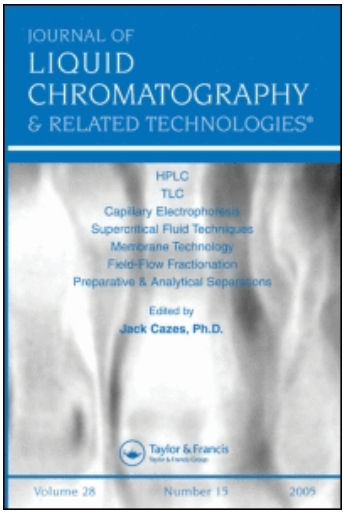

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DETERMINATION OF ADDITIVES IN COSMETICS BY MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY

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ABSTRACT

A micellar electrokinetic capillary chromatography (MECC) method suitable for the determination of antioxidants and preservatives permitted in cosmetic products is described. Unlike reversed phase high performance liquid chromatography (HPLC) this method simultaneously separates ionic solutes (sorbic acid and benzoic acid) and non-polar components (butylated hydroxytoluene and dodecyl gallate). A buffer consisting of 15 mM sodium dodecyl sulfate, 35 mM sodium cholate, 10 mM boric acid, and 10 mM sodium tetraborate adjusted to pH 9.5 and an 80 cm (total length) bare fused silica column gave good separations. The determination of additives in some cosmetic products was performed using both MECC and HPLC. There was good agreement between the methods.

INTRODUCTION

Additives including antioxidants and preservatives are often added to cosmetic products, drugs, and food to protect them from deterioration. Several methods including high performance liquid chromatography (HPLC), gas chromatography (GC), and capillary electrophoresis (CE) have been reported for the separate analysis of antioxidants and preservatives.[1-6] More recently, HPLC methods that simultaneously determine antioxidants and preservatives in food,

pharmaceutical formulations, and cosmetics have been reported.[7,8] Gagliardi et al., using reversed phase (RP) HPLC, resolved a wide range of antioxidants and preservatives, however, this method is not suitable for the simultaneous determination of the more polar ionisable additives BA and SoA.[8] Ion-pair chromatography is usually required for the separation of ionic solutes on a reversed phase column. Chen and Fu, using an ion-pair method, simultaneously determined a range of additives including sorbic acid (SoA) and benzoic acid (BA).[7] However, the non-polar additives such as butylated hydroxytoluene (BHT) and dodecylgallate (DG), permitted additives in cosmetic products, were not included in the mixture. Recently, the simultaneous determination of antioxidants, preservatives, and sweeteners in food by MECC was reported.[9] However, this method does not include in it separation additives commonly added to cosmetics such as propyl paraben (PP) and salicylic acid (SA). This paper describes an MECC method for the determination of additives (antioxidants and preservatives) present in cosmetic products. Additives in cosmetic products are quantitatively determined using this MECC method and by HPLC.

EXPERIMENTAL

Chemicals

The additives comprising *p*-hydroxybenzoic acid methyl ester (methyl paraben, MP), *p*-hydroxybenzoic acid ethyl ester (ethyl paraben, EP), *p*-hydroxybenzoic acid propyl ester (propyl paraben, PP) *p*-hydroxybenzoic acid butyl ester (butyl paraben, BP), sorbic acid (SoA), salicylic acid (SA), benzoic acid (BA), propyl gallate (PG), octyl gallate (OG), dodecyl gallate (DG), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) were purchased from Sigma, Australia and used as received. Sodium cholate (SC), hexadecyltrimethylammonium bromide (CTAB), α-hydroxyisobutyric acid, HPLC grade acetonitrile, and HPLC grade formic acid were also purchased from Sigma, Australia. HPLC grade methanol, analytical reagent grade disodium tetraborate, boric acid, potassium hydroxide (KOH), and sodium dodecyl sulfate (SDS) were purchased from BDH chemicals, Poole, England. The cosmetic products were purchased locally.

Standards and Samples

An additive mixture consisting of approximately 600 μg/mL of BHA and BHT and 200 μg/mL of the remaining additives in 80:20 acetonitrile: water was used to determine the optimal MECC separating conditions. For quantitative analysis standard mixes in the range 0.01 – 0.2 mg/mL were prepared for HPLC analysis and in the range 0.05 – 1.0 mg/mL for MECC analysis.

