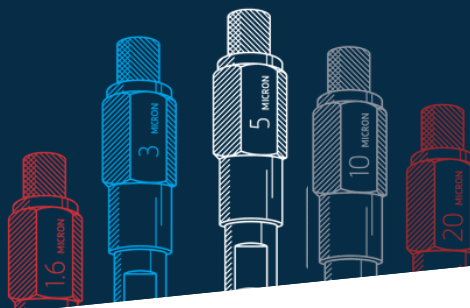


Vaast®: An Innovative, Single Column Solution for the Chiral and Achiral Separation of 21 Natural Amino Acids

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INTRODUCTION

Quantitative analysis of amino acids (AAs) is central to applications ranging from biopharmaceutical development to clinical diagnostics, food sciences and metabolomics. These applications require analytical methods capable of profiling AAs with precision across a wide dynamic range, maintaining inter-laboratory reproducibility^{1,2,3}. However, most often investigations remain blind to chirality although it is well-known that quite often enantiomers have different biological or metabolic function.

Despite major advances, the enantioselective analysis of AAs remains a significant analytical challenge due to the diversity of their structures and polarities. The introduction of efficient derivatization strategies and highly selective chromatographic systems are often required to achieve adequate resolution and sensitivity^{4,5}. However, there is no universal solution for their resolution and many of the existing methods have significant limitations: long run times, limited robustness, use of 2 (or more) columns with associated difficulties to validate, time-sensitive chemistries, inter-lab variability and/or the need for specialized instrumentation^{6,7,8,9,10}.

Despite decades of refinement, no single chromatographic method currently provides comprehensive, reproducible analysis of all natural AA enantiomers within a single run.

To address these limitations, the Vaast® column from DAICEL was developed as a unified solution for chiral AA analysis¹¹. This chiral ion-exchanger column, supported on sub-2 µm silica particles, enables the simultaneous separation of D/L-forms of 21 natural AA (20 proteinogenic plus homoserine) in five minutes with a single LC-MS compatible run. This undescribed achievement of Vaast, ensured with an easy and robust pre-column derivatization, provides consistent reproducibility and will be a guarantee for the method transfer across laboratories and instrument platforms. Importantly, it allows simultaneous resolution of chiral and achiral AAs in one standardized workflow, offering a simplified yet comprehensive analytical approach.

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-AAs 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. In all cases, the corresponding D and L-isomers of each AA pair (with the exception of glycine which is not chiral) were baseline resolved. The 21 AAs eluted in less than 5 minutes (Figure 1), with D-enantiomers eluting first for all pairs, except for Proline.

One very important observation emerged from this analysis: Vaast's ability to handle a very diverse set of AAs – spanning hydrophobic, 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 AAs – 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 AAs, 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	m/z	RRT1	RRT2	Rs
Isoleucine	302.2	0.57 (D)	0.82 (L)	8.5
Valine	288.1	0.60 (D)	0.87 (L)	8.6
Leucine	302.2	0.62 (D)	0.76 (L)	5.0
Proline	286.1	0.68 (L)	0.75 (D)	2.1
Phenylalanine	336.1	0.75 (D)	0.94 (L)	7.9
Methionine	320.1	0.78 (D)	0.93 (L)	6.1
Alanine	260.1	0.80 (D)	0.92 (L)	4.6
Threonine	290.1	0.86 (D)	1.09 (L)	7.9
Homoserine	290.1	0.91 (D)	1.03 (L)	6.0
Tryptophan	375.1	0.92 (D)	1.27 (L)	11.0
Tyrosine	352.1	0.93 (D)	1.07 (L)	7.1
Cysteine	349.1	0.95 (D)	1.06 (L)	2.6
Serine	276.1	0.96 (D)	1.16 (L)	9.2
Glutamine	317.1	0.98 (D)	1.07 (L)	5.1
Glycine	246.1	1.00		Achiral
Asparagine	303.1	1.02 (D)	1.25 (L)	8.7
Lysine	487.2	1.13 (D)	1.25 (L)	4.2
Histidine	326.1	1.14 (D)	1.43 (L)	7.2
Glutamic Acid	318.1	1.17 (D)	1.25 (L)	2.9
Aspartic Acid	304.1	1.19 (D)	1.29 (L)	2.4
Arginine	345.2	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. The exact (calculated) m/z value of the protonated species is provided when using MS detection (Cys in its AQC-Cys-IAA form and Lys in its bis-AQC-Lys form). $RRT = \text{Relative retention time} = RT / RT_{\text{glycine}}$

