Application Note

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

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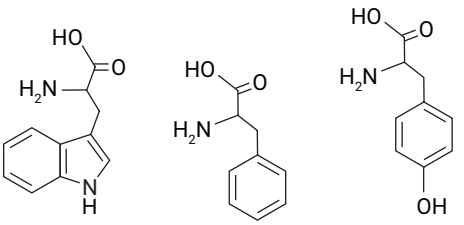
Abstract

The chiral separation of a series of underivatized aromatic 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 was monitored using an ELSD detector or a UV detector. The L-enantiomer eluted 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). Three of the amino acids have aromatic properties and as such can easily be detected using a diode array detector. These include tryptophan, phenylalanine, and tyrosine. Figure 1 shows the structures of these amino acids.



Tryptophan Phenylalanine Tyrosine

Figure 1. Aromatic amino acids.

Experimental

An Agilent 1290 Infinity II 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 tryptophan, phenylalanine, and tyrosine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Individual L-enantiomers were purchased from Agilent Technologies.

Solutions of tryptophan and phenylalanine 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. Due to solubility issues, the tyrosine samples were prepared in a similar fashion except at 50% of the concentrations of tryptophan or phenylalanine. 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).

Table 1. Instrument configuration.

Agilent 1290 Infinity II LC System	
Agilent 1290 Infinity 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 Infinity II DAD (G7117B)	• 10 mm flow cell 1.0 µL V(σ)(p/n G4212-6008) • 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

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 improved performance is primarily due to a shorter mass transfer distance and substantially narrower 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 more easily be 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, 2B, and 2C pairs of aromatic amino acid enantiomers were separated on an InfinityLab Poroshell 120 Chiral-T column and detected with an ELSD detector. The separations were achieved in four minutes or less with baseline resolution for all compounds.

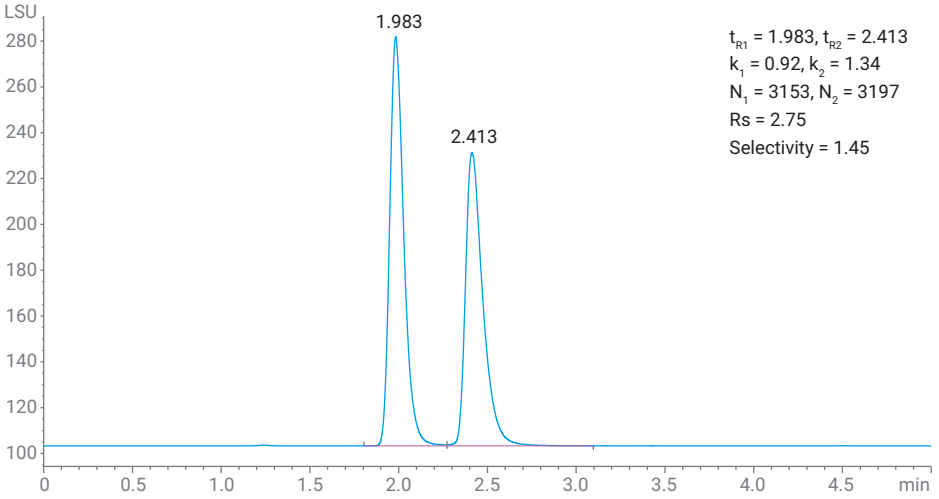


Figure 2A. Separation of tryptophan enantiomers.

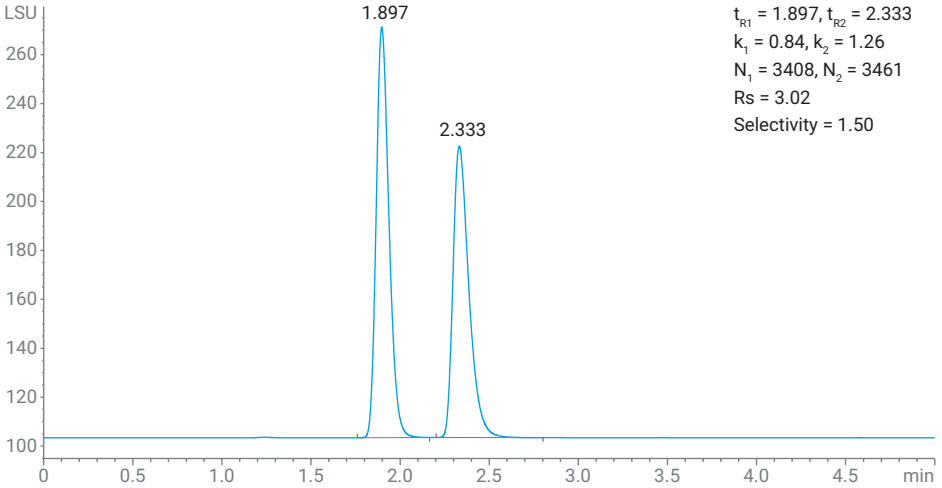


Figure 2B. Separation of phenylalanine enantiomers.

