Faculty of Pharmacy, Université de Nantes, France

Study of the efficacy of 18 sun filters authorized in European Union tested *in vitro*

C. Couteau, M. Pommier, E. Paparis, L. J. M. Coiffard

Received November 6, 2006, accepted November 30, 2006

Prof. Laurence Coiffard, Université de Nantes, Nantes Atlantique Universités, SMAB, EA2160, Faculty of Pharmacy, 1 rue G. Veil-BP 53508, Nantes, F-44000 France
laurence.coiffard@univ-nantes.fr

Pharmazie 62: 449–452 (2007) *doi: 10.1691/ph.2007.6.6247*

In this work, the authors study the influence of filter concentration on the SPF in a topical product measured *in vitro*. Firstly, the method was adapted by determining that a mass of 15 mg of product must be applied on the PMMA (polymethylmethacrylate) plate in order to have the best correlation with results obtained *in vivo*. By using the highest concentration allowed by European legislation, the following ranking was drawn up in ascending order of efficacy: 3-Benzylidene camphor (1.66) < oxybenzone (3.01) < octylsalicylate (3.12) < PABA (3.36) < polysilicone 15 (3.64) < methylene bis-benzotriazolyl tetramethylbutylphenol (3.68) < PEG25 PABA (3.81) < benzophenone-4 (3.85) < 4-methylbenzylidene camphor (4.22) < homosalate (4.33) < octyltriazone (7.80) < phenylbenzimidazole sulfonic acid (8.31) < octyldimethyl PABA (8.71) < octocrylene (10.41) < octylmethoxycinnamate (10.42) < diethylhexylbutamidotriazone (12.58) < isoamylparamethoxycinnamate (15.14) < anisotriazine (20.0).

1. Introduction

Although ultraviolet radiation has some beneficial effects, it is above all the cause of numerous harmful effects on our skin. These effects can arise either a short or long time after exposure. Short term effects, such as actinic erythema, can thus be distinguished from medium term effects, such as solar elastosis and photoimmunosuppression, and also from long term effects, such as photocarcinogenesis (Farmer and Naylor 1996, 1997; Nagashima et al. 1999; Vanquerp et al. 1999; Bayerl et al. 1995). Amongst the ways of fighting these effects are external photoprotection which includes photoprotection through clothing and topical photoprotection (Marks 1999; Morison 2003; Rosen 2003; El Sayed et al. 2006). Sun filters are regulated and each one must comply to a maximum authorized concentration in the finished product. The efficacy of the latter is characterized by the Sun Protection Factor (SPF). The SPF is the ratio of the ultraviolet energy required to produce a minimal erythema on sunscreen protected skin to the energy required to produce the same erythema on unprotected skin (Diffey 2001). It is determined by *in vivo* or *in vitro* methods (Ferrero et al. 2003; Diffey et al. 2000).

For our part, we firstly adapted the protocol *in vitro* in order to obtain a perfect correlation with the *in vivo* method, our adaptation being drawn up using the protocol suggested by the Colipa. Secondly, we studied 18 filters authorized in Europe and we assessed their performances concerning SPF.

2. Investigations, results and discussion

The physical characteristics of the filters which were studied are presented in Table 1. The spectra were presented in Fig. 1. All of the filters which were studied show at least a maximum of absorption situated in the UVB. The molar extinction coefficient has been calculated for each one (Table 1).

The technique of determining the SPF *in vitro* offers numerous advantages, in particular from an ethical point of view, as irradiation of healthy subjects is thus avoided, as well as from an economic angle.

The optimal mass of product to be spread out on the plate was determined by correlating the results obtained *in vitro* with the cream locking in 8% of homosalate with the results obtained *in vivo* with the same cream (Colipa 2006), using the protocol recommended by the Colipa (the European Cosmetic Toiletry and Perfumery Association). The different masses which were tested range from 15 to 35 mg. The SPF is assessed *in vivo* at an internationally accepted application of 2 mg/cm^2 but many studies have shown that people apply between 0.5 and 1.5 mg/cm^2 of product only (Ferrero et al. 2003). As for the filter, homosalate was chosen as it is the standard recommended by the FDA (FDA 1978). The FDA states that the SPF of the standard preparation locking in 8% of homosalate is 4.47 ± 1.279. Following the tests carried out on this standard preparation, it was proved that a quantity of 15 mg (Table 2) is required for the best correlation (Student t-test, n = 27, P = 0.95) with the values obtained *in vivo* (Table 3). Subsequent tests were thus all carried out using this mass.

