BUREAU OF INDIAN STANDARDS

Draft Indian Standard

Cosmetics — Sun Protection Test Methods — *In vivo* Determination of Sun Protection Factor (SPF) [ISO 24444 : 2019, MOD]

(First Revision of IS 17494*)*

(ICS No. 71.100.70)

Cosmetic Sectional Committee	Last date for comment is
PCD 19	**16 September 2022**

FOREWORD
(Formal Clauses shall be added later)

This Indian Standard was published in 2021 which was identical with ISO 24444 : 2019 'Cosmetics — Sun protection test methods — *In vivo* determination of sun protection factor (SPF)' issued by the International Organization for Standardization (ISO).

The committee responsible for the formulation of this standard observed that the average ITA° range prescribed in ISO 24444 : 2019 'Cosmetics — Sun protection test methods — *In vivo* determination of sun protection factor (SPF)' was 41° to 55°, whereas the average ITA° in Indian Population ranges from ITA -30° to 50°. Thus, the committee decided to revise the standard with the modification in the Average ITA° range.

During this revision, the major changes are:

1. In clause **5.1.2,** the average ITA° of the subjects making up a test panel has been modified between 28° and 41°

2. Designation of the Indian Standard has been modified from Identical Adoption to Indigenous Standard.

The composition of the Committee, responsible for the formulation of this standard is given at Annex H (will be added later).

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 2022 'Rules for rounding off numerical values (*second revision*).' The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Introduction

The level of sun protection provided by sunscreen products has traditionally been estimated using the sun protection factor or SPF test, which uses the erythemal response of the skin to ultraviolet (UV) radiation. The SPF is a ratio calculated from the energies required to induce a minimum erythemal response with and without sunscreen product applied to the skin of human test subjects. It uses ultraviolet radiation usually from an artificial source.

Different standard methods are available and described in ISO/TR 26369 Since the publication of the first version of this document, harmonization has been achieved in many member countries. The objective of this updated version is to further improve reproducibility between test sites, so as to obtain the same SPF value

1 Scope

This document specifies a method for the in vivo determination of the sun protection factor (SPF) of sunscreen products. It is applicable to products that contain any component able to absorb, reflect or scatter ultraviolet (UV) rays and which are intended to be placed in contact with human skin.

This document provides a basis for the evaluation of sunscreen products for the protection of human skin against erythema induced by solar ultraviolet rays.

2 Normative references

There are no normative references in this document.

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:

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

b) IEC Electropedia: available at http://www.electropedia.org/

3.1 Ultraviolet radiation UVR

Electromagnetic radiation in the range of 290 nm to 400 nm

3.1.1 *Ultraviolet B UVB*

Electromagnetic radiation in the range of 290 nm to 320 nm

3.1.2 *Ultraviolet A UVA*

Electromagnetic radiation in the range of 320 nm to 400 nm

NOTE − UVA II = 320 nm to 340 nm; UVA I = 340 nm to 400 nm.

3.1.3 *Erythemal effective irradiance*

E_{er} Radiometric quantity derived by multiplying the spectral irradiance $E(\lambda)$ of the solar simulator with the erythema action spectrum $s_{er}(\lambda)$ at each wavelength λ and integrating over wavelength range of 290 nm to 400 nm

$E_{er} = \int_{290}^{400} E(\lambda)\, s_{er}(\lambda)\, d\lambda$ unit: W/m^2 (eff.)

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3.1.4 Erythemal effective radiant exposure erythemal dose

H_{er} radiometric quantity defined as time integral of erythemal effective irradiance $E_{er}(t)$

$H_{er} = \int_t Eer(t)dt$ unit: J/m^2 (eff.)

3.2 Erythema

Reddening of the skin caused by UV radiation

3.3 Sunscreen Products

Products 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* (3.2) and other ultraviolet induced damage.

3.4 Minimal Erythemal Dose MED
lowest *erythemal effective radiant exposure* (*H*er) (3.1.4) that produces the first perceptible unambiguous erythema with defined borders appearing over more than 50 % of UV exposure subsite, 16 h to 24 h after UV exposure.

NOTE − Annex F contains visual references and guidance for the acceptable MED appearance.

3.4.1 MED_u

Minimal erythemal dose on unprotected skin.

3.4.1.1 $MEDi_u$

Minimal erythemal dose of an individual subject on unprotected skin.

3.4.2 MED_p

Minimal erythemal dose on product protected skin

3.4.2.1 MED_{ip}

Minimal erythemal dose of an individual subject on protected skin

3.5 Individual sun protection factor SPF_i
Ratio of the individual minimal erythemal dose on product protected skin (MEDip) to the (individual) minimal erythemal dose on unprotected skin (MEDiu) of the same subject:

$$SPF_i = \frac{\text{MED ip}}{\text{MED iu}}$$

NOTE − SPFi is expressed to one decimal place by truncation.

3.6 Sun protection factor of a product SPF

Arithmetic mean of all valid individual SPFi values obtained from all subjects in the test

NOTE − SPF is expressed to one decimal place by truncation.

3.7 Test Area

Area for testing on the back between the scapula line and the waist

NOTE – Skeletal protrusions and extreme areas of curvature should be avoided.

3.8 Test Site

Area of the skin where a product is applied or the site used for the determination of the unprotected MED

3.9 Exposure sub-sites

Areas of skin that are exposed to UV-irradiation within a test site.

3.10 Individual typology angle ITA°

Value characterizing the skin colour of the subject as measured by a skin contact reflectance
Spectrophotometer or skin colourimeter

NOTE − Refer to Annex E for the detailed requirements of the equipment/measurement.

4 GENERAL PRINCIPLE

The SPF test method is a laboratory method that utilizes a xenon arc lamp solar simulator (or equivalent) of defined and known output to determine the protection provided by sunscreen products on human skin against erythema induced by solar ultraviolet rays.

The test shall be restricted to the area of the back of selected human subjects.

A section of each subject's skin is exposed to ultraviolet light without any protection while another (different) section is exposed after application of the sunscreen product under test. One further section is exposed after application of an SPF reference sunscreen formulation, which is used for validation of the procedure.

To determine the sun protection factor, incremental series of delayed erythemal responses are induced on a number of small sub-sites on the skin. These responses are visually assessed for presence of erythema 16 h to 24 h after UV radiation, by the judgment of a trained and competent evaluator.

The individual minimal erythemal dose for unprotected skin (MED_{iu}) and the individual MED obtained after application of a sunscreen product (MED_{ip}) shall be determined on the same subject on the same day. An individual sun protection factor (SPF_i) for each subject tested is calculated as the ratio of individual MED on product protected skin divided by the individual MED on unprotected skin, as in the formula given in **3.5**.

The sun protection factor for the product (SPF) is the arithmetic mean of all valid SPF_i results from each subject in the test expressed to one decimal place.

5 TEST SUBJECTS

5.1 Selection of the test subjects

5.1.1 *General*
There are strict requirements governing the inclusion and non-inclusion of test subjects which

should be adhered to. The criteria shall be as set out in Annex A.

5.1.2 *Skin colour of the test subjects*

Test subjects included in the SPF test shall have an ITA° value of at least 28° by colourimetric methods (*see* Annexes **A** and **E**) and be untanned on the test area.

The average of the subjects making up a test panel shall have an ITA° between 41° and 55°. When possible, there should be subjects with ITA°s in each of the three ITA° bands, 28° to 40°, 41° to 55°, and >56°. Where this is not possible, there shall be at least three individuals in each of two of the three ITA° bands described in the previous sentence.

A trained and competent scientist or technician should examine each subject to ensure that there is no condition which might put the subject at risk and that the outcome of the test cannot be compromised by adverse skin conditions such as sun damage, pigmentation marks and previous history of abnormal response to the sun (*see* Annex **A**).

The test sites intended for UV exposure shall be free from blemishes and hair, and have an even colour tone with no variation in ITA° greater than **5**° from each other or the MED_u test area.

5.1.3 *Age restriction*

Test subjects below the locally regulated age of consent or older than 70 years shall not be included in the SPF test panel.

5.1.4 *Frequency of participation in tests*

Subjects may participate in a test provided that at least 8 weeks have elapsed since they participated in a previous UV exposure study (i.e. SPF, UVA-PF, photoallergy, phototoxicity test), and all skin tanned marks from that previous test have cleared from the test sites on the back and are no longer visible.

5.1.5 *Ethics and consent*

All testing shall be done in accordance with the Declaration of Helsinki. Any national regulations regarding human studies should also be taken into account. Informed, written (signature) consent shall be obtained from all test subjects and retained.

5.2 Number of test subjects

The minimum number of valid SPF_i results shall be 10 and the maximum number of valid SPF_i results shall be 20. In order to achieve between 10 and 20 valid results, a maximum of five individual invalid results may be excluded from the calculation of the mean SPF. For the test to be considered valid for the first 10 subjects, the resulting range of the 95 % CI of the mean shall be within ±17 %. Consequently, the actual number of test subjects used will fall between a minimum of 10 and a maximum of 25 subjects (i.e. a maximum of 20 valid results plus 5 rejected invalid results).

Results may only be declared invalid and excluded from the calculation of the mean SPF according to **9.5.3** or because of non-compliance with the related protocol.

In order to determine the number of test subjects, the 95 % confidence interval (95 % CI) on the mean SPF shall be taken into account. A minimum of 10 subjects shall be tested. The test shall be considered valid for the first 10 subjects if the resulting range of the 95 % CI of the mean SPF shall be within ±17 % of the mean SPF. If it is not within ±17 % of the mean SPF, the number of subjects shall be increased stepwise from the minimum number of 10 until the 95 % CI statistical

criterion is met (up to a maximum of 20 valid results from a maximum of 25 subjects tested). If the statistical criterion has not been met after 20 valid results from a maximum of 25 subjects, then the test shall be rejected. For details on statistical definitions, sequential procedure and calculations, refer to Annex D.

6 APPARATUS AND MATERIALS — SOURCE OF ULTRAVIOLET RADIATION

6.1 General

The artificial light source used shall comply with the source spectral specifications as described in **6.2** and Annex B. A xenon arc solar simulator with appropriate filters shall be used.

6.2 Quality of ultraviolet radiation

6.2.1 The solar UV simulator shall emit a continuous spectrum with no gaps or extreme peaks of emission in the UV region. The output from the solar UV simulator shall be stable, uniform across the whole output beam and suitably filtered to create a spectral quality that complies with the required acceptance limits (*see* Table B.1).

6.2.2 To ensure that appropriate amounts of UVA radiation are included in the spectrum of the solar UV simulator, the total radiometric proportion of the UVA II (320 nm to 340 nm) irradiance of the simulator shall be ≥20 % of the total UV (290 nm to 400 nm) irradiance. Additionally, the UVA I region (340 nm to 400 nm) irradiance shall be ≥60 % of the total UV irradiance.

6.2.3 The source spectral specification is described in terms of cumulative erythemal effective irradiance by successive wavelength bands from <290 nm up to 400 nm. The erythemal effective irradiance of each wavelength band is expressed as a percentage of the total erythemal effective irradiance from <290 nm to 400 nm, or as the percentage relative cumulative erythemal effectiveness (% RCEE). The definition and calculation of % RCEE values is described in Annex B and the acceptance limits are given in Table B.1.

6.3 Total irradiance (UV, visible and near infrared rays)

If total irradiance is too intense, an excessive feeling of heat or pain may be induced in the irradiated skin of subjects and heat induced erythema may result. Therefore, total irradiance shall not exceed 1 600 W/m^2. When total irradiance is <1 600 W/m^2, it shall still be confirmed, prior to conducting an SPF test, that the irradiance to be used (UV, visible and near-infrared rays) will not induce an excessive feeling of heat in the skin. 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.

6.4 Uniformity of beam

6.4.1 *General*

Uniformity of the beam shall be measured depending on the solar simulator type using either UV sensitive film or UV sensor methods (*see* **6.4.2** and **6.4.3**). Solar simulators with large beams (>1,3 cm diameter) or with multiple output ports shall be measured at least every 6 months, or when any modifications are made to the lamp optical components, or when non-uniform erythema spots are seen in test subsites. Solar simulators with a single output port beam (≤1,3 cm diameter) shall be measured at least every 1 month, or when any modifications are made to the lamp optical components, or when non-uniform erythema spots are seen in test subsites.

Uniformity measurements may be conducted using UV sensitive paper that darkens with exposure, or by using a UV sensor that is smaller in active area compared to the beam size by a

ratio of at least 1:4.8 with sufficient measurements to cover more than 75 % of the beam area.

Measurements are to be made using the orientation of the source output as used for subject exposures.

6.4.2 *Film densitometry*

Exposure doses of the UV sensitive film shall be calibrated to achieve film darkening (converted to grey scale) to a density in the mid-range of the scale (on a 0 to 255 range of black to white). A series of exposures shall be used to determine the mid-range density exposure using a calibrated scanning measurement device with at least 600 dots per inch (dpi) resolution. Exposures can be modified by use of neutral density filters or exposure times to achieve this level of exposure for uniformity measurements. Areas to be measured shall be the same as those diagrammed below (*see* Figures **1** and **2**). Films are to be scanned for density values, and average values for each area of the beam as outlined above shall be calculated, and beam uniformity calculated as per Formula (**1**) (*see* **6.4.4.3**).

6.4.3 *UV sensor*

Alternatively, a small aperture (quadrant) UV sensor with a mechanical alignment fixture may be used to measure sub-sections of the output beam intensity as outlined below and the beam uniformity calculated as per Formula (1) (*see* **6.4.4.3**).

6.4.4 *Large beam source*

When a large-beam UV source is used to simultaneously expose several sub-sites (i.e. at least two sub- sites) within an irradiation series by varying the exposure time, the intensity of the beam shall be as uniform as possible. A UV film densitometry method or a UV radiometer method may be used. The minimum number of sample sites of equal area within the beam (Area of Interest – AOI) to be assessed shall be determined by dividing the area of the beam by 6.45. (For example, if the beam is 232 cm^2 in area, then the minimum number of measurements shall be 36).

