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Nota de aplicación

Amino Acid Analysis using Andrew+ Automated Preparation

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Abstract

The objective of this application note is to demonstrate the equivalency and robustness of manual preparations of AccQ●Tag labelled amino acids to those prepared using the Andrew+ liquid handling robot with amino acid standard kits.

Introduction

Amino acids are the most basic components that make up proteins, thus making them essential components of cell culture media and food stuffs. Monitoring and optimizing the amino acid components of bioreactor media is essential for ensuring the best growing conditions for the cells. Likewise, it is necessary to confirm that food products meet specified requirements. Therefore, the analysis of amino acids is a critical routine process.

The preparation and analysis of samples is a time-consuming process that can dominate an analyst's time in the laboratory. Automated laboratory preparation systems provide the flexibility of freeing analysts time for other tasks, resulting in a more efficient way of time management. Waters has created automated sample preparation protocols for the Andrew Alliance Andrew+ platform in conjunction with the AccQ●Tag Ultra Derivatization Automation Kit (p/n: 186009232) (Figure 1) and amino acid standard kits. The AccQ●Tag Ultra Derivatization Kit for automation scales up reagent volumes necessary for use with automation systems due to their increased dead volume requirements. The reagent volumes provided allow for the preparation of up to 96 samples in a 3 x 32 sample format.

Figure 1. AccQ●Tag Ultra Derivatization Automation Kit.

A food and feed kit containing 21 amino acids and an amino acid cell culture standard kit containing 26 amino acids (Table 1) is available. Protocols for the Andrew+ liquid handling robot are stored in OneLab, a cloud-based software with an intuitive user-friendly graphical interface. Cell culture and food and feed amino acid standards

are used in this application note to demonstrate the results obtained from both manual and automated sample preparations.

Amino acid	Cell culture standard kit	Food and feed standard kit	Internal standard
	p/n: 186009300	p/n: 186009299	p/n: 186009301
Alanine	x	x	
Arginine	x	x	
Aspartic acid	x	x	
Cystine	x	x	
Glutamic acid	x	x	
Glycine	x	x	
Histidine	x	x	
Isoleucine	x	x	
Leucine	x	x	
Lysine	x	x	
Methionine	x	x	
Phenylalanine	x	x	
Proline	x	x	
Serine	x	x	
Threonine	x	x	
Tyrosine	x	x	
Valine	x	x	
Taurine	x	x	
HydroxyProline	x		
Asparagine	x		
Glutamine	x		
GABA (γ-Aminobutyric acid)	x		
Tryptophan	x		
Ornithine	x		
AABA (α-Aminobutyric acid)	x	x	
HydroxyLysine	x		
Methionine sulfone		x	
Cysteic acid		x	
Norvaline			x

Table 1. Amino acid composition of Cell Culture Standard Kit.

Experimental

The workflow consisted of manual or automated sample preparation followed by LC analysis and data processing in Empower Software.

