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Photoprotective efficacy and photostability of fifteen sunscreen products having the same label SPF subjected to natural sunlight

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ABSTRACT

The first objective of this study is to show how different can be photoprotection by sunscreens with an identical SPF given on the packaging, when subjected to sunlight radiation. The second objective is to highlight the need for global harmonization of photostability testing and UVA protection labelling. Fifteen products with various combinations of UV filters marketed in Europe were assessed based on transmission measurements of 0.75 mg cm^{-2} layer covered onto polymethylmethacrylate plate roughness 2 μm. Two absolute UV spectroscopic indices (*in vitro* SPF, UVA-PF), four well-known relative UVA indices: the UVA-PF/SPF ratio and critical wavelength by European Commission (EC); UVA/UVB ratio by Boots Star Rating system; UVA1/UV ratio by FDA Proposed Ruling and one new relative indices the Spectral Uniformity Index (SUI) by Diffey, were compared before and after sunlight exposure with dose about 42 SEDs. The UVA-PF values before exposure proved a high degree of variation among samples. After exposure only five sunscreens observed UVA protection standard by EC and the same products showed compliance with the first UVA rating by Boots system (three stars). According to the UVA1/UV ratio, except for one product, all sunscreens manifested certain UVA protection level (low, medium or high). In compliance with criteria of new rating proposed by Diffey, exactly all fifteen sunscreens gave some UVA rating exhibited as SUI (low, medium or high). These results mean that the different UVA protection indices can exhibit various data and be confusing for consumer. Photostability of each product was assessed with three indices: the Area under curve (Auc) Index for the total UV range, and UVB, UVA, UVA2, UVA1 range separately; the residual effectiveness of *in vitro* SPF and UVA-PF. All fifteen sunscreens were photostable in the UVB region. Seven products exhibited photoinstability in the total UV range (290–400 nm); all of them contained a combination of the ethylhexyl methoxycinnamate (EHMC) and butyl methoxydibenzoylmethane (BMBM) together with other UV filters. Eight products lacked their stability in the UVA1 range (340–400 nm) thus confirmed that photodegradation of some current sunscreens is primarily problem of this region. The most photoinstability showed sunscreens S1 (EHMC, BMBM and phenylbenzimidazole sulphonic acid) and S6 (EHMC, BMBM, phenylbenzimidazole sulphonic acid and ethylhexyl triazone); Auc-UVA1 Index was 0.15 only. Excellent UVA1 photostability showed sunscreen S8 (EHMC, EHT and methylene bis-benzotriazolyl tetramethylbutylphenol); Auc-UVA1 Index was of 1.00. Three sunscreens showed very good UVA1 photostability (Auc-UVA1 Index ranged from 0.98 to 0.93). The fact that these products applied only in the layer of 0.75 mg cm^{-2} were photostable under the sunlight dose, which corresponds to layer of 2 mg cm^{-2}, is proof of their quality. Comparison of the residual effectiveness of *in vitro* SPF and UVA-PF values with the Auc-Index showed that methods give a similar ranking of the sunscreens' photostability.

1. Introduction

Over the past decade awareness of the detrimental effects of unprotected ultraviolet (UV) exposure has increased and, as a result, consumers are seeking higher levels of protection. The use of sunscreen products is important part of a photoprotection strategy. However, some sunscreens seem to provide much less protection than expected. There are two cardinal problems.

Since the biological endpoint for the determination of the Sun Protection Factor (SPF) is the UV erythema, the SPF label is indicator only for a protection against erythemally effective solar UV, largely confined to the UVB (290–320 nm) and partially short-wavelength UVA (320–340 nm) radiation (Diffey et al., 2000; EC, 2006; Stanfield

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et al., 2010). The SPF value does not provide any information regarding protection against long-wavelength UVA1 (340–400 nm) that harmful effects began to be suspected in the 1990s. Major consequence of cumulative UVA radiation is the production of reactive oxygen species which can also induce cancer, for instance generating oxidized DNA base derivatives, and altered tumour suppressor genes, like p53 (Seité et al., 2000; Vielhaber et al., 2006; Velasco et al., 2008). Furthermore, scientific findings also confirm that UVA radiation causes immunosuppression, skin aging, and various other photo-disorders (WHO, 2006). Declaration of a SPF value of sunscreen that does not offer UVA protection, may give a false feeling of safety, as skin reddening as an alarm signal is delayed. Consumer therefore may become encouraged to a prolonged stay in the sun, but exposure to UVA is increased (Bernerd et al., 2003; Haywood et al., 2003; Fourtanier et al., 2006; Moyal and Fourtanier, 2008; Mendrok-Edinger et al., 2009; Autier, 2009). In order to have preventive characteristics against both UVB and UVA radiation, a majority of new commercial sun care products on the global market contain not only UVB, but also one or more UVA filters.

However, it has been clearly demonstrated that not all UVA and UVB absorbers are sufficiently photostable. Within UV exposure some of them may change spectral performance or act as photooxidants via generation of free radicals and reactive oxygen species alone or in combination with others substances when coming into direct contact with the skin (Herzog and Sommer, 2000; Damiani et al., 2007; Gaspar and Campos, 2007; Herzog et al., 2009). Hence the photochemical instability represents a further significant problem of sunscreens. High SPF value implies the long-lasting photoprotection. But, this is only guaranteed when the UV filters remain stable throughout the entire period of exposure to sunlight implied by the value of SPF or if their metabolites have a comparable protective effect. Thus, the production of photostable products is extremely important. Unfortunately, most sunscreens on the market do not have a photostability label, making them difficult to compare.

There is a considerable literature on measurement of photostability behaviour of individual UV filters and final sunscreen products upon UV irradiation with various solar simulator apparatus based on xenon arc lamps or fluorescent lamps usually (e.g. Stokes and Diffey, 1999; Herzog and Sommer, 2000; Cambon et al., 2001; Maier et al., 2001, 2005; Serpone et al., 2002; Marrot et al., 2004; Loden et al., 2005; Dondi et al., 2006; Gaspar and Campos, 2006, 2007, 2010; Hojerová et al., 2006; Couteau et al., 2007, 2009; Gonzales et al., 2007; Moyal and Fourtanier, 2008; Venditti et al., 2008; Herzog et al., 2009; Sehedic et al., 2009; Scalia et al., 2010).

When people are outdoors, they are not exposed to only UVB or UVA radiation but are exposed to full solar radiation. Sunlight reaching the Earth's surface is a mixture of UVA, UVB, visible and infrared radiation. Since is polychromatic, its ultimate effects on the human skin are results of not only action of each wavelength individually but also synergistic or antagonistic interactions between the different solar wavelengths (Cho et al., 2008). In addition, biological effects against which people may wish to be protected are caused by all wavelengths in the solar radiation spectrum.

Laboratory test with artificial UV light source that separates the effects of UVA and UVB from themselves and other radiations does not produce the level of sunscreen instability as does the natural sunlight. Furthermore, the spectrum of sunlight is changing continuously (Diffey, 2002). Intensity of the solar radiation at the Earth's surface depends strongly on varying sun angles due to latitude, season, time of day, and whether, the total amount of ozone in the atmosphere, etc. (TRLI, 2003; AFSSE et al., 2005; Lim and Rigel, 2007).

Regrettably, to date, only a very few papers appear to focus on the sunscreens' photoinstability upon outdoor sunlight exposure. Stokes and Diffey (1999) exposed four sunscreens to natural sunlight and solar simulator radiation using roughened quartz and excised human epidermis substrates. They evaluated no significant difference between the results obtained for samples irradiated. However, Gonzales et al. (2007) by *in vitro* observations compared photostability of seven sunscreens having various SPF after sun and artificial exposures and showed that the degradation same of sunscreens is more pronounced when exposed to the sunlight than when the same amount of energy is irradiated from the UV-lamps. Moyal et al. (2002) described the *in vivo* photostability measurement of sunscreens upon sun exposure using diffuse reflectance spectroscopy of the skin with and without product. Stephens and Colon (2010) studied the impact of natural sunlight on the photoprotection performance of one SPF-50 sunscreen treaded on the back of 32 volunteers by skin erythema and pigment darkening. Not long ago Sayre and Dowdy (2010) published the interesting study about examination of solar simulators used for the determination of sunscreen UVA efficacy. They showed that the solar simulator commonly used for UVA sunscreen efficacy testing has insufficient UVA1 emission. Ergo sunscreens tested with different solar simulators should produce different efficacy labels; by authors this finding is concurs with the FDA's opinion.

