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Cosmetics — Sun protection test method — Determination of sunscreen UVA photoprotection in vitro

Cosmétiques — Méthodes d'essai de protection solaire — Détermination in vitro de la photoprotection UVA

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Foreword

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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.

ISO 24443 was prepared by Technical Committee ISO/TC 217, *Cosmetics*, WG 7, in collaboration with Technical Committee CEN/TC 392.

Introduction

Cosmetics — Sun protection test method — Cosmetics — Sun protection test method — 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 of this measurement procedure can be used for other computations as necessary for local regulatory authorities. These include calculation of Ultraviolet-A Protection Factor (UVA-PF) (correlating with *in vivo* UVA-PF from 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 and thus is not entirely *in vitro* methodology.

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

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 17025, *General requirements for the competence of testing and calibration laboratories.*

3 Terms and definitions

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

2.1
***in vitro* UVA protection factor**
(UVA-PF)
in vitro UVA protection factor of a sun protection product against UVA radiation which can be obtained from *in vivo* clinical testing or derived mathematically with *in vitro* spectral modelling

2.2
***in vitro* calculation of SPF**
$SPF_{in\ vitro}$
protection factor of a sunprotection 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

2.5
action spectrum, for PPD
$P(\lambda)$
relative effects of individual spectral bands of an exposure source for a persistent pigment response

2.6
monochromatic absorbance
A_λ
sunscreen absorbance at wavelength λ related to the sunscreen transmittance (T_λ) by:

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

where transmittance (T_λ) is the fraction of incident irradiance transmitted by the sunscreen film.

2.7
irradiance
I
fluence rate per unit area, units W m^{-2}; for a defined range of wavelengths, for example from 290 to 400 nm for UVA + UVB irradiance or from 320 to 400 nm for UVA irradiance

2.8
spectral irradiance for SPF testing or PPD testing
$I(\lambda)$
irradiance per unit wavelength, $I(\lambda)$, units W m^{-2} nm^{-1}

2.9
spectrophotometer
instrument that measures absorbance (or transmission) properties of a test media as a function of wavelength

2.10
spectroradiometer
instrument that measures spectral irradiance (intensity in dimensions of W/unit area/nm) of electromagnetic sources (in this standard limited to the ultraviolet, visible and short infrared ranges)

2.11
radiometer
instrument to measure broad band irradiance (intensity in dimensions of W/unit area) of electromagnetic sources (in this standard limited to the ultraviolet, visible and short infrared ranges)

4 General principles

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 procedure of the mathematical modelling has been empirically derived to correlate with human *in vivo* (Persistent Pigment Darkening) test results.

5 Apparatus

5.1 UV Spectrophotometer (specifications)

The UV spectrophotometer wavelength range shall span the primary waveband of 290 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 PMMA substrate, without and with 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 cm² in order to reduce the variability between readings and to compensate for the lack of uniformity in 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 290-400 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/cm².

(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.)

5.2 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

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[5] as defined by COLIPA (1994)[6] or in DIN 67501 (1999)[7]. The UV irradiance must be within the following acceptance limits (measured at sample distance).

Table 1 — Title

UV exposure source specifications as measured with a spectroradiometer	
Total UV irradiance (290 to 400 nm)	(40 – 200 W/m^2)
Irradiance ratio of $UVA_{(320 \text{ to } 400 \text{ nm})}$ to $UVB_{(290 \text{ to } 320 \text{ nm})}$	8 - 22

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 is measured and not just the surrounding air temperature. To maintain samples not higher than 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 temperature below 35°C and warming devices to maintain samples at or above 25°.

5.4 Monitoring of the UV exposure source

The emission of the UV exposure source used for exposure shall be checked (at least) every 18 months or after 3000 hours of lamp running time by a suitably qualified expert for compliance with the given acceptance limits. 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 UVA Radiometer used to monitor the test sample irradiation

If a UVA radiometer is used, this device must have been suitably calibrated. This requires that it is calibrated to the spectroradiometer used to measure the exposure source (as during annual solar simulator calibration). Calibration must be conducted in terms of UVA irradiance (Wm^{-2} 320-400 nm) and must be at the same level at which the test plates will be 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.

5.6 Substrate / Plate

The substrate / plate is the material to which the test product is to be applied. For this method, polymethylmethacrylate (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, and the specifications and preparation of this type of plate[9] is described in Annex D. The size of the substrate should be chosen such that the application area is not less than 16 cm^2.

