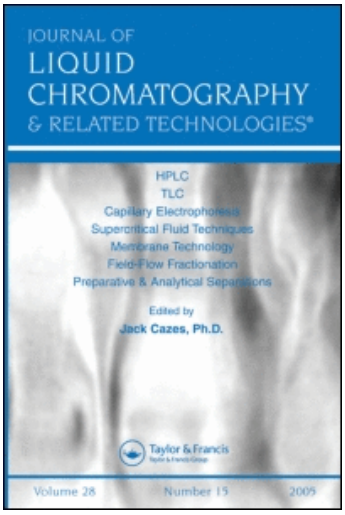


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

IDENTIFICATION AND DETERMINATION OF POLYPHENOLS IN TEA BY LIQUID CHROMATOGRAPHY WITH MULTI-CHANNEL ELECTROCHEMICAL DETECTION

Hong Long[a]; Yongxin Zhu[a]; Tiehua Huang[a]; L. A. Coury[a]; Peter T. Kissinger[a]
[a] Bioanalytical Systems, Inc., West Lafayette, IN, U.S.A.

Online publication date: 30 April 2001

To cite this Article Long, Hong , Zhu, Yongxin , Huang, Tiehua , Coury, L. A. and Kissinger, Peter T.(2001) 'IDENTIFICATION AND DETERMINATION OF POLYPHENOLS IN TEA BY LIQUID CHROMATOGRAPHY WITH MULTI-CHANNEL ELECTROCHEMICAL DETECTION', Journal of Liquid Chromatography & Related Technologies, 24: 8, 1105 — 1114
To link to this Article: DOI: 10.1081/JLC-100103434
URL: http://dx.doi.org/10.1081/JLC-100103434

J. LIQ. CHROM. & REL. TECHNOL., 24(8), 1105–1114 (2001)

IDENTIFICATION AND DETERMINATION OF POLYPHENOLS IN TEA BY LIQUID CHROMATOGRAPHY WITH MULTI-CHANNEL ELECTROCHEMICAL DETECTION

Hong Long, Yongxin Zhu, Tiehua Huang, L. A. Coury, and Peter T. Kissinger*

Bioanalytical Systems, Inc., 2701 Kent Avenue,
West Lafayette, IN 47906, USA

ABSTRACT

A liquid chromatography method with multi-channel electrochemical detection is reported for the identification and determination of polyphenols in green tea, black tea, and extracted green tea powder. Following a simple aqueous extraction, (−)-epigallocatechin (EGC), (+)-catechin (C), (−)-epicatechin (EC), (−)-epigallocatechin gallate (EGCG), (−)-gallocatechin gallate (GCG), (−)-gallocatechin (GC), (−)-epicatechin gallate (ECG), and (−)-catechin gallate (CG) were successfully separated by reversed phase liquid chromatography within 25 min.

The detection limits (S/N=3) of the polyphenols studied were 0.1–1.5 ng/mL at a potential of +900mV. The calibration curve was linear over the range of 1–1000 ng/mL for EGC, C, EC, EGCG, GCG, and GC, 5–1000 ng/mL for ECG and CG. Using this method, the concentration of these eight polyphenols in five

* Corresponding author.

different brands of green tea, one brand of black tea, and extracted green tea powder were determined.

INTRODUCTION

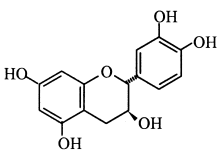
Tea is one of the most highly consumed beverages in the world. Drinking tea, especially green tea, is widely thought to benefit human health. Tea contains substantial amounts of polyphenols (tea catechins) that have unique biological activities and may be responsible for many of its health benefits.[1] Eight major catechins known to display biological activity are found in tea (Figure 1). These catechins are strong antioxidants[2,3] and have been reported to exhibit numerous biological activities including inhibition of carcinogenesis,[4–6] tumorigenesis,[7,8] and angiogenesis.[9]

Drs. James and Dorothy Morré at Purdue University have found that tea catechins appear to belong to a larger class of antioxidants that block a specific cancer cell surface protein (designated as tNOX) that is associated with cell enlargement.[10] The phenolic compounds capsaicin and vanillylamine were shown to belong to this group,[11] as was the aniline derivative N-(4-methylphenylsulfonyl-N'-chlorophenylurea.[12] When tNOX sites are blocked by an antioxidant, the cancerous cells fail to grow, and undergo programmed cell death (apoptosis) within a few days.[12] There is no affect on normal, healthy cells, which all lack tNOX on their surfaces.

