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Determination of phenolic antioxidants by micellar electrokinetic capillary chromatography with electrochemical detection

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Abstract

A new and efficient method for the determination of synthetic phenolic antioxidants (SPAs) has been developed by using micellar electrokinetic capillary chromatography (MECC) with electrochemical detection. Under the optimum conditions, all analytes were successfully separated within 13 min at the separation voltage of 18 kV in a 20 mmol/L borate running buffer (pH 7.4) containing 25 mmol/L sodium dodecyl sulfate. The excellent linearity was obtained in the concentration range from 5.0×10^{-4} to 2.0×10^{-6} mol/L and the detection limits ($S/N = 3$) of propyl gallate (PG), *tert*-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) range from 2.9×10^{-7} to 2.7×10^{-6} mol/L. This method has been proved to be effective and successfully applied for the determination of SPA in food products, providing a promising and convenient entry to monitor the superscale use of phenolic antioxidants.

Keywords: Phenolic antioxidants; Electrochemical detection; Micellar electrokinetic capillary chromatography

1. Introduction

The synthetic phenolic antioxidants propyl gallate (PG), *tert*-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) are frequently used to prevent food, pharmaceutical, and other commercial products from oxidative rancidity (IARC, 1986). Various studies have shown that they could enter human body through the intake of foods, pharmaceutical, etc. Therefore, the use of these additives is subject to regulations which defines the permitted compounds and their concentration limits. In European, the antioxidants mentioned above are strictly regulated to use in foodstuffs, BHA is permitted in bouillons, gravies, dehydrated meat and dehydrated soups individually or combined with PG up to a maximum limit of 200 mg/kg, expressed on the fat content of the product and BHT is not permitted in these foods but it may be used in fats and oils. In the United States, TBHQ is permitted and can be used alone or in combination with BHA and/or BHT up to 200 mg/kg of fat (Burdock, 1997). TBHQ is also permitted in Australia, Brazil, New Zealand and Philippines (Karovičová & Šimko, 2000b). Recently, people have also found that excess use of these artificial antioxidants may cause a loss of nourishment and even produce toxic substances to harm people's health (Chung, 1999; Safer & Al-Nughamish, 1999; Tryphonas, Lacroix, Lok, Jee, & Clayson, 1999). However, due to the variety of possible sample matrixes, complexity, the low concentration

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levels and mutual interference of the similar chemical properties, the analysis of antioxidants is limited (Karovičová & Šimko, 2000a, 2000b). So far, the methods for the quantitative analysis of these antioxidant mixtures have been developed included GC (AOAC, 1984), kinetic methodology (Aguilar-Caballons, Gómez-Hens, & Pérez-Bendito, 1997; Aguilar-Caballons, Gómez-Hens, & Pérez-Bendito, 2000), flow-injection (Garca & Ortiz, 1998; Yanez-Sadeno, Pingarron, & Polo-Diez, 1991), HPLC (Karovičová & Šimko, 2000a, 2000b; McCabe & Acworth, 1998; Rustan, Damiano, & Lesgards, 1993), and voltammetry (Ni, Wang, & Kokot, 2000; Ruiz, Calvo, & Pingarrón, 1994). Most of them suffer from interference problems, long analysis time, and low resolution. Therefore, there are still genuine needs to establish an effective and convenient method for analytical monitoring of degenerative products, the use of prohibited antioxidants and the excess use of permitted antioxidants.

Capillary electrophoresis has been the focus of much current analytical separation techniques due to its celerity, efficiency, reproducibility, ultra-small sample volume and ease of clearing up the contaminants. Combined with electrochemical detection, capillary electrophoresis will be more useful for its additional high sensitivity and good selectivity. To the best of our knowledge, the method for the determination of phenolic antioxidants by using micellar electrokinetic capillary chromatography with electrochemical (MECC-ED) has not been reported yet. In this work, we reported a sensitive and reliable method for the simultaneous determination of PG, TBHQ, BHA, and BHT in food products by MECC-ED. The molecular structures of above ingredients are shown in Fig. 1.

