Waters™

Applikationsbericht

Instrument Considerations for Successful Adaptation of Amino Acid Analysis Methods from an ACQUITY™ UPLC™ System to an ACQUITY Premier Binary Fixed Loop System

Kimberly Martin, Jennifer Simeone, Paula Hong

Waters Corporation

Abstract

Amino acid analysis is performed regularly for a wide range of samples including hydrolyzed proteins, cell culture, as well as food and feed samples. Waters™ created an ACQUITY UPLC AAA Solution which includes a pre-column derivatization kit, sub-2 µm columns and pre-packaged mobile phases suitable for UPLC analyses.[2] With the advancement of new instrumentation, it is important to be able to migrate methods to newer systems. This study will demonstrate the considerations for adaptation of this pre-column derivatization method from the ACQUITY UPLC to the ACQUITY Premier Binary Fixed Loop System. After adaptation and optimization of the separation method, performance will be demonstrated, including linearity, repeatability, reproducibility, limit of quantitation, and limit of detection.

Benefits

- ACQUITY Premier Binary System provides exceptional precision for challenging gradients and increased speed for high throughput analysis

- All critical performance characteristics are maintained, including peak shape, resolution, linearity, limit of quantification, and intermediate precision after method adaptation
- AccQ•Tag™ Ultra Chemistry Kit, which includes column, standards, eluents, and derivatization kit allows for fast, reliable and reproducible amino acid derivatization, separation, and quantification
- The ACQUITY Premier Binary System features MaxPeak High Performance Surfaces (HPS) Technology, which increases analyte recovery, sensitivity, and reproducibility by minimizing analyte/surface interactions that can lead to sample losses

Introduction

Analysis of amino acids (AAA) can be very challenging due to low or complete lack of UV absorbance and the wide range of chemical properties of amino acids. Given these challenges, pre-column derivatization using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) followed by a reversed-phase separation has been found to be a highly reproducible technique for quantitative analysis of amino acids using a UHPLC/UPLC System.[1] However, new systems are constantly being introduced and migration of the amino acid solution while maintaining method performance is crucial. In this work, a legacy method developed on the ACQUITY UPLC will be migrated to a newer LC platform, the ACQUITY Premier Binary Fixed Loop System, which includes some design differences that may impact the method migration. Some method modifications were required to account for these differences and the final method conditions yielded nearly identical results when compared to the original method run on the ACQUITY UPLC System.

Experimental

All calibration standards were prepared from Waters Amino Acid Standard (p/n: WAT088122 < https://www.waters.com/nextgen/global/shop/standards--reagents/wat088122-amino-acid-standard-accq-tag-pico-tag-accq-tag-ultra.html>) using norvaline (p/n: 186009301 < https://www.waters.com/nextgen/global/shop/standards--reagents/186009301-amino-acid-internal-standard-norvaline.html>) as the internal standard and 0.1 N HCl as the diluent. The internal standard stock was prepared at 2500 µM in 0.1 N HCl. The final concentration of the calibrants were 1, 5, 10, 20, 50, 100, 200, and 500 µM for all

amino acids (except cysteine which was present in ½ the concentration) and 250 µM for norvaline (internal standard).[3] The precision sample was prepared at 500 µM (250 µM cysteine). Norvaline was kept constant at 250 µM.

LC Conditions

LC systems:	ACQUITY UPLC System with passive preheating and in-line filter ACQUITY Premier Binary Fixed Loop System with active column preheating and in-line filter
Detection:	ACQUITY UPLC System– ACQUITY TUV Detector with 10 mm UPLC Analytical Flow Cell and CH-A Column Heater ACQUITY Premier Binary Fixed Loop System – ACQUITY TUV Detector with 10 mm UPLC Analytical Flow Cell and CH-A Column Heater
Wavelength:	260 nm
Sampling rate:	10 Hz
Vials:	LCGC certified clear glass 12 x 32 mm screw neck vial, total recovery with cap and PTFE/Silicone septum (not pre-slit) (p/n: 186000384C)
Column(s):	AccQ•Tag Ultra C_{18}, 1.7 µm 2.1 x 100 mm (p/n: 186003837)
Column temperature:	55 °C (for hydrolysate & food and feed), 60 °C (for cell culture) on the ACQUITY UPLC System 55 °C (for hydrolysate & food and feed), 50 °C (for

	cell culture) on the ACQUITY Premier Fixed Loop System
Sample temperature:	20 °C
Injection volume:	1 µL (ACQUITY UPLC System) and 0.5 µL (ACQUITY Premier Binary Fixed Loop System)
Injection mode	Partial Loop Needle Overflow (PLNO)
Flow rate:	0.7 mL/min
Mobile phase A:	1:20 dilution of AccQ•Tag Eluent A (p/n: 186003838) for hydrolysate & food and feed 1:10 dilution of AccQ•Tag Eluent A for cell culture
Mobile phase B:	AccQ•Tag Ultra Eluent B (p/n: 186003839)
Weak needle wash:	95:5 (v/v) Water:Acetonitrile (ACQUITY UPLC and ACQUITY Premier Binary Fixed Loop Systems)
Strong needle wash:	5:95 (v/v) Water:Acetonitrile (ACQUITY UPLC System) 95:5 (v/v) Water:Acetonitrile (ACQUITY Premier Binary Fixed Loop System)
Seal wash:	50:50 (v/v) Water:Acetonitrile (ACQUITY UPLC and ACQUITY Premier Binary Fixed Loop Systems)

