

In-vitro testing to assess the UVA protection performance of sun care products

Members of the DGK Task Force “Sun Protection”

Keywords

UVA protection, in-vitro test method, sunscreen, *Critical Wavelength*, Broad Spectrum Rating

Synopsis

The UVA protection of sunscreens is an issue of raising importance due to the increasing knowledge about the UVA-induced skin damage. In Europe there is no officially accepted method available to determine the degree of UVA protection. Therefore the DGK (German Society of Cosmetic Chemistry) task force “Sun Protection” investigated the applicability of a method which was originally described by B.L. Diffey in 1994 [1]. The UVA protection is calculated as Critical Wavelength or as UVA/UVB-Ratio. In contrast to other approaches, these indices define the UVA protection in relation to the UVB protection. As relative parameters they indicate the broadness of the UV protection.

Six laboratories participated in these investigations. Several improvements or harmonizations concerning instrumentation and sample application were necessary to get after all very good reproducibility. In order to simulate the outdoor situation and to cover possible light induced changes of the sunscreen a *irradiation* step was integrated resulting in a slightly increased standard deviation in the data from different laboratories. The sensitivity of the method has been found to be sufficient to clearly differentiate between sunscreens providing high and low UVA protection. In general, we found a suitable method, which should also be considered in the official discussion about the determination of the UVA protection in the future.

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Introduction

Overexposure to sunlight, particularly to UV rays, has harmful effects on human skin. A number of recent studies showed, that in addition to the most damaging UVB irradiation (290-320nm) also UVA irradiation (320-400nm) causes skin damage [2,3,4]. Alterations occur on the biochemical, the cellular and on the functional level. The effects of UVA are mainly mediated by free radicals, e.g. reactive oxygen species. The visible signs are often the result of long-term, accumulative reactions. UVA induced premature skin aging is a process of many years. Sunscreens should protect the skin against the effects of both, short-wavelength UVB and long-wavelength UVA. The sun protection factor (SPF), which is measured according to the *COLIPA standard protocol* (1995) [5] indicates the efficacy of sunburn or UVB protection but does not adequately cover the UVA part of the light. Up to now several *in-vivo* methods have been described, the most prominent are immediate pigment darkening (IPD) and persistent pigment darkening (PPD) [6,7].

They all have in common that they rely on biological endpoints. The concern is justified, how relevant are these endpoints in view of the protection against cumulative, long-term skin damage?

The action spectra for such damages are not known yet and the real endpoints are reached only after many years of solar exposure, which means not accessible for testing. Therefore, all existing *in-vivo* tests have to be considered as substitutes for the real situation. On the other hand, all these *in-vivo* models do take into account some photochemical changes of the product during the test exposure as it is the case with the well established SPF determination [8,9].

The question may then be raised : Is it possible to design an *in-vitro* method, which is easy to handle (no animals, no volunteers), more reproducible than a biological (statistical) endpoint and able to account for photochemical during exposure.

In this paper, an approach is proposed and described, including results of a ringtest, which consists of the following:

- *sample preparation* = application of a thin layer of the test product on a suitable and UV-transparent support.
- *sample exposure* to a defined dose of simulated sunlight
- *transmission measurement* of the (pre-)irradiated sample

The resulting *transmission profile* reflects the efficacy of the test product in the entire spectral range and can be interpreted in terms of UVA protection *during use* by:

- *Critical Wavelength* (λ_c) [1]
- UVA/UVB ratio (Star Rating) [10]

Materials and methods

Transmission measurement devices

The technical descriptions of the measurement devices for each participating lab are given in (*Table I*). There are different instrument suppliers and correspondingly there are differences concerning the pathway of the UV-rays and the irradiation of the sample during the measurement. As schematically depicted in *Fig. 1* when, using single beam instruments, the sample is exposed to the polychromatic light of a cooled xenon arc lamp. The spectrum of the light is adapted to sunlight by an appropriate filter. After passing the sample, the light is separated into distinct wavelengths by a monochromator and analyzed by a photomultiplier. Dual beam spectrometers (*Fig. 2*) are equipped with two light sources, a Deuterium-lamp for the UVB-region and a tungsten-halogen-lamp for UVA light. The light passes first through a double monochromator. A beamsplitter and a chopper leads the monochromatic beam either through the sample or through the reference. Monochromatic irradiation avoids possible artificial heat effects.

In all labs, spectrometers equipped with a light collecting integration sphere were used as required for measuring light-scattering films accurately. The sample holders are designed for minimal distance between sample surface and the diffuse reflecting inner surface of the sphere. The significant loss of light intensity due to the integrating device is compensated by the use of a highly sensitive detector system.

