Synthesis of Stable Brush-Type Zirconia Based Chiral Stationary Phases for Enantiomer Separations

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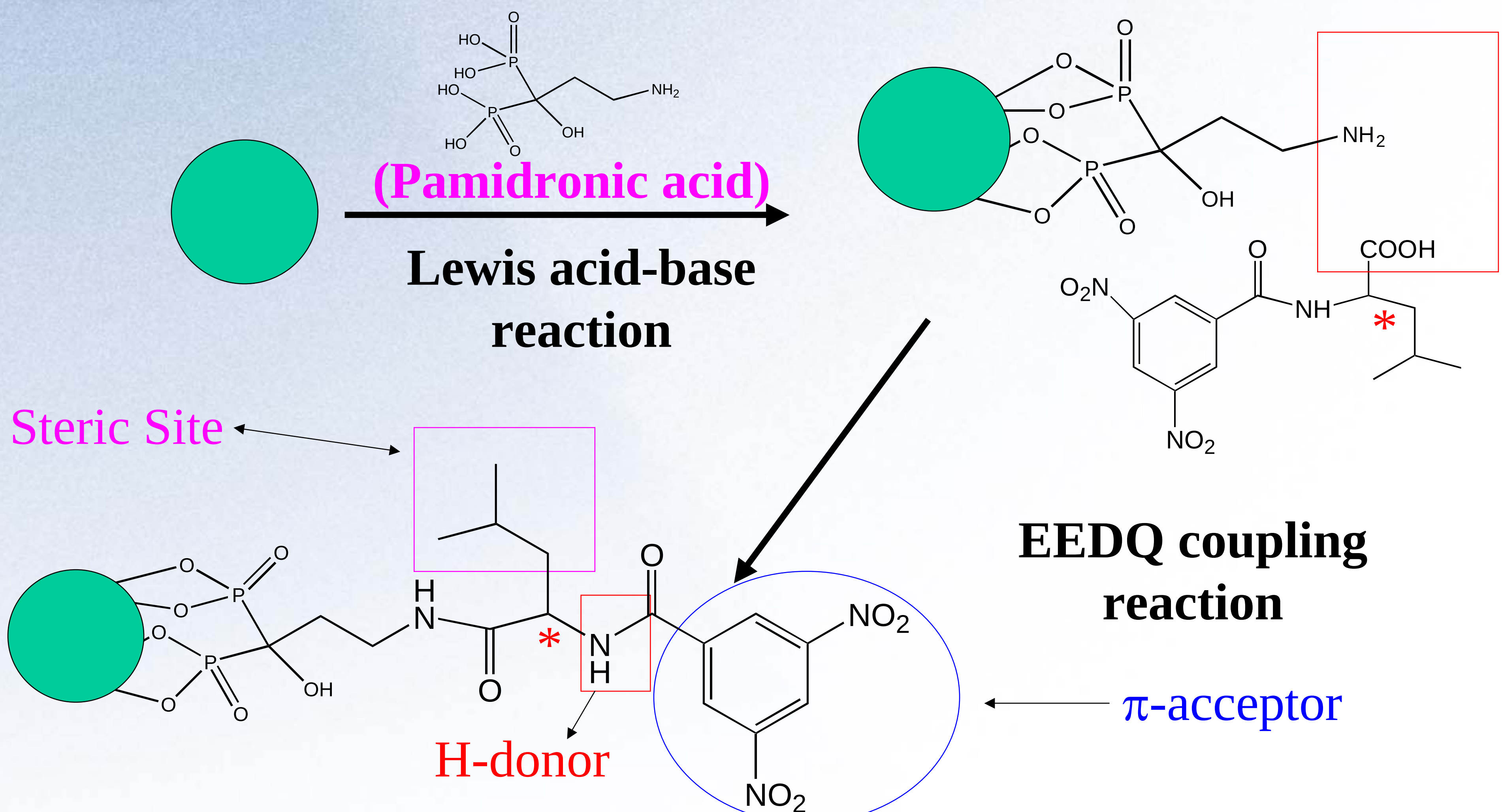
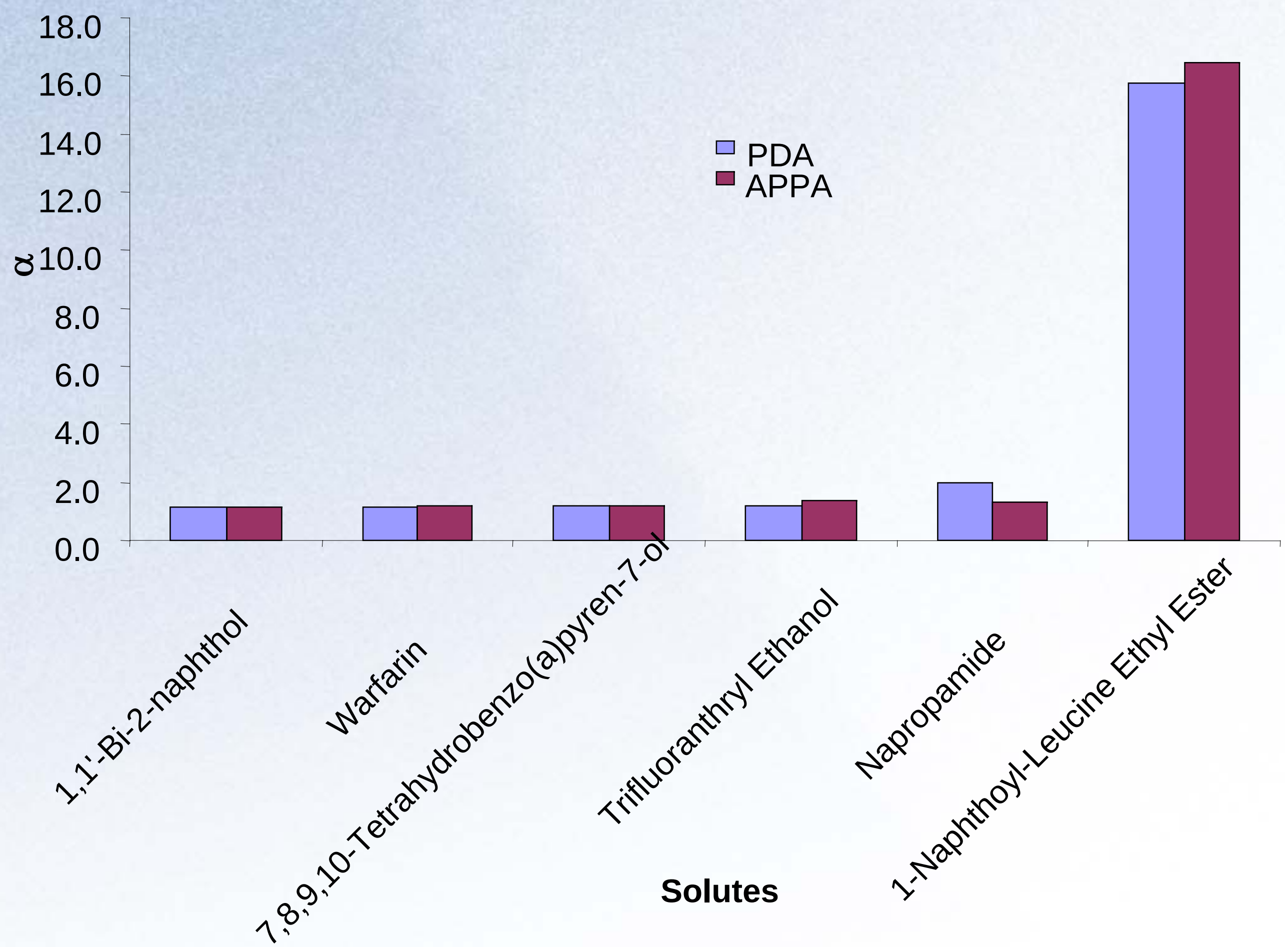
Goal-To Make Zirconia Based Chiral Stationary Phases for Fast Chiral Selector Screening

