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Determination of sunscreen UVA photoprotection *in vitro*

Détermination in vitro de la photoprotection UVA

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

Determination of sunscreen UVA photoprotection *in vitro*

1 Scope

This International Standard specifies an *in vitro* procedure to characterize the UVA protection of sunscreen products. Specifications are given to enable determination of the spectral absorbance characteristics of UVA protection in a reproducible manner.

In order to determine relevant UVA protection parameters, the method has been created to provide a UV spectral absorbance curve from which a number of calculations and evaluations can be undertaken. Results from this measurement procedure can be used for other computations, as required by local regulatory authorities. These include calculation of the Ultraviolet-A protection factor (UVAPF) [correlating with *in vivo* UVAPF from the persistent pigment darkening (PPD) testing procedure], critical wavelength and UVA absorbance proportionality. These computations are optional and relate to local sunscreen product labelling requirements. This method relies on the use of *in vivo* SPF results for scaling the UV absorbance curve.

This International Standard is not applicable to powder products such as pressed powder and loose powder products.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
***in vitro* UVA protection factor**
UVAPF
in vitro UVA protection factor of a sun protection product against UVA radiation, which can be derived mathematically with *in vitro* spectral modelling

2.2
***in vitro* calculation of SPF**
$SPF_{in\ vitro}$
protection factor of a sun protection product against erythema-inducing radiation calculated with spectral modelling

2.3
action spectrum for erythema
$E(\lambda)$
relative effects of individual spectral bands of an exposure source for an erythema response

NOTE See References [1] and [2].

2.4
action spectrum for PPD
$P(\lambda)$
relative effects of individual spectral bands of an exposure source for a persistent pigment response

NOTE See References [3] and [4].

2.5
monochromatic absorbance
A_λ
sunscreen absorbance at wavelength, λ, related to the sunscreen transmittance, T_λ, by

$$A_\lambda = -\log(T_\lambda)$$

where transmittance, T_λ, is the fraction of incident irradiance transmitted by the sunscreen film

2.6
irradiance
I
fluence rate per unit area, expressed in W/m^2, for a defined range of wavelengths

EXAMPLE From 290 nm to 400 nm for UVA + UVB irradiance; from 320 nm to 400 nm for UVA irradiance.

2.7
spectral irradiance for SPF testing or PPD testing
$I(\lambda)$
irradiance per unit wavelength, $I(\lambda)$, expressed in W/m^2/nm

2.8
spectrophotometer
instrument that measures absorbance (or transmission) properties of a test medium as a function of wavelength

2.9
spectroradiometer
instrument that measures spectral irradiance (intensity in watts per unit area per nanometre) of electromagnetic sources

NOTE Limited to ultraviolet, visible and short infrared ranges in this International Standard.

2.10
radiometer
instrument that measures broad band irradiance (intensity in watts per unit area) of electromagnetic sources

NOTE Limited to ultraviolet, visible and short infrared ranges in this International Standard.

3 Principle

The test is based on the assessment of UV-transmittance through a thin film of sunscreen sample spread on a roughened substrate, before and after exposure to a controlled dose of radiation from a defined UV exposure source. Because of the several variables that cannot be controlled with typical thin film spectroscopic techniques, each set of sunscreen transmission data is mathematically adjusted so that the *in vitro* SPF data yield the same measured *in vivo* SPF value that was determined by *in vivo* testing. Samples are then exposed to a specific measured dose of UV radiation to account for the photostability characteristics of the test product. The resulting spectral absorbance data have been shown to be a useful representation of both the width and height of the UVA protection characteristics of the sunscreen product being tested. The mathematical modelling procedure has been empirically derived to correlate with human *in vivo* (persistent pigment darkening) test results.

4 Apparatus

4.1 UV spectrophotometer specifications

The UV spectrophotometer wavelength range shall span the primary waveband of 290 nm to 400 nm. The wavelength increment step shall be 1 nm.

A UV spectrophotometer that does not have a monochromator after the test sample should employ a fluorescence rejection filter.

The UV spectrophotometer input optics should be designed for diffuse illumination and/or diffuse collection of the transmitted irradiance through the roughened polymethylmethacrylate (PMMA) substrate, with and without the sunscreen layer spread on its surface. The size of the diameter of the entrance port of the UV spectrophotometer probe shall be smaller than the size of the light spot to be measured at the sample level (in order to account for stray light). The area of each reading site should be at least 0,5 cm^2 in order to reduce the variability between readings and to compensate for the lack of uniformity in the product layer. The wavelength should be accurate to within 1 nm, as checked using a holmium-doped filter (see Annex A). The ability of an instrument to accurately measure absorbance is limited by the sensitivity of the instrument. The minimum

required dynamic range for this methodology is 2,2 absorbance units as determined according to Annex A. The maximum measured absorbance should be within the dynamic range of the device used. If the test measurements yield absorbance curves that exceed the determined upper limit of the UV spectrophotometer, the product should be re-tested using an instrument with increased sensitivity and dynamic range.