6 Test Method

6.1 Outline of the test procedure

The test procedure comprises seven steps:

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

6.1.2 Conduct blank measurements of a glycerine treated plate for the reference "blank" for the subsequent absorbance measurements

6.1.3. In vitro absorbance measurement(s) of the sunscreen product spread on PMMA plate, prior to any UV irradiation. Acquisition of initial UV absorbance spectrum with A0(λ) data.

6.1.4 Mathematical adjustment of the initial UV absorbance spectrum using coefficient 'C' (see calculation below) to achieve an in vitro SPF (no UV dose) equal to the in vivo SPF. Initial UVA-PF0 is calculated using A0(λ) and C.

6.1.5 A single UV exposure dose D is calculated, equal to 1.2 x UVA-PF0 in J/cm^2.

6.1.6 In vitro absorbance measurement of the sunscreen product after UV exposure. Acquisition of second UV spectrum with A(λ) data.

6.1.7 Mathematical adjustment of the second absorbance spectrum (following UV exposure) and by multiplying with the same 'C' coefficient, previously determined in step 4. The resulting absorbance curve is the final adjusted absorbance value.

NOTE for calculations- UV absorbance values shall be used.

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 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.

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 microliters of glycerin on the roughened side of the plate. Choose the amount of glycerin such that the entire surface is just completely covered (approximately 15μl for a 50 x 50mm 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. (Many spectrophotometers have "Baseline" functions to automatically incorporate this baseline measurement into the calculations of subsequent absorbance measurements.)

6.4 Sample application

Sunscreen product is applied to a new untreated roughened PMMA plate (roughened side upper-most) by weight, at an density of 1.3mg/cm^2. To ensure dose accuracy / repeatability, the application area should be not less than 16cm^2. Application dose may be determined via weight loss measurements of the pipette before and after application of the product, or alternatively, it may be applied based on volumetric measurements with considerations of the specific gravity of the test sample. A positive-displacement automatic pipette should be used for this purpose when possible

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 product shall be cleaned between different test products.

After depositing the sunscreen product 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 seconds.

This treated sample shall be allowed to dry for at least 30 minutes 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).

6.5 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 to 400 nm, in 1nm steps. One or more observation(s) of absorbance may be made per plate and the mean value shall be determined for each plate.

6.6 Number of determinations

At least 4 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 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 ($SPF_{in\ vitro}$), "C" value, initial UVA-PF($UVA\text{-}PF_0$), and UV exposure dose

6.7.1 Determination of initial calculated SPF (SPFin vitro)

The UV solar simulator radiation source (UV-SSR source) spectrum (Annex C) (I(λ)) is multiplied with the corresponding erythema action spectrum sensitivity value (*E*(λ)) (Annex C) at that wavelength to yield the sunburning effective energy at that wavelength. The resulting sunburning effective irradiance is integrated over the 290 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 the in vitro SPF ($SPF_{in\ vitro}$):

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

where

$E(\lambda)$ = Erythema action spectrum (CIE-1987) (see Annex C);

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

$A_0(\lambda)$ = Mean monochromatic absorbance of the test product layer *before* UV exposure;

$d\lambda$ = Wavelength step (1nm).

NOTE This calculated SPF value cannot be used 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. This is accomplished in an iterative calculation process. The initial absorbance values multiplied by this "C" value becomes the adjusted sunscreen absorbance curve that is used for determination of the initial UVA-PF_0 value, and the exposure dose. Calculation of the adjusted *in vitro* SPF ($SPF_{in\ vitro,adj}$) and determination of the coefficient of adjustment 'C' is by Equation 2:

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

where

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

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

The "C" value typically lies between 0.8 and 1.6 for valid interpretation. If it is outside this range, new samples should be prepared to validate the original observations. If it is outside this range, new samples should be prepared to validate the original observations (however the application density should not be changed from 1.3mg/cm^2). 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.

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 *in vitro* SPF. The intensity spectrum for a UVA radiation source (I(λ)) (as described in Annex C) is multiplied at each wavelength with the Persistent Pigment Darkening action spectrum sensitivity values (P(λ)) to yield the pigment darkening energy at that wavelength. The resulting pigment darkening effective irradiance is integrated over the 320 to 400nm range. The initial absorbance values from the test product at each wavelength is 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* UVA-PF_0 value:

$$UVA\text{-}PF_0 = \frac{\int_{\lambda=320nm}^{\lambda=400nm} P(\lambda)*I(\lambda)*d\lambda}{\int_{\lambda=320nm}^{\lambda=400nm} P(\lambda)*I(\lambda)*10^{-A_0(\lambda)*C}*d\lambda} \quad (3)$$

where

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

$I(\lambda)$ = Spectral irradiance received from the UVA source (UVA 320-400nm for PPD testing) (see Annex C);

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

C = Coefficient of adjustment previously determined in equation (2);

$d\lambda$ = Wavelength step (1 nm).

