



ZirChrom®

Performance of ZirChrom®-Chiral Zirconia-based CSPs

Richard Henry, Ph.D.*, Bingwen Yan, Ph.D.*, Clayton McNeff, Ph.D.* and Thomas Hoyer, Ph.D.**
*ZirChrom Separations, Inc.
**University of Minnesota

Technical Bulletin # 314

High-performance liquid chromatography has become the dominant method for the analytical and preparative separation of chiral pharmaceuticals. However, no current chiral stationary phase uses zirconia or inorganic oxides other than silica as a substrate. A promising new route to preparing chiral stationary phases using a zirconia substrate has been developed (See ZirChrom Technical Bulletin #313). The following demonstrates the performance of these new versatile, durable, and efficient zirconia-based chiral HPLC columns is comparable to that of conventional silica phases.

Introduction

The zirconia-based chiral phases employed in this study were synthesized using a two-step approach to provide a more robust and flexible platform for chiral stationary phase (CSP) design when compared to silica gel. Columns produced in this manner were compared to silica columns having analogous chiral selectors and found to have similar resolving power for the selected probe enantiomers. Most importantly, the chemisorbed chiral selectors on zirconia were found to be stable enough for extended routine use; however, they were completely removed by washing with a high pH (>pH 12) aqueous solution and could be easily regenerated. Results shown in Figures 1–3 for the zirconia-based CSP columns are comparable to the performance of silica-based columns having a similar chiral selector.

Experimental

Brush-type CSPs were selected for initial experiments due to their ease of synthesis, wide scope of applicability, and large body of available silica-based separations data for comparison. The separation conditions were as follows:

Column: See Figures, All 100 mm x 4.6 mm columns
Mobile Phase: See Figures
Temperature: 30 °C with Metalox™ 200-C column heater
Flow Rate: 1.0 ml/min.
Injection Vol.: 0.5 µl
Pressure Drop: 195 bar
Detection: UV at 254 nm

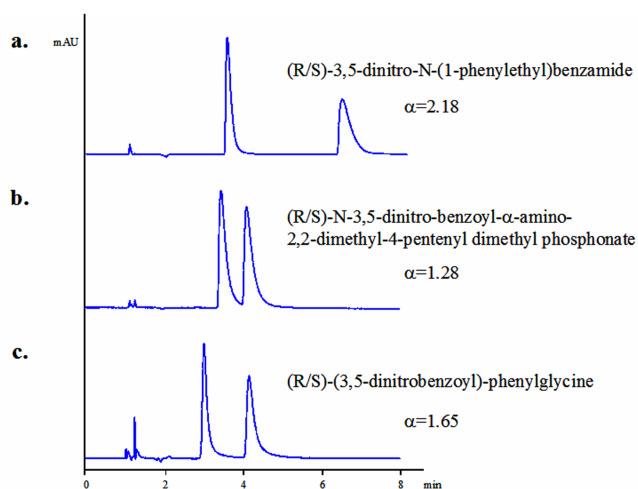


Figure 1: ZirChrom®-Chiral(R)NESA CSP column (part # ZRC02-1046) Selectivity Examples. Conditions: a) and b) 88.9/11/0.1 hexane/isopropanol/TFA (trifluoroacetic acid), c) 75/15/9/1 hexane/methanol/isopropanol/TFA.

