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# Analysis of Amino Acid Content in Commercially Available Supplements


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## PURPOSE

With the increase popularity of energy supplements, there is a need to monitor amino acid content in these products to ensure product safety. However, these products are controlled by varying regulatory agencies from country to country, each with different safety requirements [1,2]. While the medicinal properties of amino acids in supplements is unknown, the label claim of these supplements is assumed to be accurate. To quantify amino acids in these supplements, pre-column derivatization methods are often of value to minimize the interference of other components present in the formulation that may impact quantitation.

It is important to have sound methodologies for quantitating amino acids in supplement samples. The methodology showcased in this work allows for reliable quantitation of amino acids in supplement samples ranging from powdered drink mixes to supplement tablets.

## OBJECTIVE

Using a pre-column derivatization HPLC methodology for amino acid analysis, to quantitatively measure amino acid content in energy supplements and compare to label claim.

## METHOD(S)

All calibration standards were prepared from Waters Cell Culture Standard Kit (p/n: 186009300) and Waters Food and Feed Standard Kit (p/n: 186009299) using norvaline (p/n: 186009301) as the internal standard and 0.1 N HCl as the diluent [3]. Beta Alanine, Citrulline and Glutamic Acid standards were purchased from Millipore Sigma (Beta Alanine p/n: PHR1349, L-Citrulline p/n: PHR3191, Glutamic Acid p/n: PHR1107). The internal standard stock was prepared at 2500 µM in 0.1 N HCl. The final concentration of the calibrants varied as the samples varied in amino acid content and 250 µM for norvaline (internal standard).

Additional method modifications were required to optimize the scaled method [4].

### LC Conditions:

LC systems:
- Arc™ HPLC System with 30 cm CHC with passive preheater
- ACQUITY™ Arc™ System with CH-A Column heater

Detection:
- Arc HPLC -2489 UV Detector with 10 mm HPLC Analytical Flow Cell
- ACQUITY Arc - 2489 UV Detector with low dispersion 10 mm UHPLC flow cell

Wavelength: 260 nm
Column: AccQ•Tag™ Ultra C18, 2.5 µm 4.6 x 150 mm Column
Column temperature for Hydrolysate and Cell Culture: 43 °C
Column temperature for Food and Feed: 50 °C
Sample temperature: 20 °C
Injection volume:
- 3 µL (Arc HPLC System)
- 2 µL (ACQUITY Arc System)

Flow rate: 1.5 mL/min
Mobile phase A: AccQ•Tag Ultra Eluent A
Mobile phase B: 90:10 (v/v) Water:AccQ•Tag Ultra Eluent B
Mobile phase C: Milli-Q™ Water
Mobile phase D: AccQ•Tag Ultra Eluent B

Gradient Table

| Time (min) | %A | %B | %C | %D | Curve |
|---|---|---|---|---|---|
| Initial | 10.0 | 0.0 | 90.0 | 0.0 | Initial |
| 0.36 | 10.0 | 0.0 | 90.0 | 0.0 | 11 |
| 19.67 | 9.0 | 80.0 | 11.0 | 0.0 | 7 |
| 25.07 | 8.0 | 16.0 | 60.0 | 16.0 | 7 |
| 25.74 | 8.0 | 16.0 | 58.0 | 18.0 | 6 |
| 27.05 | 7.8 | 0.0 | 70.9 | 21.3 | 6 |
| 28.20 | 4.0 | 0.0 | 36.3 | 59.7 | 6 |
| 30.08 | 4.0 | 0.0 | 36.3 | 59.7 | 6 |
| 30.38 | 10.0 | 0.0 | 90.0 | 0.0 | 6 |
| 35.48 | 10.0 | 0.0 | 90.0 | 0.0 | 6 |

## RESULT(S)

Several energy supplements were purchased and tested to quantify the amino acid content. Amino acid analysis derivatization was performed using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC), followed by HPLC/UHPLC separation of the derivatives with reversed-phase liquid chromatography. Results were compared to the label claim of the products, including two powdered drink mix supplements and one amino acid supplement tablet. Any interfering peaks were identified through mass spectrometry (MS) and adjustments were made to allow for quantitation of all amino acids in the supplements.

The samples were prepared as follows:

Sample 1 (Amino Acid Supplement Tablet) was crushed using a mortar and pestle and diluted in 0.1N HCl in water. Sample #1 was then placed onto a shaker for 30 mins and centrifuged at 2000 rpm for 10 mins. The supernatant was subsequently collected in a clean centrifuge tube.

Sample 2 (Powdered Drink Mix Supplement) and Sample 3 (Powdered Drink Mix Supplement) were both weighed up in 0.1N HCl in water. Each sample was stirred on a stir plate with a stir bar on medium speed for 30 minutes and then centrifuged at 2000 rpm for 10 mins. The supernatant for each was then collected in a clean centrifuge tube and diluted **1:10** in 0.1N HCl.

Sample 4 (Powdered Drink Mix Supplement) was prepared as described above for sample 2 and 3 however, the supernatant was then collected in a clean centrifuge tube and diluted **1:100** in 0.1N HCl.

All of the samples were then derivatized using Waters standard derivatization protocol. Individual amino acid content was only provided for sample 4. Based on the results, the weight of total amino acids was calculated and compared to the label claim.

