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Cosmetics — Sun protection test methods — In vitro determination of sun protection factor (SPF)

Cosmétiques — Méthodes d'essai de protection solaire — Détermination in vitro du facteur de protection solaire (FPS)

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Foreword

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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).

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

Chronic exposure to solar ultraviolet radiation (UVR) is the main environmental source of damage to human skin. Consumer protection against exposure to solar UVB and UVA radiation is, therefore, an important public health issue. The use of sunscreens is a critical part of holistic programs of consumer UVR protection, including the use of appropriate clothing, hats and minimising exposure to the sun around its zenith.

The in vivo sun protection factor (SPF) is historically measured by an in vivo method (see ISO 24444) to communicate the amplitude of protection offered by sunscreens from erythemally-effective solar UVR.[1][2] In recent years, additional test methods have been developed to measure the breadth of protection from solar UVR, namely the in vivo human persistent pigment darkening (PPD) test[3] (and associated UVA-PF) and an in vitro equivalent.[4][5][6][7]

Invasive methods based on tests conducted on human beings are ethically problematic, time-consuming and very costly. Therefore, it has for long been a desire to develop an in vitro SPF test method,[8][9][10][11][12][13][14][15][16][17] recognising the potential advantages of such methodology, including:

a) the use of a non-human model,

b) the significant improvements in speed and cost,

c) the improved repeatability and reproducibility,

d) the elimination of technically-challenging procedures (e.g., MED determination) and

e) the use of a method which is significantly more amenable to continuous improvement.

This in vitro SPF method is based on UVR transmittance spectroscopy, whereby spectrophotometric measurement of UVR transmission through appropriate UVR-transparent substrates, allows prediction of in vivo SPF values.[18][19][20][21][22] This in vitro SPF method revealed a strong reproducibility and correlation with in vivo SPF values.[23][24][25]

Cosmetics — Sun protection test methods — In vitro determination of sun protection factor (SPF)

1 Scope

This document specifies a method for the in vitro determination of sun protection factor (SPF). This method is applicable to sunscreen products in form of an emulsion or alcoholic one-phase formulation, excluding in form of a loose or compressed powder or stick. Specifications are given to enable determination of the spectral absorbance characteristics of SPF protection in a reproducible manner.

Use of this method is strictly for the determination of a static sun protection factor. It is not applicable for the determination of water-resistance properties of a sun protection product.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 24444, *Cosmetics — Sun protection test methods — In vivo determination of the sun protection factor (SPF)*

3 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
sunscreen product
product containing any component able to absorb, reflect or scatter UV rays, which are intended to be placed on the surface of human skin with the purpose of protecting against erythema and other ultraviolet induced damage

3.2
emulsion
fine dispersion of minute droplets of one liquid in other(s) in which it is not soluble or miscible

3.3
in vitro sun protection factor
$SPF_{in\ vitro}$
protection factor of a sun protection product against erythema-inducing radiation calculated with spectral modelling between 290 nm and 400 nm

3.4
reference solar spectrum
$I_{sol}(\lambda)$
spectral irradiance of mid-summer sunlight in the spectral range of 290 nm to 400 nm, at a latitude of 40 °N, a solar zenith angle of 20° and an ozone layer thickness of 0,305 cm, as defined in Annex A

3.5
solar UVR simulator
solar ultraviolet radiation simulator
light source emitting a continuous spectrum [S(λ)] with no gaps or extreme peaks of emission in the UV region

Note 1 to entry: The solar simulator has a spectral quality that complies with the required acceptance limits in Annex A.

3.6
erythemal action spectrum
***E*(λ)**
relative effects of individual spectral bands of an exposure source for an erythema response

Note 1 to entry: The symbol for the erythemal action spectrum is defined as $s_{er}(\lambda)$ in ISO/CIE 17166 and $E(\lambda)$ in the ISO 24443.

Note 2 to entry: This entry was numbered 17-401 in CIE S 017:2011.

[SOURCE: CIE-ILV 17-26-065]

3.7
spectrophotometer
instrument for measuring the ratio of 2 values of a radiometric quantity at the same wavelength

Note 1 to entry: This entry was numbered 17-1235 in CIE S 017:2011.

[SOURCE: CIE-ILV 17-25-008]

3.8
monochromatic absorbance
***A*(λ)**
sunscreen absorbance at wavelength λ calculated as logarithm to base 10 of the reciprocal of the spectral internal transmittance, $T(\lambda)$

$$A(\lambda) = -\log 10\ T(\lambda)$$

Note 1 to entry: This entry was numbered 17-1207 in CIE S 017:2011.

[SOURCE: CIE-ILV 17-24-090]

3.9
irradiance at a point of surface
***I*(λ)**
quotient of the radiant flux $d\Phi e$ incident on an element of the surface containing the point, by the area dA of that element

Note 1 to entry: Expressed in $W \cdot m^{-2}$.

Note 2 to entry: Note that the symbol for the irradiance is defined as E in CIE-ILV 017:2020 but because it could be confused with the symbol used in ISO 24443:2021 for the erythemal action spectrum, here we use $I(\lambda)$.

Note 3 to entry: This entry was numbered 17-608 in CIE S 017:2011.

[SOURCE: CIE-ILV 17-21-053]

3.10
spectroradiometer
instrument for measuring radiometric quantities in narrow wavelength intervals over a given spectral region

Note 1 to entry: This entry was numbered 17-1236 in CIE S 017:2011.

[SOURCE: CIE-ILV 17-25-007]

3.11
radiometer
instrument for measuring the intensity of electromagnetic radiation (UV radiation specifically for this standard)

Note 1 to entry: In the context of this document, a UV radiometer measures the irradiance for the UV spectral range from 290 nm to 400 nm.

Note 2 to entry: This entry was numbered 17-1031 in CIE S 017:2011.

[SOURCE: CIE-ILV 17-25-006]

3.12
reference sunscreen formula
product used to validate the testing procedure

3.13
dose
UV exposure dose ($J \cdot m^{-2}$) for pre-irradiation of sunscreen products

Note 1 to entry: The UV dose is the product of UV intensity (expressed as energy per unit surface area) and time.

3.14
plate
piece of polymethylmethracylate (PMMA) on which test product is to be applied for absorbance measurements

Note 1 to entry: See Annex B.

3.15
erythemal irradiance
$I_{ER}(\lambda)$
effective irradiance computed from the product of the spectral irradiance, $I(\lambda)$ and the erythema spectral weighting function, $s_{er}(\lambda)$

Note 1 to entry: Expressed in $W \cdot m^{-2}$.

Note 2 to entry: This entry was numbered 17-403 in CIE S 017:2011.

[SOURCE: CIE-ILV 17-26-067]

3.16
UVB
electromagnetic radiation in the range of 290 nm to 320 nm

3.17
UVA
electromagnetic radiation in the range of 320 nm to 400 nm

3.18
UVA-I
electromagnetic radiation in the range of 340 nm to 400 nm

3.19
UVA-II
electromagnetic radiation in the range of 320 nm to 340 nm

3.20
percentage relative cumulative erythemal effectiveness
% RCEE
description of the spectral distribution of the solar simulator in terms of cumulative erythemal effective irradiance by successive wavelength bands, as defined in Annex A

4 Principles

The test is based on the assessment of UV-transmittance through a thin film of sunscreen spread on at least three moulded PMMA plates and on at least three sandblasted surface PMMA plates, before and after exposure to a controlled dose of radiation from a solar simulator. Samples submitted for testing should not have a SPF or UVA-PF target or other protection category description.

5 Reagents and/or materials

5.1 Sample substrate — Double plate

Moulded and sandblasted PMMA plates shall be used for sunscreen application according to this method (see Annex B).

5.2 Reference sunscreen

The formulae details and manufacturing instructions for the reference formulations are given in Annex C. At least once a month, the following reference standards shall be tested: P2 or P3 reference standard, and P5 or P6 reference standard, and P8 reference standard. The results shall be within the respective acceptance ranges given in Table C.1, Annex C.

5.3 Finger-cot

Finger-cots should be manufactured from untextured and un-powdered latex. As example, for a probe as F.2, a finger-cot of a medium size should be used. If alternative finger-cots are used, a validated equivalent result shall be demonstrated in this method. The finger-cot should be tight on the robot finger probe without ripples or breaks where product can get caught.

5.4 Blank

Glycerin or a modified glycerin solution (see Table B.1), or white petroleum in accordance with Annex D shall be used for blank measurement.

6 Apparatus

6.1 Spectrophotometers

6.1.1 Specification

The spectrophotometer shall span the spectral range from 290 nm to 400 nm. The wavelength increment step shall be 1 nm. A spectrophotometer that does not use a monochromator to analyse the reflected or transmitted radiation from the test sample should employ a fluorescence rejection filter. Its input optics should be designed for diffuse illumination and/or diffuse collection of the transmitted irradiance through the roughened polymethylmethacrylate (PMMA)plate, with and without the sunscreen layer spread on its surface. The size of the diameter of the entrance port of the spectrophotometer probe should 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 shall 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 E). 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 in accordance with Annex E. 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.

In order to minimise scatter, the distance between the closest side of the plate and the emission source or the integrating sphere should not be more than 2 mm. The plate shall be positioned in a horizontal plane during all steps including UV measurement steps.