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.700	99.9	0.1	Initial
0.54	0.700	99.9	0.1	6
5.74	0.700	90.9	9.1	7
7.74	0.700	78.8	21.2	6
8.04	0.700	40.4	59.6	6
8.05	0.700	10.0	90.0	6
8.64	0.700	10.0	90.0	6
8.73	0.700	99.9	0.1	6
9.50	0.700	99.9	0.1	6

Data Management

Chromatography data system: Empower™ 3, FR 3.7.0

Results and Discussion

The separation of a wide range of amino acids is challenging as slight changes in temperature, gradient delivery, and other conditions can impact retentivity and selectivity. Given these challenges, differences across LC Systems can require optimization of the method on a system-by-system basis. For these studies, the method conditions for the separation of amino acids from protein hydrolysate were adapted from the separation on a 2.1 x 100 mm from the amino acid total solution on the ACQUITY UPLC System.[1] Pre-column derivatization of amino acids was performed using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC), followed by separation of the derivatives with reversed-phase liquid chromatography.[1]

Instrument Considerations

The instrument method from the ACQUITY UPLC AccQ▪Tag Ultra Solution was tested on the ACQUITY Premier Binary Fixed Loop System. Adjustment of the method was required to achieve adequate resolution and chromatographic performance. To assess the final method conditions, both the ACQUITY UPLC and ACQUITY Premier Binary Fixed Loop Systems were tested. Both systems consist of binary pumps, fixed loop sample managers, and TUV (tunable wavelength) detectors. Differences included passive preheating on the ACQUITY UPLC System and active column preheating on the ACQUITY Premier Binary Fixed Loop System, along with slight differences in system dispersion.

Method Optimization of HPLC Amino Acid Separation

To ensure no differences could be attributed to the standards and samples, the standards and samples were prepared, derivatized, pooled, and split between the two systems. Mobile phases were also made up in large batches and split between the two systems. The same column was used between the two systems to reduce any column variability.

Direct migration of the method with no adjustments resulted in peak distortion of histidine on the ACQUITY Premier Binary Fixed Loop System, likely due to strong solvent effects caused by lower system dispersion (Figure 1). The ACQUITY Premier Binary Systems offer the needed sensitivity for amino acid analysis, precision for challenging gradients, and increased speed for high-throughput analysis.[2] Several method adjustments were evaluated including changing the strong needle wash from 5:95 water:acetonitrile to 95:5 water:acetonitrile.

Finally, adjustment of the injection volume was examined. After derivatization, the injection solvent contains a relatively high amount of organic (20%) when compared to the method starting conditions, therefore decreasing the injection volume could improve histidine peak shape. The ACQUITY Premier Binary Fixed Loop System produced significantly greater peak distortion for histidine above 0.5 µL, thus for this system a 0.5 µl injection volume was determined to be acceptable (Figure 2). Sensitivity was also evaluated due to the decrease of the injection volume by 50%.

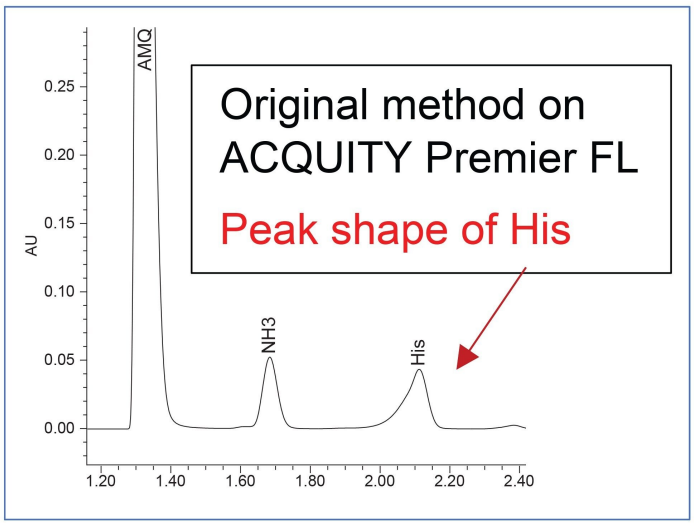

Figure 1. Injection volume determination of the Protein Hydrolysate AA Standard (500 µM) chromatogram on the ACQUITY Premier Binary Fixed Loop System.

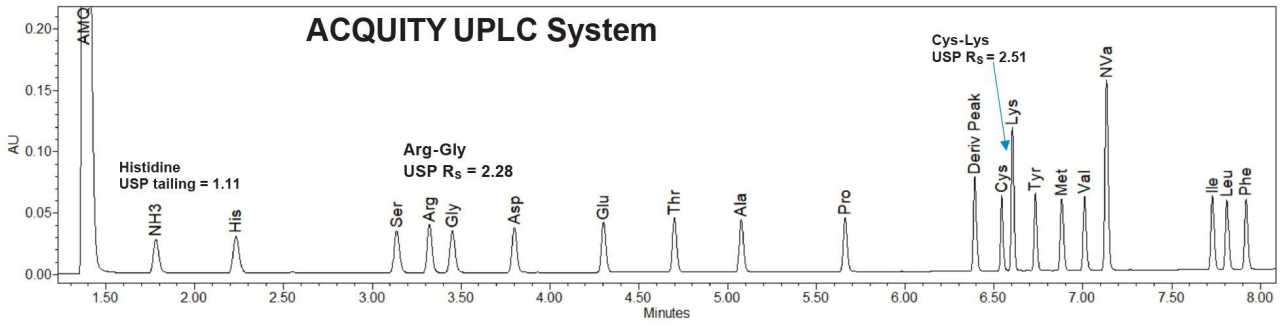

Figure 2. Protein Hydrolysate AA Standard (500 µM) chromatogram on the ACQUITY UPLC System.

