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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.

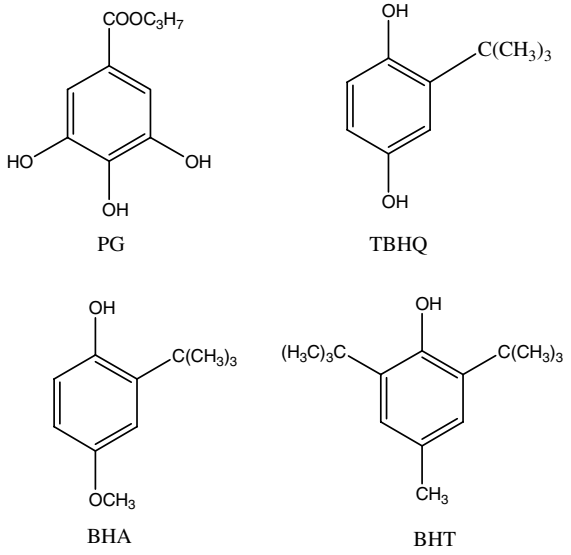

Fig. 1. Molecular structures of PG, TBHQ, BHA, and BHT.

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