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Short communication

Comparison of high-performance liquid chromatography and capillary zone electrophoresis for the determination of parabens in a cosmetic product

L. Labat *, E. Kummer, P. Dallet, J.P. Dubost

Laboratoire Chimie Analytique, Faculté de Pharmacie, 3 Place de la Victoire, F-33076 Bordeaux Cedex, France

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Abstract

A high-performance liquid chromatographic method (HPLC) and a capillary zone electrophoresis method (CZE) have been developed for the analysis of methylparaben, ethylparaben, propylparaben and butylparaben in a commercial cosmetic product. A very simple extraction procedure with acidified diethylether was developed. The HPLC method involved a C18 reversed-phase column and a gradient of methanol and water-acetic acid (1%). Electrophoretic separation was performed on a fused-silica capillary with a mixed 15 mM tetraborate buffer (pH 9.2) and methanol (85:15, v/v). The calibration curves were linear from 1 to 40 μg/ml in HPLC and from 5 to 200 μg/ml in CZE. The limit of detection in CZE (0.21 μg/ml) was higher than in HPLC (0.05 μg/ml). Repeatability and intermediate precision were satisfactory for both methods (RSD values $<3.23\%$ in HPLC and $<3.26\%$ in CZE). Only HPLC allowed the separation of butylparaben isomeric forms when CZE analysis was less time and reagents consuming. These results suggest that HPLC and CZE coupled with a simple extraction process are both suitable for parabens determination in cosmetic products.

Keywords: Preservatives, cosmetics, parabens; High-performance liquid chromatography; Capillary zone electrophoresis

1. Introduction

In the near future, cosmetic industries are going to have to comply with the sixth amendment to the 76/768/EEC Council Directive, which regulates preservatives use in cosmetic products. Alone or in combination with other compounds, esters of 4-hydroxybenzoic acid, such as methyl, ethyl, propyl and butyl 4-hydroxybenzoate, commonly known as parabens, are well suited for the preservation of cosmetics [1]. EEC directive permits their use with a maximum concentration for each one of 0.4% (w/w) and total maximum concentration of 0.8% (w/w), expressed as *p*-hydroxy-

* Corresponding author.
E-mail address: laurence.labat@chimana.u-bordeaux2.fr (L. Labat).

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benzoic acid. Therefore, developing appropriate methods to extract, separate and determine preservatives is obvious [2].

Extraction of additives from various matrices (foods, cosmetic products) has been previously achieved by solvent or solid-phase extraction [3–6]. However, most of them are time consuming and poor recoveries occur for some additives.

For parabens separation and determination, a large number of chromatographic procedures have been previously reported [6,7], mainly using high performance liquid chromatography (HPLC) [8–11]. In recent years, capillary zone electrophoresis (CZE) has found growing application for drug determination in various pharmaceutical preparations [12]. Nevertheless, only few electrophoretic studies have been developed for the determination of additives in food or pharmaceutical products [13,14].

A large number of reports have shown good agreement between CZE and HPLC data [13]. The use of CZE or HPLC for a particular application is very dependent upon the relative merits of each technique to the individual assay. Often, CZE allows reduced analysis time and is less solvent and sample consuming than HPLC [15,16].

This study presents a HPLC method and a CZE method for the simultaneous separation and determination of the four esters of 4-hydroxybenzoic acid in a cosmetic shampoo with an easy sample extraction using acidified diethyl ether, prior to injection. Both chromatographic and electrophoretic methods have been successfully applied for quality control analysis in the cosmetic preparation.

2. Materials and methods

2.1. Reagents and cosmetic products

Methylparaben (MP), ethylparaben (EP), propylparaben (PP) and butylparaben (BP) were purchased from Sigma Aldrich (St Quentin Fallavier, France). A stock solution was prepared by dissolving each paraben in methanol (20 mg/ml). For linearity studies, it was diluted as appropriate with HPLC mobile phase (concentrations ranging from 1 to 40 μg/ml) or with CZE migration buffer (concentrations ranging from 5 to 200 μg/ml). Glacial acetic acid and sodium tetraborate were purchased from Riedel-de Haën (Seelze, Germany), 2-phenoxyethanol from Sigma Chemical Co (St Louis, USA), methanol and diethyl ether from Fluka Chemie (Buchs, Switzerland). All chemicals were of analytical grade.

The cosmetic product analysed consisted of a foam shampoo.

