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Application News

No. L544

High Performance Liquid Chromatography

Simple and Quick Analysis of Theanine in Tea by Automatic Pre-Column Derivatization Method

Theanine, which has an *umami* taste, accounts for more than half of the free amino acids in tea and is an important component that determines the flavor of tea. It is known that there is a strong correlation between theanine content and tea quality, especially for green tea. Theanine also has various physiological effects, such as relaxing effect, stress-reducing effect, premenstrual syndrome (PMS) alleviation, and so on. With the recent health food boom, theanine is getting attention day by day.

Application News L529A introduced amino acid analysis by the automatic pre-column derivatization method using the co-injection mode of the i-Series. This article introduces an example of simple and quick analysis of theanine and other major amino acids, by the same method as L529A using the Nexera™ Series.

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Simultaneous Analysis of Amino Acids

In the Nexera Series, an automatic pretreatment function is provided in the autosampler, and its co-injection mode was used here. Primary amino acids were derivatized into fluorescent substances in the needle by using *o*-phtalaldehyde (OPA) to analyze. Fig. 1 shows the setting of the co-injection mode.

Fig. 2 shows the chromatogram of an amino acid mixed standard solution that contains theanine and 19 proteinogenic amino acids except proline. All 20 amino acids were separated within 15 min. The analytical conditions are shown in Table 1 to Table 4.

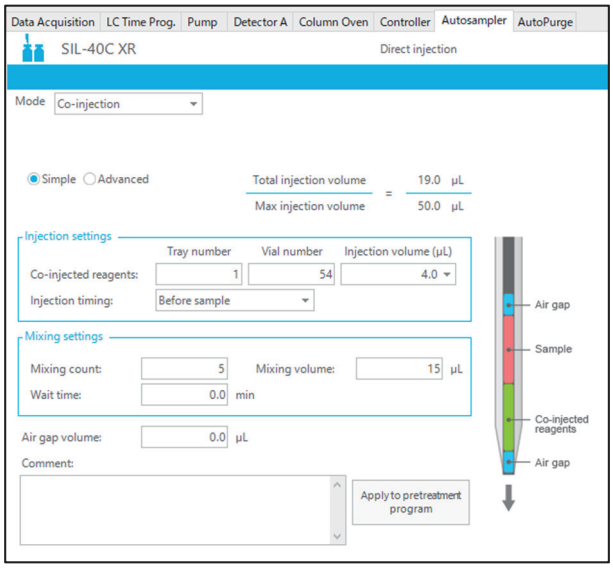


Fig. 1 Pretreatment (Co-Injection) Setting Screen of Nexera Series

Table 1 Mobile Phase Preparation Method

- 20 mmol/L (sodium) acetate buffer (pH6)
  Add 2.67 g of sodium acetate trihydrate and 41 µL of acetic acid into 1 L of ultrapure water.
- 20 mmol/L (sodium) acetate buffer (pH5) containing 0.5 mmol/L EDTA-2Na
  Add 0.19 g of EDTA-2Na, 2.03 g of sodium acetate trihydrate, and 308 µL of acetic acid into 1 L of ultrapure water.

Table 2 Derivatizing Reagent Preparation Method

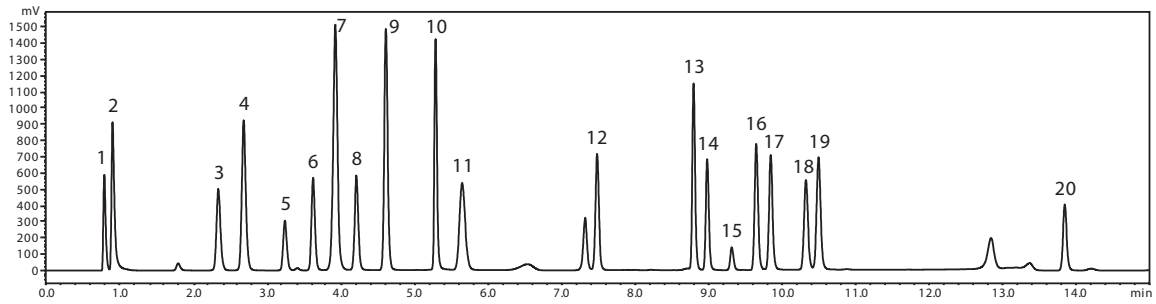
- 0.1 mol/L borate buffer (pH9)
  Add 0.62 g of boric acid and 0.20 g of sodium hydroxide into 100 mL of ultrapure water.
- MPA Reagent
  Add 10 µL of 3-mercaptopropionic acid into 10 mL of 0.1 mol/L borate buffer.
- OPA Regent
  Add 0.3 mL of ethanol into 10 mg of *o*-phthalaldehyde and dissolve completely. Then add 0.7 mL of 0.1 mol/L borate buffer and 4 mL of ultrapure water.
- MPA / OPA Solution
  Mix equal volume of MPA regent and OPA regent.

