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 in Figure 1 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, 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.

Figure 1: Chirex ligand exchange phase 3126.

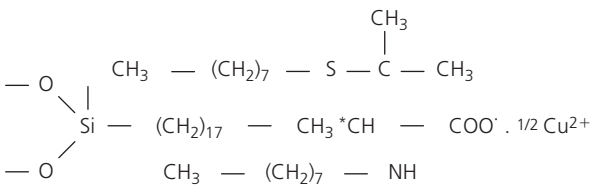
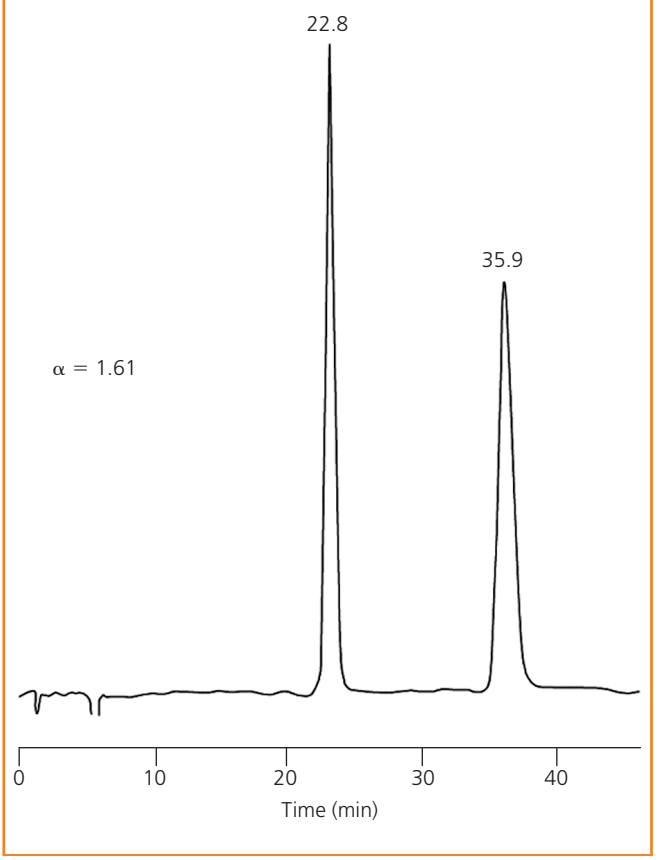


Figure 2: Chiral HPLC of 2-Hydroxybutyric Acid (separation factor alpha = 1.61). Column: 150 mm × 4.6 mm i.d. Chirex 3126; mobile phase: 2 mM copper (II) sulphate in water/methanol (85:15); flow-rate: 1 mL/min; detector: UV at 254 nm.



