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ORIGINAL ARTICLE

Sunscreens effectiveness are not altered by concomitant use of moisturizing creams: An ultraviolet reflectance photography study

María Victoria de Gálvez[1] | José Aguilera[1] | María Leal[1] | Cristina Sánchez-Roldán[1] | Enrique Herrera-Ceballos[1,2]

[1]Photobiological Dermatology Laboratory, Medical Research Center, Department of Dermatology and Medicine, Faculty of Medicine, University of Malaga, Málaga, Spain

[2]Dermatology Service, Universitary Clinical Hospital Virgen de la Victoria, Málaga, Spain

Correspondence
José Aguilera, Photobiological Dermatology Laboratory, Medical Research Center, Department of Dermatology and Medicine, Faculty of Medicine, University of Malaga, 29190-Málaga Spain.
Email: jaguilera@uma.es

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Summary

Background: Sunscreens are widely used to protect the skin against the harmful effects of solar radiation. It is not known whether solar protection factor of a sunscreen is altered by the concomitant use of other cosmetic products.

Objectives: The aim of this study was to analyze changes in the protective effect of different commercial and ISO standards sunscreens with high SPF applied shortly before and after application of non-sunscreens galenic formulas type moisturizing creams.

Methods: ISO 24444:2019 standard sunscreens, which claimed SPF 16 and 63, as well as 4 different claimed SPF 50 and 50+ commercial sunscreens were prepared and applied in different sequential order to the back of 25 volunteers and compared with different commercial moisturizing formulas. Ultraviolet (UV) reflectance photography followed by image analysis was used to compare untreated skin and skin treated with moisturizing creams alone and combined with sunscreens.

Results: The UV reflectance analysis showed no significant changes of the skin color reflectance treated with moisturizing cream compared with untreated skin. Application of the sunscreen formulations were associated with a 35% - 70% decrease in color related to the in vivo expected SPF, indicating significant UV absorption for all sunscreen formulas. All standard and commercial sunscreens showed no significant differences in UV reflection color level when combined with the different moisturizing creams applied before or after the sunscreen.

Conclusions: Effectiveness of low- and high-protection sunscreens were not altered by the concomitant use of a moisturizing creams applied shortly before and after the sunscreens.

KEYWORDS
moisturizing formula, sun protection, sunscreens, ultraviolet reflectance photography

María Victoria de Gálvez and José Aguilera equally contributed to this work.

1 | INTRODUCTION

Sunlight has numerous health benefits: it increases serotonin levels, improving mood, favors the healing of certain skin disorders, and intervenes in vitamin D synthesis.[1,2] Cumulative lifetime exposure to solar radiation, however, can have deleterious health consequences, such as premature aging and skin cancer. The incidence of these disorders is increasing due to greater sun exposure and longer life expectancy.

Topical sunscreens are one of the most used measures worldwide to prevent skin damage caused by overexposure to the sun's rays, such as sunburn and skin cancer. Sunscreens contain substances that absorb UV radiation and may additionally reflect UV rays in case of UV filters in particulate form. These products offer broad protection against UVA and UVB radiation. Their use complements other elements that protect against direct exposure to sunlight, such as sunglasses, dark clothing, and hats.[3-7]

Sun protection as claimed is achieved by applying the quantity of sunscreen recommended in ISO24444/EC recommendation, which is 2 mg/cm².[8,9] Smaller quantities decrease the degree of protection offered. In addition, the cream or lotion needs to be reapplied every 2 hours to maintain its ability to block the sun's rays. This protective ability is indicated by a numerical index known as *sun protection factor* (SPF), which is the ratio between the minimum erythematous dose of the skin with and without sunscreen to measured 24 hours after irradiation.[8]

Daily sunscreen use is increasingly recommended throughout the year, particularly in places with high levels of UV radiation such as the Mediterranean.[10] Spain, for example, has very high solar irradiance rates, but sunscreen use during the practice of sport and other outdoor activities is on the rise.[11]

Sunscreens are formulated using organic and mineral filters as sunscreens and combined with other active compounds as antioxidant for a second line of protection against oxidative damage related to UV overexposure. The excipients of the formula will enhance cosmetic appeal, safety, and performance. The protective effect (SPF and UVAPF) of sunscreens is assessed in in vivo and in vitro tests that are standardized across different countries. Europe regulation of SPF in under the standard ISO 24444:2019 while in USA is under FDA rules.[12,13] UVAPF is under the standard ISO 24443:2012.[14] However, many people now use sunscreens in combination with other cosmetic products such as moisturizing creams to prevent photoaging and ensure hydrated, healthy skin.[15] Moisturizers are generally applied combined with sunscreen because they contain more active ingredients designed to nourish and revitalize the skin. It is not known, however, whether the combined use of sunscreens and cosmetic products affects the SPF. Moreover, there are differing opinions among the general population about whether it is better to apply these products before or after the sunscreen.