The trend curves allowed us to obtain equations from which we can forecast the SPF according to the filter concentration (Table 4). Figs. 2 and 3 show the curve obtained for homosalate and anisotriazine respectively. It can be stated that for certain filters, like homosalate, the curve obtained is asymptotic. The SPF reaches a maximum value

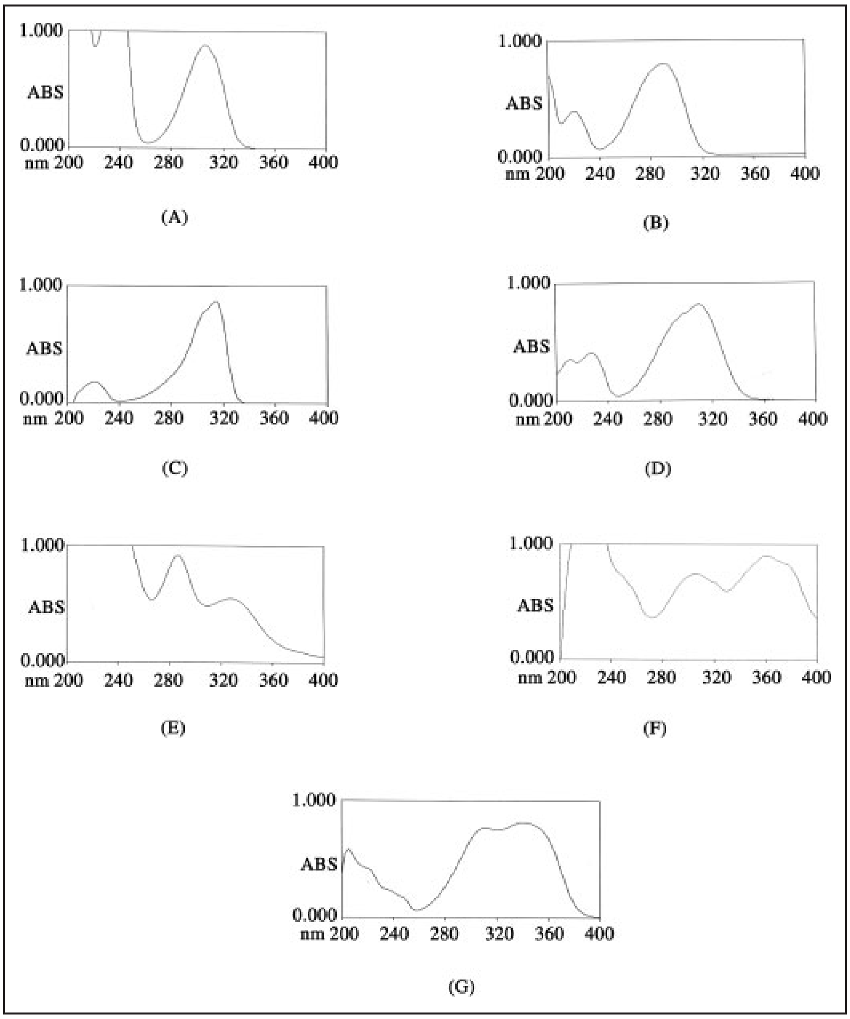

Fig. 1:
Spectra of some filters studied with narrow spectrums ((A) homosalate; (B) PABA; (C) octyltriazone; (D) octylmethoxycinnamate) and broad spectra ((E) benzophenone-4; (F) methylene bisbenzotriazolyl tetramethylbutylphenol, (G) anisotriazine)