After adjustment of the injection volumes for each system, chromatographic performance was compared using the hydrolysate standard (Table 1). Both systems were found to have acceptable USP resolution (>2.0) and peak shape for the hydrolysate amino acids (Figures 2 and 3).

ACQUITY Classic UPLC System USP resolution and tailing		
Compound	USP resolution	USP tailing
His	7.41	1.11
Ser	15.23	1.14
Arg	3.55	1.16
Gly	2.51	1.14
Asp	6.72	1.14
Glu	10.74	1.15
Thr	9.06	1.16
Ala	8.70	1.16
Pro	14.01	1.16
Cys	5.28	1.17
Lys	2.28	1.18
Tyr	4.48	1.16
Met	4.64	1.17
Val	3.91	1.21
Ile	18.26	1.17
Leu	2.47	1.18
Phe	3.20	1.17

ACQUITY Premier Binary FL System USP resolution and tailing		
Compound	USP resolution	USP tailing
His	5.38	1.03
Ser	10.71	1.10
Arg	2.67	1.12
Gly	2.12	1.11
Asp	4.90	1.10
Glu	8.75	1.11
Thr	7.74	1.12
Ala	7.20	1.12
Pro	12.3	1.13
Cys	4.49	1.17
Lys	2.21	1.16
Tyr	2.28	1.12
Met	4.11	1.13
Val	3.69	1.17
Ile	15.89	1.15
Leu	2.12	1.14
Phe	2.46	1.14

Table 1. Hydrolysate Standard (500 µM) UPS resolution and tailing results on both the ACQUITY UPLC and the ACQUITY Premier Binary Fixed Loop Systems.

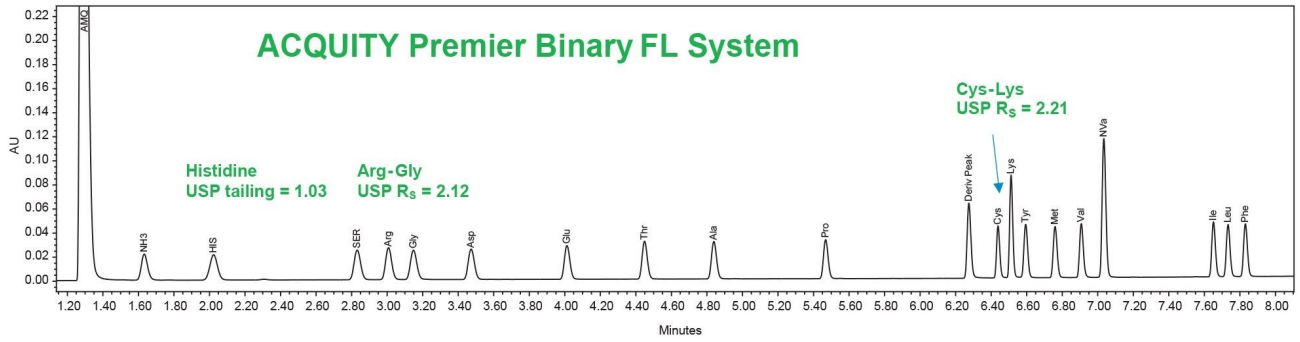

Figure 3. Protein Hydrolysate AA Standard (500 µM) on the ACQUITY Premier Binary Fixed Loop System.

Verification of Amino Acid Analysis on ACQUITY UPLC and ACQUITY Premier Binary Fixed Loop Systems

Using the adjusted injection volume (0.5 uL), each system was evaluated using the protein AA hydrolysate

standard to ensure adequate repeatability for both retention time and peak area. The results were comparable across both binary systems. All retention %RSDs were ≤0.5% for the 500 pmol standard (25 pmol on column) for six replicate injections.

The area precision for each system also produced comparable results. The ACQUITY UPLC System produced area RSDs from 0.2% to 0.4% for all the hydrolysate amino acids. For the ACQUITY Premier Binary Fixed Loop System produced area %RSDs were 1.1% to 1.4% for the hydrolysates. Although slightly lower area counts and higher %RSDs were observed on the ACQUITY Premier Binary Fixed Loop System, both systems demonstrated good area repeatability. Results for both retention time and peak area reproducibility are shown in tables 2 and 3.

ACQUITY UPLC System			
Compound	**RT**	**STDEV**	**%RSD**
His	2.227	0.004	0.2
Ser	3.136	0.001	0.0
Arg	3.321	0.001	0.0
Gly	3.450	0.001	0.0
Asp	3.800	0.001	0.0
Glu	4.304	0.001	0.0
Thr	4.701	0.002	0.0
Ala	5.077	0.002	0.0
Pro	5.662	0.001	0.0
Cys	6.544	0.001	0.0
Lys	6.605	0.001	0.0
Tyr	6.734	0.001	0.0
Met	6.882	0.001	0.0
Val	7.010	0.001	0.0
Ile	7.731	0.001	0.0
Leu	7.812	0.001	0.0
Phe	7.920	0.001	0.0

ACQUITY Premier Binary FL System			
Compound	**RT**	**STDEV**	**%RSD**
His	2.033	0.007	0.3
Ser	2.847	0.013	0.5
Arg	3.022	0.012	0.4
Gly	3.160	0.01	0.2
Asp	3.482	0.008	0.1
Glu	4.018	0.004	0.1
Thr	4.453	0.003	0.0
Ala	4.843	0.002	0.0
Pro	5.471	0.002	0.0
Cys	6.440	0.002	0.0
Lys	6.513	0.002	0.0
Tyr	6.596	0.002	0.0
Met	6.761	0.002	0.0
Val	6.909	0.002	0.0
Ile	7.653	0.002	0.0
Leu	7.753	0.002	0.0
Phe	7.832	0.002	0.0

Table 2. Protein Hydrolysate AA Standard (500 µM) retention time results on the ACQUITY UPLC and ACQUITY Premier Binary Fixed Loop Systems.