The lamp in the UV spectrophotometer that is used to measure the transmittance shall emit continuous radiation over the range of 290 nm to 400 nm, and the level of irradiance should be sufficiently low, so that the photostability of the product is not unduly challenged (a xenon flash lamp is a convenient solution). Therefore the UV dose during one measurement cycle should not exceed 0,2 J/cm^2.

NOTE A UV spectrophotometer is used to measure the absorbance properties of the sunscreen on the test plates. A spectroradiometer is used to measure the spectral energy distribution and intensity of the UV exposure source or the UV spectrophotometer during the absorbance measurement of the sunscreen on the test plate.

4.2 Calibration of the UV spectrophotometer

The UV spectrophotometer shall be validated at regular intervals (recommended at least every month) by measurements of reference materials.

A three-fold test is required, as described in Annex A:

— dynamic range of the UV spectrophotometer;

— linearity test of the UV spectrophotometer;

— wavelength accuracy test.

4.3 Calibration of the UV exposure source

The spectral irradiance at the exposure plane of the UV exposure source that is used for irradiation (to take into account any photoinstability) shall be as similar as possible to the irradiance at ground level under a standard zenith sun[5] as defined by COLIPA[6] or in DIN 67501[7]. The UV irradiance shall be within the following acceptance limits (measured at sample distance).

Table 1 — UV exposure source specifications

UV exposure source specifications as measured with a spectroradiometer	
Total UV irradiance (290 nm to 400 nm)	40 W/m^2 to 200 W/m^2
Irradiance ratio of UVA[a] to UVB[b]	8:22
[a] 320 nm to 400 nm.	
[b] 290 nm to 320 nm.	

The UV exposure source device should have the ability to maintain samples within the range of 25 °C to 35 °C. It is important that the temperature of the sample itself be measured and not just the surrounding air temperature. To maintain samples at a temperature less than or equal to 35 °C, a filter system that particularly reduces IR radiation should be used to achieve the specified temperature range. Cooling trays for the sample plates or ventilators should be used to maintain a temperature below 35 °C and warming devices to maintain samples at or above 25 °C.

4.4 Monitoring of the UV exposure source

The emission of the UV exposure source used for exposure shall be checked for compliance with the given acceptance limits by a suitably qualified expert (at least) every 18 months, or after 3 000 hours of lamp running time. The inspection should be conducted with a spectroradiometer that has been calibrated against a standard lamp that is traceable to a national or an international calibration standard. In addition to the spectroradiometric inspection, the intensity of the UV exposure source used for exposure shall be checked prior to each use. This can be done using either a spectroradiometer or a radiometer with sensitivity in the UVA, calibrated for the

same UV exposure source spectrum used for the exposure step of the procedure, applying the coefficient of calibration to adjust for variance between the UVA radiometer and the reference spectroradiometer.

4.5 Calibration of the UVA radiometer used to monitor the test sample irradiation

If a UVA radiometer is used, this device shall have been suitably calibrated. This requires that it be calibrated to the spectroradiometer used to measure the exposure source (as during annual solar simulator calibration). Calibration shall be conducted in terms of UVA irradiance (320 nm to 400 nm) and shall be at the same level at which the test plates are exposed. Once calibrated with the spectroradiometer, the UVA radiometer may be used to determine the UV doses to be used during the exposure procedure on a day-to-day basis. Annex B provides the step-by-step calibration procedure.

4.6 Substrate/plate

The substrate/plate is the material to which the test product is to be applied. For this method, PMMA plates with one rough side of the substrate are to be used and are commercially available. One specific plate has been validated for this test method; the specifications and preparation of this type of plate[9] are described in Annex D. The size of the substrate should be chosen such that the application area is not less than 16 cm^2.

5 Test method

5.1 Outline of the test procedure

5.1.1 Conduct the calibration and validation of the test equipment, including the UV spectrophotometer used for transmission/absorbance measurements and the UVA radiometer (or spectroradiometer) used to measure the UV exposure source, and verify the transmission properties of the test plates as described in Annex D.

