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Cosmetics — Determination of sunscreen UVA photoprotection in vitro

Cosmétiques — Détermination in vitro de la photoprotection UVA

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 217 *Cosmetics,* in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 392, *Cosmetics,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO 24443:2012), which has been technically revised.

The main changes compared to the previous edition are as follows:

— acceptance of moulded and sandblasted PMMA plates, according to specifications described in Annex D;

— product application fitted to 1,2mg/cm^2 for sandblasted plates;

— description of application gesture according to tested products;

— introduction of a new high UVA PF standard P8;

— introduction of critical wavelength calculation;

— calculation of coefficient "*C*"accepted from in vivo screening SPF, with specific conditions based on SEM and percentage of variability, and new range proposed from 0,6 to 1,6;

— limitation of UVA irradiation dose to 36 J/cm^2.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document specifies the procedure to determine the ultraviolet protection factor (UVA-PF) of a sunscreen product using the in vitro UVA-PF according to the principles recommended by the European Cosmetic and Perfumery Association (COLIPA) in 2011. The outcome of this test method can be used to determine the UVA classification of topical sunscreen products according to local regulatory requirements.

Topical sunscreen products are primarily rated and labelled according to their ability to protect against sunburn, using a test method to determine the in vivo sun protection factor (see ISO 24444). This rating evaluates filtration of sunburn generating radiation across the electromagnetic UV spectrum (290 nm to 400 nm). However, knowledge of the sun protection factor (SPF) rating does not provide explicit information on the magnitude of the protection provided specifically in the UVA range of the spectrum (320 nm to 400 nm), as it is possible to have high SPF products with very modest UVA protection (e.g. SPF 50 with a UVA-PF of only 3 to 4). There is a demand among medical professionals, as well as knowledgeable consumers, to have fuller information on the UVA protection provided by their sunscreen product, in addition to the SPF, in order to make a more informed choice of product, providing a more balanced and broader-spectrum protection. Moreover, there is also a demand to prevent UVA-induced darkening of the skin from a cultural point of view even without sunburn. The UVA-PF value of a product provides information on the magnitude of the protection provided explicitly in the UVA portion of the spectrum, independent of the SPF values.

The test method outlined in this document is derived primarily from the in vitro UVA-PF test method as developed by COLIPA.

Cosmetics — Determination of sunscreen UVA photoprotection in vitro

1 Scope

This document 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 an UV spectral absorbance curve from which a number of calculations and evaluations can be undertaken. These include calculation of the Ultraviolet-A protection factor (UVA-PF) [correlating with in vivo UVA-PF 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 static in vivo SPF results for scaling the UV absorbance curve.

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

2 Normative references

There are no normative references in this document.

3 Terms, definitions, symbols and abbreviated terms

3.1 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1.1
UV
ultraviolet radiation
electromagnetic radiation in the range of 290 nm to 400 nm

3.1.2
UVB
ultraviolet B
electromagnetic radiation in the range of 290 nm to 320 nm

3.1.3
UVA
ultraviolet A
electromagnetic radiation in the range of 320 nm to 400 nm

Note 1 to entry: UVA II = 320 nm to 340 nm; UVA I = 340 nm to 400 nm.

3.1.4
spectral absorbance
ai(λ)
logarithm to base 10 of the reciprocal of the spectral internal transmittance, $ai(\lambda)=-\log 10\ \tau i(\lambda)$

Note 1 to entry: In the context of this standard the absorption or transmission of sunscreen is used.

3.1.5
irradiance
I
fluence rate per unit area, expressed in W/m^2, for a defined range of wavelengths

Note 1 to entry: From 290 nm to 400 nm for UVA + UV-B irradiance; from 320 nm to 400 nm for UVA irradiance.

3.1.6
spectral irradiance
I(λ)
irradiance (3.1.5) per unit wavelength, $I(\lambda)$, expressed in $W/m^2/nm$

Note 1 to entry: Spectral irradiance can refer to PPD testing or SPF testing.

3.1.7
spectrophotometer
equipment for measuring the reflection or transmission properties of a material as a function of wavelength limited to ultraviolet, visible and short infrared ranges in this document

3.1.8
spectroradiometer
device designed to measure the spectral density of illuminants

3.1.9
radiometer
device for measuring the radiant flux (power) of electromagnetic radiation

3.1.10
product reference sunscreen
reference sunscreen product used to validate the testing procedure

3.1.11
solar simulator
equipment used to simulate the solar irradiance and spectrum

3.1.12
plate
substrate
material to which the test product is to be applied

3.2 Symbols and abbreviated terms

3.2.1
UVA-PF
in vitro ultraviolet A protection factor
in vitro UVA protection factor of a sun protection product against UVA radiation, which can be derived mathematically with in vitro spectral modelling

3.2.2
$SPF_{in\ vitro}$
in vitro sun protection factor
in vitro protection factor of a sun protection product against erythema-inducing radiation calculated with spectral modelling

3.2.3
critical wavelength
CWL
λc
wavelength at which the area under the absorbance curve represents 90 % of the total area under the curve in the UV region

Note 1 to entry: Calculated from spectral data.

3.2.4
erythema action spectrum
$E(\lambda)$
relative effects of individual spectral bands of an exposure source for an erythema response

3.2.5
PPD action spectrum
$P(\lambda)$
relative effects of individual spectral bands of an exposure source for a persistent pigment response

4 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 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. As in vivo method can raise ethical consideration, any alternative SPF method, published as an ISO method, may be used.

Samples are 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.

5 Apparatus

5.1 Spectrophotometer specifications

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

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

The 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 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 spectrophotometer, the product should be re-tested using an instrument with increased sensitivity and dynamic range.

The lamp in the 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 lamp is a convenient solution).

Therefore, the UV dose during one measurement cycle should not exceed 0,2 J/cm^2.

NOTE A 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 spectrophotometer during the absorbance measurement of the sunscreen on the test plate.

Coupled with an UV source, the spectroradiometer can give similar results to a spectrophotometer.

5.2 Calibration of the spectrophotometer

The spectrophotometer shall be validated every month by measurements of reference materials.

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

— dynamic range of the spectrophotometer;

— linearity test of the spectrophotometer;

— wavelength accuracy test.

5.3 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[1]. As defined by COLIPA[2], the reference standard sun has a total irradiance of 51,4 W/m^2 to 63,7 W/m^2 and a UVA to UVB irradiance ratio of 16,9 to 17,5.