2.2. Chromatographic conditions

2.2.1. Apparatus

HPLC was performed using a Merck liquid chromatograph (Darmstadt, Germany) equipped with a L-6200 solvent delivery pump, a L-4000 ultraviolet detector operating at 260 nm and a D-2000 Chromato Integrator. Injections were performed manually with a 20 μl Rheodyne model 7125 injector (Rheodyne, Cotati, USA).

2.2.2. HPLC conditions

A Merck reversed-phase Lichrospher C18 column (125 × 4 mm I.D., 5 μm) was used. The solvent system used was a gradient of methanol (A) and water-acetic acid (1%) (B). The gradient was as follows: 0 min: 35% A; 13 min: 60% A; 25 min: 60% A. Fifteen minutes were necessary for column conditioning. Flow rate was 1.0 ml/min. The determinations were performed at room temperature.

2.3. Electrophoretic conditions

2.3.1. Apparatus

CZE analysis was carried out on a Spectra Phoresis 1000 (ThermoQuest, Les Ulis, France) capillary electrophoresis system. Fused-silica capillary tube (ThermoQuest) (70 cm × 75 μm I.D) was used with an effective length of 63 cm. Temperature of the capillary tube during electrophoresis was maintained at 40°C by a thermostating system. Samples were injected using the hydrodynamic mode with an injection time of 2.5 s (15 nl). All analysis were performed at an applied voltage of 20 kV (with a typical current of 35 μA). The

electrophoretic zones were detected at 295 nm with an UV-Vis detector. The electropherograms were recorded and integrated with Spectraphoresis software (ThermoQuest).

2.3.2. Running buffer

The buffer was 15 mM sodium tetraborate (pH 9.2) with methanol (85:15, v/v). It was filtered through a 0.45 μm membrane filter.

2.3.3. Preparation of the capillary

The capillary column was stored under air. Each day before starting analysis, it needed a three phases conditioning cycle: (1) purging at 40°C with water (5 min), 0.1M NaOH (5 min) and water (5 min); (2) equilibration with running buffer at 40°C (15 min); (3) blank injection of running buffer. Before each sample injection, the capillary was washed with 0.1M NaOH (2 min) and running buffer (4 min). On shut-down, the capillary was flushed with 0.1M NaOH (2 min), followed by flushing with water (5 min) and purging with air (2 min).

2.4. Sample preparation

Ten millilitres of acidified ether (ether-acetic acid, 1%) were added to approximately 1 g of foam mixture accurately weighed. The preservatives were extracted by ultrasonication (Ultrasound bath, Bandelin, Sonorex RK 52) for 5 min and stirring for 2 min (vortex, IKA-Labotechnik). This extraction procedure was repeated twice. Organic phases were pooled and evaporated (Rotavap 94200, Bioblock Scientific, Illkirch, France). In CZE, the extract was solubilized in a mixture of methanol/borate buffer (17:3, v/v) and was filtered through a 0.45 μm membrane filter. In HPLC, the extract was solubilized in methanol (20 ml) and diluted with mobile phase (1:4, v/v). This solution was filtered and an aliquot was injected onto the column.

2.5. Calculation

All the CZE results are obtained using normalized areas (area/migration time).

3. Results and discussion

3.1. HPLC and CZE conditions and extraction method

3.1.1. HPLC

Fig. 1 shows a sample solution chromatogram. In addition to MP, EP, PP and BP, there are two other peaks related to phenoxyethanol and isobutylparaben (iso-BP), usually encountered in cosmetic products. The use of a gradient was necessary to achieve the separation of all solutes resulting in a time consuming analysis (40 min) compared to other HPLC methods described in literature [17–19].

3.1.2. CZE

The electropherogram of the same sample solution is presented in Fig. 1. Tetraborate buffer (pH 9.2) with 15% of methanol allowed to separate phenoxyethanol and the four paraben derivatives. Organic modifier was added to slow the electroosmotic flow: migration times were lengthened, but different migration velocities for BP and iso-BP isomers could not be obtained. Under these conditions, the preservatives were in phenate form absorbing in ultraviolet at a higher λ max of 295 nm. The migration time of electroosmotic flow measured by a neutral marker, i.e. phenoxyethanol at $\lambda = 200$ nm was 5.85 min. When using freshly prepared solutions, no hydrolysis in *p*-hydroxybenzoic acid was detected. Since these esters have similar p*K*a values, their migration velocities particularly depend on their molecular masses. An ester in the anionic form, with a lower molecular mass like MP (i.e. a higher charge-to-mass ratio) has a higher electrophoretic mobility to anode and will be last detected at the cathode. BP and iso-BP have similar p*K*a values and same molecular mass leading to the same migration time (Fig. 1). In literature, a method using cyclodextrins has been investigated for BP and iso-BP separation [14].

