HelioScreen

HelioNews

News about In Vitro Sun Protection Testing

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Editorial

The proposal of a worldwide accepted In Vitro SPF method is still a perennial debate despite years of discussion, improvements and questioning about the conditions to follow in order to reach an acceptable solution. They are huge discussions on the statistics to compare In Vitro and In Vivo values but I am still convinced the most important problem is we did not have the "gold" value (means the "real" one) on the products we used to demonstrate the performance of any new proposal yet.

The correlation In Vitro/In Vivo is still the only way to evaluate the pertinence of any new in vitro method and everybody knows about the poor variability of In Vivo method and it does not allow reaching any satisfactory solution. Additionally, the first steps to ensure as far as possible both the reliability of the In Vivo values to be compared (several results from different laboratories for each products) and the reproducibility of In Vitro values obtained (i.e. automatic spreading) are still not always respected.

So it could be quite a long while again in order to get an "acceptable" method by any country and anyone. It will surely cost a bit more expansive to be correctly equipped and keep with the rules. At that time,-this is my strong feeling- we will realize we had get it for years but the devil was in the details…

Dominique Lutz, CEO Scientist Manager

A NEW PROPOSAL FOR EXTRAPOLATION OF IN VITRO UVAPF ACCORDING TO ISO 24443:2012 USING LABELED SPF OR SCREENING IN VIVO SPF VALUE

INTRODUCTION

In contrary to the Colipa Guideline 2011 method for the in vitro determination of the UVA protection using the Labeled in vivo SPF for adjustment, the Measured in vivo SPF value (based on minimum 10 valid volunteers) shall be used in the ISO 24443:2012 standard to adjust the value to obtain correlated and relevant in vitro UVAPF value.

Unfortunately, sometimes it seems difficult to obtain this Measured in vivo SPF value before to perform the in vitro test. Then, the Labeled in vivo SPF or the Screening in vivo SPF value (on 3-5 volunteers) is used and can lead to to irrelevant results. Obviously, even if the in vitro UVAPF has been measured by using the Labeled in vivo SPF value, a new measurement shall be performed using the Measured in vivo SPF value (full test on minimum 10 vounteers) to be in total compliance with the standard. This fact can lead to unconfortable situation from all actors because the test has to be done twice.

Therefore, in this paper, based on the in vitro UVA-PF assessed using the Labeled in vivo SPF value, we propose a mathematical approach to estimate the in vitro UVAPF without using the Measured in vivo SPF value. As an evidence, this approach could be used with Screening in vivo SPF value too.

METHOD

According to the ISO 24443:2012 standard, the following simplified protocol has been respected:

1	Conduct equipments and consummables conformity check including a reference « blank » measurement of a glycerine treated plate for the subsequent absorbance measurements.
2	Sunscreen product is applied on untreated roughened Molded PMMA HD6 plate at 1.3 mg/cm² (several replicates).
3	Sunscreen product is spread by means of a robotic appliance according to a defined protocol. The sample is allowed to dry during minimum 30 min in the dark at a specified temperature (25 - 35 °C).
4	First In Vitro absorbance measurements of the sunscreen product before UV exposure. Acquisition of UV absorbance spectrum A0(λ) from 290 nm to 400 nm.
5	Mathematical adjustment of the initial UV spectrum using coefficient «C» to achieve an In Vitro SPF equals to the measured In Vivo SPF. Then initial UVA protection (UVAPF0) is calculated using A0(λ) and C.
6	A single UV exposure dose D (J/cm²) is calcultated according to equation $D = UVAPF0 \times D_0$. D_0 being fixed at 1.2 J/cm² UVA.
7	Second In Vitro absorbance measurements of the sunscreen product after UV exposure. Acquisition of a second spectrum with A(λ) from 290 nm to 400 nm.
8	Calculation of the In Vitro UVA protection factor (UVAPF) using coefficient «C» and UV absorbance spectrum A(λ).

In complement, the same protocol has been used by remplacing, in the «Step 5», the Measured in vivo SPF by the Labeled in vivo SPF. Finally, according to the Simulation choice, new steps could be added and described here after.

