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Evaluation of the photostability of different UV filter combinations in a sunscreen

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Abstract

Development of photostable sunscreens is extremely important to preserve the UV protective capacity and to prevent the reactive intermediates of photounstable filter substances behaving as photo-oxidants when coming into direct contact with the skin. Thus, the objective of this study was to evaluate the photostability of four different UV filter combinations in a sunscreen by using HPLC analysis and spectrophotometry. The formulations that were investigated included four different UV filter combinations often used in SPF 15 sunscreens. The UV filter combinations were: octyl methoxycinnamate (OMC), benzophenone-3 (BP-3) and octyl salicylate (OS) (formulation 1); OMC, avobenzone (AVB) and 4-methylbenzilidene camphor (MBC) (formulation 2); OMC, BP-3 and octocrylene (OC) (formulation 3); OMC, AVB and OC (formulation 4). In the photostability studies, 40 mg of each formulation were spread onto a glass plate and left to dry before exposure to different UVA/UVB irradiation. Exposed samples were then immersed in isopropanol and the dried film dissolved ultrasonically. The filter components in the resulting solution were quantified by HPLC analysis with detection at 325 nm and by spectrophotometry. In this study, the four UV filter combinations showed different photostability profiles and the best one was formulation 3 (OMC, BP-3 and OC), followed by formulations 4, 1 and 2. In addition, OC improved the photostability of OMC, AVB and BP-3.

Keywords: Sunscreens; Photostability; Octyl methoxycinnamate; Avobenzone

1. Introduction

Sunscreens have been recommended by dermatologists for a long time as a protective measure against excessive amounts of sunlight to prevent UV induced erythema and also because many studies show that regular use of sunscreens contribute to the prevention of skin photodamage (Tarras-Wahlberg et al., 1999).

Although recently recommended sunscreens have a better UVA protective capacity, the absorption spectra of some sunscreens do change upon irradiation with UV radiation. If the absorption decreases while the shape of the absorption curve remains the same, there will be an increase in the amount of the same spectrum UV radiation reaching the basal epidermal cell layers. However, if the shape of the absorption spectrum also changes, leading to high UVA exposure, the situation becomes more critical. The risk of melanoma development is enhanced, since the action spectrum for the induction of malignant melanoma is shifted towards UVA (Tarras-Wahlberg et al., 1999). Hence, in order to guarantee constant efficacy of the product throughout the exposure period, the UV filters used should not be altered by UV sunlight (Chatelain and Gabard, 2001; Vanquerp et al., 1999).

Another reason for the development of photostable sunscreens as part of the product efficacy evaluation (Cambon et al., 2001) is that the reactive intermediates of photounstable filter substances come into direct contact with the skin, where they may behave as photo-oxidants or may also promote phototoxic or photoallergic contact dermatitis. The interaction of photodegradation products with sunscreen excipients or skin components like sebum may lead to the formation of new molecules with unknown toxicological properties (Cambon et al., 2001; Deleo et al., 1992; Rieger, 1997; Schrader et al., 1994; Gerhard et al., 2001).

In the early 1980s, a few sunscreen photostability studies began examining the benzilidene camphor sunscreens (Beck et

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al., 1981; Deflandre and Lang, 1988), and then *p*-amino benzoic acid and its derivatives (Allen et al., 1996). During the 1990s, the photostability of the dibenzoylmethane UVA sunscreens was studied (Tarras-Wahlberg et al., 1999; Chatelain and Gabard, 2001).

Several groups have reported work using UV filters in solution, in polymer films, in liquid films, on glass or stratum corneum and even on humans in vivo (Berset et al., 1996), but in most of them the photochemistry of sunscreen agents was examined in dilute solutions, which may not be particularly relevant. In thin films and in the skin, the photochemistry of photounstable sunscreens is more complex than in dilute solutions, consequently photochemistry studies based on thin films are more similar and relevant to practical applications than the ones that are done in dilute solutions. In 1995, the European Cosmetic Toiletry and Perfumery Association (COLIPA) task group published a collaborative initial test for photostability of four sunscreen agents used in products tested in liquid films on glass surfaces (Berset et al., 1996).

The behavior of sunscreens is not predictable from the photostability of its individual filter. Thus, it is also important to evaluate the combinations of filters used in the formulation (Deflandre and Lang, 1988; Schwack and Rudolph, 1995; Vanquerp et al., 1999).

