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Application News

No. L525

High Performance Liquid Chromatography

Analysis of Amino Acids in Green Tea Using Automatic Pretreatment Function of Integrated LC System Prominence™-i

Green tea, that has been commonly enjoyed by Japanese people since centuries ago has also attracted attention as a health food in recent years. Among the amino acids contained in green tea, the content of theanine is the largest.[1] Theanine is the main *umami* (flavor enhancing) compounds in green tea, and is also expected to have various functional effects including relieving feelings of stress, aiding sleep, etc. In addition to theanine, green tea also contains a number of other compounds that are of interest as *umami* useful compounds for health.

This article focuses on four compounds of *sencha* (middle grade green tea) and *hojicha* (roasted green tea), the *umami* compounds theanine and glutamic acid (Glu), and the health-related compounds arginine (Arg) and γ-aminobutyric acid (GABA), and introduces an analysis by fluorescence derivatization with o-phthalaldehyde (OPA) using the automatic pretreatment function of the integrated LC system Prominence™-i (hereinafter, Prominence-i).

Y. Osaka

Automatic Pretreatment Function

The Prominence-i (LC-2030C) originally equips an automatic pretreatment function using an autosampler and the three modes of "Dilution," "Addition" and "Co-Injection" are provided as templates. Here, the "Co-Injection" function will be introduced. It enables aspiration of samples from multiple vials in designated order, and also makes it possible to perform mixing and set the standing time. Fig. 1 shows the screen for setting pretreatment (co-injection) conditions, and Fig. 2 shows the sequence of operations involving the conditions set in Fig. 1. In this way, even sequential aspirations of reagents can be set easily.

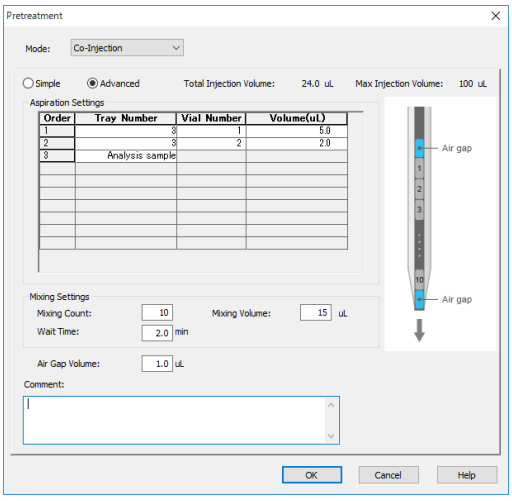

Fig. 1 Pretreatment (Co-injection) Setting Screen of Prominence-i (LC-2030C)

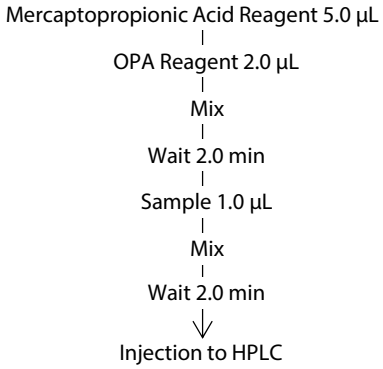

Fig. 2 Flow of Automatic Pre-column Derivatization by Prominence-i

Table 1 Reagents Used in Derivatization

● Mercaptopropionic Acid Reagent
Add 10 µL of 3 - mercaptopropionic acid into 10 mL of 0.1 mol/L borate buffer.
● OPA Reagent
Add 0.3 mL of ethanol into 10 mg of o - phthalaldehyde and dissolve completely. Then add 0.7 mL of 0.1 mol/L borate buffer and 4 mL of ultrapure water.

Extraction of Components

The astringency and fragrance of green tea change depending on the steeping temperature. High grade green tea like *gyokuro* is generally steeped at a low temperature to suppress their astringency, while hojicha (roasted green tea) is steeped at a high temperature to enjoy their fragrance. Here, 50 mL of water at 20, 40, 60, 80 or 95 °C was added to 1 g of tea leaves, that were then stirred by hand for 30 sec and allowed to steep. Then, the *sencha* was diluted 10 times and the *hojicha* was diluted 5 times with a 0.1 mol/L of hydrochloric acid.

Analysis Results

Table 2 shows the analytical conditions. Fig. 3 shows chromatograms of the components extracted from the *sencha* and *hojicha* at 95 °C.

Table 2 Analytical Conditions

Column	:	Shim-pack™ GIST C18 100 mmL. × 3.0 mmI.D., 3.0 µm
Guard column	:	10 mmL. × 3.0 mmI.D., 3.0 µm
Mobile phase	:	A) 20 mmol/L Potassium phosphate buffer (pH 6.5) B) Water/Acetonitrile/Methanol = 150/450/400
Flow rate	:	1.0 mL/min
Time program	:	B Conc. 5 % (0 min) → 40 % (7 min) → 98 % (7.5 min)
Column temp.	:	25 °C
Injection volume	:	1 µL
Detection	:	Fluorescence detector (Ex. 350 nm, Em. 450 nm)