Therefore, 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]	11-22
[a] 320 nm to 400 nm.	
[b] 290 nm to 320 nm.	

In broad-beam UV-sources, spectra from different locations under the beam shall be recorded over at least 5 different locations (a location is defined for each plate) in order to account for uniformity.

The uniformity shall be ≥ 90 % as calculated by Formula (1):

$$U = (1-(\max-\min)/(\bar{X})) \quad (1)$$

where

U is the uniformity in percentage;

$\bar{X}$ is the average.

If the uniformity is less than 90 %, then optical components should be adjusted or appropriate compensation for different irradiance shall be made in the exposure time on each plate.

The UV exposure source device should have the ability to maintain samples within the range of 27 °C (±2 °C) to 32 °C (±2 °C). It is important that the temperature of the sample itself on the plate shall be measured and not just the surrounding air temperature. Therefore, the measurement of the temperature shall be on plate level.

To maintain samples at required temperature, a filter system that particularly reduces infrared radiation shall be used to achieve the specified temperature range. Cooling trays for the sample plates or ventilators shall be used to maintain a temperature lower than 32 °C (±2 °C) and warming devices to maintain samples at or above 27 °C (±2 °C).

Measurement should be made using a sensor that is traceable to a national or an international calibration standard, within the range of use.

5.4 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 12 months, or 2 500 h 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.

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

If a UVA radiometer is used, this device shall be suitably calibrated. This requires that it is calibrated with the UVA irradiance measurement results of 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) in accordance with Annex B 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.

5.6 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 shall be used and are commercially available. The size of the substrate should be chosen such that the application area is not less than 16 cm^2.

The specifications and preparation of this type of plate[9][18] are described in Annex D.

6 Test method

6.1 Outline of the test procedure

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

6.1.2 Conduct blank measurements of a glycerin-treated or Vaseline®[1)]-treated plate for the reference "blank", which will be used in the subsequent absorbance measurements.

6.1.3 Conduct in vitro absorbance measurements of the sunscreen product spread on a PMMA plate, prior to any UV irradiation. Acquire the initial mAF spectrum with $A_0(\lambda)$ data, where mAF (=10^A)

6.1.4 Conduct the mathematical adjustment of the initial UV absorbance spectrum using coefficient "*C*" [see Formula (2) the calculation in 6.7.2] to achieve an in vitro SPF (no UV dose) equal to the measured static in vivo SPF. Initial UVA-PF_0 is calculated using $A_0(\lambda)$ and C. A single UV exposure dose, *D*, is calculated, equal to 1,2 × UVA-PF_0 in J/cm^2, for each plate.

6.1.5 Conduct UV exposure of the same sample as in 6.1.3, according to the calculated UV exposure dose *D*.

6.1.6 Measure the in vitro absorbance of the sunscreen product after UV exposure. Acquire the second UV spectrum with A(λ) data.

6.1.7 Conduct the mathematical adjustment of the second mAF spectrum (following UV exposure) by multiplying with the same coefficient "*C*", previously determined in 6.1.4. The resulting absorbance curve is the final adjusted absorbance values.

6.2 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 spectrophotometer/spectroradiometer to be used for the test procedure. Validation of the UV properties of the test PMMA plates batch shall also be conducted as described in Annex D.

6.3 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/Vaseline® on the roughened side of the plate. Choose the amount of glycerine/Vaseline® such that the entire surface is just completely covered (approximately 15 µl for a 50 mm × 50 mm plate).

Any excess of glycerine/Vaseline® should be avoided. Measure the absorbance through this "blank" plate and use this as the baseline measurement for subsequent absorbance measurements.

Measurements shall be performed on same type of plate as the one used for the product (molded or sandblasted) and same batch.

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

1) Vaseline® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

6.4 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 (±1,6 %) for molded plates and 1,2 mg/cm^2 (±1,5 %) for sandblasted plates.

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.

Plates should be weighed after application phase for any non-volatile product.

The sunscreen is applied as at least twelve 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 shall 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.

Deposit and weighing shall not take more than 30 s.

The first test plate applied should not be used for all the measurements, but to adjust the quantity.

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 human fingertip or mechanical fingertip.

Spreading should be completed in a two-phase process:

First, the product should be spread on the whole area of the plate, using circular movements with a minimum of four passages from the top to the bottom of the plate. At the end of the first pass, a turn of the plate has to be done (¼ turn) to alternate passages, with minimal pressure and repeat this movement three times at least (about 30 s).

Then the sample should be rubbed on the plate surface using alternating horizontal and vertical strokes repeated at least three times alternate passages with a moderate but increased pressure. The second phase should last about 30 s with increased moderate pressure.

For alcoholic or oil products, application should be adapted as follows:

First, the product should be spread on the whole area of the plate, using circular movements with a minimum of three passages from the top to the bottom of the plate. At the end of the first pass, a turn of the plate has to be done (¼ turn) to alternate passages, with minimal pressure and repeat this movement two times at least (about 20 s to 25 s).

Then the sample should be rubbed on the plate surface using alternating horizontal and vertical strokes repeated at least two times alternate passages with a moderate but increased pressure. The second phase should last about 20s with increased moderate pressure.

For all kinds of products, the treated sample shall be allowed to dry for 30 min to 60 min in the dark at the same temperature under UV exposure conditions (i.e. if UV source exposure conditions will be 30 °C, then the drying conditions should also be at 30 °C; or if the UV source exposure conditions will be 27 °C, then the drying conditions should also be 27 °C).

Spray products provided in a pressurized container shall first be degassed by puncturing a very small pinhole in the container to relieve all of the pressure, and then allowed to rest for at least 24 h at room temperature before accessing the liquid for testing.

6.5 Absorbance measurements of the product-treated plate

The product-treated plate is placed in the light-path of the 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.

6.6 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 UVA-PF value, until the 95 % CI is less than 17 % of the mean UVA-PF value. Calculation procedures for this are described in Annex F.

6.7 Determination of initial calculated SPF ($SPF_{in\ vitro,0}$), "*C*" value, initial UVA-PF ($UVA\text{-}PF_0$), and UV exposure dose

6.7.1 Determination of initial in vitro SPF ($SPF_{in\ vitro,0}$)

The UV solar simulator radiation 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 $SPF_{in\ vitro,0}$ is shown in Formula (2):

$$SPF_{in\ vitro,0} = \frac{\int_{\lambda=290}^{\lambda=400} E(\lambda)\times I(\lambda)\times d\lambda}{\int_{\lambda=290}^{\lambda=400} E(\lambda)\times I(\lambda)\times 10^{-A_0(\lambda)C}\times d\lambda} \quad (2)$$

where

$E(\lambda)$ 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 increment (in step of1 nm).

