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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.

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# 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<sub>*in vitro*</sub>**

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**

$A_\lambda$

sunscreen absorbance at wavelength,  $\lambda$ , related to the sunscreen transmittance,  $T_\lambda$ , by

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

where transmittance,  $T_\lambda$ , 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  $0,5\text{ cm}^2$  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<sup>2</sup>.

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<sup>[5]</sup> as defined by COLIPA<sup>[6]</sup> or in DIN 67501<sup>[7]</sup>. 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 <sup>2</sup> to 200 W/m <sup>2</sup>
Irradiance ratio of UVA <sup>a</sup> to UVB <sup>b</sup>	8:22
<sup>a</sup> 320 nm to 400 nm.	
<sup>b</sup> 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<sup>9)</sup> are described in Annex D. The size of the substrate should be chosen such that the application area is not less than 16 cm<sup>2</sup>.

### 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<sub>0</sub> is calculated using  $A_0(\lambda)$  and C.
- 5.1.5 A single UV exposure dose,  $D$ , is calculated, equal to  $1,2 \times \text{UVAPF}_0$  in J/cm<sup>2</sup>.
- 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<sup>2</sup>. To ensure dose accuracy and repeatability, the application area should be not less than 16 cm<sup>2</sup>. 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 erythral 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<sup>[1]</sup> (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<sub>0</sub> 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<sup>[10][11]</sup>.

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<sub>0</sub>)

The initial UVAPF<sub>0</sub> 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<sub>0</sub> value:

$$\text{UVAPF}_0 = \frac{\int_{\lambda=320}^{\lambda=400} P(\lambda) \times I(\lambda) \times d\lambda}{\int_{\lambda=320}^{\lambda=400} P(\lambda) \times I(\lambda) \times 10^{-A_0(\lambda)C} \times d\lambda} \quad (3)$$

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<sub>0</sub> value multiplied by a factor of 1,2, in Joules/cm<sup>2</sup>:

$$D = \text{UVAPF}_0 \times 1,2 \quad (4)$$

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<sup>2</sup> factor is based on ISO ring test validation study results<sup>[8]</sup>.

### 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.

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

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);
- $A_e(\lambda)$  is the mean monochromatic absorbance of the test product layer after UV exposure;
- $C$  is the coefficient of adjustment, previously determined in Equation (2);
- $d\lambda$  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.

## 7 Reference sunscreen formula S2

The method is controlled by the use of a reference sunscreen formulation to verify the test procedure. Reference S2 sunscreen formula as described in Annex E shall be used. The UVAPF test results of the reference S2 shall lie between the upper and lower limits, as determined from *in vivo* testing results listed below, or else the test is invalid and shall be repeated. SPF 16 is to be used as the *in vivo* SPF value for S2 for computation purposes.

**Table 2 — Limits of UVAPF testing results**

Parameter	Lower limit	Upper limit
UVAPF	10,7	14,7

The frequency of testing of the S2 standard shall be in accordance with the user's internal procedures and/or ISO/IEC 17025.

## 8 Test report

The test report on the determination of the absorbance spectrum of a sun protection product should contain at least the following information:

- a) description of the instruments used, manufacturer and instrument model with the system calibration summary in this International Standard as per the format in A.6;
- b) the calibration factor "Y" used to adjust the UVA radiometer measurement with the reference spectroradiometer measurement of the UV exposure source (4.5 and B.3.10);
- c) plate manufacturer and batch code;
- d) mean UV absorbance values at each 1 nm wavelength increment for the test sample (a graph of absorbance values, pre-exposure and post-exposure may be provided);
- e) statement of the measured *in vivo* sun protection factor (SPF) used for calculations;
- f) constant "C";
- g) UVA irradiance ( $W/m^2$ ) and mean UVA exposure dose used to irradiate the test sample;
- h) reference data for Reference S2 material with date of testing;
- i) sample identification;
- j) identification of the individual conducting the test;
- k) other informative calculations derived from the absorbance values [see 8 d)] may be reported.

## Annex A (normative)

### Calibration of UV spectrophotometer and plate transmission test

#### A.1 General

This procedure describes the requirements for wavelength accuracy, linearity and dynamic range of the UV spectrophotometer. For clarity, and in order to standardize the report format, a spreadsheet is provided as part of this International Standard.

