HIGH THROUGHPUT PREPARATION OF AMINO ACIDS USING WATERS TECHNOLOGIES ACCQ-TAG ULTRA AUTOMATION KIT AND CELL CULTURE STANDARDS

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OVERVIEW

- Provide a time saving easy to use solution with comparable results to current manual preparation option.
- Development of an automation compatible kit and scripts available on three automation platforms.
- A 96 sample preparation of amino acid cell culture samples including creation of calibrator series.

INTRODUCTION

The production of biopharmaceuticals requires constant monitoring of media components such as amino acids to ensure optimal cell growth conditions. A new automation compatible AccQ-Tag Ultra reagent Kit has been developed in parallel with a Cell Culture Standards Kit that enables the analysis of 26 amino acids. In conjunction with the automation kit, three automation scripts have been created on the Andrew+, Hamilton Microlab star, and Tecan Freedom EVO 100/4 providing a highly efficient and rapid preparation method.

Figure 1: Automated Amino analysis solution with AccQTag ultra kit and cell culture standard

METHODS

OVERALL WORKFLOW

A set of panels containing 26 cell culture amino acids were prepared manually, on the Hamilton ML Star and the Tecan Freedom EVO 100/4. The Andrew + platform was used to prepare Hydrolysate Standard samples. The resulting samples were analyzed using a Waters ACQUITY® UPLC® H-Class and H-Class Bio System TUV. Results were processed in Empower 3 and transferred to excel for further analysis.

Table 1. Method conditions for Amino acid Analysis

AccQ-Tag Profiling method - Cell culture	
LC System:	ACQUITY® UPLC® H-Class and H-Class Bio System TUV
Sample temp:	20˚C
Analytical Column Temp:	43˚C
Flow rate:	700 µL/min
Injection Volume:	1µL
Column	AccQTag Ultra Column 2.1 x 100 mm, 1.7µm
UV detection	260 nm
Mobile Phase A	100% AccQ Tag Ultra eluent A concentrate
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

AUTOMATION SCRIPT OPTIMISATION

A number of options were considered during the creation and optimisation of the scripts on all automation platforms. The following features and optimisations were added to the scripts:

- Optional sample concentration dilution included via import worklist function.
- Optional dilution of cell culture standard to create a calibration series.
- Minimal user interventions included (only at start and end of script). User inputs for well start and number of samples.
- Barcode scanning included to allow sample IDs to be recorded into excel. (Hamilton and Tecan)
- Parameters within the "liquid classes" were deliberately adjusted to ensure optimal outcome. (Hamilton and Tecan)
- Deck loading instructions or user prompts added to script to direct user.
- Sample preparation steps remain the same with or without Internal standard by adjusting initial reagent preparation steps.

AUTOMATION KIT DEVELOPMENT

- Automation compatible reagent kit designed in a 3 x 32 sample format, to account for extra reagent volumes required for platforms with liquid detection capabilities.
- Labware adapted from vials used in manual preparation to a 96 well plate format for ease of plate transfer and heating steps.
- Cell culture standard is provided in a two vial format which can be combined to give 26 amino acids at 500µM in 500µl to create a series of 7 calibrators.
- Cell culture standard kit in lyophilised format to allow for enhanced stability.
- Norvaline internal standard option included with excess volume for automation.

CHROMATOGRAPHY

Chromatography was assessed to ensure acceptable peak shape and baseline resolution for all 26 analytes in the cell culture standard.

