

Simultaneous Determination of Sodium Benzoate and *p*-Hydroxybenzoate Esters Using High-performance Capillary Electrophoresis

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Abstract

A rapid and simple capillary zone electrophoresis (CZE) method was developed for the simultaneous determination of sodium benzoate, potassium sorbate, sodium dehydroacetate, and the parabens, i.e. ethyl, *n*-propyl, and *n*-butyl *p*-hydroxybenzoic esters, which are used in several processed foods as preservatives. The compounds were well separated from each other on a fused silica capillary utilizing a 20 mM sodium tetraborate buffer (pH 9.65) and UV detection at 200 nm. The total analysis time was less than seven minutes per sample. Furthermore, sodium benzoate and the five parabens, i.e. ethyl, isopropyl, *n*-propyl, isobutyl and *n*-butyl *p*-hydroxybenzoic esters, permitted as food preservatives in Japan were well separated from each other in less than eight minutes using a micellar electrokinetic capillary electrophoresis (MEKC) method. Concentrations of sodium benzoate and *n*-butyl paraben in several soft drinks were determined using the CZE method and the levels of sodium benzoate and *n*-butyl paraben in the samples were in good agreement with those determined by the HPLC procedure.

Introduction

Recent findings from an *in vitro* yeast-based estrogen assay showed that the four most widely used parabens, i.e. methyl, ethyl, propyl and butyl *p*-hydroxybenzoate, were all found to be weakly estrogenic with the most potent (butylparaben) being 10,000-fold less potent than 17 β -estradiol [1]. Also, in uterotrophic assays, subcutaneous administration of butylparaben at 600 mg kg⁻¹ body weight per day produced a weak oestrogenic response in immature Wistar rats [2]. Therefore, the safety of these chemicals should be reassessed.

By calculating preservative concentrations in foods, daily intake of *p*-hydroxybenzoic acid in Japan in the fiscal year 1996 was estimated to be 1.06 mg per person and is several times less than those of benzoic acid (BA) and sorbic acid (SOA) [3]. There are five parabens, i.e. ethyl paraben (EP), isopropyl paraben (iPP), *n*-propyl paraben (nPP), isobutyl paraben (iBP) and *n*-butyl paraben (nBP), listed in the Japanese Food Standards Code, so the concentration of each paraben in foods should be investigated. In actual practice more than two kinds of preservatives, for example, BA-Na and some other parabens, are added to

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foods. Therefore, developing appropriate analytical methods for the simultaneous determination of BA-Na and the parabens is necessary.

A number of methods for the quantitative determination of preservatives in foods using high-performance liquid chromatography (HPLC) are the most common analytical procedures for the simultaneous determination of BA, SOA and parabens in foods and beverages [4-8]. Gas chromatography (GC) can also be used for determining preservatives, and there is a report on the utilization for a gas chromatography-mass spectrometry (GC-MS) method, which makes possible the simultaneous quantification of BA, SOA and parabens in foods and beverages [9]. High-performance capillary electrophoresis (CE) has developed rapidly, and possesses the advantages of high separation efficiency and short analysis time. Furthermore, it requires less solvent and sample than HPLC [10]. Because of these advantages, the use of CE for analyzing preservatives in food has been reported over the recent decade, and several analytical CE methods have been developed to determine preservatives in food products, including capillary zone electrophoresis (CZE) for the determination of BA [11], SOA [12] and micellar electrokinetic capillary electrophoresis (MEKC) for the determination of BA and /or SOA [13-14]. By using a cyclodextrin modified CE method, the nine preservatives, methyl *p*-hydroxybenzoate (MP), EP, iPP, nPP, iBP, nBP, BA, SOA and *p*-hydroxybenzoic acid, in a mixed standard solution were separated within nine minutes, but more than two preservatives were not simultaneously determined in a food [15]. Using a mixed MEKC method, four preservatives (MP, SOA, EP and BA) were also migrated singly, and SOA or BA was individually determined in a food. However, the simultaneous determination of SOA or BA plus other parabens was not reported [16].

