Application Note

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Chiral Analysis of Hydrophobic Amino Acids with Agilent InfinityLab Poroshell 120 Chiral-T Column

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Abstract

The chiral separation of a series of underivatized aliphatic amino acids was performed using an Agilent InfinityLab Poroshell 120 Chiral-T column using a methanol/ammonium formate buffer mobile phase. The separation of these D- and L-enantiomers is monitored using an ELSD detector. The L-enantiomer elutes first in all four cases.

Introduction

Amino acids are organic compounds containing amine (-NH_2) and carboxyl (-COOH) functional groups, along with a side chain (R group) specific to each amino acid. The 20 amino acids that function as building blocks of proteins are classified as standard. Of these 20 standard amino acids, 19 possess chiral centers. Most naturally occurring amino acids are L-stereoisomers, although a few D-amino acids occur in bacterial envelopes and in some antibiotics. Because of their biological significance, amino acids are important in nutrition, and are commonly used in nutritional supplements, fertilizers, feed, and food technology. Industrial uses include the production of drugs and biodegradable plastics,

Amino acids can also be classified by properties derived from their side chains. These properties include polar (neutral, basic, and acidic) and hydrophobic (aromatic and aliphatic). Hydrophobicity increases with increasing number of C atoms in the hydrocarbon chain. Most aliphatic amino acids are found within protein molecules, although alanine and glycine can be inside or outside the protein molecule. Glycine, alanine, valine, leucine, and isoleucine have varied aliphatic substitutions, which lead to differences in hydrophobicity.

Superficially porous particle LC columns are a popular tool in liquid chromatography. These columns generate high efficiency at lower pressure compared to their totally porous particle column counterparts. This is primarily due to a shorter mass transfer distance and substantially narrower particle size distribution of the particles in the column. The higher efficiency can be used to speed up analyses or improve results by increasing resolution and sensitivity.

Superficially porous particles have been used on reversed-phase and hydrophilic interaction liquid chromatography (HILIC) separations. With the maturation of superficially porous particle technology, applications for further chemistries and chromatographic techniques, such as chiral separations, are becoming available.

Many chiral separations are carried out using cellulose or amylose-based chiral selection phases (CSP) using normal-phase solvents such as hexane. However, other phases are frequently sought for separation based on more common solvents such as methanol that can be more easily incorporated into a laboratory running reversed-phase methods.

Using mass spectrometry-friendly mobile phases is also desirable. This Application Note demonstrates the UHPLC performance of an InfinityLab Poroshell 120 Chiral-T (2.7 µm) column, and its ability to baseline-separate several aliphatic underivatized amino acids. Figure 1 shows these compounds.

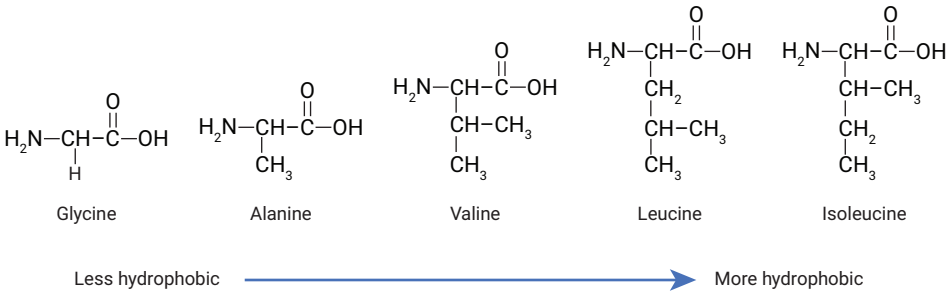

Figure 1. Structure of aliphatic amino acids.

Experimental

An Agilent 1290 Infinity LC configured for low dispersion was used for this work. Table 1 shows the experimental details. Table 2 shows the chromatographic method that was used. All compounds were injected as mixtures of enantiomers and as individual standards for identification.

Individual D-enantiomers of alanine, valine, leucine, and isoleucine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Individual L-enantiomers were purchased from Agilent. These compounds were prepared in water at 2 mg/mL.

Mixtures were prepared by mixing each enantiomer at a 1:1 ratio, yielding a concentration of 1 mg/mL of each individual enantiomer. Ammonium formate and formic acid were also from Sigma Aldrich. Methanol was purchased from Honeywell (Burdick and Jackson, Muskegon, MI, USA). Water was 0.2 µm filtered, 18 u, from a Milli-Q system (Millipore, Burlington, MA, USA).

Table 1. Instrument configuration.