6.7.4 Determination of the UV exposure dose

The UV exposure dose is the initial *in vitro* UVA-PF (UVA-PF_0) value multiplied by a factor 1.2 * UVA-PF_0 in Joules/cm^2.

$$D = \text{UVA-PF}_0 \times 1.2 \text{ J/cm}^2 \quad (4)$$

The sample is exposed to full spectrum UV radiation but the dose is being defined by the UVA content. (The 1.2J/cm^2 factor is based on ISO ring test validation study results[8]).

6.8 UV exposure

Expose the sample plates to the radiation from the UV exposure source. During the exposure the samples should be maintained at between 25°C and 35°C 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.

Attention: Personnel working with this irradiator system should be protected adequately against UV rays (e.g. glasses, gloves, etc.).

6.9 Measurement of final adjusted absorbance spectrum

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

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

where

A_e = Mean monochromatic absorbance of the test product layer after UV exposure;

A_f = Mean final monochromatic absorbance of the test product.

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

For calculation of acceptable variance, the UVA-PF is to be calculated according to Equation 5 for each individual plate using the single observation value or the mean of multiple observations on that plate.

$$\text{UVA-PF} = \frac{\int_{\lambda=320nm}^{\lambda=400nm} P(\lambda) * I(\lambda) * d\lambda}{\int_{\lambda=320nm}^{\lambda=400nm} P(\lambda) * I(\lambda) * 10^{-A_e(\lambda)*C} * d\lambda} \quad (5)$$

where

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

$I(\lambda)$ = Spectral irradiance received from the UVA source (UVA 320-400nm for PPD testing) (see Annex C);

$A_e(\lambda)$ = Mean monochromatic absorbance of the test product layer after UV exposure;

C = Coefficient of adjustment previously determined in equation (2);

$d\lambda$ = Wavelength step (1 nm).

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

7 Procedure using ISO 24443 spreadsheet

These calculations (6.1.4 through 6.1.8), can be performed automatically using the ISO 24443 Calculation spreadsheet using the following steps.

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

7.2 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.

7.3 The UV exposure irradiance and exposure time will be reported on the “Results #” tab for each individual plate.

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

7.5 Measure the absorbance of the each of the individual UV exposed plates. The measurements conducted after UV exposure should be on the same spot(s) as measured before the UV exposure.

7.6 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.

7.7 The “RESULTS (Plate #)” spreadsheet tab will show the results data for each individual plate.

7.8 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% Confidence Interval 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 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.

8 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 UVA-PF test results of the reference S2 must lie between the upper and lower limits as determined from *in vivo* testing results listed below, or the test is invalid and the test must be repeated. SPF 16 is to be used as the in vivo SPF value for S2 for computation purposes.

Table 2 — Title

Parameter	Lower Limit	Upper Limit
UVA-PF	10.7	14.7

The frequency of the testing of the S2 standard shall be in accordance to the users internal procedures and/or ISO 17025 Quality Management Standard.

9 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 ISO 24443 System Calibration Summary as per the format in Annex A.5;

b) The calibration factor “Y” used to adjust the UVA radiometer measurement with the reference spectroradiometer measurement of the UV exposure source (Section 4.5 and Annex B (B.3.10));

c) Plate manufacturer, and batch code;

d) Mean UV absorbance values at each 1nm 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 individual conducting the test;

k) Other informative calculations derived the absorbance values (8.c) may be reported.

Annex A
(normative)

Calibration of UV Spectrophotometer and Plate Transmission Test

A.1 Introduction

This procedure describes the requirements for wavelength accuracy, linearity and dynamic range of the UV Spectrophotometer. For clarity and in order to standardise the report format, a spreadsheet is provided as part of this ISO 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 so as to provide absolute wavelength calibration using absorbance peak at 361nm.