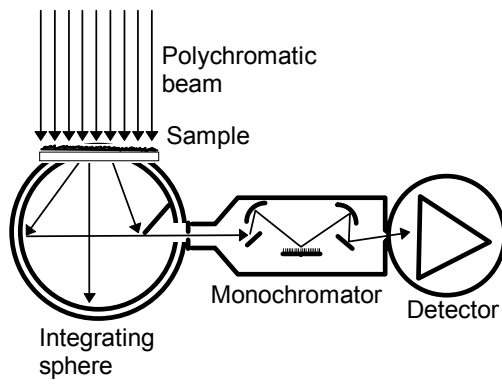


Figure 1. Polychromatic measurement

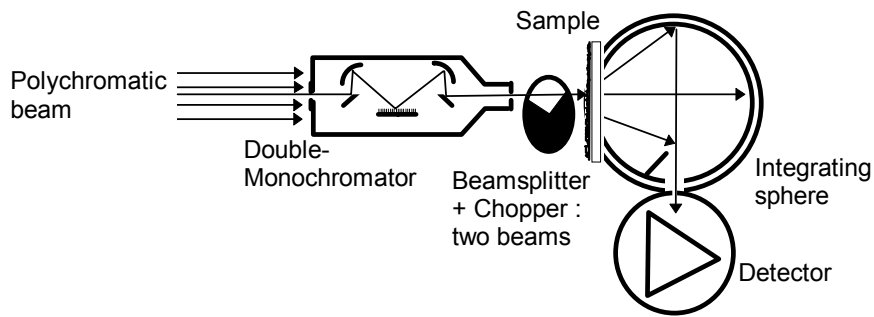


Figure 2. Monochromatic measurement

Laboratory Code	Instrument / Type	kind of beam	light source	Integrating device	detection system
LAB1	Optometrics SPF290S	single beam, polychromatic	Xenon arc 125W	Integrating sphere	monochromator + photomultiplier
LAB2	Varian, Cary 3	double beam, monochromatic	Deuterium D2 / Halogen H1	Integrating sphere	double - monochromator before sample + photomultiplier
LAB3	Optometrics SPF290	single beam, polychromatic	Xenon arc 125W	Integrating sphere and quartz diffuser	monochromator + photomultiplier
LAB4	Optometrics SPF290	single beam, polychromatic	Xenon arc 125W	Integrating sphere	monochromator + photomultiplier
LAB5	Perkin Elmer, Lambda 16	double beam, monochromatic	Deuterium D2 / Halogen	Integrating sphere	double-monochromator before sample + photomultiplier
LAB6	Dr. Kockott	single beam, polychromatic	Xenon arc 150W	Integrating sphere or dispersing disc	grating , spectral detector or integral detector with sensitivity adjusted to erythral action spectrum

Table I. Measurement devices

Irradiation devices

All labs, except lab 6, carried out the *irradiation* of the samples with a Heraeus Suntester (Atlas, Material Testing Technology B.V., Germany). The suntester is equipped with a horizontal Xenon arc lamp and a parabolically formed closed chamber for irradiation. The bottom plate of this room is a water cooled sample tray. An additional air cooling system is integrated.

To obtain an emission spectrum that is very similar to real sun, the light source is filtered by a defined glass filter. The supplier of the instrument recently developed a new filter called "solar standard"-filter, transforming the emission spectrum similar to the sun spectrum [11]. All labs were provided with such a filter immediately before starting the measurements. The suntester is also equipped routinely with an infrared filter to avoid heating of the samples within the chamber. An example for the Heraeus suntester spectrum compared to the standard sun spectrum is given in *Fig. 3*.

The measurement device in laboratory 6 is constructed in a specific way: The sample is irradiated with the same sun-like emission spectrum used for the transmission

measurements. The response of the detector over the wavelength range has been adjusted to the erythemal effectiveness of human skin by a spectroradiometric alignment. Hence the detector readings give erythemal equivalent values. To ensure equal UV doses in all laboratories, single irradiation devices were recorded in the wavelength band from 290 to 400nm by one spectroradiometer (MSS 2040-UV, Dr. Kockott, UV-Technik, Hanau, Germany). Based on this standardized measurement, the individual irradiation time for 1MED was calculated for each laboratory.

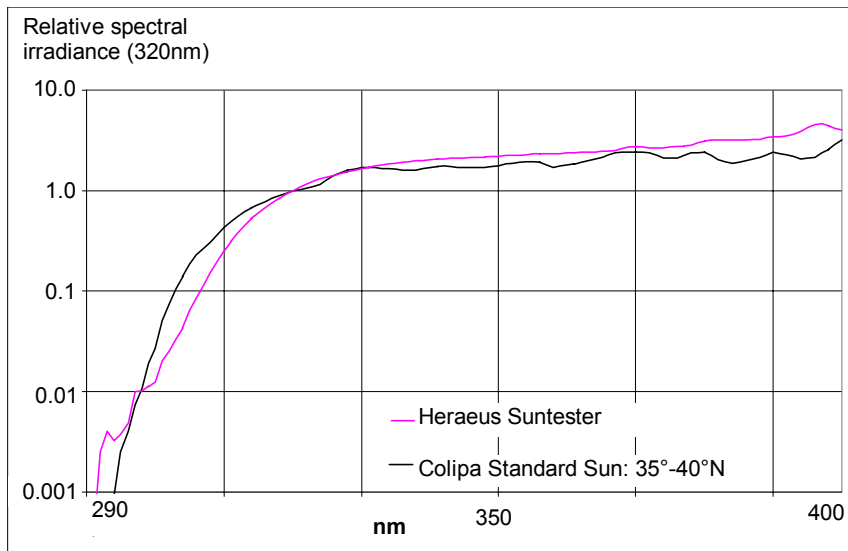


Figure 3. Relative spectral irradiance of the Heraeus Suntester CPS+(320nm)