Notwithstanding, the confounding effects of filter combinations on sunscreen product formula development, the toxicological implications of sunscreen photoinstability alone provide sufficient importance to further attention in this topic.

Thus, the objective of this study was to evaluate and compare the photostability of four different UV filter combinations in a sunscreen by using HPLC analysis and spectrophotometry.

2. Materials and methods

2.1. Formulations studied

Four UV filter combinations often used in SPF 15 sunscreen products were chosen for this study. The combined UV filters were added to a formulation containing 4% of a blend of ammonium acryloyldimethyl-taurate/VP copolymer and trilaureth-4 phosphate, 0.1% of dissodium EDTA, 0.05% of BHT, 5% of propyleneglycol, 2% of volatile silicone, 0.8% of phenoxyethanol and parabens and distilled water. The combinations were: 7% of octyl methoxycinnamate (OMC), 2% of benzophenone-3 (BP-3) and 1.5% of octyl salicylate (OS) (formulation 1) (Gaspar and Maia Campos, 2003); 10% of OMC, 2% of avobenzone (AVB) and 2% of 4-methylbenzilidene camphor (MBC) (formulation 2); 7% of OMC, 4% of BP-3 and 5% of octocrylene (OC) (formulation 3); 5% of OMC, 2% of AVB and 7% of OC (formulation 4).

2.2. Photostability studies

In these studies, formulation samples were irradiated and evaluated by two in vitro methodologies, HPLC analysis and spectrophotometry. For this, 40 mg of each formulation were spread onto a 10 cm^2 (approximately 4 mg/cm^2) area of a glass plate and left to dry for 30 min before exposure to different UVA/UVB irradiations (280–400 nm) from a 96,000 Oriel 150 W Xenon arc solar simulator (Oriel Corporation, Stratford, CT). The radiation was filtered through a dichroic mirror (280–400 nm) and a WG 305 long pass filter, which allows no passage of light less than 280 nm. Irradiance, which was approximately 20 mW/cm^2, was measured at 290 nm with a 70260 Oriel Radiant Power Meter equipped with a silicon probe (coupled with a 1 in. fused silica metallic neutral density filter-optical density 3.0) (Berset et al., 1996; Marginean Lazar et al., 1997).

Glass plates containing dried formulations were exposed to three different UV doses (30, 60 and 120 min of a 20 mW/cm^2 UV radiation). For each exposed plate, a duplicate plate serving as a negative (non-irradiated) control was kept in a dark place at 30 °C. Three replicate pairs of samples were prepared.

Exposed samples (formulations 1–4 and the vehicle without UV filters) were then immersed in 50 mL of isopropanol and the dried films dissolved ultrasonically. The UV filters in this solution were quantified by HPLC analysis (Shimatzu) on a C18 column (5 μm ODS, 250 mm × 4 mm), with methanol:water (88:12, v/v) as mobile phase and detected at 325 nm, and by a Hitachi U-2001 spectrophotometer (280–400 nm). For spectrophotometric evaluation, samples were diluted (1:4, v/v) and the ratio of the mean UVA (320–400 nm) to the mean UVB (280–320 nm) absorbances was calculated as (Diffey, 1994):

$$\frac{\int_{320}^{400} A(\lambda)d\lambda / \int_{320}^{400} d\lambda}{\int_{280}^{320} A(\lambda)d\lambda / \int_{280}^{320} d\lambda}$$

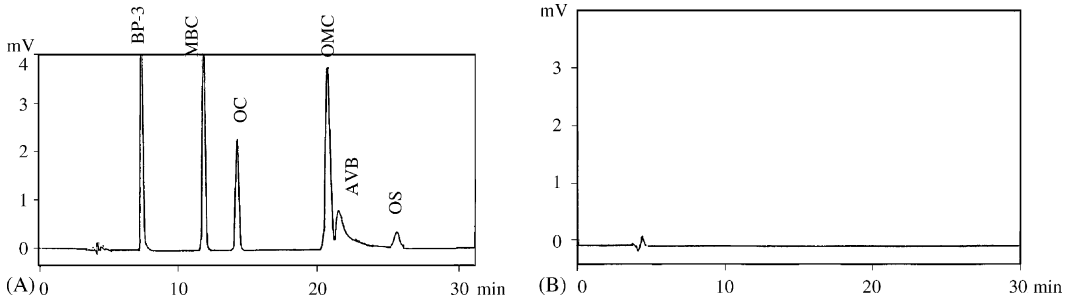

Fig. 1. HPLC chromatographic analysis of (A) an isopropanol solution of the six sunscreen agents studied. Peaks: benzophenone-3, 4-methylbenzilidene camphor, octocrylene, octyl methoxycinnamate, avobenzone and octyl salicylate and (B) placebo. Detection at 325 nm; 5 mm C18 column; isocratic elution methanol:water (88:12, v/v), flow rate 0.8 mL min.

