

UPLC-MS/MS Analysis of 45 Amino Acids Using the Kairos Amino Acid Kit for Biomedical Research

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APPLICATION BENEFITS

- Analytical selectivity of the chromatographic method provides separation of isobaric species
- Fast analytical run times (<10 minutes)
- Confidence in peak detection
- Greater flexibility, system can be used for other analyses

WATERS SOLUTIONS

[Kairos™ Amino Acid Kit](#)

[AccQ•Tag™ Ultra "3x" Derivatization Kit](#)

[CORTECS™ UPLC™ C₁₈](#)

[1.6 µm, 2.1 x 150 mm Column](#)

[ACQUITY™ UPLC I-Class System \(FL\)](#)

[Xevo™ TQ-S micro](#)

[MassLynx™ Mass Spectrometry Software](#)

[TargetLynx™ Application Manager](#)

KEYWORDS

Kairos Amino Acid Kit, amino acids, Xevo TQ-S micro, AccQ•Tag Ultra

INTRODUCTION

The research only Kairos Amino Acid Kit is designed for biomedical researchers to enable the analysis of up to 45 biologically relevant amino acids in less than 10 minutes. The kit calibrators have been value assigned using higher order reference material where available to provide added confidence to your results. Metrological traceability of the Kairos Amino Acid Kit calibrators were traceable to National Metrology Institute of Japan (NMIJ CRM 6011a-6018a, 6022a) for 9 amino acids and further 8 were traceable to National Institute of Standards and Technology (NIST SRM 2389a), the remaining amino acids were gravimetrically prepared using TraceCERT standards, where available, from Sigma-Aldrich.

Here we describe the use of the Kairos Amino Acid Kit for analysis of 45 amino acids in solution. Chromatographic separation was performed using an ACQUITY UPLC I-Class System using a CORTECS UPLC C₁₈ Column, followed by detection on a Xevo TQ-S micro System (Figure 1). Performance of the kit and analytical system was assessed in solution, in addition, NIST SRM 2389a material and matrix samples (urine and plasma) were analyzed for biomedical research purposes.



Figure 1. The Waters ACQUITY UPLC I-Class/Xevo TQ-S micro System.

The chromatographic conditions used allowed for the separation of leucine, methylhistidine, alanine and aminobutyric acid isobaric species.

EXPERIMENTAL

Reagent Kit

Kairos Amino Acid Kit (p/n: [176004379](#))

LC conditions

System:	ACQUITY UPLC I-Class (FL) with Column Heater (CH-A)
Needle/loop:	10 µL/10 µL
Column:	CORTECS UPLC C ₁₈ , 1.6 µm, 2.1 × 150 mm (p/n: 186007096)
Pre-column:	In-line filter (p/n: 205000343)
Mobile phase A:	Water with 0.1% formic acid
Mobile phase B:	Acetonitrile with 0.1% formic acid
Weak wash solvent:	Mobile phase A
Strong wash solvent:	Mobile phase B
Column temp.:	55 °C
Injection volume:	2 µL
Flow rate:	0.5 mL/min
Gradient:	See Table 1

Method conditions

Table 1. Gradient table for the separation of the amino acids.

Time (min)	%A	%B	Curve
Initial	99	1	Initial
1	99	1	6
2	87	13	6
5.5	85	15	6
6.5	5	95	6
7.5	5	95	6
7.6	99	1	6

Run time: 9 minutes

MS Conditions

System:	Xevo TQ-S micro
Acquisition mode:	Multiple Reaction Monitoring (MRM) (see Appendix Table A and B for details)
Polarity:	ESI+
Capillary:	2.0 kV
Source temp.:	150 °C
Desolvation temp.:	1000 °C
Cone:	30 V

Data management

MassLynx v4.1 Software with TargetLynx Application Manager

Kit reconstitution

To reconstitute the freeze-dried Kairos Amino Acid Kit, follow the Care and Use Manual (p/n: [720005448EN](#)). Calibrators and QCs were reconstituted using 2 mL of 0.1 M HCl and mixed at room temperature for a minimum of 30 minutes, ensuring all material is fully dissolved.

