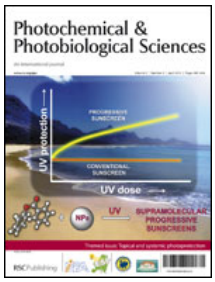

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UVA protection labeling and *in vitro* testing methods

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The importance of adequate UVA protection is apparent with improved understanding of UVA-induced skin damage. This has led to the development of new sunscreen ingredients. A number of regulatory bodies or experts from the industry and academia have proposed methods to assess the efficacy of sunscreens against UVA radiation. In addition different proposals have been made regarding the labeling for UVA protection. The purpose of this paper is to describe several *in vitro* methods for measuring UVA protection of sunscreen products and to consider their validity. The different proposals in terms of UVA labeling are also presented and discussed. This review illustrates the need for standardization of the measurement conditions and harmonization to convey to consumers the most appropriate information on UVA protection.

Introduction

Human exposure to ultraviolet (UV) radiation from sunlight can cause many adverse effects. The UVB range of the solar spectrum (290–320 nm) is generally thought to be the most harmful radiation that impacts skin at the earth level. More recent studies have shown that UVA radiation (320–400 nm) can cause a number of detrimental effects in human skin.[1–7] Increasing concern about these effects has led to the development of sunscreens which attenuate UVA exposure effectively[8–13] and raised the need for methods to properly evaluate the level of protection against UVA afforded by sunscreen products.

A number of methods based on both *in vivo* and *in vitro* techniques have been proposed together with different ways to label the UVA protection.

Background

Persistent pigment darkening (PPD) skin response has been selected for development of a standardized *in vivo* protocol.[14–15] It has been demonstrated that when using this protocol the results are reproducible[16–17] for a wide range of products and UVA protection levels (UVAPF ranging from 4 to 28).

The Japan Cosmetic Industry Association (JCIA) adopted the PPD method as the official method for assessing UVA efficacy of sunscreen products in January 1996.[18] Korea and China also adopted this method as standard in 2001 and 2007, respectively.[19,20] The PPD method was recommended by the European Commission in September 2006[21] and it has been recently proposed by the United States FDA in the sunscreen monograph amendment.[22] The method has been recognized by these various countries or regulatory bodies with some minor differences. Finally, the PPD method is currently in the process of standardization by the International Organization for Standardization (ISO).

Photo-instability of sunscreens under UV exposure is a well-known and common phenomenon.[23] An important point in the assessment of protection against UV radiation is the challenge of taking into account the degree of product photostability during the test to avoid overestimation of the UVA protection level of products which are not photostable. The *in vivo* PPD method involves exposure to UVA doses challenging the photostability of sunscreen products.[15,24]

In Japan, Korea and China,[18–20] UVA protection labeling is based on categories depending on measured *in vivo* PPD UVAPF value. There are three categories (PA+, PA++, PA+++) as shown in Table 1. The highest category is reached when UVAPF is at least 8.

In Europe, in September 2006, the European Commission recommended a UVAPF/SPF ratio as high as at least 1/3.[21] The UVAPF should be determined using the *in vivo* PPD method or any *in vitro* method able to provide equivalent results. Measured UVAPF values and labeled SPF are used for the ratio calculation. To label SPF 50+ (the maximum in Europe), measured SPF must be at least 60; therefore UVAPF has to be at least 20.

In August 2007, the United States FDA[22] proposed in its amendment to the final monograph a labeling of UVA protection level based on *both in vivo* UVAPF and an *in vitro* evaluation method. Final labeling is driven by the lowest category from the data of the two methods. Four levels of protection have been proposed based on *in vivo* UVAPF value as shown in Table 2. UVA protection level is labeled by 1 to 4 stars. Below the first category (low, 1 star), the FDA proposed mentioning "no UVA protection". The highest category is reached when UVAPF is at least 12 and also when the *in vitro* ratio is greater than 0.95 (both parameters must be achieved).

