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Nota de aplicación

UPLC-MS Analysis of 20 Amino Acids Using the Kairos Amino Acid Kit for Biomedical Research

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Abstract

This application note describes the use of the Kairos Amino Acid Kit for analysis of 20 amino acids in solution. Kairos Amino Acid Kit enables biomedical researchers to achieve trusted results through a single flexible kit that allows them to accurately quantitate 20 amino acids within their normal physiological range in less than 10 minutes using the ACQUITY UPLC/ACQUITY QDa System.

Benefits

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

Introduction

The research only Kairos Amino Acid Kit (p/n: 176004379) 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 in your results. Metrological traceability of the Kairos Amino Acid Kit Calibrators (p/n: 186009048) were traceable to National Metrology Institute of Japan (NMIJ CRM 6011a-6018a, 6022a) for 9 amino acids and a further 8 were traceable to National Institute of Standards and Technology (NIST SRM 2389a), the remaining amino acids were gravimetrically prepared using TraceCERT standards from Sigma-Aldrich.

Here we describe the use of the Kairos Amino Acid Kit for analysis of 20 amino acids in solution. Chromatographic separation was performed using an ACQUITY UPLC H-Class System using a CORTECS UPLC C_{18} 1.6 µm, 2.1 × 150 mm Column (p/n: 186007096), followed by detection on an ACQUITY QDa Mass Detector, designed for simplicity with automated set-up (Figure 1). Performance of the kit and analytical system was assessed in solution and NIST SRM 2389a material analyzed for biomedical research purposes.

The chromatographic conditions used allowed for the separation of leucine and alanine isobaric species.

Figure 1. The Waters ACQUITY UPLC H-Class System/ACQUITY QDa Mass Detector System.

Experimental

Reagent Kit

Kairos Amino Acid Kit (p/n: 176004379)

LC conditions

System:	ACQUITY UPLC H-Class Column Heater (CH-A)
Needle:	15 µL

Column:	CORTECS UPLC C_{18} 2.1 x 150 mm, 1.6 µm (p/n: 186007096)
Pre-column:	ACQUITY UPLC Column In-line Filter Kit (p/n: 205000343)
Mobile phase A:	Water with 0.1% formic acid
Mobile phase B:	Acetonitrile with 0.1% formic acid
Needle wash solvent:	Mobile phase B
Purge solvent:	Mobile phase A
Column temp.:	55 °C
Injection volume:	2 µL
Flow rate:	0.5 mL/min
Gradient:	See Table 1
Run time:	9 minutes

MS conditions

System:	ACQUITY QDa Mass Detector
Acquisition mode:	Single Ion Recording (SIR) (see Tables 2 and 3 for details)
Polarity:	ESI+

Capillary:	0.8 kV
Source temp.:	120 °C
Desolvation temp.:	600 °C
Cone:	10 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 (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 make 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 (720005448EN). 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 "3X" 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 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 °C

Step 14	Inject 2 µL

For precision and accuracy studies, panel samples were gravimetrically prepared in 0.1 M HCl at 20, 150, 400, 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.

Method conditions

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

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

Analyte	SIR (*m/z*)	Calibration range µM
Alanine	260.2	5–4000
Glycine	246.2	5–4000
Isoleucine	302.2	5–4000
Leucine	302.2	5–4000
Phenylalanine	346.2	5–4000
Valine	288.2	5–4000
Threonine	290.2	5–4000
Serine	276.2	5–4000
Tyrosine	352.2	5–4000
Glutamine	317.2	5–4000
Arginine	345.2	5–1000
Lysine	244.1	5–1000
Proline	286.2	5–1000
Aspartic Acid	304.2	5–1000
Cystine	291.1	2.5–500
Glutamic Acid	318.2	5–1000
Histidine	326.2	5–1000
Methionine	320.2	5–1000
Tryptophan	375.2	5–1000
Asparagine	303.2	5–1000

Table 2. SIR parameters for the amino acids and calibrator concentration range.

Table 3. SIR parameters for the internal standards.

Results and Discussion

No significant system carryover (detector response was ≤20% of Calibrator 1) was observed from high concentration samples into subsequent blank injections.

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 ≤12.0%CV and the between day precision performance was ≤14.7%CV and the mean within day and between day precision performance was less than ≤11.3% CV as shown in Table 4.

Analyte	Within-day precision (%CV)	Between-day precision (%CV)
Alanine	6.0	6.1
Glycine	4.9	7.2
Isoleucine	1.4	2.0
Leucine	1.6	1.9
Phenylalanine	2.2	2.5
Valine	2.1	2.8
Arginine	8.1	9.3
Lysine	4.3	6.1
Proline	3.7	4.9
Serine	6.6	7.9
Threonine	5.8	7.1
Tyrosine	2.6	3.2
Aspartic acid	7.4	8.5
Cystine	5.5	7.7
Glutamic acid	5.5	6.1
Histidine	5.6	6.9
Methionine	2.5	2.8
Asparagine	9.1	11.3
Tryptophan	1.7	2.0
Glutamine	7.1	9.9

Table 4. Mean within-day and between day precision for the analysis of 20 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 20 amino acids analyzed (Table 5). 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.