NOTE This calculated SPF value cannot be used, as an expected SPF, neither as an in vitro SPF result.

6.7.2 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. In vivo SPF can be used from the mean SPF obtained from screening test (at least 5 valid subjects) or full test (at least 10 valid subjects), measured by a published ISO method.

Extrapolation from screening is possible if final (full test) has a SEM not greater than 3,8 and the variability of the in vivo SPF shall not exceed 17 %.

As in vivo method can raise ethical consideration, any alternative SPF method, published as an ISO method, may be used.

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 UVA-PF_0 value, and the exposure dose. Formula (3) shows the calculation of the adjusted in vitro SPF ($SPF_{in\ vitro,adj}$) and determination of the coefficient of adjustment "*C*":

$$SPF_{in\ vitro,adj} = \frac{\int_{\lambda=290}^{\lambda=400} E(\lambda)\times I(\lambda)\times d\lambda}{\int_{\lambda=290}^{\lambda=400} E(\lambda)\times I(\lambda)\times 10^{-A_0(\lambda)C}\times d\lambda} \tag{3}$$

where $E(\lambda)$, $I(\lambda)$, $A_0(\lambda)$ and $d(\lambda)$ are as defined in Formula (2).

This calculation is based on Lambert-Beer's law 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].

As for tested product and reference sunscreen products S2 or P8, the "*C*" value shall lie between 0,6 and 1,6 for valid interpretation, with absorbance curves presenting no distortion (see Annex G). If it is outside this range, the plate is rejected, and new plate shall be prepared by:

— adaptation of the application procedure, in remaining in accordance with all application specifications;

— increasing the temperature, in remaining in accordance with all temperature specifications;

— changing the type of plate, in remaining in accordance with all substrate/plate specifications.

6.7.3 Determination of initial UVA protection factor before UV exposure (UVA-PF_0)

The initial UVA-PF_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 $SPF_{in\ vitro,0}$.

The intensity spectrum for a UVA radiation source, $I(\lambda)$, 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 Formula (4). The ratio of these two integrals is the initial in vitro UVA-PF_0 value:

$$UVA\text{-}PF_0 = \frac{\int_{\lambda=320}^{\lambda=400} P(\lambda)\times I(\lambda)\times d\lambda}{\int_{\lambda=320}^{\lambda=400} P(\lambda)\times I(\lambda)\times 10^{-A_0(\lambda)}\times d\lambda} \tag{4}$$

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 Formula (3);

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

6.7.4 Determination of the UV exposure dose

The UV exposure dose, *D*, is the UVA-PF_0 value multiplied by a factor of 1,2, in J/cm^2 shown as Formula (5):

$$D = \text{UVA} - \text{PF}_0 \times 1,2 \tag{5}$$

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

Pre-Irradiation dose should be limited at a maximum of 36 J/cm2 (UVA-PF_0 maximum 30).

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

6.8 UV exposure of sample plates

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 shall be maintained at a temperature between 27 °C (±2 °C) and 32 °C (±2 °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).

After the UV exposure, re-measure the absorbance of the test samples on the same spots as measured before the UV exposure, as in 6.5. The final absorbance values are equal to the observed absorbance values after the UV exposure, multiplied by the "*C*" value determined using Formula (3).

See Formula (6)

$$A_f(\lambda) = A_e(\lambda) \times C \tag{6}$$

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.

6.9 Calculation of UVA-PF of plates after UV exposure of the sample

The UVA-PF shall be calculated according to Formula (7) for each individual plate, using the single observation value or the mean of multiple observations on that plate.

$$UVA\text{-}PF = \frac{\int_{\lambda=320}^{\lambda=400} P(\lambda) \times I(\lambda) \times d\lambda}{\int_{\lambda=320}^{\lambda=400} P(\lambda) \times I(\lambda) \times 10^{-A_e(\lambda) \times C} \times d\lambda} \tag{7}$$

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 Formula (3);

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

6.10 Calculation of critical wavelength of plates after UV exposure of the sample

The critical wavelength "λc" is the wavelength at which the area under the absorbance curve represents 90 % of the total area under the curve in the UV region. This is expressed mathematically as Formula (8):

$$\int_{\lambda=290}^{\lambda c} A_e(\lambda)\times d\lambda = 0,9\times\int_{\lambda=290}^{\lambda=400} A_e(\lambda)\times d\lambda \quad (8)$$

where

$Ae(\lambda)$ is the mean absorbance at each wavelength after exposure;

$d(\lambda)$ is the wavelength interval between measurements.

The critical wavelength is expressed as a whole number (truncation) and with nanometre (nm) unit.

Other protection parameters may be calculated from the final absorbance curve in 6.9 as desired.

7 Procedure using the spreadsheet in this document

The calculations given from Formula 2 to Formula 8 can be performed automatically using the calculation spreadsheet "ISO 24443 – UVA Calculation.xls" given in https://standards.iso.org/iso/24443/ed-2/en and the following steps.

7.1 The sheet "How to use" summarizes the use of this spreadsheet.

7.2 Enter the date, operator identification, product and batch identification, measured SPF of the test product, the spectrophotometer type, plate type and batch number, 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 yellow cells on "SPF AND PPD" tab.

7.3 Measure and input the mAF spectrum data (from 290 nm to 400 nm) for each unirradiated sample plate into their respective location in spreadsheet tab named "RAW mAF Data".

7.4 Return to "SPF AND PPD" tab and press the "ADJUSTMENT: "C" CALCULATION" button to calculate the "C" coefficient, and the UV exposure irradiance and exposure time are reported for each individual plate.

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

7.6 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.

7.7 Input the post-UV exposure mAF measurements for each plate into their respective location in spreadsheet tab named "RAW mAF Data".

7.8 When the full data input for the first four valid plates is complete, the "SPF AND PPD" tab spreadsheet will give the summary results for the test sample. If the 95 % CI of the UVA-PF values is less than 17 % of the mean UVA-PF, no further plates are required and the final results are displayed in graphic and tabular form. Otherwise, additional samples should be added sequentially as above until the test criterion is satisfied.

