UV/Vis Spectrometry

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Sunscreen Testing According to COLIPA 2011/FDA Final Rule 2011 Using UV/Vis LAMBDA Spectrophotometers

Introduction

Sunlight striking the Earth is composed of 50% infrared, 40% visible, and 10% UV. Of the UV component, 9.5% is UVA and 0.5% is UVB.

Research has shown that sun exposure can have both acute and chronic consequences for the skin. Making up 95% of UV radiation, UVA radiation (320-400 nm) can penetrate the epidermis and cause indirect DNA damage as a result of the production of reactive oxygen species (ROS) as well as single strand breaks in DNA and DNA–protein crosslinks, dermal damage, and increased collagen degradation. Although UVB radiation (290-320 nm) is mostly absorbed in the epidermis, it is more cytotoxic and mutagenic, directly inducing DNA damage. UVA radiations role in photo-aging is significant. Due to deep penetration, UVA radiation is responsible for epidermal and dermal damages.
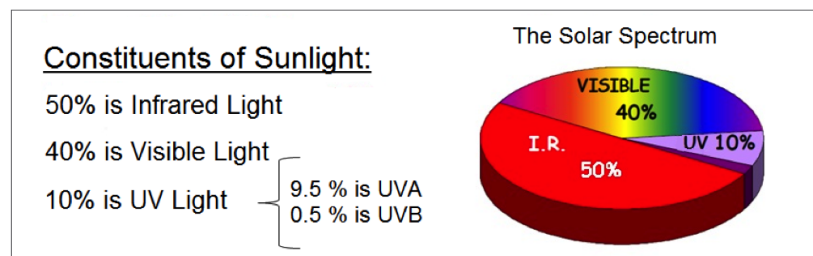

Figure 1. A diagram illustrating the components of sunlight that hits the earth, of which 50% is infrared, 40% is visible, and 10% is UV. UV represents the most energy-intense component from the sun radiation.

The sun protection factor (SPF) is supposed to allow the consumer to directly compare the protection afforded by sunscreens. Sun protection products are generally divided into four protection classes.

Table 1. Sun protection products are generally divided into four protection classes, termed Low, Moderate, High and Very High, related to the level of their SPF label claims.

Label SPF	Protection Class
6	Low
10	
15	Moderate
20	
30	High
50	
50+	Very High

Products with sun protection factors below six are no longer classified as sunscreens because the main purpose of the product (sun protection) is not achieved due to the low protective effect. Also "sun-block" claims used earlier is likewise no longer used in order to prevent misunderstanding by consumers.

There is a problem in only using the SPF value to describe sunscreen effectiveness. The SPF primarily affected by UVB radiation and is not a significant indicator of protection against UVA radiation. Since harmful effects of UVA have been demonstrated, there is growing demand for the method of measuring the level of protection against UVA (UVA Protection factor, UVA-PF).

Increasing concern about the UVA effects has led to the need for standardized methods to properly evaluate the level of protection afforded by sunscreen products

A number of methods, developed in Europe, Australia, Germany, UK, and the United States (ISO, AS/NZS, DIN, UK, FDA) based on both in vivo and in vitro techniques have been proposed together with different ways to label the level of UVA protection. Unfortunately, there has been little synchronization of the methods being developed worldwide for uniform labeling of UVA effectiveness. The current major vitro protocols are listed to the right.

COLIPA 2011 and FDA Final Rule 2011

The two protocols to be discussed in this paper are COLIPA 2011 and the FDA Final Rule 2011. For determination of the UVA protection factor, COLIPA in 2011 (the European Cosmetic, Toiletry and Perfumery Association) has published a guideline for an in vitro test. The FDA Final Rule 2011 test protocol parameters are aligned to COLIPA 2011 and allow for compliance for both to be derived from the same test.

According to both FDA and EU directives, sunscreens must provide, in addition to UVB protection, also protection against UVA radiation. The UVA protection factor (UVA-PF) should equal at least 1/3 the sun protection factor, as determined by the in vivo PPD (persistent pigment darkening) method or an equivalent degree of protection obtained by any in vitro method. Also an in vitro Critical Wavelength value of greater than 370 nm is specified, in order to satisfy requirements for broad-spectrum UVB / UVA protection and associated labeling.