Internal standard was reconstituted using 2 mL of water and mix at room temperature for 10 minutes, ensuring all material is fully dissolved. The contents of the vial were transferred to a volumetric flask and made up to 10 mL using 10% sulfosalicylic acid supplied in the kit.

Reagent preparation

To prepare the Kairos Amino Acid Kit reagents, Borate Buffer, and AccQ•Tag Ultra "3X" Derivatization Kit reagent, follow the Care and Use Manual.

Borate buffer preparation: Add 430 µL of 0.5 M NaOH aq. to the 6 mL of borate buffer provided in the derivatization kit (Reagent 1) *Expiry: 3 months. Storage: Room temperature*

AccQ•Tag Ultra "3X" reagent: Reconstitute AccQ•Tag Ultra "3X" (Reagent 2B) in 1.5 mL acetonitrile (Reagent 2A). Heat for 10 minutes at 55 °C and vortex. *Expiry 5 days. Storage: Room Temperature. AccQ•Tag Ultra should be stored in the desiccator once reconstituted.*

Sample preparation

- Step 1 Add 50 μ L of sample to 1.5 mL Eppendorf
- Step 2 Add 50 μ L of 50 μ M Internal Standard
- Step 3 Vortex mix for 5 seconds
- Step 4 Add 50 μ L of water
- Step 5 Vortex mix for 5 seconds
- Step 6 For high concentration samples only – Add 1000 μ L of diluent (0.1 M HCl)
- Step 7 Centrifuge for 15 minutes at 9000 g
- Step 8 Add 70 μ L of Borate buffer to maximum recovery vial
- Step 9 Add 10 μ L of supernatant into Borate buffer and pipette mix
- Step 10 Add 20 μ L of AccQ-Tag Ultra “3X” reagent
- Step 11 Vortex for 5 seconds
- Step 12 Allow sample to stand at room temperature for 1 min
- Step 13 Heat for 10 minutes at 55 °C
- Step 14 Inject 2 μ L

For precision and accuracy studies, panel samples were gravimetrically prepared in 0.1 M HCl at 20, 150, 400, and 700 μ M for all amino acids and an additional high concentration panel at 2500 μ M was prepared for alanine, glycine, isoleucine, leucine, phenylalanine, valine, serine, threonine, tyrosine, and glutamine.

RESULTS AND DISCUSSION

No significant system carryover (detector response was \leq 20% of Calibrator 1) was observed from high concentration samples into subsequent blank injections for all amino acids.

Precision was determined by preparing and analyzing the panel samples in replicates of five over three separate days (n=15), within day and between day precision performance (%CV) were calculated for each panel. Each panel within-day precision was \leq 6.9% CV and the between-day precision performance was \leq 16.3% CV (\leq 13.9% CV when argininosuccinic acid was excluded) and the mean within-day and between-day precision performance was less than \leq 13.9% CV as shown in Table 2.

Table 2. Mean within- and between-day precision for the analysis of 45 amino acids in solution.

Analyte	Within-day precision (%CV)	Between-day precision (%CV)
Alanine	1.9	2.3
Glycine	2.3	2.9
Isoleucine	1.3	2.0
Leucine	1.1	2.6
Phenylalanine	1.1	1.6
Valine	1.2	1.9
Serine	1.6	2.6
Threonine	2.0	3.1
Tyrosine	1.3	2.2
Glutamine	2.0	5.2
Arginine	4.1	4.8
Lysine	1.9	2.1
Proline	2.0	2.9
Aspartic Acid	2.6	3.8
Cystine	1.7	2.1
Glutamic Acid	2.3	2.4
Histidine	3.0	3.6
Methionine	1.4	1.7
Asparagine	2.3	2.9
Tryptophan	1.1	1.5
Hydroxylysine	2.6	4.8
Sacrosine	2.4	2.7
Beta Alanine	2.5	3.7
α -aminobutyric acid	3.3	4.7
γ -aminobutyric acid	2.7	3.7
Alpha Amino adipic acid	2.2	2.7
Kynurenine	2.8	3.9
Homocitrulline	2.5	3.4
Citrulline	2.6	3.2
Taurine	2.1	3.8
Allo-Isoleucine	1.7	5.5
Phosphoethanolamine	3.3	4.1
Homocystine	1.5	1.9
Glycyl Proline	2.7	3.2
Anserine	4.4	6.6
L-Ornithine	3.9	6.4
Ethanolamine	2.3	2.9
β -aminoisobutyric acid	2.4	5.0
Hydroxyproline	3.8	5.3
S-Sulfocysteine	2.4	2.6
Cystathionine	2.9	7.4
3-Methyl histidine	5.0	6.5
1-Methyl-histidine	4.4	5.4
Carnosine	4.0	4.5
Argininosuccinic acid	3.9	13.9