Some soft drinks with BA and one or two parabens added were sold in Japan. This paper describes both a CZE method and a MEKC method for the simultaneous determination of preservatives, including parabens permitted as food additives in Japan. The concentrations of BA-Na and nBP in several beverages were determined, and were compared with those obtained with HPLC to confirm the possibility using the proposed method for the simultaneous determination of preservatives.

Experimentals

1. Reagents

All reagents used were of analytical-reagent or HPLC grade. BA-Na, SOA-K, DHA-Na, sodium tetraborate, sodium dodecyl sulfate (SDS) and potassium phosphate were purchased from Wako Pure Chemical Industries (Osaka, Japan), distilled water and sodium hydroxide were from Nacalai Tesque Inc. (Kyoto, Japan) and the parabens were from Tokyo Kasei (Tokyo, Japan). Beverages were purchased locally.

2. Standards and Samples

Stock solutions of each preservative were prepared by dissolving them in a mixture of methyl alcohol-water (1:1, v/v) at concentrations of 5 mM. The solutions were then diluted with 50% methanol for analysis. All standard solutions were filtered through a 0.2 μ m membrane filter (Nihon Millipore, Tokyo, Japan) and then degassed in an ultrasonic bath for 10 min.

Samples of beverages containing preservatives were degassed in an ultrasonic bath and then homogenized using a vortex mixer for 5 min with distilled water of 10-fold for the CZE and 50% methanol of 100-fold for the HPLC, respectively. The diluted solutions were centrifuging at 1,630 g for ten min at 5 °C. The supernatant was filtered through a 0.2 μ m membrane filter and degassed in an ultrasonic bath before analysis.

To test spiking recovery, two samples of beverages were spiked with BA-Na and nBP solutions at three levels. Analyses were done in triplicate. If any peaks of BA-Na and nBP in the untreated samples were observed, each value for the untreated samples was subtracted from that of the spiked samples.

3. Calibration and Calculations

Calibration standards covering a range from 0.1 to 0.5 mM were prepared. Calibration curves were obtained by plotting peak areas versus concentration. Preservatives in sample solutions were calculated from peak areas.

4. Apparatus and Operating Conditions for CE

The CZE and MEKC methods were performed on a CAPI 3001 capillary electrophoresis system (Otsuka Electronics, Osaka, Japan) equipped with a multi-wavelength photodiode-array detector. Fused-silica capillaries (Otsuka Electronics, Osaka, Japan, 75 μ m I. D. \times 50 cm length, 37.5 cm to detector) were employed. The capillary was prepared daily by serially washing with 50% methanol (20 min), water (10 min), 0.1 M sodium hydroxide (5 min), water (10 min) and finally with the running buffer (10 min). Running buffers used were a 20 mM sodium tetraborate for the CZE analysis and the same tetraborate buffer combined with 30 mM SDS for the MEKC analysis, with both buffers adjusted to pH 9.35 with sodium hydroxide. Each buffer was filtered through the 0.2 μ m membrane filter and then degassed in an ultrasonic bath for 10 min. The samples were injected by raising the sample vial 15 mm above the level for 30 s. The calculated injection volume was ca.10 nl. The samples were run at 25 $^{\circ}$ C with an applied voltage of 15 kV. The detection wavelength was set at 200 nm.

5. High-performance Liquid Chromatography

To determine the concentrations of BA-Na and the parabens in samples, a Shimadzu Class LC-10 liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with an SPD-M10 AVP diode array detector was used. A mixed solution of 20 mM KH_2PO_4 (pH 3.3) containing 5 mM sodium 1-decansulfonate/ CH_3CN at a ratio of 3:1 used as the mobile phase for the BA-Na analysis, and a ratio of 1:1 was used for the parabens analysis. Conditions were as follows: A stainless-steel column, ϕ 4.6 mm \times 150 mm, packed with octadecyl-silanzed silica-gel (TSK gel, ODS-80TM, 5 μ m, Tosoh, Japan) was used. The flow-rate was 0.7

