Determination of Parabens in Cosmetics without Previous Extraction by Micellar Liquid Chromatography

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Abstract

A simple and rapid micellar liquid chromatography method for the determination of *p*-hydroxybenzoic acid esters (methyl-, ethyl-, *n*-propyl-, and *n*-butylparaben) in cosmetics (bath foam, milk lotion, hand cream, cream base, and shampoo) is described. The samples are solved with *n*-propanol, further diluted with more *n*-propanol or with an aqueous sodium dodecyl sulphate (SDS) micellar solution, and injected. Separations are performed with a micellar mobile phase containing 0.1M SDS, 2.5% *n*-propanol, 10mM phosphate (pH 3), and with an octadecyl silica column (C_{18}). Calibrations are linear (correlation coefficient $r > 0.999$) and the limits of detection range from 0.03 to 0.3 ng paraben. The determination of parabens at concentrations well below the levels used in cosmetic formulations is possible. Repeatabilities range from 0.2 to 1.1% for 35 ng paraben.

Introduction

Preservatives and antimicrobial agents are used in cosmetics to protect the health of the consumer and to maintain the potency and stability of the product formulation. *p*-Hydroxybenzoic acid esters (parabens) have been widely used as preservatives in cosmetics because of their broad antimicrobial spectrum with relatively low toxicity, good stability, and nonvolatility (1). The European Economic Community Council Directive on cosmetics lists 0.4 or 0.8% (as free acid) as the maximum concentrations for *p*-hydroxybenzoic acid and its esters, depending on whether a single paraben or a combination of them is used (2).

To determine parabens in cosmetics, thin-layer chromatography (3), gas chromatography with previous derivatization (4), and high-performance liquid chromatography (5–8) methods have been described. Usually, prior extraction is required. Methanol (6) or tetrahydrofuran (7) in acid media has been proposed. Liquid–liquid extraction with diethyl ether followed by solid-phase extraction with Sep-Pak Florisil cartridges has also been recommended (5).

The ability of micelles to solubilize is one of the most important properties that allows for the quantitation of analytes in complex matrices without previous extraction. Furthermore, with a micellar mobile phase, the direct injection of the samples into the liquid chromatograph and the separation of hydrophilic and hydrophobic compounds in a single chromatogram is possible. A micellar liquid chromatography (MLC) method for the determination of sunscreen agents in skin lotions without previous extraction was described by Tomasella et al. (9).

In this work, a rapid and simple MLC method for the determination of *p*-hydroxybenzoic acid esters (methyl-, ethyl-, *n*-propyl-, and *n*-butylparaben) in cosmetics (bath foam, milk lotion, hand cream, cream base, and shampoo) is described. The samples are completely solved in *n*-propanol, adequately diluted, and injected. Emulsions were not formed, and excellent baselines were obtained in all cases.

Experimental

Apparatus

A chromatograph (1050 series, Hewlett-Packard, Palo Alto, CA) with an isocratic pump, a Rheodyne (Cotati, CA) valve, a Scharlau (Barcelona, Spain) Spherisorb ODS-2 guard column (C_{18}, 35 × 4.6-mm i.d., 10-µm particle size), and an analytical column (Scharlau, C_{18}, 125 × 4-mm i.d., 5-µm particle size) were used. The flow rate was 1 mL/min. Detection was performed at 280 nm. Data were acquired through an integrator (Hewlett-Packard 3396A series) on a PC computer provided with the PEAK '96 software (Hewlett-Packard, Avondale, PA). Chromatographic parameters were measured with the MICHROM soft-

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Table I. Capacity Factors, Efficiencies, and Asymmetries for the Parabens

Compound	Medium	*k**	*N*	*B/A*
Methylparaben	micellar	4.6	2860	1.1
	n-propanol	4.3	760	0.3
Ethylparaben	micellar	6.7	2800	1.2
	n-propanol	6.4	750	0.3
n-Propylparaben	micellar	9.5	2720	1.2
	n-propanol	9.0	890	0.4
n-Butylparaben	micellar	12.5	2740	1.1
	n-propanol	11.8	1250	0.5

* *k*, capacity factor.
† *N* (efficiency) was calculated according to Foley-Dorsey equation for skewed peaks (12).
‡ *A* and *B* are the distances between the center and the leading and tailing edge of the chromatographic peak, respectively, measured at 10% of peak height.

ware package (10). The dead volume was established from the first significant baseline deviation (11).