The *in vitro* methods are all based on the same principle of transmission measurements after application of the product on a substrate. However, these methods involve different qualities of

Table 1 Method for expressing UVA protection recommended by Japan, Korea and China

PFA value	PA (protection grade of UVA)
2–<4	PA+
4–<8	PA++
≥8	PA+++

L'Oréal Recherche, Asnières, France

Table 2 United States FDA proposal for UVA labeling

In vitro UVA1/UV proportion		*In vivo* PPD (persistent pigment darkening)	
0–0.2	No UVA protection	0–2	No UVA protection
0.2–0.39	*	2–<4	*
0.4–0.69	**	4–<8	**
0.7–0.95	***	8–<12	***
>0.95	****	≥12	****

substrate, amount of product applied and UV dose for sample exposure. An approach to measure UVA protection using a thin film technique was proposed by Diffey and Robson.[25] This technique, by requiring only a relative measure of the UVA *vs.* UVB absorbance of the sunscreen product, eliminates the need for an absolute absorbance measure which is much more operator dependent. The method assesses the "flatness" of spectral absorbance of the product over the UVB and UVA range. Product absorbance is summed in the UVB and UVA ranges and the UVA/UVB absorbance ratio is calculated. The UVA/UVB absorbance ratio is calculated according to eqn (a):

$$\text{UVA/UVB ratio} = \frac{\int_{320}^{400} \lg[1/T(\lambda)]\,d\lambda \Big/ \int_{320}^{400} d\lambda}{\int_{290}^{320} \lg[1/T(\lambda)]\,d\lambda \Big/ \int_{290}^{320} d\lambda} \qquad \text{(a)}$$

The Boots company has proposed classifying products into classes. In the 2004 version,[26] 5 classes based on the ratio values were distinguished (Table 3A). Specifications on critical components such as substrate, application rate to be used and measurement device were left open which may strongly influence test results. Potential photo-instability of the sunscreen product was not taken into account in the method.

However, since the European Commission has recommended consideration of photostability, a new version was recently delivered.[27] In this new guideline, a range of roughness (from 2 μm to 4 μm) for the plates is recommended and the amount of product to be applied on the plate must be 1 mg cm^{-2}. The UV dose of exposure is set at 17.5 J cm^{-2} from a solar simulator with an irradiance level range from 45 W m^{-2} to 75 W m^{-2}. The labeling system is now based on the initial ratio of absorbance before UV exposure and the final ratio of absorbance as shown in Table 3B. Accordingly, the values used to define the limits of the 5 categories from 1 star to 5 stars were changed.

The choice of the UV dose to take into account potential photo-degradation is another parameter which needs to be carefully considered to properly reflect the situation in real life.

The *in vitro* FDA proposal[22] is based on the same principle of transmission measurements through a sunscreen film. However the substrate proposed is a roughened quartz plate with undefined roughness and the amount of product applied is 2 mg cm^{-2}. The FDA introduced an irradiation step with a UV dose specified as two thirds of the SPF × 2 J cm^{-2} (2 J cm^{-2} is standard minimal erythemal dose (MED)). Finally the FDA proposed calculation of an absorbance ratio defined as ratio of UVA1 (340 to 400 nm) absorbance to total UV (290–400 nm) absorbance.

The FDA defines 4 categories based on UVA1/UV ratio as shown in Table 2. This proposal contains several technical elements that are inappropriate for accurate measurement. The roughness of the plate is very important for reliable data,[28] and it is difficult to ensure reproducible roughness when quartz plates are used. The amount of applied product, 2 mg cm^{-2}, is excessive for the most common spectrophotometers, especially for high SPF. The UVB spectrum is flattened leading to overestimation of UVA1/UV ratio.

The UV dose proposed by the FDA to irradiate the sample may be adequate in relation to the 2 mg cm^{-2} sunscreen product applied but should be lowered when applying a lower amount of product. The choice of plate roughness can also alter photo-degradation. The source of UV exposure should also be carefully defined to ensure reproducible results.

The UVA1/UV ratio criterion was proposed by the FDA to ensure that the long UVA (UVA1) part of UVA spectrum was sufficiently covered when UVA protection was assessed by the *in vivo* PPD method. The final category to be labelled is the lowest category of either *in vitro* or *in vivo* results. However, these

Table 3 Description of the 2004 (A) and 2008 (B) Boots star ratio system and UVA/UVB limits for each category

A

UVA : UVB ratio	Rating
<0.2	No star
0.22–<0.42	*
0.42–<0.62	**
0.62–<0.82	***
0.82–<0.92	****
≥0.92	*****

B

		Initial mean UVA : UVB ratio			
		0.0–0.59	0.6–0.79	0.8–0.89	≥0.9
Post-exposure mean UVA : UVB ratio	**0.0–0.56**	No rating	No rating	No rating	No rating
	0.57–0.75	No rating	***	***	***
	0.76–0.85	No rating	***	****	****
	≥0.86	No rating	***	****	*****

in vitro categories have been defined based on a method which is not well defined, therefore the values should be reconsidered depending on the conditions of measurement which have to be validated.

