Waters™

Application Note

Comparable Chromatographic Performance for Amino Acid Analysis Using the AccQ▪Tag Ultra VanGuard Pre-Column

Kenneth D. Berthelette, Jennifer M. Nguyen, Ning Zhang, Paula Hong, Jennifer Simeone

Waters Corporation

Abstract

The analysis of complex matrices can cause column performance issues when performed routinely. In plasma, cell culture media, or food samples, the presence of endogenous compounds like lipids or proteins can cause the inlet frit of the column to become blocked over time, leading to increased column pressure and decreased column lifetime. One way to mitigate this is to use guard columns, which can be routinely changed to protect the analytical column from fouling. The work shown here compares the chromatographic performance of the AccQ▪Tag Ultra Column with and without an AccQ▪Tag Ultra guard for the analysis of neat cell culture media standard.

Benefits

- AccQ▪Tag VanGuard Pre-Column may increase analytical column lifetime
- Comparable chromatographic performance obtained with and without pre-column

Introduction

Amino acid analysis (AAA) is used in a variety of different industries, each with different sample preparation. Analysis of cell culture media, protein content in food and feeds, and protein hydrolysis all present unique matrix challenges. Depending on the methodology, the presence of lipids, proteins, sugars, and other compounds in these matrices can cause deleterious effects on the analytical column. While filtration or other sample preparation strategies could help mitigate these effects, added steps in a complex sample preparation (*i.e.* derivatization technique) are not desired. In these cases, a guard column installed just before the analytical column in the sample flow path may serve to protect the analytical column.

Guard columns, such as the new AccQ▪Tag Ultra Pre-Column, are short columns of packed stationary phase that are installed at the inlet of the AccQ▪Tag Ultra analytical column. Once installed, these guard columns are the first to be subjected to the matrix and the first to get fouled due to endogenous matrix components. Guard columns are replaced periodically, thereby preventing the analytical column from getting fouled. Several examples showing the use of guard columns have been previously published.[1-2] While guard columns can be extremely helpful, slight changes in the chromatographic results due to the added stationary phase in the flow path as well as the added system dispersion may be observed. The work shown here compares the separation of a derivatized cell culture media standard analyzed on the AccQ▪Tag Ultra Column with and without a guard column.

Experimental

Sample Description

Amino Acid Cell Culture Standard Kit (p/n: 186009300 < https://www.waters.com/nextgen/global/shop/standards--reagents/186009300-amino-acid-cell-culture-standard-kit.html>) prepared and derivatized as outlined in the Amino Acid Standard Kits Care and Use Manual.[3]

LC Conditions

LC systems:	ACQUITY UPLC H-Class with Column Manager (CM), Column Manager Aux (CM-Aux), and PDA Detector
Detection:	UV @ 260 nm
Column(s):	AccQ•Tag Ultra C_{18} Column, 1.7 µm, 2.1 x 100 mm (p/n: 186003837) AccQ•Tag Ultra C_{18}, 1.7 µm, VanGuard Pre-Column, 2.1 x 5 mm (p/n: 186009955)
Column temp.:	43 °C
Sample temp.:	20 °C
Injection volume:	0.5 µL
Flow rate:	0.7 mL/min
Mobile phase A:	AccQ•Tag Eluent A (p/n: 186003838)
Mobile phase B:	90:10 (v/v) Water:AccQ•Tag Ultra Eluent B
Mobile phase C:	Milli-Q Water
Mobile phase D:	AccQ•Tag Eluent B (p/n: 186003839)
Sample manager wash:	95:5 (v/v) Water:acetonitrile
Sample manager purge:	95:5 (v/v) Water:acetonitrile

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	%C	%D	Curve
0.00	0.7	10.0	0.0	90.0	0.0	–
0.29	0.7	9.9	0.0	90.1	0.0	11
5.49	0.7	9.0	80.0	11.0	0.0	7
7.10	0.7	8.0	15.6	57.9	18.5	6
7.30	0.7	8.0	15.6	57.9	18.5	6
7.69	0.7	7.8	0.0	70.9	21.3	6
7.99	0.7	4.0	0.0	36.3	59.7	6
8.59	0.7	4.0	0.0	36.3	59.7	6
8.68	0.7	10.0	0.0	90.0	0.0	6
10.20	0.7	10.0	0.0	90.0	0.0	6