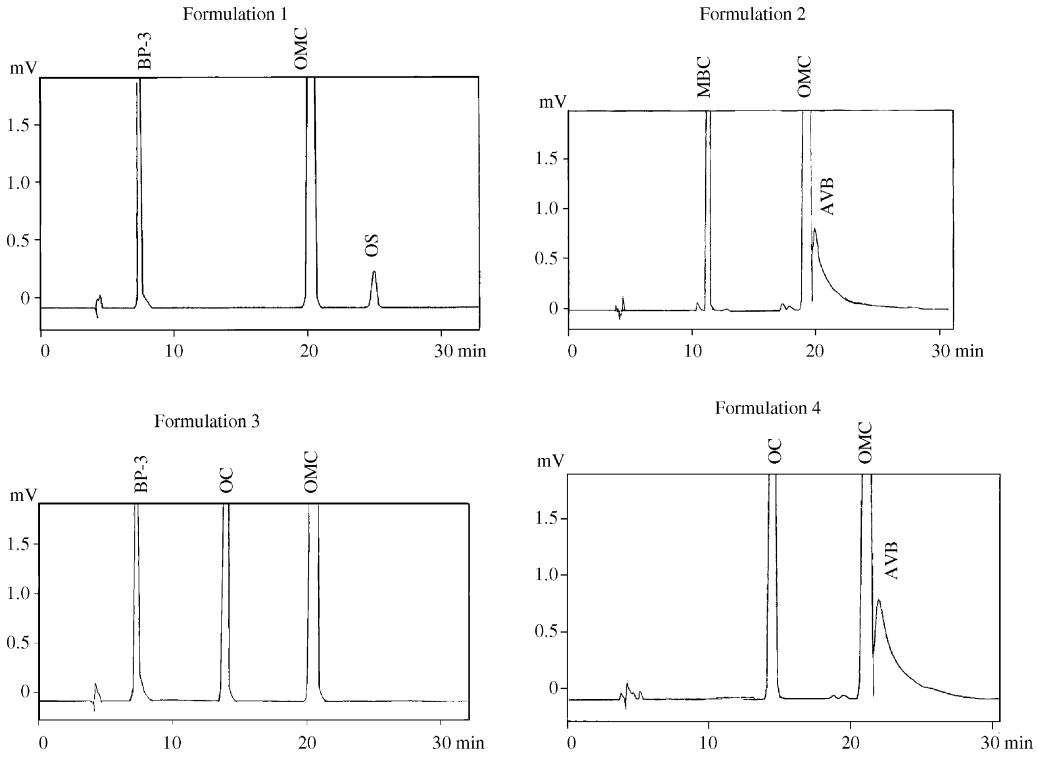

Fig. 2. Chromatographic profiles (HPLC) of formulation 1 (benzophenone-3, octyl methoxycinnamate and octyl salicylate), formulation 2 (4-methylbenzilidene camphor, octyl methoxycinnamate and avobenzone), formulation 3 (benzophenone-3, octocrylene and octyl methoxycinnamate) and formulation 4 (octocrylene, octyl methoxycinnamate and avobenzone). Conditions similar to Fig. 1.