ACQUITY UPLC System			
Compound	Area	STDEV	%RSD
His	70963	271	0.4
Ser	71370	301	0.4
Arg	72056	277	0.4
Gly	70113	294	0.4
Asp	69427	290	0.4
Glu	68344	279	0.4
Thr	73016	355	0.4
Ala	70509	287	0.4
Pro	66910	272	0.4
Cys	62622	223	0.4
Lys	119509	262	0.2
Tyr	74423	270	0.4
Met	73611	280	0.4
Val	74763	290	0.4
Ile	74390	260	0.3
Leu	71872	224	0.3
Phe	74181	170	0.2

ACQUITY Premier Binary FL System			
Compound	Area	STDEV	%RSD
His	63664	676	1.1
Ser	63638	768	1.2
Arg	64242	780	1.2
Gly	62684	770	1.2
Asp	61767	719	1.2
Glu	61013	727	1.2
Thr	65161	753	1.2
Ala	63006	753	1.2
Pro	59552	706	1.2
Cys	55843	711	1.3
Lys	106898	1343	1.3
Tyr	67302	939	1.4
Met	65815	819	1.2
Val	66808	793	1.2
Ile	66632	784	1.2
Leu	64313	730	1.1
Phe	66970	733	1.1

Table 3. Protein Hydrolysate AA Standard (500 µM) retention time and area repeatability results on the ACQUITY UPLC and the ACQUITY Premier Binary Fixed Loop Systems.

Intermediate Precision for Retention Time and Area RSD

In addition to repeatability, intermediate precision is essential for analysis and quantitation of amino acids. Both the ACQUITY UPLC and ACQUITY Premier Binary Fixed Loop Systems were tested over three days with six samples tested per day. An internal standard (norvaline) was used to normalize differences in sample preparation. The analysis evaluated the intra-day precision for six injections and the aggregate precision across all three days for a total of 18 injections.

The results on both systems demonstrated high intra-day retention time precision and area precision. All retention times were within 1.5% RSD over the course of three days, with a precision that is well within the expected retention time range for each system. The peak area %RSD were within 1.8% within one day and 2.2% across all three days. The results demonstrate the reproducibility of the analysis on both the ACQUITY UPLC and

ACQUITY Premier Binary Fixed Loop Systems.

Retention time

	ACQUITY UPLC System				ACQUITY Premier Binary FL System			
Compound	Day 1 RT RSD	Day 2 RT RSD	Day 3 RT RSD	Interday RT RSD	Day 1 RT RSD	Day 2 RT RSD	Day 3 RT RSD	Interday RT RSD
His	**0.30**	0.40	0.60	1.50	**0.30**	0.10	0.30	0.90
Ser	**0.20**	0.20	0.30	1.10	**0.20**	0.00	0.20	0.60
Arg	**0.10**	0.20	0.20	0.90	**0.20**	0.00	0.10	0.60
Gly	**0.10**	0.20	0.20	0.90	**0.10**	0.00	0.10	0.50
Asp	**0.10**	0.20	0.20	0.80	**0.10**	0.00	0.10	0.50
Glu	**0.10**	0.10	0.10	0.60	**0.10**	0.00	0.00	0.30
Thr	**0.00**	0.10	0.10	0.40	**0.00**	0.00	0.00	0.30
Ala	**0.00**	0.10	0.00	0.40	**0.00**	0.00	0.00	0.20
Pro	**0.00**	0.10	0.00	0.30	**0.00**	0.00	0.00	0.20
Cys	**0.00**	0.00	0.00	0.10	**0.00**	0.00	0.00	0.10
Lys	**0.00**	0.00	0.00	0.10	**0.00**	0.00	0.00	0.10
Tyr	**0.00**	0.00	0.00	0.20	**0.00**	0.00	0.00	0.10
Met	**0.00**	0.00	0.00	0.10	**0.00**	0.00	0.00	0.10
Val	**0.00**	0.00	0.00	0.10	**0.00**	0.00	0.00	0.10
Ile	**0.00**	0.00	0.00	0.10	**0.00**	0.00	0.00	0.10
Leu	**0.00**	0.00	0.00	0.10	**0.00**	0.00	0.00	0.10
Phe	**0.00**	0.00	0.00	0.10	**0.00**	0.00	0.00	0.10

