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Cosmetics — Determination of sunscreen UVA photoprotection in vitro

Cosmétiques — Détermination in vitro de la photoprotection UVA

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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

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

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

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

- acceptance of moulded and sandblasted PMMA plates, according to specifications described in [Annex D](#);
- product application fitted to 1,2mg/cm² for sandblasted plates;
- description of application gesture according to tested products;
- introduction of a new high UVA PF standard P8;
- introduction of critical wavelength calculation;
- calculation of coefficient "C" accepted from in vivo screening SPF, with specific conditions based on SEM and percentage of variability, and new range proposed from 0,6 to 1,6;
- limitation of UVA irradiation dose to 36 J/cm².

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

Introduction

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

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

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

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Cosmetics — Determination of sunscreen UVA photoprotection in vitro

1 Scope

This document specifies an in vitro procedure to characterize the UVA protection of sunscreen products. Specifications are given to enable determination of the spectral absorbance characteristics of UVA protection in a reproducible manner.

In order to determine relevant UVA protection parameters, the method has been created to provide an UV spectral absorbance curve from which a number of calculations and evaluations can be undertaken. These include calculation of the Ultraviolet-A protection factor (UVA-PF) [correlating with in vivo UVA-PF from the persistent pigment darkening (PPD) testing procedure], critical wavelength and UVA absorbance proportionality. These computations are optional and relate to local sunscreen product labelling requirements. This method relies on the use of static in vivo SPF results for scaling the UV absorbance curve.

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

2 Normative references

There are no normative references in this document.

3 Terms, definitions, symbols and abbreviated terms

3.1 Terms and definitions

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

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

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1.1

UV

ultraviolet radiation

electromagnetic radiation in the range of 290 nm to 400 nm

3.1.2

UVB

ultraviolet B

electromagnetic radiation in the range of 290 nm to 320 nm

3.1.3

UVA

ultraviolet A

electromagnetic radiation in the range of 320 nm to 400 nm

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

3.1.4

spectral absorbance

$a_i(\lambda)$

logarithm to base 10 of the reciprocal of the spectral internal transmittance, $a_i(\lambda) = -\log_{10} \tau_i(\lambda)$

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

3.1.5

irradiance

I

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

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

3.1.6

spectral irradiance

$I(\lambda)$

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

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

3.1.7

spectrophotometer

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

3.1.8

spectroradiometer

device designed to measure the spectral density of illuminants

3.1.9

radiometer

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

3.1.10

product reference sunscreen

reference sunscreen product used to validate the testing procedure

3.1.11

solar simulator

equipment used to simulate the solar irradiance and spectrum

3.1.12

plate

substrate

material to which the test product is to be applied

3.2 Symbols and abbreviated terms

3.2.1

UVA-PF

in vitro ultraviolet A protection factor

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

3.2.2

SPF_{in vitro}

in vitro sun protection factor

in vitro protection factor of a sun protection product against erythema-inducing radiation calculated with spectral modelling

3.2.3 critical wavelength CWL

λ_c

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

Note 1 to entry: Calculated from spectral data.

3.2.4 erythema action spectrum

$E(\lambda)$

relative effects of individual spectral bands of an exposure source for an erythema response

3.2.5 PPD action spectrum

$P(\lambda)$

relative effects of individual spectral bands of an exposure source for a persistent pigment response

4 Principle

The test is based on the assessment of UV-transmittance through a thin film of sunscreen sample spread on a roughened substrate, before and after exposure to a controlled dose of radiation from a defined UV exposure source.

Because of several variables that cannot be controlled with typical thin film spectroscopic techniques, each set of sunscreen transmission data is mathematically adjusted so that the in vitro SPF data yield the same measured in vivo SPF value that was determined by in vivo testing. As in vivo method can raise ethical consideration, any alternative SPF method, published as an ISO method, may be used.

Samples are exposed to a specific measured dose of UV radiation to account for the photostability characteristics of the test product.

The resulting spectral absorbance data have been shown to be a useful representation of both the width and height of the UVA protection characteristics of the sunscreen product being tested. The mathematical modelling procedure has been empirically derived to correlate with human in vivo (persistent pigment darkening) test results.

