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Skin Penetration and Sun Protection Factor of Five UV Filters: Effect of the Vehicle

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Key Words
UV filters · Sunscreen · Penetration · Vehicle · Human skin · Stratum corneum · Sun protection factor

Abstract
To gain information about efficacy and safety of sunscreens, we compared the skin penetration of ultraviolet (UV) filters from two vehicles, i.e. an oil-in-water (O/W) emulsion gel and petrolatum jelly both in vitro and in vivo, as well as the corresponding pharmacological effect, i.e. the sun protection factor (SPF) in vivo. The UV filters studied were benzophenone-3 (BPH), ethylhexyl methoxycinnamate (EHM), butyl methoxydibenzoyl methane, ethylhexyl salicylate and homosalate. The human skin penetration of these five chemicals from the two vehicles was determined both in vitro using Franz cells and in vivo using a standardized tape-stripping method. The SPF of the two sunscreens was determined in vivo following the COLIPA guidelines. In vitro none of the filters permeated through the skin after 6 h of product application and very little could be found in the skin. BPH and EHM were the only UV filters found in the dermis (both after 30 min and 6 h). An effect of the vehicle could be noticed only for BPH after 30 min in the dermis and 6 h in both dermis and epidermis. In vivo, no differences in the amount of individual UV filters (in % of the applied dose) in the 15 first strips of the stratum corneum (SC) were found following 30 min of application of the formulations; however, the amount of UV filters that were retained in the SC was significantly higher (around 3 times) with the O/W emulsion gel than with the petrolatum jelly. This difference between the two vehicles was also of consequence for the SPF in vivo measured 30 min after application of the products (SPF ≅ 18 with the O/W emulsion gel com-

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pared to SPF ≅ 10 with the petrolatum jelly). By choosing the right vehicle or optimizing it, not only sunscreen products can be significantly improved in terms of pharmacological efficacy but the potential toxicological risk associated with the skin penetration of UV filters may be significantly reduced.

Introduction

The increased awareness of protection against skin cancer has led to a worldwide rise in the usage of topically applied chemical sunscreen agents [1, 2]. Sunscreen agents (i.e. UV filters) are widely incorporated into skin products designed for daily use in the form of emulsions, gels, oils, lipsticks with an adequate sun protection factor and high substantivity. The desirable site of action of the UV filters is restricted to the skin surface or to the uppermost part of the stratum corneum. However, it has been demonstrated that penetration into skin, permeation through skin and retention of UV filters in the skin from topical products can differ significantly between formulations used. Treffel and Gabard [3] showed that sunscreen agents were better retained in the stratum corneum using an emulsion-type formulation than using petrolatum jelly. Marginean Lazar et al. [4] demonstrated differences in UV filter penetration among various emulsion-type formulations. During a market product survey, Jiang et al. [5] also found that diffusion of UV filters across the epidermis varied significantly with formulation type. Despite extensive usage of sunscreen products, so far moderate attention has been paid to the potential permeation of the UV filters through skin and the possible subsequent toxic effect [6]. Hayden et al. [7] found that in humans up to 2% of an applied dose of benzophenone-3 and its metabolites were excreted in the urine following topical application of a commercially available product.

Modern sun protection products contain a variety of UV filters to (a) broaden the sun protection range, (b) increase the sun protection factor and (c) reduce the concentration of particular UV filters with regard to their toxicological risk [8]. It is evident that current and future vehicle development is aimed at formulating vehicles that support penetration of the UV filters only into the uppermost part of the stratum corneum. At the same time the UV filters should be retained at this location and permeation through the skin should be prevented.

To date, investigations on sunscreen products often address isolated problems such as vehicle effects, percutaneous absorption, risk assessment or sun protection capacity. Many studies are carried out in vitro but studies that allow to link data from several methods remain erratic [3, 9]. Against this background we investigated the penetration into human skin of five UV filters simultaneously from two different vehicles in vitro and in vivo. Subsequently, the sun protection factor of the two preparations was determined in vivo according to current guidelines [10].

