

THE HIGH THROUGHPUT PREPARATION OF AMINO ACIDS USING WATERS TECHNOLOGIES NEW KAIROS AMINO ACID 500+ AUTOMATION KIT ON THE FREEDOM EVO 100 PLATFORM

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OVERVIEW

- Provide a time efficient all in one solution for the high throughput preparation of 45 amino acids.
- Development of an automation compatible kit and script on the Freedom EVO 100 platform.
- Resulting in 96 sample preparation in under two hours with an average CV of <2.6% across the panel set.

INTRODUCTION

The manual preparation of large sample batches can be time consuming and laborious, often requiring technical expertise. The development of an automation compatible kit and script for high throughput (n=48/96) amino acid sample preparation on the Freedom EVO 100 platform seeks to remove these aspects of manual preparation. Therefore providing a robust, reproducible and time efficient solution to high throughput amino acid sample preparation.

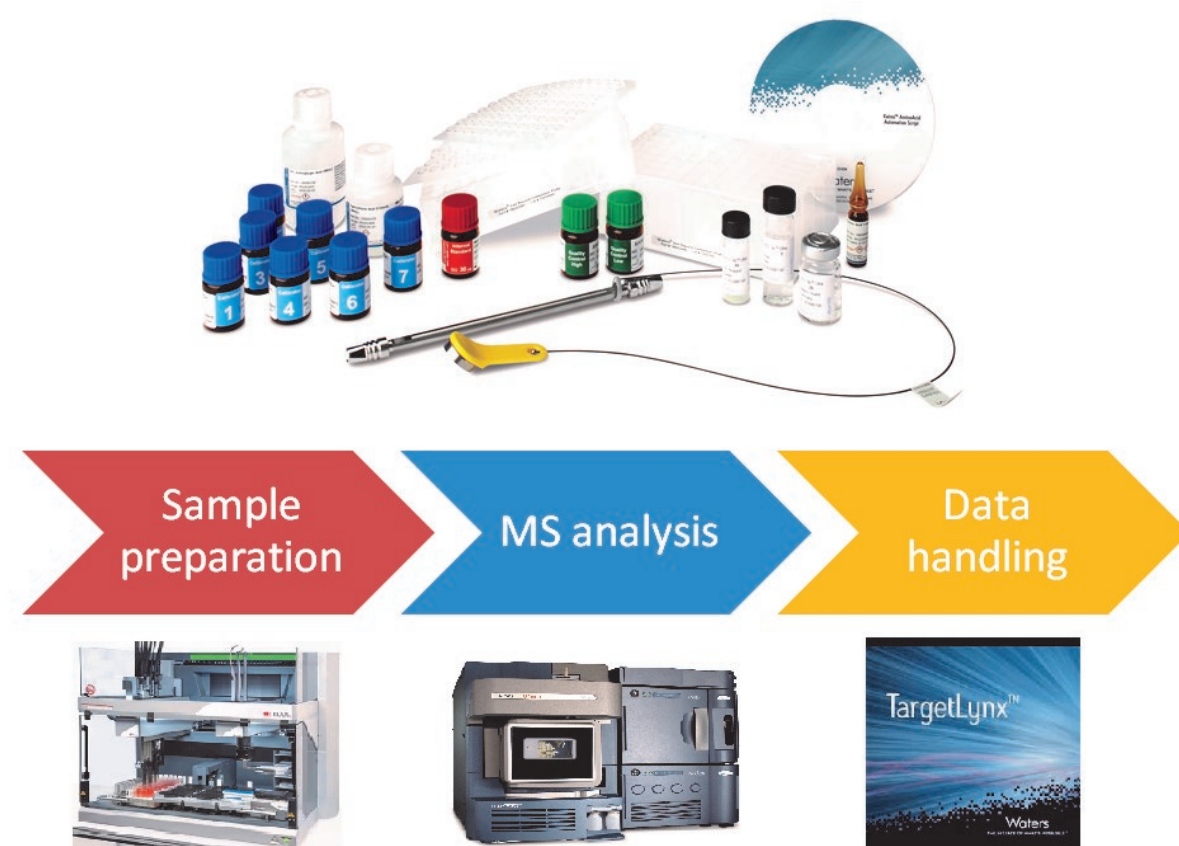


Figure 1. The Kairos Amino Acid 500+ automation kit and the overall workflow.

METHODS

OVERALL WORKFLOW

- Amino acid stock samples were prepared using the Waters Kairos amino acid 500+ automation kit on the Tecan Freedom EVO 100 platform.
- The prepared samples were analysed using a Waters ACQUITY UPLC I-Class and XEVO TQ-S micro.

