JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. 2003, 51, 1141−1147 1141

Fast Determination of Catechins and Xanthines in Tea Beverages by Micellar Electrokinetic Chromatography

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Antioxidant properties and stimulating effects of green tea are related to its content of cathechins and xanthines; tea quality evaluation is based on organoleptic tests and on the presence of those components. In this work, by a MEKC method, eight cathechins and three xanthines were quantified in some tea-based beverages. The best separation was realized using a phosphate−borate running buffer, with sodium dodecyl sulfate as micellar agent. A 40 cm capillary, a temperature of 29 °C, a voltage of 30 kV, and UV detection at 200 nm were used. The method showed a very good sensitivity (limit of detection ranging from 0.0011 to 0.0051 μg/mL) and was applied to real tea samples to characterize their antioxidant content. Statistical studies were performed and showed a satisfactory reliability of the data.

KEYWORDS: Capillary electrophoresis; tea leaves; polyphenols; catechins; xanthines

INTRODUCTION

Tea is one of the most widely consumed beverages in the world. Its popularity is related either to its taste or to evidence of a relationship between tea consumption and prevention of several forms of human diseases (*1*−*8*).

Tea is made from tender leaves of different varieties of *Camellia sinensis*. Many kinds of tea are produced, although these can be classified principally into three types: green (unfermented), oolong (semifermented), and black (fully fermented). Green tea is an excellent source of polyphenolic antioxidants, which are mostly represented by a group of compounds having a flavan-3-olic structure and which are generally defined as green tea catechins (GTCs). The catechins that are mainly represented in green tea are seven: (−)-gallocatechin (GC), (−)-epigallocatechin (EGC), (+)-catechin (C), (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EC), (−)-gallocatechingallate (GCG), and (−)-epicatechingallate (ECG). These substances show in vivo and in vitro a strong antioxidant activity and various properties, on human health, such as antibacterial, antiviral, antiallergic, antiatherogenic, and anticarcinogenic activities as described in the literature (*1*−*8*). In recent works tea intake was associated with a lower risk of myocardial infarction (*7*), and the role of tea catechins was investigated in the prevention of atherosclerosis in apoprotein E-deficient mice (*8*).

Whereas green tea is made by inactivating the enzymes in fresh leaves to prevent the enzymatic oxidation of catechins, black tea is made by polyphenol oxidase catalyzed oxidation of fresh leaves. This fermentation process results in the oxidation of simple polyphenols to more complex condensed molecules, which give black tea its typical color and strong, astringent, flavor. Traditionally, the pigments of black tea are divided into orange theaflavins (TFs) and brownish thearubigins (TRs). Oolong tea is a semifermented product; its characteristics are intermediate between those of green and black teas.

The stimulating effects of tea are due to its natural xanthines content, as caffeine (Caf), theobromine (TB), and theophylline (TP). The xanthines have a wide range of therapeutic activity: theophylline is a bronchodilator, respiration stimulant, and smooth muscle relaxant; caffeine is a central nervous system stimulant; and theobromine and theophylline are diuretics. Tea leaves contain caffeine and lower amounts of theobromine, whereas their theophylline content is negligible. These alkaloids have been mainly analyzed by HPLC (*9*−*11*), but this method is time-consuming, and samples must be pretreated to reduce interference from other compounds.

Amounts of these 11 compounds (cathechins and xanthines) vary according to the type of species, climate, and cultivation and also the technologies applied during the extraction, concentration, and preservation process. The quality control of tea extracts and tea beverages must include the evaluation of the total amount of catechins and xanthines, and the quantitation of each isomer, by rapid and reproducible procedures.

This work purposes a rapid determination of eight tea catechins and three methylxanthines (caffeine, theophylline, and theobromine) in different commercial products and tea drinks, by micellar electrokinetic capillary chromatography. Several methods exist in the literature for the separation of eight tea catechins and three xanthines by HPLC and HPCE (*11*−*22*),

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10.1021/jf020907b CCC: $25.00 © 2003 American Chemical Society
Published on Web 01/31/2003

but the method optimized here led to complete and simultaneous separation of these 11 compounds, in a very short time (within 4.5 min). First of all, the performances of the MECK-UV method proposed have been evaluated on a standard mixture and, then, on a green tea extract (GTE), purified and concentrated in catechins; the procedure was finally applied to another six tea sample beverages.

The method is demonstrated to be simple, fast (~12 min for a whole analysis), reliable, and sensitive and did not require any sample cleanup pretreatment.

