

Milan, 31/05/2023

**IN VITRO METHOD for the DETERMINATION of SUNSCREEN UVA
PHOTOPROTECTION and CRITICAL WAVELENGTH VALUES
ISO 24443:2021 Method**

METHOD: Ref. E16C rev. 6

CUSTOMER: **THE NUDE ALCHEMIST**
24c Essex St. Phillipstown
Christchurch 8011 (New Zealand)

PRODUCT: **SPF30 THE NUDE ALCHEMIST**
Ref. LAB: 139/23/01 – 151/23

STARTING DATE OF THE STUDY: 31/05/2023

COMPLETION DATE: 31/05/2023

ETHICAL AND QUALITY CRITERIA

The current study was carried out in compliance with the quality assurance system requirements.

REFERENCES

The data given in this report are exclusively related to the tested sample. This report can be only in full reproduced only with the permission.

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1. SAMPLE DATA SHEET

SAMPLE REF.: **SPF30 THE NUDE ALCHEMIST**

Ref. LAB: 139/23/01 – 151/23

SAMPLE ARRIVAL DATE: 10/05/2023

EXECUTION DATE: 31/05/2023

PRODUCT:

- PHYSICAL FORM: cream
- COLOUR: white

QUALITATIVE FORMULA:

- Known / yes /
- Other information / /

OTHER INFORMATION RELATED TO THE PRODUCT SAFETY: None.

IN VIVO TEST INFORMATION:

SPF result: 33.7 (30/05/2023)

Method: ISO 24444:2019

Test type: FULL TEST

FILE: 1 sample with the code number **Ref. LAB: 139/23/01 – 151/23** and the study findings will be kept in our files for one year and ten years respectively. After these periods, the sample and the findings report will be discarded, unless otherwise required by the client.

**IN VITRO METHOD for the DETERMINATION of SUNSCREEN UVA PHOTOPROTECTION and
CRITICAL WAVELENGTH VALUES
ISO 24443:2021 Method
(REF. E16C)**

2. PRINCIPLE OF THE METHOD

The aim of the method is to provide an *in vitro* test to measure the UVA protection provided by sunscreen products as well as their critical wavelength.

The method provides the *in vitro* UVA protection factors (UVAPF), which are shown to correlate with *in vivo* UVA protection factors determined by the Persistent Pigment Darkening (PPD) method.

The test is based on the assessment of UV-transmittance through a thin film of sunscreen sample spread on a roughened substrate (PMMA plate), before and after exposure to a controlled dose of UV radiation (290-400 nm) from a defined UV source. All the sunscreen transmission data are adjusted by first converting to absorbance data (before and after UV exposure) and then by multiplying by the same coefficient.

The coefficient (coefficient C) is iteratively determined from the non-exposed sample's absorbance data to provide a calculated *in vitro* SPF value equal to the *in vivo* measured SPF.

The sunscreen sample is exposed to an irradiation dose proportional to the initial UVA protection factor UVAPF₀, calculated from the adjusted absorbance data of the non exposed sample.

Using this method, the **UVA protection factor (UVAPF)**, the **UVAPF/SPF_{label} ratio** and the **Critical Wavelength** of the tested sunscreen product can be obtained.

All these parameters are calculated from the absorbance data of the sample exposed to UV rays.

3. INSTRUMENTS AND MATERIALS

PMMA plates (PolyMethylMethacrylate), square shaped (50x50 mm), with one side roughened and one side smooth are used as a substrate. The product is applied to the rough side of the plates. For this method, both moulded and sandblasted PMMA are accepted and the topographic parameters of the plates are described below:

Moulded PMMA plate

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

Sandblasted PMMA plate

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

In the present study the HD6 PMMA plates were used (see page 16).

Labsphere UV-2000S Ultraviolet Transmittance Analyzer operates by measuring the diffuse transmittance of a carefully prepared sample as a function of wavelength in the ultraviolet spectrum (290-400 nm).

The UV-2000S takes advantage of an optical device known as an integrating sphere and its ability to collect light transmitted in all directions after passing through a sample substrate.

Atlas Sun Test CPS+ is used as a light source for the UV-irradiation (filters for the irradiance 56052371 e 56052059 – total UV irradiance: 40 - 200 W/m² in the wavelengths range 290-400nm).