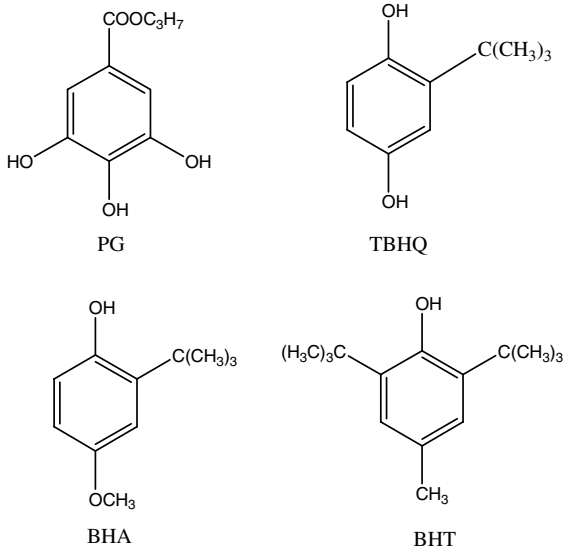

Fig. 1. Molecular structures of PG, TBHQ, BHA, and BHT.

2. Experimental

2.1. Reagent and solutions

BHA, BHT, PG and TBHQ were purchased from Sigma (St. Louis, MO, USA) and were all used as received. All chemicals were of analytical grade.

Stock solutions of all analytes (1.0×10^{-2} mol/L each) were prepared in anhydrous ethanol (A.R. grade), and were diluted to the desired concentration with the running buffer (20 mmol/L H_3BO_3–$Na_2B_4O_7$ buffer and sodium dodecyl sulphate (SDS) ranging from 5 to 30 mmol/L with pH value 7.4). Before use, all solutions were filtered through 0.22 μm nylon filters.

2.2. Apparatus

A CE-ED system has been described previously (Chen, Ye, & Cheng, 2000). A ±30 kV high-voltage power supply (Shanghai Institute of Nuclear Research, China) provided a separation voltage between the ends of the capillary. The inlet end of the capillary was held at a positive potential and the outlet end of capillary was maintained at ground. The separations were proceeded in a 75 cm length of 25 μm i.d. and 360 μm o.d. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA). Samples were all injected electrokinetically, applying 18 kV for 6 s.

A three-electrode electrochemical cell consisting of a laboratory-made 300 μm diameter carbon disc working electrode, a platinum auxiliary electrode and a saturated calomel electrode (SCE) as the reference electrode, was used in combination with a BAS LC-4C amperometric detector (Bioanalytical Systems Inc., West Lafayette, IN, USA). The carbon disc electrode was made of a piece of 300 μm diameter graphite rod from polishing technique as descried in a previous report (Gao, Chu, & Ye, 2002). Before use, the surface of the carbon-disk electrode was successively polished with emery paper and alumina power, sonicated in doubly distilled water, and finally was positioned carefully opposite the outlet of the capillary with the aid of a micromanipulator (CORRECT, Tokyo, Japan) and arranged in a wall-jet configuration (Zhang, Cao, & Ye, 2001). The electropherograms were recorded using a chart record (Shanghai Dahua Instrumental Factory, China). A YS 38-1000 220V alternate constant-voltage power supply (Shanghai Instrumental Transformer Factory, Shanghai, China) was employed to suppress the voltage fluctuation of the power line. The whole system was assembled in a air-conditioned room at 25 °C in order to minimize the effects of external noise sources.

2.3. Sample preparation

Accurate amount of samples (about 1.5 g of vegetable oil, 1 g of mushroom cream or fish soup) was extracted

with 2 mL anhydrous ethanol for 30 min in an ultrasonic bath. The extract was then diluted with running buffer by a factor of 2, after filtered through 0.22 μm nylon filters, the filtrate was injected directly to the CE-ED system for analysis. Before use, all solutions were stored in a 4 °C refrigerator.