6.4.4.1 UV film densitometry method: The UV sensitive film at least as large as the beam shall be exposed by the entire beam so that the entire beam fits inside the borders of the film.

6.4.4.2 UV Radiometer method: A UV radiometer sensor may be used to sample the beam intensity at multiple sites. Measurements shall be made at equally distributed points.

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

$$\text{Uniformity } \% = (1-(\max-\min)/(\text{average}))\ \% \qquad (1)$$

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

6.4.5 *Small beam source*

For a small beam UV source, which exposes sub-sites individually, the beam intensity uniformity shall be as measured. A UV sensitive film densitometry method or a UV radiometer method may be used.

6.4.5.1 *Single output device*

For a single output device, five equal size areas of the beam intensity shall be measured to assess the uniformity within the beam as shown in Figure 1. The uniformity shall be ≥90 % as calculated by Formula (1).

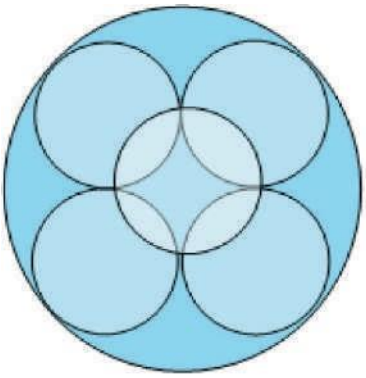


Fig. 1 — Single output device

6.4.5.2 *Multiple output device*

For a multiple output device, the intensity uniformity of each output beam shall be determined by measuring at least 4 circles of equal area of each output beam (*see* Figure 2), as calculated Formula (1).

The average uniformity of all beams for the multiple output device shall be ≥90 %

If the uniformity is less than prescribed above, then adjustments to the lamp optical system shall be made to bring the uniformity within the limits above.

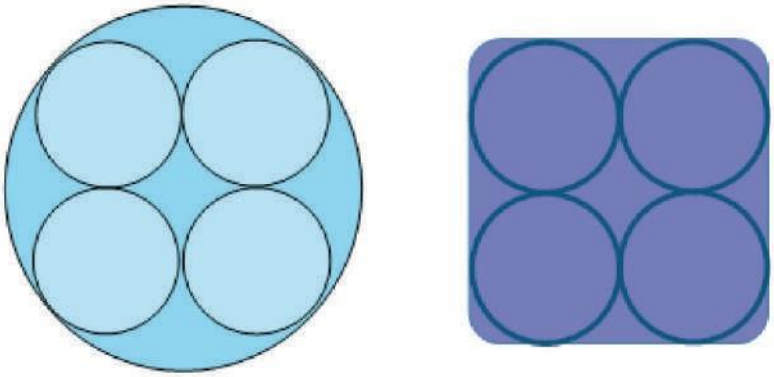


Fig. 2 — Multiple output device

7 MAINTENANCE AND MONITORING THE UV SOLAR SIMULATOR OUTPUT

7.1 Spectroradiometry

There shall be a spectroradiometric check of the spectrum of each solar simulator output port (UVA and UVB) and intensity made by the laboratory at least once every 12 months or after 2 500 h of lamp running time and after changing any significant physical (optical) component (including the bulb) of the solar simulator. The simple use of specific filters is not in itself adequate assurance that the UV output is of the correct quality. This periodical inspection should be conducted by a trained, competent, and suitably qualified person (internal or external) using a spectroradiometer that has been calibrated against a standard lamp that is traceable to a

national or an international calibration standard, with a band width of 2 nm or smaller and having a dynamic range of at least 5 decades which is usually met by spectroradiometers equipped with double monochromator. Measurements shall be recorded at 1 nm increments.

Optical alignment fixtures shall be used to assure accurate radiometer alignment and reproduction of the simulator output at the same optical reference plane measured with the spectroradiometer.

Detailed instructions for ensuring correct lamp output are given in Annex B.

7.2 Radiometry

Prior to making any measurements of the simulator output with a radiometric device, the front surface of the radiometer sensor shall be cleaned with a dry cotton cloth, and the optical tips of the light guides from the xenon source shall be cleaned with alcohol or optical cleaning fluid with lint-free cloth to remove any visible or invisible materials or residual sunscreen.

Before UV exposure of each test site, the UV irradiance shall be measured and recorded with an erythema weighted radiometer cross-calibrated against a spectroradiometric measurement of the solar simulator output as detailed in **7.1**. Optical alignment shall be configured to ensure accurate radiometer alignment and reproduction of the simulator output at the same optical reference plane measured with the spectroradiometer. A calibration factor *Y* for each radiometer shall be determined by Formula **(2)**:

$$Y = \frac{Eersp}{Eerr}$$

where

Y = is the calibration factor for each radiometer;

E_{ersp} = is the erythema effective irradiance E_{er} (W/m^2 erythemal weighted) of the solar simulator as measured by a spectroradiometer;

E_{err} = is the erythema weighted irradiance E_{er} (W/m^2) of the solar simulator as measured by the radiometer.

The UV exposure time (in seconds) for a given test shall be calculated using Formula (3):

$$t = \frac{Her}{Eesrp} = \frac{Her}{Y \times Eer}$$

where

t = is the time, in seconds, for the UV exposures for a given test;

H_{er} = are the desired doses;

E_{ersp} = is the erythema effective irradiance as measured by spectroradiometer;

E_{err} = is the erythema effective irradiance as measured by radiometer;

Y = is the calibration factor.

Output intensity should be measured before exposure of each test site in order to ensure the correct intensity is applied for each exposure. Where the solar simulator is capable of continuous monitoring of output intensity, it should be measured during the exposure of the test subjects.

The average intensity of the solar simulator as measured by the calibrated radiometer shall be included on the test study report (W/m^2 eff.), as well as the doses (J/m^2 eff.) for the MED_{iu} and MED_{ip} for each subject.

8 Reference sunscreen formulations

8.1 General

The method is controlled by the use of one of five reference sunscreen formulations to verify the test procedure. Therefore, one of the prescribed reference formulations shall be measured on the same day as products are tested. Whether a low or high SPF reference formulation is to be used depends on the expected SPF of the test products.

8.2 Reference standard to be used

8.2.1 Preliminary testing: When testing is being done on a preliminary basis, such as for product development investigations, any reference standard listed in Annex C may be used for each subject.

8.2.2 Establishment of SPF for product claim: When testing is conducted for the purpose of supporting a label claim of a product intended for market the following reference standards shall be used for testing with the test product:

8.2.2.1 SPF Claim ≤24: P2 or P3 reference standard (all subjects);

8.2.2.2 SPF ≥25 but less than SPF 50: P5 or P6 reference standard (on at least 5 subjects) and P2 or P3 on remaining subjects;

8.2.2.3 SPF ≥50: P8 reference standard (on at least 5 subjects) and P2 or P3 on the remaining subjects.

Additional subjects may be added as necessary to achieve means for the reference standards that are within the acceptance range.

Assignment of the reference standards to be used on specific subjects shall be randomized.

If P5, P6, or P8 reference standard is used on a subject, there is no necessity to include a lower SPF reference standard on that subject even though there may be lower SPF test products included in the same test. (Only one SPF reference standard sunscreen shall be required on each subject). Acceptance SPF ranges for the reference standard sunscreens are shown in Annex C. If the mean SPF of the reference standard sunscreens obtained in any test do not fall within their acceptance limits shown in Annex C for that reference standard, then the entire test (i.e. all test products) shall be rejected.

The formulae details and manufacturing instructions for the reference formulations are given in Annex C.

9 Procedure

9.1 Main steps

9.1.1 *Acclimatization period for the skin*;

9.1.2 *Determination of ITA° on the back of the subject*;

9.1.3 *Delineation of test sites on the back of the subject*;

9.1.4 *Weighing of the product for application to the test site*;

9.1.5 *Application of the product to the test site*;

9.1.6 *Waiting period before UV exposure to the test site*;

9.1.7 *UV exposure*;

9.1.8 *MED assessment*;

9.1.9 *Calculations*.

9.2 Test conditions

Product application, UV exposures and MED assessment should be carried out in stable conditions, with the room temperature maintained between (23 ± 3) °C.

9.3 Position of the test subjects

Product shall be applied to subjects in the same position as will be utilized for the irradiation procedure (sitting or prone). Powder and products which may flow (very low viscosity liquids) should be tested in the prone position to prevent the samples from falling off the surface.

9.4 Product application

The amount of product applied and the uniformity of spreading on the test sites affect the magnitude and variability of the test results. It is therefore very important to follow the procedures set out in 9.4.1 to 9.4.9.

9.4.1 The test sites intended for UV exposure shall be free from blemishes and hair, and have an even colour tone with no variation in ITA° greater than 5° from each other or the MED_u test area. When necessary, hair shall be shaved more than three days prior to the test, but not thereafter. If necessary, hair may be clipped or cut with scissors on the test day.

9.4.2 The minimum total area for a test site for product application shall be 30 cm^2 and the maximum shall be 60 cm^2.

9.4.3 The positions of the test products and reference sunscreen test sites shall be distributed randomly on the backs of subjects over the whole test group in order to reduce error arising from anatomical differences in skin. The unprotected test site used to determine MED_u shall be randomized as one of the test sites across the test area and across subjects.

9.4.4 There shall be a minimum distance of 1 cm between the borders of adjacent test sites.

9.4.5 Before product application, the test area may be cleaned by using a dry cotton pad or equivalent.

9.4.6 The test sites shall be delineated by a method which does not interfere with the test or harm the subject such as skin marker and/or a template made from non-absorbent material. The skin marker shall be indelible so as to be discernible at the time of MED evaluations 16 h to 24 h post UV-exposure.

9.4.7 *Amount of product applied*

9.4.7.1 The amount of test product and reference sunscreen formulation applied to the skin after spreading shall be (2,00 ± 0,05) mg/cm^2.

9.4.7.2 The balance used to weigh the products should be capable of weighing to the nearest 0,1 mg.

9.4.7.3 All products shall be homogeneous and shall be shaken before weighing, to ensure uniform dispersion.

9.4.7.4 When handling the product during weighing or before application to the skin, take appropriate measures to prevent evaporative loss of the volatile components. It is important that the total quantity of weighed product is transferred to the product application site. The amount of product to be applied shall be weighed in a syringe or in another device such as a watch glass. A method of weighing by loss is required.

9.4.8 Mode of delivery

It is recommended to practice application and spreading of the test materials on a subject (not in the test) to determine the best procedure to obtain uniform product spreading. The use of a finger cot (i.e. latex, nitrile, etc.) shall be required except in cases when use of a finger cot interferes with even application of the product. A new finger cot shall be used for each new application of product and shall not be pre-saturated with the test product. When a naked finger, is used a maximum of 2.1 mg/cm^2 (additional 5 %) shall be applied to the test area to account for the additional area of the application finger, and the finger shall be cleaned between product applications with an alcohol wipe.

9.4.8.1 Product application technique

The application technique to be used is dependent on the product type.

Form	Recommended application method
Lotion	Method A
Cream	Method A
Oil	Method A
Liquid	Method A
Gel	Method A
Stick	Method B
Balm	Method B
Aerosol spray	Degas then Method A
Pump spray	Method A
Roll on	Method A or B
Powder	Method C
Foaming Formulations	Method D

Method A: Fluid products. To aid uniform coverage, droplets (at least 15 per 30 cm^2, 30 per 60 cm^2) of the product should be deposited within the test site using a syringe/pipette at one time, then spread over the whole test site, first with circular movements to gather the droplets and second in horizontal and vertical directions using light pressure as shown in Figure 3. It is recommended that during the whole process, the application finger stays in contact with the skin.

Spray products provided in a pressurized container should first be degassed by puncturing a very small pinhole in the container to relieve all of the pressure. Degassing shall be done with appropriate safety precautions by securing the can within a ventilated safety hood with appropriate personnel safety equipment. The degassed can shall be allowed to rest for 24 h at room temperature when the product shall be decanted into a separate closed container with minimal headspace to minimize evaporation.

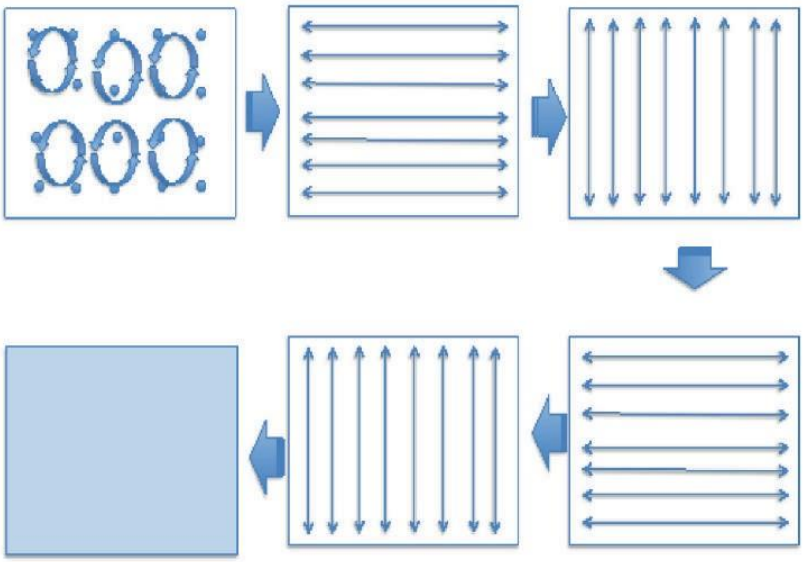


Fig 3 — Application techniques for Methods A and B

Method B: Non-flowing viscous liquids and semi-solids. Test product should be measured into a weigh boat and applied by finger in multiple areas of the test site, first with circular movements to gather the material and second in horizontal and vertical directions using light pressure as in Fig 3. It is recommended that during the whole process, the application finger stays in contact with the skin.