5 Apparatus

5.1 Spectrophotometer specifications

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

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

The spectrophotometer input optics should be designed for diffuse illumination and/or diffuse collection of the transmitted irradiance through the roughened polymethylmethacrylate (PMMA) substrate, with and without the sunscreen layer spread on its surface.

The size of the diameter of the entrance port of the spectrophotometer probe shall be smaller than the size of the light spot to be measured at the sample level (in order to account for stray light).

The area of each reading site should be at least 0,5 cm² in order to reduce the variability between readings and to compensate for the lack of uniformity in the product layer.

The wavelength should be accurate to within 1 nm, as checked using a holmium-doped filter (see [Annex A](#)). The ability of an instrument to accurately measure absorbance is limited by the sensitivity of the instrument. The minimum required dynamic range for this methodology is 2,2 absorbance units as determined according to [Annex A](#).

The maximum measured absorbance should be within the dynamic range of the device used. If the test measurements yield absorbance curves that exceed the determined upper limit of the spectrophotometer, the product should be re-tested using an instrument with increased sensitivity and dynamic range.

The lamp in the spectrophotometer that is used to measure the transmittance shall emit continuous radiation over the range of 290 nm to 400 nm, and the level of irradiance should be sufficiently low, so that the photostability of the product is not unduly challenged (a xenon lamp is a convenient solution).

Therefore, the UV dose during one measurement cycle should not exceed 0,2 J/cm².

NOTE A spectrophotometer is used to measure the absorbance properties of the sunscreen on the test plates. A spectroradiometer is used to measure the spectral energy distribution and intensity of the UV exposure source or the spectrophotometer during the absorbance measurement of the sunscreen on the test plate.

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

5.2 Calibration of the spectrophotometer

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

A three-fold test is required, as described in [Annex A](#):

- dynamic range of the spectrophotometer;
- linearity test of the spectrophotometer; [ISO/FDIS 24443](https://standards.iteh.ai/catalog/standards/sist/26aaa94c-4117-474c-9895-d8deda7a7b2b/iso-fdis-24443)
- wavelength accuracy test. <https://standards.iteh.ai/catalog/standards/sist/26aaa94c-4117-474c-9895-d8deda7a7b2b/iso-fdis-24443>

5.3 Calibration of the UV exposure source

The spectral irradiance at the exposure plane of the UV exposure source that is used for irradiation (to take into account any photoinstability) shall be as similar as possible to the irradiance at ground level under a standard zenith sun.^[1] As defined by COLIPA,^[2] the reference standard sun has a total irradiance of 51,4 W/m² to 63,7 W/m² and a UVA to UVB irradiance ratio of 16,9 to 17,5.

Therefore, the UV irradiance shall be within the following acceptance limits (measured at sample distance).

Table 1 — UV exposure source specifications

UV exposure source specifications as measured with a spectroradiometer	
Total UV irradiance (290 nm to 400 nm)	40 W/m ² to 200 W/m ²
Irradiance ratio of UVA ^a to UVB ^b	11-22
^a 320 nm to 400 nm.	
^b 290 nm to 320 nm.	

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

The uniformity shall be ≥ 90 % as calculated by [Formula \(1\)](#):

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

where

U is the uniformity in percentage;

\bar{X} is the average.

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

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

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

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

5.4 Monitoring of the UV exposure source

The emission of the UV exposure source used for exposure shall be checked for compliance with the given acceptance limits by a suitably qualified expert (at least) every 12 months, or 2 500 h of lamp running time. The inspection should be conducted with a spectroradiometer that has been calibrated against a standard lamp that is traceable to a national or an international calibration standard. In addition to the spectroradiometric inspection, the intensity of the UV exposure source used for exposure shall be checked prior to each use.

This can be done using either a spectroradiometer or a radiometer with sensitivity in the UVA, calibrated for the same UV exposure source spectrum used for the exposure step of the procedure, applying the coefficient of calibration to adjust for variance between the UVA radiometer and the reference spectroradiometer.

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

If a UVA radiometer is used, this device shall be suitably calibrated. This requires that it is calibrated with the UVA irradiance measurement results of the spectroradiometer used to measure the exposure source (as during annual solar simulator calibration).