SPF Testing – Current In Vitro Protocols

AS/NZS 2604 Broad Spectrum (2012) The new version of this is based on the test method described in ISO 24443 Determination of Sunscreen UVA Protection In vitro. UVAPF Ratio and Critical Wavelength requirements are calculated in order to arrive at Broad Spectrum compliance.

ISO -24443 (2012) Determination of Sunscreen UVA Protection in vitro. Determines both UVAPF Ratio and Broad Spectrum Compliance. Compliance with AS/NZS 2604.

SO 24442 (2012) This method is being adopted as the harmonized method for determination of UVAPF in vitro and for use in AS/NZS 2604 and Cosmetics Europe, ASEAN and other regions.

UVA-UVB Ratio (2010) Absorption of a 1.3 mg/square cm film is measured between 290 nm and 400 nm. The ratio of areas under the curve between 290 - 320 (UVB region) is compared with the area under the curve between 320 nm and 400 nm. Pre irradiation of the sample is required. (Calculated as TPF x UVA/UVB). Various substrates can be nominated.

Boots Star Rating (2011) The method used by Boots in the UK (not mandated). Absorption of a 1 mg/square cm film is measured between 290 nm and 400 nm. Pre irradiation of the sample is required. Rating scale is 3 to 5 stars . More stars means more protection (by ratio) in the UVA area.

FDA, Final Rule 2011 The current proposed method for the USA. Absorption of a 0.75 mg/square cm film is measured between 290 nm and 400 nm. The critical wavelength is the point where 90% of the area under the curve lies, starting at the UVB end. Pre irradiation of the sample is required.

DIN 67502 (UVA Balance) The methodology is based on that described in the German Standard DIN 67502. The SPF is determined using the values provided in the CIE. The SPF is applied in order to correct the values obtained in vitro. The PPD are derived by applying the values from the PPD Action Spectrum given in the standard.

COLIPA (UVAPF) (2011) UVAPF/SPF Ratio and Critical Wavelength are calculated from this measurement technique. Compliance with E.U. requirements is also reported.

In FDA Final Rule 2011 the claim of "Broad Spectrum" is allowed when the SPF is 15 or more, and when the Critical Wavelength reaches 370 nm. Note that the FDA has aligned their method with the COLIPA UVA method (2009) but has not yet updated to COLIPA (2011).

In FDA Final Rule 2011 sunscreens that do not pass the broad-spectrum test (<370 CW) and broad-spectrum sunscreens with an SPF value between 2 and 14 can only claim to help prevent sunburn and must carry a warning on their label that reads "Skin Cancer/Skin Aging Alert."

In this paper, the COLIPA 2011 procedure will be presented as an example. COLIPA has introduced a number of terms in their procedure which are defined below. These terms include UVAPF, UVAPF(0), SPF in vitro, and Critical Wavelength.

Choice of Substrate

The ideal substrate for in vitro SPF needs to be fairly transparent to the ultraviolet and simulate the porosity and texture of human skin, the in vitro substrate. In the past,

choices of recommended substrates included 3M Transpore™ Tape, Vitro-Skin™, and Polyvinyl Chloride Film (Saran Wrap). Though these substrates could all give viable results, they all had drawbacks, as there were issues in consistent sample preparation with these materials.

According to the most recent FDA and EU directives, molded polymethylmethacrylate (PMMA) plates are now recommended as the substrate of choice. These plates are exclusively made by HelioScreen Laboratories (http://helioscreen.fr/en/substrats/Helioplate_HD). The recommended HELIOPLATE HD 6 (50 mmX50 mm) about 6 microns roughness is in compliance with ISO 24443, COLIPA Rev. 2011, and FDA Final Rule 2011. The exclusive supplier of the HELIOPLATE HD 6 in the United States is Labsphere (http://labsphere.com/products/instruments/uv-transmittance-analyzer/plates-for-sunscreen-testing.aspx). A picture of the PMMA plate is shown below against a small tube of commercial sunscreen to illustrate the diffuse characteristics of the plate's pebbled surface.

COLIPA 2011 Instrumentation Requirements

The COLIPA 2011 procedure defines the instrumentation requirements for sunscreen testing:

- The wavelength range measured by the spectrophotometer must span the primary waveband of interest, that is, 290 nm to 400 nm with a digital data interval of 1 nm.

