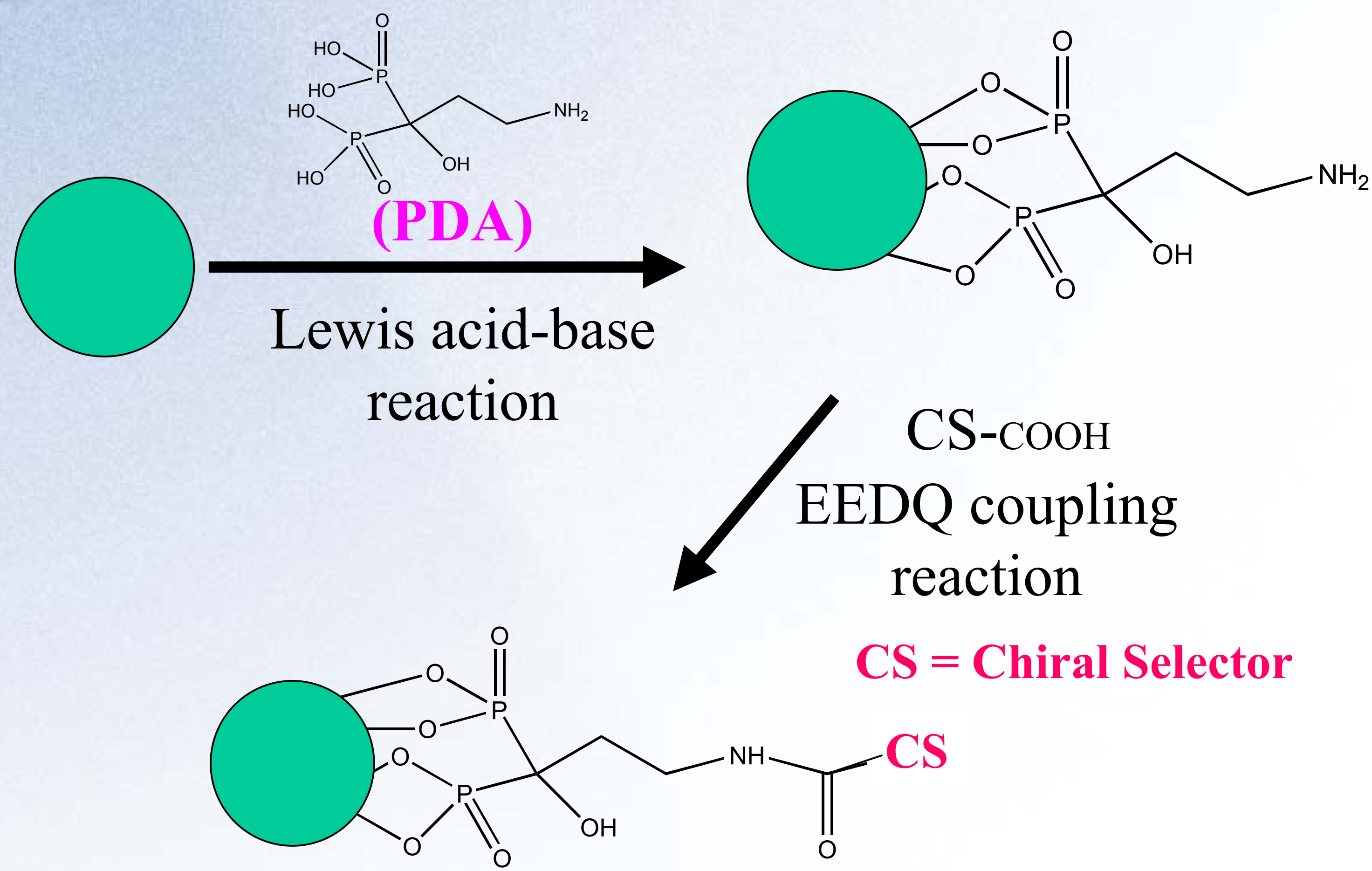
Bidentate anchor

Pamidronic acid (PDA)[1]

(Phase II Anchor)

1. Phase II SBIR (NIH).

Zirconia CSP 2-Step Synthesis with Bidentate Anchor (PDA)



Chiral Selectors Evaluated[1]

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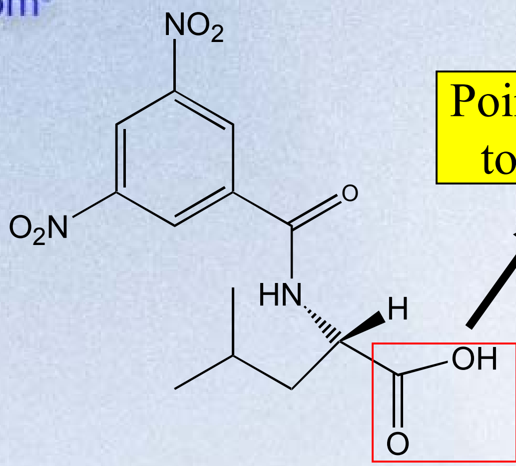
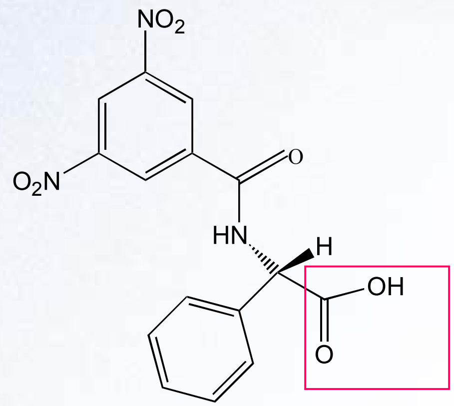


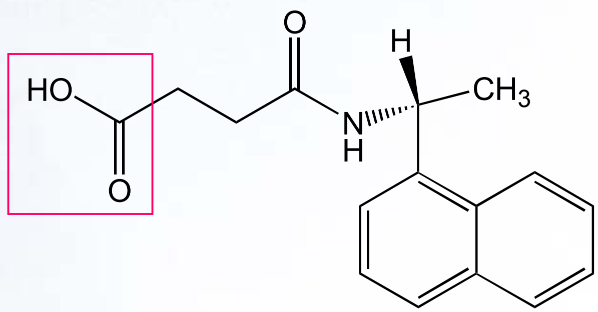
(S)-DNB-L-Leucine

[(S)-Leu]

Point of Attachment to Anchor Group



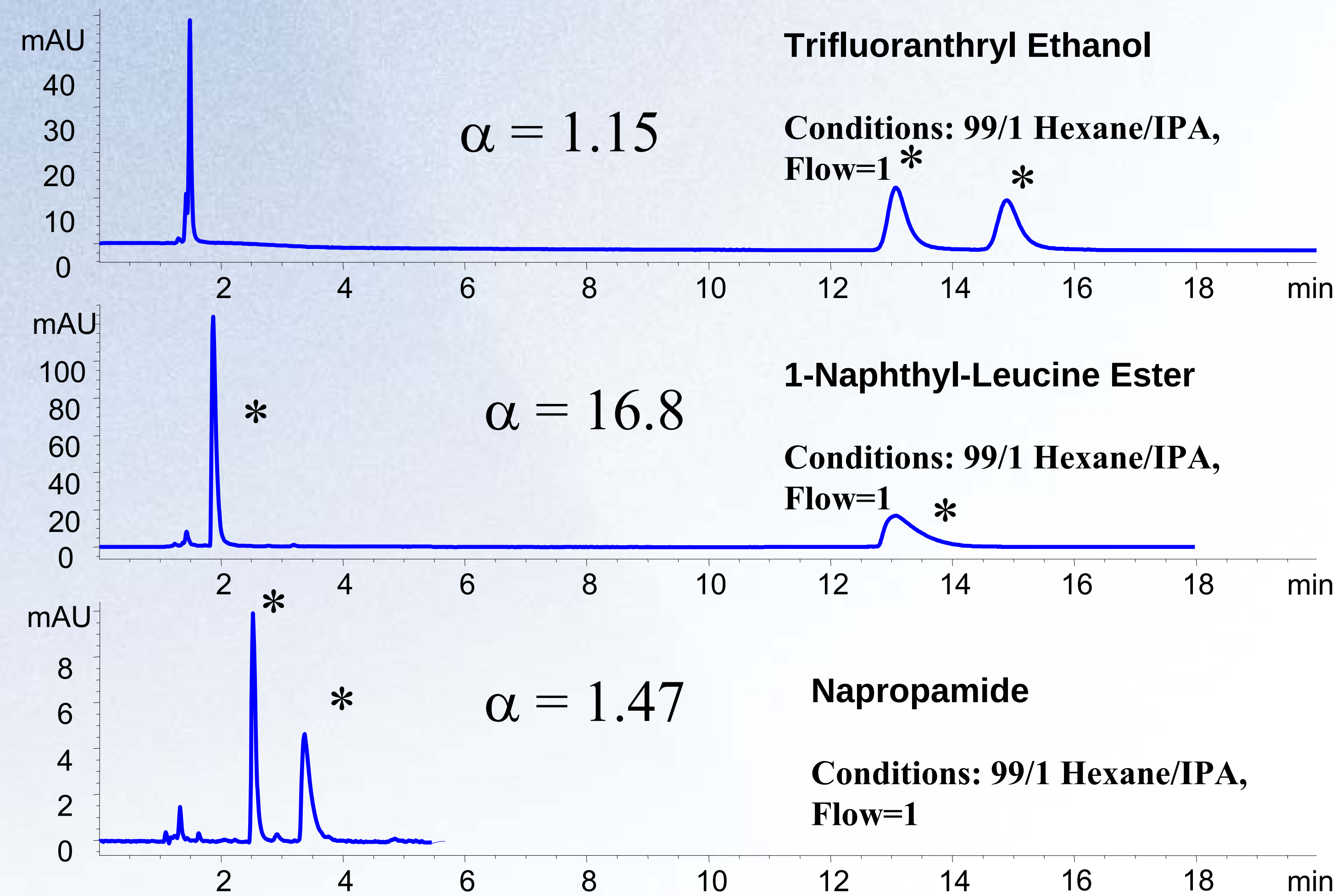
(S)-DNB-L-Phenylglycine
[(S)-PG]



(S)-N-[1-(1-naphthyl)ethyl]succinamic acid
[(S)-NESA]

1. Phase II SBIR (NIH)

Chiral Separation on Zr (S)-Leu (π-acceptor phase)



Chiral Separation on Zr (R)-PG (Another π-acceptor phase)

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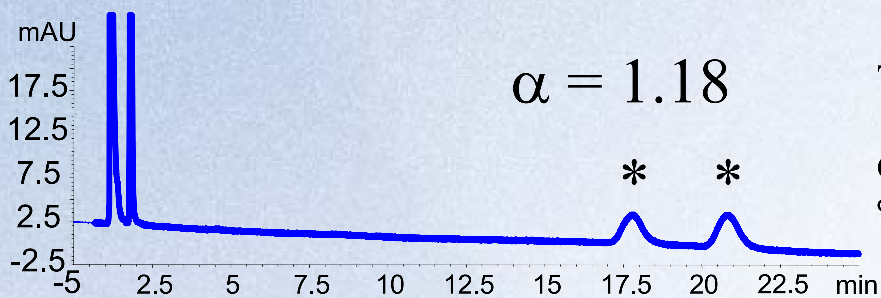
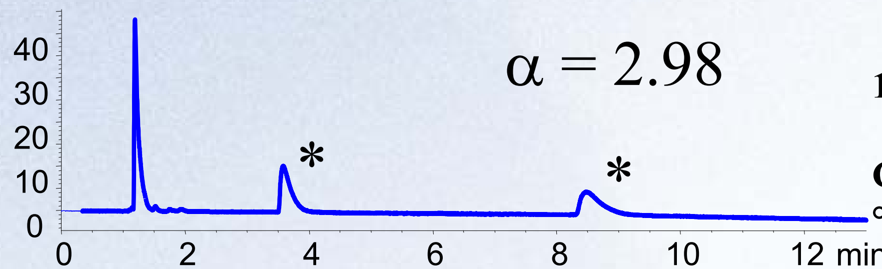