8 Product reference sunscreen

8.1 Formula S2

The method is controlled by using a reference sunscreen formulation to verify the test procedure. Product reference S2 sunscreen formula as described in Annex E shall be used. The UVA-PF test results of the product 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 UVA-PF testing results

Parameter	Lower limit	Upper limit
UVA-PF	10,7	14,7
CWL (nm)	378	382

The frequency of testing of the product reference S2 shall be once a month, as for spectrophotometer calibration.

8.2 Standard P8

The method is controlled by using a reference sunscreen formulation to verify the test procedure. Product reference P8 sunscreen formula as described in Annex E shall be used when expected UVA-PF are of 20 or more. The UVA-PF test results of the product reference P8 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 63,1 is to be used as the in vivo SPF value for P8 for computation purposes.

Table 3 — Limits of UVA-PF testing results

Parameter	Lower limit	Upper limit
UVA-PF	19,1	23,1
CWL (nm)	378	381

The frequency of testing of the product reference P8 shall be once a month, as for spectrophotometer calibration.

9 Test report

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

a) description of the instruments used, the manufacturer and instrument model with the system calibration summary in this document 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 (see 5.5 and B.3.10);

c) plate manufacturer and batch code, and specification of the profile as defined in Annex D;

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 shall be provided);

e) statement of the measured in vivo sun protection factor (SPF) used for calculations, with specification of the method and as a screening or full test;

f) constant "*C*";

g) UVA irradiance (W/m^2) and mean UVA exposure dose used to irradiate the test sample;

h) reference data for product reference S2 / P8 material with date of testing;

i) sample identification and date of the test;

j) identification of the individual conducting the test;

k) mean UVA-PF_0 and mean UVA-PF, expressed to one decimal (truncation), with standard deviation, 95 % CI and 17 % of mean;

l) critical wavelength expressed as a whole number (truncation) and in nanometre (nm) unit;

m) individual data for each plate;

n) other informative calculations derived from the absorbance values [see Clause 9 d)] may be reported.

Annex A
(normative)

Calibration of 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 given in https://standards.iso.org/iso/24443/ed-2/en as part of this document.

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 The tab "How to use" summarizes the use of the spreadsheet "ISO 24443 - UV Spectcalib.xls" given in https://standards.iso.org/iso/24443/ed-2/en.

A.2.2.2 Enter the date, operator identification, UV analyser model, supplier and serial #, Holmium model, supplier and batch # into the test spreadsheet on the yellow cells on "Spectro Control" tab.

A.2.2.3 Place the holmium oxide filter in the sample path and scan the absorbance in the range between 290 nm and 400 nm. Measure against air in the blank light path. Repeat the scan for three replicates. Accumulate the data and transfer absorbance values to the "Manual Data" tab in cells C8 to E118. The results will automatically appear in the system calibration summary sheet given in https://standards.iso.org/iso/24443/ed-2/en (see Figure A.2) and be similar to Figure A.1 below.

A.2.2.4 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 Figure A.1 below. The reported peak wavelength shall be either 360 nm, 361 nm or 362 nm, or else the instrument shall be recalibrated to achieve one of these wavelength values.

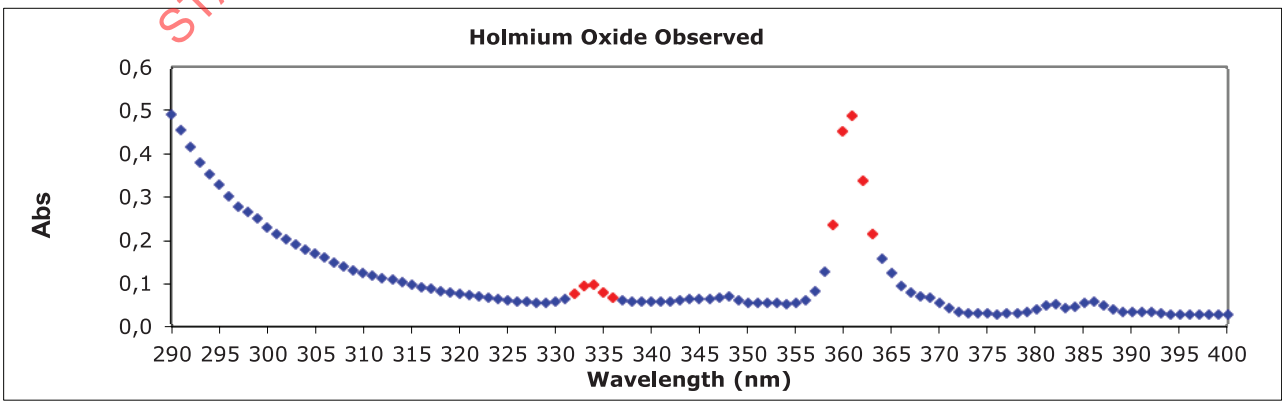


Figure A.1 — Holmium oxide actual

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 interlaboratory purposes.

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 the second plate as Slide B.

A.3.2 Linearity assessment

A.3.2.1 The tab "How to use" summarizes the use of the spreadsheet "ISO 24443 - UV Spectcalib.xls" given in https://standards.iso.org/iso/24443/ed-2/en.

A.3.2.2 Enter the date, operator identification, UV analyser model, supplier and serial #, Kit model, supplier and batch # into the test spreadsheet on the yellow cells on "Spectro Control" tab. Place the slide A in the light path of the spectrophotometer. Measure against air in the blank light path. Run two duplicates and transfer data to the "Manual Data" tab, cells H8:I118.

A.3.2.3 Place the slide B in the light path of the spectrophotometer. Measure against air in the blank light path. Run two duplicates and transfer data to the "Manual Data" tab, cells **J8:K118.**

A.3.2.4 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 400 nm). Run two duplicates and transfer data to the "Manual Data" tab, cells L8:M118.

A.3.2.5 The results will automatically appear in the "Spectro Control" tab of ISO 24443 - UV Spectcalib. xls given in https://standards.iso.org/iso/24443/ed-2/en (see Figure A.2).

A.4 Dynamic absorbance range limit determination

Spectcalib.xls, given in https://standards.iso.org/iso/24443/ed-2/en, also calculates the maximum absorbance range limit of the 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 document (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.

The moulded plate shall record > 60 % transmission at 290 nm, > 69 % at 300 nm, and > 81 % at 320 nm.