Materials and Methods

Chemicals and Formulation

Benzophenone-3 (BPH), ethylhexyl methoxycinnamate (EHM) and ethylhexyl salicylate (EHS) were from Haarmann & Reimer GmbH (Holzminden, Germany), butyl methoxydibenzoyl methane (BMDM) from Hoffmann-La Roche Ltd (Basel, Switzerland) and homosalate (H) from Merck (Germany). Bovine serum albumin (BSA) and sodium chloride were from Fluka (Buchs, Switzerland), Tween-80 from Selectchemie AG (Switzerland), acetic acid from Merck and methanol HPLC grade from Labscan Ltd (Dublin, Ireland). Human full-thickness skin, free from subcutaneous fat and other extraneous tissue, was obtained

Table 1. Physicochemical properties of UV filters used

INCI name	Molecular formula	Molecular weight	Solubility in receptor fluid[1], μg/ml	log k_{oct}[2]	Absorption maxima nm
Benzophenone-3	$C_{14}H_{12}O_3$	228	134	3.58 [3], 2.63 [16]	288–325
Ethylhexyl methoxycinnamate	$C_{18}H_{26}O_3$	290	172	5.96 [3], 5.65 [16] 6.40 [17]	307
Butyl methoxydibenzoyl methane	$C_{20}H_{22}O_3$	310	99	4.68 [18]	358
Ethylhexyl salicylate	$C_{15}H_{22}O_3$	250	213	6.02 [18], 6.19 [17]	311
Homosalate	$C_{16}H_{22}O_3$	262	301		306

1 NaCl 0.9%/BSA 1.5%.
2 Octanol/water partition coefficient from references.

from 3 Caucasian women undergoing breast reduction or abdominal surgery at the local hospital and stored frozen until required (–18°C). Two sunscreen formulations were prepared containing 5% BPH, 7.5% EHM, 2% BMDM, 5% EHS and 5% H. Formulation 1 was an O/W emulsion – water (60%), ethanol, phospholipids, carbopol, sorbitol, triethanolamine, cetyl alcohol, amphisol, silicone, tocopherol and preservatives – and formulation 2 was petrolatum jelly (vaselinum album, PhHelv. VII).

In vitro Penetration

Human full-thickness skin was mounted in a conventional static Franz diffusion cells (Crown Glass, Somerville, N.J., USA) with a receptor volume of 12.4 ml. The receptor compartment (n = 4) was filled with an aqueous solution containing NaCl (0.9%) and BSA (1.5%), thermostated at 34°C and stirred by a Teflon-coated magnetic bar at 600 rpm. The integrity of the full-thickness skin was examined by measuring the transepidermal water loss (TEWL) using the Tewameter TM 210 (Courage & Khazaka, Germany). The cell allowed 1.76 cm^2 skin to be exposed to the formulation at room temperature (22°C). 3.0 ± 0.4 mg/cm^2 sunscreen product were applied to the skin for either a period of 30 min or 6 h. At the end of the experiment, 1 ml of receptor fluid was removed from the cell and analyzed (assessment of possible permeation of UV filters). The UV filters were readily soluble in the receptor fluid as determined by HPLC (table 1). The skin surface was washed twice with cotton swabs and 2 ml methanol/water (60/40) containing 0.5% Tween-80. The skin was then removed from the cells, epidermis and dermis separated by the hot plate method (60°C for 2 min) and punch biopsies (8 mm) from the tissue samples were taken. The skin tissue was powdered in a steel ball grinder mill (Retsch, Haan, Germany) under liquid nitrogen. The sunscreen agents were extracted from the powder with 2 ml methanol, filtered through a 0.2-μm titan filter (Scientific Res. Inc., Eatontown, N.J., USA) and quantified by HPLC (extraction recovery was >95%). The total recovery for the UV filters was measured at 85–95% and complies with the COLIPA guidelines [11].

