Behind the Claim

SUNSCREEN TESTING: ethics, limits and critical points

Sunscreens are very complex products, the formulation of which requires technical expertise. They are classified as cosmetics or OTC drugs according to the different markets and regulations: cosmetics in Europe, OTCs in the USA, Japan, Australia and Canada. In Canada, they can also be defined as Natural Products (a way in between cosmetics and drugs) if they only contain mineral filters (TiO2 or ZnO). Different regulations describe the labeling features, the allowed claims and the testing protocols.

The **SPF** (Sun Protection Factor) test is both an efficacy and a safety assay. In Europe, the testing method is described by the ISO 24444:2010 standard, while in USA and Canada the reference method is FDA CFR21 part 201 (subpart G, section 201.327). These are the most used methods required in the EU and North American markets.

Basically, the testing methods aim to measure the Sun protection factor against UVB rays. The methods consist in the controlled induction of an erythema in vivo on healthy human volunteers, and in the comparison between erythema intensity in protected vs unprotected skin.

ISO and FDA protocols are partly harmonized, but different in some critical points. Both methods foresee a similar preliminary evaluation of the subjects and equivalent conditions for sample preparation and spreading and for the irradiation specifications. The most important differences concern the statistical analysis and hence the interpretation of the experimental data. The FDA method is always more restrictive and with the same data series the resulting SPF is lower when compared to the ISO result. The only way to obtain a similar result (but never identical) is increasing the number of subjects in the panel from the minimum required of 10 to at least 13 or more.

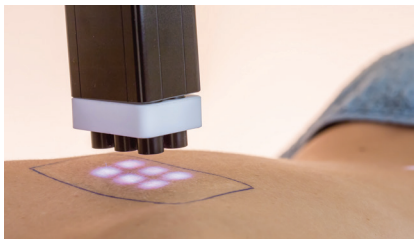
The UV irradiation must be performed with an energy level that is considered safe, considering the maximum cumulative irradiation that a volunteer can be exposed to in the year (a clearance period between two sessions of at least two months is required, so every volunteer can safely undergo a maximum of six irradiations/year). A specific equipment (Solar simulator) is used and the UV spectra of the xenon lamp ranges from 290 to 400 nm, encompassing both UVB (290-330 nm) and UVA (331-400 nm) rays. This test is designed to check proto-protection only in the UVB range, but it will expose volunteers to UVA emission also to better simulate what happens during real sun exposure, as the solar radiation is mainly composed by UVA (>90%). Recent scientific literature also describes the contribute of UVA to skin erythema, that was previously underestimated, and many companies are correctly formulating sunscreens with a much higher UVA/UVB ratio (up to 1:1). Different *in vivo* protocols and in vitro ISO standards describe the methods to specifically check the UVA protection.

More recently, companies have started to claim additional wavelenghts for protection and started formulating sunscreens accordingly. These include **UVC** rays, which generally do not reach the earth surface but may where the ozone layer is thinner, high energy visible (**HEV**) light, which has the capacity of penetrating the skin down to the dermis and may have a role in aging effects and **NIR** (near infra-red) radiation, which may damage skin collagen content via an increase in MMP-1 activity according to several scientific publications. To test protection against these wavelenghts of the light spectrum is possible, even if official methods are not available.

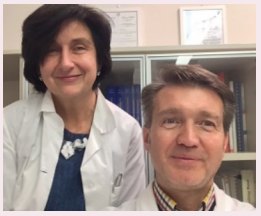
The right performance of ISO 24444:2010 and FDA CFR21 SPF tests is subject to a high variability due to the technician's handling, to laboratory procedures and equipment, to the conditions of the volunteer's skin, and also to the tested sunscreen formulation. As an example, since results are UV-induced erythema, the reading of the results is made the day after the exposure (16-24 h is the time to develop the skin erythema). In this situation, any soothing ingredient in the sample will probably reduce the intensity of the erythema, leading to an over-estimation of the SPF protection given by the sunscreens in the formula. An *in vitro* test totally replacing the *in vivo* test would allow to avoid such false results, and will also be ethically much more acceptable. The regulation in Europe is going in this direction, and a technical committee is trying to develop a similar spectrophotometric method, highly predictive and reproducible, to replace the current ISO 24444:2010. A validated standard is anyway still far, and will require several years to be enacted.

The **UVA** protection of sunscreens in Europe (marked on the label by the circled UVA symbol) and in North America (stated on the label by the claim "**Broad spectrum**") is evaluated by *in vitro* tests according to ISO 24443:2012 and to FDA CFR21 part 201 (subpart G, section 201.327) respectively. These two protocols are deeply different in experimental conditions, notwithstanding they employ similar equipment and materials. The lack of harmonization is today a still unresolved issue and companies that are selling sunscreens on these two different markets must perform both the assays despite their same goal. These tests give as a result in terms of the absolute protection towards UVA in proportion to the overall SPF and/or an indication of the UVA/UVB balance by means of the critical wavelength (λ_c) value. Additionally, the sample is exposed to an intense preliminary photostress in a Suntest chamber, and hence this assay is also a good indication of the product's photo-stability. If a severe degradation of the sunscreens occurs in this preliminary step, the UVA test cannot proceed any further.