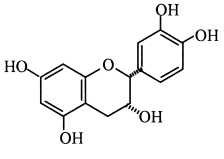
Our laboratory is interested in studying the absorption, metabolism, and excretion of a variety of nutraceutical compounds, including antioxidants. The interactions such substances may have with synthetic drugs and other xenobiotics is potentially important. One aspect of our work involves a collaboration with the Morré's group at Purdue to develop robust and sensitive analytical assays for tea catechin compounds.

Since increasing attention has been paid to the beneficial function of tea polyphenols, it is important to establish methods to determine the various catechins in both tea leaves and biological samples after administration of tea or tea catechins. Methods have been reported for determination of catechins in tea[13–17] and biological samples.[18–21] LC methods are the most widely utilized. Recently, successful analysis of green tea catechins has been developed using capillary electrophoresis (CE).[22] Kang et al. reported a RP-LC method to prepare EGCG from Korean green tea.[23] Most LC methods used gradient elution with UV detection. Few methods have resolved the EGCG cis-isomer from the GCG trans-isomer.

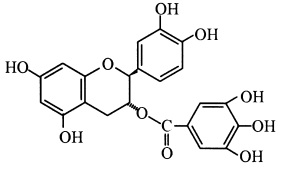
LC/electrochemistry (LCEC) has been shown to be selective and sensitive for the determination of phenolic compounds in natural sources.[24–26] In this paper, an isocratic reversed phase LC method with multi-channel electrochemical detection is reported for the separation of eight catechins in tea, including the EC trans-isomer from the C cis-isomer and the EGCG cis-isomer from the GCG trans-isomer.



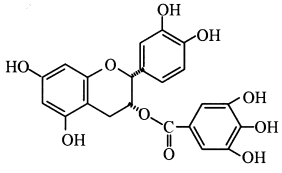
(+) - Catechin (C)



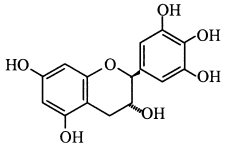
(-) - Epicatechin (EC)



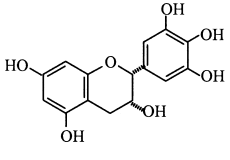
(-) - Catechin gallate (CG)



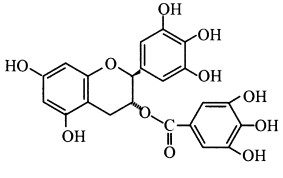
(-) - Epicatechin gallate (ECG)



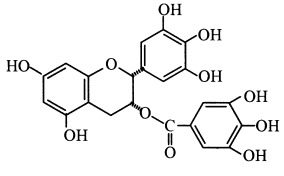
(-) - Gallocatechin (GC)



(-) - Epigallocatechin (EGC)



(-) - Gallocatechin gallate (GCG)



(-) - Epigallocatechin gallate

Figure 1. Structures of tea polyphenols.

EXPERIMENTAL

Apparatus

The LCEC system was composed of a chromatographic pump (PM-92e, BAS, West Lafayette, IN, USA) coupled with a Rheodyne injection valve (Model 7125), a 5 μm C_8 column (PEEK, 150 × 2.0 mm, BAS), and a multi-channel amperometric detector (Epsilon^{tm}, BAS) coupled to four glassy carbon working electrodes in a radial flow thin-layer cell.[27] Potentials were referenced to a Ag/AgCl (BAS) electrode. Data were acquired and integrated with BAS Chrom-Graph version 9.35 chromatography software.

Chemicals and Reagents

(−)-Epigallocatechin (EGC), (+)-catechin (C), (−)-epicatechin (EC), (−)-epigallocatechin gallate (EGCG), (−)-gallocatechin gallate (GCG), (−)-gallocatechin (GC), (−)-epicatechin gallate (ECG), and (−)-catechin gallate (CG) were purchased from Sigma (St. Louis, MO). Acetonitrile was of HPLC grade (Burdick &Jackon, Muskegon, MI).