In vivo Penetration

In vivo penetration of the UV filters was determined by tape-stripping as previously described [12]. After informed consent, 6 healthy volunteers aged 25–53 years participated in this study. The trial was approved by the local Ethics Committee. Briefly, 2 mg/cm^2 sunscreen product was applied to areas (2 × 2 cm) on the volar side of the forearm. The sunscreen products were randomly allocated to areas on the left or right arm on the upper or lower part of the forearm. 30 min after application the remaining product was removed from the skin with two dry cotton swabs and the skin was tape-stripped 16 times with D-Squames (CuDerm, Dallas, Tex., USA). The tapes were applied to the skin with a constant pressure 0.365 N/cm^2. Strip No. 1 was measured separately, strips No. 2–6, No. 7–11 and No. 12–16 were pooled and the UV filters were extracted with methanol (extraction recovery >97%) and subsequently quantified by HPLC. UV filters in strip No. 1 were considered as not penetrated. The average overall recovery of the UV filters was around

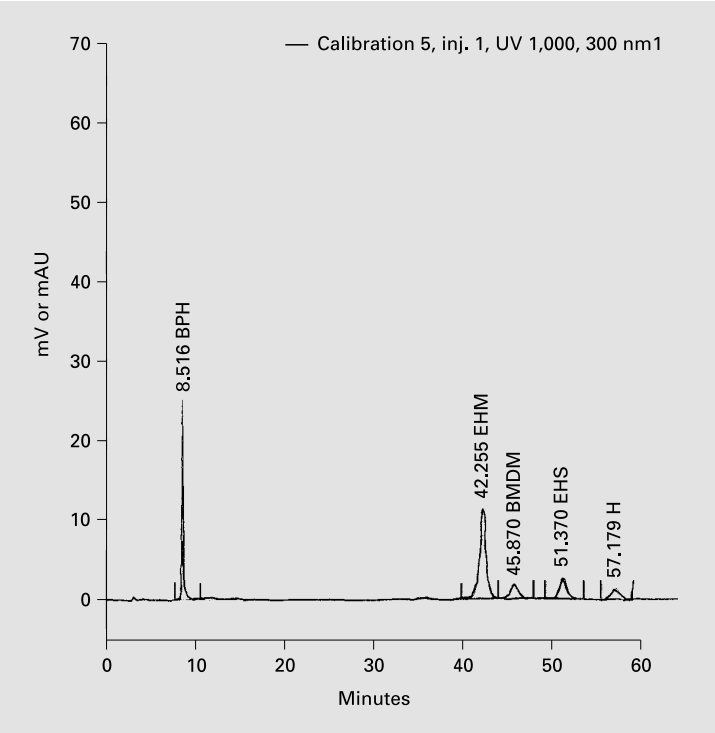


Fig. 1. HPLC chromatogram of the five UV filters (BPH, EHM, BMDM, EHS and H).

83% for the emulsion gel and 93% for the petrolatum jelly. This was satisfactory considering the fact that the SC was stripped only 16 times.

Sun Protection Factor (SPF)

The SPF was determined according to the COLIPA guidelines 30 min after application of the formulations on the back of 6 volunteers using a multiport solar ultraviolet simulator (Model 601, Solar Light Corp., Philadelphia, Pa., USA) [10].

HPLC Analysis

A method was developed to quantify the five UV filters simultaneously by HPLC (Thermo Finnigan, USA). A 250 × 4 mm Nucleosil C18, HD, 3-µm column (Macherey & Nagel, Oensingen, Switzerland) was used at a constant temperature of 10°C. The flow rate of the mobile phase – methanol/water/acetic acid (83/17/0.01, v/v) was 0.4 ml/min and the detection wavelength was 300 nm. Samples were injected via a 10-µl loop. Peak-area ratios were computed using the Chromquest Chromatography software (Thermoquest Inc., San Jose, Calif., USA) and calibration curves were obtained from least squares linear regression established from four calibration points for each UV filter. Using a diode-array detector (UV-6000, Thermo Finnigan, USA), the limit of quantification (LOQ) obtained was 0.1 µg/ml for all the UV filters and the limit of detection (LOD) was around 0.01 µg/ml. A typical chromatogram is shown in figure 1.