Agilent 1290 Infinity LC System	
Agilent 1290 Infinity II flexible pump (G7104A)	
Agilent 1290 Infinity autosampler (G4226A)	• Seat assembly, ultralow dispersion, for Agilent 1290 Infinity autosampler G4226A (p/n G4226-87030) • Autosampler and heater: capillary, stainless steel, 0.075 × 220 mm, (p/n 5067-4784) • Vial, screw top, amber with write-on spot, certified, 2 mL, 100/pk (p/n 5182-0716) • Cap, screw, blue, PTFE/red silicone septa, 100/pk (p/n 5182-0717)
Agilent 1290 Infinity II multicolumn thermostat (MCT) (G7116B)	• Ultralow dispersion heater (p/n G7116-60021) • Heater and column: InfinityLab Quick Connect assembly, 105 mm, 0.075 mm (p/n 5067-5961) • Column and ELSD capillary, stainless steel, 0.075 × 220 mm, SV/SLV (p/n 5067-4784)
Agilent 1290 Infinity II evaporative light scattering detector (G7102A)	• Evaporator temperature: 30 °C • Nebulizer temperature: 30 °C • Gas flow rate: 1 SLM • 40 Hz
Agilent OpenLab CDS, version C.01.07	

Table 2. LC method conditions.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 Chiral-T, 2.1 × 100 mm, 2.7 µm (p/n 685775-603)
Mobile phase	Premix 70/30 methanol/ammonium formate, pH 3.0, 25 mM
Flow rate	0.21 m/min
Temperature (column)	30 °C
Injection volume	1 µL
Sample concentration	2 mg/mL in water

Results and discussion

The chromatograms in Figures 2A to 2D show four pairs of aliphatic amino acid enantiomers were baseline resolved on an InfinityLab Poroshell 120 Chiral-T column. The separations were achieved in four minutes or less with baseline resolution for all compounds. The InfinityLab Poroshell 120 Chiral T uses a glycopeptide stationary phase covalently bonded to a robust superficially porous particle. While it is also capable of operation in normal-phase solvents, this column has been found to be stable in a wide variety of reversed-phase mobile phases found in most laboratories.

Teicoplanin is an amphoteric glycopeptide containing both ionizable acidic and basic groups. Thus, they can be positively charged, negatively charged, or neutral depending on the pH of the mobile phase. This allows for ionic interactions involved in chiral recognition when separating ionic compounds using this class of CSP. This is thought to play a major role in chiral recognition for this class of CSPs. Other possible interactions involved with the use of antibiotics as CSPs for chiral recognition include hydrogen bonding, steric, dipole–dipole, and π–π interactions as well as hydrophobic interactions. These interactions may take place in different combinations that are determined by the properties of an individual analyte and the mobile-phase mode used. Each separation mode provides simultaneous but different interactions for chiral recognition.

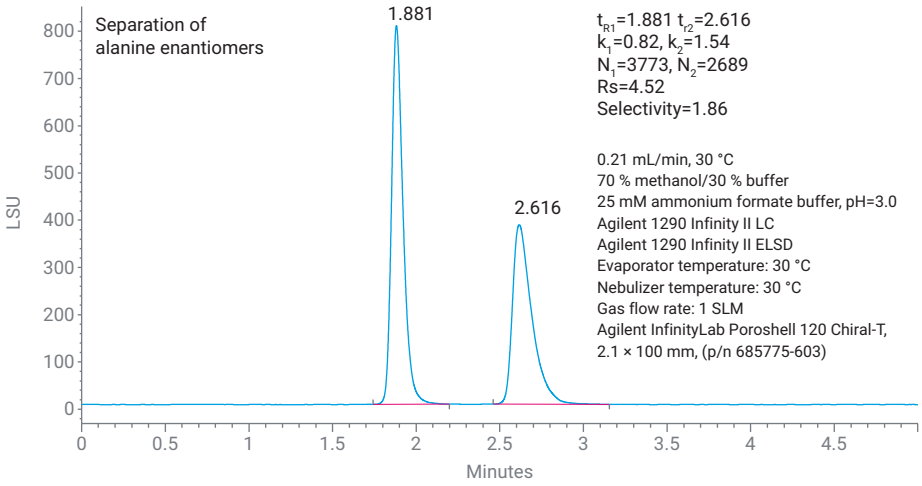

Figure 2A. Separation of alanine enantiomers.