- Why Zirconia?
- Synthetic Approach
  - Building a zirconia-based CSP
  - Proof of concept
- Chiral Separations on Zirconia Based CSPs
- Stability Study
- Column Regeneration
- Conclusion – Careful selection of an anchor group results in a stable CSP that can be stripped off and reattached under high pH condition. This offers the possibility of regeneration or use for chiral selector screening.

Example of Lewis Acid-Base Modified Zirconia CSPs

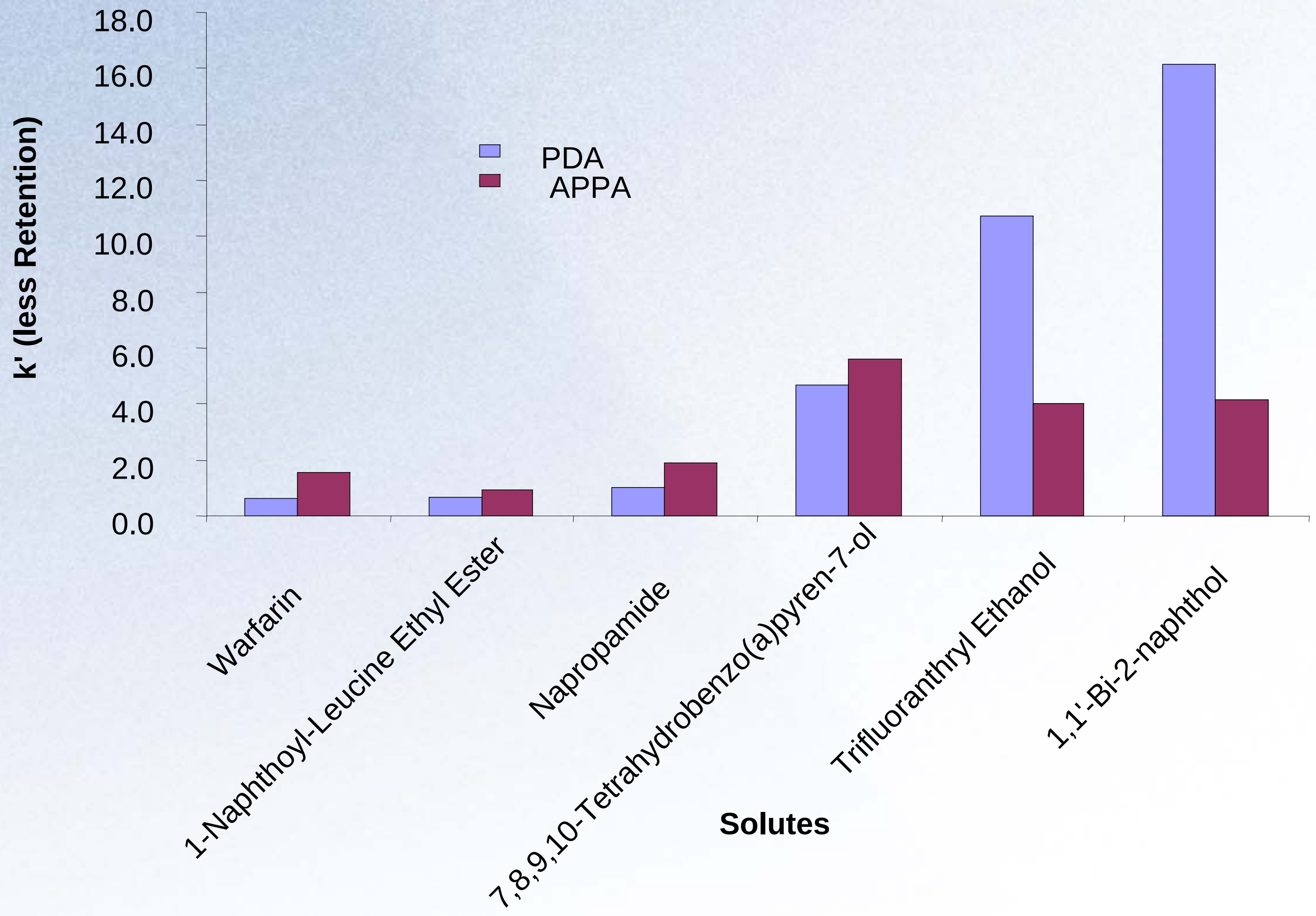