In our previous published photostability study (Hojerová et al., 2007) the transmittance spectrum of eleven sunscreen products using roughened quartz substrates were investigated before and after irradiation with the xenon arc solar simulator. We confirmed that some UV filters commonly used in sunscreens are photoinstable and we also showed that a reproducible *in vitro* measurement of degradation rates is possible. However, in this study we performed the UVA efficacy and photostability of fifteen sunscreens having the same SPF applied onto polymethylmethacrylate (PMMA) plates against to outdoor natural sunlight exposure with a known erythemal dose-rate.

2. Materials and methods

2.1. Material tested

Fifteen commercial sunscreen emulsions (S1–15) currently available on the European market were investigated. The products were selected on the basis of equal presented value SPF 20, but with a different representation of photoactive substances, as indicated by the product's ingredients on theirs packaging (Table 1). Nine formulations contained only organic UV filters (2–4), five formulations had a combination of organic (2–5) and inorganic (1) filters, and one formulation contained only inorganic filters (2). The two most represented UV filters were ethylhexyl methoxycinnamate (in 13 sunscreens) and butyl methoxydibenzoylmethane (in 12 sunscreens).

2.2. Substrate and sunscreen products application

Although the *in vivo* measurement of the sunscreens' SPF is well globally established by the International Sun Protection Factor Test Method (COLIPA, 2006), several different *in vitro* methods have proposed to assess the efficacy of products against UVA radiation and their photostability (Osterwalder and Herzog, 2010).

Experimental analyses in this paper were carried out according to the COLIPA (the European Cosmetic Products Trade Association) method for *in vitro* determination of UVA protection (COLIPA, 2009) with minor modifications. The original COLIPA method is based on an assessment of the UV transmittance of a thin film (0.75 mg cm^{-2}) of sunscreen sample spread onto a roughened PMMA Plexiglas plate (50 mm × 50 mm) after exposure to a single UV dose of 1.2 times the initial UVA-PF (in J m^{-2}) from a defined UV source. By Ferrero et al. (2010) these roughened PMMA plates are preferred substrates in

Table 1
The product form and the photoactive compounds in investigated sunscreen products having the same label SPF 20.

Sunscreen	Product form	Photoactive compounds (in INCI nomenclature) mainly protection against	
		UVB (290–320 nm)/UVA2 (320–340 nm)	UVA1 (320–400 nm)/broad-spectrum
S1	Milk o/w	EHMC, PBSA	BMBM
S2	Milk w/o	EHMC, PBSA, OCR	BMBM
S3	Spray emulsion o/w	OCR, EHS, HMS	BMBM
S4	Soft cream o/w	EHMC	BEMT
S5	Milk o/w	EHMC, PBSA	BMBM
S6	Milk w/o	EHMC, PBSA, EHT	BMBM
S7	Baby lotion o/w	EHMC	BEMT, BMBM
S8	Soft cream o/w	EHMC, EHT	MBBT
S9	Soft cream o/w	EHMC, MBC	BMBM, BP-4
S10	Hard cream w/o	–	TiO_2, ZnO
S11	Milk w/o	EHMC, PBSA, OCR	BMBM, MBBT, TiO_2
S12	Spray emulsion o/w	EHMC	MBBT, TiO_2
S13	Soft cream o/w	EHMC, EHT	BMBM, BEMT, TiO_2
S14	Milk w/o	EHMC	BMBM, TiO_2
S15	Soft cream o/w	OCR, EHT	BMBM, TDSA, DTS, TiO_2

BEMT: bis-ethylhexyloxyphenol methoxyphenyl triazine (CAS 187393-00-6); BMBM: butyl methoxydibenzoylmethane (CAS 70356-09-1); BP-4: benzophenone-4 (CAS 4065-45-6); DTS: drometrizole trisiloxane (CAS 155633-54-8); EHMC: ethylhexyl methoxycinnamate (CAS 5466-77-3); EHS: ethylhexyl salicylate (CAS 118-60-5); EHT: ethylhexyl triazone (CAS 88122-99-0); HMS: homosalate (CAS 118-56-9); MBBT: methylene bis-benzotriazolyl tetramethylbutylphenol (CAS 103597-45-1); MBC: methylbenzylidene camphor (CAS 38102-62-4/36861-47-9); OCR: octocrylene (CAS 6197-30-4); PBSA: phenylbenzimidazole sulphonic acid (CAS 27503-81-7); TDSA: terephthalidene dicamphor sulfonic acid (CAS 90457-82-2); TiO_2: titanium dioxide (CAS 13463-67-7); ZnO: zinc oxide (CAS 8051-03-4); S1-15: sunscreen number; CAS: chemical abstracts service number; INCI: international nomenclature of cosmetic ingredients.

the most recent European publications or methods in the spectroscopic *in vitro* UVA and SPF assessment. Owing to the dimension of our spectrophotometer's cell-holders (10 mm × 45 mm × 10 mm), the original PMMA plates (50 mm × 50 mm × 2.5 mm) were cut to pieces (10 mm × 40 mm × 2.5 mm) using laser (Technical laboratory of Slovak University of Technology, Bratislava, Slovak Republic). Product in the form of several small drops was applied by a self-displacing pipette onto the roughened side of the PMMA plate (Schönberg GmbH, Munich, Germany, a roughness of approximately 2 μm) and uniformly distributed with a latex-gloved finger (which was not pre-saturated with sunscreen). To ensure the correct application rate 0.75 mg cm^{-2} ± 5%, the amount of sunscreen applied was adopted weighing the plate before and immediately after applying the product. Three plates were prepared for each product to be tested. The product film was allowed to dry in the dark under ambient conditions (22 ± 2 °C) for 15–30 min before exposure.

Garoli et al. (2009) showed, that the PMMA substrates are also suitable for photostability testing, but there is not clear optimum thickness of the layer of sunscreen onto these plates. SPF value stated on the package is determined by the irradiation of sunscreens applied on the human skin at 2 mg cm^{-2}. This quantity of product is the first condition for ensuring adequate photoprotection. However, it is known that consumers are usually applied to their skin less than 2 mg cm^{-2} but for staying safe in the sunlight they are calculated on the basis of the SPF value stated on the packaging. By Kim et al. (2010) in real life the amount of sunscreen applied is only on average 0.5 mg cm^{-2} (ranging from 0.39 to 0.79 mg cm^{-2}), independent of skin type. In this photostability assay, we used the same amount as in the determination of *in vitro* UVA (0.75 mg cm^{-2}), because we consider it more realistic than the amount of 2 mg cm^{-2}.

2.3. Spectrophotometric measurements

Transmission measurements of sunscreens S1–9 contained a combination only of organic UV filters (Table 1), were taken using a two-beam UV/Vis spectrophotometer Shimadzu UV-1800 without an integrating sphere (wavelength accuracy: ±0.3 nm, wavelength repeatability: less than ±0.1 nm, photometric repeatability: less than ±0.001 Abs (1 Abs), less than ±0.003 Abs (2.0 Abs). The UV spectra of sunscreens S10–15 contained inorganic UV filters, which scatter light, were recorded using a two-beam UV/VIS/NIR Spectrophotometer Shimadzu UV-3600 with UV/VIS integrating sphere (wavelength accuracy in UV/VIS region: ±0.2 nm, wavelength repeatability in UV/VIS region: less than ±0.08 nm, noise: 0.00005 Abs or less (500 nm). Both spectrophotometers were operated with UV-Probe PC software (Shimadzu, Kyoto, Japan).

PMMA plate covered with sunscreen was inserted vertically into the first cell-holder of the spectrophotometer. A UV transparent glycerine-treated PMMA plate, used to obtain the blank transmittance, was inserted vertically into the second cell holder. UV transmission measurements (from 290 to 400 nm at 1 nm intervals) were recorded on each plate at a five different positions per plate by soft hitch up and invert of its. The average UV transmission data at each wavelength T_λ was taken for the calculation. There did not register any product film streaming on the plate during the transmission measurements.