Kairos Amino Acid LC Method

Column: CORTECS UPLC C₁₈, 1.6 µm, 2.1 x 150 mm (p/n: 186007096)
Temperature: 55 °C
Mobile phase A: Water + 0.1 % Formic Acid (LC-MS grade water is recommended)
Mobile phase B: Acetonitrile + 0.1% Formic Acid (LC-MS grade ACN is recommended)
Flow rate: 0.5 mL/min
Injection volume: 2 µL of the derivatized sample from step 2
Gradient:

Gradient Timetable

Time (min)	Flow Rate (mL/min)	Mobile Phase A (%)	Mobile Phase B (%)	Curve
0.0	0.5	99%	1%	-
1.0	0.5	99%	1%	6
2.0	0.5	87%	13%	6
5.5	0.5	85%	15%	6
6.5	0.5	5%	95%	6
7.5	0.5	5%	95%	6
7.6	0.5	99%	1%	6
9.0	0.5	99%	1%	6

Seal Wash: 80% Water: 20% Methanol
Weak Needle Wash/Purge Wash: Water + 0.1 % Formic Acid
Strong Needle Wash/Wash: Acetonitrile + 0.1% Formic Acid

Figure 2. Method conditions for Amino acid analysis using Xevo TQ-S micro.

AUTOMATION SCRIPT FEATURES

- Barcode scanning is included in the script to allow for sample ID tracking.
- File convertor option to allow for transfer of sample information from Tecan to Masslynx software.
- Minimal user interventions (centrifuge intervention during script) including user inputs for well start and number of samples.
- Automation friendly volumes used with a minimum volume of 10µL.
- Parameters within the "liquid classes" were deliberately adjusted to ensure optimal outcome.
- Dilution step included for high concentration samples.

AUTOMATION KIT DEVELOPMENT

- Automation compatible kit designed to account for extra reagent/solvent volumes required for platforms with liquid detection capabilities.
- Labware adapted for use with automation platforms for ease of plate transfer and efficient heating steps.
- All in one solution option provided to include calibrators, QCs, internal standard, required reagents, the Tecan platform script and consumables.

RESULTS

AUTOMATION VS MANUAL PREPARATION

Sample preparation was performed both with the manual method and the automated method using the same reagents across three days. Results show that the manual and automated methods are both comparable across all amino acids. The main essential and non essential amino acids are displayed below. The linearity of the sample runs was also assessed. All runs had an r^2 value ≥ 0.99 and all points in the calibration line were below 20% (Cal 1) and 15% (Cal 2-7).

