

# Technical Report

## Analysis of Tea Beverages Using Catechin Analysis Kit

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### Abstract:

The catechin analysis kit, developed in collaboration with the National Agriculture and Food Research Organization (NARO), provides validated test methods for catechins in tea leaves and green tea drinks from sample pre-treatment to HPLC measurement. You can accurately quantitate the content of 11 catechins (including methylated catechins) and caffeine in tea leaves. This article summarizes the results of the catechins quantitation in several beverages: green tea, black tea, and oolong tea.

**Keywords:** Catechin, Methylated Catechin, Tea Leaves, Tea Beverages, Catechin Analysis Kit, Nexera™ XR

### 1. Catechin Analysis Kit



NARO and Shimadzu are researching simple and accurate analytical methods for the analysis and quantitation of functional ingredients in foods and ensuring their public acceptance. The catechin analysis kit was developed in collaboration with NARO and provides validated test methods for catechins in green tea, from sample pre-treatment to measurement by HPLC. The kit includes an HPLC column, an instruction manual that describes a series of steps from sample pre-treatment to HPLC measurement, an LC method file, and a report template. It enables users to quickly start an analysis, regardless of their experience with catechin analysis. In this article, we present examples of the application of the catechin analysis kit to various tea beverages.

### 2. Analysis of a Standard Sample

The kit can analyze 11 catechins, including 3 methylated catechins and caffeine, for a total of 12 compounds. Fig. 1 shows the chromatogram of standard sample. All 12 compounds were separated in about 24 minutes. The analytical conditions are shown in Table 1. Calibration curves for each compound were prepared within the ranges shown in Table 2, and all the calibration curves gave good linearity with a contribution ratio ( $r^2$ ) of 0.9999 or more.

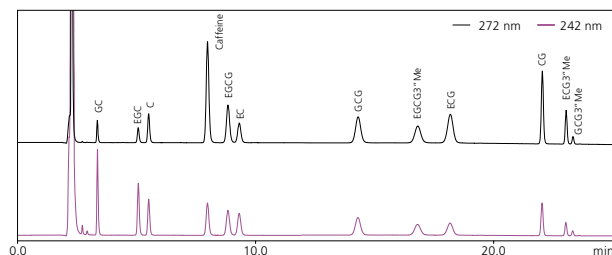


Fig. 1 Chromatogram of a Standard Sample

Table 1 Analytical Conditions

System	: Nexera XR
Column	: Shim-pack™ GISS C18 (150 mm x 4.6 mm I.D., 3 μm)
Mobile phase	: A) 0.2% Phosphoric acid in H <sub>2</sub> O B) Methanol
Gradient	: 16%B (0-15 min) – 45%B (26 min) – 90%B (26.01-30 min) – 16%B (30.01-35 min)
Mixer	: MR180 μL II
Flow rate	: 0.85 mL/min
Column temp.	: 55 °C
Injection vol.	: 10 μL
Detection	: PDA 242 nm (GC, EGC), 272 nm (others)

Table 2 Range of Calibration Curve and its Contribution Ratio ( $r^2$ )

Compound	Range (mg/L)	Contribution Ratio ( $r^2$ )
GC	0.5-19.6	> 0.9999
EGC	0.5-19.3	> 0.9999
C	0.5-9.8	> 0.9999
EGCG	0.9-18.8	> 0.9999
EC	0.5-10.8	> 0.9999
GCG	0.9-18.1	> 0.9999
EGCG3*Me	0.4-8.3	> 0.9999
ECG	0.5-9.8	> 0.9999
CG	0.4-8.8	> 0.9999
ECG3*Me	0.2-4.2	> 0.9999
GCG3*Me	0.2-2.1	> 0.9999
Caffeine	10.0-100.2	> 0.9999

### 3. Sample Pre-treatment Protocol

Commercially available green, black, and oolong tea were pretreated according to the procedure in Fig. 2 and subjected to HPLC analysis. However, the catechins may be adsorbed depending on the material of the membrane or the housing of the membrane filters, so prior verification of the recovery is required to evaluate the inertness of the filters. The TORAST™ Disc \* (hydrophilic PRFE) employed for the current protocol was confirmed to be unaffected and remained fully inert after conditioning the filter with 0.5 mL of the sample. An aqueous solution containing ascorbic acid and EDTA was used as the diluent to prevent sample degradation.

\*P/N: GLCTD-HPTFE1345

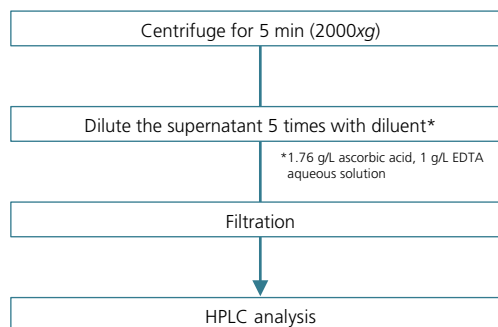


Fig. 2 Pre-treatment Protocol

## 4. Analysis of Samples

Chromatograms of green, black, and oolong tea drinks are shown in Fig. 3, and the quantitative results are shown in Table 3. Although none of the samples contained methylated catechins, green tea contained more catechins than black and oolong teas. It is inferred that the catechins in black tea and oolong tea were converted into theaflavins or theasinensins during fermentation. In addition, non-epi-form catechins, which are rarely found in tea leaves, were detected in all tea varieties. It is thought that epi-form catechins were isomerized to non-epi-form during heat sterilization.<sup>1)</sup> In this way, this kit enables a variety of scientific insights into catechins.

