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Applikationsbericht

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

Padhraic Rossiter, Jaime Salcedo Dominguez, Jennifer Warren, Norma Breen, Lisa J. Calton

Waters Corporation

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Abstract

This study describes 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 C_{18} Column, followed by detection on a Xevo TQ-S micro System.

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.

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

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 C_{18} 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 x 150 mm
Pre-column:	In-line filter
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

Method conditions

Time (min)	%A	%B	Curve
Initial	99	1	Initial

Time (min)	%A	%B	Curve
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

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

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" Derivitization 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 µL of sample to 1.5 mL Eppendorf

Step 2 Add 50 µL of 50 µM Internal Standard

Step 3 Vortex mix for 5 seconds

Step 4 Add 50 µL of water

Step 5 Vortex mix for 5 seconds

Step 6 For high concentration samples only – Add 1000 µL of diluent (0.1 M HCl)

Step 7 Centrifuge for 15 minutes at 9000 g

Step 8 Add 70 µL of Borate buffer to maximum recovery vial

Step 9 Add 10 µL of supernatant into Borate buffer and pipette mix

Step 10 Add 20 µL 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 ÅãC

Step 14 Inject 2 µL

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

Results and Discussion

No significant system carryover (detector response was ≤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 ≤6.9% CV and the between-day precision performance was ≤16.3% CV (≤13.9% CV when argininosuccinic acid was excluded) and the mean within-day and between-day precision performance was less than ≤13.9% CV as shown in Table 2.

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 Aminoadipic 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

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

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 ±15% of the nominal value, except for the LLOQ (within ±20%). At least 75% of the calibrators fulfilled this criterion, with the exception of the 3 analytes highlighted in red (hydroxyproline, cystathionine, argininosuccinic acid).

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

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

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 ≥10:1 and the precision performance of the replicates was ≤20% CV. The LOQ for all 45 amino acids analyzed were ≤5 µM with the exception of S-sulfocysteine, argininosuccinic acid (20 µM), and carnosine (15 µM), as shown in Table 4.

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
Sacrosine	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

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

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 ≤ ±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 ≤ ±10.0% bias (range -10.0 to +3.7%), for the analytes present in the material, all data is summarized in Table 5.

Compound	Accuracy summary (% bias)						
	20 µM	150 µM	400 µM	700 µM	2500 µM	Mean	NIST 400 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

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

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.

