Application Note

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Chiral Analysis of Sulfur Amino Acids with Agilent InfinityLab Poroshell 120 Chiral-T Columns

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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 with 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. Of the 21 amino acids that function as building blocks of proteins, 20 are encoded in the genetic code with triplet codons and 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) or, in this case, the inclusion of a sulfur molecule.

Experimental

An Agilent 1290 Infinity II LC configured for low dispersion and an Agilent InfinityLab Poroshell Chiral-T column were 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 cysteine and methionine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Individual L-enantiomers were purchased from Agilent Technologies. 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 for 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 µ, from a Milli-Q system (Millipore, Burlington, MA, USA).

Results and discussion

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 difference in performance is primarily due to a shorter mass transfer distance and the 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.

Table 1. Instrument configuration.

Agilent 1290 Infinity II LC	
Agilent 1290 II Flexible Pump (G7104A)	
Agilent 1290 Infinity Autosampler (G4226A)	• 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)	• Agilent InfinityLab Quick Connect heat exchanger, ultralow dispersion (p/n G7116-60021) • Heater and column: Agilent InfinityLab Quick Connect assembly, 0.075 × 105 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 ELSD (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, 2.7µm (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

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, which 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.

The chromatograms in Figures 2A and B show that two pairs of sulfur-containing amino acid enantiomers were separated 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 column uses a glycopeptide stationary phase covalently bonded to a robust superficially porous particle. While it can also be used in other LC modes such as normal-phase or SFC, this column has been found to be stable in mobile phases commonly used in reversed-phase LC.

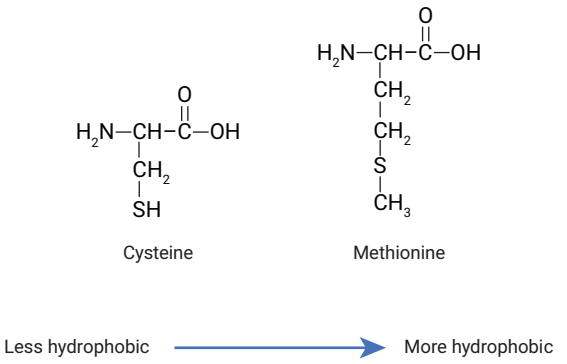

Figure 1. Structure of sulfur-containing amino acids.

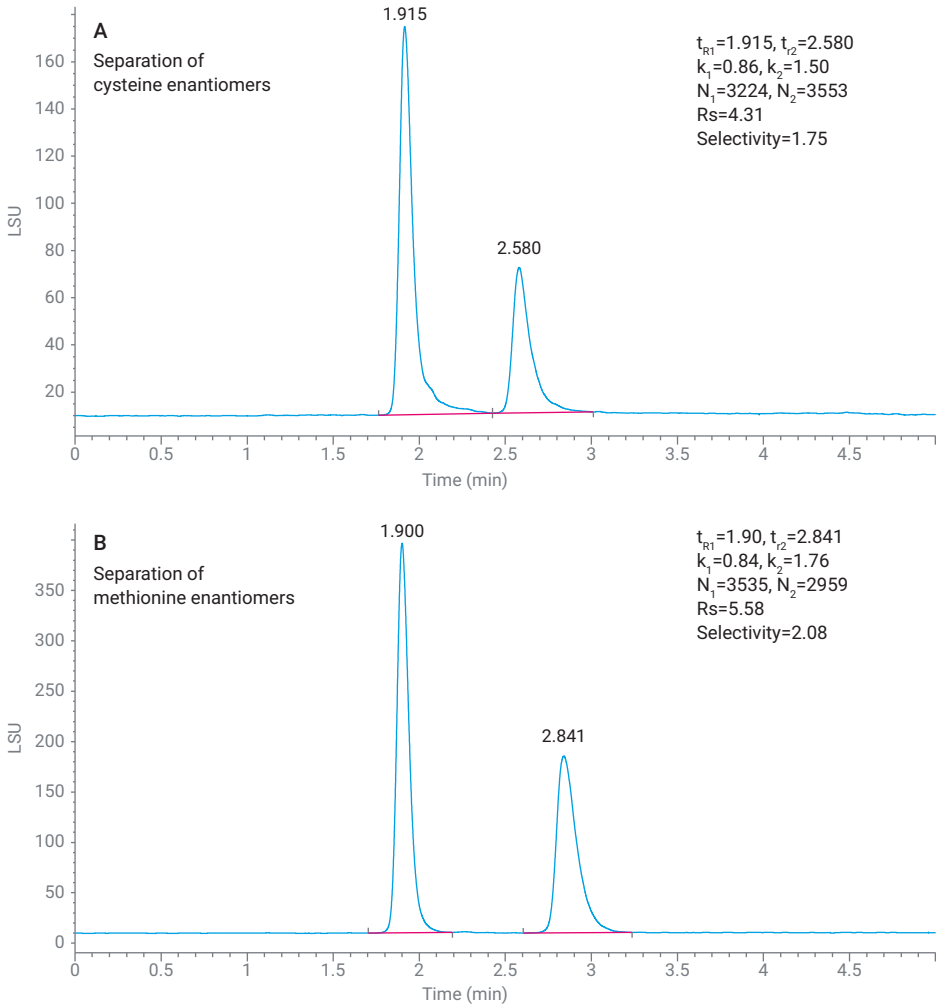

Figure 2. Separation of (A) cysteine and (B) methionine enantiomers.

Conclusion

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

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

Compound	k_1	k_2	Rs	Selectivity
Cysteine	0.86	1.50	4.31	1.75
Methionine	0.84	1.76	5.58	2.08

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