Short communication

Simultaneous analysis of individual catechins and caffeine in green tea

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Abstract

A simple and fast high performance liquid chromatography analysis method for eight tea catechins and caffeine using an ODS column and a water–acetonitrile–phosphoric acid mobile phase system was developed. The catechins, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, catechin, catechin gallate, gallocatechin and gallocatechin gallate, and caffeine were separated by an acetonitrile gradient within 20 min. The detection limit of this method was approximately 0.2 ng for all nine compounds and the quantitation curves were linear between 2 ng to 2 μg. Some Japanese green tea samples were analyzed using this method. No extraneous peaks interfered with the analysis and the detection limit of each compound was less than 0.02% of the dry weight of the tea.

1. Introduction

Catechins are a group of polyphenol compounds found in the leaves of the green tea *Camellia sinensis*. These compounds may be contained in up to 30% of the dry weight of the leaf [1] and are an important factor in the taste of the tea [2]. High levels of catechins may render the tea bitter and may affect its astringency. In the black teas, the polymerized catechins such as theaflavins and thearubigins [3] that result from the fermentation process are important factors in determining the overall quality of the tea [4]. Caffeine is a plant alkaloid contained in some popular beverages like tea, coffee and cocoa and is known for its stimulatory effect. Recently,

interest in the relationship between food components and human health has become widespread, both within the scientific community [5] and among the general public. Of the many polyphenol compounds found in food commodities, tea catechins, particularly those found in green tea, have been found to possess anti-mutagenic [6] and anti-tumorigenic [7] properties and may exert prophylactic effects against hypertension [8]. Previously, the structural similarity of the various tea catechins (Fig. 1) made the quantitation and analysis of individual catechins difficult [9–11]. As each catechin possesses distinct properties, a simple and rapid method that could be used for analysis of individual catechins in a complex mixture would be advantageous.

Therefore we have developed a relatively simple and quick high-performance liquid chromatography
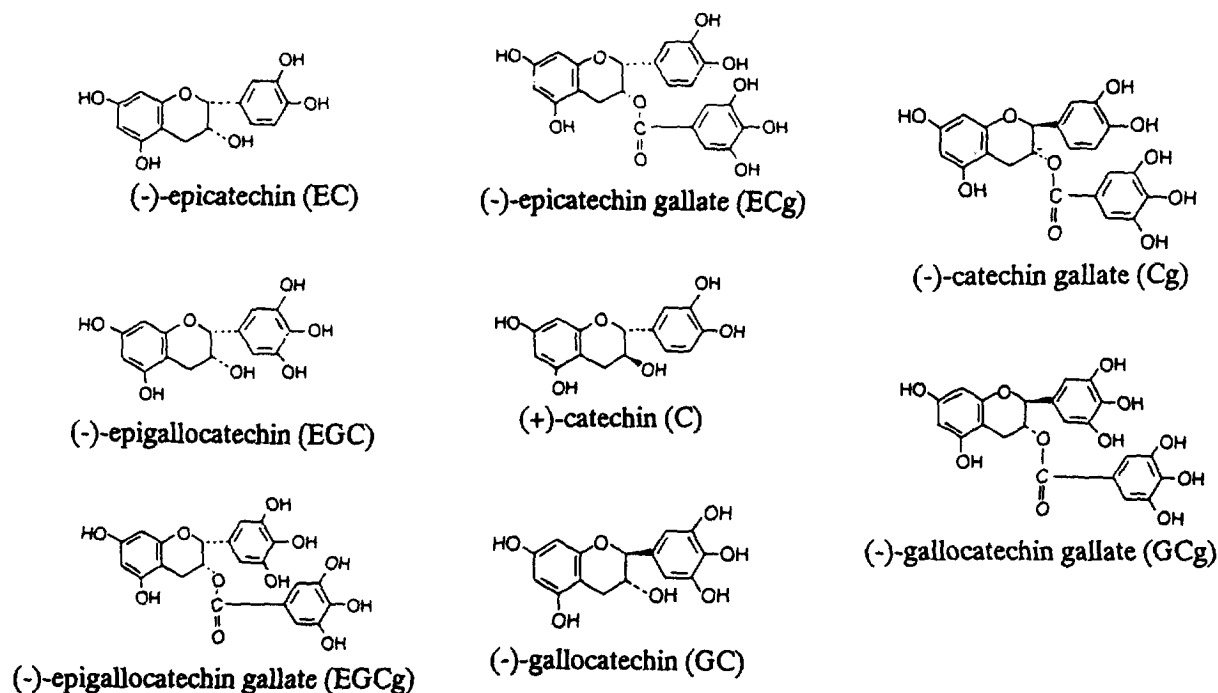
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Fig. 1. Structure of catechins.