Table 3 Analytical Conditions

Column	: Shim-pack™ XR-ODS II 100 mmL.×3 mmI.D., 2.2 µm
Mode	: Low pressure gradient
Mobile phase	: A) 20 mmol/L (sodium) acetate buffer (pH6) B) Water / Acetonitrile = 1 / 9 C) 20 mmol/L (sodium) acetate buffer (pH5) containing 0.5 mmol/L EDTA-2Na
Flow rate	: 1.0 mL/min
Column temp.	: 40 °C
Injection volume	: 1 µL
Detection	: Fluorescence detector Ex. 350 nm, Em. 450 nm

Table 4 Time Program

Time (min)	A.conc	B.conc	C.conc
0	95	5	0
0.2	93	7	0
1	93	7	0
4	87	13	0
5	0	15	85
7.5	0	30	70
12	0	35	65
14	0	45	55
14.01	0	95	5
17	0	95	5
17.01	95	5	0
19.5	95	5	0



1, Asp 2, Glu 3, Asn 4, Ser 5, Gln 6, His 7, Gly 8, Thr 9, Arg 10, Ala 11, Theanine 12, Tyr 13, Met 14, Val 15, Cys2 16, Trp 17, Phe 18, Ile 19, Leu 20, Lys

Fig. 2 Chromatogram of Amino Acid Mixed Standard Solution (25 µmol/L Each)

Linearity and Repeatability

Calibration curves for theanine, aspartic acid, glutamic acid, serine, glutamine, and arginine, which are the major amino acids in tea, were prepared from the chromatogram of the amino acid mixed standard solution. Calibration curves for each of these compounds were prepared in the concentration ranges of 0.25, 1.25, 2.5, 12.5 and 25 µmol/L, and their linearity (R^2) was evaluated. Area repeatability was also evaluated by a repeated analysis (n=6) for 12.5 µmol/L. Table 5 shows these results.

Table 5 Linearity of Calibration Curves and Repeatability of Each Amino Acid

Amino acids	Linearity (R^2)	Repeatability (%RSD)
Theanine	0.9999	0.37
Aspartic acid	0.9998	0.61
Glutamic acid	0.9997	0.55
Serine	0.9998	1.26
Glutamine	0.9999	0.59
Arginine	0.9999	0.72

Example of Analysis of Teas

Each of the tea-leaves was extracted by the general methods to prepare analytical samples except green tea (PET bottle). These samples were diluted 50 times with ultrapure water, filtered with a 0.22 µm membrane filter, and then used in the analysis.

Fig. 3 shows the quantitative analysis results for the amino acids in the 5 types of tea, and Fig. 4 shows the chromatograms of teas. Judging from these data, the content of amino acids varies in accordance with the type of tea.

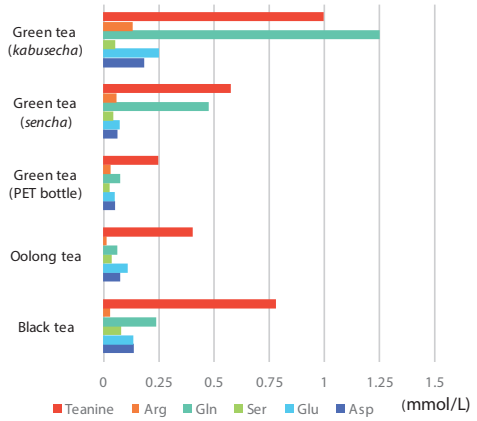


Fig. 3 Contents of Amino Acids in Teas

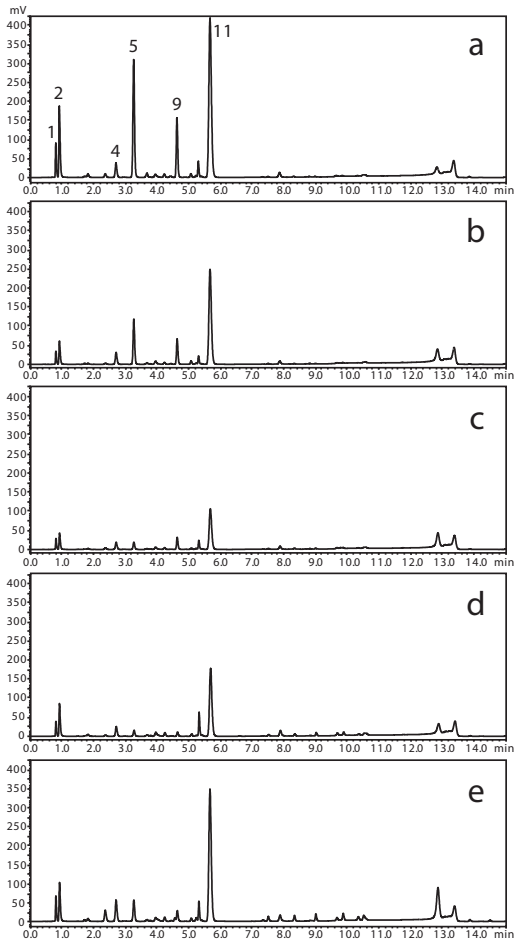


Fig. 4 Chromatograms of Teas
a: Green Tea (*kabusecha*), b: Green Tea (*sencha*), c: Green Tea (PET bottle), d: Oolong Tea, e: Black Tea

Conclusion

In this analysis, derivatization of amino acids was performed in the needle by using the co-injection mode installed in the Nexera Series as a standard feature. As a result, troublesome pretreatment operation and a complex equipment configuration are not necessary. Thus, it has become possible to perform simple and quick analyses of amino acids.

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