INSTRUCTION MANUAL FOR *Vaast® COLUMN*

Please read this instruction manual completely before using this column.

THIS INSTRUCTION SHEET IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS.

Column Description

The Vaast® column is packed with an ionic chiral stationary phase based on DAICEL proprietary technology and specifically developed to achieve the simultaneous enantioselective analysis of 21 natural amino acids, after AQC (6 aminoquinolyl-N-hydroxysuccinimidyl carbamate) pre-column derivatization, with both UV and MS-detection.

The following column instructions will refer to this specific application as well as to other applications for the resolution of acidic and amphoteric molecules that are also feasible using this column. Therefore, more general considerations will also be given.

The chiral selector is immobilized onto 1.7 µm spherical silica gel. This column is stable to all common chromatographic solvents.

Shipping Solvent: **100% MeOH**

All columns have been pre-tested before packaging. Test parameters and results, as well as the Column Lot Number, were included with the column when purchased.

Operating Conditions

Column Characteristics ①	**100mm * 2.1mm i.d.** 1.7 µm particle size
Flow Rate Direction	As indicated on the column label
Typical Flow Rate	0.2-0.8 mL/min
Temperature	10-50°C
Pressure Limitation ②	600 bar (8700 psi)
Column Fitting ③	Finger tight fittings compatible with high pressure are recommended (eg Waters™Three-Piece Finger-Tight Fitting, Agilent InfinityLab Quick Connect and Quick Turn fittings, Thermo Scientific™ Viper™ Fingertight fittings)

① Although this column can be used with an HPLC system, it is highly recommended to use a UHPLC system to benefit from the best separation performance of the column.

② The column pressure is the total pressure minus the system pressure. At a given temperature, the column back pressure is linearly proportional to the flow rate.

③ It is highly recommended to use suitable fittings. Improperly matched fittings can result in increased void volume, in significant peak broadening and decreased column performance. This effect is much more pronounced on the sub-2 µm particle size columns, compared to larger particle sizes. Additionally, unsuitable fitting may also lead to solvent leakages or column hardware damage.

Method Parameters

A – Simultaneous Enantioselective Analysis of 21 AQC-derivatized Amino Acids

This column is optimized for the analysis of AQC-derivatized amino acids. Prior to the analytical runs, the amino acid samples should be derivatized using the Vaast® Reagent Kit Amine Derivatization, which include the products needed to perform this derivatization. See separate document, Vaast® Derivatization Protocol.

The optimized chromatographic conditions are:

MOBILE PHASE	DESCRIPTION	PREPARATION STEPS
A	10 mM Formic Acid + 10 mM Ammonium Formate in Acetonitrile/Water (93/7; v/v)	1. Mix 930 mL Acetonitrile + 70 mL Water 2. Add 0.38 mL Formic Acid 3. Add 0.63 g Ammonium Formate 4. Mix until dissolved 5. Degas ≥10 min in an ultrasonic bath
B	50 mM Formic Acid + 50 mM Ammonium Formate in Methanol/Acetonitrile (75/25; v/v)	1. Mix 750 mL Methanol + 250 mL Acetonitrile 2. Add 1.88 mL Formic Acid 3. Add 3.15 g Ammonium Formate 4. Mix until dissolved 5. Degas ≥10 min in an ultrasonic bath

CHEMICALS NEEDED :
Acetonitrile (ACN), HPLC grade : CAS RN = 75-05-8
Methanol (MeOH), HPLC grade : CAS RN = 67-56-1
Purified water, grade 2
Formic acid (FA), >98%, ACS reagent : CAS RN = 64-18-6
Ammonium formate (NH_4FA), >99%, HPLC : CAS RN = 540-69-2
N.B. – all chemicals can be purchased in an LC-MS grade if required

Gradient Conditions		
Time (min)	**Mobile Phase A (%)**	**Mobile Phase 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
Temperature	50°C
UV-detection	254 nm (max. absorbance for AQC-amino acids)
MS-detection	These operating conditions are compatible with MS-detection. As a reference the following parameters were used on a Waters™ Acquity I-Class UPLC + QDa Performance MS Mode: ESI positive (ESI+) Capillary voltage: 1.0 kV Cone voltage: 15 V Probe temperature: 600 °C Sampling rate: 15 points/sec Gain: 1

References to enantiomeric resolution and retention behavior achieved for each amino acid can be found in Annex 1 of this document.

