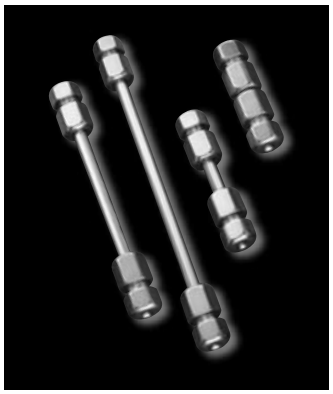
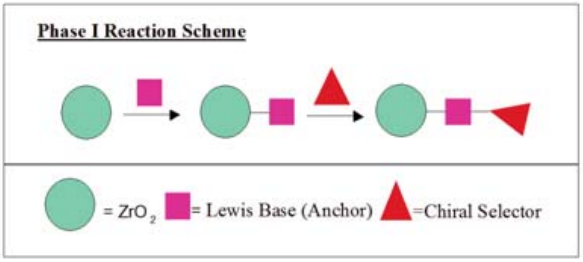
Method Development with ZirChrom®-Chiral

ZirChrom Separations, Inc. is pleased to announce the arrival of a full line of zirconia-based chiral HPLC phases, ZirChrom®-Chiral. These new patent-pending chiral stationary phases incorporate the unsurpassed chemical and mechanical stability of zirconia with the flexibility of Lewis acid/base anchored chiral selectors. This combination creates a CSP that is reproducible, durable and can be regenerated.

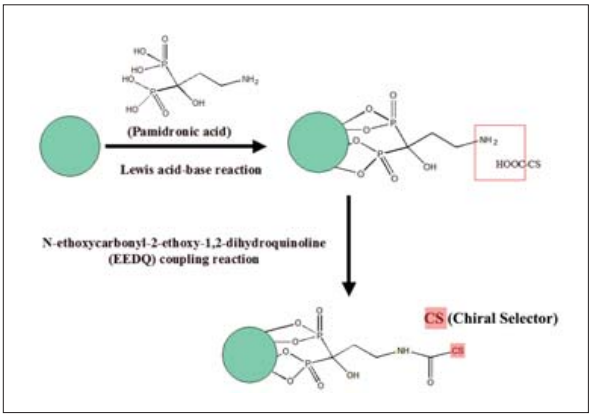
The surface chemistry of zirconia is very different from silica gel due to the presence of a high population of strong Lewis acid (Zr+4) sites. The synthesis of the ZirChrom®-Chiral phases capitalizes on the presence of Lewis acid sites on the surface of the zirconia to provide a more robust and chemically flexible platform for CSP design.

This novel approach to the production of chiral stationary phases on zirconia that has been developed that offers significant method development advantages over other platforms. Zirconium atoms on the surface of zirconia (zirconium dioxide) particles act as strong Lewis acid sites that allow for facile attachment of chiral stationary phases by a tethering group having strong electron donor chelating properties. ZirChrom®-Chiral phases are synthesized using a simple two-step approach: 1) attach an appropriate tethering group to the zirconia surface through a Lewis acid-base reaction, and 2) covalently attach the desired CSP to the tethering group using amide bond formation chemistry.





Typical two-step chemical reaction involving the addition of a reactive chelator (pamidronic acid) followed by EEDQ amide bond formation with a chiral carboxylic acid reagent.



Example of a typical chemical reaction used to tether a chiral selector to the zirconia surface.

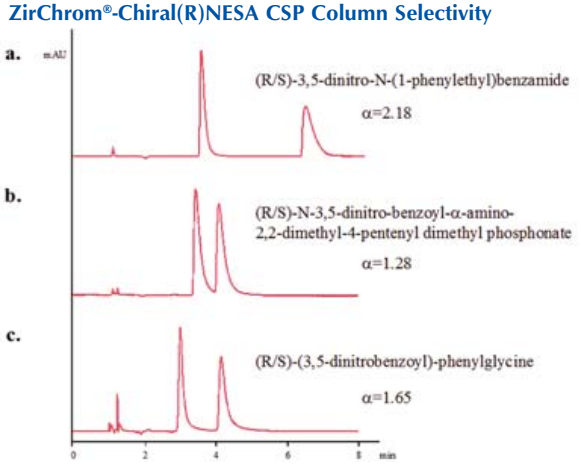
PACKING	USE	PART
ZirChrom®-Chiral(S)LEU	Pirkle Chiral Phase Pi Acceptor	ZRC01
ZirChrom®-Chiral(R)NESA	Pirkle Chiral Phase Pi Donor	ZRC02
ZirChrom®-Chiral(S)NESA	Pirkle Chiral Phase Pi Donor	ZRC03
ZirChrom®-Chiral(S)PG	Pirkle Chiral Phase Pi Acceptor	ZRC04
ZirChrom®-Chiral(R)PG	Pirkle Chiral Phase Pi Acceptor	ZRC05
ZirChrom®-CelluloZe	Carbohydrate Chiral Phase	ZRC06