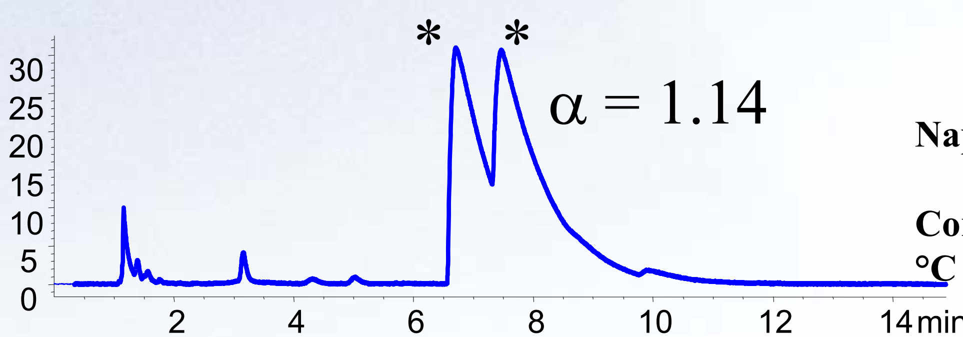
Trifluoranthryl Ethanol

Conditions: 99/1 Hexane/IPA, Flow=1, 30 °C



1-Naphthyl-Leucine Ester

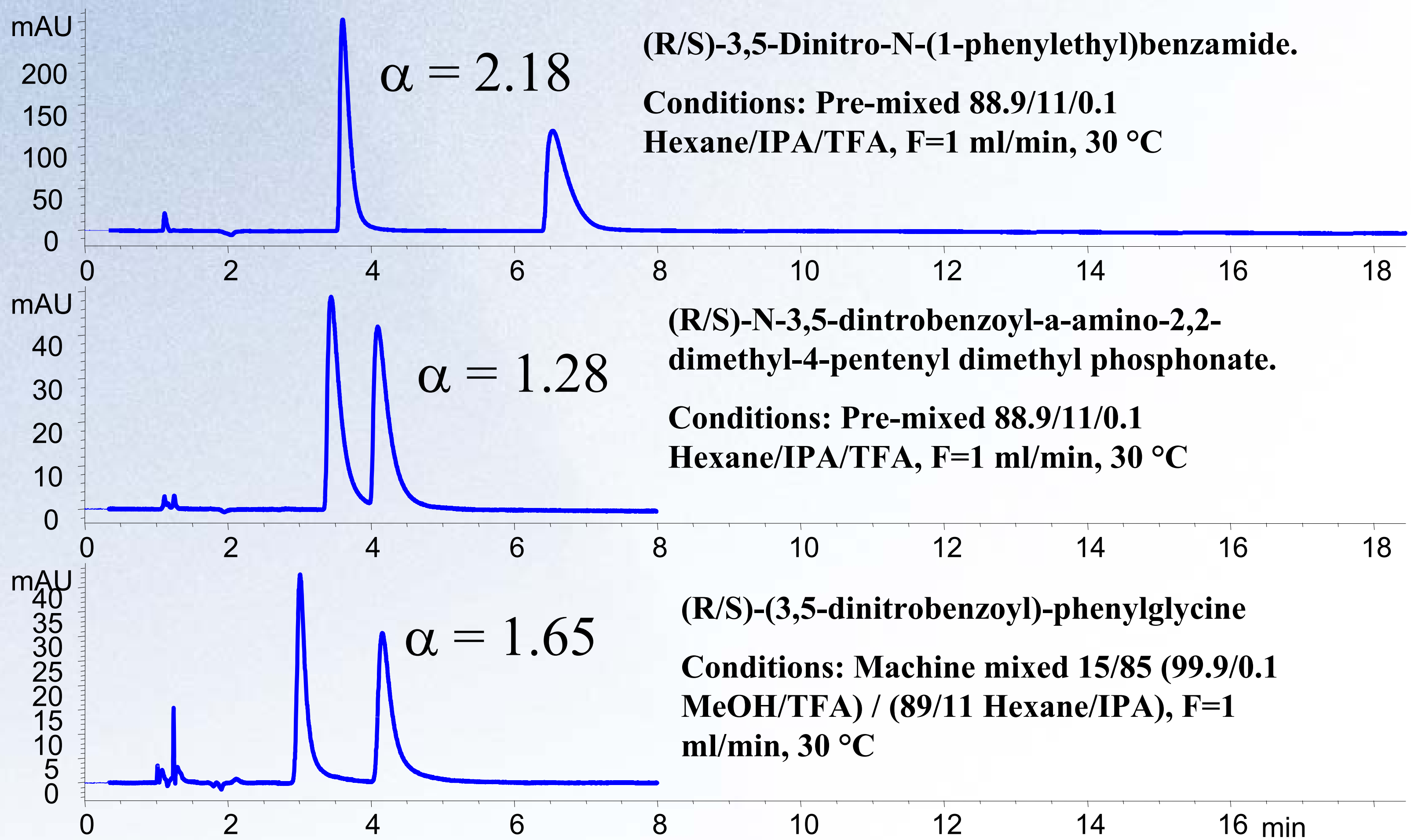
Conditions: 99/1 Hexane/IPA, Flow=1, 30 °C



Napropamide

Conditions: 99/1 Hexane/IPA, Flow=1, 30 °C

Chiral Separations on Zr (S)-NESA (π- donor phase)



(R/S)-3,5-Dinitro-N-(1-phenylethyl)benzamide.

Conditions: Pre-mixed 88.9/11/0.1 Hexane/IPA/TFA, F=1 ml/min, 30 °C

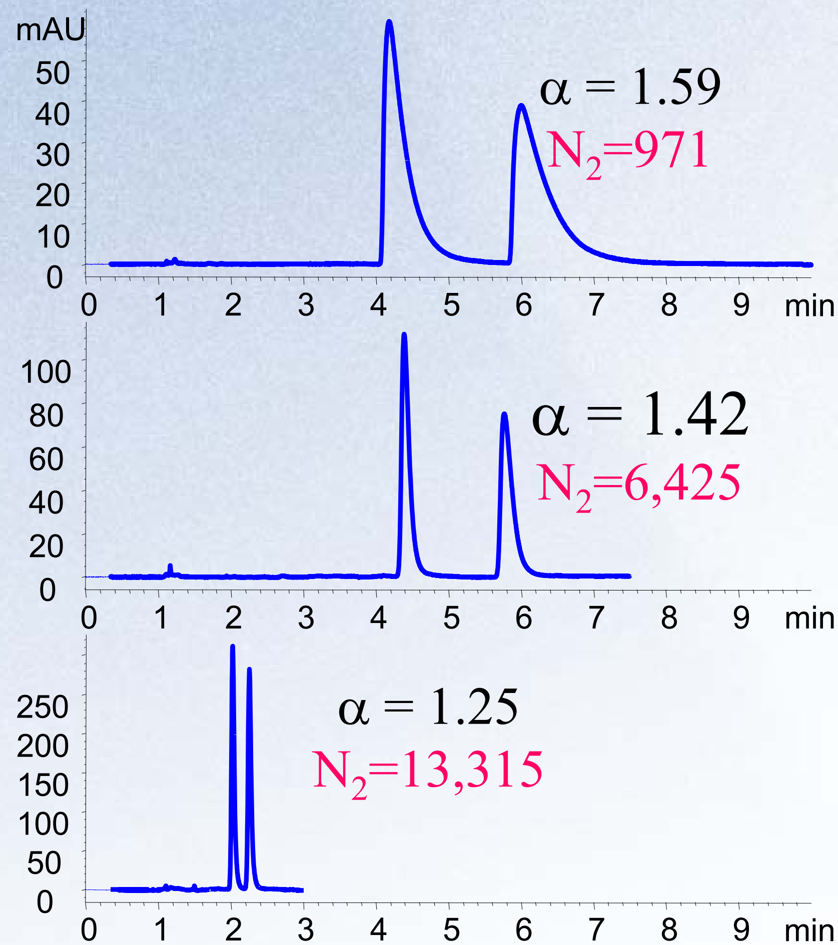
(R/S)-N-3,5-dintrobenzoyl-a-amino-2,2-dimethyl-4-pentenyl dimethyl phosphonate.

Conditions: Pre-mixed 88.9/11/0.1 Hexane/IPA/TFA, F=1 ml/min, 30 °C

(R/S)-(3,5-dinitrobenzoyl)-phenylglycine

Conditions: Machine mixed 15/85 (99.9/0.1 MeOH/TFA) / (89/11 Hexane/IPA), F=1 ml/min, 30 °C

Methanol Effect on Zr (S)-NESA



Sample: (R/S)-N-3,5-dintrobenzoyl-a-amino-2,2-dimethyl-4-pentenyl dimethyl phosphonate

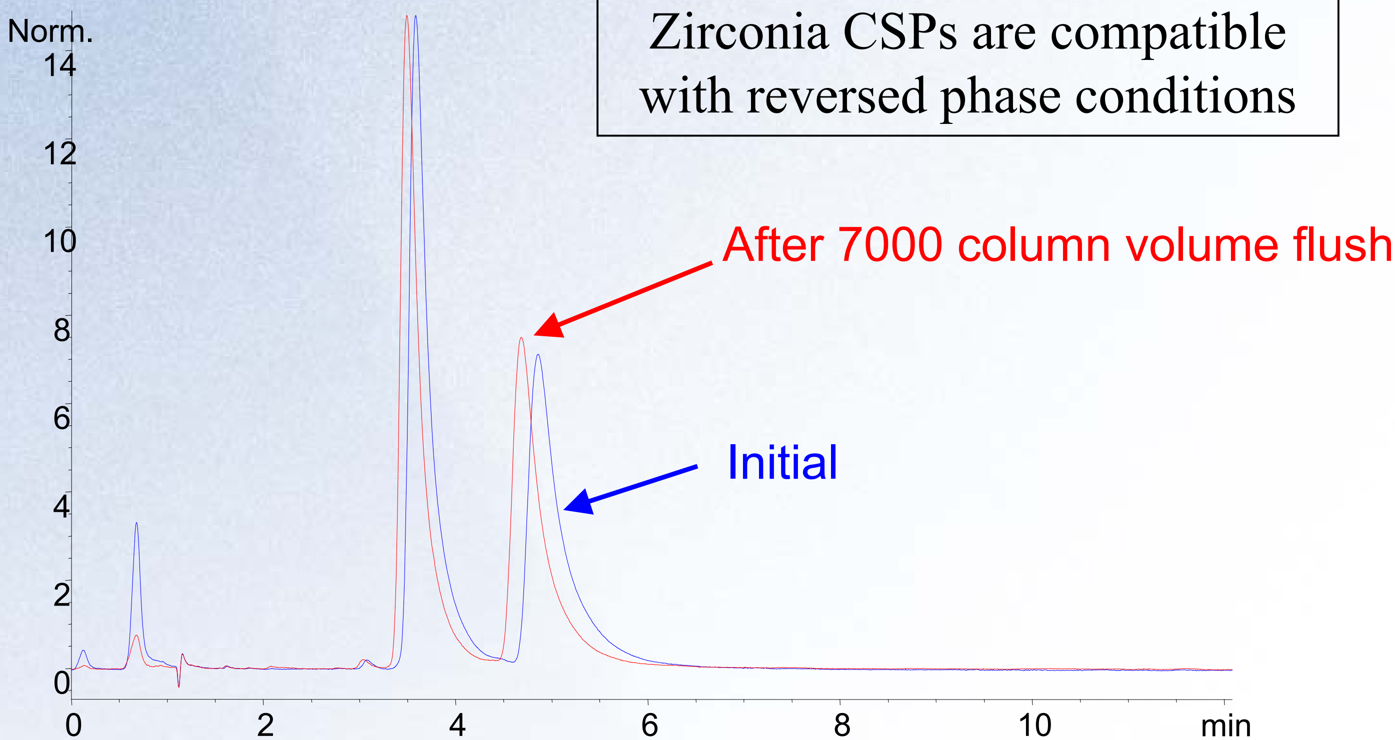
Conditions: 89/11 Hexane/IPA, F=1 ml/min, 30 °C.

Conditions: 90 / 2 / 8 (99/1 Hexane/IPA) / MeOH / (70/30 Hexane/IPA), F=1 ml/min, 30 °C

Conditions: 80 / 10 / 10 (99/1 Hexane/IPA) / MeOH / (70/30 Hexane/IPA), F=1 ml/min, 30 °C

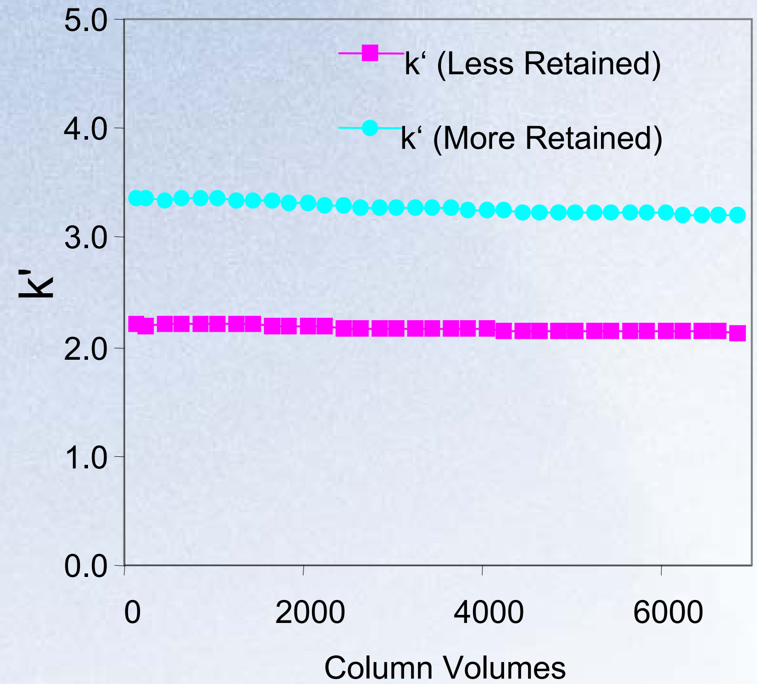
Stability of Zr-(S)-NESA at pH 2

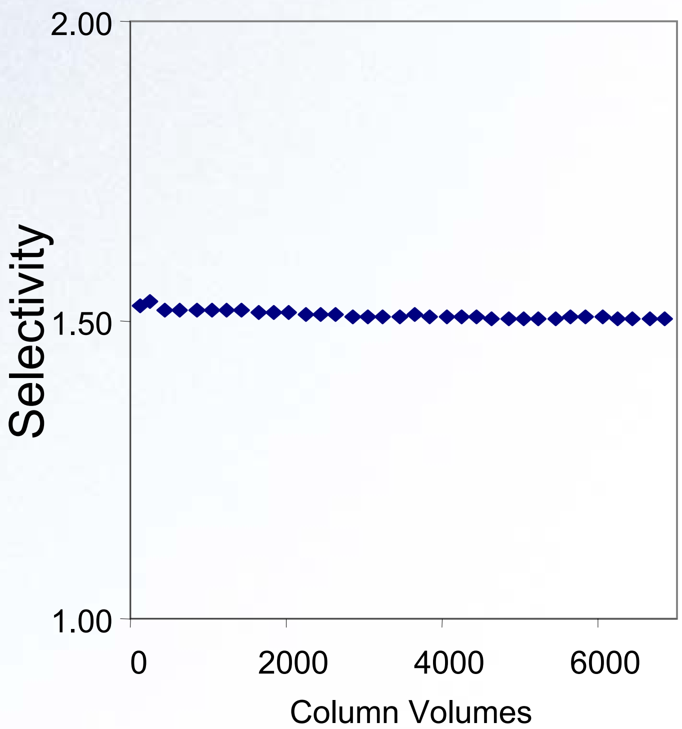
Zirconia CSPs are compatible with reversed phase conditions



Column ID: ZrCSP051605C, Mobile phase: 15/85 ACN/0.01 mM TFA pH 2, Temperature: 30 °C. Injection volume: 5 ul, Wavelength: 254 nm. Probe solutes:(R/S)-3,5-dinitro-N-(1-phenylethyl)benzamide.