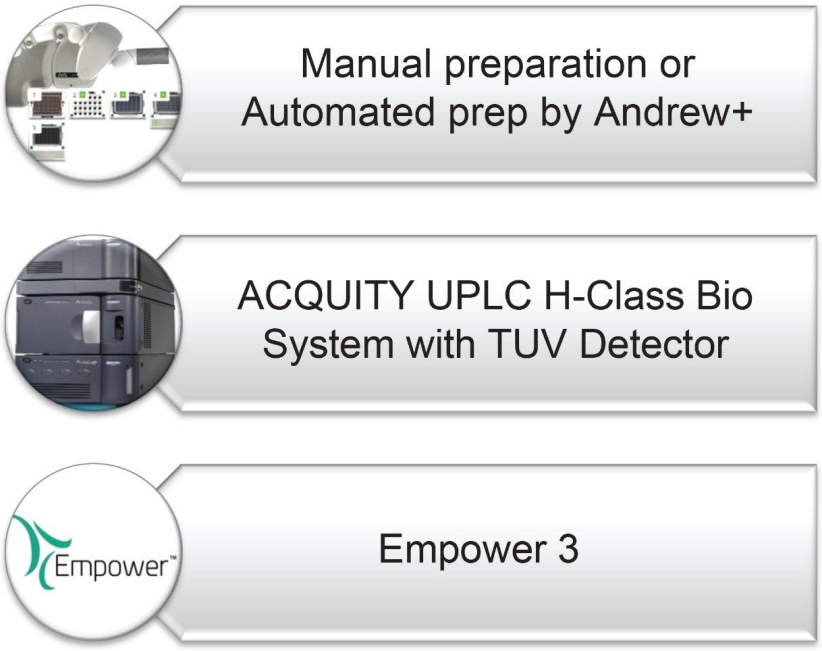

Figure 2. Systems and software used in the AccQ●Tag workflow.

Analytical Method Conditions

LC system:	ACQUITY UPLC H-Class Bio with TUV
Sample temp.:	20 °C
Analytical column temp.:	43 °C (cell culture), 49 °C (food and feed)
Flow rate:	700 µL/min
Injection volume:	1 µL
Column:	AccQ•Tag Ultra, 1.7 µm, 2.1 × 100 mm
UV detection:	260 nm
Mobile phase A:	100% AccQ•Tag Ultra Eluent A
Mobile phase B:	90:10 water, AccQ•Tag Ultra Eluent B
Mobile phase C:	100% HPLC-grade water
Mobile phase D:	100% AccQ•Tag Ultra Eluent B

Table 2. AccQ●Tag profiling method for cell culture/food and feed.

Design Factors

Protocol features

A set of three sample preparation protocols (32, 64, and 96 samples) were created for the Andrew+ liquid handling robot based on the AccQ●Tag derivatization automation kit. In addition, calibration line and reagent preparation protocols are available in OneLab, a protocol design and execution software for connected devices. The calibration line protocol provides the ability to perform dilution of standards with a reference range of 500 µM to 0.5 µM (cystine 250 µM to 0.25 µM). The resulting diluted standards can then be used with the sample preparation protocols as a 7-point calibration line.

The reagent preparation protocol can be used in conjunction with Pipette+ connected electronic pipettes to prepare reagents and standards for the sample preparation and derivatization protocol. There is also the flexibility to include the Norvaline Internal Standard (p/n: 186009301) when preparing samples.

Labware

The manual preparation of amino acid samples with the AccQ●Tag Derivatization Kit was performed using Waters Total Recovery Vials. In order to make this automation compatible, the total recovery glass vials were replaced with a 96-well Lo Bind PCR plate (p/n: 0030129555). This labware was also appropriate for use with the Shaker+, Peltier+ and gripper devices during preparation. Testing was conducted to support the labware change and no impact to product performance was detected.

Experimental Design

Manual and Andrew+ sample preparations were performed for the food and feed and cell culture standard preparations. The AccQ●Tag Ultra Derivatization automation kits were used along with a minimum of two different column lots and AccQ●Tag Ultra mobile phase eluents. Three solvent panels (0.1 M HCl) were created at levels spanning the concentration range (10 µM, 200 µM, and 400 µM) for both food and feed and cell culture. These panels contained the relevant amino acids to assess preparation performance.

Results and Discussion

The automated preparation method using the Andrew+ platform was assessed and compared to the manual preparation method for robustness and equivalency. Performance characteristics were monitored across three concentration levels (10 µM, 200 µM, and 400 µM) to determine the accuracy and precision (retention time, analyte peak area, and concentration) as well as the linearity of these results. A total of 18 samples were assessed using the AccQ●Tag Ultra 32-sample protocol with six preparations at each level. Panels were prepared in singlicate and injected in duplicate. The first injection was used in the calculation of the results. The duplicate injection is only analyzed as a backup in case of instrument issues. Norvaline internal standard was used in all experiments. The use of a Norvaline internal standard best compensates for the variability generated in sample hydrolysis and amino acid analysis.