2.4. Sunlight exposure conditions

Every sunscreen product under study was exposed for the same time to a natural sunlight. The exposure was effectuated outdoors during a clear sunny day in late June, in Bratislava city; latitude 48°17′N, longitude 17°12′E, altitude 292 m asl (Pribullová and Chmelík, 2008). Maximum of the daily temperature was 33 °C. The sun exposure intensity was not measured on the experimental place immediately. It was assessed by two ways: 1. approximately a day before the experimental day according to the UV Index forecast; 2. more accurately after the experimental day, according to the precise readings of the Minimal Erythema Doses (MEDs) per hour during all exposure time. Both data were provided from the Slovak Hydrometeorological Institute (SHMU). The total UV energy (the exposure dose) over the experimental period was calculated from these data.

2.4.1. Sunlight exposure dose using the UV Index forecast

The UV Index is a unit-less quantity defined as the daily maximum of the dose-rate, i.e., the integral over wavelength of the solar spectral irradiance reaching the Earth's surface, weighted by the erythemal action spectrum (WHO, 2002). It is currently widely used in many operational weather forecasts for informing the general public about the UV radiation levels. The UV Index forecast for the experimental day by SHMU (SHMU, 2010a) was 7.8. The daily-UV

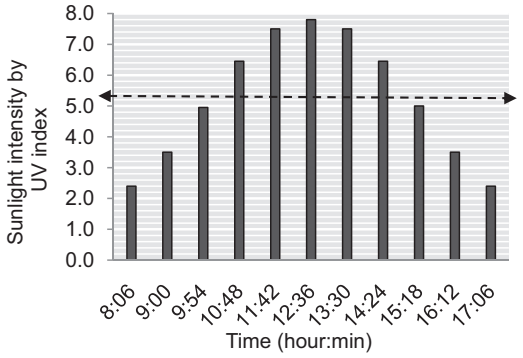

Fig. 1. Histograms displaying the sunlight intensity according to the forecast of a daily-UV Index-course for experimental day in late June in Bratislava, Slovak Republic. Data were obtained from the Slovak Hydrometeorological Institute (SHMU, 2010a). Time period of sunlight exposure was 8:00–17:30 h; UV Index maximal: 7.8. The dashed line shows the mean UV Index (value of 5.3) during the period of sunlight exposure.

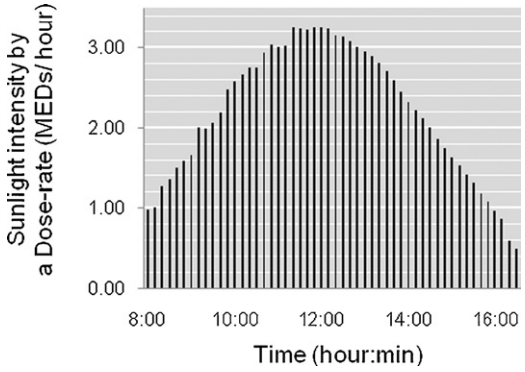

Fig. 2. Histograms displaying the sunlight intensity according to the dose-rate in the Minimal Erythema Doses (MEDs) per hour at intervals of 10 min during the period of sunlight exposure (8:00–17:30 h). Data were obtained from the Slovak Hydrometeorological Institute (SHMU, 2010b).

Index-course forecast for this day is showed in Fig. 1. According to these readings, regardless of layer thickness, we determined the time of exposure to sunlight, as consumers would be calculated according to SPF values indicated on the packaging of the product.

All forty five PMMA plates covered with 0.75 mg cm^{-2} of sunscreen (three for each product) were placed horizontally outdoors in direct sunlight in period of time 8:00 h and 17:30 h (i.e., 9.50 h of the summer-time) on the pad of black paper to avoid the multiple scattered and reflected rays. The mean UV Index throughout the exposure time was 5.3 (Fig. 1). An average daily exposure dose to the sun expressed by SEDs (Standard Erythema Doses) per hour, was 4.41 SEDs per hour (AFSSE et al., 2005), i.e. 41.90 SEDs (rounded 42 SEDs) per 9.50 h. By CIE (1998) one SED value is equivalent to eryhemal effective (Eeff) radiant exposure of 100 J m^{-2}. Based on this fact, we considered that the Eeff solar UV exposure dose reaching the surface of PMMA plates was about 4200 J m^{-2}. As the UVB radiation according to the CIE action spectrum is normalized at 297 nm and with the MED defined 210 J m^{-2} of EAS-weighted UV energy (CIE, 1998), we conjectured, that the sunscreens were exposed about 20 values of the MED. Naturally, when testing the sunscreen photostability should be considered that the dose of sunlight would answer the protective layer 2 mg cm^{-2} but not 0.75 mg cm^{-2} layer only, as has been used. After sunlight exposure the transmission measurements of each plate were taken alike as before.

2.4.2. Sunlight exposure dose using the precise readings of the erytemal dose-rate

The dose-rate is expressed in MEDs per hour EAS-weighted irradiance, where a MED is the amount of sun exposure which causes barely perceptible skin sunburn redness (erythema). For a MED scale factor of 1.0 (the base, or default, value) a dose-rate of 4.3 MEDs per hour is equivalent to an UV Index of 10. Stated another way, the base MED rate is 3/7 of the UV Index value (SHMU, 2010a).

The precise data of the Eeff sunlight dose-rate (in MEDs per hour) at intervals of one minute directly appointed in Bratislava during the experimental day were acquired from SHMU (2010b) for this research work purpose. The values of MEDs/h during the experimental period at intervals of ten minutes are displayed in Fig. 2. At 8:00 a.m. the dose rate was 0.982 MEDs/h, the maximum was 3.399 MEDs/h at 11:55, and at the end of exposure the dose-rate value was 0.260 MEDs/h. The mean of the dose-rate throughout exposure time was 2.027 MEDs/h. So, the total sun Eeff UV dose over the whole experimental period was 19.26 MEDs. It is clearly showed, that the Eeff sun exposure dose calculated according to UV Index forecast (MEDs value of 20) and according to dose-rate (MEDs value of 19.26) are the same nearly.

2.5. Study of UVA protection efficacy

In order to quantify and characterize the photoprotection properties of sunscreen products before and after sunlight exposure, two criteria by Ferrero et al. (2006) were considered: the first one were absolute indices, the second one were relative indices.

2.5.1. Absolute indices: SPF and UVA-PF

The most used absolute indices *in vitro* are two protection factors: the SPF and UVA-PF. We did not use the Colipa UVA *in vitro* method for the calculation of the UVA-PF using the labelled SPF. The values of the SPF and UVA-PF we calculated using a spectral irradiance of clear midday midsummer terrestrial sunlight for Southern Europe at 40°N (McKinlay and Diffey, 1987), because we did not obtain these readings for Central Europe, where Bratislava city is localized at 48°17′N.

Values of the *in vitro* SPF and UVA Protection Factor (UVA-PF) before and after sunlight exposure for each individual plate (SPF_i or UVA-PF_i) were derived from the mean UV transmission data at each wavelength with Eqs. (1) and (2) respectively. Final the *in vitro* SPF and UVA-PF values before and after exposure for each sunscreen under study were the mean three SPF_i or UVA-PF_i values respectively.

$$\text{In vitro SPF}_i = \frac{\int_{290}^{400} E_\lambda I_\lambda \, d\lambda}{\int_{290}^{400} E_\lambda I_\lambda T_\lambda \, d\lambda} \quad (1)$$

where E_λ: erythema action spectrum (CIE, 1998) at wavelength λ; I_λ: the spectral irradiance of sunlight expected for a clear sky at noon in midsummer for a latitude of 40°N (solar altitude 70°) at wavelength λ (McKinlay and Diffey, 1987); T_λ: measured transmittance of the sunscreen layer at wavelength λ; SPF_i: SPF of sunscreen on an individual PMMA plate calculated to comply with Eq. (2).

$$\text{UVA-PF}_i = \frac{\int_{320}^{400} P_\lambda I_\lambda \, d\lambda}{\int_{320}^{400} P_\lambda I_\lambda T_\lambda \, d\lambda} \quad (2)$$

where P_λ: PPD action spectrum (COLIPA, 2009) at wavelength λ; I_λ: the spectral irradiance of sunlight expected for a clear sky at noon in midsummer for a latitude of 40°N at wavelength λ (McKinlay and Diffey, 1987); T_λ: measured transmittance of the sunscreen layer at wavelength λ; UVA-PF_i: UVA-PF of sunscreen on an individual PMMA plate calculated to comply with Eq. (2).

