Chiral Resolution of Enantiomeric Drugs and Biochemicals by Ligand Exchange HPLC

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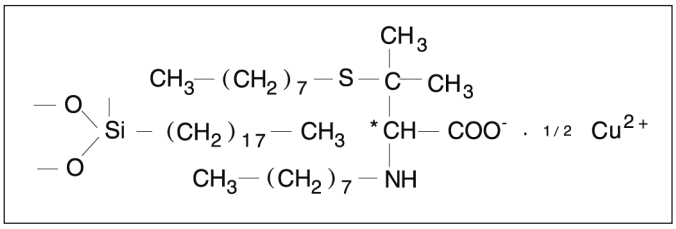
Introduction

In this Technical Note three HPLC chiral stationary phases (CSPs) based on the ligand exchange (LE) separation mode are profiled. These phases are highly effective for the direct resolution of a wide variety of optically active compounds of biological importance. Not only are these three of the most useful phases for separating the enantiomers of α- and β-amino acids and hydroxy acids, but they also show high enantioselectivity for other copper-chelate-forming compounds such as amino alcohols, diamines, dicarboxylic acids, amino lactames and dipeptides.



Shown above is Chirex phase 3126, a stationary phase containing a chiral selector ligand that has been adsorbed onto a reversed phase packing and complexed with a copper ion. The selector ligand is tightly bound (by hydrophobic attraction) to the packing and is part of the stationary phase. The LE separation mechanism is based on the formation of a reversible diastereomeric metal complex between the chiral selector ligand (the CSP) and the chiral solute ligand by coordination with a metal ion, usually copper. Because the selector ligand is chiral, the stereochemistry of the chiral solutes will determine the elution order; the enantiomer which forms the most energetically-stable complex with the CSP will be the one that is retained the longest. Chirex phase 3126 was highlighted previously in Technical Note TN-1005.

Two other, unique chiral LE phases are also profiled in this Note. The three phases combine to offer chiral chromatographers exceptional utility for the direct resolution of enantiomers that are typically difficult to separate.

Instrumentation & Equipment

Analyses were performed using an HP 1100 LC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump, in-line degasser, multi-wavelength detector, and autosampler. HP Chemstation software was used for the data analysis. The HPLC columns used for the analysis are Chirex brand (Phenomenex, Torrance, CA, USA) and Sumichiral OA chiral columns (Sumika Chemical Analysis Service, Ltd., Japan). See Ordering Information. Standards were purchased from Sigma (St. Louis, MO), Aldrich (Milwaukee, WI), or Fluka (Ronkonkoma, NY), depending on availability.

Results & Discussion

Typically run in the ***reversed phase mode***, retention times on these LE phases are easily controlled through the addition of small amounts of organic modifiers. High concentrations of organic modifiers are generally not permitted due to the possible stripping of the hydrophobically-bound ligand from the reversed phase support. However, for most compounds the use of organic modifiers will be minimal; rapid elution with ample resolution is often a hallmark of these separations. Moreover, chiral separations are almost always performed without the need for derivatization – a distinct advantage over other types of chiral columns.

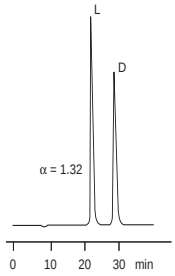
The columns are quite stable and long-lived, yielding reproducible data under routine conditions. From the applications shown below, it is obvious the inherent enantioselectivity for certain types of compounds is exceptional.

Chirex 3216 (D)-Penicillamine - An excellent phase for the direct resolution of D- and L- α-amino acids and α-hydroxy acids. It is more retentive than the other two CSPs.

