

Instrumental (*In-vitro*) UVA Measurement and Validation

John Staton - Dermatest Pty Ltd

Although considered as therapeutic products in many parts of the world including Australia, it is interesting to observe that the major developments in sunscreens, their formulation, safety and efficacy have come primarily from the area of cosmetic science. It is rare to find a conference dedicated to OTC technology incorporating a workshop or technical session devoted to this product group, whilst in the cosmetic technology arena, issues surrounding SPF and UVA are now a regular component of equivalent conferences.

Whilst the scientists involved in the cosmetic arena are very much focused on formulation and improvement to cosmetic stability, performance and safety, the consideration of analytical methodology is somewhat a lower priority than it is for main stream therapeutics.

The presentations that Dr Nearn and I will make today are intended to outline the state of the art in relation to only one aspect of sunscreen technology - that of the measurement of UVA in relation to both claim justification and safety.

Primarily, the objective is to cover the background and rationale for the proposal by ASMI to be put before the sunscreen expert group of the Standards Association of Australia. This committee is the body responsible for the formulation of the standard which, in this country is taken up by our Therapeutic Goods Administration regulators and compliance forms part of the legal requirement for marketing approval.

As Dr Nearn is to cover the aspects of in-vivo methodology, theoretical aspects of SPF and UVA determination and to outline the proposal itself and interpretation into labelling, I am restricting

my part of the presentation only the discussion of the instrumentation which would appear to be appropriate to the test methodology.

Instrumental UVA Measurement - requirements of the current Australian Standard

It should be noted that compliance with this part of this standard is not mandatory for all sunscreens. It applies only for those wishing to make the additional “Broad Spectrum” claim. There are currently 3 options for determination of UVA, for support of the claim , provided by the AS/NZS 2604 Standard. These cover:

1. A solution method appropriate for those product which completely, or almost completely dissolve in a specified organic solvent diluent ...**or**

1

2. a thin film method for those products which are not solvent clearly soluble, primarily the inorganic or so called physical sunscreens...

or

3. a method involving an even thinner film which is placed on an optically flat quartz plate before an integrating sphere device or a spectroradiometer.

All three methods involve the measurement of the transmission spectrum in the near ultraviolet within the range of 320 to 360 nm. In each alternative a “pass-fail” upper limit of transmission is applied. If the transmission exceeds this limit at any point of the range scanned, then a broad spectrum claim cannot be made for the product. As well, a broad spectrum claim cannot be made if the SPF is below 4.

Methods 1 and 2 have appeared in the standard since 1986, whilst

Method 3 was introduced in the 1993 revision. Thus, it can be seen that these earlier methods precede the newer in-vivo methods. It should also be noted that, at the time of the inclusion of these methods, the maximum permitted SPF within the Standard was 15, rather than 30 as it now is.

Since these methods are instrumental, they are fast and comparatively cheap to perform. They also provide a simple go - no go, rather than a scale of UVA performance.

Since there has been no revision of these methods for close to 10 years it can be said that the state of the art in relation to sunscreen formulation as it impacts on the absorption of UVA has somewhat advanced. In principle, we believe that the time is now appropriate for a review of the methodology, intended to support greater efficacy and safety.

In arriving at the options for the Australian Standard, the Sunscreen Sub-Committee of ASMI has considered several *in-vitro* methods in use in other countries, in particular Europe and USA. I will therefore now discuss the instrumental aspects of those methods, no doubt familiar to many of you.

Boots Star Rating

Based on a method originally proposed and published by Diffey and Robson (1) in 1989, this method was taken to the market place by the Boots Company in the UK in the form of the so called "Boots Star Rating" .

As shown in fig 1, the method involves the measurement of transmission over the range of 290 to 400 nm, and after conversion to Absorption, the calculation of the ratio of UVA to UVB. The measurements are made at 5nm increments and the mean is determined for the UVB and UVA parts of the curve. 12 readings are taken at various points over the sample.

Fig 1 “Boots Star” Measurement of UVA/UVB Ratio

The instrument described as being appropriate for determination by this method incorporates a xenon arc lamp as the light source, polychromatic light and a photomultiplier detector. The sample is presented as a thin film on Transpore™ tape. The methodology is fully described in a publication of the Boots Company (2) .

Critical Wavelength

Another methodology proposed by Diffey (3) , Critical Wavelength represents primarily a manipulation of the same data provided by the UVA/UVB star measurement described above. However, in this technique the “critical” wavelength is defined as the point on the spectrum at which 90% of the total area under the curve occurs, when calculated beginning at the UVB end (290 nm). This point must be at least at 370 nm.

