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Cosmetics — Sun protection test methods — *In vivo* determination of the sun protection factor (SPF)

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

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 24444 was prepared by Technical Committee ISO/TC 217, *Cosmetics*.

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 volunteers. It uses ultraviolet radiation usually from an artificial source.

Different standard methods are available and described in the technical report ISO/TR 26369[4].

These standards are similar by some parameters but different by others. Differences can lead to discrepancy of results. Harmonization is therefore necessary to get the same SPF value for a single product whatever the country in which it is tested.

Cosmetics — Sun protection test methods — *In vivo* determination of the sun protection factor (SPF)

1 Scope

This International Standard specifies a method for the *in vivo* determination of the sun protection factor (SPF) of sunscreen products. This International standard 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.

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

2 Terms and definitions

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

2.1
ultraviolet radiation
UVR
electromagnetic radiation in the range of 290 nm to 400 nm

2.1.1
ultraviolet B
UVB
electromagnetic radiation in the range of 290 nm to 320 nm

2.1.2
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.

2.2
erythema
reddening of the skin caused by UV radiation

2.3
sunscreen products
products containing any component able to absorb, reflect or scatter UV rays, which are intended to be placed in contact with human skin

2.4
minimal erythemal dose
MED
lowest dose of ultraviolet radiation (UVR) that produces the first perceptible unambiguous erythema with defined borders appearing over most of the field of UV exposure, 16 h to 24 h after UV exposure

2.4.1
MEDu
MED on unprotected skin

2.4.2
MEDp
MED on product protected skin

2.5
individual sun protection factor
SPFi
ratio of the minimal erythemal dose on product protected skin (MEDp) to the minimal erythemal dose on unprotected skin (MEDu) of the same subject:

$$SPFi = \frac{MED\,(protected\,skin)}{MED\,(unprotected\,skin)} = \frac{MEDp}{MEDu}$$

NOTE SPFi is expressed to one decimal place (see 7.1).

2.6
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 (see 7.2).

2.7
test area
back between the scapula line and the waist

2.8
test site
site where a product is applied or the site used for the determination of the unprotected MED

2.9
exposure sub-sites
skin exposed spots

2.10
individual typology angle
ITA°
value characterizing the skin colour of the subject

3 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 is 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 and 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 redness 16 h to 24 h after UV radiation, by the judgment of a competent evaluator.

The minimal erythemal dose (MED) for unprotected skin (MEDu) and the MED obtained after application of a sunscreen product (i.e. the MED for product protected skin, MEDp) shall be determined on the same subject on the same day. An individual sun protection factor (SPFi) for each subject tested is calculated as the ratio of individual MED on product protected skin divided by the individual MED on unprotected skin i.e. MEDp/MEDu.

The sun protection factor for the product (SPF) is the arithmetic mean of all valid SPFi results from each subject in the test.

4 Test subjects

4.1 Selection of the test subjects

4.1.1 General

For subject inclusion and non inclusion criteria, refer to Annex A.

4.1.2 Skin phototype of the test subjects

Test subjects included in the SPF test shall be only phototypes I, II or III according to Fitzpatrick[7] or shall have an ITA° value $>$ 28° by colorimetric methods (see Annexes A and E) and be untanned on the test area. An SPF test should not contain subjects who are all of the same phototype.

A 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).

4.1.3 Age restriction

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

4.1.4 Frequency of participation in tests

Since a sufficient interval after a previous test is needed in order to allow for reversal of skin tanning resulting from that previous test, a test site that has been exposed to UV should not be used in a subsequent test before two months have elapsed and the site is clear.

4.1.5 Ethics and consent

All testing shall be done in accordance with the Declaration of Helsinki[8] and National Regulations regarding human studies, if any.

Informed, written (signature) consent shall be obtained from all test subjects.

4.2 Number of test subjects

The minimum number of valid SPFi results shall be 10 and the maximum number of valid SPFi 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. 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 6.7.4 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 is 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.

4.3 Test area

The back is the chosen anatomical region for the test area. The individual product test sites and the unprotected test site shall be delineated within the region between the scapula line and the waist.

Skeletal protrusions and extreme areas of curvature should be avoided.

5 Apparatus and materials

5.1 Source of ultraviolet radiation

5.1.1 General

The artificial light source used shall comply with the source spectral specifications as described in 5.1.2 and Annex B. A xenon arc solar simulator with appropriate filters is recommended.

5.1.2 Quality of ultraviolet radiation

5.1.2.1 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 (particularly important for a single large-beam) and suitably filtered to create a spectral quality that complies with the required acceptance limits (see Table B.1).

5.1.2.2 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 $\geqslant$ 20 % of the total UV (290 nm to 400 nm) irradiance. Additionally, the UVA I region (340 nm to 400 nm) irradiance shall be $\geqslant$ 60 % of the total UV irradiance.

5.1.2.3 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 $<$ 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.

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

If total irradiance is strong, an excessive feeling of heat or pain may be induced in the irradiated skin of subjects. 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.

5.1.4 Uniformity of beam

5.1.4.1 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. The minimum beam irradiance, at any sub-site, shall be no more than 10 % lower than

the maximum beam irradiance at any sub-site. If the variation exceeds 10 %, then appropriate compensation for different irradiance should be made in the exposure time on each sub-site.

5.1.4.2 For a small beam UV source, which exposes sub-sites individually, the erythema generated following exposure shall be as uniform as possible. An uneven erythema in unprotected skin (such as a half-moon shape) indicates that the irradiance is not uniform and the delivery system shall be corrected.