In the critical wavelength method proposal, the absorbance of the thin film of the sunscreen is integrated (summed) from 290 nm across the UV wavelengths until the sum reaches 90% of total absorbance of the sunscreen in the ultraviolet range (290–400 nm). The wavelength at which the summed absorbance reaches 90% of total absorbance is defined as the "critical wavelength" and is considered to be a measure of the breadth of sunscreen protection.[29] Sunscreen products are then classified as "broad spectrum" having a significant part of their absorbance in the UVA, when the critical wavelength is longer than 370 nm. The critical wavelength λ_c is defined according to eqn (b):

$$\int_{290}^{\lambda_c} \lg[1/T(\lambda)]\,\mathrm{d}\lambda = 0.9\int_{290}^{400} \lg[1/T(\lambda)]\,\mathrm{d}\lambda \qquad \text{(b)}$$

Combining both the *in vivo* PPD method for measuring the level of UVA protection and the critical wavelength method to measure the broadness of UVA absorbance with a minimum critical wavelength ≥370 nm has been proposed by the European Commission for UVA protection assessment of sunscreen products.[21]

A modified critical wavelength method including a pre-irradiation step to take into account the photochemical behaviour of the sunscreen product was published.[30] 1 mg cm^{-2} sunscreen product is applied onto a synthetic collagen (Vitro-skin) as substrate and exposed to one third of product SPF (*e.g.* for an SPF 15, pre-irradiation was 5 J cm^{-2}) or to increasing UV doses, from 0 to 30 J cm^{-2}. With photo-unstable products it was shown that there was a decrease in the critical wavelength value when UV dose increased. This study emphasized that taking into account sunscreen product photo-instability was of importance. However, it is difficult to validate the right UV dose to be used to properly reflect *in vivo* conditions, which are obviously not standardized.

Ferrero *et al.*[28] studied the reproducibility of the UVA/UVB absorbance ratio and of critical wavelength measurements using different substrates. The two parameters showed a smaller variation compared to absolute criteria variation but significant in relation to roughness of the substrate plate used. For the same amount of product applied (1 mg cm^{-2}), higher UVA/UVB ratios and higher critical wavelength values were found when plates with higher roughness were used. Larger variability was found for the UVA/UVB index ratio than for critical wavelength. The amount of sunscreen applied and substrate roughness are two important parameters to be carefully set to ensure reproducibility of the results. Variability of the results can lead to a difference in the rating and consequent difference on labelling information.

An *in vitro* PPD method has been developed and data was compared to that from *in vivo* PPD.[31] Transmission through the sunscreen-coated sample was cross-multiplied by the UVA source emission spectrum and PPD action spectrum. A product amount of 0.75 mg cm^{-2} was applied on a polymethylmethacrylate (PMMA) plexiglass plate with a roughness of $S_a = 2$ µm.

To reduce operator effect, a calibration step was incorporated into the computation process (eqn (c) and (d). Spectral absorbance was adjusted by a scalar multiplier so that the predicted SPF matched the SPF measured *in vivo* yielding an "absolute" absorbance curve from which UVA protection can be predicted (eqn (e)).

$$\mathrm{SPF}_{in\,vitro} = \frac{\int_{\lambda=290\mathrm{nm}}^{\lambda=400\mathrm{nm}} E(\lambda)I(\lambda)\,\mathrm{d}\lambda}{\int_{\lambda=290\mathrm{nm}}^{\lambda=400\mathrm{nm}} E(\lambda)I(\lambda)\times 10^{-A_0(\lambda)}\,\mathrm{d}\lambda} \qquad \text{(c)}$$

$$\mathrm{SPF}_{in\,vitro,\,\mathrm{adj}} = \mathrm{SPF\ label} = \frac{\int_{\lambda=290\mathrm{nm}}^{\lambda=400\mathrm{nm}} E(\lambda)I(\lambda)\,\mathrm{d}\lambda}{\int_{\lambda=290\mathrm{nm}}^{\lambda=400\mathrm{nm}} E(\lambda)I(\lambda)\times 10^{-A_0(\lambda)C}\,\mathrm{d}\lambda} \qquad \text{(d)}$$

$$\mathrm{UVAPF}_0 = \frac{\int_{\lambda=320\mathrm{nm}}^{\lambda=400\mathrm{nm}} P(\lambda)I(\lambda)\,\mathrm{d}\lambda}{\int_{\lambda=320\mathrm{nm}}^{\lambda=400\mathrm{nm}} P(\lambda)I(\lambda)\times 10^{-A_0(\lambda)C}\,\mathrm{d}\lambda} \qquad \text{(e)}$$

This method showed good reproducibility and a good correlation with data from *in vivo* PPD method for photostable products.