Selectivity Comparison Between PDA Anchored Zr (S)-Leu and APPA Anchored (S)-Leu



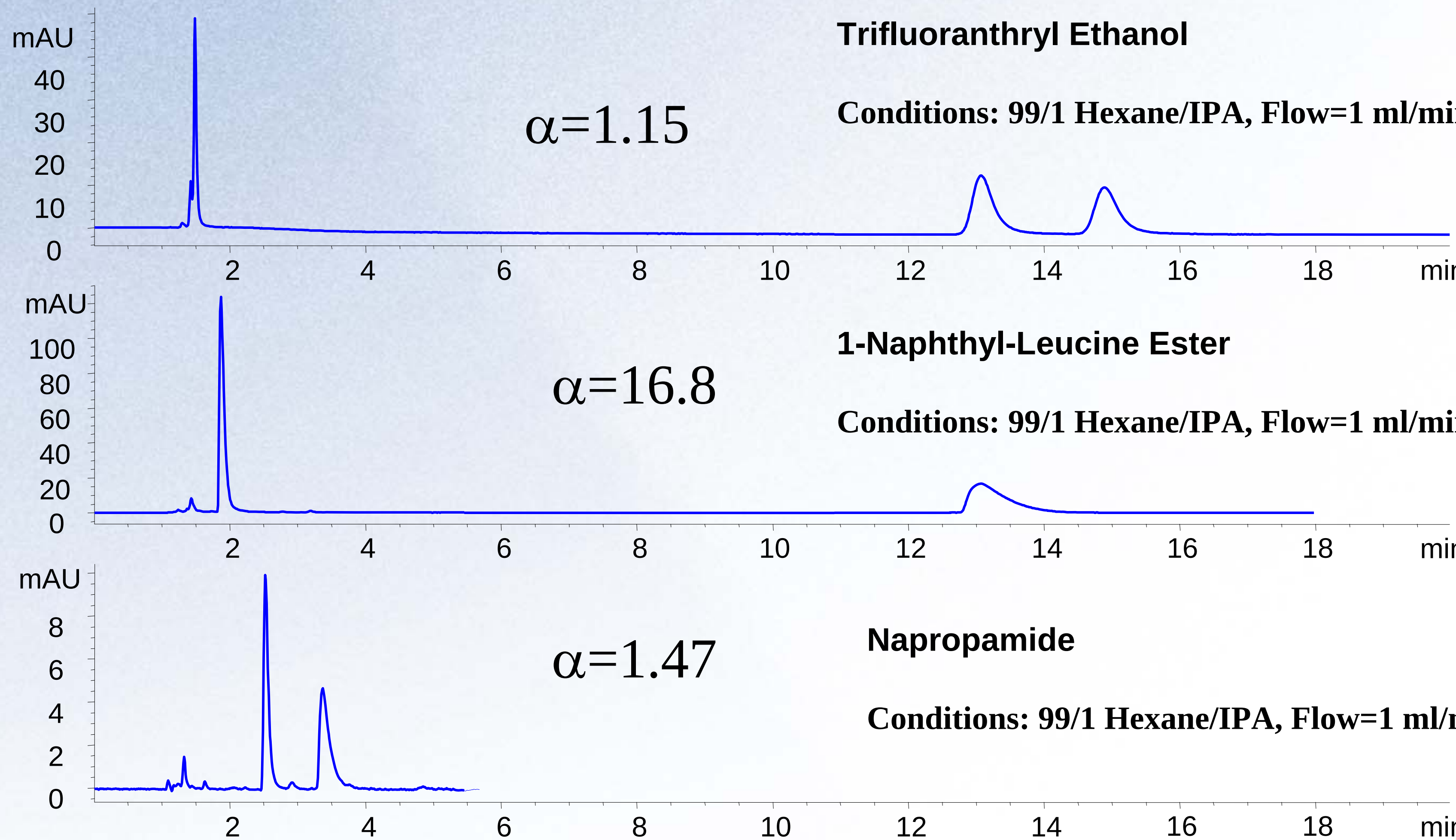
Selectivity for both anchors is very similar.

Retention Comparison Between PDA Anchored Zr (S)-Leu and APPA Anchored (S)-Leu



Retention for both anchors is different.

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



α=1.15

Trifluoranthryl Ethanol

Conditions: 99/1 Hexane/IPA, Flow=1 ml/min

α=16.8

1-Naphthyl-Leucine Ester

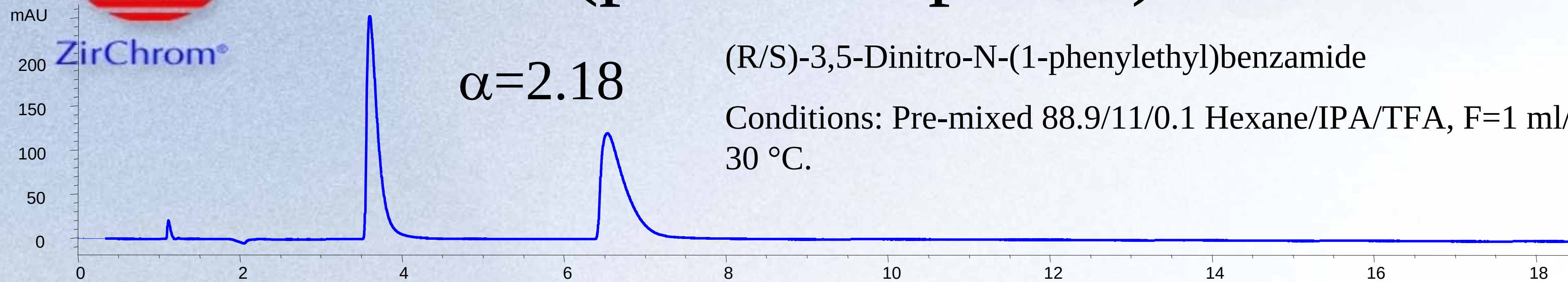
Conditions: 99/1 Hexane/IPA, Flow=1 ml/min