5.1.5 Maintenance and monitoring the UV solar simulator output

5.1.5.1 Radiometry

Before UV exposure of each test site, the UV irradiance should be measured and recorded with a radiometer calibrated against a spectroradiometric measurement of the solar simulator output.

5.1.5.2 Spectroradiometry

It is recommended that a complete spectroradiometric check (UVA and UVB) of output spectrum and intensity be made by the laboratory at least once every 18 months or after 3 000 h of lamp running time and after changing any significant physical (optical) component of the solar simulator. This periodical inspection should be conducted by a competent and suitably qualified person.

The simple use of specific filters is not in itself adequate assurance that the UV output is of the correct quality. Detailed instructions for ensuring correct lamp output are given in Annex B.

5.2 Reference sunscreen formulations

5.2.1 General

The method is controlled by the use of one of three 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.

5.2.2 Expected SPF < SPF 20

Any one of the following reference sunscreen formulations shall be used: P2, P3 or P7.

If a high SPF reference formulation is used, there is no necessity to also include the low SPF reference formulation in the test even though there may be low SPF test products.

5.2.3 Expected SPF ⩾ SPF 20

One of the following reference sunscreen formulations shall be used: P2 or P3.

If a high SPF reference formulation is used, there is no necessity to also include the low SPF reference formulation in the test even though there may be low SPF test products.

5.2.4 Acceptance SPF limits for the reference sunscreen formulations

Acceptance SPF ranges for the reference sunscreens are shown in Annex C. If the mean SPF obtained in any test does not fall within the acceptance limits of the reference values then the entire test (i.e. all test products) shall be rejected. If the 95 % confidence interval on the mean SPF for the reference sunscreen falls outside a range defined by the mean reference sunscreen SPF ±17 %, then the entire test (i.e. all test products) shall be rejected.

5.2.5 Formulae and preparation of the reference sunscreen formulations

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

6 Procedure

6.1 Main steps

— delineation of test sites on the back of the subject;

— weighing of the product;

— application of the product;

— waiting period before UV exposure;

— UV exposure;

— MED assessment;

— calculations.

6.2 Test conditions

Product application, UV exposures and MED assessment should be carried out in stable conditions, with the room temperature maintained between (22 ± 4) °C.

6.3 Position of the test subjects

All steps in the procedure shall be performed in the same position: an upright, seated or prone position.

Powder should be tested in the prone position to prevent the samples from falling off the surface.

6.4 Procedure for product application

6.4.1 General

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 recommendations set out in 6.4.2 to 6.4.5.

6.4.2 Test sites and product application

6.4.2.1 The test sites intended for UV exposure shall be free from blemishes and have an even colour tone.

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

6.4.2.3 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 MEDu should be randomized as one of the test sites across the test area and across subjects.

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

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

6.4.2.6 The test sites shall be delineated by a method which does not interfere with the test or harm the subject e.g. skin marker and/or a template made from non-absorbent material.

6.4.3 Amount of product applied

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

6.4.3.2 The balance used to weigh the products should be capable of weighing to the nearest 0,000 1 g, i.e. to the nearest 0,1 mg.

6.4.3.3 All products should be homogeneous and should be shaken if necessary, before weighing, to ensure uniform dispersion.

6.4.3.4 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.

6.4.3.5 The amount of product to be applied is weighed in a syringe or in another device such as a watch glass. A method of weighing by loss is strongly recommended.

6.4.4 Mode of delivery

6.4.4.1 General

The use of a finger cot is optional but is recommended. When employed, a new finger cot shall be used for each new application of product and should not be pre-saturated with the test product. When a naked finger is used, the finger should be cleaned between product applications.

6.4.4.2 Liquid type products (e.g. lotions, liquids, milks, creams, sprays and sticks)

6.4.4.2.1 To aid uniform coverage, droplets (approximately 15 per 30 cm^2, 30 per 60 cm^2) of the product should be deposited within the test site using a syringe/pipette, then spread over the whole test site using light pressure.

6.4.4.2.2 Spreading time should be in the range of (35 ± 15) s depending on the surface and ease of spreading of the product.

6.4.4.3 Powders

6.4.4.3.1 In the case of powder products, aliquots of powder should be transferred to the skin in a grid-like manner, using a spatula or finger.

6.4.4.3.2 The accumulated powder is 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.

6.4.4.3.3 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.

NOTE Powders present a unique form of cosmetic product. The modified method for these, described above, takes into account the need to present a reproducible application on the skin.

6.4.5 Drying time between application and UV exposure

Exposure of the test site to the sequence of UV doses shall start 15 min to 30 min after the application of the product(s). 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.

6.5 Procedure for UV exposure

6.5.1 Exposure sub-sites or skin exposed spots

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

6.5.1.2 The minimum area of each exposure sub-site is 0,5 cm^2.

6.5.1.3 The minimum distance between borders of each exposure sub-site (spots) shall be at least 0,8 cm.

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

6.5.1.5 The minimum number of exposure sub-sites used shall be five for unprotected MED (MEDu) and five for protected MED (MEDp).

6.5.2 Provisional MEDu

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

6.5.3 Estimated/anticipated MEDu

The MEDu can be estimated by colorimetric technique (ITA°) without UV exposure (Annex E) or predicted by an experienced technician (i.e. history of the subjects) (anticipated MEDu).

6.5.4 MEDu

For each subject, the unprotected MEDu shall be determined on the same day as the test product protected MEDp.