3. Results and discussion

3.1. Effect of the applied potential to the working electrode

In amperometric detection, the potential applied to the working electrode directly affects the sensitivity, detection limit and stability of this detection method. Therefore, hydrodynamic voltammertry experiment was conducted to obtain optimum detection. As shown in Fig. 2, the peak current of TBHQ does not have obvious changes, while, the peak current of other three analytes increases rapidly when the applied potential exceed +50 mV for PG, +200 mV for BHA, and +300 mV for BHT, respectively. However, when applied potential is greater than +950 mV (vs. SCE), although the peak current of the analytes still have certain increase, both the baseline noise and the background current increase substantially, which is a big disadvantage for sensitive and stable detection. Therefore, the potential applied to the working electrode is maintained at +950 mV (vs. SCE), where the background current is not too high and the signal-to-noise ($S/N = 3$) ratio is the highest.

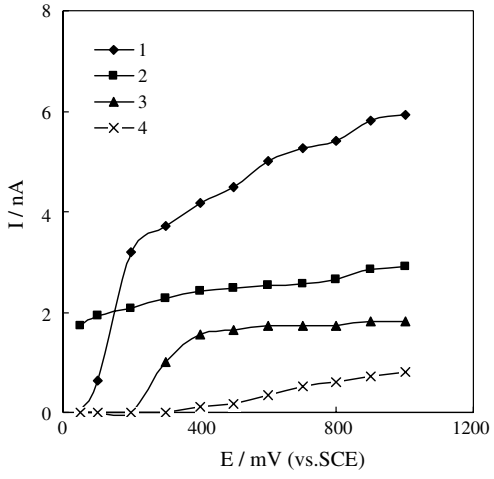

Fig. 2. Hydrodynamic voltammograms (HDVs) of PG (1), TBHQ (2), BHA (3) and BHT (4) in MECC-ED. Fused-silica capillary: 25 μm i.d. × 75 cm; working electrode: 300 μm diameter carbon disk electrode; running buffer: 25 mmol/L SDS in 20 mmol/L (BB, pH 7.4); separation voltage: 18 kV injection time: 18 kV/6 s; concentrations of four analytes, 5.0×10^{-5} mol/L each.

3.2. Effects of the pH and the SDS concentration

In CE, the separation relies on the differential electrophoretic mobility of the analytes, i.e., relies on the degree of dissociation and the molecular size. In MECC, however, both the difference in electrophoretic mobility, and the partition of analytes between the running buffer and the "pseudo-stationary phase" – micelle play important role in sample separation. Whether electrophoresis or partition mechanism dominates the separation process mainly depends on the acidity of the running buffer, hence, the pH value strongly influences the separation.

The migration time of all analytes increases with the increasing pH value. Moreover, higher pH value results in longer analysis time, and the analytes are more susceptible to oxidation. At pH 7.4, the four analytes can be well separated. Therefore, pH 7.4 was selected as the optimum pH value for this work.

In addition to the pH value, the concentration of the SDS is another important parameter. It is well known that the SDS concentration is related to pseudo-retention factors of the solutes. As we can see from Fig. 3, at fixed pH values, pseudo-retention time of all analytes increases with increasing SDS concentration. When SDS concentration exceeds 16 mmol/L, the elution order of TBHQ and PG is switched, this is because the hydrophobicity of TBHQ is higher than that of PG. When SDS concentration value is greater than 25 mmol/L, baseline separation of the analytes can be obtained. SDS concentration (25 mmol/L) was finally chosen for shorter analysis time. Besides, the effect of the running buffer concentration on migration time has also studied, and the optimum running buffer concentration is 20 mmol/L (pH 7.4) in this work.