Microbore, Semi-Prep and Prep Formats Available–see Page 24

ZIRCHROM®-CHIRAL

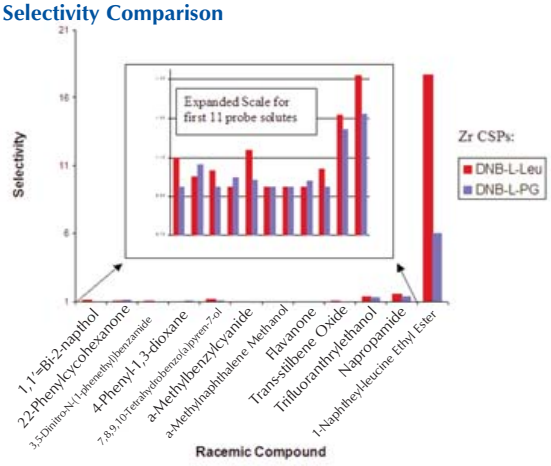
ZirChrom®-Chiral columns were compared to silica columns having analogous chiral selectors and found to have similar resolving power for the selected probe enantiomers. Most importantly, the chemisorbed chiral selectors on ZirChrom®-Chiral were found to be stable enough for extended routine use; however, they could be completely removed by washing with a high pH (>pH 12) aqueous solution and could be easily regenerated.

ZirChrom®-Chiral(R)NESA CSP Column Selectivity



LC CONDITIONS
Mobile phase: |A| Hexane
|B| Methanol
|C| Isopropanol
|D| TFA
Isocratic Elution: a)&b) 88.9/0/11/0.1 A/B/C/D, c) 75/15/9/1 A/B/C/D
Flow rate: 1.0 ml/min
Temperature: 30 °C
Column: ZirChrom®-Chiral(S)NESA, 100 x 4.6 mm i.d. (**part# ZRC02-1046**)

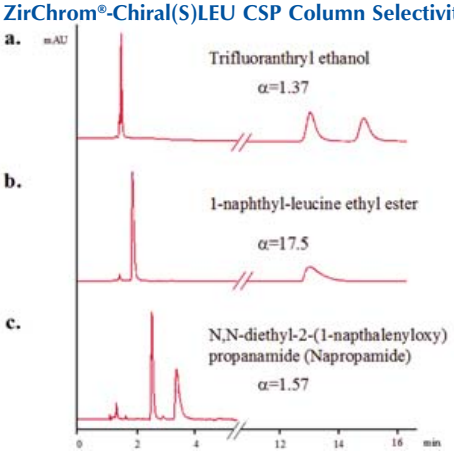
ZirChrom®-Chiral(S)LEU & ZirChrom®-Chiral(S)PG Selectivity Comparison



LC CONDITIONS
Mobile phase: |A| Hexane
|B| Isopropanol
Isocratic Elution: 99/1 A/B
Flow rate: 1.0 ml/min
Temperature: 30 °C
Columns: ZirChrom®-Chiral(S)LEU, 100 x 4.6 mm i.d. (**part# ZRC01-1046**)
ZirChrom®-Chiral(S)PG, 100 x 4.6 mm i.d. (**part# ZRC04-1046**)

Selectivity is compared for 12 probe solute enantiomers on zirconia (S)-dintrobenzoyl-L-leucine CSP and zirconia (S)-dinitrobenzoyl-L-phenylglycine CSP. As expected, changing the chiral selector had a significant effect on the resolution of 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.

ZirChrom®-Chiral(S)LEU CSP Column Selectivity



LC CONDITIONS
Mobile phase: |A| Hexane
|B| Isopropanol
Isocratic Elution: 99/1 A/B
Flow rate: 1.0 ml/min
Temperature: 30 °C
Column: ZirChrom®-Chiral(S)LEU, 100 x 4.6 mm i.d.
(**part# ZRC01-1046**)

Introducing ZirChrom®-Chiral: A Revolutionary New Suite of Phases for Chiral Separations

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 #313

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. We present here a new technique for the synthesis of zirconia-based chiral phases. This promising new route to preparing chiral stationary phases combines the chemical and mechanical stability of zirconia substrate with flexible, durable, and efficient chiral selectors to create a new novel line of chiral HPLC columns. The work here was funded by the NIH SBIR phase I and II grant program (Grant Number 2R44HL070334-02A2).

Introduction

Zirconia has many attractive properties for HPLC, including spherical particle shape and narrow size distribution. Additionally, it exhibits unsurpassed chemical and mechanical stability. Its surface chemistry is very different from silica gel due to the presence of a high population of strong Lewis acid (Zr^{+4}) sites (Figure 1). The following method exploits these strong Lewis acid sites on the surface in a two step approach to provide a more robust and flexible platform for CSP design when compared to silica gel.

