Direct Separation of Chiral Amino Acids, Hydroxy Acids and Dipeptides by Ligand Exchange

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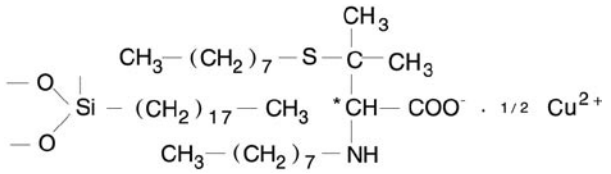
Introduction

In this Application Note the direct resolution of chiral amino acids, hydroxy acids and dipeptides are highlighted. Chirex ligand exchange phase 3126 is an excellent tool for the separation of free, underivatized amino acids. This chiral stationary phase (CSP) also shows high utility for the direct resolution of alpha hydroxy acids and dipeptides. Examples of each of these compound groups are given.



Shown above is Chirex phase 3126, which contains a chiral selector ligand D-penicillamine 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 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.

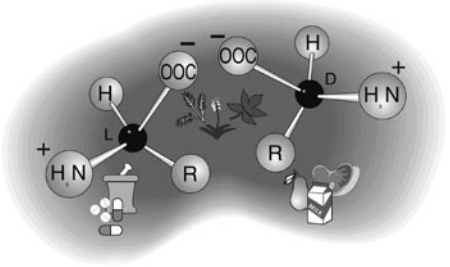
Instrumentation & Equipment

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 column used for the analysis was Chirex 3126 D-Penicillamine 150 x 4.6mm (Phenomenex, Torrance, CA, USA Order No.: 00F-3126-E0). Standards were purchased from Sigma (St. Louis, MO), Aldrich (Milwaukee, WI), or Fluka (Ronkonkoma, NY) chemical companies, depending on availability.

Results & Discussion

Separations on Chirex 3126 are typically run in the ***reversed phase*** mode. Analyte retention times 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 organics modifiers will be minimal; rapid elution with ample resolution is often a hallmark of these separations. Moreover, chiral separations on this ligand exchange column are performed without the need for a time-consuming chemical derivatization.

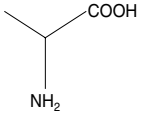
Chirex phase 3126 is quite stable and long-lived, yielding reproducible data under routine conditions. From the applications shown below, it is obvious the inherent enantioselectivity for a variety of alpha amino acids, alpha hydroxy acids and dipeptides is excellent. Table 1 provides a more complete list of applications using Chirex phase 3126. Call us for more details on any of these applications or to discuss your specific application needs.



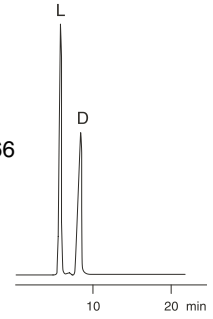
Free Amino Acids

App ID 14004 | Alanine

Column:	Chirex 3126
Size:	150 x 4.6mm ID
Order No.:	00F-3126-E0
Mobile Phase:	1 mM Copper (II) sulfate in water
Flow Rate:	1.0mL/min
Detector:	UV @ 254nm

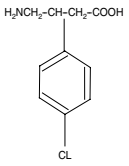


α=1.66

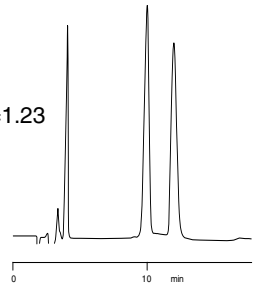


App ID 13785 | Baclofen

Column:	Chirex 3126
Size:	150 x 4.6mm ID
Order No.:	00F-3126-E0
Mobile Phase:	2 mM Copper (II) sulfate in water/Isopropanol (85:15)
Flow Rate:	1.0mL/min
Detector:	UV @ 254nm



α=1.23

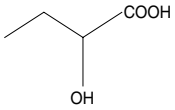


Hydroxy Acids

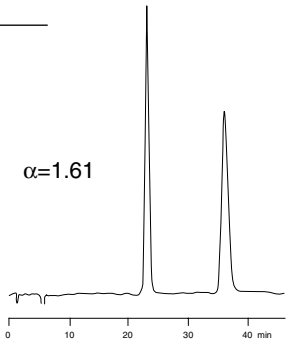
App ID 14098

2-Hydroxybutyric Acid

Column: Chirex 3126
Size: 150 x 4.6mm ID
Order No.: 00F-3126-E0
Mobile Phase: 2 mM Copper (II) sulfate in water/Methanol (85:15)
Flow Rate: 1.0mL/min
Detector: UV @ 254nm



