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Technical note

High-performance liquid chromatographic method for the comparison of the photostability of five sunscreen agents

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Abstract

Sunscreen agents are commonly used in cosmetic products to filter out noxious radiation in sunlight. A convenient high-performance liquid chromatographic (HPLC) method for the quantification of five sunscreens after irradiation has been selected. We used this analytical method to compare the photostability of benzophenone-3, PEG-25 PABA, octyl dimethyl PABA, 4-methylbenzylidene camphor and butyl methoxydibenzoylmethane, at levels in the range of 25–60 μM . The assay was carried out, using a C_8 column with a methanol–water mobile phase. The detector was set at a wavelength of 300 nm. The assay was linear with the following limits: 0.2 $\mu\text{g ml}^{-1}$ for benzophenone-3, 1 $\mu\text{g ml}^{-1}$ for PEG-25 PABA, 0.15 $\mu\text{g ml}^{-1}$ for octyl dimethyl PABA, 0.1 $\mu\text{g ml}^{-1}$ for methylbenzylidene camphor and 0.05 $\mu\text{g ml}^{-1}$ for butyl methoxydibenzoylmethane. The half-lives calculated indicate a very good photostability of the sunscreens studied and permit to classify amongst themselves. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Sunscreen agents; 2-Hydroxy-4-methoxybenzophenone; 4-Methylbenzylidene camphor; Octyl dimethyl PABA; PEG-25 PABA; Butyl methoxydibenzoylmethane

1. Introduction

Photoaging and carcinogenesis are long-term consequences of repeated exposures [1–3]. The photo-protection includes all methods to prevent UV radiation (UVR) damage to the skin, including sunscreen [4]. The molecules employed in cosmetic products to protect skin from sun are classed as physical blockers, which interrupt the path of UV light by scattering or reflection and chemical sunscreen [5–10]. This study was aiming at compar-

ing the photostability of butyl methoxydibenzoylmethane with that of other sunscreen agents using a HPLC method.

2. Experimental

2.1. Chemicals

PEG-25 PABA (tradename, Uvinul P25) and 2-hydroxy-4 methoxybenzophenone (tradename, Uvinul M40) were obtained from BASF (Ludwigshafen, Germany). 3-(4-Methylbenzylidene)camphor (tradename, Eusolex 6300), 4-*t*-butyl-4'-methoxy-

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dibenzoyl methane (tradename, Eusolex 9020) and *N,N*-dimethyl-4-aminobenzoic acid-2-ethyl hexyl ester (tradename, Eusolex 6007) were obtained from Merck (Darmstadt, Germany). The structures are shown in Fig. 1. All other chemicals used were of analytical-reagent grade. Water, applied throughout the study, was purified by an Autostill 4000X (Jencons) apparatus. Demineralized, deionised water was obtained from MilliQ system (Millipore).

Methanol (HPLC reagent grade) and acetic acid (AnalaR grade) were purchased from Merck.

2.2. Experimental protocol

PEG-25 PABA was dissolved in water and benzophenone-3, methylbenzylidene camphor, butyl methoxydibenzoylmethane and octyl dimethyl PABA were dissolved in ethanol to give 37.9, 61.4, 35.4,