6.5.5 Incremental progression of UV dose

6.5.5.1 For the unprotected site, the range of UV doses applied shall be established using the subject's provisional MEDu, the estimated MEDu or the anticipated MEDu. A minimum of five sub-sites centered on or close to the provisional/estimated MEDu shall be exposed with incremental UV doses using a recommended geometric progression of 1,25 ×. Other geometric progressions of less than 1,25 × may be used (e.g. 1,2; 1,15; 1,12) but should be consistent throughout the test.

6.5.5.2 For the product protected sites, the UV doses delivered are defined by the expected MEDp, which is the multiple of the expected SPF of the test product and the provisional MEDu for the subject. A minimum of five sub-sites centered on or close to the expected MEDp shall be exposed with incremental UV doses using a recommended geometric progression of 1,25 ×. Other geometric progressions may be used (e.g. 1,2; 1,15; 1,12). A maximum geometric progression of 1,15 shall be used for expected SPF > 25. Smaller geometric progressions (e.g. 1,12) may be used but shall also be consistent throughout the exposure sequence.

6.6 Product removal

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

6.7 Procedure for MED assessment

6.7.1 General

The minimal erythemal dose for unprotected skin (MEDu), that for test product protected skin (MEDp) and the MEDp for the reference sunscreen formulation, shall all be determined on the same day.

6.7.2 Time of assessment of MED

The MED shall be assessed when the erythemal response is optimal, i.e. 20 h ± 4 h after UV exposure (between 16 h and 24 h). 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 exposure to the test area will invalidate the whole test.

6.7.3 MED assessment

6.7.3.1 The MED 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.

6.7.3.2 Visual assessment should be performed in sufficient and uniform illumination. At least 450 lux are recommended.

6.7.3.3 The determination of MEDs shall be carried out in a room with matt, neutral wall colours.

6.7.3.4 Erythemal responses shall be observed in a "blind" manner. The observers of erythemal responses on any subjects should 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.

6.7.4 Data rejection criteria

Test data are deemed invalid and shall be rejected under the following circumstances:

— the series of UV exposures on a subject fails to elicit an erythemal response on any sub-site, 20 h ± 4 h after exposure;

— erythemal responses within an exposure series are randomly absent 20 h ± 4 h after exposure;

— all sub-sites in the exposure series show an erythemal response 20 h ± 4 h after exposure.

When one of the above criteria applies to the exposure series on unprotected skin or to the reference sunscreen formulation exposure sites, then all data for all products on that subject are invalid and shall be rejected.

When one of the above rejection criteria applies to a test product treated exposure series, then all data for that test product on that subject are invalid and shall be rejected.

If invalid data (whether MEDu or MEDp) have to be rejected for any one product on more than five subjects, then the whole test for that product is invalid and shall be rejected.

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

Any additional exposure to the test area will invalidate the whole test.

6.7.5 Expression of MEDs

MEDs shall be expressed in terms of energy ($J \cdot m^{-2}$), or MED units or time (seconds). Units of time may only be used where the flux rate of the solar simulator is constant throughout the test and has been recorded in units of radiant flux.

All irradiance measurements made for a specific study shall be made using the same radiometer, previously calibrated against a spectroradiometric measurement.

7 Calculation of the sun protection factor and statistics

7.1 Calculation of the individual SPF (SPFi)

The SPFi of both the reference sunscreen and the product under test for each subject is calculated as below.

$$\text{SPFi} = \frac{\text{MED(protected skin)}}{\text{MED(unprotected skin)}} = \frac{\text{MEDp}}{\text{MEDu}}$$

SPFi is expressed to one decimal place.

7.2 Calculation of product SPF

The SPF result for the test product and for the reference sunscreen formulation is calculated as the arithmetical mean of all valid individual SPFi values.

The minimum number of valid SPFi values shall be ten and the maximum number of valid SPFi values twenty. A maximum of five results may be excluded from the calculation of the mean SPF, but each exclusion shall be justified. A sixth invalid result automatically invalidates the whole test for that test product and no SPF can be calculated for it.

SPF is expressed to one decimal place.

7.3 Statistical criterion

The statistical criterion for all SPF measurements is that the 95 % confidence interval on the mean SPF measured shall fall within a range of ±17 % of the measured mean SPF. This applies to test products and reference sunscreen products.

Consequently, the actual number of subjects tested is 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.

A minimum of ten valid results is only sufficient if the statistical criterion is fulfilled. If not, the number of subjects is increased from ten until the statistical criterion is met up to a maximum of twenty valid results.

The full statistical procedure for this calculation is described in Annex D.

7.4 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 and shall comply with the ±17 % CI statistical criterion described in 7.3.

8 Test report

The test report shall contain at least the following information:

a) product identifier and expected SPF;

b) subject information (number, name or identification code, skin phototype or ITA° value, age and gender);

c) characterization of the UV source (% RCEE compliance and intensity in units of radiant flux);

d) reference sunscreen used;

e) individual MED for unprotected skin, test product protected skin and reference sunscreen protected skin;

f) individual SPFi values expressed to one decimal place, including all valid data and rejected data for the test product and for the reference sunscreen;

g) mean SPF values, standard deviation on the mean and 95 % CI;

h) protocol deviations if any;

i) identification, by subject, of the technician who conducted the test;

j) date of the test.

Annex A
(normative)

Selection criteria for the test subjects

A.1 Rationale

Experience has shown that, as skin melanization increases (from skin phototype I to IV), the exposure dose needed to generate erythema also increases and the SPF tends to decrease. In addition, comparing subjects of the same phototypes (I to IV) untanned and then after sun tanning, led to the same conclusion. These observations suggest that only skin phototypes I to III should be utilized in the SPF test and that the inclusion of tanned subjects with these phototypes should be avoided.