The lamp in the spectrophotometer (or spectroradiometer) that is used to measure the absorbance shall emit a continuous spectrum of radiation (with no gaps or extreme peaks of emission in the UV region) 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, wherein the UV dose during one measurement cycle should not exceed 0,2 J·cm^{-2}.

6.1.2 Monitoring

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

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

— dynamic range of the spectrophotometer;

— linearity test of the spectrophotometer;

— wavelength trueness test;

— absolute transmission trueness.

6.2 Automatic positive-displacement pipette

Positive displacement pipettes, micro-pipettes, automatic pipettes or any similar device should use piston-driven displacement and shall be capable of delivering accurate and repeatable aliquots of approximately 1,6 mg to 1,8 mg of a sunscreen product (as described in 7.3.1).

NOTE 1,6 mg to 1,8 mg correspond to approximately 1,6 µl to 1,8 µl, respectively.

6.3 Analytical balance

A laboratory balance with at least 10^{-4} g precision shall be used.

6.4 Robot

6.4.1 Specifications

The robot shall be in accordance with Annex F and shall have:

a) positional repeatability of at least ±0,1 mm in *x*, *y* and *z* axes,

b) degrees of freedom equal to at least 6 rotating joints,

c) a payload of at least 0,5 kg,

d) a vertical force (*z* axis), measured in the centre of the plate (with the finger tool and finger cot, without *x* and *y* axis movement), of (6,0 ± 0,5) N.

6.4.2 Monitoring

The robotic appliance shall be checked by a suitably qualified expert at regular intervals (at least every twelve months) to ensure compliance to the mechanical and spreading specifications given in 6.4.1.

The finger tool shall be replaced after every cycle of 400 spreading operations or when damaged (e.g. cracks, etc.).

6.5 Solar simulator

6.5.1 General

A xenon arc solar simulator with appropriate filters should be used and shall conform with the spectral specifications described in Table A.1 and Figure A.1. It shall be able to maintain a stable plate temperature of (27 ± 2) °C.

6.5.2 Quality of solar simulator radiation

The output from the solar UVR simulator shall be continuous, uniform, stable, with no gaps or extreme peaks of emission in the UVR region and suitably filtered to create a spectral quality that complies with the required acceptance limits (see Table A.1).

To ensure that appropriate amounts of UVA radiation are included in the spectrum of the solar UV simulator, the total radiometric proportion of UVA-II irradiance of the simulator (320 nm to 340 nm) shall be ≥ 20,0 % of total UVR irradiance (290 nm to 400 nm) in accordance with ISO 24444 which requires the same solar irradiance. Additionally, UVA-I region (340 nm to 400 nm) irradiance shall be ≥ 60 % of total UVR irradiance. The source spectral specification is described in terms of cumulative erythemal effective irradiance by successive wavelength bands, 290 nm to 400 nm. The erythemal effective irradiance of each wavelength band is expressed as a percentage of total erythemal effective irradiance, 290 nm to 400 nm, or as percentage relative cumulative erythemal effectiveness (%RCEE). The calculation of %RCEE values shall be in accordance with Annex A, where acceptance limits are shown in Table A.1.

Total irradiance shall not exceed 200 $W \cdot m^{-2}$. The output of the solar simulator shall be measured with a broad-spectrum sensor (capable of measuring between 280 nm and 1 600 nm) calibrated against a standard reference source over the range of 280 nm to 1 600 nm. Alternatively, the source may be measured with a calibrated spectroradiometer over this same wavelength range to determine the total irradiance.

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 (and a new beam uniformity control shall be performed) or appropriate compensation for different irradiance shall be made in the exposure time on each plate.

6.5.3 Maintenance and monitoring the solar simulator

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 and after changing any significant physical (optical) component of the solar simulator (including the bulb only if the bulb was not already previously calibrated with the associated solar simulator). 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. Prior to the UV exposure of sample, the UV intensity of the exposure source output shall be measured and recorded with a spectroradiometer (as detailed in 6.1) or an erythema weighted radiometer cross-calibrated against a spectroradiometric measurement of each assigned solar simulator output as detailed in 6.5.2. Optical alignment shall be configured to ensure accurate radiometer alignment and reproduction of the assigned simulator output at the same optical reference

plane measured with the spectroradiometer. A calibration factor Y for each radiometer with assigned solar simulator output shall be determined by Formula (2):

$$Y = I_{ersp}/I_{err} \quad (2)$$

where

Y is the calibration factor for each radiometer;

I_{ersp} is $I(\lambda) \times s_{er}(\lambda)$ measured by the spectroradiometer;

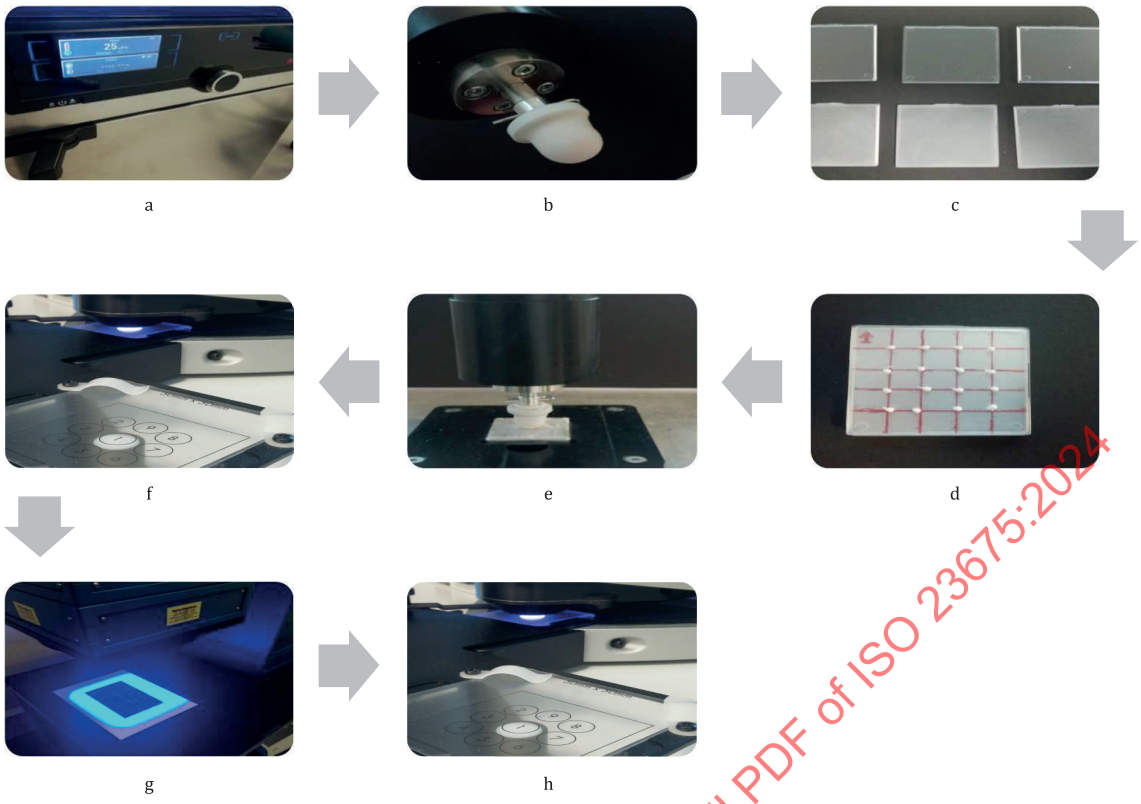
I_{err} is $I(\lambda) \times s_{er}(\lambda)$ measured by the radiometer.

7 Procedure

7.1 Outline of the test procedure

1) Preparation of reagents and materials.
2) Product application on plates and robot automatic spreading.
3) Measurement of initial absorbance using two plate types (290 nm to 400 nm).
4) Calculation of initial in vitro SPF.
5) Calculation of irradiation dose (based on initial in vitro SPF).
6) Irradiation with calculated dose.
7) Measurement of final post-irradiation absorbance using two plate types (290 nm to 400 nm).
8) Calculation of final in vitro SPF.

See Figure 1.



a Incubation at (27 ± 2) °C, at least 12 h before.
b Finger tool and finger cot installation.
c At least three moulded PMMA plates and three sandblasted plates.
d Product deposit.
e Spreading and drying step.
f First absorbance measurements.
g UV exposure.
h Second absorbance measurements.

Figure 1 — Key steps of the method

7.2 Preparation of reagents and materials

7.2.1 Plate preparation and handling

Three (at least) moulded PMMA plates and three (at least) sandblasted plates shall be used, each in accordance with the specifications in Annex B.

Plates and product shall be stored, in the dark, at (27 ± 2) °C for at least twelve hours before the start of the test.

The surface of sandblasted plates should be cleaned with a dry, antistatic microfibre cloth.

The plates should be handled carefully by holding them by the edges, avoiding finger contact with the surface.

A reference mark should be made on edge of each plate, outside of spreading area, to ensure the whole measurement process proceeds in the same order with plate placed in same orientation each time.

The plates shall be used without additional treatment on surface (chemical and/or physical). The plates shall be used only once.