Results

In vitro Penetration

The in vitro penetration data are shown in table 2. After 30 min and 6 h, BPH and to a lesser extent EHM were detected in the dermis. The other UV filters were not detectable in the dermis. Penetration of BPH into dermis was more pronounced from petrolatum. Penetration of all UV filters – except BPH – into epidermis after 30 min and 6 h was com-

Table 2. Penetration in vitro of five UV filters 30 min and 6 h after product application; μg/cm²; mean, n = 4 (% of the applied dose)

UV filter	Vehicle	Epidermis		Dermis	
		30 min	6 h	30 min	6 h
BPH					
	Emulsion gel	0.5 (0.3)	1.1 (0.6)	0.9 (0.5)	1.3 (0.7)
	Petrolatum	0.6 (0.3)	2.8 (1.5)	1.6 (1.1)	3.6 (2.0)
EHM	Emulsion gel	0.5 (0.2)	0.4 (0.2)	0.3 (0.1)	0.2 (0.1)
	Petrolatum	0.4 (0.1)	0.9 (0.3)	0.5 (0.2)	0.2 (0.1)
BMDM	Emulsion gel	0.1 (0.1)	0.1 (0.1)	0	0
	Petrolatum	0.1 (0.2)	0.2 (0.2)	0	0
EHS	Emulsion gel	0.4 (0.2)	0.4 (0.2)	0	0
	Petrolatum	0.4 (0.2)	0.6 (0.3)	0	0
H	Emulsion gel	0.4 (0.2)	0.3 (0.2)	0	0
	Petrolatum	0.4 (0.2)	0.6 (0.3)	0	0

parable. None of the UV filters did permeate through the skin after 6 h application.

In vivo Penetration

The in vivo penetration data are shown in table 3 and figure 2. The data show a clear vehicle effect on penetration of the UV filters into the stratum corneum. The effect of the emulsion gel formulation was more pronounced in the upper part (strips 2–6) than in the deeper parts (strips 7–11 and 12–16, respectively) of the stratum corneum. The total amount of UV filters penetrating into the stratum corneum (strips 2–16) from the emulsion gel formulation was significantly higher (table 3). The average penetrated percentage of the dose applied was similar for each UV filter (25.8% for the emulsion gel formulation and 10.3% for the petrolatum jelly formulation).