The aim of this study was to determine, using non-invasive procedure as ultraviolet reflection photography, how the use of a moisturizing cream applied shortly before or after a sunscreen product affects the SPF.

Summary statement

One of the key aspects of skin photoprotection is the use of sunscreens that covers with maximal efficacy all the solar UV spectrum, and their efficacy on skin can be altered by different circumstances. By means of UV reflectance analysis followed image analysis we were able to show that effectiveness of low- and high-protection sunscreens, both commercial and formulas based on ISO standards, were not altered by the concomitant use of moisturizing creams applied shortly before and after the sunscreens.

2 | MATERIALS AND METHOD

2.1 | Study design

We performed a comparative quasi-experimental study in which we compared the UV radiation absorption capacity of different sunscreen formulations before and after the application of a moisturizing cream. The study was performed in the Dermatological Photobiology Laboratory of the Medical Research Center at the University of Malaga in Malaga, Spain. The products were tested in 25 healthy volunteers (15 women and 10 men) aged between 19 and 50 years with Fitzpatrick skin type II-III. The volunteers gave their written consent to participate in the study after they had been informed following the ethical principles of the declaration of Helsinki.

2.2 | Sunscreen and moisturizing products and in vitro SPF testing

Different combinations of commercial sunscreens with different commercial moisturizing products were tested. The combinations are included in table 1. For a control of known SPF we used the ISO 24444:2019 reference sunscreens products P2 and P8, which are sunscreens formulations that offer SPF of 16.1 and 63, respectively.[12] Both sunscreens references were provided by the company Solar Light (Solar Light Company Inc Glenside), with certification of their filter combination concentrations by the company. Control moisturizing cream for combination with P2 and P8 SPF standards were prepared using the same excipient formula as that used for the sunscreens but without the UV filters. The spectral transmittance as well as the spectral absorbance of the formulas were estimated according to European guidelines following the standard ISO 24443:2020 for in vitro UVA protection factor.[14] Briefly, transmittance spectra was determined by evenly spreading 1.3 mg/cm-2 of the product over PMMA plates of a surface of 5 × 5 cm^2 (Schönberg, Hamburg, Germany). The plate had a roughness simulating that of real skin relief, as indicated by the aforementioned ISO regulation. After 15 minutes in the darkness, the sample was placed on the sensor (Ulbrich sphere type) of a Macam SR-2210 double

TABLE 1 List of combinations of sunscreens products and moisturizing formulas

Combination	Sunscreen	Moinsturizing
C 1	Anthelios 50+ Shaka Fluid Laroche Posay	Lipicar Hydrating Laroche Posay
C 2	Eucerin 50 + Sensitive Protect	Eucerin Sensitive Relief After sun
C 3	Helicare 360 Water gel 50+	Endocare Essential Hidrating Gel Cream
C 4	Isdin Fusion Water SPF 50	Isdin After sun hydrating Lotion

monochromator spectroradiometer (Macam), and illuminated with a 300 W Oriel solar simulator (Oriel, Newport Corporation). Spectral transmittance spectrum was analyzed at 1 nm intervals in the range 290-400 nm, referred to the spectral transmittance of the blank PMMA plate coated with glycerol. The spectral transmittance and absorbance of the formulas with SPF 16.1 (P2 standard) and SPF 63 (P8 standard) are shown in Figure 1. It is confirmed that any of the moisturizing creams used in the present study did not absorb any UV radiation and had similar transmission properties to the glycerol used as blank in the ISO.

The products were applied to rectangular areas measuring 5×1 cm^2 in the left and right paravertebral areas of the back as observed in Figure 2. Five rectangles were marked out in each area using a thin strip of Transpore 3 M tape with a width of 0.5 cm measured with a ruler (Figure 2A,B). To compare the optical properties and the effect of combination of sunscreens with moisturizing formula, the high SPF (ISO P8 standard SPF 63) sunscreens formulation was applied to the left area of the back and the SPF 16 (ISO P2 standard) formulation was applied to the right area. In each case, a quantity of 2 mg cm^{-2} (total, 10 mg of cream) was spread evenly onto the corresponding rectangle for 1 minute using a fingertip. Rectangle 1 was untreated. Rectangle 2 was treated with moisturizing cream only, rectangle 3 with sunscreen only, rectangle 4 with sunscreen followed by moisturizing cream 10 minutes later, and rectangle 5 with moisturizing cream followed by sunscreen 10 minutes later (Figure 2A).