7.2.2 Finger cot

7.2.2.1 Preparation

A latex finger-cot is first placed on the robot finger probe. It is important to verify that there are no creases in the finger-cot after fitting and to verify that the finger cot remains firmly in place and does not break during the spreading procedure. If creases should appear on the finger-cot or if it breaks during the spreading procedure, it shall be replaced, and the plate currently being spread shall be discarded.

7.2.2.2 Saturation

To ensure saturation of the finger cot, a standard spreading procedure is first performed with a moulded PMMA plate, using the same protocol as described in 7.3. This plate shall be discarded and is not part of the final calculation. The same finger cot can be used for the complete set of plates of one product test, unless it gets damaged in the process. Each time that there is a change in finger cot, the saturation procedure shall be repeated.

7.3 Product application on plates and robot automatic spreading

7.3.1 Weighing of product and application on plates

Liquid sunscreen products shall be shaken well before application to the plates.

Plates shall, first of all, be placed onto the weighing pan of an analytical balance (with the roughened side uppermost), in order to check the weight of the plate before and after application of product.

Using an automatic positive-displacement pipette capable of dispensing repeated identical aliquots of product:

— moulded plate: 1,3 mg.cm^{-2} (±1,6 %) of product is applied to each plate;[17][19];

— sandblasted plate: 1,2 mg.cm^{-2} (±1,5 %) of the same product is applied to each plate.[17][19]

NOTE 1 For viscous/gelled products for which the use of an automatic volumetric pipette would be difficult, the sampling speed can be reduced or the end of the tip can be cut to widen the opening. If these measurements remain insufficient, another tool can be used, such as a spatula by applying a minimum of 12 points (ideally 16 points) evenly.

For the alcoholic one phase formulations, the application dose shall be determined while reducing the evaporation lost during application.

NOTE 2 For application of the alcoholic one phase formulations, when the type of plates allows it, the plates can be tared by superimposing 2 plates on top of each other (plate A-2 on the top of the plate A-1). The product is applied on the plate below (plate A-1), then immediately covered by the one above (plate A-2) to limit evaporation during weighing control. The 2 plates (plate A-1 and plate A-2) are then placed on the robot platform and the top plate (plate A-2) is removed just as the spreading process begins.

Spray products supplied in a pressure container shall first be sprayed in a jar or be degassed by puncturing a very small pinhole in the container to relieve all of the pressure, then left to stand for at least 24 h at room temperature before accessing the liquid to be tested.

Spreading area is defined as the whole area of the plate. Droplet application should be uniform, achieved with at least 16 equally-spaced droplets of approximately 1,6 mg to 1,8 mg each (depending on the density of the applied products; as described in the diagram here after for example) as Figure 2.

NOTE 3 1,6 mg to 1,8 mg correspond to approximately 1,6 µl to 1,8 µl, respectively.

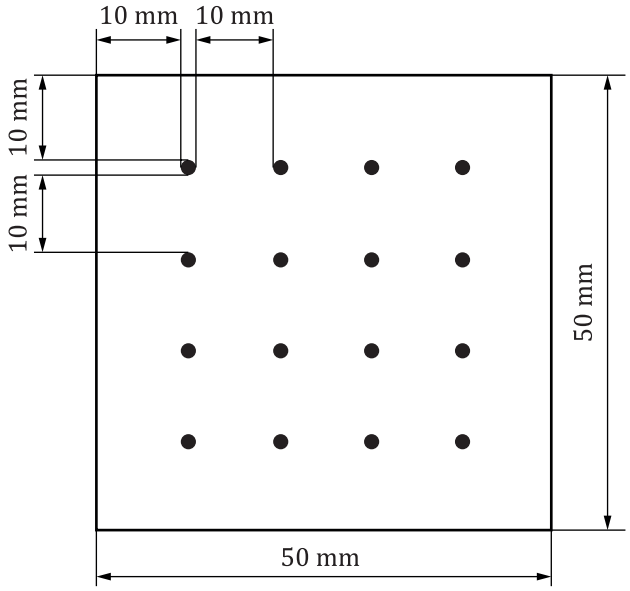


Figure 2 — Diagram of droplet application

If the applied weight is too low, up to four more equal droplets can be added to the plate in a uniform manner, to achieve the desired target final weight. If the applied weight is too high, product can be removed carefully using the pipette tip, until the desired weight is achieved.

Deposit and weighing shall not take more than 30 s. After the sunscreen product is deposited on the surface of the plate, it shall be spread immediately over the whole surface.

7.3.2 Automatic spreading

Once the product is deposited on plates (refer to 7.3.1), each plate is immediately placed on the measurement stage of the robot and the automated spreading sequence starts (see Figure F.1 and Figure F.2). For each plate, the time between the start of droplet application and placing the plate on the robot measurement stage shall be no more than 30 s. The spreading should be completed within one minute after placing of the plate on the robot measurement stage.

Immediately after the robot application sequence stops, the plates shall be placed in a dark environment for at least 30 min (up to a maximum of 60 min) at (27 ± 2) °C.

7.4 Measurement of initial absorbance using two plate types (290 nm to 400 nm)

7.4.1 Blank measurement

It is necessary to first determine the absorbance of UVR radiation through "blank" PMMA plates to establish the baseline for the measurement device. Therefore, a "blank" plate should be prepared for both moulded and sandblasted plates by spreading a few microlitres of glycerine/white petroleum on the roughened side of the plate. This "blank" measurement should be done at least with each new batch of plates. The amount of glycerine/modified glycerine solution/white petroleum should be such that the entire surface is completely covered without excess (approximately 15 mg for a 50 mm × 50 mm ± 4 % plate) and spread manually or by the robot using a new finger cot. UVR transmission through plates spread in this way should be measured using the spectrophotometer and used as the "blank" or subsequent spectrophotometric measurement (many spectrophotometers have a function to automatically account for baseline values in subsequent measurements).

7.4.2 Initial absorbance measurement

An equal number of moulded and sandblasted plates shall be used. A minimum of three valid plates of each type is required, and a maximum of 10 valid plates of each type shall be used.

The absorption spectrum (290 nm to 400 nm) of product films spread on either moulded or sandblasted plates is measured using a calibrated spectrophotometer.

If a large beam of at least 16 cm^2 is used, measurements should be taken at one centre location within the plate. If a small beam is used, then at least 5 (ideally 9) equally-spaced and non-overlapping locations on the plate shall be used, within a total area of at least 16 cm^2 centred around the centre of the plate.

Initial absorbance (290 nm to 400 nm) shall be measured after at least 30 min and no more than 60 min after product spreading, using the spectrophotometric procedure described above. Care shall be taken to measure absorbance on the same plate locations (using a standard template and the reference mark).

For each plate location and each wavelength, wherever the measured absorbance is above 2,2 (i.e. monochromatic protection factor is above 158), it shall be capped at 2,2.

Pairing of plate shall be performed by pairing the 1st moulded plate with the 1st sandblasted plate, the 2nd moulded plate with the 2nd sandblasted plate and the 3rd moulded plate with the 3rd sandblasted plate and shall be kept during the whole protocol.

Initial absorbance data from both plate types (i.e. called a pair) are then combined to yield a single term describing initial absorbance, using Formula (3):

$$A_{Initial,i}(\lambda) = C_{Moulded} * min\left\{2,2; A^{pre-irradiation}_{Moulded,i}(\lambda)\right\} + C_{Sandblasted} * min\left\{2,2; A^{pre-irradiation}_{Sandblasted,i}(\lambda)\right\} \quad (3)$$

where

$A^{pre-irradiation}_{Moulded,i}(\lambda)$ is the absorbance of sunscreen on the moulded plate before UV exposure;

$C_{Moulded}$ is 0,225 for emulsion and 0,000 for alcoholic one-phase formulation;

$A^{pre-irradiation}_{Sandblasted,i}(\lambda)$ is the absorbance of sunscreen on the sandblasted plate before UV exposure;

$C_{Sandblasted}$ is 0,800 for emulsion and 0,800 for alcoholic one-phase formulation .

This step shall be conducted separately for each pair.

7.4.3 Calculation of pre-irradiation in vitro SPF_i

For each pair of plate, pre-irradiation in vitro SPF_i values are calculated from $A_{Initial,i}(\lambda)$ as expressed above, using Formula (4):

$$SPF_{in\ vitro,pre-irradiation,i} = \frac{\int_{290}^{400} E(\lambda) I_{sol}(\lambda) d\lambda}{\int_{290}^{400} E(\lambda) I_{sol}(\lambda) 10^{-A_{Initial,i}(\lambda)} d\lambda} \quad (4)$$

where

$E(\lambda)$ is the CIE erythemal action spectrum[26];

$I_{sol}(\lambda)$ is the Reference solar spectrum;

$A_{Initial,i}(\lambda)$ is the mean monochromatic absorbance of the test product layer before UV exposure from Formula (1);

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

This step shall be conducted for each pair separately.

The pre-irradiation in vitro SPF_i is rounded to one decimal place.

7.5 Calculation of irradiation dose (based on pre-irradiation in vitro SPF_i)

Sun filter photo-stability is an important parameter that influences results in in vivo testing and this, therefore, shall be taken into account in in vitro testing.