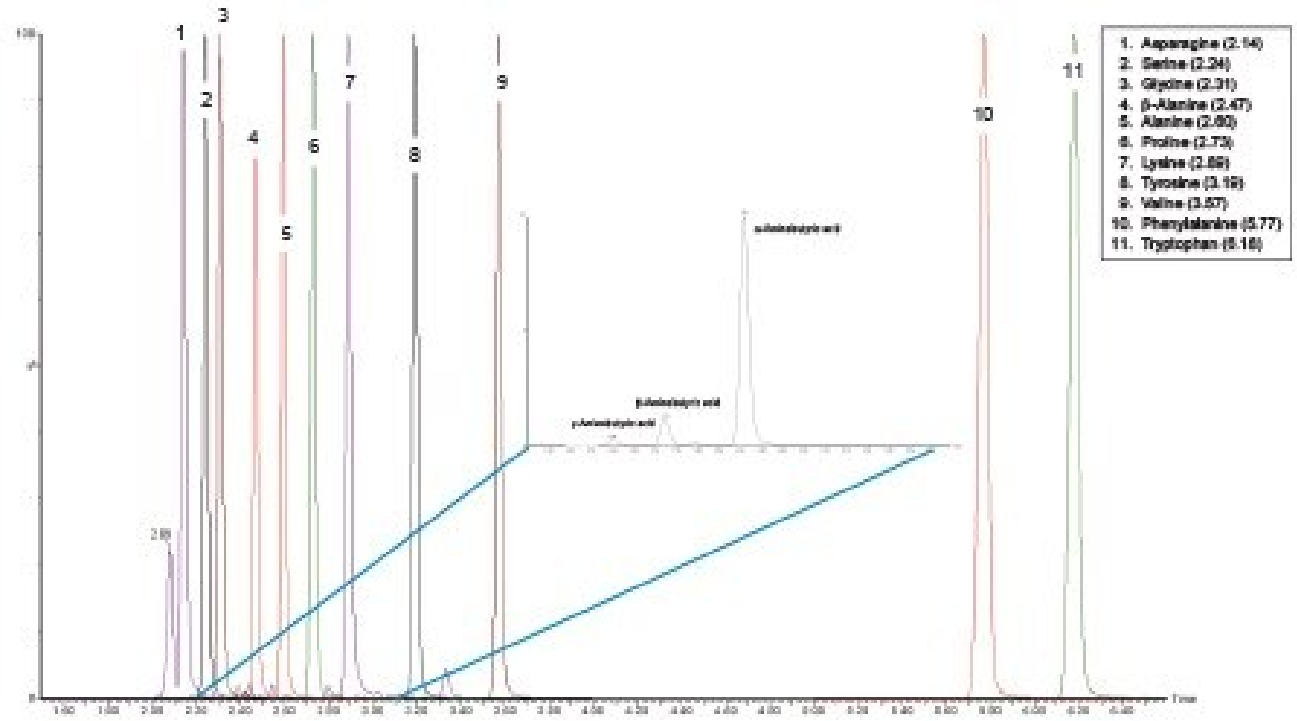
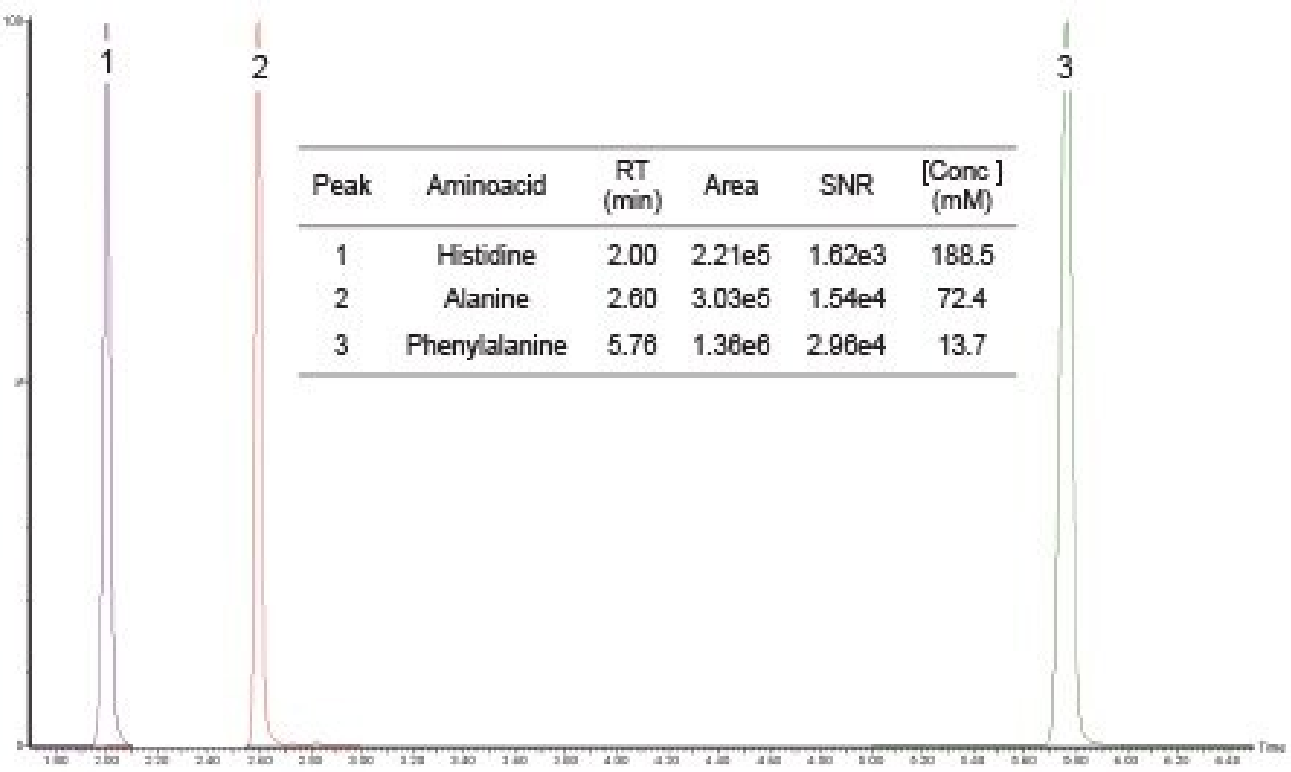


Figure 2. Total Ion Chromatogram (TIC) for 11 amino acids present in a plasma sample and inset a chromatogram to show the separation of gamma-aminobutyric acid, beta-aminoisobutyric acid, and alpha-aminobutyric acid in the same plasma sample.



Peak	Aminoacid	RT (min)	Area	SNR	[Conc.] (mM)
1	Histidine	2.00	2.21e5	1.62e3	188.5
2	Alanine	2.60	3.03e5	1.54e4	72.4
3	Phenylalanine	5.76	1.36e6	2.96e4	13.7

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.

Conclusion

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" Derivitization 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.

Featured Products

ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

Xevo TQ-S micro Triple Quadrupole Mass Spectrometry <https://www.waters.com/134798856>

MassLynx <https://www.waters.com/513164>

TargetLynx <https://www.waters.com/513791>

Available for purchase online:

Kairos Amino Acid Kit <https://www.waters.com/waters/en_US/Kairos-Amino-Acid-Analysis-Kit-for-physiological-fluids/nav.htm?cid=135004655>

AccQ▪Tag Ultra “3x” Derivitization Kit <https://www.waters.com/waters/partDetail.htm?partNumber=186004535>

CORTECS UPLC C18 Column, 90Å, 1.6 µm, 2.1 mm X 150 mm <https://www.waters.com/waters/partDetail.htm?partNumber=186007096>

ACQUITY UPLC Column In-Line Filter Kit <https://www.waters.com/waters/partDetail.htm?partNumber=205000343>

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