α=1.47

Napropamide

Conditions: 99/1 Hexane/IPA, Flow=1 ml/min

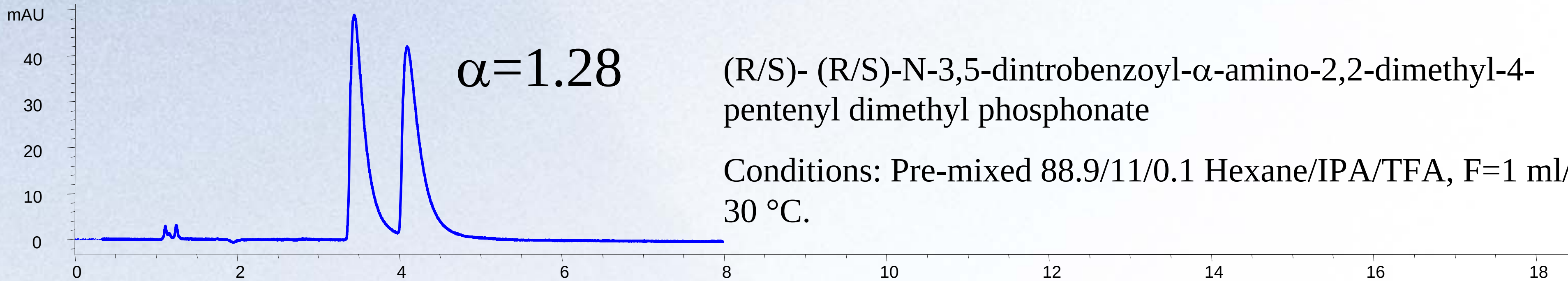
Chiral Separations on Zr (S)-NESA (pi-donor phase)



α=2.18

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

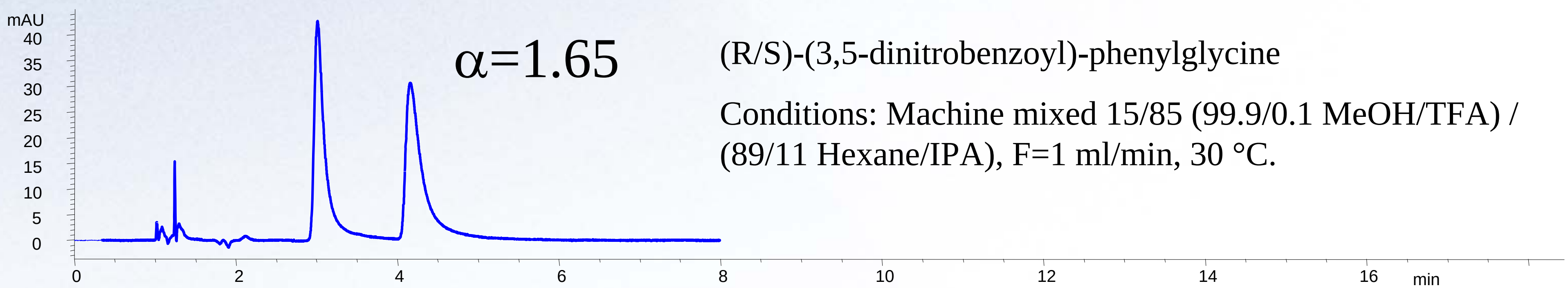
Conditions: Pre-mixed 88.9/11/0.1 Hexane/IPA/TFA, F=1 ml/min, 30 °C.



α=1.28

(R/S)- (R/S)-N-3,5-dintrobenzoyl-α-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.



α=1.65

(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.

Mobile Phase Effect of adding MeOH on Separation of (R/S)-N-3,5-dintrobenzoyl-α-amino-2,2-dimethyl-4-pentenyl dimethyl phosphonate on Zr (S)-NESA