To assess linearity, the Kairos Amino Acid Kit calibrators were analyzed each day over three days. Regression analysis demonstrated a linear fit using 1/x weighting across the concentration range for the 45 amino acids analyzed (Table 3). For each amino acid, the coefficient of determination (r^2) was >0.99 where the back calculated concentrations of the calibrators were within $\pm 15\%$ of the nominal value, except for the LLOQ (within $\pm 20\%$). At least 75% of the calibrators fulfilled this criterion, with the exception of the 3 analytes highlighted in red (hydroxyproline, cystathionine, argininosuccinic acid).

Table 3. Linearity of the Kairos Amino Acid Kit in solution. * denotes deviation at LLOQ.

Analyte	Day 1		Day 2		Day 3	
	r^2	Max dev (%)	r^2	Max dev (%)	r^2	Max dev (%)
Alanine	0.999	-12.6	0.999	-13.5	0.999	-14.3
Glycine	1.000	-16.2*	0.999	-18.5*	1.000	-11.0
Isoleucine	0.998	-17.6*	0.999	-10.8	1.000	-9.8
Leucine	0.999	-17.5*	1.000	-4.9	1.000	-13.4
Phenylalanine	1.000	-12.2	1.000	-3.9	1.000	-13.5
Valine	0.999	-17.5*	1.000	-8.7	1.000	-9.5
Serine	1.000	-7.7	0.999	-16.4*	1.000	-10.5
Threonine	1.000	-12.5	0.999	-10.4	0.998	-14.7
Tyrosine	1.000	-11.7	1.000	-10.9	0.998	-10.0
Glutamine	1.000	-10.2	0.999	-16.5*	0.998	-9.4
Arginine	1.000	-4.5	0.996	13.6	0.996	9.4
Lysine	0.999	-14.0	1.000	-3.1	0.999	-17.4*
Proline	1.000	-6.7	1.000	9.6	0.998	-14.2
Aspartic Acid	0.998	-11.3	1.000	-4.1	0.999	-5.3
Cystine	0.998	8.7	0.999	9.3	0.999	10.7
Glutamic Acid	1.000	-8.5	1.000	4.5	0.998	6.5
Histidine	0.998	-16.0*	0.999	-11.8	1.000	-2.6
Methionine	1.000	3.8	1.000	3.7	1.000	2.2
Asparagine	1.000	-10.6	0.999	-6.6	0.999	-10.8
Tryptophan	1.000	-6.3	1.000	5.2	1.000	-6.0
Hydroxylysine	0.999	9.2	0.999	-7.1	0.997	8.6
Sacrosine	0.998	-11.9	0.998	7.5	0.999	-10.7
Beta Alanine	0.999	5.9	0.999	-5.5	0.999	6.5
α -aminobutyric acid	0.998	15.3*	1.000	-11.5	0.997	-13.1
γ -aminobutyric acid	0.993	13.5	0.994	11.8	0.996	10.1
Aminoadipic acid	1.000	-2.6	0.997	-8.3	1.000	2.9
Kynurenine	0.999	10.8	1.000	-6.8	0.999	-17.7*
Homocitrulline	0.999	-5.0	1.000	-3.9	0.999	-4.9
Citrulline	0.998	13.5	0.998	7.6	1.000	-10.3
Taurine	0.999	15.2*	0.998	19.3*	0.999	12.2
Allo-Isoleucine	0.995	10.7	0.994	-14.0	0.990	-14.6
Phosphoethanolamine	0.999	4.2	0.999	-6.0	0.999	5.3
Homocystine	0.999	-4.3	0.999	14.1	0.999	4.8
Glycyl Proline	0.999	-5.6	0.999	-6.0	0.998	13.0
Anserine	0.999	6.8	0.994	11.5	0.997	15.0
L-Ornithine	0.999	13.3	1.000	4.9	0.998	-9.9
Ethanolamine	1.000	-9.1	0.999	-9.0	1.000	4.5
β -aminoisobutyric acid	0.990	14.2	0.992	-12.6	0.997	-14.3
Hydroxyproline	0.990	-16.4*	0.997	-11.6	0.999	12.5
S-Sulfocysteine	1.000	-10.8	0.998	-12.5	0.999	6.0
Cystathionine	0.993	-14.6	0.990	13.8	0.996	18.1
3-Methyl histidine	0.999	17.2*	0.996	15.0	0.999	-5.2
1-Methyl-histidine	0.999	18.4*	0.998	15.8*	0.997	11.6
Carnosine	1.000	-12.4	0.998	-14.3	0.995	-14.2
Argininosuccinic acid	1.000	-4.7	0.992	-13.8	0.998	-11.7