#### A.2 Wavelength accuracy

##### A.2.1 Holmium oxide filter

The filter should be no more than 3 mm in thickness and dosed with holmium oxide in order to provide absolute wavelength calibration using an absorbance peak of 361 nm.

##### A.2.2 Method

**A.2.2.1** Place the holmium oxide filter in the sample path and scan the absorbance in the range between 290 nm and 430 nm. Measure against air in the blank light path. Repeat the scan for 3 replicates. Accumulate the data and transfer absorbance values to the "Holmium Wavelength Accuracy" tab in the attached spreadsheet "ISO 24443 UVSpectcalib.xls" in Columns B to D. Click on the macro button at cell "P28" to activate the peak check function. The results will automatically appear in the system calibration summary sheet in this International Standard (see Figure A.2) and be similar to Figure A.1 below.

**A.2.2.2** The deviations of the measured band from the reference value in the UV range of the instrument should not exceed 1 nm. An example of a measured calibration spectrum is shown in the graph below. The reported peak wavelength shall be either 360, 361 or 362, or else the instrument shall be recalibrated to achieve one of these wavelength values.

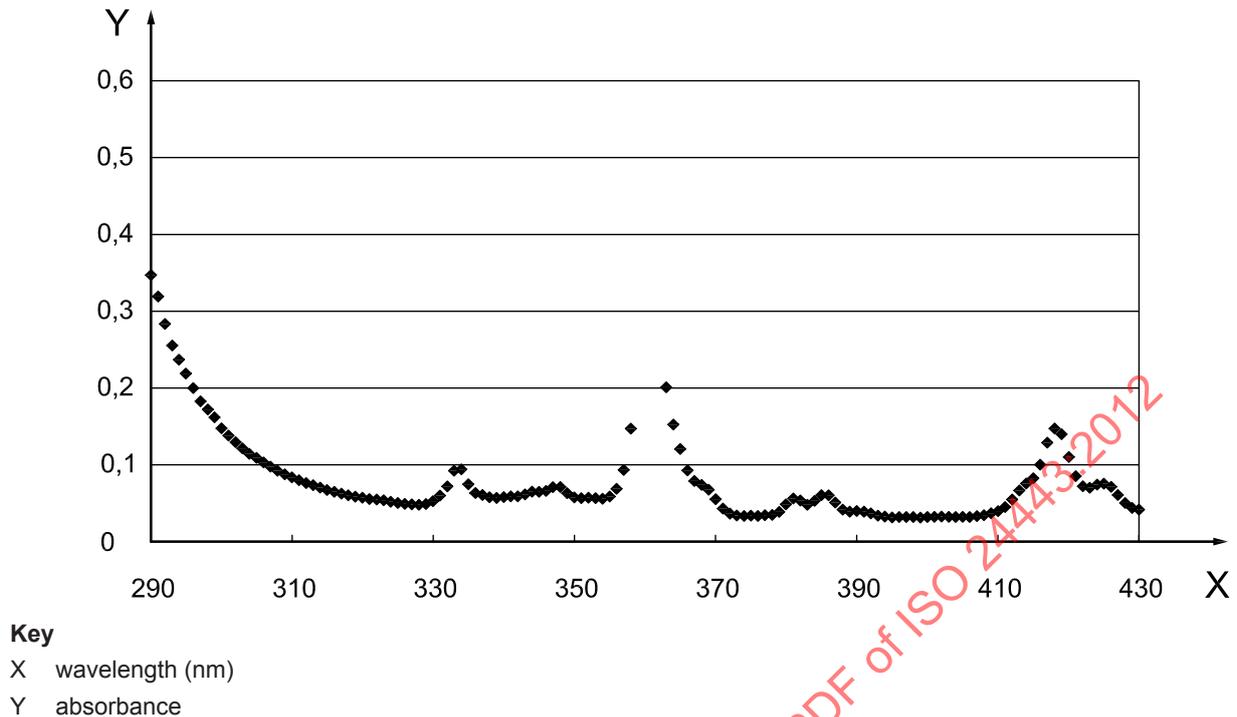


Figure A.1 — Holmium oxide actual

### A.3 Linearity

#### A.3.1 Standard reference plates

The plates are cut from a large sheet of standard cast, UV-stabilized PMMA (helping ensure the same optical properties for each plate). The plates are made in a way as to match the absorption spectra of a range of common sunscreens. The casting process enables a very homogeneous distribution of UV absorbing material, relative to a manually applied film of a test emulsion.