Reagent grade water was prepared from in-house deionized water using a NANOpure system (Barnstead/Thermolyne, Dubuque, IA). Chloroacetic acid (MCAA) of analytical grade was purchased from Aldrich (Milwaukee, WI).

Luan Guapian, Longjin, and Maofeng are three brands of green tea from China. Suntory and Dynasty are two brands of green tea from Japan and Hoji-cha is a black tea from Japan. Sunphenon is one brand of extracted green tea powder from Japan.

Preparation of Standard Stock Solutions

Stock solutions of EGC, C, EC, EGCG, GCG, GC, ECG, and CG were prepared in 10 mM HCl at a concentration of 1.0 mg/mL. Less concentrated solutions were prepared, as needed, by dilution with distilled water. The stock solutions were kept in the dark at 4°C when not in use.

Sample Preparation

The tea samples were prepared using an aqueous extraction procedure. A 50 mg sample of dry tea leaves was steeped in 10 mL 80°C water for 10 minutes, ultrasonicated a further 10 minutes, filtered through a 0.45 μm Nylon filter,

diluted 1000 times, and injected. The extracted green tea powder was dissolved in distilled water, filtered, diluted, and injected.

Preparation of Calibration Curve Solutions

To quantify the eight catechins in tea, a calibration curve was prepared for each by diluting the 1.0 mg/mL stock solution with distilled water to yield final concentrations of 1, 5, 10, 50, 100, 200, 400, 600, 800, 1000 ng/mL. A 20 μL volume of standard was injected into the chromatograph.

RESULTS AND DISCUSSION

Extraction Efficiency

In most papers concerned with the amounts of polyphenols in tea leaves, the "infusion method" (brewing tea leaves with hot water) was used. Another paper determined EGCG in various tea samples after extraction with 50% methanol.[28] We compared brewing with water and extracting with 50% and 100% methanol. Results showed that the brewing method has the highest efficiency. It is also consistent with the way tea is prepared in daily life.

More catechins are extracted into water from tea leaves as brewing time increases, as reported by Bronner and Beecher.[15] So, in order to get the highest extraction efficiency in a reasonable time, in this work, tea samples were ultrasonicated (10 minutes) after brewing (10 minutes).

Multi-channel Electrochemical Detection

In most previous reports on analysis of tea catechins, UV detection was employed. These compounds are all electroactive reducing agents, so they can be detected more selectively by electrochemical detection. Dual-channel electrochemical detection has been used for the identification of antioxidants.[24] Figure 2 shows the hydrodynamic voltammogram (HDV) of EGCG in the mobile phase (pH=2.8). The other seven polyphenols show a similar pattern.

A four electrode detector was used to identify and quantitate the catechins in different tea samples. Using a radial flow pattern, an equal flow with the same concentration of the analyte passed over all four electrodes in the thin-layer channel.[29] The potentials were set at four different values. Monitoring four potentials gives a better voltammetric characterization of the catechins. By comparing ratios at different energies between standards and samples, one can confirm the

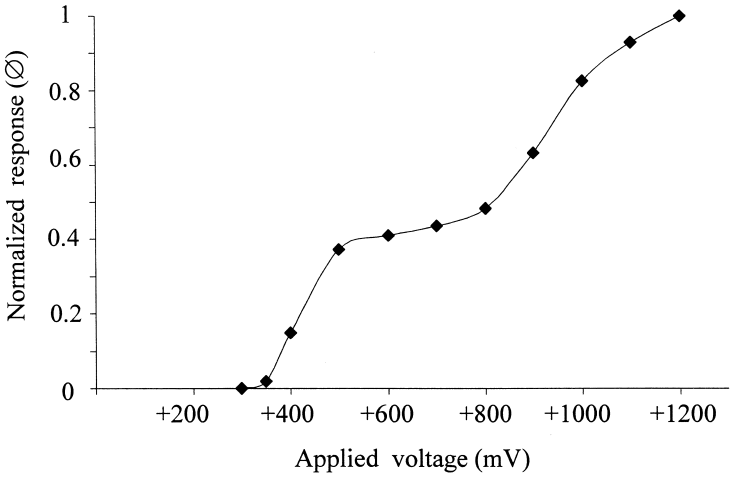


Figure 2. Hydrodynamic voltammogram of EGCG in the mobile phase of choice.

peak identity and assure peak purity using analogous techniques to those for diode array UV detection. Table 1 lists some of the ratios.