Sensitivity was assessed by the analysis of samples, prepared in 0.1 M HCl over the range 1–20 μM , in replicates of five on one day. The LLOQ for each analyte was determined when the signal-to-noise ratio of the analyte peak was $\geq 10:1$ and the precision performance of the replicates was $\leq 20\%$ CV. The LOQ for all 45 amino acids analyzed were $\leq 5 \mu\text{M}$ with the exception of S-sulfocysteine, argininosuccinic acid (20 μM), and carnosine (15 μM), as shown in Table 4.

Table 4. Sensitivity of the Kairos Amino Acid Kit in solution using the ACQUITY UPLC I-Class and Xevo TQ-S micro System.

Analyte	LOQ (μM)	Precision (%CV)	Accuracy (% Bias)	Signal-to-noise ratio
Alanine	2	6.7	15.0	793
Glycine	2	9.6	5.0	5016
Isoleucine	2	2.6	15.0	5060
Leucine	5	2.8	18.0	9034
Phenylalanine	2	2.6	15.0	1580854
Valine	4	3.3	17.5	1659
Serine	4	6.9	7.5	3531
Threonine	4	3.6	20.0	2841491
Tyrosine	4	1.6	15.0	13818
Glutamine	2	4.2	0.0	1677
Arginine	1	10.5	20.0	120492
Lysine	1	7.9	10.0	331
Proline	2	3.0	7.5	3803
Aspartic Acid	4	1.6	15.0	431
Cystine	0.5	8.9	0.0	466
Glutamic Acid	2	6.3	20.0	1444
Histidine	1	3.7	20.0	73036
Methionine	2	0.0	15.0	11671
Asparagine	3	5.0	13.3	6016373
Tryptophan	1	5.0	10.0	5548
Hydroxylysine	1	0.0	0.0	380
Sarcosine	3	7.0	20.0	1111
Beta Alanine	1	13.7	20.0	2396
α -aminobutyric acid	1	0.0	10.0	172
γ -aminobutyric acid	3	5.1	0.0	2868
Aminoadipic acid	2	3.2	15.0	1713
Kynurenine	2	2.8	20.0	896
Homocitrulline	4	1.7	20.0	529
Citrulline	4	2.5	12.5	451
Taurine	1	3.7	20.0	566
Allo-Isoleucine	4	3.4	10.0	14005
Phosphoethanolamine	2	3.4	20.0	109
Homocystine	1	0.0	20.0	2353
Glycyl Proline	1	4.6	20.0	170
Anserine	5	1.7	6.0	105
L-Ornithine	3	8.3	16.7	1683
Ethanolamine	4	1.3	12.5	1206
β -aminoisobutyric acid	1	5.0	10.0	305
Hydroxyproline	2	7.6	0.0	1237
S-Sulfocysteine	20	2.4	15.2	15907
Cystathionine	2	0.0	10.0	2939
3-Methyl histidine	3	4.3	10.0	26531
1-Methyl-histidine	4	5.2	20.0	123785
Carnosine	15	4.3	18.0	1562983
Argininosuccinic acid	20	3.8	15.2	88840

Accuracy was assessed through the analysis of the independently prepared panel samples as well as NIST SRM 2389a which was gravimetrically diluted to a concentration of 400 μM prior to analysis. All Panel samples were $\leq \pm 20.4\%$ bias (range -18.5 to +20.4%) for the concentration ranges tested, with the exception of the three analytes highlighted in red (hydroxyproline, cystathionine, argininosuccinic acid). The diluted NIST SRM 2389a calculated concentrations were $\leq \pm 10.0\%$ bias (range -10.0 to +3.7%), for the analytes present in the material, all data is summarized in Table 5.