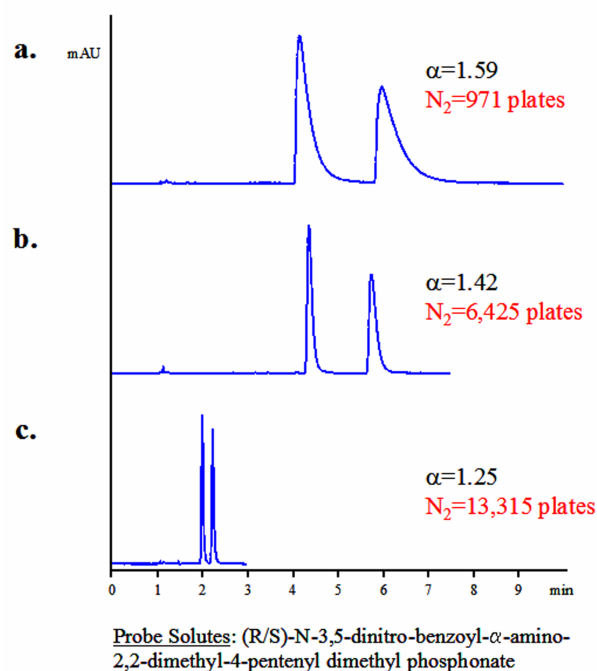


Figure 2: Effect of mobile phase changes on ZirChrom®-Chiral(R)NESA CSP column (part # ZRC02-1046). Conditions: a) 89/11 hexane/isopropanol, b) 95/2/3 hexane/methanol/isopropanol, c) 87/10/3 hexane/methanol/isopropanol.

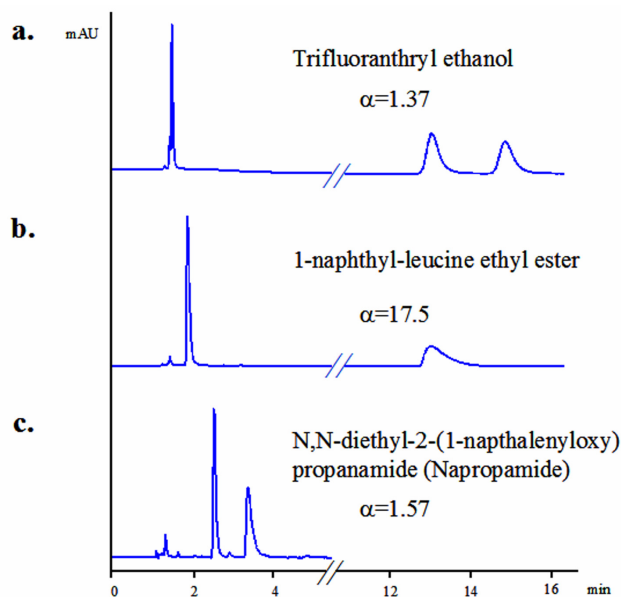


Figure 3: ZirChrom[®]-Chiral(S)LEU CSP column (part # ZRC01-1046) Selectivity Examples. Conditions: 99/1 hexane/isopropanol (all runs).

ZirChrom[®]-Chiral stationary phases are available in packed columns with a growing variety of chiral selectors. Currently, ZirChrom offers five different chiral columns in the ZirChrom[®]-Chiral line:

- ZirChrom[®]-Chiral(S)LEU; Chiral Selector(CS): (S)-3,5-dinitrobenzoyl-leucine, Part# ZRC01
- ZirChrom[®]-Chiral(R)NESA; CS: (R)-N-[1-(1-naphthyl)ethyl]succinamic acid, Part# ZRC02
- ZirChrom[®]-Chiral(S)NESA; CS: (S)-N-[1-(1-naphthyl)ethyl]succinamic acid, Part# ZRC03
- ZirChrom[®]-Chiral(S)PG; CS: (S)-3,5-dinitrobenzoyl-phenylglycine, Part# ZRC04
- ZirChrom[®]-Chiral(R)PG; CS: (R)-3,5-dinitrobenzoyl-phenylglycine, Part# ZRC05

Product development is underway for a single, highly stable zirconia column plus a kit of pure CSP coating reagents that will allow users to easily remove and replace chiral selectors by reproducible and simple methods. Chiral selectors with multiple chiral centers, featuring both π -donor and π -acceptor groups, are also under development, as are chiral selectors based on polysaccharides.

Please contact ZirChrom technical support at 1-866-STABLE-1 or support@zirchrom.com for more information regarding this exciting new technology.

References

(1) American Laboratory, 37, No. 21, pp 22-4 (October 2005).