Because of their stable and standardized absorption and diffuse-scattering properties, they are very suitable as “reference emulsions” to check and compare instruments used for *in vitro* determination of UV protection, for intra- as well as inter-laboratory purposes.

#### A.3.2 Linearity assessment

Select two of the transparent UV-stabilized PMMA reference plates. The absorbance peak of these reference plates at 340 nm shall be between 1,1 and 1,5 absorbance units (AU).

Designate the first plate as Slide A and place it in the light path of the UV spectrophotometer. Measure against air in the blank light path. Run a duplicate (290 nm to 380 nm) and transfer data to the “Linearity Test” spreadsheet tab, cells B8:C98.

Designate the second plate as Slide B and place it in the light path of the UV spectrophotometer. Measure against air in the blank light path. Run in duplicate and transfer data to the “Linearity Test” spreadsheet tab, cells D8:E98. Place both slides (A and B) on top of each other with their roughened sides towards one another into the light path and measure the combined absorbance (290 nm to 380 nm). Measure against air in the blank light path. Run 4 replicates and transfers data to the “Linearity Test” spreadsheet tab, cells F8:I98. The results will automatically appear in the system calibration summary sheet in this International Standard (see Figure A.2).

### A.4 Dynamic absorbance range limit determination

The spreadsheet will also calculate the maximum absorbance range limit of the UV spectrophotometer, based on deviation from additivity of the two plates. When the deviation exceeds 0,1 AU, the dynamic range limit is determined and the results will automatically appear in the system calibration summary sheet in this International Standard (see Figure A.2). The minimum range limit is 2,2 AU.

### A.5 PMMA test plate qualification

#### A.5.1 General

The PMMA plates used as substrate for the sunscreen testing shall pass minimum transmission specifications.

#### A.5.2 Method

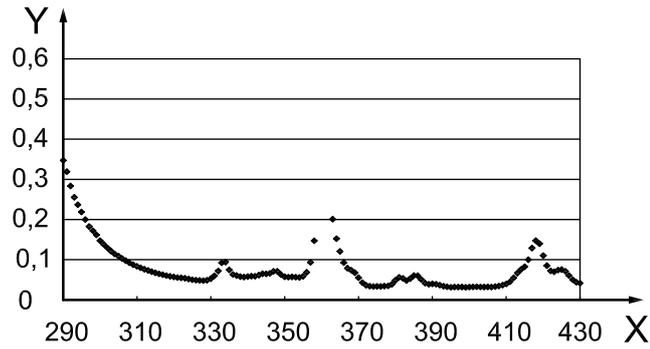
Set the baseline of the UV spectrophotometer with an air blank (no sample). Apply approximately 15 mg of glycerin or modified glycerin solution to the rough surface of the PMMA plate to make the surface clear using a finger tip. Remove any excess glycerin with the fingertip. Place the prepared plate in the measurement position and measure the absorbance (or % transmission) of the plate. Record and transfer the data to the spreadsheet tab marked "Plate Transmission" in the appropriate column B for absorbance values or column C for % transmission values. The plate shall record > 60 % transmission at 290 nm, > 69 % at 300 nm, and > 81 % at 320 nm. The results will automatically appear in the system calibration summary sheet in this International Standard as shown in Figure A.2.

### A.6 Reporting

The results of the calibration should be recorded in the format indicated on the spreadsheet "Summary".

Instrument:	0	Tested by:	0
Date:	March 31, 2011	Sig:	0
Plate Transmission Test	Plate Manuf:	0	Lot #: 0
nm	Limits		
290	>60%	65.5%	Pass
300	>69%	70.9%	Pass
320	>81%	82.1%	Pass
Spectrophotometer Wavelength Accuracy			
		Peak 1	
Reference Wavelength		361	
Measured Wavelength		361	
Peak Value		0.433	
Limit ±1		TRUE	

Figure A.2 — System calibration summary sheet (1 of 2)