Method C: Powders. Aliquots of powder should be transferred to the skin in a grid-like manner, using a spatula, sponge, or finger.

The accumulated powder shall be tapped and then spread over the whole test site using a finger with or without a finger cot. Alternatively, the tip of a pre-loaded cosmetic applicator puff may be used instead of a finger. In this case, it is important to verify that (2.00 ± 0.05) mg/cm^2 of test powder product remains on the skin after spreading, by weighing the powder remaining on the tip of the applicator puff.

Purified water or another suitable solvent that has no UV protection properties may be applied on the skin before the powder application to help the sample adhere to the application site. Water or solvent should not transform the powder into a paste and thus influence its SPF value.

Method D: Foaming formulations. For samples which are presented as foams and where the contents cannot be extracted or dispensed other than as a foam, the test product should be measured into a weighing boat and then the sample allowed to degas or deaerate until they can be easily applied to the skin. Application will be subsequently accomplished following Method B.

9.4.8.2 Spreading

Spreading time should be in the range of (35 ± 15) s depending on the surface and ease of spreading of the product. Volatile liquids should be spread without delay.

9.4.9 Evaluation of application uniformity

After application is completed, and before commencement of the UV exposure doses, the application shall be checked with an ultraviolet-A "Woods" lamp with at least 6W of power, that is capable to visualize the uniformity of the application. If noticeable non-uniformity or streaking of the product is noted, the test site shall be rejected and may not be used for the test. If another test site is available, a new application may be attempted.

9.4.10 Drying time between application and UV exposure

Exposure of the first test site to the sequence of UV doses shall start 15 min to 30 min after the product is applied. Any extraneous exposure of the test sites to UV light (artificial or natural) shall be avoided during this period and for a period of 24 h after exposure.

9.4.11 Exposure sub-sites

9.4.9.11.1 Where a template is used to demarcate the exposure sub-sites (such as large-beam UV solar simulator), the template should be of non-absorbent material.

9.4.9.11.2 The minimum area of each exposure sub-site shall be 0.5 cm^2.

9.4.9.11.3 The minimum distance between borders of each exposure sub-site (spots) shall be at least 0.8 cm.

9.4.9.11.4 The distance between any exposure sub-site and any edge of the test site shall be at least 1 cm.

9.4.9.11.5 The minimum number of exposure sub-sites used shall be five for unprotected MED (MED_u) and five for protected MED (MED_p).

9.4.12 Provisional MED_{iu}

Before starting the main test, it may be necessary to determine a provisional $MEDi_u$ in order to centre the UV dose ranges for the exposures of MED_{iu} and MED_{ip}. A provisional MED_{iu} is a pre-test in which the MED_{iu} of a subject should be determined prior to establishing the test MED_{iu}. This is performed by applying a preliminary series of UV exposures up to one week before the test. The

provisional MED_{iu} shall be determined by the colometric ITA° technique (see Annex E) using the UV dose range in Table E.1.

9.4.13 Estimated MED_{iu}

If the provisional MED_{iu} has not been determined before the product test day, the MED_{iu} can be estimated by colourimetric technique (ITA°) without UV exposure (see Annex E) on the same day as the test. The estimated MED_{iu} exposure dose shall be determined using the colometric ITA° technique (see Annex E) and the UV dose range from Table E.1. Otherwise, use the provisional MED_{iu} previously determined in **9.4.12**.

For each subject, the unprotected MED_{iu} shall be determined on the same day as the test product protected MED_{ip}.

9.4.14 Incremental progression of UV dose

9.4.14.1 For the unprotected site, the range of UV doses applied shall be established using the subject's provisional MED_{iu}, or the estimated MED_{iu} based on the ITA° and Annex E. A minimum of five sub-sites centered on or close to the provisional/estimated MED_{iu} shall be exposed with incremental UV doses using a recommended geometric progression of 1,25×. Other geometric progressions of less than 1,25x may be used (such as 1.2; 1.15; 1.12) but shall be consistent throughout the test (same progression used for unprotected and protected sites). Exposure times may be rounded to the closest integer seconds.

9.4.14.2 For the product protected sites, the UV doses delivered are defined by the expected MED_{ip}, which is the multiple of the expected SPF of the test product (as determined by the test sponsor or previous data) and the provisional or estimated MED_{iu} for the subject. A minimum of five sub-sites centred on or close to the expected MED_{ip} shall be exposed with incremental UV doses using a recommended geometric progression of 1,25x. Other geometric progressions may be used (such as 1.2, 1.15 or 1.12). A maximum geometric progress of 1.15 shall be used for expected SPF ≥25. Smaller geometric progressions (such as 1.12 may be used but shall also be used but shall also be consistent throughout the exposure procedure (same progression used for unprotected and protected sites).

The expected value of the SPF may be changed during the testing of the product between test subjects as requested by the test sponsor or the laboratory management to prevent test failures or overexposure of subjects.

9.4.15 Product removal

After UV exposures, reference and test products may be gently removed, using an appropriate means.

9.5 Procedure for MED assessment

9.5.1 General

The minimal erythemal dose for individual unprotected skin (MED_{iu}), for the test product individual protected skin (MED_{ip}), and the MED_{ip} for the reference sunscreen formulation, shall all be determined on the same day.

9.5.2 Time of assessment of MED

The MED(s) shall be assessed 20 h ± 4 h after UV exposure (between 16 h and 24 h) as measured from the end of the last exposure period. During the time interval between UV exposure and MED assessment, the subject shall avoid any extra UV exposure (artificial UV light or sunlight) to the exposed area. Any additional UV exposure (natural or artificial) to the test area of an individual will invalidate the data from that individual and that data shall be rejected from the test results and not count against the total allowable rejected subjects.

9.5.2.1 The MEDs shall be assessed visually. The observer's eyesight should have been checked for

normal colour vision. A yearly check of acuity of vision is recommended.

9.5.2.2 Visual assessment should be performed in sufficient and uniform illumination. At least 450 lux in the plane parallel with the back of the test subject is required using a lamp with a colour temperature of 6 500°K.

9.5.2.3 The determination of MED(s) shall be carried out in a room with matt, neutral wall colours.

9.5.2.4 Erythemal responses shall be observed in a "blind" manner. The observers of erythemal responses on any subjects shall not be the same persons as the ones who performed product application and exposure. The observers shall be not aware of the test design (randomization of test sites) on that subject.

9.5.2.5 *Grading scale for the MED_{is}*

Unprotected UV exposed sites and protected UV exposed sites should be graded with the same reference scale and same visual references as shown in Annex F:

9.5.2.5.1 0: no erythema present;

9.5.4.5.2 0.5: ambiguous erythema, and/or no clear border, and/or not filling more than 50 % of the exposure subsite;

9.5.2.5.3 1: Perceptible unambiguous erythema with defined borders filling more than 50 % of the exposure subsite (MED if it is the lowest exposure dose with grade 1);

9.5.2.5.4 2: Moderate to intense erythema.

9.5.2.6 *Pigmentation responses*

The responses observed at the exposure sites may be pink/red (erythema), grey/brown (pigmentation), or a mixture of both. If the exposure site has grey/brown colouration, the surface of the skin shall be lightly pressed with a glass slide to determine if there is also erythema present. If erythema is present, there will be a slight blanching of the any redness of the skin with the pressure and colour will return after removal of the pressure. The site shall be scored as having unambiguous erythema provided that the returning redness in the exposed sub-site is more than that of the unexposed surrounding skin, and the erythema occurs over more than 50% of the exposure sub-site. Pigmentation responses (with no erythema) shall not be considered as a qualifying response for MED_u or MED_p.

9.5.3 Data rejection criteria

Test data are deemed invalid and shall be rejected according to the circumstances specified in Table 1.

Table 1 — Data rejection criteria

Observation	MED_{iu}	MED_{ip}	**Reference standard**
No grade of at least 1 for any exposed sub sites[a]	Data for subject is rejected Does not count against total allowable rejected number of subjects	Data for test product is rejected. Does not count against total allowable rejected number of subjects	Data for subject is rejected Failure counts against allowable rejected number of subjects
All test subsites show erythema of at least grade 1[b]	Data for subject is rejected Does not count against number of total allowable rejections	Data for test product is rejected Counts against number of total allowable rejections	Data for subject is rejected Counts against number of total allowable rejections

erythemal respon-se(s) is (are) absent for exposures higher than the determined MED	Data for subject is rejected Does not count against number of total allowable rejections	Data for subject is rejected Counts against number of total allowable rejections	Data for subject is rejected Counts against number of total allowable rejections
Non-compliance of the subject d OR	Data for subject is rejected Does not count against number of total	Data for subject is rejected Does not count against number of total	Data for subject is rejected Does not count against number of

Observations definitions:

1 No Grade of at least 1 for any exposed sub: All exposed subsites have grades of 0, or 0,5, and no qualifying MED (Grade 1) is observed.

2 All test sites show erythema of at least Grade 1: No sites have grades of 0 or 0,5, and a MED response cannot be established.

3 Erythemal response(s) is (are) absent for exposures higher than the determined MED dose (randomly absent): A Grade of 0 is observed at an exposure dose higher than the determined MED, (randomly absent or illogical sequence).

4 Non-compliance of the subject: Subject does not follow instructions during or after the treatment or UV exposures that could affect the outcome of the test (wipes sunscreen treated areas during application or exposures, medicates with anti-inflammatory drugs, exposes treatment areas to UV light (sunlight or other UV source), irritates treated area, etc.).

5 Technical failure: Failure of equipment or procedures during the treatment phases of the procedure (for example: incorrect lamp intensity or fluctuations, incorrect exposure times, incorrect site application of sunscreen, and similar reasons) that would jeopardize the integrity of the treatments and conclusions.

9.5.3 Test failure criteria

If data have to be rejected for the test product on more than five subjects, then the whole test for that product shall be invalid and shall be rejected.

If data have to be rejected for the reference sunscreen on more than five subjects, then the whole test shall be invalid and shall be rejected.

9.5.4 Expression of MEDs

MEDs shall be expressed in terms of energy J/m^2 eff. (integers).

All irradiance measurements shall use a radiometer previously cross calibrated against a spectroradiometric measurement weighted with the erythema action spectrum, or a spectroradiometer measurement weighted with the erythema action spectrum to determine the J/m^2 eff.

10 Calculation of the sun protection factor and statistics

10.1 Calculation of the individual SPF (SPF_i)

The SPF_i of both the reference sunscreen and the product under test for each subject shall be calculated as shown in Formula (4), and expressed to one decimal place by truncation.

$$SPF_i = \frac{MEDip}{MEDiu}$$

MED_{ip} = MED of sunscreen protected skin for an individual;

MED_{iu} = MED of unprotected skin for an individual.

10.2 Calculation of product SPF

The SPF result for the test product and for the reference sunscreen formulation shall be calculated as the arithmetic mean of all valid individual SPF_i values.

The minimum number of valid SPF_i values shall be ten and the maximum number of valid SPF_i values twenty. A maximum of five results may be excluded from the calculation of the mean SPF, but each exclusion shall be justified according to **9.5.3** or if protocol non-compliance has occurred. A sixth invalid result automatically invalidates the whole test for that test product and no SPF can be calculated for it.

SPF shall be expressed to one decimal place by truncation.

10.3 Statistical criterion

The statistical criterion for all SPF measurements is that the 95 % confidence interval on the mean SPF measured shall comply with the ±17 % CI criteria of the measured mean SPF. This applies to test products and reference sunscreen products.

Consequently, the actual number of subjects tested shall be defined as the number (minimum ten) required to produce a mean test product SPF with a 95 % confidence interval (CI) which falls within a range of ±17 % of the measured mean SPF for the tested product and a mean reference product SPF which has a 95 % CI which falls within the range of ±17 % of the measured mean SPF for the reference sunscreen formulation.

10.4 Validation of the test

The mean SPF of the reference sunscreen formulation used in the test shall fall within the acceptance limits shown in Annex C.

11 TEST REPORT

11.1 Overview

The test report shall contain at least the following information.

11.2 General information

11.2.1 Identification of the testing laboratory.

11.2.2 A reference to this test method.

11.2.3 Product identifier and expected SPF.

11.2.4 Any instructions compliant with this document given by the sponsor for the application of the product (i.e. fingercot or not, latex or nitrile, pretreatment for powders, etc.).

11.2.5 Any specific instructions not compliant with this document given by the sponsor for the application of the product. For example, special preparation of sample prior to application, such as recombining 2 phase systems.

11.2.6 The geometric progression used for the individual MED.

11.2.7 Commencement and ending dates of the test.

11.2.8 Identification of the reference sunscreen used and evidence of compliance with the acceptance range for this sunscreen according to the limits described in C.1.

11.2.9 A reference to latest calibration and statement of compliance document (date and provider) of solar simulators used in the test.

11.2.10 Mean SPF value expressed in one decimal place (truncated), standard deviation on the mean, and 95 % CI as a number and as a %, and 17 % of the mean SPF.

11.2.11 Protocol deviations if any.

11.3 Data in tabular form for each test subject

Annex G provides a template for reporting the following data.

11.3.1 The subject number in sequence.

11.3.2 Identification by subject, of the technicians who applied, exposed, and evaluated responses during the test.

11.3.3 The subject ITA° value.

11.3.4 The dates of UV exposures for each subject.

11.3.5 Identification by code number, of each subject;

11.3.6 The intensity of the solar simulator output in Watts/m2 erythemally effective (Eer) irradiance. In the case of a multiport device, this should be the value for the highest intensity port.

11.3.7 Seconds of exposure for the MEDiu, MEDip, and MEDip for reference standard.

11.3.8 Individual MED for unprotected skin, test product protected skin and reference sunscreen protected skin, reported as J/m^2 eff. (no decimal).

11.3.9 Individual SPF_i values expressed in one decimal place (truncated), including all valid data and rejected data for the test product and for the reference sunscreen.