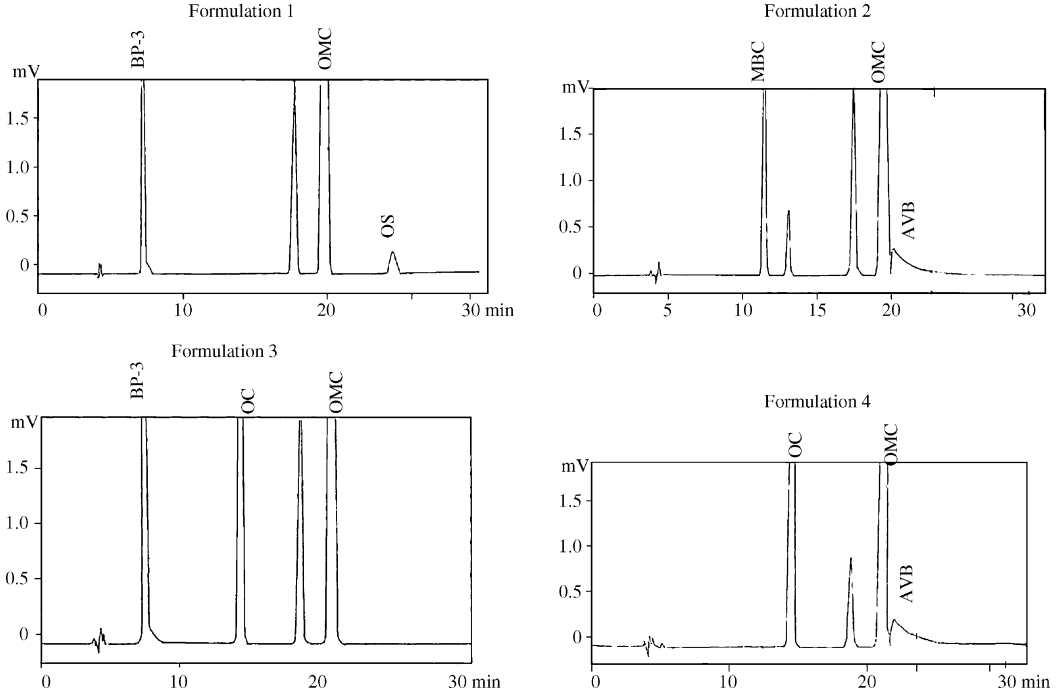

Fig. 3. Chromatographic profiles (HPLC) of formulations 1, 2, 3 and 4 after 60 min UVA/UVB irradiation. Formulation 1 (BP-3, OMC and OS), formulation 2 (MBC, OMC and AVB), formulation 3 (BP-3, OC and OMC) and formulation 4 (OC, OMC and AVB). Conditions similar to Fig. 1. Observe unidentified peaks after irradiation of all formulations (this figure and Fig. 2).

Results obtained were statistically analyzed using Kruskal–Wallis, a non-parametric test.

3. Results and discussion

The chromatographic separation of the UV filters on C18 columns was optimized with regard to the mobile phase. Isocratic elution with 100% methanol was found to produce good resolution and a short time analysis (8 min) but the separation of OMC and AVB could not be accomplished. However, isocratic elution with a methanol:water (88:12, v/v) mobile phase gave good results in separating the six UV filters under study (Fig. 1A) in a relatively shorter time (27 min) than in other gradient analysis reports (Vanquerp et al., 1999; Meijer and Loden, 1995).

The chromatographic profiles of formulations both exposed and not exposed to 60 min UVA/UVB irradiation are shown in Figs. 3 and 2, respectively.

Our results were validated in terms of linearity, precision and accuracy. The correlation coefficients were all above 0.999. The precision (CV) was between 3.6 and 8.4. The accuracy values were low (−10.5 to −18.1) because a small amount of the formulation was lost when it was spread onto the glass plate, but the procedure was kept because it is currently used for the photostability evaluation of sunscreens.

The formulations studied showed variation in stability, which emphasizes the fact that photostability studies are very important to guarantee the efficacy of a sunscreen. Also, the chromatographic assay appears to be a convenient method to obtain data about this class of cosmetic ingredients (Vanquerp et al., 1999).