Table 1: Characteristics of the filters studied

Nom INCI	λ_{max} (solvent)	ε ($L \cdot mol^{-1}cm^{-1}$)
PABA	290.0 (Ethanol)	18255
Homosalate	306.0 (Ethanol)	4416
Phenylbenzimidazolez sulfonic acid	305.5 (Water, neutralisation by NaOH)	24100
Benzophenone-3	287.5 (Ethanol)	13992
Octocrylene	304.0 (Ethanol)	12308
Octyl methoxycinnamate	310.0 (Ethanol)	26250
PEG-25 PABA	307.0 (Ethanol)	23882
Isoamyl p-methoxycinnamate	310.0 (Ethanol)	27679
Octyltriazone	314.5 (Ethanol)	131497
Diethylhexylbutamidotriazone	310.5 (Ethanol)	167199
4-Methylbenzylidene camphor	301.0 (Ethanol)	22827
3-Benzylidene camphor	291.5 (Ethanol)	31073
Octylsalicylate	306.0 (Ethanol)	5607
Octyl dimethyl PABA	312.0 (Ethanol)	29211
Benzophenone-4	287.5 (Water, neutralisation by NaOH)	11520
Methylene bis-benzotriazolyl tetramethylbutylphenol	305.5 (Water)	12326
Anisotriazine	310.0 (Ethanol)	405302
Polysilicone 15	312.5 (Ethanol)	24726

of about 4 as soon as concentration is at 6%. Thus, it is not at all necessary to use this filter at the maximum authorized level. This is the case for the vast majority of filters studied. For anisotriazine and octocrylene, the SPF is directly proportional to filter concentration, in the range of concentrations studied, that is to say between 0 and 10%.

The study of SPF according to concentration allowed us to class the filters in ascending order of efficacy according to: Polysilicone 15 < 3-benzylidene camphor < methylene bis-benzotriazolyl tetramethylbutylphenol < PEG 25 PABA < homosalase < oxybenzone < octylsalicylate < PABA < benzophenone 4 < 4-methylbenzylidene camphor < phenylbenzimidazole sulfonic acid < octylmethoxycinna-

Table 2: SPF obtained *in vitro* according to the mass spread out

Concentration in homosalate (%) Mass applied (mg)	2	4	6	8	10
15	1.95 ± 0.05	3.24 ± 0.33	4.25 ± 0.69	4.63 ± 0.61	5.12 ± 0.72
20	2.45 ± 0.24	4.05 ± 0.68	5.18 ± 0.84	5.52 ± 0.89	6.20 ± 0.99
25	2.70 ± 0.28	4.80 ± 0.68	6.04 ± 0.93	6.42 ± 1.02	7.22 ± 1.15
30	2.96 ± 0.30	5.43 ± 0.77	6.83 ± 1.07	7.56 ± 1.09	8.32 ± 1.25

Table 3: SPF obtained *in vivo* using the Colipa method with different concentrations of homosalate

Concentrations in homosalate (%) Mass applied (mg/cm^2)	2	4	6	8	10
2	2.07 ± 0.52	3.39 ± 0.85	4.37 ± 1.10	4.68 ± 1.17	5.16 ± 1.29

Table 4: Trend curves of the 18 filters studied

INCI name	Trend curves SPF = f©	Correlation coefficient r	SPF calculated at the maximum dose of use
PABA	$y = -0.1964 x^2 + 1.6184 x + 0.1829$	0.990	3.36
Homosalate	$y = -0.045 x^2 + 0.872 x + 0.059$	0.998	4.30
Oxybenzone	$y = -0.0041 x^2 + 0.0788 x + 2.636$	0.976	3.01
Phenylbenzimidazole sulfonic acid	$y = -0.098 x^2 + 1.788 x + 0.276$	0.992	8.31
Octocrylene	$y = 0{,}951 x + 0{,}907$	0.986	10.44
Octyl methoxycinnamate	$y = -0.074 x^2 + 1.741 x + 0.432$	0.985	10.42
PEG-25 PABA	$y = -0.031 x^2 + 0.668 x + 0.265$	0.979	3.85
Isoamyl p-methoxycinnamate	$y = -0.093 x^2 + 2.455 x - 0.145$	0.991	15.10
Octyl triazone	$y = -0.0334 x^2 + 3.132 x + 0.485$	0.984	15.31
Diethylhexylbutamidotriazone	$y = -0.0608 x^2 + 1.8326 x + 0.3364$	0.997	12.58
4-Methylbenzylidene camphor	$y = -0.344 x^2 + 2.415 x + 0.057$	0.997	4.22
3-Benzylidene camphor	$y = -0.03 x^2 + 0.525 x + 0.7325$	0.999	1.66
Octylsalicylate	$y = -0.094 x^2 + 1.063 x + 0.158$	0.989	3.12
Octyl dimethyl PABA	$y = -0.179 x^2 + 2.507 x + 0.087$	0.997	8.69
Benzophenone-4 et 5	$y = -0.158 x^2 + 1.521 x + 0.199$	0.989	3.85
Methylene bis-benzotriazolyl tetramethylbutylphenol	$y = -0.033 x^2 + 0.684 x + 0.161$	0.989	3.70
Anisotriazine	$y = 1.941 x + 0.585$	0.997	20.0
Polysilicone 15	$y = -0.033 x^2 + 0.678 x + 0.161$	0.991	3.64

