

Amino acids are building blocks of proteins and essential amino acids need to be obtained from diet as they cannot be synthesized by our bodies. Amino acid supplements are popular among athletes and bodybuilders and are sold in the form of pills, powders and drinks. They usually also contain vitamins, electrolytes, herbs, flavorings and other additives.

Analysis of these supplements is important to confirm the label claims and to maintain industry standards.

Amino Acid Analysis with post-column derivatization is a very sensitive, reproducible and rugged method and it has been a preferred approach for laboratories running biological samples, protein, peptides and foods analysis. Pickering Laboratories Inc. products for Amino Acid Analysis include post-column derivatization instruments, columns, eluants, reagents and standards. All products are designed to work together to deliver optimum results for any chosen sample.

The amino acids present in the product determine if Sodium or Lithium columns and buffers need to be used. Lithium columns have longer run time but have higher selectivity and are able to separate more amino acids than Sodium columns. Pickering Laboratories will help you to identify the best method for your Amino Acid Analysis.

Sample Preparation

Grind 10 pills to fine powder before taking a sample for analysis. Mix all the powdered supplements thoroughly. Sample size depends on concentrations of amino acids present.

To finely ground sample (0.05 g – 0.2 g) add 100 mL of Na220. Mix well and sonicate for 10 min. Filter through 0.45 µm filter and inject.

Method

Analytical Conditions

Column System: High-efficiency Sodium cation-exchange column, 4.6 x 110 mm, Catalog Number 1154110T

Flow rate: 0.6 mL/min

Mobile Phase: See method in Table 1

Injection Volume: 10-50 µL

Post-Column Conditions

Post-Column System: Onyx PCX or Pinnacle PCX

Reactor Volume: 0.5 mL

Reactor Temperature: 130 °C

Column Temperature: See method in Table 2

Flow Rate: 0.3 mL/min

Table 1. HPLC Program for Column 1154110T

Time	Na315, %	Na425, %	Na640, %	RG011, %
0	100	0	0	0
4.0	100	0	0	0
15.0	0	100	0	0
16.0	0	0	100	0
31.0	0	0	100	0
31.1	0	0	0	100
33.0	0	0	0	100
33.1	100	0	0	0
40	100	0	0	0

Table 2. Column Oven Program

Time	Temp, °C
0	46
4	46
9	70
32	70
33	46

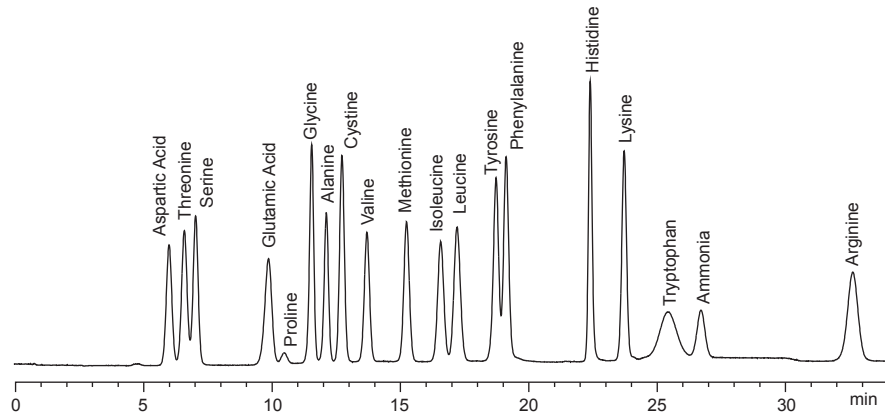


Fig 1. Chromatogram of amino acids standard using sodium column 1154110T

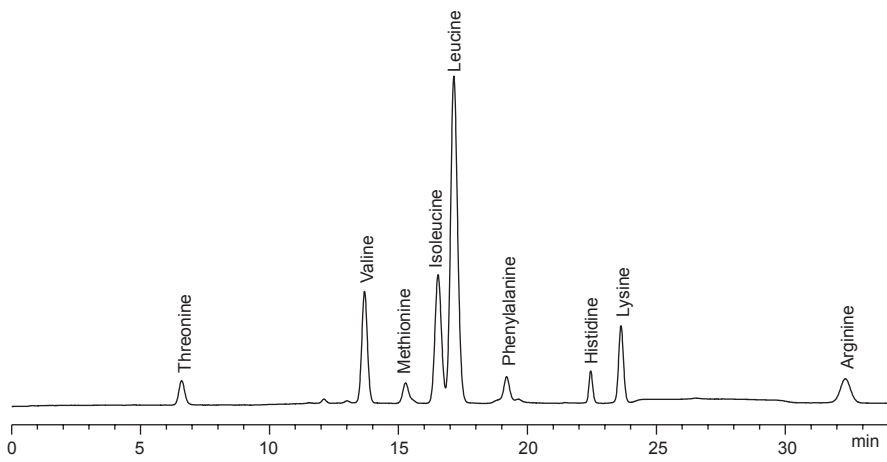


Fig 2. Chromatogram of a sample of commercial training supplement using sodium column 1154110T