5.1.2 Conduct blank measurements of a glycerin-treated plate for the reference "blank", which will be used in the subsequent absorbance measurements.

5.1.3 Conduct *in vitro* absorbance measurements of the sunscreen product spread on a PMMA plate, prior to any UV irradiation. Acquire the initial UV absorbance spectrum with $A_0(\lambda)$ data.

5.1.4 Conduct the mathematical adjustment of the initial UV absorbance spectrum using coefficient "C" (see the calculation in 5.7.2) to achieve an *in vitro* SPF (no UV dose) equal to the *in vivo* SPF. Initial $UVAPF_0$ is calculated using $A_0(\lambda)$ and C.

5.1.5 A single UV exposure dose, D, is calculated, equal to 1,2 × $UVAPF_0$ in J/cm^2.

5.1.6 Conduct UV exposure of the same sample as in 5.1.3, according to the calculated UV exposure dose D.

5.1.7 Measure the *in vitro* absorbance of the sunscreen product after UV exposure. Acquire the second UV spectrum with $A(\lambda)$ data.

5.1.8 Conduct the mathematical adjustment of the second absorbance spectrum (following UV exposure) by multiplying with the same "C" coefficient, previously determined in 5.1.4. The resulting absorbance curve is the final adjusted absorbance values.

NOTE For calculations, UV absorbance values shall be used.

5.2 Equipment calibration and validation of test plates

Test procedures as described in Annex A are to be completed to validate the wavelength accuracy, linearity and absorbance limits of the UV spectrophotometer/spectroradiometer to be used for the test procedure. Validation of the UV properties of the test PMMA plates shall also be conducted as described in Annex D.

5.3 Absorption measurements through the plate

It is necessary to first determine the absorbance of UV radiation through a “blank” PMMA plate. Prepare a “blank” plate by spreading a few microlitres of glycerin on the roughened side of the plate. Choose the amount of glycerin such that the entire surface is just completely covered (approximately 15 µl for a 50 × 50 mm plate). Any excess of glycerin should be avoided. Measure the absorbance through this “blank” plate and use this as the baseline measurement for subsequent absorbance measurements.

NOTE Many spectrophotometers have “baseline” functions to automatically incorporate this baseline measurement into the calculations of subsequent absorbance measurements.

5.4 Sample application

The sunscreen product is applied to a new untreated roughened PMMA plate (with the roughened side uppermost) by mass, at an application rate of 1,3 mg/cm^2. To ensure dose accuracy and repeatability, the application area should be not less than 16 cm^2. The application dose may be determined by measuring the mass loss of the pipette before and after application of the product; alternatively, it may be applied based on volumetric measurements with consideration of the specific gravity of the test sample. Where possible, a positive-displacement automatic pipette should be used for this purpose.

The sunscreen is applied as a large number of small droplets of approximate equal volume, distributed evenly over the whole surface of the plate. Finger cots should not be used to spread the product on the plate. The fingertip used for spreading should be dipped into the test product and then wiped to remove excess product before spreading the test product applied to the plate. The fingertip used to spread the product shall be cleaned between applications of different test products.

After the sunscreen product is deposited on the surface of the plate, it shall be spread immediately over the whole surface using light strokes with a fingertip (without finger cot). Spreading should be completed in a two-phase process. First, the product should be distributed over the whole area as quickly as possible (less than 30 seconds) using small circular motions with minimal pressure. Then the sample should be rubbed on the plate surface using alternating horizontal and vertical strokes with increased moderate pressure. The second phase should take 20 to 30 s.

This treated sample shall be allowed to dry for at least 30 min in the dark at the same temperature that will be experienced under the UV exposure conditions (i.e. if UV source exposure conditions will be 35 °C, then the drying conditions should also be at 35 °C; or if the UV source exposure conditions will be 25 °C, then the drying conditions should also be 25 °C).

5.5 Absorbance measurements of the product-treated plate

The product-treated plate is placed in the light-path of the UV spectrophotometer and the absorbance of UV radiation through the sample is determined for each wavelength, from 290 nm to 400 nm, in 1 nm steps. One or more observations of absorbance may be made per plate and the mean value shall be determined for each plate.

5.6 Number of determinations

At least four plates prepared with the test sunscreen shall be used to establish the protection aspects of the test sample. Additional plates shall be added to the sampling if the 95 % confidence interval (CI) is greater than 17 % of the mean value of the UVAPF value, until the 95 % CI is less than 17 % of the mean UVAPF value. Calculation procedures for this are described in Annex F.