ZirChrom Separations, Inc.
617 Pierce Street, Anoka, MN 55303
1-866-STABLE-1
support@zirchrom.com

Visit www.zirchrom.com for more application notes using ultra-stable, high efficiency ZirChrom columns.



Effect of Chiral Selector on Performance of ZirChrom[®]-Chiral CSPs

Richard Henry, Ph.D.*, Bingwen Yan, Ph.D.*, Clayton McNeff, Ph.D.* and Thomas Hoyer, Ph.D.**

*ZirChrom Separations, Inc.
**University of Minnesota

Technical Bulletin # 315

High-performance liquid chromatography has become the dominant method for the analytical and preparative separation of chiral pharmaceuticals. However, no current chiral stationary phase uses zirconia or inorganic oxides other than silica as a substrate. A promising new route to preparing chiral stationary phases using a zirconia substrate has been developed (See ZirChrom Technical Bulletin #313). The following demonstrates the effect of chiral selector change on the performance of these new versatile, durable, and efficient ZirChrom[®]-Chiral zirconia-based chiral stationary phases (CSP).

Introduction

Many of the challenges faced in chiral method development stem from the inability to quickly and cost effectively analyze the performance of a set of chiral selectors for a given racemic set. In this application we demonstrate the effects of chiral selector choice on a set of enantiomer pairs:

1. 1,1'-Bi-2-naphthol
2. 2-Phenylcyclohexanone
3. 3,5-Dinitro-N-(1-phenethyl)benzamide
4. 4-Phenyl-1,3-dioxane
5. 7,8,9,10-Tetrahydrobenzo(a)pyren-7-ol
6. *a*-Methylbenzylcyanide
7. *a*-Methylnaphthalene Methanol
8. Flavanone
9. Trans-stilbene oxide
10. Trifluoranthrylethanol
11. Napropamide
12. 1-Naphthyl-leucine ethyl ester

In these experiments the only change made to the stationary phase was the chiral selector, thus minimizing the influence of any other stationary phase effects on selectivity.

Experimental

Twelve sets of racemic probe solutes were injected on a ZirChrom[®]-Chiral(S)LEU and a ZirChrom[®]-Chiral(S)PG column for selectivity comparison. The separation conditions were as follows:

Columns: ZirChrom[®]-Chiral(S)LEU and ZirChrom[®]-Chiral(S)PG, 100 mm x 4.6 mm i.d.
(Part #: ZRC01-1046 and ZRC04-1046)
Mobile Phase: 99/1 hexane/isopropanol
Temperature: 30 °C with Metalox[™] 200-C column heater
Flow Rate: 1.0 ml/min.
Injection Vol.: 0.5 μ l
Pressure Drop: 195 bar
Detection: UV at 254 nm

Selectivity is compared in *Figure 1* for 12 probe solute enantiomers on ZirChrom[®]-Chiral(S)LEU and ZirChrom[®]-Chiral(S)PG columns. As expected, changing the chiral selector had a significant effect on the resolution of the enantiomers. This ability to change chiral selectors on the same column can reduce the influence of other column factors and allow the focus to be placed on choosing the best chiral selector during method development.

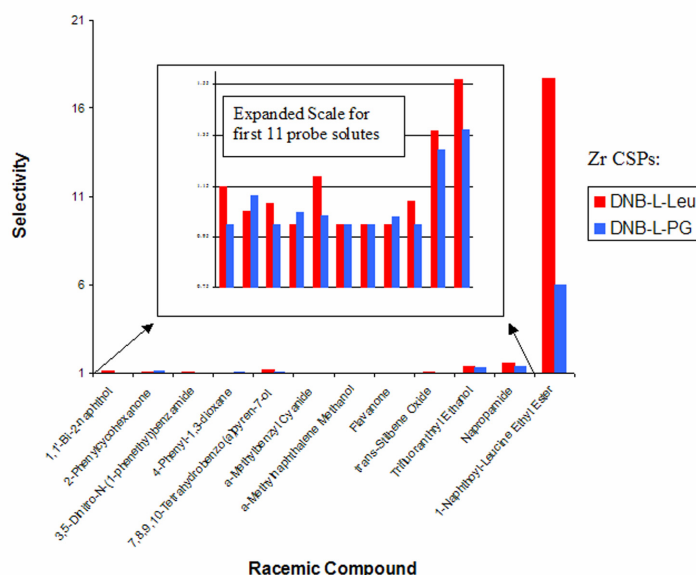


Figure 1: Selectivity comparison between ZirChrom[®]-Chiral(S)LEU and ZirChrom[®]-Chiral(S)PG CSP columns.