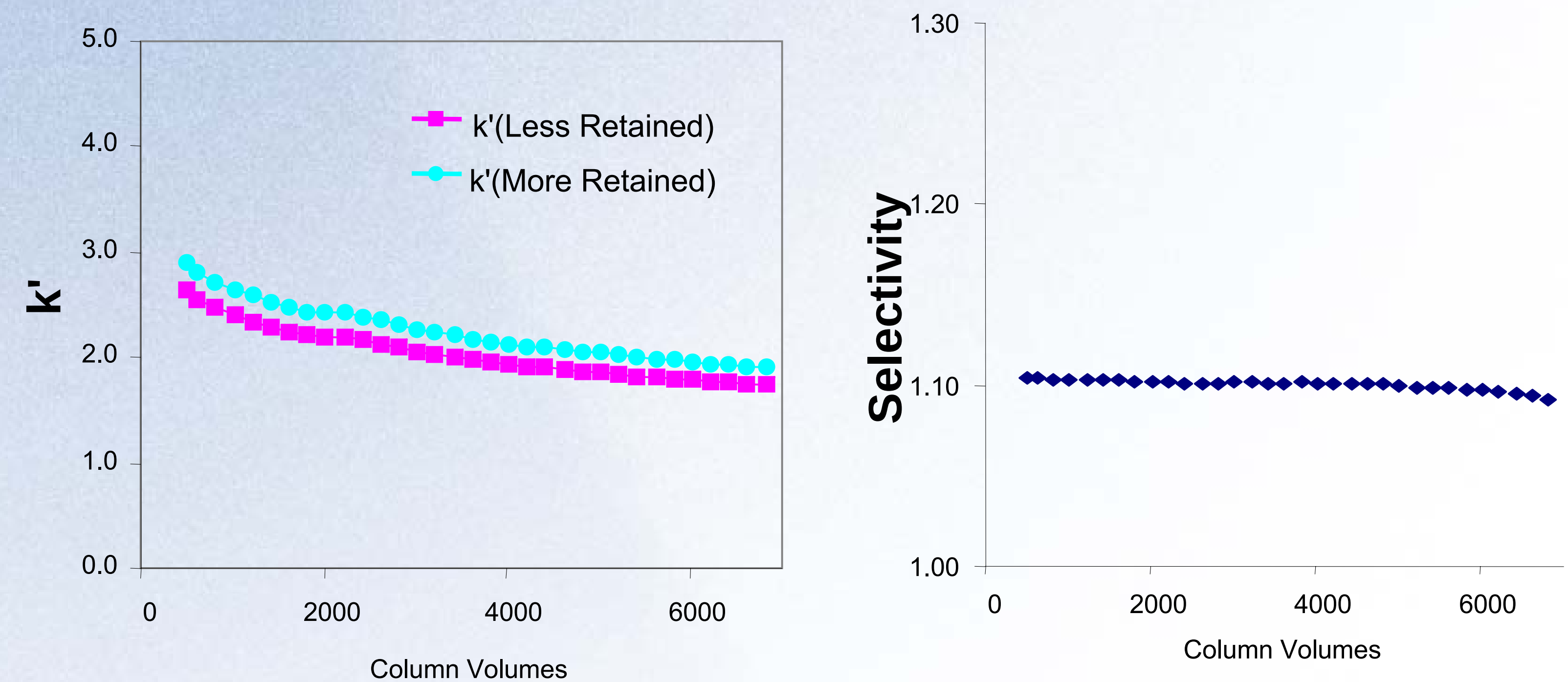
Stability of Zr-(S)-NESA at pH 2





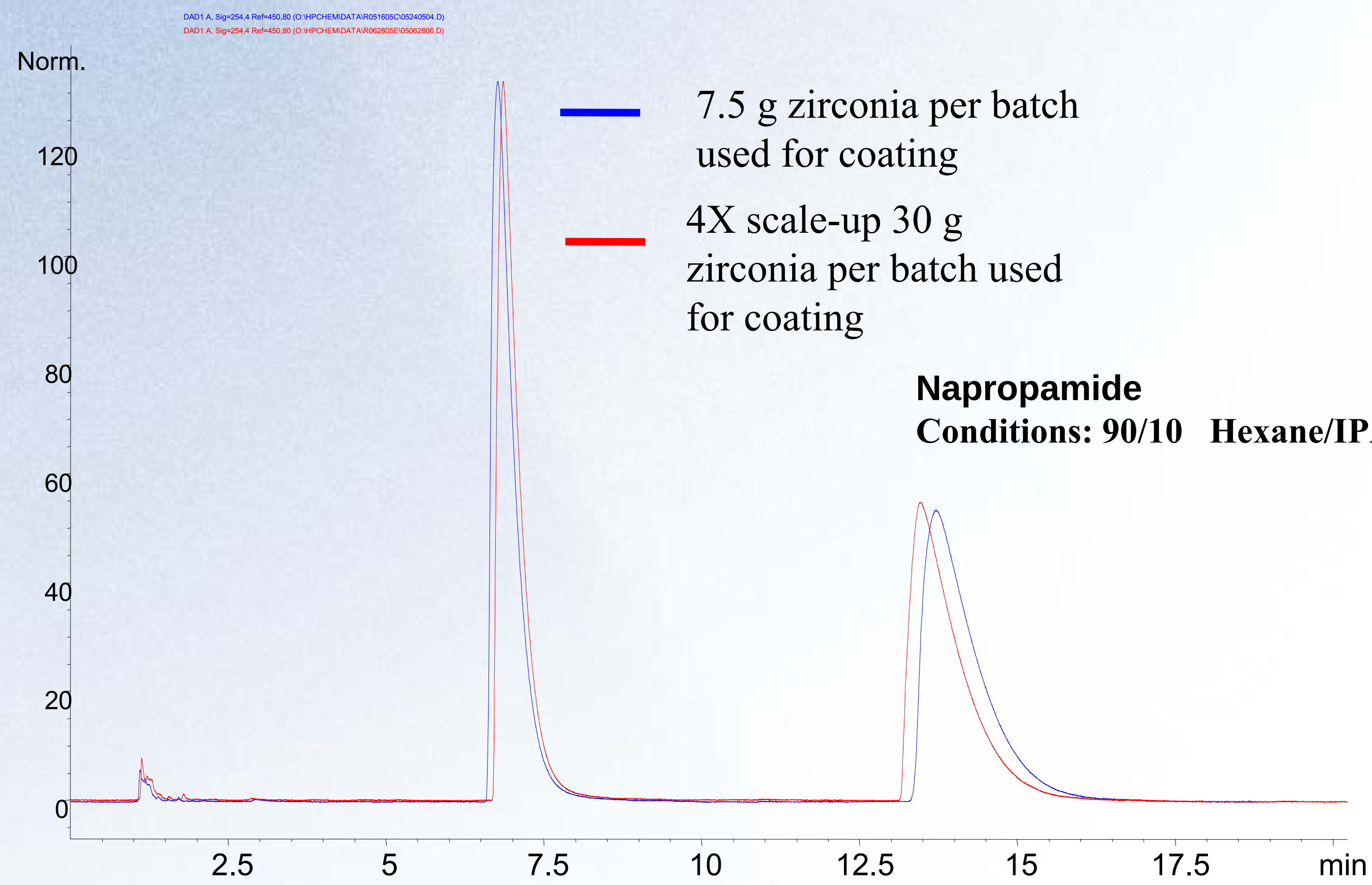
Column ID: ZrCSP051605C, Mobile phase: 15/85 ACN/0.01 mM TFA pH 2, Temperature: 30 °C. Injection volume: 5 ul, Wavelength: 254 nm. Probe solutes:(R/S)-3,5-dinitro-N-(1-phenylethyl)benzamide.

Stability of Zr-(S)-DNB-Leu at pH 8



Column ID: ZrCSP032805A, Mobile phase: 15/85 ACN/5 mM ammonium hydrogencarbonate pH 8.0, Temperature: 30 ^{o}C. Injection volume: 5 ul, Wavelength: 254 nm. Probe solutes:(R/S)-2, 2, 2-trifluoro-1-(9-anthryl)ethanol

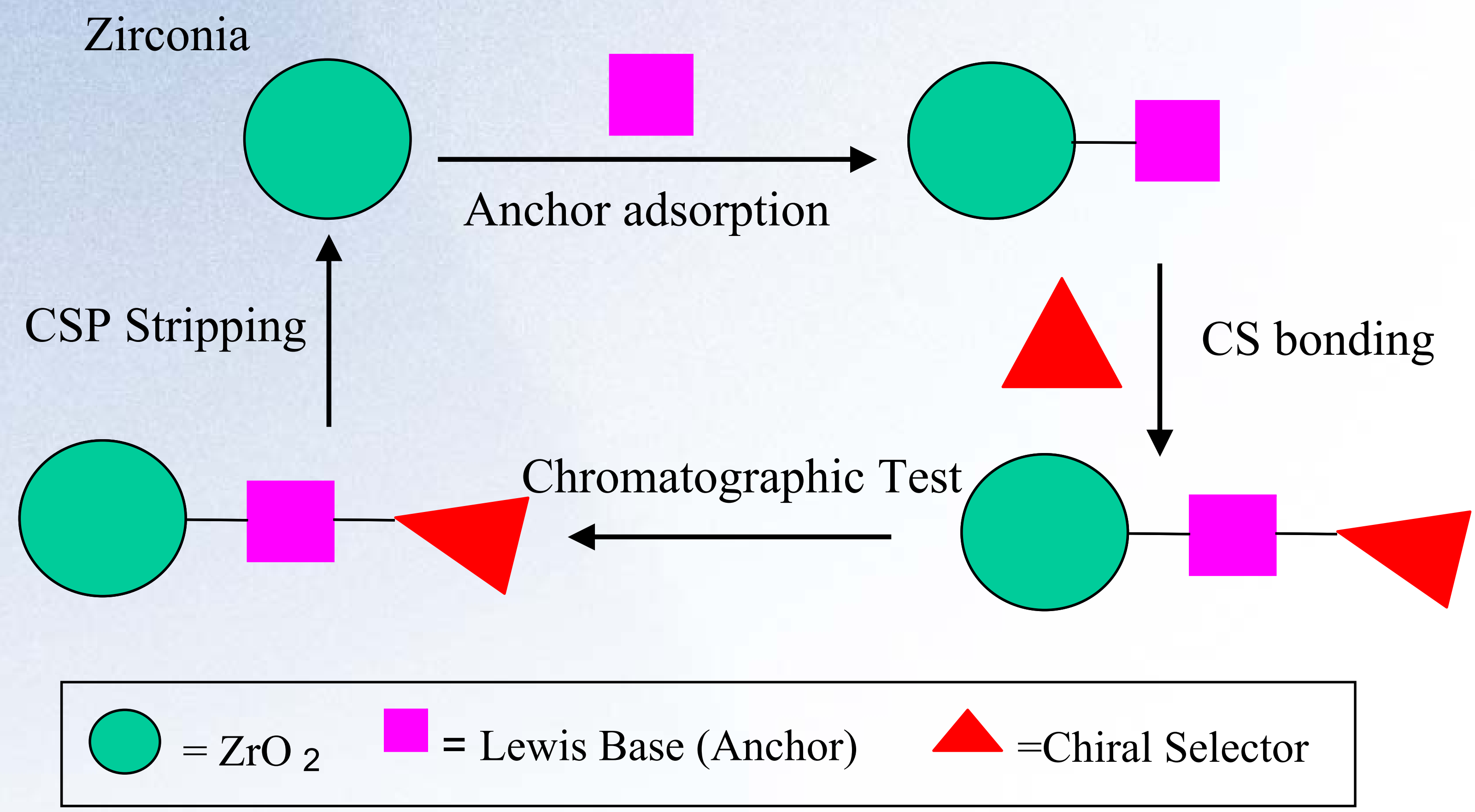
Production Scale-up of Zr (S)-NESA



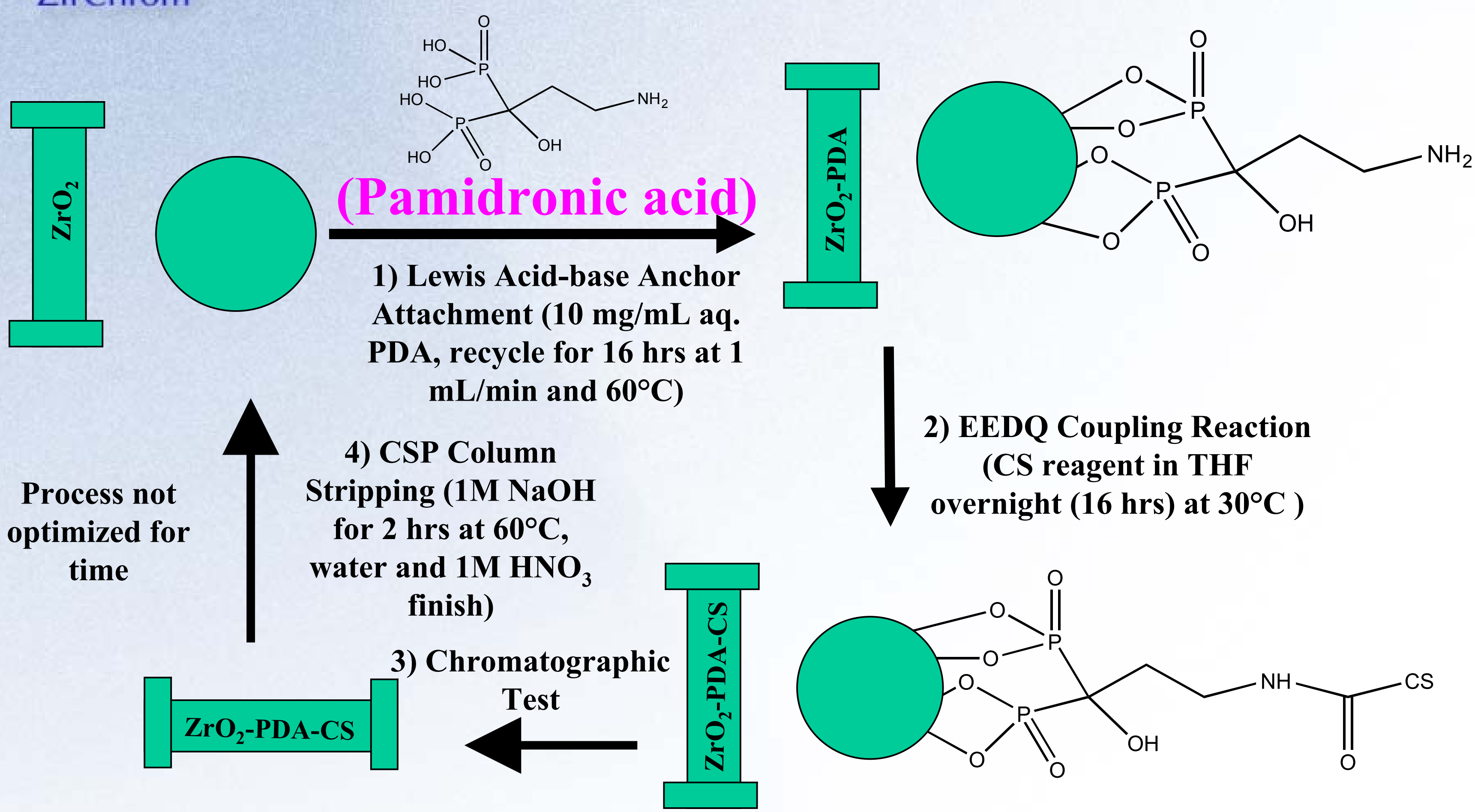
Napropamide

Conditions: 90/10 Hexane/IPA, Flow=1

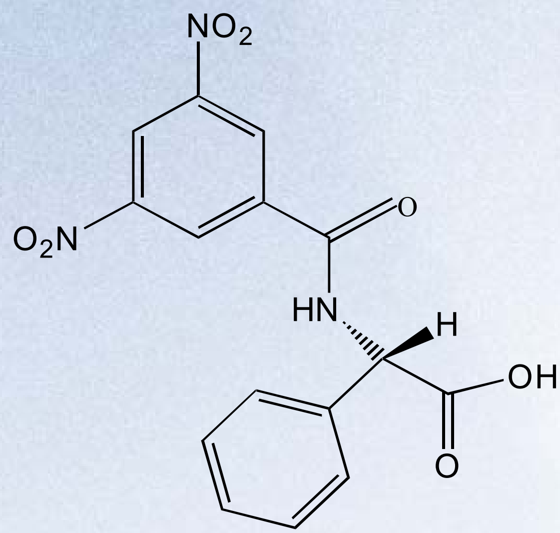
2-Step Synthesis of Zirconia CSPs for Chiral Selector Screening



