Chiral Separations of Pharmaceuticals and Other Compounds on Lewis Acid-Base Anchored Zirconia Chiral Stationary Phases

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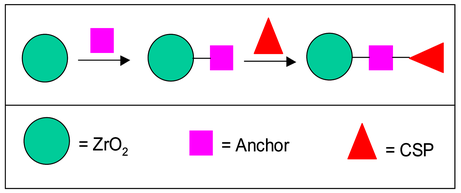
Synthesis of Lewis Acid-Base Modified Zirconia CSPs



General method for attaching CSPs to zirconia by Lewis acid-base anchors.

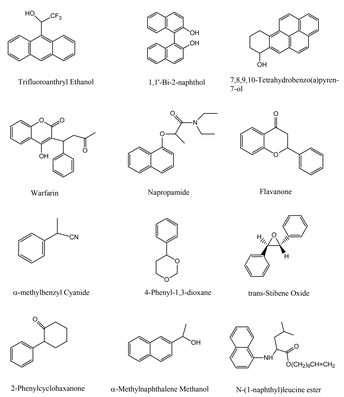
- Three different anchor groups:
  - APPA (aminopropylphosphonic acid)
  - DHNP (Dihydroxynorephedrine)
  - ASPA (Aspartic acid).
- These anchor groups were used to bond:
  - DNP-PG (3,5-dinitrobenzoyl-phenylglycine)
  - DNB-LEU (3,5-dinitrobenzoyl-Leucine)
  - DNB-PRO (3,5-dinitrobenzoyl-proline)
  - NAP-VAL (Naphthoyl-valine)
  - 1 or 2- NAP-LEU (Naphthoyl-leucine)
  - NAP (naproxen).

List of Zirconia and Silica CSPs Studied

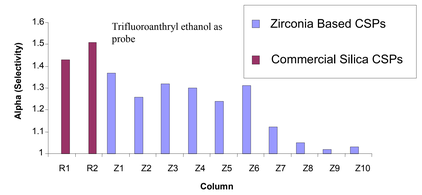
Column	CSP[a]	Anchor	Column Legnth (mm)	Normed V_0 (ml)	N/meter [b]	Stability Rank [c.]
Z1	DNB-Leu	APPA	100	1.07	54600	5
Z2	DNB-Leu	Aspartic acid	100	1.08	70900	4
Z3	DNB-Leu	DHNP	100	1.11	49800	3
Z4	DNG-PG	APPA	100	1.08	53000	5
Z5	DNB-PG	Aspartic acid	100	1.08	63500	4
Z6	DNB-PG	DHNP	100	1.14	52400	3
Z7	DNB-Pro	DHNP	100	1.07	86000	3
Z8	NAP-Leu	APPA	100	1.07	88900	5
Z9	NAP-Val	DHNP	100	1.00	15800	3
Z10	Naproxen	APPA	100	1.08	26000	5
R1	DNB-PG	None	250	1.10	75000	~4
R2	DNB-Leu	None	250	1.14	102000	~4

a. DNB = dinitrobenzoyl, PG = phenylglycine, PRO = proline
b. Theoretical plates per meter (N/meter) for butylbenzene as a solute.
c. Stability Rank: 1 = Unstable, 2 = Stable in 100% IPA mobile phase, 3 = Stable in 100% MeOH mobile phase, 4 = Stable in 0.5% trifluoroacetic acid in IPA/Hexane (1/99), 5 = Stable in 40 mM tetrabutylammonium hydroxide in IPA/Hexane (15/85)

Chiral Probe Solutes Used in This Study

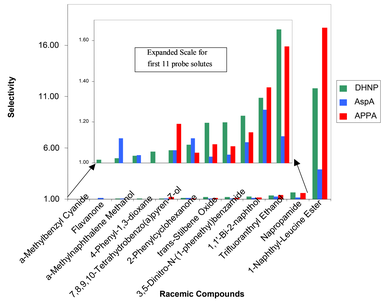


Chromatographic Comparison of Zirconia and Silica CSPs



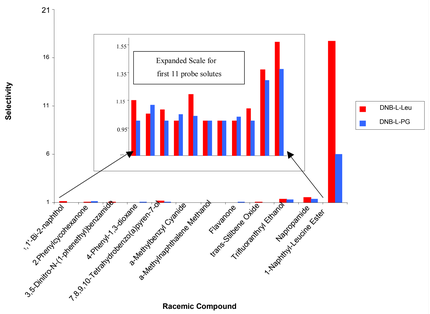
For this probe solute the new silica-based CSPs were better able to separate the enantiomers, whereas in other cases the zirconia-based CSPs were more selective.