Table 5. Accuracy of the Kairos Amino Acid Kit in solution.

Compound	Accuracy summary (% bias)						Mean	NIST 400 μM
	20 μM	150 μM	400 μM	700 μM	2500 μM			
Alanine	4.6	1.3	-0.4	-1.0	-5.2	-0.1	-0.4	
Glycine	3.2	1.8	-0.9	0.8	-6.4	-0.3	-2.0	
Isoleucine	0.2	2.1	14.1	0.8	-0.6	3.3	3.7	
Leucine	0.7	3.4	2.7	1.6	-1.5	1.4	3.0	
Phenylalanine	0.3	1.1	-0.6	-0.5	-4.0	-0.8	-1.4	
Valine	1.2	3.4	3.1	1.1	-0.9	1.6	2.4	
Serine	2.9	2.7	-0.9	0.3	-5.0	0.0	-2.0	
Threonine	7.5	5.6	-0.1	3.5	-4.6	2.4	-1.6	
Tyrosine	5.8	1.7	-6.8	-0.1	-9.5	-1.8	-6.2	
Glutamine	15.8	12.0	8.6	10.9	4.8	10.4	N/A	
Arginine	0.0	-2.0	1.7	-1.5	N/A	-0.5	-5.3	
Lysine	2.6	-1.6	-6.8	-6.2	N/A	-3.0	-8.0	
Proline	4.8	2.2	-1.3	-0.7	N/A	1.2	-7.8	
Aspartic Acid	9.0	3.5	-2.3	2.2	N/A	3.1	-3.3	
Cystine	-1.0	1.4	0.5	0.8	N/A	0.4	-0.1	
Glutamic Acid	8.5	4.2	-0.3	2.3	N/A	3.7	-10.0	
Histidine	-1.3	0.3	-0.4	-3.3	N/A	-1.2	-1.5	
Methionine	1.3	1.7	0.0	-0.6	N/A	0.6	-0.3	
Asparagine	4.1	0.1	-5.2	-0.1	N/A	-0.3	N/A	
Tryptophan	0.3	-1.4	-3.0	-2.7	N/A	-1.7	N/A	
Hydroxylysine	7.8	7.2	7.2	3.1	N/A	6.3	N/A	
Sacrosine	-0.7	-0.4	7.2	-7.1	N/A	-0.2	N/A	
Beta Alanine	-0.9	1.8	12.0	-5.2	N/A	1.9	N/A	
α -aminobutyric acid	-7.2	3.1	7.2	2.2	N/A	1.3	N/A	
γ -aminobutyric acid	N/A	5.1	-13.0	1.9	N/A	-2.0	N/A	
Aminoadipic acid	3.3	2.4	9.5	-3.9	N/A	2.8	N/A	
Kynurenine	0.4	1.3	10.1	2.3	N/A	3.5	N/A	
Homocitrulline	-4.7	6.1	12.0	0.0	N/A	3.3	N/A	
Citrulline	-10.6	0.0	5.9	-9.0	N/A	-3.4	N/A	
Taurine	19.9	8.8	11.7	-6.7	N/A	8.4	N/A	
Allo-Isoleucine	11.7	-10.9	-15.7	-1.8	N/A	-4.2	N/A	
Phosphoethanolamine	-0.1	2.8	-3.2	-12.2	N/A	-3.2	N/A	
Homocystine	N/A	7.7	6.3	-4.2	N/A	3.3	N/A	
Glycyl Proline	15.5	15.2	16.6	4.0	N/A	12.8	N/A	
Anserine	-29.7	-4.4	3.1	-9.9	N/A	-3.8	N/A	
L-Ornithine	17.1	2.8	-3.0	-2.8	N/A	3.5	N/A	
Ethanolamine	5.2	7.9	6.2	1.2	N/A	5.1	N/A	
β -aminoisobutyric acid	N/A	5.9	-18.5	9.0	N/A	11.4	N/A	
Hydroxyproline	25.7	-4.9	-22.8	-1.5	N/A	N/A	N/A	
S-Sulfocysteine	15.2	12.2	20.4	2.7	N/A	12.6	N/A	
Cystathionine	46.6	-0.2	-29.2	5.8	N/A	N/A	N/A	
3-Methyl histidine	N/A	-12.7	4.6	-14.5	N/A	-7.6	N/A	
1-Methyl-histidine	N/A	-11.0	-5.0	-14.9	N/A	-10.3	N/A	
Carnosine	9.6	4.2	1.3	-0.9	N/A	3.5	N/A	
Argininosuccinic acid	15.2	21.5	34.3	2.6	N/A	N/A	N/A	