- The wavelength of the spectrophotometer should be accurate to within 1 nm.

- The spectrophotometer optics should be designed for the diffuse collection of the transmitted irradiance through the roughened PMMA substrate, both with and without the sunscreen layer spread on its surface. An integrating sphere is recommended and, when used, smaller fractional port areas compared with total sphere wall area will lead to greater accuracy.

- The minimum required dynamic range is at least 2.2 absorbance units. The maximum measured absorbance should be within 90% of the dynamic range of the device used. As a consequence, the UV-Vis spectrometer must be designed for efficient stray-light rejection (e.g., as provided by a double monochromator).

Figure 2. Pictured against a small tube of commercial sunscreen is the recommended PMMA substrate for SPF testing, the HELIOPLATE HD 6 (50 mm x 50 mm, 6 microns).

The port area integrating sphere requirement, in particular, will exclude smaller integrating sphere (i.e., less that 100 mm in diameter), because the smaller sphere will have port area ratios in the 8-10% range. Conversely, a 150 mm integrating sphere will have a port area ratio of 1-2%. Accurate transmittance measurements of solid samples with a normal spectrophotometer can be problematic without an integrating sphere accessory, particularly if the samples are scattering such as the PMMA plates. The broad angle collection of light (as illustrated in the diagram below) provided by an integrating sphere accessory compensates for light loss due to sample scatter. Instead of measuring the PMMA plate in the normal sample compartment, where the detector is unable to detect the scattered light (= inaccurate absorbance), use of an integrating sphere accessory allows the scattered light from the PMMA plate to be collected and measured (= accurate absorbance).
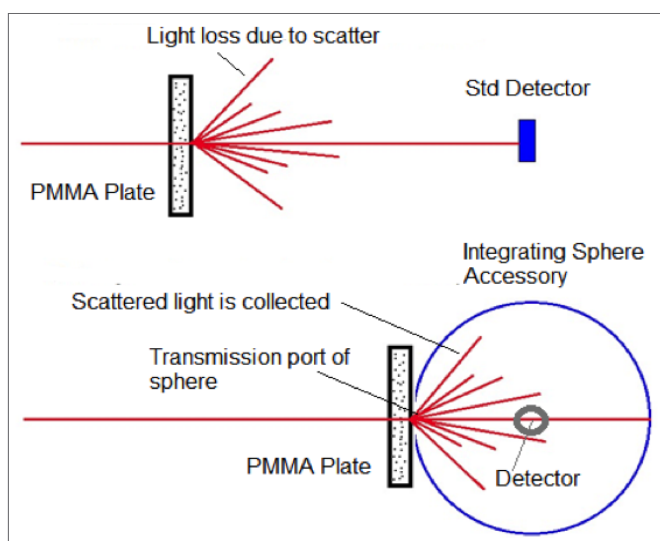

Figure 3. An integrating sphere accessory is required for SPF testing. Pictured is a normal sample compartment transmission configuration (top figure), where due to light scatter measurement errors can occur. The broad angle collection of light provided by an integrating sphere (bottom figure) compensates for light scatter, providing an accurate transmission reading.

In compliance with the recommendations of COLIPA, the PerkinElmer LAMBDA 650 fitted with a 150 mm Spectralon (Labsphere) sphere is an ideal in vitro sunscreen measurement system. The LAMBDA 650 is a double monochromator UV/Vis with a wavelength range of 900 nm to 190 nm, having an ultra-low stray light level of 0.0001%T. The dynamic range is specified at +/- 6A, with a wavelength accuracy of +/- 0.15 nm. A picture of the LAMBDA 650 fitted with a Labsphere 150 mm integrating sphere is shown below, along with a top view diagram of the sphere.

