Determination of Phenolic Disinfectants in Consumer Products by Capillary Electrophoresis with Amperometric Detection

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Abstract

Numerous disinfection products are widely used in daily life to kill pathogenic microorganisms. However, most disinfectants are organic compounds that might be hazardous to the environment and humans when used excessively. Phenolic disinfectants in disinfection products are investigated using a high-performance capillary electrophoresis-amperometric detection method. Under the optimum conditions, five commonly used disinfectants can be well-separated within 19 min at the separation voltage of 18 kV in a 80 mmol/L borax running buffer (pH 9.2), and adequate extraction was obtained with ethanol for the determination of the five compounds. Satisfactory recovery (93.5–106.0%), intra-day repeatability of the peak current (< 2.9%), and detection limits (1.6×10^{-7} – 3.8×10^{-8} g/mL) for the method are achieved. This proposed procedure is successfully used to analyze different samples of disinfection products.

Introduction

In recent years, the consciousness of healthcare and environment protection has become increasingly popular worldwide. In daily life, all kinds of disinfection products are used more and more as they can kill pathogenic microorganisms. However, most chemical disinfectants are organic compounds that are potentially hazardous to the environment and humans when used excessively. For example, triclosan (TCS), one of most popular and effective disinfectants, can inhibit bacterial growth, eliminate carbuncle furunculosis, and promote skin metabolism, and possesses deodorization function, which has been used in medical and daily products in over fifty countries (1–3). However, the pharmacological and toxicological experimental results indicated that TCS could not only inhibit the synthesis of bacterial fatty acid (4,5) but affect the fat metabolism in mammal (6) and also interfere with carbohydrate metabolism in rats (7). Therefore, it is necessary to develop a simple, economical, and efficient method for the analysis of disinfectants in consumer products to supervise the quality and their environmental impact of these products.

Phenolic disinfectants have long-standing use history and are widely applied for its advantages of broad spectrum, high efficiency, low toxicity, and long validity. However, there exists no standard method for detecting phenolic disinfectants contained in disinfection technical specifications (2006 edition) promulgated by Ministry of Health of the People's Republic of China. So far, only a few reports can be found for the analysis of phenolic disinfectants in consumer products, including high-performance liquid chromatography (HPLC) (8–12), UV spectrophotometry (9,13), and capillary electrophoresis (CE) (14–17). Most of these methods rely on photoabsorption detection, and the sensitivity is relatively low.

CE is becoming increasingly recognized as an important analytical separation technique due to its speed, efficiency, reproducibility, ultra-small sample volume, and ease of clearing up the contaminants. In combination with amperometric detection (AD), CE-AD offers high sensitivity and good selectivity for electroactive species (18–20). The major objectives of this investiga-

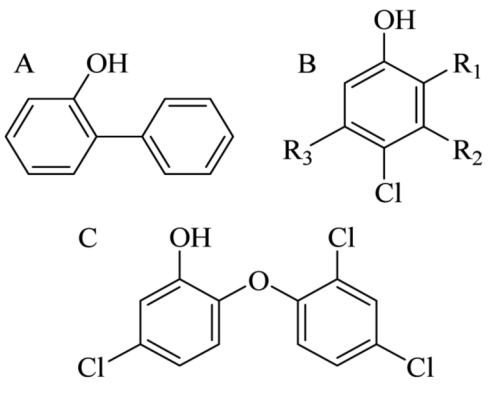

Figure 1. Molecular structures of (A) OPP, (B) PCMX (R_1 = H, R_2, R_3 = CH_3); PCMC (R_1, R_3 = H, R_2 = CH_3); DCMX (R_1 = Cl, R_2, R_3 = CH_3), and (C) TCS.

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tion were to develop a CE-AD method that has been used to simultaneously detect five phenolic disinfectants (Figure 1), namely 2-hydroxybiphenyl (OPP), 4-chloro-3,5-dimethylphenol (PCMX), 4-chloro-3-methylphenol (PCMC), TCS, and 3,5-dimethyl-2,4-dichlorophenol (DCMX) in consumer products.

Experimental

Apparatus

The laboratory-built CE-AD system has been constructed in this work and is similar to that described previously (15). A ±30 kV high-voltage power supply provided separation voltage between the ends of the capillary. The inlet end of the capillary was held at a positive potential, and the outlet end was maintained at ground. A fused-silica capillary (75 cm × 25-µm i.d.) (Polymicro Technologies, Phoenix, AZ) was used for the separation. Samples were all injected electrokinetically, applying 18 kV for 6 s.