Analysis of a urine and plasma sample allowed for the analysis of amino acids at physiological levels. Figure 2, shows the Total Ion Chromatogram (TIC) for 11 amino acids present in a plasma sample and the chromatogram inset, highlights the chromatographic separation of gamma-aminobutyric acid, beta-aminoisobutyric acid, and alpha-aminobutyric acid from the same plasma sample.

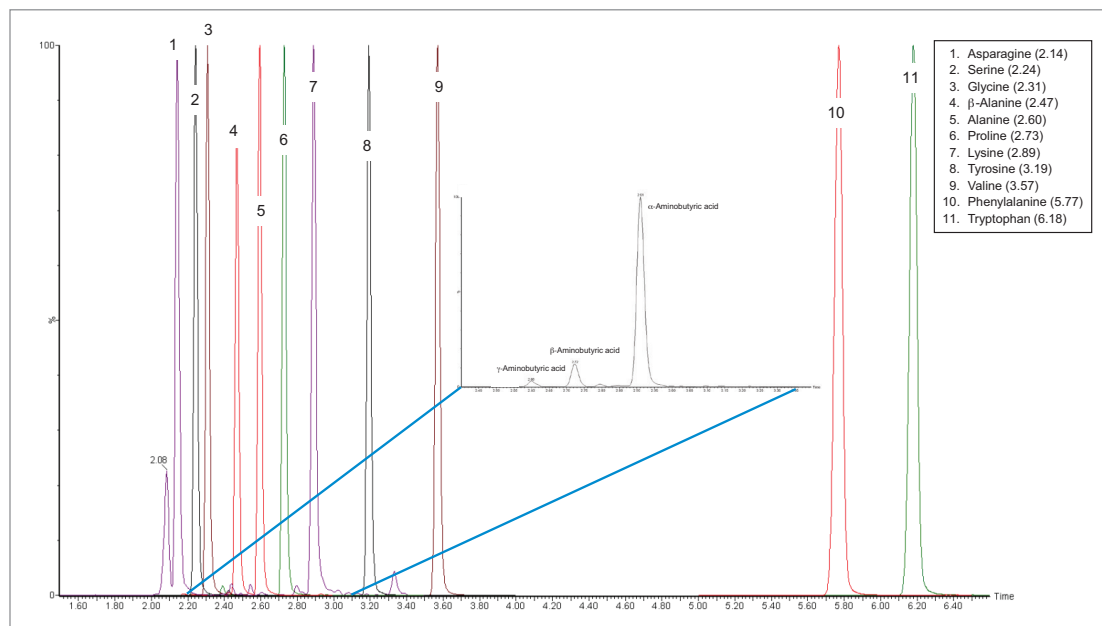


Figure 2. Total Ion Chromatogram (TIC) for 11 amino acids present in a plasma sample and inset a chromatogram to show the separation of gamma-aminobutyric acid, beta-aminoisobutyric acid, and alpha-aminobutyric acid in the same plasma sample.

Figure 3 shows the chromatography and quantitation of histidine, alanine and phenylalanine in a urine sample.

