

Quantitative Analysis of Catechins in Tea Leaves

In collaboration with the National Agriculture and Food Research Organization,



Shimadzu Corporation has been developing a simple, quick and accurate method of analyzing functional components in agricultural and food products.

This report introduces a quantitative method for catechins analysis in tea leaves and presents the results obtained in two kinds of them. Catechins, a kind of polyphenols, are classified into flavanols which are a group of flavonoid compounds. There are four main green tea catechins: epigallocatechin gallate, epigallocatechin, epicatechin gallate and epicatechin. In this report, the catechins shown in Table 1, including these green tea ones, were analyzed.

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Table 1 Target Compounds

| Compound | Abbreviation |
|---|--------------|
| Catechin | C |
| Epicatechin | EC |
| Gallocatechin | GC |
| Epigallocatechin | EGC |
| Catechin gallate | CG |
| Epicatechin gallate | ECG |
| Gallocatechin gallate | GCG |
| Epigallocatechin gallate | EGCG |
| Epicatechin 3-O-(3"-O-methyl)gallate | ECG3"Me |
| Epigallocatechin 3-O-(3"-O-methyl)gallate | EGCG3"Me |
| Caffeine | — |

Sample Pretreatment

The extraction was performed in the reference of methods for lutein analysis by Japanese Agricultural Standards (JAS) 1), 3). The workflow is shown in Fig. 1. The extract obtained from crushed tea leaves using 0.2% phosphoric acid aqueous solution, was then diluted 10 times in water to obtain the analysis sample.

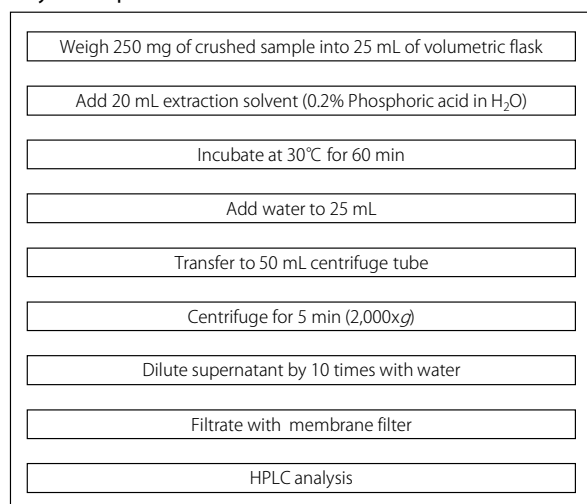


Fig. 1 Pretreatment Workflow

Analytical Conditions

The analytical conditions were determined in the reference of methods specified by JAS^{1), 2), 3)}. The analytical conditions are shown in Table 2.

Table 2 Analytical Conditions

| | |
|------------------|--|
| System | : Nexera™ X3 |
| Column | : Shim-pack™ GIST C18 (150 mm × 4.6 mm I.D., 3 μm P/N : 227-30011-07) |
| Mobile phases | : A) 0.2% Phosphoric acid in H ₂ O B) MeOH/Acetonitrile=15 : 5 (v/v) |
| Gradient Program | : B conc. 20% (0-10 min) - 35% (12.5-20 min) - 70% (20.01-25 min) - 20% (25.01-30 min) |
| Flow rate | : 0.8 mL/min |
| Column Temp. | : 40 °C |
| Injection volume | : 10 μL |
| Detection | : PDA 242 nm (GC, EGC), 272 nm (others) |

Analysis Results of Standards

The linearities were determined by the standards analysis. Fig. 2 shows the calibration curves and Fig. 3 shows representative chromatograms. Table 3 shows the dynamic range and the coefficients of determination. Good linearities were obtained with a coefficient of determination (R²) ≥ 0.999 for all compounds.

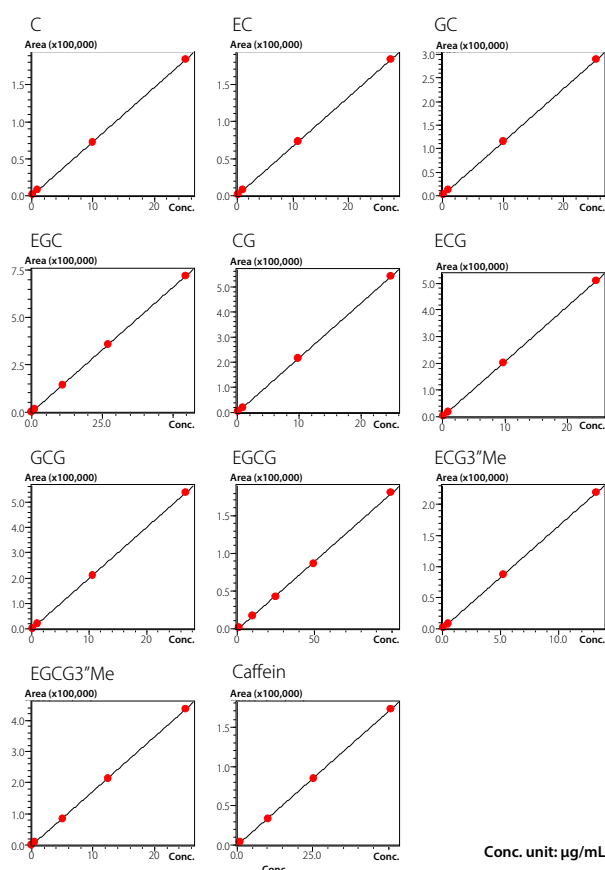


Fig. 2 Calibration Curves

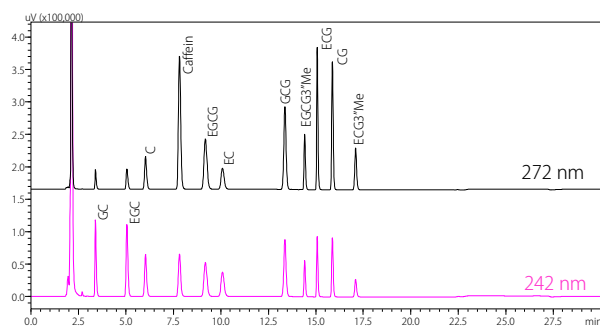


Fig. 3 Chromatograms of Standard Samples

Table 3 Linear range and Coefficient of determination (R²)