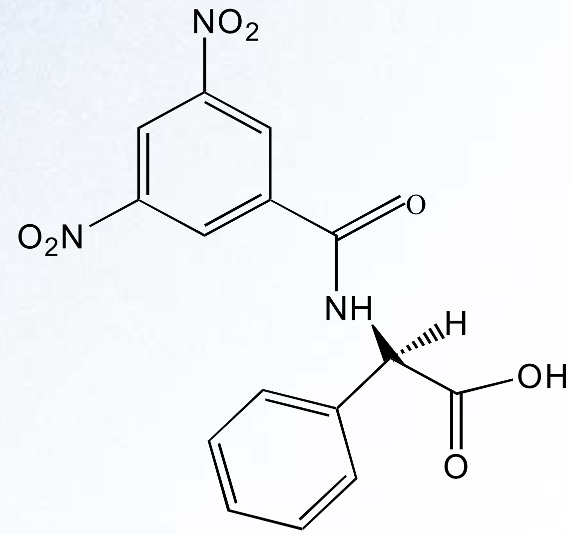
2-Step Online Zirconia CSP Synthesis for Chiral Screening



Changing Chiral Selectors



(S)-DNB-L-Phenylglycine (S-PG)



(R)-DNB-L-Phenylglycine (R-PG)

Pamidronic acid derivatives

Stripping Experiment: (S)-PG CSP

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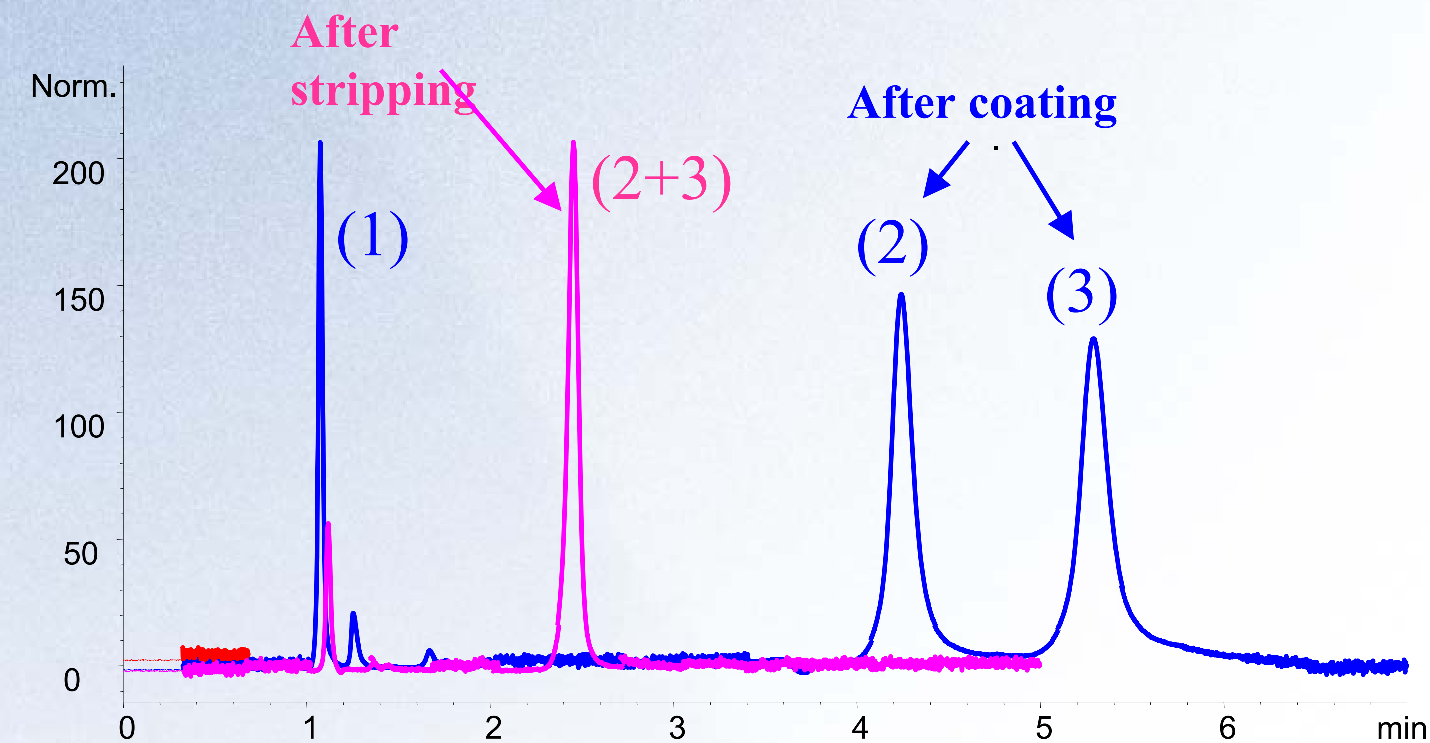
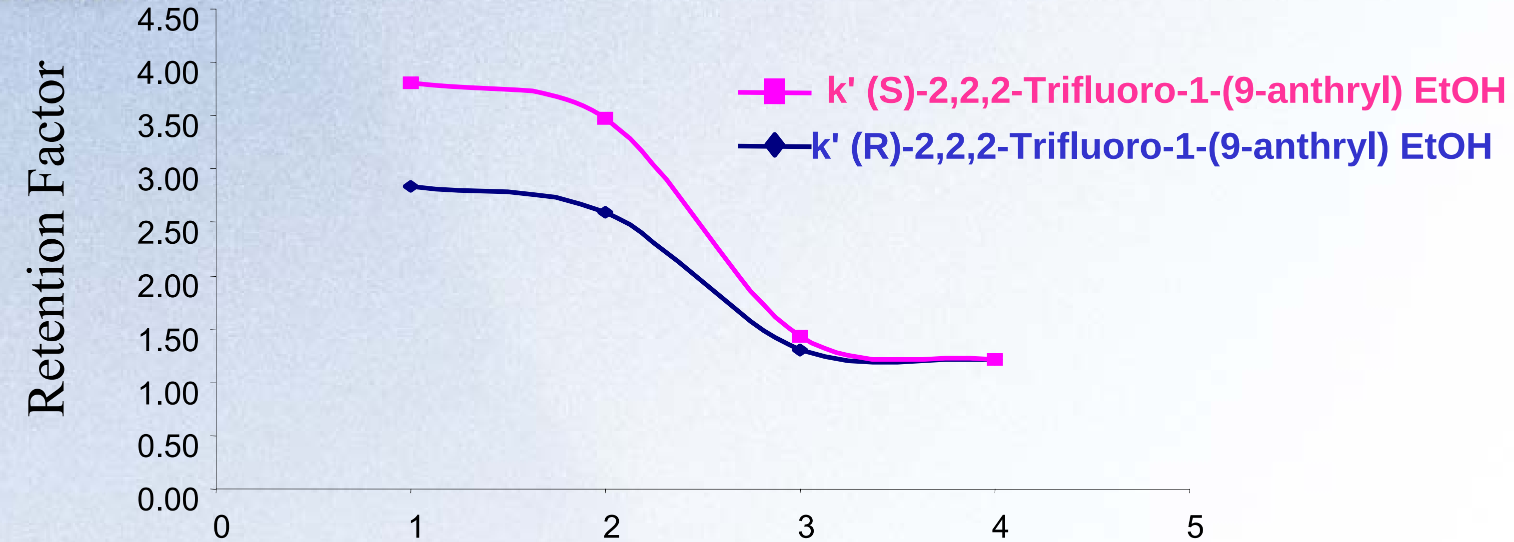


Pre-mixed 98/0.5/1.5 Hexane/TFA/IPA, flow rate=1 ml/min, ambient temperature, 254 nm, Column: ZirChrom PDA-(S)-PG, S/N SPG122005D (100 × 4.6 mm, 3 µm, Running HPLC coated on PHASE110805A, batch#: 52-132). Solute: (1) 1,3,5-Tri-t-butyl-benzene, (2) (S)-2,2,2-Trifluoro-1-(9-anthryl) ethanol , (3) (R)-2,2,2-Trifluoro-1-(9-anthryl) ethanol 5 µl injection.

Changes During (S)-PG Stripping



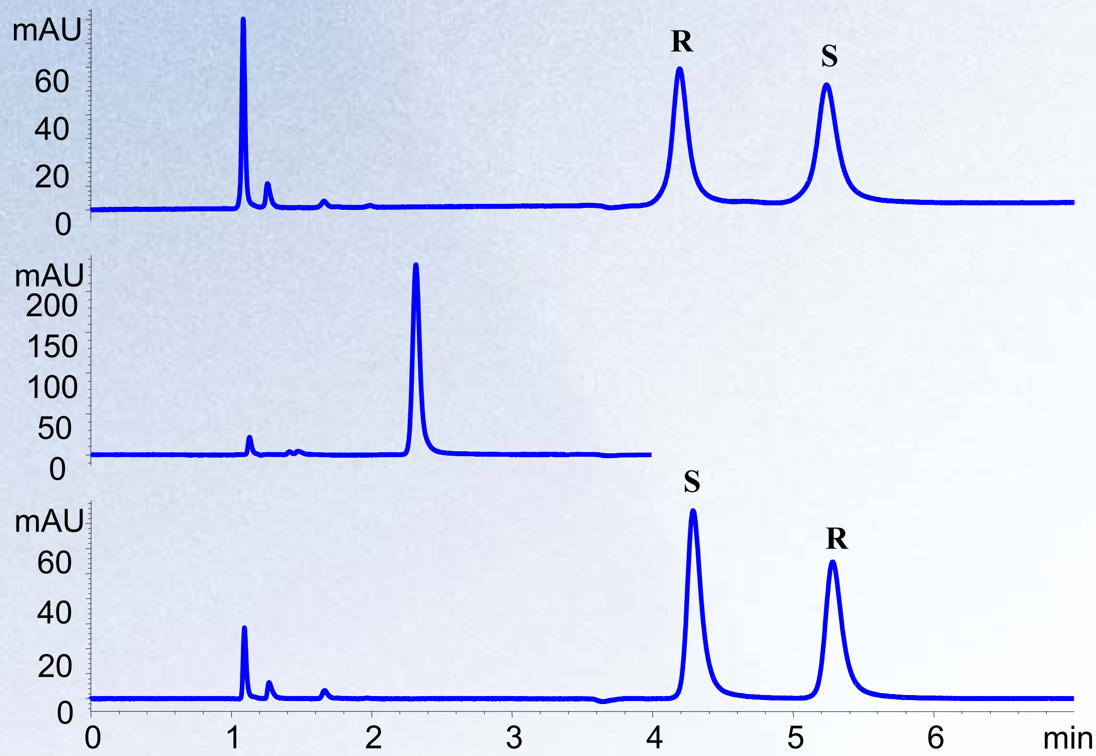
1- Original column.

2- Column flushed with 15/85 ACN/pH 12 NH_4OH for 10 column volumes, then 10 column volumes of water, 10 column volumes of 1.0 M nitric acid, and 10 column volumes of water.

3- Column then flushed with 50 column volumes of 20/80 ACN/ 1 M NaOH, then 10 column volumes of water, 10 column volumes of 1 M nitric acid and 10 column volumes of water.

4- Column then flushed with 20/80 ACN/ 1 M NaOH for 50 column volumes at 60 ^{o}C, then flushed with 10 column volumes of water, 10 column volumes of 1 M nitric acid, and 10 column volumes of water.

Changing (S) to (R)-Phenylglycine CSP on Same Zr Column



2-Step Load (S)-PG CS

k'(less) = 2.84

k'(more) = 3.81

α = 1.34

Strip (S)-PG CS
No separation.