The sandblasted plate shall record > 72 % transmission at 290 nm, > 75 % at 300 nm, and > 81 % at 320 nm.

A.5.2 Method

A.5.2.1 The tab "How to use" summarizes the use of the spreadsheet "ISO 24443 - UV Spectcalib.xls" given in https://standards.iso.org/iso/24443/ed-2/en.

A.5.2.2 Enter the date, operator identification, UV analyser model, supplier and serial #, Substrate type, Substrate model, supplier and batch # into the test spreadsheet on the yellow cells on "Plate Transmission" tab.

A.5.2.3 Set the baseline of the spectrophotometer with an air blank (no sample). Apply approximately 15 mg of glycerin or modified glycerine/Vaseline® solution to the rough surface of the PMMA plate to make the surface clear using a fingertip. Remove any excess glycerine/Vaseline® with the fingertip.

A.5.2.4 Place the prepared plate in the measurement position and measure three replicates of the % transmission of the plate. Record and transfer the data to the spreadsheet tab marked "Manual Data" in the appropriate column R8:T118.

A.5.2.5 The results will automatically appear in the "Plate Transmission" tab in this document according to substrate type.

A.6 Reporting

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

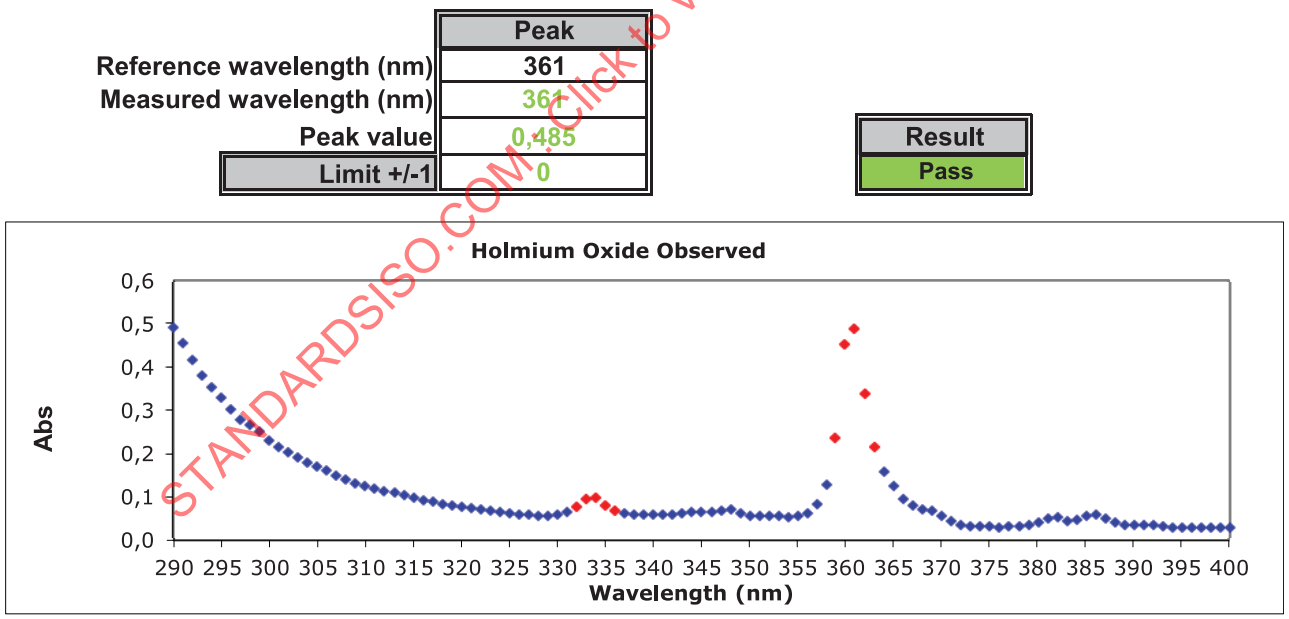
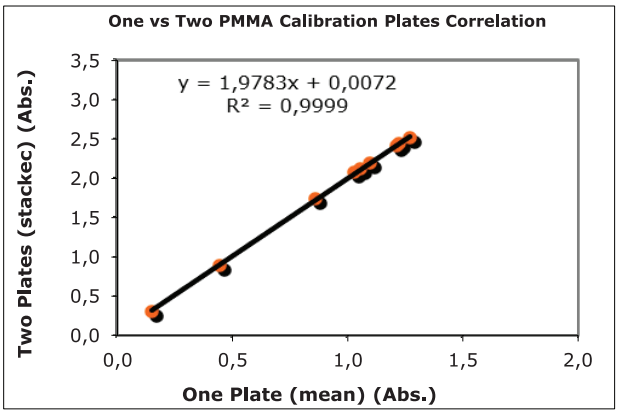
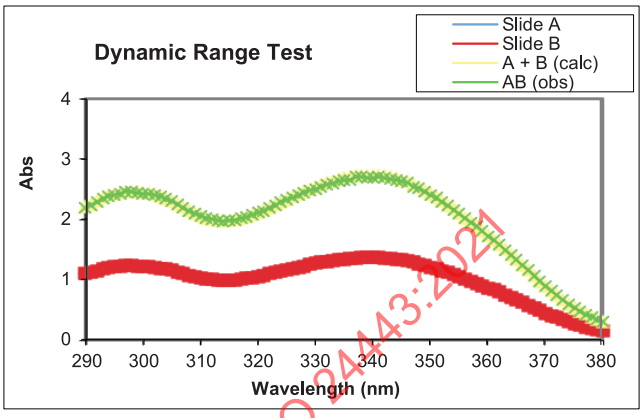


Figure A.2 — System calibration summary sheet (1 of 2)

	Limit	Measurement	Result
Additivity (DO)	2,2	2,71	Pass
Linearity (%)	85	99,3	Pass
Abs of a plate (340 nm)	1,1 - 1,5	1,37	Pass



a) One versus two PMMA calibration plates correlation



b) Dynamic range test

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

Annex B
(normative)

Radiometer calibration to spectroradiometric irradiance procedure

B.1 Purpose

The purpose of this procedure 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 national or an international calibration standard 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 min.

B.3.4 Scan the spectral irradiance of the source with the spectroradiometer over the range of 290 nm to 400 nm 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 $IUVA_{spec}$.

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 $IUVA_{rad}$.