Correlation studies between the individual SPF of sun protective products and the colorimetric skin characteristics of the subjects' skin at the time of the SPF determination showed that SPF begins to significantly decrease when the individual typology angle (ITA°) of the subjects falls under the value of about 28° (i.e. from "intermediate" skin colour category to "tanned" category). These findings justify the exclusion of skin phototype IV or "tan/mat" skin colour category.

Measuring the skin colour in the CIE 1976 ($L^*a^*b^*$) colour space[6] and characterizing this colour by the ITA° value at the time of the SPF test may allow the selection of subjects according to their actual response to UV light at that moment.

A.2 Selection criteria for the test subjects

A.2.1 Skin phototypes

Subjects should be selected using the Fitzpatrick[7] skin phototype or colorimetric ITA° value. The skin phototype of subjects shall be Fitzpatrick type I, II, III or the colorimetric ITA° value of subjects shall be greater than 28°.

— The Fitzpatrick skin phototype definitions are based on the first 30 min to 45 min of sun exposure after a winter season of no sun exposure, i.e.:

Type I : Always burns easily: never tans

Type II : Always burns easily: tans minimally

Type III : Burns moderately: tans gradually

Type IV : Burns minimally: always tans well

Type V : Rarely burns: tans profusely

Type VI : Never burns; deeply pigmented

— Colorimetric ITA values and skin colour categories are defined by the colorimetric descriptors of Chardon et al.[10] 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 = {arc tangent [(L^* – 50)/b^*]}180/3,141 6

A.2.2 Medical and ethical considerations

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 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.

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.

When there is some doubt on the provisional SPF value of the test product, a screening should first be performed. To protect the subjects, it is recommended to start with a lower SPF value and to increase it progressively.

SPF measurements should not impart harmful, long-lasting effects on human volunteers. Tests have to be performed by competent personnel in order to avoid any damage to the skin of the volunteers involved in the test.

Prior to starting any test, the study supervisor must possess adequate information on the product to be tested.

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) children and persons below the age of consent or > 70 years;

b) pregnant or lactating women;

c) subjects using medication with photo-sensitizing potential;

d) subjects using anti-inflammatory medication;

e) subjects with dermatological conditions;

f) subjects with a history of abnormal response to the sun;

g) subjects accustomed to using tanning beds;

h) subjects having had sun exposure on the back area in the previous four weeks prior to SPF testing;

i) subjects having marks, blemishes or nevi or presenting existing sun damage in the test area;

j) subjects having excessive hair in the area of the test.

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

There shall be a sufficient interval between two successive UV exposures to the same test site for resolution of discoloration resulting from previous tests, i.e. not less than two months.

Annex B
(normative)

Definition of the UV solar simulator output

B.1 Introduction

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, e.g. 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 fulfill 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, it is recommended that UV solar simulators utilizing a xenon arc lamp, filtered with a dichroic UV filter to minimize IR radiation, and UV shaping filters such as Schott WG320 and UG11/1mm or equivalent filters be used.

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 ⩾ 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. The spectroradiometer should be fitted with a double monochromator and its resolution bandwidth should be $\leqslant$ 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 (eg 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 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 suitable warm-up time (typically 10 min) should 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 (1999) 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 E at each wavelength is calculated in relative units from the following formulae:

$E = 1{,}0$ for wavelengths 250 nm $< \lambda \leqslant$ 298 nm

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

$E = 10^{\,0{,}015\,(139-\lambda)}$ for wavelengths 328 nm $< \lambda \leqslant$ 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 $\geqslant$ 60 % UVA I (340 nm to 400 nm) and $\geqslant$ 20 % UVA II (320 nm to 340 nm).

The total irradiance of the source should 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		Eryth. A.S. (CIE-1999)	Spectral eryth. effic.	Interval eryth. effic.	Cumulative eryth. effic.	Sol. Sim. % RCEE	RCEE accept. range	
W.L. λ nm	Irradiance {S} $W \cdot m^{-2} \cdot nm^{-1}$	Normalized to 320 nm	{E}	{$E \times S$}	1/2{$E \times S$}dl	Sum{$E \times S$}	Sum{$E \times S$}/T	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	1,00E+00	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	1,00E+00	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				
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–01	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–01	3,01E–01				
312	5,384E+00	6,19E–01	4,83E–02	2,60E–01	2,73E–01				

Table B.2 (*continued*)