After calculation of pre-irradiation in vitro SPF_i, the irradiation dose Dx (of full-spectrum solar-simulated radiation, expressed in $J \cdot m^{-2}$ eff) is calculated using Formula (5):

$$Dx = 0{,}25 \times SPF_{\text{in vitropre-irradiation}} \times 210 \quad (5)$$

This step shall be conducted for each pair separately.

7.6 Irradiation with calculated dose

A warm-up time of at least 20 min shall be allowed for the UV simulator to stabilise before starting.

The intensity of the UV exposure source used for exposure shall be checked at the level of the plate as described in 6.5.2. The measured intensity is used to determine the exposure time of the plates in order to achieve an irradiation with Dx.

Immediately (up to maximum 30 minutes) after initial absorbance measurement, irradiate each pair of sunscreen-treated plates with its associated irradiation dose (calculated in Formula (5) of full-spectrum solar-simulated radiation using a solar simulator (see Annex A), at a stable, sample-level temperature of (27 ± 2) °C.

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).

7.7 Measurement of post-irradiation absorbance using two plate types

Post-irradiation absorbance (290 nm to 400 nm) is measured immediately (up to maximum 30 minutes) after UV exposure using the spectrophotometric procedure described above. Care should be taken to measure absorbance on the same plate locations used in 7.4.2 (using the same standard plate template and reference mark location).

For each plate location and each wavelength, wherever the measured absorbance is above 2,2 (i.e. monochromatic protection factor is above 158) it shall be capped at 2,2.

The final absorbance value spectrum shall be used in 7.8.

7.8 Calculation of post-irradiation in vitro SPF_i

Post-irradiation in vitro SPF_i value of each pair is calculated using the Formula (6). Pairing of plate shall be kept unchanged from 7.4.2 by pairing the 1st moulded plate with the 1st sandblasted plate, the 2nd moulded plate with the 2nd sandblasted plate and the 3rd moulded plate with the 3rd sandblasted plate, i.e. at least 3 combinations:

$$SPF_{\text{in vitro},post-irradiation,i} = \frac{\int_{290}^{400} E(\lambda) I_{sol}(\lambda) d\lambda}{\int_{290}^{400} E(\lambda) I_{sol}(\lambda) 10^{-A_{Final,i}(\lambda)} d\lambda} \quad (6)$$

where $E(\lambda)$, $I_{sol}(\lambda)$ and $d\lambda$ are defined in Formula (4);

$$A_{Final,i}(\lambda) = C_{Moulded} * min\left\{2{,}2; A^{post-irradiation}_{Moulded,i}(\lambda)\right\} + C_{Sandblasted} * min\left\{2{,}2; A^{post-irradiation}_{Sandblasted,i}(\lambda)\right\}$$

with

C_{Moulded} and $C_{\text{Sandblasted}}$ are the correction factors defined in Formula (3);

$A^{post-irradiation}_{Moulded,i}(\lambda)$ is the absorbance of the exposed moulded plate;

$A^{post-irradiation}_{Sandblasted,i}(\lambda)$ is the absorbance of the exposed sandblasted plate.

The post-irradiation in vitro SPF_i is rounded to one decimal place.

7.9 Calculation of final in vitro SPF_i of each pair of plates

The final in vitro SPF_i of each pair of plates is then calculated using the following Formula (7) or Formula (8):

$$If\ SPF_{\text{in vitro,post-irradiation,i}} \geq 3{,}5 : SPF_{\text{in vitro,post-irradiation,final,i}} = \frac{\sqrt{SPF_{\text{in vitro,post-irradiation,i}}} - 1{,}457}{0{,}107} \quad (7)$$

$$If\ SPF_{\text{in vitro,post-irradiation,i}} < 3{,}5 : SPF_{\text{in vitro,post-irradiation, final,i}} = SPF_{\text{in vitro,post-irradiation,i}} \quad (8)$$

NOTE This model is based on ring test characterization study results.

The final in vitro SPF_i is rounded to one decimal place.

7.10 Calculation of final in vitro SPF of the product

7.10.1 General

The final in vitro SPF of the product is the arithmetical mean of the individual plate pairs. It is calculated using the total number (n) of pairs used, expressed to one decimal point (truncation):

$$SPF_{\text{in vitro,final}} = \left(\sum SPF_{\text{in vitro,final,i}} \right) / n$$

The final in vitro SPF is rounded to one decimal place .

The standard deviation (s) is defined thus:

$$s = \sqrt{\left[\left(\sum \left(SPF_{\text{in vitro,final,i}} \right)^2 - \left(\left(\sum SPF_{\text{in vitro,final,i}} \right)^2 / n \right) \right) / (n-1) \right]}$$

7.10.2 Validation of final in vitro SPF

At least three pairs of treated plates shall be used to establish the final in vitro SPF value of the test sample. If the 95 % confidence interval (CI) of the calculated final in vitro SPF value is greater than 17 % of the mean, additional pairs of treated plates can be added until the 95 % CI is reduced to below 17 % of the mean. If this criterion is not fulfilled after ten valid pairs, then the entire test shall be rejected and repeated.

The 95 % confidence interval (95 % CI) of the mean final in vitro SPF is expressed as:

$$95\ \%\ CI = (SPF_{in\ vitro,final} - c)\ \text{to}\ (SPF_{in\ vitro,final} + c)$$

c is calculated as:

$$c = (t) \times SEM = (t) \times s/\sqrt{n}$$

The percentage half-range confidence interval is calculated as:

$$CI[\%] = 100 \times c/SPF_{in\ vitro,final}$$

where

SEM is the standard error of the mean;

n is the total number of pairs of plates used;

t is the t value from the "two-sided" student-t distribution table at a probability level $p = 0{,}05$ and with degrees of freedom $v = (n - 1)$.

8 Test report

Test reports describing the determination of in vitro SPF of a sunscreen product using this method shall, at a minimum, contain the following information:

a) name and address of testing facility;

b) identification of the individual conducting the test;

c) reference to this test method;

d) sample identification and date of the testing;

e) name of company and product references (e.g., batch identification/other reference(s) to identify the product);

f) constant "C" for moulded (0,225 for emulsion and 0,000 for alcoholic one-phase-formulation) and sandblasted (0,800 for emulsion and 0,800 for alcoholic one-phase formulation) plate;

g) detailed description of instruments used, manufacturer and instrument model with the system calibration summary in this document;

h) detailed information on test plates used (plate manufacturer, batch code, confirmation of adherence to plate certificate ref to Annex B);

i) the calibration factor "*Y*" used to adjust the radiometer measurement with the reference spectroradiometer measurement of the UV exposure source (see 6.5.2);

j) UV irradiance (MED/hr) and irradiation dose value *Dx* (MED) used to irradiate the test sample;

k) individual data for each pair (pre-exposure and post-exposure);

l) mean final in vitro *SPF* of the tested products, rounded to one decimal place, standard deviation on the mean, and 95 % CI as a number and as a %, and 17 % of the mean final in vitro SPF;

m) mean UV absorbance values at each 1 nm wavelength increment for the test sample;

n) statistical data (e.g., Confidence Interval, standard deviation);

o) final in vitro SPF results (rounded to one decimal place) of three reference sunscreen formulations tested within the last 30 days prior to the test of the test sample (see 5.2), with standard deviation and Confidence Interval and accompanying statement that the reference standard SPF results fall within the respective acceptance limits for the standards as per Table C.1 in Annex C;

p) protocol deviation if any.

The report can be generated automatically using the calculation spreadsheet "ISO-23675_SPF-Calculation.xls", which is available at https://standards.iso.org/iso/23675/ed-1/en/.

Annex A
(normative)

UV exposure and erythemal action spectra and solar simulator UV spectrum

Table A.1 — UV exposure and erythemal action spectra and solar simulator UV spectrum