11.3.10 An indication if the SPF_i value was rejected (Y or N?).

11.3.11 Mean SPF values, standard deviation on the mean, and 95 % CI as a number and as a %, and 17 % of the mean SPF.

11.4 Statistics for the test products

After completion of at least 10 valid test subjects, calculations and statistics as described in Annex D and at least:

11.4.1 M*ean SPF of the test product truncated to one decimal place;*

11.4.2 *Standard deviation (s) calculation*;

11.4.3 *Confidence interval (c) calculation*;

11.4.4 *CI [%]*;

11.4.5 *95 % CI*;

11.4.6 *17 % of the mean SPF calculation*;

11.4.7 *A statement that the test study complies with the statistical validations.*

ANNEX A
(Normative)
SELECTION CRITERIA FOR THE TEST SUBJECTS

A-1 RATIONALE

Experience has shown that utilization of skin phototyping is problematic, and unable to adequately distinguish appropriate subjects for SPF testing and for estimating skin sensitivity to sunburn. In contrast, use of the ITA° value has been shown to be a useful quantitative measure to choose test subjects for SPF testing, and for predicting their MED_u values. This approach has been used for many year by many different laboratories. Their experience has been utilized to establish the limits of useful range of ITA° values for SPF testing and to estimate MED_u values. This document references only the use of ITA° values for qualifying subjects for the SPF test.

A-2 SELECTION CRITERIA FOR THE TEST SUBJECTS

A-2.1 Skin colour

Subjects shall be selected using the colourimetric ITA° value. The skin of subjects shall have a colourimetric ITA° value of subjects of ≥28°.

Colourimetric ITA° values and skin colour categories are defined by the colourimetric descriptors of Chardon et al. using the CIE (1976) *L*a*b** colour space.

Skin colour categories	**ITA° values ranges**
Very light	>55°
Light	>41° to 55°
Intermediate	>28° to 41°
Tan (or matt)	>10° to 28°
Brown	>−30° to 10°
Black	≤−30°

Where

$$ITA° = \{\text{arc tangent } [(L^* - 50)/b^*]\}180/3{,}141\ 6.$$

A-2.2 Medical and ethical considerations

Subjects should be adequately informed of the aims and potential risk (direct or secondary effects) of the study and any discomfort they may experience. Each subject shall give a written agreement to participate in SPF tests.

It is recommended that new subjects first be interviewed by a health professional to establish their medical status and suitability prior to inclusion in the subject panel.

Subjects should be checked visually by a trained and competent scientist or technician before participating in a study. Their skin colour shall be uniform over the whole test area without pigmentation, nevi, or the like and no sunburn (erythema) shall be present on the test area.

When there is some doubt on the provisional SPF value of the test product, a screening should first be performed. In order to protect the subjects a lower SPF value should be used on two or three subjects and increased progressively on the other subjects. Data from these tests may be included in the final results provided they comply with all other requirements for a valid test result.

SPF measurements should be designed to minimize any harmful, long-lasting effects on human test subjects. Tests shall be performed by trained and competent personnel in order to avoid any damage to the skin of the test subjects involved in the test.

A-2.3 Non-inclusion criteria

All non-inclusion criteria shall be checked before testing.

The following conditions shall automatically disallow inclusion of a subject in the test group:

A-2.3.1 Children and persons below the locally legal age of consent or >70 years;

A-2.3.2 Pregnant or lactating women;

A-2.3.3 Subjects using medication with photo-sensitizing potential;

A-2.3.4 Subjects using anti-inflammatory medication;

A-2.3.5 Subjects with systemic dermatological conditions (including dysplastic nevi);

A-2.3.6 Subjects with a history of abnormal response to the sun;

A-2.3.7 Subjects who have used tanning beds in the previous eight weeks prior to SPF testing;

A-2.3.8 Subjects having had sun exposure on the back area in the previous eight weeks prior to SPF testing;

A-2.3.9 Subjects having marks, blemishes or nevi in the test area;

A-2.3.10 Subjects presenting with existing sun damage in the test area;

A-2.3.11 Subjects having excessive hair in the area on the test on the day of testing (may be shaved up to 3 days prior to the test day);

A-2.3.12 Subjects having skeletal protrusions and extreme areas of curvature in the test area.

A-2.4 Frequency of subject participation (interval between two tests)

Subjects may participate in a test provided that at least 8 weeks have elapsed since they participate in a previous UV exposure study (i.e. SPF, UVA-PF, Photoallergy, Phototoxicity test), and all skin tanned marks from previous tests have cleared from the test sites on the back and are no longer visible.

ANNEX B
(Normative)
Definition of the UV solar simulator output

B.1 GENERAL

The aim of these specifications is to define practical criteria for defining and measuring the spectral compliance of UV solar simulators used for SPF determination, such as xenon arc lamp.

B-2 RATIONALE FOR SPECIFICATIONS

B-2.1 UV range

Because UV rays are responsible for most of the sun's damaging effects on skin, the erythemal protective efficiency of sunscreen products is tested within this range of wavelengths. Therefore, the definition of the spectrum of the UV solar simulator is limited to the terrestrial UV-wavelengths, i.e. from 290 nm to 400 nm.

Wavelengths below this range (<290 nm) do not occur in terrestrial sunlight and should be excluded, whilst those above this range (>400 nm) may cause undesirable side effects (particularly thermal effects) and should be removed using appropriate devices.

B-2.2 Sun UV spectra

Measured solar spectra have been published taking into account different geographical latitudes and altitudes, and variations due to year, season, time of day and ozone content.

For the purpose of this method, a set of selected representative spectra were compiled.

B-2.3 Erythemal balance between wavelengths

The erythema induced by sunlight UV in unprotected human skin is mainly generated by wavelengths between 290 nm and 320 nm, with a maximum effectiveness around 308 nm. For this reason, some previous attempts to standardize UV solar simulator output concentrated on UVB wavelengths alone. However, when a high SPF product is tested, the erythemal contribution from UVA wavelengths can become important, especially if the sun product protects predominantly in the UVB wavelengths. Therefore, it is necessary to include all UVA and UVB wavelengths when standardizing the UV solar simulator output.

B-2.4 Test criteria

The accuracy of the SPF measured is dependent on the absorbance characteristics of the sunscreen filtering system to be tested in conjunction with the source spectrum. Therefore, it is important to define the source by the spectral distribution of its erythemal efficacy as well as its overall spectral irradiance characteristics.

Thus, the source spectral specification is described in terms of cumulative erythemal effectiveness by successive wavelength bands from 290 nm up to 400 nm. The erythemal effectiveness of each wavelength band is expressed as a percentage of the total erythemal effectiveness from less than 290 nm to 400 nm, or as the percentage relative cumulative erythemal effectiveness (% RCEE). Wavelengths below 290 nm should be excluded from any source by appropriate filters. Wavelengths above 400 nm should be limited as much as possible and are not included in the calculation of % RCEE.

Since RCEE values and the distribution of the UVA proportions of the UV spectrum are calculated as relative percentages, the spectral irradiance need not be measured in absolute energy units; however absolute irradiance measurements are needed to determine the total irradiance of the source.

B.2.5 Solar simulator and filtration

A lamp that produces a continuous spectrum can readily be adapted to fulfil the % RCEE acceptance limits for the output between 290 nm and 400 nm by using specific optical filters. To ensure uniformity in spectral shape in SPF testing, UV solar simulators utilizing a xenon arc lamp, shall be filtered with a dichroic UV filter to minimize IR radiation, and UV shaping filters such as WG320 and UG11 or equivalent filters.

The simple use of the recommended filters is not, in itself, an adequate assurance that the UV output is of the correct quality and so the spectral output shall be confirmed by spectroradiometric measurement.

B.2.6 UV solar simulator acceptance limits

The limits prescribed in terms of % RCEE values are shown in Table B.1. They have been determined from the measured spectral outputs of actual UV solar simulators.

B-3 MODE OF OPERATION

B-3.1 UV solar simulator acceptance limits

The % RCEE limit values are given in Table B.1. The actual % RCEE values, for an individual solar simulator, calculated from Spectroradiometric measurements, shall fall within the limits listed in columns 2 and 3 of Table B.1 and those also reported in Table B.2, columns 9 and 10.

These practical limits take into account the uncertainty in Spectroradiometric measurements and in optical components of the solar simulators. They have been defined and restricted as tightly as possible.

Table B.1 — % RCEE acceptance limits for the UV solar simulator output

Spectral range nm	**Measured % RCEE**	
	Lower limit	Upper limit
<290		<0.1
290 to 300	1.0	8.0
290 to 310	49.0	65.0
290 to 320	85.0	90.0
290 to 330	91.5	95.5
290 to 340	94.0	97.0
290 to 400	99.9	100.0

To ensure that appropriate amounts of UVA radiation are included in the spectrum of the solar simulator, the total radiometric proportion of the UVA II (320 nm to 340 nm) irradiance of the simulator shall be ≥20 % of the total UV (290 nm to 400 nm) irradiance. Additionally, the UVA I region (340 nm to 400 nm) irradiance shall be ≥60 % of the total UV irradiance.

B-3.2 Quality of the UV solar simulator output

B-3.2.1 Spectroradiometric measurements

The output spectrum of the UV solar simulator, including all filters and optical components, shall be measured with a spectroradiometer that has been calibrated against a standard lamp that is traceable to a national or an international calibration standard. The spectroradiometer should be fitted with a double monochromator and its bandwidth should be ≤2 nm (1 nm is recommended) in order to ensure that all energies are represented in an amplitude range of at least 5 decades. Measurements shall be made in steps not exceeding the bandwidth.

The instrument shall have been calibrated against standard light sources for its response to spectral irradiances, for its wavelength accuracy (e.g. mercury lamp) and for linearity of signal responses at all wavelengths over an irradiance range covering the actual source measurement range.

The units of source irradiance should be in actual spectral energy ($W/m^2 \cdot nm$, $mW/cm^2 \cdot nm$).

B-3.2.2 Radiometric measurements

The UV irradiance of the solar simulator is controlled with a radiometer that has been previously cross-calibrated for this source spectrum against the spectroradiometric measurement (see B.3.2.1).

A UV dose is the result of multiplying the UV source irradiance by the exposure duration. When a large-beam UV solar simulator is used, allowing simultaneous exposure of several sub-sites by varying the exposure time, the uniformity in beam irradiance should be as high as possible. This uniformity can be measured with the radiometer. The range of irradiance variation over all the exposure sub-sites should be less than 10 %. If the variation exceeds 10 %, then appropriate compensation for different irradiance levels should be made in the exposure time on each sub-site. Solar simulators with light guides or multiple small beams, exposing all sub-sites for the same duration but with varied irradiance values should be checked to ensure that each beam or guide generates uniform erythemal responses.

A warm-up time of at least 20 min shall be allowed for the UV solar simulator to stabilize before starting exposures. This is to ensure a consistent irradiance over the whole exposure period.

B-3.2.3 Calculation of percentage relative cumulative erythemal effectiveness (% RCEE)

An example of calculations for a xenon arc UV solar simulator that complies with the output specifications is given in Table B.2.

The measured spectral irradiance of the UV solar simulator (Table B.2: column 2) is multiplied by the CIE (1998) standard skin erythemal action spectrum (column 4) to obtain the spectral erythemal effectiveness of the UV solar simulator (column 5).

The CIE (1999) erythemal effectiveness at each wavelength is calculated in relative units from the following formulae:

S_{er} = 1.0 for wavelengths 250 nm < $\lambda \leq$ 298 nm

$S_{er} = 10^{0,094\,(298-\lambda)}$ for wavelengths 298 nm < $\lambda \leq$ 328 nm

$S_{er} = 10^{0,015\,(140-\lambda)}$ for wavelengths 328 nm < $\lambda \leq$ 400 nm

The spectral erythemal effectiveness values (column 5) of the UV solar simulator spectrum are then integrated from 290 nm to the various successive reference wavelengths (300 nm, 310 nm, 320 nm, 330 nm, 340 nm, 350 nm and 400 nm) in order to produce the cumulative erythemal effectiveness for each wavelength band (column 7) and the total erythemal effectiveness calculated up to 400 nm (*T* value, last row, column 6 or 7). Integration can be performed by approximation techniques such as the trapezium or rectangle methods using a spreadsheet, applying wavelength intervals of 1 nm. The example shown uses the trapezium method to calculate the areas of each 1 nm interval from 280 nm to 400 nm (column 6), which are then summed to each reference wavelength to give the cumulative erythemal effectiveness value (column 7). Finally, the percentage relative cumulative erythemal effectiveness (% RCEE, column 8) is calculated at the reference wavelengths as the percentage ratio of the cumulative erythemal effectiveness (column 7) at each of these wavelengths to the total integrated value at 400 nm (*T* value, column 7).

B-3.3 Evaluating compliance

For each reference waveband, the % RCEE values of the source (Table B.2, column 8) shall comply with those specified in Table B.1 (or in Table B.2, columns 9 and 10). All values shall lie within the acceptance limits. If the UV solar simulator spectrum is outside the limits in any of the wavebands, then the filtration needs to be adjusted to comply with the spectral output specifications.

In addition, the solar simulator spectrum shall include less than 0.1 % of UVB-RCEE below 290 nm and, to ensure that the solar simulator contains the correct balance of UVA : UVB, the output from the lamp system should contain ≥60 % UVA I (340 nm to 400 nm) and ≥20 % UVA II (320 nm to 340 nm).
The total irradiance of the source shall be measured.

B-3.4 Adjusting UV solar simulator output

If the output spectrum of the UV solar simulator needs to be adjusted to fit the acceptance specifications, this may be achieved either by checking the xenon lamp's elapsed life and replacing it if necessary, or by adapting the spectral shaping filters within the UV solar simulator, particularly the thickness of the short cut-off filter.

If the total irradiance of the UV solar simulator exceeds 1 600 W/m^2, the irradiance can usually be reduced by lowering the electrical current supplying the xenon lamp, provided that the current remains in the normal operational stability range. If total irradiance is adjusted in this way, then the quality of the emission spectrum should be checked again to ensure that the acceptance specifications are met.