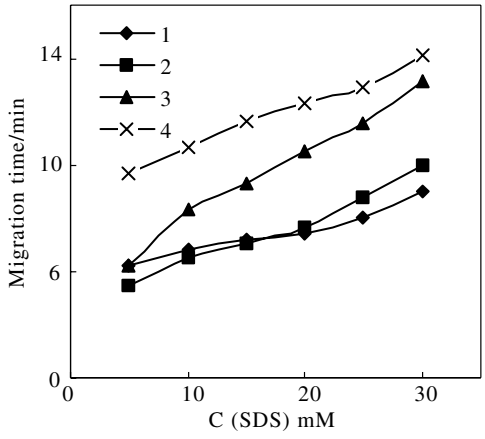

Fig. 3. Effect of SDS concentration on the migration time of the analytes. Working potential was +950 mV (vs. SCE); other experimental conditions and labels are the same as in Fig. 2.

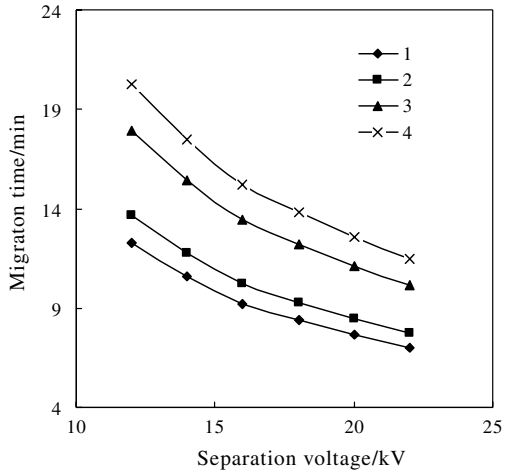

Fig. 4. Effect of separation voltage on the migration time of the analytes. Experimental conditions and labels are the same as in Fig. 3.

3.3. Effect of separation voltage and injection time

For a given capillary length, the separation voltage determines the electric field strength, which affects both the velocity of electrosmotic flow (EOF) and the migration velocity of the analytes, which in turn determines the migration time of the analytes. As shown in Fig. 4, higher separation voltage gives shorter migration time for all analytes. However, when the separation voltage exceeds 20 kV, baseline noise becomes larger. Therefore, the optimum separation voltage selected is 18 kV, at which good separation can be obtained for all analytes within 13 min.

The injection time determining the amount of sampling affects both peak current and peak shape. The effect of injection time on peak current is studied by varying injection time from 2 to 10 s at 18 kV. As expected, the peak current increases with increasing sampling time. When the injection time is longer than 8 s, peak current nearly levels off and peak broadening becomes more severe. In this experiment, 6 s (18 kV) is selected as the optimum injection time.

Through the experiments above, the optimum separation conditions for PG, TBHQ, BHA, and BHT have been decided. The typical electropherogram for a standard mixture solution of the four analytes is shown in Fig. 5(a), from which we can see good separation can be achieved within 13 min.

3.4. Reproducibility, linearity, detection limits and recovery

The reproducibility of the peak current is estimated by making repetitive injections of a standard mixture solution (1.0×10^{-4} mol/L for each analyte) under the selected optimum conditions. The relative standard deviations (RSDs) of the peak current are 0.62%, 0.76%, 2.0%, 2.0% for PG, TBHQ, BHA, and BHT, respectively ($n = 7$). The high reproducibility indicates that this method is accurate and stable.