Reagents

Nanopure deionized water (Barnstead deionizer, Sybron, Boston, MA), sodium dodecyl sulphate (SDS) (99%, Merck, Darmstadt, Germany), analytical-grade sodium dihydrogenphosphate monohydrate, hydrochloric acid (Panreac, Barcelona, Spain), and *n*-propanol (Scharlau) were used. Stock solutions containing 0.1% (w/w) methyl-, ethyl-, *n*-propyl-, and *n*-butylparaben (purum grade, Fluka, Buchs, Switzerland) in *n*-propanol were prepared.

The recommended mobile phase contained 0.1M SDS, 2.5% (v/v) *n*-propanol, and 0.01M NaH_2PO_4–H_20; the pH was adjusted to 3 with

Table II. Analytical Figures of Merit for the Analysis of Parabens

Compound	Medium	*b* ± *s**	*a* ± *s**	*r*	RSD (%)	LOD (ng)
Methylparaben	micellar	53.32 ± 0.10	0.006 ± 0.003	0.99999	0.22	0.03
	n-propanol	42 ± 1	0.00 ± 0.04	0.9991	1.13	0.09
Ethylparaben	micellar	48.82 ± 0.04	0.0005 ± 0.0013	0.999999	0.24	0.05
	n-propanol	39.2 ± 0.6	–0.02 ± 0.02	0.9998	0.87	0.1
n-Propylparaben	micellar	44.91 ± 0.11	0.004 ± 0.003	0.99999	0.17	0.07
	n-propanol	34.6 ± 0.4	–0.005 ± 0.011	0.9999	0.84	0.2
n-Butylparaben	micellar	42.32 ± 0.09	–0.005 ± 0.003	0.99999	0.41	0.1
	n-propanol	32.4 ± 0.6	–0.02 ± 0.02	0.9996	0.64	0.3

* Calibration parameters, y(area) = $b \bullet x$ (µg) + a (n =5); s, standard deviation.
† Repeatability (as relative standard deviation) for 35 ng paraben (n = 5).
‡ LOD, limit of detection.

Table III. Recoveries of Parabens Added to Commercial Cosmetic Products

	Recovery (%) ± *s*			
Sample*	Methylparaben	Ethylparaben	*n*-Propylparaben	*n*-Butylparaben
Bath foam	100.0 ± 0.8	100.0 ± 0.2	101.0 ± 0.4	99.0 ± 0.4
Body milk	102.0 ± 0.4	99.0 ± 0.2	100.0 ± 0.8	98.0 ± 0.3
Cream base 1	101.0 ± 0.9	100.0 ± 1.1	98 ± 2	102 ± 2
Cream base 2	104.0 ± 0.9	105.0 ± 0.5	109.0 ± 0.6	93.1 ± 0.8
Hand cream	101.0 ± 1.0	93.0 ± 1.5	95.0 ± 0.4	97.1 ± 1.1
Shampoo 1	101.0 ± 0.2	100.0 ± 0.3	100.0 ± 0.7	100.0 ± 0.6
Shampoo 2	101.0 ± 0.3	100.0 ± 0.4	100.0 ± 0.8	100.0 ± 0.6

* 0.1% (w/w) of each paraben was added to the samples.
† Mean and standard deviation calculated over 3 determinations.

0.1M HCl before the addition of *n*-propanol. Mobile phases and solutions for injection were filtered through nylon membranes (0.45-µm pore size, MSI, Westboro, MA).

Sample preparation and calibration procedures

A test portion of approximately 0.2 g of sample was weighed into a 50-mL glass tube fitted with a screw cap, and 25 g *n*-propanol was added. The tube was closed and shaken. If necessary, the mixture was gently heated in a water bath until any lipid phase was melted and dissolved. A 5.0-mL aliquot was diluted to 50 mL in a volumetric flask with either aqueous 0.1M SDS or *n*-propanol, depending on the cosmetic formulation, and 20-µL aliquots were injected. When the sample contained fat-soluble excipients in large concen- trations (as with the hand cream and cream bases), an emulsion was formed by dilution with aqueous 0.1M SDS; in this case, *n*-propanol was used to further dilute the sample.