2.3 | UV photography

All the application zones (rectangles 1-5) were photographed using a UV camera (Figure 2C). The photographs were taken with a Canon EOS 1100D digital reflex camera (Canon Co.) fitted with two flashes with a Schott UG11 UV filter (Schott AG, Jena, Germany) to emit light in the region of 300-400 nm. The camera was positioned 24 cm from the volunteers' backs and all the photographs were taken in darkness. The camera was controlled by Clear Stone MD photography software (Digidmedys Co.). Six photographs were taken for each volunteer and saved for subsequent image analysis. In order to test if there is changes on UV reflection properties of products in the skin, rectangles 4 and 5 were photographed twice to test the effect of the combined use of moisturizer and sunscreen as follows: the first photograph was taken 10 minutes after the application of the first product and the second photograph was taken 10 minutes after the addition of the second product.

2.4 | Image analysis

All photographs were processed in ImageJ, an open-source software program for processing multidimensional scientific images. For each UV photograph (ie, for each treatment), the program's color histogram was used to measure color reflectance as a function of the tonality of pixels in a selected area of the skin (Figure 2C). The histogram has a tonal range of 0 to 255, were 0 is the darkest color (maximum absorbance) and 255 is the lightest color (minimum absorbance). In our case, 255 was blue. An example of the image analysis from the rectangle in Figure 2C is shown in Figure 2D.

In order to analyze the sensitivity of the UV reflectance level depending on different level of sunscreen applied, 5 different quantities (0.5-1-1.5-2-2.5 mg/cm^2) of ISO 24444:2019 P8 standard were applied in the skin of three volunteers and the pixel color intensity (0-255 pixel intensity level) from the black/blue level was analyzed after taking an UV photography of the volunteer skin. The image analysis showed an exponential degree in the UV reflectance levels that reveals a high level of sensitivity of the method (Figure 3).

2.5 | Statistical analysis

The tone values resulting from the analysis of the UV photographs were analyzed in terms of percentage differences in each rectangle with respect to the untreated zone. Mean differences and standard deviations were then calculated for the group of volunteers and the effects of the moisturizing cream were compared by analysis of variance followed by Bonferroni correction for post hoc pairwise comparisons. Differences between treatments were considered statistically significant when $P < .05$. Data were analyzed in Microsoft Excel 2019 and statistical differences were calculated using the IBM SPSS 20 software package.

3 | RESULTS

The SPF 16 and 63 formulations both showed a different level of UV light absorption as observed in the different blue/black level of UV photographs (Figures 2Cand4).

The square corresponding to the moisturizing cream did not changed significantly ($P > .05$) to color level observed in the non-treated skin (reference of 100%) (Figure 4A,B).

In the squares corresponding to only sunscreen application, the mean difference in black/blue color was around 67% for the SPF 63

