

TLC Separation of Methylated (–)-Epigallocatechin-3-Gallate

RYSZARD AMAROWICZ¹, ANNA MARYNIAK¹ and FEREDOON SHAHIDI²

¹Department of Food Chemistry, Division of Food Science, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland;

²Department of Biochemistry, Memorial University of Newfoundland, St. John's, Canada

Abstract

AMAROWICZ R., MARYNIAK A., SHAHIDI F. (2005): TLC separation of methylated (–)-epigallocatechin-3-gallate. Czech J. Food Sci., 23: 36–39.

Methylated EGCG was separated from the crude extract using Sephadex LH-20 column chromatography with methanol as the mobile phase, and a semi-preparative HPLC method with water-dimethylformamide-methanol-acetic acid (157:40:2:1, v/v/v/v) as the mobile phase. The chemical structure of the separated catechin was confirmed by ESI-MS in the negative-ion mode. Three different mobile phases were used for silica gel and reversed phase TLC of EGCG and methylated EGCG. R_f values of both catechins were calculated and are reported. In the normal phase, the best condition of separation was with chloroform-methanol-water (65:35:10; v/v/v; lower phase) being used as the mobile phase. On octadecylsilylated silica gel plates, the phase water-acetonitrile-methanol-acetic acid (79.5:18:2:0.5; v/v/v/v) offered the best separation of catechins.

Keywords: green tea; methylated epigallocatechin gallate; TLC

Leaves of green tea are a rich source of such catechins as (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), and (–)-epigallocatechin-3-gallate (EGCG). The activity of these compounds has been reported by several research groups as being related to antioxidant activity (AMAROWICZ & SHAHIDI 1995; CHEN & HO 1995; GUO *et al.* 1999) and antimutagenic potential (YEN & CHEN 1995). Catechins also possess anticarcinogenic (BUSHAN 1998; DREOSTI *et al.* 1997; HOLLMAN *et al.* 1999) and antimicrobial properties (FUKAI *et al.* 1991; AMAROWICZ *et al.* 2000).

Methylated catechins were isolated from different anatomical parts of several plants (MONACHE *et al.* 1967, 1976; KAMAL *et al.* 1982; MATSUZAKI & HARA 1985; MORIMOTO *et al.* 1985). The presence of methylated EGCG (Figure 1) in green tea was noted by SAIJO (1982), SANO *et al.* (1999), AMAROWICZ and SHAHIDI (2003).

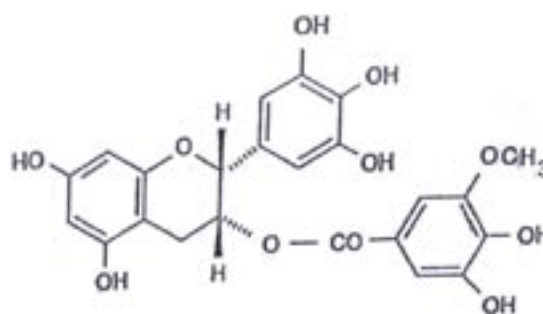


Figure 1. Chemical structure of methylated EGCG

For the preparation of standard methylated EGCG, thin layer chromatography can serve as a useful method for monitoring column chromatography. Therefore, several mobile phases were compared in terms of the separation of methylated EGCG by TLC procedures.

MATERIAL AND METHODS

Materials. Chinese green tea leaves were obtained from Anhui Province (Chinese People's Republic).

Extraction of crude catechins. Crude catechins were extracted from 50 g of green tea leaves using 500 ml of hot water (80°C) under stirring over a 1 h period (PRICE & SPITZER 1993).

Separation and purification of methylated EGCG. Crude catechins (0.8 g) were dissolved in 8 ml of methanol and applied on a Sephadex LH-20 column (60 × 3.0 cm) equilibrated with HPLC grade methanol (water content < 0.03%); fractions (10 ml) were collected using a fraction collector. Catechins eluted from the column were monitored by thin layer chromatography (TLC) on silica gel plates (Sigma, St. Louis, MD) with a chloroform-methanol-water (65:35:10, v/v/v, lower phase) mobile phase (TANIZAWA *et al.* 1984), and with vanillin-hydrochloric reagent for the detection (AMAROWICZ & SHAHIDI 1996). For the separation of catechins from a Sephadex LH-20 fraction which showed the presence of EGCG, a semi-preparative Shimadzu HPLC system was used: LC-6A pump, SPD-6AV UV-VIS spectrophotometric detector, SCL-6B system controller and CR 501 Chromatopac. The conditions of separation were as follows: semi-preparative Hilbar pre-packed column RT (10 × 250 mm) with Lichrosorb RP-18 (7 µm) (Merck, Darmstadt, Germany); mobile phase water-dimethylformamide-methanol-acetic acid (157:40:2:1, v/v/v/v) (HOEFLER & COGGON 1976); flow rate 3 ml/min; injection volume 500 µl; the detector was set at 280 nm.