2-Step Load (R)-PG CS

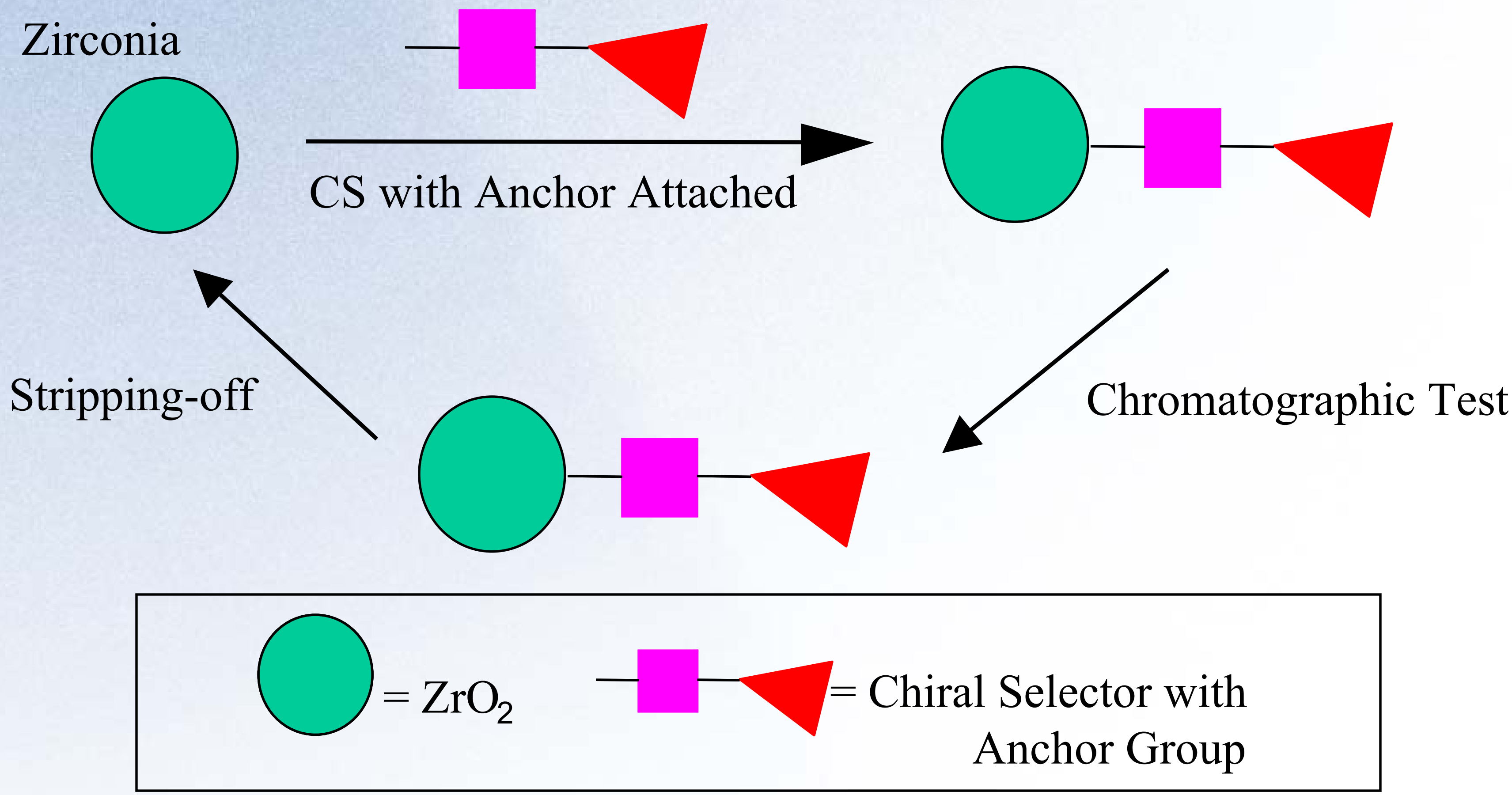
k'(less) = 2.92

k'(more) = 3.83

α = 1.34

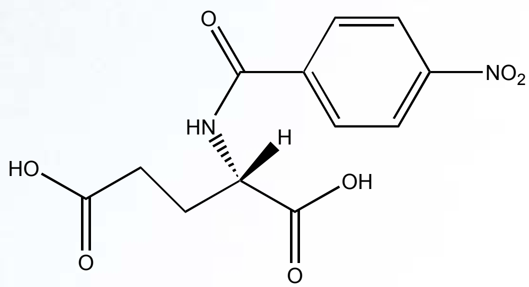
Pre-mixed 98/0.5/1.5 Hexane/TFA/IPA, F=1 ml/min, rm °C, 254 nm, Column: ZirChrom PDA-(S)-PG, S/N SPG122005D and ZirChrom PDA-(R)-PG, S/N RPG020806A (100 × 4.6 mm, 3 µm, Running HPLC coated on PHASE110805A, batch#: 52-132). Solute: 1,3,5-Tri-t-butyl-benzene, (R orS)-2,2,2-Trifluoro-1-(9-anthryl) EtOH. 5 µl injection.

1-Step Synthesis of Zirconia CSPs for Fast Chiral Screening



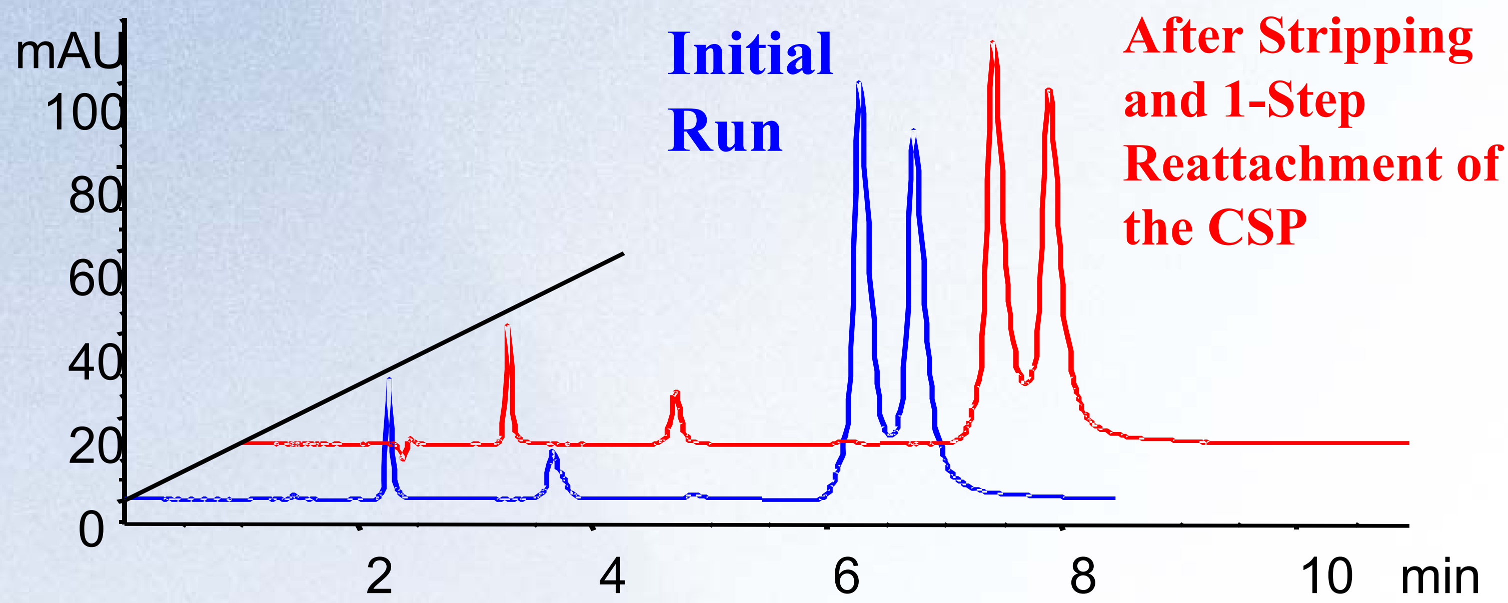
Example 1-Step Attachment and Detachment Cycle

- Pass a solution of 20 mM N-(4-nitrobenzoyl)-L-glutamic acid (CSP) in tetrahydrofuran for 10 minutes at a column temperature of 60°C and a flow rate of 1 mL/min .
- Flush column with 100% THF for 10 minutes at 2 mL/min at ambient temperature.
- Separate a racemic solution of (±)-2,2,2-trifluoro-1-(9-anthyl)ethanol.
- Strip the CSP by flushing the column with a 50 mM solution of tetramethylammonium hydroxide solution (pH 12) for 20 minutes at 60°C using a flow rate of 1 mL/min.
- Repeat procedure using the same CSP



N-(4-nitrobenzoyl)-L-glutamic acid

Glutamic Acid Proof of Concept



Comparison between the initial and final separation of (±)-2,2,2-trifluoro-1-(9-anthyl)ethanol leucine ester during a single CSP screening cycle. Chromatographic conditions: mobile phase: 99/1 hexane/IPA; flow rate: 1 ml/min; temperature: 30 ºC, solute concentration = 1mg/mL, 5 µL injection.

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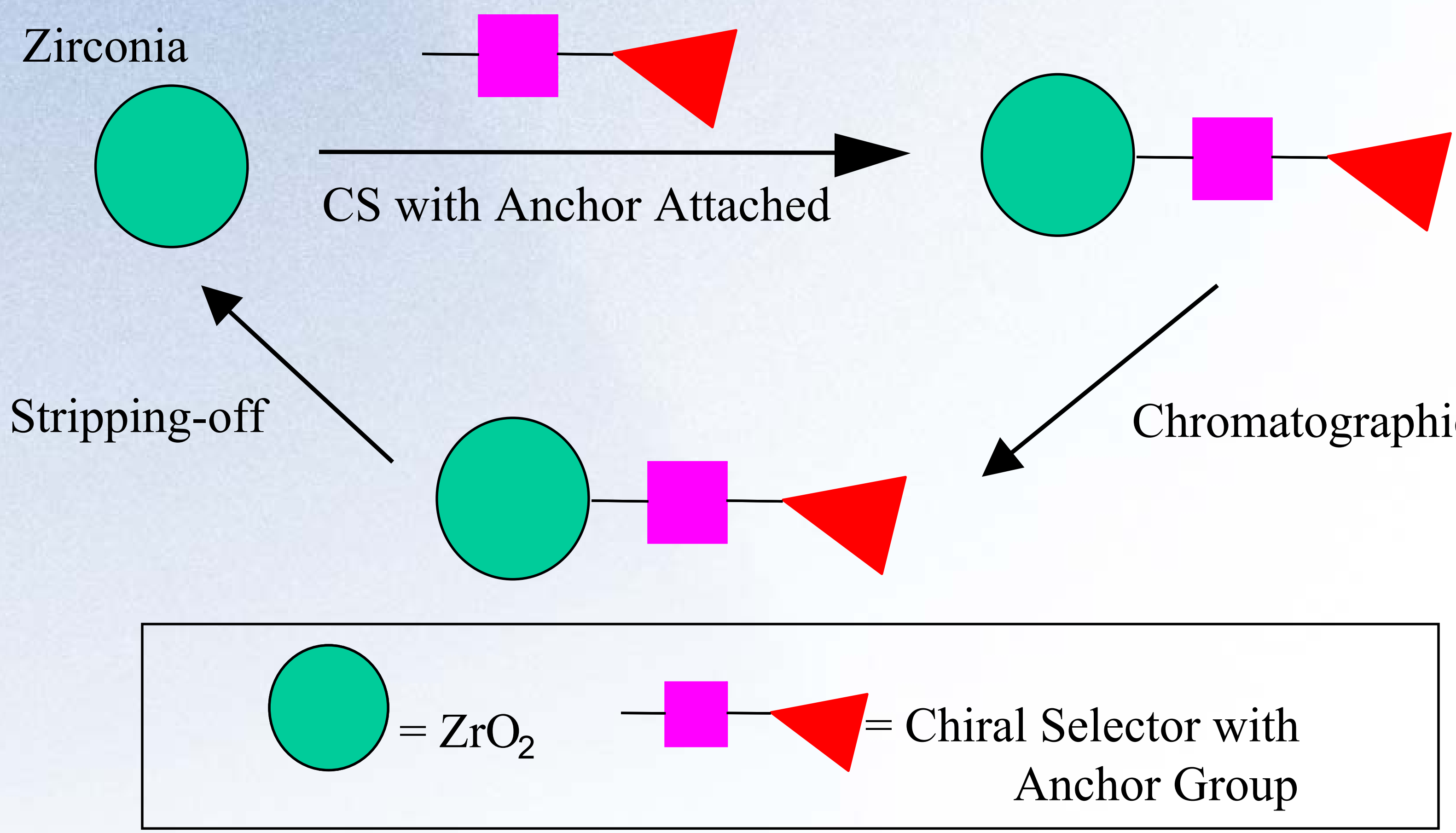
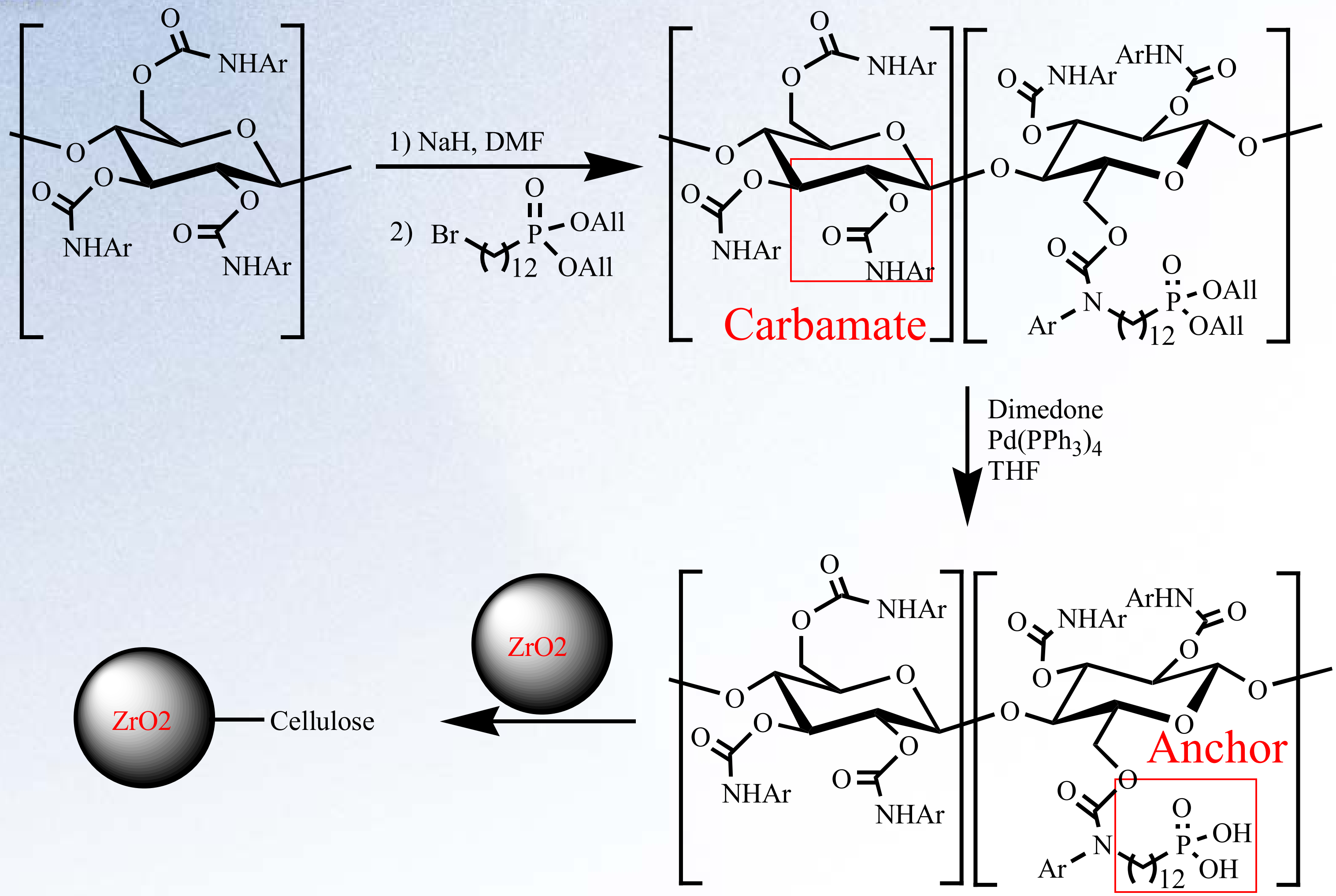
Development of a New Class of Regenerable Cellulosic Coated Zirconia Stationary Phases