The design of the CE-AD detector was based on the end-column approach in which the working electrode was simply placed at the outlet of the separation capillary, and detection was carried out in the same solution reservoir that contains the grounding electrode for CE instrument. A carbon-disk electrode with 300-µm diameter was employed as the working electrode. Before use, the surface of the carbon-disk electrode was polished with emery sandpaper, sonicated in deionized water, and then positioned carefully opposite the capillary outlet with the aid of an Oriel Model 14901 micropositioner (Stratford, CT). A three-electrode cell system consisting of a carbon-disk working electrode, a platinum auxiliary electrode, and a saturated calomel electrode (SCE) reference electrode were used in combination with a BAS LC-4C amperometric detector (Biochemical System, West Lafayette, IN). The electropherograms were recorded using a chart recorder (Shanghai Dahua Instrument factory, Shanghai, China).

Chemical and reagents

The standard compounds of OPP, PCMX, PCMC, TCS, and DCMX were purchased from Sigma (St. Louis, MO), and they were all used as received. All real-life samples, including hand wash, mouthrinse, clothing disinfection liquid, and lotion, were purchased from a supermarket in Shanghai, China.

Stock solutions of five analytes (1.0×10^{-3} g/mL each) were prepared in A.R.-grade anhydrous ethanol, stored in the dark at 4°C, and diluted to the desired concentration with the running buffer (H_3BO_3–$Na_2B_4O_7$ buffer with pH value 8.2–9.66). Water used in the experiments was twice-distilled water, and other reagents were analytical grade. Before use, all solutions were filtered through 0.22-µm nylon filters.

Sample preparation

Appropriate amount of each sample was extracted with 10 mL anhydrous ethanol for 30 min in an ultrasonic bath, 10 min centrifugation (speed of 6000 rpm), then sent through a 0.22-µm syringe filter. The filtrate from the diluted solution can be run directly for the CE-AD analysis after being diluted to the desired concentrations with the running buffer. And the dilution multiple of samples depends on the content of analytes in the real samples, so that the concentration of analytes is within the linear range of detection. Before use, all the sample solutions were stored in the refrigerator at 4°C.

Electrophoresis procedure

Electrophoresis separation was carried out on a laboratory-built CE-AD system using running buffer 80 mmol/L borax buffer (pH 9.2). The applied potential to the working electrode was selected at +950 mV (versus SCE), and the injection time was 6 s (18 kV). The analytes can be well-separated within 19 min at the separation voltage of 18 kV. All experiments were performed at room temperature.

Results and Discussion

Optimum of analytical procedure

Effect of the potential applied to the working electrode

In AD, the potential applied to the working electrode directly affects the sensitivity, detection limit, and stability of this method. Therefore, hydrodynamic voltammetry was investigated to obtain optimum detection results. As shown in Figure 2, when the applied potential exceeds +400 mV (versus SCE), all analytes can generate oxidation current at the working electrode, and the oxidation currents of OPP and PCMC increase rapidly. When the applied potential is greater than +1000 mV (versus SCE), both the baseline noise and the background current increase very strongly, resulting in an unstable baseline which is a disadvantage for sensitive and stable detection. Therefore the applied potential to the working electrode was maintained at +950 mV (versus SCE) where the background current is not too high and

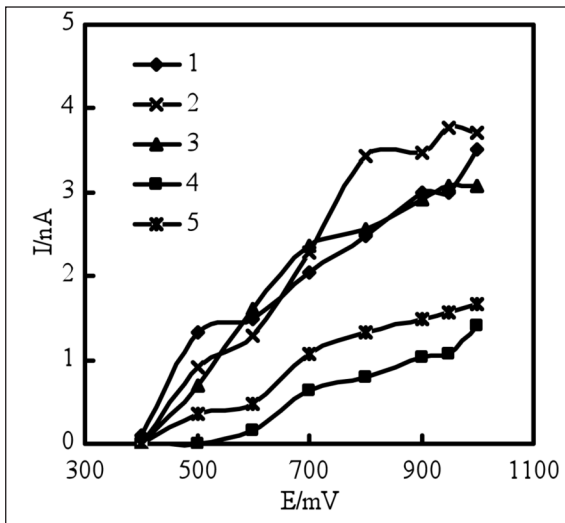

Figure 2. Hydrodynamic voltammograms (HDVs) of 1, OPP; 2, PCMX; 3, PCMC; 4, TCS; and 5, DCMX in CE-AD. Fused-silica capillary: 25 µm i.d. × 75 cm; working electrode: 300 µm diameter carbon disk electrode; running buffer: 70 mmol/L (pH 9.2); separation voltage: 18 kV; injection time: 6 s (at 18 kV); concentrations of five analytes: 1, OPP; and 3, PCMC; 1.0×10^{-5} g/mL, 2, PCMX; 4, TCS; and 5, DCMX, 2.0×10^{-5} g/mL.