Instrumentation Requirements - Validating Photometric Linearity

COLIPA states clearly that the photometric linearity of the UV/Vis spectrophotometer being used for sunscreen resting needs to be validated to at least 2.2 A. Because the dynamic range and photometric linearity of a UV/Vis spectrophotometer are reduced significantly when an integrating sphere is installed, photometric linearity needs to be verified with the complete spectrophotometer sphere system.
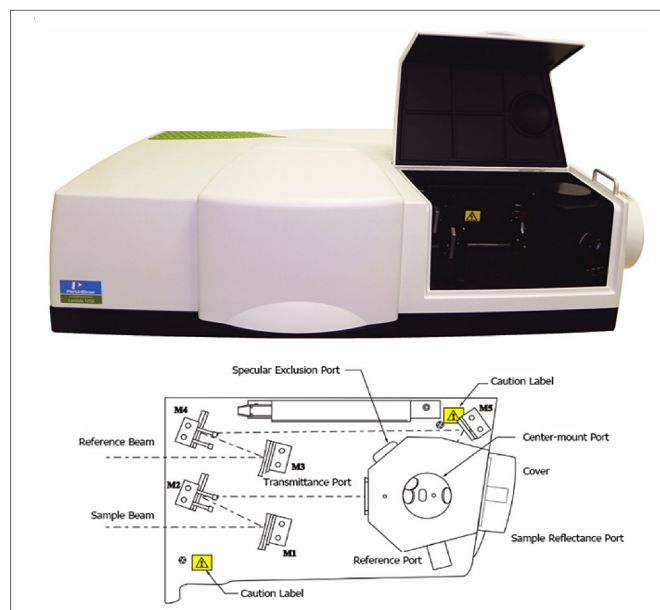

Figure 4. In compliance with the recommendations of COLIPA, the PerkinElmer LAMBDA 650 fitted with a 150 mm Spectralon (Labsphere) sphere is an ideal in vitro sunscreen measurement system. Pictured here is a LAMBDA 650 fitted with the 150 mm sphere accessory, and a top down view of the sphere design.

COLIPA in Appendix II describes the use of two calibration PMMA plates, stacked together, for testing the linearity of the spectrometer. These calibration plates contain UV-absorbing substances and are made in such a way as to match the absorption spectra of a range of common commercial sun-screens. The plates (supplied by Schonberg GmbH – order number 951) are cut from a large sheet of a standard cast, UV-stabilized Plexiglas which helps ensure the same optical properties for each plate.

Photometric linearity can also be established by using other test materials. In lieu of using the PMMA calibration plates, serial dilutions of potassium dichromate (NIST procedure) can be used to check the linearity of the spectrometer over the sunscreen test range. A linearity test using serial dilutions of potassium dichromate performed on a LAMBDA 650 with 150 mm integrating sphere (results shown below) demonstrates linearity well past 2.2 A with a calculated correlation coefficient of 0.9992.

