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SHORT COMMUNICATION

Effect of Packing Size on Chomatographic Separation of Catechin Compounds in Green Tea

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Abstract–EGCG (Epigallocatechin Gallate), one of the catechin compounds abundant mainly in green tea, has chemopreventive effects against carcinogenesis. The extract at 50 °C water from the powder of green tea was partitioned with chloroform and ethyl acetate. The resulting solution was further purified on three different columns, 4.6×250 mm (40/63 μm), 4.6×250 mm (15 μm), 3.9×300 mm (10 μm), in order to separate EGCG among catechin compounds from green tea. In a given packing size, the composition of the binary mobile phase, water and acetonitrile, with 0.1% acetic acid was changed to operate in optimized experimental conditions. In a packed column of 40/63 μm packing, catechin compounds were not well separated. With packing sizes of 15 and 10 μm, the resolution of EGCG among catechin compounds was better, and maximum injection volume to separate EGCG purely was 100 and 480 μl, respectively. The study on the effect of packing size may be utilized for preparative chromatographic separation.

Key words: Green Tea, Epigallocatechin Gallate, Packing Size, Chromatography

INTRODUCTION

Recently, the demand for the green tea has increased due to health concerns and personal preference. Although its chemical composition varies with growing conditions, climate and location, the principal catechin compounds included in green tea are (+)catechin (C), (−)epigallocatechin gallate (EGCG), (−)epigallocatechin (EGC), (−)epicatechin gallate (ECG), and (−)epicatechin (EC). These catechin compounds have been proven to have a variety of physiological functions affecting the duodenum [Choi et al., 1993], colon [Yamane et al., 1991], skin [Santosh et al., 1993], gastric, lung, breast [Valcic et al., 1996], esophageal, pancreatic and prostate cancer. Industries and governmental research institutes have actively studied ways to produce highly pure catechin compounds on a commercial scale. These compounds have also been reported to have a variety of other effects, such as preventing dementia, inhibiting the AIDS virus, offering microwave protection and inhibiting environmental hormones. The most interesting substance in catechin compounds of green tea is EGCG (Epigallocatechin Gallate). Among these catechin compounds, EGCG has the strongest cancer preventive activities. EGCG is indentificated as a non-toxic anti-cancer agent killing only various cancer cells except normal cells [Ahmad et al., 1997]. Therefore the research for EGCG as well as other catechin compounds and the effect of green tea is progressing actively.

Goto et al. [1996] extracted green tea with acetonitrile-water (1 : 1, v/v) and analyzed it by HPLC with mobile phase of water-acetonitrile-85% phosphoric acid (95.45 : 4.5 : 0.05, v/v), water-acetonitrile-85% phosphoric acid (49.95 : 50.0 : 0.05, v/v) with Develosil ODS-HG column. Bronner and Beecher [1998] partitioned and quantified catechin compounds contained in green tea, jasmine tea, and red tea with mobile phase of acetonitrile-acetate, acetonitrile-ascorbate, methanol-acetate by C_{18} column. Lee et al. [1992] concentrated them after extracting green tea at 80 °C hot water and separated them to HPLC with 20% methanol and 80% acetonitrile as mobile phase after partitioning into ethyl acetate. Kang et al. [1999] partitioned with chloroform and ethyl acetate after extracting green tea at 50 °C water and separated with mobile phase of 0.1% acetic acid in water/acetonitrile, 87/13 (vol%) using RP-HPLC with μ-Bondapak column.

The major factors affecting the resolution of components in a chromatographic separation are the particle size of packing as well as mobile phase compositions. The particle size is especially dependent on the application, analytical or preparative separation. In this research, the effect of particle sizes was investigated for preparative work in order to separate pure EGCG among catechin compounds from green tea.

EXPERIMENTAL

1. Chemicals

The green tea used in this experiment was cultivated at Bosung (Chonnam, Korea, 1997) and purchased from a domestic market. The standard chemicals of (+)catechin (C), (−)epigallocatechin gallate (EGCG), (−)epigallocatechin (EGC), (−)epicatechin gallate (ECG), (−)epicatechin (EC) were purchased from Janssen Chimica and Sigma Co. The extra-pure grade solvents of methanol, acetonitrile were purchased from J. T. Baker (Phillipsburg NJ, U.S.A.). The water was distilled and deionized prior to use.

2. Extraction and Pretreatment

Initially, catechin compounds from green tea were extracted by distilled water. Five grams dry Korean green tea was weighed and placed in a 500 ml triangle flask with 150 ml distilled water. Then, the extract was filtered and concentrated to 30 ml with a rotary evaporator (Resona technics, Switzerland). The extract was partitioned with an equal volume of chloroform to eliminate impurities. Ca-

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techin compounds were extracted from the water layer after an equal volume of ethyl acetate was added.