Area

	ACQUITY UPLC System				ACQUITY Premier Binary FL System			
Compound	Day 1 area RSD	Day 2 area RSD	Day 3 area RSD	Interday area RSD	Day 1 area RSD	Day 2 area RSD	Day 3 area RSD	Interday area RSD
His	**0.30**	0.40	0.60	**1.50**	**0.30**	0.10	0.30	**0.90**
Ser	**0.20**	0.20	0.30	**1.10**	**0.20**	0.00	0.20	**0.60**
Arg	**0.10**	0.20	0.20	**0.90**	**0.20**	0.00	0.10	**0.60**
Gly	**0.10**	0.20	0.20	**0.90**	**0.10**	0.00	0.10	**0.50**
Asp	**0.10**	0.20	0.20	**0.80**	**0.10**	0.00	0.10	**0.50**
Glu	**0.10**	0.10	0.10	**0.60**	**0.10**	0.00	0.00	**0.30**
Thr	**0.00**	0.10	0.10	**0.40**	**0.00**	0.00	0.00	**0.30**
Ala	**0.00**	0.10	0.00	**0.40**	**0.00**	0.00	0.00	**0.20**
Pro	**0.00**	0.10	0.00	**0.30**	**0.00**	0.00	0.00	**0.20**
Cys	**0.00**	0.00	0.00	**0.10**	**0.00**	0.00	0.00	**0.10**
Lys	**0.00**	0.00	0.00	**0.10**	**0.00**	0.00	0.00	**0.10**
Tyr	**0.00**	0.00	0.00	**0.20**	**0.00**	0.00	0.00	**0.10**
Met	**0.00**	0.00	0.00	**0.10**	**0.00**	0.00	0.00	**0.10**
Val	**0.00**	0.00	0.00	**0.10**	**0.00**	0.00	0.00	**0.10**
Ile	**0.00**	0.00	0.00	**0.10**	**0.00**	0.00	0.00	**0.10**
Leu	**0.00**	0.00	0.00	**0.10**	**0.00**	0.00	0.00	**0.10**
Phe	**0.00**	0.00	0.00	**0.10**	**0.00**	0.00	0.00	**0.10**

Table 4. Intermediate precision for retention time and area reproducibility on ACQUITY Arc and ACQUITY Premier Binary Fixed Loop Systems.

Linearity and Limit of Detection/Limit of Quantitation

To ensure accurate quantitation of all the amino acids, linearity as well as limit of detection (LOD) and limit of

quantitation (LOQ) were evaluated for both the ACQUITY UPLC System and the ACQUITY Premier Binary Fixed Loop System. Linearity was determined by injecting eight concentration levels of hydrolysate standard ranging from 1 µM to 500 µM. The calibration curves for both instruments resulted in an acceptable R^2 of >0.99 for all amino acids. Given the distribution of standards across the dynamic range, a weighting of 1/x was used for the calibration curve. The limit of quantitation (LOQ), as defined by signal-to-noise equal to or greater than 10:1, was 1 µM for both systems. All values above the LOQ exhibited deviations of <17% and deviations of <30% at the LOQ. Despite the differences in injection volume, the limit of detection (LOD) was determined to be 0.5 µM based on a signal to noise ratio of 3:1 for both instruments.

ACQUITY UPLC System									
		% Deviation at concentration level (µM)							
Compound	R^2	2	5	10	20	50	100	200	500
His	0.9997	9.34	-0.28	5.37	-0.37	-4.45	-3.93	1.88	0.43
Ser	0.9986	-9.18	-4.86	-1.98	1.20	5.11	13.06	-0.51	-2.84
Arg	0.9997	0.69	1.28	2.28	-3.29	-5.99	4.46	1.31	-0.75
Gly	0.9998	6.87	-7.40	-1.89	-0.99	0.47	2.78	1.06	-0.90
Asp	0.9997	-5.64	-2.80	4.94	0.99	1.29	-0.54	3.11	-1.35
Glu	0.9998	-6.94	3.39	5.04	0.55	-2.72	-1.53	2.92	-0.72
Thr	0.9998	-0.78	1.44	2.63	-0.05	-2.49	-2.84	2.22	-0.13
Ala	0.9998	-1.18	-1.57	3.26	0.81	-1.29	-1.97	2.49	-0.55
Pro	0.9998	4.01	-2.63	2.71	-0.77	-2.81	-2.77	2.38	-0.13
Cys	0.9998	5.69	-1.17	2.94	-2.53	-3.97	-3.37	2.18	0.23
Lys	0.9998	-2.33	-1.04	5.83	-1.16	-1.25	-2.02	2.47	-0.51
Tyr	0.9998	2.51	0.31	3.38	-1.50	-3.56	-3.33	1.97	0.21
Met	0.9995	2.95	1.56	2.53	-1.12	-5.04	-3.23	2.05	0.30
Val	0.9998	-4.81	0.71	6.92	0.69	-2.87	-2.56	2.14	-0.21
Ile	0.9998	0.56	0.27	3.61	-1.08	-3.00	-2.59	2.41	-0.18
Leu	0.9998	0.38	1.08	2.21	-0.28	-3.07	-2.52	2.40	-0.19
Phe	0.9998	3.88	-0.40	2.24	-0.87	-3.96	-3.10	2.02	0.18