Building a COLIPA/FDA Final Rule Method in UVWinlab V6

The software (UVWinLab V6) that operates with the LAMBDA 650 can be programmed to build a suitable method for sunscreen testing according to COLIPA/FDA Final Rule. A few key points: 1) three different PMMA plates are prepared for each sunscreen compliance test, and 2) adhering to COLIPA recommendations each prepared plate is measured as (4) replicates, with each replicate representing a different beam spot location on the plate (typically this involved rotating the plate on the transmission port of the sphere 90 degrees for each replicate), and 3) the prepared PMMA plates need to be measured before irradiation by a solar simulator, and repeated after irradiation. Therefore, the best way to proceed building a method is to have a sample sequence programmed for measuring the PMMA plates before irradiation, after which the session task is saved into the database of UVWinLab V6, and when irradiation is completed, the task is
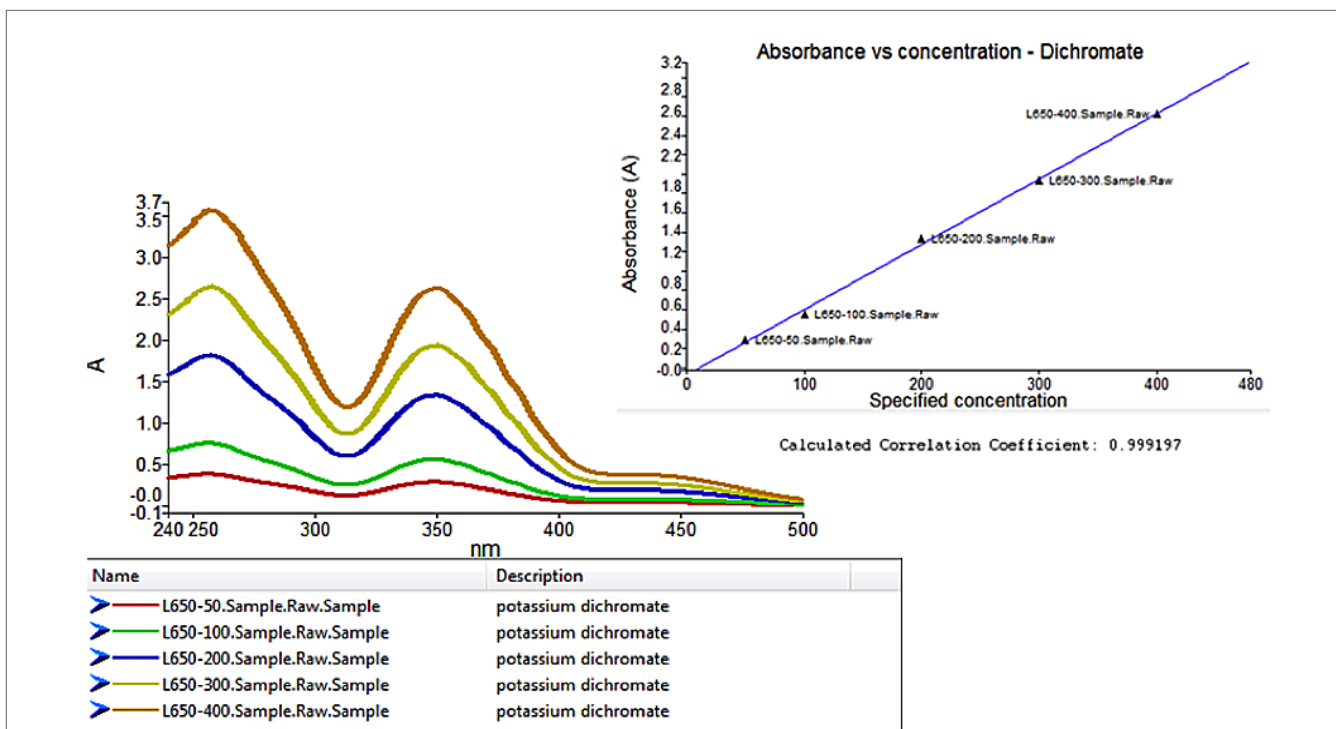
Figure 5. Serial dilutions of potassium dichromate (NIST procedure) is used to verify the linearity of the spectrometer over the sunscreen test range. In this figure, serial dilutions of potassium dichromate were measured on a LAMBDA 650 and 150 mm integrating sphere. A correlation coefficient of 0.9992 demonstrates compliance to the COLIPA linearity recommendations.

opened and continued to measure the post-irradiated plates. In that way all data is available for the SPF calculations and final report.

The steps involved to create a method include:

1. Open the LAMBDA 650 Default Scan method. Set the scan range from 400 nm to 290 nm, with an ordinate mode of %T. Enter a data interval of 1 nm. Enter A bandpass of 2 nm, and an integration time between 0.20 and 0.32 seconds, as shown in example with the LAMBDA 650.

2. Click on the Sample Info section to build the sample sequence. Two columns are added to the sample table: the first is column called SPF Label (which is the stated product SPF value is entered), and a column called Sample Tag (where the replicate sequence is identified as a "before" irradiation" or "after" irradiation for each plate/replicate). An example sample table is shown in Figure 7. Note: because the sample info is saved with the method, this sample sequence needs to be defined only once. The sample ID will automatically increment for each sunscreen compliance test.

3. The COLIPA 2011 SPF-UVAPF calculations can either be

programmed directly into the method, or if preferred, the resulting spectral data can be exported in CSV format to allow data calculations to be processed off-line in Excel. The example here will illustrate where to enter the necessary equations under the Processing section of the method to allow SPF-UVAPF calculations to be embedded in the method. By clicking on the Processing section of the method, equations can be sequentially defined and then added. The equations can be used for arithmetic calculations (i.e., a spectrum multiplied by a factor), or external COLIPA 2011 SPF-UVAPF weighting tables can be "browsed" into the method for required spectral transformations. In an example Equation 7 shown in Figure 8, two COLIPA defined weighting tables are browsed into the method to allow use in further calculations. In this example the COLIPA defined equation for SPF (in vitro) is programmed in a sequence of five equations, calculating both arithmetic functions and spectral transformations using browsed weighting tables.