MATERIALS AND METHODS

Instrumentation. Electrophoretic analysis of GTCs and methylxanthines was carried out on a Beckman P/ACE 5000 model equipped with a UV–vis detector, an autosampler with two rotating trays, and a cartridge interface with a cooling option (Beckman Instruments, Inc., Fullerton, CA). Data acquisition and processing were accomplished using a PC equipped with Beckman P/ACE Station software. The capillary cartridge contained undeactivated fused silica tubing (50 μm i.d. × 375 μm o.d.) supplied from Beckman. Total capillary length was 47 cm, whereas effective length was 40 cm.

Reagents and Chemicals. Catechin standards (−)-gallocatechin (GC, 98%), (+)-catechin (C, 98%), (−)-epigallocatechin (EGC, 98%), (−)-epigallocatechin-3-gallate (EGCG, 95%), (−)-epicatechin (EC, purity not specified), (−)-epicatechingallate (ECG, 98%), (−)-gallocatechingallate (GCG), theophylline (TP, 1,3-dimethylxanthine, 99%), theobromine (TB, 3,7-dimethylxanthine, 99%), and gallic acid (GA, 99%) were purchased from Sigma Chemical Co. (St. Louis, MO). Anhydrous caffeine (Caf, 1,3,7-trimethylxanthine, 98%) was purchased from Carlo Erba (Milan, Italy). Sodium dodecyl sulfate for HPCE, water for HPCE, 0.1 M sodium hydroxide, 1 M sodium hydroxide, and 0.1 M hydrochloric acid were from Fluka (Neu-Ulm, Switzerland). Potassium dihydrogen phosphate and formic acid were purchased from Carlo Erba Reagents (Milan, Italy). HPLC grade water, HPLC grade acetone, and HPCE grade 50 mM sodium tetraborate (pH 9.3) were from Merck (Darmstadt, Germany).

Standard Mixture and Tea Sample Preparation. A standard mixture was prepared by mixing 100 μL of each single standard solution, having the following concentration: 500 μg/mL TB, 1000 μg/mL TP, 1000 μg/mL Caf, 500 μg/mL C, 200 μg/mL GC, 1000 μg/mL EGC, 1000 μg/mL EGCG, 1000 μg/mL GCG, 1000 μg/mL ECG, 1000 μg/mL EC, and 1000 μg/mL GA.

The samples used in this work were diluted, or prepared, as follows.

Green tea extract (GTE, Greenselect) was kindly donated by Indena (Milan, Italy) and was defined, by the productor, as an aqueous spray-dried extract with a caffeine content <0.1% (w/w, HPLC determination), having a polyphenol content >60% (w/w, HPLC determination), expressed as EGCG, and not less than 40% of EGCG. A solution of GTE (1000 μg/mL) was prepared by dissolving 1 mg of extract in 1 mL of an HPLC grade water/formic acid solution (99.7:0.3, v/v).

Three bottled green tea drinks (A–C), randomly selected, were purchased in a local market.

Two infusions of green tea leaves (D) and one of black tea leaves (E), purchased in a local market, were prepared by leaving one tea bag (Da), corresponding at 2 g of minced leaves, or 2 g of fresh dried tea leaves (Db and E) in 250 mL of oligomineral boiling water, for 5 min. These were similar to the brewing conditions for a cup of tea. Finally, the samples were diluted two times, with distilled water.

All of the diluted samples were added, before injection, with acetone (1%, v/v) as a marker because it does not interact with the micelles.

Method Evaluation. Each single standard of cathechin, or xanthine, was dissolved in HPLC grade water/formic acid (99.7:0.3, v/v), to obtain a range of concentrations useful for the calibration curve, from 5 to 1000 μg/mL. Each point of the calibration graph corresponded to the mean value, obtained from three standard injections. Detection limits (LODs) were estimated from the calibration curves (signal/noise, or S/N, =3).

The intra-assay precision (repeatability) was calculated from 10 consecutive determinations of the same GTE sample (GTE1); the interassay precision was calculated from five different samples. Both statistical parameters were reassessed 1 week later by using the same samples.