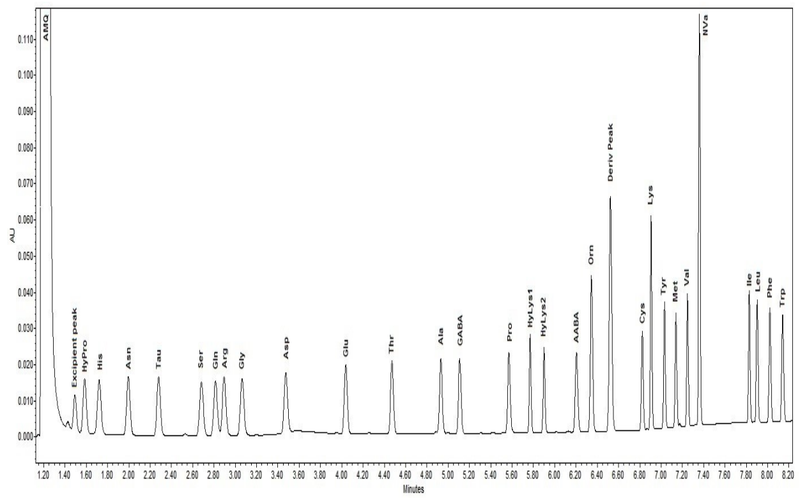

Figure 2. Separation of 10 pmols of the cell culture standard spiked with 23.5 pmols of Nva on column

PRECISION

Sample preparation was performed on both the Hamilton ML Star and the Tecan Freedom EVO 100 using the same reagent lot across three days. Samples on the Andrew+ were from a single preparation of 8 samples and were run in triplicate. One day of manual sample preparation was performed for comparison. Within run precision data is shown for Hamilton and Tecan results for comparison with manual (n=9) see Table 3 . The mean % CV of each cell culture standard amino acid is ≤ 3.9%.

Table 2. Overall precision results for 26 cell culture standard amino acids (n=9 for Hamilton, Tecan, and Manual; n=8 for Andrew+) using four preparation methods with internal standard.

Analyte	Hamilton	Andrew +	Tecan	Manual
	Mean %CV	Mean %CV	Mean %CV	Mean % CV
Hydroxyproline	1.1	N/A	2.6	3.8
Histidine	1.2	3.6	2.6	3.0
Asparagine	1.5	N/A	2.9	1.6
Taurine	1.3	N/A	2.6	1.2
Serine	1.3	3.4	2.6	1.5
Glutamine	1.4	N/A	2.6	1.2
Arginine	1.3	3.4	2.6	1.2
Glycine	1.3	3.7	2.6	1.5
Aspartic Acid	2.6	3.9	2.2	1.5
Glutamic Acid	2.3	3.7	2.5	1.2
Threonine	1.3	3.4	2.5	1.3
Alanine	2.2	3.6	2.5	1.5
Gamma Amino Butyric Acid	3.3	N/A	2.2	1.2
Proline	1.4	3.4	2.6	1.6
Hydroxylysine 1	1.4	N/A	2.6	1.4
Hydroxylysine 2	1.3	N/A	2.7	1.1
Alpha Amino Butyric Acid	2.0	N/A	2.6	1.4
Ornithine	2.3	N/A	2.5	1.2
Cystine	1.2	3.4	2.6	1.3
Lysine	2.8	3.7	2.4	1.3
Tyrosine	1.2	3.4	2.6	1.2
Methionine	1.4	3.4	2.6	1.2
Valine	1.6	3.5	2.6	1.2
Isoleucine	1.6	3.4	2.6	1.3
Leucine	1.7	3.4	2.6	2.1
Phenylalanine	1.3	3.4	2.6	1.2
Tryptophan	1.2	N/A	2.6	1.2

ACCURACY

Sample preparation was performed on both the Hamilton ML Star and the Tecan Freedom EVO 100 using the same reagent lot across three days. Overall accuracy for all amino acids <15% on both the Hamilton and Tecan platforms. Analyses not performed on the Andrew+ platform.

LINEARITY

Linearity was assessed over three analytical runs across three days. All runs had correlation coefficient value of ≥0.99 with all calibration points below 20% (Cal 1) and 15% (Cal 2-7) deviation. Analyses not performed on the Andrew+ platform.

CONCLUSION

- **An automation compatible reagent Kit providing flexible 3 x 32 sample format.**
- **The precision of each cell culture standard amino acid is ≤3.9% (Table 2).**
- **Amino acid analysis scripts available on the Andrew+, Hamilton, and Tecan automation platforms.**
- **Comparable to the current manual preparation option (Table 2).**
- **The results on the Andrew+ platform are preliminary and require further optimization.**

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