To purify EGCG among catechin compounds, the three different packings of 10, 15, 40/63 μm were in-house packed by a vacuum pump.

3. Chromatography

The HPLC system in this experiment was as follows: Waters Model 600S liquid chromatograph (Waters Associates, Milford, MA, U.S.A.) equipped with the Waters 616 Multisolvent Delivery System with 2486 Dual absorbance, and injector (2 ml sample loop) of Rheodyne. The data acquisition system was Millennium[32] (Waters Co.) installed in a PC. The mobile phases of water, methanol, acetonitrile (ACN), ethyl acetate and acetic acid were experimented. The three chromatographic columns experimented included, first, μ-Bondapak (10 μm, 3.9×300 mm, Waters Co.); the other two empty columns (4.6×250 mm) were packed by 15 μm (Merck Co.) and 40/63 μm (YMC-GEL Co.).

RESULTS AND DISCUSSION

Catechin compounds contained in Korean green tea were extracted in 50 °C water for four hours dipping with 300 rpm of agitation speed. To remove unnecessary components, the extract was purified by mixing with chloroform followed by ethyl acetate. It was reported that the extract through partition process needed a purification process which used preparative column in order to increase and concentrate the degree of purity greatly [Chung et al., 1998]. To investigate the effects of packing size on the resolution of EGCG, the target component in this work, from catechin compounds, three different sizes of packings were used: the particle sizes of packing were changed to 10, 15, and 40/63 μm [Jung and Row, 1998]. Particles larger than 10 μm are reported to be suitable for preparative work [Row, 1999; Row and Lee, 1997]. Table 1 shows the details of packings used in this experiment. To separate EGCG from ethyl acetate layer, a chromatographic column packed with 40/63 μm was used. Catechin compounds were not well resolved, so the mixtures of (+)C, EC, and EGCG were coeluted in isocratic mode. In Fig. 1, the gradient mode was implemented. The first mobile phase composition was 0.1% acetic acid in water/acetonitrile, 95/5 (vol%), the second mobile phase was linearly changed to 90/10 (vol%) for 10 min, the third was 80/20 (vol%) for 20 min, and the fourth was 75/25 (vol%) for 130 min. The retention times of the three catechin compounds were shorter, and the resolutions were improved. However, only with the packing of 40/63 μm, was EGCG difficult to isolate. For preparative separation, this packing might be utilized as a pretreatment step.

The smaller packing of 15 μm was isocratically experimented in Fig. 2. Compared to 40/63 μm packings, the resolution of EGCG

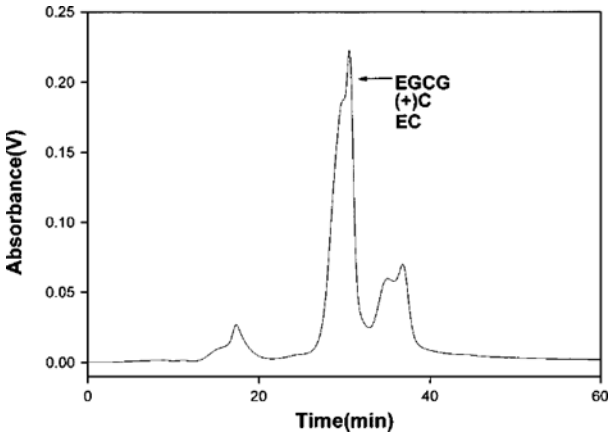

Fig. 1. Chromatographic separation of catechin compounds from green tea.
(40-63 μm column, 0.1% acetic acid in water/ACN=95/5 (vol%), after 10 min-90/10, after 20 min-80/20, after 130 min-75/25, 1.0 ml/min, 10 μl injection volume)

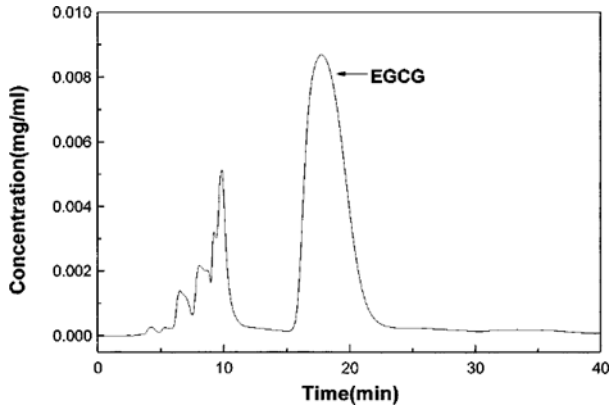

Fig. 2. Chromatographic separation of catechin compounds from green tea.
(15 μm column, 0.1% acetic acid in water/ACN/ethyl acetate= 87/12/1 (vol%), 1.0 ml/min, 10 μl injection volume)

was better and the retention time was shorter in 15 μm packing due to the high pressure exerted on the chromatographic column. Adding ethyl acetate in the mobile phase of acetic acid-containing solution resolved EGCG from the neighboring peaks. The mobile phase composition was 0.1% acetic acid in water/acetonitrile/ethyl acetate, 87/12/1 (vol%) in isocratic mode. This experimental condition could be used in preparative HPLC separation. Fig. 3 shows the resolution of EC and EGCG with the injection volumes of 10, 20, 50 and 100 μl. The resolution defined by the ratio of the difference in retention times to the average peak widths were 1.92,