The **analytical balance (ME204T/00, Mettler Toledo or Radwag Mod. AS 220.X2 PLUS)** is used to weight the product. It determines up to 4 decimal numbers and has a sensitivity of 0.1 mg.

4. METHOD

STEP 1: *in vitro* transmission measurement of the sunscreen product spread on PMMA plate, prior to any UV irradiation. A standard dose of the product (equal to 1.3 mg/cm^2 for HD6 and 1.2 mg/cm^2 for SB6) is applied on the substrate, a PMMA plate.

The sunscreen is spread onto at least four PMMA plates for each sunscreen sample and then at least 5 transmission measurements are performed for each plate.

Acquisition of initial UV transmission spectrum with $A_0(\lambda)$ data.

STEP 2: mathematical adjustment of the initial UV spectrum using coefficient 'C' to achieve an *in vitro* SPF equal to the *in vivo* SPF. It is recommended that C falls within a range between 0.6 and 1.6.

Initial $UVAPF_0$ is calculated using $A_0(\lambda)$ and C.

STEP 3: a single UV dose **D** is calculated, proportional to $UVAPF_0$.

STEP 4: UV exposure of the samples, according to the calculated UV dose **D**.

STEP 5: *in vitro* transmission measurement of the sunscreen product after UV exposure. Acquisition of second UV spectrum with $A(\lambda)$ data.

STEP 6: mathematical adjustment of the second spectrum (following UV exposure) according to the same C coefficient, previously determined in step 2. Calculation of the *in vitro* UVA protection factor $UVAPF$ after irradiation using $A(\lambda)$ and C. Calculation of Critical Wavelength value.

5. REFERENCE SUNSCREEN

The method must be checked regularly by the use of a reference sunscreen formulations to verify the test procedure.

The formulations used are described in Annex E of ISO 24443:2021.

Product reference sunscreen Formulation	Mean	Mean	Acceptance limits	
	SPF	UVAPF	Lower limit	Upper limit
S2	16,0	12,7	10,7	14,7
P8	63,1	21,1	19,1	23,1

If the UVA-PF of the standard formulation does not fall in the reference range of values the test is deemed invalid and must be repeated.

Reference P8 sunscreen formula should be used when expected UVA-PF is of 20 or more.

In the present study the standard S2 was used.

6. CALCULATIONS

1. Calculation of the *in vitro* SPF

$$\textit{in vitro} \text{ SPF} = \frac{\int_{290 \text{ nm}}^{400 \text{ nm}} E(\lambda) \times I(\lambda) \times d\lambda}{\int_{290 \text{ nm}}^{400 \text{ nm}} E(\lambda) \times I(\lambda) \times 10^{-A_0(\lambda)} \times d\lambda}$$

where:

$E(\lambda)$ = erythema action spectrum (CIE-1987)

$I(\lambda)$ = spectral irradiance received from the UV source (290-400 nm)

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

$d\lambda$ = wavelength step (1 nm)

2. Calculation of the coefficient C

$$\text{SPF } in \text{ vitro, adj} = \text{SPF } in \text{ vivo} = \frac{\int_{290 \text{ nm}}^{400 \text{ nm}} E(\lambda) \times I(\lambda) \times d\lambda}{\int_{290 \text{ nm}}^{400 \text{ nm}} E(\lambda) \times I(\lambda) \times 10^{-A_0(\lambda) \times C} \times d\lambda}$$

3. Calculation of the UVAPF₀ before UV exposure

$$\text{UVAPF}_0 = \frac{\int_{320 \text{ nm}}^{400 \text{ nm}} P(\lambda) \times I(\lambda) \times d\lambda}{\int_{320 \text{ nm}}^{400 \text{ nm}} P(\lambda) \times I(\lambda) \times 10^{-A_0(\lambda) \times C} \times d\lambda}$$

$P(\lambda)$ = PPD action spectrum

$I(\lambda)$ = spectral irradiance received from the UV source (UVA 320-400 nm)

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

C = coefficient C

$d\lambda$ = wavelength step (1 nm)

4. Calculation of the UV exposure dose D

The single UVA dose **D** is proportional to the UVAPF₀ value.

