REPORT No.121354/17/JSHR/Z1

(Replaces the report no. 121354/17/JSHR made on 19.05.2017)

Client:		Sample (according to declaration of the Client)
BIOSELECT CHARMANI – CHRISTOULAKIS VAT: EL 099984857 4, PAPANIKOLAOU STR, RETHYMNO, CRETE, PO BOX 74100		**BIOSELECT OLIVE SUN CREAM FOR FACE & BODY SPF 30**
Received on:	03.04.2017	
Analysis completed on:	18.05.2017	
Report date:	23.05.2017	

DETERMINATION OF SUNSCREEN UVA PHOTOPROTECTION IN VITRO

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SCOPE OF TEST COMPLIANT WITH:

- INTERNATIONAL STANDARD ISO 24443:2012

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Content of the report:

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1. THE BASIS OF THE STUDY

- Test samples delivered by the Client.
- The qualitative composition of the product delivered by the Client.
- Declaration of conformity composition of the product.
- The result of microbiology purity of the product declared by the Client.
- Negative test results dermatological of the product delivered by the Client (Report no.: SI.01.C_2014/168).
- The results of in vivo determination of the Sun Protection Factor (SPF) (Report no.: 121348/17/JSHR).

2. SUBJECT OF TEST

No.	Parameter	Description
1.	Appearance	Emulsion
2.	Color	Creamy
3.	Fragrance	Characteristic for used fragrance composition
4.	Packaging	Repackaging covered with a label containing the name of the product

Picture 1: Sample no. 121354/17/JSHR - subject of study.

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3. QUALITATIVE COMPOSITION OF THE PRODUCT

Aqua (Water), Ethylhexyl Methoxycinnamate, Octocrylene, Butyl Methoxydibenzoylmethane, C12-15 Alkyl Benzoate, Cetearyl Alcohol, Potassium Cetyl Phosphate, Glycerin, Dibutyl Adipate, Diethylamino Hydroxybenzoyl Hexyl Benzoate, Aloe Barbadensis Leaf Juice, Simmondsia Chinensis (Jojoba) Seed Oil, Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine, Olea Europaea (Olive) Fruit Oil, Benzyl Alcohol, Butyrospermum Parkii (Shea) Butter, Glyceryl Stearate, Cetearyl Glucoside, Glyceryl Undecylenate, PEG-100 Stearate, Helianthus Annuus (Sunflower) Seed Oil, Origanum Dictamnus Flower/Leaf/Stem Extract, Parfum, Xanthan Gum, Echinacea Purpurea Root Extract, Potassium Sorbate, Dehydroacetic Acid, Rosmarinus Officinalis (Rosemary) Leaf Extract, Tetrasodium Glutamate Diacetate.

The Client is responsible for compliance with the declared qualitative composition of the product and microbiological purity of sent samples for testing.

4. AIM OF THE TEST

The present International Standard specifies is performed to assess the in vitro UVA factor on 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 parameters, the methods has been created to provide a UV spectral absorbance curve from which a number of calculations and evaluations can be undertaken. Results from this measurement procedure can be used for other computations, as required by local regulatory authorities. These include calculation of the Ultraviolet-A protection factor, critical wavelength and UVA absorbance proportionality. These computations are optional and relate to local sunscreen product labeling requirements. This method relies on the use of in vivo SPF results for scaling the UV absorbance curve.

5. SAMPLES AND TESTING CONDITIONS

Upon arrival, all products are registered on the HAMILTON LIMS System and kept at room temperature (unless otherwise requested). The test is performed in an air-conditioned room, with the room temperature maintained at 22 ± 4°C and the relative humidity 50 ± 10%.

6. SUBSTRATE AND INSTRUMENT FEATURES

The substrate/plate is MOLDED PMMA plates (PolyMethylMethacrylate Plexiglas$^{TM)}$ with one side of the substrate roughened. A quantitative of sunscreen sample was applied and distributed as homogenous as possible on the PMMA plates. The sample was spotted evenly across the plate surface with microsyringe.
The principle of the analysis is a transmission measurement. The glycerine on the reference substrate serves as a "blank" emulsion (placebo) which contains no-light absorbing or scattering compounds and reduces artificial scattering by the roughened, dry surface much the same as a placebo.