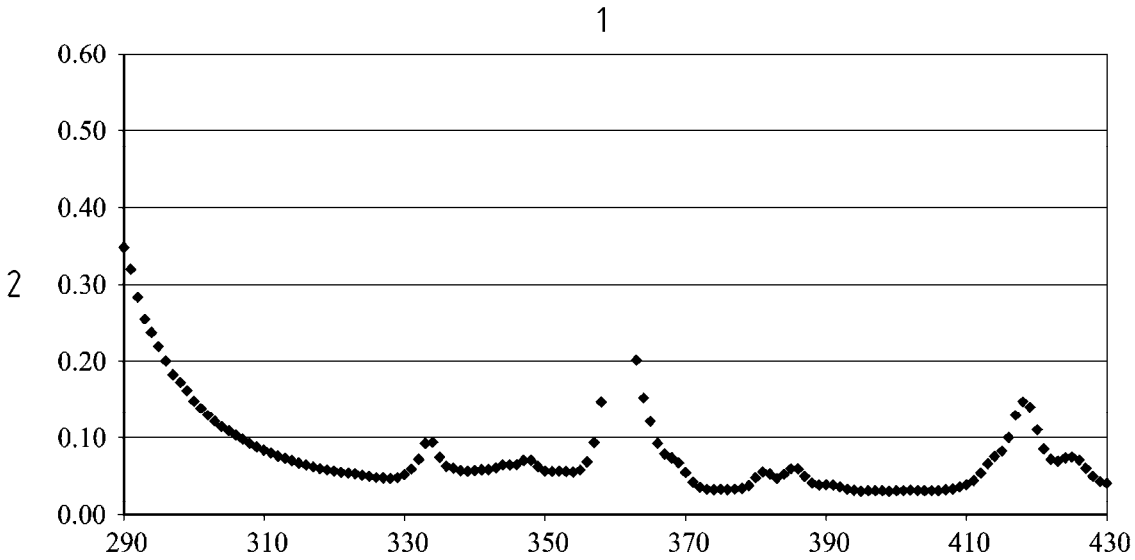
A.2.2 Method

A.2.2.1

Place the Holmium oxide filter in the sample path and scan the absorbance in the range between 270 and 400nm. Measure against air in the blank light path. Repeat the scan for 3 replicates. Accumulate the data and transfer Absorbance values to tab “Holmium Wavelength Accuracy” in the attached spreadsheet “ISO 24443UVSpectcalib.xls” in Columns B thru D. Click on the macro button at cell “P28” to activate the peak check function. The results will automatically appear in the ISO 24443 System Calibration Summary sheet (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 must be either 360, 361, or 362, or the instrument must be recalibrated to achieve one of these wavelength values.



Key

1 Holmium Oxide Actual

2 Abs

Figure A.1 — Title

A.3 Linearity

A.3.1 Standard reference plates

The plates are cut from a large sheet of a standard cast, UV-stabilised Polymethylmethacrylate (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 standardised 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 - UV stabilized. The absorbance peak of these reference plates at 340nm shall be at least 1.1, Absorbance Units but lower than 1.5 Absorbance Units.

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 to 380 nm) and transfer data to spreadsheet tab marked “Linearity Test” into 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 spreadsheet tab marked “Linearity Test” into cells D8:E98. Place both slides (A+B) on top of each other with their roughened sides towards one another into the light path and measure the combined absorbance (290 to 380 nm). Measure against air in the blank light path. Run 4 replicates and transfer data to spreadsheet tab marked “Linearity Test” into cells F8:I98. The results will automatically appear in the ISO 24443 System Calibration Summary sheet (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 addictively 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 ISO 24443 System Calibration Summary sheet (Figure A.2). The Minimum Range Limit is 2.2 AU.

A.5 PMMA Test Plate Qualification

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

A.5.1 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 must record >60% transmission at 290nm, >69% at 300nm, and >81% at 320nm. The results will automatically appear in the ISO 24443 System Calibration Summary sheet as shown in Figure A.2.

A.6 Reporting

The results of the calibration should be recorded in the standardised format as indicated on the spreadsheet ”Summary”.