The irradiation with the dose D allows to achieve a good correlation between the *in vitro* UVAPF and *in vivo* PPD values.

$$D = \text{UVAPF}_0 \times D_0 \text{ J/cm}^2$$

$$D_0 = \text{unit UVA dose per unit UVAPF}_0 = 1.2 \text{ J/cm}^{-2} \text{ UVA}$$

5. Calculation of **UVAPF** of plates after UV irradiation of the sample

$$\text{UVAPF} = \frac{\int_{320 \text{ nm}}^{400 \text{ nm}} P(\lambda) \times I(\lambda) \times d\lambda}{\int_{320 \text{ nm}}^{400 \text{ nm}} P(\lambda) \times I(\lambda) \times 10^{-A(\lambda) \times C} \times d\lambda}$$

$A(\lambda)$ = mean monochromatic absorbance of the test product layer after UV exposure.

6. Calculation of **UVAPF** of the product

It is the mean of the UVAPF's of at least four individual plates. If the confidence interval exceeds 17% of the mean UVAPF value, then further plates have to be prepared until the statistical requirement is reached.

7. Calculation of the ratio **UVAPF/SPF**

$$\text{Ratio} = \frac{\text{UVAPF}}{\text{SPF}_{\text{label}}}$$

8. Calculation of **Critical Wavelength value**

It is that wavelength where the area under the absorbance spectrum for the irradiated product from 290 nm to λ_c is 90% of the integral of the absorbance spectrum from 290 nm to 400 nm.

$$\int_{290 \text{ nm}}^{\lambda_c} A(\lambda) d\lambda = 0.9 \int_{290 \text{ nm}}^{400 \text{ nm}} A(\lambda) d\lambda$$

7. RESULTS AND GRAPH

Substrate: 4 PMMA plates HD6 by Helioscreen, batch 637 date 27/03/2022

Coefficient of calibration radiometer versus spectroradiometer: 2.04

Exposure temperature (at plates level): 29°C

Raw UVA irradiance: 25.88 W/m²

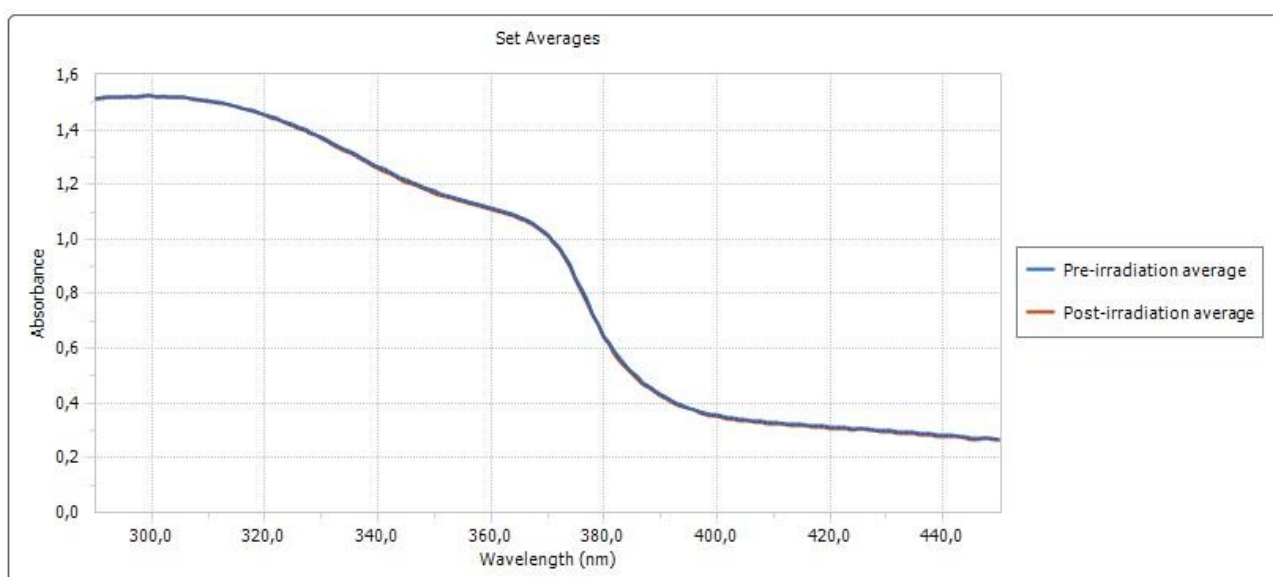
UV Dose: 14.06 J/cm²

UVAPF obtained for S2 reference standard: 13.4 ± 0.2 (05/05/2023)

Table 1: results of the in vitro test.

