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Application News

No. L583A

High Performance Liquid Chromatography

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.

M. Kawashima

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 a mixed solution of phosphoric acid and ethanol, was then diluted 10 times in water to obtain the analysis sample.

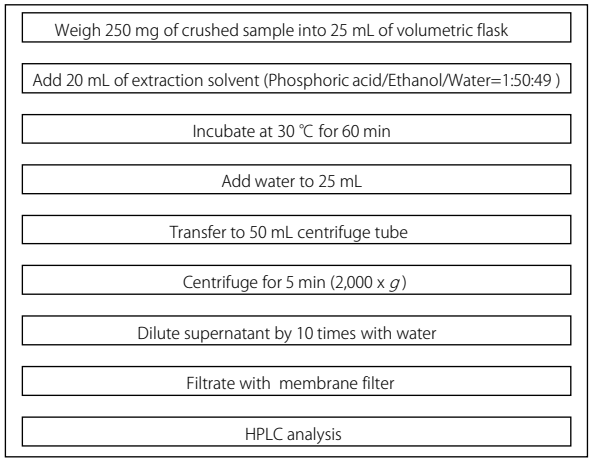


Fig. 1 Pretreatment Workflow

Analytical Conditions

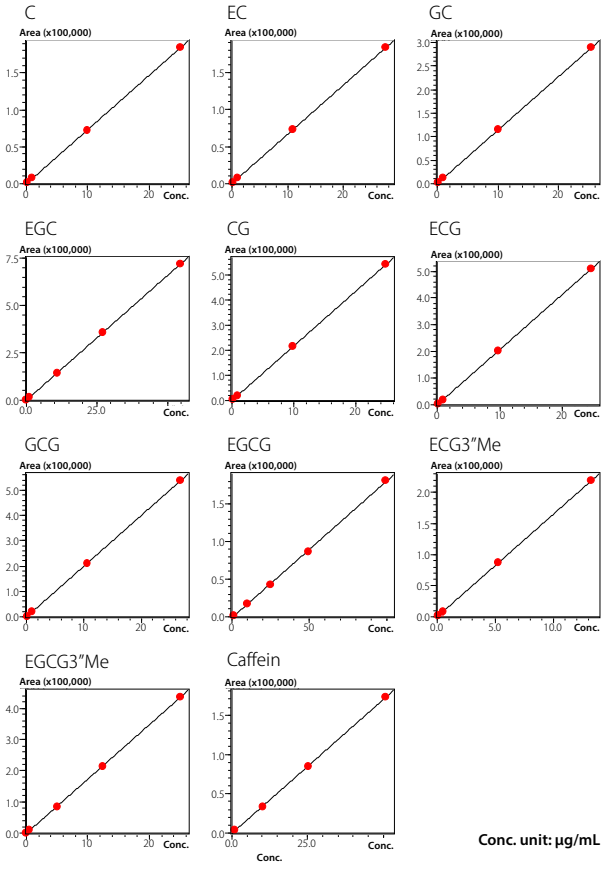
The analytical conditions were determined in the reference of methods specified by JAS[1), 2), 3)]. The analytical conditions are shown in Table 2.

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 H2O 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 (R^2) ≥ 0.999 for all compounds.



Conc. unit: µg/mL

Fig. 2 Calibration Curves

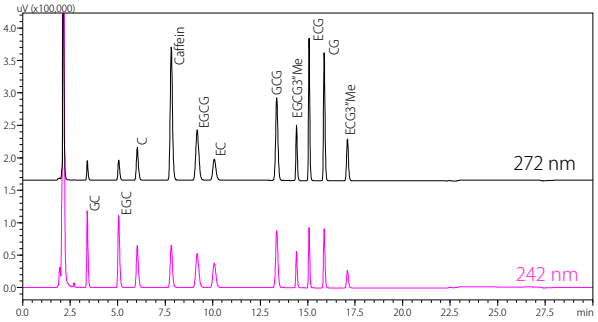


Fig. 3 Chromatograms of Standard Samples

Table 3 Linear range and Coefficient of determination (R^2)

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.

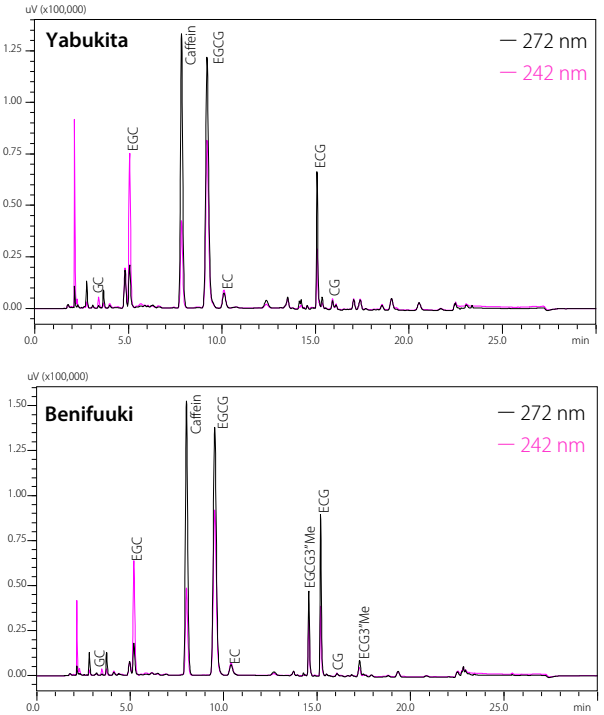


Fig. 4 Chromatograms of Tea Leaf Extracts

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>
1) Japanese Agricultural Standards. Determination of the O-methylated Catechin in 'Benifuuki' Green Tea (Camellia sinensis L.) — High-performance liquid chromatographic method (JAS 0002)
2) 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)
3) 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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