Results & Discussion

Therefore, 15 sunscreen products have been tested with here below the different results:

		Measured in vivo SPF					Labeled in vivo SPF				
Product	Type	Measured in vivo SPF	UVAPF Before UV Not adjusted	D dose (J/cm²)	UVAPF After UV Not ajusted	Final UVAPF Adjusted	Labeled in vivo SPF	UVAPF Before UV Not adjusted	D dose (J/cm²)	UVAPF After UV Not ajusted	Final UVAPF Adjusted
P1	Colored Cream	40.0	14.3	27.2	13.9	21.9	30	14.3	21.4	14.1	17.6
P2	Colored Cream	71.1	34.6	39.7	29.2	28.0	60	34.6	34.6	29.9	25.1
P3	Milk	100.0	55.9	38.6	57.0	32.8	60	55.9	26.6	56.9	22.6
P4	Oil	63.5	10.1	33.2	11.0	31.9	60	10.1	31.9	10.6	28.8
P5	Colored Cream	17.3	7.5	13.2	4.5	5.9	15	7.5	11.7	4.5	5.5
P6	Gel	35.3	17.7	24.4	13.8	15.7	30	17.7	21.5	14.1	14.2
P7	Emulsion	100.0	40.5	45.0	24.2	22.6	60	40.5	30.6	29.8	19.3
P8	Emulsion	11.8	6.5	8.6	4.5	4.9	10	6.5	7.6	4.6	4.5
P9	Emulsion	17.9	4.8	13.2	4.1	8.6	15	4.8	11.4	4.1	7.6
P10	Emulsion	22.2	4.6	16.6	5.1	16.5	20	4.6	15.2	5.0	14.5
P11	Cream	93.2	21.8	29.6	15.2	17.0	60	21.8	22.0	16.0	13.7
P12	Emulsion	71.4	43.5	36.9	40.5	29.0	60	43.5	32.2	41.2	25.7
P13	Cream	65.9	38.1	39.9	39.1	34.2	60	38.1	37.0	38.7	31.4
P14	Foundation	43.5	16.8	10.7	17.2	9.1	30	16.8	8.6	17.4	7.3
P15	Foundation	40.5	13.6	11.7	13.5	9.7	30	13.6	9.7	13.8	8.2

First, it is possible to observe that the Final UVAPF value using the Labeled in vivo SPF value (unofficial method in Blue) are generally irrelevant (always lower with a gap between 0.3 and 10.2) compared to the Final UVAPF value using the Measured in vivo SPF value (official method in Orange). Based on the correlation between the «Final UVAPF Adjusted - Measured in vivo SPF» and the «Final UVAF Adjusted - Labeled in vivo SPF», the coefficient of determination $r^2 = 0.962$ and the RMSE = 3.597.

Therefore, as the result doesn't really fit with the Measured final UVAPF value, we decided to test 3 different simulations in order to obtain a better accuracy with examples here below to illustrate the principle:

Simulation 1

Alternive approach (1) using the Labeled in vivo SPF to calculate the UV dose D, (2) to measure UVAPF after irradiation not adjusted and (3) to use the Measured in vivo SPF value to adjust the absorbance curve to obtain finally an in vitro UVAPF value.

Example 1. Simulation 1 with a single UV dose for product P11 using Measured in vivo SPF for extrapolation

As explained here above, in this Simulation 1, we just use the Labeled in vivo SPF to calculate the UV dose D, (2) to measure UVAPF after irradiation not adjusted and (3) to use the Measured in vivo SPF value to adjust the absorbance curve to obtain finally an in vitro UVAPF value equals to 17.9. By means of this approach, the Simulated final UVAPF is very close to the Measured final UVAPF equals to 17.0.

Simulation 2 & Simulation 3

The First-Order Decay Kinetics (F-ODK) equation is used to simulate data using respectively a single (D) or two UV exposure doses (2xD) based on Labeled in vivo SPF. In order to forecast the potential photo-stability behavior during irradiation step, the following F-ODK's equation was used:

$$\text{Simulated In Vitro UVAPF After UV Not adjusted} = \theta_1 \times \text{Exp}(-\theta_2 * \text{Dose}) + \theta_3$$

where θ_1, θ_2 and θ_3 are constants to be determined using Excel solver in order to fit the measured values. To estimate the relevance of the model using F-ODK's equation, the lower RMSE (Root Mean Square Error) was considered by comparing simulated vs. measured values of the «UVAPF After irradiation Not adjusted».
Finally, a second factor (called «C1 factor») is calculated on the absorbance curve to adjust the «Measured UVAPF After irradiation Not Adjusted» to the «Simulated UVAPF After irradiation Not Adjusted» and a factor (called «C2 factor») used on the absorbance curve to adjust the in vitro SPF to the in vivo SPF is applied too.