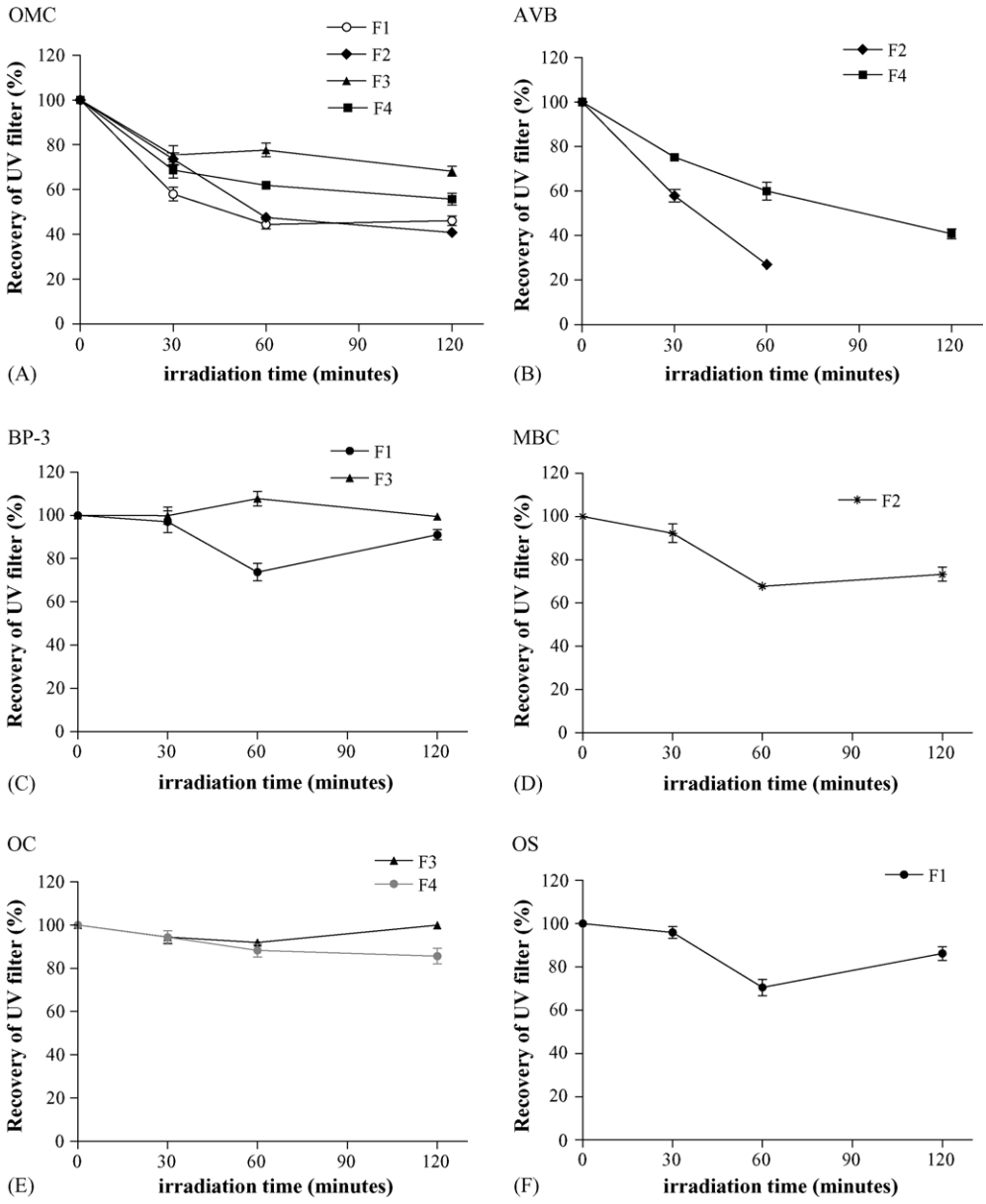

Fig. 4. Recovery of the UV filters: (A) OMC, (B) AVB, (C) BP-3, (D) MBC, (E) OC and (F) OS contained in the formulations under study, after 0, 30, 60 and 120 min UVA/UVB irradiation, which were expressed as percentage of the initial filter amount (negative control).

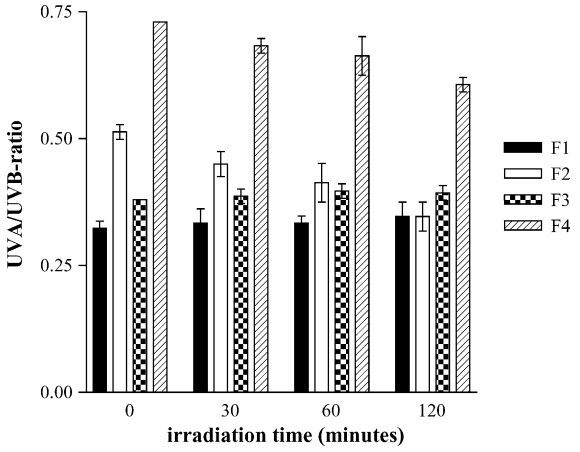

Fig. 5. UVA/UVB absorption ratio for formulations 1, 2, 3 and 4, after 0 (negative control), 30, 60 and 120 min UVA/UVB irradiation.