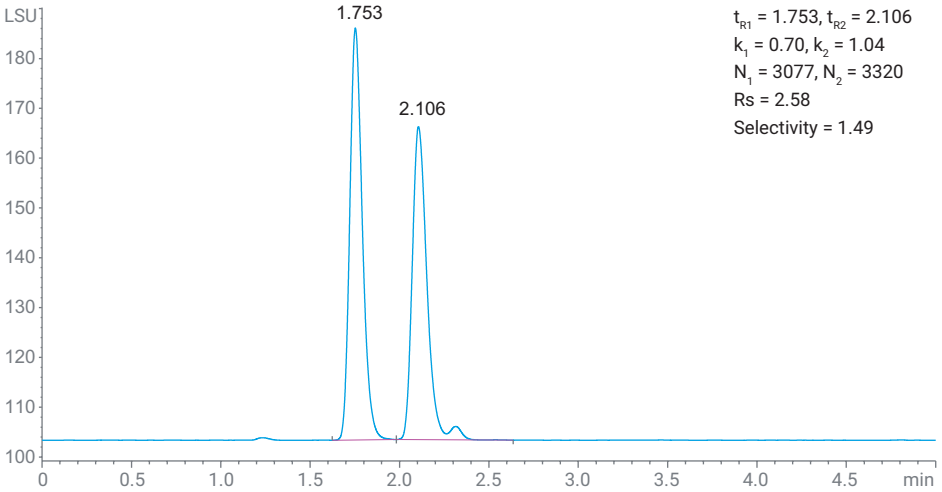


Figure 2C. Separation of tyrosine enantiomers.

In a separate experiment, chromatograms were collected using a diode array detector at 220 nm. Figures 3A, 3B, and 3C show these chromatograms. The spectra were then collected for each of the three compounds. Figures 4A, 4B, and 4C show these UV spectra. Final chromatograms are shown at the UV maximum above 250 nm for each in Figures 5A, 5B, and 5C. The ELSD shows near universal response to all compounds while the UV response in other figures can be used as a more discriminating tool for the analysis of amino acids. Since diode array UV detectors are more common in laboratories, this analysis can be carried out more readily.

The InfinityLab Poroshell 120 Chiral-T column uses a glycopeptide stationary phase covalently bonded to a robust superficially porous particle. While also used in other LC modes such as normal-phase or SFC, this column has been found to be stable in several mobile phases commonly used in reversed-phase LC.

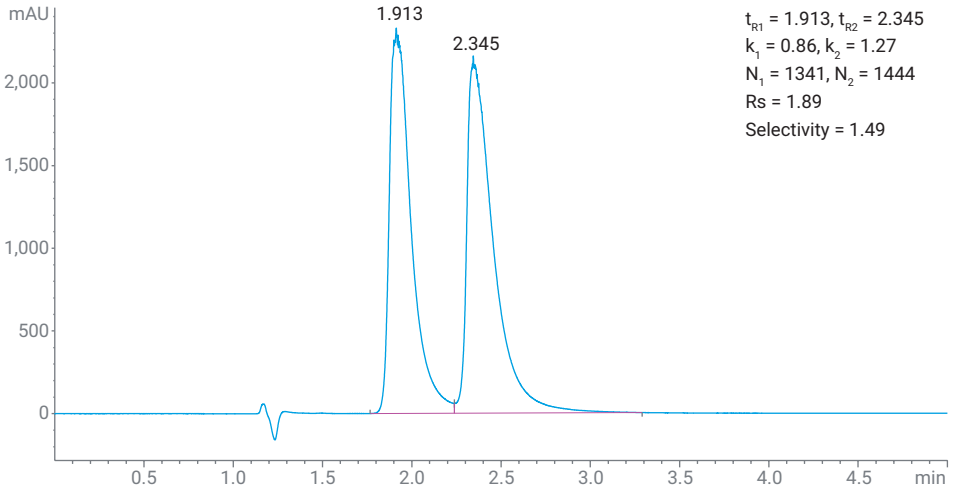


Figure 3A. Separation of tryptophan enantiomers (UV 220).