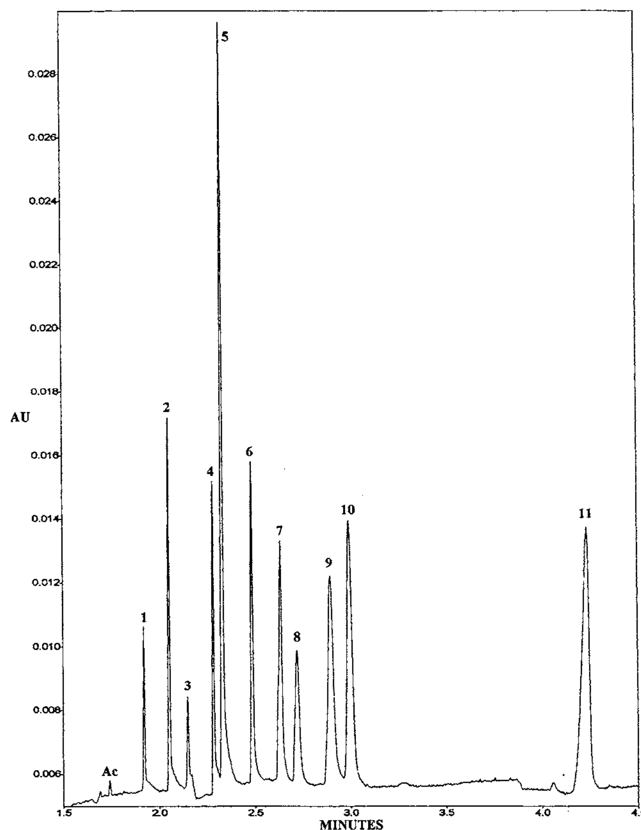

Figure 1. Electropherogram of the standard mixture. Peaks: Ac, acetone; 1, TB; 2, TP; 3, GC; 4, Caf; 5, C; 6, EGC; 7, EGCG; 8, GCG; 9, ECG; 10, EC; 11, GA. Running buffer: 10 mM KH_2PO_4, 8.3 mM sodium tetraborate, 66.7 mM SDS at pH = 7. Separation conditions: capillary, 47 cm (40 cm effective length) × 50 μm i.d. undeactivated fused silica; applied voltage, 30 kV; temperature, 29 °C; detection, 200 nm.

Micellar Electrokinetic Capillary Chromatography. New capillaries were conditioned by flushing a 1 M sodium hydroxide solution, for 5 min, a 0.1 M sodium hydroxide solution for 5 min, HPCE grade water for 5 min, and, finally, the running buffer for 5 min. The capillaries not in use were stored in water to prevent buffer crystallization. The running buffer was daily prepared by mixing three parts of 20 mM potassium dihydrogen phosphate (solid salt dissolved in HPLC grade water), one part of 50 mM sodium tetraborate, and two parts of 200 mM sodium dodecyl sulfate (solid salt dissolved in HPLC grade water). The mixture solution was adjusted at pH 7 (to minimize catechin degradation in basic conditions), by adding a 0.1 M hydrochloric acid, filtered through a cellulose acetate 0.2 μm syringe filter (Orange Scientific, Waterloo, Belgium), and then sonicated, for 10 min, in an ultrasonic bath.

Samples were hydrodynamically injected in the anodic end at low-pressure mode (0.5 psi) for 1 s.

Every electrophoretic run were carried out at 30 kV, for 5 min, maintaining the temperature of the capillary at 29 °C, resulting in a current of ~85 μA. Before each run, the capillary was rinsed at high pressure (20 psi), consecutively with a 0.1 M NaOH solution for 2 min and HPCE grade water for 2 min, and re-equilibrated with running buffer for 2 min. After each electrophoretic cycle, the capillary was rinsed with HPCE grade water for 2 min. Every step of washing was performed at 29 °C. The running buffer was changed after three electrophoretic cycles. The electrophoretic run is between the injection of the sample and the end of the applying of voltage, whereas the electrophoretic cycle is the sum of the electrophoretic run and washing steps.

UV detection was performed at 200 nm; rise time was set at 0.17 s.

Table 1. Calibration Curve Equations, Corresponding Correlation Coefficients, and Limits of Detection of Standard Compounds

analyte	concn[a] (μg/mL)	calibration eq[b]	slope error[c]	r^d	r^e	LOD[f] (μg/mL)
TB	5, 10, 50, 100, 200	$A = 89.855c - 135.94$	2.12	0.993	0.999	0.0017
TP	5, 10, 50, 100, 200	$A = 88.557c - 793.73$	1.30	0.997	0.995	0.0011
GC	5, 10, 50, 100, 200	$A = 131.22c - 30.759$	2.19	0.997	0.993	0.0013
Caf	5, 10, 50, 100, 200	$A = 76.866c + 247.11$	0.79	0.998	0.985	0.0019
C	5, 10, 50, 100	$A = 141.97c - 38.37$	3.41	0.992	0.998	0.0012
EGC	10, 50, 100, 200, 500, 1000	$A = 101.81c + 270.66$	0.70	0.999	0.996	0.0017
EGCG	10, 50, 100, 200, 500	$A = 112.11c + 1169.4$	2.56	0.999	0.998	0.0018
GCG	5, 10, 50, 100, 200, 500	$A = 100.51c - 850.26$	1.92	0.994	0.993	0.0033
ECG	10, 50, 100, 200, 500	$A = 180.95c - 1086.1$	1.23	0.999	0.995	0.0023
EC	10, 50, 100, 200, 500, 1000	$A = 166.31c - 469.18$	1.63	0.999	0.995	0.0023
GA	5, 10, 50, 100, 200	$A = 134.06c - 499.39$	0.73	0.999	0.997	0.0051

[a] Three injections for each. [b] Using peak area. [c] Standard error of the calibration curve slope. [d] Correlation coefficient of the calibration curves, using peak area. [e] Correlation coefficient of the calibration curves, using peak height. [f] Using peak height.