In addition to the difficulties met with the *in vitro* methods described above, another factor of variability and limited correlation with *in vivo* test results is photo-instability of the test product. Some filters (notably dibenzoylmethane derivatives) are chemically broken down under UV exposure resulting in reduced ability to absorb UV radiation. Throughout their use, the protection provided by these unstable products continuously diminishes, making it difficult to assess the overall "protection" provided by the sunscreen as it strongly depends on exposure dose. One way to include this variable into *in vitro* test methods is to measure the absorption properties on a pre-irradiated sample. The choice of exposure dose is somewhat arbitrary, yet critical to test outcome. The recent *in vitro* test proposal by the European Industry Association (Colipa)[32] involves a pre-irradiation step in the procedure to account for this sunscreen-specific weak point. The UV dose to be used has been defined based on computation from data obtained using different UV doses.[33] Finally the UVA dose D is defined from the initial UVAPF_0 as obtained before UV exposure by calculation multiplied by 1.2 J cm^{-2} (eqn (f)):

$$D = \mathrm{UVAPF}_0 \times 1.2\ \mathrm{J\ cm^{-2}} \qquad \text{(f)}$$

e.g. for a $\mathrm{UVAPF}_0 = 10$, the UVA dose will be 10×1.2 J cm^{-2} = 12 J cm^{-2}.

After irradiation, the *in vitro* spectrum is measured again on the same plate and is processed using the previously determined constant C, and the UVA-PF is calculated using the transformed spectrum according to eqn (g).

$$\mathrm{UVAPF}_0 = \frac{\int_{\lambda=320\mathrm{nm}}^{\lambda=400\mathrm{nm}} P(\lambda)I(\lambda)\,\mathrm{d}\lambda}{\int_{\lambda=320\mathrm{nm}}^{\lambda=400\mathrm{nm}} P(\lambda)I(\lambda)\times 10^{-A_0(\lambda)C}\,\mathrm{d}\lambda} \qquad \text{(g)}$$

This *in vitro* UVA method has been validated against the *in vivo* PPD method for photostable and photo-unstable products. Good reproducibility between laboratories and good correlation with the *in vivo* method has been found. Adjusting the UV absorbance curve to the same SPF is obviously the best way to

limit the high variability in terms of amplitude. Colipa issued a revision of the guidelines,[34] including the critical wavelength determination after UV exposure, in the same conditions as the UVAPF determination.

Variability of this adjusted *in vitro* UVAPF has been observed when the plate roughness is not well controlled.[28] Are plates with high roughness better than plates with low roughness? It is a question under consideration by the ISO sun protection methods working group.

Defining the conditions of measurement (roughness definition, amount of product, UV dose used) is essential for the relevance and reliability of the results. To illustrate that point, we performed studies using plates with different roughness, different amounts of product and different pre-irradiation UV doses.

In addition, because the results are linked to the conditions of measurement, it appears that defining categories for labelling before setting relevant conditions of measurement is questionable. We have compared the different proposed criteria for UVA protection assessment in different conditions of measurement.

Materials and methods

The measurements were performed using a Vitro skan spectro-radiometer (Jobin Yvon, France), which is equipped with two double monochromators and fulfills all requirements of Colipa guidelines.[32]

The UV irradiation source was a Suntest (Atlas, France) with an irradiance of 7.5 mW cm^{-2} and a UV spectrum compliant with the specification (UVA/UVB irradiance ratio = 20.3). The radiometer used to measure UVA irradiance at plate level before each exposure was cross-calibrated with UVA irradiance measured with the spectro-radiometer in order to ensure that the UV doses of exposure were absolute UV doses to avoid overestimation or underestimation of photo-degradation.

Four studies were performed. The conditions of measurements for these studies are described in Table 4.

Study 1

In the first study, we compared the conditions of measurement described in the Boots method[27] (condition #1) with those described in the Colipa Guidelines[32] (condition #2). Four marketed products from SPF 8 to 50+ including different types of filtering systems (photostable or not) were tested.