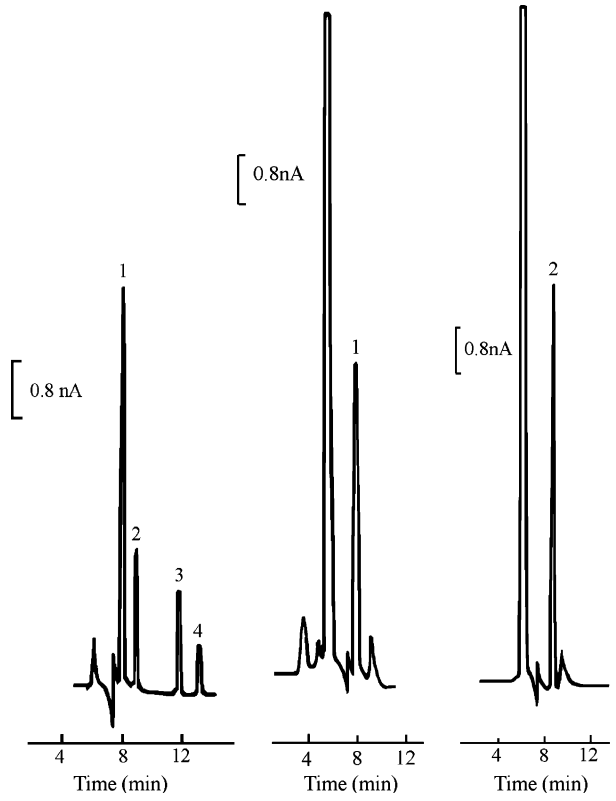

Fig. 5. Electropherograms of the standard mixture solution (a), sample of mushroom cream (b), and sample of fish soup (c). Experimental conditions and labels are the same as in Fig. 3. Peak identification: (1) PG; (2) TBHQ; (3) BHA; (4) BHT.

To determine the linearity of PG, TBHQ, BHA, and BHT, a series of the standard mixture solutions containing 2.0×10^{-6} to 5.0×10^{-3} mol/L of each analyte were tested. The determination limit is evaluated on the basis of a signal-to-noise ratio of 3. The results of regression analysis on calibration curves and detection limits are presented in Table 1, from which we can see the detection limit of MECC-ED is much higher than that of HPLC-UV method.

To further evaluate the precision and accuracy of the method the recovery experiment under the optimum conditions were also conducted with the real samples ($n = 3$). Recovery is determined by standard addition method and the results are listed in Table 2.

3.5. Application and discussion

Under the optimum conditions, MECC-ED is employed for the determination of PG, TBHQ, BHA, and BHT, typical electropherograms for real samples are shown in Figs. 5(b) and (c). By standard addition method and comparing migration times of analytes with

Table 1
The results of regression analysis on calibration curves and the detection limits[a]

Compound	Regression equation $Y = aX + b$[b]	Correlation coefficient	Linear range ($\times 10^{-4}$ mol/L)	Detection limit ($\times 10^{-6}$ mol/L)
PG	$Y = 9.36 \times 10^4 X - 0.1368$	0.9995	0.02–5	0.29
TBHQ	$Y = 3.19 \times 10^4 X + 0.0404$	0.9992	0.02–2	0.80
BHA	$Y = 2.73 \times 10^4 X - 0.0873$	0.9999	0.05–5	1.0
BHT	$Y = 1.54 \times 10^4 X - 0.0720$	0.9994	0.05–2	2.7

[a] MECC-ED conditions are the same as in Fig. 3.
[b] In the regression equation, the X value is the concentration of analytes (mol/L), the Y value is the peak current (nA).

Table 2
Assay results and recovery of antioxidants in food[a] (mg/kg)

Sample	PG	TBHQ	BHA	BHT
Vegetable oil	1.03×10^2 (99.1%)[b]	N.F.[c] (95.8%)	N.F. (96.5%)	N.F. (92.1%)
Mushroom cream	2.17×10^3(101.8%)	N.F. (99.7%)	N.F. (97.4%)	N.F. (94.3%)
Fish soup	N.F. (98.7%)	7.35×10^3 (95.4%)	N.F. (102.3%)	N.F. (95.9%)

[a] MECC-ED conditions are the same as in Fig. 3.
[b] The data in parentheses refer to the recovery rate.
[c] N.F. refers to not found.

those of the standard mixture solution (Fig. 5(a)), antioxidants content in food products can be determined. The experimental results of real samples are presented in Table 2.

4. Conclusions

The satisfactory analytical results show that this method for the determination of PG, TBHQ, BHA, and BHT by using micellar electrokinetic capillary chromatography with electrochemical (MECC-ED) is accurate, sensitive, convenient, and dependable, which provides an alternative and convenient means for fast monitoring of antioxidants in real-world food products.

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