HIGH THROUGHPUT SAMPLE PREPARATION IN AMINO ACID ANALYSIS

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INTRODUCTION

The analysis of amino acids is an essential technique in areas such as the food and drink industry to ensure products meet requirements. An automation compatible AccQ-Tag Ultra reagent Kit has been developed in parallel with a Food and Feed Standards Kit that enables the analysis of 21 amino acids. In conjunction with the automation kit, a new 32 sample protocol on Andrew+ has been created in addition to two previously released automation scripts on the Hamilton Microlab star and Tecan Freedom EVO 100/4 providing a highly efficient and time saving preparation method.

A: AccQ●Tag Ultra Eluents
B:AccQ●Tag Ultra, 1.7 µm, 2.1 X 100 mm Column;
C:AccQ●Tag Ultra Derivatization Automation Kit;
D:Amino Acid Food and Feed Standard Kit;
E: Internal NVa std
F:Autommaiton Scripts
G:96-well collection plate
H:Cap Mat

Figure 1: Waters amino acid analysis automation solution

- Automation compatible reagent kit designed in a 3 x 32 sample format, containing extra reagent volume required for automation platforms.
- Labware adapted from vials used in manual preparation to a 96 well plate format for ease of plate transfer and heating steps.
- Food and Feed standard is provided in a two vial format which can be combined to give 21 amino acids at 500µM in 500µl to create a series of 7 calibrators.
- Food and Feed standard kit in lyophilised format to allow for enhanced stability.
- Norvaline internal standard option included with excess volume for automation.

HAMILTON

TECAN.

Figure 2: Amino acid analysis automation platforms: Tecan, Hamilton, and Andrew +

Automation scripts for amino acid analysis using AccQTag Ultra reagents are developed and optimized for all three automation platforms with the following features:

- 96 samples: Run time < 1hr
- Minimal user interventions included (only at start and end of script).
- User inputs for starting well and number of samples;
- Optional sample concentration normalization;
- Optional standards serial dilution to create a calibration curve;
- Optional internal standard spiking;
- Derivatization;
- Barcode scanning included to allow sample IDs to be recorded into excel. (Hamilton and Tecan);
- Deck loading instructions or user prompts added to Care and Use Manual to direct user.

METHODS

Table 1. LC Conditions

AccQ-Tag Profiling method - Food and Feed	
LC System	ACQUITY® UPLC® H-Class and H-Class Bio System TUV
Sample temp	20˚C
Analytical Column Temp	49˚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

RESULTS

A set of three panels spanning the concentration range (10µM, 200µM and 400µM) containing 21 food and feed amino acids were prepared along with a 7 point cali-bration l on the Andrew+. The Andrew+32 sample protocol was performed to prepare 6 single preparations from each panel. The same preparation was also performed manually for comparison.

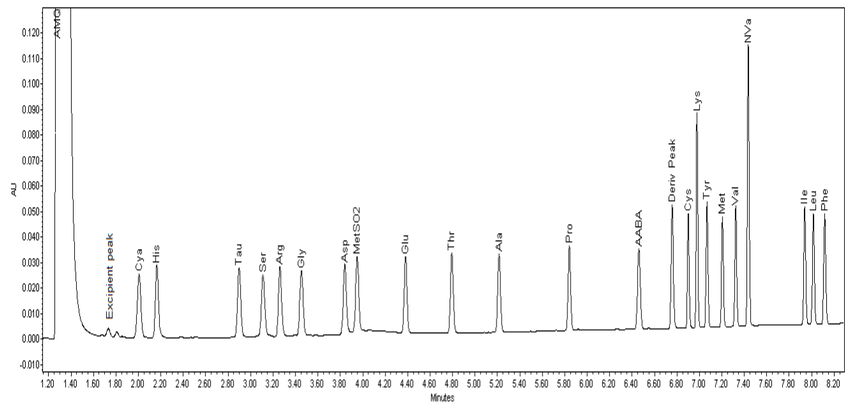

Figure 3. A Representative Chromatogram of 10 pmols of the Food and Feed standard

PRECISION

Precision was previously assessed using the high throughput automation platforms (Tecan Freedom EVO 100/4 and the Hamilton Microlab Star). An example of the Food and Feed preparation of a 200µM panel for high throughput is shown below with data obtained on a Tecan Freedom EVO 100/4. The run contained three single preparations of a spiked panel (200µM) with three replicate injections of each. The comparison data shown below for manual and Andrew preparations with a 200µM panel were performed using 6 single preparations with 1 injection for each prep.