1	2	3	4	5	6	7	8	9	10
	UV Source		Eryth. A.S. (CIE-1999)	Spectral eryth. effic.	Interval eryth. effic.	Cumulative eryth. effic.	Sol. Sim. % RCEE	RCEE accept. range	
W.L. λ nm	Irradiance {S} $W \cdot m^{-2} \cdot nm^{-1}$	Normalized to 320 nm	{E}	{$E \times S$}	1/2{$E \times S$}dl	Sum{$E \times S$}	Sum{$E \times S$}/T	Lower limit	Upper limit
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**	**87,4 %**	**85,0 %**	**90,0 %**
321	8,988E+00	1,03E+00	6,89E–03	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	1,46E–03	1,52E–02	1,73E–02				
329	1,060E+01	1,22E+00	1,41E–03	1,50E–02	1,51E–02				
330	**1,071E+01**	**1,23E+00**	**1,36E–03**	**1,46E–02**	**1,48E–02**	**5,35E+00**	**93,3 %**	**91,5 %**	**95,5 %**
331	1,085E+01	1,25E+00	1,32E–03	1,43E–02	1,45E–02				
332	1,099E+01	1,26E+00	1,27E–03	1,40E–02	1,42E–02				
333	1,108E+01	1,27E+00	1,23E–03	1,36E–02	1,38E–02				
334	1,120E+01	1,29E+00	1,19E–03	1,33E–02	1,35E–02				
335	1,127E+01	1,29E+00	1,15E–03	1,29E–02	1,31E–02				
336	1,135E+01	1,30E+00	1,11E–03	1,26E–02	1,28E–02				
337	1,143E+01	1,31E+00	1,07E–03	1,22E–02	1,24E–02				
338	1,149E+01	1,32E+00	1,04E–03	1,19E–02	1,21E–02				
339	1,160E+01	1,33E+00	1,00E–03	1,16E–02	1,18E–02				
340	**1,166E+01**	**1,34E+00**	**9,66E–04**	**1,13E–02**	**1,14E–02**	**5,48E+00**	**95,5 %**	**94 %**	**97,0 %**
341	1,176E+01	1,35E+00	9,33E–04	1,10E–02	1,11E–02				
342	1,185E+01	1,36E+00	9,02E–04	1,07E–02	1,08E–02				
343	1,189E+01	1,37E+00	8,71E–04	1,04E–02	1,05E–02				
344	1,194E+01	1,37E+00	8,41E–04	1,00E–02	1,02E–02				
345	1,196E+01	1,37E+00	8,13E–04	9,72E–03	9,88E–03				

Table B.2 (*continued*)

1	2	3	4	5	6	7	8	9	10
	UV Source		Eryth. A.S. (CIE-1999)	Spectral eryth. effic.	Interval eryth. effic.	Cumulative eryth. effic.	Sol. Sim. % RCEE	RCEE accept. range	
W.L. λ nm	Irradiance {S} $W \cdot m^{-2} \cdot nm^{-1}$	Normalized to 320 nm	{E}	{$E \times S$}	1/2{$E \times S$}dl	Sum{$E \times S$}	Sum{$E \times S$}/T	Lower limit	Upper limit
346	1,200E+01	1,38E+00	7,85E–04	9,42E–03	9,57E–03				
347	1,204E+01	1,38E+00	7,59E–04	9,14E–03	9,28E–03				
348	1,212E+01	1,39E+00	7,33E–04	8,88E–03	9,01E–03				
349	1,215E+01	1,40E+00	7,08E–04	8,60E–03	8,74E–03				
350	**1,220E+01**	**1,40E+00**	**6,84E–04**	**8,34E–03**	**8,47E–03**	**5,57E+00**	**97,2 %**		
351	1,224E+01	1,41E+00	6,61E–04	8,09E–03	8,22E–03				
352	1,230E+01	1,41E+00	6,38E–04	7,85E–03	7,97E–03				
353	1,231E+01	1,42E+00	6,17E–04	7,59E–03	7,72E–03				
354	1,229E+01	1,41E+00	5,96E–04	7,32E–03	7,46E–03				
355	1,234E+01	1,42E+00	5,75E–04	7,10E–03	7,21E–03				
356	1,233E+01	1,42E+00	5,56E–04	6,85E–03	6,98E–03				
357	1,232E+01	1,42E+00	5,37E–04	6,62E–03	6,73E–03				
358	1,234E+01	1,42E+00	5,19E–04	6,40E–03	6,51E–03				
359	1,234E+01	1,42E+00	5,01E–04	6,19E–03	6,29E–03				
360	**1,233E+01**	**1,42E+00**	**4,84E–04**	**5,97E–03**	**6,08E–03**	**5,64E+00**	**98,5 %**		
361	1,230E+01	1,41E+00	4,68E–04	5,75E–03	5,86E–03				
362	1,225E+01	1,41E+00	4,52E–04	5,54E–03	5,64E–03				
363	1,217E+01	1,40E+00	4,37E–04	5,31E–03	5,42E–03				
364	1,212E+01	1,39E+00	4,22E–04	5,11E–03	5,21E–03				
365	1,200E+01	1,38E+00	4,07E–04	4,89E–03	5,00E–03				
366	1,183E+01	1,36E+00	3,94E–04	4,66E–03	4,77E–03				
367	1,171E+01	1,35E+00	3,80E–04	4,45E–03	4,55E–03				
368	1,153E+01	1,33E+00	3,67E–04	4,24E–03	4,34E–03				
369	1,130E+01	1,30E+00	3,55E–04	4,01E–03	4,12E–03				
370	**1,102E+01**	**1,27E+00**	**3,43E–04**	**3,78E–03**	**3,89E–03**	**5,69E+00**	**99,3 %**		
371	1,073E+01	1,23E+00	3,31E–04	3,55E–03	3,66E–03				
372	1,042E+01	1,20E+00	3,20E–04	3,33E–03	3,44E–03				
373	1,005E+01	1,16E+00	3,09E–04	3,11E–03	3,22E–03				
374	9,649E+00	1,11E+00	2,99E–04	2,88E–03	2,99E–03				
375	9,370E+00	1,08E+00	2,88E–04	2,70E–03	2,79E–03				
376	8,977E+00	1,03E+00	2,79E–04	2,50E–03	2,60E–03				
377	8,597E+00	9,88E–01	2,69E–04	2,31E–03	2,41E–03				
378	8,195E+00	9,42E–01	2,60E–04	2,13E–03	2,22E–03				

Table B.2 (*continued*)