ZirChrom now offers a full line of chiral phases. ZirChrom chiral phases utilize a fully renewable platform and offer unique selectivity, high efficiency, and excellent stability. Please contact ZirChrom technical support at 1-866-STABLE-1 or support@zirchrom.com for more information regarding this exciting new technology.

References

(1) American Laboratory, 37, No. 21, pp 22-4 (October 2005).

ZirChrom Separations, Inc.
617 Pierce Street, Anoka, MN 55303
1-866-STABLE-1
support@zirchrom.com

Visit www.zirchrom.com for more application notes using ultra-stable, high efficiency ZirChrom columns.



ZirChrom®

Stability and Reproducibility of ZirChrom®-Chiral(S)LEU

Richard Henry, Ph.D.*, Bingwen Yan, Ph.D.*, Clayton McNeff, Ph.D.* and Thomas Hoye, Ph.D.**.
*ZirChrom Separations, Inc.
**University of Minnesota.

Technical Bulletin # 316

High-performance liquid chromatography has become the dominant method for the analytical and preparative separation of chiral pharmaceuticals. A promising new route to preparing chiral stationary phases using a zirconia substrate has been developed (See ZirChrom Technical Bulletin #313). The following demonstrates the stability and reproducibility of these new versatile, durable, and efficient ZirChrom®-Chiral zirconia-based chiral stationary phases (CSP).

Introduction

In the initial portion of this work examines the effect of tethering agent on the stability of the chiral phase was examined. A two-step reaction scheme was employed using three different types of Lewis base tethering agents (APPA= aminopropylphosphonic acid; DHNP= dihydroxynorephedrine; ASPA = aspartic acid). The phases were then washed with solvents that would be used in analytical mobile phases. The second half of the research investigates the lot to lot reproducibility of most promising of these tethering groups, APPA.

Experimental

The stability and reproducibility of the ZirChrom®-Chiral(S)LEU column was investigated. The separation conditions were as follows:

Column: ZirChrom®-Chiral(S)LEU, 100mm x 4.6mm i.d. (Part #: ZRC01-1046)
Mobile Phase: See Figures
Temperature: 30 °C with Metalox™ 200-C column heater
Flow Rate: 1.0 ml/min.
Injection Vol.: 0.5 µl
Pressure Drop: 195 bar
Detection: UV at 254 nm

Figure 1 confirms, as expected, that the choice of tethering groups has a great effect on column stability.

Aminopropylphosphonic acid (APPA) was the most stable among the three tethering groups tested.

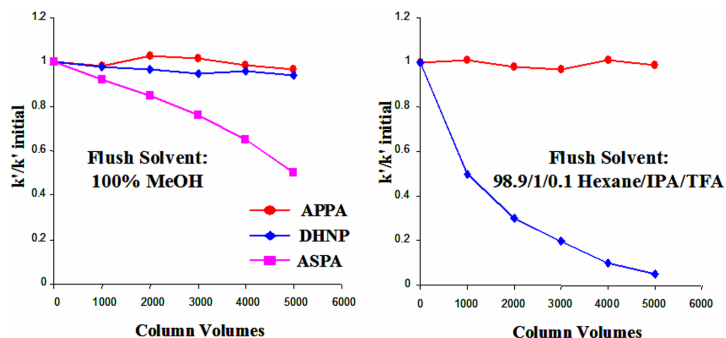


Figure 1: Stability comparison of ZirChrom®-Chiral(S)LEU tethering groups.