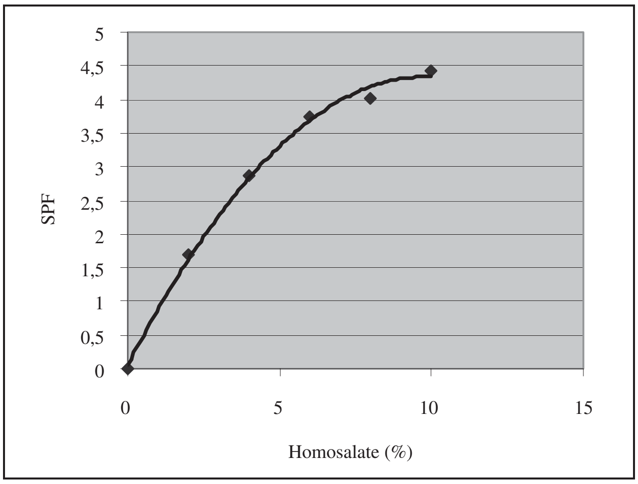

Fig. 2: SPF according to the concentration for homosalate (Eusolex HMS®)

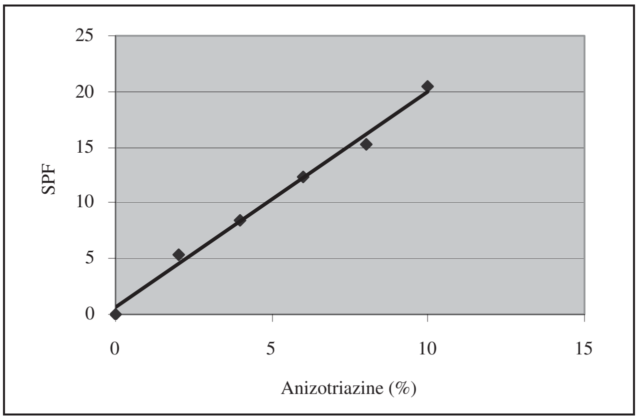

Fig. 3: SPF according to the concentration for anisotriazine (Tinosorb S®)

mate < octyldimethyl PABA < diethylhexylbutamidotriazone < isoamyl p-methoxycinnamate < octyltriazone < anisotriazine. Salicylates with low molecular coefficients of extinction are poor sunscreens. Triazines prove to be the most effective agents in the photoprotection with respect to UVB. This is the case particularly with anisotriazine which with its very strong coefficient of extinction is the most effective filter studied.

3. Experimental

3.1. Chemicals

27 chemical filters are currently authorized in the European Union. This number comprises 26 chemical filters and one screen (TiO_2). In this list, 5 filters (Mexoryl®) can be found, patented by L'Oréal. Our work consisted in studying the efficacy of the anti-UVB chemical filters by drawing up the SPF = f(C) trend curve for each filter. The filters tested are shown in Table 5. Each of these filters has been included in ascending order up to a limit of its maximum concentration for use, in a previously described O/W emulsion (Couteau et al. in press).

Table 5: Origin of the chemical UV-B filters studied

INCI name	Trade name	Supplier
PABA	PABA	Merck
Homosalate	Eusolex HMS	Merck
Oxybenzone	Néohéliopan BB	Symrise
Phenylbenzimidazole sulfonic acid	Eusolex 232	Merck
Octocrylene	Uvinul N539T	BASF
Octyl methoxycinnamate	Eusolex 2292	Merck
PEG-25 PABA	Uvinul P25	BASF
Isoamyl p-methoxycinnamate	Néohéliopan E1000	Symrise
Octyl triazone	Uvinul T150	BASF
Diethylhexylbutamidotriazone	Uvasorb HEB	3V Sigma
4-Methylbenzylidene camphor	Néohéliopan MBC	Symrise
3-Benzylidene camphor	Unisol S22	Unipex
Octylsalicylate	Dermoblock OS	Alzo
Octyl dimethyl PABA	Eusolex 6007	Merck
Benzophenone-4 et 5	Uvinul MS 40 (4)	BASF
Methylene bis-benzotriazolyl tetramethylbutylphenol	Tinosorb M	Ciba
Anisotriazine	Tinosorb S	Ciba
Polysilicone 15	Parsol SLX	Roche