Mass spectrum of methylated EGCG. The electrospray ionization (ESI) mass spectrum (negative-ion mode) was recorded with a Micromass VG-Quattro II quadrupole-hexapole-quadrupole mass spectrometer (Micro-mass, Cheshire, UK).

¹H-NMR spectrum of methylated EGCG. The ¹H-NMR spectrum of methylated EGCG was obtained at 300 MHz with a GN-300 spectrometer (General Electric, Paolo Alto, CA, USA) and recorded at room temperature in DMSO-d₆. Chemical shifts were reported relative to tetramethylsilane as the internal standard.

TLC analysis. Silica gel (layer thickness 0.20 mm; Sigma) and C₁₈-silanised silica gel (layer thickness 0.20 mm; Merck) plates without the activation procedure were used for TLC analysis. Chromatograms were developed using various mobile phase systems (Table 1) in a chromatographic chamber (12 × 10 × 8 cm; Sigma) until the solvent front advanced 8 cm. The spots were located on the plate under UV lamp.

RESULTS AND DISCUSSION

The ES-MS spectrum of the separated compound was characterised by a negative ion [M-H]⁻ *m/z* 471 (Figure 2). Therefore, the MW of the separated compound was 472. The same MW was attributed to epigallocatechin-3-(3-*O*-methylgallate) which was separated by SAIJO (1982). In the ¹H-NMR spectrum, the singlet at 3.76 ppm was assigned to the methoxy group attached to the carbon atom at position 3 in the gallolyl ring. SAIJO (1982) observed in his study that the ¹H-NMR spectrum of epigallocatechin-3-(3-*O*-methylgallate) indicated the presence of a methoxy group at δ 3.82 ppm.

On applying TLC plates with silica gel and mobile phase No. 1–3 (listed in Table 1) for the separation of EGCG and methylated EGCG, the greatest differences between R_f of these compounds were obtained with phase 3 (chloroform-methanol-water; 65:35:10; v/v/v; lower phase) (Figure 3). For all phases used, the R_f value of EGCG was lower than that of methylated EGCG. This observation is in accordance with the theoretical expectation

Table 1. Condition of TLC separation of EGCG and methylated EGCG

No.	Composition of mobile phase	Plate	Literature
1	ethyl acetate-water-formic acid		NONAKA <i>et al.</i> (1983) (90:5:5; v/v/v)
2	toluene-acetone-formic acid	Silica	LEA (1978) (30:30:10; v/v/v)
3	chloroform-methanol-water		AMAROWICZ <i>et al.</i> (2003) (65:35:10; v/v/v; lower phase)
4	water-acetonitrile-methanol-acetic acid		SAIJO (1982) (79.5:18:2:0.5; v/v/v/v)
5	water-dimethylformamide-methanol-acetic acid	C ₁₈	HOEFLER and COGGON (1976) (157:40:2:1; v/v/v/v)
6	water-acetone-tetrahydrofuran		MATSUZAKI and HARA (1985) (78:12:10; v/v/v)

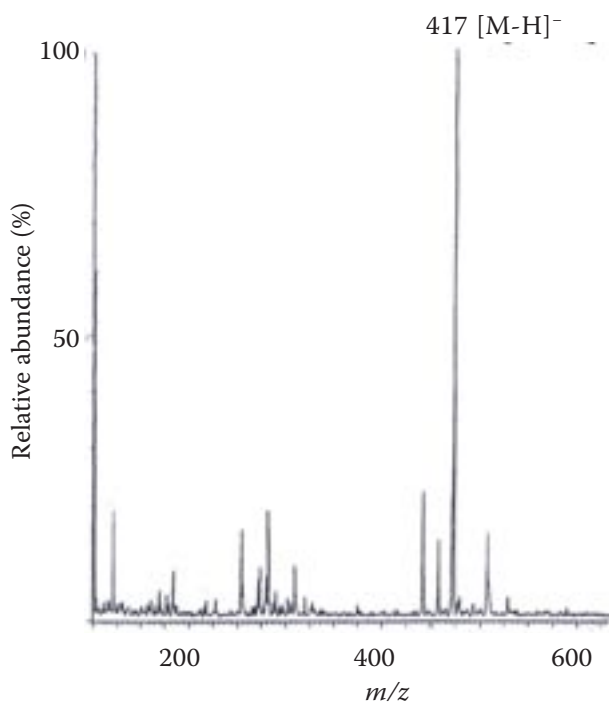


Figure 2. ESI-MS spectra of methylated EGCG

because methylation of the hydroxyl group in the EGCG molecule reduces its polarity.