Table B.2 — Example of calculation — Xenon-arc UV source and RCEE values

1	2	3	4	5	6	7	8	9	10
	UV Source Irradiance {E} W·m^{-2} · nm^{-1}	**Normalized** to 320 nm	Eryth. A.S. (CIE-1999) $\{s_{er}\}$	**Spectral eryth. effic.** $\{E \times s_{er}\}$	**Interval eryth. effic.** 1/2$\{E \times s_{er}\}$dl	**Cumulative eryth. effic.** Sum$\{E \times s_{er}\}$	**Sol. Sim. % RCEE** Sum$\{E \times s_{er}\}/T$	RCEE accept.	
W.L. λ nm								**Lower limit**	**Upper limit**
280	**1.523E−05**	**1.75E−06**	**1.00E+00**	**1.52E−05**					
281	1.848E−05	2.12E−06	1.00E+.00	1.85E−05	1.69E−05				
282	2.904E−05	3.34E−06	1.00E00	2.90E−05	2.38E−05				
283	1.878E−05	2.16E−06	1.00E+00	1.88E−05	2.39E−05				
284	2.139E−05	2.46E−06	1.00E+00	2.14E−05	2.01E−05				
285	2.837E−05	3.26E−06	1.00E+00	2.84E−05	2.49E−05				
286	2.935E−05	3.37E−06	1.00E+00	2.94E−05	2.89E−05				
287	2.627E−05	3.02E−06	1.00E+00	2.63E−05	2.78E−05				
288	2.927E−05	3.36E−06	1.00E+00	2.93E−05	2.78E−05				
289	4.308E−05	4.95E−06	1.00E+00	4.31E−05	3.62E−05				
290	**4.405E−05**	**5.06E−06**	**1.00E+00**	**4.40E−05**	**4.36E−05**	**2.74E−04**	**0.00 %**	**—**	**< 0.1 %**
291	5.500E−05	6.32E−06	1.00E00	5.50E−5	4.95E−05				
292	8.279E−05	9.52E−06	1.00E+00	8.28E−05	6.89E−05				
293	2.379E−04	2.73E−05	1.00E+00	2.38E−04	1.60E−04				
294	8.219E−04	9.45E−05	1.00E+00	8.22E−04	5.30E−04				
295	2.685E−03	3.09E−04	1.00E+00	2.68E−03	1.75E−03				

296	8.029E−03	9.23E−04	1.00E+00	8.03E−03	5.36E−03				
297	2.102E−02	2.42E−03	1.00E+00	2.10E−02	1.45E−02				
298	5.030E−02	5.78E−03	1.00E+00	5.03E−02	3.57E−02				
299	1.041E−01	1.20E−02	8.05E−01	8.39E−02	6.71E−02				

S_{er} is the erythemal effectiveness.

E is the source irradiance.

W.L. is the wavelength λ of the source.

Table B.2 *(continued)*

1	2	3	4	5	6	7	8	9	10
W.L λ nm	**UV Source Irradiance** {E} W·m^{-2} · nm^{-1}	**Normalized** to 320 nm	Eryth. A.S. (CIE-1999) $\{s_{er}\}$	**Spectral eryth. effic.** $\{E \times s_{er}\}$	**Interval eryth. effic.** 1/2$\{E \times s_{er}\}$dl	**Cumulative eryth. effic.** · Sum $\{E \times s_{er}\}$	**Sol. Sim. % RCEE** Sum$\{E \times s_{er}\}/T$	RCEE accept. range **Lower limit**	**Upper limit**
300	**1.886E−01**	**2.17E−02**	**6.49E−01**	**1.22E−01**	**1.03E−01**	**2.29E−01**	**4.0 %**	**1 %**	**8.0 %**
301	3.352E−01	3.85E−02	5.22E−0	1.75E−01	1.49E−01				
302	5.358E−01	6.16E−02	4.21E−01	2.25E−01	2.00E-01				
303	8.051E−01	9.25E−02	3.39E−01	2.73E−01	2.49E−01				
304	1.126E+00	1.29E−01	2.73E−01	3.07E−01	2.90E−01				
305	1.563E+00	1.80E−01	2.20E−01	3.43E−01	3.25E−01				
306	2.009E+00	2.31E−01	1.77E−01	3.56E−01	3.50E−01				
307	2.576E+00	2.96E−01	1.43E−01	3.67E−01	3.61E−01				
308	3.081E+00	3.54E−01	1.15E−01	3.54E−01	3.60E−01				
309	3.700E+00	4.25E−01	9.25E−02	3.42E−01	3.48E−01				
310	**4.248E+00**	**4.88E−01**	**7.45E−02**	**3.16E−01**	**3.29E−01**	**3.19E+00**	**55.7 %**	**49.0 %**	**65.0 %**
311	4.769E+00	5.48E−01	6.00E−02	2.86E−0	3.01E−01				
312	5.384E+00	6.19E−01	4.83E−02	2.60E−01	2.73E−01				
313	5.978E+00	6.87E−01	3.89E−02	2.33E−01	2.46E−01				
314	6.399E+00	7.36E−01	3.13E−02	2.01E−01	2.17E−01				
315	6.896E+00	7.93E−01	2.52E−02	1.74E−01	1.87E−01				
316	7.250E+00	8.33E−01	2.03E−02	1.47E−01	1.61E−01				
317	7.731E+00	8.89E−01	1.64E−02	1.27E−01	1.37E−01				
318	8.060E+00	9.26E−01	1.32E−02	1.06E−01	1.16E−01				
319	8.338E+00	9.58E−01	1.06E−02	8.85E−02	9.74E−02				
320	**8.700E+00**	**1.00E+00**	**8.55E−03**	**7.44E−02**	**8.15E−02**	**5.01E+00**	**87.0 %**	**85.0 %**	**90.0 %**
321	8.988E+00	1.03E+00	6.89E−0	6.19E−02	6.81E−02				
322	9.320E+00	1.07E+00	5.55E−03	5.17E−02	5.68E−02				
323	9.547E+00	1.10E+00	4.47E−03	4.26E−02	4.72E−02				
324	9.755E+00	1.12E+00	3.60E−03	3.51E−02	3.89E−02				
325	9.913E+00	1.14E+00	2.90E−03	2.87E−02	3.19E−02				
326	1.015E+01	1.17E+00	2.33E−03	2.37E−02	2.62E−02				
327	1.029E+01	1.18E+00	1.88E−03	1.93E−02	2.15E−02				
328	1.042E+01	1.20E+00	1.51E-03	1.58E-02	1.76E-02				
329	1.060E+01	1.22E+00	1.46E-03	1.55E-02	1.56E-02				

330	**1.071E+01**	**1.23E+00**	**1.41E-03**	**1.51E-02**	**1.53E-02**	**5.35E+00**	**92.9%**	**91.5 %**	**95.5 %**
331	1.085E+01	1.25E+00	1.36E-03	1.48E-02	1.50E-02				
332	1.099E+01	1.26E+00	1.32E-03	1.45E-02	1.46E-02				
333	1.108E+01	1.27E+00	1.27E-03	1.41E-02	1.43E-02				
334	1.120E+01	1.29E+00	1.23E-03	1.38E-02	1.39E-02				
335	1.127E+01	1.29E+00	1.19E-03	1.34E-02	1.36E-02				
336	1.135E+01	1.30E+00	1.15E-03	1.30E-02	1.32E-02				
337	1.143E+01	1.31E+00	1.11E-03	1.27E-02	1.29E-02				
338	1.149E+01	1.32E+00	1.07E-03	1.23E-02	1.25E-02				
339	1.160E+01	1.33E+00	1.04E-03	1.20E-02	1.22E-02				
340	**1.166E+01**	**1.34E+00**	**1.00E-03**	**1.17E-02**	**1.18E-02**	**5.48E+00**	**95.2 %**	**94 %**	**97.0 %**

s_{er} is the erythemal effectiveness.

E is the source irradiance.

Table B.2 *(continued)*

1	2	3	4	5	6	7	8	9	10
W.L λ nm	**UV Source Irradiance** {E} $W \cdot m^{-2} \cdot nm^{-1}$	**Normalized** to 320 nm	Eryth. A.S. (CIE-1999) $\{s_{er}\}$	**Spectral eryth. effic.** $\{E \times s_{er}\}$	**Interval eryth. effic.** $1/2\{E \times s_{er}\}dl$	**Cumulative eryth. effic.** Sum$\{E \times s_{er}\}$	**Sol. Sim. % RCEE** Sum$\{E \times s_{er}\}/T$	RCEE accept. **Lower limit**	**Upper limit**
341	1.176E+01	1.35E+00	9.66E-04	1.14E-02	1.15E-02				
342	1.185E+01	1.36E+00	9.33E-04	1.11E-02	1.12E-02				
343	1.189E+01	1.37E+00	9.02E-04	1.07E-02	1.09E-02				
344	1.194E+01	1.37E+00	8.71E-04	1.04E-02	1.06E-02				
345	1.196E+01	1.37E+00	8.41E-04	1.01E-02	1.02E-02				
346	1.200E+01	1.38E+00	8.13E-04	9.75E-03	9.91E-03				
347	1.204E+01	1.38E+00	7.85E-04	9.45E-03	9.60E-03				
348	1.212E+01	1.39E+00	7.59E-04	9.19E-03	9.32E-03				
349	1.215E+01	1.40E+00	7.33E-04	8.90E-03	9.05E-03				
350	**1.220E+01**	**1.40E+00**	**7.08E-04**	**8.64E-03**	**8.77E-03**	**5.57E+00**	**97.2 %**		
351	1.224E+01	1.41E+00	6.84E-04	8.37E-03	8.50E-03				
352	1.230E+01	1.41E+00	6.61E-04	8.13E-03	8.25E-03				
353	1.231E+01	1.42E+00	6.38E-04	7.86E-03	7.99E-03				
354	1.229E+01	1.41E+00	6.17E-04	7.58E-03	7.72E-03				
355	1.234E+01	1.42E+00	5.96E-04	7.35E-03	7.46E-03				
356	1.233E+01	1.42E+00	5.75E-04	7.10E-03	7.22E-03				
357	1.232E+01	1.42E+00	5.56E-04	6.85E-03	6.97E-03				
358	1.234E+01	1.42E+00	5.37E-04	6.63E-03	6.74E-03				
359	1.234E+01	1.42E+00	5.19E-04	6.40E-03	6.51E-03				
360	**1.233E+01**	**1.42E+00**	**5.01E-04**	**6.18E-03**	**6.29E-03**	**5.64E+00**	**98.5 %**		
361	1.230E+01	1.41E+00	4.84E-04	5.96E-03	6.07E-03				
362	1.225E+01	1.41E+00	4.68E-04	5.73E-03	5.84E-03				

363	1.217E+01	1.40E+0	4.52E-04	5.50E-03	5.61E-03				
364	1.12E+01	1.39E+0	4.37E-04	5.29E-03	5.39E-03				
365	1.200E+01	1.38E+0	4.22E-04	5.06E-03	5.18E-03				
366	1.183E+01	1.36E+0	4.07E-04	4.82E-03	4.94E-03				
367	1.171E+01	1.35E+0	3.94E-04	4.61E-03	4.71E-03				
368	1.153E+01	1.33E+0	3.80E-04	4.38E-03	4.50E-03				
369	1.130E+01	1.30E+0	3.67E-04	4.15E-03	4.27E-03				
370	**1.102E+01**	**1.27E+0 0**	**3.55E-04**	**3.91E-03**	**4.03E-03**	**5.69E+0 0**	**99.3 %**		
371	1.073E+01	1.23E+0	3.43E-04	3.68E-03	3.79E-03				
372	1.042E+01	1.20E+0	3.31E-04	3.45E-03	3.56E-03				
373	1.005E+01	1.16E+0	3.20E-04	3.21E-03	3.33E-03				
374	9.649E+00	1.11E+0	3.09E-04	2.98E-03	3.10E-03				
375	9.370E+00	1.08E+0	2.99E-04	2.80E-03	2.89E-03				
376	8.977E+00	1.03E+0	2.88E-04	2.59E-03	2.69E-03				
377	8.597E+00	9.88E−0	2.79E-04	2.40E-03	2.49E-03				
378	8.195E+00	9.42E−0	2.69E-04	2.21E-03	2.30E-03				
379	7.707E+00	8.86E−0	2.60E-04	2.00E-03	2.10E-03				
380	**7,176E+00**	**8,25E−0 1**	**2,51E-04**	**1,80E-03**	**1,90E-03**	**5,72E+0 0**	**99,8 %**		
381	6,703E+00	7,70E−0 1	2,43E-04	1,63E-03	1,71E-03				

s_{er} is the erythemal effectiveness.

E is the source irradiance.

