COGENT 2.0
Diamond Hydride™

L-THEANINE IN GREEN TEA

Food & Beverage Application

Extended Application Note

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L-Theanine Structure

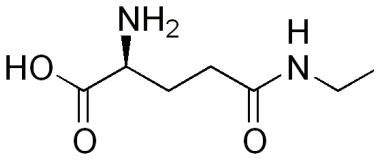

Figure 1

Gradient:

Time (min.)	%B
0	95
1	95
8	50
9	95

Post Time: 4 min
Flow Rate: 0.3 mL/min
Injection Volume: 2.0µL
Detection: UV 200 nm

Introduction

L-Theanine is a naturally occurring amino acid analog found in certain fungi and plants. In green tea, it is present at varying concentrations depending on several factors in preparation, although typically values around 10 mg/L are reported. In a 250 mL cup of tea, this translates to a dose of about 40 mg L-theanine.

As a psychoactive compound, L-theanine can cross the blood–brain barrier and exhibits a number of potentially beneficial effects. Some reported examples include increased focus and alertness, alleviated anxiety, and elevated mood. Sometimes it is taken by itself as a work-out supplement.

Due to the interesting properties of this compound, there is a growing need to study its pharmacological effects. However, there are some difficulties with its analysis due to its structure (**Figure 1**). The compound does not have significant UV absorption which makes adequate sensitivity in UV-based analyses problematic. Furthermore, the compound is polar and would not be suited to conventional reversed phase HPLC analyses.

To this end, we investigated the use of a Diamond Hydride 2.ō™ column for the study of L-theanine in green tea. The column has two important properties which should address the issues discussed earlier. First, it is designed for use in the Aqueous Normal Phase (ANP) mode in which compounds are retained on the basis of polarity. Second, the particle diameter is much lower than standard 4 or 5µm phases. It is a near-UHPLC phase with an average particle diameter of 2.2µm. This leads to increased efficiency of analyte peaks and therefore greater peak height. Hence, the sensitivity can be increased without resorting to lengthy and unreliable derivatization procedures. A smaller particle size column can also be used to obtain greater analysis speed and hence higher throughput.

Experimental

Materials

L-Theanine bulk powder (99.3% purity by assay) was purchased from a commercially available source online. Green tea packets were purchased from a local supermarket. Formic acid (FA) was from EMD (Gibbstown, NJ, USA). Deionized water (DI H_2O) was prepared on a Milli-Q™ purification system from Millipore (Bedford, MA, USA). Acetonitrile (HPLC grade) was obtained from GFS Chemicals, Inc. (Powell, OH, USA).

Instrumentation

A Hewlett–Packard (Palo Alto, CA, USA) 1100 HPLC system consisting of an autosampler, degasser, binary pump, and variable wavelength UV detector was used. The system was interfaced with Agilent Chemstation™ (Santa Clara, CA, USA) software. The analytical column was 2.1 x 50 mm and packed with a Diamond Hydride 2.ō™ stationary phase (MicroSolv Technology Corporation, Leland, NC, USA). The particle diameter was 2.2µm and the pore size was 120Å. The binary mobile phase solvents were A: DI H_2O + 0.1% formic acid and B: acetonitrile + 0.1% formic acid.

Sample Preparation

A stock solution of L-theanine was prepared by weighing 50.0 mg bulk powder and quantitatively transferring to a 25 mL volumetric flask. A portion of 50/50 solvent A/solvent B was added and the flask was sonicated for 10 min. Then it was diluted to mark, capped, and mixed thoroughly. Appropriate aliquots were transferred to a series of volumetric flasks to obtain concentrations in the range 2–200 ppm

Concentration (ppm)	Volume (mL)	Aliquot of Stock (µL)
2.0	100	100
5.0	100	250
10.0	100	500
50.0	10	250
200.0	10	1000

The green tea sample was prepared by boiling 250 mL bottled water and steeping the tea packet in the water for a period of 5 min. These conditions are meant to approximate those used in a typical home preparation rather than a laboratory setting (e.g. bottled water instead of DI H_2O, steeping instead of sonicating, etc.). The green tea liquid was then filtered with a 0.45µm nylon syringe filter (MicroSolv Technology Corporation, Leland, NC, USA).

A spiked green tea sample was also prepared in which filtrate obtained in the same manner as above was used as a diluent in a 10 mL volumetric flask. A 250µL spike of the L-theanine stock solution was added and the liquid was allowed to cool to room temperature before diluting to mark.