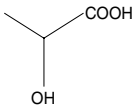
α=1.61



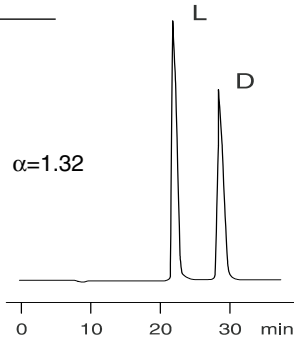
App ID 14094

Lactic Acid

Column: Chirex 3126
Size: 150 x 4.6mm ID
Order No.: 00F-3126-E0
Mobile Phase: 1 mM Copper (II) sulfate in water
Flow Rate: 1.0mL/min
Detector: UV @ 254nm



α=1.32

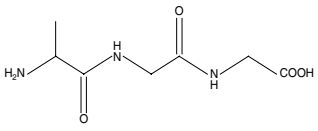


Dipeptides

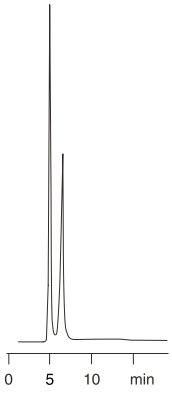
App ID 14082

Alanylglycyl-glycine

Column: Chirex 3126
Size: 150 x 4.6mm ID
Order No.: 00F-3126-E0
Mobile Phase: 1 mM Copper (II) sulfate in water
Flow Rate: 1.0mL/min
Detector: UV @ 254nm



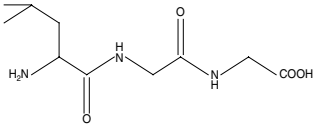
α=1.62



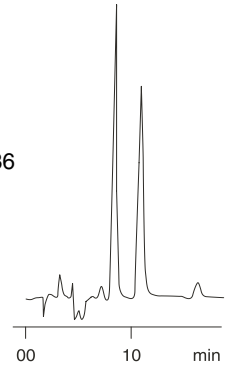
App ID 14083

Leucylglycyl-glycine

Column: Chirex 3126
Size: 150 x 4.6mm ID
Order No.: 00F-3126-E0
Mobile Phase: 2 mM Copper (II) sulfate in water/Methanol (70:30)
Flow Rate: 1.0mL/min
Detector: UV @ 254nm



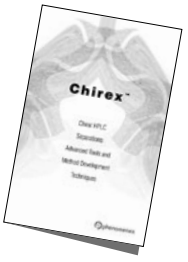
α=1.36



Compounds	Alpha Factor	App ID No.
Alanine	1.66	14004
Alanylglycine	2.26	14080
Alanylglycyl-glycine	1.62	14082
Alloisoleucine	1.67	14038
Allothreonine	1.19	14046
Arginine	2.15	14027
Asparagine	1.10	14049
Aspartic acid	1.42	14019
Baclofen	1.23	13785
p-Boronophenylalanine	1.36	13790
2-amino-n-Butyric acid	1.80	14034
Cystine	2.47	14085
2,6-Diaminopimelic acid	2.77	14066
3-(3,4-Dihydroxyphenyl)-alanine (DOPA)	1.22	13750
Glutamic acid	1.11	14047
Glutamine	1.71	14022
Glycylalanine	1.78	14079
Glycylvaline	1.69	14081
Histidine	1.32	13745
Isoleucine	1.70	14035
Leucine	1.56	14009
Leucylglycyl-glycine	1.36	14083
Lysine	1.83	14018
Methionine	1.42	14024
alpha-Methyl Leucine	1.59	14457
alpha-Methyl Tryptophan	1.18	14456
Naphthylglycine	1.42	13789
Norvaline	1.95	14029
Ornithine	1.38	14041
Phenylalanine	1.44	13740
Phenylglycine	1.78	13748
Pipecolic acid	1.77	14031
Proline	2.50	14011
Serine	1.17	14016
Threonine	1.20	14043
dl-Threo-3-phenylserine	1.15	13787
Tryptophan	1.11	13737
Tyrosine	1.34	13743
Valine	1.91	14006

If you would like more information on these ligand exchange columns or any specific application 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***.



Ordering Information

Order No.	Description
00F-3126-E0-TN	Chirex 3126 (D)-Penicillamine, 150 x 4.6mm

2509_L

CHIREX™

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