Table 2. Repeatability Study Realized Using the Standard Mixture ($n = 6$)

	area			MT[a]			RMT[b]		
analyte	mean	SD	%RSD	mean	SD	%RSD	mean	SD	%RSD
TB	35.5	2.30	**6.47**	1.92	0.007	**0.34**	0.91	0.001	**0.06**
TP	76.5	5.17	**6.79**	2.05	0.006	**0.31**	0.85	0.001	**0.08**
GC	12.9	0.44	**3.56**	2.13	0.004	**0.17**	0.82	0.003	**0.40**
Caf	79.9	1.48	**1.86**	2.28	0.009	**0.40**	0.76	0.001	**0.12**
C	139.5	8.37	**6.01**	2.32	0.007	**0.30**	0.75	0.000	**0.06**
EGC	81.15	4.29	**5.29**	2.50	0.017	**0.68**	0.70	0.003	**0.40**
EGCG	67.37	5.64	**7.06**	2.62	0.005	**0.19**	0.66	0.001	**0.15**
GCG	75.13	2.96	**3.94**	2.72	0.015	**0.55**	0.64	0.002	**0.28**
ECG	82.49	4.19	**5.09**	2.92	0.027	**0.93**	0.59	0.004	**0.66**
EC	97.12	4.47	**4.61**	3.03	0.023	**0.76**	0.57	0.004	**0.67**
GA	41.56	1.27	**3.07**	4.12	0.012	**0.29**	0.42	0.002	**0.50**
TOT	676.05	27.39	**3.97**						

[a] Migration time. [b] Relative migration time (acetone as reference compound).

RESULTS AND DISCUSSION

Optimization of the MEKC Method. The optimization of the MEKC method was performed using a green tea extract (GTE) containing eight main components: GC, C, EGC, EGCG, GCG, EC, ECG, and GA. From a previous work (*23*), performed in similar conditions (30 kV, 30 °C, 34.3 cm of effective length) on a ThermoQuest SpectraPHORESIS ULTRA capillary electrophoresis, we found that the best compromise, in terms of buffer composition, for the highest resolution, was obtained with a running buffer solution at pH 7, containing 10 mM potassium dihydrogenphosphate, 3.3 mM sodium tetraborate, and 66.7 mM sodium dodecyl sulfate. However, using the same conditions on a Beckman P/ACE 5000, employed in the present paper, the separation of the eight compounds was not satisfactory, being affected by the coelution of EGC and EGCG. Because many of the soluble species of tea are polyhydroxylated and they can undergo complexation with tetraborate, the increasing tetraborate concentration is known to be an additional tool to improve resolution of negatively charged species (*16*, *24*, *25*). After many trials (results not shown), we found that a different running buffer solution, constituted of 10 mM potassium dihydrogenphosphate, 8.3 mM sodium tetraborate, and 66.7 mM sodium dodecyl sulfate, adjusted at pH 7, with a borate concentration 2.5 times higher than the former, permitted the best separation conditions. In these conditions, the only polyphenol that underwent an increase of migration time, due to the borate raising, was gallic acid, the last compound eluted. Probably, its small dimension and high number of hydroxyl groups promoted a preferential complexation with tetraborate molecules that caused new larger negatively charged species. However, as shown in **Figure 1**, the migration time of gallic acid was ∼4.2 min, and a good separation of all 11 compounds considered was achieved within 4.5 min. It is possible to note that the only two compounds not completely separated at the baseline were caffeine and catechin; however, their content, in real samples, allows a correct quantification. Other parameters that may influence separation were investigated, as temperature and applied voltage, during running. Raising the temperature and the applied voltage lead to an increase in peak efficiency, but on the other hand, it might cause an increase in mobility of all the species and that may lead to overlapping of several peaks. The best compromise on reasonable migration times, without loss of resolution (**Figure 1**), was achieved at 30 kV and 29 °C, as shown in **Figure 1**.

Reliability and Repeatability Study: GTE Analysis. First of all, we carried out our repeatability study on a standard compounds mixture and, subsequently, on a green tea extract (GTE).