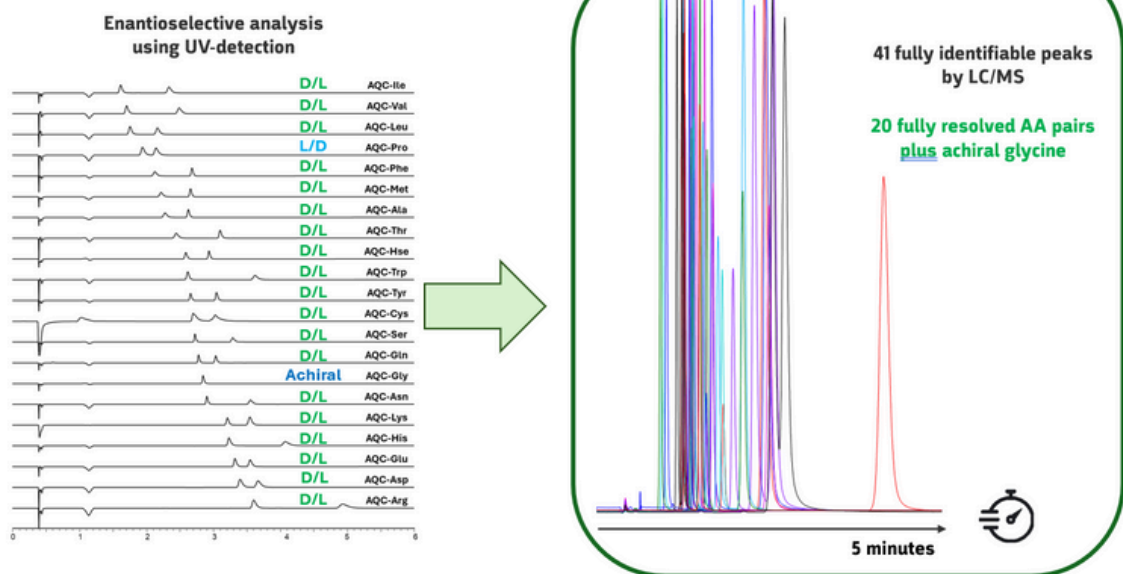


Figure 1: UV chromatograms at 254 nm (intensity vs LC retention time) under HPLC conditions of the 21 AQC-derivatized AAs on the Vaast column (1.7 μ m, 100 x 2.1 mm) and corresponding overlay of the 41 peaks fully identifiable by LC/MS. Gradient conditions as described in Experimental Section.

CONCLUSIONS

Vaast sets a new standard in amino acid analysis. It's the first and only single-method solution that can separate 21 natural amino acids—including both enantiomeric forms, as AQC-derivatives—in just 5 minutes, all within one streamlined LC-MS compatible workflow.

Designed for versatility, Vaast works seamlessly with LC-MS detection, while its pre-column derivatization also enables UV-based determination of enantiomeric pairs. Moreover, although optimized for the described 21 AAs, its recognition capabilities go beyond to other AAs (e.g., allo-amino acids) and different AA-derivatives (e.g., Fmoc, etc).

For researchers in biopharma, peptide synthesis, food analysis and biological or clinical sciences, Vaast delivers what others can't: speed, reproducibility and comprehensive coverage in one robust method.

REFERENCES

- Phipps, W. S., Kim, S. H., Ma, Q., & Hage, D. S. (2019). Quantitative amino acid analysis: Comparing ion-exchange and LC-MS methods. *Journal of Mass Spectrometry*, 54(12), 996-1007. <https://doi.org/10.1002/jms.4448>.
- Sharer, J. D., Milis, M. A., & Bennett, M. J. (2018). Amino acid analysis in clinical laboratories: Ion-exchange chromatography and beyond. *Genetics in Medicine*, 20(1), 3-12. <https://doi.org/10.1038/gim.2017.103>.
- Biochrom. (n.d.). *Biochrom 30+ amino acid analyzer: Applications and methods*. Cambridge, UK: Biochrom Ltd.
- Calderón C, Lämmerhofer M. Enantioselective metabolomics by liquid chromatography-mass spectrometry. *J Pharm Biomed Anal* 2022;207:114430. doi:10.1016/j.jpba.2021.114430.
- Rebane R, Leito I. Derivatization for success: a tutorial review of liquid chromatography mass spectrometry method optimization for determining amino compounds. *Anal Chim Acta*. 2025;1370:344354. doi:10.1016/j.aca.2025.344354
- Du S, Wang Y, Weatherly CA, Holden K, Armstrong DW. Variations of L- and D-amino acid levels in the brain of wild-type and mutant mice lacking D-amino acid oxidase activity. *Anal Bioanal Chem*. 2018;410(12):2971-2979. doi:10.1007/s00216-018-0979-9
- Karongo R, Ge M, Geibel C, Horak J, Lämmerhofer M. Enantioselective multiple heart cutting online two-dimensional liquid chromatography-mass spectrometry of all proteinogenic amino acids with second dimension chiral separations in one-minute time scales on a chiral tandem column. *Anal Chim Acta*. 2021;1180:338858. doi:10.1016/j.aca.2021.338858
- Karongo R, Horak J, Lämmerhofer M. Comprehensive Online Reversed-Phase x Chiral Two-Dimensional Liquid Chromatography-Mass Spectrometry with Data-Independent Sequential Window Acquisition of All Theoretical Fragment-Ion Spectra-Acquisition for Untargeted Enantioselective Amino Acid Analysis. *Anal Chem*. 2022;94(49):17063-17072. doi:10.1021/acs.analchem.2c03042
- Nagata Y, Akita T, Ishii C, Oyaide M, Mita M, Ide T, Hamase K. Development of an enantioselective three-dimensional HPLC system for the determination of alanine, valine, isoleucine, allo-isoleucine and leucine in human plasma and urine. *J Pharm Biomed Anal Open*. 2023;100013. doi:10.1016/j.jpba.2023.100013
- Jaag SJ, Valadbeigi Y, Causon T, Gross H, Lämmerhofer M. Three-Minute Enantioselective Amino Acid Analysis by Ultra-High-Performance Liquid Chromatography Drift Tube Ion Mobility-Mass Spectrometry Using a Chiral Core-Shell Tandem Column Approach. *Anal Chem*. 2024;96(6):2666-2675. doi:10.1021/acs.analchem.3c05426
- Falatas, A., Hausser, N., Kinderstuth, L., Schaeffer, M., & Franco, P. (2025). Method for the simultaneous enantioselective analysis of 21 natural amino acids and its application to analytics of fluorenylmethoxycarbonyl-derivatives under liquid chromatography conditions. *Journal of Chromatography Open* 8,(2025) 100286. <https://doi.org/10.1016/j.jcoa.2025.100286>