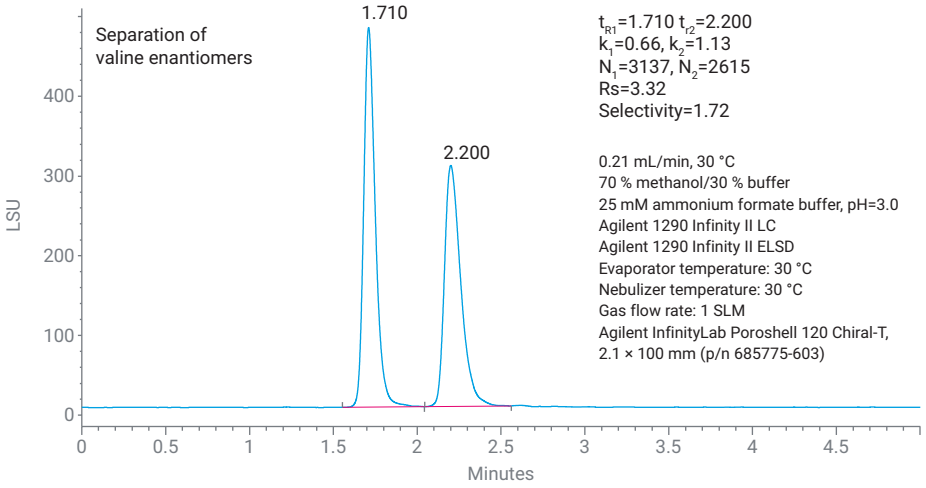

Figure 2B. Separation of valine enantiomers.

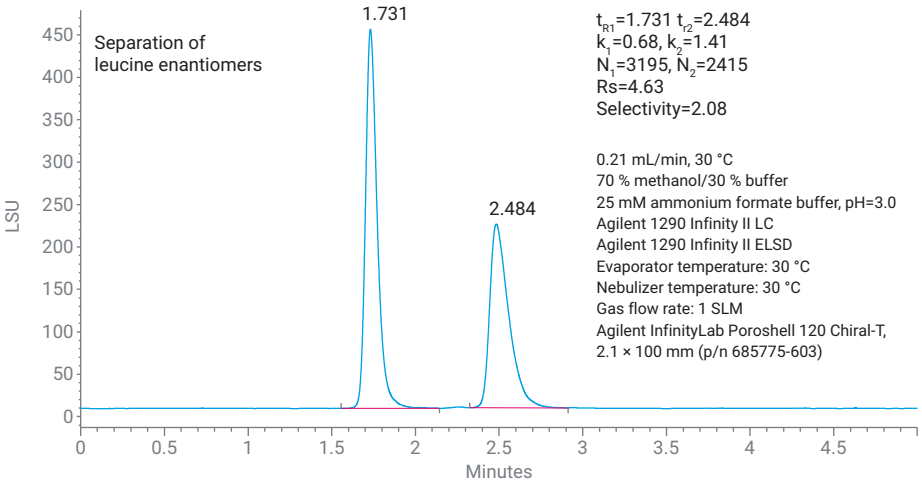

Figure 2C. Separation of leucine enantiomers.

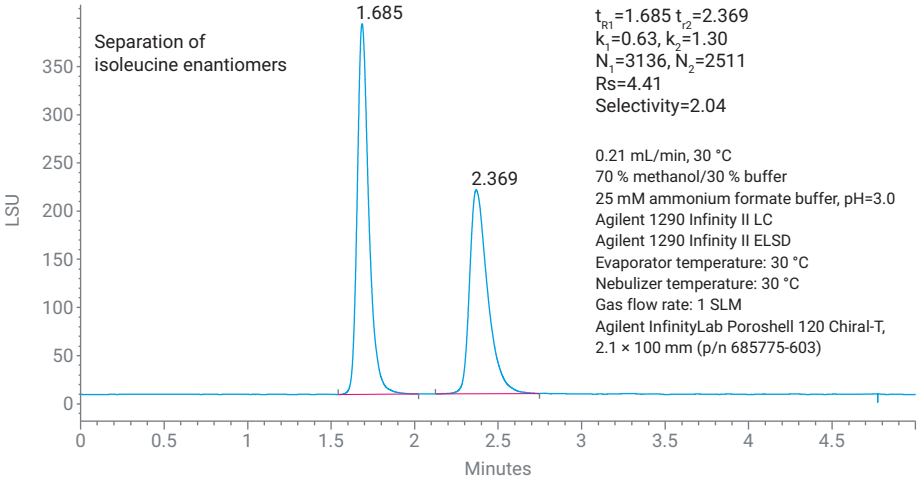

Figure 2D. Separation of isoleucine enantiomers.

Table 3. Summary of chromatographic data for chiral aliphatic amino acid separation.

Compound	k_1	k_2	Rs	Selectivity
Alanine	0.82	1.54	4.52	1.86
Valine	0.66	1.13	3.32	1.72
Leucine	0.68	1.41	4.63	2.07
Isoleucine	0.63	1.30	4.41	2.04

Conclusion

The Agilent InfinityLab Poroshell 120 Chiral-T column provides a robust method for the separation of aliphatic amino acid enantiomers. This column offers good resolution and peak shape for all compounds studied.

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