Sun Protection Factor

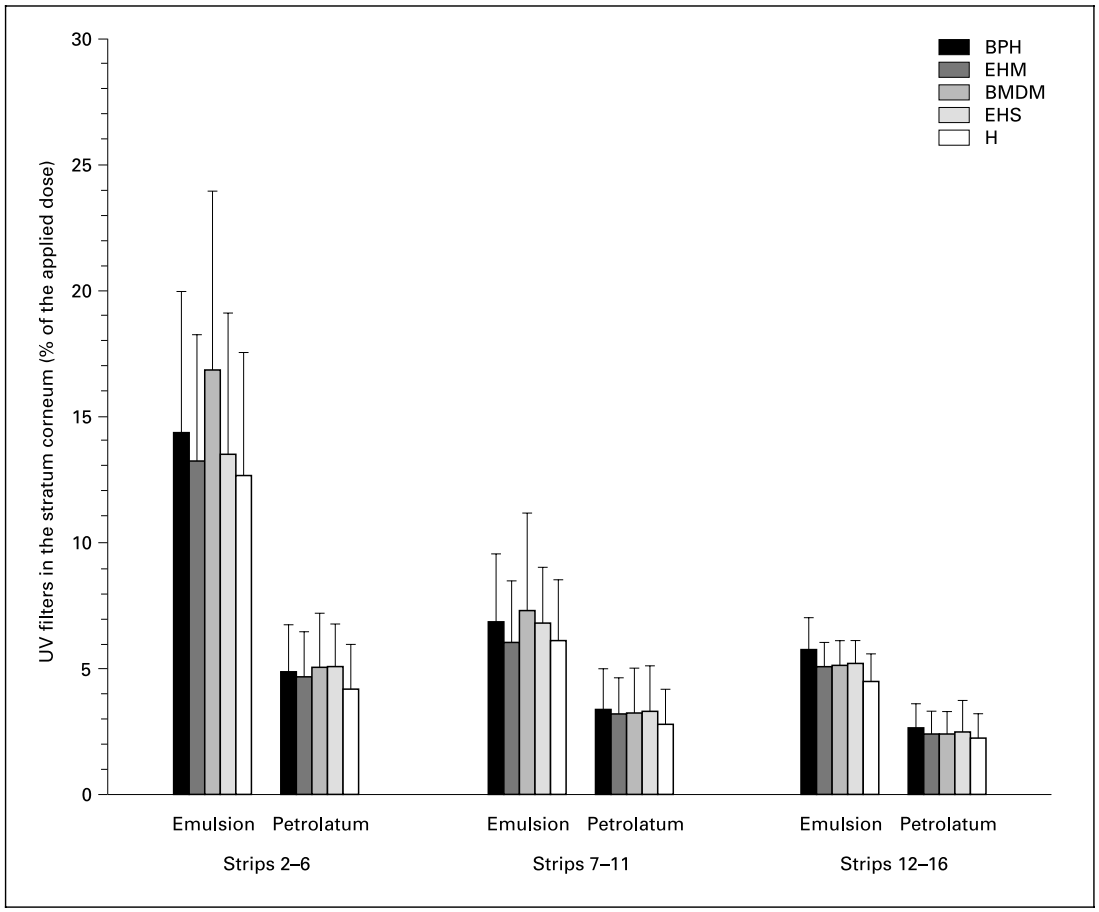
As shown in figure 3, the SPF values of the two formulations (18.2 ± 6.0 for the emulsion gel formulation and 9.9 ± 1.2 for the petrolatum jelly formulation) were significantly different.

Table 3. UV filters present in strips 2–16 of the SC 30 min after application of the sunscreens; μg/cm²; mean ± SD, n = 6 (% of the applied dose)

UV filter	Amount, μg/cm² (% applied dose)	
	emulsion gel	petroleum
BPH	29.9 ± 6.6 (26.9)	9.8 ± 4.0 (10.7)
EHM	40.2 ± 8.9 (24.1)	13.8 ± 5.1 (10.0)
BMDM	12.9 ± 3.5 (29.2)	3.9 ± 1.7 (10.6)
EHS	28.4 ± 6.6 (25.6)	10.1 ± 3.5 (11.0)
H	25.7 ± 6.4 (23.2)	8.3 ± 3.6 (9.0)

Discussion

The present data confirm earlier investigations on the in vitro penetration of BPH and EHM [3]. Penetration of these UV filters was vehicle-dependent and both compounds were detected in the dermis already 30 min after product application (BPH and to a much lesser extent EHM). The polar properties of UV filters may be responsible for these findings. Both in vitro and in vivo investigations on BPH [in vitro, 13, 14] support these results.