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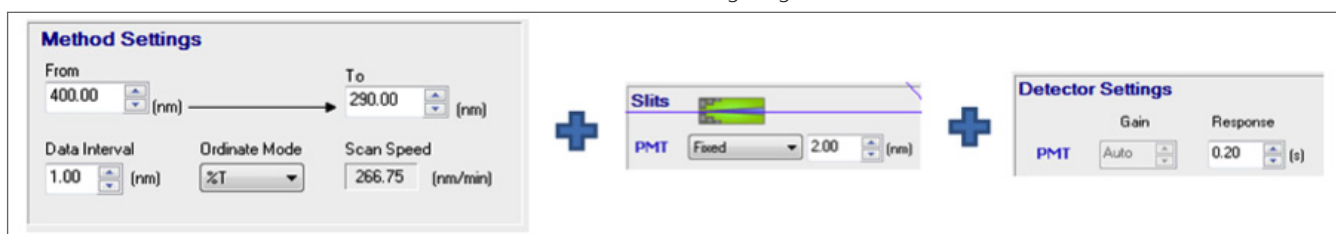

Figure 6. To build a SPF testing method in UVWinLab V6, the scan range, data interval, ordinate mode, slit, and response time settings are entered as illustrated here.

Figure 7. An example SPF sample table is shown here. Two columns are added to the table: the first is called SPF Label (where the stated product SPF value is entered) , and the second column called Sample Tag (where the replicate sequence is identified as "before" irradiation or "after" irradiation).

Figure 8. Equations can be defined in the Processing section of UVWinLab V6 to allow SPF calculations to be embedded in the method. The equations can be used for arithmetic calculations or external weighting tables can be "browsed" into the method for required spectral transformations. In the example below, two COLIPA 2011 defined weighting tables are browsed into the method for use in further calculations.

$$SPF_{in\ vitro} = \frac{\int_{\lambda=290nm}^{\lambda=400nm} E(\lambda)*I(\lambda)*d\lambda}{\int_{\lambda=290nm}^{\lambda=400nm} E(\lambda)*I(\lambda)*10^{-A_0(\lambda)}*d\lambda}$$

where:

$E(\lambda)$ = Erythema action spectrum (CIE- 1987)
$I(\lambda)$ = Spectral irradiance of the UV source (SSR for SPF testing)
$A_0(\lambda)$ = Mean monochromatic absorbance measurements per plate of the test product layer *before* UV exposure
$d\lambda$ = Wavelength step (1 nm)

Equation	
1	erythema-400-290.asc*"UV-SSR-400-290.asc"
2	[before1+before2+before3+before4]/4
3	[after1+after2+after3+after4]/4
2	Mean[Equation1]
3	Equation1*[Equation2/100]
4	Mean[Equation3]
5	Equation2/Equation4 ——————→ **SPF (in vitro)**

Figure 9. Illustrated in this example are the equations needed for the calculation of SPF (in vitro). The COLIPA defined Erythema Action Spectrum, the PPD Action Spectrum, the UVA and the UV-SSR Spectral Irradiances are entered into the method by browsing to the location for these files.

The final step in creating a sunscreen test method is to define the report output needed. UVWinlab V6 has a powerful report builder called Communiqué as part of the software which allows custom report templates to be built according to user specifications. Objects containing spectra and calculations being outputted from the method are dragged and dropped on the report form, which can then be sized and positions can be adjusted. An example report template is shown in Figure 10. Once the report template is defined, it can be saved and then linked to a method. Once a method is completed, it can be saved for easy recall and execution.

An Example Sequence

There are four basic steps in executing a sunscreen compliance test.

Step 1. In vitro transmission measurement of the sunscreen product spread on a PMMA plate, prior to UV irradiation. In this step are the acquisition of initial UV spectra, from replicates of three plate preparations, with four different beam spot locations per plate.

1. Complete the sample table as shown earlier. The product labeled SPF effectiveness needs to be put into a column, as well as assigning Before/After sample tags to the replicate sequence.

Figure 10. Using the built-in Communiqué Report Builder of UVWinLab V6, an appropriate report is created to display the spectra and the calculated protection values.