Table 1 shows statistical parameters of standard compounds calibration curves. Detection limits ranged from 0.0011 (μg/mL for TP) to 0.0051 (μg/mL for GA), which demonstrates the high sensitivity of capillary electrophoresis in the determination of catechins and xanthines (*14*, *17*, *19*, *20*).

To evaluate the precision of this method, the standard mixture was consecutively injected six times. The relative standard deviations (%RSD) ranged from 1.86 (Caf) to 7.06 (EGCG) being 3.97 the %RSD of total of the 11 compounds. The migration time %RSD was $<1\%$, whereas the relative migration time %RSD, using acetone as marker, ranged from 0.06% (TB, C) to 0.67% (EC) (see **Table 2**).

We also carried out a wider repeatability study on the GTE samples. As previously mentioned, the intra-assay precision

Table 3. Repeatability of GTE Samples

	first evaluation						after 1 week of evaluation					
	intra-assay ($n = 10$)			interassay ($n = 5$)			intra-assay ($n = 10$)			interassay ($n = 5$)		
analyte	mean[a]	SD	%RSD	mean[a]	SD	%RSD	mean[a]	SD	%RSD	mean[a]	SD	%RSD
						A. Quantification						
GC	**1.18**	0.07	5.64	**1.16**	0.11	9.44	**1.19**	0.13	10.72	**1.20**	0.03	2.75
C	**0.74**	0.04	5.63	**0.72**	0.04	5.00	**0.76**	0.03	4.27	**0.75**	0.01	1.03
EGC	**10.54**	0.91	8.6	**10.78**	0.75	6.92	**10.30**	0.78	7.57	**10.73**	0.25	2.36
EGCG	**35.60**	2.62	7.36	**34.75**	2.01	5.79	**37.18**	1.75	4.70	**37.26**	1.09	2.92
GCG	**1.14**	0.12	10.58	**1.20**	0.10	8.72	**1.15**	0.13	11.11	**1.22**	0.09	7.27
ECG	**8.98**	0.77	8.57	**8.86**	0.30	3.43	**9.72**	0.47	4.86	**9.78**	0.50	5.13
EC	**5.04**	0.42	8.37	**4.92**	0.28	5.76	**5.31**	0.13	2.50	**5.46**	0.13	2.42
GA	**0.07**	0.00	39.4	**0.10**	0.03	30.53	**0.08**	0.03	40.81	**0.11**	0.04	39.17
total	**63.06**	3.73	**5.92**	**62.41**	3.46	**5.54**	**65.45**	2.49	**3.81**	**66.55**	1.68	**2.53**
						B. Migration Time						
GC	**2.12**	0.01	**0.66**	**2.12**	0.01	**0.24**	**2.15**	0.02	**0.72**	**2.14**	0.01	**0.43**
C	**2.29**	0.02	**0.67**	**2.29**	0.01	**0.23**	**2.34**	0.02	**0.76**	**2.32**	0.01	**0.40**
EGC	**2.44**	0.02	**0.74**	**2.44**	0.01	**0.22**	**2.50**	0.02	**0.90**	**2.48**	0.01	**0.40**
EGCG	**2.58**	0.02	**0.83**	**2.58**	0.01	**0.26**	**2.64**	0.02	**0.84**	**2.62**	0.01	**0.52**
GCG	**2.65**	0.02	**0.92**	**2.65**	0.00	**0.16**	**2.71**	0.03	**0.93**	**2.69**	0.02	**0.67**
ECG	**2.82**	0.03	**0.94**	**2.82**	0.01	**0.23**	**2.90**	0.03	**1.07**	**2.88**	0.01	**0.48**
EC	**2.93**	0.03	**1.01**	**2.93**	0.01	**0.22**	**3.01**	0.04	**1.27**	**3.00**	0.01	**0.48**
GA	**4.08**	0.06	**1.41**	**4.02**	0.03	**0.65**	**4.11**	0.05	**1.13**	**4.06**	0.03	**0.75**
						C. Relative Migration Time						
GC	**0.81**	0.00	**0.29**	**0.81**	0.00	**0.08**	**0.81**	0.00	**0.35**	**0.81**	0.00	**0.29**
C	**0.75**	0.00	**0.19**	**0.75**	0.00	**0.05**	**0.75**	0.00	**0.17**	**0.75**	0.00	**0.26**
EGC	**0.71**	0.00	**0.30**	**0.71**	0.00	**0.06**	**0.70**	0.00	**0.28**	**0.70**	0.00	**0.30**
EGCG	**0.67**	0.00	**0.39**	**0.67**	0.00	**0.09**	**0.66**	0.00	**0.31**	**0.66**	0.00	**0.32**
GCG	**0.65**	0.00	**0.40**	**0.65**	0.00	**0.11**	**0.65**	0.00	**0.30**	**0.65**	0.00	**0.23**
ECG	**0.61**	0.00	**0.47**	**0.61**	0.00	**0.06**	**0.60**	0.00	**0.44**	**0.60**	0.00	**0.34**
EC	**0.59**	0.00	**0.62**	**0.59**	0.00	**0.11**	**0.58**	0.00	**0.64**	**0.58**	0.00	**0.40**
GA	**0.42**	0.01	**1.16**	**0.43**	0.00	**0.67**	**0.42**	0.00	**0.78**	**0.43**	0.00	**0.56**

[a] Expressed in mg/100 mg of extract.