To analyze the alterations that occurred in the formulations under study after UVA/UVB irradiation, and choose the most photostable one, the recovery of the six studied UV filters contained in the formulations was plotted on graphs against irradiation times as shown in Fig. 4. In terms of OMC recovery (all formulations studied contained OMC), formulation 3 was the most photostable (OMC, BP-3 and OC), followed by formulation 4 (OMC, AVB and OC), formulation 1 (OMC, BP-3 and OS) and formulation 2 (OMC, AVB and MBC), respectively (Fig. 4A). Formulation 4 was more photostable than formulation 2 in terms of AVB by the same criteria (Fig. 4B) and in terms of BP-3, formulation 3 was more photostable than formulation 1, and finally, in terms of OC, formulation 3 was more photostable than formulation 4.

OMC alone undergoes a *cis*-/*trans*-isomerization mechanism that cannot really be considered as photoinstability but rather a very efficient way of dispersing the absorbed energy, and it has been regarded as relatively photostable in some studies (Butt and Christensen, 2000; Chatelain and Gabard, 2001). However, following irradiation AVB and OMC react with each other to form cycloaddition products and perhaps other photoadducts, which may explain why formulation 3 was more photostable than formulations 4 and 2 (Chatelain and Gabard, 2001). OC and MBC can stabilize AVB as they have triplet energy similar to AVB (55-59 kcal/mol) (Sayre et al., 2005; Chatelain and Gabard, 2001).

The results obtained in the spectrophotometric analysis (Fig. 5) showed that formulation 4 had a higher UVA/UVB absorption ratio than formulations 1, 2 and 3. When formulation 4 was irradiated, this ratio was reduced but still statistically higher than the others. In addition, formulation 3 was the most photostable.

Formulation 4 had lower reduction of UVA/UVB absorption ratio (4.6 for 30 min, 6.3 for 60 min and 13.2 for 120 min of irradiation) than formulation 2 (6.4 for 30 min, 10.3 for 60 min and 16.7 for 120 min of irradiation), which indicates that F4 had a lower reduction in UVA absorption capacity than F2.

Considering UV filter interactions, we observed that octocrylene was more effective than MBC in stabilizing OMC in the presence of AVB, as formulation 4 (OMC, AVB and OC) showed higher photostability than formulation 2 (OMC, AVB and MBC). It was also possible to confirm OC as a good UV stabilizer by showing that formulation 3, which contained OMC, BP-3 and OC, was more photostable than formulation 1, which contained OMC, BP-3 in association with OS.

These evaluations, based on the methods described, are more suitable than analysis based on absorption spectroscopy alone, which can lead to misinterpretations. Therefore, separation techniques such as HPLC or GC analysis, etc., should complement photostability studies (Berset et al., 1996).

4. Conclusions

In the photostability studies, the four UV filter combinations presented different photostability profiles, the best one being formulation 3 (OMC, BP-3 and OC) followed by formulations 4, 1 and 2. In addition, filter interaction was also seen with OC improving the photostability of OMC, AVB and BP-3. These formulations containing OC also maintained a high UVA/UVB absorption ratio when irradiated for 120 min. Since maintaining the UVA absorption capacity is important to prevent erythema and to reduce the subsequent risk of melanoma development, formulations 3 and 4 containing OC have superior performance compared to formulation 1 and 2 that did not contain OC. The HPLC analysis proposed was adequate for the simultaneous determination of the six studied sunscreen UV filters. The extraction procedure was efficient, showing good precision. However, a small amount of the formulation was lost when it was spread onto the glass plate, reducing the accuracy of the extraction procedure. The UVA/UVB absorption ratio had a good correlation to HPLC analysis since in terms of avobenzone, formulation 4 was more photostable than formulation 2 and also had lower reduction in the UVA/UVB absorption ratio than formulation 2, which indicates that formulation 4 had a lower reduction in UVA absorption capacity than formulation 2. Formulation 4 had the highest UVA absorption; however, formulation 3 was the most photostable and was judged to have the best performance overall, since photounstable products can cause phototoxic or photoallergic contact dermatitis. In addition, the reduction in UVA absorption can lead to a high UVA exposure, enhancing the risk of melanoma development.