The additives were extracted from the pharmaceutical preparations using a method described and validated by Gagliardi et al.[8] Approximately 2 g of sample was mixed with 10 mL of a 1:1 methanol:acetonitrile mixture and sonicated for 10 minutes. The samples were made up to volume, usually 15.0 mL, and filtered prior to analysis. The samples were diluted by a factor of two for HPLC analysis.

Apparatus and Conditions

Micellar electrokinetic capillary chromatography separations were carried out using a Waters Quanta 4000 system equipped with a 60 cm (52 cm effective length) or an 80 cm (72 cm effective length) x 75 μm ID fused silica capillary (Polymicro Technology, Phoenix, AZ, USA). The capillary was conditioned daily by washing first with 0.5 M KOH (15 min), then with water (15 min) and finally with the running buffer (15 min). Between each run the capillary was conditioned with 0.5 M KOH, water, and buffer each for two minutes. The samples and standards were injected by hydrodynamic injection for 10 sec unless otherwise stated. The samples were run at 25°C and with an applied voltage of 18 kV. The detection wavelength was 214 nm. The separation buffers consisted of (a) 10 mM sodium tetraborate, 10 mM boric acid, 35 mM SC, and 15 mM SDS and 10 % methanol (pH 9.3)[9] and (b) 10 mM sodium tetraborate, 10 mM boric acid, 35 mM SC, and 15 mM SDS (pH 9.5).

High performance liquid chromatography separations were carried out using a Varian 9010 gradient pump fitted with a Varian 9050 variable wavelength UV-VIS detector and a Varian auto-sampler fitted with a 10 μL Rheodyne loop. The additives were separated on an Altima C18, 5 μm column (150 mm x 4.6 mm, Alltech, Australia) using the ion-pair method described by Chen and Fu.[7] The mobile phase consisted of acetonitrile – 50 mM α-hydroxyisobutyric acid in the ratio 2.2:3.4 (pH 4.5) and containing 2.5 mM CTAB. Detection was at 233 nm and the flow rate was 1.0 mL/min. The additives were also separated on an Altima C18, 5 μm column (250 mm x 4.6 mm, Alltech, Australia) using the method modified after Gagliardi et al.[8] The mobile phase consisted of methanol (A), water with 3% formic acid (B), and acetonitrile (C). The following conditions of gradient elution were used: Initial conditions 30% A, 50% B, 20%C; in 0-15 minutes 30-60% A, 50-20% B, 20 % C; in 15-25 minutes 60-70% A, 20-10% B, 20% C, 25-27 isocratic mode. Detection was at 280 nm and the flow rate was 1.0 mL/min.

Calculations

Theoretical plates were calculated using the following equation:

$$N = 5.45\ (t_r/w_{1/2})^2$$

where t_r is the migration or retention time and w = width of the peak at half peak height.

Limits of detection were calculated based on a signal-to-noise ratio of 3.

RESULTS AND DISCUSSION

Separation of the additive mixture using a method developed for the analysis of additives in food[9] failed to resolve this mixture. Propyl paraben and ethyl paraben coeluted. Figure 1 shows full separation of the antioxidants and preservatives by MECC using a buffer system consisting of 35 mM SC, 15 mM SDS, 10 mM boric acid, and 10 mM sodium tetraborate (pH 9.5) and using an 80 cm