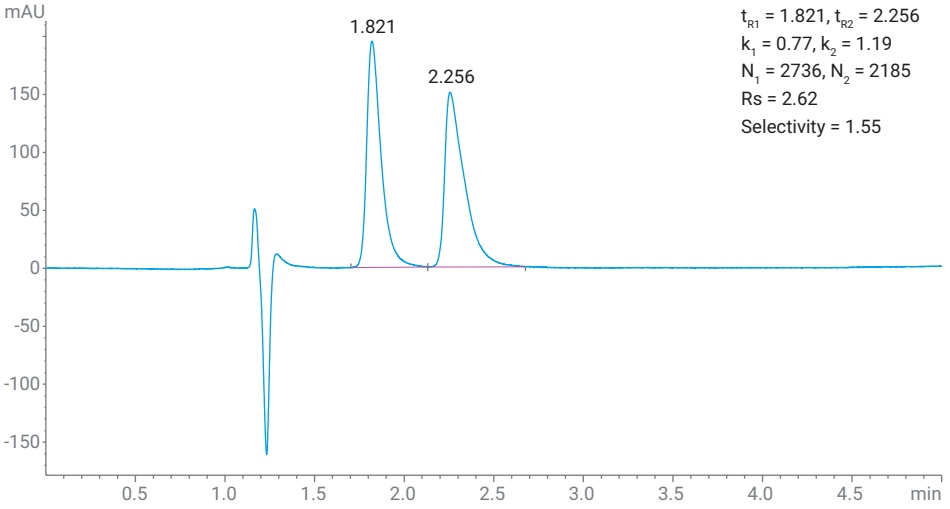


Figure 3B. Separation of phenylalanine enantiomers (UV 220).

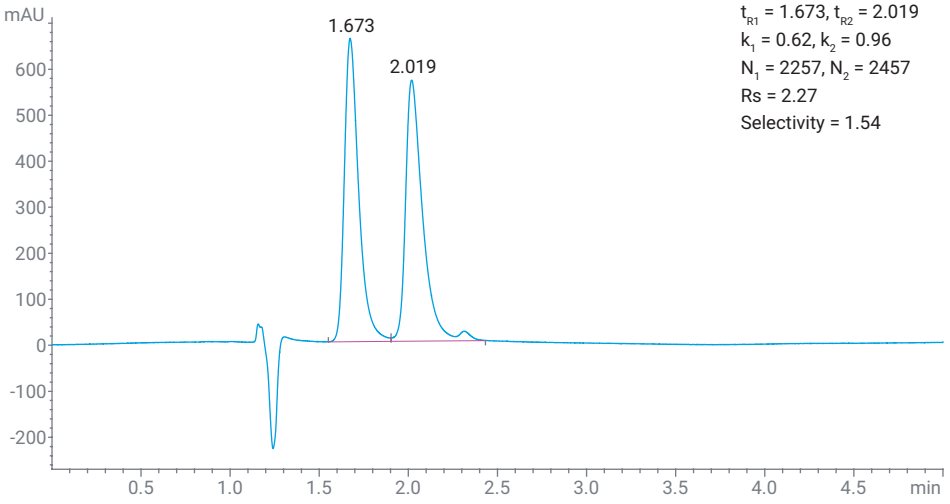


Figure 3C. Separation of tyrosine enantiomers (UV 220).

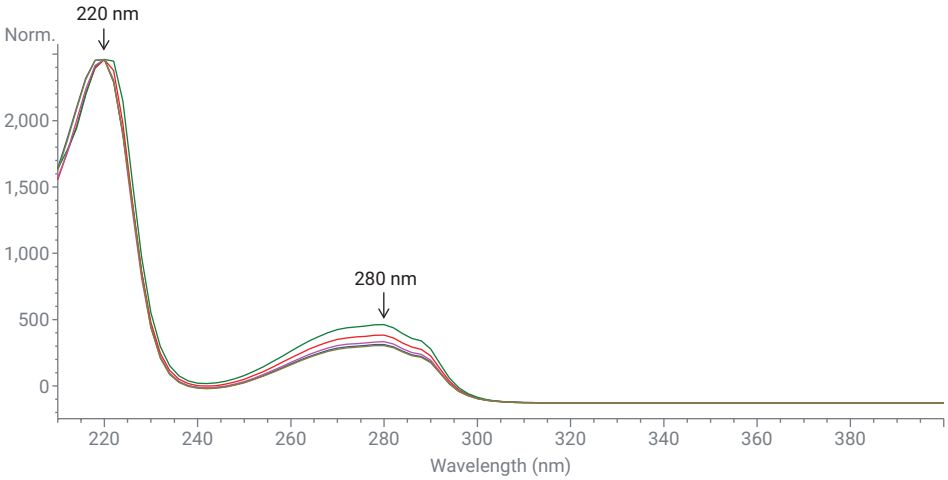


Figure 4A. UV spectrum of tryptophan.