Effect of Anchor on Separations by Zirconia-based CSPs



- The anchor type has relatively little effect on the ability of a given chiral selector to achieve a separation.
- Data shown is for DNB-L-Leu based Zirconia phases

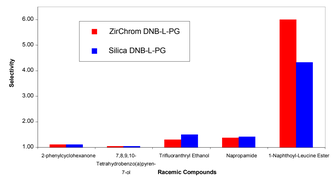
Effect of Selector on Separations by Zirconia-based CSPs



- There was very little effect between DNB-LLEU and DNB-L-PG chiral selectors except for the separation of 1-naphthyl-leucine ester.

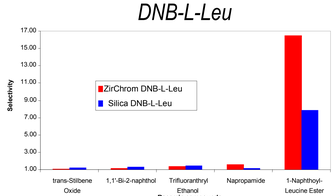
Direct Comparison of Zirconia and Silica based CSPs

DNB-L-PG



Study uses hexane/Isopropanol mixtures where the amount of isopropanol was adjusted to keep retention factors the same on both the zirconia and silica based columns. No mobile phase additives were used. Other LC conditions used were: flow rate = 2 ml/min, 30°C, UV detection at 254nm, column dimensions 10cm x 4.6 mm id (zirconia columns) and 25cm x 4.6 mm id (silica columns).

DNB-L-Leu



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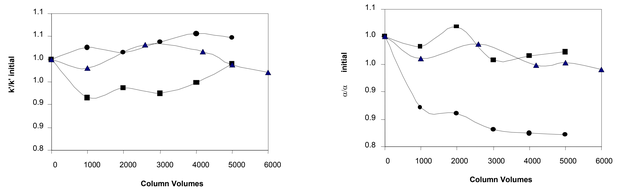
Both the zirconia and silica based phases showed comparable separation factors for the enantiomers, except for napropamide and 1-naphthylleucine ester, which were much better separated on zirconia-based CSPs.

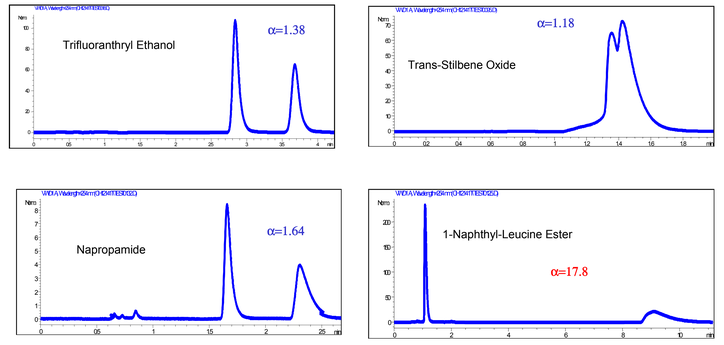
Zirconia-Based CSP Column Stability



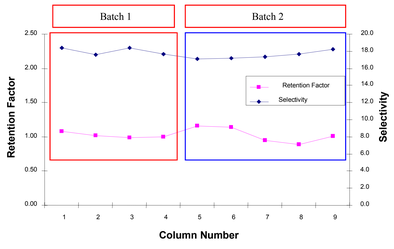
- ***Mobile phase: 99/1 Hexane/IPA; Test solute: Trifluoranthryl Ethanol (same for all phases).***
- **DNBLeu-APPA** (▲) column challenge conditions = flushing eluent: 100% IPA (1000 column volumes), 100%MeOH (3200 column volumes), 49.25/49.25/0.5 Hexane/IPA/TFA (800 column volumes), and 85/15 ACN/50 mM tetrabutyl ammonium hydroxide in MeOH (1000 column volumes)
- **DNBPG-AspA** (●) column challenge conditions = Flushing eluent: 50/50 IPA/Hexane (3000 column volumes) and 80/20 CH_2Cl_2/40mM NH_4OAc in IPA (2000 column volumes).
- **DNBPG-DHNP** (■) column challenge conditions = Flushing eluent: 80/20 CH_2Cl_2/40mM NH4OAc in IPA (3000 column volumes) and 100% MeOH (2000 column volumes). All column dimensions: 4.6X100.

The APPA anchor proved to be vastly more stable than either of the other two anchors.

Sample Separations for DNB-L-Leu/APPA Zirconia CSP



Batch-to-Batch Reproducibility



Batch-to-batch and column-to-column reproducibility study of DNB-L-LEU attached to zirconia via APPA using (R or S)-naphthylleucine ester probe solutes. Retention Factor is shown for R-naphthylleucine ester.

Conclusions

- APPA is the best anchor in terms of column stability.
- APPA anchored zirconia based CSPs have better chemical stability compared to the analogous silica based CSPs.
- Zirconia based CSPs have comparable chromatographic performance compared to the commercial silica based CSPs for a wide range of chiral compounds.
- Column to column and batch to batch reproducibility for the zirconia-based CSPs are very good.

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