B.3.9 Calculate the calibration factor using Formula (B.1):

$$Y = IUV\ A_{spec} / IUV\ A_{rad} \quad \text{(B.1)}$$

B.3.10 Subsequent measurements of the UV exposure source by the UVA radiometer shall be multiplied by Y for calibrated UVA irradiance ($UVA_{cal,irr}$):

$$UVA_{cal,irr} = IUV\ A_{rad} \times Y \quad \text{(B.2)}$$

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.

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/m²/nm	UVA radiation source W/m²/nm
290	—	1,00E+00	8,741E-06	—
291	—	1,00E+00	1,450E-05	—
292	—	1,00E+00	2,659E-05	—
293	—	1,00E+00	4,574E-05	—
294	—	1,00E+00	1,006E-04	—
295	—	1,00E+00	2,589E-04	—
296	—	1,00E+00	7,035E-04	-
297	—	1,00E+00	1,678E-03	—
298	—	1,00E+00	3,727E-03	—
299	—	8,05E−01	7,938E-03	—
300	—	6,49E−01	1,478E-02	—
301	—	5,22E−01	2,514E-02	—
302	—	4,21E−01	4,176E-02	—
303	—	3,39E−01	6,223E-02	—
304	—	2,73E−01	8,690E-02	—
305	—	2,20E−01	1,216E-01	—
306	—	1,77E−01	1,615E-01	—
307	—	1,43E−01	1,989E-01	—
308	—	1,15E−01	2,483E-01	—
309	—	9,25E−02	2,894E-01	—
310	—	7,45E−02	3,358E-01	—
311	—	6,00E−02	3,872E-01	—
312	—	4,83E−02	4,311E-01	—
313	—	3,89E−02	4,884E-01	—
314	—	3,13E−02	5,121E-01	—
315	—	2,52E−02	5,567E-01	—
316	—	2,03E−02	5,957E-01	—
317	—	1,64E−02	6,256E-01	—
318	—	1,32E−02	6,565E-01	—
319	—	1,06E−02	6,879E-01	—
320	1,000E+00	8,55E−03	7,236E-01	4,843E-06
321	9,750E-01	6,89E−03	7,371E-01	8,466E-06
322	9,500E-01	5,55E−03	7,677E-01	1,356E-05
323	9,250E-01	4,47E−03	7,955E-01	2,074E-05

Table C.1 *(continued)*

Wavelength nm	PPD action spectrum	Erythema action spectrum	UV-SSR source W/m²/nm	UVA radiation source W/m²/nm
324	9,000E-01	3,60E−03	7,987E-01	3,032E-05
325	8,750E-01	2,90E−03	8,290E-01	4,294E-05
326	8,500E-01	2,33E−03	8,435E-01	5,738E-05
327	8,250E-01	1,88E−03	8,559E-01	7,601E-05
328	8,000E-01	1,51E-03	8,791E-01	9,845E-05
329	7,750E-01	1,46E-03	8,951E-01	1,215E-04
330	7,500E-01	1,41E-03	9,010E-01	1,506E-04
331	7,250E-01	1,36E-03	9,161E-01	1,811E-04
332	7,000E-01	1,32E-03	9,434E-01	2,132E-04
333	6,750E-01	1,27E-03	9,444E-01	2,444E-04
334	6,500E-01	1,23E-03	9,432E-01	2,833E-04
335	6,250E-01	1,19E-03	9,571E-01	3,186E-04
336	6,000E-01	1,15E-03	9,663E-01	3,589E-04
337	5,750E-01	1,11E-03	9,771E-01	3,980E-04
338	5,500E-01	1,07E-03	9,770E-01	4,387E-04
339	5,250E-01	1,04E-03	9,967E-01	4,778E-04
340	5,000E-01	1,00E-03	9,939E-01	5,198E-04
341	4,938E-01	9,66E-04	1,007E+00	5,608E-04
342	4,876E-01	9,33E-04	1,012E+00	5,998E-04
343	4,814E-01	9,02E-04	1,011E+00	6,384E-04
344	4,752E-01	8,71E-04	1,021E+00	6,739E-04
345	4,690E-01	8,41E-04	1,025E+00	7,123E-04
346	4,628E-01	8,13E-04	1,033E+00	7,468E-04
347	4,566E-01	7,85E-04	1,034E+00	7,784E-04
348	4,504E-01	7,59E-04	1,040E+00	8,180E-04
349	4,442E-01	7,33E-04	1,027E+00	8,427E-04
350	4,380E-01	7,08E-04	1,045E+00	8,754E-04
351	4,318E-01	6,84E-04	1,042E+00	9,044E-04
352	4,256E-01	6,61E-04	1,040E+00	9,288E-04
353	4,194E-01	6,38E-04	1,039E+00	9,486E-04
354	4,132E-01	6,17E-04	1,043E+00	9,733E-04
355	4,070E-01	5,96E-04	1,046E+00	9,863E-04
356	4,008E-01	5,75E-04	1,035E+00	1,009E-03
357	3,946E-01	5,56E-04	1,039E+00	1,028E-03
358	3,884E-01	5,37E-04	1,027E+00	1,045E-03
359	3,822E-01	5,19E-04	1,035E+00	1,062E-03
360	3,760E-01	5,01E-04	1,037E+00	1,078E-03
361	3,698E-01	4,84E-04	1,025E+00	1,086E-03
362	3,636E-01	4,68E-04	1,023E+00	1,098E-03
363	3,574E-01	4,52E-04	1,016E+00	1,095E-03
364	3,512E-01	4,37E-04	9,984E-01	1,100E-03
365	3,450E-01	4,22E-04	9,960E-01	1,100E-03
366	3,388E-01	4,07E-04	9,674E-01	1,093E-03