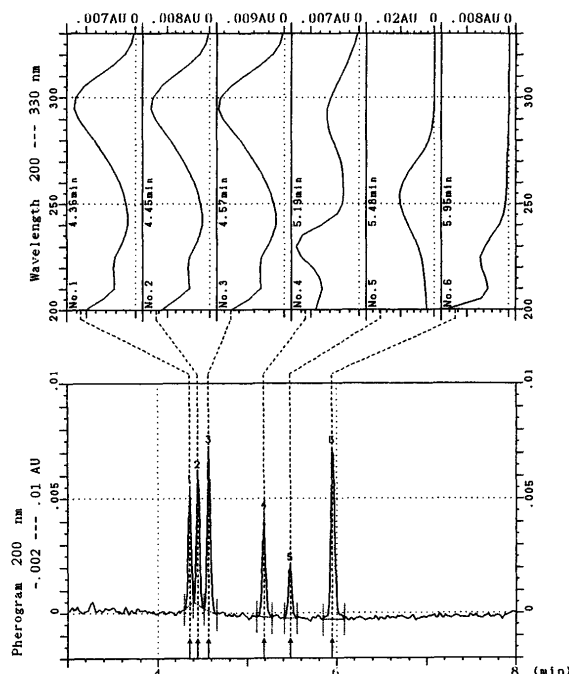


Fig. 1 Electropherogram and UV spectra of mixed solution of preservatives in 50% methanol analyzed by the CZE method.

No.1: *n*-butyl *p*-hydroxybenzoate, No.2: *n*-propyl *p*-hydroxybenzoate, No.3: ethyl *p*-hydroxybenzoate, No.4: sodium dehydroacetate, No.5: potassium sorbate and No.6: sodium benzoate.

Running buffer: a 20 mM sodium tetraborate. The concentration of each solute was 0.1 mM.

ml min⁻¹, producing a pressure of 56-60 kg cm⁻² in the separation procedure. The temperature was set at 40 °C. The injection volume was 20 µl.

Results and Discussion

A standard solution containing six preservatives, i.e. BA-Na, DHA-Na, SOA-K, EP, n-PP and n-BP was analyzed using the CZE method. Fig. 1 shows an electropherogram and individual absorption spectra of six preservatives with detection at 200 nm. Each preservative showed distinct separation within seven minutes after injection. Isopropyl and isobutyl parabens migrated with the same velocity as n-propyl and n-butyl parabens, respectively, so separation of the isomers of the two parabens could not be achieved with the CZE method.

A standard solution containing six preservatives, i.e. BA-Na and the five parabens, EP, iPP, nPP, iBP and nBP, was analyzed using the MEKC method. Fig. 2 shows an electropherogram and individual absorption spectra of the six preservatives in the standard solution with detection at 200 nm. Each preservative showed distinct separation within eight minutes after injection. Various concentrations of SDS were tried and separation of the isomers of the parabens was found to be best at a concentration of 30 mM SDS. In addition, the shape of the UV spectrum of the five parabens was divided into three groups, i.e. EP, the propyl parabens and the butyl parabens. Thus, separation of the isomers was achieved with the MEKC.

The method was first validated with standard solutions at different concentrations ranging from 0.1 to 0.5 mM to determine linearity. Table 1 lists average migration times, limits of detection (LOD), slopes, intercepts and correlation coefficients of the calibration graphs of the analytes. Migration times for the CZE and MEKC methods ranged from 4.33 to 6.00 min and 5.52 to 7.28 min, respectively. LOD was set

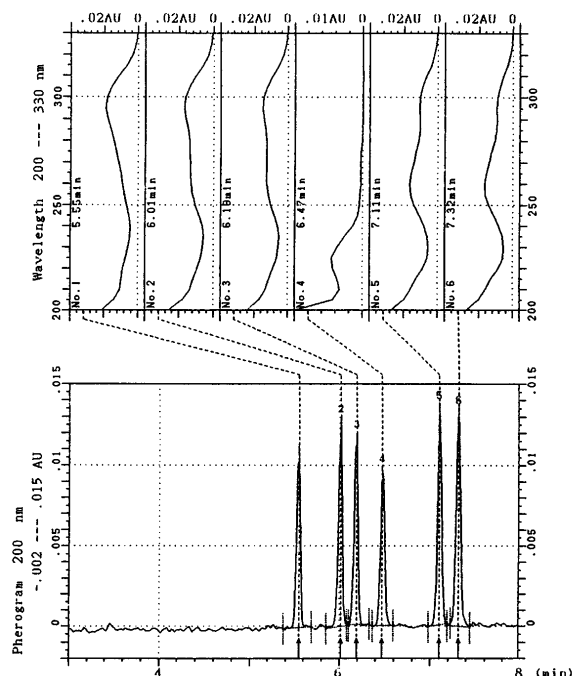


Fig. 2 Electropherogram and UV spectra of mixed solution of preservatives in 50% methanol analyzed by the MEKC method.