A New Approach to Making Chiral Stationary Phases

The two-step approach utilized in this research involved first attaching an appropriate tethering group, such as pamidronic acid, to the zirconia surface through a Lewis acid-base reaction, and then covalently attaching the desired CSP to the tethering group using amide bond formation chemistry.[1] This general approach allows for the flexible, durable and efficient functionalization of zirconia with a wide variety of chiral selectors. 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. An illustration of the two-step reaction scheme is shown in *Figure 2*, and a typical chemical reaction is shown in *Figure 3* using pamidronic acid as a very strong tethering agent.

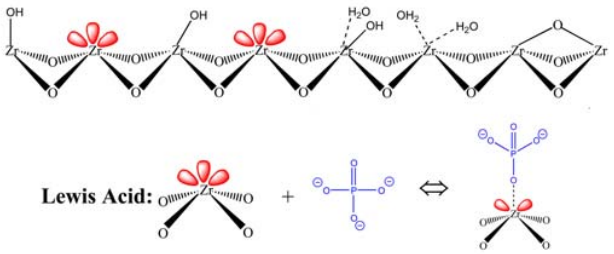


Figure 1: Surface Chemistry of Zirconia particles.

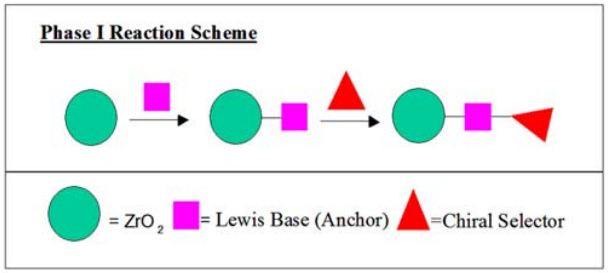


Figure 2: Phase I reaction scheme. A reactive tethering group is attached first, and a chiral selector molecule is attached to the tethering group by an amide bond formation reaction.[1]

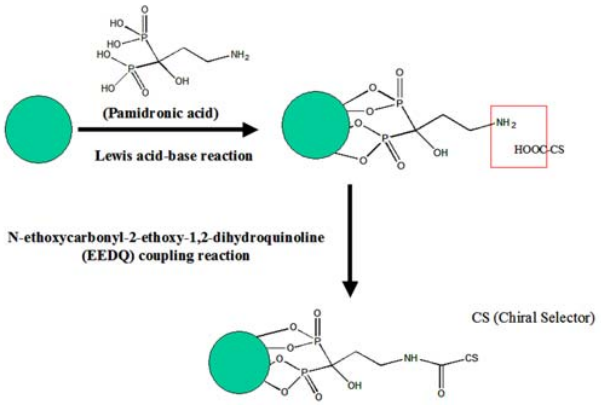


Figure 3: General two-step chemical modification involving the addition of a reactive chelator (pamidronic acid) followed by EEDQ amide bond formation with a chiral carboxylic acid reagent.[1]

Durable, efficient CSP columns were successfully prepared in Phase I research by the two-step reaction scheme using the chiral selectors shown in *Figure 4*.

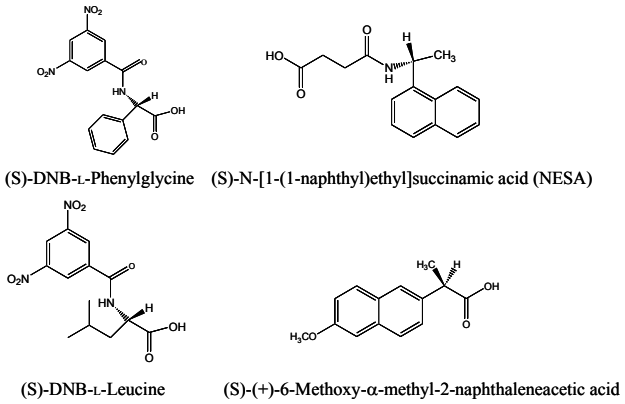


Figure 4: Chiral selector molecules that were evaluated during ZirChrom®-Chiral research.

During phase I research, the tethering group was allowed to react with the unmodified zirconia particles, and the chiral selector was covalently attached to the amino-modified zirconia using the N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) coupling reaction that is commonly employed for peptide synthesis.[1] 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 (See ZirChrom Technical Bulletin 314). The chemisorbed chiral selectors on zirconia were found to be stable enough for extended routine use. Most importantly, the selectors could be completely removed by washing with a high pH (>pH 12) aqueous solution and could be easily regenerated.

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) Yang A, Gehring A, Li T. J Chromatogr A 2000; 878:165–70.

(2) 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.