Extending Routine Amino Acid Analysis with Simultaneous Quantitative and Chiral UHPLC Separation

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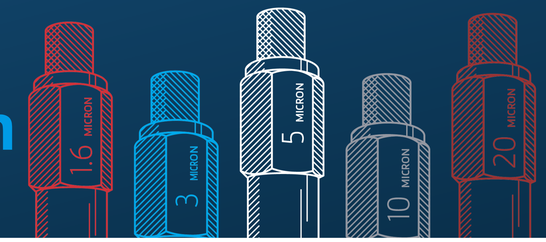

Introduction

Amino acid analysis is widely used for nutritional labeling, protein quality assessment, ingredient verification, and food quality control. Current standardized methods provide reliable quantitative amino acid profiles but generally do not distinguish between L- and D-enantiomers. D-amino acids may arise through fermentation, processing, aging, microbial activity, or adulteration, making stereochemical information potentially valuable for food characterization and quality assessment.

Objective: Evaluate Vaast™, an ion-exchanger chiral stationary phase, as an extension of the standard AQC-based workflow for routine essential amino acid analysis with complementary stereochemical information.

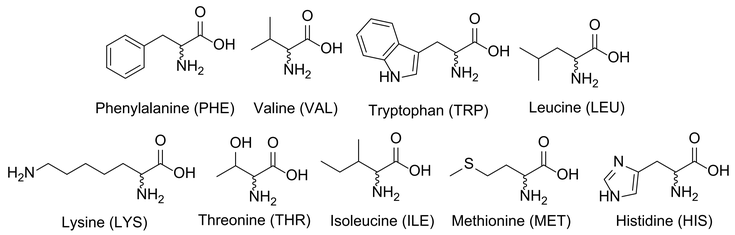

Figure 1:Nine Nutritional Essential Amino Acids (shown as racemic)

Experimental

Chromatographic Conditions for the Separation of Essential Amino Acids		
Method	**Isocratic**	**Gradient**
Column	Vaast (100 mm L x 2.1 mm i.d., 1.7 µm)	
Mobile Phase A	90-10-2 = MeOH-ACN-Water + 10 mM Ammonium Formate + 10 mM Formic Acid	93-7 = ACN-Water + 10 mM Ammonium Formate + 10 mM Formic Acid
Mobile Phase B	N/A	75-25 = MeOH-ACN + 50 mM Ammonium Formate + 50 mM Formic Acid
Flow Rate	0.21 ml/min	
Detection	DAD, 254 nm (Ref. 450 nm)	
Temperature	60°C	50°C
Injection Vol.	2 µl	
Analysis Time	20 mins	30 mins

1. **AOAC Official Method 2018.06.** *Total Amino Acids in Infant Formulas and Adult Nutritionals by UHPLC-UV.*

2. Friedman, M. **Chemistry, nutrition, and microbiology of D-amino acids**. *J. Agric. Food Chem.* **1999**, 47, 3457–3479. DOI: 10.1021/jf990080u.

Gradient Conditions		
Time (min)	**Mobile Phase A (%)**	**Mobile Phase B (%)**
0	90	10
2.0	90	10
22.00	10	90
24.00	10	90
24.01	90	10
30.00	90	10

Standard Preparation: The nine essential amino acid standards were purchased from Sigma-Aldrich as individual L-isomers and DL-racemates. Standards were prepared in 0.1 M HCl at 8 mM prior to AQC derivatization.

Sample Preparation: The pooled contents of five commercial EAA supplement capsules (2.620 g) were extracted in 500 mL of 0.1 M HCl with sonication for 10 min. Following centrifugation, the supernatant was diluted 1:10 in 0.1 M HCl prior to AQC derivatization.

AQC Derivatization: Standards and samples were derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) according to the Daicel AQC amino acid derivatization protocol prior to UHPLC analysis.

Instrumentation: Agilent 1290 Infinity II system equipped with DAD.

Results and Discussion

Separation of Nine Essential L-Amino Acids: All nine essential L-amino acids were separated using an isocratic method following AQC derivatization. Analysis of a commercial EAA supplement demonstrated retention-time correspondence with the amino acid standards (Fig. 2).

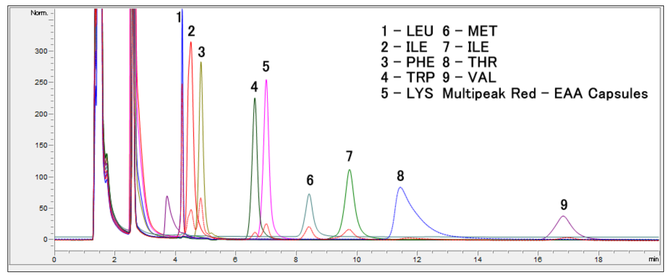

Figure 2: Separation of Nine Essential L-Amino Acids Overlaid with Commercial Sample

The same isocratic conditions also provided enantiomeric resolution of individual DL-amino acid standards, demonstrating that chiral recognition does not require gradient elution (Fig. 3 and 4).

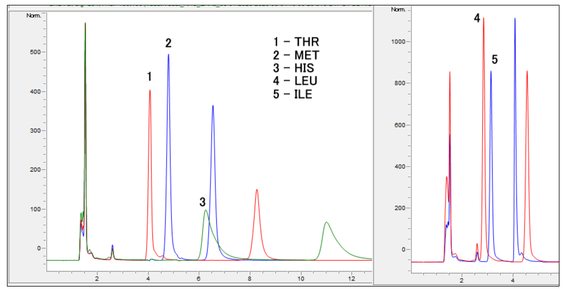

Figure 3: Representative Enantiomeric Separations Under Iso. Conditions (THR, MET, HIS, LEU, ILE)

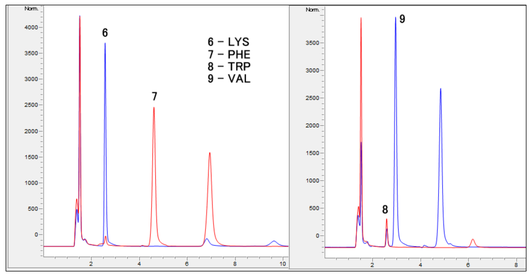

Figure 4: Representative Enantiomeric Separations Under Iso. Conditions (LYS, PHE, TRP, VAL)

Gradient Optimization for Simultaneous Chiral Analysis: Gradient optimization improved selectivity for the combined nine-racemate mixture, allowing 14 of 18 expected stereoisomer peaks to be distinguished (Fig. 5). Residual overlap was consistent with cross-analyte coelution rather than a lack of enantiomeric selectivity.

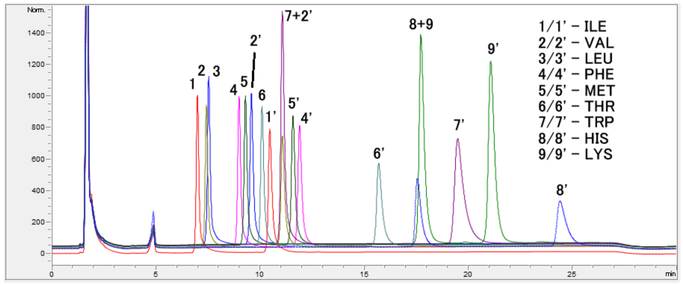

Figure 5: Gradient Optimization for the Separation of Nine Racemic Essential Amino Acids

Conclusions

- Rapid isocratic separation resolved nine essential L-amino acids while retaining chiral recognition of individual D/L pairs.
- Gradient optimization resolved 14 of 18 stereoisomer peaks in the combined racemic mixture, demonstrating expanded stereochemical capability.

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