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DETERMINATION OF SUNSCREEN UVA PHOTOPROTECTION

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ABSTRACT
The level of sun protection of cosmetic products has been traditionally expressed as sun protection factor (SPF) defined as the ratio of the minimal erythemal dose on product protected skin to the minimal erythemal dose on unprotected skin. The SPF value is primarily affected by UVB radiation and may not be a significant indicator of protection against UVA radiation. Since harmful effects of UVA have been demonstrated recently, there are growing demand for the method of measuring the level of protection against UVA (UVA Protection factor, UVA-PF). The aim of this study was to verify the procedure described in the Draft ISO Standard (ISO/WD 24443 on Determination of Sunscreen UVA Photoprotection In vitro) and to compare results obtained in vitro with the outcome of SPF and UVA-PF determination in vivo. When selecting samples, particular attention was paid to formulations with problematical mode of application. The results confirmed acceptable agreement between the values obtained in vitro and in vivo, especially for samples with SPF lower than 20.
Keywords: Sunscreen, SPF, UVA-PF, UV filters

1. INTRODUCTION
The level of sun protection has traditionally been estimated using the sun protection factor or SPF test, which utilises the erythemal response of the skin to ultraviolet (UV) radiation. Since the SPF number is influenced primarily by UVB wavelengths, however, it is not necessarily a sufficient indicator of a sunscreen product's protection against UVA exposure [1].
In recent years, the harmful effects of the UVA wavelengths of sunlight have been more thoroughly established. Its contribution to the induction of erythema is about 15% of the corresponding effect of the overall solar UV radiation [2]. It was also shown recently that UVA radiation leads to increased levels of the p53 gene product in human skin, which is an indicator for mutations and, therefore, for risk of skin cancer [3]. With this understanding arose the need, not only for sun protection products that were effective against UVA wavelengths, but also for a common test method for measuring UVA protection levels.
In their Recommendation of 22nd September 2006, on the efficacy of sunscreen products and the claims made relating thereto (2006/247/EC), the European Commission included a requirement for UVA protection comprising both (a) a UVA-PF of 1/3 the SPF as determined by the *in vivo* PPD

(persistent pigment darkening) method or an equivalent degree of protection obtained by any *in vitro* method and (b) an *in vitro* Critical Wavelength value of greater than 370 nm, in order to satisfy requirements for broad-spectrum UVB / UVA protection and associated labeling. The critical wavelength λc value for the test product is defined as that wavelength where the area under the absorbance spectrum for the irradiated product (obtained using the method described below) from 290 nm to λc is 90% of the integral of the absorbance spectrum from 290 nm to 400 nm.
In vitro method for the determination of UVA-PF is based on an assessment of UV transmittance of a thin film of sunscreen sample spread on a roughened substrate after exposure to a control dose of UV radiation from a defined UV source [4]. Due to the current lack of inter-laboratory reproducibility of absolute *in vitro* UV measurements, each set of sunscreen transmission data is adjusted by first converting to absorption data (before and after UV exposure) and then by multiplying by a correction coefficient. This coefficient is determined iteratively from the non-exposed sample's absorbance data to provide a calculated *in vitro* SPF value equal to the labelled (*in vivo*) SPF.
The sunscreen sample is exposed to an irradiation dose proportional to an initial UVA protection factor UVA-PF_0, calculated from the corrected absorbance data of the nonexposed sample. Both the final *in vitro* UVA-PF and *in vitro* Critical Wavelength value, λc, are calculated from the absorbance data of the UV exposed sample [5,6].

2.METHODS

Sunscreen products were applied to the roughened polymethylmethacrylate (PMMA) plates as a large number of droplets of approximately equal volume and spread immediately over the whole surface. Spreading was completed in a two phase process comprising of quick distribution of the sample without pressure and rubbing into the rough plate surface using pressure. Applied samples were subsequently equilibrated for at least 15 min in the dark to facilitate formation of standard stabilised product film. The refference plate was prepared using glycerine.
The transmittance was measured using the spectrophotometer UV-VIS Cary 100 (Varian, Australia) with the wavelenght range 290-400 nm with an increment wavelenght step of 1 nm. The area of each measurement was 0.5 cm and the dynamic range was 2.2 absorbance units. The dose of UV delivered during one measurement cycle did not exceed 0.2 J/cm^2.
The general procedure consisted of several steps. At first, the transmission of the sunscreen product spread on the PMMA plate was measured prior to UV irradiation and initial UV spectrum was mathematicaly adjusted using coefficient of adjustment *Co* to achieve an *in vitro* SPF equal to the labelled SPF (*in vivo*). It is recommended that *Co* falls within a range between 0.8 and 1.2. The values of $UVAPF_0$ and a single UV dose *D* were calculated according to the COLIPA guideline [6]. Then the sample was exposed to UV irradiation according to the calculated UV dose *D* and the transmission of the sunscreen product was measured after UV exposure. These data were also adjusted according to the same coefficient *Co* and *in vitro* UVA protection factor (UVA-PF) after irradiation was calculated.
In Tab. 1. all samples of sunscreen products are listed and characterized by SPF labelled by the manufacturer, the type of emulsion and UV filters present in each product.