ACQUITY Premier Binary FL System									
		% Deviation @ concentration level (µM)							
Compound	R^2	2	5	10	20	50	100	200	500
His	0.9989	-1.96	-2.44	0.81	2.64	0.08	-2.91	5.48	-1.71
Ser	0.9983	-8.05	-4.61	5.40	3.55	1.41	-1.78	6.73	-2.65
Arg	0.9989	-3.97	-0.87	0.18	2.74	0.51	-2.25	5.58	-1.92
Gly	0.9985	-20.56	-3.13	13.23	7.83	1.98	-2.15	5.03	-2.25
Asp	0.9901	-24.11	-3.36	8.47	12.24	-2.51	-0.32	16.32	-6.74
Glu	0.9924	-23.76	-5.69	3.70	9.21	7.60	2.89	12.79	-6.74
Thr	0.9981	-10.60	-1.02	2.20	4.63	2.26	-1.65	7.09	-2.91
Ala	0.9954	-18.88	-3.42	4.43	6.87	5.25	0.42	10.33	-4.99
Pro	0.9978	-9.40	-1.88	1.13	4.59	2.55	-1.37	7.53	-3.14
Cys	0.9982	-18.98	-8.51	15.36	9.83	1.90	-2.49	5.20	-2.31
Lys	0.9924	-28.81	-8.37	10.23	11.37	7.83	1.92	12.45	-6.61
Tyr	0.9989	-8.71	-3.00	6.54	3.67	-0.11	-2.25	5.12	-1.80
Met	0.9987	-5.03	-5.78	5.58	3.72	0.00	-2.31	5.92	-2.09
Val	0.9971	-18.32	-4.77	4.41	13.04	2.76	-0.82	7.60	-3.64
Ile	0.9977	-12.32	-1.79	2.71	5.66	2.68	-1.32	7.66	-3.28
Leu	0.9977	-10.19	-2.58	2.02	4.99	2.72	-1.36	7.64	-3.23
Phe	0.9990	-7.03	0.34	1.69	3.61	0.48	-2.46	5.14	-1.77

Table 5. Protein Hydrolysate AA Standard (2–500 µM) R^2 and % deviation at concentration level linearity results

on the ACQUITY UPLC and the ACQUITY Premier Binary Fixed Loop Systems.

Multi-day concentration % deviation from nominal and concentration RSDs were calculated. Low % deviation and low RSDs were observed across the three days for both systems.

Concentration % deviation from nominal

	ACQUITY UPLC System				ACQUITY Premier Binary FL System			
Compound	Day 1	Day 2	Day 3	Interday	Day 1	Day 2	Day 3	Interday
His	1.1	-0.1	-0.7	**0.1**	-0.8	-1.5	-0.5	**-0.9**
Ser	1.6	0.4	-0.2	**0.6**	-1.0	-1.2	-0.5	**-0.9**
Arg	1.6	0.5	-0.2	**0.6**	-1.0	-1.5	-0.5	**-1.0**
Gly	1.5	0.7	-0.1	**0.7**	-0.9	-1.3	-0.5	**-0.9**
Asp	1.7	0.3	-0.2	**0.6**	-0.9	-1.6	-0.5	**-1.0**
Glu	1.6	0.3	-0.2	**0.6**	-0.9	-1.6	-0.5	**-1.0**
Thr	1.6	0.2	-0.1	**0.6**	-0.8	-1.5	-0.5	**-0.9**
Ala	1.5	0.2	-0.1	**0.5**	-0.9	-1.6	-0.5	**-1.0**
Pro	1.6	0.6	-0.2	**0.6**	-0.9	-1.6	-0.5	**-1.0**
Cys	1.5	0.3	-0.1	**0.6**	-0.9	-1.6	-0.5	**-1.0**
Lys	1.4	0.5	0.0	**0.6**	-0.9	-1.7	-0.5	**-1.0**
Tyr	1.6	0.4	-0.2	**0.6**	-1.1	-1.5	-0.5	**-1.0**
Met	1.6	0.4	-0.2	**0.6**	-0.9	-1.5	-0.5	**-1.0**
Val	1.6	0.3	-0.2	**0.6**	-0.9	-1.5	-0.5	**-1.0**
Ile	1.5	0.4	-0.2	**0.6**	-0.9	-1.6	-0.5	**-1.0**
Leu	1.5	0.4	-0.2	**0.5**	-0.9	-1.6	-0.5	**-1.0**
Phe	1.1	0.4	-0.3	**0.4**	-0.9	-1.6	-0.5	**-1.0**

Concentration RSD

	ACQUITY UPLC System				ACQUITY Premier Binary FL System			
Compound	Day 1	Day 2	Day 3	Interday	Day 1	Day 2	Day 3	Interday
His	1.8	1.4	0.6	**1.3**	1	1.5	1	**1.1**
Ser	1.8	0.8	0.4	**1.1**	1.1	1.5	1.1	**1.2**
Arg	1.7	0.9	0.4	**1.1**	1.1	1.5	1.1	**1.2**
Gly	1.8	1.2	0.4	**1.1**	1.1	1.6	1.1	**1.2**
Asp	1.8	0.7	0.5	**1.1**	1	1.5	1.1	**1.1**
Glu	1.8	0.8	0.5	**1.1**	1.1	1.5	1.1	**1.1**
Thr	1.8	0.7	0.5	**1.1**	1.1	1.5	1.1	**1.1**
Ala	1.8	0.7	0.4	**1.1**	1	1.5	1.1	**1.2**
Pro	1.8	0.8	0.5	**1.1**	1.1	1.5	1.1	**1.2**
Cys	1.6	0.7	0.4	**1**	1	1.5	1.1	**1.2**
Lys	1.6	0.7	0.3	**1**	1	1.5	1.1	**1.2**
Tyr	1.7	0.7	0.4	**1**	1.1	1.5	1.1	**1.2**
Met	1.7	0.7	0.4	**1**	1	1.5	1.1	**1.2**
Val	1.7	0.7	0.4	**1**	1.1	1.5	1.1	**1.2**
Ile	1.7	0.7	0.5	**1.1**	1.1	1.5	1.1	**1.2**
Leu	1.7	0.7	0.5	**1.1**	1	1.5	1.1	**1.2**
Phe	1.6	0.7	0.5	**1**	1.1	1.5	1.1	**1.2**

Table 6. Protein Hydrolysate AA Standard (1–500 µM) R^2 concentration % deviation from nominal and concentration RSD linearity results on the ACQUITY UPLC and the ACQUITY Premier Binary Fixed Loop Systems.

