Comparison between Capillary Electrophoresis and High-Performance Liquid Chromatography Separation of Food Grade Antioxidants†

Clifford A. Hall III, An Zhu, and Michael G. Zeece*

Department of Food Science and Technology, University of Nebraska—Lincoln, Lincoln, Nebraska 68583-0919

Four major food grade antioxidants, propyl gallate (PG), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone (TBHQ), were separated using micellar electrokinetic capillary chromatography (MEKC). The separation was completed with excellent resolution and efficiency within 6 min and picomole amounts of the antioxidants were detectable using UV absorption. In contrast, reversed-phase high-performance liquid chromatography separation was not as efficient and required larger sample amounts and longer separation time.

INTRODUCTION

Aromatic antioxidants are widely used in food systems for prevention of lipid oxidation. Several synthetic antioxidants based on phenolic structures with varying degrees of hydroxylation and side-chain substitutions are commonly employed. Those approved by the Food and Drug Administration include propyl gallate (PG), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone (TBHQ) (Haumann, 1990). Indyk and Woollard (1986) reported that BHT, BHA, and TBHQ represented greater than 90% of the synthetic antioxidants in commercial use in edible oils. Because of their critical role in the inhibition of oxidative deterioration of food products, various means of analysis have been developed for their quantitation including gas chromatography and high-performance liquid chromatography (HPLC) (Indyk and Woollard, 1986).

Recently, the technique of capillary electrophoresis (CE) with its various modes of separation has been shown to be faster, more sensitive, and capable of higher resolution in separating many compounds than HPLC (Ewing et al., 1989). Free solution capillary electrophoresis (FSCE) separates compounds on the basis of charge and thus could be utilized for separation of phenolic antioxidants under alkaline conditions (Kuhr, 1993). In addition, incorporation of the anionic detergent sodium dodecyl sulfate (SDS) into CE has been shown to improve separation of compounds with both polar and hydrophobic character (Terabe et al., 1984; Cohen et al., 1987; Ewing et al., 1989). In this mode of separation, SDS is included at sufficient concentrations to maintain micellar structures. The presence of SDS micelles in the separation buffer creates a system in which the solute can partition between the micelle and the aqueous phase, thereby adding an extra dimension of selectivity. The technique has been coined micellar electrokinetic capillary chromatography (MEKC) by Terabe et al. (1984). Ong et al. (1991) used MEKC to evaluate catechol and catecholamine separation using SDS in a phosphate–borate buffer. Ong et al. (1990) separated 11 phenolic compounds through MEKC using SDS in a phosphate buffer at pH 6.6, 7.0, and 7.5. These two studies indicated that the separation of the synthesis antioxidants is feasible.

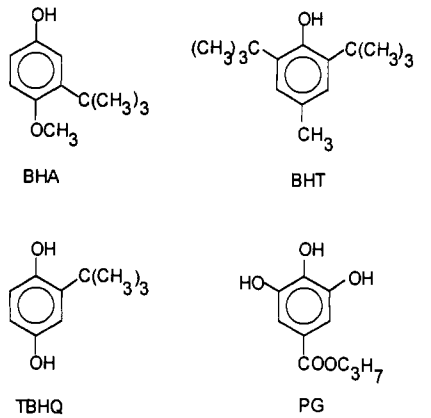

Figure 1. Structures of synthetic antioxidants: butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ), and propyl gallate (PG).

The purpose of this study was to evaluate the use of CE for the separation of synthetic phenolic antioxidants commonly used in food systems. Specifically, the objectives were (1) to compare FSCE and MEKC techniques for the separation of four primary synthetic antioxidants (PG, BHA, BHT, and TBHQ) used in food applications and (2) to compare capillary electrophoretic techniques with HPLC analysis of the synthetic antioxidants.

MATERIALS AND METHODS

Synthetic Antioxidants. Propyl gallate (PG), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone (TBHQ) were obtained from Eastman Kodak (Kingsport, TN) and were dissolved in methanol at concentrations of 1 mM for electrophoretic techniques and 10 mM for HPLC using the mobile phase (acetonitrile–water–2-propanol 62:34:4.0%). The structures of these antioxidants are similar and shown in Figure 1.

Reversed-Phase High-Performance Liquid Chromatography. RP-HPLC separation was performed with a Waters (Milford, MA) Model 625 HPLC using a Waters μ-Bondpak C_{18} column (3.9 mm × 300 mm) at ambient temperature followed by detection at 214 nm with a Waters Model 440 UV detector and extended wavelength module. Chromatographic data were recorded and analyzed with a Spectra Physics (San Jose, CA) Model 4270 integrator. A set of mixed and individual standards was used in these separation at a concentration of 10 mM. The sample injection loop was 20 μL, and the total amount of sample injected was approximately 200 nmol. A mobile phase of acetonitrile–distilled water–2-propanol (62:34:4.0%) previously filtered through a microporous filter (0.45 μm) was used as the solvent system, and solvent flow rate was 1.0 mL/min.