Lot reproducibility studies shown in Figure 2 confirm that a chiral selector can be attached reproducibly to the zirconia surface using the APPA tethering group in a two-step reaction scheme. Further research (data not shown) indicates that the choice of tethering group can also have a minor effect on chiral selectivity.

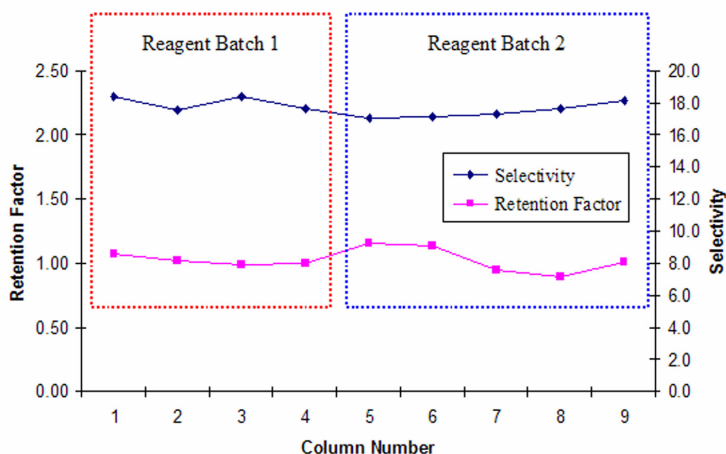


Figure 2: QC tests for two different batches of zirconia with APPA-tethered ZirChrom®-Chiral(S)LEU and nine columns packed with these two batches; probe solutes were (R/S)-naphthylleucine ethyl ester; retention factor is shown for R-naphthylleucine ethyl ester. Mobile Phase: 99/1 hexane/isopropanol.

ZirChrom now offers a full line of chiral phases. ZirChrom chiral phases utilize a fully renewable platform and offer unique selectivity, high efficiency, and excellent stability. Please contact ZirChrom technical support at 1-866-STABLE-1 or support@zirchrom.com for more information regarding this exciting new technology.

References

(1) American Laboratory, 37, No. 21, pp 22-4 (October 2005).

ZirChrom Separations, Inc.
617 Pierce Street, Anoka, MN 55303
1-866-STABLE-1
support@zirchrom.com

Visit www.zirchrom.com for more application notes using ultra-stable, high efficiency ZirChrom columns.



ZirChrom®

Online Regeneration of ZirChrom®-Chiral Zirconia-based CSP

Richard Henry, Ph.D.*, Bingwen Yan, Ph.D.*, Clayton McNeff, Ph.D.* and Thomas Hoye, Ph.D.**.

*ZirChrom Separations, Inc.

**University of Minnesota

Technical Bulletin # 317

High-performance liquid chromatography has become the dominant method for the analytical and preparative separation of chiral pharmaceuticals. However, no current chiral stationary phase uses zirconia or inorganic oxides other than silica as a substrate. A promising new route to preparing chiral stationary phases using a zirconia substrate has been developed. The following details the reaction scheme and reproducibility of the online regeneration of these new versatile, durable, and efficient ZirChrom®-Chiral zirconia-based chiral stationary phases (CSP).

Introduction

In this application note we present a method for the online regeneration of zirconia-based chiral columns. The phase is created using a simple single-step reaction scheme. The reproducibility of column regeneration is demonstrated by measuring the separating capability of the phase is examined before and after the stripping procedure as well as after regeneration. This new technology, targeted for future commercialization, will allow users to rapidly evaluate the suitability of different chiral selectors for resolving enantiomers with a single, highly stable zirconia column plus a kit of pure CSP coating reagents.

Experimental

The separation conditions were as follows:

Columns: ZirChrom-PHASE, 100 mm x 4.6 mm i.d.
(Part Number: ZR02-1046)
Mobile Phase: See Figures
Temperature: 30 °C with Metalox™ 200-C column heater
Flow Rate: 1.0 ml/min.
Injection Vol.: 0.5 µl
Pressure Drop: 195 bar
Detection: UV at 254 nm

Figure 1 illustrates the single-step reaction scheme that enables chiral selectors to be easily removed and replaced by simple, low-temperature reactions.