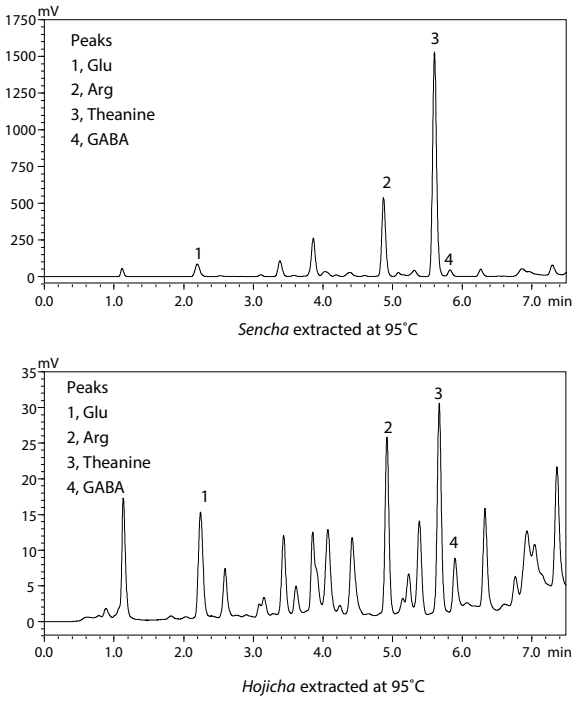

Fig. 3 Chromatograms of *Sencha* and *Hojicha*

Linearity of Calibration Curves

Table 3 shows the concentrations of the standards analyzed under the conditions in Table 2. Fig. 4 shows the calibration curves for each compound. Excellent linearity with a coefficient of determination R^2 = 0.999 or higher was obtained for all compounds.

Table 3 Linearity of Calibration Curves of Components

Compound	Calibration Point (µmol/L)
Glu/Arg/GABA	0.5, 1.0, 5.0, 10, 50
Theanine	0.5, 1.0, 5.0, 10, 50, 100

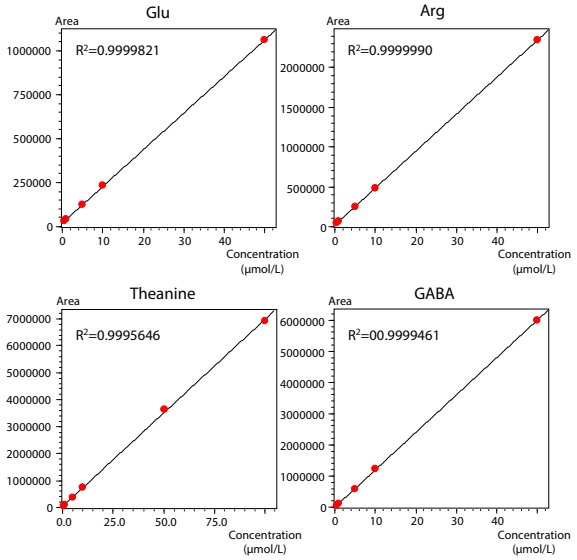

Fig. 4 Calibration Curves of Components

Quantification Results

The concentrations (µg/mL) of target compounds in *sencha* and *hojicha* were calculated based on the calibration curves. The results are shown in Fig. 5 and Fig. 6. *Sencha* is generally considered to have a strong *umami* flavor, while *hojicha* has a strong fragrance, and the results clearly confirmed that *sencha* had higher contents of the *umami* components theanine and glutamic acid.

Steeping at around 60 °C is recommended for *gyokuro* tea in order to enjoy its *umami* flavor, but at least in these results, a larger amount of theanine was extracted at 95 °C. However, as extraction of the astringent catechins increased with temperature,[(2)] it can be understood from these results that a temperature of around 60 °C is suitable for extracting the *umami* flavor while suppressing astringency.

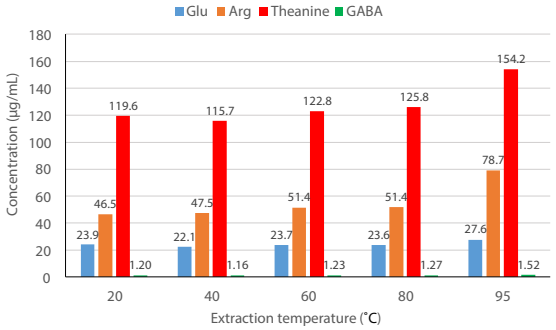

Fig. 5 Results of Extraction of Sencha at Various Temperatures

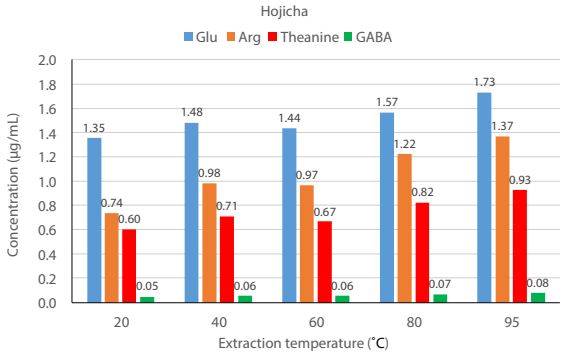

Fig. 6 Results of Extraction of Hojicha at Various Temperatures

Conclusion

As shown in this article, simple analysis of pre-column derivatized amino acids is possible using the automatic pretreatment function of Prominence-i. Since the labeling reaction is performed within the needle (sample loop), it is not necessary to prepare empty vials for the mixing. In addition, because all the derivatized samples are introduced into the column, increased sensitivity can be expected with smaller sample and reagent consumption than in the case of vial mixing.

[References]

(1) Kozo Ishigaki, Bioscience, Biotechnology, and Biochemistry, Vol. 19, No. 5 (1981), 278-285

(2) Shigemi Ikeda, Tea Research Journal, No. 37 (1972), 69-78

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