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PROTOCOL

***In Vitro* Determination of the UVA Protection Provided By
Sunscreen Products**

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Protocol Number: SRL2008-XXX

Objectives:

1. To determine compliance with the EC recommendation on the efficacy of sunscreen products [1], by measuring the UVAPF/SPF ratio of a sunscreen product after irradiation with a prescribed dose of UV, according to the Colipa Guideline of 2007 [2], and the critical wavelength method of Diffey [3].
2. To determine the Boots Star Rating [4]
3. To determine the UVA protection rating according to the FDA proposed monograph amendment of 2007. [5]
4. To evaluate photostability [6,7]

Test Product:

Sponsor:

Investigator: Joseph W. Stanfield

Introduction:

The Commission of the European Communities [1] has recommended that sunscreen products have a UVA protection factor of at least 1/3 the SPF and a critical wavelength of at least 370 nm, as obtained by the method of Diffey et al.[2] The UVA protection factor is may be measured *in vitro* according to the Colipa Guideline of 2007. [3] The essential aspects of the guideline include the following:

Step 1: *In vitro* measurement of the initial UV transmittance spectrum, $A_0(\lambda)$, of a fixed amount of the sunscreen product spread on a suitable substrate, prior to UV irradiation.

Step 2: Mathematical adjustment of the initial UV transmittance spectrum to achieve an *in vitro* SPF equal to the labelled SPF (*in vivo*). The adjustment is accomplished using a multiplication factor, C. The initial UVA protection factor, $UVAPF_0$, is calculated using the adjusted UV transmittance spectrum.

Step 3: A single UV dose D is calculated as $1.2 \times UVAPF_0$.

Step 4: UV exposure of the sample according to the calculated UV dose, D.

Step 5: *In vitro* measurement of the UV transmittance spectrum $A(\lambda)$ of the sunscreen product after UV exposure.

Step 6: Mathematical adjustment of the second UV transmittance spectrum (following UV exposure) using the factor C, determined in step 2 and calculation of the *in vitro* UVA protection factor UVAPF after irradiation using the adjusted second UV transmittance spectrum.

The critical wavelength is then determined by converting the second adjusted UV transmittance spectrum (Step 6 above) to an absorbance spectrum and measuring the cumulative area under the curve at each wavelength to determine the wavelength at which the area is 90 percent of the total.[3]

The Boots Star rating is determined by converting the first adjusted UV transmittance spectrum (Step 2 above) to an absorbance spectrum and determining the ratio of the mean UVA absorbance (320-400 nm)

to the mean UVB absorbance (290-320 nm). The rating is assigned as follows [4]:

Mean UVA Absorbance/Mean UVB Absorbance	Rating
>0.2	1 Star
>0.4	2 Stars
>0.6	3 Stars
>0.8	4 Stars
>0.9	5 Stars

The UVA protection rating according to the FDA proposed monograph amendment of 2007 is determined from the UVAPF measured on human subjects and the ratio of the mean UVAI absorbance (340-400 nm) to the mean total UV absorbance (290-400 nm) measured in vitro after irradiation with a UV dose of 2/3 the SPF in MEDs. One MED is defined as a typical minimal erythema dose of 20 effective mJ/cm². [5]

The UVAPF determined in vitro according to the Colipa guideline of 2007 provides an estimate of the UVAPF measured on human subjects.

The proposed UVA protection categories based on UVAPF are:

UVAPF	Rating
≥2	Low
≥4	Medium
≥8	High
≥12	Highest

The proposed UVA protection categories based on mean UVAI absorbance/mean UV absorbance are:

Mean UVA Absorbance/Mean UVB Absorbance	Rating
≥0.2	Low
≥0.4	Medium
≥0.7	High
>0.95	Highest

In the FDA proposal, the actual product label uses the lower category of the two categories determined above.

Finally the photostability is evaluated according to the proposed method of Stanfield [6,7] using continuous UV irradiation of a sunscreen film that has been applied to a substrate in the same amount as used in Step 1 above. The substrate is placed over the aperture of detector that measures the erythema effective irradiance in MED/hr, where the MED is 20 effective mJ/cm.² Applied and transmitted dose are measured during irradiation until an effective applied UV dose in MEDs near the SPF of the sunscreen is reached. The effective UV dose transmitted by the sunscreen is then plotted against the applied effective UV dose, and the following least squares curve fit equation is computed for the UV transmittance curve, using a Microsoft Excel[®] spreadsheet (Microsoft, Redmond, WA):

$$y = \alpha x^{\beta}$$

Where **y** is the transmitted UV dose in MEDs and **x** is the applied UV dose in MEDs.

The exponent, β , is an index of photostability: If β is equal to 1 the relationship between the applied and transmitted doses is linear, and the sunscreen is considered photostable; if β is substantially greater than 1, the transmitted UV dose increases with applied dose, and the sunscreen is considered photolabile.

References:

1. The Commission of the European Communities. Commission Recommendation of 22 September 2006 on the efficacy of sunscreen products and the claims made relating thereto. Official Journal of the European Union, L265/39, 26.9.2006.
2. Colipa Project Team IV, *In vitro* Photoprotection Methods, Method for the *in vitro* Determination of UVA Protection Provided by Sunscreen Products. Guideline, 2007.
3. BL Diffey, PR Tanner, PJ Matts, JF Nash. In vitro assessment of the broad-spectrum ultraviolet protection of sunscreen products. *J Amer Acad Dermatol* 43:1024-35, 2000.
4. Brown M. Of Cassiopeia and five stars. 2010-A Sun Odyssey. London, June 2005.
5. U. S. Food and Drug Administration. Sunscreen Drug Products for Over-the-Counter Human Use; Proposed Amendment of Final Monograph; Proposed Rule; 21CFR Parts 347 and 352. Federal

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