Table 2. Analysis of Food and Feed 200µM solvent panel using high through-put Tecan, manual and Andrew+ preparations. *Hamilton data is available upon request.

	%CV of Aminno Acid Conc.		
Amino Acid	Tecan	Manual	Andrew +
Cya	2.3	2.5	0.9
His	2.3	2.8	1.0
Tau	2.2	2.8	1.0
Ser	2.3	2.8	0.8
Arg	2.2	2.9	1.0
Gly	2.2	2.8	1.0
Asp	2.2	2.7	1.2
MetSO2	2.2	2.9	0.8
Glu	2.2	2.8	1.0
Thr	2.2	2.9	0.8
Ala	2.2	2.8	0.9
Pro	2.2	2.8	0.8
AABA	2.4	2.8	0.8
Cys	2.2	2.8	1.0
Lys	2.2	2.8	1.2
Tyr	2.2	2.9	1.0
Met	2.2	2.8	0.9
Val	2.2	2.9	0.8
Ile	2.2	2.9	0.8
Leu	2.2	2.8	0.8
Phe	2.2	2.8	1.0

ACCURACY

A set of solvent panels spanning different concentration levels (10µM, 200µM and 400µM) containing 21 Food and Feed amino acids were prepared both manually and with the Andrew+ using the newly developed 32 sample protocol. The mean %Recovery across all Food and Feed amino acids at each con-centration level (10µM, 200µM and 400µM) on the Andrew+ was determined. Results for recovery were within ±10% for both manual and automation.

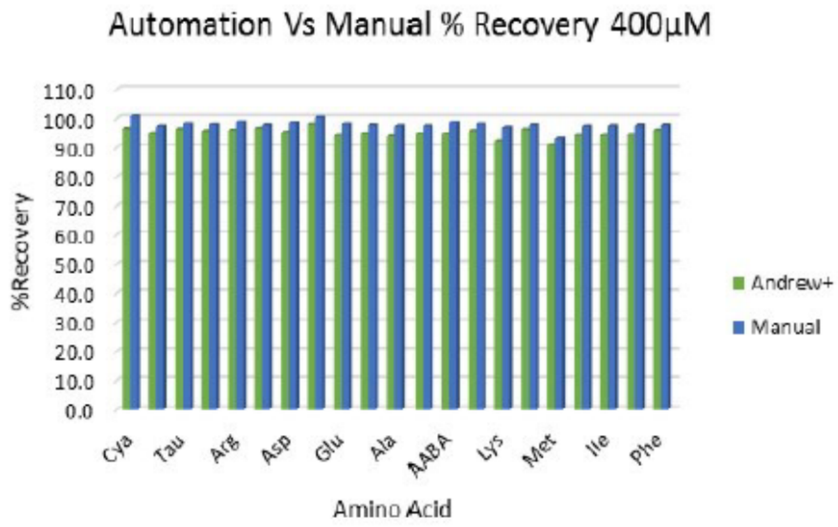

Figure 4. Comparison of %Recovery at concentration level of 400µM for both manual and Andrew+ preparations. * %Recovery data at concentration levels of 10µM and 200µM are available upon request.

LINEARITY

Linearity was assessed using the food and feed standard prepared at seven concentration levels for each amino acid across a range of 0.5–500 µM (cystine 0.25–250 µM). All analytical runs were assessed for linearity. All runs had a correlation coefficient value of ≥ 0.995.

Table 3. Comparison of Linearity for both manual and Andrew + preparations.

	Linearity R^2	
	Manual	Andrew
Cya	0.999653	0.999597
His	0.998973	0.999581
Tau	0.998871	0.999701
Ser	0.998914	0.999263
Arg	0.997997	0.999451
Gly	0.998944	0.99967
Asp	0.998228	0.998057
MetSO2	0.999086	0.999624
Glu	0.998358	0.998394
Thr	0.998949	0.999534
Ala	0.998741	0.999002
Pro	0.999046	0.999399
AABA	0.998936	0.99922
Cys	0.998788	0.99964
Lys	0.998473	0.998545
Tyr	0.998983	0.999628
Met	0.998533	0.99956
Val	0.998534	0.99941
Ile	0.998962	0.999393
Leu	0.999033	0.99944
Phe	0.999006	0.999629

CONCLUSIONS

- **Amino acid analysis scripts available on the Andrew+, Hamilton, and Tecan automation platforms. Results are comparable to the current manual preparation option with better %RSD.**
- **Automated sample prep greatly improves testing efficiency, reduced the risks of contamination and human error, and allow analysts work remotely.**
- **Ease the analyst training and facilitate method transfer between labortories.**

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