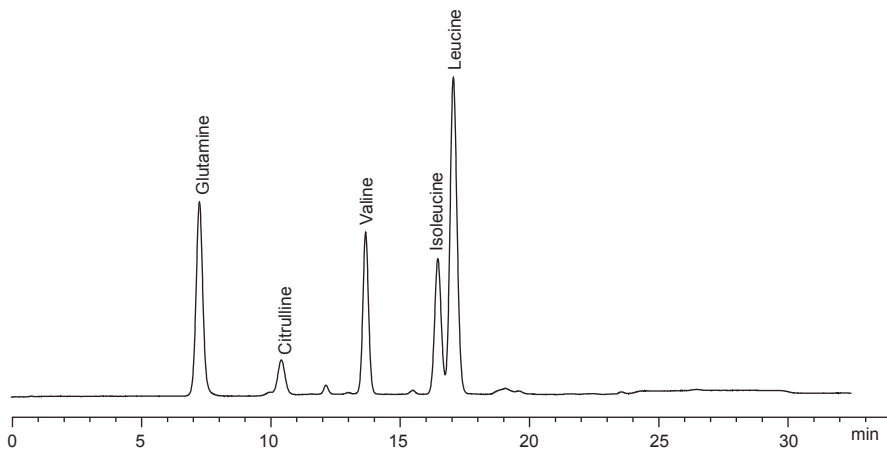


Fig 3. Chromatogram of the a sample of sport recovery drink using sodium column 1154110T

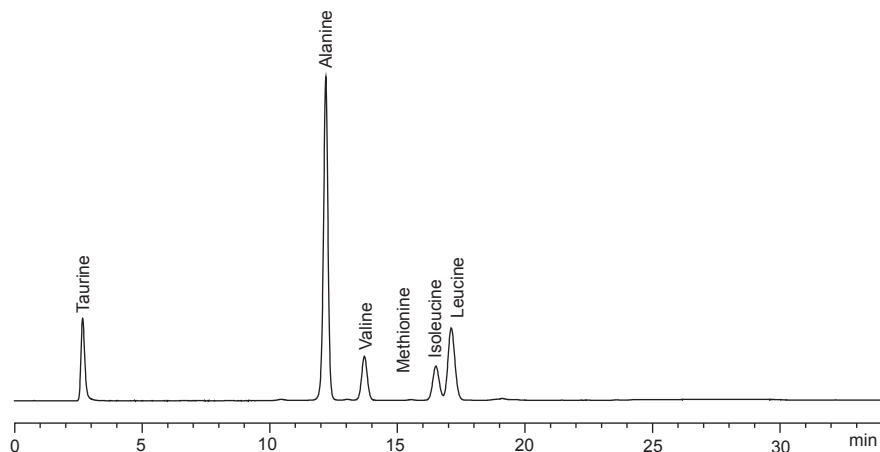


Fig 4. Chromatogram of a commercial sport performance drink using sodium column 1154110T

Method for Lithium Column

Analytical Conditions

Column: High-efficiency Lithium cation-exchange column, 4.6 x 75 mm, Catalog Number 0354675T

Flow rate: .55 mL/min

Mobile Phase: See method in Table 3

Injection Volume: 50 uL

Post-column Conditions

Post-Column System: Onyx PCX or Pinnacle PCX

Reactor Volume: 0.5 mL

Reagent: Trione®

Reactor Temperature: 130 °C

Column Temperature: See method in Table 4

Flow Rate: 0.3 mL/min

Detection: UV/VIS 570 nm for primary amino acids, 440 nm for secondary amino acids

Table 3. HPLC Program for Column 0354675T

Time	1700-1125, %	Li365, %	Li375, %	RG003, %
0	100	0	0	0
15	100	0	0	0
35	40	60	0	0
38	0	100	0	0
43	0	100	0	0
43.1	0	0	100	0
57	0	0	100	0
57.1	0	0	0	100
60	0	0	0	100
60.1	100	0	0	0
72	100	0	0	0