The calibration standards containing 0.5–2.5 µg/mL of each paraben were prepared by diluting the stock solutions in two steps, the first with *n*-propanol and the second according to the sample dilution procedure with either aqueous 0.1M SDS (final *n*-propanol content was 10%, v) or with more *n*-propanol.

Results and Discussion

Optimization studies

Parabens show a main absorption maximum at 254 nm and a

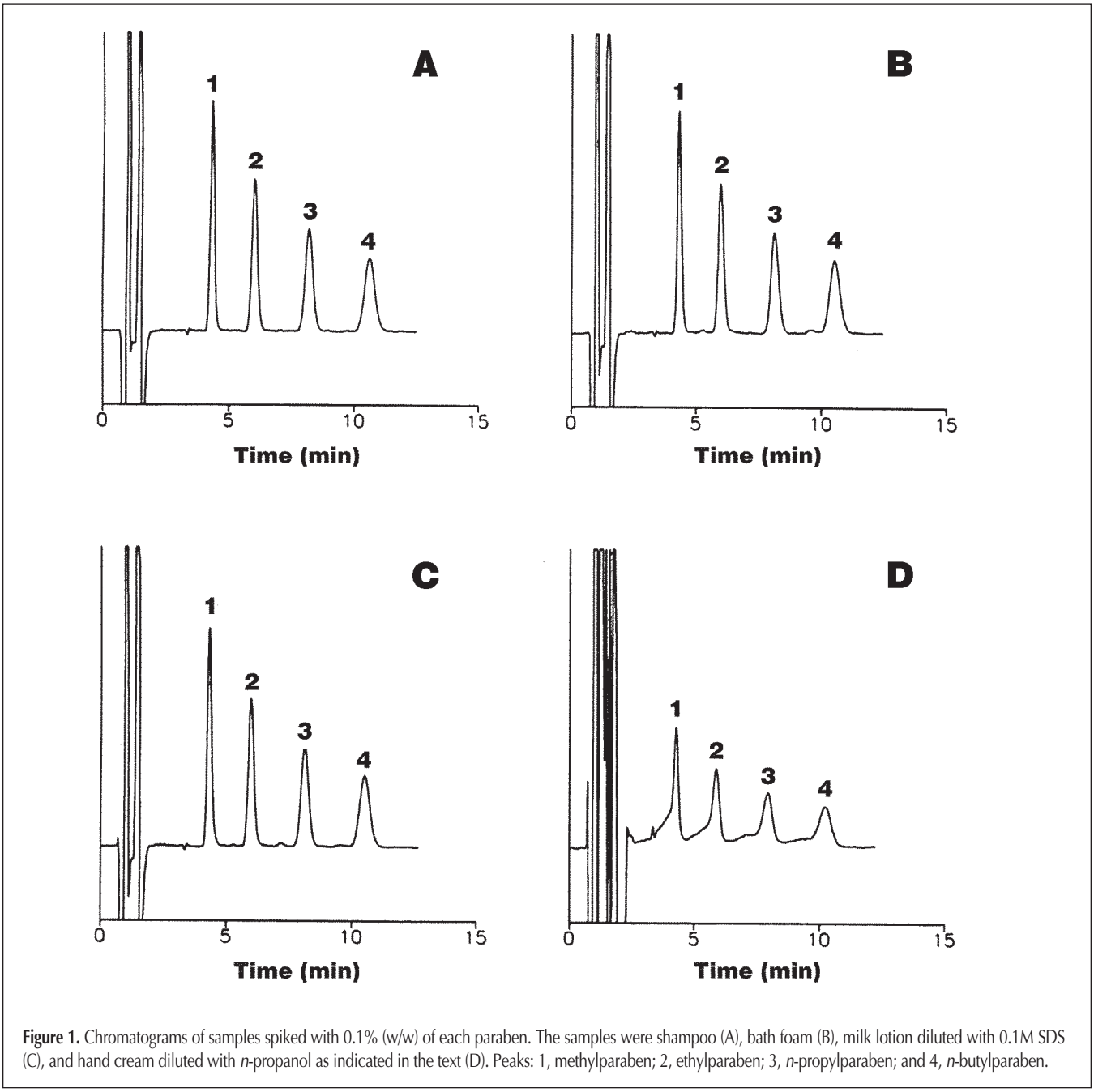

Figure 1. Chromatograms of samples spiked with 0.1% (w/w) of each paraben. The samples were shampoo (A), bath foam (B), milk lotion diluted with 0.1M SDS (C), and hand cream diluted with *n*-propanol as indicated in the text (D). Peaks: 1, methylparaben; 2, ethylparaben; 3, *n*-propylparaben; and 4, *n*-butylparaben.

narrow secondary maximum at 288 nm followed by a steep slope. To improve the selectivity and reproducibility, the minimum between the two maxima, 280 nm, was selected. At this wavelength, the relative sensitivity with respect to the main maximum was 0.8.

In Figure 1, chromatograms of several spiked samples prepared in both 0.1M SDS with 10% (v) *n*-propanol (Figure 1A, B, and C) and *n*-propanol (Figure 1D) are shown. The chromatograms were indistinguishable from those obtained with the corresponding standard solutions prepared in the same media. Good resolution for the parabens was achieved in less than 12 min. Efficiencies, asymmetries, and capacity factors are given in Table I. In comparison to samples diluted with 0.1M SDS, samples diluted with only *n*-propanol produced asymmetric peaks and lower efficiencies, owing to a fronting of the peaks; however, paraben quantitation was not hindered.

Figures of merit

The calibration parameters and figures of merit are given in Table II. Excellent linearity (correlation coefficient $r > 0.999$) was obtained. The limits of detection (LODs) were calculated as three times the standard deviation of the baseline s divided by the slope of the calibration curves obtained from peak heights; s was calculated from the baseline peak-to-peak width that was taken as 5 s (13). The LODs were less than 0.1 and 0.3 ng paraben for the standards prepared with 0.1M SDS and with *n*-propanol, respectively. With these values, the determination of parabens at concentrations well below the levels used in cosmetic formulations was possible. Repeatabilities (as relative standard deviations) for 35 ng injected paraben ranged from 0.2 to 1.1%.