(HPLC) analysis method in which caffeine and several catechins may be analyzed simultaneously. The following eight catechins, epicatechin (EC), epicatechin gallate (ECg) epigallotechin (EGC), epigallocatechin gallate (EGCg), catechin (C), gallocatechin (GC), gallocatechin gallate (GCg) and catechin gallate (Cg), and caffeine may be quantitated by this method.

2. Experimental

2.1. High-performance liquid chromatography

The HPLC system consisted of Shimadzu LC-6A pumps with a two pump gradient system (Shimadzu, Kyoto, Japan), a Shimadzu SPD-M10A diode array detector (200–300 nm), an SSC3500 column oven (Senshu Kagaku, Tokyo, Japan), a Rheodyne 7125 sample injector with a 20-μl sample loop (Rheodyne, Cotati, CA, USA). The column was a De-velosil ODS-HG column (150×4.6 mm, Nomura Chemical Co., Seto, Japan) equipped with a guard

column (10×4 mm, Nomura). The flow-rate of the mobile phase was 1 ml/min. A Class M10A software (Shimadzu) was used for both the operation of the detector and for data processing. The purity of the peaks was calculated by the Class M10A using measured spectra.

2.2. Chemicals and samples

Eight catechin standards (EC, ECg, EGC, EGCg, C, GC, GCg and Cg) and a partially purified green tea extract (Polyphenon 60, 60% catechin content) were donated by Food Research Laboratories, Mitsui Norin (Fujieda, Japan). Caffeine was purchased from Wako (Osaka, Japan). The acetonitrile used for the mobile phase was of HPLC grade; all the other chemicals were of analytical reagent grade and were used without further purification.

Green tea samples, Sencha and Matcha, were purchased from standard markets. Sencha samples were milled by a Cyclon Sample Mill (Udy, USA) with a 1-mm mesh screen. The Matcha tea, which is a powdered green tea, was extracted without further

manipulation. Green tea samples were extracted by the method of Suematsu et al. [12] with slight modifications. The samples (500 mg) were extracted with 100 ml of acetonitrile–water (1:1, v/v) at room temperature for 40 min with constant shaking. The extract was filtered through a filter cartridge (DIS-MIC 13HP, Advantec Toyo, Tokyo, Japan) and diluted five-fold with water before HPLC analysis.

3. Results and discussion

3.1. Separation conditions of standard catechins

Several acetonitrile aqueous-based mobile phases were tested, including water–acetonitile in combination with acetic acid, phosphate buffer, a phosphoric acid system, methanol–acetic acid, methanol–phosphate buffer and ethylacetate–phosporic acid. Eventually, it was found that a water–acetonitrile–phosphoric acid solvent system with two step linear gradients of acetonitrile concentration successfully separated all nine chemicals within 20 min (Fig. 2B). The mobile phase compositions used were: (A) Water–acetonitrile–85% phosphoric acid (95.45:4.5:0.05, v/v); (B) water–acetonitrile–85% phosphoric acid (49.95:50.0:0.05, v/v). The solvent composition started at 90% solvent A and 10% solvent B and was maintained for 5 min, then linearly increased to 30% solvent B in 3 min. This

condition was maintained for 2 min followed by a linear increase of solvent B to 80% in 5 min. The final conditions were held for an additional 5 min.

Over the course of these experiments, it became evident that the temperature of the column oven strongly affected the separation. As the column temperature increased, the retention times of all nine chemicals decreased, but the magnitude of the change varied for each compound (Fig. 2). For convenience, 40°C was selected as the standard oven temperature for subsequent analyses, as satisfactory separation of all nine compounds was achieved at this temperature.

3.2. Quantitative analysis

Using the Polyphenon 60 solution, the repeatability of this analytical method was tested. For all eight catechins and caffeine, the C.V. value of the retention time was less than 0.5% and for quantitation was less than 2.5% for five injections. At 231 nm, the calibration curves of the eight catechins were linear from 2 to 2000 ng. For caffeine, linearity over this range was obtained at 274 nm. The minimum detection limit was approximately 0.2 ng for all nine compounds. The sensitivity of the analysis could be enhanced three-fold by using a wavelength of 207 nm for the detection, however, the linearity of the calibration curves was not comparable to the curves generated at 231 nm. They were linear only up to 500 ng. In addition, the baseline of the chromatograms shifted with the concentration of acetonitrile at the shorter wavelength and the potential for interference by other chemicals in complex mixtures could not be ignored. Thus, we have selected 231 nm as the appropriate wavelength for the detection and quantitation of catechins in tea leaves.