The run time required in CZE for each analysis was 16 min (migration time = 10 min and conditioning time before each injection = 6 min).

3.1.3. Extraction method

Under their acidic form, parabens are freely soluble in methanol and diethyl ether resulting in the choice of acidified diethyl ether for the extraction procedure. According to Fig. 1, it is highly selective, since no parasite peaks appeared in the chromatogram or the electropherogram.

3.2. Validation of HPLC and CZE methods

3.2.1. Linearity and limit of detection (LOD)

Linearity was checked by performing triplicate injections at five standard concentrations 1, 5, 10, 20, 40 μg/ml in HPLC and 5, 10, 50, 100, 200 μg/ml in CZE. The ranges are different for the

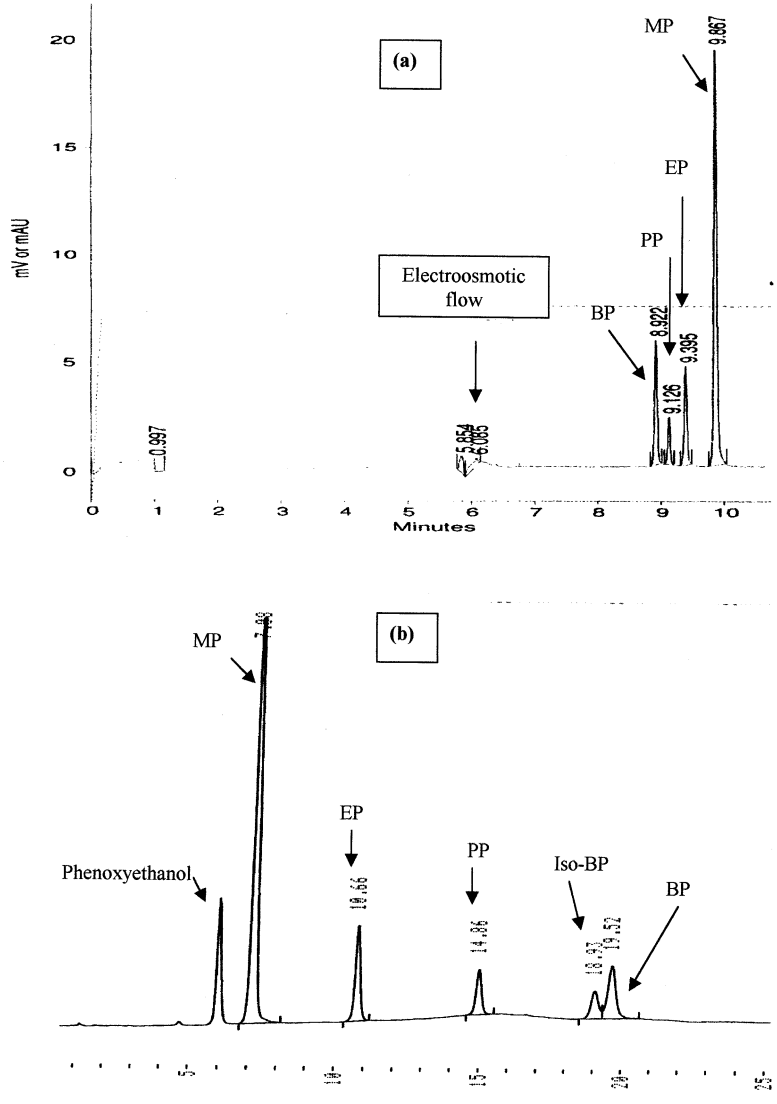

Fig. 1. Electropherogram (a) and chromatogram (b) of a shampoo extract of parabens.