Separation of Additional Amino Acid Standards

Separation of additional amino acid standards was performed on the ACQUITY Premier Binary Fixed Loop System with a 0.5 µL injection volume. To achieve adequate separation, the cell culture standard required a column temperature of 50 °C. Food and feed standard separation was at 55 °C.

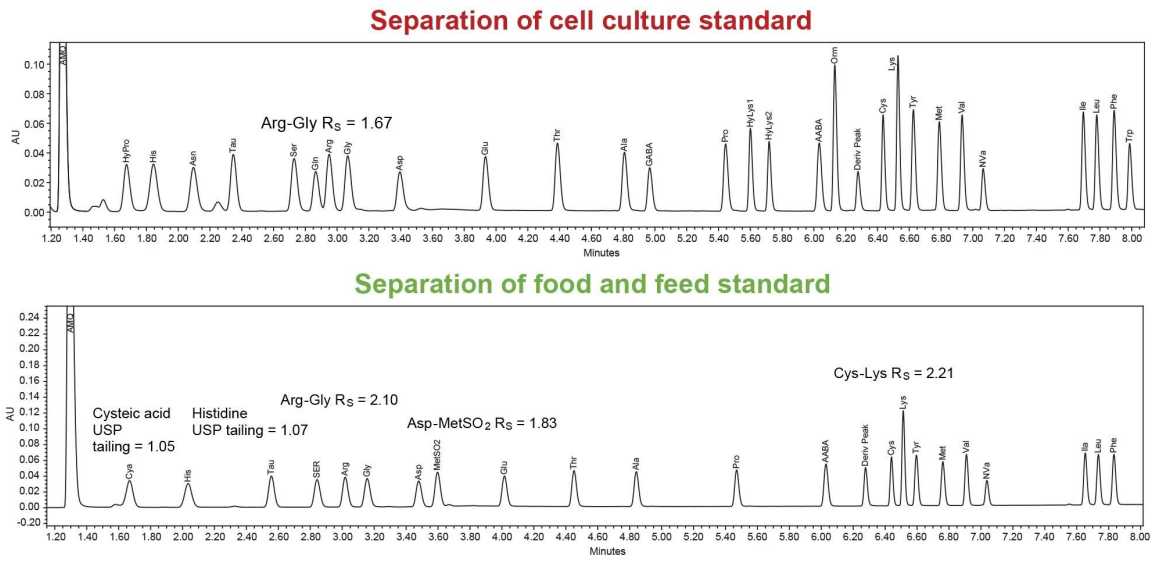

Figure 4. Cell culture and Food and Feed AA Standards (500 µM) on the ACQUITY Premier Binary Fixed Loop System.

As many customers need to analyze both hydroxyproline and cysteic acid in samples, the food and feed standard was spiked with hydroxyproline. Both amino acids elute early in the gradient, at <3% organic, and may require a binary pump for reproducible separation. Successful separation was achieved and is shown in Figure 5.

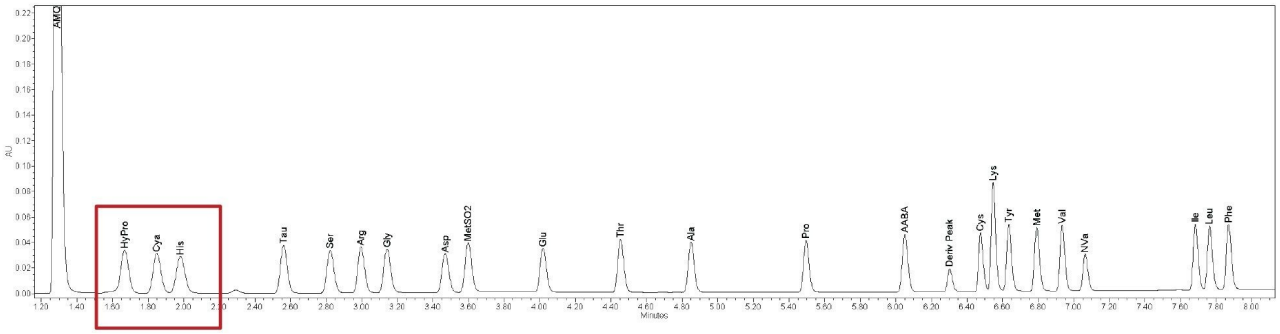

Figure 5. Protein Hydrolysate AA Standard (500 µM) food and feed standard spiked with hydroxyproline on the ACQUITY Premier Binary Fixed Loop System.

Quantitative Analysis of Energy Drink

Quantitative analysis of an amino acid powdered drink mix was performed on the ACQUITY UPLC and ACQUITY Premier Binary Fixed Loop Systems. The method provided comparable results for the amino acid content in the powdered drink mix sample. The chromatograms for the standard and the sample are shown in Figure 6 for the ACQUITY Premier Binary Fixed loop System. The low % difference between the two systems indicates high quantitative reproducibility (Table 7).