UVAPF values for the 4 plates	11.5	11.6	11.7	11.3
UVAPF mean value (post-irradiation)	11.5			
Standard deviation	0.2			
95% CI (<17%)	2.4%			
UVAPF₀ mean value (pre-irradiation)	11.7			
SPF_{in vivo}	33.7			
SPF_{label}	30			
UVAPF / SPF_{label} (> 0.33)	0.38			
Coefficient C (0.6-1.6)	1.0			
λ_c (nm >370)	375			

Graph 1: the graph represents the mean values of the absorbance readings of the 4 plates, before and after UV irradiation.



8. CONCLUSIONS

The evaluation of the UVA photoprotection of the product **SPF30 THE NUDE ALCHEMIST, Ref. LAB: 139/23/01 – 151/23** was performed under the experimental conditions described above in this report.

Such evaluation was conducted in accordance with Labanalysis' analysis criteria and its specific know-how and follows accepted international guidelines for cosmetic evaluation.

Observations

The European Commission in their Recommendation of September 22nd 2006 on the efficacy of sunscreen products and claims (2006/647/EC) include a requirement for UVA protection comprising both:

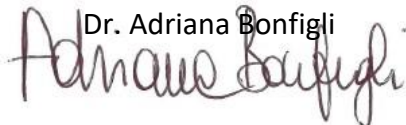
- the mean UVA-PF of a solar product must be at least 1/3 of the labelled SPF
- the Critical Wavelength must be at least 370 nm

On the basis of the obtained results we can draw the following conclusions:

The product passes the test.