Table B.2 *(continued)*

1	2	3	4	5	6	7	8	9	10
W.L λ nm	**UV Source Irradiance** {E} W·m^{-2} · nm^{-1}	**Normalized** to 320 nm	Eryth. A.S. (CIE-1999) $\{s_{er}\}$	**Spectral eryth. effic.** $\{E \times s_{er}\}$	**Interval eryth. effic.** 1/2$\{E \times s_{er}\}$dl	**Cumulative eryth. effic.** Sum$\{E \times s_{er}\}$	**Sol. Sim. % RCEE** Sum$\{E \times s_{er}\}/T$	RCEE **Lower limit**	RCEE **Upper limit**
382	6.147E+00	7.07E−01	2.34E-04	1.44E-03	1.53E-03				
383	5.577E+00	6.41E−01	2.26E-04	1.26E-03	1.35E-03				
384	4.994E+00	5.74E−01	2.19E-04	1.09E-03	1.18E-03				
385	4.423E+00	5.08E−01	2.11E-04	9.35E-04	1.01E-03				
386	3.860E+00	4.44E−01	2.04E-04	7.88E-04	8.61E-04				
387	3.348E+00	3.85E−01	1.97E-04	6.60E-04	7.24E-04				
388	2.846E+00	3.27E−01	1.91E-04	5.42E-04	6.01E-04				
389	2,389E+00	2,75E−01	1,84E-04	4,40E-04	4,91E-04				
390	**1.996E+00**	**2.29E−01**	**1.78E-04**	**3.55E-04**	**3.97E-04**	**5.73E+00**	**100.0 %**		
391	1.626E+00	1.87E−01	1.72E-04	2.79E-04	3.17E-04				
392	1.297E+00	1.49E−01	1.66E-04	2.15E-04	2.47E-04				

393	1.016E+00	1.17E−01	1.60E-04	1.63E-04	1.89E-04				
394	7.810E−01	8.98E−02	1.55E-04	1.21E-04	1.42E-04				
395	5.916E−01	6.80E−02	1.50E-04	8.85E-05	1.05E-04				
396	4.438E−01	5.10E−02	1.45E-04	6.41E-05	7.63E-05				
397	3.247E−01	3.73E−02	1.40E-04	4.53E-05	5.47E-05				
398	2.312E−01	2.66E−02	1.35E-04	3.12E-05	3.83E-05				
399	1.593E−01	1.83E−02	1.30E-04	2.08E-05	2.60E-05				
400	**1.073E−01**	**1.23E−02**	**1.26E-04**	**1.35E-05**	**1.71E-05**	**5.73E+00**	**100.0 %**	**99.9**	**100.0 %**
	UV irrad (W·m^{-2}): 8.03*E*+02		UVe irrad. (W·m^{-2}·ery), *T* : **5.76*E*+00**			**Conclusion**: Complies			
s_{er} is the erythemal effectiveness. *E* is the source irradiance.									

ANNEX C
(Normative)
SPF REFERENCE SUNSCREEN FORMULATIONS

C-1 MEAN SPF AND ACCEPTANCE LIMITS FOR REFERENCE SUNSCREEN FORMULATIONS

Table C.1

Reference sunscreen formulatio	Mean SPF	Acceptance limits	
		Lower	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 16 REFERENCE STANDARD

C-2.1 Ingredients

Ingredients		**Mass fraction (%)**
Phase 1	lanolin	4.5
	Theobroma cacao (cocoa) seed butter	2.0
	Glyceryl monostearate SE	3.0
	stearic acid	2.0
	Ethylhexyldimethyl PABA (CAS 21245-02-3) (2-ethylhexyl-4-(dimethylamino)-benzoate)	7.0
	Benzophenone-3 (CAS 131-57-7)	3.0
Phase 2	Water	71.6
	Sorbitol (liquid 70 %)	5.0
	Triethanolamine (99 %)	1.0
	Methylparaben	0.3
	Propylparaben	0.1
Phase 3:	benzyl alcohol	0.5

C-2.2 Manufacturing process

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

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

C-2.2.3 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

Appearance: white/yellowish fluid emulsion

pH: 8.0 ± 0.5

Viscosity (20 °C): range of values: 19 000 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.

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 (*see* 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
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 (%mass fraction) = $\frac{A}{A_{std}} \times \frac{C}{1000} \times \frac{50}{M}$

Where

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:

C-2.5.1 The standard coefficient of variation shall be ≤2.5 %;

C-2.5.2 Recovery value shall be 100 % ± 5 % for all actives;

C-2.5.3 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 15 REFERENCE STANDARD

C-3.1 Ingredients

Ingredients		**Mass fraction (%)**
Phase 1	Cetearyl alcohol	2.205
	PEG-40 castor oil	0.63
	sodium cetostearyl sulphate	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 sulphonic acid (CAS 27503-81-7) (2-phenylbenzimidazole-5-sulphonic 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

C-3.3.1 *Appearance: white to slightly yellowish emulsion*

C-3.3.2 *pH:* 7.5 ± 0.5

C-3.3.3 *Density* (20 °C): 0.970 ± 0.05 g/cm^3

C-3.3.4 Viscosity (20 °C): range of values: 2 000 mPa·s to 4 000 mPa·s [Brookfield®[1)] rotating viscometer, RV type, spindle 4, speed 20 r/min (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-3.6 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-3.6.1 *Chemicals/reagents*

C-3.6.2 *Phenylbenzimidazole sulfonic acid,* production raw material; various suppliers.

C-3.6.3 *Butyl methoxydibenzoylmethane*, production raw material; various suppliers.

C-3.6.4 *Ethylhexyl methoxycinnamate*, production raw material; various suppliers.

C-3.6.5 *Methanol*, HPLC grade.

C-3.6.6 *Water*, fresh distilled.

C-3.6.7 *Glacial acetic acid*, analar or higher purity.

C-3.6.8 *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-3.7 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.

C-3.7.1 butyl methoxydibenzoylmethane 10 mg

C-3.7.2 ethylhexyl methoxycinnamate 75 mg

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

C-3.8 Mixed working standard.

Pipette 5 ml of the mixed standard (*see* C.3.7) into a 50 ml volumetric flask and make up to volume with the solution in accordance with C.3.6.8.

C-3.9 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 according to C.3.6.8
	Flowrate	1.5 ml/min
Detector	Type	UV
	Wavelength	308 nm [or 254 mm for fixed wavelength detection (less sensitivity, less specific)]
Data:	Quantification	peak area

C-3.10 Sample preparation

C-3.10.1 Using an analytical balance weigh approximately 1 g of formulation, to the nearest 0.1 mg, into a 50 ml volumetric flask.

C-3.10.2 Add methanol to dissolve the sample and make up to volume.

C-3.10.3 Ultrasonicate the flask for 5 min and shake to completely mix the sample.

C-3.10.4 Pipette 1 ml into a 10 ml graduated tube and make up to volume with HPLC mobile phase.

C-3.10.5 Analyse the sample and mixed working standard by reverse phase HPLC.

C-3.11 Quality control

C-3.11.1 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-3.11.2 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-3.12 Calculations

$$\text{Analyte (\%mass fraction)} = \frac{A}{A_{\text{std}}} \times \frac{C}{1000} \times \frac{50}{M}$$

A = Peak area in sample extract;

C = Mass concentration of analyte in working standard in milligrams per litre;

A_{std} = Analyte peak area in working standard;

M = Mass of the sample expressed in grams.

C-3.13 Acceptance criteria

The analytical results are acceptable if the following are achieved:

C-3.13.1 the standard coefficient of variation is ≤2.5 %;

C-3.13.2 recovery value is 100 % ± 5 % for all actives;

C-3.13.3 no interfering chromatographic peaks in the sample placebo or working solvent;

C-3.13.4 storage and expiry.

C-3.14 Storage and expiration

C-3.14.1 Store the reference material at 20 °C in a vessel protected from light.

C-3.14.2 The package label shall include an expiration date provided by the manufacturer specifications.

C-4 P5 SPF 30 Reference Standard

C-4.1 Ingredients

Ingredient		% Weight of
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 Alkyl Acrylates Crosspolymer	0.20
D	Water	1.00
	Triethanolamine (99 %)	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 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

C-4.3.1 Color: white/slightly off-white

C-4.3.2 Odor: characteristic

C-4.3.3 Appearance: smooth lotion

C-4.3.4 pH: 5.5 ± 0.5

C-4.3.5 Viscosity (20 °C): 77,000 cPs ± 10 % (Brookfield LV with heliopath, spindle F, 12 r/min, reading after 60 seconds)

C-4.3.6 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 16344analytical method.

C-4.6 Acceptance criteria

The analytical results are acceptable if the following are achieved:

C-4.6.1the standard coefficient of variation is ≤2.5 %;

C-4.6.2 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 43 Reference Standard

C-5.1 Ingredients

Phase 1	**(Oil Phase)**	**Mass fraction (%)**
	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	58.00
	Disodium EDTA	0.20
	Chlorphenesin	0.30
	Phenoxyethanol	1.00
Phase 3		
	Water and Acrylates/Beheneth-25 Methacrylates Copolymer (28-33 % 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
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 $mPas^{-1}$ to 19 000 $mPas^{-1}$ using Brookfield DVIII Ultra, Spindle RV-5 at 10 r/min.

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 analytical method.

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 SPF 63 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 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 (23-28 % 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 $mPas^{-1}$ to 15 000 $mPas^{-1}$ using Brookfield DVIII Ultra, Spindle RV-5 at 10 r/min.

C-6.3.4 Density: 0.97 g/cm^3 to 1 g/cm^3.

C-6.4 Analytical method

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

C.6.4 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)
CALCULATIONS AND STATISTICS

D-1 GENERAL EQUATIONS

D-1.1 Individual sun protection factor (SPF_i)

The SPF_i of each product on each subject shall be calculated from the individual MED on unprotected skin (MED_{ui}) and the individual MED on product protected skin (MED_{pi}) according to Formula (D.1):

$$SPF_i = \frac{MEDpi}{MEDui} \quad (D.1)$$

D-1.2 Product sun protection factor (SPF)

The SPF of the product shall be the arithmetic mean of the individual SPF_i values obtained from the total number, *n*, of subjects with valid results, expressed to one decimal point, as shown in Formula (D.2):

$$SPF = \frac{(\sum SPFi)}{n} \quad (D.2)$$

Its standard deviation, *s*, is given by Formula (D.2):

$$S = \sqrt{\left\{ \frac{\left[\Sigma \left(SPF^2 \right) \right] - \left[\frac{\left(\Sigma SPF_i \right)^2}{n} \right]}{(n-1)} \right\}} \quad (D.3)$$

D-1.3 95 % confidence interval

The 95 % confidence interval (95 % CI) for the mean SPF shall be expressed by Formula (D.4):

$$95\,\%\ \text{CI} = SPF - c \text{ to } SPF + c \quad (D.4)$$

Where, *c* is calculated as shown in Formulae (D.5) and (D.6):

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

$$c = \frac{t \times s}{\sqrt{n}} \quad (D.5)$$

$$CI[\%] = \frac{100 \times c}{SPF} \quad (D.6)$$

where

SEM = Standard error of the mean;
n = Total number of subjects used;

t = Value from the "two-sided" Student-t distribution Table D.1 at a probability level p = 0.05 and with degrees of freedom $v = (n - 1)$.

Table D.1 — Student-*t* distribution

n	10	11	12	13	14	15	16	17	18	19	20
t	2,262	2,228	2,201	2,179	2,160	2,145	2,131	2,120	2,110	2,101	2,093

NOTE − For spreadsheet calculation, t can be modelled by: $t = 2.03 + \frac{12.7}{n^{1,75}}$ (for n≥4)

D-2 EXPEMENTAL CALCULATION PROCEDURE

D-2.1 Sequential Procedure

An SPF test is begun by testing the product on an initial panel of n' subjects (n' shall be at least 10). The individual sun protection factors (SPF_i) for the product on each subject are then calculated according to Formula (D.1).

From these individual SPF_i values, a provisional mean sun protection factor for the initial n' subjects ($SPF_{n'}$) is calculated according to Formula (D.2), together with a provisional 95 % confidence interval (95 %$CI_{n'}$) using Formulae (D.4), (D.5) and (D.6) and Table D.1, i.e.:

$$SPF_{n'} = \frac{(\sum SPFi)}{n'}$$

95 %$CI_{n'}$ = $SPF_{n'} - c_{n'}$ to $SPF_{n'} + c_{n'}$

where

$c_{n'}$ is calculated as:

$$c_{n'} = \frac{t_n \times s_{n'}}{\sqrt{n}}$$

where

$s_{n'}$ = standard deviation from the first n' subjects calculated according to Formulae (D.7) and (D.8):

$$s_{n'} = \sqrt{\left\{ \frac{\left[\sum (SPF^2) \right] - \left[\frac{(\sum SPF_i)^2}{n'} \right]}{(n'-1)} \right\}} \quad \text{(D.7)}$$

$$CI_{n'}[\%] = \frac{100 \times c_{n'}}{SPF_{n'}} \quad \text{(D.8)}$$

If the calculated provisional $CI_{n'}$[%] is greater than 17 % of the provisional mean $SPF_{n'}$ value, then testing of the product shall continue on additional subjects until the provisional $CI_{n'}$[%] is ≤17 % of the mean provisional SPF.

If this criterion is not fulfilled after twenty valid subjects, then the entire test shall be repeated.

D-2.2 Predicted number of subjects, *n**

If the $CI_{n'}$[%] on the provisional $SPF_{n'}$ is greater than 0,17 $SPF_{n'}$, then the predicted, likely total number of subjects, *n**, necessary to meet the statistical criterion can be estimated according to Formula (D.9) and rounded up to the nearest integer:

$$n' = \left(\frac{t_{n'} \times s_{n'}}{c_{n'}}\right)^2$$

\

where

$t_{n'}$ = *t* statistic from Table D.1, with n' results;

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

$c_{n'}$ = 17 % of mean $SPF_{n'}$, representing the required confidence interval.

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

$$n^* = \left(\frac{2.262\, s_{n'}}{0.17\, SPF_{n'}}\right)^2$$

i.e.

$$n^* = \left(\frac{13.30\, s_{n'}}{SPF_{n'}}\right)^2$$

D-3 Examples

D-3.1 Example 1

Table D.2 is an example of a table gathering data, calculations and results. When data are entered in spreadsheet software, all calculations can be performed automatically.