The UVA-PF/SPF ratio before and after sunlight exposure for each sunscreen tested was calculated using the *in vivo* SPF (SPF label) and

final UVA-PF with Eq. (3):

$$\text{UVA-PF/SPF ratio} = \frac{\text{UVA-PF}}{\text{SPF}_{\text{label}}} \quad (3)$$

2.5.2. Relative indices

Relative indices are indicators that reflect a ratio of the UV absorbing efficacy of the sunscreen in some UV region thus eliminating the need for an absolute absorbance measure. However, the photoprotection calculated according to these indices is strictly based on the absorption properties of the product and includes neither a source spectrum nor a biological endpoint (action spectrum) (Dippe et al., 2005; Ferrero et al., 2006; Moyal, 2008, 2010). Regardless of this, a number of regulatory bodies or experts from the industry and academia have proposed various such relative measurements by *in vitro* methods. To assess the degree of UV protection of sunscreens against sun radiation in our experimental conditions the relative indices were calculated by four ways.

2.5.2.1. UVA-PF/SPF ratio and critical wavelength according to the European Commission. In order to ensure a related protection against UVB and UVA radiation, the EC (2006) recommended for all sunscreen products marketed in European Union a UVA-PF at least 1/3 of labelled SPF and a critical wavelength (CW) of at least 370 nm. Such sunscreen can be signposted by a UVA symbol: the letters "UVA" inside a circle (COLIPA, 2007). Consecutively in 2007, companies began progressively phasing in the UVA symbol on product packaging across Europe. The experimental details of the original COLIPA method (2007 and 2009 revised)COLIPA (2007), (2009) are described in Section 2.2 of this paper. To determine compliance of sunscreens tested in this study with EC recommendation, these two indices were assessed.

The CW for each individual plate covered with sunscreen (λc_i) before and after sunlight exposure was appointed using Eq. (4). For this purpose the average UV transmission data at each wavelength (T_λ) from three individual plates for each sunscreen were converted into absorbance values (A_λ). Final CW value before and after exposure for each sunscreen tested was the mean three λc_i values.

$$\int_{290}^{\lambda_{ci}} A_\lambda \, d\lambda = 0.9 \int_{290}^{400} A_\lambda \, d\lambda \quad (4)$$

where A_λ: monochromatic absorbance calculated from transmittance at wavelength λ; λc_i: the critical wavelength of sunscreen on an individual PMMA plate calculated to comply with the equation.

2.5.2.2. UVA/UVB absorbance ratio according to the Boots Star Rating system. Another *in vitro* approach to express UVA protection is the Boots Star Rating system (BOOTS, 2008), originally conceived by Diffey in 1991 (Diffey, 1994). It is mainly used for quantify the UVA protection performance of sunscreens in the United Kingdom. By this method PMMA plates covered with 1 mg cm^{-2} of sunscreen are used. The ratio of the mean UVA absorbance to the mean UVB absorbance is calculated for both before and after irradiation with a fixed dose of UV light of 17.5 J cm^{-2} using xenon arc solar simulator. According to UVA/UVB absorbance ratio, calculated with Eq. (6), sunscreens are allocated: *No star* (<0.60 before irradiation, <0.56 after irradiation); *Three stars* (>0.60 before, >0.57 after); *Four stars* (>0.80 before, >0.76 after); *Five stars* (>0.90 before, >0.86 after). In this study we estimated the UVA/UVB absorbance ratio before and after sunlight exposure under given experimental conditions.

$$\text{UVA/UVB ratio} = \frac{\int_{320}^{400} A_\lambda \, d\lambda / \int_{320}^{400} d\lambda}{\int_{290}^{320} A_\lambda \, d\lambda / \int_{290}^{320} d\lambda} \quad (5)$$

2.5.2.3. UVA1/UV absorbance ratio according to the FDA Proposed Ruling. According to the Food and Drug Administration proposal (FDA, 2007), the UVA protection must be determined using the *in vivo* PPD method. In addition for the broadness a ratio of the mean UVA1 absorbance to the mean the total UV absorbance (UVA1/UV ratio) was also proposed. By FDA ruling optical-grade quartz roughened plates covered with 2 mg cm^{-2} of sunscreen are used. UVA1/UV absorbance ratios are calculated after pre-irradiation with a UV dose specified as two-thirds of the SPF in MEDs with defined solar simulator. 1 MED in this method is 200 J m^{-2} of EAS-weighted UV energy. According UVA1/UV absorbance ratio sunscreens are allocated to one from four categories: *Low* (≥0.20); *Medium* (≥0.40); *High* (≥0.70); *Highest* (>0.95).

The UVA1/UV absorbance ratio for each sunscreen tested under conditions used in this study was calculated before and after sunlight exposure according to Eq. (6):

$$\text{UVA1/UV ratio} = \frac{\int_{340}^{400} A_\lambda \, d\lambda / \int_{340}^{400} d\lambda}{\int_{290}^{400} A_\lambda \, d\lambda / \int_{290}^{400} d\lambda} \quad (6)$$

2.5.2.4. SUI according to new proposal method by Diffey (2009). The Spectral Uniformity Index (SUI) is a new index for rating of UVA sunscreen protection that has been recently introduced by Diffey (2009). The principle of this *in vitro* method is based on closeness of fit between measured and flat spectral profiles. A SUI value according to the original Diffey method is derived from the spectral absorbance data after irradiation under defined conditions according to Eq. (7). Suggested rating by Diffey is: *Low* (<2); *Medium* (<5); *High* (<12); *Very high* (≥12).

This new proposal method by Diffey was used for assessment of the UVA efficacy of sunscreens tested under conditions in this study with Eq. (8):

$$\text{SUI} = \frac{\sum_{290}^{380} A_\lambda}{\sum_{290}^{380} \left| A_\lambda - \hat{A} \right|} \quad (7)$$

where A_λ: absorbance calculated from transmittance at wavelength λ; $\hat{A}$: the average absorbance across the spectral region 290–380 nm (Diffey, 2009).

Basically, all three last indices (UVA/UVB ratio, UVA1/UV ratio and SUI) are different mathematical manipulations of the same data set only (Diffey, 2009).

2.6. Study of photostability

In order to evaluate the sunscreens' photostability or photoinstability after sunlight exposure, two criteria by Garoli et al. (2008) were considered: the first one is based on the Area under curve Index, the second one is the residual percentage of two protection factors.

2.6.1. The Area under curve Index

The relative indices of the Area under curve (Auc) and the Area under curve Index (Auc-UV Index) were used as adopted by Gonzales et al. (2007). The Auc for the total UV spectrum (290–400 nm), Auc for UVB (290–320 nm), Auc over the entire UVA waveband (320–400 nm), Auc for UVA2 (320–340 nm) and Auc for UVA1 (340–400 nm) region was computed for each plate covered with sunscreen before and after sun exposure. The calculations used the following Eqs. (8)–(12):

$$\text{Auc-UV} = \int_{290}^{400} A_\lambda \, d\lambda \quad (8)$$

$$\text{Auc-UVB} = \frac{1}{2} A_{320} + \int_{290}^{319} A_\lambda \, d\lambda \quad (9)$$

$$\text{Auc-UVA} = \frac{1}{2}A_{320} + \int_{321}^{400} A_\lambda \, d\lambda \quad (10)$$

$$\text{Auc-UVA2} = \frac{1}{2}A_{320} + \frac{1}{2}A_{340} + \int_{321}^{339} A_\lambda \, d\lambda \quad (11)$$

$$\text{Auc-UVA1} = \frac{1}{2}A_{340} + \int_{341}^{400} A_\lambda \, d\lambda \quad (12)$$

Finally, to compare the photoprotection of each sunscreen before and after sun exposure, the Auc Index for the total UV and each region (UVB, UVA, UVA2 and UVA1) was calculated using Eq. (13):

$$\text{Auc Index} = \frac{\text{Auc}_{\text{after}}}{\text{Auc}_{\text{before}}} \quad (13)$$

Maier et al. (2005) used the difference between the spectral transmission before and after defined UV exposure as an indicator of photostability. In their study a sunscreen product was claimed photostable if the mean difference of transmission values was at least 95%. According to the drugs stability testing by Meunier (1981), Couteau et al. (2007) considered that a sunscreen product is stable when it preserves 90% of its effectiveness. By Gonzales et al. (2007) a sunscreen product was considered photostable if the Auc Index was ≥0.80. Adopting a last criterion, we indicated as photostable such a sunscreen which the Auc Index was at least 0.80.