B – Further Method Development

In general, this column exhibits high versatility and can be applied for the analysis of a wide range of molecules, including other natural and unnatural amino acids (after AQC-derivatization), and other protected amino acids.

The above conditions can for example be applied for the chiral analysis of Fmoc-protected amino acids, (see 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**, 100286. https://doi.org/10.1016/j.jcoa.2025.100286)

If the simultaneous enantioselective resolution of all 21 amino acids is not required, method parameter adjustments are possible to adapt to the application goals. This can include:

- Adapt the gradient characteristics based on the retention times of the targeted amino acids. Isocratic conditions are also possible.
- The ratio of water in the eluent can be increased up to 10%. Water usually reduces retention and may help with chiral recognition and MS sensitivity. Increased water percentage will result in higher viscosity, thus the flow rate will have to be decreased to keep the column pressure within the recommended range.
- The concentration of the modifiers (acid, base, salt) can be modulated between 2 mM and 50 mM, but solubility in the solvent mixtures should be confirmed. If increased amount of salt is used, it is highly recommended the eluent be filtered to remove insoluble salt, which if introduced to the column, will result in blocked frits and an increase in column pressure. For a high concentration of salt, it helps to dissolve the salt first in the aqueous component and then add the organic modifier.
- Flow rates can be reduced to increase the Rs between target analytes.
- If UV-detection is used instead of MS-detection, alternative additives can be utilized. Formic acid can be replaced with acetic or phosphoric acid. Ammonium Formate can be replaced with Ammonium Acetate or organic amines like Diethylamine or Triethylamine.

For investigational purposes, a recommended starting mobile phase would be the one used for the QC-release of the column: 50 mM Formic Acid + 50 mM Ammonium Formate in Methanol/Water 98/2.

The column can be also used in SFC mode – please refer to the separate SFC instruction manual.

Column Care / Maintenance

Before running the analysis, equilibrate the system and column at decreased flow rate (0.2 mL/min) for 20 min (~ 12 column volumes) with the MP composition of 90% of A and 10% of B at T=50°C, followed by a flow rate of 0.8mL/min for 40 min (~90 column volumes) with the MP composition of 90% of A and 10% of B at T=50°C.

If possible, samples should preferably be dissolved in the eluent or in one of its main components. In case of a gradient, the mobile phase composition at the beginning of the run should be used.

Attention should be given to proper preparation of biological samples. Injecting only clear and homogeneous solutions will significantly increase column lifetime. It is highly recommended to filter solutions prior to injection through a membrane filter of approximately 0.5 µm porosity.

An in-line filter may be added to extend column lifetime. This will add an extra void volume that will slightly alter the intrinsic column performance. A compromise should then be found between meticulous sample preparation and column protection.

After exhaustive use, the column can be flushed with Acetonitrile/Water 50/50 or Methanol/Water 50/50 at 0.4mL/min for 40 min (~50 column volumes) at T=40°C.

Column Storage

For column storage, remove the acidic or basic additives, as well as the salts by flushing the column with several column volumes of Acetonitrile/Water 50/50 at 0.4mL/min and 40°C.

Columns can then be stored with ends capped in the additive-free mobile phase, or the shipping solvent, at room temperature.

Operating this column in accordance with the guidelines outlined here will result in a long column life.

⇨ If you have any questions about the use of these columns, or encounter a problem, contact:

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Annex 1: Analytical results for the simultaneous analysis of 21 natural amino acids

Relative retention times of AQC-AA derivatives analyzed on Vaast® column, compared with AQC-Gly, under gradient conditions detailed in page 2. 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 = Relative retention time = RT / $RT_{glycine}$

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