Table C.1 *(continued)*

Wavelength nm	PPD action spectrum	Erythema action spectrum	UV-SSR source W/m²/nm	UVA radiation source W/m²/nm
367	3,326E-01	3,94E-04	9,648E-01	1,087E-03
368	3,264E-01	3,80E-04	9,389E-01	1,082E-03
369	3,202E-01	3,67E-04	9,191E-01	1,071E-03
370	3,140E-01	3,55E-04	8,977E-01	1,048E-03
371	3,078E-01	3,43E-04	8,725E-01	1,026E-03
372	3,016E-01	3,31E-04	8,473E-01	9,953E-04
373	2,954E-01	3,20E-04	8,123E-01	9,703E-04
374	2,892E-01	3,09E-04	7,840E-01	9,367E-04
375	2,830E-01	2,99E-04	7,416E-01	9,057E-04
376	2,768E-01	2,88E-04	7,148E-01	8,757E-04
377	2,706E-01	2,79E-04	6,687E-01	8,428E-04
378	2,644E-01	2,69E-04	6,280E-01	8,058E-04
379	2,582E-01	2,60E-04	5,863E-01	7,613E-04
380	2,520E-01	2,51E-04	5,341E-01	7,105E-04
381	2,458E-01	2,43E-04	4,925E-01	6,655E-04
382	2,396E-01	2,34E-04	4,482E-01	6,115E-04
383	2,334E-01	2,26E-04	3,932E-01	5,561E-04
384	2,272E-01	2,19E-04	3,428E-01	4,990E-04
385	2,210E-01	2,11E-04	2,985E-01	4,434E-04
386	2,148E-01	2,04E-04	2,567E-01	3,876E-04
387	2,086E-01	1,97E-04	2,148E-01	3,363E-04
388	2,024E-01	1,91E-04	1,800E-01	2,868E-04
389	1,962E-01	1,84E-04	1,486E-01	2,408E-04
390	1,900E-01	1,78E-04	1,193E-01	2,012E-04
391	1,838E-01	1,72E-04	9,403E-02	1,640E-04
392	1,776E-01	1,66E-04	7,273E-02	1,311E-04
393	1,714E-01	1,60E-04	5,532E-02	1,028E-04
394	1,652E-01	1,55E-04	4,010E-02	7,897E-05
395	1,590E-01	1,50E-04	2,885E-02	5,975E-05
396	1,528E-01	1,45E-04	2,068E-02	4,455E-05
397	1,466E-01	1,40E-04	1,400E-02	3,259E-05
398	1,404E-01	1,35E-04	9,510E-03	2,302E-05
399	1,342E-01	1,30E-04	6,194E-03	1,581E-05
400	1,280E-01	1,26E-04	4,172E-03	1,045E-05

The reference sun has a total UV irradiance of 51,4 to 63,7 W/m²[6] and[7] and a UVA to UVB irradiance ratio of 16,9 to 17,5.

Annex D
(normative)

PMMA substrate plate surface specifications

D.1 Substrate plate type

A PMMA plate with a molded 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. Other plates, as sand blasted ones, may be used as far as they fit the profile parameters described in D.2.

D.2 Surface profile of substrate plate

The surface profile characteristics of the substrates were measured based on several batches under specific criteria as recommended in this subclause.

Profilometer

— Non-contact surface topographic analysis consisting of an optical sensor, a motion controller, an x-y translation stage, and microtopography software.

— Optical sensor based on a white light chromatic aberration principle is recommended which allows for a high resolution of at least 10 nm vertically and 1 µm horizontally.

Measurement

— A surface area of at least X = 10 mm and Y = 5mm with at least 15-µm intervals.

— A speed of at least 1 000 µm/s is recommended according to sensor type and frequency.

Analysis operators

— Fill in non-measured points by a smooth shape calculated from the neighbours.

— Leveling method by least square plane by substraction.

— Conversion to a serie by extraction west-east of all surface profiles for 2D profile parameters.

Gaussian filters of 0,8 mm should be used according to profilometer characteristics.

Profile parameters

Ra (µm): The mean arithmetic deviation of the roughness profile.

Rv (µm): The maximum depth of profile valleys within a sampling length.

Rdq (°): The root-mean-square slope of the profile within a sampling length.

A1 ($\mu m^2.mm^{-1}$): The upper area, i.e. the area of the rest overs of the peaks extending above an average profile ± kernel.

Ssc ($L.\mu m^{-1}$): The arithmetic mean summit curvature of the surface, which indicates the meanform of peaks and valleys.

Vvv (m m^3.m^{-2}): The volume of void in the valleys, i.e., the volume of rest overs of valleys extending below an average profile ± kernel.

Substrate plate profile parameters

By using the criteria described in D.2, specifications for reproducibility of plate-to-plate shall respect the topographic parameters described in the control chart below.

Alternative specifications of topographic parameters may be obtained by using the same technology principle of the profilometer (non-contact surface topographic analysis) but with other (i) optical sensor, (ii) measurement conditions, (iii) analysis operators or (iv) microtopography software. These alternative control charts can be considered as valid only after the user has demonstrated, based on several batches, that the alternative is suitable and produces equivalent and correlated measurement results to those achieved using original specifications[17],[18].

Moulded PMMA plate

Ra (µm) = 4,853 ± 0,501

Rv (µm) = 13,042 ± 0,989

Rdq (°) = 11,122 ± 2,032

A1 (µm^2/mm) = 239,750 ± 70,165

Ssc (1/µm) = 0,033 ± 0,021

Vvv (mm^3/mm^2) = 1,044,10-4 ± 9,76,10-5

Sandblasted PMMA plate

Ra (µm) = 4,188 ± 0,514

Rv (µm) = 11,402 ± 2,499

Rdq (°) = 11,004 ± 1,938

A1 (µm^2/mm) = 238,252 ± 72,663

Ssc (1/µm) = 0,032 ± 0,015

Vvv (mm^3/mm^2) = 8,701,10-4 ± 2,325,10-4

D.3 Substrate plate optical characteristics

D.3.1 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, or Vaseline®.

Table D.2 — Modified glycerin solution

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

D.3.2 Method

D.3.2.1 Prepare a standard PMMA blank plate by applying approximately 15 mg of pure glycerin or modified glycerin solution or Vaseline® 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/Vaseline® with a bare fingertip.

D.3.2.2 Place the plate in the light path of the spectrophotometer. Measure transmittance (290 nm to 400 nm) against air (with no plate) as the reference light path.

D.3.3 Minimum transmission values

Limits for the treated molded PMMA plate transmission values are:

290 nm: > 60 % T

300 nm: > 69 % T

320 nm: > 81 % T

Limits for the treated sandblasted PMMA plate transmission values are:

290 nm: > 60 % T

300 nm: > 69 % T

320 nm: > 81 % T

Annex E
(normative)

Product reference sunscreen formulations

E.1 Mean UVA-PF and acceptance limits for product reference sunscreen formulation

Table E.1 provides the mean and acceptance range for the product reference sunscreen S2 and P8 used for the purposes of validating the test procedures of this test method.