Sunscreens

Four sunscreens were chosen for this investigation, covering most of the sun protection range common to European consumers. Standard P1 contains only an UVB filter, the other formulations contain at least one organic UVA filter, and one sample (OTC2) contains also micropigments. For more details see table II.

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P1 ist keine repräsentative Probe des europäischen Marktes

Sample	Labeled SPF	Type of emulsion	UV-filter system
Standard P1, DIN Std. K17N	4	O/W	2.7% Octyl Methoxycinnamate
Standard P3 Colipa Standard C202/101	15	O/W	3.0% Octyl Methoxycinnamate 2.8% Phenylbenzimidazole Sulfonic Acid 0.5% Butyl Methoxydibenzoylmethane
OTC1	20	O/W	5.5% Octyl Methoxycinnamate 3.0% Phenylbenzimidazole Sulfonic Acid 2.5% Butyl Methoxydibenzoylmethane
OTC2	30	O/W	4.1% Titanium dioxide 4.0% Methylbenzylidene Camphor 2.0% Butyl Methoxydibenzoylmethane 0.5% Terephthalylidene dicamphorsulfonic acid

Table II. Sunscreens

Substrates

Roughened quartz plates were produced individually by the following procedure: On a smooth and hard surface, i.e. a glass pane, a spoonful of grinding powder (Carborundum (SiC), quality: green, grain: FEPA 100) is mixed with a few milliliters of water. This paste will be crossed out on the pane. The surface of a quartz plate is moved with gentle pressure on this paste layer, in form of an eight, to achieve a regular roughening pattern. This grinding should be continued until the surface looks completely frosted. The grinding material is then flushed with water from the roughened plate. After drying the transmission at 400nm will be determined to control of the roughening process.

Roughened quartz plates could be used at least five times without repeating further roughening. For cleaning purposes the plates were put for several hours into laboratory detergent solution. Subsequently they were rinsed with water and then immersed in isopropanol for several minutes. The cleaning was controlled by transmission measurement. Instead of a real structured spectrum, there should be only a flat line ($T\%_{300nm}$ approx. $0.95 \cdot T\%_{400nm}$). Micropigments like TiO_2 and ZnO were often adsorbed persistently to the rugged surface, even after extensive cleaning. They had to be removed by new grinding.

Transpore tape (3M Company Health Care, Maine, USA) was carefully affixed to polished quartz plates (Quality: Spectrosil B) to avoid air bubbles between tape and plate.

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statt ZnO_2

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Umstellung

Sample application

Different amounts of products were used in this study, as described in detail below. The routine application started with saturating the (latex-)gloved finger for about one

minute with the product. Before applying the sample, the weight of the plate was recorded. The sample had to be spotted evenly across the plate surface with an appropriate device like a micro-syringe or a self-displacing pipette. Immediately after spotting the applied amount was weighted. The weight was to lie within +/-10% of the calculated weight, depending on the size of the plate. The applied spots were distributed with the gloved finger as evenly as possible.

Thus at least six plates per product were prepared (three reference plates and three plates for exposure to UV light).

The plates are equilibrated in the dark for 15 minutes, before they were measured or irradiated according to the study design.

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Irradiation

The samples were positioned in the Heraeus suntester on the cooled tray in a distance of 21 cm to the lamp surface. To compensate possible flux inhomogeneities of the lamp, the samples were moved carefully for the duration of the irradiation. The time of irradiation was set individually, corresponding to the SPF of the product, and was calculated by the following formula:

Equation 1

$$DURATION \quad [\text{min}] = \frac{MED \times SPF \times \Theta}{E_{UV\ ery} \times 60}$$

- SPF : Sun protection factor of the sunscreen under test
 Θ : erythemal dose of the standard sun (250 J x m⁻²) (12)
 $E_{UV\ ery}$: Erythemal irradiance of the light source (W x m⁻²)

As already mentioned above, the erythemal irradiance of each lamp was determined by a standardized procedure using the MSS 2040-UV (Dr. Kockott). The resulting data are shown in *Table III*. The time to reach 1 MED ranged from 20 to 35 minutes, even when instruments from the same supplier with the same filter equipment were used, indicating that this might be a very crucial parameter.