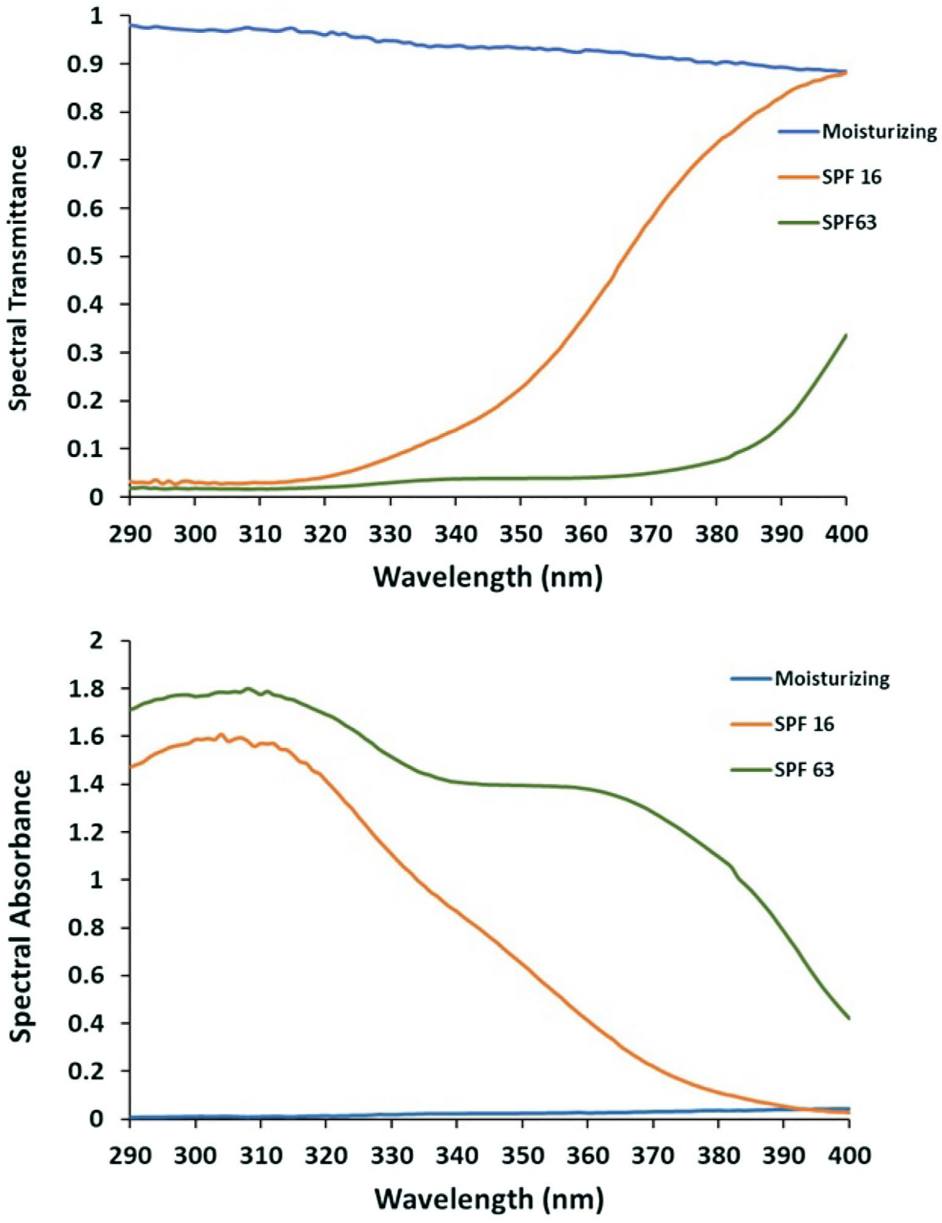

FIGURE 1 Transmittance and absorbance of UV radiation offered by a base formula (with no UV filters) compared the ISO 2444:2019 standards P2 and P8 to sunscreens with an expected Solar Protection Factor of 16 and 63

formulation and approximately 35% for the SPF 16 formulation with respect to non-treated skin both at the beginning and after 10 min ($P < .01$). No significant differences were observed between values of color level between sunscreen alone compared to concomitant application of moisturizing formula before and after of sunscreen application.

Results of the combination of commercial moisturizing creams and sunscreens are shown in Figure 5. For each combination, a photography example of skin UV reflection color is shown. All the formulas that confer SPF of 50+ (Figure 4A-C) and SPF 50 (Figure 4D) showed decrease in blue/black color level until around 37 −47% with respect to non-treated skin 10 min after application of sunscreen alone. The application of moisturizing formulas, that do not confer any UV absorption in all cases of sunscreens/moisturizing combinations as observed in the lateral UV photos, resulted in not significant changes ($P > .05$) with respect to the application of sunscreen alone, independently of mineral or organic filter combination. Moreover, no significant differences were observed in the treatments when sunscreen formula was applied before or after the moisturizing cream in all cases ($P > .05$) (Figure 4A-E).

4 | DISCUSSION

Sunscreens containing mineral and organic filters to absorb or reflect different types of UV radiation (UVB and UVA) are widely used to protect against the harmful effects of excessive UV exposure.[9] The higher a sunscreen's SPF, the longer it takes for the sun's rays to