Responsible for the laboratory

Dr. Adriana Bonfigli



Responsible for the evaluation

Dr. Monica Prigioni

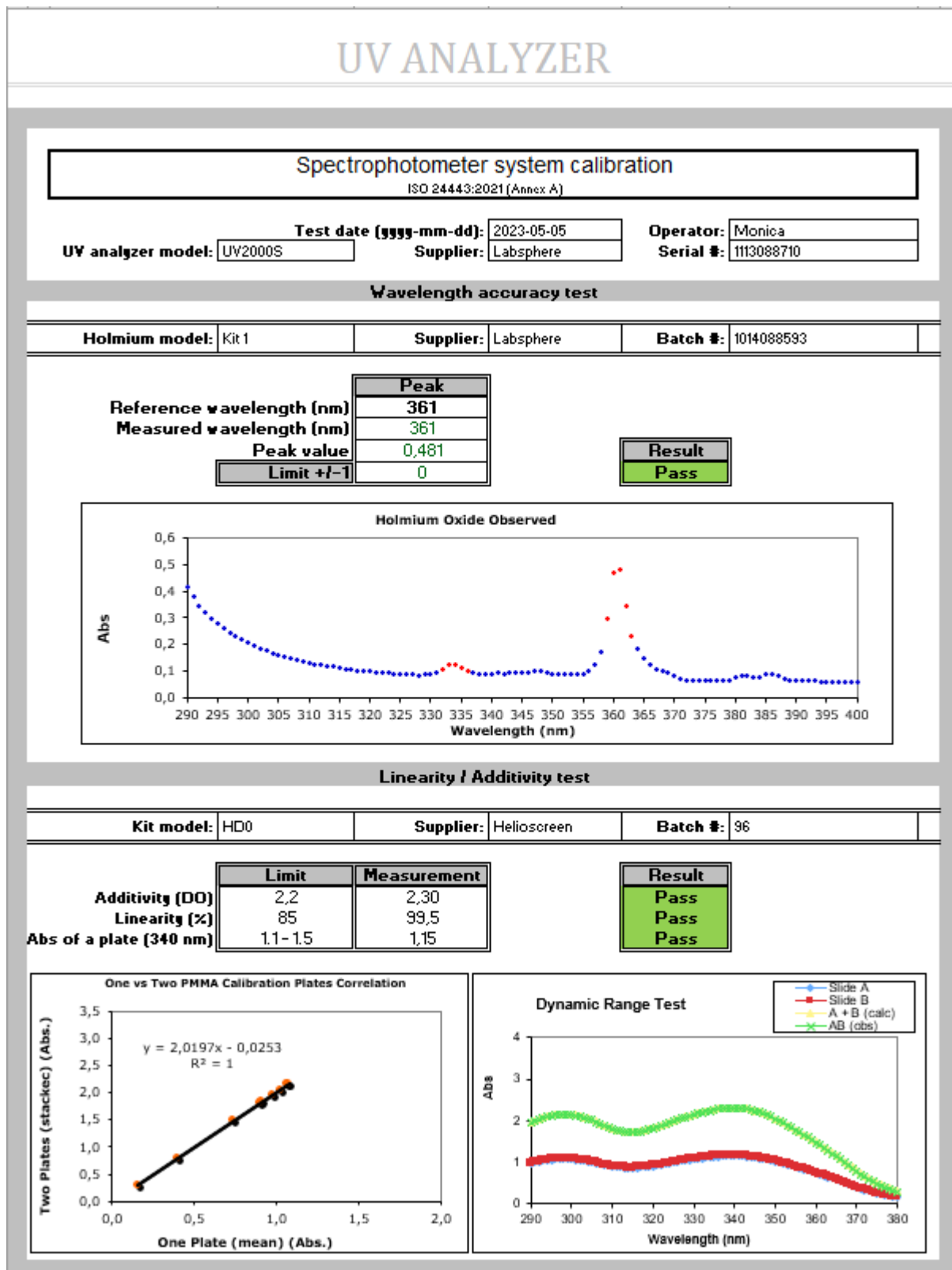


9. BIBLIOGRAPHY


International Standard ISO 24443:2021: Determination of sunscreen UVA photoprotection in vitro.

Glantz S A. Statistica per discipline bio-mediche. Seconda edizione. McGraw-Hill Libri Italia. 1988.

10. SYSTEM CALIBRATION SUMMARY SHEET




11. SOLAR SIMULATOR CALIBRATION SUMMARY SHEET




opto.cal

optical calibration + engineering




Swiss Calibration and Testing Services
Schweizerischer Kalibrier- und Prüfstellendienst
Services Suisse d'étalonnage et d'essai
Servizi Svizzeri di taratura e di prova

SCS 0053



Accreditation N°
Akkreditierungs-Nr.:
N° d'accréditation
accredited according to

STS 0612



ILAC-MRA

Calibration and Testing Laboratory accredited by the Swiss Accreditation Service
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Laboratoire d'étalonnage et de tests accrédité par le service suisse d'accréditation

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N° d'accréditation
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The Swiss Accreditation Service SAS is one of the signatories to the EA Multilateral Agreement for the recognition of calibration certificates and to the ILAC Mutual Recognition Arrangement (www.sas.ch; www.european-accreditation.org; www.ilac.org)

SCS

CALIBRATION and TEST REPORT
KALIBRIER - und PRÜFBERICHT
RAPPORT d'ETALONNAGE et d'ESSAI

STS

Certificate N° Zertifikat Nr. N° de certificat	22401 -2	Page Seite Page	of 1 von 6 de
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Customer Auftraggeber Client	LabAnalysis S.r.l. IT-20125 Milano	Order N°, date Auftrags-Nr. - Datum N° de comm, date	Email 07.09.22
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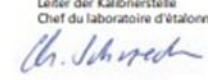
Device under test (DUT) Gegenstand, Objekt	Solar Simulator
Model / Serial N° Typ / Serien Nr Type / N° de serie	Suntest CPS+ / sn 0712021
Manufacturer Hersteller, Fabricant	Atlas MTT GmbH
Accessories Zubehör Accessoires	Filter "B" (UV special glass) UV-Radiometer PM2100/sn8661 with Detector UVA/sn9101
Remarks Bemerkungen Remarques	Test according to ISO 24443:2021 / Boots star system criteria, On-Site- Calibration: LabAnalysis S.r.l., Sala in vitro, IT-20125 Milano
Date of calibration Kalibrierdatum Date d'étalonnage	11.10.2022

This calibration certificate documents the traceability to national standards, which realize the physical units of measurements (SI).
Dieses Kalibrierzertifikat dokumentiert die Rückverfolgbarkeit auf nationale Normale zur Darstellung der physikal. Einheiten (SI).
Ce certificat d'étalonnage confirme le raccordement aux étalons nationaux qui matérialisent les grandeurs physiques (SI).