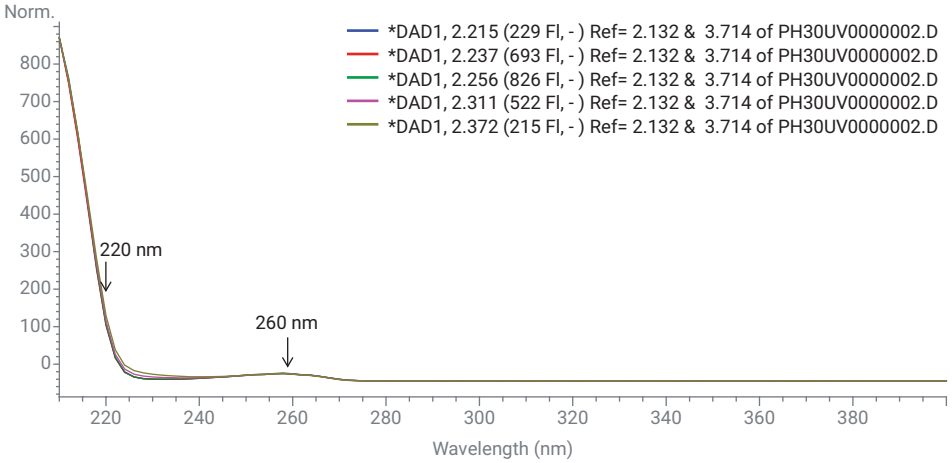


Figure 4B. UV spectrum of phenylalanine.

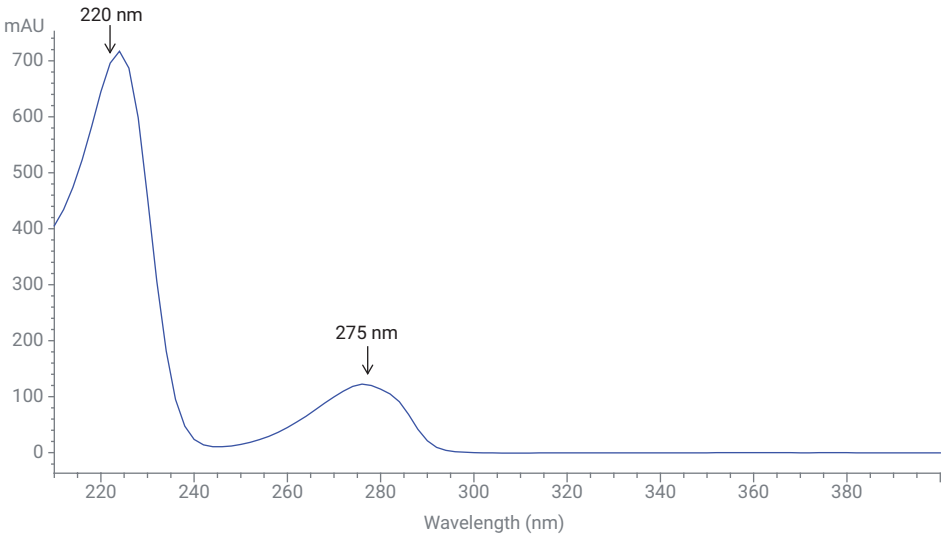


Figure 4C. UV spectrum of tyrosine.