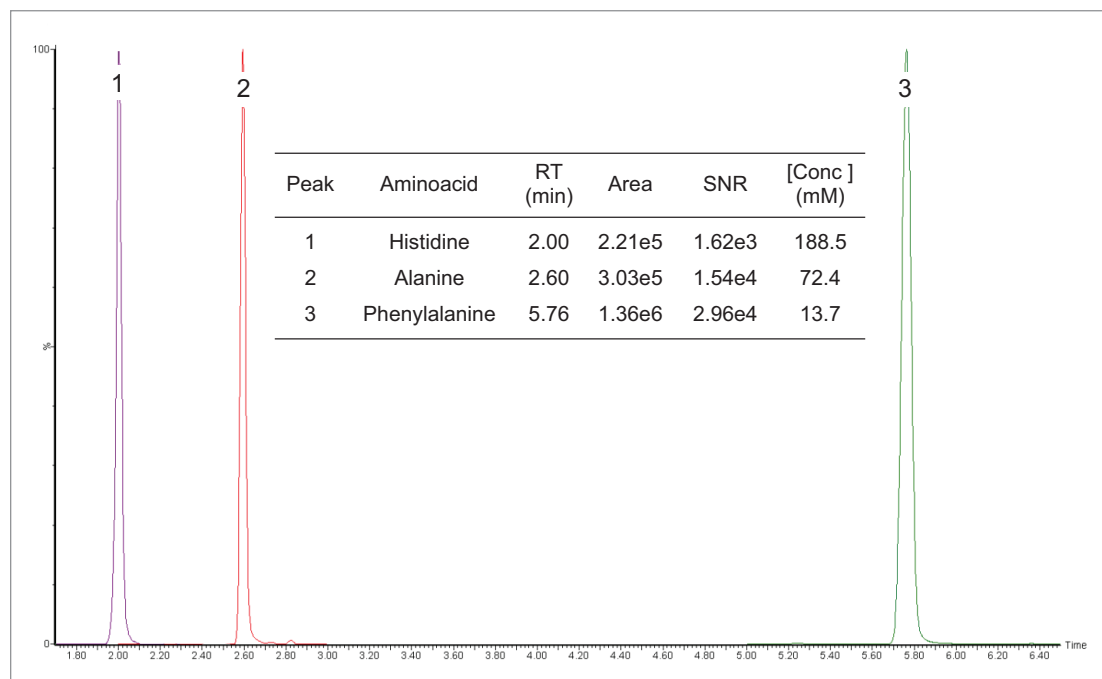


Figure 3. Chromatogram to show the quantitation of histidine (188.5 μM), alanine (72.4 μM) and phenylalanine (13.7 μM) in a urine sample. Signal to noise ratios (SNR) and peak areas are shown.

CONCLUSIONS

Kairos Amino Acid Kit enables biomedical researchers to achieve trusted results through a single flexible kit that allows them to accurately quantitate 42 amino acids within their normal physiological range in less than 10 minutes using the ACQUITY UPLC I-Class/Xevo TQ-S micro System.

Using AccQ-Tag Ultra "3X" Derivatization Kit to derivatize the samples allows for a simple, robust, and fast reversed-phase UPLC analysis of the amino acids without the need for mobile-phase buffers or ion-pair reagents. This means that you do not need a dedicated system and so the ACQUITY UPLC I-Class/Xevo TQ-S micro System can be used for other analyses. The derivatized samples are stable and in less than 10 minutes, the chromatographic conditions deliver the separation of isobaric amino acids giving you confidence in peak identification.

The method described in the research only Kairos Amino Acid Kit allows for the quantitative analysis of 42 amino acids in solution using an ACQUITY UPLC I-Class/Xevo TQ-S micro System with good precision, linearity, sensitivity, and accuracy. The remaining three analytes; hydroxyproline, cystathionine, and argininosuccinic acid present in the kit are for semi-quantitative analysis only.

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Appendix

Table A. MRM parameters for the amino acids analytes.