Table D.3 shows the results for product EX1 with expected SPF 10. After ten subjects had been exposed,
the results were:

D-3.1.1 $SPF_{n'}$ = 11.4

D-3.1.2 $s_{n'}$ = 2.4

D-3.1.3 $c_{n'}$ = 1.7

D-3.1.4 95 %$CI_{n'}$ = 9.7 to 13.1

D-3.1.5 $CI_{n'}$[%] = 15.1 %

Since the $CI_{n'}$[%] was smaller than 17 %, no further testing was necessary and the final SPF of the product EX1 was:

D-3.1.6 SPF = 11.4 with CI[%] = 15.1 %

D-3.2 Example 2

Table D.3 shows the results for product EX2 with expected SPF 20. After ten subjects had been exposed, the results were:

D-3.2.1 $SPF_{n'}$ = 21.3

D-3.2.2 $s_{n'}$ = 6.0

D-3.2.3 $c_{n'}$ = 4.3

D-3.2.4 95 %$CI_{n'}$ = 17.0 to 25.6

D-3.2.5 $CI_{n'}$ [%] = 20.3 %

The relative variation of the results was higher than in Example 1 and the statistical criterion was not met ($CI_{n'}$ [%] was greater than 17 %). The test had to be continued and the likely total number, *n*, of subjects necessary was calculated as shown in Formula (D.10):

$$n = \left(\frac{t_{n'} \times s_{n'}}{c_{n'}}\right)^2 = \left(\frac{2{,}262 \times 6{,}0}{3{,}61}\right) = 14$$

Therefore, five subjects were added and the newly calculated provisional results were:

— SPF_{15} = 21.2

— s_{15} = 6.2

— c_{15} = 3.4 with n = 15 and t_{15} = 2.145

— 95 %CI_{15} = 17.8 to 24.6

— CI $[\%]_{15}$ = 16.2 %

The criterion was met after the fifteenth subject ($CI_{n'}$ [%] smaller than 17 % of the mean SPF) and the final SPF of product EX2 was:

— SPF = 21.2 with CI[%] = 16.2 %

Table D.2 — Example of calculation with 10 subjects (expected SPF 10)

ISO 24444 (20) Test Method																		
Laboratory:																		
Test Product Description:					**Dose Increments:**						**Start Date:**		**End Date:**					**UV source:**
	TEST			**SIM**	**TEST SUBJECTS**						**RESULTS**							**CONCLUSION**
Subj.	Exposur	Applied Read		Sim EE (highest)	Subject	Skin	MEDu		MEDp		SPFi	Reject? Y? or N?	$SPF_{n'}$	$s_{n'}$	$c_{n'}$	$CI_{n'}$[%]	n	
N°	date	by	by	W/m2 eff.	code	ITA°	mm:ss	J/m2 eff	mm:ss	J/m2 eff					0.17	(100.$c_{n'}$/ $SPF_{n'}$)		$CI_{n'}$[%] ≤ 17 % ?
1				8.0		56.0	00:24	190	06:03	2 900	15.3		—	—	—	—	—	—
2				8.0		48.6	00:36	290	07:42	3 700	12.8							—
3				8.0		58.1	00:29	230	04:48	2 300	10.0							—
4				8.0		43.5	00:46	370	08:45	4 200	11.4							—
5				8.0		44.0	00:36	290	08:48	2 300	7.9							—
6				8.0		42.7	00:29	230	06:03	2 900	12.6							—
7				8.0		34.9	00:57	460	07:42	3 700	8.0							—
8				8.0		57.0	00:24	190	04:25	2 600	13.7							—
9				8.0		54.8	00:36	290	07:42	3 700	12.8							—
10				8.0		45.3	00:29	230	08:48	2 300	10.0		**11.4**	2.4	**1.73**	15.1 %	8	Complies
11																		
12																		
13																		
14																		
15																		
16																		
17																		
18																		
19																		
20																		

FINAL RESULT:		**Mean SPF = 11.4**		**s = 2.4**	**c = 1.7**	**CI[%] = 15.1 %**	**95 % CI: 9.7-13.1 (*n* = 10)**

Table D.3 — Example of calculation with 15 subjects (expected SPF 20)

ISO 24444 (20) Test Method																		
Laboratory:																		
Test Product Description:					**Dose Increments:**						**Start**			**End Date:**			**UV source:**	
	TEST			**SIM**	**TEST SUBJECTS**						**RESULTS**						**CONCLUSI**	
Subj.	Exposure	Applied	Rea	Sim EE (highest)	Subject	Skin	MEDu		MEDp		SPFi	Rejected? $SPF_{n'}$		$s_{n'}$	$c_{n'}$	$CI_{n'}$[%]	n	
N°	date	by	by	W/m^2e	code	ITA°	mm:s	J/m^2	mm:s	J/m^2		Y? or N?			0,17	$(100.c_{n'}/SPFn')$		$CI_{n'}$[%] =<
1				8.0		56.4°	00:24	190	07:54	3 799	20.0		—	—	—	—	—	—
2				8.0		42.5°	00:34	269	13:57	6 694	24.9							—
3				8.0		50.6°	00:28	220	11:29	5 512	25.0							—
4				8.0		32.8°	00:42	336	09:01	4 329	12.9							—
5				8.0		45.1°	00:32	253	13:06	6 290	24.9							—
6				8.0		47.9°	00:30	241	12:32	6 014	25.0							—
7				8.0		29.4°	00:45	361	12:07	5 816	16.1							—
8				8.0		54.3°	00:25	201	05:18	2 548	12.7							—
9				8.0		43.3°	00:33	264	17:12	8 256	31.3							—
10				8.0		59.9°	00:22	173	07:11	3 447	19.9		21.3	6.0	4.31	20.3 %	14	Does not
11				8.0		35.0°	00:40	320	8:36	4 123	12.9		20.5	6.3	4.20	20.5%	17	Does not
12				8.0		48.8°	00:29	231	14:57	7 173	31.1		21.4	6.7	4.26	19.9 %	18	Does not
13				8.0		36.5°	00:39	309	16:06	7 724	25.0		21.7	6.5	3.92	18.1 %	16	Does not
14				8.0		47.1°	00:30	241	7:58	3 825	15.9		21.2	6.4	3.71	17.5 %	16	Does not
15				8.0		38.1°	00:37	298	12:36	5 929	19.9		21.2	6.2	3.43	16.2 %	15	Complies
16																		
17																		
18																		
19																		
20																		
FINAL RESULT:					**Mean SPF =**		**21.2**			**s =**	**6.2**		**c =**	**3.4**	**CI[%] = 16.2 %**		**95 % CI:**	**95 %Cl: 17.8 – 24.6 (*n* = 15)**

ANNEX E
(Normative)

COLOURIMETRIC DETERMINATION OF SKIN COLOUR TYPING AND PREDICTION OF THE MINIMAL ERYTHEMAL DOSE (MED) WITHOUT UV EXPOSURE

E-1 GENERAL

CIE normalized tristimulus colourimetry and spectrocolourimetry, using the *L*a*b**/*L**CH colour spaces, have long been internationally accepted and validated. They are routinely used to analyse colours in a way that is strictly correlated with human vision (10).

Skin colour, as characterized by the individual typology angle (ITA°), appears to be particularly helpful when pre-selecting subjects (*see* Annex A). In addition, these procedures may be suitable for the prediction of a subject's minimal erythemal dose (MED) without UV exposure. The determination of the sun protection factor of sunscreens requires a preliminary estimation of the MED of the subjects who will be exposed to a source of UVA-UVB rays. The MED may vary considerably among subjects, depending upon the existing melanotic status of the skin. MED_u values as a function of ITA° have been collected from 13 SPF testing laboratories representing over 9 000 test subjects and are summarized in Figure E.1. These data form the basis for the MED_u predictions for test subjects.

The traditional Fitzpatrick phototype classification is based on the subject's experience of his/her own sensitivity to actinic erythema and the ability to tan after a first sun exposure. However, this classification provides a subjective and unchanging indication of the skin's sensitivity to UV, which does not take into account the level of melanization of the subject's skin. This can lead to misinterpretation of the UV-sensitivity of the subject and to the use of inappropriate UV doses when determining the MED. Therefore, it is often considered prudent to predetermine the likely MED for any subject prior to the evaluation on the protected skin.

Measuring skin colour in the *L*a*b** system as defined by the "Commission Internationale de l'Eclairage", allows the melanotic status of the skin, at the time of testing, to be taken into account, and also allows the MED of the subject to be pre-evaluated with more precision.

E-2 APPARATUS

The measurement equipment is a skin contact reflectance spectrophotometer and/or colourimeter with a view of at least 8mm diameter which utilizes the *L*a*b** colour space and complies with CIE recommendations.

E-2.1 A lamp for illumination of the back of the subject shall have a colour temperature of 6 500 K.

E-3 Mode of operation

E-3.1 For reliable colour measurements on skin, allow subjects to rest for at least 10 min with the skin uncovered, until elimination of contact or stress-related redness and marks.

E-3.2 During measurements, care should be taken to apply the cone aperture of the reflectance colourimeter sensing head so that it just makes contact with the skin, without any pressure. This is critical as undue pressure may cause a "blanching" effect in the skin which may lead to seriously inaccurate measurements. An ergonomic position should be adopted and preliminary training may be necessary,

until a standard deviation smaller than 0.2 (0.1 typically) on the *L**, *a** or *b** co-ordinate can be obtained by repeated triplicate measurements on the same skin area.

E-4 Skin colour typing

E-4.1 For this purpose absolute *L**, *a** and *b** values should be recorded. The reflectance colourimeter aperture should not be reduced by use of any form of diaphragm.

E-4.2 Calibrate the skin contact reflectance spectrophotometer and/or colourimeter as per manufacturer's instructions. Perform *L*a*b** colourimetric measurements on the test sites where products will be applied and exposed later in the test. Calculate the mean *L**, *a**, *b** value (at least three measurements on each test site of each subject).

E-4.3 Calculate the individual typology angle, ITA°, on the mean *L** and *b** values using Formula (E.1):

$$\text{ITA°} = \left(arc\ tangent\ \frac{L*-50}{b*}\right)\frac{180}{\pi} \qquad \text{(E.1)}$$

where the arc tangent is expressed in radians. Round the value off and express ITA° to the nearest integer.

E-4.4 The difference in average ITA° values between test sites used on a subject shall be less than 5.

E-5 Determining exposure range for preliminary MEDu by ITA°

E-5.1 Perform at least three *L*a*b** measurements according to E.4.2. on the area of the subject's back

where the MED_u will be determined. Calculate the mean *L*a*b** values.

E-5.2 Calculate the individual typology angles (ITA°) according to E.4.3.

E-5.3 Formula (E.2) shall be used to calculate the midpoint dose for the preliminary MED_u:

$$\text{Midpoint } MED_u \text{ Dose in } (J/m^2 \text{ eff}) = (0.051 * (ITA°\text{^}2)) - 10{,}718 * ITA + 629.32 \qquad \text{(E.2)}$$

Alternatively, the preliminary midpoint MED_u can be estimated by ITA° range in Table E.1 below and the associated dose to be used as the midpoint for the individual. This exposure series shall be used to expose the test individual to determine the preliminary MED_u prior to beginning the test.

E-5.4 For a 6-exposure sequence, the midpoint exposure dose from Table E.1 shall be midway between the 3rd and 4th exposure doses, and for a 5-exposure dose sequence, the midpoint exposure dose from Table E.1 shall be the middle (3rd) exposure dose.

Table E.1 — Preliminary MED_u midpoint exposure doses

ITA° value of individual	Midpoint dose J/m^2 eff.
28° to 30°	361
31° to 35°	331
36° to 40°	296
41° to 45°	263
46° to 50°	232
51° to 55°	205
56° to 60°	179
61° to 65°	157
≥ 66°	136

EXAMPLE 1 For individual with ITA° of 50º the mid-point exposure (midway between site 3 and 4) for the MEDu is 232 J/m^2 eff. The exposure sequence calculated for 6 exposure test subsites using 12 % dose increments would be:

1st subsite	2nd subsite	3rd subsite	4th subsite	5th subsite	6th subsite
174	195	219	246	275	308

EXAMPLE 2 For the same individual with ITA° value of 50º the exposure series sequence for a 5-exposure test subsites using 12 % dose increments would be:

1st subsite	2nd subsite	3rd subsite	4th subsite	5th subsite
185	207	232	260	291

EXAMPLE 3 For the same individual with ITA° value of 50° the exposure series sequence for a 5-exposure test subsites using 25 % increments would be:

1st subsite	2nd subsite	3rd subsite	4th subsite	5th subsite
148	186	232	290	363

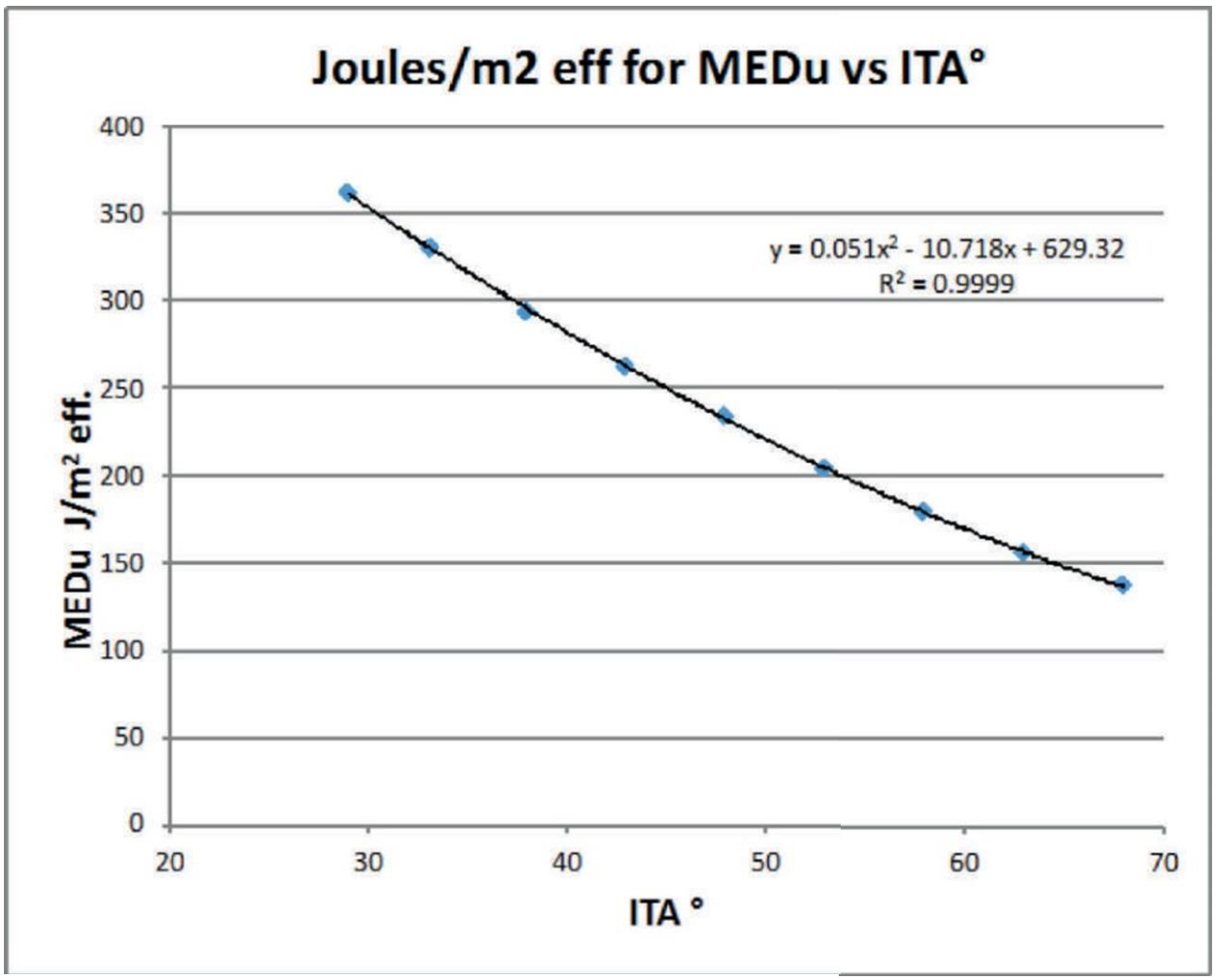


Figure E.1 — Estimation of MED_u

Figure E.1_depicts the relationship between the ITA angle and the unprotected MED value based on data from over 9 500 test subjects from laboratories across the globe.