This newer method also stipulates that the sample be pre-irradiated in order to ensure that the effects of photo degradation that would impact on the product during SPF testing, or in-use, be taken account of. Apart from this addition, it is recognised that this critical wavelength value can be determined by using the same equipment as described for the previous method. It should also be noted that the sample is not pre-irradiated in the measurement instrument. This must be conducted using a Xenon arc light source.

| | |

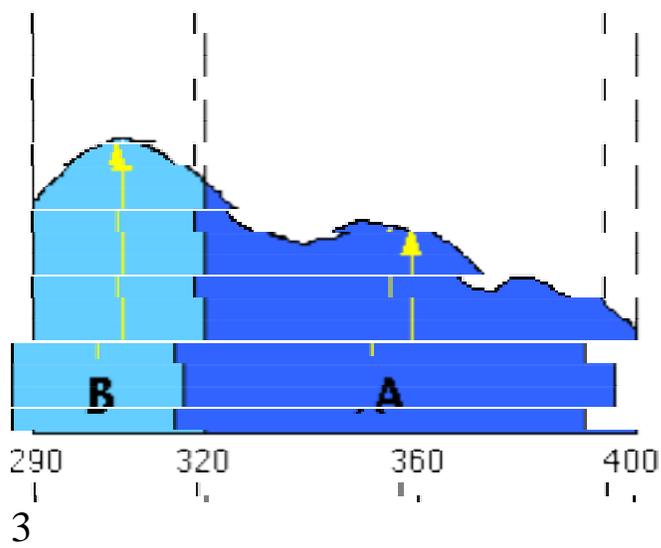
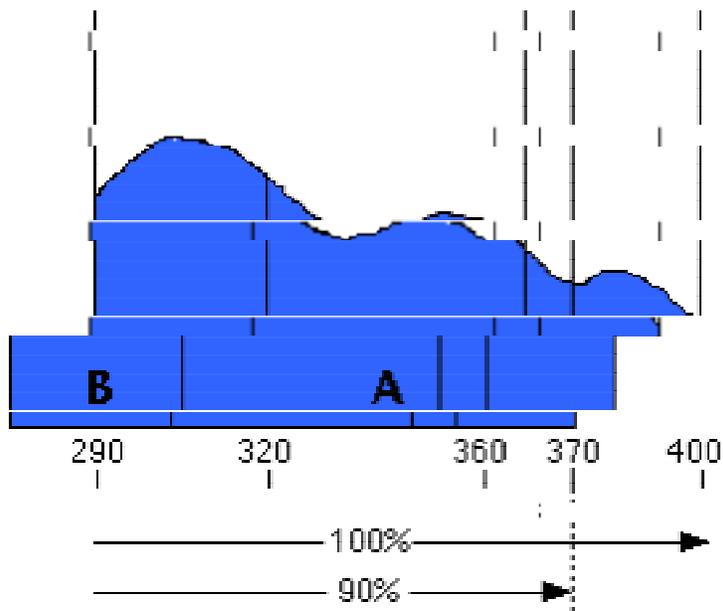


Fig 2 Critical Wavelength Determination

| | |



As can be seen from the above, both of these methods, now in wide usage, involve the presentation of the sample as a thin film. This contrasts with method 1 and 2 of the current Australian Standard.

If we are to consider the development of a thin film method in line with international developments, then we should now consider the physical factors which may impact on this aspect of the technique.

Issues impacting on determination by thin film methods.

The first issue which must be considered is the selection of film thickness. For the in-vivo SPF test, the internationally consistent application rate is 2 mg/ sq cm. Measurement of SPF at this film thickness is, from experience, practical for application to the skin of test subjects and reproducible in terms of providing an even application over a test area.

There are those who would argue in support of a lower application rate, both for SPF and for UVA measurements. However, the considerations are very much the same for both techniques. That is, a lower application rate has an impact on reproducibility, particularly in relation to ruggedness - variations from lab to lab. Certainly, the appearance of the spectral curve varies with thickness, and such variation is not consistent from product to product.

Instrument resolution must be suitable. The light sources used in the spectrophotometer and spectroradiometers generates continuous output over the target spectrum, but the intensity

4

is much greater at the 400 nm end. The instrument must therefore be capable of providing sufficient resolution over the entire range.

Photostability of the product in thin film will impact on the measurement of UVA performance. Whilst it can be said that it is not a prerequisite for a sunscreen formulation to be photostable in order to provide SPF performance, instrumental UVA measurement will give reduced performance where photodegrading formulations are being tested.

Forward scatter of light by inorganic physical screens and by excipients is also a consideration. The instruments which incorporate an integrating sphere are specifically designed to capture this additional transmitted light and include it in the measurement.

In view of this latter consideration, it would appear that the most appropriate instruments of measurement of UVA would be those which include an integrating sphere in their optical configuration. The sphere is internally coated with highly reflective Barium Sulphate .

A number of systems are commercially available and vary in design as shown in fig 3 to 5.

Fig 3 Integrating Sphere SPF Analyser - External Polychromatic Light Source

In this system the lamp emits at low intensity from a Xenon Arc source. The sample is irradiated for a short period and the attenuation approximates sunlight.