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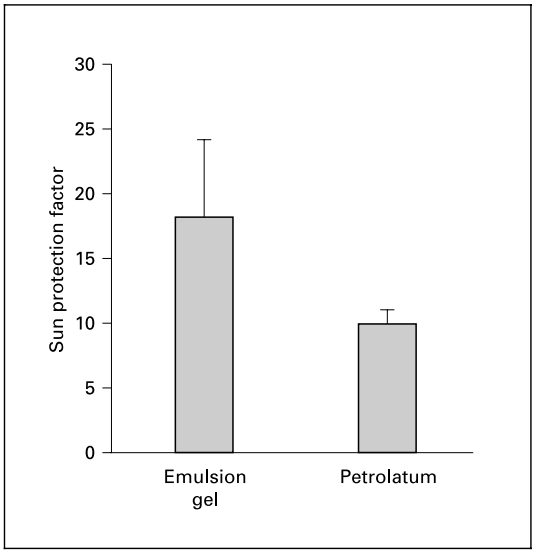

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Fig. 2. Concentration profile of the UV filters present in the stratum corneum in vivo 30 min after application of the emulsion gel or petrolatum jelly. The UV filter amounts in strips 2–6, 7–11 and 12–16 of the stratum corneum expressed as percentage of the applied dose are shown (mean ± SD, n = 6).

Fig. 3. Sun protection factor measurements in vivo 30 min after application of sunscreens containing the same amount of UV filters in different vehicles (mean ± SD, n = 6).

On the other hand, the more lipophilic UV filters tend to be absent in the hydrophilic dermis and to accumulate in the more lipophilic stratum corneum [5]. The possibility of an insufficient solubility of the UV filters in the receptor medium that could be responsible for the above-described phenomenon has been excluded by using a receptor medium in which they were sufficiently soluble to guarantee permanent sink conditions (table 1).

The in vivo penetration data show a clear dependence on vehicle properties. The concentration of the UV filters in the upper part of the stratum corneum (strips 2–6) was significantly higher after application of the emulsion gel formulation than after application of the petrolatum jelly formulation. In the deeper parts of the stratum corneum (strips 7–11 and 12–16) the UV filters concentrations – delivered from the emulsion gel formulation – were significantly lower but still higher than those achieved with the petrolatum jelly formulation. After application of the petrolatum jelly formulation, UV filter concentrations in all parts of the stratum corneum (strips 2–6, 7–11 and 12–16) were low and tended to decrease slightly with increasing depth.

Which of the properties of the vehicle is responsible for the present results remain to a certain extent speculative. However, one may argue that, first, the ingredients of the emulsion gel formulation that have penetrated into the stratum corneum increase solubility of the UV filters therein. Second, the emulsion gel formulation supports an efficient partitioning of UV filters into the stratum corneum. Both could be responsible for the high amount of UV filters in the upper part of the stratum corneum (strips 2–6). The petrolatum jelly formulation possibly hampers these mechanisms. Data in favor of this interpretation have been published for salicylic acid delivered to the skin from an emulsion-type formulation and from a petrolatum jelly formulation [9]. Different product spreadability [15] as well as dramatic changes in the formulation occurring after application of the emulsion gel formulation (e.g. water evaporation) possibly increasing the thermodynamic activity of the UV filters could also explain their efficient delivery to the upper part of the stratum corneum.

The question whether UV filters acts *on* or *in* the skin has so far not been fully answered. Despite the fact that an answer would be a key to improve formulations of sun protection products, many publications carefully avoid addressing the question. Treffel and Gabard [3] have shown that removal of nonpenetrated sunscreen formulation from the skin prior to the determination of the SPF reduces the protection factor significantly (approximately factor 2.8 in the SPF range 5–15) showing that the product on the skin does actively contribute to the total SPF. It was also shown that the amount UV filters delivered from two different formulations into the skin were directly related to the SPF determined after removal of nonpenetrated sunscreen formulation.

In the present study the emulsion gel formulation delivered a higher amount of UV filters to the stratum corneum than the petrolatum jelly formulation. As a result, the SPF of the emulsion gel formulation was significantly higher. From our data and data currently available, one may conclude that the total SPF of a sun protection product is the sum of the protective properties of the formulation on the skin and the protective properties of the UV filters that have penetrated into the stratum corneum. These findings underline the importance of formulation into which the UV filters have been incorporated.

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