ISO 24443 System Calibration Summary

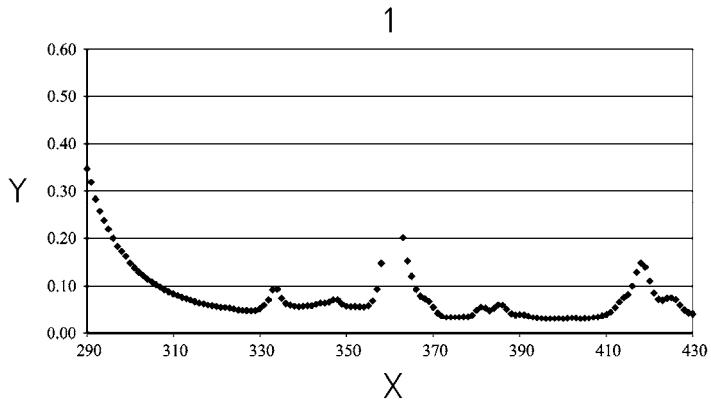
Instrument:	0	**Tested by:**	0
Date:	March 31, 2011	**Sig:**	0

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



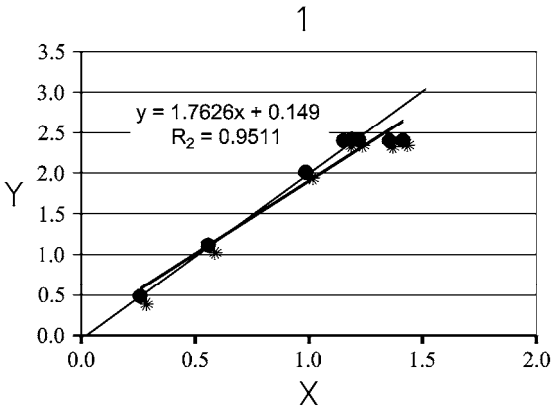
a)

Key

1 Holmium Oxide Observed
X Wavelength
Y Abs

Spectrophotometer Linearity Test

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



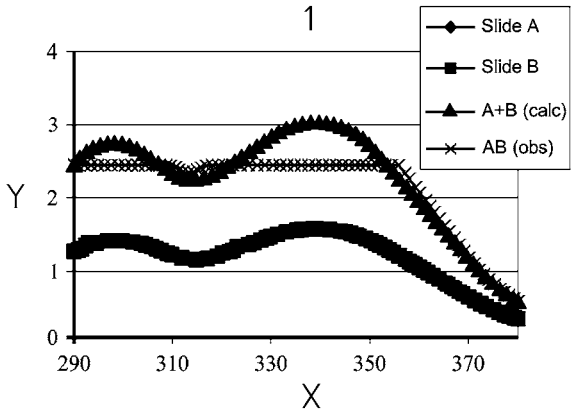
b)

Key

1 One vs Two PMMA Calibration Plates Correlation

X One Plate (mean) (abs.)

Y Two Plates (stackec) (Abs.)



c)

Key

1 Dynamic Range Test

X Wavelength

Y Absorbance

Figure A.2 – Title

Annex B
(normative)

Radiometer calibration to spectroradiometric irradiance procedure

B.1 Purpose

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 to 400nm. 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 to 400nm 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 to 400nm 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 to 400nm in 1 nm increments.

B.3.5 Integrate the spectral irradiance from 320 to 400nm to determine the total UVA spectroradiometric irradiance of the source at the exposure plane of the PMMA plate samples. This will be designated $IUVA_{spec}$.

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

B.3.7 Place the UVA radiometer to be calibrated in the same position as the spectroradiometer such that the calibration reference plane of the radiometer is at 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 will be designated $IUVA_{rad}$.

B.3.9 Calculate the Calibration Factor Y = $IUVA_{spec/}$. $IUVA_{rad}$

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

Calibrated UVA irradiance = $IUVA_{rad}$ * Y

B.3.11 The calibration correction factor “Y” can be entered directly into the ISO In Vitro UVA Test Spreadsheet and 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 – Title

Wavelength nm	**PPD action spectrum**	**Erythema action spectrum**	**UV-SSR source** $W.m^{-2}nm^{-1}$	**UVA radiation source** $W.m^{-2}nm^{-1}$
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	-

"to be continued"

Table C.1 *(continued)*

Wavelength nm	**PPD action spectrum**	**Erythema action spectrum**	**UV-SSR source** $W.m^{-2}nm^{-1}$	**UVA radiation source** $W.m^{-2}nm^{-1}$
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
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

"to be continued"

Table C.1 *(continued)*

Wavelength nm	**PPD action spectrum**	**Erythema action spectrum**	**UV-SSR source** $W.m^{-2}nm^{-1}$	**UVA radiation source** $W.m^{-2}nm^{-1}$
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
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

"to be continued"

Table C.1 *(concluded)*

Wavelength nm	**PPD action spectrum**	**Erythema action spectrum**	**UV-SSR source** $W.m^{-2}nm^{-1}$	**UVA radiation source** $W.m^{-2}nm^{-1}$
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^2 (Colipa 1994[6]/DIN 67501[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 polymethylmethacrylate (PMMA) plate with a molded surface roughness that has the following surface parameters within the upper and lower limit values was qualified for use for this *in vitro* UVA test method via ring testing.