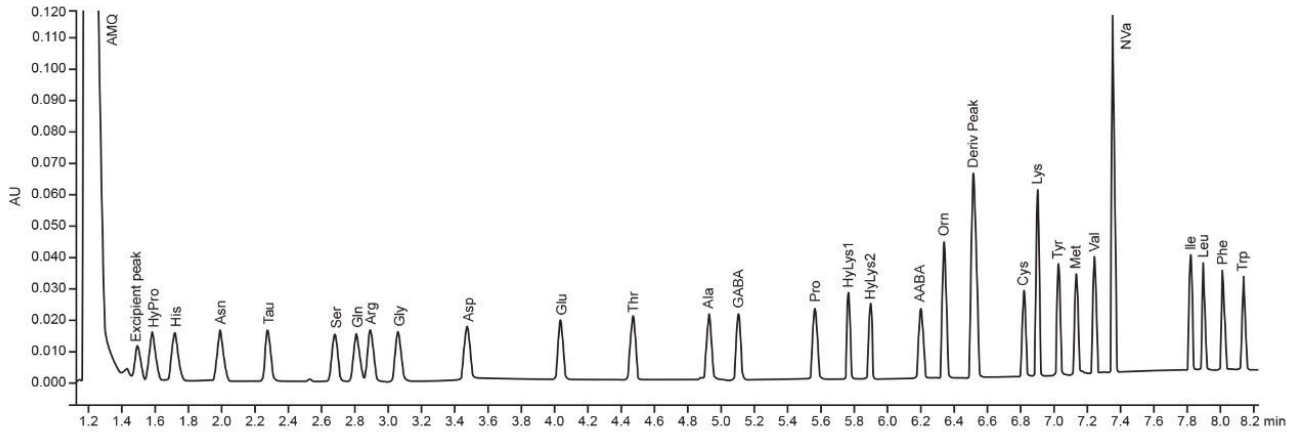

Figure 3. Separation of 10 pmols of the cell culture standard spiked with 23.5 pmols of Nva on column.

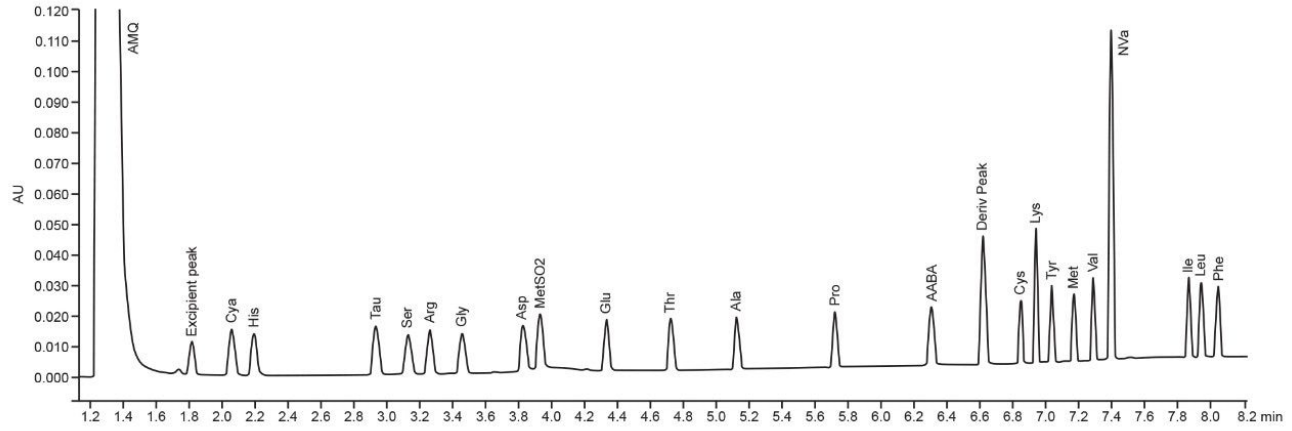

Figure 4. Separation of 10 pmols of the food and feed standard spiked with 23.5 pmols of Nva on column.

Precision

To demonstrate the repeatability of the automated sample preparation in comparison to the manual preparation, the %CV for each concentration level was determined. The maximum mean %CV across all amino acids and all concentration levels for cell culture Andrew+ and manual preparation was 2.0% and 2.3%, respectively. The maximum mean %CV across all amino acids and all concentration levels for food and feed Andrew+ was 1.7% and for manual it was 2.8%. The data in Tables 3 and 4 indicate comparable precision between Andrew+ and

manual preparation.

