

Theanine is a neurologically active amino acid found in tea plants. Theanine is a dominant amino acid in green tea and is responsible for its unique pleasant taste as well as known relaxation effect. Theanine also has been proven to reduce physical and mental stress, and improve cognition and mood.

Cation-exchange chromatography using post-column Ninhydrin reagent Trione and UV detection has shown unmatched reproducibility and selectivity in the analysis of free amino acids in complex matrices. The Pinnacle PCX post-column derivatization system allows shortening run times by utilizing column temperature gradients.

We introduce a simple, fast and robust method for analysis of Theanine. The same column and solutions could be used for extended analysis to determine other free amino acids in tea leaves.

## Method

### Sample Preparation

Homogenize 2 g of dry tea leaves with 25 mL of Li220 for 5 min. Centrifuge and filter through 0.45  $\mu$ m Nylon filter.

### Analytical Conditions

**Column:** High-efficiency Lithium cation-exchange column, 4.6x75 mm, Catalog number 0354675T

**Flow Rate:** 0.55 mL/min

**Mobile Phase:** see method

**Injection Volume:** 10  $\mu$ L

### Post-Column Conditions

**Post-Column System:** Onyx PCX or Pinnacle PCX

**Reactor Volume:** 0.5 mL

**Reactor Temperature:** 130  $^{\circ}$ C

**Reagent:** Trione Ninhydrin reagent

**Flow Rate:** 0.3 mL/min

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

HPLC Gradient			
Time (Min)	1700-1125, %	Li365, %	Rg003, %
0	100	0	0
10	100	0	0
19	40	60	0
19.1	0	0	100
24	0	0	100
24.1	100	0	0
36	100	0	0

Column Oven Program	
Time (Min)	Temperature, $^{\circ}$ C
0	34
6	34
17	65
19	34

Run Time: 24 Min  
Equilibration Time: 12 Min

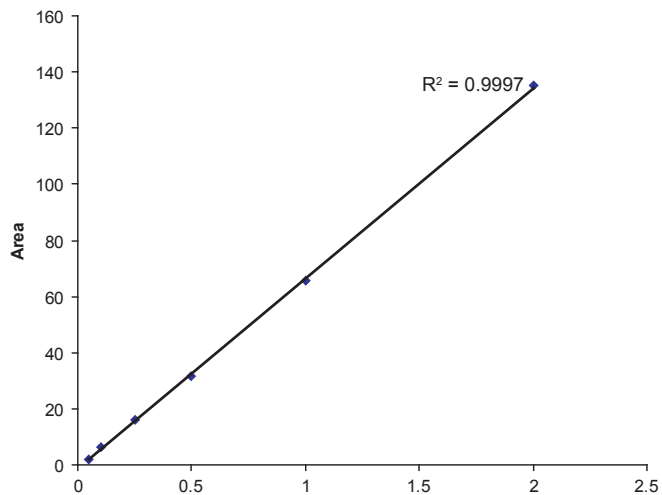


Fig 1. Calibration curve for Theanine.  
Concentration range 0.05-2  $\mu\text{mole/mL}$

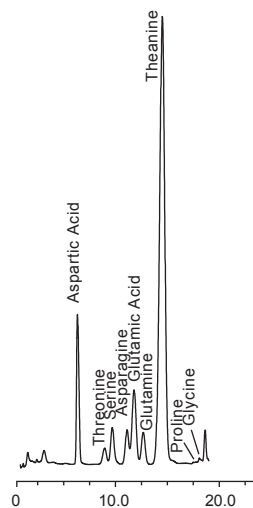


Fig 2. Chromatogram of green tea sample

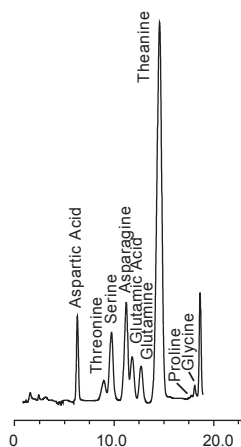


Fig 3. Chromatogram of white tea sample