Example 2. Simulation 2 with a single UV dose for product P11 using F-ODK's equation for extrapolation

First, based on the irradiation dose D using Labeled in vivo SPF value, Blue values were used to calculate the F-ODK's equation $\theta_1 = 12.366$, $\theta_2 = 0.029$ and $\theta_3 = 9.424$ to obtain a RMSE = 0.000 and to simulate the Orange and Red values. Second, the Green value based on an irradiation dose using the Measured in vivo SPF value and the previous F-OK's equation was calculated too and finally used to calculate the «C1 factor» = 0.967 (to adjust the Red value). Third, this «C1 factor» was used in complement to the «C2 factor» to simulate a Final UVAPF value = 16.4. By means of this approach, the Simulated final UVAPF is very close to the Measured final UVAPF equals to 17.0.

	No irradiation	Irradiation dose D using Labeled in vivo SPF value	Irradiation dose D using Measured in vivo SPF value
UV DOSE (J/cm²)	0.0	22.0	29.5
Measured In vitro UVAPF not adjusted	21.8	16.0	-
Simulation 1 F-O DK's In vitro UVAPF not adjusted	21.8	16.0	14.7

Example 3. Simulation 3 with two UV doses (D and 2xD) for product P11 using F-ODK's equation for extrapolation

First, based on the irradiation doses D and 2xD using Labeled in vivo SPF value, Blue values were used to calculate the F-ODK's equation $\theta_1 = 11.555$, $\theta_2 = 0.032$ and $\theta_3 = 10.235$ to obtain a RMSE = 0.000 and to simulate the Orange and Red values. Second, the Green value based on an irradiation dose using the Measured in vivo SPF value and the previous F-OK's equation was calculated too and finally used to calculate the «C1 factor» = 0.969 (to adjust the Red value). Third, this «C1 factor» was used in complement to the «C2 factor» to simulate a Final UVAPF value = 16.5. By means of this approach, the Simulated final UVAPF is very close to the Measured final UVAPF equals to 17.0.

	No irradiation	Irradiation dose D using Labeled in vivo SPF value	Irradiation dose 2xD using Labeled in vivo SPF value	Irradiation dose D using Measured in vivo SPF value
UV DOSE (J/cm²)	0.0	22.0	44.0	29.6
Measured In vitro UVAPF not adjusted	21.8	16.0	13.1	-
Simulation 2 F-O DK's In vitro UVAPF not adjusted	21.8	16.0	13.1	14.8

Considering different simulations, the table here below presents the results:

	Measured in vivo SPF		Labeled in vivo SPF		Simulation 1	Simulation 2		Simulation 3	
Product	Measured in vivo SPF	Final UVAPF - Measured in vivo SPF	Labeled in vivo SPF	Final UVAPF - Labeled in vivo SPF	Simulated final UVAPF with Measured in vivo SPF	Simulated final UVAPF with 1 dose D	C1 factor	Simulated final UVAPF with 2 doses D and 2xD	C1 factor
P1	40.0	21.9	30	17.6	22.2	22.1	0.949	22.0	0.948
P2	71.1	28.0	60	25.1	28.6	28.1	0.994	27.4	0.994
P3	100.0	32.8	60	22.6	32.8	32.8	1.000	32.5	0.998
P4	63.5	31.9	60	28.8	30.1	29.1	0.989	29.4	0.993
P5	17.3	5.9	15	5.5	6.0	5.8	0.973	6.8	0.989
P6	35.3	15.7	30	14.2	16.0	15.6	0.989	15.6	0.990
P7	100.0	22.6	60	19.3	27.7	24.8	0.965	24.2	0.958
P8	11.8	4.9	10	4.5	6.4	4.9	0.977	6.2	0.979
P9	17.9	8.6	15	7.6	6.6	7.3	0.907	10.0	0.908
P10	22.2	16.5	20	14.5	15.9	14.8	0.973	15.0	0.978
P11	93.2	17.0	60	13.7	17.9	16.4	0.967	16.5	0.969
P12	71.4	29.0	60	25.7	29.4	29.2	0.998	28.9	0.997
P13	65.9	34.2	60	31.4	33.9	33.6	0.998	33.7	0.998
P14	43.5	9.1	30	7.3	9.2	9.0	0.995	9.1	0.995
P15	40.5	9.7	30	8.2	9.9	9.8	0.996	9.8	0.996