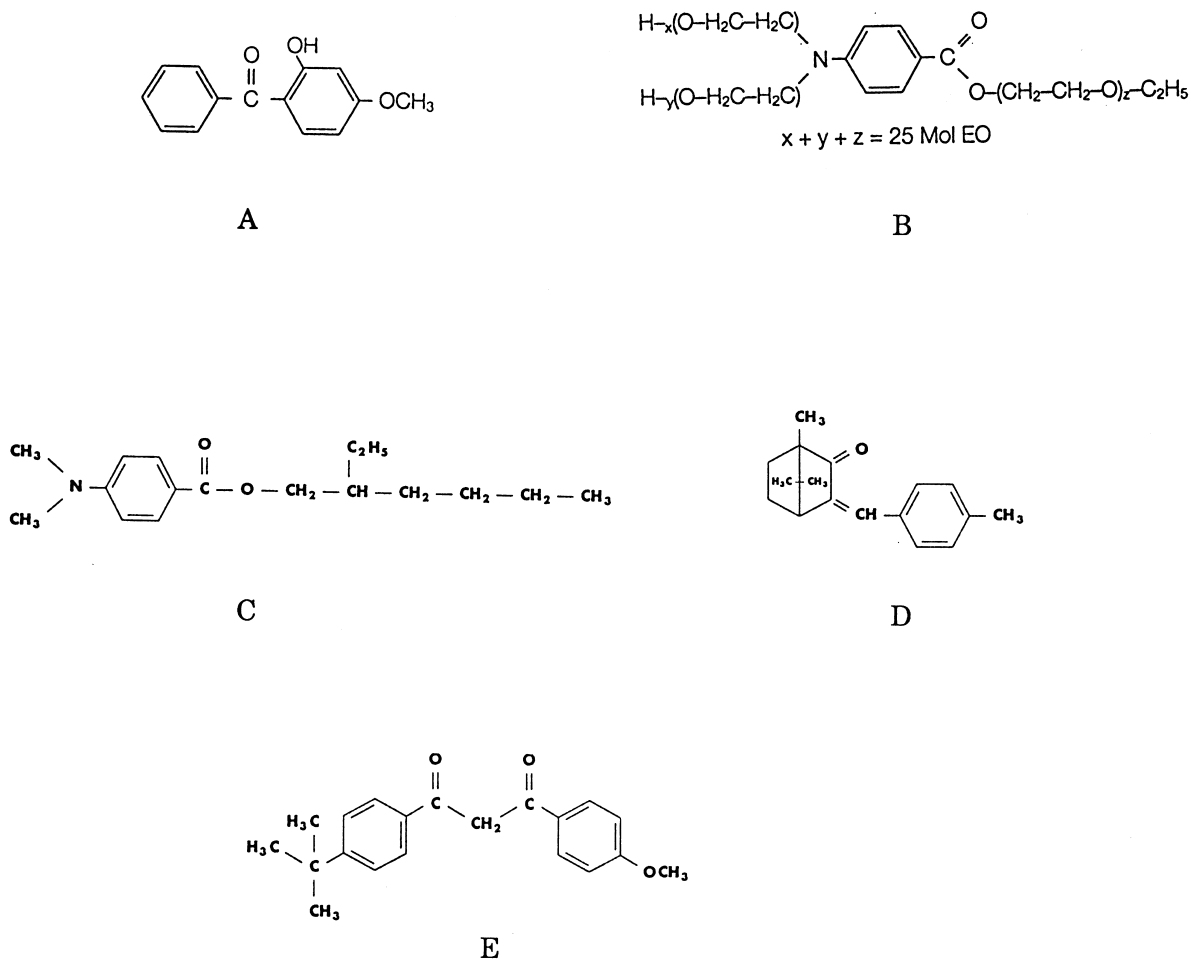


Fig. 1. Chemical structure of sunscreen agents, wavelengths of maximum absorbance (λ_{\max}) and values of molar absorptivity (ϵ). (A) 2-Hydroxy-4-methoxybenzophenone (INCI name, benzophenone-3; molar formula, $C_{14}H_{12}O_3$; molecular weight, 228.24 g mol⁻¹; λ_{\max} = 287 nm; ϵ = 15 300 M⁻¹ cm⁻¹). (B) 4-Bis-(polyethoxy)-aminobenzoic acid-polyethoxyethyl ester (INCI name, PEG-25 PABA; molar formula, $C_{59}H_{111}NO_{27}$; molecular weight, 1266.50 g mol⁻¹; λ_{\max} = 308 nm; ϵ = 47 400 M⁻¹ cm⁻¹). (C) *N,N*-Dimethyl-4-aminobenzoic acid-2-ethyl hexyl ester (INCI name, octyl dimethyl PABA; molar formula, $C_{17}H_{27}NO_2$; molecular weight, 277.41 g mol⁻¹; λ_{\max} = 310 nm; ϵ = 89 000 M⁻¹ cm⁻¹). (D) 3-(4-Methylbenzylidene)camphor (INCI name, 4-methylbenzylidene camphor; molar formula, $C_{18}H_{22}O$; molecular weight, 254.37 g mol⁻¹; λ_{\max} = 301 nm; ϵ = 53 600 M⁻¹ cm⁻¹). (E) 4-*t*-Butyl-4'-methoxydibenzoylmethane (INCI name, butyl methoxydibenzoylmethane; molar formula, $C_{20}H_{22}O_3$; molecular weight, 310.39 g mol⁻¹, λ_{\max} = 358 nm; ϵ = 86 000 M⁻¹ cm⁻¹).

25.8 and 32.5 μM stock solutions, respectively, which were stored at 4°C.