Analytical Method Development

Several mobile phase combinations of MCAA, methanol, and/or acetonitrile were studied. It was found that acetonitrile gave a better separation than methanol. Better peak shape resulted from lowering the buffer pH. An isocratic reversed phase separation was optimized for the eight catechins using a mobile phase containing 20 mM MCAA (pH=2.8) and 11% acetonitrile. All tea samples tested exhibited a similar chromatographic pattern. Figure 3 shows chromatograms at +800 mV for the standard mixture and one tea sample (Sunphenon).

Limit of Detection and Calibration

The detection limit (S/N=3) and linear range of the eight catechins were investigated. The detection limit for all analytes was 0.1–1.5 ng/mL at a potential of +900 mV. Calibration was performed over 1–1000 ng/mL for EGC, C, EC, EGCG, GCG, and GC, 5–1000 ng/mL for ECG and CG. The calibration curves

Table 1. Ratios of Peak Area at Different Applied Potentials

	STD	1	2	3	4	5	6	7
				A_{400mV}/A_{900mV}				
EGC	0.36	0.37	0.37	0.36	0.38	0.36	0.37	0.37
EC	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
EGCG	0.26	0.28	0.27	0.28	0.26	0.26	0.27	0.26
GCG	0.28	0.30	0.30	0.27	0.29	0.27	0.28	0.27
ECG	0.03	*	0.01	*	0.02	*	0.02	0.03
				A_{600mV}/A_{900mV}				
EGC	0.50	0.51	0.52	0.53	0.52	0.50	0.53	0.51
C	0.62	0.63	0.63	0.63	0.64	0.61	0.63	0.60
EC	0.68	0.67	0.66	0.67	0.67	0.66	0.69	0.70
EGCG	0.74	0.75	0.76	0.75	0.73	0.73	0.72	0.73
GCG	0.60	0.60	0.59	0.58	0.60	0.61	0.59	0.62
GC	0.77	0.78	0.75	0.79	0.75	*	*	0.77
ECG	0.65	0.64	0.64	0.65	0.64	0.66	0.67	0.66
CG	0.63	*	*	*	*	0.62	*	0.62
				A_{800mV}/A_{900mV}				
EGC	0.67	0.68	0.66	0.65	0.69	0.69	0.68	0.67
C	0.91	0.93	0.91	0.90	0.91	0.92	0.93	0.92
EC	0.72	0.72	0.71	0.71	0.72	0.73	0.72	0.73
EGCG	0.92	0.93	0.92	0.92	0.91	0.93	0.91	0.93
GCG	0.99	0.98	0.99	0.98	0.97	0.97	0.98	0.99
GC	0.97	0.98	0.95	0.95	0.98	*	*	0.98
ECG	0.78	0.76	0.77	0.79	0.77	0.79	0.79	0.79
CG	0.77	*	*	*	*	0.76	*	0.76

* Not detected; 1: Luan Guapian; 2: Longjin; 3: Maofeng; 4: Suntory; 5: Hoji-cha (black tea); 6: Dynasty; 7: Sunphenon.

were obtained by linear regression of peak height versus concentration. Correlation coefficients r^2 were over 0.9996 for all compounds.

Quantitative Determination of the Polyphenols

The concentration of polyphenols in green tea, black tea, and green tea powder was determined by comparison with a standard solution (Table 2), RSD (n=3) to be less than 5.0%. EGCG and C were determined at +400 mV while the others were determined at +900 mV at the same time. It can be seen that EGCG contributed the most to tea catechins. Differences in the content of polyphenols