Laboratory	time for 1 MED (min)	erythema irradiance (mW/m ²)
Lab1	20	205.5
Lab2	21	202.8
Lab3	28	147.2
Lab4	27	156.2
Lab5	21	198.5
Lab6	Erythema detection	-
Mean	25	171.6

Table III. Minimal erythema dose of the irradiation light source

Measurement

As described by different authors [13,14,15], the principle of the analysis is a *transmission* measurement. In case of the measurements on Transpore tape, the substrate without any test product serves as a reference. Concerning the roughened quartz plates, preceding studies showed that suitable reference plates are prepared by spreading a thin film of glycerin across the surface. The glycerin serves as a “blank”-emulsion, not containing any light absorbing or scattering a the similar way a placebo would do. Samples were measured at least at four different positions per plate.

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Calculations

Conversion of Rawdata

Prior to the calculation the measured signals have to be converted to absorbance, taking also into account the electrical noise (I_{backgr}).

Equation 2

$$A = \log(MPF) = \log\left(\frac{I_{ref} - I_{backgr}}{I_{sample} - I_{backgr}}\right)$$

where :

A = Absorbance

I_{ref} = Intensity of the transmission signal through the substrate without sunscreen

I_{sample} = Intensity of the transmission signal through the substrate with applied sunscreen

MPF = 1/Transmission

Critical Wavelength

The Critical Wavelength (λ_c) describes the broadness of the protection throughout the whole UV (290 - 320 nm). To calculate the *Critical Wavelength*, the area under the absorbance curve (AUC) is set at 100%.

The *Critical Wavelength* λ_c is the wavelength at which 90% of the AUC (starting from 290nm) is reached. The following formula is applied in correspondence to Diffey (1994) [1]:

Equation 3

$$\int_{290nm}^{\lambda_c} A(\lambda) * \delta\lambda = 0,9 * \int_{290nm}^{400nm} A(\lambda) * \delta\lambda$$

UVA Star Rating

The UVA/UVB Ratio R is defined as the ratio of the mean absorbances from two distinctive wavelength ranges: UVA (320-400nm) and UVB (290- 320nm):

Equation 4

$$R = \frac{\int_{320nm}^{400nm} A(\lambda) * \delta\lambda / \int_{320nm}^{400nm} \delta\lambda}{\int_{290nm}^{320nm} A(\lambda) * \delta\lambda / \int_{290nm}^{320nm} \delta\lambda}$$

where : A= Absorbance

λ_c = the wavelength, where the area under the absorbance spectrum reaches 90% of the whole area in the range of 290 to 400nm

Raw data generated by some instruments in 5nm-steps were interpolated to 1nm-intervals before calculating λ_c

Sun Protection Factor

The *in-vitro* sun protection factor (SPF) is calculated by Equation 5 :

Equation 5

$$SPF = \frac{\int_{290nm}^{400nm} E(\lambda) * s(\lambda) * \delta(\lambda)}{\int_{290nm}^{400nm} E(\lambda) * s(\lambda) * \delta(\lambda) / MPF(\lambda)}$$

where $E(\lambda)$ = Irradiance of the "Standard"-sun at wavelength λ
 $s(\lambda)$ = erythemal effectiveness at wavelength λ
 MPF = monochromatic protection factor ($1/T$)

Results and discussion

Substrates

To get reproducible and standardized transmission measurements it is required that the substrate can be supplied or be prepared in the same quality and in a sufficient quantity. In this study we chose Transpore tape that is used already for years in similar investigations and quartz plates that are roughened individually in the participating labs.

Concerning the roughening of the quartz plates, experimental data prove that this process can be done in a very reproducible manner. For example, measurements of the transmission at 400nm of plates prepared by eight different technicians resulted in a standard deviation of only 2.1%. Repeated grinding after several uses provides also standardized quality of the plates.

For comparison reasons, both, Transpore tape and roughened quartz plate, were used in parallel to determine the *Critical Wavelength* λ_c of the product OTC1 before and after exposure to UV light. The tested sun products did not show significant differences on quartz or Transpore before irradiation but behaved differently during irradiation: the *Critical Wavelength* drops significantly more on the quartz plates compared to the Transpore tape (Fig. 4). Obviously, the Transpore tape interacts with the sunscreen. Therefore the tape is inappropriate if an irradiation is intended. Based on these findings the group decided to continue with the roughened quartz plates.