App ID 14094

Lactic Acid

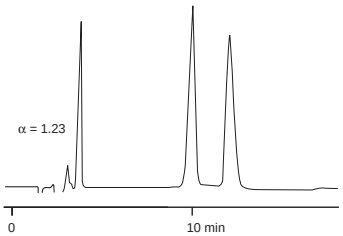
Column:	Chirex 3126
Dimensions:	150 x 4.6mm
Order No.:	00F-3126-E0
Mobile Phase:	1mM Copper (II) sulfate in water
Flow Rate:	1mL/min
Detector:	UV @ 254nm



App ID 13785

Baclofen

Column:	Chirex 3126
Dimensions:	150 x 4.6mm
Order No.:	00F-3126-E0
Mobile Phase:	2mM Copper (II) sulfate in water / Isopropanol (85:15)
Flow Rate:	1mL/min
Detector:	UV @ 254nm

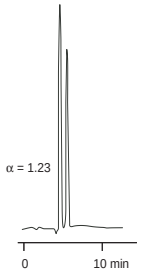


Sumichiral OA-5500 Chiral Schiff Base - This phase offers superior separation of α-amino acids, α-amino alcohols and various biogenic amines. Faster separations are often obtained compared with the D-Penicillamine CSP Chirex 3126.

App ID 5618

Norphenylephrine

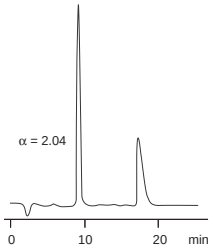
Column:	Sumichiral OA-5500
Dimensions:	150 x 4.6mm
Order No.:	CH0-1861
Mobile Phase:	2mM Copper (II) sulfate in water
Flow Rate:	1mL/min
Detector:	UV @ 254nm



App ID 5619

Phenylalaninol

Column:	Sumichiral OA-5500
Dimensions:	150 x 4.6mm
Order No.:	CH0-1861
Mobile Phase:	1mM Copper (II) sulfate in water
Flow Rate:	1mL/min
Detector:	UV @ 254nm

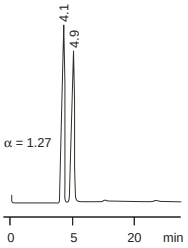


Sumichiral OA-6100 L-Tartaric acid-mono-L-valine (S)-1-(α-naphthyl) ethylamide - This phase provides exceptional selectivity and fast separations for the enantiomers of β-amino acids and β-hydroxy acids.

App ID 12813

3-Aminobutyric acid

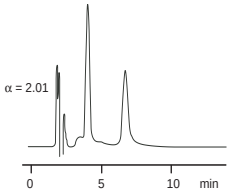
Column:	Sumichiral OA-6100
Dimensions:	150 x 4.6mm
Order No.:	CH0-1863
Mobile Phase:	1mM Copper (II) acetate in water
Flow Rate:	1mL/min
Detector:	UV @ 254nm



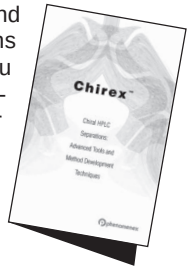
App ID 5620

DOPA

Column:	Sumichiral OA-6100
Dimensions:	150 x 4.6mm
Order No.:	CH0-1863
Mobile Phase:	1mM Copper (II) sulfate in water / Acetonitrile (95:5)
Flow Rate:	2mL/min
Detector:	UV @ 280nm



If you would like more information on these ligand exchange columns or any of the applications listed, please contact Phenomenex. Also, if you are new to chiral HPLC or are doing method development work call us today to reserve your ***FREE*** copy of our 70-page ***Guidebook to Chiral HPLC Method Development***.



Order Information:

Chirex Ligand-Exchange Type Phase, 150 x 4.6mm ID

Order No.	Description
00F-3126-E0	Chirex phase 3126 (D)-Penicillamine, 150 x 4.6mm ID
CH0-1861	Sumichiral OA-5500 Chiral Schiff Base, 150 x 4.6mm
CH0-1863	Sumichiral OA-6100 L-Tartaric acid-mono-L-valine (S)-1-(α-naphthyl)ethylamide, 150 x 4.6mm ID

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