1	2	3	4	5	6	7	8	9	10
	UV Source		Eryth. A.S. (CIE-1999)	Spectral eryth. effic.	Interval eryth. effic.	Cumulative eryth. effic.	Sol. Sim. % RCEE	RCEE accept. range	
W.L. λ nm	Irradiance {S} W·m^{-2}·nm^{-1}	Normalized to 320 nm	{E}	{E×S}	1/2{E×S}dl	Sum{E×S}	Sum{E×S}/T	Lower limit	Upper limit
379	7,707E+00	8,86E–01	2,51E–04	1,94E–03	2,03E–03				
380	**7,176E+00**	**8,25E–01**	**2,43E–04**	**1,74E–03**	**1,84E–03**	**5,72E+00**	**99,8 %**		
381	6,703E+00	7,70E–01	2,34E–04	1,57E–03	1,66E–03				
382	6,147E+00	7,07E–01	2,26E–04	1,39E–03	1,48E–03				
383	5,577E+00	6,41E–01	2,19E–04	1,22E–03	1,31E–03				
384	4,994E+00	5,74E–01	2,11E–04	1,06E–03	1,14E–03				
385	4,423E+00	5,08E–01	2,04E–04	9,03E–04	9,79E–04				
386	3,860E+00	4,44E–01	1,97E–04	7,61E–04	8,32E–04				
387	3,348E+00	3,85E–01	1,91E–04	6,38E–04	7,00E–04				
388	2,846E+00	3,27E–01	1,84E–04	5,24E–04	5,81E–04				
389	2,389E+00	2,75E–01	1,78E–04	4,25E–04	4,74E–04				
390	**1,996E+00**	**2,29E–01**	**1,72E–04**	**3,43E–04**	**3,84E–04**	**5,73E+00**	**100,0 %**		
391	1,626E+00	1,87E–01	1,66E–04	2,70E–04	3,06E–04				
392	1,297E+00	1,49E–01	1,60E–04	2,08E–04	2,39E–04				
393	1,016E+00	1,17E–01	1,55E–04	1,57E–04	1,83E–04				
394	7,810E–01	8,98E–02	1,50E–04	1,17E–04	1,37E–04				
395	5,916E–01	6,80E–02	1,45E–04	8,55E–05	1,01E–04				
396	4,438E–01	5,10E–02	1,40E–04	6,20E–05	7,37E–05				
397	3,247E–01	3,73E–02	1,35E–04	4,38E–05	5,29E–05				
398	2,312E–01	2,66E–02	1,30E–04	3,01E–05	3,70E–05				
399	1,593E–01	1,83E–02	1,26E–04	2,01E–05	2,51E–05				
400	**1,073E–01**	**1,23E–02**	**1,22E–04**	**1,31E–05**	**1,66E–05**	**5,73E+00**	**100,0 %**	**99,9**	**100,0 %**
	UV irrad (W·m^{-2}): 8,03E+02		UVe irrad. (W·m^{-2}·ery), T : **5,73E+00**			**Conclusion:** Complies			

E is the erythemal effectiveness.

S is the source irradiance.

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

Annex C
(normative)

SPF reference sunscreen formulations

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

Reference sunscreen formulation	Mean SPF	SD	Acceptance limits (Mean ±2SD)	
			Lower limit	Upper limit
P2	16,1	1,2	13,7	18,5
P3	15,7	1	13,7	17,7
P7	4,4	0,2	4,0	4,8

C.2 P2 high SPF reference formula

C.2.1 Ingredients		mass fraction (%)
Phase 1:	lanolin	4,5
	theobroma cacao (cocoa) seed butter	2,0
	glyceryl monostearate	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	1,0
	methylparaben	0,3
	propylparaben	0,1
Phase 3:	benzyl alcohol	0,5

C.2.2 Manufacturing process

— melt the ingredients of phase 1 and mix using a propeller agitator at 77 °C to 82 °C until uniform;

— mix phase 2 using a propeller agitator, at 77 °C to 82 °C;

— 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;

— 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;

— 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 Storage and expiry

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

C.2.5 Analytical data

C.2.5.1 Principle

The formulation is sampled gravimetrically and dissolved in methanol, in which the analytes are soluble. The solution is 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.2.5.2 Chemicals/reagents

C.2.5.2.1 **Benzophenone-3**, production raw material, various suppliers.

C.2.5.2.2 **Ethylhexyldimethyl PABA**, production raw material, various suppliers.

C.2.5.2.3 **Methanol**, HPLC grade.

C.2.5.2.4 **Water**, fresh distilled.

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1) The RV type Brookfield rotating viscometer is the trade name of a product supplied by Brookfield Engineering Laboratories. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

C.2.5.2.5 Glacial acetic acid, of high purity.

C.2.5.2.6 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 um PTFE membrane filter.

C.2.5.2.7 Mixed standard

Accurately weigh 30 mg of benzophenone-3 (C.2.5.2.1) 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.2.5.2.8 Mixed working standard

Pipette 5 ml of mixed standard (C.2.5.2.7) into a 50 ml volumetric flask and make to volume with solution in accordance with C.2.5.2.6.

C.2.5.3 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.2.5.2.6.
	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.2.5.4 Sample preparation

C.2.5.4.1 Using an analytical balance, weigh approximately 1 g of formulation, to the nearest 0,1 mg, into a 50 ml volumetric flask.

C.2.5.4.2 Add methanol (C.2.5.2.3) to dissolve the sample and make up to volume.

C.2.5.4.3 Ultrasonicate the flask for 5 min and shake to completely mix the sample.

C.2.5.4.4 Pipette 1 ml into a 10 ml graduated tube and make up to volume with HPLC mobile phase.

C.2.5.4.5 Analyse the sample and mixed working standard (C.2.5.2.8) by reverse phase HPLC.