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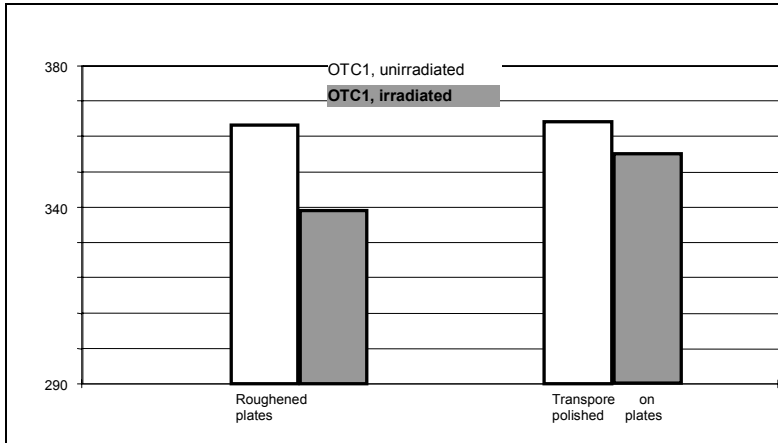


Figure 4. OTC1 : Influence of Transpore tape on the ratio of unirradiated-/irradiated samples

Equilibration Time

After application of an emulsion, water and other volatile components can evaporate, influencing the remaining product film and of course its absorption or transmission characteristics. For routine purposes, samples should be equilibrated for an adequate time to ensure stable and comparable product conditions during the determinations. To study the changes of a sunscreen, we recorded the weight and the transmission (MPF spectrum) on several time intervals after spreading. The data for OTC1 are given in Fig. 5 and Fig. 6.

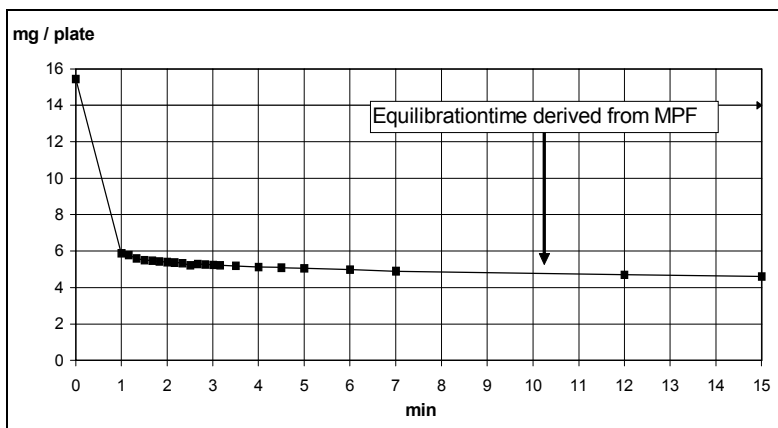


Figure 5. Change of film weight over time for OTC1 (LF20)

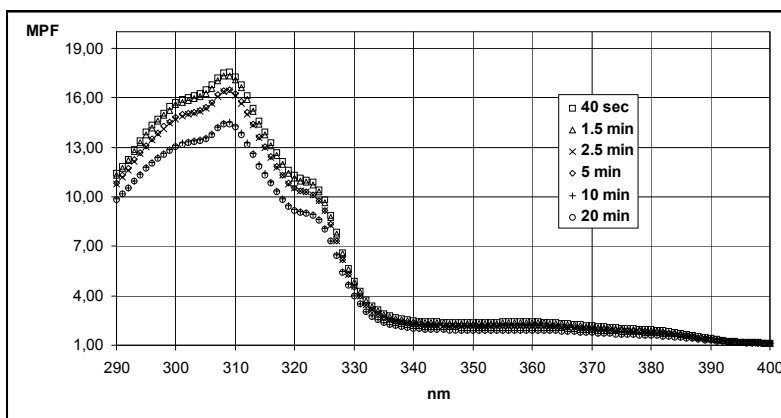


Figure 6. Equilibration of OTC1 (SPF20), change of the spectrum

It is obvious that the changes of the weight occur mainly within the first minute and that the residual amount of sunscreen remains quite stable afterwards. There is also a drop in the monochromatic protection factors during 10 minutes after application, but no difference is detected between 10 and 20 minutes indicating a stable condition of the product film. The equilibration time for the standard procedure was set at 15 minutes.

Sample application

Different amounts of sample, ranging from 0.75 to 2.0 mg/cm² were applied to roughened quartz plates in order to determine the appropriate amount for transmission measurement, taking into account that the method should be suitable for highly protecting products. These products filter the transmitted light very significantly, and false data may result due to the limitations of the detector system. The tests run with OTC2 the sample with the highest protection capacities of the samples used in this study.

The *Critical Wavelength* (λ_c) decreased with decreasing amount of application (Table IV). Even though the differences in this parameter do not appear very dramatic, the view at the transmission curves indicates that the measurement of this SPF 30 product gets close to the detector limit as recognized by the uneven curve in the high MPF range (Fig. 7). But there is not only a change in the level of the protection factors but also in the profile. As shown in Fig. 8, the shape of the peak in the UVB moves with increasing amount to longer wavelength.