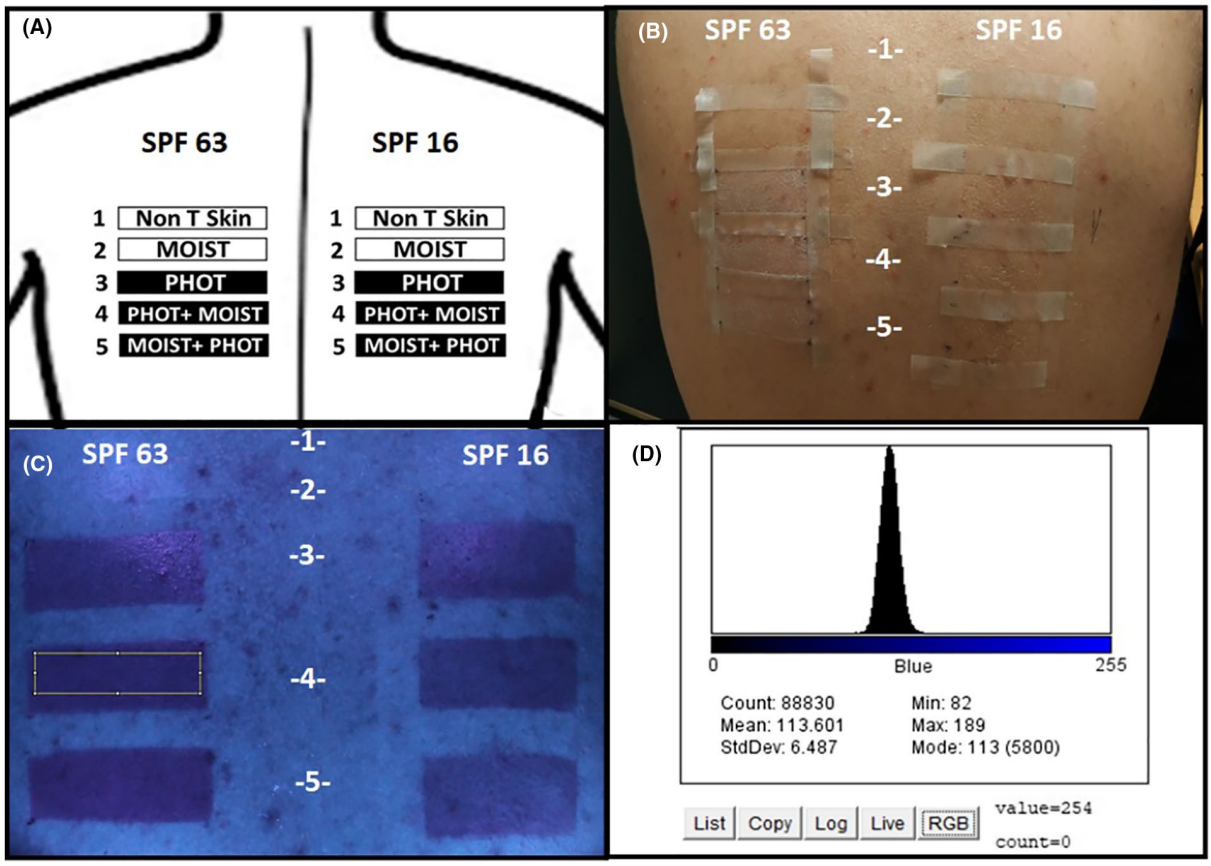

FIGURE 2 A, Graph showing application of sunscreens and moisturizing cream to the right and left areas of the back. The same protocol was used in both areas to analyze color differences between a sunscreen with an expected sun protection factor (SPF) of 63 (left) and a sunscreen with an SPF of 16 (right). Rectangle 1, untreated skin; rectangle 2, moisturizing cream; rectangle 3, sunscreen only; rectangle 4, sunscreen followed by moisturizing cream; and rectangle 5, moisturizing cream followed by sunscreen. B, UV photograph of skin showing rectangles for different treatments marked with 3 M Transpore film. C, UV photograph 10 min after application of treatments. The yellow rectangle shows the area selected for light reflection image analysis. D, Pixel distribution for the rectangle shown in C