C.2.5.5 Quality control

C.2.5.5.1 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.2.5.5.2 Analyse three mixed working standards (C.2.5.2.8) by reverse phase HPLC and calculate the coefficient of variation of the analyte peak areas.

C.2.5.6 Calculations

$$\text{Analyte (\% mass fraction)} = \frac{A}{A_{\text{std}}} \times \frac{C}{1\,000} \times \frac{50}{m}$$

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.2.5.7 Acceptance criteria

The analytical results are acceptable if the following are achieved:

a) the standard coefficient of variation is $\leqslant$ 2,5 %;

b) recovery value is 100 % $\pm$ 5 % for all actives;

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

C.3 P3 high SPF reference formula

C.3.1 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

— heat phase 1 to 75 °C to 80 °C and heat phase 2 °C to 80 °C (if necessary increase heat until solution is clear and cool to 75 °C to 80 °C);

— add phase 1 to phase 2 while stirring phase 2;

— to prepare phase 3, disperse carbomer in water by stirring with a rotor/stator disperser, then add sodium hydroxide for neutralization;

— add phase 3 to phases 1 and 2 while stirring and homogenize for about 3 min;

— adjust pH with sodium hydroxide or lactic acid and stir until completely cool;

— compensate for water loss and homogenize.

C.3.3 Physicochemical data

Appearance: white to slightly yellowish emulsion

pH: 7,5 ± 0,5

Density (20 °C): 0,970 ± 0,05 g/cm^3

Viscosity (20 °C): range of values: 2 000 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

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

C.3.5 Analytical data

C.3.5.1 Principle

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

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

C.3.5.2 Chemicals/reagents

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

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

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

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

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

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

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

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

C.3.5.2.8 **Mixed standard**

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

butyl methoxydibenzoylmethane	10 mg
ethylhexyl methoxycinnamate	75 mg

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

C.3.5.2.9 **Mixed working standard**

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

C.3.5.3 **Apparatus — HPLC**

Injector:	Injection volume	10,0 µl
Column:	Type	reverse phase C8 5 µm
		4,6 mm × 250 mm or equivalent
	Mobile phase	solution according to C.3.5.2.7
	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.3.5.4 **Sample preparation**

C.3.5.4.1 Using an analytical balance weigh approximately 1 g of formulation, to the nearest 0,1 mg, into a 50 ml volumetric flask.

C.3.5.4.2 Add methanol to dissolve the sample and make up to volume.

C.3.5.4.3 Ultrasonicate the flask for 5 min and shake to completely mix the sample.

C.3.5.4.4 Pipette 1 ml into a 10 ml graduated tube and make up to volume with HPLC mobile phase.

C.3.5.4.5 Analyse the sample and mixed working standard by reverse phase HPLC.

C.3.5.5 Quality control

C.3.5.5.1 Analyse a sample of HPLC mobile phase and a placebo, if available, prepared according to the method reverse phase HPLC, in order to confirm the absence of interfering chromatographic peaks.

C.3.5.5.2 Analyse three mixed working standards (C.3.5.2.9) by reverse phase HPLC and calculate the coefficient of variation of the analyte peak areas.

C.3.5.6 Calculations

$$\text{Analyte (\% mass fraction)} = \frac{A}{A_{\text{std}}} \times \frac{C}{1\,000} \times \frac{50}{m}$$

where

A is the peak area in sample extract;

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

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

m is the mass of the sample expressed in grams.

C.3.5.7 Acceptance criteria

The analytical results are acceptable if the following are achieved:

a) the standard coefficient of variation is $\leqslant$ 2,5 %;

b) recovery value is 100 % $\pm$ 5 % for all actives;

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

C.4 P7: low SPF reference formula

C.4.1 Ingredients		mass fraction (%)
Phase 1:	lanolin	5,00
	homosalate (CAS 118-56-9)	8,00
	petrolatum	2,50
	stearic acid	4,00
	propylparaben	0,05
Phase 2:	methylparaben	0,10
	disodium EDTA	0,05
	propylene glycol	5,00
	triethanolamine	1,00
	water	74,30

C.4.2 Manufacturing process and analytical controls

Heat phase 1 and phase 2 separately to 77 °C and 82 °C respectively, with constant stirring, until the contents of each part are solubilized.

Add phase 1 slowly to phase 2 while stirring. Continue stirring until the emulsion formed is cooled to room temperature (15 °C to 30 °C). Add sufficient purified water to obtain 100 g of standard sunscreen preparation.

C.4.3 Physicochemical data

pH: 8,0 ± 0,5

Density (20 °C): (0,970 ± 0,05) g/cm^3

Viscosity (20 °C): range of values: 1 000 mPa·s to 3 000 mPa·s [Brookfield®[1)] rotating viscosimeter, RV type, spindle 2, speed 10 r/min (0,167 5^{-1}), rotation time 60 s]

NOTE The above values are specific to the material used.

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 data

C.4.5.1 Principle

The formulation is sampled gravimetrically and dissolved in methanol. The solution is analysed by reverse phase HPLC.

The concentration of homosalate in the sample is determined by quantification against an external standard solution of analyte raw materials.

C.4.5.2 Chemicals/reagents

C.4.5.2.1 **Homosalate**, production raw material (various suppliers).

C.4.5.2.2 **Methanol**, HPLC grade.

C.4.5.2.3 **Water**, freshly distilled.

C.4.5.2.4 **800 mg/l standard**

Accurately weigh 40 mg of homosalate into a 50 ml volumetric flask, dissolve and make up to volume with methanol.

