

Determination of Theanine in Tea

Theanine is one of the amino acids contained in tea, and is well known as the component providing flavor and sweetness to tea. Various studies about the effects of theanine indicate that this substance suppresses the neurological excitation caused by caffeine, and that it can benefit to lower the blood

pressure.

Theanine can be analyzed simultaneously with other amino acids by using OPA (o-phthalaldehyde) as the reaction reagent with the post-column fluorescent derivitization method. Such an analysis is introduced here, using the Shimadzu amino acid analysis system.

■ Simultaneous Determination of Theanine and other 37 Amino Acids

After separation of theanine and the other amino acids using a Li type cation replacement column, sodium hypochlorite (in order to open-ring the secondary amines such as proline) and OPA reagent are added to convert them to fluorescent compounds, and detection is performed using a fluorescence detector.

Fig. 1 shows the chromatogram of a standard solution containing theanine and other 37 amino acids. Firstly 100μL each of commercial amino acid standard

solutions (Waco Pure Chemical Industries, AN II type and B type) and 2.5μmol/mL of glutamine, L-asparagine, L-tryptophan and L-theanine solutions were pipetted, and then mobile phase solution was added to bring the total volume to 10mL. Of this, 20μL was injected for analysis. (100-times dilution of standard solution). Fig. 2 shows the structural formula for L-theanine, and Table 1 shows the analytical conditions.

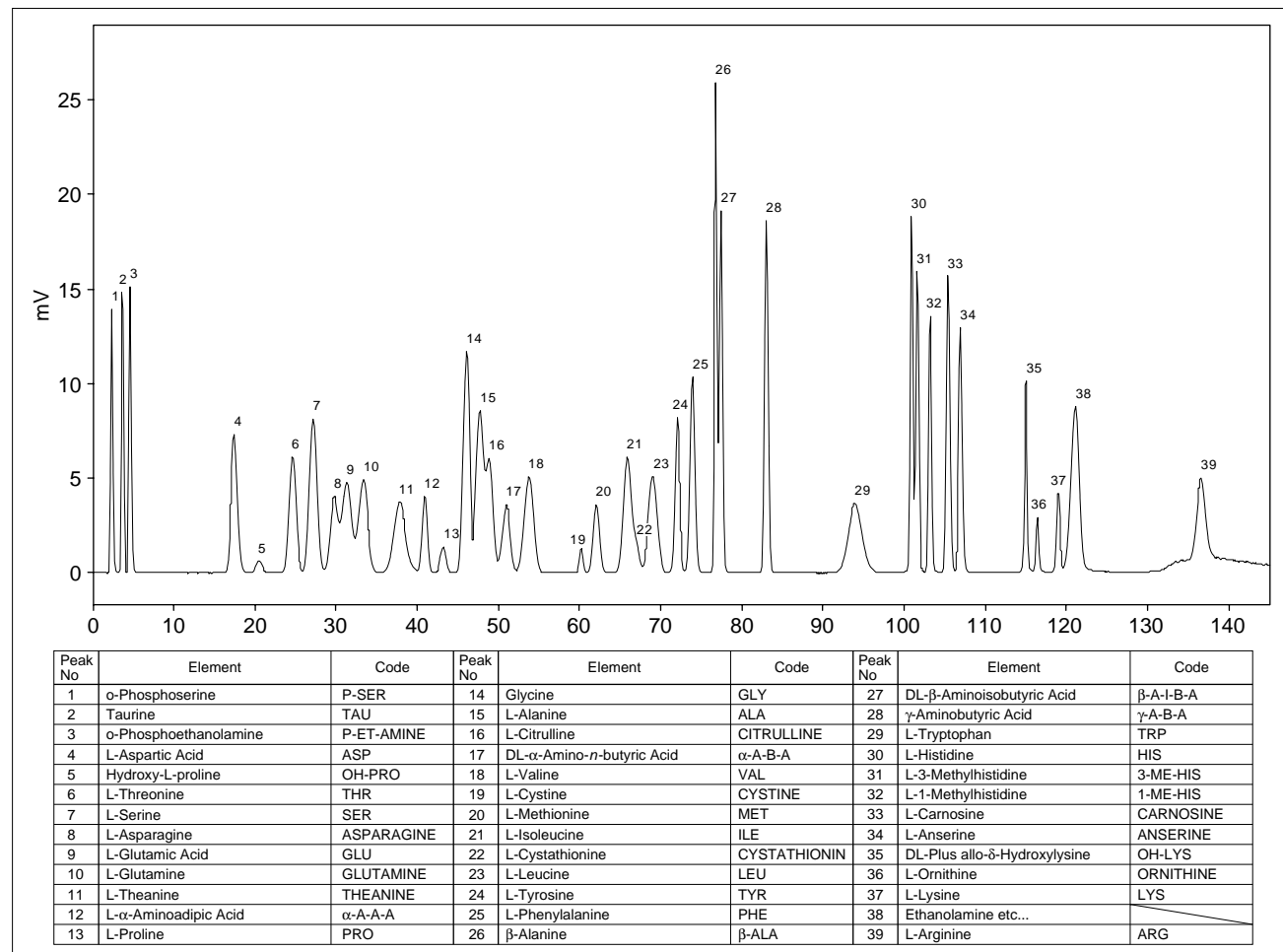
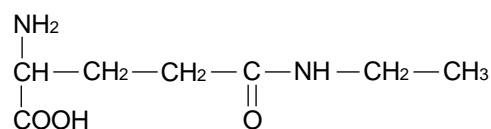