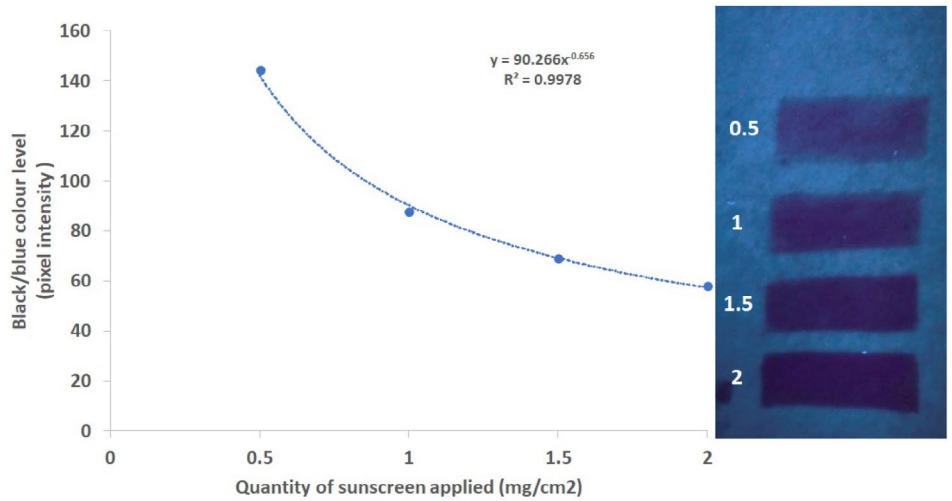

FIGURE 3 Changes in UV reflectance in terms of pixel intensity of the ISO 2444:2019 standard P8 applied at different quantities per surface of skin (0.5, 1, 1.5, 2 mg/cm^2). The figure pixel intensity levels on corresponds to each rectangle of right photo after image analysis calculations

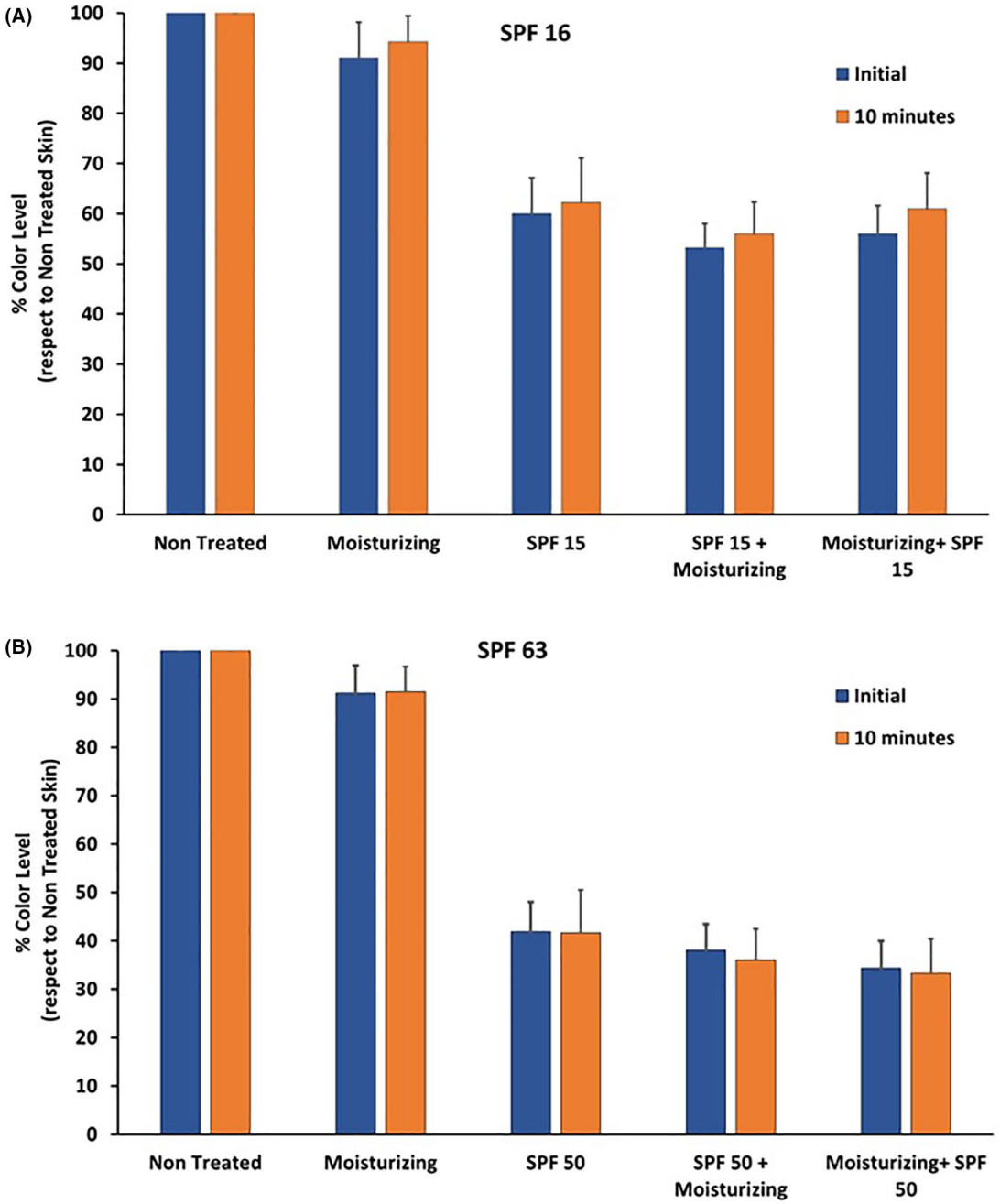

FIGURE 4 Data from UV reflection image analysis on the back of a volunteer treated with a sunscreen with moisturizing formula and sunscreens formulas of SPF 16 (right) and SPF 63 (left). A, 10 min after application of moisturizing formula in rectangle 2, sunscreen alone in rectangle 3, sunscreen (previous to moisturizing formula) in rectangle 4 and moisturizing (previous to sunscreen) in rectangle 5. B, 10 min after application of the moisturizing formula in rectangle 4 and sunscreen formula in rectangle 5