Table IV. λ_c and applied amount

mg/cm ²	λ_c
0.75	375
1.5	376
2.0	377

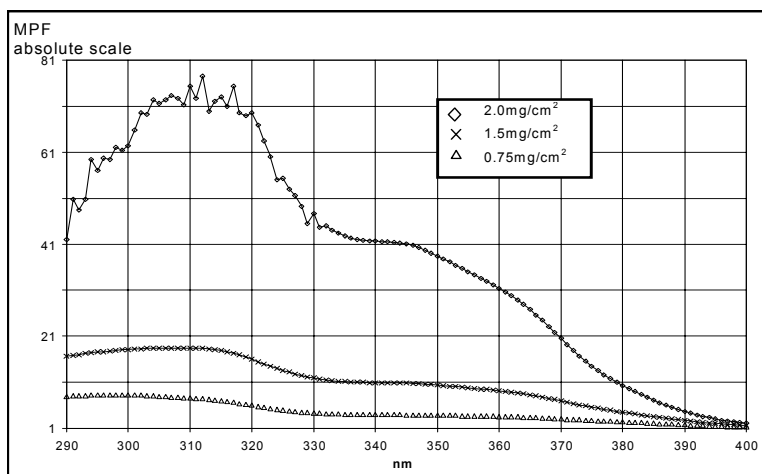


Figure 7. OTC2: Spectrum distortions due to spectrometer limitations: Influence of the film thickness

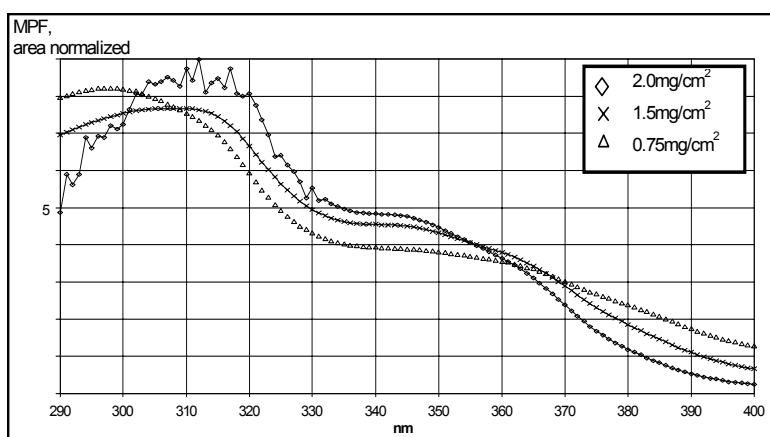


Figure 8. OTC2: Spectrum distortions due to spectrometer limitations: Influence of the film thickness. For comparison of the spectra shapes, the areas under the curves have put on a level with each other.

0.75 mg/cm² were set as the appropriate application amount. But the questions arose at this point, if this amount can be applied homogeneously and does the spreading with a gloved finger or by different technicians influence the measurement significantly.

Accordingly ten technicians weighted 0.75 mg/cm² of the standard product P3 to roughened quartz plates, following a mutually agreed practical guidance. The plates were prepared freshly and controlled by transmission measurements in advance of this study. The measurements at six different positions per plate were done with the same spectrometer. The *in-vitro* SPF's were calculated as the absolute parameter of the protection level and were then compared with the relative parameters, λ_c . Fig. 9 shows a representative result for one plate.

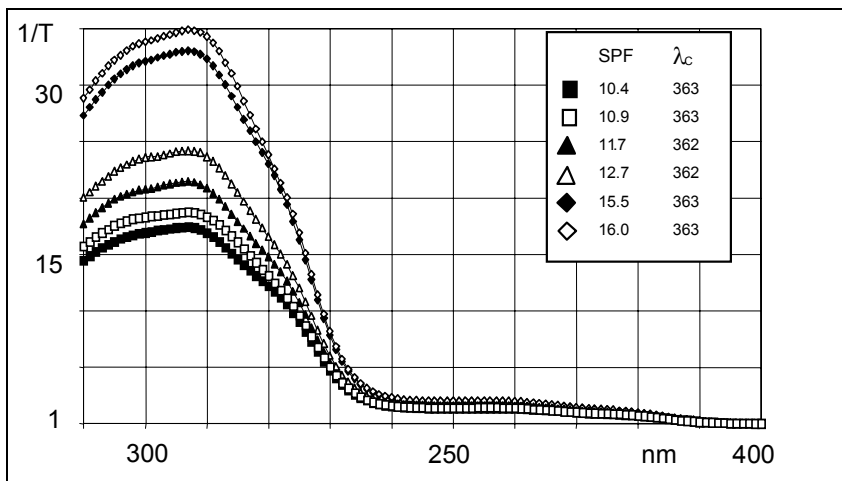


Figure 9. Standard P3: MPF's measured at 6 points on a single plate

Even when the technicians concentrated on the target of a homogeneous distribution of the sample on the plate, the resulting SPF values pointed to very significant differences in the film thickness which occur either on a single plate or among plates handled by different technicians. The variability of the results may depend on such factors as applied pressure and the spreading time. The residual film differs not only in thickness but also in weight as shown in the *Table V*, where the effective sample amount is the amount of the sample after drying and equilibrating. The differences range between 2.7 to 4.4 mg per plate. As expected, this dramatic difference of approx. 60% affects the SPF value (*Table V*), and renders this method unsuitable for SPF determination at this time.