The measurements, the uncertainties with confidence probability and the calibration methods are given on the following pages and are part of the certificate.
Messresultate, Messunsicherheiten mit Vertrauensbereich und Messverfahren sind auf den folgenden Seiten aufgeführt und Teil dieses Zertifikates.
Les résultats, les incertitudes avec le niveau de confiance et les méthodes de mesure sont donnés aux pages suivantes et font partie du certificat

Stamp and date
Stempel und Datum
opto.cal gmbh
SCS-Kalibrierlabor
Chemin des Vies 24
CH-2812 Movelier
18.10.2022

Head of the Calibration laboratory
Leiter der Kalibrierstelle
Chef du laboratoire d'étalonnage



Christoph Schroeder

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S. 1 / 6

12. STANDARD PRODUCT

Standard S2

ISO 24443 : In vitro determination of the UVA photoprotection (2021)

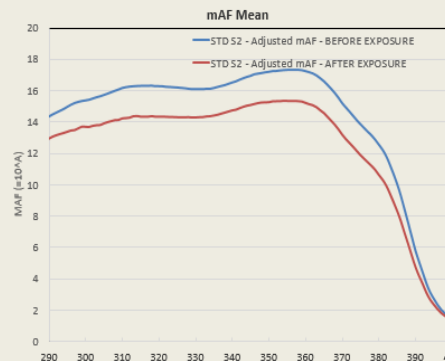
Date	05/05/2023	Spectrophotometer	Absphere UV2000
Operator	Massia	Plate type	PMMAHD6
Product	STD S2	Plate Batch Nb	569
Batch		Nb of plate	4
Measured SPF	16	Nb of Valid Results	4

Conditioning temperature (°C)	27
Exposure temperature (°C)	27,3

RESET

ADJUSTMENT : "C" CALCULATION

Results per plate:	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6	Plate 7	Plate 8	Plate 9	Plate 10	Mean	SD
Adjustment coefficient	Valid	Valid	Valid	Valid							1,459	0,050
Raw SPF0	7,1	7,0	6,3	6,3							6,7	0,4
Adjusted SPF0	16,0	16,0	16,0	16,0							16,0	0,0
UVAPF0	15,3	15,4	15,3	15,4							15,3	0,0
Critical wavelength (nm)	381,7	381,8	381,7	381,9							381,8	0,1
D ₀ (J/cm ²)	18,4	18,4	18,4	18,4							18,4	0,0




mAF Mean

— STD S2 - Adjusted mAF - BEFORE EXPOSURE
— STD S2 - Adjusted mAF - AFTER EXPOSURE

Solar simulator exposure duration (hh:mm:ss)											
Raw UVA irradiance (W/m ²)	25,59										
** Irradiance Correction factor	2,04										
Corrected UVA Irradiance (W/m ²)	12,5936										

Results per plates:	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6	Plate 7	Plate 8	Plate 9	Plate 10	Mean	SD	c (=1*σ/μ)	CI [X]	95% CI
Final results	13,2	13,4	13,4	13,4							13,4	0,2	0,3	2,1%	[13,1 - 13,7]
Critical wavelength (nm)	381,1	381,2	381,1	381,2							381,1	0,1			