| Compound | Linear range (µg/mL) | Coefficient of determination (R ²) |
|----------|----------------------|--|
| C | 0.100 - 25.05 | 0.9999 |
| EC | 0.110 - 27.5 | 0.9999 |
| GC | 0.101 - 25.3 | 0.9999 |
| EGC | 0.109 - 54.5 | 0.9999 |
| CG | 0.0990 - 24.75 | 0.9999 |
| ECG | 0.0982 - 24.55 | 0.9999 |
| GCG | 0.107 - 26.75 | 0.9999 |
| EGCG | 0.992 - 99.2 | 0.9993 |
| ECG3*Me | 0.053 - 13.25 | 0.9999 |
| EGCG3*Me | 0.050 - 25 | 0.9998 |
| Caffein | 1.012 - 50.6 | 0.9999 |

Repeatability Test Results of Tea Leaf Extracts

Seven extracts were prepared from two kinds of tea (Yabukita, Benifuuki) and repeatability test was performed to confirm validity. Table 4 shows the results.

Table 4 Repeatability Test Results (n=7)

| Compound | Repeatability (%RSD) | |
|----------|----------------------|-----------|
| | Yabukita | Benifuuki |
| C | 1.30 | 1.15 |
| EC | 0.89 | 1.21 |
| GC | 1.19 | 1.21 |
| EGC | 0.82 | 0.87 |
| CG | < LLOQ | < LLOQ |
| ECG | 1.01 | 1.19 |
| GCG | < LLOQ | < LLOQ |
| EGCG | 0.98 | 1.15 |
| ECG3*Me | < LLOQ | 1.90 |
| EGCG3*Me | < LLOQ | 1.45 |
| Caffein | 0.83 | 1.12 |

Quantitative Results for Tea Leaves

The extracts of two kinds of tea (Yabukita, Benifuuki) were analyzed to determine the content of catechins. Fig. 4 shows the chromatograms and Table 5 shows the calculated content of each catechin in tea leaves.

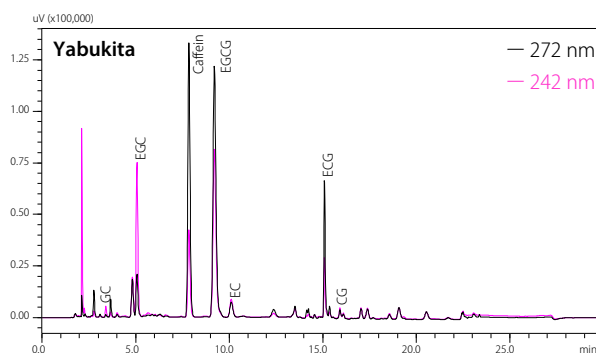


Fig. 4 Chromatograms of Tea Leaf Extracts

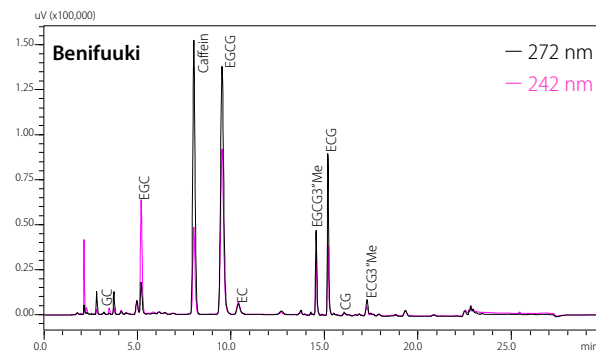


Table 5 Catechin Content in Tea Leaves

| Component | Content (g/100g) | |
|-----------|------------------|-----------|
| | Yabukita | Benifuuki |
| C | 0.03 | 0.13 |
| EC | 1.26 | 1.12 |
| GC | 0.25 | 0.16 |
| EGC | 3.69 | 3.21 |
| CG | < LLOQ | < LLOQ |
| ECG | 1.62 | 2.15 |
| GCG | < LLOQ | < LLOQ |
| EGCG | 7.70 | 8.83 |
| ECG3*Me | < LLOQ | 0.44 |
| EGCG3*Me | < LLOQ | 1.39 |
| Caffein | 3.30 | 3.85 |

Conclusion

- Using Nexera series, simultaneous analysis of 11 catechins was performed.
- The catechins quantification results show a difference in content depending on the kind of tea leaves.

<References>

- Japanese Agricultural Standards. Determination of the O-methylated Catechin in 'Benifuuki' Green Tea (*Camellia sinensis* L.) — High-performance liquid chromatographic method (JAS 0002)
- Mari Maeda-Yamamoto. Analytical Method of Green Tea Catechins (including Isomer Catechins), Food Functionality Evaluation Manual (IV) (<http://fmric.or.jp/ffd/kinousei-hyoka4.html>)
- Hideki Horie, Mari Maeda-Yamamoto, Tomomi Ujihara and Katsunori Kohata. Extraction of Tea Catechins for Chemical Analysis. Tea Research Journal. 94, 60-64 (2002)

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