The total amino acid content for sample #1 was equal to 5000 mg per five (5) tablets. For samples #2 & #3, the total amino acid content was 5000 mg per serving. For sample #4, the total amino acid content was 5000 mg per serving, which was also broken down into individual amino acid content in Table 2.

Initial method conditions were used to quantify most amino acids, however, co-elution of citrulline and glutamic acid in samples #2 & #3 was identified by mass spectrometry. Following mass spectrometry identification of glutamic acid and citrulline, quantitation was performed using a TUV detector. The chromatographic separation for quantitation is shown in Figure 1. Subsequently, method conditions were altered to allow for quantitation of all the amino acids in two analyses.

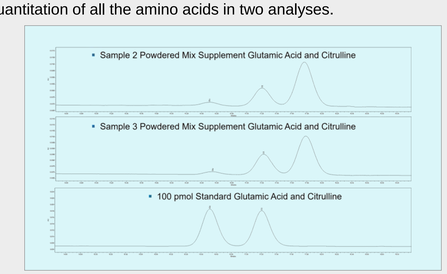


*Figure 1. Chromatograms for Amino Acid Supplement Samples #2 & 3 of Glutamic Acid and Citrulline Quantitation using a TUV detector.*

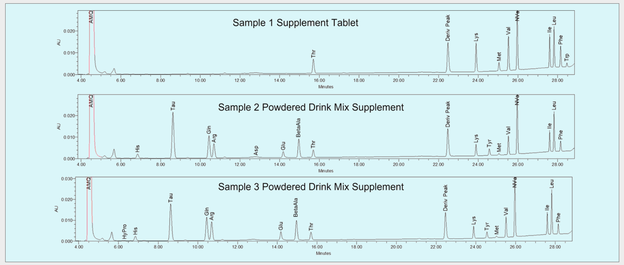


*Figure 2. Amino Acid Supplement Sample 1 – 3 Chromatograms.*

| Arc HPLC System | | | |
|---|---|---|---|
| Sample results (g) | | | |
| Compound | Sample 1 Supplement Tablet | Sample 2 Powdered Drink Mix Supplement | Sample 3 Powdered Drink Mix Supplement |
| Sum Quantitated in Sample | 1.047 | 6.306 | 6.115 |
| Label Claim (g) | 1.000 | 5.758 | 5.714 |
| Difference between Label Claim vs. Results | -4.46% | -8.69% | -6.55% |

| ACQUITY Arc System | | | |
|---|---|---|---|
| Sample results (g) | | | |
| Compound | Sample 1 Supplement Tablet | Sample 2 Powdered Drink Mix Supplement | Sample 3 Powdered Drink Mix Supplement |
| Sum Quantitated in Sample | 1.008 | 6.310 | 5.796 |
| Label Claim (g) | 1.000 | 5.758 | 5.714 |
| Difference between Label Claim vs. Results | -0.79% | -5.04% | -1.41% |

*Table 1. ACQUITY Arc and Arc HPLC Systems amino acid supplement sample for samples 1-3 for the quantitated results vs. label claims.*

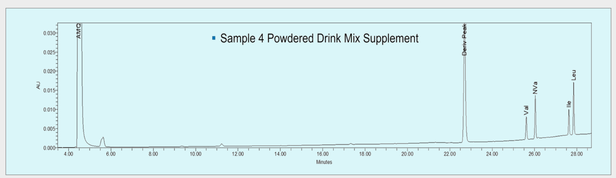


*Figure 3. Amino Acid Supplement Sample 4 Chromatogram.*

| ACQUITY Arc System | | | |
|---|---|---|---|
| Individual Compound Sample Results (g) | | | |
| Compound | Sample 4 Powdered Drink Mix Supplement in Weighed Sample Amount | Sample 4 Powdered Drink Mix Supplement | Difference between Label Claim vs. Results |
| Val | 1.46 | 1.44 | 1.3% |
| Ile | 1.46 | 1.40 | 4.4% |
| Leu | 3.17 | 3.13 | 1.4% |

| ACQUITY Arc System | |
|---|---|
| Total Sample Results (g) | |
| Compound | Sample 4 Powdered Drink Mix Supplement |
| Sum Quantitated in Sample | 5.969 |
| Label Claim (g) | 6.094 |
| Difference between Label Claim vs. Results | 2.09% |

*Table 2. ACQUITY Arc System amino acid supplement sample #4 for the quantitated results vs. label claims.*

## CONCLUSION(S)

Analysis of amino acids in food and supplements is critical to ensure product safety and accuracy of the product's label claim. While there are a range of amino acids, not all components may be separated under standard conditions. In this example, to facilitate method development, existing methodologies can be scaled and further optimized to create a robust method for the analysis of a wide range of amino acids. The results demonstrate the ability to quantify amino acid content in numerous energy supplements.

In this work, the method required column temperature adjustments for the food and feed standard to obtain optimal resolution of critical pairs along with identification and separation of glutamic acid and citrulline using the Waters ACQUITY QDa™ Mass Detector. With these adjustments and adaptations, the method shows results that demonstrate the ability to quantify amino acid content in numerous energy supplements.



## REFERENCES


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3. Amino Acid Standard Kits Care and Use Manual. Waters Care and Use Manual, 72000663EN.
4. Kimberly Martin, Kenneth D. Berthelette, Paula Hong. Instrument Considerations for Reliable Amino Acid Analysis Using AccQ•Tag™ Ultra $C_{18}$ 2.5 µm Column. Waters Application Note, 720007678EN, 2022.


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