Cell culture concentration %CV (N = 6)						
	Andrew +			Manual		
Analyte	10 µM	200 µM	400 µM	10 µM	200 µM	400 µM
HyPro	0.9	1.0	2.4	2.0	1.5	1.9
His	1.1	1.1	2.3	2.4	1.4	1.6
Asn	1.3	1.1	2.2	2.3	2.2	3.1
Tau	1.3	1.1	2.5	2.0	1.5	1.6
Ser	0.9	1.1	1.8	2.4	1.5	1.7
Gln	2.2	1.1	2.1	1.9	1.4	1.4
Arg	1.2	1.1	2.2	2.5	1.9	1.8
Gly	1.3	1.1	2.3	2.7	1.5	1.6
Asp	1.7	1.8	1.8	2.4	1.8	3.3
Glu	1.4	1.6	1.6	2.4	1.7	2.9
Thr	1.5	1.5	2.1	2.4	1.5	1.6
Ala	1.9	1.5	1.5	2.1	1.7	2.5
GABA	2.1*	2.3	2.1	2.4	2.0	4.3
Pro	1.7	1.2	1.6	2.4	1.5	1.8
HyLys1	3.8	1.2	1.7	2.3	1.5	1.7
HyLys2	1.2	1.2	1.7	2.3	1.5	1.7
AABA	1.5	1.3	1.5	2.4	1.6	2.1
Orn	1.5	1.5	1.8	2.3	1.6	2.4
Cys	1.2*	1.1	2.3	2.5	1.5	1.6
Lys	1.8	1.9	2.0	2.3	1.7	3.1
Tyr	1.0	1.1	2.6	2.4	1.5	1.6
Met	1.2	1.0	2.0	2.7	1.6	1.8
Val	1.3	1.2	1.6	2.3	1.5	1.8
Ile	1.3	1.2	1.6	2.1	1.5	1.8
Leu	1.1	1.2	1.6	2.5	1.5	1.8
Phe	1.2	1.2	2.6	2.2	1.5	1.6
Trp	1.1	1.2	2.9	2.3	1.5	1.7

Table 3. Cell culture %CV Andrew+ and manual preparation across 10 µM, 200 µM, and 400 µM solvent panels.

**A second injection was used due to integration error.*

Food and feed concentration %CV (N = 6)						
	Andrew +			Manual		
Analyte	10 µM	200 µM	400 µM	10 µM	200 µM	400 µM
Cya	0.6	0.9	2.3	2.1	2.5	1.6
His	0.2	1.0	2.4	0.4	2.8	1.5
Tau	0.9	1.0	2.5	1.4	2.8	1.5
Ser	0.7	0.8	1.6	2.0	2.8	1.5
Arg	0.5	1.0	2.3	0.6	2.9	1.7
Gly	1.8	1.0	2.1	1.1	2.8	1.6
Asp	0.5	1.2	1.2	1.0	2.7	1.7
MetSO2	0.9	0.8	2.1	1.1	2.9	1.5
Glu	0.3	1.0	0.9	0.6	2.8	1.6
Thr	0.6	0.8	1.5	1.4	2.9	1.5
Ala	0.4	0.9	0.9	0.9	2.8	1.6
Pro	0.5	0.8	1.2	1.3	2.8	1.5
AABA	0.3	0.8	0.9	0.6	2.8	1.5
Cys	0.4	1.0	2.3	0.7	2.8	1.5
Lys	0.4	1.2	1.4	0.7	2.8	1.7
Tyr	0.6	1.0	2.6	0.8	2.9	1.5
Met	0.3	0.9	1.9	0.8	2.8	1.7
Val	1.5	0.8	1.1	2.2	2.9	1.5
Ile	0.3	0.8	1.1	0.9	2.9	1.5
Leu	0.4	0.8	1.2	0.8	2.8	1.6
Phe	0.5	1.0	2.6	0.8	2.8	1.5

Table 4. Food and feed %CV for Andrew+ and manual preparation across 10 µM, 200 µM, and 400 µM solvent panels.