Data Management

Chromatography software: Empower 3 Feature Release 4

Results and Discussion

Protecting an analytical column is a prudent way to extend column lifetime. One of the easiest ways to protect an analytical column is to use a guard column, or pre-column. These short columns use the same stationary phase as the analytical column and are installed directly before the analytical column in the system flow path. As complex matrices are analyzed, the guard column will be the first to foul due to components in the sample matrix like proteins or lipids. This prevents the analytical column from being fouled. Guard columns are also meant to be changed out periodically to ensure the analytical column is protected.

While the use of guard columns has clear benefits, the added stationary phase and additional connection in the sample flow path can impact the analytical results and system pressure. (Figure 1) shows the system pressure of an ACQUITY UPLC H-Class when running the AccQ▪Tag Ultra Column with and without the AccQ▪Tag Ultra Pre-Column installed.

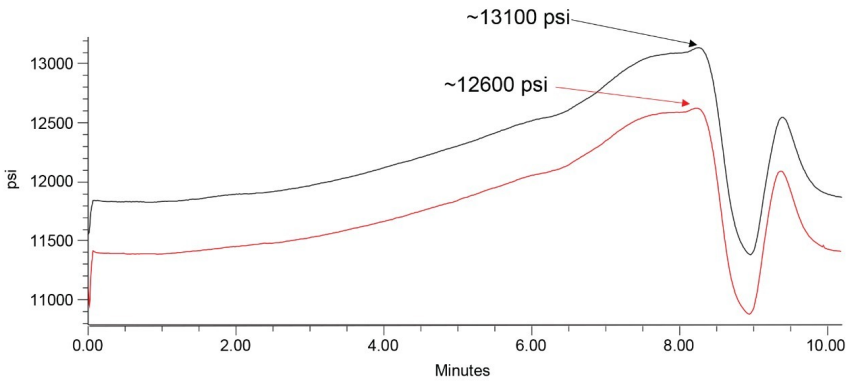

Figure 1. System pressure of an ACQUITY UPLC H-Class running the gradient conditions outlined above on an AccQ▪Tag Ultra Column with (black) and without (red) AccQ▪Tag Ultra Pre-Column installed.

Under these test conditions, the use of the AccQ▪Tag Ultra Pre-Column leads to a ~500 psi increase in system pressure. This is because the pre-column adds a 5 mm packed bed to the system, resulting in the 4% increase in pressure. To assess the effect of the pre-column on chromatographic performance, the cell culture media standard was tested and analyzed using the AccQ▪Tag Ultra derivatization kit and analyzed on the AccQ▪Tag Ultra Column with and without a guard column (Figure 2).