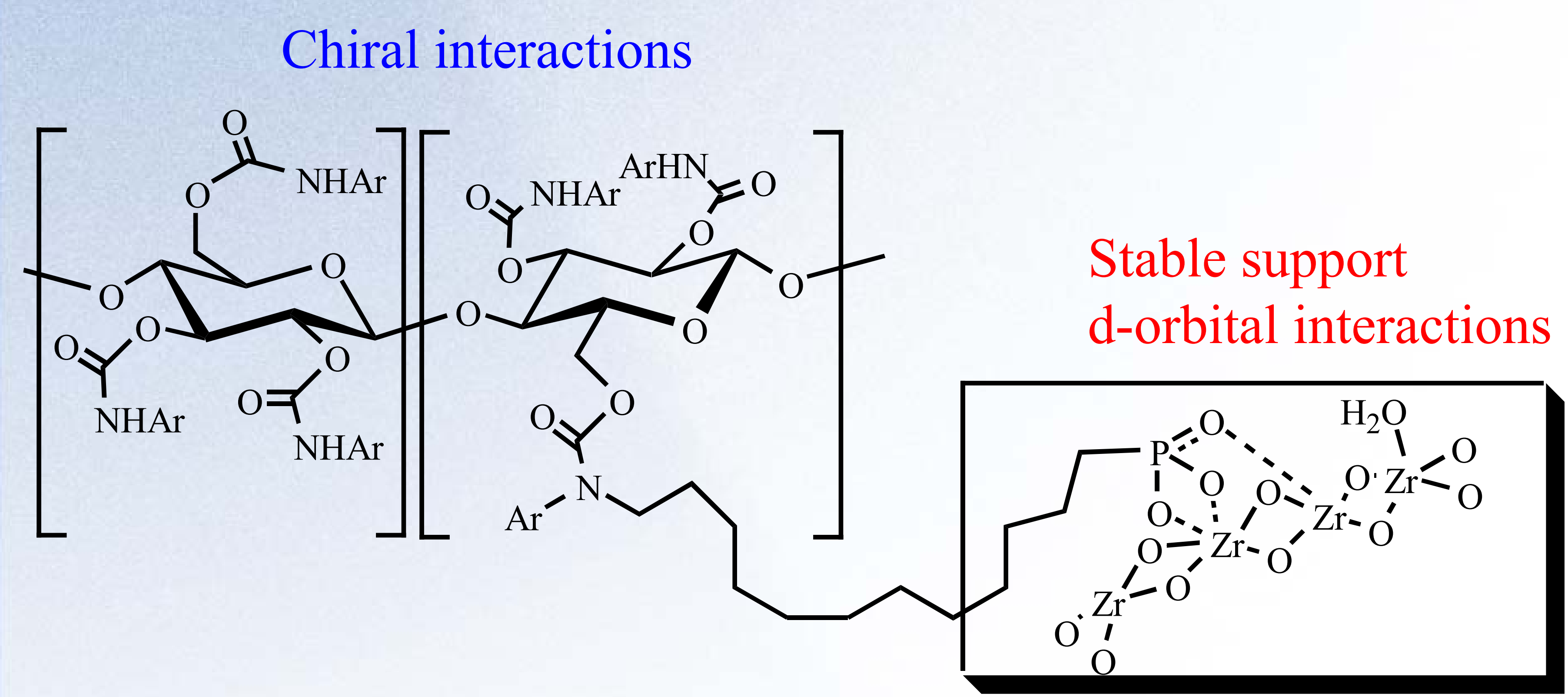
1-Step Synthesis of Zirconia CSPs for Fast Chiral Screening



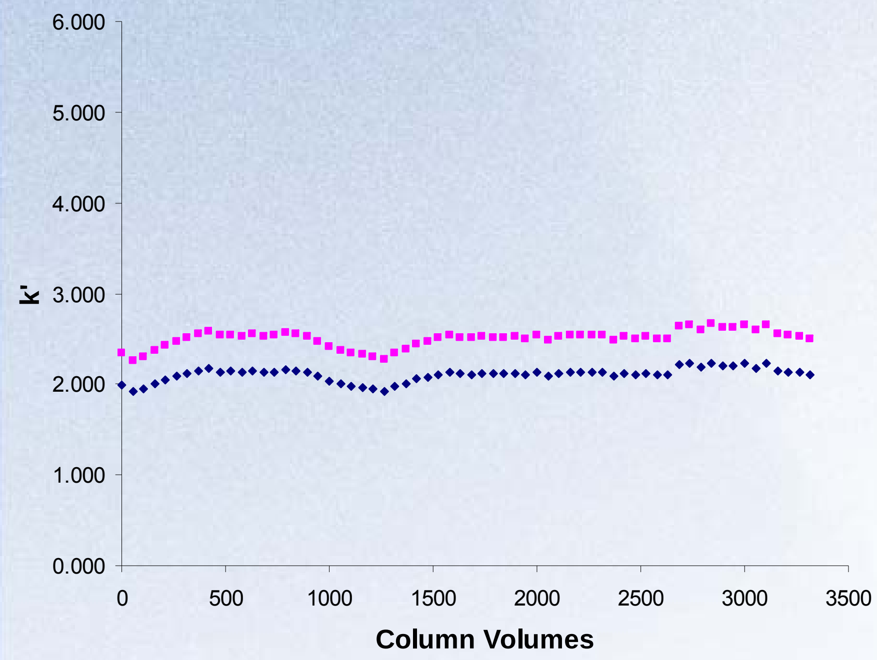
Phosphonate Modified Cellulose Based CSP on Zirconia

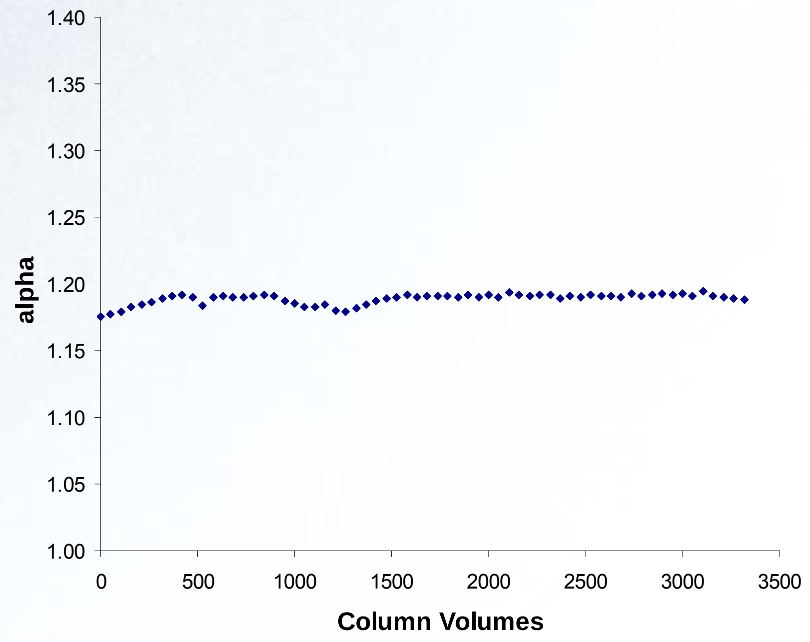


Cellulose Surface Chemistry



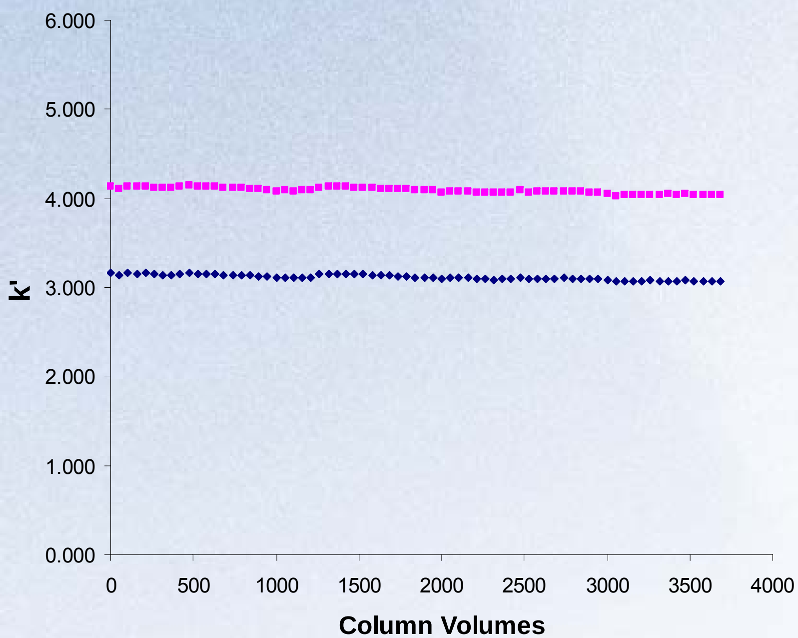
Cellulose – pH 2 Stability

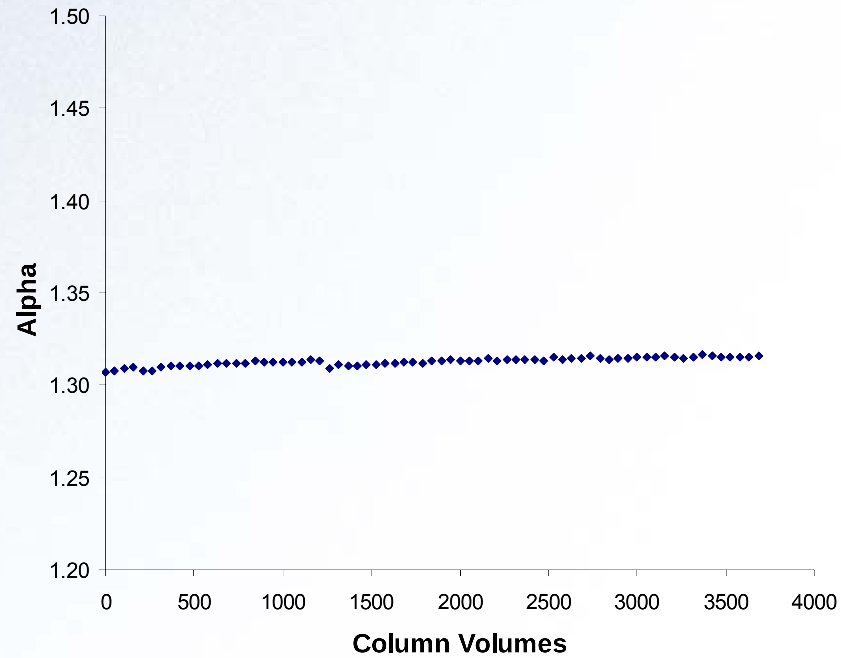




Column ID: R020907W, Mobile phase: 20/80 ACN/0.01 M TFA pH 2, Temperature: 30 °C. Injection volume: 5 ul, Wavelength: 254 nm. Probe solutes: Benzoin.

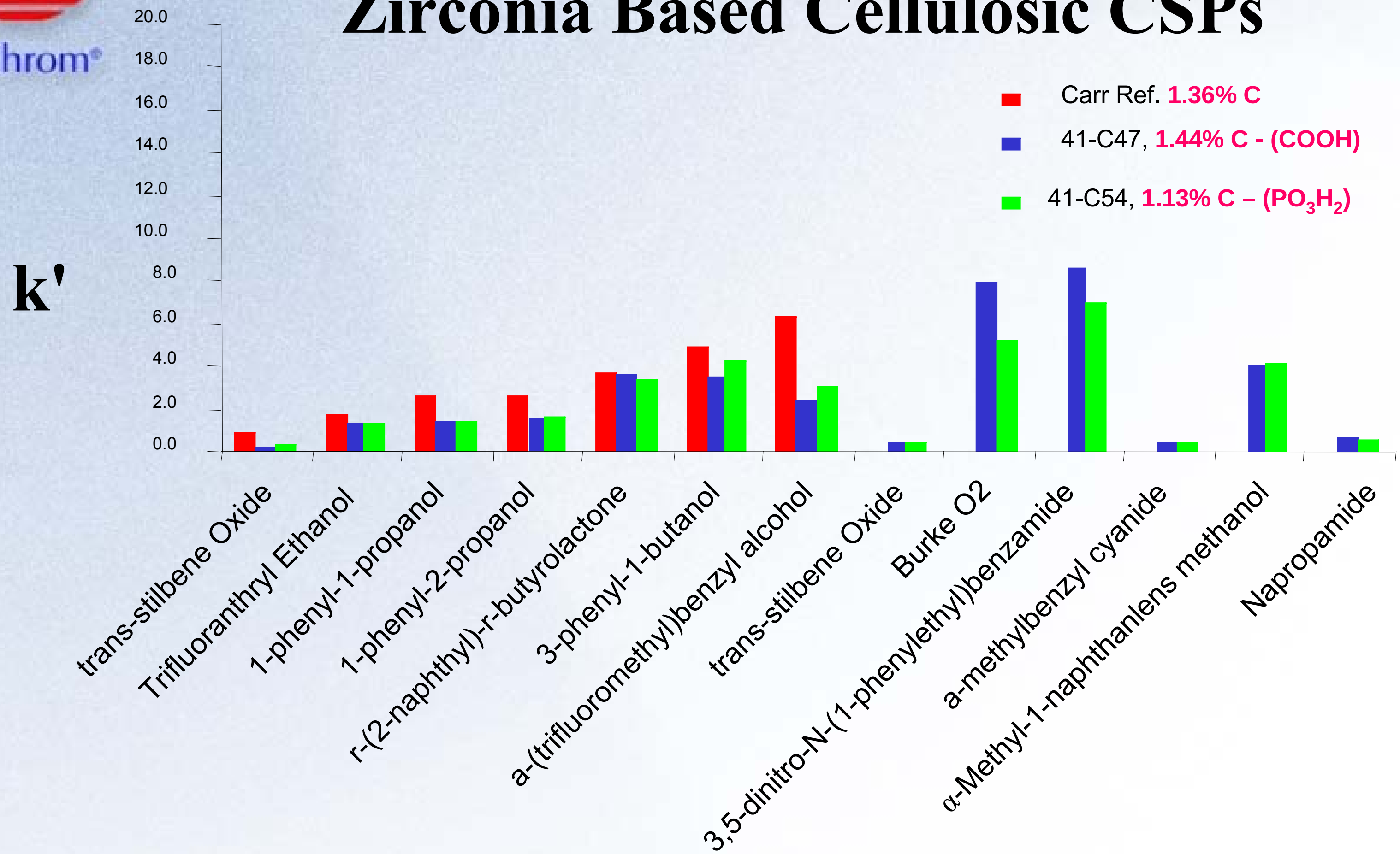
Cellulose – pH 8 Stability





Column ID: R031607W, Mobile phase: 35/65 ACN/5mM NH4HCO3
pH 8, Temperature: 30 °C. Injection volume: 5 ul, Wavelength: 254 nm.
Probe solute: (RS)-trifluroanthrylethanol.