2.6.2. The residual percentage of two protection factors

According to Garoli et al. (2008), a certain parameter of sunscreens' photostability can be a percentage variation of the *in vitro* SPF value after irradiation compared to the *in vitro* SPF value before. They considered as photostable such sunscreen which has percentage variation of SPF <20%. The same criterion we used in this study for SPF and very wilfully for the *in vitro* UVA-PF value, too.

Therefore, the sunscreen photostability (or lack thereof) under our experimental conditions was expressed as the percentage effectiveness after exposure of both protection factors: percentage of the SPF *in vitro* (% $\text{SPF}_{\text{eff.}}$) and percentage of the UVA-PF (% $\text{UVA-PF}_{\text{eff.}}$), calculated according to Eq. (14) and (15), respectively. Thus, we considered as photostable product with % $\text{SPF}_{\text{eff.}}$ and % $\text{UVA-PF}_{\text{eff.}}$ at least 80.

$$\%\text{SPF}_{\text{eff.}} = \frac{\text{in vitro SPF}_{\text{after}}}{\text{in vitro SPF}_{\text{before}}} \times 100 \quad (14)$$

$$\%\text{UVA} - \text{PF}_{\text{eff.}} = \frac{\text{UVA-PF}_{\text{after}}}{\text{UVA-PF}_{\text{before}}} \times 100 \quad (15)$$

3. Results and discussion

3.1. Photoprotection efficacy of sunscreens before exposure

The absorption profiles of sunscreens S1–15 before exposure represented by full line in Fig. 3 shows that sunscreens having the same SPF of 20 on the label have significantly different shape of the UV absorption spectra and therefore products do not provide the same level of UV protection. The values of two protection factors (*in vitro* SPF and UVA-PF) of all products before and after sunlight exposure are reported in Table 2. The *in vitro* SPF values before exposure were in the range from 15.4 ± 1.4 to 22.9 ± 3.0. However, the UVA-PF values before exposure showed a high degree of variation among sunscreens tested. Only seven sunscreens (S2, S3, S7, S8, S10, S13 and S15) were in compliance with a current standard on UVA protection by EC (2006). They are reported in bold characters in Table 2. These products have demonstrated the value of UVA-PF in the range from 7.2 to 13.1, which fulfilled the condition at least 1/3 of the SPF value (20) stated on the packaging (i.e., the value of UVA-PF 6.67).

But eight of the remaining products (S1, S4, S6, S9, S11, S12 and S14), did not reach even marginal value UVA-PF before radiation recommended by the European Commission (UVA-PF values ranged only from 3.3 to 6.5). These products provide photoprotection against UVB radiation in particular.

Comparison of the CW values to UVA-PF values before exposure (Table 2) confirmed earlier findings by some scientists (Forestier, 1999; Rudolph, 2004; Dippe et al., 2005; Moyal, 2008; Velasco et al., 2008), that the CW may well describe the width of the spectrum photoprotection, but is not suitable for measuring the intensity of protection.

Among the twelve sunscreens (bold numbers in Table 2), whose CW values before exposure were at least 370 nm, only seven products (listed above), met the minimum requirements for the UVA-PF value according to the European Commission. The problem with this method is that the value of CW is relying only on the shape of the absorption spectrum of ultraviolet radiation and not its amplitude. Therefore, two products with very different UVA protection, e.g. S14 (UVA-PF value of 5.8) and S13 (UVA-PF value of 13.1) exhibited nearly identical CW values of 372 nm or 373 nm, respectively.

3.2. Photoprotection efficacy of sunscreens after exposure

Four different *in vitro* indices for quantification of the UVA protection after sunlight exposure of sunscreens were compared. Three of them (UVA-PF/SPF ratio, UVA/UVB absorbance ratio and UVA1/UV absorbance ratio) were used according to three current methods (EC, 2006; BOOTS, 2008; FDA, 2007), respectively. One new relative indices (SUI) was used as proposed by Diffey (2009). All four tests are based on the measurement of UV transmittance through a sunscreen film applied to an artificial substrate with appropriate pre-irradiation of the sunscreen product. Regrettably, however, the conditions of methods are not the same. Since the analyses of sunscreens tested in this paper were carried out under given experimental conditions, a UV protection performance expressed by these four relative indices was afforded approximately only.

3.2.1. Indices by the European Commission recommendation

As can be observed in Table 3, among the fifteen sunscreens only five (S3, S8, S10, S13 and S15) were convenient according to the current UVA standards (EC, 2006) over the whole sun exposure. Given that UVA-PF/SPF ratio values, calculated according to Eq. (3), was at least 0.33 (ranging from 0.33 to 0.47) and the CW was at least 370 nm (ranging from 370 to 381 nm), these five sunscreens can be indicated on the packaging symbol of protection against UVA radiation (COLIPA, 2007, 2009).

The best efficacy against UVA radiation over the whole exposure time showed product S8 (UVA-PF/SPF ratio of 0.47 and CW value of 381 nm) with photoactive compounds ethylhexyl methoxycinnamate (EHMC), ethylhexyl triazone (eht) and methylene bis-benzotriazolyl tetramethylbutylphenol (MBBT). But the level of UVA protection provided by ten products (S1, S2, S4–S7, S9, S11, S12 and S14) was insufficient (UVA-PF/SPF ratios ranged from 0.10 to 0.24 and CW value ranged from 333 to 369 nm); these products therefore give a false feeling of safety. The poorest level of UVA protection during sunlight exposure exhibited sunscreen S6 (UVA-PF/SPF ratio of 0.10 and CW value of 333 nm) with photoactive compounds EHMC, phenylbenzimidazole sulphonic acid (PBSA), EHT and Butyl methoxydibenzoylmethane (BMBM).

3.2.2. Indices by the Boots Star Rating system

The same five products (S3, S8, S10, S13 and S15) those were convenient according to EC indices, showed compliance with the