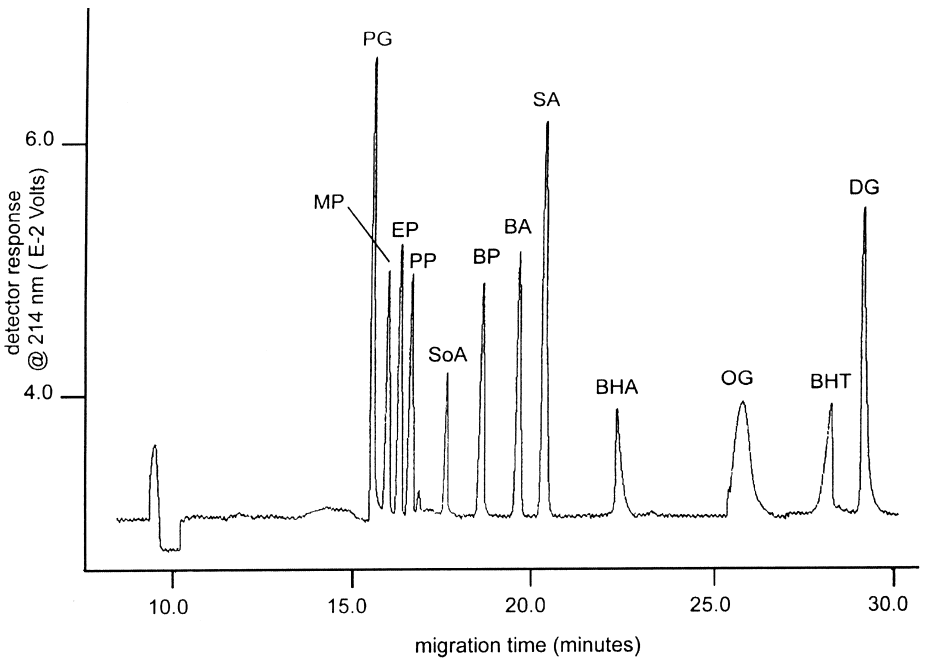

Figure 1. Electropherogram showing the separation of methyl paraben (MP), ethyl paraben, (EP), propyl paraben (PP), butyl paraben (BP), sorbic acid (SoA), salicylic acid (SA), benzoic acid (BA), propyl gallate (PG), octyl gallate (OG), dodecyl gallate (DG) butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) using a buffer consisting of 35 mM SC, 15 mM SDS, 10 mM boric acid and 10 mM borate (pH 9.5). Injection time 2 s.

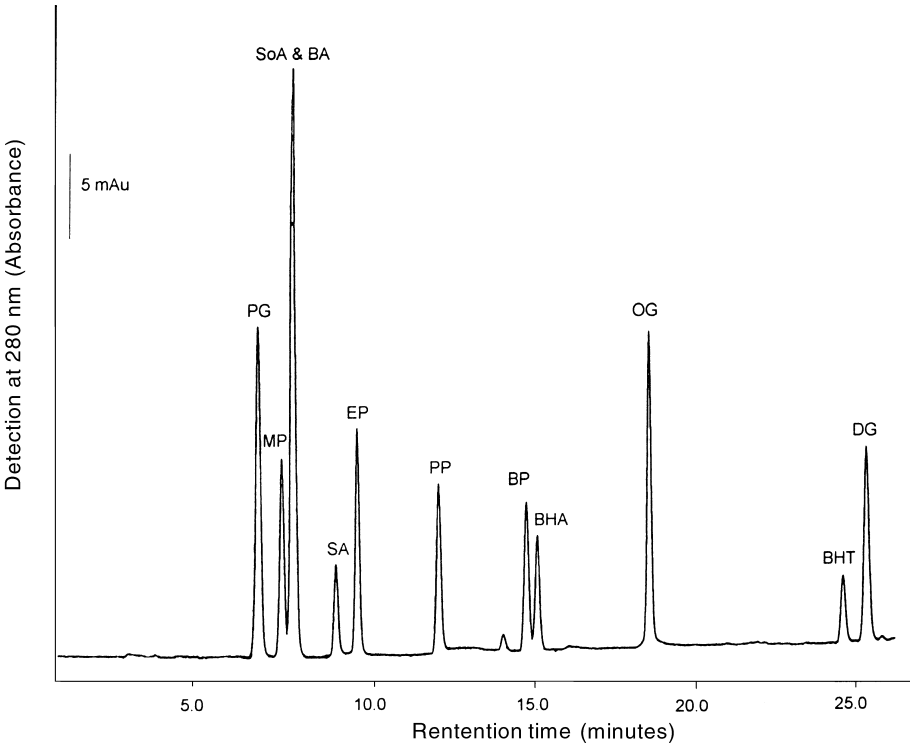

Figure 2. HPLC chromatogram showing the separation of the additive mixture using a C18 analytical column and a mobile phase consisting of acetonitrile/water/methanol/formic acid run in gradient mode (see Text for full details). See Figure 1 for peak identification.