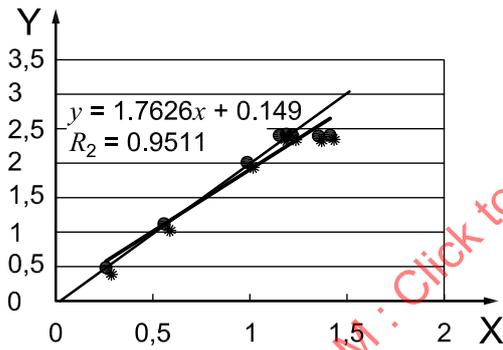
Holmium oxide observed

**Key**

X wavelength (nm)  
Y absorbance

Spectrophotometer Linearity Test

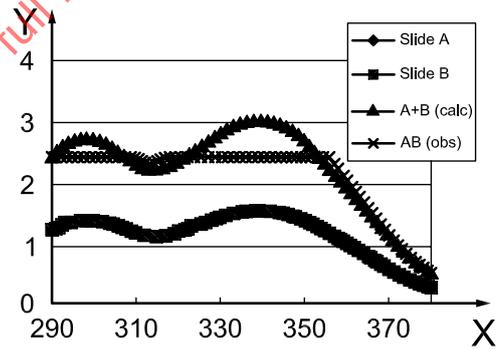
			Limit
Dynamic Range Limit	2.41	Pass	2.2
Linearity Limit	92.50	Pass	85



a) One versus two PMMA calibration plates correlation

**Key**

X one plate (mean) (absorbance)  
Y two plates (stacked) (absorbance)



b) Dynamic range test

**Key**

X wavelength (nm)  
Y absorbance

NOTE calc = calculated; obs = observed.

Figure A.2 — System calibration summary sheet (2 of 2)

## Annex B (normative)

### Radiometer calibration to spectroradiometric irradiance procedure

#### B.1 Purpose

The purpose is to calibrate the radiometer used to measure the UV exposure source for accurate dose exposures for the photostability challenge step in the *in vitro* UVA sunscreen testing protocol.

#### B.2 Summary of procedure

A spectroradiometric irradiance measurement is first conducted on the UV exposure source over the UV range of 290 nm to 400 nm. The UVA radiometer probe being cross calibrated is placed in the same exposure position as the spectroradiometer and a measurement of the irradiance at the plane of exposure is conducted. Taking the spectroradiometric irradiance data, the energy at each wavelength, in 1 nm intervals, is integrated from 320 nm to 400 nm to yield the spectroradiometric UVA intensity. The spectroradiometric UVA intensity is divided by the irradiance measurement from the UVA radiometer to yield a calibration factor,  $Y$ , that is used to multiply all subsequent UVA radiometer measurements of the UVA source used in this exercise.

#### B.3 Step-by-step procedure

**B.3.1** A spectroradiometer with current calibration traceable to a certified irradiance standard source over the range of 290 nm to 400 nm is required.

**B.3.2** Place the reference entrance optics of the spectroradiometer into position at the plane of exposure of the test PMMA plates.

**B.3.3** Turn on the UV exposure source and allow it to warm up for at least 20 minutes.

**B.3.4** Scan the spectral irradiance of the source with the spectroradiometer over the range of 290 nm to 400nm in 1 nm increments.

**B.3.5** Integrate the spectral irradiance from 320 nm to 400 nm in order to determine the total UVA spectroradiometric irradiance of the source at the exposure plane of the PMMA plate samples. This is designated  $I_{UVA_{spec}}$ .

**B.3.6** Move the spectroradiometer probe away from the source.

**B.3.7** Place the UVA radiometer that is to be calibrated in the same position as the spectroradiometer so that the calibration reference plane of the radiometer is in the same position as the spectroradiometer reference entrance optics being illuminated by the UV exposure source.

**B.3.8** Measure the irradiance of the source with the UVA radiometer. This is designated  $I_{UVA_{rad}}$ .

**B.3.9** Calculate the calibration factor:

$$Y = I_{UVA_{spec}}/I_{UVA_{rad}}$$

**B.3.10** Subsequent measurements of the UV exposure source by the UVA radiometer shall be multiplied by  $Y$  for calibrated UVA irradiance.

$$\text{Calibrated UVA irradiance} = IUVA_{\text{rad}} \times Y$$

**B.3.11** The calibration correction factor  $Y$  can be entered directly into the ISO *in vitro* UVA test spreadsheet. The raw radiometer value (without calibration correction) is also entered on this same tab to calculate the calibrated irradiance value for the UV exposure step.