C.4.5.3 Apparatus — HPLC

Injector:	Injection volume	10,0 µl
Column:	Type	reverse phase C18 5 µm 4,6 mm × 250 mm or equivalent
	Mobile phase	methanol
	Flowrate	1,5 ml/min
Detector:	Type	UV
	Wavelength	308 nm[1)] [or 254 nm for fixed wavelength detection (less sensitive, less specific)]
Data:	Quantification	peak area

C.4.5.4 Sample preparation

C.4.5.4.1 Using an analytical balance weigh approximately 0,5 g of formulation, to the nearest 0,1 mg, into a 50 ml volumetric flask.

C.4.5.4.2 Add methanol to dissolve the sample and make up to volume.

C.4.5.4.3 Ultrasonicate the flask for 5 min and shake to completely mix the sample.

C.4.5.4.4 Analyse the sample of 800 mg/l standard by reverse phase HPLC.

C.4.5.5 Quality control

C.4.5.5.1 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.4.5.5.2 Analyse three 800 mg/l standard by reverse phase HPLC and calculate the coefficient of variation of the analyte peak areas.

C.4.5.6 Calculations

$$\text{Analyte}\,(\%\ \text{mass fraction}) = \frac{A}{A_{\text{std}}} \times \frac{C}{1\,000} \times \frac{50}{m}$$

where

A is the peak area in sample extract;

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

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

m is the mass of the sample expressed in grams.

C.4.5.7 Acceptance criteria

The analytical results are acceptable if the following are achieved:

a) the standard coefficient of variation is $\leqslant$ 2,5 %

b) recovery value is 100 % $\pm$ 5 % for all actives

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

Annex D
(normative)

Calculations and statistics

D.1 General equations

D.1.1 Individual sun protection factor (SPFi)

The SPFi of each product on each subject is calculated from the individual MED on unprotected skin (MEDu) and the individual MED on product protected skin (MEDp) according to the equation:

$$\text{SPFi} = \frac{\text{MEDp}}{\text{MEDu}} \tag{D.1}$$

D.1.2 Product sun protection factor (SPF)

The SPF of the product is the arithmetical mean of the individual SPFi values obtained from the total number, n, of subjects used, expressed to one decimal point:

$$\text{SPF} = \frac{(\Sigma\text{SPFi})}{n} \tag{D.2}$$

Its standard deviation, s, is given by:

$$s = \sqrt{\left\{\frac{\left[\Sigma\left(\text{SPFi}^2\right)\right] - \left[\frac{(\Sigma\text{SPFi})^2}{n}\right]}{(n-1)}\right\}} \tag{D.3}$$

D.1.3 95 % confidence interval

The 95 % confidence interval (95 %CI) for the mean SPF is expressed by:

$$95\ \%\text{CI} = \text{SPF} - c \text{ to SPF} + c \tag{D.4}$$

where c is calculated as:

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

$$c = \frac{t \times s}{\sqrt{n}} \tag{D.5}$$

$$\text{CI}[\%] = \frac{100 \times c}{\text{SPF}} \tag{D.6}$$

and where

SEM is the standard error of the mean;

n is the total number of subjects used;

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

Table D.1 — Student-t distribution

n	10	11	12	13	14	15	16	17	18	19	20
t **value**	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 value can be modelled by: $t = 2{,}03 + \frac{12{,}7}{n^{1{,}75}}$ (for $n \geqslant 4$).

D.2 Experimental 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 (SPFi) for the product on each subject are then calculated according to equation (D.1).

From these individual SPFi values, a provisional mean sun protection factor for the initial n' subjects ($\mathrm{SPF}_{n'}$) is calculated according to equation (D.2), together with a provisional 95 % confidence interval (95 %$\mathrm{CI}_{n'}$) using equations (D.4), (D.5) and Table D.1, i.e.:

$$\mathrm{SPF}_{n'} = \frac{(\Sigma \mathrm{SPFi})}{n'}$$

$$95\ \%\mathrm{CI}_{n'} = \mathrm{SPF}_{n'} - c_{n'} \text{ to } \mathrm{SPF}_{n'} + c_{n'}$$

where $c_{n'}$ is calculated as:

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

and where $s_{n'}$ is the standard deviation from the first n' subjects calculated according to equation (D.3):

$$s_{n'} = \sqrt{\left\{ \frac{\left[\Sigma\left(\mathrm{SPFi}^2\right)\right] - \left[\frac{(\Sigma \mathrm{SPFi})^2}{n'}\right]}{(n' - 1)} \right\}}$$

$$\mathrm{CI}_{n'}[\%] = \frac{100 \times c_{n'}}{\mathrm{SPF}_{n'}}$$

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 $\leqslant$ 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 the following formula and rounded up to the nearest integer:

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

where

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

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

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

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

$$n^* = \left(\frac{2{,}262 \, s_{n'}}{0{,}17 \, \mathrm{SPF}_{n'}} \right)^2$$

i.e.

$$n^* = \left(\frac{13{,}30 \, s_{n'}}{\mathrm{SPF}_{n'}} \right)^2$$

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

$SPF_{n'}$ = 11,4

$s_{n'}$ = 2,4

$c_{n'}$ = 1,73

95 %$CI_{n'}$ = 9,7 to 13,1

$CI_{n'}$[%] = 15,1 %

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

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:

$SPF_{n'}$ = 21,3

$s_{n'}$ = 6,0

$c_{n'}$ = 4,3

95 %$CI_{n'}$ = 17,0 to 25,6

$CI_{n'}$ [%] = 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 % of the mean SPF). The test had to be continued and the likely total number, n, of subjects necessary was calculated as:

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

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 %