The distance covered by catechins in chromatography on the plate with octadecylsilylated silica gel was much lower than that recorded for the TLC in the normal phase. Phase 4 (water-acetonitrile-methanol-acetic acid; 79.5:18:2:0.5; v/v/v/v) offered the best separation of EGCG and methylated EGCG (Table 2, Figure 3): the R_f values of EGCG and methylated EGCG were 0.36 and 0.23, respectively. For phases No. 4–6 the reversed effect was noted, i.e. the R_f values of EGCG were higher than those of methylated EGCG.

Table 2. R_f values of TLC separation of EGCG and methylated EGCG

Number of mobile phase	Plate	EGCG	Methylated EGCG
1		0.88	0.92
2	Silica	0.61	0.68
3		0.62	0.73
4		0.36	0.23
5	C ₁₈	0.23	0.15
6		0.20	0.14

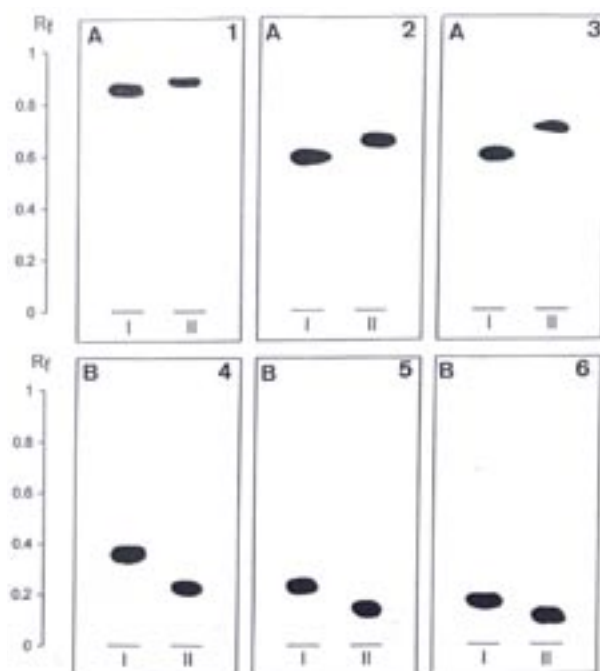


Figure 3. TLC chromatograms of EGCG and methylated EGCG (A – silica gel plates; B – reversed phase plates; 1–6 – mobile phases reported in Table 1)

In conclusion, due to the best separation results and from the economical point of view (silica gel TLC plates are cheaper than C₁₈ plates), we recommend silica gel TLC with chloroform-methanol-water (65:35:10) as the mobile phase as the best method for monitoring column chromatography during the separation of methylated EGCG from the crude extract of catechins in green tea.

References

- AMAROWICZ R., SHAHIDI F. (1995): Antioxidant activity of green tea catechins in a β -carotene-linoleate model system. *Journal of Food Lipids*, **2**: 47–56.
- AMAROWICZ R., SHAHIDI F. (1996): A rapid chromatographic method for separation of individual catechins from green tea. *Food Research International*, **29**: 71–76.
- AMAROWICZ R., PEGG R.B., BAUTISTA D.A. (2000): Antibacterial activity of green tea polyphenols against *Escherichia coli* K12. *Nahrung/Food*, **44**: 60–62.
- AMAROWICZ R., SHAHIDI F. (2003): Presence of two forms of methylated (–)-epigallocatechin-3-gallate in green tea. *Nahrung/Food*, **47**: 21–23.
- AMAROWICZ R., SHAHIDI F., WICZKOWSKI W. (2003): Separation of individual catechins from green tea using silica gel column chromatography and HPLC. *Journal of Food Lipids*, **10**: 165–177.