induce erythema. In this study, the first of its type to our knowledge, we have shown that the application of a moisturizing cream shortly before and after sunscreens of low and high Solar Protection Factors did not diminish the ability of the sunscreens to block UV radiation.

For a sunscreen to be effective, it needs to be applied generously (and where appropriate reapplied every 2 hours) to clean and dry skin.[8] The reality is, however, that many people do not follow these recommendations and in addition they apply sunscreen over other cosmetic products.

We analyzed the absorption capacity of the sunscreens tested in our study after 10 minutes (and not the 30 minutes recommended by guidelines to ensure stability), as a recent study by our group found that optimal sunscreen protection was achieved just 10 minutes after application.[16]

UV photography has been used for many years to detect sun-induced skin damage invisible to the naked eye and recently is being used for sunscreens performance and potential both in vitro and in vivo.[17] Apart from its clinical value, UV reflectance imaging

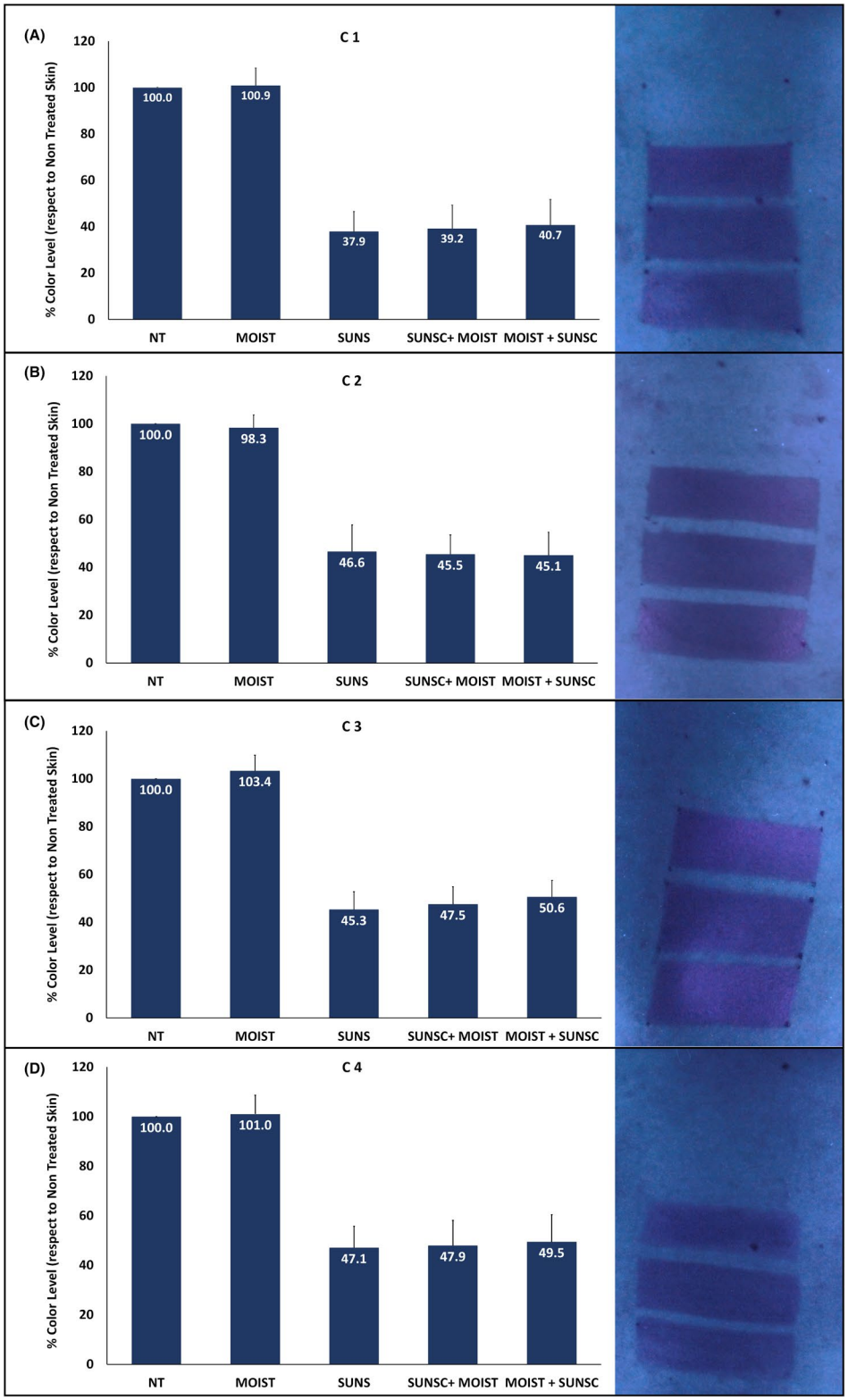

FIGURE 5 Data from UV reflection image analysis on the back of the volunteers treated with 4 combinations of sunscreens with moisturizing formulas. Combinations are shown in table 1. A, C1, B, C2, C, C3, and D, C4. Data are expressed in terms of mean black/blue color values (%) observed in UV reflectance analysis of skin. 100% untreated skin values were used as reference values. For each combination treatments, the UV reflectance image is shown on the right of each figure

is a useful tool for raising awareness about the deleterious effects of excessive sun exposure and the importance of proper protection. [18,19] As we demonstrate in this and previous works, the use of image analysis with high sensibility software for color levels in image pixels increase the accuracy of non-invasive techniques for UV effect on human skin. Recently Diffey et al demonstrate with UV photography on human skin and imaging analysis systems how effective can be along the day a simple application of cosmetics with SPF 15.[20]

As both sunscreen and skincare products have grown in popularity, it is only logical that they are frequently used together. However, little is known about how their combined application affects the protection provided by the sunscreen. Thus, the main goal of our work has been demonstrated. There is no alteration to the UV absorption properties, and finally to no changes in the SPF of the sunscreen formulas when combined to other skin care products. Therefore, user can be sure in the performance of this this skin cancer prevention tool for their daily use. Analysis of the UV photographs from our study shows that the moisturizing cream applied shortly before and after the two sunscreen formulations did not alter their protective ability. So, when application protocols are correct and time between application