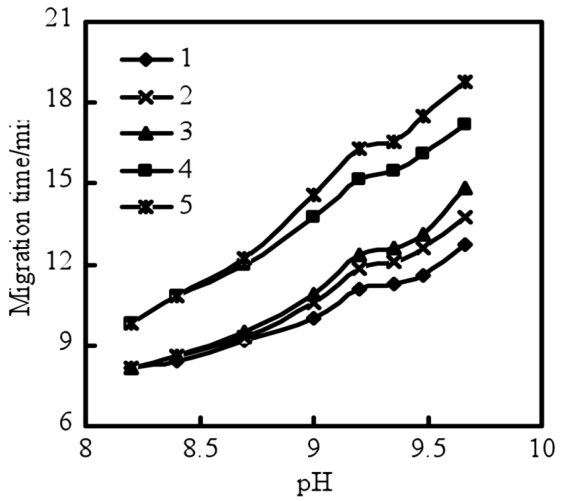

Figure 3. Effect of the running buffer pH on migration time of analytes. Working electrode potential is +950 mV (versus SCE). Other experimental conditions are the same as in Figure 2.

the signal-to-noise ratio is the highest. Moreover, the working electrode showed good stability and high reproducibility at this optimum potential.

Effects of the pH value and concentration of running buffer

The effect of the running buffer pH on the migration time and resolution of the analytes was investigated in the pH range of 8.2–9.66 (Figure 3). When pH was lower than 9.0, satisfactory separation of most analytes could not be achieved. When pH was higher than 9.2, five analytes could be baseline separated; however, higher pH value results in long analysis time also makes analytes more susceptible to oxidation. Therefore, pH 9.2 was selected as the optimum pH value.

Besides the pH value, the running buffer concentration was also an important parameter. The effect of the running buffer concentration on migration time and resolution was also studied ranging from 20 to 100 mM, and 80 mM borax buffer (pH 9.2) was chosen as the running buffer concentration in considering the peak current, resolution, analytical time, and buffer capacity.