Green tea samples (500 mg) were extracted with 100 ml of acetonitrile–water (1:1, v/v) and were diluted with water. Before analysis, the extracts were filtered through a cartridge-type sample filtration unit with a polytetrafluoroethylene (PTFE) membrane that had a hydrophilic surface. Several types of filtration membranes were utilized and, as shown in Table 1, the composition of the filter affected the efficiency of recovery. For our purpose, the PTFE membrane was the most efficient.
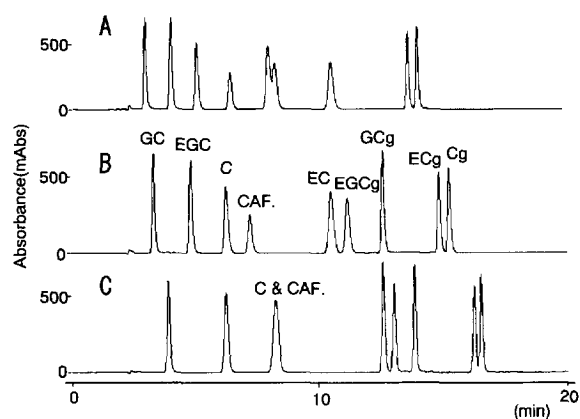

Fig. 2. Chromatographic analysis of standard catechins and caffeine and the effect of column temperature on their separation. (A) At 50°C; (B) at 40°C, standard analysis condition; (C) at 30°C.

Table 1
Recovery of major catechins and caffeine after filtration[a] (%)

Group[b]	Membrane material	EC	EGC	ECg	EGCg	Caffeine
A	PTFE[c]	100.5	101	100.5	100	101
A	PTFE	100	99.5	99.5	100	100
A	PVDF[d]	98	97.5	21	43.5	99.5
A	RC[e]	95.5	93.5	93	91.5	100
B	PTFE	101	101	101	101	101
B	PVDF	98	98.5	95	95.5	98
B	RC	99	99	99.5	99	99

[a] A 2-ml volume of a Polyphenon 60 solution (15 mg/100 ml) was filtered through each cartridge and the recovery of each chemical was measured by HPLC.
[b] Group A; The sample was dissolved in a 5% acetonitrile solution. Group B; The sample was dissolved in a 50% acetonitrile solution.
[c] Polytetrafluoroethylene with a hydrophilic coating.
[d] Polyvinyldifluoride.
[e] Regenerated cellulose.

3.3. Analysis of green tea samples

Two different types of green tea, both high and low grades for each tea, were analyzed for individual catechins and caffeine content for a total of eighteen samples (Table 2). Matcha, the tea traditionally used in the Japanese tea ceremony, is produced by growing the plants under a sunshade. Sencha, the most popular type of Japanese green tea, is grown in an outdoor field.

In general, the catechin content was higher in Sencha than in Matcha tea and the lower grade teas contained more catechins and less caffeine than the higher grade teas. Detectable amounts of C were found in most of these teas samples. In addition, the Sencha samples contained detectable amounts of Cg, GC and GCg, whereas the Matcha tea samples did not. Chromatograms of these samples are shown in Fig. 3. No extraneous peaks interfered with the
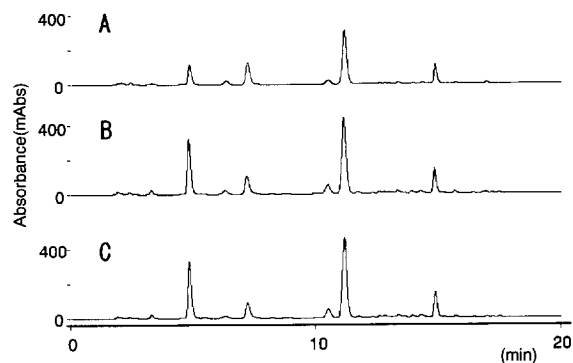

Fig. 3. Chromatographic analysis of green tea extracts at a wavelength of 231 nm. (A) High grade Matcha; (B) High grade Sencha; (C) Low grade Sencha.

chromatographic analysis of the extracts. The purity of each catechin peak in the tea samples was more than 98%, as calculated from the peak spectrum. The minimum quantitation limits for each catechin and

Table 2
Analysis of four major catechins and caffein in green tea

Samples	Number of samples	Dry weight (%)[a]				
		EC	EGC	ECg	EGCg	Caffeine
HG[b] Matcha	3	0.45	1.64	1.45	5.94	3.87
LG[c] Matcha	3	0.67	2.67	1.54	7.51	3.53
HG Sencha	6	1.11	3.95	1.65	7.89	3.16
LG Sencha	6	1.10	4.39	1.73	9.26	3.07

[a] Values given are the avarage of three or six samples.
[b] High grade.
[c] Low grade.

for caffeine was less than 0.02% of the dry weight of the tea, which is more than adequate for the analysis of catechins and caffeine in green teas.

Acknowledgments

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