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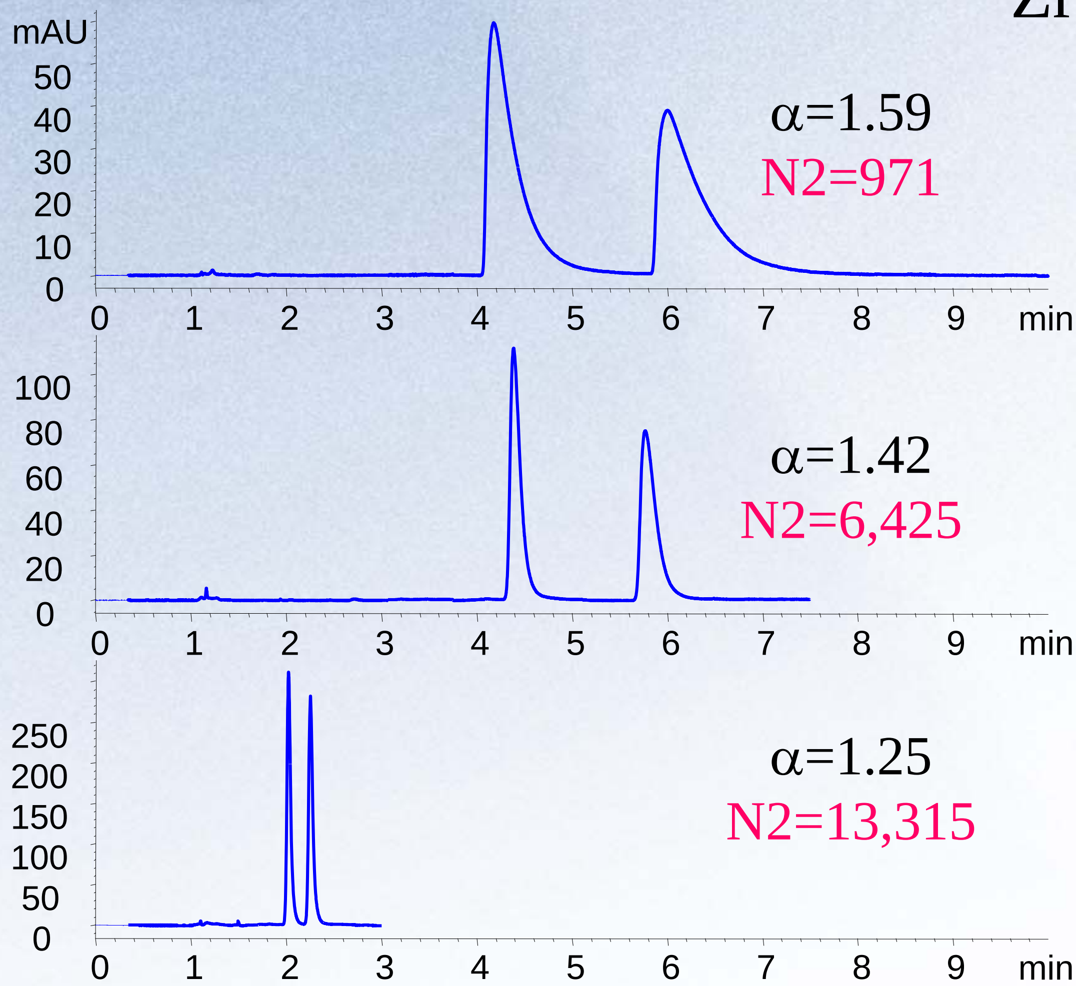
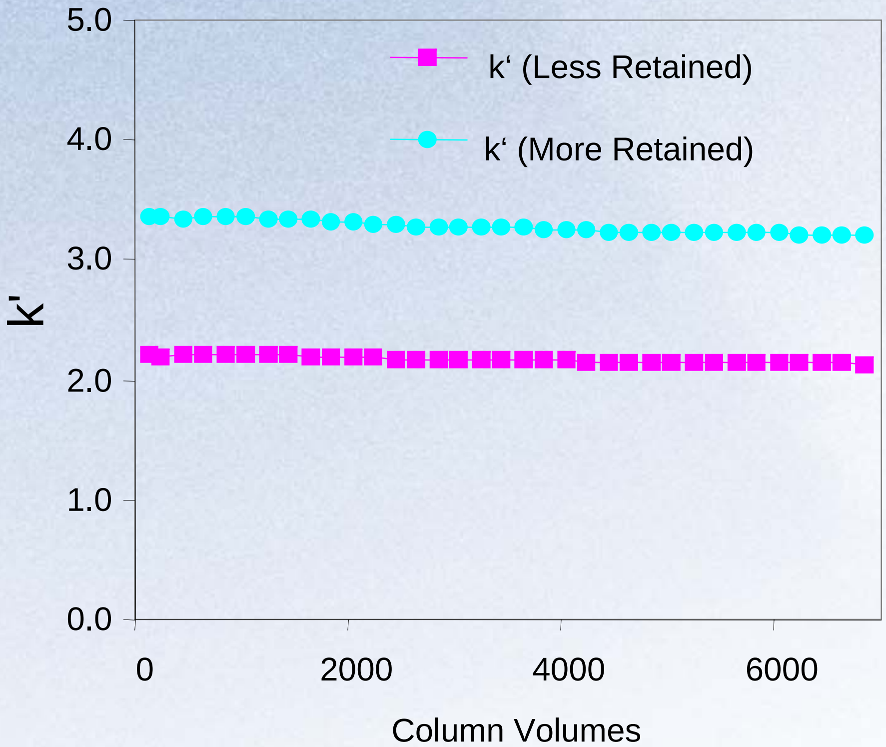


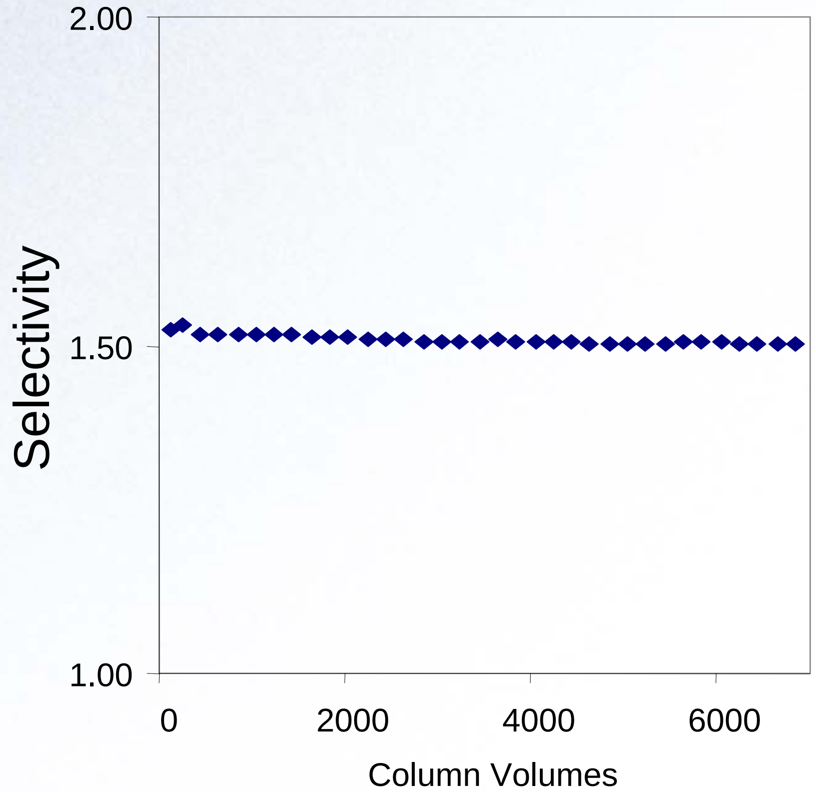
Conditions: 89/11 Hexane/IPA, F=1 ml/min, 30 °C.