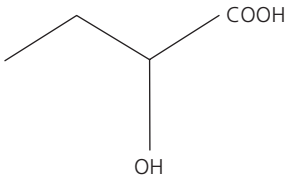
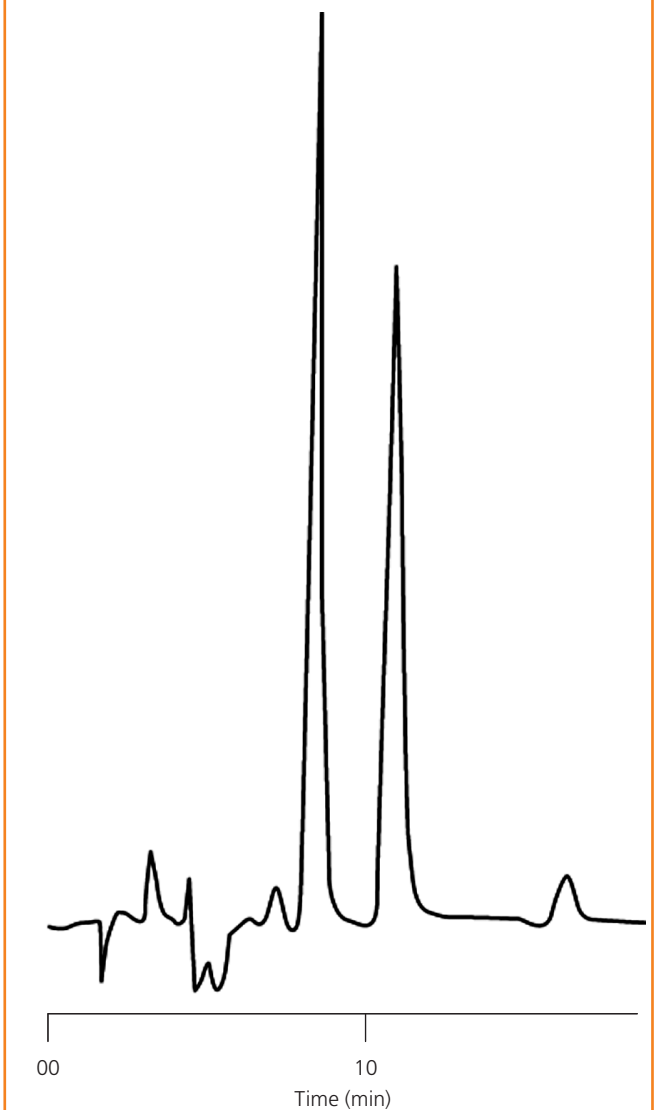


Figure 3: Chiral HPLC of Leucylglycyl-glycine (separation factor alpha = 1.36). Column: 150 mm × 4.6 mm i.d. Chirex 3126; mobile phase: 2 mM Copper (II) sulphate in water/methanol (70:30); flow-rate: 1 mL/min; detector: UV at 254 nm.



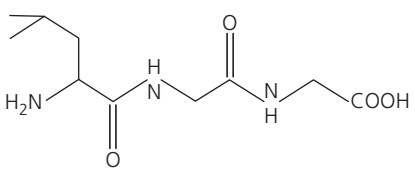
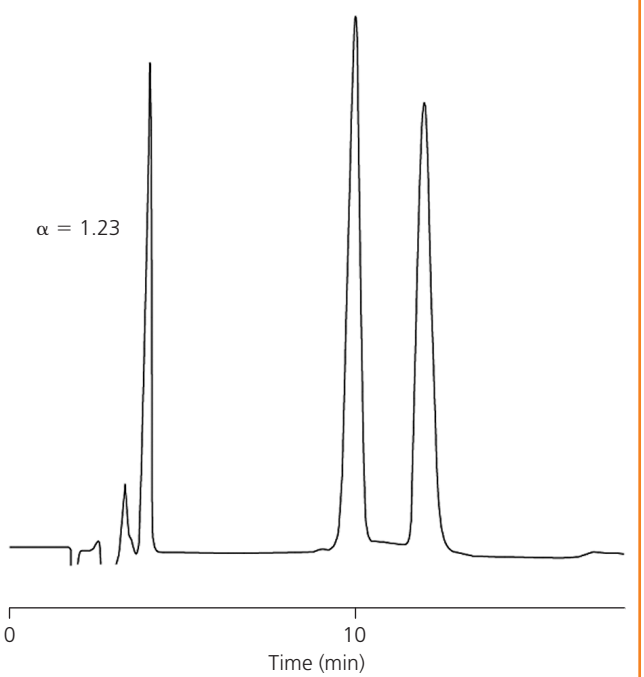
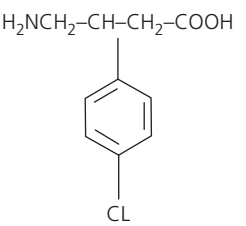


Figure 4: Chiral HPLC of Baclofen (separation factor alpha = 1.23). Column: 150 mm × 4.6 mm i.d. Chirex 3126; mobile phase: 2 mM copper (II) sulphate in water/isopropanol (85:15); flow-rate: 1 mL/min; detector: UV at 254 nm.





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.

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