Annex F
(informative)

Visual guidance for erythema grading

F-1 Visual appearance of erythema

The acceptance or rejection of qualifying erythema reactions for determination of the MED is critical to uniform determination of the SPF of a sunscreen product. The grading scale for the MED_i is described.

F-1.1 Visual appearance of erythema should be performed in sufficient and uniform illumination. At least 450 lux in the plane parallel with the back of the test subject should be provided by a lamp with a continuous emission in the visible spectrum with a colour temperature of 6 500°K. Incandescent light bulbs or Light Emitting Diode (LED) lamps are recommended and can be found in this colour temperature range. Fluorescent lamps may not be used as the sole source for the visual assessment.

F-1.2 The determination of MED(s) shall be carried out in a room with matte, neutral wall colours.

F-1.3 Erythemal responses shall be observed in a "blind" manner (with exception of the provisional MED_{iu}). The observers of erythemal responses on any subjects shall not be the same persons as the ones who perform product application and exposure. The observers shall not be aware of the test design (randomization of the test sites) on that subject.

The grading scale for UV exposed test subsites shall be:

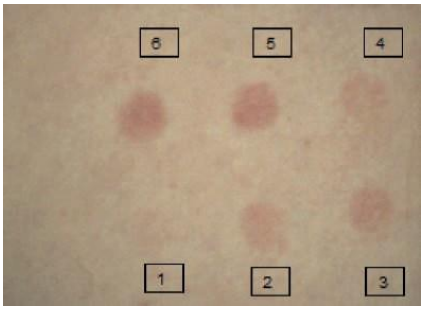
F-1.3.1 0: *No erythema present*

F-1.3.2 0.5: *ambiguous erythema, and/or no clear border, and/or not filling more than 50 % of the exposure subsite*

F-1.3.3 1: *Perceptible unambiguous erythema with defined borders filling more than 50 % of the exposure subsite (MED if it is the lowest exposure dose with grade 1)*

F-1.3.4 2: *Moderate to intense erythema*

Evaluation shall be done on each subsite individually using the definition of MED_i. The evaluation has to be done on the presence or absence of the erythema and not on the intensity. An illogical progression but with erythema (≥Grade 0.5) present does not have to be discarded. Examples are given for guidance.



Key

Subsite 1: ambiguous erythema, no clear border Grade 0.5

Subsite 2: unambiguous erythema, >50 % of area, clear border: Grade 1 = **MED**

Subsite 3: unambiguous erythema, >50 % of area, clear border: Grade 1

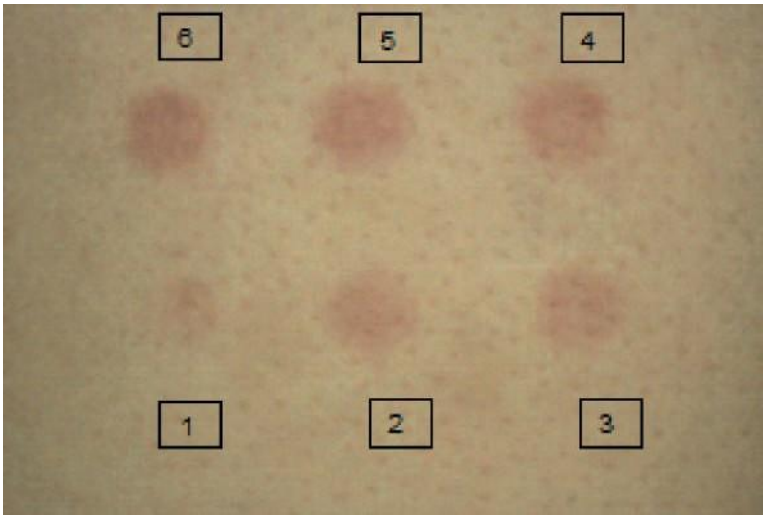
Subsite 4: unambiguous erythema, >50 % of area, clear border: Grade 1

Subsite 5: unambiguous erythema, >50 % of area, clear border: Grade 2

Subsite 6: unambiguous erythema, >50 % of area, clear border: Grade 2

NOTE The MED_i is taken on subsite 2.

Figure F.1 — Skin Response Example 1



Key

Subsite 1: unambiguous erythema, >50 % of area, clear border: Grade 1

Subsite 2: unambiguous erythema, >50 % of area, clear border: Grade 1

Subsite 3: unambiguous erythema, >50 % of area, clear border: Grade 1

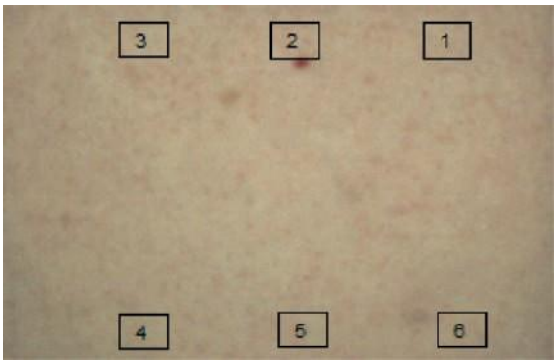
Subsite 4: unambiguous erythema, >50 % of area, clear border: Grade 2

Subsite 5: unambiguous erythema, >50 % of area, clear border: Grade 2

Subsite 6: unambiguous erythema, >50 % of area, clear border: Grade 2

NOTE − Rejection because all subsites present an erythema.

Figure F.2 — Skin Response Example 2

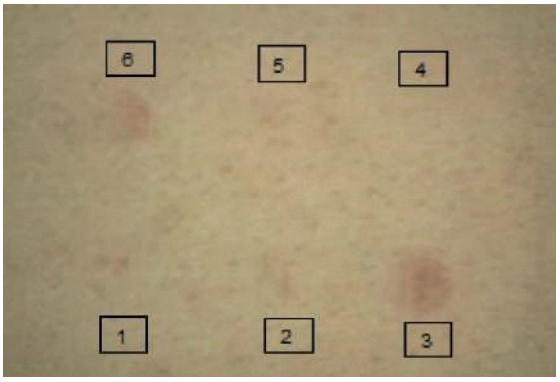


Key

Subsite 1: no erythema: Grade 0
Subsite 2: no erythema: Grade 0
Subsite 3: no erythema: Grade 0
Subsite 4: no erythema: Grade 0
Subsite 5: no erythema: Grade 0
Subsite 6: no erythema: Grade 0

NOTE − Rejection because no erythema on any subsites

Figure F.3 — Skin Response Example 3

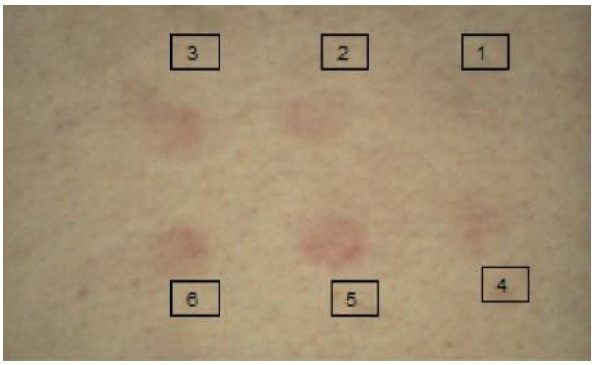


Key

Subsite 1: no erythema: Grade 0
Subsite 2: no erythema: Grade 0
Subsite 3: unambiguous erythema, >50 % of area, clear border: Grade 1
Subsite 4: no erythema: Grade 0
Subsite 5: no erythema: Grade 0
Subsite 6: unambiguous erythema, > 50 % of area, clear border: Grade 1

NOTE − Rejection because illogical progression.

Figure F.4 — Skin Response Example 4



Key

Subsite 1: no erythema: Grade 0

Subsite 2: unambiguous erythema, >50 % of area, clear border: Grade 1 = **MED**

Subsite 3: unambiguous erythema, >50 % of area, clear border: Grade 1

Subsite 4: unambiguous erythema, >50 % of area, clear border: Grade 1

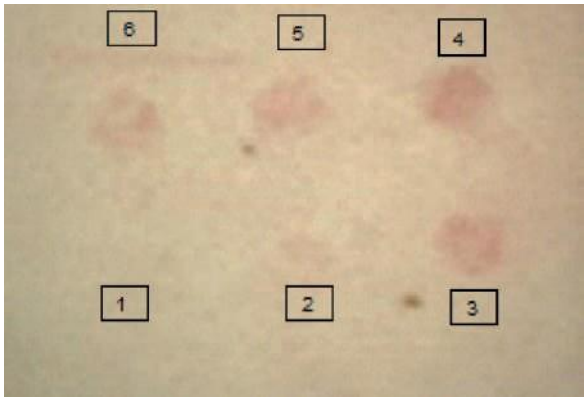
Subsite 5: unambiguous erythema, >50 % of area, clear border: Grade 2

Subsite 6: unambiguous erythema, >50 % of area, clear border: Grade 1

NOTE − MED_i is taken on subsite 2.

Figure F.5 — Skin Response Example 5

The evaluation has been done on the presence or absence of the spot not on the intensity. An illogical progression but with erythema present does not have to be discarded.



Key

Subsite 1: no erythema: Grade 0

Subsite 2: no erythema: Grade 0

Subsite 3: unambiguous erythema, >50 % of area, clear border: Grade 1= **MED**

Subsite 4: unambiguous erythema, >50 % of area, clear border: Grade 2

Subsite 5: unambiguous erythema, >50 % of area, clear border: Grade 1

Subsite 6: unambiguous erythema, <50 % of area, clear border: Grade 0.5

NOTE − MED_i is taken on subsite 3. An illogical progression but with erythema of at least Grade 0.5 present does not have to be discarded. (If subsite 6 were scored as Grade 0, then it would need to be discarded as an illogical progression).

Figure F.6 — Skin Response Example 6

Annex G

(Normative)

Sample report form

Table G.1 — SPF Test Report (All Data)

ISO 24444 (20) Test						**Laboratory:**								**Report Date:**								
Test Product Description:						**Dose Increments:**																
	TEST				**SIM**	**TEST SUBJECTS**																
Subj.	Exposu	Appl.	Exp		Sim EE	Subject	Skin	MEDu		MEDp		SFPi	Rej.	Ref. Standard				Reference Standard				
N°	date	by	by	by	W/m2	code	ITA°	secon	J/m2ef	secon	J/m2			P#	sec-onds	J/m2	SPF	P#	second	J/m2 ff	SPF	
1																						
2																						
3																						
4																						
5																						
6																						
7																						
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16																						
17																						
18																						
19																						
20																						

FINAL RESULT

Test Product Avg. SPF Std. Dev. 95%CI %17% of

Mean P Avg. SPF

P Avg. SPF

Table G.2 — SPF Test Report Valid Data (only)

ISO 24444 (20) Test								**Laboratory:**							**Report Date:**						
Test Product Description:								**Dose Increments:**													
	TEST				**SIM**	**TEST SUBJECTS**															
Subj.	Exposure	Appl. Read		Exp	Sim EE (highest)	Subject	Skin	MEDu		MEDp		SFPi	Rej.?	Ref. Standard				Reference Standard			
N°	date	by	by	by	W/m^2 eff.	code	ITA°	seconds	J/m^{2}eff	se	J/m^2			P#	sec-onds	J/m^2	SPF	P#	seconds	J/m^2 eff	SPF

FINAL RESULT

Test Product Avg. SPF	Std. Dev. 95%CI	%17% of Mean P Avg. SPF P Avg. SPF

Table G.3 — SPF Test Report Invalid Data (only)

ISO 24444 (20) Test						**Laboratory:**								**Report Date:**
Test Product Description:						**Dose Increments:**								
	TEST				**SIM**	**TEST SUBJECTS**								
Subj.	Exposu	Appl.	Exp		Sim EE	Subject	Skin	MEDu		MEDp		SFPi	Rej.	
N°	date	by	by	by	W/m2	code	ITA°	secon	J/m2ef	secon	J/m2			Reason for Rejection

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