Study 2

In the second study, condition #1 described in the Boots method[27] was compared to condition #3 using the same fixed UV dose of 17.5 J cm^{-2} but with a different plate (higher roughness) and a higher amount of product. Five marketed photostable products from SPF 30 to 50+ have been tested.

In studies 1 and 2, two *in vitro* criteria were calculated, the UVAPF and the ratio of absorbance UVA/UVB. Then labeling according to the Boots method was defined based on the UVA/UVB absorbance ratio values and the ratio of protection factors SPF/UVAPF values were calculated.

Study 3

In study 3, we compared condition #2 (Colipa method) and condition #4 using a different plate (higher roughness) and a higher amount of product. In condition #4, the irradiation dose was set to 1/3 SPF × 2 J cm^{-2}, as proposed some years ago for the critical wavelength method.[30] Condition #4 is an adaptation of the method proposed by the FDA.[22] The amount of product was set to 1 mg cm^{-2} instead of 2 mg cm^{-2} to avoid any saturation in the spectrophotometric measurements. The UV dose is also lower than those proposed by the FDA (1/3 instead of 2/3 × SPF × 2J cm^{-2}) to take into account the lower thickness of the product layer on the plate. *In vitro* criteria were calculated, the critical wavelength value and the UVA1/UV ratio as proposed by the FDA.[22] The *in vivo* UVAPF using the PPD method[18] was also determined. Five marketed products from SPF 20 to 50+ including different types of UVA filter have been tested.

Study 4

In study 4, condition #2 was compared to condition #5 using a different type of plate and a higher rate of product but the same principle for the sample exposure (1.2 J cm^{-2} × $UVAPF_0$). Five products from SPF 5 to 50+ including different types of UVA filter have been tested. Some products are photostable, some others are photo-unstable.

Five *in vitro* criteria were calculated: the *in vitro* UVAPF, the critical wavelength value, the UVA1/UV ratio, the UVA/UVB ratio and the spectral uniformity index (SUI), which has been recently published by Diffey.[35] The SUI is calculated from

$$\sum_{290}^{380} A\lambda \Big/ \sum_{290}^{380} \left| A\lambda - \hat{A} \right|$$

where $A\lambda$ denotes the *in vitro* spectral absorbance at each wavelength and $\hat{A}$ the average spectral absorbance over the spectral range 290–380 nm.

Table 4 Conditions of measurements used in the different studies. Study 1: comparison of conditions #1 and #2; study 2: comparison of conditions #1 and #3; study 3: comparison of conditions #2 and #4; study 4: comparison of conditions #2 and #5

	Condition #1, Boots	Condition #2, Colipa	Condition #3	Condition #4	Condition #5
Plate	Sandblasted, roughness 2μm	Sandblasted, roughness 2μm	Moulded, roughness 6μm	Sandblasted, roughness 5–6 μm	Moulded, roughness 6μm
Amount of product	1 mg cm^{-2}	0.75 mg cm^{-2}	1.3 mg cm^{-2}	1 mg cm^{-2}	1.3 mg cm^{-2}
UV dose	17.5 J cm^{-2}	1.2 J cm^{-2} × $UVAPF_0$	17.5 J cm^{-2}	1/3 SPF × 2 J cm^{-2}	1.2 J cm^{-2} × $UVAPF_0$

Table 5 Results of study 1: comparison of conditions #1 and #2. Influence on *in vitro* UVAPF, SPF/UVAPF ratio, UVA/UVB Boots ratio and number of stars

		In vitro UVAPF			UVA/UVB ratio			SPF/UVAPF ratio			UV dose/J cm^{-2}	
Product	SPF	#1	#2	#1/#2	#1	#2	#1/#2	#1	#2	#1/#2	#1	#2
1.A	8	6.6	7.3	0.9	0.83 ****	0.86 *****	0.97	1.2	1.1	1.09	17.5	10
1.B	25	18.3	15.2	1.2	0.85 ****	0.84 ****	1.01	1.4	1.6	0.88	17.5	25.5
1.C	50+	33	21.5	1.5	0.80 ****	0.73 ***	1.1	1.8	2.8	0.64	17.5	43
1.D	50+	15.4	13.9	1.1	0.63 ***	0.61 ***	1.03	3.9	4.3	0.91	17.5	20.3
Mean				1.18			1.03			0.88		
SD				0.25			0.05			0.18		
%CV				21.2%			4.9%			20.5%		

Results

Study 1

The results obtained on the 4 products are reported in Table 5. The *in vitro* UVAPF values are similar in the two conditions of measurements for products 1.A, 1.B and 1.D. The *in vitro* UVAPF values are different for product 1.C: UVAPF 33 in condition #1 (Boots method conditions) and UVAPF 21.5 in condition #2 (Colipa method conditions). In terms of labeling, the ratio of absorbance is modified depending on the conditions of measurements and the number of stars is changed for 2 products. In the Colipa conditions, one product (1.A) got a higher number of stars (5 instead of 4) and one product (1.C) a lower number of stars (3 instead of 4).