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

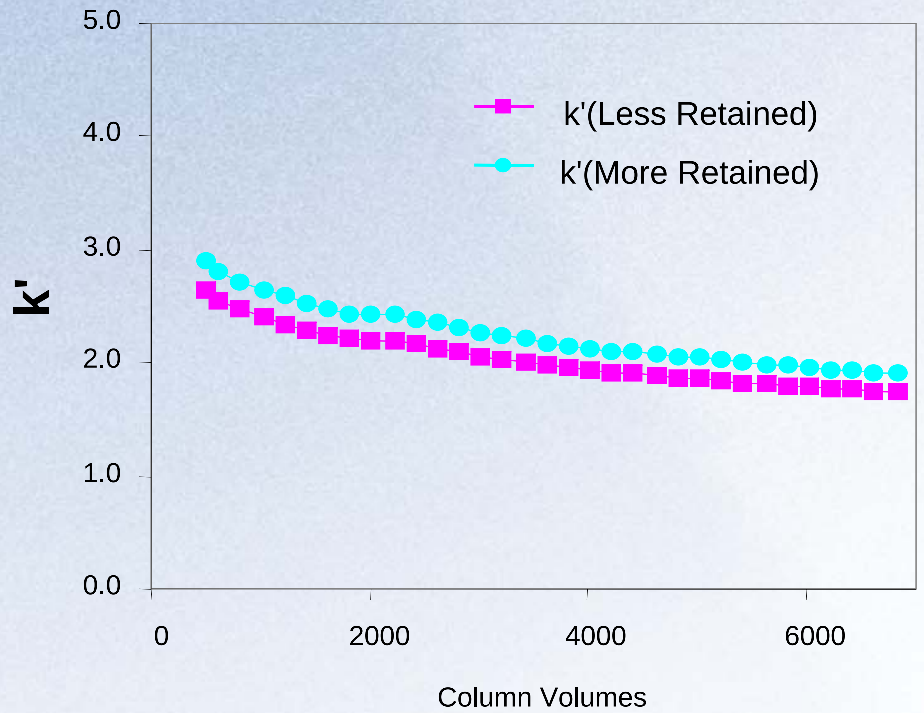
Stability of Zr-(S)-NESA at pH 2

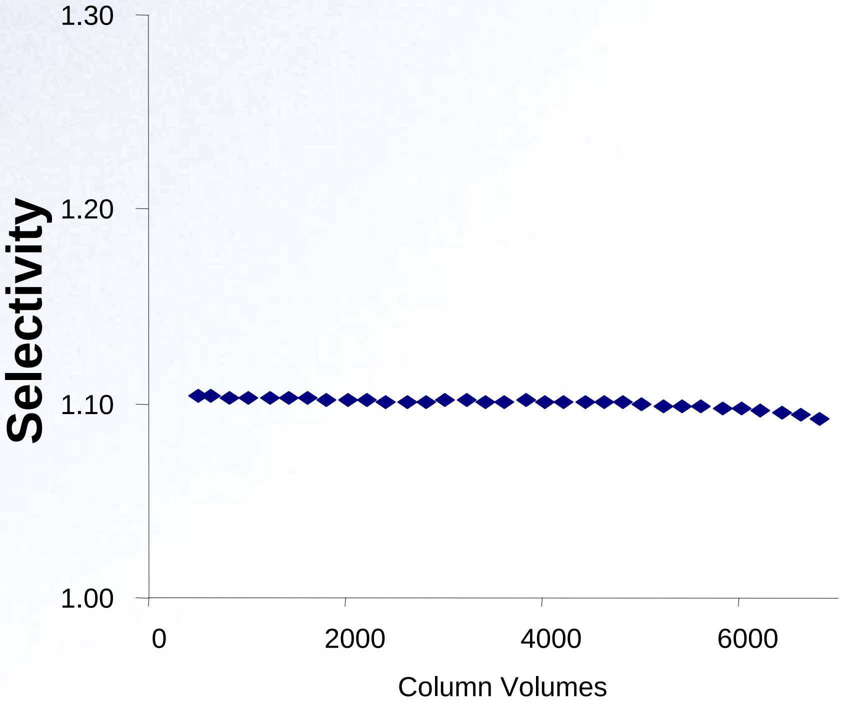




Column ID: ZrCSP051605C, Mobile phase: 15/85 ACN/0.01 mM TFA pH 2,
Temperature: 30 ^{o}C, Injection volume: 5 ul, Wavelength: 254 nm.
Probe solutes:(R/S)-3,5-dinitro-N-(1-phenylethyl)benzamide