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**Annex C**  
(normative)

**Computation values: PPD and erythema action spectra and UVA and UV-SSR spectral irradiances**

**Table C.1 — PPD and erythema action spectra and UVA and UV-SSR spectral irradiances**

Wavelength nm	PPD action spectrum	Erythema action spectrum	UV-SSR source W/m <sup>2</sup> /nm	UVA radiation source W/m <sup>2</sup> /nm
290	—	1,0000E+00	8,741E-06	—
291	—	1,0000E+00	1,450E-05	—
292	—	1,0000E+00	2,659E-05	—
293	—	1,0000E+00	4,574E-05	—
294	—	1,0000E+00	1,006E-04	—
295	—	1,0000E+00	2,589E-04	—
296	—	1,0000E+00	7,035E-04	-
297	—	1,0000E+00	1,678E-03	—
298	—	1,0000E+00	3,727E-03	—
299	—	8,0538E-01	7,938E-03	—
300	—	6,4863E-01	1,478E-02	—
301	—	5,2240E-01	2,514E-02	—
302	—	4,2073E-01	4,176E-02	—
303	—	3,3884E-01	6,223E-02	—
304	—	2,7290E-01	8,690E-02	—
305	—	2,1979E-01	1,216E-01	—
306	—	1,7701E-01	1,615E-01	—
307	—	1,4256E-01	1,989E-01	—
308	—	1,1482E-01	2,483E-01	—
309	—	9,2470E-02	2,894E-01	—
310	—	7,4473E-02	3,358E-01	—
311	—	5,9979E-02	3,872E-01	—
312	—	4,8306E-02	4,311E-01	—
313	—	3,8905E-02	4,884E-01	—
314	—	3,1333E-02	5,121E-01	—
315	—	2,5235E-02	5,567E-01	—
316	—	2,0324E-02	5,957E-01	—
317	—	1,6368E-02	6,256E-01	—
318	—	1,3183E-02	6,565E-01	—
319	—	1,0617E-02	6,879E-01	—
320	1,000E+00	8,5507E-03	7,236E-01	4,843E-06
321	9,750E-01	6,8865E-03	7,371E-01	8,466E-06
322	9,500E-01	5,5463E-03	7,677E-01	1,356E-05
323	9,250E-01	4,4668E-03	7,955E-01	2,074E-05
324	9,000E-01	3,5975E-03	7,987E-01	3,032E-05

Table C.1 (continued)

Wavelength nm	PPD action spectrum	Erythema action spectrum	UV-SSR source W/m <sup>2</sup> /nm	UVA radiation source W/m <sup>2</sup> /nm
325	8,750E-01	2,8973E-03	8,290E-01	4,294E-05
326	8,500E-01	2,3335E-03	8,435E-01	5,738E-05
327	8,250E-01	1,8793E-03	8,559E-01	7,601E-05
328	8,000E-01	1,5136E-03	8,791E-01	9,845E-05
329	7,750E-01	1,4125E-03	8,951E-01	1,215E-04
330	7,500E-01	1,3646E-03	9,010E-01	1,506E-04
331	7,250E-01	1,3183E-03	9,161E-01	1,811E-04
332	7,000E-01	1,2735E-03	9,434E-01	2,132E-04
333	6,750E-01	1,2303E-03	9,444E-01	2,444E-04
334	6,500E-01	1,1885E-03	9,432E-01	2,833E-04
335	6,250E-01	1,1482E-03	9,571E-01	3,186E-04
336	6,000E-01	1,1092E-03	9,663E-01	3,589E-04
337	5,750E-01	1,0715E-03	9,771E-01	3,980E-04
338	5,500E-01	1,0351E-03	9,770E-01	4,387E-04
339	5,250E-01	1,0000E-03	9,967E-01	4,778E-04
340	5,000E-01	9,6605E-04	9,939E-01	5,198E-04
341	4,938E-01	9,3325E-04	1,007E+00	5,608E-04
342	4,876E-01	9,0157E-04	1,012E+00	5,998E-04
343	4,814E-01	8,7096E-04	1,011E+00	6,384E-04
344	4,752E-01	8,4140E-04	1,021E+00	6,739E-04
345	4,690E-01	8,1283E-04	1,025E+00	7,123E-04
346	4,628E-01	7,8524E-04	1,033E+00	7,468E-04
347	4,566E-01	7,5858E-04	1,034E+00	7,784E-04
348	4,504E-01	7,3282E-04	1,040E+00	8,180E-04
349	4,442E-01	7,0795E-04	1,027E+00	8,427E-04
350	4,380E-01	6,8391E-04	1,045E+00	8,754E-04
351	4,318E-01	6,6069E-04	1,042E+00	9,044E-04
352	4,256E-01	6,3826E-04	1,040E+00	9,288E-04
353	4,194E-01	6,1660E-04	1,039E+00	9,486E-04
354	4,132E-01	5,9566E-04	1,043E+00	9,733E-04
355	4,070E-01	5,7544E-04	1,046E+00	9,863E-04
356	4,008E-01	5,5590E-04	1,035E+00	1,009E-03
357	3,946E-01	5,3703E-04	1,039E+00	1,028E-03
358	3,884E-01	5,1880E-04	1,027E+00	1,045E-03
359	3,822E-01	5,0119E-04	1,035E+00	1,062E-03
360	3,760E-01	4,8417E-04	1,037E+00	1,078E-03
361	3,698E-01	4,6774E-04	1,025E+00	1,086E-03
362	3,636E-01	4,5186E-04	1,023E+00	1,098E-03
363	3,574E-01	4,3652E-04	1,016E+00	1,095E-03
364	3,512E-01	4,2170E-04	9,984E-01	1,100E-03
365	3,450E-01	4,0738E-04	9,960E-01	1,100E-03
366	3,388E-01	3,9355E-04	9,674E-01	1,093E-03
367	3,326E-01	3,8019E-04	9,648E-01	1,087E-03