As also shown in Table II, sensitivities (as peak areas) decreased about 22% when solutions in *n*-propanol rather than micellar solutions were injected. Sensitivity losses seemed to be caused by the lower efficiencies.

Analysis of the samples

Cosmetic samples (bath foam, milk lotion, hand cream, cream bases, and shampoos) obtained from local outlets were spiked with 0.1% (w) of each paraben, and the recommended procedure was applied. As shown in Table III, quantitative recoveries with excellent precision were obtained. No deterioration of the column was detected after more than 300 injections.

The contents of the samples were as follows: bath foam consisted of water, sodium laureth sulfate, cocamidopropyl betaine, sodium chloride, parfum, cocamide DEA, glycerin, styrene/acrylates copolymer, nonoxynol-40, nonoxynol-9, nonoxynol-4 sodium sulphate, oat (*Avena sativa*) extract, wheat (*Triticum vulgare*) extract, propylene glycol, PEG-40, hydrogenated castor (*Ricinus communis*) oil, formaldehyde, phosphoric acid, and C.I. 19140; body milk consisted of water, paraffinum liquidum, glycerin, PEG-55 lanolin, ceteareth-6, stearyl alcohol, sodium hidroxide, lactic acid, carbomer, and sodium lactate; cream base 1 consisted of stearyl-stareth-10 alcohol, isooctodecylic ester, myristic ester and vaseline; cream base 2 consisted of ceteareth-10, beeswax, stearyl heptanoate, cetyl octanoate, spermaceti, myristoyl, dimethicone, mineral oil, and lanolin oil; hand cream consisted of water, paraffinum liquidum, glycerin, ceresin, isohexadecane, lanolin alcohol, paraffin, decyl oleate, magnesium sulfate, octyldodecanol, aluminum stereates, citric acid, and panthenol; shampoo 1 consisted of water, cocamidopropyl betaine, sulphuric ester of polyetoxil fatty alcohol, PEG 20 glycerilmonococoate, polietilenicol 6000 distearate, and quaternium 15; shampoo 2 consisted of water, polysorbate-20, sodium laureth sulfate, disodium lauroamphodiacetate, PEG-150 distearate, lauryl betaine, polyquaternium-10, quaternium 15, benzyl alcohol, citric acid, parfum, C.I. 47005, and C.I. 15985.