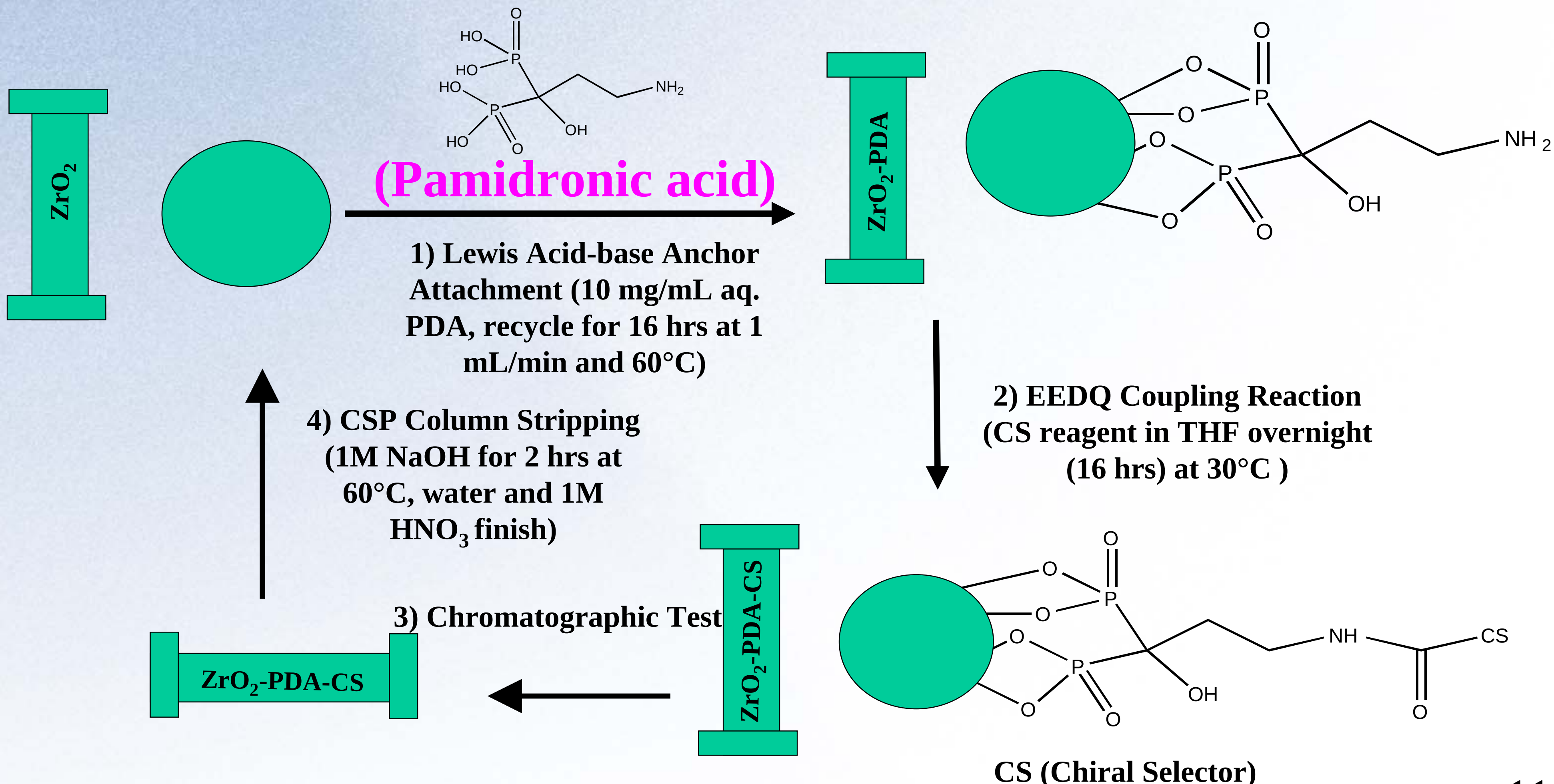
Stability of Zr-(S)-DNB-Leu at pH 8



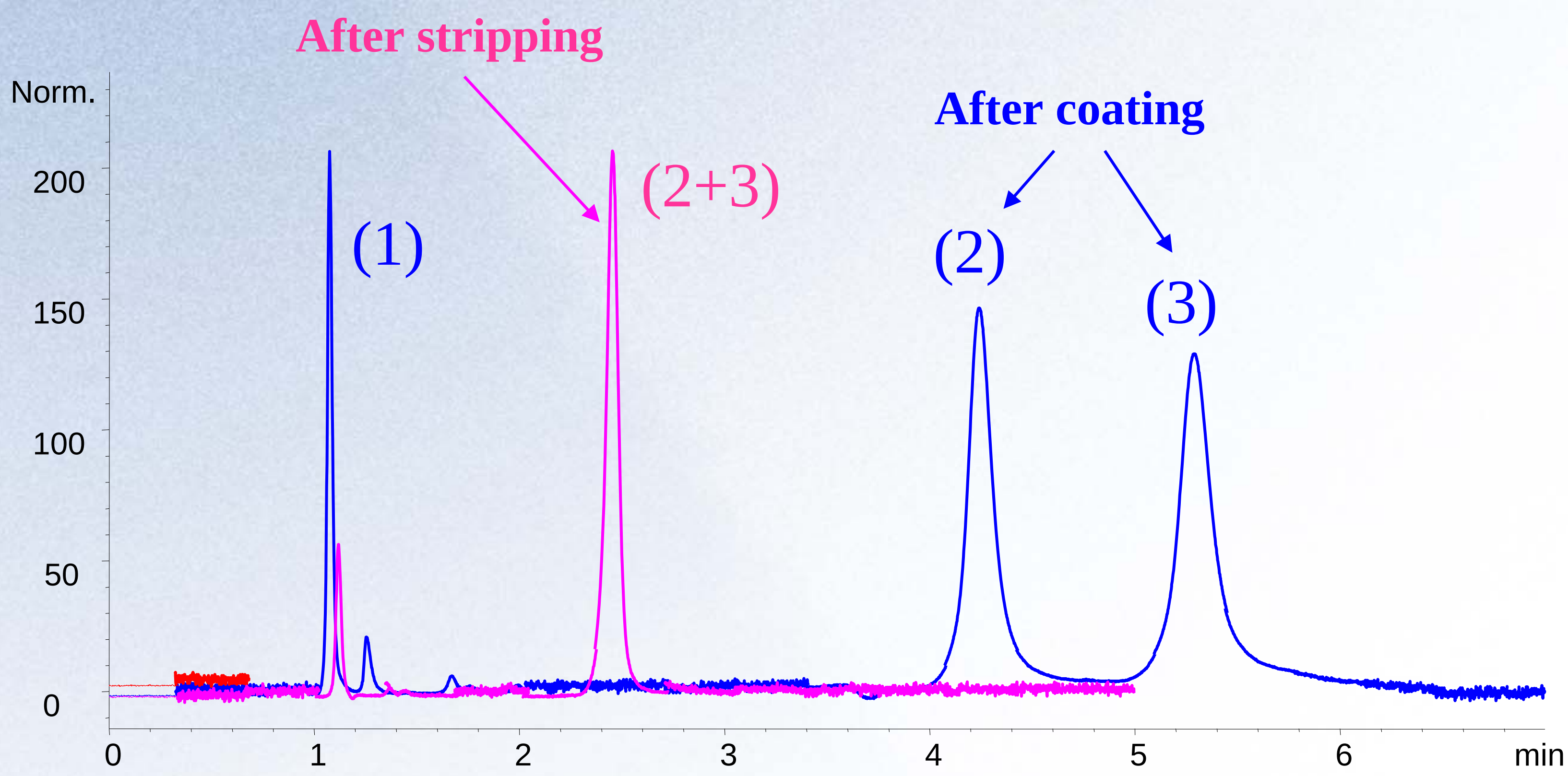


Column ID: ZrCSP032805A, Mobile phase: 15/85 ACN/5 mM ammonium hydrogencarbonate pH 8.0, Temperature: 30 ^{o}C, Injection volume: 5 ul, Wavelength: 254 nm.
Probe solutes:(R/S)-2, 2, 2-trifluoro-1-(9-anthryl)ethanol