When looking at the ratio SPF/UVAPF, the values are similar in both conditions except for product 1.C for which the UVAPF is significantly influenced by the conditions of measurements; then the ratio is higher in the Colipa conditions. The conclusion in terms of compliance with the European recommendation is unchanged when the two conditions are compared. It was noticed that one product (1.D) having 3 stars using the Boots classification does not comply with the European recommendation (ratio SPF/UVAPF $\leq$ 3).

Study 2

All results are reported in Table 6. The *in vitro* UVAPF values are similar between the two conditions of measurements for the first three products and higher for two products (2.D and 2.E) when using condition #3. The ratio SPF/UVAPF is therefore lower for these two products but the conclusion on compliance is unchanged. The ratio of absorbance UVA/UVB is increased for all products using condition #3 and the number of stars is increased (4 stars instead of 3) for three products (2.C, 2.D, 2.E)

Study 3

The results are reported in Table 7. All products had an *in vivo* UVAPF higher than 12; they reached the 4 stars category according to the FDA classification based on *in vivo* UVAPF data and *in vitro* UVAPF obtained in the two conditions of measurements.

The *in vitro* UVAPF results are similar using the two conditions for 3 of the 5 products. Globally, condition #4 gave higher UVAPF values. The correlation with the *in vivo* data is good in both conditions, a UVAPF/SPF ratio of at least 1/3 is not reached for one of the products (3.D) with condition #2, whereas the 1/3 ratio is reached for all products using the *in vivo* data or condition #4.

In both conditions #2 and #4, all products had a critical wavelength value higher than 370 nm.

The ratio UVA1/UV is also influenced by the measurement conditions, with higher ratio values in condition #4. However, in all cases in both conditions, the labeling remains at 3 stars (0.7 to 0.95).

Study 4

All results are reported in Table 8. For each of the calculated indices, it is quite obvious that the value depends on the conditions of measurement. All indices are higher when condition #5 is used. The *in vitro* UVAPF values obtained in condition #5 are closer to *in vivo* UVAPF values than values obtained in condition #2.

All products tested have an *in vivo* UVAPF/SPF ratio higher than 1/3 and they have a critical wavelength higher than 370 nm in both conditions #2 and #5, in compliance with the EC recommendation. In condition #2, product 4.D has an *in vitro* UVAPF that does not comply with the 1/3 criteria but it is compliant when using condition #5. The UVA1/UV ratio values are in most cases higher in condition #5. However, the highest category (UVA1/UV $>$ 0.95) is only reached with one product (4.A), which has the lowest SPF and UVAPF values. This product contains 5% avobenzone but not photostabilized.

The UVA/UVB ratio is also influenced by the conditions of measurement. The values increased in condition #5, leading in some cases to a different rating (higher number of stars). Products 4.A and 4.B reach the 5 stars category, however only 4.A, with a low SPF, reaches the highest FDA category (UVA1/UV $>$ 0.95).

The SUI values highly depend on the conditions. In condition #2, only 2 products can be rated as "very high" (SUI $\geq$ 12) according to Diffey's proposal instead of all products in condition #5. In the latter conditions, SUI values are far from 12 for most of the products.