Table E.1 — Mean UVA-PF and acceptance limits for product reference sunscreen formulation

Product reference sunscreen	Mean	Mean	Acceptance limits	
Formulation	SPF	UVAPF	Lower limit	Upper limit
S2	16,0	12,7	10,7	14,7
P8	63,1	21,1	19,1	23,1

E.2 Formula and preparation for product reference sunscreen S2

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

Table E.2 — S2 product reference sunscreen

Ingredients	% mass of total mass
Phase 1 (aqueous)	
Water	62,43
Propylene glycol	1,00
Xanthan gum	0,60
Carbomer	0,15
Disodium EDTA	0,08
Phase 2 (oil)	
Octocrylene	3,00
Butylmethoxy dibenzoylmethane	5,00
Ethylhexyl methoxycinnamate	3,00
Bis-ethylhexyloxyphenol-methoxyphenyl triazine	2,00
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)	Appropriate amount
Ethylparaben (and)	Appropriate amount

Table E.2 *(continued)*

Ingredients	% mass of total mass
Butylparaben (and)	Appropriate amount
Propylparaben	Appropriate amount
Phase 3	
Cyclopentasiloxane	2,00
Triethanolamine	0,23

E.2.1 Manufacturing process

E.2.1.1 General

The manufacturing process requires that the following five steps be undertaken.

E.2.1.2 Heat phase 1 and phase 2 separately up to 75 °C.

E.2.1.3 Add oily phase 2 slowly into aqueous phase 1 while stirring phase 1.

E.2.1.4 Cool to 40 °C while stirring.

E.2.1.5 Add phase 3 to phase 1 and 2 while stirring.

E.2.1.6 Compensate water loss and homogenize.

E.2.2 Specifications

The S2 product reference sunscreen specifications for an acceptable batch are as follows.

— Colour: white to slightly yellow.

— pH: 6,5 ± 0,5.

— Density: 0,96 to 1 g/cm^3.

— Viscosity: 7 000 to 12 000 (Brookfield DV-II, Helipath Mobile, Spindle B[2)]), 20 r/min for 60s.

E.2.3 Storage and expiry

The storage conditions and expiration date for the product reference sunscreen S2 are 12 months at <30 °C from the fabrication date in a glass container or closed package and protected from light.

E.2.4 Analytical data

E.2.4.1 General

The methodology for validation of the sunscreen content of the S2 product reference sunscreen is described below.

E.2.4.2 Principle

The formulation is sampled gravimetrically and dissolved in ethanol, in which the analytes are soluble. The solution is filtrated and chromatographed on microparticulate silica gel column, using a mix

2) Brookfield DV-II, Helipath Mobile, Spindle B is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

water/ethanol as mobile phase. The concentrations of the analytes in the sample are determined by quantification against a mixed external standard solution of analyte raw materials

E.2.4.3 Chemicals/reagents

The chemicals and reagents for the analytical methods used in this procedure are:

— Absolute ethanol (HPLC grade);

— Ultrapure water (HPLC grade);

— Phosphoric acid, 85 % analytical purity;

— Ethylhexyl methoxycinnamate;

— Butyl methoxydibenzoylmethane;

— Octocrylene;

— Bis-ethylhexyloxyphenol methoxyphenyl triazine.

E.2.4.4 Apparatus (high-pressure liquid chromatography)

The apparatus necessary to conduct the analytical measurements of the sunscreens in the S2 product reference sunscreen are as follows.

— **Injector**, with an injection volume of 10,0 µl.

— **Column**, e.g. Waters Symmetry Shield C18[3], 5 µm, with a length of 150 mm, an inner diameter of 4,6 mm and a flow rate of 1,2 ml/min[4].

The gradient of the column should be as follows: 37 % A and 63 % B for 0 min to 12 min, 100 % B for 12 min to 22 min, 100 % B for 22 min to 25 min, 37 % A and 63 % B for 25 min to 26 min, and 37 % and 63 % B for 26 min to 30 min.

— **Eluent A**, ultrapure water acidified with H_3PO_4.

— **Eluent B**, absolute ethanol (HLPC grade).

— **Detector**, of type UV wavelength, 312 nm.

— **Data**, i.e. quantification of peak area.

E.2.4.5 Method

The following steps are taken to measure the active sunscreen in the S2 product reference sunscreen.

E.2.4.5.1 Using an analytical balance, weigh approximately 50 mg of formulation, to the nearest 0,1 mg, into a 25 ml volumetric flask**.**

E.2.4.5.2 Complete to volume with ethanol.

E.2.4.5.3 Shake with a vortex and, in the case of a non-liquid formulation, sonicate with an ultrasonic bath until homogenization occurs.

3) Waters Symmetry Shield C18 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

4) The gradient of the column should be as follows: 37 % A and 63 % B for 0 to 12 min, 100 % B for 12 to 22 min, 100 % B for 22 to 25 min, 37 % A and 63 % B for 25 to 26 min, and 37 % and 63 % B for 26 to 30 min.

E.2.4.5.4 Filter through a 0,45 µm PVDF disc filter.

E.2.4.5.5 Analyse the standard and mixed working standard by reverse-phase HPLC.

E.2.4.6 Quality control

The following steps are taken to validate the quality of the analytical method.

E.2.4.6.1 Analyse a sample of HPLC mobile phase and a placebo, if available, prepared as per E.2.4.5, by reverse-phase HPLC, to confirm the absence of interfering chromatographic peaks.

E.2.4.6.2 Inject a standard solution three times by reverse-phase HPLC and calculate the coefficient of variation of the analysis peak areas.

E.2.4.7 Calculations

Analyte percentage is calculated using Formula (E.1):

$$\frac{M \times h \times 2,5}{P \times H} \tag{E.1}$$

where

M is the mass of analyte, expressed in mg;

P is the mass of sample, expressed in mg;

h is the area of analyte peak of the sample;

H is the area of analyte peak from standardization.

E.2.4.8 Acceptance criteria

The analytical results are acceptable if the following conditions are achieved:

— the standard coefficient of variation is ≤ 2,5 %;

— the recovery value is 95 % to 105 % of the formula amount;

— there are no interfering chromatographic peaks in the sample placebo or working solvent.

E.3 P8 High product reference sunscreen

The formulation and preparation procedure in this subclause describe the quantitative measures and mixing procedures necessary to prepare the P8 product reference sunscreen used to validate the testing procedure.