* Author to whom correspondence should be addressed.

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Capillary Electrophoresis. Free solution capillary electrophoresis and MEKC experiments were performed with an ISCO (Lincoln, NE) Model 3850 capillary electrophoretic system. Electropherogram data were recorded and analyzed using ISCO's Chem Research data management computer software. A voltage of 24 kV was used for all separations and peak detection completed at 214 nm. The column was an untreated fused silica capillary tube 50 μm in i.d. and 70 cm (50 cm to detector) in length. All samples were loaded by pressure differential created by elevating the capillary inlet, immersed in sample, 14 cm above the outlet for 3 s. A set of mixed and individual samples was prepared at a concentration of 1 mM, and approximately 2 nL of solute was loaded under these conditions. Therefore, total sample loaded onto the column was 1–2 pmol.

Free solution capillary electrophoresis was performed using a 50 mM sodium borate buffer (pH 9.5). Micellar electrokinetic capillary chromatography was performed using the same 50 mM sodium borate buffer (pH 9.5) with three levels of SDS (20, 50, and 70 mM).

Theoretical plate (i.e., peak) efficiencies were calculated using the equation

$$N = 5.54(t_R/W_h)^2$$

where t_R is the retention time (in seconds) and W_h is the width (in seconds) at half the maximum peak height (Wallingford and Ewing, 1988).

RESULTS AND DISCUSSION

Both normal-phase and reversed-phase HPLC methods have been utilized for the analysis of antioxidants. Indyk and Woollard (1986) reported a normal-phase HPLC technique using two mobile phase systems: (1) hexane–2-propanol and (2) hexane–methylene chloride–acetonitrile. A limitation of these two systems was that the more polar antioxidants, such as PG, were not separated effectively. Anderson and Niekerk (1987) reported a normal-phase HPLC technique using hexane–1,4-dioxane–acetonitrile to separate synthetic antioxidants. This technique was successful at separating the more polar antioxidants but was not useful in separating BHT from the oil sample. Page (1979, 1983) separated the polar as well as the nonpolar antioxidants using reversed-phase HPLC and a gradient elution technique.

We performed RP-HPLC using isocratic elution and separated both the relatively polar (PG) and the less polar (BHT, BHA, and TBHQ) antioxidants in under 25 min (Figure 2). This separation compares favorably with that reported by Page (1979), who demonstrated the separation of both types of antioxidant in less than 15 min using gradient elution.

However, RP-HPLC separation of these compounds was not very efficient under conditions employed here. The peak efficiencies (i.e., theoretical plates, Table 1) for all four antioxidants were less than 5000, and two of the antioxidants (PG and TBHQ) were not completely resolved. In addition, the lower limit of measurement with our detector was approximately 10 nmol.

Several normal-phase HPLC techniques were attempted including isopropyl alcohol in hexane (2:98 v/v), but the separation of the nonpolar antioxidants, in particular BHT, was not effective (results not shown).

CE separations were performed with much lower amounts of analyte. The hydrostatic method of injection described above typically resulted in loading of approximately 2 nL of analyte solution. Since analyte solutions were prepared at a concentration of 1 mM, the amount of compound loaded was typically 2 pmol.

The technique of FSCE was not very successful in resolving these four antioxidants, and only two peaks were observed (Figure 3). The first peak consisted of PG and

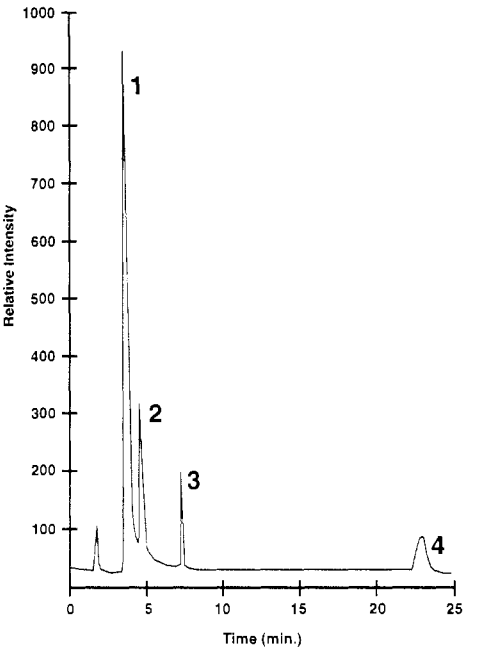

Figure 2. RP-HPLC separation of food antioxidants. Approximately 200 nmol of (1) PG, (2) TBHQ, (3) BHA, and (4) BHT was loaded and separated isocratically with acetonitrile–water–isopropyl alcohol (62:34:4) on a C_{18} column. Flow rate was 1.0 mL/min.