Until now, contrary to the thermal stability, there is no internationally fixed testing conditions for photostability testing. Our experimental conditions were the following: solutions of various sunscreens studied were enclosed in spectrophotometer tubes and exposed to the light source in the light-stability cabinet (Original Hanau, No. 7011, Original Hanau Quartzlampen GmbH). The intensity of UV-A and UV-B was measured with an Osram apparatus (Centra-UV-Meßgerät). This intensity was maintained at 6.45 and 1.47 mW cm^{-2} for UV-A and UV-B, respectively. All tubes containing sunscreen solutions were covered with aluminium foil before exposure in order to eliminate the influence of heat generated by the light within the cabinet. The analysis was carried out on triplicate samples.

The absorbance spectra of the five sunscreen agents were analyzed between 200 and 400 nm with a Hitachi UV-visible, double-beam spectrophotometer, model U-2000. Slit width was fixed at 2 nm. Solutions were recorded in 1-cm path-length quartz cells over the 200–400 nm range ($\Delta\lambda=2.3$ nm). The scan speed was 400 nm min^{-1} .

The HPLC system was composed of a Waters Model 6000 A pump (Waters Millipore, St Quentin,

France), a Waters Lambda Max model 481 LC variable-wavelength detector and a Merck D-2500 model integrator (Merck, France). A 5-mm Nucleosil 100-5C₈ HD column (250×4 mm I.D.) was used. The column was eluted with a mobile phase which was a gradient of methanol and acetic acid (1% in water). The percentage of methanol increased from 80 to 100% in 10 min. The solvents were filtered separately through a 0.45- μm filter (Millipore) and mixed in the desired proportions. The flow-rate was 1.0 ml min^{-1} . The detector attenuation was 0.2 AUFS. Ten μl were injected into the HPLC column.

Analysis of variance (ANOVA) and least-significant differences (L.S.D.) ($P<0.05$) were computed from the results of the three replicate samples.

3. Results and discussion

The spectra of all the sunscreens studied are shown in Fig. 2. A chromatogram is shown in Fig. 3. The characteristics of the chromatographic assay are shown in Table 1. Excellent linearity was obtained for all molecules. There was no significant difference between day-to-day analysis (slopes evaluation, $P<0.05$). Detection limits (LOD_S) determined as higher

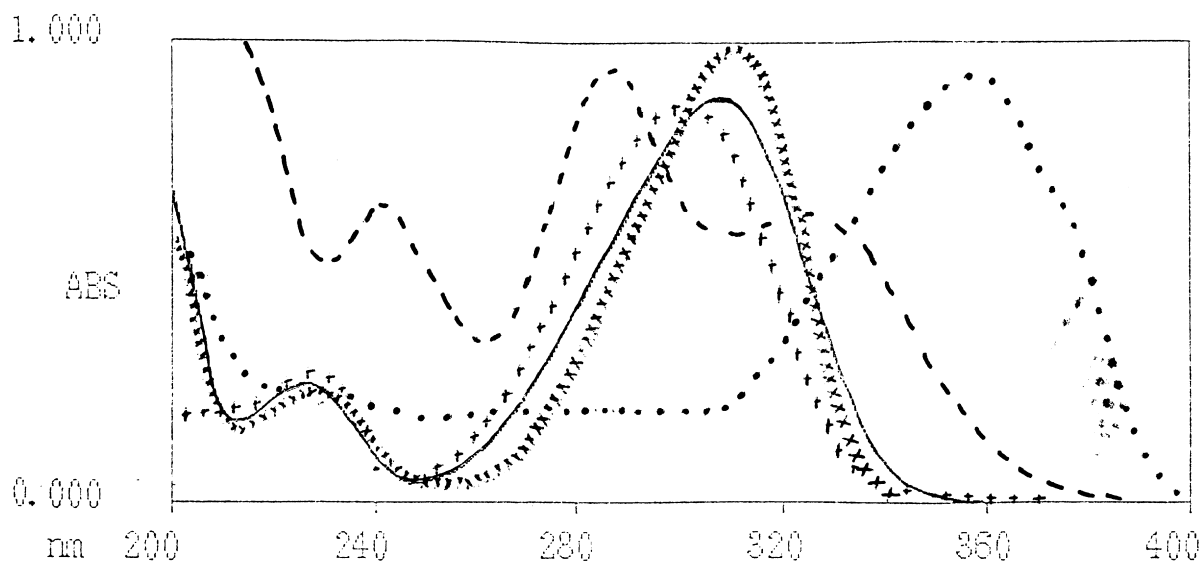


Fig. 2. Absorbance spectra of sunscreen agents: PEG-25 PABA (—); benzophenone-3 (---); methylbenzylidene camphor (+ + +), butyl methoxydibenzoylmethane (···) and octyl dimethyl PABA (× × ×).