Accuracy

Accuracy was assessed at concentrations of 10 µM, 200 µM, and 400 µM using six preparations at each concentration level. The average concentration of the six preparations is used to calculate the difference from the target value in order to find the %Recovery. The %Recovery for each amino acid for both cell culture and food and feed was within 10% of the target concentration (Tables 5 and 6). This recovery data demonstrates the suitability of Andrew+ as a great time-saving alternative to manually derivatizing amino acids.

Cell culture %recovery (N = 6)						
	Andrew+			Manual		
Analyte	10 µM	200 µM	400 µM	10 µM	200 µM	400 µM
HyPro	93.7	94.1	97.2	102.2	105.8	108.6
His	95.5	93.8	95.3	104.7	103.4	102.5
Asn	93.1	94.3	96.7	101.9	108.6	107.0
Tau	90.0	93.1	96.0	103.6	105.8	106.3
Ser	95.9	96.1	95.9	102.1	104.3	102.7
Gln	95.0	92.6	94.8	102.9	105.5	104.5
Arg	94.3	94.3	95.7	102.9	104.6	102.5
Gly	94.1	93.5	95.8	101.2	102.8	102.9
Asp	96.8	97.8	95.8	101.7	107.6	102.6
Glu	97.8	97.8	95.0	104.8	107.0	102.0
Thr	91.2	95.0	95.3	100.3	103.8	102.2
Ala	97.3	97.8	96.6	94.7	106.9	100.2
GABA	100.6	98.4	96.3	111.1	113.6	107.4
Pro	95.8	94.7	95.3	97.8	103.1	102.3
HyLys1	95.3	98.1	99.0	112.1	114.6	114.6
HyLys2	94.7	95.5	96.2	111.7	113.3	113.0
AABA	93.4	95.1	95.9	101.0	107.1	106.9
Orn	100.7	95.7	95.2	105.6	107.8	106.3
Cys	98.6	93.6	95.4	100.9	103.2	102.8
Lys	98.0	96.1	94.2	103.9	105.8	102.0
Tyr	93.4	93.1	95.5	100.6	102.6	102.7
Met	92.2	92.6	90.5	100.4	102.3	99.9
Val	95.0	94.6	95.2	101.9	103.5	102.4
Ile	94.1	94.5	95.6	102.4	103.3	102.8
Leu	99.0	94.4	94.9	102.4	103.3	102.1
Phe	93.0	93.3	95.8	100.6	103.0	102.8
Trp	94.1	93.4	96.0	102.7	107.1	106.6

Table 5. Cell culture amino acid standard %Recovery from target values.

Food and feed %Recovery (N = 6)						
	Andrew+			Manual		
Analyte	10 µM	200 µM	400 µM	10 µM	200 µM	400 µM
Cya	98.4	93.2	96.4	97.1	102.8	100.7
His	98.1	92.0	95.0	100.7	100.3	97.2
Tau	98.7	93.0	96.3	106.9	101.4	98.1
Ser	100.7	94.3	95.6	102.3	101.1	97.7
Arg	98.3	93.1	95.9	102.9	102.5	98.8
Gly	99.3	92.8	96.5	101.7	100.5	97.6
Asp	101.8	96.3	95.2	101.1	102.8	98.6
MetSO2	105.7	93.8	97.8	99.1	102.4	100.3
Glu	102.6	95.6	94.1	102.4	102.6	98.0
Thr	103.4	93.1	94.8	102.0	100.8	97.5
Ala	101.8	94.2	93.9	101.9	101.2	97.3
Pro	100.0	93.3	94.7	100.9	100.5	97.2
AABA	101.2	94.2	94.7	102.5	102.1	98.7
Cys	99.4	92.6	95.7	102.3	101.3	97.9
Lys	101.4	93.9	92.3	101.6	101.3	96.8
Tyr	97.9	92.5	96.2	102.6	100.7	97.6
Met	97.2	91.0	91.1	99.3	99.1	93.1
Val	101.7	93.0	94.1	93.7	100.7	97.2
Ile	99.7	92.8	94.2	101.6	100.5	97.3
Leu	99.5	92.8	94.3	101.0	100.5	97.5
Phe	97.9	92.3	95.9	101.0	100.6	97.5

Table 6. Food and feed amino acid standard %Recovery from target values.