Table 1. Peak Efficiency Values for HPLC and MEKC Separation of Food Antioxidants

	peak efficiency (theoretical plates)[a]			
		MEKC		
antioxidant	HPLC	20 mM	50 mM	70 mM
PG	426 ± 33	63992 ± 997	63144 ± 9970	54229 ± 82
TBHQ	1118 ± 223	4388 ± 779	3339 ± 539	2639 ± 374
BHA	1870 ± 168	52601 ± 4402	52684 ± 18979	14758 ± 7357
BHT	4017 ± 2165	34271 ± 4214	53103 ± 12167	14917 ± 857

[a] Theoretical plate data represent means ± SD of seven determinations.

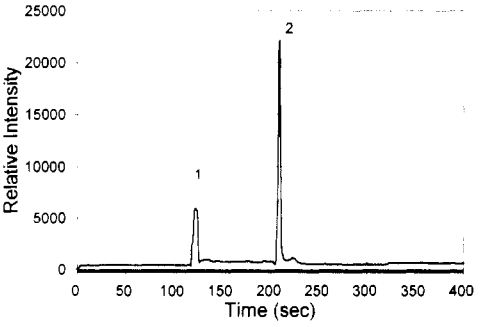

Figure 3. Free solution capillary electrophoresis electropherogram of food antioxidants. Separation of 2 pmol of (1) PG and TBHQ and (2) BHA and BHT using a 50 mM sodium borate buffer at pH 9.5. The voltage applied was 24 kV.

TBHQ followed by comigrating BHA and BHT. A possible explanation for the incomplete separation of BHA and BHT may be found in their degree of ionization. The hydroxyl group pK values for BHA and BHT have not been reported; however, they can be assumed to be close to 10.5, which has been demonstrated for a family of structurally similar phenols. Therefore, at pH 9.5 they can both be considered to be weakly (approximately 10%) ionized. Similarly, PG and TBHQ belong to a family of gallates and hydroquinones with pK values of about 11.6 and therefore have little or no charge at pH 9.5. The lack

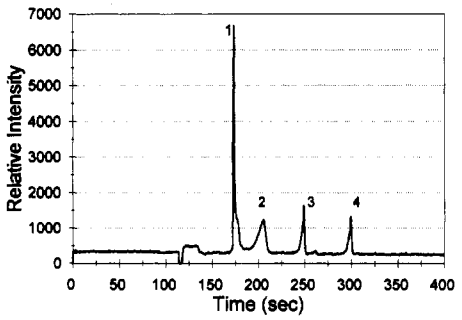

Figure 4. Micellar electrokinetic capillary chromatography electropherogram of food antioxidants. Separation of 2 pmol of (1) PG, (2) TBHQ, (3) BHA, and (4) BHT using 50 mM SDS in a 50 mM sodium borate buffer at pH 9.5. The applied voltage was 24 kV.

of negative charge for PG and TBHQ relative to BHA and BHT results in their migration ahead of the BHA/BHT peak.

Next, separation of these antioxidants was performed using the MEKC technique. The critical micellar concentration for SDS has been reported as 8 mM (Wallingford and Ewing, 1988), and all separations performed here were above that level. Addition of SDS at a concentration of 50 mM to the borate buffer (pH 9.5) resulted in resolution of all four antioxidants and a substantial increase in peak efficiencies (with the exception of TBHQ) compared to RP-HPLC (Figure 4 and Table 1). Increasing the concentration of SDS to 70 mM (separation not shown) resulted in lower efficiencies for all four antioxidants (Table 1) due to the zone broadening. Decreasing the SDS concentration to 20 mM (separation not shown) resulted in a substantial decline in efficiency for separation of BHT while the efficiency for the others was essentially unchanged (Table 1). Lowering SDS concentration to 20 mM also resulted in reversing the order of the migration between PG and TBHQ. A possible explanation for this observation could be an interaction with borate ions. Borate buffers have been reported to electrostatically interact with 1,2-diol compounds (Wallingford and Ewing, 1988). Therefore, a possible reason for PG's migration after TBHQ with lower concentrations of SDS could be its interaction with borate ions, migrating in the opposite direction.

The order of migration for the four antioxidants, in the presence of 50 or 70 mM SDS, occurs in the order of decreasing polarity (i.e., PG eluted first followed by TBHQ, BHA, and, lastly, BHT). This may have resulted from differential interaction with SDS micelles. For example, the least polar BHT (most hydrophobic) would partition to the greatest extent with SDS micelles, resulting in the slowest migration.

In summary, MEKC separated picomole levels of the four antioxidants, of various polarities, with very good resolution. This mode of separation shows excellent potential for resolving a variety of synthetic and natural antioxidants important to food quality. Further, CE in general shows excellent potential for becoming an analytical methodology complimentary to HPLC. CE has unique advantages in resolution while avoiding the use of organic solvents. As with most new technologies, its cost can be expected to decline.

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