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.

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

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