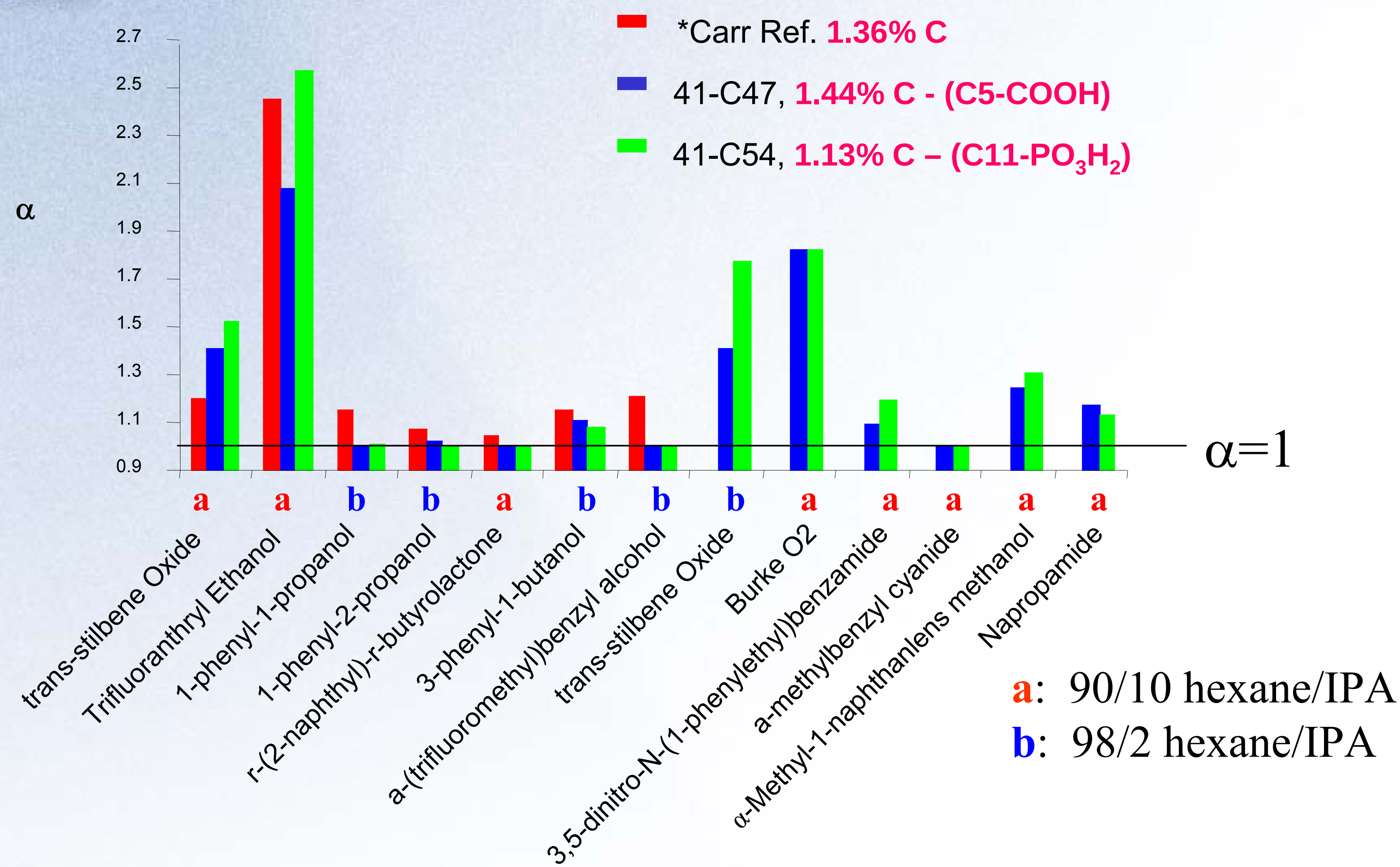
Retention Comparison of Hexanoic Acid Modified and Undecylphosphonic Acid Zirconia Based Cellulosic CSPs



(1) Generally k' on the new CSP is lower than previous published work (Carr, et al., Anal. Chem., 71 (1999) 3013-3021).

(2) Batch C54 is slightly less retentive than C47.

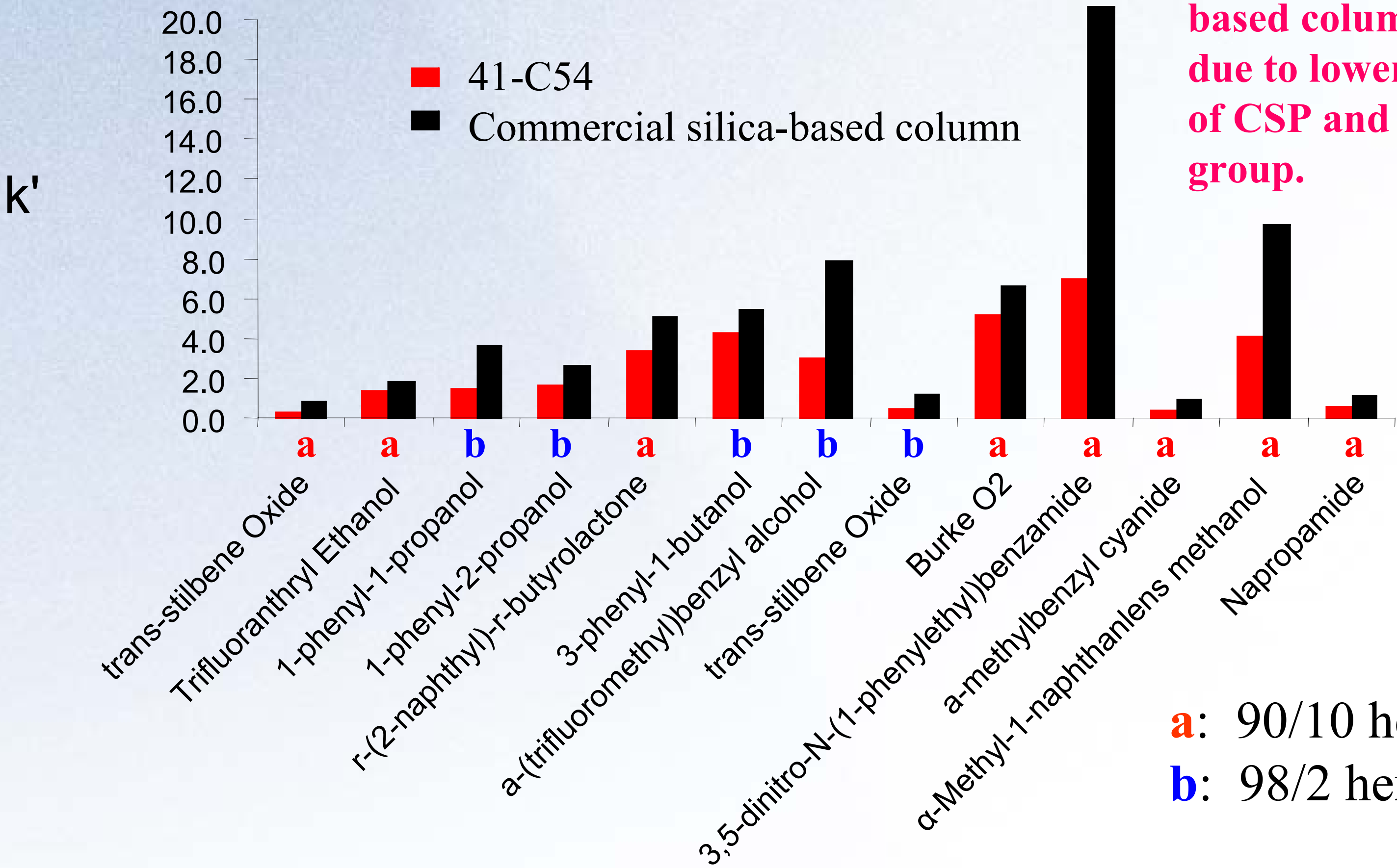
Selectivity Comparison of Previous and New Zirconia Based Cellulosic CSPs



*Data of α from Carr, et al., Anal. Chem., 71 (1999) 3013-3021

Retention Comparison Between Alkylphenyl Modified Cellulosic CSPs and Commercial Silica CSPs

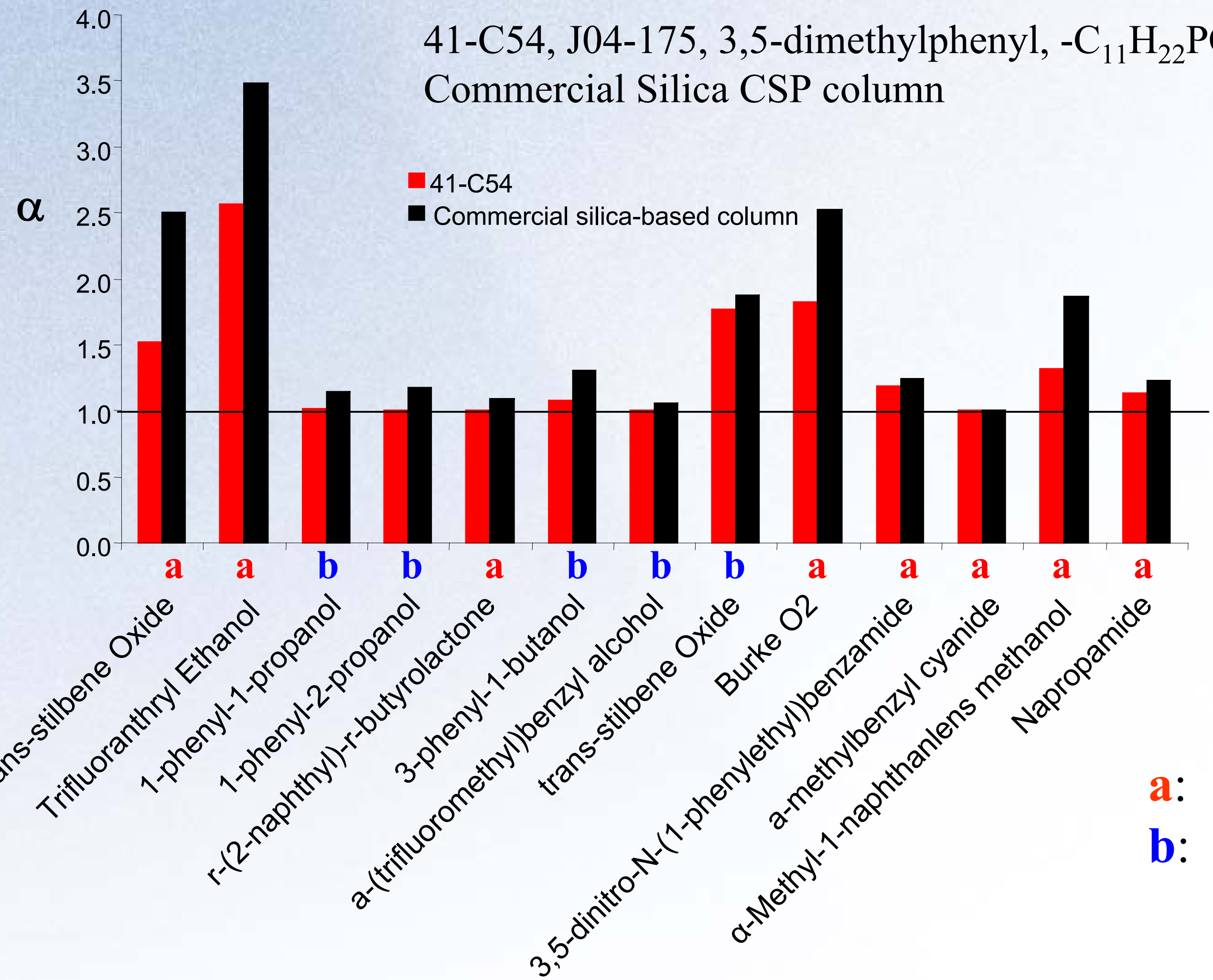
41-C54, J04-175, 3,5-dimethylphenyl, $-C_{11}H_{22}PO_3H$
Commercial Silica CSP column



New phase has less retention than commercial Silica-based column likely due to lower loading of CSP and anchor group.

a: 90/10 hexane/IPA
b: 98/2 hexane/IPA

Selectivity Comparison Between Dimethylphenyl Carbamate Modified Cellulosic CSPs and Commercial Silica CSPs



Undecylphenyl carbamate modified cellulosic CSP has good selectivity compared to a commercial silica column.

a: 90/10 hexane/IPA
b: 98/2 hexane/IPA

Effect of Ionic Strength on the Separation of Basic Chiral Phamaceuticals on Undecylphosphonic Acid Modified Cellulosic CSPs

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41-C54, J04-175, 3,5-dimethylphenyl, $-C_{11}H_{22}PO_3H$

Ion Strength/ Selectivity	Ammonium Acetate in IPA (mM)			
	200	100	80	40
Pindolol	2.87	2.10	1.79	1.30
Propranolol	1.55	1.53	1.35	1.10
Atenolol	1.26	1.12	1.09	1.00
Nadolol	1.00	1.00	1.00	1.00

Increasing ammonium acetate increases enantio-selectivity.

LC Conditions: Agilent 1100 with chemstation, flow rate 0.5 mL/min., UV 254, mobile phase = 100% IPA with specified concentration of ammonium acetate, Temperature = ambient, column dimension 10 cm x 4.6 mm id, 3 micron particles.