2-Step Online Zirconia CSP Synthesis for Chiral Screening

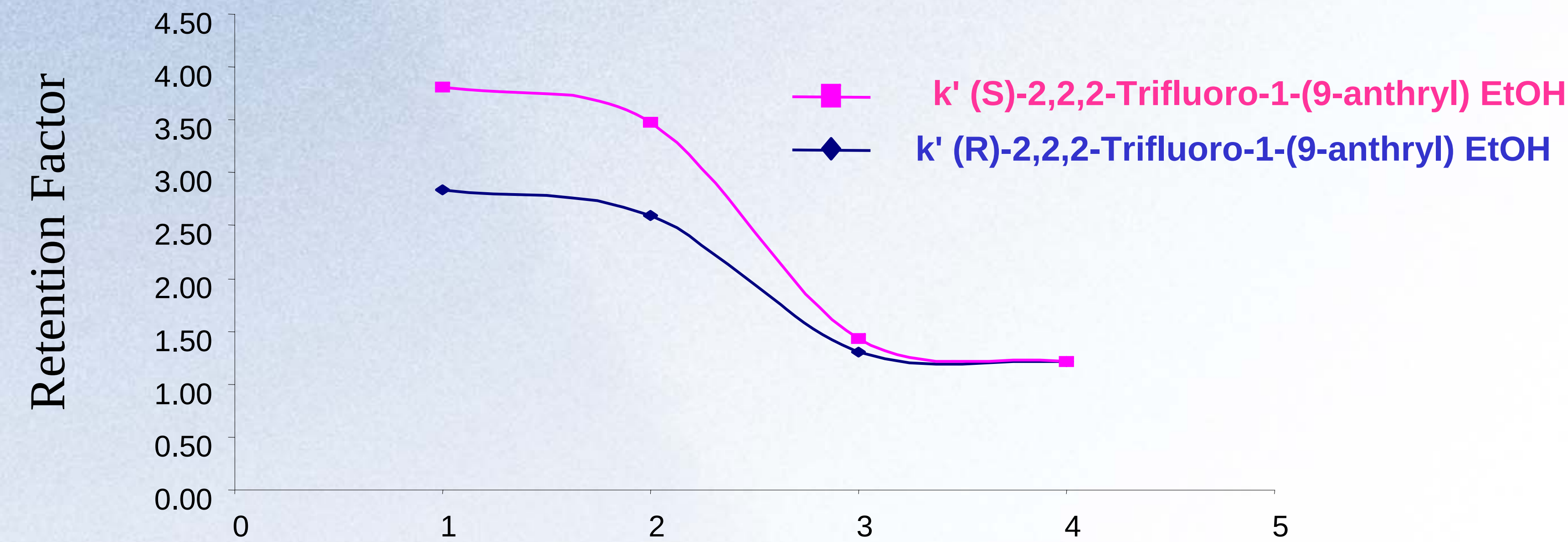


Stripping of (S)-PG CSP



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



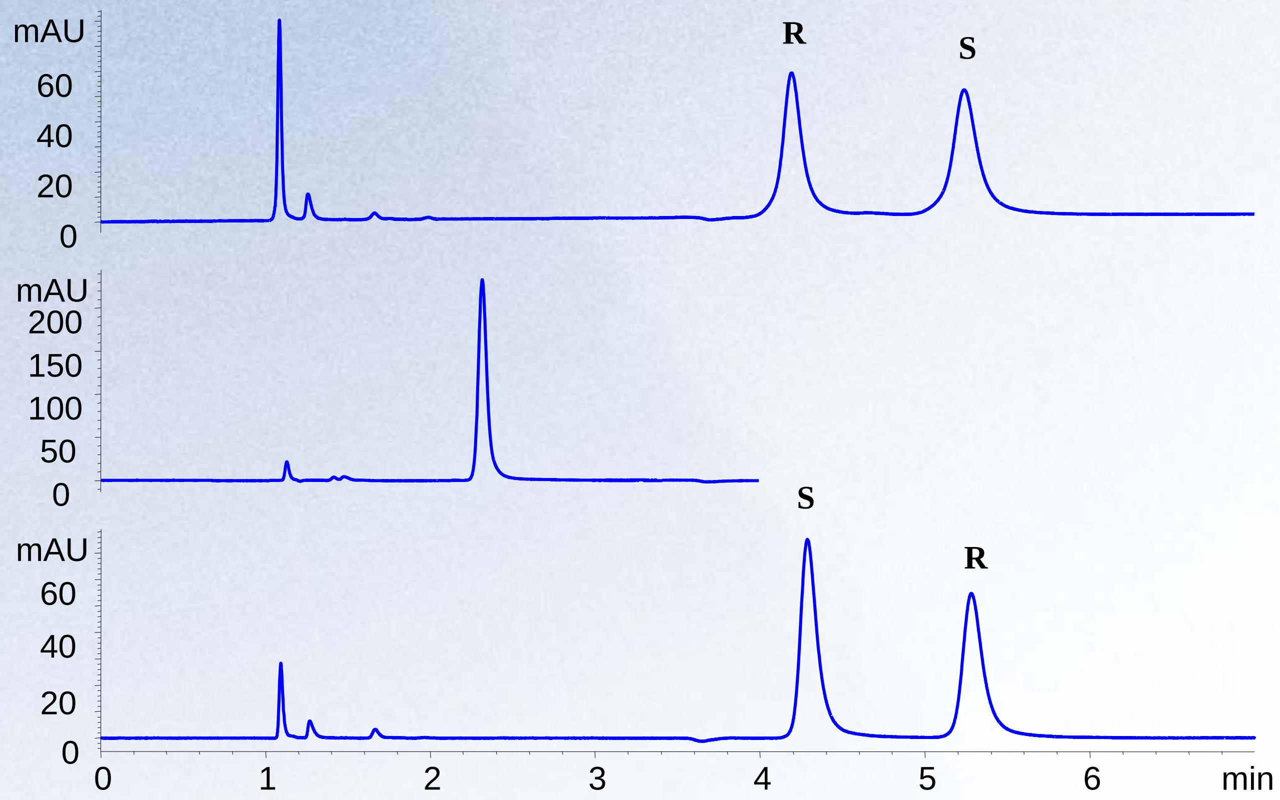
1- Original column

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 ºC, 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, 30 °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)

Conclusions

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- Five new CSPs were attached to zirconia using the PDA anchor, including:

  π–acceptors: Zr (S)-Leu, Zr (R)-PG, and Zr (S)-PG

  π–donors: Zr (R)-NESA, Zr (S)-NESA
- The new Zirconia-based CSPs were found to be ***fairly stable*** in reversed-phase mobile phase from pH 2 to pH 8.
- Zirconia based CSPs have the potential to regenerate the chiral stationary phase online.
- Acknowledgement: ***National Institutes of Health Grant*** (Phase II SBIR) 2R44HL070334-02A2.

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