D.2 Surface profile of substrate

Measure the surface profile characteristics of the substrate[9], covering at least a surface area of 10 mm x 5 mm in 15-µm intervals. Non-contact surface topographic analysis may be conducted using a lab 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 um horizontally.

Surface profile parameters: Surface profile parameters are to be calculated by the mean of the different profiles (R_a, R_v, R_{dq}, A1, SSc, Vw) analyzed.

D.3 Plate profile parameters

PMMA plates can be prepared with using a molding process having surface characteristics that meet the following measurement parameters, as measured with instrumentation[9]:

Table D.1 — Target and Upper and Lower Limits

Parameter	Ra	Rv	Rdq	A1	SSc	Vw
Target value	4,853	13,042	11,122	239,750	0,033	1,044E-6
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 also Annex A.4)

Representative samples of each lot of PMMA plates are to be tested for transmission properties to assure compliance. The profiled surface of the test plate is to be treated with pure glycerin or a modified glycerin solution (below):

Modified Glycerin Solution

Ingredient	**%w/w**
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 15mg 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 against air (with no plate) as the reference light path. (290 to 400 nm).

D.4.3 Minimum transmission values

Limits for the treated plate transmission values are:

290 mn : >60 %T

300 nm : >69 %T

320 nm : >81 %T

Annex E
(normative)

UVA reference sunscreen S2

E.1 Mean UVA-PF 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. (see section 7.0 of the test method)

Table E.1 — Title

Reference Sunscreen Formulation	Mean SPF	Mean UVA-PF	Acceptance limits	
			Lower limit	Upper limit
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.

		%w/w
Phase 1 (Aqueous):		
	water	62.43
	propylene glycol	1.00
	xanthan gum	0.60
	Carbomer (Carbopol Ultrez 10)	0.15
	disodium EDTA	0.08
Phase 2. (Oil)		
	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	
Phase 3		
	cyclopentasiloxane	2.00
	triethanolamine	0.23

E.3 Manufacturing Process

The manufacturing process requires the following 5 steps:

E.3.1 Heat Phase 1 and phase 2 separately up to 75°C.

E.3.2 Add oily phase 2 slowly into aqueous phase 1 while stirring phase 1.

E.3.3 Cool to 40°C while stirring.

E.3.4 Add phase 3 to phase 1&2 while stirring.

E.3.5 Compensate water loss and homogenise.

E.4 Specifications

The S2 reference sunscreen specifications for an acceptable batch are the following:

E.4.1 Color: white to slightly yellow.

E.4.2 pH: 6.5 +/- 0.5.

E.4.3 Density: 0.96 to 1 g/cm$^{3.}$

E.4.4 Viscosity: 7000 to 12000 (Brookfield DV-II , Helipath Mobile, Spindle B, 20 RPM, time of assessment 60 sec).

E.5 Storage and expiry

The storage conditions and expiration date for the reference sunscreen S2 are:

12 months at 20°C from the fabrication date, in glass container or closed package and protected from light.

E.6 Analytical data

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

E.6.1 Principle

The formulation is sampled gravimetrically and dissolved in Ethanol, in which the analytes are soluble. Solution is filtrated and chromatographed on microparticulate silica gel column, using a mix 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.6.2 Chemicals / Reagents

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

E.6.2.1 Absolute ethanol HPLC grade.

E.6.2.2 Ultrapure water HPLC grade.

E.6.2.3 Phosphoric acid 85% p.a.

E.6.2.4 Ethyl hexyl methoxycinnamate.

E.6.2.5 Butyl methoxydibenzoylmethane.

E.6.2.6 Octocrylene.

E.6.2.7 Bis-EthylHexyloxyphenol Methoxyphenyl Triazine.