Discussion

When conditions #1 and #2 were compared, because the plate type used is the same in both conditions, the difference observed for the UVAPF can be related to the amount of product applied and to the photodegradation of the product, which is also linked

Table 6 Results of study 2: comparison of conditions #1 and #3. Influence on *in vitro* UVAPF, SPF/UVAPF ratio, UVA/UVB Boots ratio and number of stars

		In vitro UVAPF			UVA/UVB ratio			SPF/UVAPF ratio		
Product	SPF	#1	#3	#1/#3	#1	#3	#1/#3	#1	#3	#1/#3
2.A	30	10.2	10.9	0.94	0.65 ***	0.69 ****	0.94	2.9	2.8	1.04
2.B	50	22.2	23.7	0.94	0.72 ***	0.77 ***	0.94	2.3	2.1	1.1
2.C	50+	25.4	27.8	0.91	0.76 ***	0.79 ****	0.96	2.4	2.2	1.09
2.D	50+	22.6	31.4	0.72	0.73 ***	0.79 ****	0.92	2.7	1.9	1.42
2.E	50+	24.9	33.1	0.75	0.76 ***	0.81 ****	0.94	2.4	1.8	1.33
Mean				0.85			0.94			1.2
SD				0.11			0.01			0.17
%CV				12.7%			1.5%			14.0%

Table 7 Results of study 3: comparison of conditions #2 and #4 Influence on *In vitro* UVAPF, UVA1/UV FDA ratio and associated labeling and critical wavelength (λc)

			In vitro UVAPF		SPF/UVAPF ratio		UVA1/UV ratio		λ_c		UV dose/J cm^{-2}		
Product	Labelled SPF	*In vivo* UVAPF	#2	#4	#2	#4	#2	#4	#2	#4	#2	#4	UV filters
3.A	20	17.0 ****	17	17.2	1.2	1.2	0.91 ***	0.92 ***	379	380	21	13.3	Octocrylene 6% Tinosorb M 2% Tinosorb S 2% Avobenzone 3%
3.B	40	23.2 ****	17.3	23.1	2.3	1.7	0.81 ***	0.88 ***	378	381	25.3	26.7	Octocrylene 10% Mexoryl SX 3% Avobenzone 2% Titanium dioxide 5%
3.C	45	15.4 ****	14.4	17	3.1	2.6	0.76 ***	0.81 ***	375	378	18.7	30	Octocrylene 2.3% Homosalate 12% Octisalate 5% Oxybenzone 6% Avobenzone 3%
3.D	50	16.2 ****	16.8	21.1	3	2.4	0.78 ***	0.82 ***	375	378	21.5	33.3	Octocrylene 7% Homosalate 10% Octisalate 5% Oxybenzone 6% Avobenzone 3% Tinosorb S 3%
3.E	50+	26.0 ****	20.6	23.7	2.9	2.5	0.8 ***	0.85 ***	378	381	37	40	Octocrylene 10% Mexoryl SX 1.5% Mexoryl XL 2.5% Avobenzone 4% Titanium dioxide 5%

to the amount of product applied and to the UV dose used for sample exposure. In the Boots conditions, the amount of product is higher than in the Colipa conditions and the UV dose is lower than those used in the Colipa conditions, as shown in Table 5. Consequently, the photodegradation may be lowered in the Boots conditions, and this is likely the case for product 1.C, and then the UVAPF is higher.

When conditions #1 and #3 were compared, all products tested were photostable. The differences observed in terms of the ratio of UVA/UVB values and then on labeling and also in terms of UVAPF values are linked to the plate roughness and to the amount of product used. When using higher roughness and higher rate of product the UVAPF and UVA/UVB values are higher and the star rating for labeling is also improved. However, the UVA/UVB ratio shows less dependence on measurement conditions than the *in vitro* UVAPF and the ratio SPF/UVAPF.

Since the Boots method has three sub-divisions, unlike the SPF/UVAPF which has just one division (>3 or <3), there is a higher likelihood that a category will change.

When conditions #2 and #4 were compared, all products tested were photostable by composition and as shown by comparison of *in vitro* results before and after irradiation. All products contained from 2% to 4% avobenzone, which is the most efficient long UVA filter when photostabilized. In addition, some products contained Tinosorb® M, Tinosorb® S or Mexoryl® XL, able to improve UVA broadness and UVA protection level. The *in vivo* PPD UVAPF was determined using a UVA source emitting from 320 to 400 nm. The PPD action spectrum is known to cover all UVA wavelengths.[15] Even though there is a decrease in the sensitivity

Table 8 Results of study 4: comparison of conditions #2 and #5. Influence on *in vitro* UVAPF, UVA1/UV FDA ratio and related labeling, critical wavelength (λ_c), UVA/UVB ratio and related labelling and SUI