2. Background at 100%T with the blank PMMA plate on the %T port of the sphere. Remove the PMMA plate.

3. Background at 0%T by blocking the %T beam.

4. Apply a thin film of the test product, at a thickness of 1.3 mg/square cm by the recommended application technique. This involves applying a series of around 30 dots over the area of the plate and then rubbing out evenly with a finger cot which has been pre-impregnated to saturation with the same product. The white test product at some point becomes a fairly clear thin coating.

5. Place PMMA "BEFORE" plate preparation #1 on %T port of the integrating sphere. Measure four different spot locations of plate #1, rotating the PMMA plate 90 degrees for each replicate. Repeat measurements for remaining PMMA "BEFORE" plate preparations #2 and #3, again measuring four different spots per plate.

6. This completes the before irradiation sequence. The Task is then saved in UVWinlab V6 to allow the session to be continued after the plates are irradiated.

SPF (in vitro), Coefficient C, and UVAPF(0) are calculated. The spectra are mathematically adjusted for film thickness to equal the claimed SPF label claim.

Step 2. The PMMA test plates are irradiated in a Xenon Arc solar simulator, filtered to comply with the requirements of the COLIPA Protocol.

Step 3. In vitro transmission measurements of the sunscreen product after UV exposure are acquired. The acquisitions of a second set of UV spectra are performed, again on each replicate plate and different beam spot locations.

1. From the Task list in UVWinlab V6, click once to highlight the task saved from above session and select CONTINUE from the toolbar. This allows the data acquisition of the AFTER measurement plate preparations/replicates to be appended to the previously saved session.

2. Background at 100%T with the blank PMMA plate on the %T port of the sphere. Remove the PMMA plate.

3. Background at 0%T by blocking the %T beam.

4. Place PMMA "AFTER" plate preparation #1 on %T port of the integrating sphere. Measure four different spot locations of plate #1, rotating the PMMA plate 90 degrees for each replicate. Repeat measurements for remaining PMMA "BEFORE" plate preparations #2 and #3, again measuring four different spots per plate.

5. This completes the AFTER irradiation measurement sequence.

Step 4. Print the report.

UVAPF, Ratio SPF/UVAPF, and Critical Wavelength are calculated. The mean SPF(in vitro), Coefficient C, and UVAPF(0) UVAPF, Ratio SPF/UVAPF, and Critical Wavelength values from the three plate preparations/ four spot locations are calculated.

An Example Sunscreen Report

For this example the test sunscreen used was commercially obtained. SPF 30 Broad Spectrum UVA/UVB protection. Shown in Figure 11 are the combined BEFORE irradiation and the AFTER irradiation spectra acquired on a LAMBDA 650 and 150 mm integrating sphere.
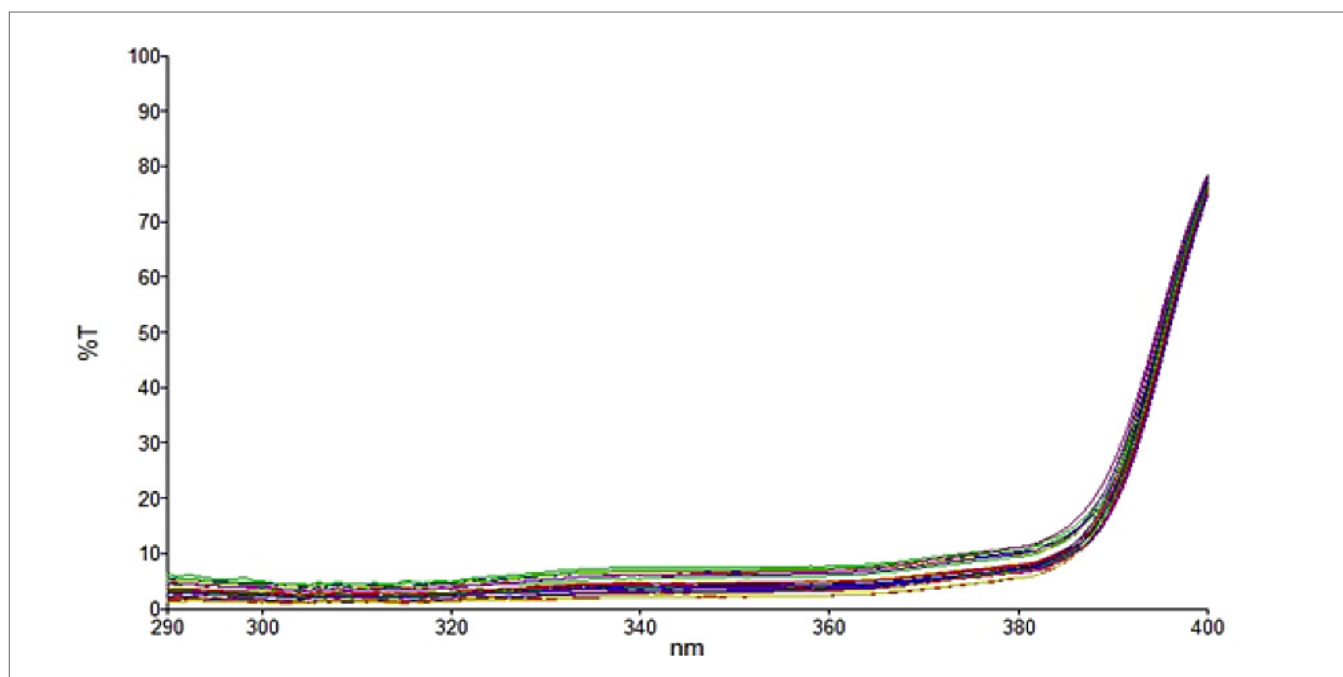