Table 4. Repeatability of Total Amount of Compounds (TACs) in GTE (Intra- and Interassays), after 1 Week

intra-assay ($n = 10$)					interassay ($n = 5$)				
$\lvert\bar{d}\rvert$[a]	SD	$\lvert t_0\rvert$	t^{α}_{n-1}[b]	c	$\lvert\bar{d}\rvert$[a]	SD	$\lvert t_0\rvert$	t^{α}_{n-1}[b]	c
2.87	3.17	2.86	1.83	S	4.14	2.69	3.44	2.13	S

[a] $\bar{d} = \sum_{i=1}^{n}(TACsINTRAorINTER_{\text{after 1 week},i} - TACsINTRAorINTER_{\text{first det er mination},i})/n$. [b] $\alpha = 0.05$ and $n - 1 = 9$ for intra-assay; $\alpha = 0.05$ and $n - 1 = 4$ for interassay. [c] S, significant; NS, not significant.

(repeatability) was calculated from 10 consecutive analyses of the same GTE sample (GTE1); the interassay precision was calculated from five different samples, having the same concentration. One week later the same samples were reinjected to verify the statistical parameters. As shown in **Table 3**, the main cathechins in GTE were GC, C, EGC, EGCG, GCG, ECG, EC, and gallic acid. **Table 3A** shows individual quantification and total content of the height compounds. %RSD of individual compounds reached, in the worst case, ∼11% (for GC and GCG); this was justifiable because the content of the last two analytes was very low. For GA, the unfavorably high value of %RSD was probably due to its low content (from 0.07 to 0.011%) as well as to its tendency to undergo complexation with tetraborate molecules. %RSD values (calculated on the total content), ranging from 2.53 to 5.92%, demonstrated a satisfactory precision of the method. For the migration times (**Table 3B**) and the relative migration times (**Table 3C**), the greatest values were 1.41 and 1.16%, respectively, confirming the goodness of the method. The slight differences between the GA migration time of the standard mixture and of the GTE sample could be due to its different concentration and to an eventual matrix effect. A higher value of intra-%RSD than inter- %RSD

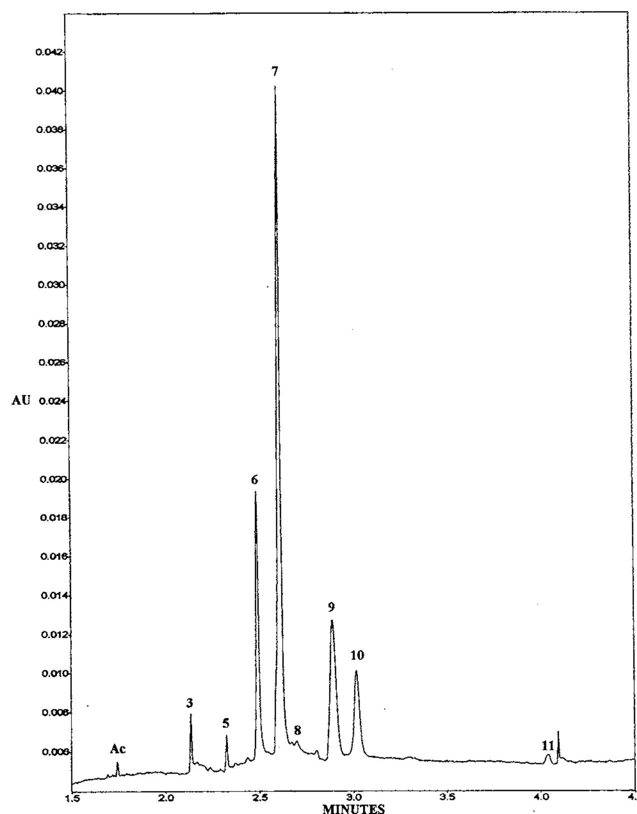

Figure 2. Electropherogram of the GTE sample. Peak identifications and conditions are as **Figure 1**.

could be explained on the basis of possible little variations in the concentration of the analytes in the GTE1 solution, during