Conclusion

A rapid and reproducible MLC procedure without previous extraction suitable for the routine analysis of parabens in cosmetics containing a wide variety of ingredients has been developed. All cosmetics were readily and completely dissolved in *n*-propanol, and upon further dilution with an SDS micellar solution or with more *n*-propanol when necessary, direct injection in a micellar mobile phase was possible. Parabens were determined with LODs which were well below the concentrations allowed in European countries. With this procedure, the determination of other additives in cosmetics and other complex matrices containing hydrophilic and hydrophobic components, including fats of vegetal and animal origin and mineral oil products, should be possible.

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References

1. M.S. Parker. *Cosmetic and Drug Preservation*, J.J. Kabara, Ed. Marcel Dekker, New York, NY, 1984, p 389.
2. European Economic Community (EEC) instruction no. 93/35. *Off. J. Eur. Comm.* (Brussels) **L1F1**: 32–37 (1993).
3. N. de Kruijf, M.A.H. Rijk, A. Pranoto-Soetardhi, and A. Schouten. Determination of preservatives in cosmetic products I. Thin-layer chromatographic procedure for the identification of presevatives in cosmetic products. *J. Chromatogr.* **410:** 395–411 (1987).
4. E. Weisenberg, B. Gershon, and J. Schoenberg. Micro-determination of *p*-hydroxybenzoic esters in pharmaceuticals and cosmetics. *J. Assoc. Off. Anal. Chem.* **60:** 56–59 (1977).
5. Y. Maeda, M. Yamamoto, K. Owada, S. Sato, T. Masui, H. Nakazawa, and M. Fujita. High-perfomance liquid chromatographic determination of six *p*-hydroxybenzoic acid esters in cosmetics using Sep-Pak Florisil cartridges for sample pretreatment. *J. Chromatogr.* **410:** 413–18 (1987).
6. N. de Kruijf, A. Schouten, M.A.H. Rijk, and A. Pranoto-Soetardhi.

Determination of preservatives in cosmetic products II. High-perfomance liquid chromatographic identification. *J. Chromatogr.* **469:** 317–28 (1989).
7. L. Gagliardi, G. Cavazzuti, L. Turchetto, F. Manna, and D. Tonelli. Determination of preservatives in cosmetic products by reversed-phase high-perfomance liquid chromatography IV. *J. Chromatogr.* **508:** 252–58 (1990).
8. L. Gagliardi, D. De-Orsi, F. Manna, and D. Tonelli. Simultaneous determination of antioxidants and preservatives in cosmetics and pharmaceutical preparations by reversed-phase HPLC. *J. Liq. Chromatogr. Relat. Technol.* **20:** 1797–1808 (1997).
9. F.P. Tomasella, P. Zuting, and L.J. Cline Love. Determination of sun-screen agents in cosmetic products by micellar liquid chromatography. *J. Chromatogr.* **587:** 325–28 (1991).
10. J.R. Torres-Lapasió, J.J. Baeza-Baeza, and M.C. García Alvarez-Coque. Global treatment of chromatographic data with MICHROM. *Anal. Chim. Acta* **348:** 187–96 (1997).
11. J.R. Torres-Lapasió, J.J. Baeza-Baeza, and M.C. Garcia Alvarez-Coque. On the measurement of dead time in micellar liquid chromatography. *J. Liq. Chromatogr. Relat. Technol.* **19:** 1205–28 (1996).
12. J.P. Foley and J.G. Dorsey. Equations for calculation of chromatographic figures of merit for ideal and skewed peaks. *Anal. Chem.* **55:** 730–37 (1983).
13. M.J. Adams. *Chemometrics in Analytical Spectroscopy.* The Royal Society of Chemistry, Cambridge, U.K., 1995, pp 32–34.

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