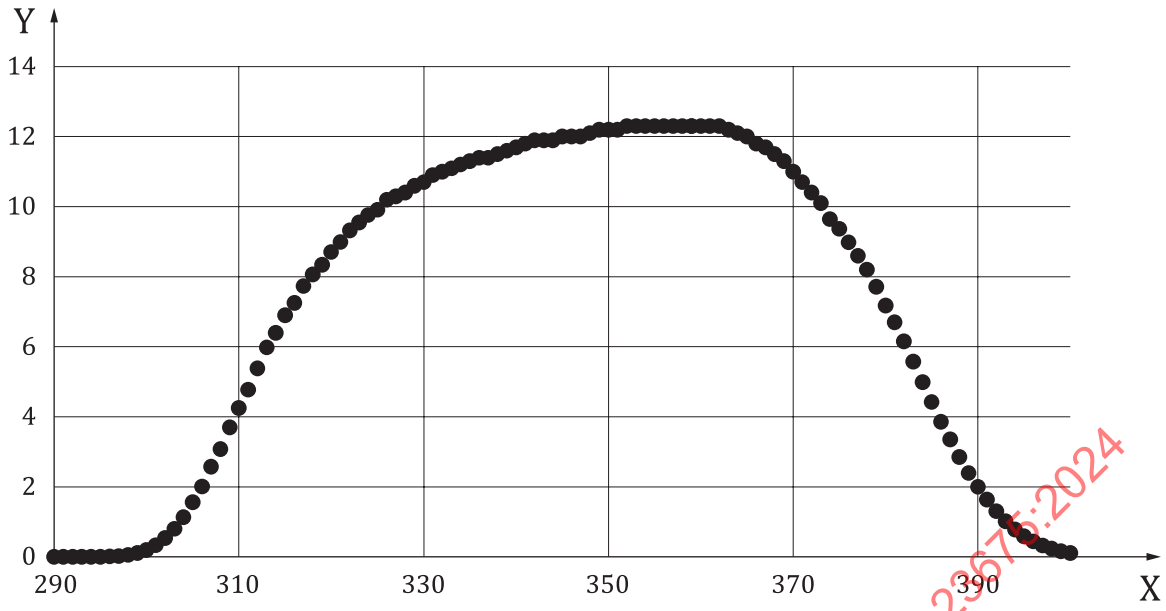
	UV calculation	Erythema	Reference solar spectrum	Sol. Sim.	RCEE accept. Range	
	Midday midsummer global irradiance at 40 °N ($W \cdot m^{-2} \cdot nm^{-1}$)	Action Spectrum	SSR ($W \cdot m^{-2} \cdot nm^{-1}$)	%RCEE	Lower	Upper
nm	$I_{sol}(\lambda)$	$E(\lambda)$	$S(\lambda)$	Sum{E*S}/T	limit	limit
290	**3,680E-06**	**1,00E+00**	**4,41E-05**	**0,00 %**	**-**	**< 0,1 %**
291	1,300E-05	1,00E+00	5,50E-05			
292	4,500E-05	1,00E+00	8,28E-05			
293	1,300E-04	1,00E+00	2,38E-04			
294	3,400E-04	1,00E+00	8,22E-04			
295	7,970E-04	1,00E+00	2,69E-03			
296	1,650E-03	1,00E+00	8,03E-03			
297	3,000E-03	1,00E+00	2,10E-02			
298	5,500E-03	1,00E+00	5,03E-02			
299	8,500E-03	8,05E-01	1,04E-01			
300	**1,280E-02**	**6,49E-01**	**1,89E-01**	**4,00 %**	**1,00 %**	**8,00 %**
301	2,100E-02	5,22E-01	3,35E-01			
302	3,000E-02	4,21E-01	5,36E-01			
303	4,100E-02	3,39E-01	8,05E-01			
304	5,300E-02	2,73E-01	1,13E+00			
305	6,510E-02	2,20E-01	1,56E+00			
306	8,400E-02	1,77E-01	2,01E+00			
307	9,700E-02	1,43E-01	2,58E+00			
308	1,200E-01	1,15E-01	3,08E+00			
309	1,450E-01	9,25E-02	3,70E+00			
310	**1,710E-01**	**7,45E-02**	**4,25E+00**	**55,70 %**	**49,00 %**	**65,00 %**
311	1,950E-01	6,00E-02	4,77E+00			
312	2,150E-01	4,83E-02	5,38E+00			
313	2,450E-01	3,89E-02	5,98E+00			
314	2,700E-01	3,13E-02	6,40E+00			
315	2,950E-01	2,52E-02	6,90E+00			
316	3,100E-01	2,03E-02	7,25E+00			
317	3,300E-01	1,64E-02	7,73E+00			
318	3,500E-01	1,32E-02	8,06E+00			
319	3,700E-01	1,06E-02	8,34E+00			
320	**3,980E-01**	**8,55E-03**	**8,70E+00**	**87,40 %**	**85,00 %**	**90,00 %**
321	4,050E-01	6,89E-03	8,99E+00			
322	4,300E-01	5,55E-03	9,32E+00			
323	4,600E-01	4,47E-03	9,55E+00			
324	5,000E-01	3,60E-03	9,76E+00			
325	5,360E-01	2,90E-03	9,91E+00			

Table A.1 *(continued)*

	UV calculation	Erythema	Reference solar spectrum	Sol. Sim.	RCEE accept. Range	
	Midday midsummer global irradiance at 40 °N ($W \cdot m^{-2} \cdot nm^{-1}$)	Action Spectrum	SSR ($W \cdot m^{-2} \cdot nm^{-1}$)	%RCEE	Lower	Upper
nm	$I_{sol}(\lambda)$	$E(\lambda)$	$S(\lambda)$	Sum{E*S}/T	limit	limit
326	5,450E-01	2,33E-03	1,02E+01			
327	5,650E-01	1,88E-03	1,03E+01			
328	6,000E-01	1,51E-03	1,04E+01			
329	6,150E-01	1,41E-03	1,06E+01			
330	**6,300E-01**	**1,36E-03**	**1,07E+01**	**93,30 %**	**91,50 %**	**95,50 %**
331	6,350E-01	1,32E-03	1,09E+01			
332	6,370E-01	1,27E-03	1,10E+01			
333	6,420E-01	1,23E-03	1,11E+01			
334	6,470E-01	1,19E-03	1,12E+01			
335	6,500E-01	1,15E-03	1,13E+01			
336	6,520E-01	1,11E-03	1,14E+01			
337	6,600E-01	1,07E-03	1,14E+01			
338	6,700E-01	1,04E-03	1,15E+01			
339	6,750E-01	1,00E-03	1,16E+01			
340	**6,800E-01**	**9,66E-04**	**1,17E+01**	**95,50 %**	**94,00 %**	**97,00 %**
341	6,820E-01	9,33E-04	1,18E+01			
342	6,840E-01	9,02E-04	1,19E+01			
343	6,860E-01	8,71E-04	1,19E+01			
344	6,880E-01	8,41E-04	1,19E+01			
345	6,900E-01	8,13E-04	1,20E+01			
346	6,920E-01	7,85E-04	1,20E+01			
347	6,940E-01	7,59E-04	1,20E+01			
348	6,960E-01	7,33E-04	1,21E+01			
349	6,980E-01	7,08E-04	1,22E+01			
350	**7,000E-01**	**6,84E-04**	**1,22E+01**	**97,20 %**		
351	7,020E-01	6,61E-04	1,22E+01			
352	7,040E-01	6,38E-04	1,23E+01			
353	7,060E-01	6,17E-04	1,23E+01			
354	7,080E-01	5,96E-04	1,23E+01			
355	7,100E-01	5,75E-04	1,23E+01			
356	7,140E-01	5,56E-04	1,23E+01			
357	7,180E-01	5,37E-04	1,23E+01			
358	7,220E-01	5,19E-04	1,23E+01			
359	7,260E-01	5,01E-04	1,23E+01			
360	**7,300E-01**	**4,84E-04**	**1,23E+01**	**98,50 %**		
361	7,340E-01	4,68E-04	1,23E+01			
362	7,380E-01	4,52E-04	1,23E+01			
363	7,420E-01	4,37E-04	1,22E+01			
364	7,460E-01	4,22E-04	1,21E+01			
365	7,500E-01	4,07E-04	1,20E+01			
366	7,530E-01	3,94E-04	1,18E+01			
367	7,600E-01	3,80E-04	1,17E+01			
368	7,700E-01	3,67E-04	1,15E+01			
369	7,750E-01	3,55E-04	1,13E+01			
370	**7,800E-01**	**3,43E-04**	**1,10E+01**	**99,30 %**		

Table A.1 *(continued)*

	UV calculation	Erythema	Reference solar spectrum	Sol. Sim.	RCEE accept. Range	
	Midday midsummer global irradiance at 40 °N ($W \cdot m^{-2} \cdot nm^{-1}$)	Action Spectrum	SSR ($W \cdot m^{-2} \cdot nm^{-1}$)	%RCEE	Lower	Upper
nm	$I_{sol}(\lambda)$	$E(\lambda)$	$S(\lambda)$	Sum{E*S}/T	limit	limit
371	7,840E-01	3,31E-04	1,07E+01			
372	7,880E-01	3,20E-04	1,04E+01			
373	7,920E-01	3,09E-04	1,01E+01			
374	7,960E-01	2,99E-04	9,65E+00			
375	8,000E-01	2,88E-04	9,37E+00			
376	8,060E-01	2,79E-04	8,98E+00			
377	8,120E-01	2,69E-04	8,60E+00			
378	8,180E-01	2,60E-04	8,20E+00			
379	8,250E-01	2,51E-04	7,71E+00			
380	**8,300E-01**	**2,43E-04**	**7,18E+00**	**99,80 %**		
381	8,350E-01	2,34E-04	6,70E+00			
382	8,400E-01	2,26E-04	6,15E+00			
383	8,480E-01	2,19E-04	5,58E+00			
384	8,550E-01	2,11E-04	4,99E+00			
385	8,600E-01	2,04E-04	4,42E+00			
386	8,650E-01	1,97E-04	3,86E+00			
387	8,750E-01	1,91E-04	3,35E+00			
388	8,820E-01	1,84E-04	2,85E+00			
389	8,860E-01	1,78E-04	2,39E+00			
390	**9,000E-01**	**1,72E-04**	**2,00E+00**	**100,00 %**		
391	9,060E-01	1,66E-04	1,63E+00			
392	9,120E-01	1,60E-04	1,30E+00			
393	9,180E-01	1,55E-04	1,02E+00			
394	9,250E-01	1,50E-04	7,81E-01			
395	9,300E-01	1,45E-04	5,92E-01			
396	9,350E-01	1,40E-04	4,44E-01			
397	9,420E-01	1,35E-04	3,25E-01			
398	9,540E-01	1,30E-04	2,31E-01			
399	9,620E-01	1,26E-04	1,59E-01			
400	**9,700E-01**	**1,22E-04**	**1,07E-01**	**100,00 %**	**99,90 %**	**100,00 %**