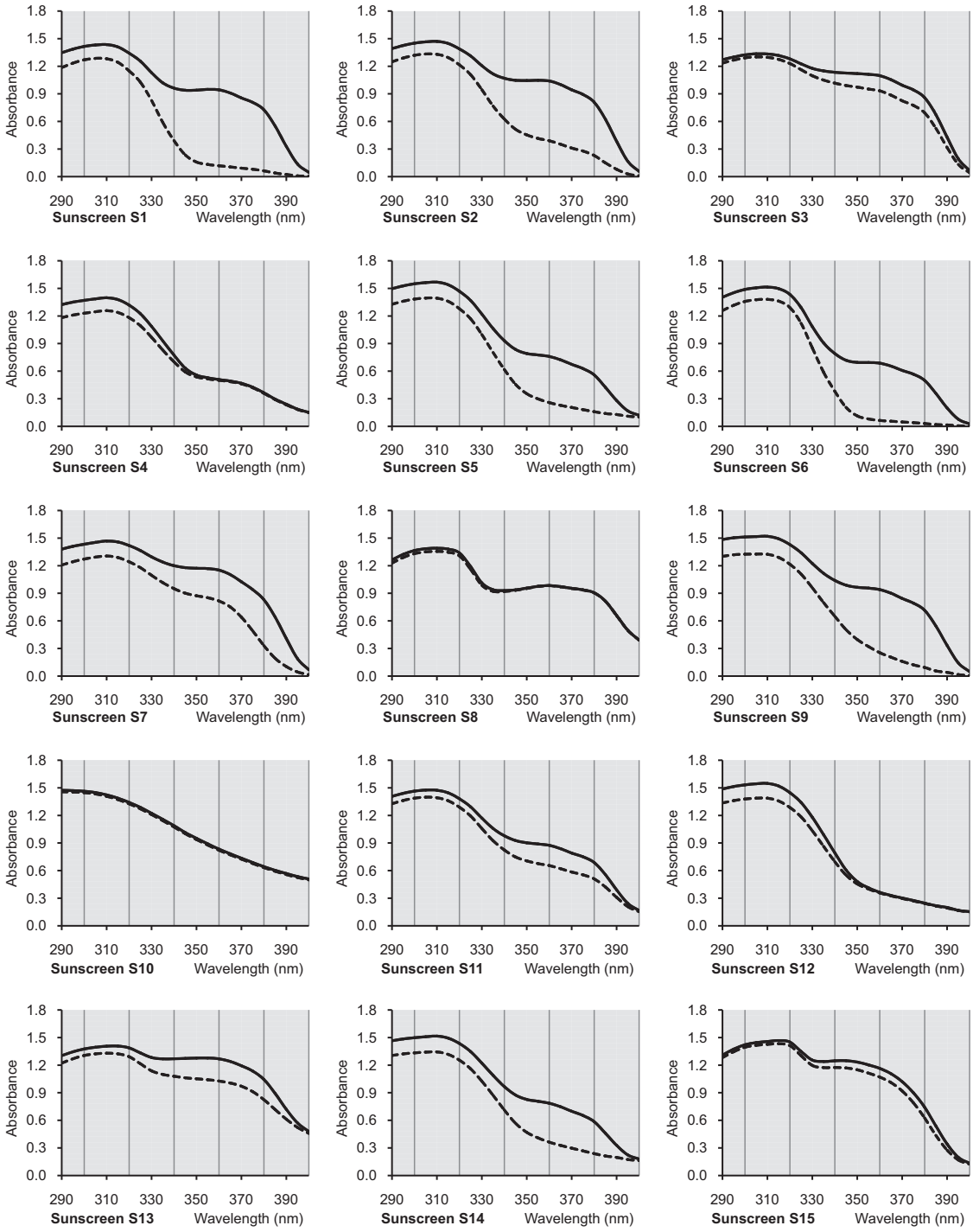

Fig. 3. Absorbance profiles of 0.75 mg cm^{-2} layer of sunscreens S1–15 having the same label SPF 20 applied to the PMMA plates roughness 2 μm. Full line: before sunlight exposure, dashed line: after sunlight exposure with erythemal effective dose of 42 SEDs approximately. The curves presented are the mean values resulting from three PMMA plates; the reproducibility of the values was found within 10%.

first UVA rating by BOOTS (2008). By their UVA/UVB absorbance ratios, calculated using Eq. (6), these five sunscreens could be label with three stars (Table 3). The best UVA protection during sunlight exposure offered product S13 (UVA/UVB ratio of 0.81 before and of 0.73 after exposure) with photoactive compounds EHMC, EHT, BMBM, bis-ethylhexyloxyphenol methoxyphenol methoxyphenyl triazine (BEMT) and titanium dioxide (TiO_2). However ten formulations did not fulfil Boots rating for any star. The poorest level of the UVA protection during exposure (UVA/UVB ratio before of 0.45, UVA/UVB ratio after of 0.22) exhibited sunscreen S6, as well as by the EC indices.

3.2.3. *Indices by the US FDA Proposed Ruling*

In contrast to above results, according to UVA1/UV absorbance ratio calculated with Eq. (7), except for one product (S6), all sunscreens manifested certain UVA protection level (Table 3). Sunscreen S1, S5 and S9 gave low UVA category. According to Garoli et al. (2008) and our opinion, the UVA1/UV absorbance ratios <0.2

Table 2
In vitro SPF, UVA-PF and critical wavelength of sunscreens having the same label SPF 20 before and after natural sunlight exposure with erythemal effective dose of 42 SEDs approximately.

Sunscreen	SPF label	*In vitro* SPF		UVA-PF		Critical wavelength (nm)	
		Before (mean ± S.D.)	After sunlight exposure (mean ±S.D.)	Before (mean ± S.D.)	After sunlight exposure (mean ±S.D.)	Before	After sunlight exposure
S1	20	19.6 ± 1.5	9.2 ± 0.9	6.2 ± 0.3	1.9 ± 0.3	**373**	337
S2	20	21.9 ± 1.8	12.3 ± 1.2	**7.2** ± 0.4	2.8 ± 0.3	**373**	358
S3	20	18.1 ± 1.2	15.9 ± 1.0	**7.7** ± 0.3	6.6 ± 0.6	**374**	**373**
S4	20	15.4 ± 1.4	12.4 ± 1.2	3.9 ± 0.2	3.7 ± 0.2	368	369
S5	20	22.4 ± 1.1	12.6 ± 1.3	5.4 ± 0.3	2.6 ± 0.2	369	354
S6	20	19.0 ± 1.4	9.8 ± 1.0	4.4 ± 0.4	1.8 ± 0.2	**367**	333
S7	20	22.9 ± 3.0	14.5 ± 1.4	**8.1** ± 0.3	4.3 ± 0.4	**373**	364
S8	20	21.2 ± 0.8	18.6 ± 1.1	**10.3** ± 0.6	**9.4** ± 0.4	**380**	**381**
S9	20	22.9 ± 1.1	11.3 ± 1.3	6.4 ± 0.3	2.4 ± 0.4	**372**	348
S10	20	21.2 ± 0.4	20.5 ± 0.7	**7.8** ± 0.3	**7.5** ± 0.4	**378**	**379**
S11	20	21.3 ± 1.1	16.7 ± 1.1	6.5 ± 0.5	4.8 ± 0.2	**375**	**372**
S12	20	17.0 ± 0.9	13.8 ± 1.0	3.3 ± 0.2	3.1 ± 0.2	360	363
S13	20	22.8 ± 1.1	18.3 ± 1.2	**13.1** ± 0.5	**9.2** ± 0.9	**373**	**370**
S14	20	21.5 ± 1.0	13.1 ± 1.3	5.8 ± 0.3	3.2 ± 0.1	**372**	364
S15	20	22.7 ± 1.2	20.6 ± 0.9	**8.0** ± 0.4	**6.9** ± 0.4	**373**	**371**

In vitro SPF value and UVA-PF value before and after exposure for each sunscreen tested is the mean three individual SPF_i values or UVA-PF_i values respectively for each PMMA plate covered with a sunscreen; S.D.: standard deviation. The bold numbers show when the UVA-PF value and the critical wavelength value satisfy the European Commission recommendation on the efficacy of sunscreen products (EC, 2006).

are too low for the claiming of the UVA protection efficacy. Sunscreen S2, S4, S7, S11, S12 and S14 exhibited medium UV category. The high category of the sunscreen S3, S8, S10, S13 and S15 corresponds to UVA claiming by EC indices and to three stars by Boots indices. No sunscreen exhibited the highest category of UVA protection.

3.2.4. Indices by the new Diffey rating proposed in 2009

According to criteria of this method, exactly all the fifteen sunscreens manifested some UVA rating exhibited as SUI (Eq. (8)), although S1, S5, S6, S9 and S12 only low rating (Table 3). In our opinion, the SUI value <2.0 for the lowest rating is to poor for the claiming of the UVA protection. In agreement with results obtained by EC, Boots and FDA indices, the poorest UVA efficacy after sunlight exposure showed sunscreen S6 (SUI value of 1.2). Sunscreen S2, S4, S7, S10, S11, S12 and S14 manifested medium UV rating that correlates strongly with FDA indices, except product S10. Sunscreen S3, S8, S13 and S15 showed high category alike according to FDA indices.

A good comparability was found between the FDA category (according to UVA1/UV absorbance rate) and new Diffey rating (according to SUI indices). In addition, three stars rating by Boots Star method corresponded with UVA claim by the EC rules and with high or sometimes medium category/rating by FDA and new Diffey ratings. These results mean that the different UVA protection indices can exhibit various data and be confusing for consumer. Therefore this is necessity to global harmonization of the determination of UVA protection.