No.1: ethyl *p*-hydroxybenzoate, No.2: isopropyl *p*-hydroxybenzoate, No.3: *n*-propyl *p*-hydroxybenzoate, No.4: sodium benzoate, No.5: isobutyl *p*-hydroxybenzoate and No.6: *n*-butyl *p*-hydroxybenzoate.

Running buffer: a 20 mM sodium tetra borate containing 30 mM SDS. The concentration of each solute is 0.1 mM.

at a signal-to-noise ratio of 3. The LODs for the CZE and MEKC methods ranged from 0.27-0.84 and 0.29-0.44 $\mu\text{g ml}^{-1}$, respectively. These were smaller or almost the same order as those determined by the HPLC [6,17]. The linearities of the calibration graphs for all analytes had correlation coefficients exceeding 0.999 in the concentration range 0.1 to 0.5 mM.

Table 1 Average migration times, limits of detection, slopes, intercepts and correlation coefficients of preservatives' calibration graphs.

Method of analysis	Compound	Migration time (min) ^a	LOD ($\mu\text{g ml}^{-1}$)	Slope	Intercept	r
CZE	n-butyl <i>p</i> -hydroxybenzoate	4.33	0.43	0.0057	-0.00006	0.9997
	n-propyl <i>p</i> -hydroxybenzoate	4.42	0.39	0.0061	-0.00004	0.9995
	Ethyl <i>p</i> -hydroxybenzoate	4.54	0.33	0.0061	-0.00002	0.9994
	Sodium dehydroacetate	5.21	0.48	0.0051	-0.00002	0.9993
	Potassium sorbate	5.51	0.84	0.0022	-0.00002	0.9991
	Sodium benzoate	6.00	0.27	0.0083	0.00001	0.9990
MEKC	Ethyl <i>p</i> -hydroxybenzoate	5.52	0.44	0.0048	0.00008	0.9991
	Isopropyl <i>p</i> -hydroxybenzoate	5.98	0.32	0.0055	0.00010	0.9995
	n-propyl <i>p</i> -hydroxybenzoate	6.16	0.32	0.0058	0.00008	0.9998
	Sodium benzoate	6.45	0.30	0.0050	0.00004	0.9993
	Isobutyl <i>p</i> -hydroxybenzoate	7.08	0.30	0.0068	0.00006	0.9996
	n-butyl <i>p</i> -hydroxybenzoate	7.28	0.29	0.0071	0.00008	0.9998

r ; Correlation coefficients of calibration graph.

^aMean (n=8).

To determine repeatability, 0.1 mM mixtures of the preservatives were injected sequentially eight times for both the CZE and MEKC methods. Intermediate precision was determined by injecting 0.1 mM mixtures of the preservatives on six successive days of analysis. The relative standard deviations (R.S.D.s) of repeatabilities and intermediate precision on migration times and on peak areas are shown in Table 2. For the single-day analyses, the R.S.D.s of the migration times for the CZE and MEKC methods were less than 0.12% and 0.69%, respectively, and those of the peak areas were less than 3.34% and 4.91%, respectively. For the successive days analyses, the R.S.D.s of the migration times for the CZE and MEKC methods were less than 1.74% and 1.18%, respectively, and those of the peak areas were less than 6.65% and 5.16%, respectively. The R.S.D.s of both migration times and peak areas for the successive day analyses were higher than the single day analyses. The reason for these higher R.S.D.s for successive analyses is that it is difficult to keep the condition of the capillary wall over several days.

Several beverages containing preservatives were analyzed using the CZE and MEKC methods. Fig. 3 shows electropherograms of two soft drinks, (a) and (b), analyzed by both methods. Two peaks, BA-Na and nBP were found in sample (a), and three peaks, BA-Na, EP and nBP, were found in sample (b) using both CZE and MEKC methods.