Figure 11. As an example the test sunscreen used was commercial sunscreen labeled Sport SPF 30 Broad Spectrum UVA/UVB protection. Shown is the BEFORE irradiation, and the AFTER irradiation scans acquired on a LAMBDA 650.

Table 2. COLIPA 2011 UVA Protection Report. COLIPA 2011 UVA protection results calculated with a UVWinlab V6 method created with the steps discussed in this paper. Using the acquired spectra of the test sunscreen shown in Figure 11, reported are the SPF (in vitro), SPF (adj), Coefficient C, UVAPF(0), UVAPF, the Ratio (label)/UVAPF, and the Critical Wavelength for each plate replicate, and the Mean of the replicates.

Plate Replicate	SPF (in vitro)	SPF (adj)	Coefficient C	UVAPF(0)	UVAPF	Ratio SPF (label)/UVAPF	Critical Wavelength (nm)
1	26.20	30	0.8719	15.70	21.40	1.40	373.8
2	24.50	30	0.8180	16.40	29.00	1.00	373.9
3	26.70	30	0.8904	16.60	22.90	1.30	371.2
Mean	25.80	30	0.8601	16.20	22.40	1.20	373.0

Shown in Table 2 are the results table report containing the COLIPA SPF-UVA-PF calculated values for the sunscreen tested

In this example, both the UVAPF values (> 1/3rd of the stated SPF), and the Critical Wavelength values (> 370 nm) comply with the labeling requirements of COLIPA.

Conclusion

All commercial sunscreen formulations with label claims at to their effectiveness, need to be tested according to accepted procedures. In this paper, we chose the procedure COLIPA 2011 (Europe), and with some differences, the FDA Final Rule 2011(USA) as procedure guidelines. Sunscreen testing requires a UV-Vis spectrophotometer fitted with a procedure compliant integrating sphere accessory, and this system is required to meet the photometric linearity range stated by COLIPA.

A LAMBDA 650 fitted with a 150 mm Labsphere integrating sphere accessory is one of the recommended systems from PerkinElmer for sunscreen testing. This double monochromator system not only exceeds the photometric linearity requirements, but the small port area ratio of the integrating sphere complies with the COLIPA recommendations.

UVWinlab V6 software allows the COLIPA SPF-UVA-PF calculations to be programmed to be part of a method. Use of Communiqué to build appropriate report templates allows customization of the final hard copy report.

References

1. Federal Register / Vol. 76, No. 117 / Friday, June 17, 2011/ Rules and Regulations. DEPARTMENT OF HEALTH AND HUMAN SERVICES. Food and Drug Administration. 21 CFR Parts 201 and 310. [Docket No. FDA–1978–N–0018] (Formerly Docket No. 1978N–0038) RIN 0910–AF43. Labeling and Effectiveness Testing; Sunscreen Drug Products for Over-the-Counter Human Use. Final Rule.

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PerkinElmer