of products in the skin are correct, no differences in UV photoprotection after sunscreen application is observed. By means of this technique, absence of sunscreen filters in formula do not offer any change of color in UV reflectance as observed in the application zones corresponding to the moisturizing cream, with similar blue/black color to zone with untreated skin. In order to confirm the UV photography observations objectively, we calculated the absorbance and transmittance of UV radiation for two ISO 24444:2019 standards (P2 with SPF of 16 and P8 with SPF 63) and compared with excipient alone as example of moisturizing formula and found that the moisturizer did not absorb UV light, confirming that it did not offer protection against the sun's rays (Figures 1Cand2). This absence of UV absorption has been also observed in all the commercial moisturizing formulas used in the study, thus, the possible changes in UV reflectance changes of the images is attributed only to changes in sunscreen performance in the skin. Moreover, we tested the sensitivity of the UV image analysis system with the use of the ISO P8 standard at different quantities in the skin (0.5-1-1.5-2 mg/cm^2) in the skin and as expected for changes in solar protection factors related to sunscreen amount per surface of skin, color reflectance is decreased exponentially while sunscreen quantity is increased. This has been previously analyzed by other authors in which decreasing quantity of the same sunscreen on skin, the SPF is decreased exponentially.[21,22] So, the ultraviolet reflectance is a sensitive system for correlating potential SPF of a formula with respect to changes in their UV absorbing properties applied on skin.

The effect of application of moisturizing formulas concomitantly to sunscreens revealed no changes in UV reflectance color have been observed when combined moisturizing formulas to the sunscreens in any of the 4 commercial combinations as well as the 2 ISO standards of low and high SPF combinations, independent of their order of application.

One limitation of our study is that it was conducted in a laboratory environment, with controlled temperature and lighting conditions and protection against loss of product (and effectiveness) due to movement or friction with clothing. The effectiveness of these creams in natural conditions may, therefore, vary.

In conclusion, the effectiveness of low- and high-protection sunscreens were not altered by the concomitant use of a moisturizing creams applied shortly before and after the sunscreens. Ultraviolet photography is a very useful tool for analyzing the effectiveness of sunscreen products.

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CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Authors confirm the absence of shared data.

ORCID

José Aguilera https://orcid.org/0000-0002-1911-111X
María Leal https://orcid.org/0000-0002-5863-495X

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