Analyte	Day 1		Day 2		Day 3	
	r^2	Max dev (%)	r^2	Max dev (%)	r^2	Max dev (%)
Alanine	0.999	10.8	0.997	14.4	0.999	11.2
Glycine	0.999	4.7	1.000	13.4	1.000	9.1
Isoleucine	1.000	3.6	1.000	5.6	1.000	3.0
Leucine	1.000	5.0	1.000	1.9	1.000	7.7
Phenylalanine	0.999	12.1	1.000	3.9	1.000	11.0
Valine	1.000	7.6	0.999	6.6	1.000	7.2
Arginine	1.000	5.9	0.999	9.0	0.998	19.7
Lysine	0.998	10.0	0.999	4.0	1.000	5.4
proline	0.999	8.6	0.998	9.7	0.999	8.4
Serine	0.996	17.5	0.999	8.3	0.999	15.1
Threonine	0.999	7.1	0.999	14.9	1.000	14.9
Tyrosine	1.000	11.7	0.999	10.3	1.000	9.9
Aspartic acid	0.999	8.0	1.000	2.8	1.000	6.7
Cystine	0.993	16.1	0.994	11.6	0.999	12.5
Glutamic acid	0.999	11.8	0.995	20.3	1.000	3.1
Histidine	0.999	8.1	0.999	15.7	0.998	8.2
Methionine	0.999	4.5	0.998	12.8	0.999	6.9
Asparagine	0.999	12.1	0.996	15.5	0.999	7.6
Tryptophan	0.999	7.8	1.000	3.2	0.999	5.1
Glutamine	0.997	18.8	0.999	14.8	1.000	4.7

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

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 LOQ 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 20 amino acids analysed were ≤4 µM and are shown in Table 6.

Analyte	LOQ (µM)	Precision (%CV)	Accuracy (% Bias)	Signal-to-noise ratio
Alanine	4	4.0	17.5	301
Glycine	3	6.8	19.3	177
Isoleucine	1	0.0	20.0	31
Leucine	1	3.8	18.0	33
Phenylalanine	2	2.4	12.0	109
Valine	1	5.3	4.0	62
Arginine	3	9.0	18.7	24
Lysine	3	2.9	16.7	223
Proline	3	1.6	15.3	153
Serine	4	8.0	19.5	69
Threonine	4	4.1	18.0	125
Tyrosine	2	0.0	10.0	137
Aspartic acid	4	3.2	19.0	97
Cystine	1.5	5.1	16.0	37
Glutamic acid	2	5.2	19.0	100
Histidine	2	9.6	14.0	11
Methionine	1	5.2	6.0	91
Asparagine	1	5.8	6.0	25
Tryptophan	3	1.6	15.3	152
Glutamine	2	7.9	4.0	20

Table 6. Sensitivity of the Kairos Amino Acid Kit in solution.

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 ≤ ±12.3% bias (range -10.2 to +12.3%) and the diluted NIST SRM 2389a calculated concentrations were ≤ ±8.9% bias (range -8.9 to +6.2%) as shown in Table 7.

Compound	Accuracy Summary (% bias)						
	20 µM	150 µM	400 µM	700 µM	2500 µM	Mean	NIST 400 µM
Alanine	-1.8	-5.0	-5.1	-5.3	-5.7	-4.6	-5.7
Glycine	-3.1	-0.1	-2.4	-2.2	-7.9	-3.2	-3.8
Isoleucine	-1.9	-1.6	-1.5	-2.7	-3.1	-2.2	-0.7
Leucine	0.8	0.1	-0.7	-0.7	-1.6	-0.4	0.3
Phenylalanine	-0.2	-2.3	-2.4	-4.6	-3.2	-2.6	-2.4
Valine	0.2	-0.9	-1.3	-2.3	-2.0	-1.3	-0.5
Arginine	4.0	8.4	7.1	9.4	N/A	7.2	6.2
Lysine	-0.4	-2.7	-1.7	-5.5	N/A	-2.6	-5.0
Proline	-3.4	-0.4	0.9	-0.7	N/A	-0.9	-2.0
Serine	-2.4	-6.2	-5.1	-10.2	-6.6	-6.1	-6.1
Threonine	1.8	2.4	5.6	1.5	-1.4	2.0	0.6
Tyrosine	1.8	1.0	1.9	0.3	0.4	1.1	2.0
Aspartic acid	3.1	1.3	4.1	1.8	N/A	2.6	-0.7
Cystine	-1.5	-7.7	-4.4	-2.2	N/A	-4.0	-0.7
Glutamic acid	2.6	7.3	6.4	1.3	N/A	4.4	-8.9
Histidine	-3.0	-1.0	2.4	-0.2	N/A	-0.4	-3.9
Methionine	-2.8	-1.2	-1.1	-2.8	N/A	-2.0	-2.6
Asparagine	5.0	2.2	2.5	-0.7	N/A	2.2	N/A
Tryptophan	-2.3	-4.3	-4.0	-3.3	N/A	-3.5	N/A
Glutamine	12.0	12.3	12.1	8.1	11.3	11.1	N/A

Table 7. Accuracy of the Kairos Amino Acid Kit in solution.

Conclusion

Kairos Amino Acid Kit enables biomedical researchers to achieve trusted results through a single flexible kit that allows them to accurately quantitate 20 amino acids within their normal physiological range in less than 10 minutes using the ACQUITY UPLC/ACQUITY QDa System.

Using AccQ•Tag Ultra "3X" Derivitization Kit to derivatize the samples allows for a simple, robust, and fast reverse 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/ACQUITY QDa 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 analysis of 20 amino acids in solution using an ACQUITY UPLC/ACQUITY QDa System with good precision, linearity, sensitivity, and accuracy.

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