Results and Discussion

In terms of developing a suitable method, one consideration was to keep the conditions LC-MS compatible. In this manner, other investigations involving LC-MS could use the method as a template for their own samples. For instance, a clinical lab may be interested in monitoring L-theanine and metabolites in plasma samples. In this case LC-MS would most likely be used. Analyses of green tea could benefit from the use of LC-MS since significantly higher sensitivity can be obtained with MS compared to UV detection.

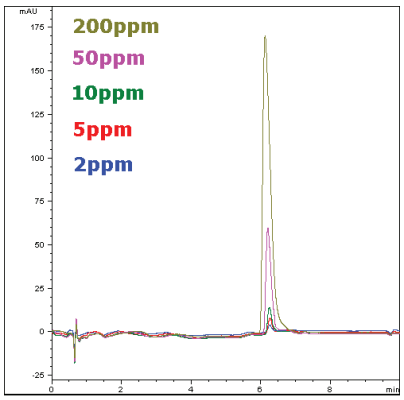

Figure 3

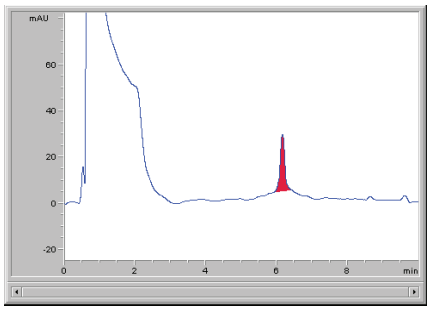

Figure 4

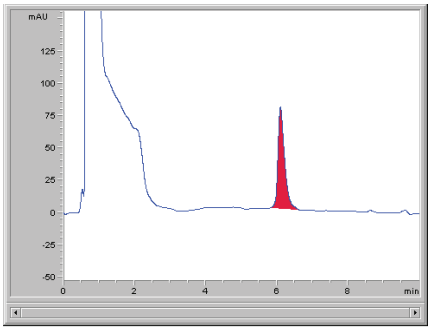

Figure 5

L-Theanine does not have significant UV absorption, but detection at a low wavelength (200 nm) was found to be suitable. With an expected sample concentration of around 10 ppm, a calibration curve in the range 2–200 ppm would be appropriate. The data showed good linearity in the studied range, with a line of best fit of y = 12.32x – 7.643 and an r^2 value of 0.99995 (see **Figure 2**).

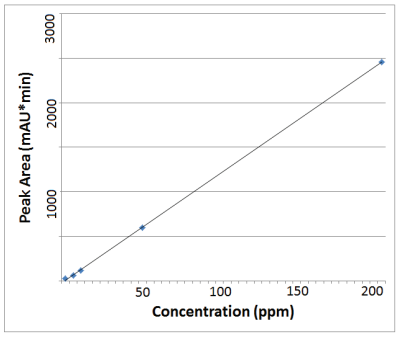

Figure 2

The chromatograms for each calibration curve standard are shown in **Figure 3**. Good peak shape and efficiency were obtained. The observed retention time could be used to assign the correct peak in the subsequent green tea injections.

Injection of the green tea sample produced an L-theanine peak which was well separated from the other UV-absorbing components in the sample. The majority of these compounds eluted near the solvent front while the highly polar L- theanine was retained by an ANP mechanism (see **Figure 4**). Correlation of the peak area with the calibration curve gave an estimate of 15.0 ppm L-theanine in the green tea sample.

The retention time of the peak matched those of the calibration curve standards. As a final confirmation of peak identity, the green tea sample was spiked with 50 ppm L- theanine. In some cases, matrix effects may cause an analyte's retention time in a sample to differ from that of a standard. A spiked solution should confirm that the correct peak was identified. The chromatogram depicting this spiked sample is shown in **Figure 5**.

The spiked sample could also be used for a standard addition estimate of concentration. Using this technique, the concentration of L-theanine in the sample was determined to be 13.5 ppm. This shows close agreement with the value of 15.0 ppm calculated using a calibration curve approach. Standard addition is considered the more accurate method since it accounts for matrix effects. This may be why the value is slightly lower than with the calibration curve method. In the sample preparation stage of the spiked tea, it is important to wait until the liquid has fully cooled to room temperature before diluting to mark. If the solution is still hot, the volume will be higher than normal, leading to too high a concentration when the flask is diluted to mark.