3.2. Ultraviolet spectrum

Each sunscreen was scanned to obtain the absorption spectra at wavelengths between 200 and 400 nm using a spectrophotometer double-beam (Hitachi UV-visible, model U-2000).

3.3. SPF determination

About 15 to 35 mg of product exactly weighed were spread on PMMA plates over the whole surface (25 cm^2) using a cot-coated finger. Three plates were prepared for each product to be tested and 9 measures were performed on each plate. Transmission measurements between 290 and 400 nm were carried out using a spectrophotometer equipped with an integrating sphere (UV Transmittance Analyzer UV1000S, Labsphere, North Sutton, USA). The standard used was the 8% homosalate standard mandated by the US Food and Drug Administration Sunscreen Monograph. The calculations for either term use the same relationship (1):

$$SPF = \sum_{290}^{400} E_\lambda I_\lambda \Delta_\lambda / \sum_{290}^{400} E_\lambda I_\lambda T_\lambda \Delta_\lambda \qquad (1)$$

where E_λ is spectral irradiance of terrestrial sunlight at λ, I_λ is erythemal action spectrum at λ and T_λ is spectral transmittance of the sample at λ (Groves et al. 1979; Diffey and Robson 1989).

References

Bayerl C, Taake S, Moll I, Jung EG (1995) Characterization of sunburn cells after exposure to ultraviolet light. Photodermatol Photoimmunol Photomed 11: 149–154.

Colipa, International Sun Protection Factor Test Method (2003).

Couteau C, Benjamen S, Fourel C, Paparis E, Coiffard LJM. Influence of the oil on the SPF measured in vitro (OCL, in press)

Diffey BL, Robson J (1989) Sun Protection Factor *in vitro*. J Soc Cosmet Chem 40: 127.

Diffey B (2001) Sunscreen isn't enough. J Photochem Photobiol B 64: 105–108.

Diffey BL, Tanner PR, Matts PJ, Nash JF (2000) In vitro assessment of the broad-spectrum ultraviolet protection of sunscreen products. J Am Acad Dermatol 43: 1024–1035.

El Sayed F, Ammoury A, Nakhle F, Dhaybi R, Marguery MC (2006) Photoprotection in teenagers. Photodermatol Photoimmunol Photomed 22: 18–21.

Farmer KC, Naylor MF (1996) Sun exposure, sunscreens, and skin cancer prevention: a year-round concern. Ann Pharmacother 30: 662–673.

FDA Department of Health and human Services Food and drug administration, USA, Sunscreen drug products for over the counter use: proposed safety, effectiveness and labelling conditions. Fed Reg 43 (166) (1978) 38206–38269.

Ferrero L, Pissavini M, Marguerie S, Zastrow L (2003) *In vitro* determination of Sun Protection Factor. J Cosmet Sci 54: 463.

Groves GA, Agin PP, Sayre RM (1979) *In vitro* and *in vivo* methods to define sunscreen protection. Australas J Dermatol 20: 112–119.

Marks R (1999) Photoprotection and prevention of melanoma. Eur J Dermatol 9: 406–412.

Morison WL (2003) Photoprotection by clothing. Dermatol Ther 16: 16–22.

Nagashima H, Hanada K, Hashimoto I (1999) Correlation of skin phototype with facial wrinkle formation. Photodermatol Photoimmunol Photomed 15: 2–6.

Naylor MF, Farmer KC (1997) The case for sunscreens. A review of their use in preventing actinic damage and neoplasia. Arch Dermatol 133: 1146.

Rosen CF (2003) Topical and systemic photoprotection. Dermatol Ther 16: 8–15.

Vanquerp V, Rodriguez C, Coiffard C, Coiffard LJM (1999) High-performance liquid chromatographic method for the comparison of the photostability of five sunscreen agents. J Chromatogr A 832: 273–277.