Table 1
Linearity and limit of detection values of the parabens in HPLC and CZE[a]

Compound	Method	Concentration range (μg/ml)	Calibration curve (slope) (a)	Correlation coefficient (r)	LOD (μg/ml)[b]
MP	HPLC	1–40	$3.84\ 10^{-5}$	0.999	0.02
EP	HPLC	1–40	$4.17\ 10^{-5}$	0.999	0.03
PP	HPLC	1–40	$4.37\ 10^{-5}$	0.999	0.03
BP	HPLC	1–40	$4.64\ 10^{-5}$	0.999	0.05
MP	CZE	5–200	$4.52\ 10^{-3}$	0.998	0.16
EP	CZE	5–200	$4.83\ 10^{-3}$	0.999	0.17
PP	CZE	5–200	$5.13\ 10^{-3}$	0.999	0.18
BP	CZE	5–200	$5.12\ 10^{-3}$	0.999	0.21

[a] MP, methylparaben; EP, ethylparaben; PP, propylparaben; BP, butylparaben; HPLC, high performance liquid chromatography; CZE, capillary zone electrophoresis.
[b] The limit of detection (LOD) is an estimation of $3 \times$ noise.

two methods owing to the better sensitivity of HPLC. Considering the experimental procedure, the upper limits are related to the maximum authorized concentration in cosmetic products. The calibration curves (peak area versus concentration in HPLC and peak area/migration time vs. concentration in CZE) were obtained using the least square regression method. The slopes (a) and the coefficients of correlation (r) are shown in Table 1 for both methods. Correlation coefficients for the linear fit are higher than 0.99.

The LODs were calculated as the amount of compound that would still give a signal three times greater than the noise of the baseline. Results are given in Table 1. LODs in HPLC have lowest values.

3.2.2. Repeatability and intermediate precision

To determine the repeatability of the method, parabens were analysed five times in the foam shampoo. The RSDs values (Table 2) show that both techniques exhibit a good repeatability.To determine the intermediate precision, the same experiments were performed during five consecutive days. The results are summarized in Table 2, indicating good values for both techniques.Repeatability and intermediate precision studies using a F test showed no significant differences at 95% confidence level, except for EP repeatability which was better in HPLC than in EC.

3.2.3. Recoveries

Parabens recoveries were investigated for the foam shampoo sample. Observed concentration values are in good agreement with the expected ones. Compared to other methods described in literature [8,16], this technique presents a high recovery. For MP, EP and PP, the recoveries data ranged from 99.50 to 104.88% in HPLC and 91.80 to 98.50% in CZE (Table 2). In the experimental CZE conditions, no separation of BP and iso-BP could be obtained. The observed concentration of BP corresponds to the total concentration of the two isomeric forms and explains the elevated recovery value of 124.85% (Table 2).

4. Conclusion

The aim of the present study was to perform simple extraction procedure in a commercial cosmetic product and to optimize separation and quantification of MP, EP, PP and BP by two analytical methods: HPLC and CZE. It involves an extraction with ether-acetic acid (1%), presenting short handling time and ease of operation, well suited for routine quality control. HPLC and CZE methods are validated in terms of linearity, limit of detection and also in terms of repeatability and intermediate precision of parabens determination after extraction.

Our proposed CZE method is quantitative, rapid and accurate. However, it presents a restric-

Table 2
Recoveries of the parabens in a foam shampoo, repeatability and intermediate precision for HPLC and CZE[a]

Compound	Method	Theoretical concentration in foam shampoo (mg/100 g)	Average of observed concentration in foam shampoo (mg/100 g)	Recoveries ($n = 5$) (%)	Repeatability ($n = 5$) (RSD%)	Intermediate precision ($n = 5$) (RSD%)
MP	HPLC	68	68.06	100.09	0.41	1.53
EP	HPLC	18	18.88	104.88	0.46	3.23
PP	HPLC	10	9.95	97.60	1.15	2.51
BP	HPLC	20	19.52	99.50	1.14	2.36
MP	CZE	68	66.86	98.32	2.06	3.19
EP	CZE	18	17.73	98.50	2.17	2.98
PP	CZE	10	9.18	91.80	1.95	3.26
BP	CZE	20	24.97	124.85	0.77	3.13

[a] Abbreviations: see Table 1 and RSD, relative standard deviation.

tion: isomers like BP and iso BP cannot be separated only by varying pH value or methanol percentage of the CZE buffer. Nevertheless, CZE could have a highly promising potential for the determination of MP, EP, PP and for the simultaneous determination of isomeric forms of BP. Then, combined with the rapidity and the cheaper characteristics of CZE, this technique could be used in routine determination of parabens mixtures in a wide range of cosmetic products.

In HPLC, all the preservatives can be separated and determined within 21 min. This method could be suited for routine quality control of commercial products containing MP, EP, PP and BP.

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