- BUSHMAN J.L. (1998): Green tea and cancer in humans: A review of the literature. *Nutrition and Cancer*, **31**: 151–159.
- CHEN C.-W., HO C.-T. (1995): Antioxidant properties of polyphenols extracted from green and black teas. *Journal of Food Lipids*, **2**: 35–46.
- DREOSTI I.E., WARGOVICH M.J., YANG C.S. (1997): Inhibition of carcinogenesis by tea: The evidence from experimental studies. *Critical Review of Food Science and Nutrition*, **37**: 761–770.
- FUKAI K., ISHIGAMI T., HARA Y. (1991): Antibacterial activity of tea polyphenols against phyto-pathogenic bacteria. *Agricultural and Biological Chemistry*, **55**: 1895–1897.
- GUO Q., ZHAO B., SHEN S., HOU J., HU J., XIN W. (1999): ESR study on the structure antioxidant activity relationship of tea catechins and their epimers. *Biochimica et Biophysica Acta (BBA) – General Subjects*, **1427**: 13–23.
- HOEFLER A.C., COGGON P. (1976): Reversed-phase high-performance liquid chromatography of tea constituents. *Journal of Chromatography*, **129**: 460–463.
- HOLLMAN P.C.H., PESKENS E.J.M., KATAN M.B. (1999): Tea flavonols in cardiovascular disease and cancer epidemiology. *Proceedings of the Society for Experimental Biology and Medicine*, **220**: 198–202.
- KAMAL G.M., GUNAHERATH B., GUNATILAKA L.A.A., SULTANBAWA U.M., BALASUBRA-MANIAM S. (1982): Dulcitol and (–)-4'-*O*-methylepigallocatechin from *Kokoona zeylanica*. *Journal of Natural Products*, **45**: 140–142.
- LEA A.G.H. (1978): The phenolics of ciders: oligomeric and polymeric procyanidins. *Journal of the Science of Food and Agriculture*, **29**: 471–479.
- MATSUZAKI T.L., HARA Y. (1985): Antioxidative activity of tea leaf catechins. *Nippon Nogeikaku Kaishi*, **59**: 129–134.
- MONACHE D.F., D'ALBUQUERQUE I.L., FERRARI F., MARINI-BETTOLO G.B. (1967): A new catechin and a dimeric proanthocyanidin from *Ouratea* sp. *Tetrahedron Letters*, **43**: 4211–4214.
- MONACHE D.F., POMPONI M., MARINI-BETTOLO G.B., D'ALBUQUERQUE I.L., DE LIMA O.G. (1976): A methylated catechin and proanthocyanidins from the *Celastraceae*. *Phytochemistry*, **15**: 573–574.
- MORIMOTO S., NONAKA G., NISHIOKA I., EZAKI N., TAKIZAWA N. (1985): Tannins and related compounds. XXIX. Seven new methyl derivatives of flavan-3-ols and a 1,3-diarylpropan-2-ol from *Cinnamomum cassia*, *C. obtusifolium* and *Lindera umbellata* var. *membranacea*. *Chemical and Pharmaceutical Bulletin (Tokyo)*, **33**: 2281–2286.
- NONAKA G., KAWAHARA O., NISHIOKA I. (1983): Tannins and related compounds. XV. A new class of dimeric flavan-3-ol gallates, theasinensins A and B, and proanthocyanidin gallates from green tea leaf. *Chemical and Pharmaceutical Bulletin (Tokyo)*, **31**: 3906–3914.
- PRICE W.E., SPITZER J.C. (1993): Variation in the amounts of individual flavanols in a range of green tea. *Food Chemistry*, **47**: 271–276.
- SAIJO R. (1982): Isolation and chemical structures of two new catechins from fresh tea leaf. *Agricultural and Biological Chemistry*, **46**: 1969–1970.
- SANO M., SUZUKI M., MIYASE T., YOSHINO K., MAEDA-YAMAMOTO M. (1999): Novel antiallergic catechin derivatives isolated from oolong tea. *Journal of Agricultural and Food Chemistry*, **47**: 1906–1910.
- TANIZAWA H., TODA S., SAZUKA Y., TANIYAMA T., NAYASHI T., ARICHI S., TAKINO Y. (1984) Natural antioxidants. I. Antioxidative components of tea leaf (*Thea sinensis* L.). *Chemical and Pharmaceutical Bulletin (Tokyo)*, **32**: 2011–2014.
- YEN G.-C., CHEN H.-Y. (1995): Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agricultural and Food Chemistry*, **43**: 27–32.

Received for publication December 7, 2004
Accepted after corrections January 13, 2005

Corresponding author:

Doc. Dr. RYSZARD AMAROWICZ, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Division of Food Science, Department of Food Chemistry, ul. Tuwima 10, P.O. Box 55, 10-718 Olsztyn, Poland
tel.: + 89 523 26 27, fax: + 89 524 01 24, e-mail: amaro@pan.olsztyn.pl