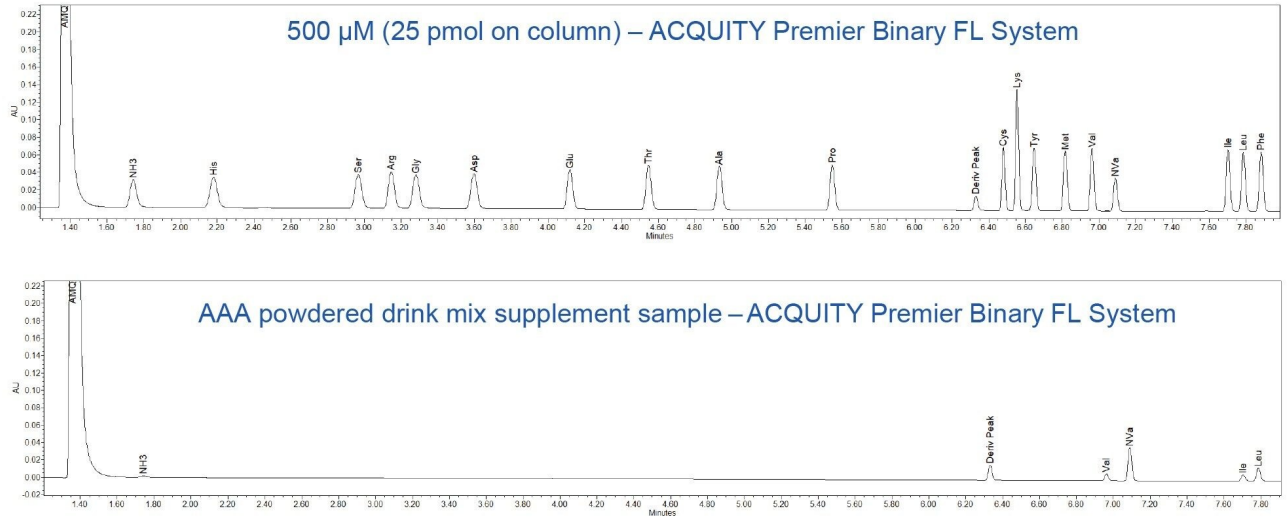

Figure 6. Protein Hydrolysate AA Standard (500 µM) and Amino Acid Powdered Drink Mix Supplement Sample Chromatograms on the ACQUITY Premier Binary Fixed Loop System.

ACQUITY UPLC System			
Amino acid	**Sample 1 powdered drink mix supplement label claim in weighed sample amount (g)**	**Sample 1 powdered drink mix supplement results (g)**	**Difference between label claim vs. results**
Val	1.40	1.42	-1.00%
Ile	1.40	1.41	-0.50%
Leu	3.04	3.12	-2.50%
Total amino acid weight in sample	5.85	5.95	-1.70%

ACQUITY Premier Binary FL System			
Amino acid	**Sample 1 powdered drink mix supplement label claim in weighed sample amount (g)**	**Sample 1 powdered drink mix supplement results (g)**	**Difference between label claim vs. results**
Val	1.40	1.41	-0.70%
Ile	1.40	1.41	-0.20%
Leu	3.04	3.02	0.70%
Total amino acid weight in sample	5.85	5.84	0.20%

Result differences between systems			
Amino acid	**Sample 1 powdered drink mix supplement ACQUITY UPLC Results (g)**	**Sample 1 powdered drink mix supplement ACQUITY Premier binary FL results (g)**	**Difference between systems results**
Val	1.42	1.41	0.70%
Ile	1.4	1.41	-0.70%
Leu	3.04	3.02	0.70%
Total amino acids	5.95	5.84	1.90%

Table 7. Amino Acid Powdered Drink Mix Sample Results on the ACQUITY UPLC and on the ACQUITY Premier Binary Fixed Loop System.

Conclusion

The AccQ•Tag Ultra method on the ACQUITY UPLC System was successfully migrated to the ACQUITY Premier Binary Fixed Loop System. The injection volume was changed from 1 µL in the original method to 0.5 µL to reduce the impact of strong solvent effects on histidine peak shape. Method performance was evaluated on both systems to ensure the results for linearity, precision, intermediate precision, LOD, and LOQ were acceptable after

the adjustment of injection volume. Both systems showed similar overall performance, which was demonstrated by low % RSDs and precise quantitative results.

Additionally, standards for cell culture and foods and feeds were also analyzed. To facilitate adequate peak shape and resolution, the cell culture method required adjustment of the column temperature from 60 °C to 50 °C. The food and feed standard spiked with hydroxyproline was successfully separated using only the decreased injection volume.

Finally, two powdered drink mix samples were analyzed using the adjusted method to compare quantitative results acquired using both systems. The results for the quantitation of the amino acid content in the powdered drink mix sample showed good agreement between the two systems and the label claims for the sample. This is indicative of high quantitative reproducibility across the two systems.

References

1. Amino Acid Analysis Application Notebook, 720006130 < https://www.waters.com/webassets/cms/library/docs/720006130en.pdf> , 2020.
2. Jennifer Simeone, Paula Hong. Instrument Considerations for Successful Adaptation of Amino Acids Analysis Methods Which Utilize Pre-Column Derivatization from an ACQUITY UPLC to an ACQUITY Premier Binary System. Waters Application Note, 720007440, 2021.
3. Amino Acid Standard Kits Care and Use Manual. Waters Care and Use Manual, 72000663. < https://www.waters.com/webassets/cms/support/docs/720006663en.pdf>

Featured Products

ACQUITY Premier System < https://www.waters.com/nextgen/global/products/chromatography/chromatography-systems/acquity-premier-system.html>

ACQUITY UPLC and ACQUITY Premier Tunable UV Detectors <https://www.waters.com/514228>

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

720008073, October 2023