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LabsphereUV-2000S (UV transmittance analyzer): it operates by measuring the diffuse transmittance of a carefully prepared sample.
Transmittance is a percent energy transmitted through the sample, relative to the incident beam. The transmittance of the measured sample is equal to the ratio of the transmitted radiative flux to the incident flux. The sample beam is generated inside the upper chamber of the optic head and direct downwards through the sample. The spectral radiance of the incident beam is sampled by a fiber optic port through the integrating sphere wall and measured by spectrograph. Ultraviolet radiation from the incident beam that is not reflected or absorbed by the sample material is collected by the lower chamber of the optic head and measured by a second spectrograph. The transmittance of the measured sample is a equal to the ratio of the transmitted radiative flux to the incident flux.

Solar Simulator.

UV source:	Xenon lamp: Solar Light type Multiport 601
	Spectrum: 290 - 400 nm
	Power of the lamp: 300 W.
	Elimination of IR and visible radiation: UG11 (1mm) and dichroic mirror.
	Radiated surface: Six holes (diameter 8 mm).
UV Light Radiometer:	Solar Light Co. DCS 2.0.
Detector:	Solar Light Co. Erythema detector PMA2108.LLG.

7. STANDARD SUNSCREEN

The method should be checked regularly by the use of reference formulation to verify the test procedure and, therefore, reference sunscreen formula S2 should be used for this purpose. The result of the reference S2 UVAPF must lie between the upper and lower limits below.

Parameter	Lower Limit	Upper Limit
UVA-PF	10.7	14.7

8. MEASUREMENT

This test combines a well established method for determining in vivo SPF and the advantages of determining relative parameters by in vitro measurements. To obtain the in vitro PPD factor, there are the following this steps:

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1. In vitro transmission measurement of the sun screen product spread on PMMA plate, prior to any UV irradiation. Acquisition of initial UV transmission spectrum with AO(l) data
2. Mathematical adjustment of the initial UV spectrum using coefficient "C" (see calculation below) to achieve an in vitro SPF (0% UV dose) equal to the labelled SPF (in vivo). Initial UVAPF0 is calculated using A0(l) and C.
3. UVAPF 0 is calculated for each plate individually (UVAPF0)1 A single UV dose D is calculated, proportional to UVAPF0.
4. UV exposure of the sample as in step 1, according to the calculated UV dose D. (step 3).
5. In vitro transmission measurement of the sunscreen product after UV exposure. Acquisition of second UV spectrum with A(l) data.
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 UVAPF after irradiation using A(l) and C.
7. UVAPF calculated post UV exposure.

9. CALCULATIONS

In vitro UVA protection calculated before UV exposure and after adjustment with in vivo SPF. $UVAPF_0$ is calculated for each plate individually. The UVAPF of the sunscreen is the mean of each plate.

9.1. Calculation in vitro SPF (SPF $_{in\ vitro}$)

Equation 1

$$SPF_{vitro} = \frac{\int_{290nm}^{400nm} E(\lambda)I(\lambda)d\lambda}{\int_{290nm}^{400nm} E(\lambda)I(\lambda)10^{-A_0(\lambda)}d\lambda}$$

Where:
E (λ) - Erythema action spectrum
I (λ) – Spectral irradiance received from the UV source (SSR for SPF testing)
A_0 (λ) – Mean monochromatic absorbance to the test product layer before UV exposure
d(λ) – Wavelength step (1nm)
NOTE: The calculated SPF value cannot be used as an SPF $_{in\ vitro}$ result.

9.2. Determination of "C" value

C is the coefficient of adjustment, iteratively determined t adjust the calculated in vitro SPF value (in vivo) SPF value. It is recommended:

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Equation 2

$$SPF_{in\_vitro,adj} = SPF_{in\_vivo} = \frac{\int_{290nm}^{400nm} E(\lambda)I(\lambda)d\lambda}{\int_{290nm}^{400nm} E(\lambda)I(\lambda)*10^{-A_0\ (\lambda)*C}d\lambda}$$

Where:
E (λ) - Erythema action spectrum
I (λ) – Spectral irradiance received from the UV source (SSR for SPF testing)
A_0 (λ) – Mean monochromatic absorbance to the test product layer before UV exposure
d(λ) – Wavelength step (1nm)
The "C" value typically lies between 0,8 and 1,6 for valid interpretation.

9.3. Determination of initial UVA protection factor before UV exposure ($UVAPF_0$)

UVAPF0 is calculated for each plate individually.