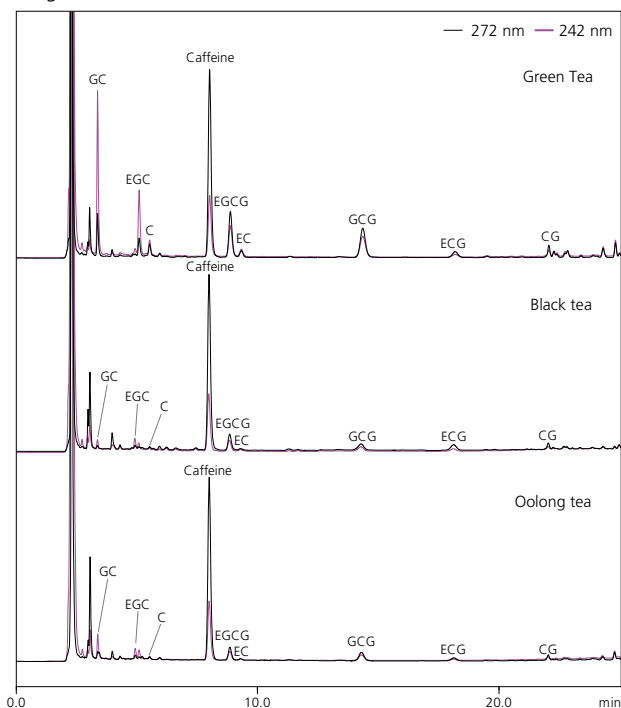


Fig. 3 Chromatograms of Various Teas

Table 3 Catechins Content of Various Teas (mg/L)

Compound	Green Tea	Black Tea	Oolong Tea
GC	87.9	5.0	15.5
EGC	40.7	6.2	9.8
C	25.8	3.5	4.1
EGCG	43.1	21.5	17.0
EC	9.5	4.9	3.2
GCG	44.5	13.2	14.8
EGCG3"Me	N.D.	N.D.	N.D.
ECG	6.6	10.4	4.5
CG	9.1	4.4	3.2
ECG3"Me	N.D.	N.D.	N.D.
GCG3"Me	N.D.	N.D.	N.D.
Total catechins	267.2	69.1	72.1
Caffeine	88.3	98.6	102.1

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## 5. Evaluation of Repeatability and Recovery Rate

For green, black, and oolong teas, we evaluated the repeatability ( $n = 6$ ) and recovery rate at 5 mg/L (ECG3 "Me and GCG" 3Me spiked 2.5 mg/L and 0.5 mg/L, respectively.). The maximum repeatability (% RSD) was 1.60 % for all samples (Table 4). The recovery rate of all samples ranged from 87.7 % to 107.2 % (Table 5). These results confirmed that this kit can be used to quantitate catechins from various tea species.

Table 4 Repeatability of Various Tea Analyses ( $n = 6$ ) (% RSD)

Compound	Green Tea	Black Tea	Oolong Tea
GC	0.07	0.27	0.28
EGC	0.93	0.63	0.45
C	0.41	0.53	0.67
EGCG	0.23	0.10	0.74
EC	0.48	1.53	1.60
GCG	0.12	0.52	0.41
EGCG3"Me	N.D.	N.D.	N.D.
ECG	0.27	0.80	0.96
CG	0.26	1.09	0.32
ECG3"Me	N.D.	N.D.	N.D.
GCG3"Me	N.D.	N.D.	N.D.
Caffeine	0.19	0.05	0.05

Table 5 Recovery Rate (%) of Catechin-Spiked Samples (5 mg/L) ( $n=3$ )

Compound	Green Tea	Black Tea	Oolong Tea
GC	104.3	99.3	98.5
EGC	101.0	105.5	101.0
C	102.0	99.7	98.6
EGCG	104.2	97.3	97.2
EC	99.2	99.1	96.5
GCG	104.7	100.2	97.1
EGCG3"Me	96.3	98.0	96.2
ECG	99.8	96.8	96.1
CG	99.4	98.8	107.2
ECG3"Me (2.5 mg/L spiked)	98.1	99.5	87.7
GCG3"Me (0.5 mg/L spiked)	99.0	103.6	106.6
Caffeine	103.2	92.6	92.0

## 6. Summary

This article has shown examples of applying the catechin analysis kit to various tea drinks. This kit can measure catechins in not only green tea but also black and oolong teas. In addition, the method files included with this kit are pre-configured to optimize peak integration and use UV spectrum similarity to prevent misidentification, thus saving analysis time. Using this catechin analysis kit, tea drinks can be easily analyzed regardless of the user's skill level.

### < References >

- 1) Mitsuaki Sano, Function of tea catechins and structural changes during cooking, Journal of the Japanese Society of Cooking, Vol. 40, No. 4, 223 to 230 (2007)