Based on the correlation between the «Final UVAPF - Measured in vivo SPF» and the «Simulation 1», the coefficient of determination $r^2 = 0.988$ and the RMSE = 1.576. Second, between the «Final UVAPF - Measured in vivo SPF» and the «Simulated final UVAPF with 1 dose D», the $r^2 = 0.994$ and the RMSE = 1.097. With the same comparison using the «Simulated final UVAPF with 2 doses D and 2xD», the $r^2 = 0.995$ and the RMSE = 1.057.

Conclusion

By these results, we can conclude that the Simulation 3 allows a better correlation with real data compared to Simulation 2 which is itself better than Simulation 1. Nevertheless, it seems not be enough significant to add a new irradiation step (i.e. 2 x D) in the process as results are more or less similar between Simulation 2 and Simulation 3. Furthermore, in both cases, the «C1 factor» should fluctuate between 0.9 - 1.1. Finally, even if in the present study the Measured in vivo SPF values are always higher than the Labeled in vivo SPF values, the same approach could be used if the Measured in vivo SPF value is lower than the Labeled in vivo SPF values or the Screening in vivo SPF value.

Therefore, in the revision of the ISO 24443:2012, in case of introduction of the possibility to use the Labeled in vivo SPF or the Screening in vivo SPF value in first instance followed by a extrapolation/corrective action when the Measured in vivo SPF value is available, the Simulation 2 approach seems relevant in terms of results and from a practical point of view. Obviously, the Measured in vivo SPF should be preferred in first instance.

Frequently Asked Questions

1. What does SPF 15 stands for/means?

The SPF (meaning Sun Protection Factor) is a measure of the level of protection against UV (mainly based on UVB, also with a consideration of UVA too). The number tells us about the proportion of UV rays blocked by sunscreen and how long the customer can expose himself to the sun before apparition of a sunburn. The SPF is always followed by a number: 15, 30, 45, 60, etc. Thus, an SPF of 30 indicates that one can, in theory, expose oneself to the sun 30 times longer without sunburn. However, it is important for the customer to choose the correct one according to several factors such as his photo-type, season, time, localisation, etc.

2. Does SPF 30 give twice sun protection than SPF 15?

Regarding this question, you can have two different approaches.

The first one based on a physical protection with a difference of UV rays stopped not equals to the double when you will get SPF 30 compared to a SPF 15.

The second one based on a biological approach with the consideration that when you will measure a SPF 30, you have twice the sun exposition to get this sunburn compared to a SPF 15.

3. How frequently one has to apply SPF product to get sun protection while going out?

The different worldwide recommendations are to reapply sunscreen products at least every two hours or as soon as you have a potential influence on this protection (such as water, sweat, clothe, etc.).

4. What is the importance of UVA protection?

The UVA pass through clouds, glass and the epidermis and unlike UVB, they are painless and can penetrate the skin very deeply, to the cells of the dermis. They can lead to sun intolerance, skin aging and cancer. Moreover, in contrary to the UVB with an immediate information (sunburn) about an overexposure informing us to avoid the sun, the UVA harmful impact is visible long time after the sun exposure and no consideration on the day by day use basis.

5. What does PA, PA+, PA++, etc. means?

The UVAPF (meaning the UVA Protection Factor and also called PFA) is similar to the SPF principle but only based on UVA protection including the pigmentation action spectrum. The higher the number, the better is the UVA protection. In the past, the JCIA proposed a method to assess this UVAPF number and a rating system for consumers guidance based on a PA+ system. In other words, a PA+ for UVAPF 2 to < 4, PA++ for UVAPF 4 to < 8, PA+++ for UVAPF 8 to < 16 and PA++++ for UVAPF ≥ 16.