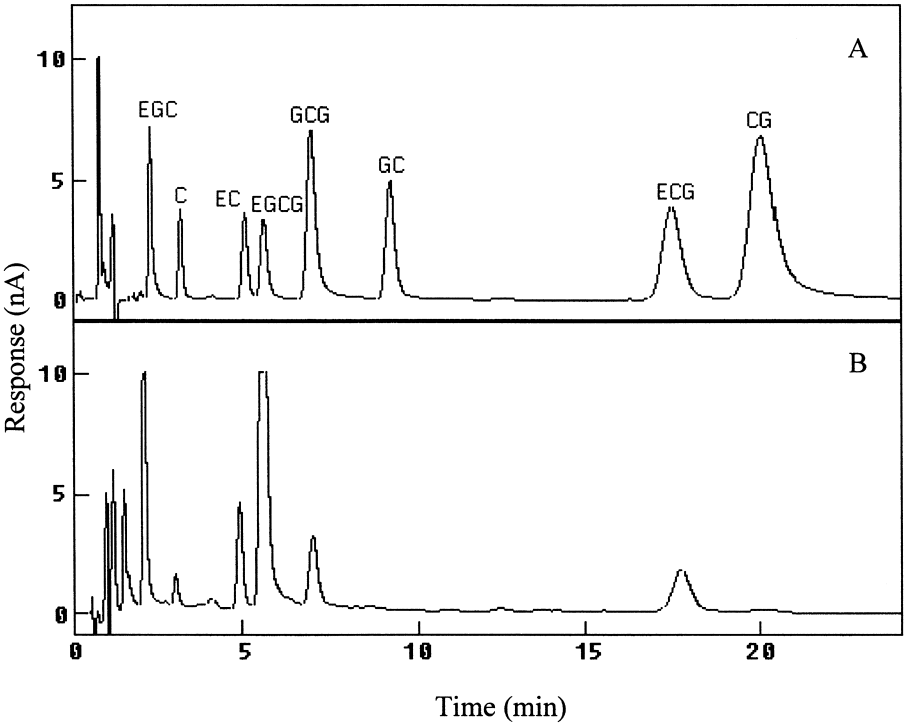


Figure 3. Chromatograms of standard mixture and one tea sample at + 800 mV. (A) Standard mixture; (B) Green tea extract: Sunphenon.

Table 2. Concentration of Polyphenols in Tea (mg/g)

Tea	EGC	C	EC	EGCG	GCG	GC	ECG	CG
1	10.0	2.0	2.8	93.9	0.8	0.3	11.5	*
2	5.1	5.4	4.2	63.2	1.8	0.8	17.9	*
3	3.6	9.0	2.5	64.7	1.1	1.4	17.3	*
4	16.5	1.3	7.2	47.8	1.2	0.2	10.6	*
5	0.7	0.8	0.6	2.6	2.3	*	1.2	1.3
6	18.3	1.2	7.7	52.4	0.7	*	11.1	*
7	19.6	4.0	18.0	95.0	9.3	0.3	22.1	2.2

* Not detected; 1: Luan Guapian; 2: Longjin; 3: Maofeng; 4: Suntory; 5: Hoji-cha (black tea); 6: Dynasty; 7: Sunphenon.

for different tea samples are expected because of species, weather, soil, and the way the tea leaves were processed. For black tea, freshly picked leaves are withered indoors and allowed to oxidize. For green tea, the leaves are not oxidized, but are steamed and parched to better preserve the natural active substance of the leaf. Green tea contains far more polyphenols than black tea and is, therefore, thought to be advantageous.

CONCLUSION

It has been demonstrated that liquid chromatography/electrochemistry with multi-channel detection is suitable for the determination of tea catechins in various tea samples. The method developed here is simple and sensitive. Eight catechins can be separated within 25 min with minimal sample pretreatment. Our goal is to adapt this methodology to blood samples so that oral absorption of tea catechins can be studied.

ACKNOWLEDGMENTS

Thanks to Dr. Kazuaki Yoshioka at the Tokyo Metropolitan Institute for Neuroscience for supplying the extracted green tea powder sample, and to Drs. James and Dorothy Morré, Purdue University, for useful discussions and for providing some tea extracts.