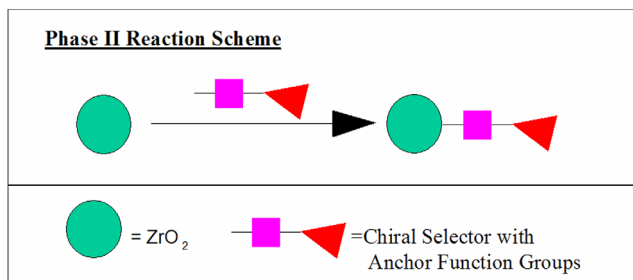


Figure 1: A chiral reagent containing an integral tethering group is attached to bare zirconia in a simple, one-step process.

A separation on a zirconia-based chiral column prepared with N-(4-nitrobenzoyl)-L-glutamic acid using a single-step reaction process is shown in Figure 2; the column was stripped and regenerated to demonstrate reproducibility of the simple, one-step loading process. After stripping, the bare zirconia column did not exhibit any retention or selectivity for the chiral analytes but resumed its initial performance after regeneration.

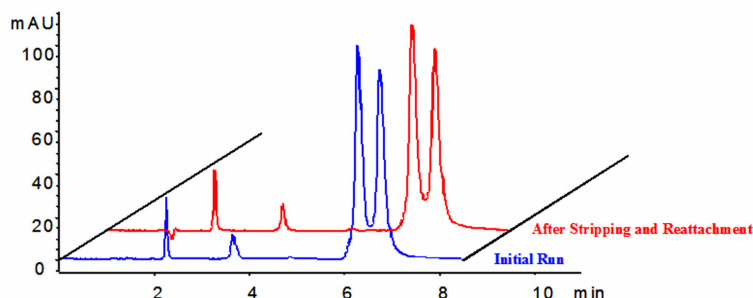


Figure 2: Comparison between the separation of (R/S)-2,2,2-trifluoro-1-(9-anthryl)ethanol before and after stripping and reattaching an N-(4-nitrobenzoyl)-L-glutamic acid (CSP) reagent according to the scheme shown in Figure 1. Mobile phase conditions: 99/1 hexane/isopropanol.

ZirChrom now offers a full line of chiral phases. ZirChrom chiral phases utilize a fully renewable platform and offer unique selectivity, high efficiency, and excellent stability. Please contact ZirChrom technical support at 1-866-STABLE-1 or support@zirchrom.com for more information regarding this exciting new technology.

References

- (1) American Laboratory, 37, No. 21, pp 22-4 (October 2005).
- (2) Phase II SBIR Grant (NIH R44 HL070334-02A2).

ZirChrom Separations, Inc.
617 Pierce Street, Anoka, MN 55303
1-866-STABLE-1
support@zirchrom.com

Visit www.zirchrom.com for more application notes using ultra-stable, high efficiency ZirChrom columns.



ZirChrom®

Performance Comparison: ZirChrom®-Chiral to Silica CSPs

Richard Henry, Ph.D.*, Bingwen Yan, Ph.D.*, Clayton McNeff, Ph.D.* and
Thomas Hoyer, Ph.D.**
*ZirChrom Separations, Inc.
**University of Minnesota

Technical Bulletin # 319

High-performance liquid chromatography is the dominant method for both analytical and preparative separation of chiral pharmaceuticals. However, no currently available commercial chiral stationary phase uses zirconia as a substrate. A promising new route to preparing chiral stationary phases using a zirconia substrate particle has been developed (See ZirChrom Technical Bulletin #313). The following demonstrates the performance of these new zirconia-based chiral HPLC columns compared to that of analogous conventional silica phases. The zirconia chiral selector attachment chemistry is extremely stable under routine use conditions with the added potential feature of selector regeneration or substitution, for chiral screening, by use of a high pH wash.