long fused silica column. Earlier work reported on the difficulty of resolving the non-polar components DG and BHT using a single micellar phase such as SDS, and showed that the inclusion of SC in the micellar phase was essential for their separation.[5] Figure 2 shows the separation of the same additive mixture using a RP HPLC method adapted after Gagliardi et al.[8] The additives were eluted in order of increasing hydrophobicity and, not surprisingly, the ionised solutes SoA and BA eluted unresolved. An ion-pair method such as that developed by Chen and Fu is necessary to determine ionic compounds.[7] Using this method, SoA and BA were easily resolved and did not elute with other additives of interest, however, under these chromatographic conditions, it was not possible to elute the non-polar components such as BHT and DG from the column. The large differences in polarity among the preservatives and antioxidants make

Table 1

Limits of Detection and Efficiency Data for Selected Compounds Separated by HPLC and MECC

	Limits of Detection (μg/mL)[a]		Theoretical Plates 1000's per Metre[b]	
Additive	MECC	HPLC	MECC	HPLC
MP	80	0.3	250	
SoA	120	0.15	225	48
BA	70	0.1	265	40
SA	50	0.7	165	
OG	60	0.2	65	
BHT	170	0.7	200	
DG	60	0.3	325	

[a] Detection wavelength for MECC was 214 nm; for HPLC was 280 and 233 nm (for SoA and BA). [b] Theoretical plate calculations are not valid for gradient HPLC systems.

the simultaneous separation by HPLC quite difficult. However, the MECC method reported here allows the simultaneous determination of both the polar solutes BA and SoA and the non-polar additives DG and BHT.

The high separating capabilities of this MECC method is reflected in the theoretical plate counts recorded for the components. Table 1 shows the theoretical plates for a number of components. Peaks were chosen to include early, intermediate, and late eluting components. Theoretical plates in excess of 150,000 was recorded for each component with the exception of OG. In fact, many components had plates in excess of 200,000. The much lower theoretical plates recorded for SoA and BA highlight the large differences in efficiency between the two techniques. The percentage relative standard deviation (% RSD) for the migration times and the peak areas of the components separated by MECC was < 0.25% and < 0.5% respectively over three runs and these results are typical for MECC separations.[5,9] HPLC separations did give superior peak retention time (% RSD 0.1%) and peak area (0.2% RSD) reproducibilities. The superior reproducibility of HPLC over MECC has previously been reported.[10]

The extracts from a number of cosmetic products were analysed in triplicate by both MECC and HPLC (Table 2). Ion-pair HPLC was used to determine amounts of BA and SoA, while RP-HPLC was used to determine amounts

Table 2

Determination of the Additive Content in Cosmetic Products

Cosmetic Product	Additive	Concentration mg/g (% RSD) MECC	HPLC
Moisturising cream	MP	1.99 (1.6)	1.84 (0.8)
	PP	0.91 (1.1)	1.0 (1.4)
Shampoo	BA	1.85 (0.5)	1.82 (0.9)
Clarifying lotion	MP	2.04 (2.0)	2.0 (2.1)
	SA	2.32 (0.1)	2.16 (1.0)
Cleansing lotion	MP	1.04 (0.53)	1.15 (0.36)
Skin toning lotion	MP	1.79 (1.1)	----
	BA	1.08 (3.8)	0.92 (2.2)
Hair conditioner	BA	0.83 (0.2)	----

for all other additives. There was good agreement between the methods with relative differences typically less than 10%. The HPLC and MECC calibration curves used for quantitative determination were linear over the concentration range covered (r^2 0.996 – 1). In all cases, the additives identified in the products were included in the ingredients list on the label of the product. In most cases, the manufacturer did not include the amounts present; however, for the moisturising cream the quantities of MP and PP present in the cream was quoted as 2.09 and 1.0 mg/g, respectively. Both MECC and HPLC were in agreement with the manufacturers quote with the exception of MP by HPLC. The low result (1.84 mg/g) obtained for MP by HPLC might be a consequence of the presence of an unknown component that is not baseline resolved from it, making it difficult to accurately determine the area under the peak. A similar problem was encountered for the analysis of MP in other products, including skin toning lotion and hair conditioner (Figure 3a). The presence of an unknown substance that partially eluted with MP made accurate quantitative analysis difficult. However, using MECC, MP was fully resolved from the other components and provides an alternative method for determining MP levels (Figure 3b).