6. How to define a good SPF product?

The sun protection performance is driven by the UV filters (absorbance, distribution, photostability, etc.) and the film forming performance (homogeneous, resistant, etc.). From the first point, in theory, the UV filters should include UVA and UVA protection, should be solubilized (organic) and well distributed into the formula (reparation of inorganic UV filters, hydrophilic and lipophilic for organic UV filters into an emulsion, etc.). For the second point, the product should be stable in time and easy to apply (meaning a homogenous film after spreading). Moreover, some studies highlight the fact that if you like your sunscreen product (perfume, feeling, texture, etc.), you will apply more product and more frequently, if not, you will avoid to use it and will get a SPF very low. In other words, it seems better to use a lovely sunscreen product with a SPF 30 instead of an unpleasant sunscreen product with a SPF 50+.

7. Which test is basic/official to measure the SPF?

Depending on the target market but currently, only in vivo methods are standardised through the ISO 24444:2010 and FDA monograph 2011 (sometimes, International SPF 2006 method is still used). Development of a harmonized in vitro SPF method is still in progress.

8. What is an official test for measuring UVA?

Depending on the target market but currently, in vivo UVAPF assessment by using the ISO 24442:2011, in vitro UVAPF and Critical Wavelength assessment by using the ISO 244443:2012, in vitro Critical Wavelength assessment by using the FDA monograph 2011 and the in vitro UVA:UVB ratio assessment by using the Boots Star rating system 2011 (sometimes, the JCIA 1999 method is still used for in vivo UVAPF assessment and the Colipa 2011 method for in vitro UVAPF and Critical Wavelenght assessment).

9. When to do pre-irradiation test for SPF? What does it mean?

It allows to take into consideration the potential photo-degradation of the product to determine the final endpoint with higher relevance. To be noted that all official methods include an UV exposure step and, without this consideration, some results can be totally different for unphotostable products. In the contrary a complete photo-stability test allows to understand its behaviour before and after an UV exposure without consideration of the final endpoint.

10. What is the significance of critical wavelength in SPF testing?

The Critical Wavelength expresses the UVB/UVA balance based on the UV absorbance curve of the sunscreen product (relative value without expression of the UVA protection level). From studies and regulations, a minimum of 370 nm is required to confirm a minimum UVA protection (the higher this number, the better is the UVA/UVB balance). This step is complementary to UVAPF assessment (absolute value expressing the level of UVA protection).

11. What tests are relevant for making 'Broad Spectrum' claim?

"Broad Spectrum" claim informs about the long UVA protection. Most of the time, it refers to the USA market and shall be assessment following the FDA monograph 2011 for in vitro Critical Wavelength.

12. What could be possible explanation to obtain different in vitro SPF values than labelled on the product?

Regarding the correlation, two points should be considered.

The first one is the relevance of the SPF labelled. Indeed, in reality, some products on the market claim the SPF value based on the (i) in vivo value, (ii) in vitro value or (iii) in silico value:

(i) For the first part, variability of the in vivo value is now worldwide recognized and can lead to different results if you test the product in one or another testing laboratory. Furthermore, obviously, it also depends of the competence and serious of the testing laboratory. Finally, even if it is not worldwide accepted, it seems that some ingredients could potentialy delay the erythema response based on a biological action without a real UV protection.

(ii) For the second part, as no official in vitro method is available, you can find any kind of values.

(iii) For the last part, some cosmetics manufacturer used the in silico simulator to calculate the UV protection based on the percentage of the UV filters in the formulae. Unfortunately, in practice, the theory can be far from the reality and can lead to clearly overestimate the UV protection.

The second one is the reliability of the in vitro method because you have to distinguish the (a) screening test and (b) the claiming test:

(a) For the first part, a screening test is used to quickly assess the SPF protection as cheaper as possible to select, eliminate or compare the different candidates. The main aim is not to obtain a good correlation but to follow the same logic with new formulation.

(b) For the second part, a claiming in vitro SPF test is of course to obtain the correlation with in vivo SPF value. In this case, steps are considered in order to obtain reproducibility and accuracy with in vivo values. Recently, a multi-substrates approach based on special process and correction factors in order to obtain a very high correlation with in vivo SPF value.