Table 3 presents the analytical recoveries for a soft drink containing BA-Na and nBP spiked at three levels with the preservatives using the CZE method. The recoveries of the preservatives using the CZE method were more than 97.1% and the R.S.D. values of the recoveries of the spiked preservatives were less than 5.0%. Table 4 lists the amounts of BA-Na and nBP in four beverages as determined by both the CZE method and the HPLC procedure. The levels of BA-Na and nBP in the samples were almost same for both

Table 2 Repeatability and intermediate precision of the results for the CZE and MEKC methods.

	R. S. D. (%)			
	Migration time		Peak area	
	CZE	MEKC	CZE	MEKC
Intra-day analysis (n=8)				
Ethyl <i>p</i> -hydroxybenzoate	0.10	0.34	1.89	4.91
Isopropyl <i>p</i> -hydroxybenzoate	ND	0.52	ND	3.24
n-propyl <i>p</i> -hydroxybenzoate	0.12	0.55	1.89	2.79
Isobutyl <i>p</i> -hydroxybenzoate	ND	0.69	ND	1.70
n-butyl <i>p</i> -hydroxybenzoate	0.08	0.67	1.98	2.80
Sodium benzoate	0.06	0.31	3.34	2.59
Inter-day analysis (n=6)				
Ethyl <i>p</i> -hydroxybenzoate	1.70	1.03	5.75	3.38
Isopropyl <i>p</i> -hydroxybenzoate	ND	1.03	ND	5.16
n-propyl <i>p</i> -hydroxybenzoate	1.69	1.02	6.09	3.50
Isobutyl <i>p</i> -hydroxybenzoate	ND	1.09	ND	2.93
n-butyl <i>p</i> -hydroxybenzoate	1.74	1.14	5.80	4.90
Sodium benzoate	1.56	1.18	6.65	5.13

The concentration of each preservative is 0.1 mM.

ND; not detectable.

methods. The R.S.D. values for the results were less than 4.5% using the CZE method. The mean values of BA-Na and nBP levels were less than the maximum allowable in soft drinks in Japan at 600 mg kg⁻¹ and 100 mg kg⁻¹, respectively. The separation of the preservatives by both the CZE and MEKC methods was much faster and simpler than by the HPLC procedure.

A number of simultaneous determinations of parabens in drugs and cosmetic products using HPLC procedures have been reported [17-22]. Also a simultaneous determination of parabens using a discontinuous electrokinetic chromatography has been reported [23], and an analysis of food additives containing parabens in standard solutions using mixed MEKC methods has been reported [16]. However, there are only a few papers using the CE method for determining parabens in foods. For example, nBP in a soy sauce has been determined using cyclodextrin-modified CE [15].

Conclusions

The CZE method has been shown to be a good method for determining BA-Na, SOA-K, DHA-Na, EP, PP and BP, simultaneously. The compounds were well separated from each other on a fused silica capillary utilizing a 20 mM sodium tetraborate buffer (pH 9.65) and UV detection at 200 nm. The total analysis time was less than seven min per sample. Furthermore, BA-Na and the five parabens, i.e. ethyl, isopropyl, n-propyl, isobutyl and n-butyl *p*-hydroxybenzoic esters were well separated from each other in less than eight min using the MEKC method. Concentrations of BA-Na and nBP in several soft drinks were determined using the CZE method and the levels of BA-Na and nBP in the samples were good agreement with those determined by the HPLC procedure. According to the experimental results, determining preservatives in sample beverages required no time-consuming extraction procedure because they could be directly analyzed