5.7 Determination of initial calculated SPF ($SPF_{in\ vitro}$), “C” value, initial UVAPF($UVAPF_0$), and UV exposure dose

5.7.1 Determination of $SPF_{in\ vitro}$

The UV solar simulator radiation (UV-SSR) source spectrum, $I(\lambda)$, (see Annex C) is multiplied with the corresponding erythema action spectrum sensitivity value, $E(\lambda)$, (see Annex C) at that wavelength to yield the sunburning effective energy at that wavelength. The resulting sunburning effective irradiance is integrated over the 290 nm to 400 nm

range. The sunscreen transmission values at each wavelength are multiplied with the erythemal effective energy at that wavelength and integrated over the same interval to yield the effective sunburning energy transmitted through the test product. The ratio of these two integrals is the *in vitro* calculated SPF value.

Calculation of $SPF_{in\ vitro}$ is shown in Equation (1):

$$SPF_{in\ vitro} = \frac{\int_{\lambda=290}^{\lambda=400} E(\lambda)\times I(\lambda)\times d\lambda}{\int_{\lambda=290}^{\lambda=400} E(\lambda)\times I(\lambda)\times 10^{-A_0(\lambda)}\times d\lambda} \quad (1)$$

where

$E(\lambda)$ is the erythema action spectrum[1] (see Annex C);

$I(\lambda)$ is the spectral irradiance received from the UV source (SSR for SPF testing) (see Annex C);

$A_0(\lambda)$ is the mean monochromatic absorbance of the test product layer before UV exposure;

$d\lambda$ is the wavelength step (1 nm).

NOTE This calculated SPF value cannot be used as an $SPF_{in\ vitro}$ result.

5.7.2 Determination of "C" value

The initial absorbance curve values are multiplied by a scalar value "C" until the *in vitro* calculated SPF values are equal to the *in vivo* measured SPF. This is accomplished in an iterative calculation process. The initial absorbance values multiplied by this "C" value become the adjusted sunscreen absorbance curve that is used for determination of the initial $UVAPF_0$ value, and the exposure dose. Equation (2) shows the calculation of the adjusted *in vitro* SPF ($SPF_{in\ vitro,adj}$) and determination of the coefficient of adjustment "C":

$$SPF_{in\ vitro,adj} = SPF_{in\ vitro} = \frac{\int_{\lambda=290}^{\lambda=400} E(\lambda)\times I(\lambda)\times d\lambda}{\int_{\lambda=290}^{\lambda=400} E(\lambda)\times I(\lambda)\times 10^{-A_0(\lambda)C}\times d\lambda} \quad (2)$$

where

$E(\lambda)$, $I(\lambda)$, $A_0(\lambda)$ and $d\lambda$ are as defined in Equation (1).

This calculation is based on Lambert-Beer's law $E = E_0\ e^{-cd}$ which is related to ideal solutions. While sunscreens in thin film do not behave as ideal solutions, this calculation has been proven satisfactory for this specific application[10][11].

The "C" value typically lies between 0,8 and 1,6 for valid interpretation. If it is outside this range, new samples should be prepared to validate the original observations. The "C" value for the reference S2 shall lie in this range 0,8 to 1,6 or the application procedure should be modified to achieve it.

5.7.3 Determination of initial UVA protection factor before UV exposure ($UVAPF_0$)

The initial $UVAPF_0$ value is calculated for the purpose of determining the UV exposure dose. It is calculated in a manner similar to the calculation of the initial $SPF_{in\ vitro}$. The intensity spectrum for a UVA radiation source, $I(\lambda)$, (as described in Annex C) is multiplied at each wavelength with the persistent pigment darkening action spectrum sensitivity values, $P(\lambda)$, to yield the pigment darkening energy at that wavelength. The resulting pigment darkening effective irradiance is integrated over the 320 nm to 400 nm range. The initial absorbance values from the test product at each wavelength are used to calculate the effective intensity at each wavelength

to yield the effective pigment darkening energy transmitted through the test product as shown in Equation (3) below. The ratio of these two integrals is the initial *in vitro* $UVAPF_0$ value:

$$UVAPF_0 = \frac{\int_{\lambda=320}^{\lambda=400} P(\lambda) \times I(\lambda) \times d\lambda}{\int_{\lambda=320}^{\lambda=400} P(\lambda) \times I(\lambda) \times 10^{-A_0(\lambda)C} \times d\lambda} \quad (3)$$

where

$P(\lambda)$ is the PPD action spectrum (see Annex C);

$I(\lambda)$ is the spectral irradiance received from the UVA source (UVA 320 nm to 400 nm for PPD testing) (see Annex C);

$A_0(\lambda)$ is the mean monochromatic absorbance of the test product layer before UV exposure;

C is the coefficient of adjustment, previously determined in Equation (2);

$d\lambda$ is the wavelength step (1 nm).