Linearity

Linearity was assessed using a cell culture standard prepared at seven concentration levels for each amino acid across a range of 0.5 µM–500 µM (Cystine 0.25 µM–250 µM). All analytical runs were assessed for linearity and all met the criteria of $r^2 > 0.995$ with no point deviation from the expected concentration by >15% for calibrators 2–7 (2.5 µM–500 µM) and >20% for calibrator 1 (0.5 µM). The data was consistent between manual and automated preparation methods and no trends were observed.

Cell culture R^2		
Amino acid	Andrew+	Manual
HyPro	0.9991	0.9990
His	0.9992	0.9997
Asn	0.9991	0.9997
Tau	0.9988	0.9997
Ser	0.9993	0.9997
Gln	0.9993	0.9998
Arg	0.9993	0.9997
Gly	0.9991	0.9997
Asp	0.9988	0.9992
Glu	0.9991	0.9995
Thr	0.9993	0.9998
Ala	0.9993	0.9997
GABA	0.9988	0.9980
Pro	0.9993	0.9997
HyLys1	0.9994	0.9997
HyLys2	0.9994	0.9997
AABA	0.9993	0.9998
Orn	0.9993	0.9998
Cys	0.9993	0.9997
Lys	0.9992	0.9996
Tyr	0.9992	0.9996
Met	0.9993	0.9997
Val	0.9993	0.9997
Ile	0.9993	0.9997
Leu	0.9994	0.9997
Phe	0.9991	0.9997
Trp	0.9992	0.9997

Table 7. R^2 values for line generated using the Waters Amino Acid Cell Culture Standard. All lines passed

acceptance criteria of having an R^2 value greater than 0.995.

Food and feed R^2		
Amino acid	Andrew+	Manual
Cya	0.9996	0.9997
His	0.9996	0.9990
Tau	0.9997	0.9989
Ser	0.9993	0.9989
Arg	0.9995	0.9980
Gly	0.9997	0.9989
Asp	0.9981	0.9982
MetSO2	0.9996	0.9991
Glu	0.9984	0.9984
Thr	0.9995	0.9989
Ala	0.9990	0.9987
Pro	0.9994	0.9990
AABA	0.9992	0.9989
Cys	0.9996	0.9988
Lys	0.9985	0.9985
Tyr	0.9996	0.9990
Met	0.9996	0.9985
Val	0.9994	0.9985
Ile	0.9994	0.9990
Leu	0.9994	0.9990
Phe	0.9996	0.9990

Table 8. R^2 values for line generated using the Waters Amino Acid Food and Feed Standard. All lines passed

acceptance criteria of having an R^2 value greater than 0.995.

Conclusion

The performance characteristics of precision, accuracy, and linearity were used to determine the equivalence of the Andrew+ preparations to manual preparations. The results indicate excellent comparability between the two sample preparation methods for the UPLC Amino Acid Analysis Solution, however there are convenient benefits to automation that must also be considered when performing a comparative analysis:

- The Andrew+ robot provides efficiency without compromising accuracy and precision with calibration line preparation and sample preparation performed in under an hour.
- The automation protocol developed requires no manual intervention during the run, taking advantage of features like the Bluetooth configured pipettes which switch between volumes and the gripper device to transfer labware, thus allowing the analyst time to perform other laboratory tasks.
- The OneLab cloud-based software allows the user to monitor the run from any internet connected computer or tablet they have available.
- The use of automation removes analyst-to-analyst variation allowing laboratories and companies to standardize analysis methods and facilitate method transfer between multiple sites.

Acknowledgements

Danielle Cullen, Niamh Stafford, Leanne Davey, Norma Breen (Waters Technologies Ireland Ltd); Steven Calciano, Ning Zhang (Waters Corporation, Milford, MA).

Featured Products

ACQUITY UPLC H-Class PLUS Bio System <https://www.waters.com/10166246>

ACQUITY UPLC Tunable UV Detector <https://www.waters.com/514228>

Automated Liquid Handling for LC & LC-MS Sample Preparation Workflows <https://www.waters.com/waters/nav.htm?cid=135070059>

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

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