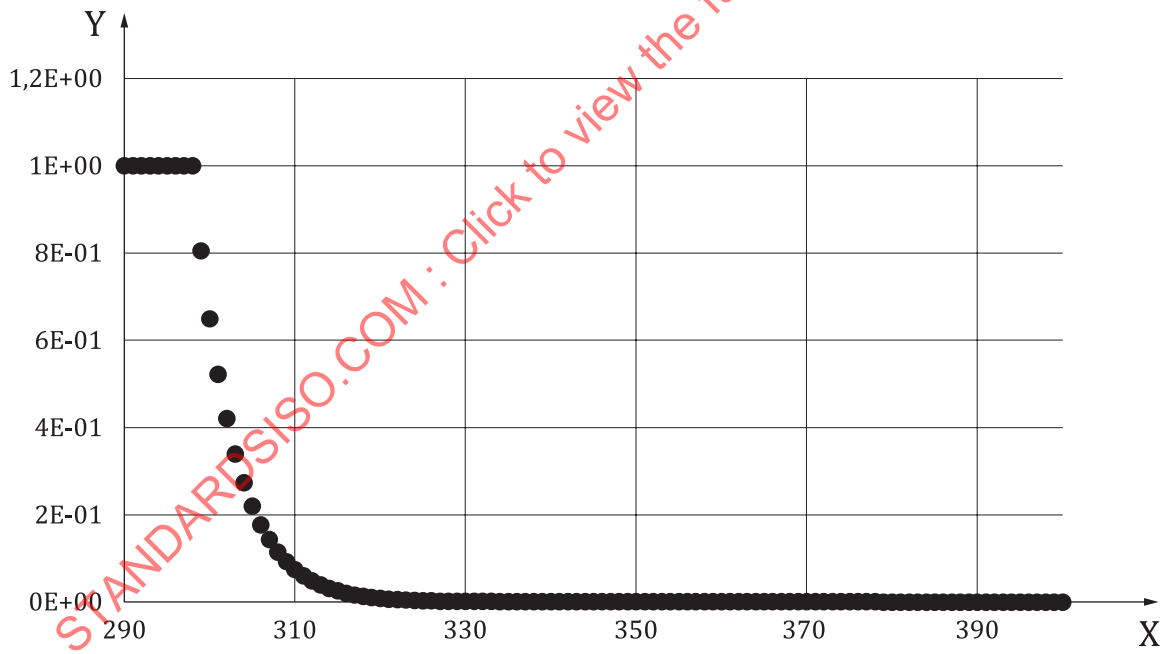


Key

X wavelength (nm)

Y SSR ($W \cdot m^{-2} \cdot nm^{-1}$)

Figure A.1 — Solar simulator UV spectrum



Key

X wavelength (nm)

Y action spectrum

Figure A.2 — Erythemal action spectrum

Annex B
(normative)

Specification sample plate

B.1 General

Moulded and sandblasted PMMA plates shall be used as the plate for sunscreen application according to this method. Samples plates shall be UVR-transparent, non-fluorescent (i.e. no detectable fluorescence when exposed to UVR and measured with a spectrophotometer), photostable and inert to all ingredients in the preparations to be tested. Furthermore, to enable the application of stable, thin films of different types in a skin-like manner, the plate shall have a standard, textured upper surface. The plate shall allow an optimal, homogeneous film to be formed after the product has been spread.

B.2 Size and surface profile of plate

B.2.1 The size of the plate shall be chosen such that the application area is not less than 16 cm^2. It is important to verify the exact dimension of the plate before performing this method and/or to check the specification in the accompanying certificate of analysis from the manufacturer.

The surface profile characteristics of the plates were determined based on several batches under specific criteria as recommended here below.

B.2.2 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.

B.2.3 Measurement

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

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

B.2.4 Analysis operators

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

Leveling method by least square plane by subtraction.

Conversion to a series 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.

B.2.5 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 mean form of peaks and valleys.

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

B.3 Specification

B.3.1 General

Correct plate specification is of primary importance to the correct deployment of this method.[15]-[20] UVR-transparent PMMA is the preferred material for test plates . Specifications for plate-to-plate reproducibility and surface roughness parameters are defined here after.

B.3.2 Moulded PMMA plate

Ra (µm)	= 4,853 ± 0,501
Rv (µm)	= 13,042 ± 0,989
Rdq (°)	= 11,122 ± 2,032
A1 ($\mu m^2\ mm^{-1}$)	= 239,750 ± 70,165
Ssc ($1/\mu m$)	= 0,033 ± 0,021
Vvv (mm^3/mm^2)	= $1{,}044 \cdot 10^{-4} \pm 9{,}76 \cdot 10^{-5}$

B.3.3 Sandblasted PMMA plate

Ra (µm)	= 4,188 ± 0,514
Rv (µm)	= 11,402 ± 2,499
Rdq (°)	= 11,004 ± 1,938
A1 ($\mu m^2\ mm^{-1}$)	= 238,252 ± 72,663
Ssc ($1/\mu m$)	= 0,032 ± 0,015
Vvv (mm^3/mm^2)	= $8{,}701 \cdot 10^{-4} \pm 2{,}325 \cdot 10^{-4}$

B.4 Plate optical characteristics

B.4.1 Transmittance specifications

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 B.1, or white petroleum.

Table B.1 — Modified glycerin solution

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

B.4.2 Method

B.4.2.1 Prepare a standard PMMA blank plate by applying approximately 15 mg of pure glycerin or modified glycerin solution or white petroleum 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/white petroleum with a bare fingertip.

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

B.4.3 Minimum transmission values

Limits for the treated moulded and sandblasted PMMA plate transmission values are:

290 nn: > 60 % T

300 nm: > 69 % T

320 nm: > 81 % T

Annex C
(normative)

SPF reference sunscreen products

C.1 Mean SPF and acceptance limits for reference sunscreen formulations

Table C.1 — Mean SPF and acceptance limits for reference sunscreen formulations

Reference sunscreen formulation	Mean SPF	Acceptance limits	
		Lower limit	Upper limit
P2	16,1	13,7	18,5
P3	15,7	13,7	17,7
P5	30,6	23,7	37,4
P6	43,0	31,0	54,9
P8	63,1	43,9	82,3

C.2 P2 SPF reference standard

C.2.1 Formula and preparation for product standard

	Ingredients	mass fraction (%)
Phase 1:	Lanolin	4,5
	Cocoa Butter	2,0
	Glyceryl Monostearate	3,0
	Stearic Acid	2,0
	Octyl Dimethyl PABA (padimate O)	7,0
	Benzophenone-3 (Oxybenzone)	3,0
Phase 2:	Water	71,6
	Sorbitol	5,0
	Triethanolamine	1,0
	Methylparaben	0,3
	Propylparaben	0,1
Phase 3:	Benzyl Alcohol	0,5

C.2.2 Manufacturing process

C.2.2.1 Step 1: Melt the ingredients of Phase 1 and mix using a propeller agitator at 77 °C to 82 °C until uniform.

C.2.2.2 Step 2: Mix Phase 2 using a propeller agitator, at 77 °C to 82 °C.

C.2.2.3 Step 3: Add the batch of step 1 to the batch of step 2 and mix until smooth and uniform; slowly cool the batch to 49 °C to 54 °C.

C.2.2.4 Add benzyl alcohol of Phase 3 to the batch of step 3; mix until uniform and continue to cool batch to 35 °C to 41 °C.

C.2.2.5 Compensate for water loss and homogenize, avoiding air entrapment; cool batch to 27 °C to 32 °C.

C.2.3 Physicochemical data[1)]

Appearance:	white/yellowish fluid emulsion
pH:	8,0 ± 0,5
Viscosity (20 °C):	range of values: 19 000 mPa·s to 33 000 mPa·s [Brookfield®[1)] rotating viscometer, RV type, helipath type, spindle B, speed 10 r/min (0,167 s^{-1}), rotation time 60 s]
	NOTE The values provided above are specific to the material used.
Density (20 °C):	0,970 ± 0,05 $g.cm^{-3}$

C.2.4 Analytical data

C.2.4.1 Principle

The formulation shall be sampled gravimetrically and dissolved in methanol, in which the analytes are soluble. The solution shall be diluted with HPLC mobile phase and analysed by reverse phase HPLC.

The concentrations of the analytes in the sample are determined by quantification against a mixed external standard solution of analyte raw materials.

C.2.4.2 Chemicals/reagents

C.2.4.2.1 **Benzophenone-3**, production raw material, various suppliers.

C.2.4.2.2 **Ethylhexyldimethyl PABA**, production raw material, various suppliers.

C.2.4.2.3 **Methanol**, HPLC grade.

C.2.4.2.4 **Water**, fresh distilled.

C.2.4.2.5 **Glacial acetic acid**, of high purity

C.2.4.2.6 **Solution**, with mass fractions of 85 % methanol and 1 % acetic acid.