Table C.1 (continued)

Wavelength nm	PPD action spectrum	Erythema action spectrum	UV-SSR source W/m <sup>2</sup> /nm	UVA radiation source W/m <sup>2</sup> /nm
368	3,264E-01	3,6728E-04	9,389E-01	1,082E-03
369	3,202E-01	3,5481E-04	9,191E-01	1,071E-03
370	3,140E-01	3,4277E-04	8,977E-01	1,048E-03
371	3,078E-01	3,3113E-04	8,725E-01	1,026E-03
372	3,016E-01	3,1989E-04	8,473E-01	9,953E-04
373	2,954E-01	3,0903E-04	8,123E-01	9,703E-04
374	2,892E-01	2,9854E-04	7,840E-01	9,367E-04
375	2,830E-01	2,8840E-04	7,416E-01	9,057E-04
376	2,768E-01	2,7861E-04	7,148E-01	8,757E-04
377	2,706E-01	2,6915E-04	6,687E-01	8,428E-04
378	2,644E-01	2,6002E-04	6,280E-01	8,058E-04
379	2,582E-01	2,5119E-04	5,863E-01	7,613E-04
380	2,520E-01	2,4266E-04	5,341E-01	7,105E-04
381	2,458E-01	2,3442E-04	4,925E-01	6,655E-04
382	2,396E-01	2,2646E-04	4,482E-01	6,115E-04
383	2,334E-01	2,1878E-04	3,932E-01	5,561E-04
384	2,272E-01	2,1135E-04	3,428E-01	4,990E-04
385	2,210E-01	2,0417E-04	2,985E-01	4,434E-04
386	2,148E-01	1,9724E-04	2,567E-01	3,876E-04
387	2,086E-01	1,9055E-04	2,148E-01	3,363E-04
388	2,024E-01	1,8408E-04	1,800E-01	2,868E-04
389	1,962E-01	1,7783E-04	1,486E-01	2,408E-04
390	1,900E-01	1,7179E-04	1,193E-01	2,012E-04
391	1,838E-01	1,6596E-04	9,403E-02	1,640E-04
392	1,776E-01	1,6032E-04	7,273E-02	1,311E-04
393	1,714E-01	1,5488E-04	5,532E-02	1,028E-04
394	1,652E-01	1,4962E-04	4,010E-02	7,897E-05
395	1,590E-01	1,4454E-04	2,885E-02	5,975E-05
396	1,528E-01	1,3964E-04	2,068E-02	4,455E-05
397	1,466E-01	1,3490E-04	1,400E-02	3,259E-05
398	1,404E-01	1,3032E-04	9,510E-03	2,302E-05
399	1,342E-01	1,2589E-04	6,194E-03	1,581E-05
400	1,280E-01	1,2162E-04	4,172E-03	1,045E-05

The reference sun has a total UV irradiance of 51,4 to 63,7 W/m<sup>2</sup>[6][7] and a UVA to UVB irradiance ratio of 16,9 to 17,5.