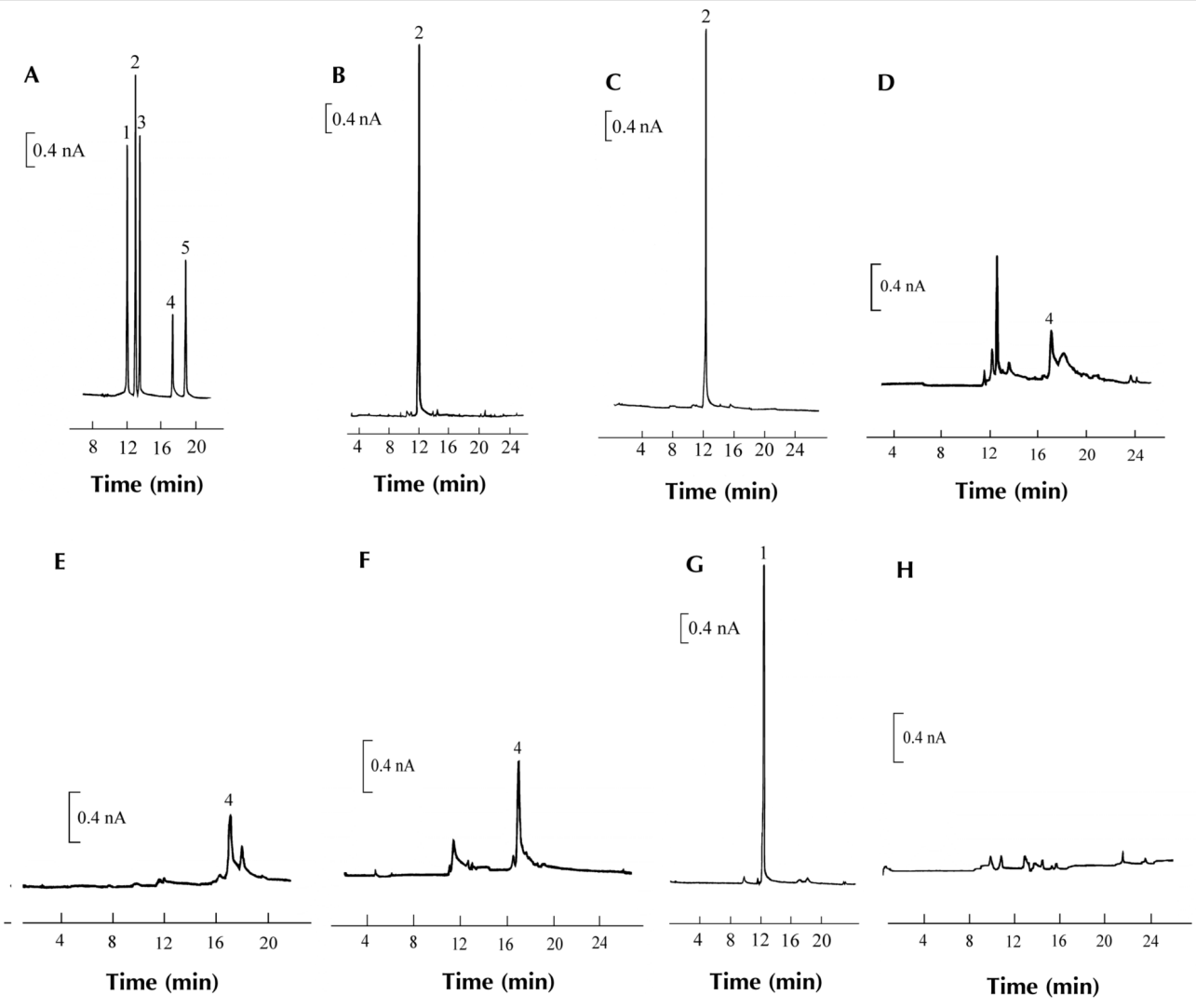

Figure 4. The electropherograms of standard mixture solution (A), clothing disinfection liquid (B), hand wash 1 (C), mouthrinse 1 (D), lotion 1 (E), lotion 2 (F), hand wash 2 (G), and mouthrinse 2 (H). The concentration of running buffer is 80 mmol/L (pH 9.2). Other experimental conditions and peak identification are the same as in Figure 3.

Effects of the separation voltage and injection time

For a given capillary length, the separation voltage determines the electric field strength, which affects both the velocity of electro-osmotic flow (EOF) and the migration velocity of the analytes, which in turn determines the migration time of the analytes. As expected, higher separation voltage gave shorter migration time for all analytes. However, when the separation voltage exceeded 20 kV, baseline noise became larger. Therefore, the optimum separation voltage selected was 18 kV, at which good separation could be obtained for all analytes within 19 min.

In our work, samples were all injected electrokinetically, which is a commonly used and widely recognized injection method in CE work. The injection time determining the amount of sampling affects both peak current and peak shape. The effect of injection time on peak current was studied by varying injection time from 2 s to 10 s at 18 kV. It was found that the peak current increased with increasing sampling time. When the injection time was longer than 6 s, peak current nearly leveled off, and peak broadening became more severe. In this experiment, 6 s (18 kV) was selected as the optimum injection time.

Through the experiments done earlier, the optimum separation and detection conditions for five phenolic disinfectants have been decided, and the typical electropherogram for a standard mixture solution of the five analytes under optimum conditions is shown in Figure 4A.

Method validations

Linearity and detection limits of the target analytes

To determine the linearity of the five analytes, a series of standard solutions from 2.0×10^{-7} to 1.0×10^{-4} g/mL were tested. Results of regression analysis on calibration curves are summarized in Table I. The peak current and concentration of each analyte were subjected to regression analysis to obtain the calibration equations and correlation coefficients, and the results showed that within the concentration range there was an excellent correlation between peak current and concentration of each analyte. The limit of detection was established based on a signal-to-noise ratio of 3, and the limits of detection of five analytes ranged from 1.6×10^{-7} to 3.8×10^{-8} g/mL (Table I).