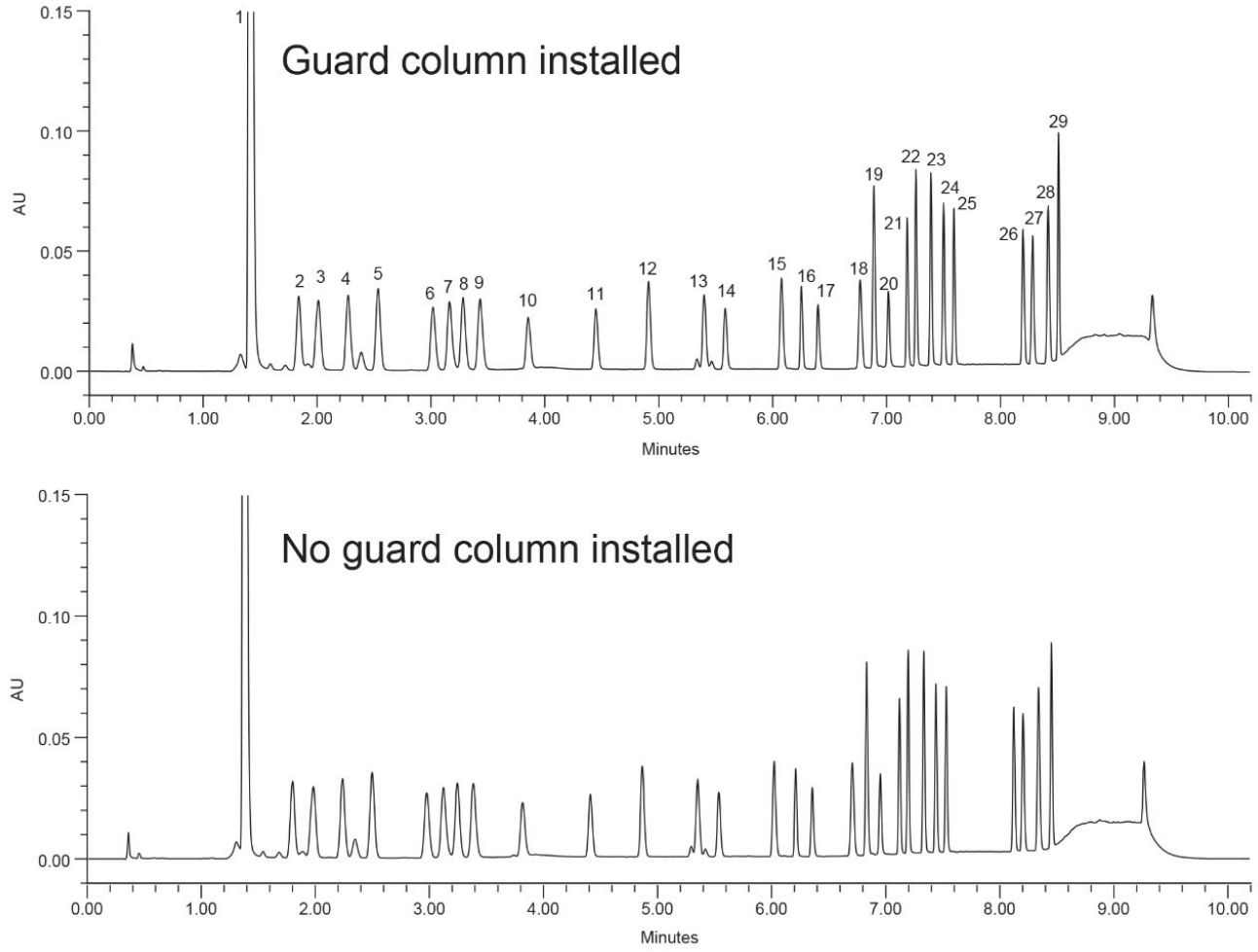

Figure 2. Analysis of the cell culture media standard on the AccQ▪Tag Ultra Column with (top) and without (bottom) guard column installed using the cell culture media gradient method on an ACQUITY UPLC H-Class with PDA detection. 1) AMQ, 2) HyPro, 3) His, 4) Asn, 5) Tau, 6) Ser, 7) Gln, 8) Arg, 9) Gly, 10) Asp, 11) Glu, 12) Thr, 13) Ala, 14) GABA, 15) Pro, 16) HyLys1, 17) HyLys2, 18) AABA, 19) Orn, 20) Derivatization peak, 21) Cys, 22) Lys, 23) Tyr, 24) Met, 25) Val, 26) Ile, 27) Leu, 28) Phe, 29) Trp.

Chromatographic performance with and without a guard column is comparable with the critical pairs of Gln/Arg (7/8) and Cys/Lys (21/22). These critical pairs are baseline resolved and have USP resolutions >1.5 with and without a pre-column installed. It should be noted that the peak between components 4 and 5 could be a partially derivatized mono-lysine, which is only present when lysine is not fully derivatized. For this work, the presence of the mono-lysine peak does not affect the results. Peak tailing, retention time, and USP resolution

values also show comparable results with and without a pre-column installed (Table 1). The added stationary phase does shift the retention times slightly; however, the difference in retention times is slight, <0.2 minutes, and within the processing method retention time windows.