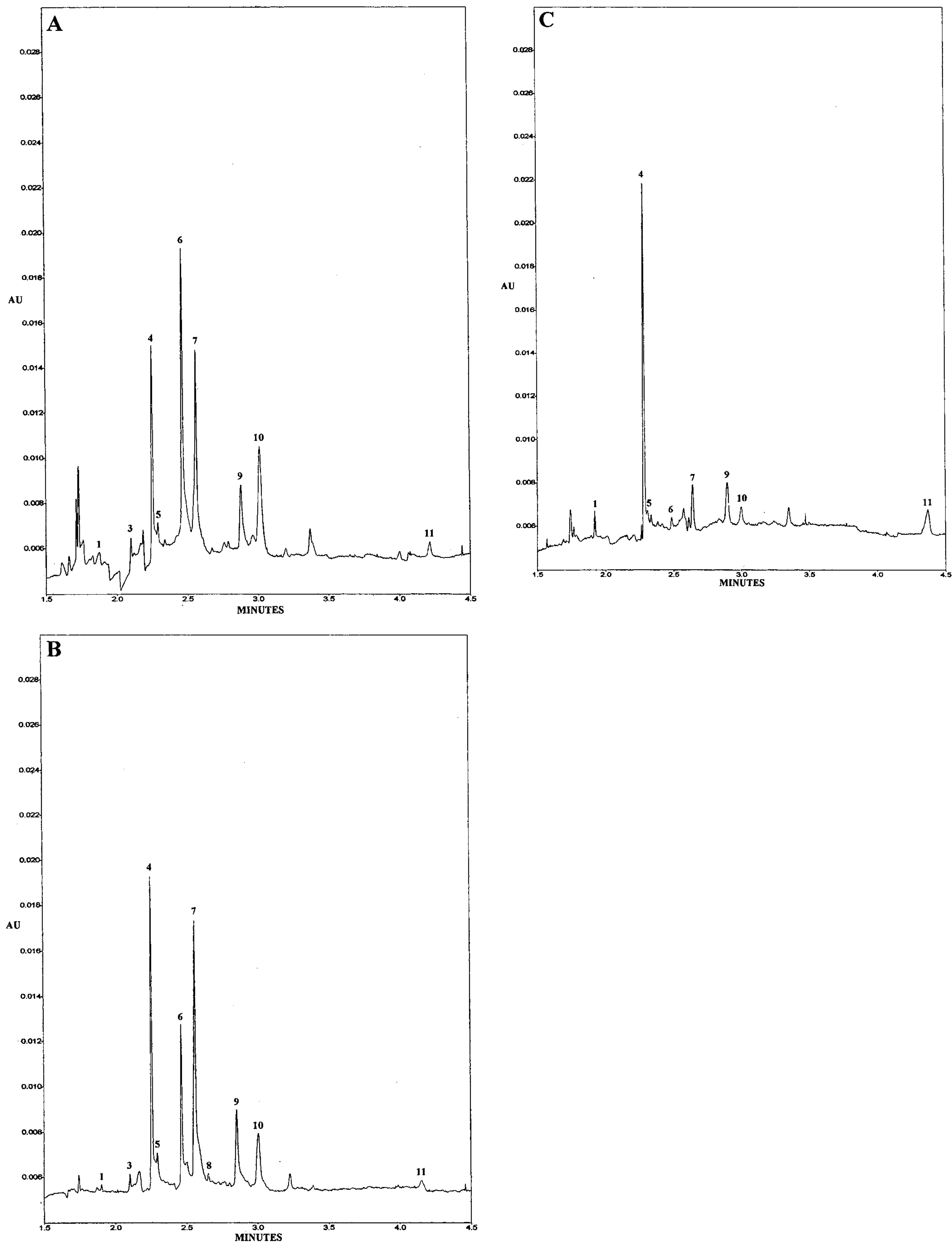

Figure 3. Electropherograms corresponding to the injection of some real samples: (A) bottled green tea; (B) green tea bag infusion (sample Da); (C) black tea dried leaves infusion (sample E). Electropherograms B and C have been obtained from the injection of a 0.4% w/v solution. Peak identifications and conditions are as **Figure 1**.