Add 10 ml of glacial acetic acid to 850 ml of methanol and make up to 1 000 ml with water. Filter under vacuum through a 0,45 µm PTFE membrane filter.

1) Brookfield® is the trademark of a product supplied by Brookfield Engineering Laboratories. 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.

C.2.4.2.7 Mixed standard

Accurately weigh 30 mg of benzophenone-3 and 70 mg of octyl dimethyl PABA into a 100 ml volumetric flask and dissolve in and make to volume with methanol. Mix well.

C.2.4.2.8 Mixed working standard

Pipette 5 ml of mixed standard (C.2.4.2.7) into a 50 ml volumetric flask and make to volume with solution in accordance with C.2.4.2.6.

Apparatus — HPLC

Injector:	Injection volume	10,0 µl
Column:	Type	reverse phase C8 5 µm 4,6 mm × 250 mm or equivalent
	Mobile phase	solution in accordance with C.2.4.2.6
	Flowrate	1,5 $ml.min^{-1}$
Detector:	Type	UV
	Wavelength	308 nm [or 254 nm for fixed wavelength detection (less sensitive, less specific)]
Data:	Quantification	peak area

C.2.4.3 Sample preparation

C.2.4.3.1 Using an analytical balance, weigh approximately 1 g of formulation, to the nearest 0,1 mg, into a 50 ml volumetric flask.

C.2.4.3.2 Add methanol (C.2.4.2.3) to dissolve the sample and make up to volume.

C.2.4.3.3 Ultrasonicate the flask for 5 min and shake to completely mix the sample.

C.2.4.3.4 Pipette 1 ml into a 10 ml graduated tube and make up to volume with HPLC mobile phase.

C.2.4.3.5 Analyse the sample and mixed working standard (C.2.4.2.8) by reverse phase HPLC.

C.2.4.4 Quality control

C.2.4.4.1 Analyse a sample of HPLC mobile phase and a placebo, if available, prepared in accordance with the method, by reverse phase HPLC, to confirm the absence of interfering chromatographic peaks.

C.2.4.4.2 Analyse three mixed working standards (C.2.4.2.8) by reverse phase HPLC and calculate the coefficient of variation of the analyte peak areas.

C.2.4.5 Calculations

Analyte is calculated following Formula (C.1).

$$X = \frac{a}{a_{\text{std}}} \times \frac{C}{1\ 000} \times \frac{50}{m} \qquad \text{(C.1)}$$

where

X is the analyte, expressed in percentage mass fraction;

a is the peak area in the sample extract;

C is the mass concentration of analyte in the working standard in milligrams per litre;

a_{std} is the analyte peak area in the working standard;

m is the mass of the sample expressed in grams.

C.2.5 Acceptance criteria

The analytical results are acceptable if the following are achieved:

a) the standard coefficient of variation shall be ≤ 2,5 %;

b) recovery value shall be 100 % ± 5 % for all actives;

c) no interfering chromatographic peaks in the sample placebo or working solvent.

C.2.6 Storage and expiration

Store the reference material at 20 °C in a vessel protected from light. The package label shall include an expiration date provided by the manufacturer specifications.

C.3 P3 SPF reference standard

C.3.1 Ingredients

	Ingredients	Mass fraction (%)
Phase 1:	cetearyl alcohol	2,205
	PEG-40 castor oil	0,63
	sodium cetostearyl sulfate	0,315
	decyl oleate	15,0
	ethylhexyl methoxycinnamate (CAS 5466-77-3)(2-ethylhexyl-4-methoxycinnamate)	3,0
	butyl methoxydibenzoylmethane (CAS 70356-09-1)	0,5
	propylparaben	0,1
Phase 2:	water	53,57
	phenylbenzimidazole sulfonic acid (CAS 27503-81-7)(2-phenylbenzimidazole-5-sulfonic acid)	2,78
	sodium hydroxide (45 % solution)	0,9
	methylparaben	0,3
	disodium EDTA	0,1

Phase 3:	water	20,0
	carbomer (grade 980)	0,3
	sodium hydroxide (45 % solution)	0,3

C.3.2 Manufacturing process

C.3.2.1 Heat Phase 1 to 75 °C to 80 °C and heat Phase 2 to 80 °C (if necessary, increase heat until solution is clear and cool to 75 °C to 80 °C).

C.3.2.2 Add Phase 1 to Phase 2 while stirring Phase 2.

C.3.2.3 Prepare phase 3, disperse carbomer in water by stirring with a rotor/stator disperser, then add sodium hydroxide for neutralization.

C.3.2.4 Add phase 3 to phases 1 and 2 while stirring and homogenize for about 3 min.

C.3.2.5 Adjust pH with sodium hydroxide or lactic acid and stir until completely cool.

C.3.2.6 Compensate for water loss and homogenize.

C.3.3 Physicochemical data

Appearance:	white to slightly yellowish emulsion
pH:	7,5 ± 0,5
Density (20 °C):	0,970 ± 0,05 g.cm^{-3}
Viscosity (20 °C):	range of values: 2 000 mPa·s to 4 000 mPa·s [Brookfield® rotating viscometer, RV type, spindle 4, speed 20 r/min^{-1} (0,333 s^{-1}), rotation time 60 s]

NOTE The values provided above are specific to the material used.

C.3.4 Storage and expiry

Store the reference material at 20 °C in a vessel protected from light. The package label shall include an expiration date provided by the manufacturer specifications.

C.3.5 Analytical data

C.3.5.1 Principle

The formulation shall be sampled gravimetrically and dissolved in methanol, in which the analytes are soluble. The solution shall be diluted with HPLC mobile phase and analysed by reverse phase HPLC.

The concentrations of the analytes in the sample are determined by quantification against a mixed external standard solution of analyte raw materials.

C.3.5.2 Chemicals/reagents

C.3.5.2.1 **Phenylbenzimidazole sulfonic acid**, production raw material; various suppliers.

C.3.5.2.2 **Butyl methoxydibenzoylmethane**, production raw material; various suppliers.

C.3.5.2.3 **Ethylhexyl methoxycinnamate**, production raw material; various suppliers.

C.3.5.2.4 **Methanol**, HPLC grade.

C.3.5.2.5 **Water**, fresh distilled.

C.3.5.2.6 **Glacial acetic acid**, analar or higher purity.

C.3.5.2.7 **Solution**, with mass fractions of 85 % methanol and 1 % acetic acid

Add 10 ml of glacial acetic acid to 850 ml of methanol and make to 1 000 ml with water. Filter under vacuum through a 0,45 µm PTFE membrane filter.

C.3.5.2.8 **Mixed standard.**

Accurately weigh 65 mg of phenylbenzimidazole sulfonic acid into a 100 ml volumetric flask and dissolve in a minimum of 0,1 M NaOH. Weigh into the flask the remaining analytes as listed and make up to volume with methanol.

— butyl methoxydibenzoylmethane 10 mg;

— ethylhexyl methoxycinnamate 75 mg.

NOTE Complete solution might not occur immediately. Mixing by ultrasonic bath and standing with time will achieve complete solution.

C.3.5.2.9 **Mixed working standard.**

Pipette 5 ml of the mixed standard (C.3.5.2.8) into a 50 ml volumetric flask and make up to volume with the solution in accordance with C.3.5.2.7.

C.3.5.3 **Apparatus — HPLC**

Injector:	Injection volume	10,0 µl
Column:	Type	reverse phase C8 5 µm
		4,6 mm × 250 mm or equivalent
		solution according to C.3.5.2.7
	Flowrate	1,5 $ml.min^{-1}$
Detector:	Type	UV
	Wavelength	308 nm [or 254 mm for fixed wavelength detection (less sensitivity, less specific)]
Data:	Quantification	peak area

C.3.5.3.1 Add methanol to dissolve the sample and make up to volume.

C.3.5.3.2 Ultrasonicate the flask for 5 min and shake to completely mix the sample.

C.3.5.3.3 Pipette 1 ml into a 10 ml graduated tube and make up to volume with HPLC mobile phase.

C.3.5.3.5 Analyse the sample and mixed working standard by reverse phase HPLC.

C.3.5.4 Quality control

C.3.5.4.1 Analyse a sample of HPLC mobile phase and a placebo, if available, prepared according to the method reverse phase HPLC, in order to confirm the absence of interfering chromatographic peaks.

C.3.5.4.2 Analyse three mixed working standards (C.3.5.2.9) by reverse phase HPLC and calculate the coefficient of variation of the analyte peak areas.

C.3.5.5 Calculations

$$X = \frac{a}{a_{\text{std}}} \times \frac{C}{1\ 000} \times \frac{50}{m} \qquad \text{(C.2)}$$

where

X is the analyte, expressed in percentage mass fraction;

a is the peak area in sample extract;

C is the mass concentration of analyte in working standard in milligrams per litre;

a_{std} is the analyte peak area in working standard;

m is the mass of the sample expressed in grams.

C.3.5.6 Acceptance criteria

The analytical results are acceptable if the following are achieved:

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

b) recovery value is 100 % ± 5 % for all actives;

c) no interfering chromatographic peaks in the sample placebo or working solvent;

d) storage and expiry.

C.3.5.7 Storage and expiration

Store the reference material at 20 °C in a vessel protected from light.