REFERENCES

1. Ahmad, N; Mukhtar, H. Nutr. Rev. **1999,** *57*, 78–83.
2. Salah, N.; Miller, N.J.; Paganga, G.; Tijburg, L.; Bolwell, G.P.; Rice-Evans, C. Arch. Biochem. Biophys. **1995,** *322*, 339–346.
3. Dreosti, I.E. Nutr. Rev. **1996,** *54*, S51–S58.
4. Agarwal, R.; Mukhtar, H. *Dietary Phytochemicals in Cancer Prevention and Treatment*; Plenum Press:New York, 1996; 35–50.
5. Mukhtar, H.; Wang, Z.Y.; Katiyar, S.K.; Agarwal, R. Prev. Med.**1992**, *21*, 351–360.
6. Kuroda, Y.; Hara, Y. Mutat. Res. **1999**, *436*, 69–97.
7. Wang, Z.Y.; Khan, W.A.; Bickers, D.R.; Mukhtar, H. Carcinogenesis **1989**, *10*, 411–415.
8. Conney, A.H.; Wang, Z.Y.; Huang, M.T.; Ho, C.T.; Yang, C.S. Prev. Med. **1992**, *21*, 361–369.
9. Cao, Y.H.; Cao, R.H. Nature **1999,** *398*, 381–382.

10. Morré, D.J. Biohcim. Biophys. Acta **1995,** *1240*, 201–208.
11. Morré, D.J.; Chueh, P.J.; Morré, D.M. Proc. Natl. Acad. Sci. USA **1995,** *92*, 1831–1835.
12. Morré, D.J.; Jacobs, E.; Sweeting, M.; de Cabo, R.; Morré, D.M. Biochim Biophys. Acta **1997,** *1325*, 117–125.
13. Khokhar, S.; Venema, D.; Hollman, P.C.; Dekker, M. W.; Jongen, Cancer Lett **1997,** *114*, 171–172.
14. Dalluge, J.J.; Nelson, B.C.; Thomas, J.B.; Sander, L.C. J. Chromatogr. A **1998,** *793*, 265–274.
15. Bronner, W.E.; Beecher, G.R. J. Chromatogr. A **1998,** *805*, 137–142.
16. Ding, M.Y.; Yang, H.J.; Xiao, Sh.Q. J. Chromatogr. A **1999,** *849*, 637–640.
17. Khokhar, S.; Venema, D.; Hollman, P.C.; Dekker, M.; Jongen, W. Cancer Lett. **1997,** *114*, 171–172.
18. Maiani, G.; Serafini, M.; Salucci, M.; Azzini, E.; Ferro-Luzzi, A. J Chromatogr. B Biomed. Sci. Appl. **1997,** *692*, 311–317.
19. Tsuchiya, H.; Sato, M.; Kato, H.; Okubo, T.; Juneja, L.R.; Kim, M. J. Chromatogr. B **1997,** *703*, 253–258.
20. Tsuchiya, H.; Sato, M.; Kato, H.; Kureshiro, H.; Takagi, N. Talanta **1998,** *46*, 717–726.
21. Dalluge, J.J.; Nelson, B.C.; Thomas, J.B.; Welch, M.J.; Sander, L.C. Rapid Commun. Mass Spectrom **1997,** *11*, 1753–1756.
22. Horie, H.; Mukai, T.; Kohata, K. J. Chromatogr. A **1997,** *758*, 332–335.
23. Kang, J.H.; Chung, S.T.; Go, J.H.; Row, K.H. J. Liquid Chrom. & Rel. Technol. **2000,** *23*, 2739–2749.
24. Roston, D.A.; Kissinger, P.T. Anal. Chem. **1981,** *53*, 1695–1699.
25. Lunte, C.E.; Kissinger, P.T. Anal. Chem. **1985,** *57*, 1546–1552.
26. Lunte, S.M.; Blankenship, K.D.; Read, S.A. Analyst **1988,** *113*, 99–102.
27. Zhu, Y.X.; Coury, L.A.; Long, H.; Duda, C.T.; Kissinger, C.B.; Kissinger, P.T. J. Liquid Chrom. & Rel. Technol. **2000,** *23*, 1555–1564.
28. Sakata, I.; Ikeuchi, M.; Maruyama, I.; Okuda, T. Yakugaku Zasshi **1991,** *111*, 790–793.
29. Solomon, B.P.; Long, H.; Zhu, Y.X.; Gunaratna, Ch.; Coury, L. Curr. Sep'ns. **2000,** *18*, 113–116.

Received October 31, 2000 Manuscript 5430
Accepted November 20, 2000