Table 5. Analysis of 11 Components in Different Real Samples ($n = 5$)

analyte	canned green tea drinks (mean ± SD)[a] A	B	C	tea infusions[b] (mean ± SD)[a] Da[e]	Db[f]	E[g]
TB	nd[c]	nd	9.60 ± 0.22	3.09 ± 0.09	tr[d]	8.23 ± 0.39
TP	tr	nd	nd	nd	nd	nd
GC	tr	5.32 ± 0.19	5.62 ± 0.61	7.01 ± 0.20	2.67 ± 0.21	nd
Caf	tr	15.84 ± 2.16	72.81 ± 3.93	100.85 ± 2.49	101.65 ± 3.00	134.09 ± 3.99
C	tr	17.80 ± 0.79	2.46 ± 0.16	8.30 ± 0.23	2.19 ± 0.31	tr
EGC	2.65 ± 0.46	3.82 ± 0.76	118.69 ± 3.51	74.50 ± 9.02	50.65 ± 10.77	tr
EGCG	tr	tr	69.45 ± 9.06	168.96 ± 17.97	115.22 ± 8.61	5.66 ± 1.08
GCG	nd	19.52 ± 0.95	nd	14.62 ± 0.70	tr	nd
ECG	nd	8.05 ± 0.30	29.81 ± 2.54	67.59 ± 6.46	32.83 ± 1.91	21.16 ± 0.56
EC	4.81 ± 0.46	4.31 ± 0.44	47.26 ± 4.63	52.49 ± 3.05	26.98 ± 2.12	8.57 ± 0.38
GA	nd	nd	tr	tr	tr	14.31 ± 0.62
total	7.46 ± 0.69	74.66 ± 3.47	346.10 ± 21.15	494.32 ± 36.70	332.19 ± 18.22	183.80 ± 4.16

[a] Expressed in μg/mL. [b] 0.4% tea leaves solution (w/v). [c] Not detectable. [d] Traces (<LOD). [e] Green tea leaves, in bag. [f] Green tea leaves. [g] Black tea leaves.

the entire test (10 injections on the same vial). This effect did not influence the interassay, because the study was performed using freshly filled vials.

Finally, the repeatability of method was calculated on the basis of the inter- and intra-assay standard deviation for the total amount of compounds (TACs, mg/100 mg of extract); the following statistical test was applied:

$$t_0 = \frac{|\bar{d}|}{\mathrm{SD}/\sqrt{n}}$$

$\bar{d}$ is defined as the average difference between the total amount of compounds of 1 week of evaluation and that of the first evaluation, SD is the standard deviation, and n is the number of replications. Finally, t_0 was compared with the tabulated Student's value (t^{α}_{n-1}) with $\alpha = 0.05$. Reliability was performed for the intra- and interassays, spaced on a week, respectively; the repeatability hypothesis was rejected when $|t_0|$ was $> t^{\alpha}_{n-1}$. The small deviation, although statistically significant (see **Table 4**), between intra- and interquantification, could be due to the slight variations in buffer composition and instability of EOF.

Analysis of Real Samples. The developed method was applied to several tea-based beverages purchased in local markets or freshly prepared in our laboratory from tea leaves; in this case, only boiling water was used to extract catechins and xanthines. Samples were then directly injected, after appropriate dilution. Representative electropherograms are shown in **Figure 3**. The resulting concentrations of the components are shown in **Table 5**.

Bottled tea drink A had the lowest content of 11 compounds dosed and bottled drink B showed a concentration ∼10 times higher. Tea drink C had a total content of catechins and xanthines comparable to that of the two green tea infusions Da and Db. The green tea bag infusion (Da) showed the greatest total content of components and, as expected, black tea leaves infusion (E) showed the greatest xanthines content (∼140 μg/mL). Theophylline has been found only in sample A (<LOD).

The differences in the total amounts of compounds found could be explained by the different varieties of tea leaves employed for the tea infusions and by the different amount of ingredients used in the preparation of canned tea drinks by the manufacturers.

Conclusion. In conclusion, under our conditions, the time for the analysis is only 12 min, including the rinsing steps. This method allows direct injection of tea samples without sample pretreatment. The analytical conditions described here have been optimized to offer an effective tool for fast and qualitative routine analysis of tea beverages. Compared to HPLC, capillary electrophoresis provides greater efficiency (normally ranging from 10^5 to 10^6 theoretical plates) and sensitivity, shorter analysis time, smaller sample volume requirements, and lower running cost (*9−11*). Thus, the MEKC method proposed can represent an alternative tool for the evaluation of tea quality.

ABBREVIATIONS USED

GTE, green tea extract; GTCs, green tea catechins; TFs, theaflavins; TRs, thearubigins; tr, traces; nd, not detectable; MT, migration time; RMT, relative migration time; Ac, acetone; GC, (−)-gallocatechin; EGC, (−)-epigallocatechin; C, (+)-catechin; EGCG, (−)-epigallocatechin-3-gallate; EC, (−)-epigallocatechin; GCG, (−)-gallocatechingallate; ECG, (−)-epicatechingallate; GA, gallic acid; TP, theophylline; TB, theobromine; Caf, caffeine; S, significant; NS, not significant.

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Received for review August 23, 2002. Revised manuscript received December 3, 2002. Accepted December 3, 2002.

JF020907B