E.7 Apparatus

The apparatus necessary to conduct the analytical measurements of the sunscreens in the S2 reference sunscreen are:

HPLC		
Injector	Injection Volume	-10.0µL
Column	Type	- Symmetry Shield C18, 5µm
	Length	- 150mm
	I.D.	- 4.6mm
	Flow rate	- 1.2 mL/min
Eluant	A – Ultrapure Water acidified with H_3PO_4	
	– Absolute Ethanol , HPLC grade B	
Gradient	0-12 min: 37% A + 63% B	
	12-22 min: 100% B	
	22-25 min: 100% B	
	25-26 min: 37% A + 63% B	
	26-30 min: 37% A + 63% B	
Detector	Type	- UV
	Wavelength	- 312nm
Data	Quantification	- Peak Area

E.8 Method

The steps to measure the sunscreen actives in the S2 reference sunscreen are:

E.8.1 Using an analytical balance weigh approximately 50mg of formulation, to the nearest 0.1mg, into a 25mL volumetric flask.

E.8.2 Dilute to volume with ethanol.

E.8.3 Shake with a vortex and in case of a non liquid formulation, sonicate with an ultrasonic bath until homogenization.

E.8.4 Filter through a 0,45 µm PVDF disc filter.

E.8.5 Analyze the standard and mixed working standard by reverse phase HPLC.

E.9 Quality control

The steps to validate the quality of the analytical method are:

E.9.1 Analyze a sample of HPLC mobile phase and a placebo, if available, prepared as per the method, by reverse phase HPLC, to confirm the absence of interfering chromatographic peaks.

E.9.2 Inject three Times a standard solution by reverse phase HPLC and calculate the coefficient of variation of the analysis peak areas.

E.10 Calculations

The calculations to determine the amount of sunscreens filters in the S2 reference sunscreen are:

$$\text{Analyte \% w/w} = \frac{M \times h \times 2.5}{P \times H}$$

M = Weight in mg of analyte;

P = Weight in mg of sample;

h = Area of analyte peak for sample;

H = Area of analyte peak for standardization.

E.11 Acceptance Criteria

The analytical results are acceptable if the following are achieved:

— The standard coefficient of variation is =< 2,5%

— Recovery value is 95% - 105% of formula amountj

— No interfering chromatographic peaks in the sample placebo or working solvent.

Annex F
(informative)

Statistical Calculations

F.1 Individual Plate Ultraviolet A Protection Factor

UVA-PFi

The individual UVA-PFi of each plate is calculated according to the spreadsheet

$$\text{UVA-PF}_i = \frac{\int_{\lambda=320nm}^{\lambda=400nm} P(\lambda) * I(\lambda) * d\lambda}{\int_{\lambda=320nm}^{\lambda=400nm} P(\lambda) * I(\lambda) * 10^{-A_e(\lambda)*C} * d\lambda} \quad \text{(F.1)}$$

F.2 Product Ultraviolet A Protection Factor

The UVA-PF of the product is the arithmetical mean of the individual plate UVA-PFi values obtained from the total number (n) of plates used, expressed to one decimal point:

$$\text{UVA-PF} = (\sum \text{UVA-PFi}) / n \quad \text{(F.2)}$$

Its standard deviation (s) is:

$$s = \sqrt{[(\sum(\text{UVA-PFi})^2 - ((\sum \text{UVA-PFi})^2 / n)) / (n - 1)]} \quad \text{(F.3)}$$

F.3 95% confidence interval

The 95% confidence interval (95 %CI) for the mean UVA-PF is expressed as:

$$95\ \%CI = (\text{UVA-PF} - c) \text{ to } (\text{UVA-PF} + c) \quad \text{(F.4)}$$

c is calculated as: $c = (\text{t value}) \times \text{SEM} = (\text{t value}) \times s / \sqrt{n}$

$$c = t \times s / \sqrt{n} \quad \text{(F.5)}$$

$$CI[\%] = 100 \times c / \text{UVA-PF} \quad \text{(F.6)}$$

where

SEM = the standard error of the mean;

N = total number of plates used;

T = t value from the “two-sided” Student-t distribution table (7) at a probability level p = 0,05 and with degrees of freedom $\nu = (n - 1)$.

N	4	5	6	7	8	9	10
t value	3.182	2.776	2.571	2.447	2.365	2.306	2.262

NOTE For spreadsheet calculation t value can be modelled by: $t = 2{,}03 + 12{,}7 / n^{1{,}75}$ (for $n \geq 4$).

F.4 Experimental calculation procedure

F.4.1 Sequential procedure

A test is begun by testing the product on an initial set of n' plates (a minimum of 4 plates are to be used). The individual UVA-PFi values for the product on each plate are then calculated according to equation E.1 above.