## Annex D (normative)

### PMMA test plate surface specifications

#### D.1 Test plate type

A PMMA plate with a moulded surface roughness having the following surface parameters within the upper and lower limit values was qualified for use for this *in vitro* UVA test method by way of ring testing.

#### D.2 Surface profile of substrate

Measure the surface profile characteristics of the substrate<sup>[9]</sup>, covering at least a surface area of 10 mm × 5 mm in 15 µm intervals. Non-contact surface topographic analysis may be conducted using a laboratory work station consisting of an optical sensor, a motion controller, an x-y translation stage and microtopography software. A sensor based on a white light chromatic aberration principle is recommended, which allows for a high resolution: 10 nm vertically and 1 µm horizontally.

Surface profile parameters are to be calculated by the mean of the different analysed profiles ( $R_a$ ,  $R_v$ ,  $R_{dq}$ ,  $A_1$ ,  $SS_c$ ,  $V_w$ ).

#### D.3 Plate profile parameters

PMMA plates can be prepared using a moulding process so that they have surface characteristics that meet the following measurement parameters, as measured using instrumentation<sup>[9]</sup>:

Table D.1 — Target and upper and lower limits

Parameter	$R_a$	$R_v$	$R_{dq}$	$A_1$	$SS_c$	$V_w$
Target value	4,853	13,042	11,122	239,750	0,033	1,044E-06
Upper limit	5,170	13,669	12,411	284,256	0,046	1,663E-06
Lower limit	4,535	12,414	9,833	195,244	0,020	4,248E-07

#### D.4 Plate optical characteristics

##### D.4.1 Transmittance specifications (see A.5)

Representative samples of each lot of PMMA plates are to be tested for transmission properties to ensure compliance. The profiled surface of the test plate is to be treated with pure glycerin or a modified glycerin solution as shown in Table D.2.

Table D.2 — Modified glycerin solution

Ingredient	%
Glycerin BP/USP/JP	90,0
Sodium lauryl sulfate (SLS) solution (1 % SLS solution in water)	10,0

#### D.4.2 Method

**D.4.2.1** Prepare a standard PMMA blank plate by applying approximately 15 mg of pure glycerin or modified glycerin solution as a thin continuous film to the rough side of the plate. The slide should be transparent after treatment. Wipe away any excess glycerin material with a bare fingertip.

**D.4.2.2** Place the plate in the light path of the UV spectrophotometer. Measure transmittance (290 nm to 400 nm) against air (with no plate) as the reference light path.

#### D.4.3 Minimum transmission values

Limits for the treated plate transmission values are:

290 nm: > 60 % T

300 nm: > 69 % T

320 nm: > 81 % T

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## Annex E (normative)

### UVA reference sunscreen S2

#### E.1 Mean UVAPF and acceptance limits for reference sunscreen formulation

Table E.1 provides the mean and acceptance range for the reference sunscreen S2 used for the purposes of validating the test procedures of this test method.

**Table E.1 — Mean UVAPF and acceptance limits for reference sunscreen formulation**

Reference sunscreen	Mean	Mean	Acceptance limits	
			Lower limit	Upper limit
Formulation	SPF	UVAPF		
S2	16,0	12,7	10,7	14,7

#### E.2 Formula and preparation for standard S2

The formulation and preparation procedure below describes the quantitative measures and mixing procedures necessary to prepare the S2 reference sunscreen used to validate the testing procedure.

**Table E.2 — S2 reference sunscreen**

Ingredients	% mass of total mass
<b>Phase 1 (aqueous)</b>	
Water	62,43
Propylene glycol	1,00
Xanthan gum	0,60
Carbomer, e.g. Carbopol Ultrez 10 <sup>a</sup>	0,15
Disodium EDTA	0,08
<b>Phase 2 (oil)</b>	
Octocrylene	3,00
Butylmethoxy dibenzoylmethane	5,00
Ethylhexyl methoxycinnamate	3,00
Bis-ethylhexyloxyphenol	2,00
Methoxyphenyl triazine	
Cetyl alcohol	1,00
Steareth-21	2,50
Steareth-2	3,00
Dicaprylyl carbonate	6,50
Decyl cocoate	6,50
Phenoxyethanol (and)	1,00
Methylparaben (and)	
Ethylparaben (and)	
Butylparaben (and)	
Propylparaben	