Calibration shall be conducted in terms of UVA irradiance (320 nm to 400 nm) in accordance with [Annex B](#) and shall be at the same level at which the test plates are exposed. Once calibrated with the spectroradiometer, the UVA radiometer may be used to determine the UV doses to be used during the exposure procedure on a day-to-day basis.

5.6 Substrate/plate

The substrate/plate is the material to which the test product is to be applied. For this method, PMMA plates with one rough side of the substrate shall be used and are commercially available. The size of the substrate should be chosen such that the application area is not less than 16 cm².

The specifications and preparation of this type of plate^{[9][18]} are described in [Annex D](#).

6 Test method

6.1 Outline of the test procedure

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

6.1.2 Conduct blank measurements of a glycerin-treated or Vaseline¹-treated plate for the reference “blank”, which will be used in the subsequent absorbance measurements.

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

6.1.4 Conduct the mathematical adjustment of the initial UV absorbance spectrum using coefficient “C” [see [formula \(2\)](#) the calculation in [6.7.2](#)] to achieve an in vitro SPF (no UV dose) equal to the measured static in vivo SPF. Initial UVA-PF₀ is calculated using $A_0(\lambda)$ and C. A single UV exposure dose, D , is calculated, equal to $1,2 \times \text{UVA-PF}_0$ in J/cm², for each plate.

6.1.5 Conduct UV exposure of the same sample as in [6.1.3](#), according to the calculated UV exposure dose D .

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

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

6.2 Equipment calibration and validation of test plates

Test procedures as described in [Annex A](#) are to be completed to validate the wavelength accuracy, linearity and absorbance limits of the spectrophotometer/spectroradiometer to be used for the test procedure. Validation of the UV properties of the test PMMA plates batch shall also be conducted as described in [Annex D](#).

6.3 Absorption measurements through the plate

It is necessary to first determine the absorbance of UV radiation through a “blank” PMMA plate. Prepare a “blank” plate by spreading a few microlitres of glycerin/Vaseline¹ on the roughened side of the plate. Choose the amount of glycerine/vaseline¹ such that the entire surface is just completely covered (approximately 15 µl for a 50 mm × 50 mm plate).

Any excess of glycerine/vaseline¹ should be avoided. Measure the absorbance through this “blank” plate and use this as the baseline measurement for subsequent absorbance measurements.

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

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

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

6.4 Sample application

The sunscreen product is applied to a new untreated roughened PMMA plate (with the roughened side uppermost) by mass, at an application rate of 1,3 mg/cm² ($\pm 1,6$ %) for molded plates and 1,2 mg/cm² ($\pm 1,5$ %) for sandblasted plates.

To ensure dose accuracy and repeatability, the application area should be not less than 16 cm².

The application dose may be determined by measuring the mass loss of the pipette before and after application of the product; alternatively, it may be applied based on volumetric measurements with consideration of the specific gravity of the test sample. Where possible, a positive-displacement automatic pipette should be used for this purpose.

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

The sunscreen is applied as at least twelve small droplets of approximate equal volume, distributed evenly over the whole surface of the plate.

Finger cots should not be used to spread the product on the plate.

The fingertip used for spreading shall be dipped into the test product and then wiped to remove excess product before spreading the test product applied to the plate. The fingertip used to spread the product shall be cleaned between applications of different test products.

Deposit and weighing shall not take more than 30 s.

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

After the sunscreen product is deposited on the surface of the plate, it shall be spread immediately over the whole surface using light strokes with human fingertip or mechanical fingertip.

Spreading should be completed in a two-phase process:

First, the product should be spread on the whole area of the plate, using circular movements with a minimum of four passages from the top to the bottom of the plate. At the end of the first pass, a turn of the plate has to be done ($\frac{1}{4}$ turn) to alternate passages, with minimal pressure and repeat this movement three times at least (about 30 s).

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

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

First, the product should be spread on the whole area of the plate, using circular movements with a minimum of three passages from the top to the bottom of the plate. At the end of the first pass, a turn of the plate has to be done ($\frac{1}{4}$ turn) to alternate passages, with minimal pressure and repeat this movement two times at least (about 20 s to 25 s).

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

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

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