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Determination of antioxidants in cosmetics by micellar electrokinetic capillary chromatography with electrochemical detection

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Abstract

A new and efficient method for the determination of antioxidants [Propyl gallate (PG), *tert*-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT)] in cosmetics has been established by using micellar electrokinetic capillary chromatography with electrochemical detection (MECC–ED). Under the optimum conditions of the method, 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 PG, TBHQ, BHA, and BHT range from 3×10^{-7} to 3×10^{-6} mol/L.

Keywords: Cosmetics; Antioxidants; Micellar electrokinetic capillary chromatography; Electrochemical detection

1. Introduction

The skin is the important external barrier which can destroy the radical-chain oxidation processes induced by radiations, smokes, pesticides, etc. [1,2] and protect us from sickness. Cosmetics are commercially available products that can not only improve the appearance of the skin but also benefit to the health of the skin because of their antioxygenation. More and more people realize that one of the most effective ingredients of cosmetics is the antioxidant which can interrupt the radical-chain processes, help the skin to repair systems, help cell rejuvenation and prevent skin-cancer [3]. Therefore, many synthetic antioxidants such as Propyl gallate (PG), *tert*-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) are used to the skin care products [4]. However, the long-term and widespread studies indicate that the superscale use of antioxidants of cosmetic can result in potential health risks associated with theirs intake [5–8].

So there are still genuine needs to establish an effective and convenient quantification method for analytically monitoring the proper use of prohibited antioxidants and the superscale use of permitted antioxidants.

In recent years, considerable interest has been attracted by the current analytical separation techniques of capillary electrophoresis because of its speed, 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, so far no people have reported the application of micellar electrokinetic capillary chromatography with electrochemical detection (MECC–ED) for the determination of PG, TBHQ, BHA, and BHT in cosmetics. The molecular structures of above ingredients were shown in Fig. 1.

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.

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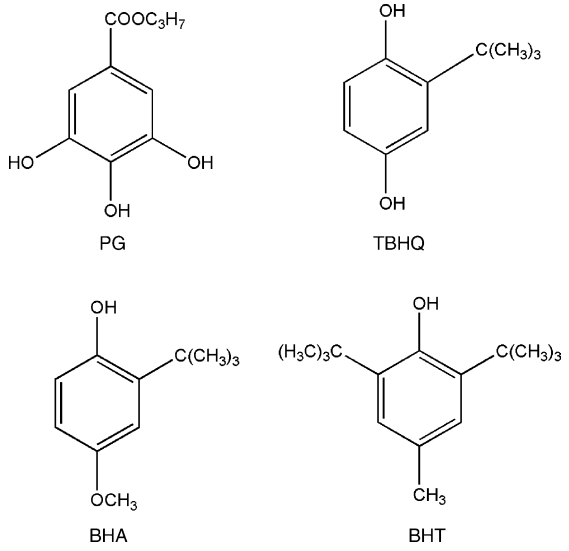

Fig. 1. Molecular structures of PG, TBHQ, BHA, and BHT.

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 [9,10]. 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 [11]. 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 [12]. The electropherograms were recorded using a chart record (Shanghai Dahua Instrumental Factory, China). A YS 38-1000, 220 V 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 variation of running buffer viscosity, which is important to the reproducibility of the experiment results.

2.3. Sample preparation

Before use, all standard solutions were stored in a refrigerator at 4 °C and were stable for at least 1 week. All solutions were diluted to the desired concentration with the running buffer (20 mmol/L borate running buffer (pH 7.4) containing 25 mmol/L sodium dodecyl sulfate). Before use, all solutions were filtered through 0.22 μm nylon filters (Shanghai Peninsula Industry co. Ltd, Shanghai, China).

Accurate amount of samples was extracted with anhydrous ethanol for 30 min in an ultrasonic bath. The extract was diluted with running buffer, after filtered through 0.22 μm nylon filters, the filtrate were injected directly to the MECC–ED system for analysis, and no significant changes of the sample solutions were observed in 24 h. All experiments were performed in a air-conditioned room at 25 °C.

3. Results and discussion

3.1. Effect of the potentials applied to the working electrode

Hydrodynamic voltammertry experiment was investigated to obtain optimum detection. 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 (versus SCE), although the peak current of the analytes still increases, 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 (versus SCE), where the background current is not too high and the signal-to-noise ($S/N = 3$) ratio is the highest.

3.2. Effects of the pH and the SDS concentration

The observed migration time of all analytes increases with the increasing pH value, and the analytes are more susceptible to oxidation in higher pH. At pH 7.4, the four analytes can be well separated, and the analytes are stable enough for analytical purpose at this pH value, 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 fac-

tors of the solutes. At a fixed pH values, pseudo-retention time of the analytes increases with increasing SDS concentration, on the other hand, the hydrophobic character (BHT > BHA > TBHQ > PG) of the analytes is becoming higher with increasing SDS concentration. The effect of SDS concentration on migration time was investigated. When SDS concentration value is greater than 25 mmol/L, baseline separation of the analytes can be obtained. 25 mmol/L SDS concentration was finally chosen for relatively 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.

3.3. Effect of separation voltage and injection time

Higher separation voltage gives shorter migration time for all analytes. However, when the separation voltage exceeds 20 kV, baseline noise becomes more pronounced. 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. 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 conditions for determining PG, TBHQ, BHA, and BHT have been decided. The typical electropherogram for a standard mixture solution of the four analytes is shown in Fig. 2A, 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, and 2.0% for PG, TBHQ, BHA, and BHT, respectively ($n = 7$). The high reproducibility indicates that this method is accurate and reliable.