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References

Allen, J.M., Gossett, C.J., Allen, S.K., 1996. Photochemical formation of singlet molecular oxygen in illuminated aqueous solutions of several commercially available sunscreen active ingredients. Chem. Res. Toxicol. 9, 605–609.

Beck, I., Deflandre, A., Lang, G., Arnaud, R., Lemaire, J., 1981. Study of the photochemical behaviour of sunscreens-benzylidene camphor and derivates. Int. J. Cosmet. Sci. 3, 139–152.

Berset, G., Gozenbach, H., Christ, R., Martin, R., Deflandre, A., Mascotto, R.E., Jolley, J.D.R., Lowell, W., Pelzer, R., Stiehm, T., 1996. Proposed protocol for determination of photostability. Part I: cosmetic UV filters. Int. J. Cosmet. Sci. 18, 167–177.

Butt, S.T., Christensen, T., 2000. Toxicity and phototoxicity of chemical sun filters. Radiat. Prot. Dosim. 91, 283–286.

Cambon, M., Issachar, N., Castelli, D., Robert, C., 2001. An in vivo method to assess the photostability of UV filters in a sunscreen. J. Cosmet. Sci. 52, 1–11.

Chatelain, E., Gabard, B., 2001. Photostabilization of butyl methoxydibenzoylmethane (avobenzone) and ethylhexyl methoxycinnamate by bis-ethylhexyloxyphenol methoxyphenyl triazine (Tinosorb S), a new UV broadband filter. Photochem. Photobiol. 74, 401–406.

Deflandre, A., Lang, G., 1988. Photostability assessment of sunscreens. Benzylidene camphor and dibenzoylmenthane derivatives. Int. J. Cosmet. Sci. 10, 53–62.

Deleo, V.A., Suarez, S.M., Maso, M.J., 1992. Photoallergic contact dermatitis. Results of photopatch testing in New York, 1985–1990. Arch. Dermatol. 128, 1513–1518.

Diffey, B.L., 1994. A method for broad spectrum classification of sunscreens. Int. J. Cosmet. Sci. 16, 47–52.

Gaspar, L.R., Maia Campos, P.M.B.G., 2003. Rheological behavior and the SPF of sunscreens. Int. J. Pharm. 250, 35–44.

Gerhard, J., Nohynek, Schaefery, H., 2001. Benefit and risk of organic ultraviolet filters. Regul. Toxicol. Pharmacol. 33, 285–299.

Marginean Lazar, G., Fructus, A.E., Baillet, A., Bocquet, J.L., Thomas, P., Marty, J.P., 1997. Sunscreens' photochemical behavior: in vivo evaluation by the stripping method. Int. J. Cosmet. Sci. 19, 87–101.

Meijer, J., Loden, M., 1995. Stability analysis of three UV-filters using HPLC. J. Liquid Chromatogr. 18, 1821–1832.

Rieger, M.M., 1997. Photostability of cosmetic ingredients on the skin. Cosmet. Toil. 112, 65–72.

Sayre, R.M., Dowdy, J.C., Gerwig, A.J., Shields, W.J., Lloyd, R.V., 2005. Unexpected photolysis of the sunscreen octinoxate in the presence of the sunscreen avobenzone. Photochem. Photobiol. 81, 452–456.

Schrader, A., Jakupovic, J., Baltes, W., 1994. Photochemical studies on *trans*-3-methylbutyl 4-methoxycinnamate. J. Soc. Cosmet. Chem. 45, 43–52.

Schwack, W., Rudolph, T., 1995. Photochemistry of dibenzoyl methane UVA filers. Part 1. J. Photochem. Photobiol. B Biol. 28, 229–234.

Tarras-Wahlberg, N., Stenhagen, G., Larko, O., Rosen, A., Wennberg, A.M., Wennerstrom, O., 1999. Changes in ultraviolet absorption of sunscreens after ultraviolet irradiation. J. Invest. Dermatol. 113, 547–553.

Vanquerp, V., Rodriguez, C., Coiffard, C., Coiffard, L.J., De Roeck-Holtzhauer, Y., 1999. High-performance liquid chromatographic method for the comparison of the photostability of five sunscreen agents. J. Chromatogr. A 832, 273–277.