			In vitro UVAPF		UVA1/UV ratio		λ_c		UVA/UVB ratio		SUI	
Product	Labelled SPF	*In vivo* UVAPF	#2	#5	#2	#5	#2	#5	#2	#5	#2	#5
4.A	5	4.3 * *	4.4	4.6	0.98 ****	0.96 ****	379	381	1.02 *****	0.95 *****	10.45	22.17
4.B	16	12.1 * * * *	9.9	12.9	0.89 ***	0.94 ***	377	382	0.85 ****	0.92 *****	24.62	60.77
4.C	30	17.8 * * * *	16.1	19.8	0.87 ***	0.91 ***	378	381	0.80 ****	0.87 *****	16.33	23.87
4.D	50+	23.2 * * * *	18	22.9	0.81 ***	0.85 ***	376	378	0.71 ***	0.75 ***	10.08	14.30
4.E	50+	28.0 * * * *	23	26.3	0.83 ***	0.86 ***	378	380	0.73 ***	0.77 ****	11.75	14.54

of PPD response in the longest UVA range of wavelengths, the majority of PPD on the skin is induced by UVA1 radiation, since the UVA1 range covers the large band from 340 to 400 nm. Using *in vitro* UVAPF data, it is possible to calculate the contribution of UVA1 to the UVAPF. For the products tested in this study, UVA1PF represents at least 92% of UVAPF, thus protection against UVA1 has a higher contribution than protection against UVA2 (320 to 340 nm).

When looking at *in vitro* results obtained in the two conditions of measurement, the UVA1/UV ratio and the critical wavelength value obtained in condition #4 are always higher than those obtained using condition #2. These results confirm those published by Ferrero *et al.*,[28] showing that a ratio of absorbance is sensitive to the conditions of measurement. Because all products are photostable, the difference observed between the two conditions is unlikely to be attributed to the difference in terms of UV dose of exposure and the range of UV doses used in condition #4 from 13 J cm^{-2} for the SPF 20 product to 40 J cm^{-2} for the SPF 50+ product is similar to the range used in condition #2 (18.7 to 37 J cm^{-2}). The higher UVAPF values in condition #4 can be attributed to the higher amount of product applied on the plate and to the plate roughness.

Even though the *in vivo* UVAPF is very high when a high concentration of avobenzone (4%) is used in combination with other UVA filters (product 3.E), UVA1/UV ratio does not reach the 4 stars category (ratio > 0.95).

Compared to product 3.C, product 3.D has a similar SPF but it contains a broad UVA filter (Tinosorb® S, 3%). The UVA1/UV ratio is increased as a result, however it does not reach a ratio higher than 0.95. The highest ratio obtained is 0.92 for product 3.A, which contains three UVA filters that absorb in the UVA1 band. This product has a SPF of 20 and thus a lower UVB absorbance compared to products with higher SPF; a higher value of UVA1/UV ratio is therefore more easily reached. These results show that when using either condition of measurements, condition #2 as proposed by Colipa or condition #4 similar to conditions proposed by Diffey *et al.*[30] for critical wavelength assessment, the highest star category (>0.95) is never reached.

When conditions #2 and #5 have been compared, a similar situation was observed. Products having 4 stars based on the *in vivo* UVAPF or *in vitro* UVAPF cannot reached the threshold (>0.95) and then can only be labeled 3 stars based on the UVA1/UV ratio values. Considering the FDA proposed requirement to label products with the lower of the two test data (*in vitro* and *in vivo*), it ensues that products with very good UVA protection afforded by combination of long UVA and short UVA filters as measured using the *in vivo* PPD method (UVA protection level of at least 15 and up to 28) must be labeled as if they were only UVAPF 8 with a 3-star rating. The result is that there is no incentive and a rather discouraging regulatory stance for manufacturers to provide UVA protection above UVAPF 8. The values of the UVA1/UV ratio limit used for category determination should be revised after technical specifications have been chosen and validated.

Conclusion

All the results presented here show that whatever the parameters assessed, absolute or relative, data depends on the conditions of measurement. However, absolute criteria such as UVAPF is more dependent than relative absorbance ratios. Rating of UVA protection can also change according to the conditions. Defining categories or ratings based on limits should not precede the validation of the method of measurement, including the choice of substrate, amount of product applied and UV dose of irradiation. The only way to validate a method is to ensure both reliability and reproducibility. In the absence of other *in vivo* UVA endpoints easily measurable with sensitivity throughout the UVA wavelength range, the *in vivo* PPD method should be chosen as a reference as has been done for the UVA *in vitro* method developed by Colipa. Validation of this method for a large number of types of product should be made to ensure a common basis of measurements for calculation of any index which can be selected for labeling UVA protection level.

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