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 results of regression analysis on calibration curves and detection limits are presented in Table 1. Determination limits are evaluated on the basis of a signal-to-noise ratio of 3. The calibration curves exhibit excellent linear behavior over the concentration range of about three orders of magnitude for all investigated compounds with the detection limits of 3×10^{-7} to 3×10^{-6} mol/L, and the correlation coefficients are in the range of 0.9992–0.9999.

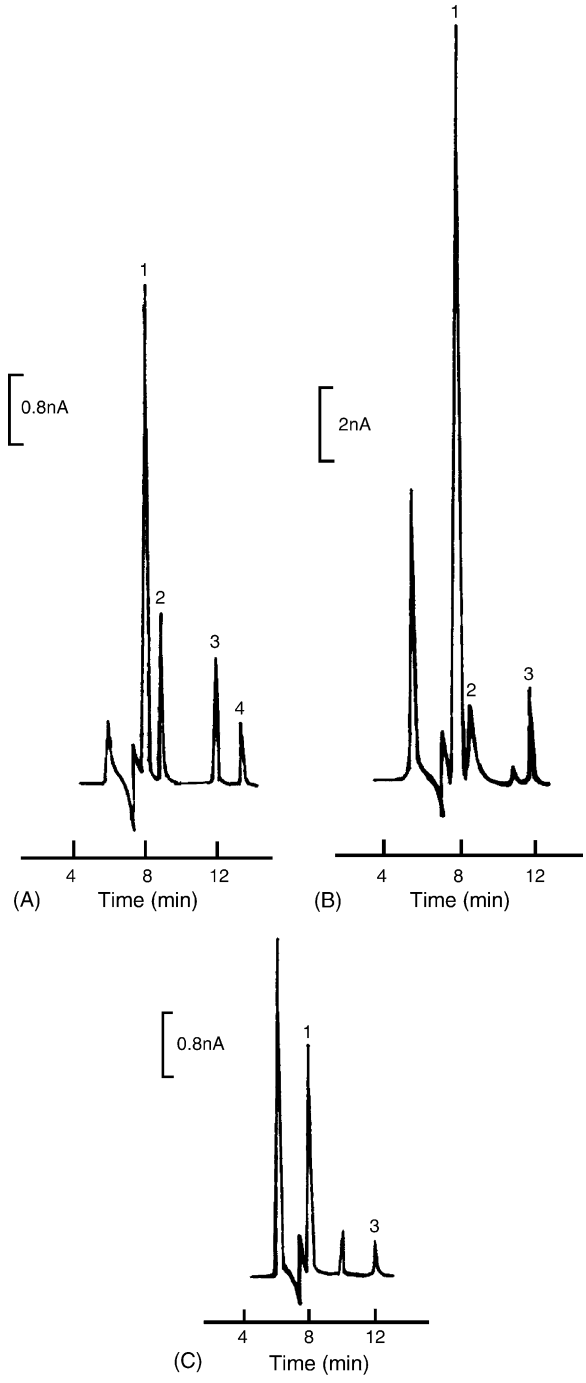

Fig. 2. Electropherograms of the standard mixture solution (A), sample of facial mask (B), and sample of bath oil (C). Peak identification: (1) PG; (2) TBHQ; (3) BHA and (4) BHT. Working potential was +950 mV (vs. SCE); fused-silica capillary: 25 μm i.d. × 75 cm; working electrode: 300 μm diameter carbon 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.

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

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	0.3
TBHQ	$Y = 3.19 \times 10^4\ X + 0.0404$	0.9992	0.02–2	0.8
BHA	$Y = 2.73 \times 10^4\ X - 0.0873$	0.9999	0.05–5	1
BHT	$Y = 1.54 \times 10^4\ X - 0.0720$	0.9994	0.05–2	3

[a] MECC–ED conditions are the same as in Fig. 2.
[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 costimatics[a] (mg/kg)

Sample	PG	TBHQ	BHA	BHT
Facial mask	3.1×10^4 (96%)[b]	3.4×10^3 (95%)	1.6×10^4 (96%)	N.F.[c] (95%)
Bath oil	2.0×10^3 (99%)	8.7×10^2 (99%)	N.F. (98%)	N.F. (96%)
Moisturing sun block lotion	3.8×10^3 (99%)	2.1×10^3 (97%)	N.F. (97%)	N.F. (99%)

[a] MECC–ED conditions are the same as in Fig. 2.
[b] The data in parentheses refer to the recovery.
[c] N.F. refers to not found.

3.5. Application and discussion

Under the optimum conditions, MECC–ED is employed for the determination of PG, TBHQ, BHA, and BHT, where the co-existing compounds produce no interference including tocopherol (V_E). Typical electropherograms for the standard mixture solution and real samples are shown in Fig. 2A–C.

Accurate amounts of PG, TBHQ, BHA, and BHT were added to the sample solution of cosmetic, and the recovery values were calculated based on the peak currents and the calibration curve. The average recoveries and experimental results of real samples for PG, TBHQ, BHA, and BHT are listed in Table 2. The results indicate this method is accurate and reliable for all analytes.

4. Conclusions

A new method for the determination of antioxidants (PG, TBHQ, BHA, and BHT) in cosmetics by using micellar electrokinetic capillary chromatography with electrochemical (MECC–ED) has been developed. The satisfactory results show that the method is quick, accurate, sensitive, convenient and effective, providing a promising and convenient entry to fast monitor the proper use of prohibited antioxidants and the superscale use of permitted antioxidants.

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