Fig.1 Chromatogram of Standard Mixture of Theanine and Other 37 Amino Acids

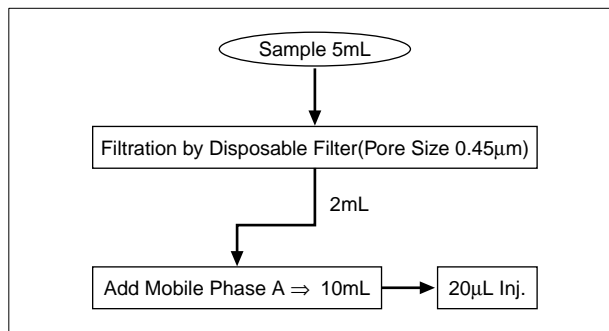
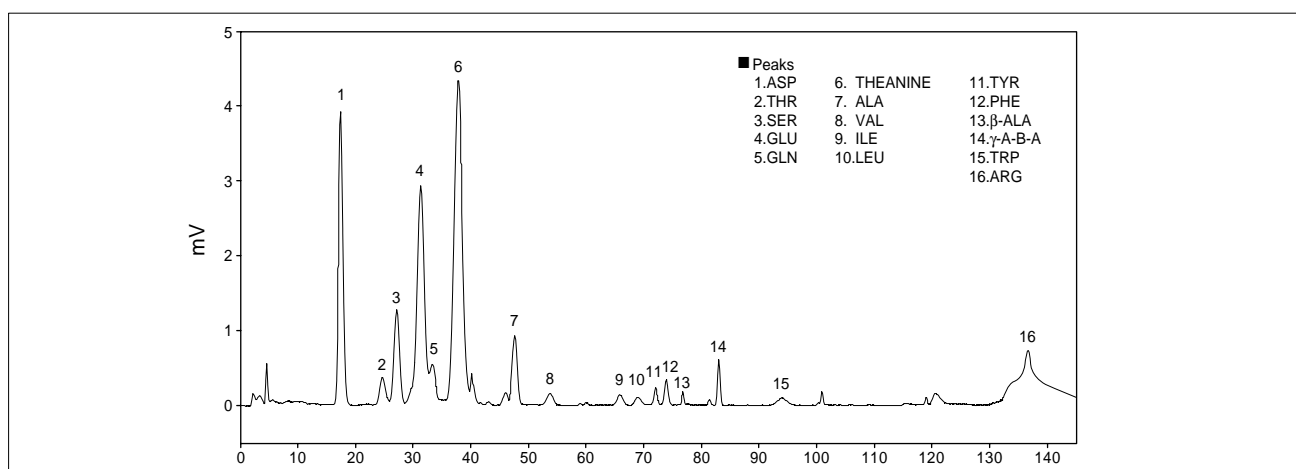
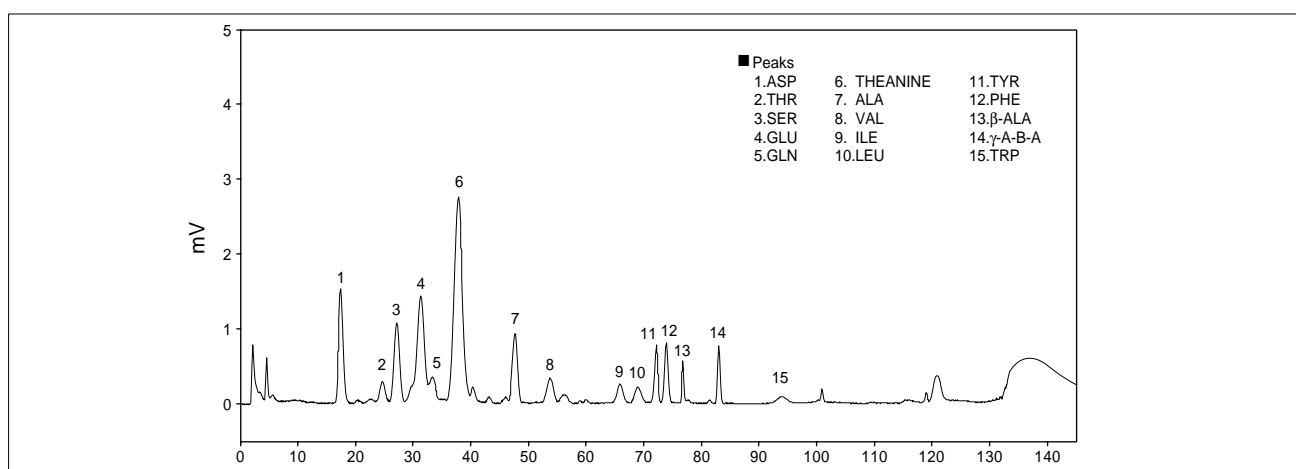
Table 1 Analytical Conditions

Column	:Shim-pack Amino-Li(100mmL.×6.0mmI.D.)
Mobile Phase	:Amino Acid Mobile Phase Kit(Li Type)
	Gradient Elution
Flow Rate of Mobile Phase	:0.6 mL/min
Column Temp.	:39°C
Reaction Reagent	:Amino Acid Reagent Kit
Flow Rate of Reagent	:0.3 mL/min
Reaction Temp.	:39°C
Detection	:RF-10A _{XL}
	Ex. at 350nm, Em. at 450nm
Injection Volume	:20μL

**Fig.2 Structure of L-Theanine**

■ Analysis of Tea Beverages

Fig. 4 and Fig. 5 show the results of the analysis of commercial green tea beverage and black tea beverage, respectively. Sample preparation was performed as shown in Fig. 3, and 20μL was injected. In addition to theanine, aspartic acid, serine, and glutamic acid, etc., were detected in both of these beverages.

**Fig.3 Sample Preparation****Fig.4 Chromatogram of Green Tea (20μL inj.)****Fig.5 Chromatogram of Black Tea (20μL inj.)**

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