Table I. The Regression Equations and Detection Limits*

Compound	Regression equation†	Correlation coefficient	Linear range (g/mL)	Detection limit (g/mL)
OPP	$y = 2.80 \times 10^5 x + 0.07$	0.9998	$5.0 \times 10^{-7} – 5.0 \times 10^{-5}$	4.0×10^{-8}
PCMX	$y = 1.75 \times 10^5 x + 0.16$	0.9996	$1.0 \times 10^{-6} – 1.0 \times 10^{-4}$	6.3×10^{-8}
PCMC	$y = 2.81 \times 10^5 x + 0.13$	0.9997	$5.0 \times 10^{-7} – 5.0 \times 10^{-5}$	3.8×10^{-8}
TCS	$y = 6.49 \times 10^4 x – 0.00$	0.9996	$1.0 \times 10^{-6} – 1.0 \times 10^{-4}$	1.6×10^{-7}
DCMX	$y = 7.99 \times 10^5 x – 0.01$	0.9992	$1.0 \times 10^{-6} – 1.0 \times 10^{-4}$	1.3×10^{-7}

* CE–AD conditions are the same as in Figure 4.
† In the regression equation, x is the concentration of analytes (g/mL), and y is the peak current (nA).

Precision

The repeatability of the peak current was estimated by making repetitive injections of a standard mixture solution (OPP and PCMC: 1.0×10^{-5} g/mL each; PCMX, TCS, and DCMX: 2.0×10^{-5} g/mL each) under the selected optimum conditions. The relative standard deviations (RSD) of peak current of analyte in intra-day were 1.1% (OPP), 1.0% (PCMX), 1.6% (PCMC), 2.9% (TCS), and 2.2% (DCMX), respectively (n = 7). The RSDs of peak current of analyte in inter-day were 2.1% (OPP), 2.9% (PCMX), 3.6% (PCMC), 4.4% (TCS), and 3.8% (DCMX), respectively (n = 5). The reproducibility data exhibited in the present study shows that it was feasible to determine the analytes based on the developed CE-AD method.

Accuracy

To evaluate the accuracy of the method, the recovery experiments under the optimum conditions were also conducted with real-world samples (n = 3). Recovery was determined by standard addition method, and the results are listed in Table II. The results indicated that the method was sufficiently accurate for the simultaneous determination of the analytes.

Table II. Results of Recovery in this Method with Real-World Sample (n = 3)*

Samples	Ingredient	Original amount (g/mL)	Added amount (g/mL)	Found (g/mL)	Recovery (%)	RSD (%)
Hand wash 2	OPP	1.45×10^{-5}	1.0×10^{-5}	2.51×10^{-5}	106.0	3.2
Lotion 1	TCS	1.04×10^{-5}	2.0×10^{-5}	2.91×10^{-5}	93.5	3.7
Laundry detergent	PCMX	1.33×10^{-5}	5.0×10^{-6}	1.84×10^{-5}	102.0	2.4
Laundry detergent	PCMX	1.33×10^{-5}	1.0×10^{-5}	2.36×10^{-5}	103.0	2.8
Laundry detergent	PCMX	1.33×10^{-5}	1.5×10^{-5}	2.78×10^{-5}	96.7	3.5

* CE-AD conditions are the same as Figure 4.

Sample analysis

Under the optimum conditions, the proposed procedure was followed for the determination of disinfectant components in different commodity samples based on CE-AD. The typical electropherograms of sample extraction are shown in Figure 4B–4H. By a standard addition method and migration time of the target analyte compared with the electropherogram of the standard mixture solution (Figure 4A), the disinfectants OPP (peak 1), PCMX (peak 2), and TCS (peak 4) have been determined in the different real-world samples, respectively, and the assay results are listed in Table III.

Conclusion

In the present study, five disinfectant components in multiform personal care products were detected simultaneously by a developed CE-AD procedure. Comparing the previously mentioned assay results obtained using the developed CE-AD method with those of HPLC-diode array detection (8,12), CE-electrochemical detection (14), and micellar electrokinetic chromatography–UV (15), the same analytes such as TCS can be obtained at the same or much lower limit of detection (1.6×10^{-7} g/mL versus 1 µg/mL–0.1 mg/L) and/or more acceptable recovery range (93.5–106.0% versus 90.0–108.2%). The analysis results indicated that CE-AD is accurate, sensitive, and

Table III. Assay Results for Different Commodity Samples (n = 3)*

Samples	Ingredients	Found (w/v)	RSD (%)
Laundry detergent	PCMX	1.66%	2.7
Hand wash 1	PCMX	0.19%	1.6
Hand wash 2	OPP	0.21%	1.2
Mouthrinse 1	TCS	0.47‰	1.9
Mouthrinse 2	–	–	–
Lotion 1	TCS	0.16%	2.8
Lotion 2	TCS	0.20%	2.5

* CE-AD conditions are the same as Figure 4.

reproducible for the determination of the above analytes, which provided an alternative method for the quantitative multi-analysis of disinfectants in consumer products.

Acknowledgments

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