Table 4. Column Oven Program

Time	Temp, °C
0	34
6	34
30	45
32	70
59	70
60	34

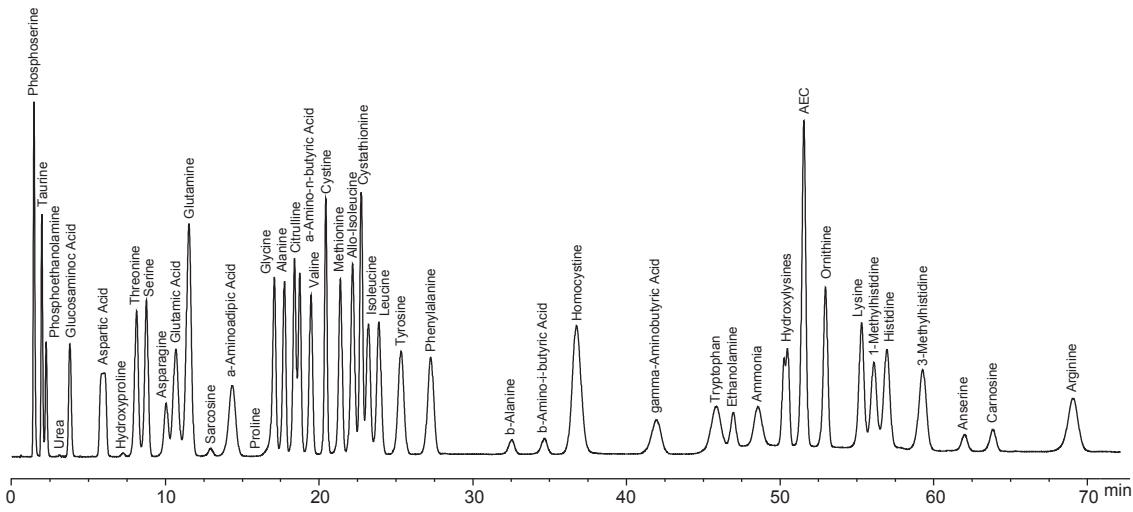


Fig 5. Chromatogram of amino acids standard using lithium column 0354675T

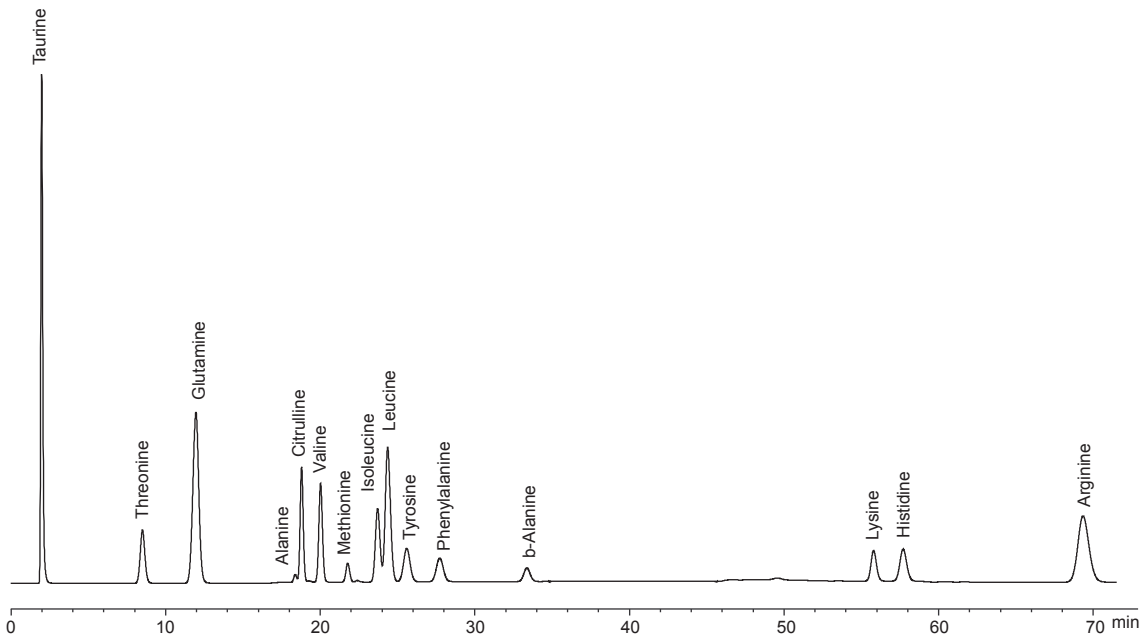


Fig 6. Chromatogram of a pre-workout amino acid supplement drink using lithium column 0354675T