In contrast to the absolute parameter (SPF) we did not observe significant variations in the relative parameters (λ_c and UVA/UVB Ratio). They remain very constant with respect to different positions on one plate, and also between plates prepared by different technicians. So, the relative parameters describe very reproducibly a sun product's photoprotective profile. The connected determination process can be considered as not being affected by handling influences.

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technician	effective sample amount	SPF	λ_c	UVA/UVB Ratio
A	2.60	6.79	360	0.28
B	2.70	4.46	357	0.25
C	2.90	7.65	361	0.28
D	3.10	12.67	363	0.32
E	3.30	10.17	361	0.28
F	3.30	8.11	360	0.27
G	3.80	11.27	363	0.31
H	4.00	11.93	364	0.33
I	4.30	11.10	361	0.30
J	4.40	9.84	363	0.32

Table V. SPF versus effective sample amount

Irradiation

It is known that some UV-filters may be partly degraded during UV exposure [16, 17] and of course this reaction may influence the protection efficacy of the sunscreen. In order to obtain information on the adequate *irradiation* dose necessary to account for light induced changes, we investigated the *Critical Wavelength* of the samples after different *irradiation* doses Fig. 10 shows the evolution of the Critical Wavelength as a function of the UV dosis.

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For the products tested the strongest influence on the *Critical Wavelength* already occurred within a time corresponding to 0.3 MEDxSPF. This dose is in good agreement with the procedures of other workgroups (CTFA, Colipa Taskforce for *in-vitro* UVA protection. In view of the reduced amount of application of 0,75mg/cm² and thus reduced film thickness, compared to an amount of 2mg/cm² in the Colipa *in-vivo* method for the determination of the SPF, this dose seems to be quite high.

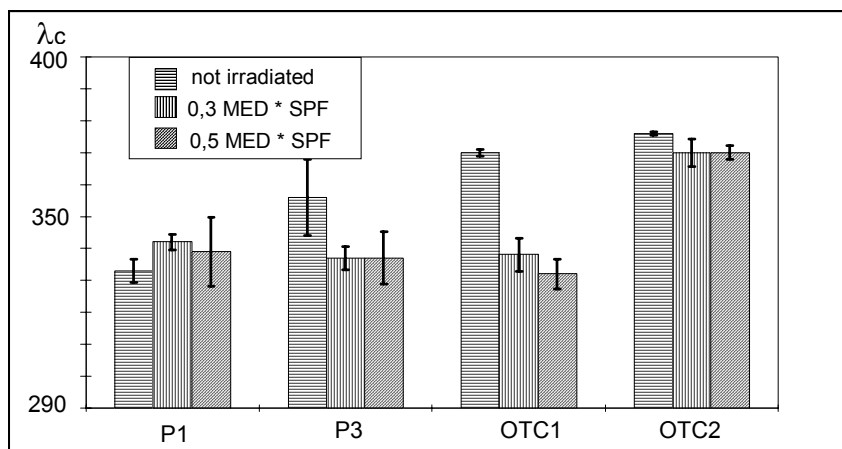


Figure 10: Influence of the *irradiation* on the Broad Spectrum Rating

UV induced changes in a sun product's transmission spectrum may of course be affected by the emission spectrum of the light source. Hence, a uniform irradiation is absolute required for comparable results. Although all test centers except one used the same irradiation equipment, the standard deviation rose significantly as consequence of the *irradiation* step.

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Therefore a standardization of the different devices by a measurement with a single spectroradiometer (MSS 2040-UV, Dr. Kockott) was initiated (see Materials and Equipment, *Irradiation*). It resulted in a marked improvement of the reproducibility. *Table VI* shows the data for the *Critical Wavelength* and the corresponding standard deviations before and after irradiation and before and after the standardization of the light sources.

Sample	Before standardization: +/- SD not irradiated	After standardization: +/- SD not irradiated	Before standardization: +/- SD irradiated	After standardization: +/- SD irradiated
Standard P1	3.7	5.2	10.7	8.6
Standard P3	1.9	2.0	8.2	3.6
OTC1	1.1	1.3	9.6	6.0
OTC2	0.5	1.2	2.1	2.2

Table VI. Standard deviations of λ_c before and after standardization

Interlaboratory ring study

Critical Wavelength

Having improved and standardized the most influencing parameters of the measurement (application amount, spreading and equilibration, irradiation a. o.), a ring test (Round Robin Test) with four samples in six labs was carried out to demonstrate the applicability of the method to describe the broadness of the UV-protection of a sunscreen. The *Critical Wavelength* was calculated as a relative parameter , to describe the UVA-protection efficacy of a sunscreen in relation to its UVB protection. The results of this index are given in *Table VII* and *Table VIII*.