Table 1. Chromatographic columns used in this experiment

Packing size	Packing material	Dimension of column	Pore size	Shape	Supplier
10 μm	Dimethyloctadecylsilyl bonded amorphous silica Methyl Alcohol	3.9×300 μm	125 Å	Irregular	Waters
15 μm	Lichrospher 100RP-18	4.6×250 μm	100 Å	Spherical	Merck
40/63 μm	ODS-120-400/230	4.6×250 μm	120 Å	Irregular	YMC-GEL

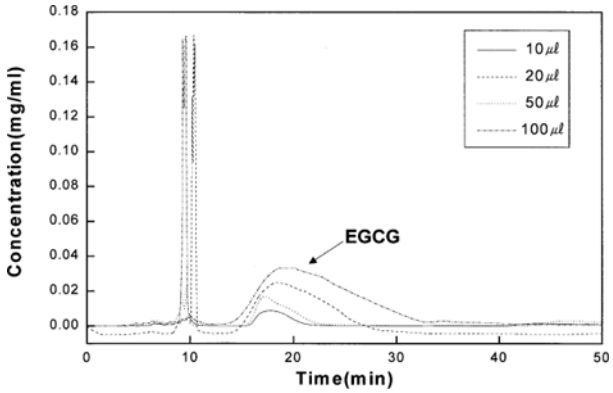

Fig. 3. Increase in the injection volume of sample.
(15 μm column, the same mobile phase in Fig. 2, 1.0 ml/min)

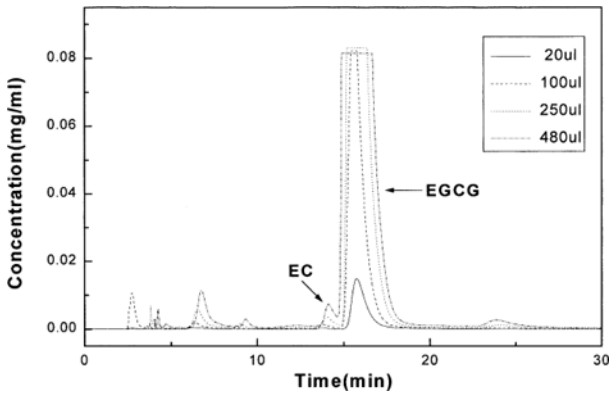

Fig. 4. Increase in the injection volume of sample
(10 μm column, 0.1% acetic acid in water/ACN=87/13 (vol %), 1.0 ml/min)

1.89, 1.06 and 1.00, respectively, and in the injection volume up to 100 μl, the EGCG was collected in a pure form. The sample in the ranges of 14-26 min in Fig. 2 was collected several times, and was vaporized to concentrate. Fig. 4 shows the resolution of EC and EGCG with the injection volumes 20, 100, 250, and 480 μl of the concentrated sample in the μ-Bondapak analytical column (10 μm). Some impurities and other catechin compounds were almost removed and pure EGCG was obtained.

In the smallest packings, the maximum injection volume to isolate EGCG purely was 480 μl. The resolutions of EGCG were deteriorated 1.29, 1.27, 0.89 and 0.76 with increase in the injection volumes of 20, 100, 250, 480 μl, respectively. This implied that the resolution and the maximum injection volume in the packing size of 10 μm were much better than that in 15 μm. Thus, the particle size of packing and sample size have a strong influence on the chromatographic separation [Row and Lee, 1992]. It was reported that the use of small and homogeneous particles has an impressive advantage towards low-cost, so-called 'preparative' particles when anthraquinone antibiotics are purified by preparative reversed-phase liquid chromatography, even under heavily over-loaded column conditions [Kulik and Fiedler, 1998].

CONCLUSION

Three different packing sizes were considered to separate preparatively EGCG in Korean green tea. With a packing size of 40/63 μm, catechin compounds were poorly separated in isocratic mode, but the operation in gradient mode showed possibility as pretreatment step. In 15 μm, the resolution of EGCG among catechin compounds was better and the maximum injection volume was 100 μl, while the injection volume was increased to 480 μl in the smallest packing, 10 μm. Depending on the purity and yield of EGCG as well as packing cost, the optimized chromatography would be slightly changed. In this work, it is suggested that, for the preparative separation of EGCG from green teas, packings of 40/63 μm be used in the pretreatment step followed by packings of 15 μm in the final separation step.

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