13. PLATES QUALITY CERTIFICATE

		Quality Certificate Moulded PMMA substrate - HD6®			
Reference: Heliplate® HD6®		Batch N°: 634-635-636-637			
Certificate N°: 2381		Control Manufacture Date: March 27, 2022			
Beginning Manufacture: March 27, 2022		Mould N°: 769			
End Manufacture: March 27, 2022		Expiration Date: March 26, 2027			
		Raw material N°: ZK031255			
DESIGNATION	Moulded PMMA plate on which the cosmetic product is applied to assess the in vitro sun protection performance • Topographic parameters compliant: ISO 24443:2021 - FDA 2011 - BOOTS STAR RATING 2011 • One unit: Overall size 50x50mm - Spreading area 47x47mm • Material: PMMA (Polymethylmethacrylate) • HS Code: 3906.10.00 - Origin: France - Manufacturer: HeliScreen				
Date Control Roughness: July 12, 2022					
The control of the roughness was performed randomly on several plates from the same batch described here above with the appliance AltSurf® A500 (ALTIMET SAS) and the software AltMap 8 (Mountains® DIGITAL SURF SARL).					
Profilometer characteristic:	- Non-contact surface topographic analysis consisting of an optical sensor, a motion controller, an x-y translation stage, and microtopography software. - Optical sensor based on a white light chromatic aberration principle which allows for a high resolution of at least 10 nm vertically and 1 µm horizontally.				
Measurement:	- A surface area of at least X = 10 mm and Y = 5mm with at least 15-µm intervals. - A speed of at least 1 000 µm/s is recommended according to sensor type and frequency.				
Analysis operators:	1 - Fill in non-measured points by a smooth shape calculated from the neighbours. 2 - Leveling method by least square plane by subtraction. 3 - Conversion to a serie by extraction west-east of all surface profiles for 2D profile parameters. - Gaussian filters of 0.8 mm should be used according to profilometer characteristics.				
RESULTS					
Profile		Measured value		Specification	
Parameter*	Unit	Mean ± SD**	ISO 24443:2021	FDA 2011	Boots Star Rating 2011
Ra	(µm)	4,582 ± 0,098	4,853 ± 0,501	OK	/
Rv	(µm)	12,302 ± 0,193	13,042 ± 0,989	OK	/
Rdq	(°)	12,515 ± 0,21	11,122 ± 2,032	OK	/
A1	(µm²/mm)	215,431 ± 12,331	239,750 ± 70,165	OK	/
Scs	(1/µm)	0,023 ± 0,004	0,033 ± 0,021	OK	/
Vvv	(mm³/mm²)	8,588E-05 ± 7,272E-07	1,044.10-4 ± 9,76.10-5	OK	/
Se	(µm)	5,77 ± 0,06	/	2,0 - 7,0	OK
					2,0 - 6,0
					OK
<small>*Ra (µm): The mean arithmetic deviation of the roughness profile; - [Rv (µm): The maximum depth of profile valleys within a sampling length]; - [Rdq (°): The root-mean-square slope of the profile within a sampling length]; - [A1 (µm²·mm⁻¹): The upper area, i.e., the area of the rest over the peaks extending above an average profile ± 1kernel]; - [Scs (1·µm⁻¹): The arithmetic mean summit curvature of the surface, which indicates the meanderform of peaks and valleys]; - [Vvv (in m³·m⁻²): The volume of void in the valleys, i.e., the volume of rest over of valleys extending below an average profile ± 1kernel] <small>**SD: Standard Deviation</small> </small>					
Date Control Optical: July 6, 2022					
The control of the optical properties was performed randomly on several plates from the same batch described here above with the spectrophotometer U/V-2000S (Labsphere).					
Spectrophotometer characteristic:	- Primary waveband of 290 nm to 400 nm with a 1 nm increment step, including an integrating sphere and a flash lamp - Reading site with at least 0,5 cm², minimum required dynamic range of 2,2 and one measurement cycle not exceed 0,2 ¼/cm²				
Measurement:	- Approximately 15 mg of vaseline/glycerin is applied and spread over the rough side of the plate to obtain a thin continuous film. - Measure transmittance (290 nm to 400 nm) of the treated plate against air (with no plate) as the reference light path.				
RESULTS					
Transmission (%T)		Measured value		Specification	
Wavelength	Unit	Mean ± SD**	ISO 24443:2021	Conclusion	
290 nm	%	69,7 ± 0,5	> 60%	OK	
300 nm	%	76,0 ± 0,3	> 69%	OK	
320 nm	%	86,9 ± 0,1	> 81%	OK	
<small>**SD: Standard Deviation</small>					
Conclusion: COMPLIANT					
The results of analysis of the profile parameters and optical properties are in compliance with the pre-established specification. Therefore, the batch here above of the Moulded PMMA plate - Heliplate HD6® is officially confirmed.					

Place: CREIL, France
 Date: December 5, 2022
 Name: Sébastien MIKSA

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