Critical Wavelength Company	Standard P1		Standard P3	
	Not irradiated	Irradiated with 0,3MED X SPF	Not irradiated	Irradiated with 0,3MED X SPF
Lab1	-	-	362	339
Lab2	335	340	359	335
Lab3	342	347	363	337
Lab4	328	361	361	335
Lab5	334	343	364	330
Lab6	332	342	359	340
Mean	334	346	361	336
Standarddev.	5.2	8.6	2.0	3.6

Table VII. Critical wavelength for standard P1 and P3

Critical Wavelength Company	OTC1		OTC2	
	Not irradiated	Irradiated with 0,3MED X SPF	Not irradiated	Irradiated with 0,3MED X SPF
Lab1	371	334	376	373
Lab2	371	331	374	373
Lab3	370	345	377	369
Lab4	370	329	377	374
Lab5	373	329	377	373
Lab6	369	335	375	369
Mean	371	334	376	372
Standarddev.	1.3	6.0	1.2	2.2

Table VIII. Critical wavelength for standard OTC1 and OTC2

The data show that the *Critical Wavelength* can be determined very reproducibly. We found in all samples excellent, small standard deviations, that are slightly increased by a *irradiation* step. The method is able to differentiate the sunscreens corresponding to their UVA protection.

Standard P1 shows a slight increase in the calculated UVA-protection parameters after irradiation. At first sight this behaviour is quite unexpected, because this special emulsion is only containing a single UVB-filter, Octyl Methoxycinnamate, and no UVA absorbing agent. But it is well known that Methoxycinnamates partially undergo a trans-cis isomeration during exposure to UV light [18, 19]. This photoisomeration walks along with a slight change in the shape of the absorption spectrum giving rise to altered protection-parameters.

In the case of the products P3 and OTC1 the *Critical Wavelength* drops significantly after *irradiation* due to a substantial loss of their UVA filtration capacity. The other

product, OTC2, shows less intensive changes which may be attributed to a slower disappearance in its UVA protection.

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UVA Star Rating

As a second relative parameter to describe the broadness of a sunscreen, we calculated also the UVA/UVB- ratio These results are shown in *Table X* and *XI*.

UVA/UVB – Ratio	Standard P1		Standard P3	
	Not irradiated	Irradiated with 0,3MED X SPF	Not irradiated	Irradiated with 0,3MED X SPF
Lab1	-	-	0.29	0.18
Lab2	0.18	0.19	0.27	0.17
Lab3	0.21	0.21	0.31	0.17
Lab4	0.13	0.21	0.29	0.17
Lab5	0.19	0.21	0.34	0.15
Lab6	0.16	0.19	0.28	0.19
Mean	0.17	0.20	0.30	0.17
Standarddev.	0.032	0.013	0.029	0.013

Table X. UVA/UVB-Ratio for standard P1 and P3

UVA/UVB – Ratio	OTC1		OTC2	
	Not irradiated	Irradiated with 0,3MED X SPF	Not irradiated	Irradiated with 0,3MED X SPF
Lab1	0.44	0.15	0.65	0.52
Lab2	0.46	0.14	0.58	0.51
Lab3	0.41	0.19	0.65	0.43
Lab4	0.43	0.14	0.65	0.54
Lab5	0.50	0.14	0.62	0.47
Lab6	0.39	0.15	0.61	0.43
Mean	0.44	0.15	0.63	0.48
Standarddev.	0.042	0.021	0.030	0.049

Table XI. UVA/UVB-Ratio for standard OTC1 and OTC2

Apparently, these data can also be determined by the proposed method very precisely. Even the tendencies in the UVA-protection concerning the composition and treatment of the samples, derived from the *Critical Wavelength*, are confirmed by the UVA/UVB-ratio.

Conclusion

We investigated the applicability of the determination of a relative UVA-protection index for four products. The studies were performed in six laboratories. They showed the steps which, require particular efforts and harmonization in order to improve the results including the product application to a specific substrate, the irradiation process and the transmission measurement. Following these improvements, it was demonstrated within a final ring study, that the two relative parameters *Critical Wavelength* and *UVA/UVB Ratio* could be measured with an inter- and intra-laboratory reproducibility, which is superior to any *in-vivo* endpoint. Because the *in-vitro* methods for UV protection are based on common transmission measurements, this methodical approach is not restricted to the above mentioned protection parameters. Although, the present study shows, that the reproducibility in measuring the *absolute* parameters, like the SPF, is quite unsatisfactory.

A choice of support between quartz and Transpore was made in favor for quartz. This is based on the observation that sunscreens and emollients interact with Transpore, making this material not suitable for samples to be exposed. The layer thickness was reduced from 2 mg/cm² to 0.75 mg/cm² for analytical reasons (dynamic range / optical density). The UV dose, 0.3 MED * SPF and the concomitant UVA radiation, is in agreement with similar approaches elsewhere. This energy applied to a reduced layer must be considered as being a very high dose. It is further crucial to carefully monitor the output of the lightsource. But still then, an additional irradiation step means to decrease the accuracy of the method. The authors believe that the proposed model is a worthwhile contribution to the ongoing debate on UVA methodology.

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