Analyte	MRM transition (<i>m/z</i>)	Collision energy (eV)	Calibration range μ M
Alanine	260.2>171.10	20	5-4000
Glycine	264.20>171.10	20	5-4000
Isoleucine	302.20>171.10	20	5-4000
Leucine	302.20>171.10	20	5-4000
Phenylalanine	336.20>171.10	20	5-4000
Valine	288.20>171.20	20	5-4000
Serine	276.20>171.10	20	5-4000
Threonine	290.20>171.10	20	5-4000
Tyrosine	352.20>171.10	20	5-4000
Glutamine	317.20>171.10	20	5-4000
Arginine	345.20>171.10	20	5-1000
Lysine	244.1>171.10	15	5-1000
Proline	286.20>171.10	20	5-1000
Aspartic Acid	304.20>171.10	20	5-1000
Cystine	291.10>171.10	15	2.5-500
Glutamic Acid	318.20>171.10	20	5-1000
Histidine	326.20>171.10	20	5-1000
Methionine	320.20>171.10	20	5-1000
Asparagine	303.20>171.10	20	5-1000
Tryptophan	375.20>171.10	20	5-1000
Hydroxylysine	252.10>171.10	15	5-1000
Sacrosine	260.20>171.10	20	5-1000
Beta Alanine	260.20>171.10	20	5-1000
α -aminobutyric acid	274.20>171.10	20	5-1000
γ -aminobutyric acid	274.20>171.10	20	5-1000
Alpha Amino adipic acid	332.20>171.10	20	5-1000
Kynurenine	379.20 >171.10	25	5-1000
Homocitrulline	360.20>171.10	20	5-1000
Citrulline	346.20>171.10	20	5-1000
Taurine	296.20>171.10	20	5-1000
Allo-Isoleucine	302.20 >171.10	20	5-1000
Phosphoethanolamine	312.20>171.10	15	5-1000
Homocystine	305.20>171.10	15	5-1000
Glycyl Proline	343.20 >171.10	20	5-1000
Anserine	411.20 >171.1	20	5-1000
L-Ornithine	237.10>171.10	15	5-1000
Ethanolamine	232.20 >171.10	20	5-1000
β -aminoisobutyric acid	274.20 >171.10	20	5-1000
Hydroxyproline	302.20>171.10	20	5-1000
S-Sulfocysteine	372.20 >171.10	20	5-1000
Cystathionine	282.10>171.10	15	5-1000
3-Methyl histidine	340.20>171.10	20	5-1000
1-Methyl-histidine	340.20>171.10	20	5-1000
Carnosine	397.20>171.10	25	5-1000
Argininosuccinic acid	461.20>171.10	30	5-1000

Table B. MRM parameters for the internal standards.

Internal standards	MRM transition (<i>m/z</i>)	Collision energy (eV)
Alanine ¹³ C ₃ ¹⁵ N	264.20>171.10	20
Glycine ¹³ C ₂ ¹⁵ N	249.20>171.10	20
Isoleucine ¹³ C ₆ ¹⁵ N	309.20>171.10	20
Leucine ¹³ C ₆ ¹⁵ N	309.20>171.10	20
Phenylalanine ¹³ C ₉ ¹⁵ N	346.20>171.10	20
Valine ¹³ C ₅ ¹⁵ N	294.20>171.10	20
Threonine ¹³ C ₄ ¹⁵ N	295.20>171.10	20
Serine ¹³ C ₃ ¹⁵ N	280.20>171.10	20
Tyrosine ¹³ C ₉ ¹⁵ N	362.20>171.10	20
Glutamine ¹³ C ₅ ¹⁵ N	324.20>171.10	20
Arginine ¹³ C ₆ ¹⁵ N ₄	355.20>171.10	20
Lysine ¹³ C ₆ ¹⁵ N ₂	248.10>171.10	15
Proline ¹³ C ₅ ¹⁵ N	292.20>171.10	20
Aspartic acid ¹³ C ₄ ¹⁵ N	309.20>171.10	20
Cystine ¹³ C ₆ ¹⁵ N ₂	295.10>171.10	15
Glutamic acid ¹³ C ₅ ¹⁵ N	324.20>171.10	20
Histidine ¹³ C ₆ ¹⁵ N ₃	335.20>171.10	20
Methionine ¹³ C ₅ ¹⁵ N	326.20>171.10	20
Tryptophan ¹³ C ₁₁ ¹⁵ N ₂	388.20>171.10	20
Asparagine ¹³ C ₄ ¹⁵ N ₂	309.20>171.10	20