Rapid Determination of Eight Catechins in Bottled Green Tea Drinks using Isocratic Elution Method/RP–HPLC–UV

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Summary

An accurate, simple and rapid isocratic elution method for the simultaneous determination of eight catechins in bottled green tea drinks using reversed-phase (RP) high performance liquid chromatography (HPLC) with ultraviolet detector (UV) and short column (100 mm×4.6 mm i. d., particle diameter 3 μm) has been studied. As a result, good linearity of the calibration curve were obtained in the concentration range from 1 mg l⁻¹ to 100 mg l⁻¹ (|r|=0.9998~0.9974). The detection limits based on S/N=3 were 0.62 mg l⁻¹ for (−)-gallocatechin (GC), 0.78 mg l⁻¹ for (−)-epigallocatechin (EGC), 0.59 mg l⁻¹ for (+)-catechin (C), 0.38 mg l⁻¹ for 7-(β-hydroxyethyl)theophylline, 0.42 mg l⁻¹ for (−)-epigallocatechin gallate (EGCg), 0.57 mg l⁻¹ for (−)-epicatechin (EC), 0.48 mg l⁻¹ for (−)-gallocatechin gallate (GCg), 0.40 mg l⁻¹ for (−)-epicatechin gallate (ECg), and 0.43 mg l⁻¹ for (−)-catechin gallate (Cg) in 5 μl injection. The eight catechins and 7-(β-hydroxyethyl) theophylline were separated by an isocratic elution method within 30 min. This isocratic elution method could be successfully applied to the rapid determination of eight catechins in bottled green tea drinks.

Key words: RP–HPLC–UV, isocratic elution, polyphenol, catechin, green tea

Introduction

Japanese green tea is one of the most popular beverages in Japan. Catechins, major polyphenol constituents of green tea, are well known because of their antioxidant activity and chemopreventive effects against cancers.¹ Due to this beneficial effect of catechins on the human health, the current interest in the health effect of green tea and investigations of natural materials as a source of chemotherapeutic agents have necessitated the develop of an accurate and rapid analytical method for the determination of catechins.

Several workers have reported the determination of catechins in green tea by reversed-phase (RP) high
performance liquid chromatography (HPLC) with ultra-violet detector (UV). The RP-HPLC-UV is currently the most useful approach for the routine analysis. Iso-cratic elution has been performed, although gradient elution is the most commonly used approach, control of the temperature being necessary to obtain an adequate resolution in rapid determination.

This paper reports an accurate, simple and rapid RP-HPLC-UV and isocratic elution method in which the eight catechins occurring in bottled green tea drinks is separated at 40 °C with a short time of analysis. The eight catechins determined were (-)-gallocatechin (GC), (-)-epigallocatechin (EGC), (+)-catechin (C), (-)-epigallocatechin gallate (EGCg), (-)-epicatechin (EC), (-)-gallocatechin gallate (GCg), (-)-epicatechin gallate (ECg), and (-)-catechin gallate (Cg). By using short separation column (100 mm × 4.6 mm i. d., particle diameter 3 μm), which was packed with the octadecyl (C18) chemically-bonded silica gel, rapid determination can be achieved.

**Experimental**

**Reagents and Standard Solutions**

HPLC-grade methanol was purchased from Kanto Kagaku Co., Ltd., Japan. HPLC-grade water (below 0.2 μS cm⁻¹), prepared using a YG221 purification system (Yamato Scientific Co., Ltd., Japan), was used to prepared all solutions. (-)-gallocatechin (GC), (-)-epigallocatechin (EGC), (+)-catechin (C), (-)-epigallocatechin gallate (EGCg), (-)-epicatechin (EC), (-)-gallocatechin gallate (GCg), (-)-epicatechin gallate (ECg), and (-)-catechin gallate (Cg) were purchased from Wako Pure Chemical Industries, Ltd., Japan for biochemical grade. 7-(β-hydroxyethyl)theophylline was purchased from ICN Biomedicals Inc., USA for biochemical grade. All other reagents used were JIS special grade (JIS K8775) purchased from Wako Pure Chemical Industries, Ltd.

A standard solution of catechins were prepared (1,000 mg l⁻¹). A quantity of catechins was dissolved in methanol was added to bring solution to the desired concentration at each stage.

**Apparatus and Chromatographic conditions**

The HPLC system was performed using a Hitachi-655A system, consisting of a 655A-11 (Hitachi, Ltd., Tokyo, Japan) pump, a Rheodyne (Cotati, CA, USA) Model 7725i syringe-loading sample injector with a injection volume 5 μl sample loop, a 655A-11 (Hitachi, Ltd.) UV spectrophotometric detector, a L-7300(Hitachi, Ltd.) column oven, and a C-R6A Chromatopac data system (Shimadzu Co., Kyoto, Japan) A detection wavelength was set at 280 nm. The separation column used throughout this study was a CAPCELL PAK C18 UG120 S3 (100 mm × 4.6 mm i. d., particle diameter 3 μm, Shiseido, Ltd., Tokyo, Japan), which was packed with the octadecyl (C18) chemically-bonded silica gel. A column temperature was set at 40 °C. The solution of methanol-water (20:80 v/v) mixture containing 0.5 % H3PO4 was employed as the mobile phase for HPLC separation. A flow rate in the column was 0.6 ml min⁻¹.