The package label shall include an expiration date provided by the manufacturer specifications.

C.4 P5 SPF30 reference standard

C.4.1 Ingredients

Ingredient		% weight of composition ($g.kg^{-1}$)
A1	Water	39,35
	Disodium EDTA	0,05
	Methylparaben	0,35
	Chlorphenesin	0,20
	Phenoxyethanol	0,70

A2	Glycerin	5,00
B1	Xanthan gum	0,01
	Butyl methoxydibenzoylmethane	3,00
	Octocrylene	10,00
	Octyl Salicylate	5,00
	Benzophenone-3	5,00
B2	PPG-2 Myristyl ether propionate	2,00
	Octyldodecyl neopentanoate	2,00
	Butyloctyl salicylate	8,00
	PVP/Eicosene copolymer	1,30
B3	Polyglyceryl-3 methyl glucose distearate	2,00
	Cetyl alcohol	0,50
	Stearic acid	1,00
	Butylparaben	0,03
C	Cyclopentasiloxane	3,00
	Acrylates/C10-30 Acrylate crosspolymer	0,20
	Water	1,00
	Triethanolamine	0,06
E	Water	10,00
	Potassium cetyl phosphate	0,25

C.4.2 Process

C.4.2.1 Combine A1 into main kettle. Heat and mix to 80 °C.

C.4.2.2 While contents in main kettle are heating, premix A2. Add A2 to main kettle when temperature is 75 °C.

C.4.2.3 Combine ingredients of B1 in side kettle #1. Mix and heat to 80 °C. Maintain heat and mix in until homogenous.

C.4.2.4 Combine ingredients of B2 in inside kettle #2. Mix and heat to 80 °C. Maintain heating and mixing until homogenous. Add B2 ingredients into kettle with B1 ingredients. Mix well.

C.4.2.5 Combine ingredients in B3 in side kettle #3. Heat and mix to 80 °C. Maintain heating and mixing until homogenous. Add ingredients in B3 into kettle #1 with ingredients of B1/B2. Mix well.

C.4.2.6 Add ingredients from kettle #1 containing B1/B2/B3 into the main kettle containing A1/A2. Start homogenization. Maintain temperature and mixing for 10 min to 15 min.

C.4.2.7 Begin cooling to room temperature while maintaining homogenization.

C.4.2.8 When mixture has cooled to 60 °C, add premix of ingredients of *C* into the main kettle. Mix until uniform.

C.4.2.9 When temperature reaches 35 °C to 40 °C, add ingredients in *D* premixture into the main kettle. Mix until uniform.

C.4.2.10 Also while the temperature is between 35 °C and 40 °C, add ingredients in *E* premixture into the main kettle. Mix until uniform.

C.4.2.11 Continue cooling to room temperature.

C.4.3 Physiochemical data

Colour:	white/slightly off-white
Odor:	characteristic
Appearance:	smooth lotion
pH:	5,5 ± 0,5
Viscosity (20 °C):	77 000 mPa.s ± 10 % (Brookfield® LV with heliopath, spindle F, 12 r/min, reading after 60 s)
Specific gravity (20 °C):	1,00 ± 0,05 $g.cm^{-3}$

C.4.4 Storage and expiry

Twelve months at 20 °C from the fabrication date, in a vessel protected from light.

C.4.5 Analytical method

UV filters present can be measured using EN 16344[28] analytical method.

C.4.6 Acceptance criteria

The analytical results are acceptable if the following are achieved:

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

b) recovery value is 100 %/5 % for all actives.

C.4.7 Storage and expiration

Store the reference material at 20 °C in a vessel protected from light. The package label shall include an expiration date provided by the manufacturer specifications.

C.5 P6 SPF reference standard

C.5.1 Ingredients

Phase 1	(Oil Phase)	
	Ceteareth-12	1,00
	Dicaprylyl carbonate	8,00
	Isopropyl palmitate	5,00
	Ethylhexyl methoxycinnamate	5,00
	Bis-Ethylhexyloxyphenol methoxyphenyl triazine	2,00
Phase 2	(Water phase)	
	Water	58,80
	Disodium EDTA	0,20
	Chlorphenesin	0,30
Phase 3	Water and Acrylates/Beheneth-25 Methacrylates Copolymer (28 % to 33 % Acrylates Beheneth-25 methacrylate copolymer)	1,50
Phase 4	Water and sodium hydroxide (30 % NaOH)	adjust to pH = 7
Phase 5	Cyclohexasiloxane, Cyclopentasiloxane	6,0
	DMDM Hydantoin	0,20
Phase 6	Methylene Bis-Benzotriazolyl Tetramethylbutylphenol (nano), water,decyl glucoside, propylene glycol, xanthan gum (50 % MBBT)	12,00

C.5.2 Process

C.5.2.1 Heat Phase 1 and Phase 2 in separate kettles up to 80 °C. Mix each phase until uniform.

C.5.2.2 Under mixer, add Phase 1 at 80 °C into Phase 2 at 80 °C.

C.5.2.3 Add immediately Phase 3 under homogenizer. Mix until homogeneous.

C.5.2.4 Adjust pH to 7 with Phase 4. Mix with homogenizer until homogeneous.

C.5.2.5 Cool down to 60 °C and add Phase 5. Mix until homogeneous

C.5.2.6 Cool down to room temperature and add Phase 6 under stirrer. Mix until homogeneous.

C.5.2.7 Adjust for water loss and homogenize, avoiding air entrapment.

C.5.3 Specifications

C.5.3.1 Appearance: White cream.

C.5.3.2 pH value (25 °C): 7,0 ± 0,3.

C.5.3.3 Viscosity: 16 000 mPa.s to 19 000 mPa.s using Brookfield® DVIII Ultra, Spindle RV-5 at 10 $r.min^{-1}$.

C.5.3.4 Density: 0,95 $g.cm^{-3}$ to 0,98 $g.cm^{-3}$.

C.5.4 Analytical method

UV filters present can be measured using EN 16344.[28]

C.5.5 Storage and expiration

Store the reference material at 20 °C in a vessel protected from light. The package label shall include an expiration date provided by the manufacturer specifications.

C.6 P8 High reference standard

C.6.1 Ingredients

Phase 1	**Oil phase**	**Mass fraction (%)**
	Ceteareth-12	1,00
	C12-15 Alkyl Benzoate	7,00
	Isopropyl palmitate	5,00
	Ethylhexyl methoxycinnamate	5,00
	Bis-Ethylhexyloxyphenol	3,00
	Methoxypheyl triazine	3,00
	Ethylhexyl salicylate	3,00
Phase 2	Water phase	
	Water	47,30
	Disodium EDTA	0,20
	Chlorphenesin	0,30
	Phenoxyethanol	1,00

Phase 3	Water and Acrylates/Beheneth-25 Methacrylates Copolymer (23% to 28 % acrylates/behenyth-25 methacrylate copolymer)	1,20
Phase 4	Water and Sodium Hydroxide (30 % NaOH)	adjust to pH = 7
Phase 5	Cyclohexasiloxane, Cyclopentasiloxane	6,0
Phase 6	Methylene Bis-Benzotriazolyl Tetramethylbutylphenol (nano), water,decyl glucoside, propylene glycol, xanthan gum (50 % MBBT)	20,00

C.6.2 Process

C.6.2.1 Heat Phase 1 and Phase 2 in separate kettles up to 80 °C. Mix each phase until uniform.

C.6.2.2 Under mixer, add Phase 1 at 80 °C into Phase 2 at 80 °C.

C.6.2.3 Add immediately Phase 3 under homogenizer. Mix until homogeneous.

C.6.2.4 Adjust pH to 7 with Phase 4. Mix with homogenizer until homogeneous.

C.6.2.5 Cool down to 60 °C and add Phase 5. Mix until homogeneous.

C.6.2.6 Cool down to room temperature and add Phase 6 under stirrer. Mix until homogeneous.

C.6.2.7 Adjust for water loss and homogenize, avoiding air entrapment.

C.6.3 Specifications

C.6.3.1 Appearance: White cream.

C.6.3.2 pH value (25 °C): 7,1 ± 0,3.

C.6.3.3 Viscosity: 12 000 mPa.s to 15 000 mPa.s using Brookfield® DVIII Ultra, Spindle RV-5 at 10 $r.min^{-1}$.

C.6.3.4 Density: 0,97-1 $g.cm^{-3}$.

C.6.4 Analytical method

UV filters present can be measured using EN 16344[28] analytical method.

C.6.5 Storage and expiration

Store the reference material at 20 °C in a vessel protected from light. The package label shall include an expiration date provided by the manufacturer specifications, and a statement “Intended for Laboratory Use Only”.

Annex D
(normative)

White petroleum and glycerin

White petroleum	
	
White petroleum[29]	
Identification	
Synonym	petrolatum, Vaseline
No CAS	8009-03-8
No CE	232-373-2
No E	E905b
Appearance	Paste colourless to white, waxy
Chemical properties	
Formula	hydrocarbons > C_{25}
Physical properties	
T° fusion	36 °C to 60 °C
T° boiling	302 °C
Solubility	Insoluble in water
Mass in volume	0,9 g·cm^{-3}
T° auto-ignition	>290 °C
Flash point	182 to 221 °C