3.3. Photostability of sunscreens after exposure

The changes in the absorbance profiles of sunscreens S1–15 after sunlight exposure compare to profiles before exposure are displayed in Fig. 2. As we mentioned in Section 2.4.1, exposed dose of sunlight radiation 42 SEDs, corresponds to protection level of SPF 20 only when the thickness of sunscreen layer is 2 mg cm^{-2}. Given that the layer of applied product 0.75 mg cm^{-2} was 2.6 times smaller than the recommended amount; all tested products were exposed to massive dose of UV radiation. But so it is unfortunately also often actually. It is likely that photoinstable products in the corresponding layer 2 mg cm^{-2} would show a smaller rate photoinstability. On the contrary, all products that withstand sunlight also under these drastic conditions, confirmed their photostability.

Table 3
Comparison of the UVA/UVB protection performance of sunscreen products having the same SPF 20 derived from the spectral absorbance data after sunlight exposure with erythemal effective dose of 42 SEDs approximately.

Sunscreen	European Commission[a] COLIPA Guideline 2009			Boots Star Rating[b]			U.S. FDA Proposed Ruling[c]		New Proposal by Diffey[d]		
	UVA-PF/SPF	CW (nm)		UVA/UVB ratio			UVA1/UV ratio		Spectral Uniformity Index SUI		
	After	After	UVA label	Before	After	Star	After	Category	Before	After	Rating
S1	0.10	337	No	0.58	0.24	No	0.20	Low	5.0	1.4	Low
S2	0.14	358	No	0.62	0.36	***	0.43	Medium	6.0	2.0	Medium
S3	0.33	373	Yes	0.70	0.63	***	0.75	High	10.3	6.5	High
S4	0.19	369	No	0.44	0.46	No	0.57	Medium	2.5	2.6	Medium
S5	0.13	354	No	0.49	0.33	No	0.37	Low	3.2	1.7	Low
S6	0.10	333	No	0.45	0.22	No	0.15	No	2.8	1.2	Low
S7	0.22	364	No	0.67	0.53	No	0.64	Medium	8.1	4.1	Medium
S8	0.47	381	Yes	0.67	0.68	***	0.83	High	6.1	6.5	High
S9	0.12	348	No	0.56	0.31	No	0.34	Low	4.5	1.7	Low

[a] Approximation by EC (2006).
[b] Approximation by Boots Ltd. (2008).
[c] Approximation by FDA (2007).
[d] Approximation by Diffey (2009).

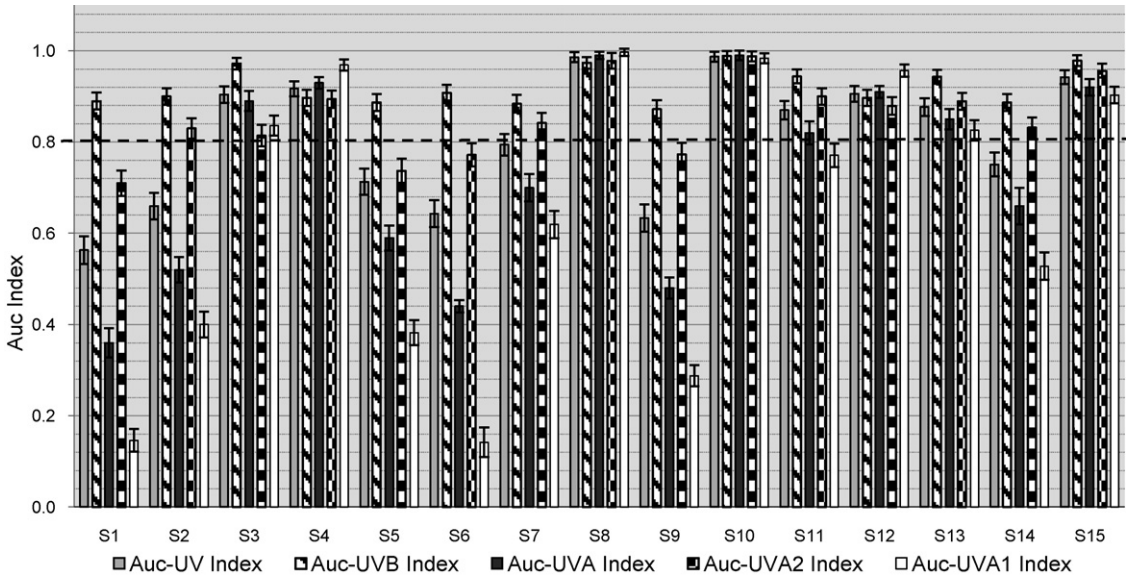

Fig. 4. Histograms displaying on the *y* axis the Area under curve Index (Auc Index) of sunscreens S1–15 having the same label SPF 20 after sunlight exposure with erythemal effective dose of 42 SEDs approximately. Auc-UV Index: the total UV range (290–400 nm), Auc-UVB Index: the UVB range (290–320 nm), Auc-UVA Index: over the whole UVA range (320–400 nm), Auc-UVA2 Index: the UVA2 range (320–340 nm), Auc-UVA1 Index: the UVA1 range (340–400 nm). The data presented are the mean three values and Standard Deviation. The dashed line shows where value Auc Index is ≥0.80; i.e. this sunscreen was considered as photostable in a given UV range.

3.3.1. Photostability by Area under curve Index

The sunscreens' photostability profiles were investigated in each UV region (in the total UV range, UVB, UVA, UVA2 and UVA1) according to Eqs. (8)–(12). The product was considered photostable in a given UV range, if the Auc Index was at least 0.80. Fig. 4 shows histograms of photostability or photoinstability in each from investigated UV ranges. As we can see UV Index values of sunscreens in the study were significantly different.

Seven sunscreens (S1, S2, S5–S7, S9 and S14) showed photoinstability in the total UV range (Auc-UV Index ranged from 0.56 to 0.79). All these photoinstable sunscreens contain the combination of EHMC and BMBM (Table 1). This result is in accordance with observations by Gaspar and Campos (2007). The absorbance profiles of these sunscreens before and after exposure (Fig. 3) as well as the Auc-UVA Index values (Fig. 4) clearly demonstrate, that the decrease of UV absorbance after exposure is evidently in the UVA range, probably due to loss in efficacy of BMBM, which is known to be photoinstable UVA filter. The mere combination of EHMC and BMBM, the two "workhorses" in UVB and UVA protection dominating the ranking of market shares in most countries (Damiani et al., 2007), has for long been reported to be spectroscopically instable if the specific stabilising molecules are absent (Sayre and Dowdy, 1999; Maier et al., 2001, 2005; Sayre et al., 2005; Dondi et al., 2006; Hojerová et al., 2007; Gonzales et al., 2007; Huong et al., 2008). Therefore, the organic UVB filters octocrylene (OCR) and methylbenzylidene camphor (MBC), and also UVA filter bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT), are used to stabilise the BMBM (Herzog and Sommer, 2000; Chatelain and Gabard, 2001; Moyal, 2008). In this study, the stability profiles in the total UV range (Fig. 4) of the sunscreens, which formulations have contained one or two of these stabilizers (Table 1), were sufficient (0.87 and 0.88) in sunscreens S11 (OCR) and S13 (BEMT), but insufficient (0.66 and 0.79) in sunscreens S2 (OCR) and S7 (BEMT).

It is noteworthy, that all fifteen sunscreens under this study were photostable in the UVB range (Auc-UVB Index ranged from 0.87 to 0.99), but seven products only (S3, S4, S8, S10, S12, S13, and S15) were photostable in both UVB and over the entire UVA waveband (320–400 nm). However, among these, solely one product (S13) contains a combination of EHMC and BMBM (also BEMT and other UV filters).

