

# Application News

## No. L543

### High Performance Liquid Chromatography

## High-Speed Analysis of Methylated Catechin in Benifuuki Green Tea

It has been reported that the functional food Benifuuki green tea contains a large amount of methylated catechin as an antiallergic functional ingredient. <sup>(1)</sup> Methylated catechin is a kind of polyphenol which is contained in tea leaves, and is a partially methylated form of epigallocatechin gallate. It has also been reported that methylated catechin alleviates the discomfort of the eyes and nose caused by house dust and other forms of dust.

In 2018, "Determination of the O-methylated Catechin in 'Benifuuki' Green Tea (*Camellia sinensis* L.) - High-performance liquid chromatographic method" <sup>(2)</sup> was established as a Japanese Agricultural Standard (JAS) based on Japan's Act on Standardization and Proper Quality Labeling of Agricultural and Forestry Products (JAS Law).

This article introduces applications of analysis of methylated catechin in Benifuuki green tea leaves based on the JAS method and its high-speed analysis, using a Nexera™ X3 and Shim-pack™ GIST Series columns.

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### ■ Analysis of Methylated Catechin Standard Solution

A methylated catechin ((-) - epigallocatechin 3-(3"-O-methyl) gallate (EGCG3"Me)) standard solution (10 mg/L) was analyzed. Tables 1 and 2 show the analytical conditions for the JAS method and the high-speed analysis, respectively. The upper part of Fig. 1 shows the chromatogram using a Shim-pack GIST C18 analytical column (particle size: 5 μm) according to the JAS method, and the bottom portion shows that using a Shim-pack GIST-HP C18 column (particle size: 3 μm) for high-speed analysis in the same Shim-pack Series.

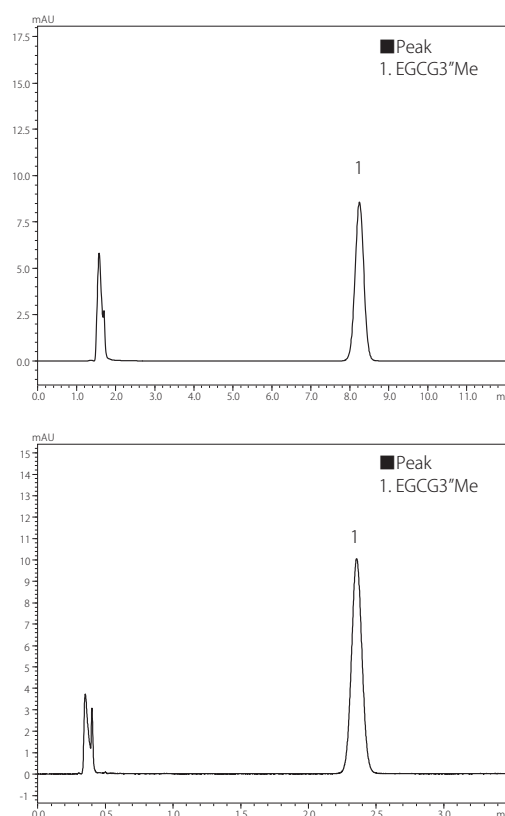
Analysis time and mobile phase consumption could be reduced to approximately 1/4 respectively using GIST-HP C18 column with the particle size of 3 μm.

**Table 1 Analytical Conditions of JAS Method-Compliant**

System	: Nexera X3
Column 1	: Shim-pack GIST C18 (150 mm L×4.6 mm I.D., 5 μm)
Flow rate	: 1.0 mL/min
Mobile phase	: A) 0.2% Phosphoric acid in water B) Methanol/Acetonitrile=18/5 (v/v)
Time Program 1	: 23%B (0-12 min) →70%B (12.01-20 min) → 23%B (20.01-30 min)
Column temp.	: 40 °C
Injection volume	: 10 μL
Detection	: UV 272 nm

**Table 2 Analytical Conditions of High-Speed Analysis**

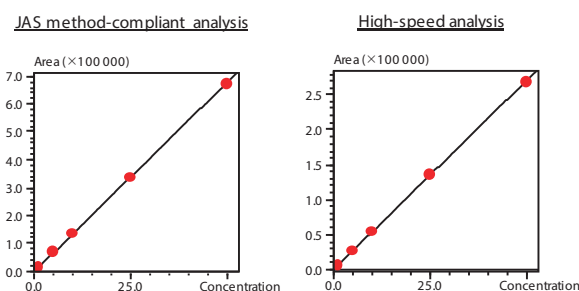
System	: Nexera X3
Column	: Shim-pack GIST-HP C18 (75 mm L×3.0 mm I.D., 3 μm)
Flow rate	: 1.0 mL/min
Mobile phase	: A) 0.2% Phosphoric acid in water B) Methanol/Acetonitrile=18/5 (v/v)
Time Program	: 23%B (0-3 min) →70%B (3.01-5 min) → 23%B (5.01-7.5 min)
Column temp.	: 40 °C
Injection volume	: 4 μL
Detection	: UV 272 nm



**Fig. 1 Chromatograms of Methylated Catechin Standard Solution**  
Upper: JAS Method-Compliant Analysis,  
Bottom: High-Speed Analysis

### ■ Calibration Curves

Fig. 2 shows the calibration curves for 1 to 50 mg/L in the respective analyses. Satisfactory linearity was obtained in both cases, with the contribution ratio  $R^2 = 0.99999$  or more.