Table 1. Characterization of sunscreen product samples

sample code	SPF labelled by manufacturer	emulsion type	UV filtres
V1	15	lotion	EHMC, MBBT, BEMT
V2	15	spray	PBSA, EHMC, EHT, MBBT, DHHB
V3	20	lotion	TDSA, BMDBM, OCR, EHT, DTS, TiO2
V4	20	aerosol	PBSA, BMDBM, EHMC, EHT, BEMT, TiO2
V5	30	crem	TiO2
V6	30	crem	EHMC, MBBT, BEMT, TiO2
V7	50+	crem	TDSA, BMDBM, OCR, DTS, TiO2
V8	50+	lotion	HMS, B3, BMDBM, OCR, EHS

HMS - Homosalate, **B3** - Benzophenone-3, **PBSA** - Phenylbenzimidazole Sulfonic Acid, **TDSA** - Terephtalidene Dicamphor Sulfonic Acid, **BMDBM** - Butyl Methoxy-dibenzouylmethane, **OCR** - Octocrylene, **EHMC** - Ethylhexyl Methoxycinnammate, **EHT** - Ethylhexyl Triazone, **DTS** - Drometrizole Trisiloxane, **EHS** - Ethylhexyl Salicylate, **MBBT** - Methylen bis-benzotriazolyl Tetramethylbutylphenol, **BEMT** - Bis-Ethylhexyloxyphenol Methoxyphenyl Ttiazine, **DHHB** - Diethylamino Hydroxybenzoyl Hexyl Benzoane

3. RESULTS

Results of SPF and UVA-PF measuring by *in vitro* method are shown in Tab. 2 along with the corresponding SPF and UVA-PF values acquired *in vivo* by a PPD (persistent pigment darkening method). In most samples a realtively good correlation between the *in vivo* and *in vitro* SPF values was achieved. In UVA-PF values a good agreement was found in V1, V2 and V3 samples, in V5, V6 and V8 the differences did not exceed 25%.The highest deviation was found in sample V7. In V4 the value of UVA-PF *in vitro* was considerably higher in comparison with *in vivo* UVA-PF value, which was probably due to aerosol form of the sample.

Table 2. Sun protection factors and UVA protection factors of studied sunscreen products

sample code	SPF labelled by manufacturer	SPF *in vivo*	SPF *in vitro*	UVA-PF *in vivo*	UVA-PF *in vitro*
V1	15	19,1	17,3	5,2	4,5
V2	15	17,3	20,2	5,8	5,6
V3	20	24,1	23,8	8,0	7,9
V4	20	24,1	26,3	7,5	12,9
V5	30	38,0	37,7	12,2	15,5
V6	30	34,1	34,4	9,2	7,1
V7	50+	60,4	46,9	22,3	12,5
V8	50+	64,3	63,0	22,2	27,3

All sunscreen samples were also evaluated for their critical wavelenght λc before UV exposure and after UV exposure. This value did not change significantly after UV exposure. Sunscreen product providing a protection against UVA radiation should have the critical wavelenght value λc greater than 370 nm. The higher this value is, the better protection against UVA is reached. All sunscreen product samples used in the study met this requirement.

Table 3. Coefficient of adjustment, critucal wavelenght and UVA/UVB ratio of studied sunscreen products

sample code	SPF labelled by manufacturer	Co	λc (static)	λc (Dx)	UVA/UVB ratio	
					without irradiation	after irradiation
V1	15	-1,040	372	373	0,51	0,54
V2	15	-0,939	372	372	0,56	0,58
V3	20	-0,978	378	375	0,73	0,66
V4	20	-0,974	377	377	0,75	0,75
V5	30	-1,004	379	379	0,75	0,73
V6	30	-1,001	373	374	0,53	0,54
V7	50+	-1,072	378	379	0,61	0,65
V8	50+	-1,020	381	381	0,82	0,79

Co...coefficient of adjustment, **λc (static)**…critical awvelenght before exposure, **λc (Dx)**…critical wavelenght after exposure

4. CONCLUSION

The aim of this study was to verify the procedure described in the Draft ISO Standard (ISO/WD 24443 on Determination of Sunscreen UVA Photoprotection In vitro) and to compare results obtained by *in vitro* method with the outcome of SPF and UVA-PF determination *in vivo*.

An agreement on acceptable level in UVA-PF values was achieved especially for the samples with SPF lower than 20. Regarding the SPF values determined both *in vivo* and *in vitro*, relatively good correlation was observed in all tested samples except for sample V7, which had 50+ SPF labelled by the manufacturer.

When selecting samples, particular attention was paid to formulations with problematical mode of application. These samples include the products in aerolos or spray form (samples V2 and V4). Although the treatment, preparation and application of these samples were more complicated, the values obtained *in vitro* are in accordance with those of *in vivo* measuring.

5. ACKNOWLEDGEMENT
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