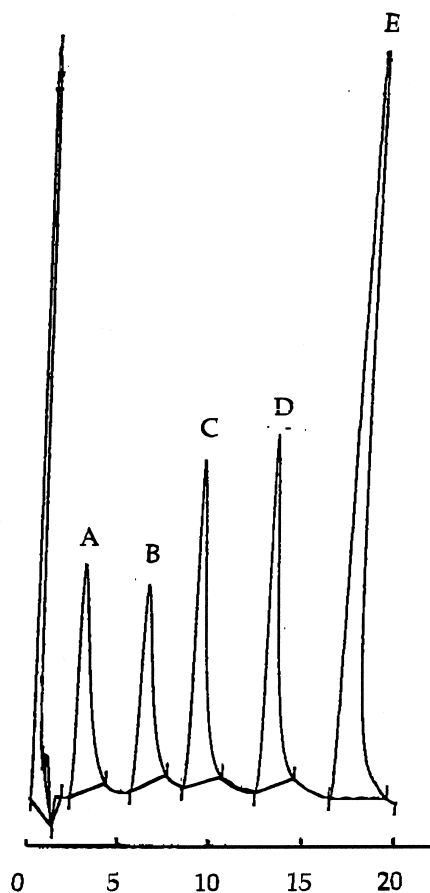


Fig. 3. Analytical HPLC analysis of five sunscreen agents. Peaks: (A) benzophenone-3; (B) methylbenzylidene camphor; (C) octyl methyl PABA; (D) butyl methoxydibenzoylmethane; (E) PEG-25 PABA.

than three times the baseline noise level ($S/N=3$), were $0.01 \mu\text{g ml}^{-1}$ for butyl methoxydibenzoylmethane, octyl dimethyl PABA, methyl-

benzylidene camphor and benzophenone-3, and $0.02 \mu\text{g ml}^{-1}$ for PEG-25 PABA.

The order of the photodegradation reaction was determined by the least-squares method linear adjustment and by calculation of correlation coefficients, in order to choose between the nul-order kinetics and first-order kinetics. The degradation rate constant was calculated from the slope of the line of the peak area of each compound versus time. The percentage of substance remaining was then calculated. The photodegradation of sunscreens in diluted aqueous solution follows apparent first-order kinetics and is described by the following equation:

$$C/C_0 = e^{-kt}$$

where C and C_0 are the concentrations of sunscreens at time t after irradiation and initially, and k is the apparent first-order degradation rate constant. This equation gives us the values of the degradation rate constants and we can deduce for each sunscreen agent the 90% shelf-life (time by which the sunscreen concentration had decreased by 10% from the initial concentration) and the predicted half-life (Table 2). Furthermore, there is no international directives concerning required half-lives. But, we consider that a molecule or a pharmaceutical product is stable at time t if its concentration is superior or equal to 90% of its initial concentration.

We can class sunscreen agents according to their photostability. In a stability increasing classification, we find: methylbenzylidene camphor < PEG-25 PABA < octyl dimethyl PABA < butyl methoxydibenzoylmethane < benzophenone-3.

The chromatographic assay appears to be a convenient method to obtain data about this class of cosmetic ingredients.

Table 1
Characteristics of the HPLC assay

Molecule studied	Regression equation ^a	Correlation coefficient (r)	Linear range ($\mu\text{g ml}^{-1}$)
Benzophenone-3	$y = 15557x - 0.020$	0.9998	2.0–15.0
PEG-25 PABA	$y = 23228x + 0.510$	0.9999	10.0–50.0
Octyl methyl PABA	$y = 30197x + 0.661$	0.9999	1.5–10.0
Methylbenzylidene camphor	$y = 23376x + 0.564$	0.9999	1.0–10.0
Butyl methoxydibenzoylmethane	$y = 35679x + 0.585$	0.9999	0.5–10.0

^a y , peak area; x , sunscreen concentration ($\mu\text{g ml}^{-1}$).

Table 2

Degradation rate constants (min^{-1}), 90% shelf-lives (min) and half-lives (min) for each sunscreen agent studied

Molecule studied	Degradation rate constant, k (min^{-1} ; \pm SEM)	90% Shelf-life (min)	Half-life (min)
Benzophenone 3	0.50 ± 0.005	2141	13 978
PEG-25 PABA	2.29 ± 0.120	422	2987
Octyl methyl PABA	2.12 ± 0.100	510	3287
Methylbenzylidene camphor	7.54 ± 0.420	108	888
Butyl methoxydibenzoylmethane	1.67 ± 0.020	661	4188

S.E.M., standard error of means of three determinations.

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