**Fig. 2 Calibration Curves**  
Left: JAS Method-Compliant Analysis,  
Right: High-Speed Analysis

### Reproducibility

Table 3 shows the coefficients of variation (%RSD) for retention time and area in analyses repeated six times using 1 mg/L of the standard solution, which is the lowest concentration of the calibration curve. In both analyses, coefficients of variation of 1% or less were obtained for both retention time and area.

**Table 3 Coefficients of Variation in Analyses Repeated Six Times (%RSD)**

Analytical condition	Column	Retention time	Area
JAS method-compliant	Shim-pack GIST C18 (particle size: 5 µm)	0.05	0.79
High-speed analysis	Shim-pack GIST-HP C18 (particle size: 3 µm)	0.02	0.53

### Analysis of Benifuuki Green Tea

The test specimen was a commercially-available Benifuuki green tea. Pretreatment was carried out referring to the JAS method.<sup>(2),(3)</sup>

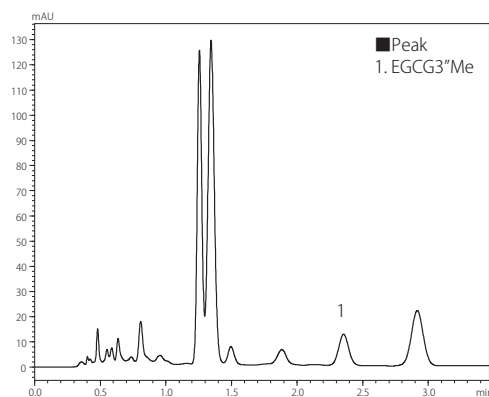
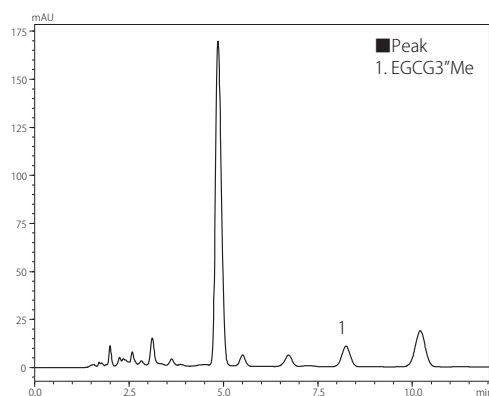
Fig. 3 shows the pretreatment protocol. Extraction from the powdered specimen was performed using a mixed solution of phosphoric acid and ethanol at 30 °C. Next, extracts were filtrated with a filter paper and a 0.2 µm membrane filter, and diluted 10 times with water prior to HPLC injection.

Fig. 4 shows the chromatograms of the Benifuuki green tea. The upper part of Fig. 4 shows the chromatogram using a Shim-pack GIST C18 analytical column (particle size: 5 µm) according to the JAS method, and the bottom portion shows that using a Shim-pack GIST-HP C18 column (particle size: 3 µm) for high-speed analysis in the same series. Methylated catechin was separated from the other compounds in both analyses.

The contents of methylated catechin were calculated from the quantitative values. As a result, the specimen contained 12 g/kg of methylated catechin in both the JAS method-compliant analysis and the high-speed analysis.

Weigh 250 mg of Benifuuki green tea (homogenized) in 25 mL of volumetric flask
Add 20 mL of phosphoric acid/ ethanol/ water (1:50:49), then stir
Stand in a water bath (60 min, 30 °C)
Allow to stand to R.T.
Add water to make 25 mL
Swing roll mixing
Filtrate with filter paper (discard initial flow)
Filtrate with membrane filter (discard initial flow)
Dilute ten times with water
HPLC

**Fig. 3 Pretreatment Protocol**



**Fig. 4 Chromatograms of Benifuuki Green Tea  
Upper: JAS Method-Compliant Analysis,  
Bottom: High-Speed Analysis**

### Conclusion

Analyses of methylated catechin in Benifuuki green tea were carried out using the Nexera Series and Shim-pack GIST C18 columns. Using a high-speed analytical column in the same series, it was possible to reduce analysis time and mobile phase consumption to approximately 1/4 respectively while maintaining the same separation performance.

In both of the analyses described above, the Benifuuki green tea used in this experiment was found to contain 12 g/kg of methylated catechin.

#### [References]

- (1) Mari Maeda-Yamamoto, Mitsuaki Sano, Nahomi Matsuda, Toshio Miyase, Keiko Kawamoto, Naoko Suzuki, Masayasu Yoshimura, Hirofumi Tachibana and Katsuhiko Hakamata, The Change of Epigallocatechin-3-O-(3-O-methyl) gallate Content in Tea of Different Varieties, Tea Seasons of Crop and Processing Method, Nippon Shokuhin Kagaku Kogaku Kaishi (published by Japanese Society for Food Science and Technology), 48, 64-68 (2001).
- (2) Japanese Agricultural Standard, Determination of the O-methylated Catechin in 'Benifuuki' Green Tea (*Camellia sinensis* L.) - High-performance liquid chromatographic method (JAS 0002), Ministry of Agriculture, Forestry and Fisheries (March 29, 2018).
- (3) Yuji Homura, Yusuke Hiejima, Takashi Kodama, Masumi Tanaka, Hideki Horie, Tadanao Suzuki and Akemi Yasui, Validation of Method for Determining O-methylated Catechin in 'Benifuuki' Green Tea (*Camellia sinensis* L.) by Interlaboratory Study, Nippon Shokuhin Kagaku Kogaku Kaishi, 63(7), 312-318 (2016).

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