Introduction

The zirconia-based chiral phases used in this work were synthesized using a two-step approach to provide a more robust and flexible platform for chiral stationary phase (CSP) design when compared to silica gel. Columns produced in this manner were compared to silica columns having analogous chiral selectors and found to have similar resolving power for the selected probe enantiomers. Most importantly, the Lewis acid-base bonded chiral selectors on zirconia were found to be stable enough for extended routine use; however, they could be completely removed by washing with a high pH (>pH 12) aqueous solution and could be then easily regenerated. Results shown in Figures 1 & 2 for the zirconia-based CSP columns are comparable to the performance of silica-based columns having an analogous chiral selector. This new approach, the synthesis of chiral stationary phases that are regenerable on-column, promises to significantly extend the useful lifetime of both analytical and preparative enantiomer separation media.

Experimental

Brush-type CSPs were selected for initial experiments due to their ease of synthesis, wide scope of applicability, and large body of available silica-based separations data for comparison. The separation conditions were as follows:

Column: See Figures, All 5 mm x 4.6 mm columns
Mobile Phase: See Figures
Temperature: 30 °C with Metalox™ 200-C column heater
Flow Rate: 1.0 ml/min.
Injection Vol.: 0.5 µl
Pressure Drop: 195 bar
Detection: UV at 254 nm

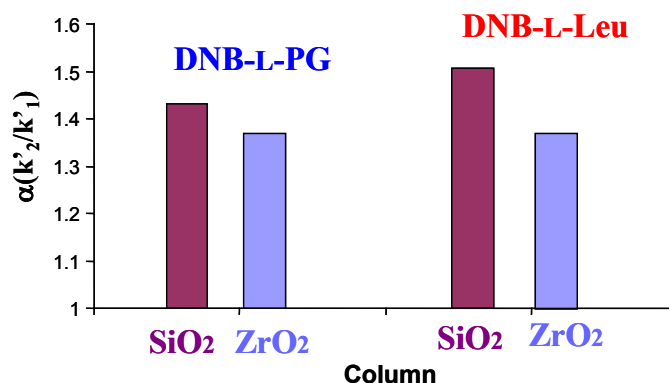


Figure 1: ZirChrom®-Chiral (S)LEU CSP column (part # ZRC01-0546) & ZirChrom®-Chiral (S)PG CSP column (part # ZRC04-0546) Selectivity Comparisons. Conditions: 99/1 hexane/isopropanol. Compound: trifluoroantrhyl ethanol.

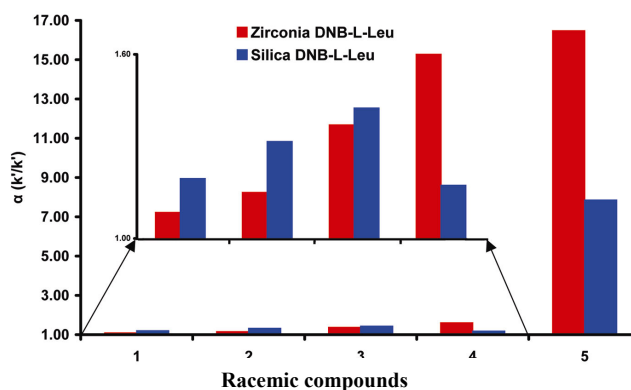


Figure 2: Comparison of ZirChrom®-Chiral (S)LEU CSP column (part # ZRC01-0546) and Silica CSP Selectivity. Conditions: 99/1Hexane/isopropanol, 1. trans-stilbene oxide, 2. 1,1'-bi-2-naphthol, 3. trifluoroantrhyl ethanol, 4. napropamide, 5. 1-naphthyl leucine ester.

Please contact ZirChrom technical support at 1-866-STABLE-1 or support@zirchrom.com for more information regarding this exciting new technology.

ZirChrom Separations, Inc.
617 Pierce Street, Anoka, MN 55303
1-866-STABLE-1
support@zirchrom.com

Visit www.zirchrom.com for more application notes using ultra-stable, high efficiency ZirChrom columns.