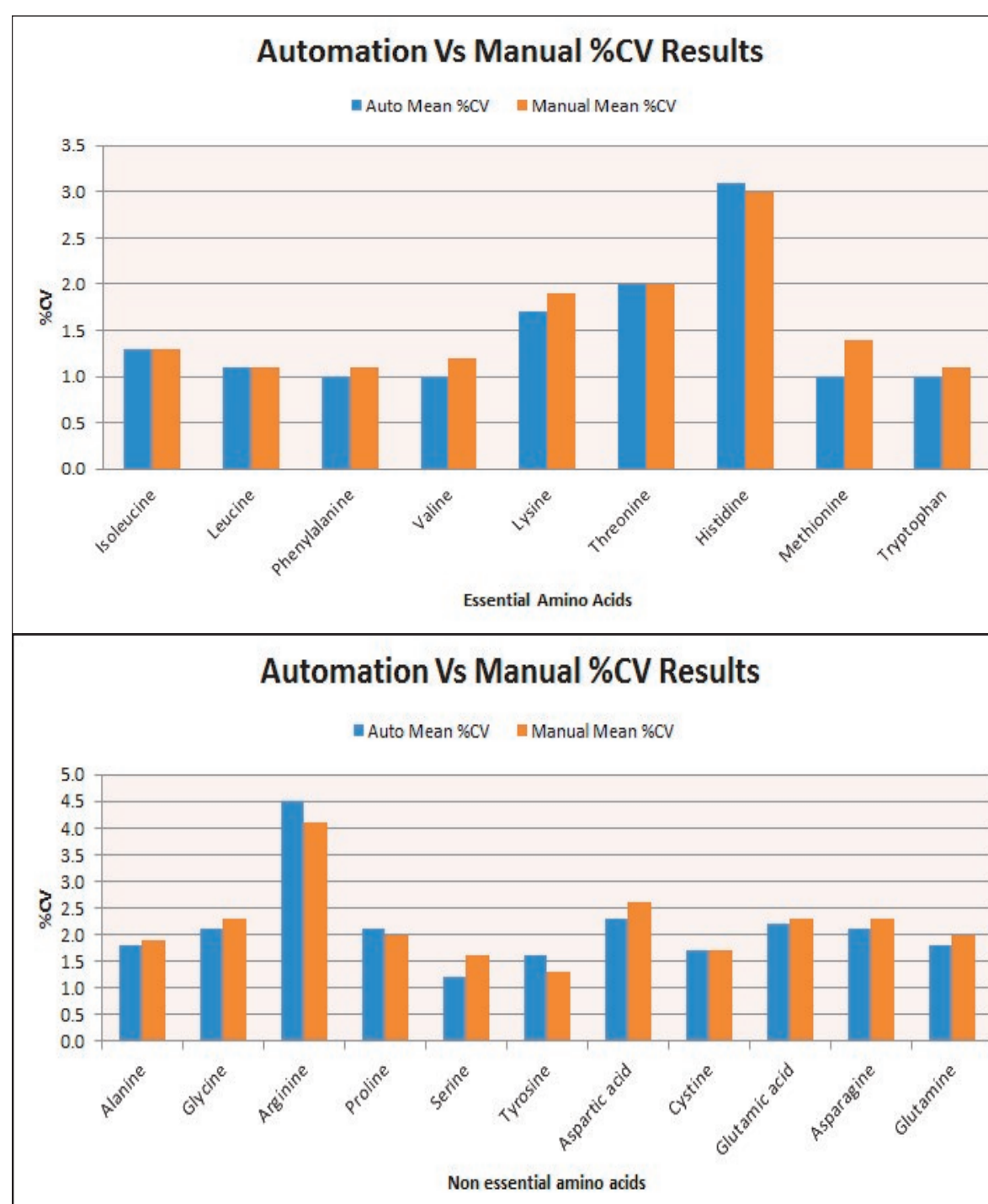


Figure 3. Comparable Manual vs. automated amino acid sample preparation. The comparison details the average %CV data across three days for five concentration levels (20µM, 150µM, 400µM, 700µM and 2500µM) for nine essential and eleven non essential amino acids.

ACCURACY

The accuracy across all amino acids in the kit was determined. The average accuracy across the three days was $\geq 87.5\%$.

Amino Acid	% Accuracy		
	Day 1	Day 2	Day 3
Alanine	96.2	97.0	96.9
Glycine	98.0	96.8	95.5
Isoleucine	98.2	98.8	98.2
Leucine	97.3	97.7	97.5
Phenylalanine	98.8	98.7	99.0
Valine	97.6	98.4	98.1
Arginine	97.6	97.3	99.1
Lysine	94.9	96.9	95.7
proline	98.3	97.7	98.3
Serine	97.2	97.8	96.2
Threonine	96.8	94.7	95.1
Tyrosine	94.9	94.1	94.1
Aspartic acid	95.5	97.1	95.8
Cystine	99.1	98.4	98.6
Glutamic acid	96.2	96.1	97.0
Histidine	98.5	99.3	98.6
Methionine	99.5	99.5	99.0
Asparagine	97.3	96.4	97.2
Tryptophan	97.5	98.4	98.4
Glutamine	86.2	91.5	84.8

Figure 4. The total average % accuracy for five concentration levels (20µM, 150µM, 400µM, 700µM and 2500µM) of 20 key essential and non essential amino acids across three days of testing is shown.

PRECISION

The mean % CV for each analyte across three days was determined. The average precision across the 3 days was $\leq 4.5\%$.

Amino Acid	% Precision		
	Day 1	Day 2	Day 3
Alanine	1.8	2.2	1.4
Glycine	2.0	2.7	1.5
Isoleucine	1.3	1.3	1.2
Leucine	1.0	1.1	1.1
Phenylalanine	1.0	1.2	0.7
Valine	1.2	0.8	0.9
Arginine	4.7	3.8	5.0
Lysine	1.5	1.9	1.6
proline	1.8	2.7	1.7
Serine	1.3	1.0	1.4
Threonine	1.5	2.3	2.1
Tyrosine	1.4	2.3	1.2
Aspartic acid	3.5	1.8	1.7
Cystine	1.8	1.9	1.3
Glutamic acid	2.2	2.7	1.8
Histidine	3.8	3.2	2.3
Methionine	0.9	1.2	0.9
Asparagine	2.0	2.4	1.9
Tryptophan	1.1	1.0	1.0
Glutamine	2.1	1.9	1.5

Figure 5. The total average % CV results for five concentration levels (20µM, 150µM, 400µM, 700µM and 2500µM) of 20 key essential and non essential amino acids across three days of testing is shown.

CONCLUSION

- An automation compatible Kit providing all necessary reagents.
- Comparable to the current manual preparation option (Figure 3).
- Further developments will include the creation of scripts on other automation platforms to be coupled with the automation compatible kit.