Equation 3

$$UVAPF_0 = \frac{\int_{320nm}^{400nm} P(\lambda)*I(\lambda)*d\lambda}{\int_{320nm}^{400nm} P(\lambda)*I(\lambda)*10^{-A_0(\lambda)*C}*d\lambda}$$

Where:
P (λ) – PPD action spectrum
I (λ) – Spectral irradiance received from the UV source (UVA 320nm to 400nm for PPD testing)
A_0 (λ) – Mean monochromatic absorbance to the test product layer after UV exposure
d(λ) – Wavelength step (1nm)
C – Coefficient of adjustment, previously determined in equation 2

9.4. Determination of the UV exposure dose "D" for sample irradiation

The UV exposure dose "D" is the $UVAPF_0$ value multiplied by a factor of 1.2 Joules/cm^2

Equation 4
$D = UVAPF_0 \times 1.2\ J/cm^2$

The sample is exposed to full spectrum UV radiation but the dose is being defined by the UVA content. The 1.2 J/cm^2 factor is based on ISO ring test validation study results.

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9.5. Calculation of UVAPF of plates after UV irradiation of sample

The UVAPF shall be calculated according to equation 5 for each individual plate, using the mean of multiple observations on the plate.

Equation 5

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

Where:
P (λ) – PPD action spectrum
I (λ) – Spectral irradiance received from the UVA source (UVA 320nm to 400nm for PPD testing)
A_e (λ) – Mean monochromatic absorbance to the test product layer after UV exposure
d(λ) – Wavelength step (1nm)
C – Coefficient of adjustment, previously determined in equation 2

9.6. Calculation of the Critical Wavelength Value λ_C (established by the E.C. Recommendation GUUE L265 22 September)

The Critical Wavelength λ_C value for the test product is defined as that wavelength where the area under the absorbance spectrum for the irradiated product (obtained using the method described above) from 290 nm to λ_C is 90% of the integral of the absorbance spectrum from 290 to 400 nm and is calculated in the following way:

$$\int_{290}^{\lambda c} A(\lambda)d\lambda = 0.9 \int_{290}^{400} A(\lambda)d\lambda$$

The final Critical Wavelength value for each tested sunscreen product is the mean of values recorded for each measured, irradiated, product-treated PMMA plate.

10.STATISTICAL CALCULATIONS

The calculation are performed automatically using the calculation spreadsheets provided by ISO International Standard. The UVAPF of the product is the arithmetical mean of the individual plate UVAPFi values obtained from at least 4 plates, expressed to one decimal point:

$$UVAPF = \frac{\sum UVAPFi}{n}$$

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It's standard deviation s is:

$$s_{n'} = \sqrt{\left\{\sum (UVAPFi)^2 - \left[(UVAPFi)^2 / n'\right]\right\} / (n'-1)}$$

The 95% Confidence interval (95% CI) for the mean UVAPF is expressed as:

95% CI = (UVAPF – C) to (UVAPF + C)

C is calculated as:

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

$$CI\ [\%] = 100 \times c / UVAPF$$

Where:
SEM – standard error of the mean
n – total number of the plates used
t – t value from "two sides" student distribution table at probability level p – 0.05 and with degrees of freedom n-1.
If the calculated provisional Cin' [%] is greater than 17% of the provisional mean UVAPFn value then testing of the product shall continue on additional plate until the provisional Cin' [%] ≤ 17% of the mean provisional UVAPF.

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11.RESULTS

ISO in vitro UVA protection test method	**Results**	
Product name	BIOSELECT OLIVE SUN CREAM FOR FACE & BODY SPF 30	
Date	18.05.2017	
SPF in vivo results	30,31	
Spectro Analyser	Labsphere 2000s	
Applied amount of product per area	1,3 mg/cm^2	
Plate manufacturer / Lot number	PMMA plates HELIOPLATE HD6 UC: 000240 No:39	
Solar simulator for UV exposure	601 Multiport Solar Simulator	
Results obtained for the reference sunscreen S2	ISO *in vitro* UVA-PF	14.2 ± 0.6
	Critical Wavelenght Value λ	379nm
Results obtained for tested product	**UVAPF Mean**	**26,51**
	UVAPF STD	0,41
	UVAPF COV	1,54%
	UVA:UVB Ratio	**87,46%**
	Lambda Critical	**383,58nm**
	Broad spectrum protection	**pass**
Use of symbol on label		**permitted**

12.SIGNATURE

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