Before performing the *in vivo* SPF test on volunteers, it is better to carry out a preliminary full range UV protection test *in vitro* to check the expected SPF of the formula. This is a quick screening test and it will reduce the R&D timing and risk of failure in the much more expensive and time-consuming *in vivo* test. Many software are also available to predict theoretically the SPF of a formula *in silico*. One of these simulators is available for free on Abich website (http://www.abich.it/it/spf-standard-e-sunscreen/sunscreen-simulator.aspx) after registering.



At the same time, it is strongly suggested to perform a **photostability** testing, by analyzing the sunscreen titration in the formula before and after an intense cycle of accelerated photo-stress and at the end of the standard accelerated aging test at high temperature. Both sun exposure and heating will strongly affect the stability of sunscreens in the finished formula and can highly compromise its efficacy on the skin even just after the application. The expiring date and the PAO for cosmetics must be defined also on the bases of such assays.
The most commonly used protocol for photostability testing requires the exposure of the sample to an irradiation equivalent to 5 MEDs in a refrigerated solar simulator, and the comparison between the sample's results before and after irradiation. The possible degradation of the sample can be calculated in terms of percentage loss of efficacy using a spectrophotometric analysis, while HPLC measurements provide a reading of the exact degradation of each single filter in the formula, a very useful information, especially in complex formulas with 4 or more filters. This in turn allows the formulator to understand whether the problem is limited to a specific filter or more than one, thus determining any crossed incompatibilities.
To better assess the safety of application, an *in vitro* test on skin-derived cells (3T3) for **phototoxicity** is available (protocol described in OECD 432) and it is strongly advised to use it as a screening device when formulating sunscreens. It measures the viability of cells by NRU (Neutral Red Uptake) with and without the sample, with and without simultaneous UV exposure and by comparison with a known Phototoxic substance.
Another important claim that is often required for sunscreens is **Water Resistance**. In Europe, an *in vivo* ISO method on volunteers to measure water resistance is in progress but it is still not available. Currently this test is performed according to the 2005 Colipa (now Cosmetics Europe) guidelines, measuring the static SPF of the investigated product before and after 2 controlled washings of 20' each (for Water-resistant products) or 4 controlled washing of 20' each (for VERY water-resistant product). Also a VERY VERY water resistant claim can be found on the label, where 2x 20'more washes have been performed in addition to the 4 x20' required by the guideline for the "very water resistant" claim. The SPF must not lose more than 50% of its value after the washes as compared to the pre-wash measurements, in order to be considered water-resistant and for the claim to be reported on the label. The product is applied on the back of the volunteers and the washings are made by immersion in a Jacuzzi bath/ pool with tap water. Between the first bath and the next one, the treated area is gently toweled. The procedure for measuring SPF is the same as for the static test, and for that reason the WR measured according to Cosmetics Europe guideline always encompass also the SPF measure. Instead, to assess the water resistance of a sunscreen according to FDA, a wash of 40' or 80' must be performed, and the resulting SPF is the number that can be stated on the label, together with the number of minutes of washing performed and allowed to maintain this value. In this case, the SPF and the WR test are made separately.
Other methods can be developed based on the standard water-resistant protocol to measure for instance **sweat resistance**, **sea water resistance** or **chlorinated water resistance**. Sweat resistance is assessed by measuring SPF before and after 2x20' or 4 x20 'sessions in a sauna. The loss of SPF must not exceed 50%. For chlorinated water and sea water resistance, that simulate the sunscreens use while bathing in the sea or in a pool, salt water and Cl supplemented water are used instead of tap water. These assay variants are not regulated by any guideline, but can be performed to support a proper claim. Sunscreens can be properly formulated, i.e. with stronger emulsifiers, to withstand sea water or Chlorinated water washing. These tests will add more safety and additional value to the tested product, even if not designed on a specific guideline.
In the R&D phase for a sunscreen, an *in vitro* predictive method to measure water resistance would be very helpful but its development encountered many technical limits due to difficulties in reproducing the interaction between the formula, the filming agents and the human epidermis.
Another interesting method that can be applied to better support the stability of the formula, is to check the **maintenance of the correct SPF value at different end-times**, i.e. at T= 4 h, T= 6 h, after the application on the skin (T= 0). This property is linked to the thermal and photo-stability of the formula and to its compatibility with the skin pH and lipidic or salt content. This will be very useful to give a more proper indication about the timing for re-applying the sunscreens, or even an indication to modify and adjust the formula in order to make it more stable
Sometimes stability issues are the reason for SPF failures, and the compatibility of each ingredient in the formula must be correctly verified before, as well as the sunscreens solubility in each phase and solvent. If the filters are not stably solubilized or not homogeneously dispersed in the finished formula, the desired SPF will not be reached. To avoid this risk, an observation of the emulsion at the microscope and a preliminary centrifuge stress to check for phase separation are strongly recommended.



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