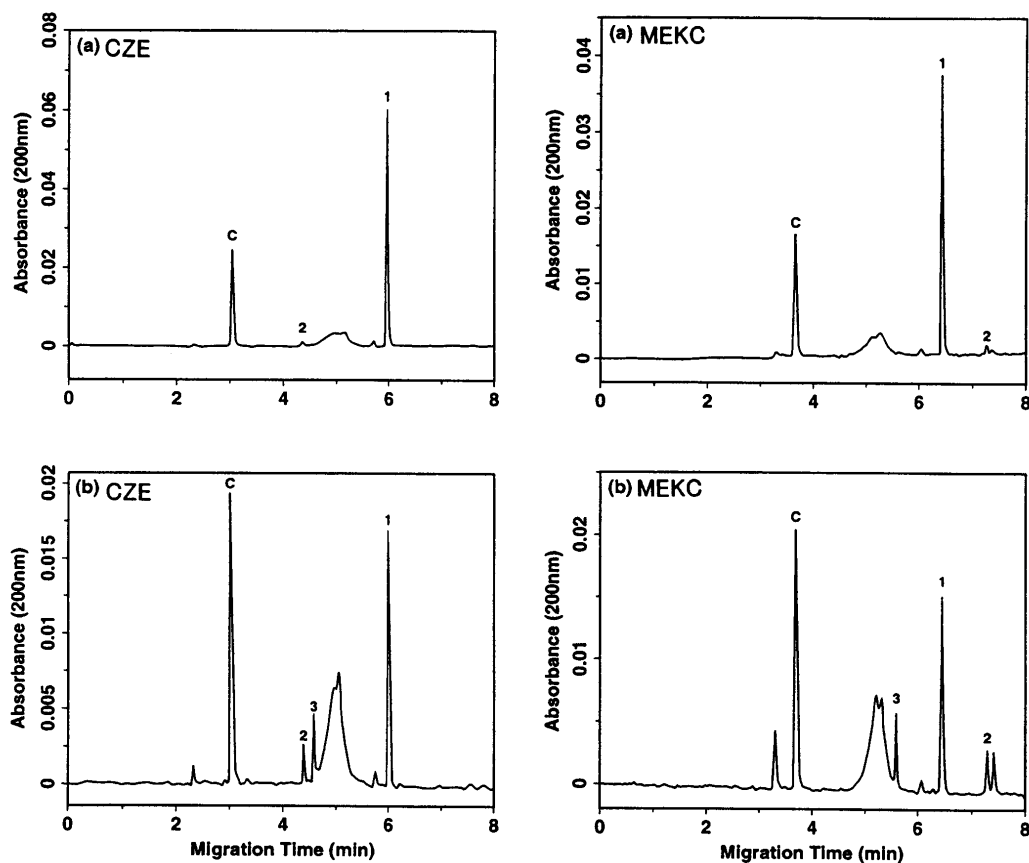


Fig. 3 Electropherograms of beverages using the CZE and the MEKC methods. No.1: sodium benzoate, No.2: n-butyl *p*-hydroxybenzoate and No. 3: ethyl *p*-hydroxybenzoate. The peak labeled c is caffeine. Beverages (a) and (b) were manufactured by different companies, and (a) was diluted five-fold and (b) was diluted ten-fold with distilled water. Other conditions were described in the text.

Table 3 Recoveries for the spiked preservatives.

Compound	Spiking level (μ M)	% Recovery (R. S. D.)
Sodium benzoate	25	99.8 (4.6)
	50	98.5 (4.3)
	100	98.1 (2.9)
n-butyl <i>p</i> -hydroxybenzoate	25	98.9 (1.8)
	50	98.0 (5.0)
	100	97.1 (3.2)

The procedures were described in the text.

by using either the CZE or the MEKC methods. Furthermore, each CE method proposed here offers a technique with high precision and rapid analysis. These CE methods require a minimal quantity of samples and thus can be useful for a routine determination of preservatives in foods.

Table 4 Determination of the amounts of the preservatives in selected beverages.

Sample ^a	Concentration ^b			
	$\mu\text{g ml}^{-1}$			
	Sodium benzoate		n-butyl <i>p</i> -hydroxybenzoate	
	CZE	HPLC	CZE	HPLC
Beverage 1	460 (2.8)	434 (3.0)	19.0 (4.5)	18.7 (6.7)
Beverage 2	460 (1.1)	462 (4.3)	16.1 (3.1)	16.7 (5.6)
Beverage 3	464 (0.8)	444 (3.2)	23.6 (4.2)	26.5 (1.9)
Beverage 4	567 (0.2)	574 (4.6)	15.8 (4.3)	17.3 (4.8)

The R.S.D.s (%) are described in the parentheses.

^a Samples are manufactured by different companies.

^b Mean of three replicates.

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