From these individual UVA-PFi values, a provisional mean UVA-PF for the initial n' plates is calculated according to equation (E2), together with a provisional 95% confidence interval ($95\%CI_{n'}$) using equations (E4), (E5) and (E6) and t-table (E7), i.e.:

$$UVA\text{-}PF_{n'} = \sum UVA\text{-}PFi / n' \quad (F.8)$$

$$95\ \%CI_{n} = UVA\text{-}PF_{n'} - c_{n'} \text{ to } UVA\text{-}PF_{n'} + c_{n'} \quad (F.9)$$

$c_{n'}$ is calculated as $c_{n'} = t_{n'} \times s_{n'} / \sqrt{n'}$ (F.10)

where $s_{n'}$ = standard deviation from the first n' plates calculated according to equation (3):

$$sn' = \sqrt{[(\sum(UVA\text{-}PFi)^2 - ((\sum UVA\text{-}PFi)^2 / n'))\ /\ (n' - 1)]} \quad (F.11)$$

$$CI_{n'}[\%] = 100 \times c_{n'} / UVA\text{-}PF_{n'} \quad (F.12)$$

If the calculated provisional $CI_{n'}[\%]$ is greater than 17 % of the provisional mean $UVA\text{-}PF_{n'}$ value, then testing of the product shall continue on additional plates until the provisional $CI_{n'}[\%]$ is $\leq$ 17 % of the mean provisional UVA-PF. If this criterion is not fulfilled after 10 valid plates, then the entire test shall be rejected and repeated.

F.4.2 Predicted number of plates

n*

If the $CI_{n'}[\%]$ on the provisional $UVA\text{-}PF_{n'}$ is greater than 0,17 $UVA\text{-}PF_{n'}$, then the predicted likely total number of plates (n*) necessary to meet the statistical criterion can be estimated according to the following formula and rounded-up to the nearest integer:

$$n^* = (t_{n'} \times s_{n'} / C_{n'})^2 \quad (F.13)$$

where

$t_{n'}$ = t statistic from t-table or equation (7), with n' results;

$s_{n'}$ = best estimate of population standard deviation (i.e. from the n' results);

$C_{n'}$ = 17 % of mean $UVA\text{-}PF_{n'}$, representing the required confidence interval.

EXAMPLE When n* is calculated after the first 10 data, then:

$$n^* = (2{,}262\ s_{n'} / 0{,}17\ UVA\text{-}PF_{n'})^2 \quad (F.14)$$

$$n^* = (13{,}30\ s_{n'} / UVA\text{-}PF_{n'})^2 \quad (F.15)$$

Bibliography

1 CIE Erythema Action Spectrum: Publication: CIE S007 / ISO 17166

2 McKinlay AF & Diffey BL. A reference action spectrum for ultraviolet induced erythema in human skin. *CIE J* (1987) 6:17–22

3 PPD Action Spectrum A.Chardon, D.Moyal, C.Hourseau Persistent pigment darkening response as a method for evaluation of Ultraviolet A protection assays. In Sunscreens, Development, Evaluation,and regulatory aspects. N.Lowe, N.Shaath, M.Pathak, eds. NY, Marcel Dekker,(1997) 559-582.

4 D.Moyal, A.Chardon, N.Kollias. UVA protection efficacy of sunscreens can be determined by the persistent pigment darkening (PPD) method (part 2). Photodermatol Photoimmunol Photomed (2000) 16:250-255.

5 Bernhard G, Mayer B, Seckmeyer G, Moise A ”Measurement of spectral solar irradiance in tropical Australia” J Geophys Res, (1997) 102:8719–8730,

6 COLIPA (European Cosmetic Toiletry and Perfumery Association). *SPF Test Method* (1994) Brussels: COLIPA.

7 DIN 67501: Evaluation of sunscreen products (1985) Berlin: Beuth

8 ISO TC217 WG7 In Vitro UVA Ring Test Report, 2009. In preparation for publication.

9 M. Pissavini, S. Marguerie, A. Dehais, L. Ferrero and L. Zastrow, Characterizing Roughness: A New Substrate to Measure SPF Cosmet. Toiletries, (2009) 9:56-62

10 Matts, P. J. *et al*., COLIPA *in vitro* UVA method: a standard and reproducible measure of sunscreen UVA protection, Int. J. Cosmet. Sci., (2010) 32(1):35-46,

11 COLIPA (European Cosmetics Association) Method for the *in vitro* determination of UVA protection provided by sunscreen products. (2009) Brussels: COLIPA