Table 1 highlights the poorer detection limits for MECC compared to HPLC. Under the conditions reported here, the limits of detection for MECC is typically 50-150 μg while for HPLC it is 0.1-0.7 μg/g. Our study indicated that additives are generally present in cosmetics at levels of 1 – 3 mg/g. Gagliardi et al. also reported additive levels in cosmetics of 1 – 5 mg/g.[9] Therefore, MECC is sensitive enough to study additives in cosmetics.

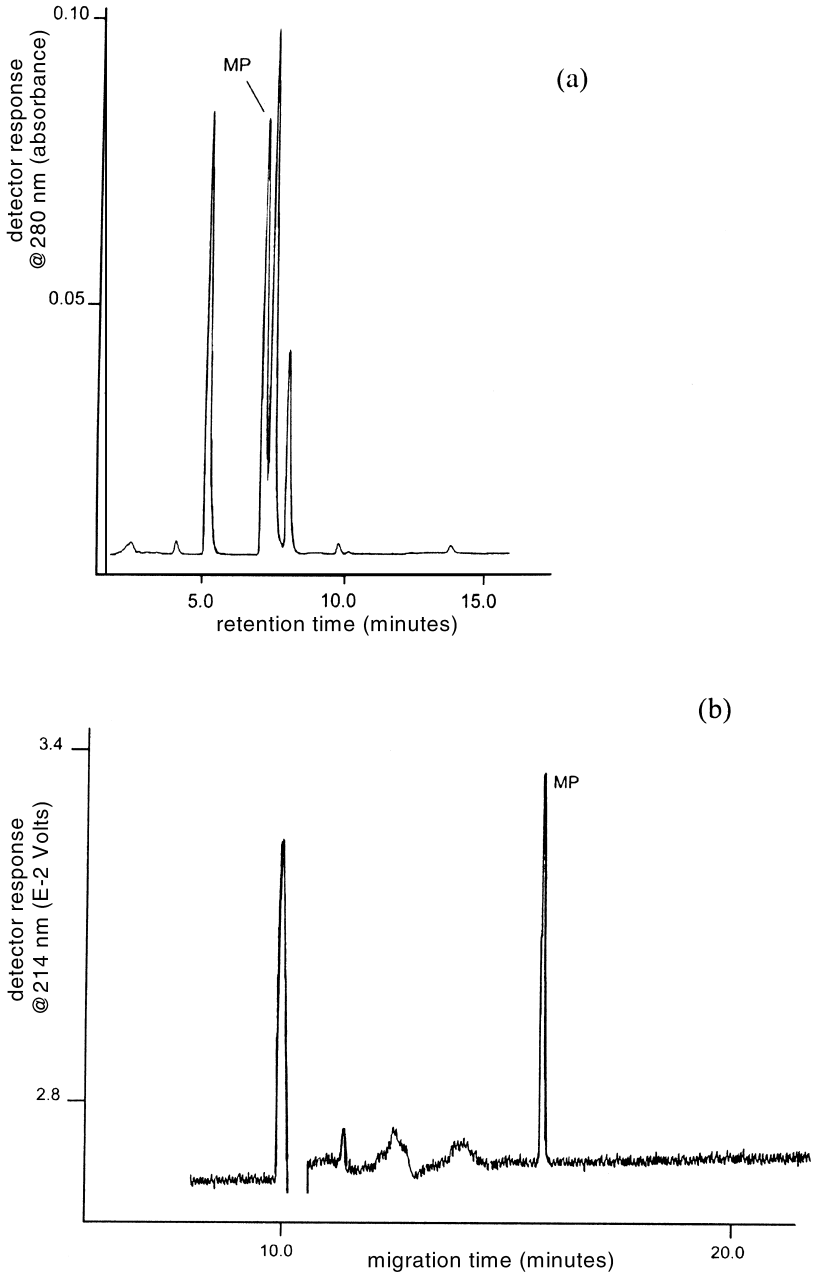

Figure 3. (a) HPLC chromatogram of a hair conditioner extract. Separation conditions as described in Figure 2. (b) Electropherogram of a hair conditioner extract. Separation conditions as described in Figure 1.

In conclusion, the MECC method described here is an effective alternative method to HPLC for the analysis of additives in cosmetics and it also provides a useful complementary technique to current HPLC methods. Unlike GC, which requires an extensive and very different sample preparation step, sample preparation for MECC analysis is similar to that for HPLC making it an attractive alternative.

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