APPLICATION NOTE

Vaast: An Innovative, Single Column Solution for the Chiral and Achiral Separation of 21 Natural Amino Acid

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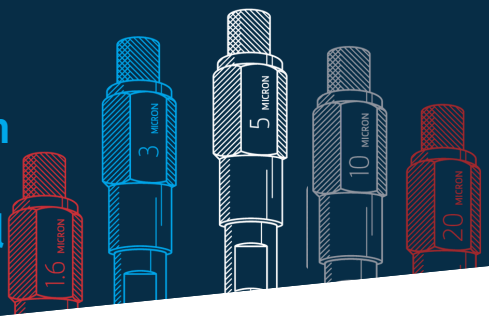

DAICEL Chiral Technologies

INTRODUCTION

Quantitative analysis of amino acids is central to applications ranging from biopharmaceutical development to clinical diagnostics, food sciences, and metabolomics. These applications require analytical methods capable of profiling amino acids with precision across a wide dynamic range, maintaining inter-laboratory reproducibility, and distinguishing enantiomers where chirality affects biological or metabolic function. Current analytical strategies for naturally occurring proteogenic amino acids largely fall into three categories, each with significant trade-offs.

Classical ion-exchange with post-column ninhydrin (IEC–NIN) offers a robust quantitation across an array of amino acids. Dedicated amino acid analyzers continue to serve as reference methods, but they often come with long run times, inflexible methods, and the need for specialized instrumentation[1, 2, 3]. Moreover, they are blind to chirality.

Reversed-phase chromatography with pre-column derivatization (commonly OPA, AQC, or FMOC) is widely adopted because it is compatible with standard HPLC/UHPLC systems and can be scaled for high throughput. However, such methods require multiple reagents to capture primary versus secondary amines, introduce time-sensitive chemistry, and are prone to inter-lab variability[4, 5].

HILIC-based LC–MS approaches without derivatization provide direct analysis and faster workflows. These methods simplify sample preparation and are increasingly used in targeted and untargeted metabolomics. Yet they can suffer from matrix effects, limited robustness across platforms, and a lack of chiral resolution, leaving an unmet need in standardization and comparability[6].

Despite decades of refinement, **no single chromatographic method currently provides comprehensive, reproducible analysis of all amino acids within a single run.** Inter-laboratory reproducibility remains a major challenge, with even validated methods showing variation when applied across sites, instruments, or software platforms[7]. This compromises the reliability of large-scale or multi-center datasets.

To address these limitations, the Vaast column from Daicel was developed as a unified solution for amino acid analysis. The method enables the simultaneous separation of 21 natural amino acids (20 proteinogenic plus homoserine) within a single LC–MS run of less than five minutes. Vaast eliminates multiple kits, avoids cumbersome derivatization, and provides consistent reproducibility across laboratories and instrument platforms. Importantly, it allows simultaneous resolution of chiral and achiral amino acids in one standardized workflow, offering a simplified yet comprehensive analytical approach[8].

EXPERIMENTAL

Chromatographic Conditions for Separation of 21 Natural Amino Acids

Column	Vaast (100 mm x 2.1 mm i.d., 1.7 µm) Part #: 72U93
Mobile Phase	A: 10 mM Formic Acid + 10 mM Ammonium Formate in Acetonitrile/Water (93/7; v/v) B: 50 mM Formic Acid + 50 mM Ammonium Formate in Methanol/Acetonitrile (75/25; v/v)
Gradient Program	Time (min) / Solvent A (%) / Solvent B (%) 0 / 90 / 10 2.1 / 75 / 25 2.5 / 0 / 100 5.8 / 0 / 100 6.0 / 90 / 10 9.0 / 90 / 10
Flow Rate	0.8 ml/min
Detection	MS
Temperature	50°C
Sample	145 pmol/µl in prepared Derivatization Solution (see Derivatization Protocol)
Injection Volume	1 µl

Screening and optimization were performed on a Waters Acquity I-Class UPLC + PDA + QDa Performance MS. The MS detector was set with the following parameters:

- Mode: ESI positive (ESI+)
- Cone voltage: 15 V
- Sampling rate: 15 points/sec
- Capillary voltage: 1.0 kV
- Probe temperature: 600 °C
- Gain: 1

All mobile phases and reagents were prepared according to the Derivatization Protocol and Mobile Phase Preparation Protocol (available as Supplementary Information).

DISCUSSION

The 21 derivatized AQC-amino acids listed in Table 1 were prepared according to the standard procedure outlined in the Derivatization Protocol and analyzed using Vaast according to the method outlined in the experimental section. As demonstrated in Table 1, in all cases, the corresponding D and L isomers of each amino acid pair (with the exception of glycine with is not chiral), were all more than baseline resolved. In all cases, except for Proline, the D enantiomer eluted first, with all amino acid pairs eluting in less than 5 minutes (Figure 1). One very important observation emerged from this analysis: Vaast's ability to handle a very diverse set of amino acids -spanning hydrophobic, The 21 derivatized AQC-amino acids listed in Table 1 were prepared aromatic, polar, acidic, and basic classes- in a single run. This

versatility enables comprehensive enantiomeric and compositional profiling in one step, eliminating the need for multiple columns or separate chiral and achiral workflows.