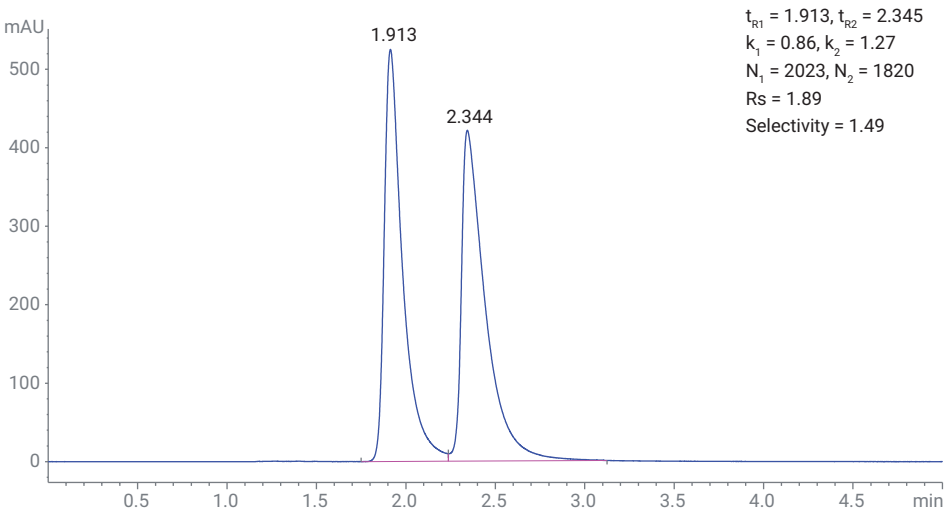


Figure 5A. Separation of tryptophan enantiomers (UV 280).

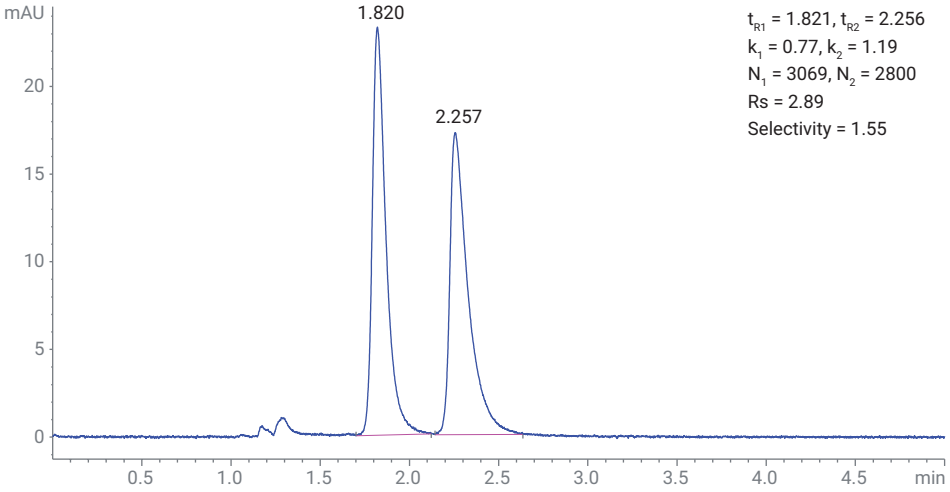


Figure 5B. Separation of phenylalanine enantiomers (UV 260).

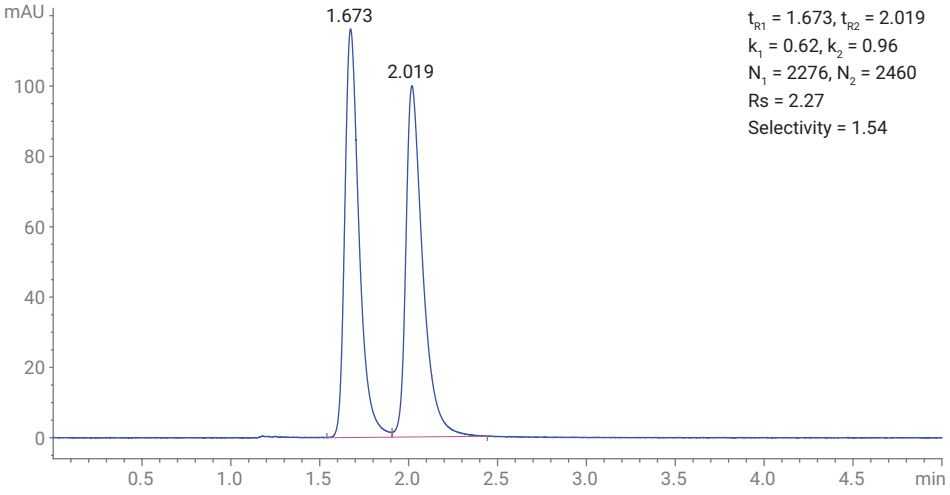


Figure 5C. Separation of tyrosine enantiomers (UV 275).

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

Compound	k_1	k_2	Rs	Selectivity
Tryptophan	0.92	1.34	2.75	1.45
Phenylalanine	0.84	1.26	3.02	1.50
Tyrosine	0.70	1.04	2.58	1.49

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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