**Samples**

Samples for this experiment, japanese green tea and oolong tea made by the Suntory, Ltd., Tokyo, Japan were collected in November 2003.

**Results and Discussion**

**Determination of Eight Catechins in a Standard Mixture Using RP-HPLC-UV**

Fig. 1 shows the chromatogram obtained under the running conditions described in the experimental section for a standard solution (100 mg l⁻¹) of eight catechins using short column (CAPCELL PAK C18 UG120 S3, 100 mm × 4.6 mm i. d., particle diameter 3 μm, Shiseido, Ltd.) (a) and general column (CAPCELL PAK C18 UG120 S5, 150 mm × 4.6 mm i. d., particle diameter 5 μm, Shiseido, Ltd.) (b).

The retention time for eight catechins under the running conditions reported herein is 30 minutes (a). This increased retention ensures base-line stability.

The concentration of eight catechins in the distilled water and methanol used for this experiment were the detection limit.

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Standard solutions of eight catechins were prepared at 1, 5, 10, 20, 50, 70, 100 mg l⁻¹. An eight catechins calibration curve, using the internal calibration curve method, was constructed from the chromatograms obtained for these seven solutions. The obtained calibration curve had good linearity (Table 1). The calibration curve were obtained in the concentration range from 1 mg l⁻¹ to 100 mg l⁻¹ (|r|=0.9998 ~0.9974).
For eight catechins and 7-(β-hydroxyethyl)theophylline by RP-HPLC-UV, the detection limits based on S/N=3 were 0.62 mg \( \text{L}^{-1} \) for GC, 0.78 mg \( \text{L}^{-1} \) for EGC, 0.59 mg \( \text{L}^{-1} \) for C, 0.38 mg \( \text{L}^{-1} \) for 7-(β-hydroxyethyl)theophylline, 0.42 mg \( \text{L}^{-1} \) for EGCg, 0.57 mg \( \text{L}^{-1} \) for EC, 0.48 mg \( \text{L}^{-1} \) for GCg, 0.40 mg \( \text{L}^{-1} \) for ECg, and 0.43 mg \( \text{L}^{-1} \) for Cg in 5 \( \mu \text{L} \) injection.

The standard solutions of eight catechins were measured 10 times, and the relative standard deviations (RSD) were determined to be 1.26–2.79 %, respectively. These values were considered to be good.

The selectivity criterion for an assay method is that the analyte peaks will have a chromatographic baseline with a suitable resolution from all the other sample components. In our case, the peaks showed resolutions (Rs) ≥ 1.50 for all the determined analytes.

**Determination of Eight Catechins in Samples Using RP-HPLC-UV**

The utility of the RP-HPLC-UV system for separation and detection of eight catechins in bottled green tea drinks was also tested. Sample preparation simulated bottled green tea drinks was analyzed directly.

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**Fig. 1** RP-HPLC-UV chromatograms of standard solution for catechins (100 mg \( \text{L}^{-1} \)) on (a) CAPCELL PAK C18 UG120 S3 (100 mm×4.6 mm i.d., 3 \( \mu \text{m} \)), (b) CAPCELL PAK C18 UG120 S5 (150 mm×4.6 mm i.d., 5 \( \mu \text{m} \))

Peaks - (0): methanol; (1): GC; (2): EGC; (3): C; (4): 7-(β-hydroxyethyl)theophylline (Internal standard substance); (5): EGCg; (6): EC; (7): GCg; (8): ECg; (9): Cg; HPLC conditions - Mobile phase: 20/80 wt % methanol-water, 0.5 % \( \text{H}_3\text{PO}_4 \); Flow rate: 0.6 mL min\(^{-1} \); Column temp.: 40 °C; Detection wavelength 280 nm; Injection volume: 5 \( \mu \text{L} \)

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**Table 1** Linearity of calibration curve with internal calibration method

| Equation | \(|r|\) |
|----------|--------|
| GC \(Y = (1.63 \times 10^2)X + (1.33 \times 10^3)\) | 0.9989 |
| EGC \(Y = (9.77 \times 10^3)X - (1.07 \times 10^5)\) | 0.9994 |
| C \(Y = (4.35 \times 10^2)X + (9.81 \times 10^2)\) | 0.9992 |
| EGCg \(Y = (9.52 \times 10^3)X - (1.26 \times 10^4)\) | 0.9996 |
| EC \(Y = (4.88 \times 10^2)X + (3.22 \times 10^2)\) | 0.9974 |
| GCg \(Y = (1.10 \times 10^1)X - (2.78 \times 10^3)\) | 0.9997 |
| ECg \(Y = (1.39 \times 10^2)X + (3.27 \times 10^4)\) | 0.9994 |
| Cg \(Y = (1.30 \times 10^2)X + (3.67 \times 10^2)\) | 0.9998 |