1. Leucine, isoleucine, and valine, all **branched chain amino acids** – despite their similar m/z values, were baseline resolved, both from their D/L pairs, but also from each other.
2. Phenylalanine, tyrosine, and tryptophan – **aromatic amino acids**, which are structurally related with only minor functional differences, were baseline resolved from their D/L pairs, and from each other.
3. Aspartic acid and glutamic acid, as well as asparagine and glutamine differing only by a single methylene group in their side chains, were resolved, confirming the column's fine selectivity for subtle structural variations.
4. Proline, - **a secondary amine** - often requiring its own separate derivatization and method, was resolved.

AQC-AA	Molecular weight (g/mol)	RRT1	RRT2	Rs
Isoleucine	301.3	0.57 (D)	0.82 (L)	8.5
Valine	287.3	0.60 (D)	0.87 (L)	8.6
Leucine	301.3	0.62 (D)	0.76 (L)	5.0
Proline	285.3	0.68 (L)	0.75 (D)	2.1
Phenylalanine	335.4	0.75 (D)	0.94 (L)	7.9
Methionine	319.4	0.78 (D)	0.93 (L)	6.1
Alanine	259.3	0.80 (D)	0.92 (L)	4.6
Threonine	289.3	0.86 (D)	1.09 (L)	7.9
Homoserine	289.3	0.91 (D)	1.03 (L)	6.0
Tryptophan	374.4	0.92 (D)	1.27 (L)	11.0
Tyrosine	351.4	0.93 (D)	1.07 (L)	7.1
Cysteine	348.2	0.96 (D)	1.16 (L)	2.6
Serine	275.3	0.96 (D)	1.16 (L)	9.2
Glutamine	316.3	0.98 (D)	1.07 (L)	5.1
Glycine	245.2	1.00		Achiral
Asparagine	302.3	1.02 (D)	1.25 (L)	8.7
Lysine	486.5	1.13 (D)	1.25 (L)	4.2
Histidine	325.3	1.14 (D)	1.43 (L)	7.2
Glutamic Acid	317.3	1.17 (D)	1.25 (L)	2.9
Aspartic Acid	303.3	1.19 (D)	1.29 (L)	2.4
Arginine	344.4	1.27 (D)	1.73 (L)	8.4

Table 1: Relative retention times of AQC-AA derivatives analysed on Vaast column, compared with AQC-Gly, under gradient conditions detailed in Experimental section. Molecular masses of the AQC-derivatives when using MS-detection. *RRT = Relative retention time = RT / $RT_{glycine}$*

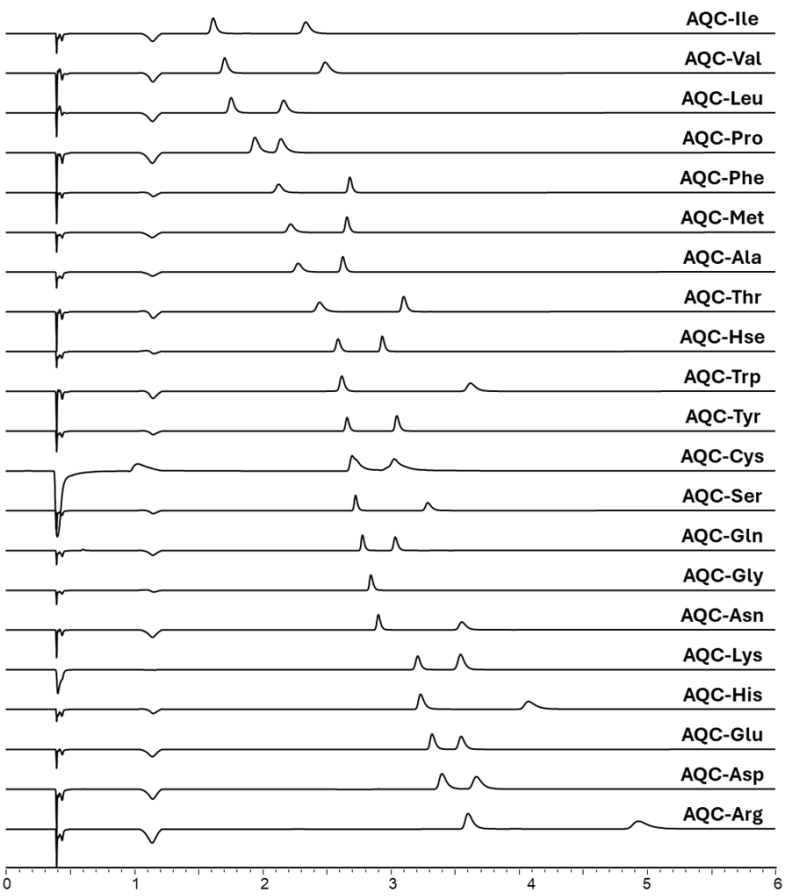

Figure 1: UV chromatogram (intensity vs LC retention time) of the 21 AQC-derivatized amino acids and Relative retention time (RRT = RT / $RT_{glycine}$).
VAAST column (1.7 µm. 100 x 2.1mm); Experimental conditions as describe above.

CONCLUSIONS

The results demonstrated that Vaast provides the first validated single-method solution capable of separating simultaneously 21 amino acids, including both enantiomeric forms, within one single workflow. Unlike/compared to ion-exchange, reversed-phase derivatization, or HILIC–MS methods, this approach eliminates the need for multiple columns, complex derivatization protocols, or split chiral/achiral analyses.

This unified solution directly addresses the long-standing needs of researchers in biopharma, metabolomics, and clinical science by delivering reproducibility, efficiency, and comprehensive coverage in a single validated method. As such, it represents a transformative advance over existing amino acid analysis solutions.

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