5.7.4 Determination of the UV exposure dose

The UV exposure dose, D, is the $UVAPF_0$ value multiplied by a factor of 1,2, in Joules/cm^2:

$$D = UVAPF_0 \times 1{,}2 \quad (4)$$

The sample is exposed to full spectrum UV radiation but the dose is being defined by the UVA content.

NOTE The 1,2 J/cm^2 factor is based on ISO ring test validation study results[8].

5.8 UV exposure

WARNING — Personnel working with this irradiator system should be protected adequately against UV rays (glasses, gloves, etc.).

Expose the sample plates to the radiation from the UV exposure source. During the exposure the samples should be maintained at a temperature between 25 °C and 35 °C, and at the same temperature used for the drying period. The PMMA plates should be fixed above a non-reflective UV background behind each plate to reduce back exposure. Ensure that the UV exposure source does not switch off while placing samples under the lamp (in this case, ensure the output irradiance is the same on restart as it was before the lamp was turned off).

5.9 Measurement of final adjusted absorbance spectrum

After the UV exposure, re-measure the absorbance of the test samples on the same spots as measured before the UV exposure, as in 5.5. The final absorbance values are equal to the observed absorbance values after the UV exposure, multiplied by the "C" value determined in 5.7.2.

$$A_f(\lambda) = A_e(\lambda)C$$

where

A_e is the mean monochromatic absorbance of the test product layer after UV exposure;

A_f is the mean final monochromatic absorbance of the test product.

5.10 Calculation of UVAPF of plates after UV exposure of the sample

The UVAPF shall be calculated according to Equation (5) for each individual plate, using the single observation value or the mean of multiple observations on that plate.

$$\mathrm{UVAPF} = \frac{\int_{\lambda=320}^{\lambda=400} P(\lambda)\times I(\lambda)\times \mathrm{d}\lambda}{\int_{\lambda=320}^{\lambda=400} P(\lambda)\times I(\lambda)\times 10^{-A_{\mathrm{e}}(\lambda)C}\times \mathrm{d}\lambda} \tag{5}$$

where

$P(\lambda)$ is the PPD action spectrum (see Annex C);

$I(\lambda)$ is the spectral irradiance received from the UVA source (UVA 320 nm to 400 nm for PPD testing) (see Annex C);

$A_{\mathrm{e}}(\lambda)$ is the mean monochromatic absorbance of the test product layer after UV exposure;

C is the coefficient of adjustment, previously determined in Equation (2);

$\mathrm{d}\lambda$ is the wavelength step (1 nm).

Other protection parameters may be calculated from the final absorbance curve in 5.9 as desired.

6 Procedure using the spreadsheet in this International Standard

6.1 The calculations given in 5.1.4 to 5.1.8 can be performed automatically using the calculation spreadsheet in this International Standard and the following steps.

6.2 Enter the name, date, operator identification and *in vivo* SPF of the test product, the spectroanalyser type, UV exposure device type, the raw UVA exposure irradiance of the UV exposure source, and the irradiance correction value "Y" (from Annex B) into the test spreadsheet on the "Start here" tab.

6.3 Measure and input the absorbance data for the first four unirradiated sample plates into the spreadsheet on the tabs named "Plate #0". Click on the "OK, data entered, let's proceed" button after each entry.

6.4 The UV exposure irradiance and exposure time are reported on the "Results #" tab for each individual plate.

6.5 Expose the sunscreen treated plate for the prescribed time to achieve the UV exposure dose for each plate.

6.6 Measure the absorbance of each of the individual UV exposed plates. The measurements conducted after UV exposure should be on the same spot or spots as measured before the UV exposure.

6.7 Input the post-UV exposure absorbance measurements for each plate into their respective spreadsheet tabs "Plate #UV". Click on the "OK, data entered, let's proceed" button.

6.8 The "RESULTS (Plate #)" spreadsheet tab will show the results data for each individual plate.

6.9 When the full data input for the first four plates is complete, the "Report" tab spreadsheet will appear giving the summary results for the test sample. If the 95 % CI of the UVAPF values is less than 17 % of the mean UVAPF, no further plates are required and the final results are displayed in graphic and tabular form. Otherwise, additional samples will need to be added sequentially as above. Additional data sheets for additional plates will appear and be completed as above until the test criterion is satisfied.