The behaviour of sunscreens clearly confirmed, that sunscreens' photoinstability is primarily a problem in the UVA1 region (340–400 nm). The worst photostability (Auc-UVA1 Index of 0.15 only) showed sunscreens S1 (EHMC, PBSA and BMBM) and S6 (EHMC, PBSA, EHT and BMBM). Another six products (S2, S5, S7, S9, S11 and S14) with insufficient photostability showed the Auc-UVA1 Index in the range from 0.29 (S9) to 0.62 (S7). By contrast, there were confirmed excellent UVA1 photostabilities of sunscreen S8 (EHMC, EHT and MBBT; Auc-UVA1 Index of 1.00), sunscreen S10 (TiO_2 and ZnO; Auc-UVA1 Index of 0.98), sunscreen S4 (EHMC and BEMT; Auc-UVA1 Index of 0.97), S12 (EHMC, MBBT and TiO_2; Auc-UVA1 Index of 0.96) and sunscreen S15 (OCR, EHT, BMBM, TDSA, DTS and TiO_2; Auc-UVA1 Index of 0.93). The fact that these products be photostable withstands even a thin layer for the enormous exposure to sunlight, is proof of their quality.

3.3.2. Photostability by the protection factors change

The values of the *in vitro* SPF or UVA-PF before and after sunlight exposure are shown in the Table 2. A loss of sunscreen's photoprotection potential was expressed as the Residual effectiveness after exposure (%) of both protection factors: the *in vitro* SPF (% $SPF_{eff.}$) and UVA-PF (% UVA-$PF_{eff.}$), calculated according to Eqs. (14) and (15) respectively. A sunscreen was considered photostable in the UV wavelength range to that protection factor (PF) is confined, when it preserved after sunlight exposure at least 80% of its PF value before exposure. The SPF is largely confined to the UVB (290–320 nm) and partially to the short-wavelength UVA2 (320–340 nm) and UVA-PF to the entire UVA waveband (320–400 nm).

The results displayed in Fig. 5 showed, that the % $SPF_{eff.}$ as well as the % UVA-$PF_{eff.}$, varied considerably. Excellent correlations were observed to compare % $SPF_{eff.}$ (Fig. 5) to the Auc-UVB Index and Auc-UVA2 Index (Fig. 4). Just the seven products that were photostable in both UVB and UVA2 regions (S3, S4, S8, S10, S12, S13, and S15) showed residual % $SPF_{eff.}$ at least 80; ranging from 80.3% (S13) to 96.7% (S10). Eighth product (S11), which was stable by Area under curve Index in the UVB and UVA2 region, showed almost satisfactory residual % $SPF_{eff.}$ of 78.4. Seven remaining sunscreens lost their SPF value; % $SPF_{eff.}$ ranging from 46.9% (S1) to 63.3% (S7). All

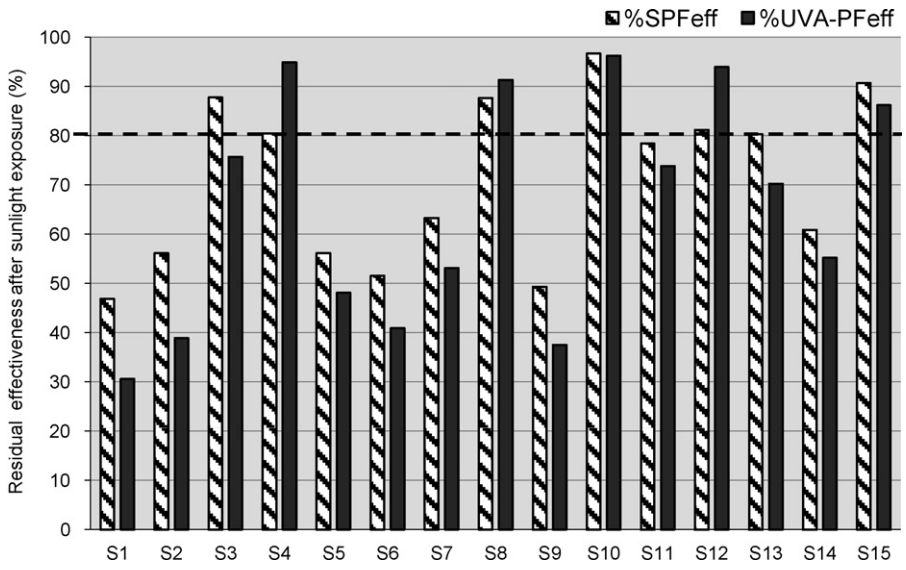

Fig. 5. Histograms displaying on the *y* axis the residual effectiveness of *in vitro* SPF (% $SPF_{eff.}$) and UVA-PF (% $UVA\text{-}PF_{eff.}$) of sunscreens S1–15 having the same label SPF 20 after sunlight exposure with erythemal effective dose of 42 SEDs approximately.
The dashed line shows where the % $SPF_{eff.}$ or % $UVA\text{-}PF_{eff.}$ is ≥0.80. This sunscreen was considered as photostabile in the UV range to that protection factor is confined; i.e. SPF parameter largely to the UVB (290–320 nm) and partially to the short-wavelength UVA (320–340 nm) and UVA-PF parameter over the whole UVA (320–400 nm) range. See Section 2.6.2 of this paper.

of them contained the combination of EHMC and BMBM (Table 1), uniformly to evaluation by the Auc-UV Index.

Merely five products (S4, S8, S10, S12 and S15) were preserved their $UVA\text{-}PF_{eff.}$ at least 80%; ranging from 86.2% (S15) to 96.2% (S10). The other two sunscreens (S3 and S13), which were stable by Area under curve Index in the UVA1 region, showed almost satisfactory % $UVA\text{-}PF_{eff.}$ of 75.7 and 70.2 respectively.

The most significant changes both indices showed sunscreens S1; % $SPF_{eff.}$ of 46.9 and % $UVA\text{-}PF_{eff.}$ of 30.6. This finding means, that approximately 53% of protection efficacy against the UVB and short wave UVA radiation as well as nearly 70% of the protection efficacy against the whole UVA radiation were lost during sunlight exposure. Comparison of the residual effectiveness of *in vitro* SPF and UVA-PF values with the Auc-Index showed that methods give a similar ranking of the sunscreens' photostability.

4. Conclusions

Medical and commercial interest in the effects of UVA radiation on skin has stimulated efforts to quantify and characterize the efficacy of sunscreen products in the broad-spectrum. However, for an appropriate protection against the UV sun radiation modern sunscreens should also maintain this effectiveness during the entire period of exposure to the sun implied by the value of the SPF. Accordingly, it is relevant to query whether sunscreen is photostable when subjected to outdoor sunlight radiation.

To assess the UVA protection effectiveness and photostability upon sun exposure of fifteen sunscreens this work used several *in vitro* UVA indices and three photostability indices, all based on substrate spectrophotometry. The results showed that the products having the same SPF on the label showed a wide variety of UVA protection level. Among fifteen products, three sunscreens only exhibited a complying UVA protection efficacy according to each UVA absolute and relative indices evaluated. Two pairs from the four *in vitro* tests used for assessing UVA protection gave results that were comparable each other. Three stars rating by Boots system (UVA/UVB ratio) were approximately in accordance to UVA claim by the EC rules (UVA-PF/SPF ratio). Similarly, a good comparability was found between the FDA category (UVA1/UV ratio) and new Diffey rating (SUI). In addition, three stars rating by Boots system was in accordance to high or sometimes medium category/rating by FDA proposed rules and new Diffey rating. It is necessary to state that this correspondence between the different calculation modes is only given when the transmission data are from the same measurement. These findings confirmed that the different UVA protection indices can exhibit various data, and so different UVA labelling. Therefore there is in particular a need for a global uniform claim on UVA protection in order to facilitate the choice of the consumer for a product protecting against both UVB and UVA radiation.

Although during the photostability study sunscreens were subjected too strong sunlight exposure time, among fifteen sunscreens, eight sunscreens appeared to be stable in all UV regions investigated and showed broad photostability. The same products exhibited also a complying UVA protection efficacy according to each absolute and relative indices used. All remaining sunscreen products showed photoinstability in some UV range upon sunlight exposure. These results emphasize a fact that evaluation of photostability is very important to guarantee the efficacy of a sunscreen. However, for the consumer is very difficult to choose the appropriate product, since the photostability varies between different brands and, moreover, mostly is not indicated on the bottle. The observations concerning the sunscreens clearly showed that their performance measurement and photoprotection label are still far from perfect.

Competing interests

The authors declare that they have no competing interests.

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