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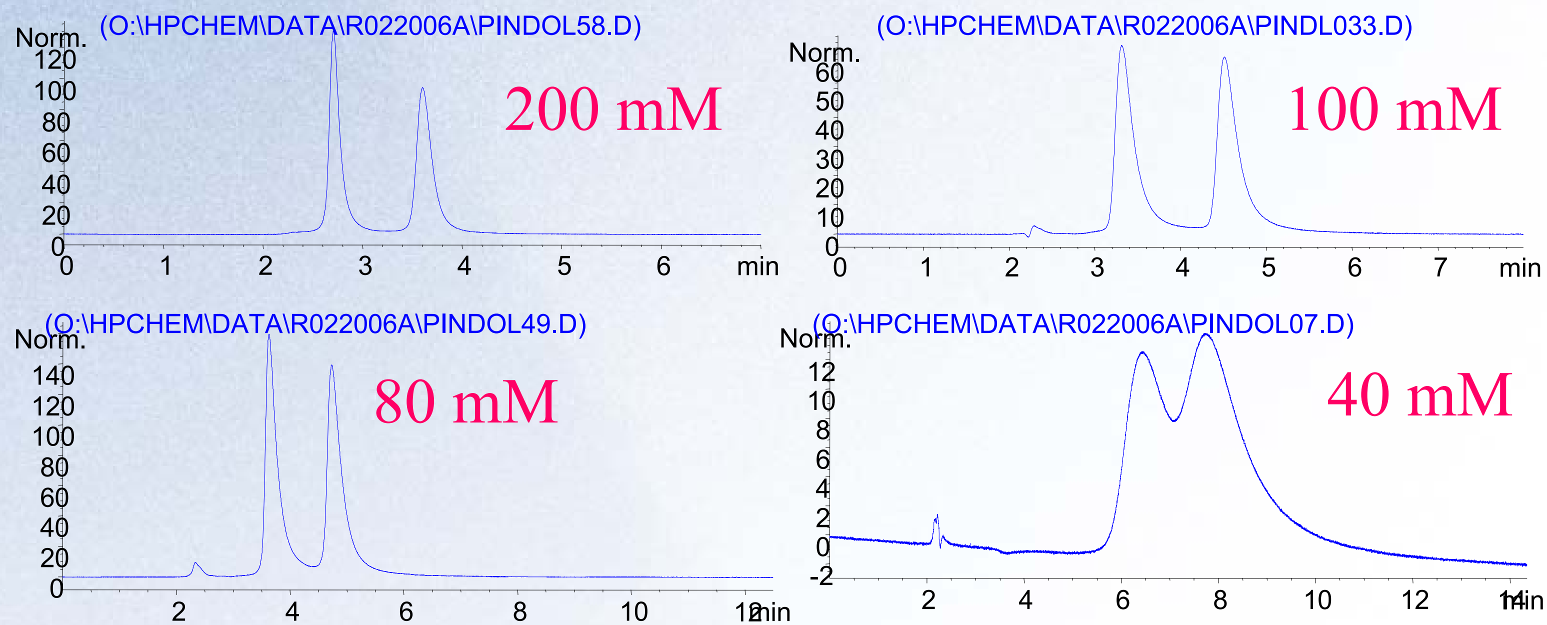
Effect of Ionic Strength on Undecylphosphonic Acid Modified Cellulosic CSPs



Increasing ammonium acetate increases the selectivity and decreases retention and improves peak shape for Pindolol. This is likely due to supression of cation-exchange retention mechanism that occurs for *basic molecules.*

Comparison of Silica and Zirconia Cellulosic Phases

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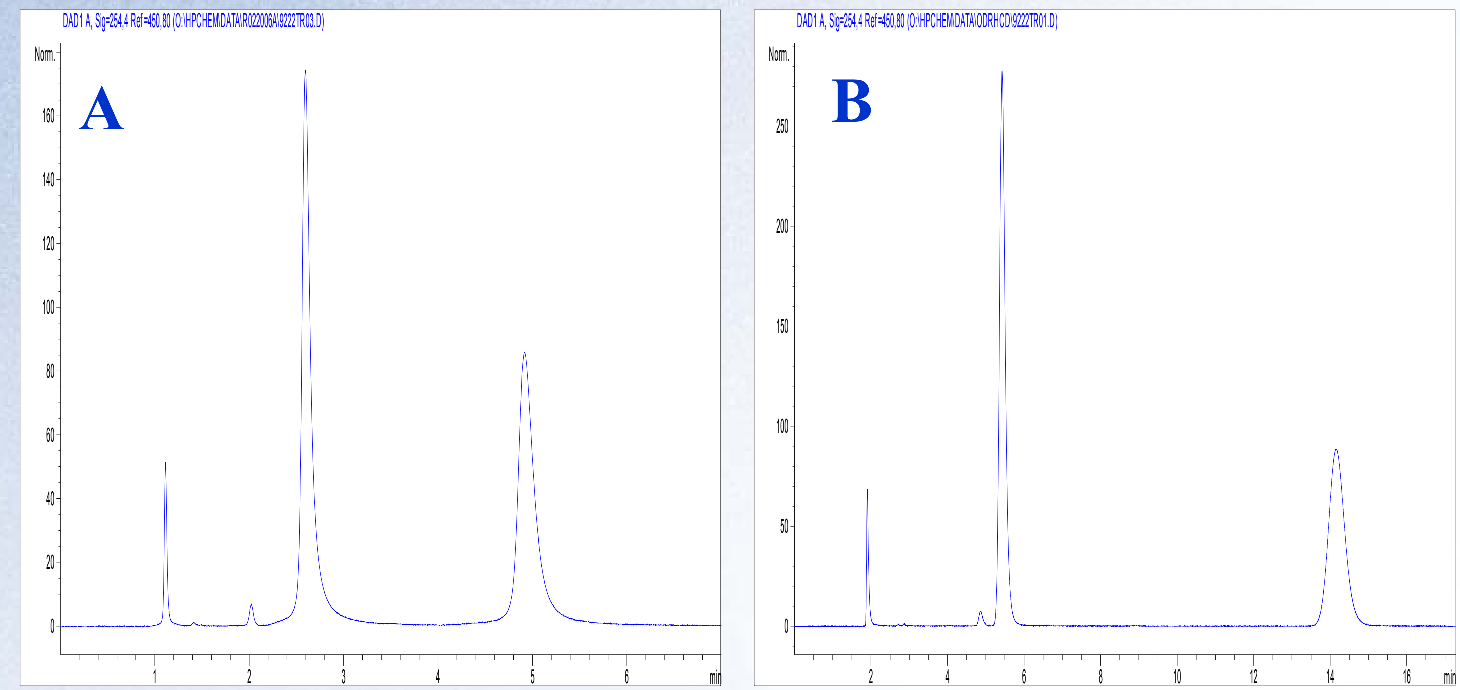
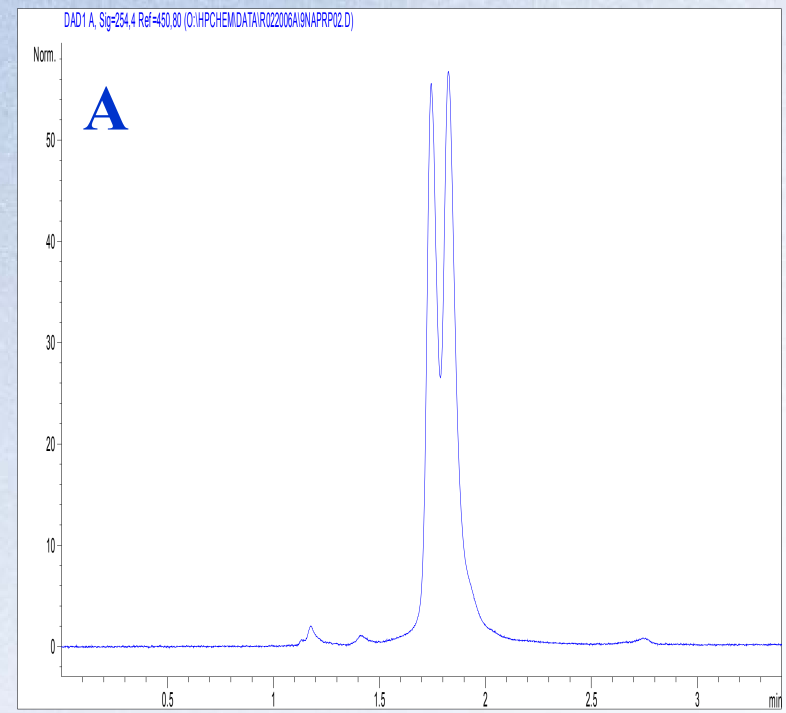
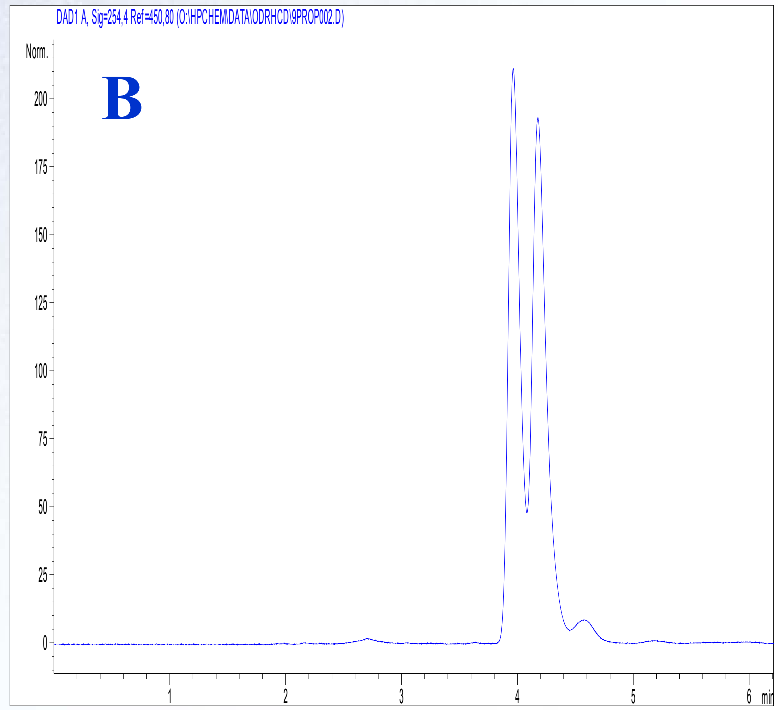


Columns, (A) **CelluloZe™** (Celu022006A), 100 × 4.6 mm, 3 µm Zirconia, (B) Silica-based column, 150 × 4.6 mm, 5 µm Silica, **Solute (RS)-(±)-2,2,2-Trifluoro-1-(9-anthryl) EtOH**, **Mobile phase** 90 / 10 Hexane / IPA, **Flow Rate**, 1 mL/min, **Column temperature**, ambient.

ZirChrom®

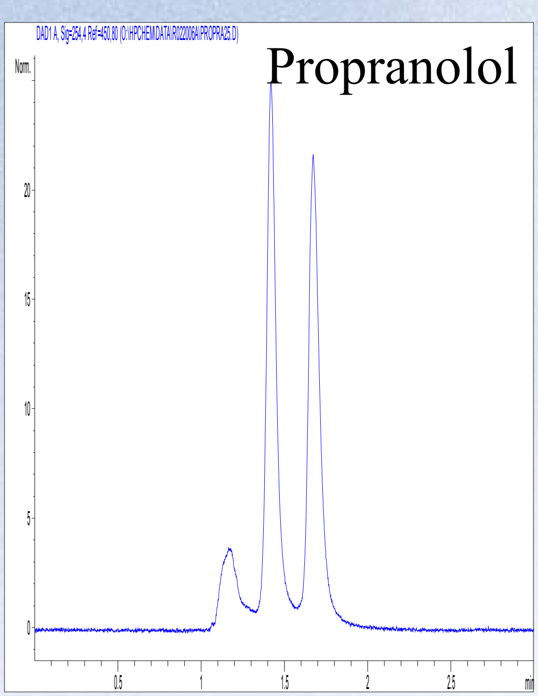
Comparison of Silica and Zirconia Cellulosic Phases

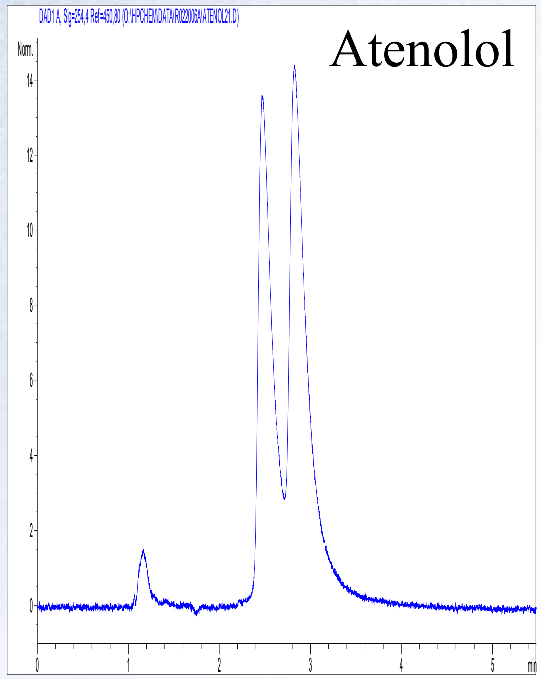


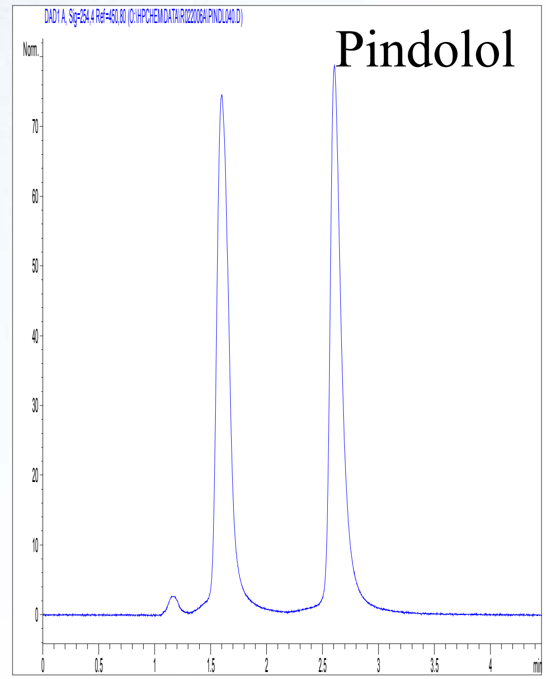


Columns, (A) **CelluloZe™** (Celu022006A), 100 × 4.6 mm, 3 µm Zirconia, (B) Silica-based column, 150 × 4.6 mm, 5 µm Silica, **Solute Napropamide**, **Mobile phase** 90 / 10 Hexane / IPA, **Flow Rate**, 1 mL/min, **Column temperature**, ambient.

Separation of Basic Drugs on Phosphonated Cellulose Zirconia







Column, **CelluloZe™** (Celu022006A), 100 × 4.6 mm, 3 µm Zirconia,
Mobile phase, = 50/50 Heptane/IPA (100 mM NH_4OAc in IPA),
Flow Rate, 1 mL/min, **Column temperature**, ambient.

Conclusions

- **Brush-type CSPs were attached to zirconia using multi-dentate pamidronic acid (PDA).**
- **Zirconia-based CSPs were shown to be reproducible, stable and have comparable chromatographic performance to commercial silica-based Brush-type CSPs for a range of chiral compounds.**
- **Zirconia-based CSPs offer the user the potential to regenerate the chiral stationary phase online.**
- **The new zirconia-based cellulosic CSPs showed similar resolving power to commercial silica-based cellulosic CSPs for selected chiral compounds; increased ionic strength improved resolution of basic chiral compounds.**

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References

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2. C. B. Castells and P. W. Carr, Chromatographia, Vol. 52, No. 9/10, November 2000, 535-542.
3. C. B. Castells and P. W. Carr, J. of Chromatogr. A (2000) 904, 17-33.

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Thanks *very much* for listening!

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