Internal standard substance (I. S.): 7-(β-hydroxyethyl)theophylline (50 mg \( \text{L}^{-1} \)); \(Y\) = peak area of standard catechins / peak area of I. S.; \(X\) = concentration of catechins (mg \( \text{L}^{-1} \)); \(|r|\) = correlation coefficient; HPLC conditions - Column: CAPCELL PAK C18 UG120 S3 (100 mm×4.6 mm i. d., 3 \( \mu \text{m} \)); Mobile phase: 20/80 wt % methanol-water, 0.5 % \( \text{H}_3\text{PO}_4 \); Flow rate: 0.6 mL min\(^{-1} \); Column temp.: 40 °C; Detection wavelength 280 nm; Injection volume: 5 \( \mu \text{L} \)
without pretreatment or extraction. A comparison of the interenal calibration method with the external calibration method, yielded comparable results. (Table 2) Fig. 2 shows the chromatogram obtained under the running conditions described in the experimental section for a bottled green tea drinks (a), (b) and a bottled oolong tea drinks (c), (d) of eight catechins using short column (CAPCELL PAK C18 UG120 S3, 100 mm×4.6 mm i. d., particle diameter 3 μm, Shiseido, Ltd.) (a), (c) and general column (CAPCELL PAK C18 UG120 S5, 150 mm×4.6 mm i. d., particle diameter 5 μm, Shiseido, Ltd.) (b), (d).

Quantitative analysis using a UV detector is challenging when analyzing the components of samples with dye disruptions from organic compounds. However, quantitative analysis of bottle green tea drinks and bottle oolong tea drinks containing minimal disruptive elements was successfully accomplished, as shown.

Table 2 Concentration of catechins in bottled green tea drinks

<table>
<thead>
<tr>
<th>Sample</th>
<th>Catechins / mg L⁻¹</th>
<th>GC</th>
<th>EGC</th>
<th>C</th>
<th>I.S.</th>
<th>EGCg</th>
<th>EC</th>
<th>GCg</th>
<th>ECg</th>
<th>Cg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green tea</td>
<td></td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>90.1</td>
<td>88.8</td>
<td>50.3</td>
<td>49.4</td>
<td>22.0</td>
<td>20.6</td>
<td>3.1</td>
<td>58.7</td>
<td>55.9</td>
<td>14.6</td>
</tr>
<tr>
<td>Oolong tea</td>
<td></td>
<td>28.1</td>
<td>27.1</td>
<td>11.4</td>
<td>10.6</td>
<td>17.5</td>
<td>16.8</td>
<td>1.2</td>
<td>33.2</td>
<td>32.1</td>
</tr>
</tbody>
</table>

a: Internal standard curve method, b: External standard curve method, Internal standard substance (I. S.): 7-(β-hydroxyethyl)theophylline (50 mg L⁻¹); a: Internal calibration method; b: External calibration method; HPLC conditions - Column: CAPCELL PAK C18 UG120 S3 (100 mm×4.6 mm i. d., 3 μm); Mobile phase: 20/80 wt % methanol-water, 0.5 % H₃PO₄; Flow rate: 0.6 ml min⁻¹; Column temp.: 40 °C; Detection wavelength 280 nm; Injection volume: 5 μl

Fig. 2  RP-HPLC-UV chromatograms of (a), (b) green tea and (c), (d) oolong tea on (a), (c) CAPCELL PAK C18 UG120 S3 (100 mm×4.6 mm i. d., 3 μm), (b), (d) CAPCELL PAK C18 UG120 S5 (150 mm×4.6 mm i. d., 5 μm)

Peaks - (1): GC; (2): EGC; (3): C; (4): 7-(β-hydroxyethyl)-theophylline; (5): EGCg; (6): EC; (7): GCg; (8): ECg; (9): Cg; HPLC-UV conditions - Mobile phase: 20/80 wt % methanol-water, 0.5 % H₃PO₄; Flow rate: 0.6 ml min⁻¹; Column temp.: 40 °C; Detection wavelength 280 nm; Injection volume: 5 μl
in the chromatograms in Fig. 2 and by the concentration of catechins reported in Table 2.

Eight catechins in bottled green tea drinks were measured 10 times, and the RSD were determined to be 1.34~2.88 %, respectively. These values were considered to be good.

This isocratic elution/RP-HPLC-UV method is considered an extremely useful routine analysis of determining the eight catechins in bottled green tea drinks in the ppm range without extraction or sample pretreatment. Therefore, as for isocratic elution method using this short column and gradient elution method in general, the measurement time is same and this isocratic method is more practical because the repeatability of the measurement value is good.

References