	AccQ•Tag Ultra Column with pre-column installed			AccQ•Tag Ultra Column without pre-column installed		
	Retention time (min)	USP resolution	USP tailing	Retention time (min)	USP resolution	USP tailing
AMQ	1.413	–	1.41	1.379	–	1.35
HyPro	1.840	6.35	1.05	1.801	6.25	1.02
His	2.012	2.17	0.99	1.983	2.23	0.94
Asn	2.273	3.25	1.12	2.239	3.14	1.08
Tau	2.536	3.48	1.09	2.498	3.45	1.07
Ser	3.018	6.28	1.12	2.977	6.19	1.10
Gln	3.163	1.85	1.27	3.123	1.82	1.24
Arg	3.282	1.56	1.10	3.245	1.55	1.07
Gly	3.431	1.94	1.19	3.385	1.79	1.17
Asp	3.853	5.58	1.23	3.817	5.57	1.35
Glu	4.448	8.78	1.14	4.411	8.49	1.12
Thr	4.910	7.59	1.14	4.866	7.36	1.11
Ala	5.398	8.37	1.15	5.351	8.24	1.15
GABA	5.583	3.31	1.14	5.537	3.30	1.12
Pro	6.077	9.41	1.15	6.022	9.19	1.12
HyLys1	6.252	3.88	1.18	6.210	4.18	1.15
HyLys2	6.398	3.62	1.18	6.356	3.64	1.15
AABA	6.768	7.85	1.14	6.707	7.51	1.12
Orn	6.888	2.64	1.18	6.832	2.77	1.15
Derivitization peak	7.016	3.25	1.22	6.952	3.09	1.17
Cys	7.180	4.47	1.15	7.121	4.65	1.14
Lys	7.257	2.34	1.16	7.196	2.29	1.14
Tyr	7.389	3.98	1.14	7.334	4.12	1.12
Met	7.500	3.18	1.14	7.440	3.06	1.11
Val	7.591	2.64	1.15	7.530	2.64	1.12
Ile	8.198	16.8	1.15	8.123	16.7	1.12
Leu	8.281	2.15	1.15	8.204	2.13	1.12
Phe	8.418	3.44	1.12	8.339	3.42	1.12
Trp	8.509	2.64	1.10	8.452	3.04	1.08

Table 1. Retention times, peak tailing, and USP resolution of the identified amino acids with and without a pre-column installed.

Overall, the chromatographic performance with and without the pre-column installed is comparable. No more than a 5% difference in retention time was observed. Peak tailing changes were also less than 5% except for Asp

which showed an improvement in peak shape of 9%. USP resolution changes were also under 5% except for HyLys1 and Trp, however the resolutions are still well above the 1.5 cutoff which indicates baseline resolution. The changes observed are minor are not significant enough to alter the separation. By using the pre-column, the AccQ•Tag Ultra Column may potentially be used for more sample analyses without the need for changing columns.

Conclusion

Using a guard column in front of the analytical column can help to protect and extend the lifetime of the analytical column. Guard columns, also called pre-columns, are packed stationary phase beds that are the first to experience any sample injected. This means if a sample has a particularly troublesome matrix that can foul a column, like cell culture media or food and feed matrices, the guard column will foul before the analytical column. Therefore, column lifetime can be extended by replacing the fouled guard column instead of the column itself.

The AccQ•Tag Ultra Pre-Column shows that comparable chromatographic performance can be achieved with and without a guard column installed. Less than a 5% difference in system pressure and retention times were observed when using a column with and without a guard column. The utility of guard columns can help to protect more expensive analytical columns while not drastically affecting chromatographic performance.

References

1. Shiner S, Delano M, Lauber M, Rzewuski S, Warren W, McLaughlin J, Byrd S. VanGuard FIT: A Breakthrough in Guard Column Performance for Challenging Chromatographic Separations. Waters Application Note, 720006500EN, 2019.
2. Koza S, Chen W. BioResolve SEC mAb Guard Columns for Production Process and Formulation Development Samples. Waters Application Note, 720006955EN, 2020.
3. Amino Acid Standard Kits Care and Use Manual. Waters Care and Use Manual, 72000663EN < https://www.waters.com/webassets/cms/support/docs/720006663en.pdf> , 2020.

Featured Products

ACQUITY UPLC H-Class PLUS System <https://www.waters.com/10138533>

ACQUITY UPLC PDA Detector <https://www.waters.com/514225>

Empower Chromatography Data System <https://www.waters.com/10190669>

720007460, December 2021