5

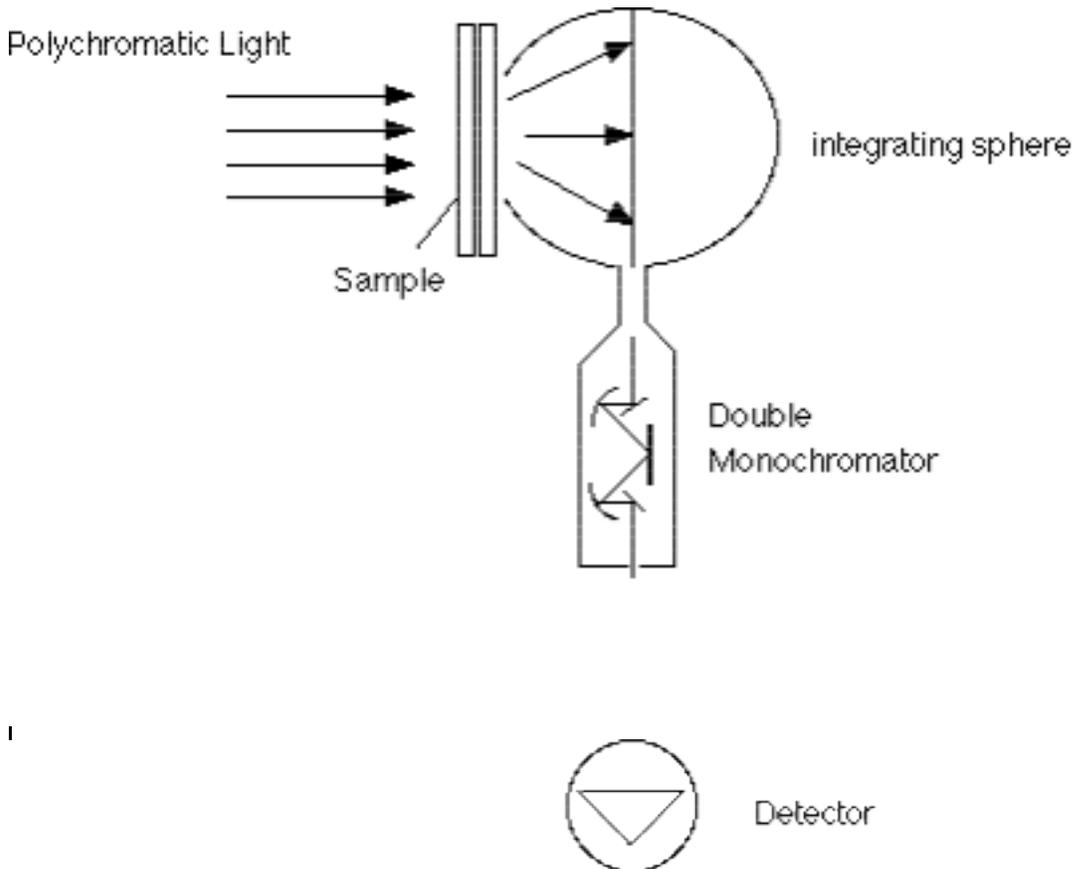
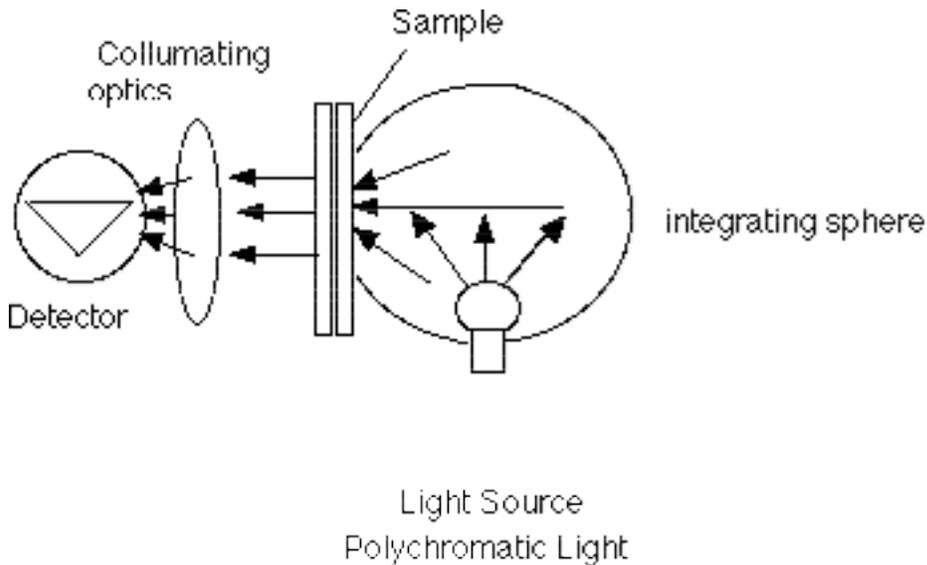


Fig 4 Integrating Sphere SPF Analyser - Internalised

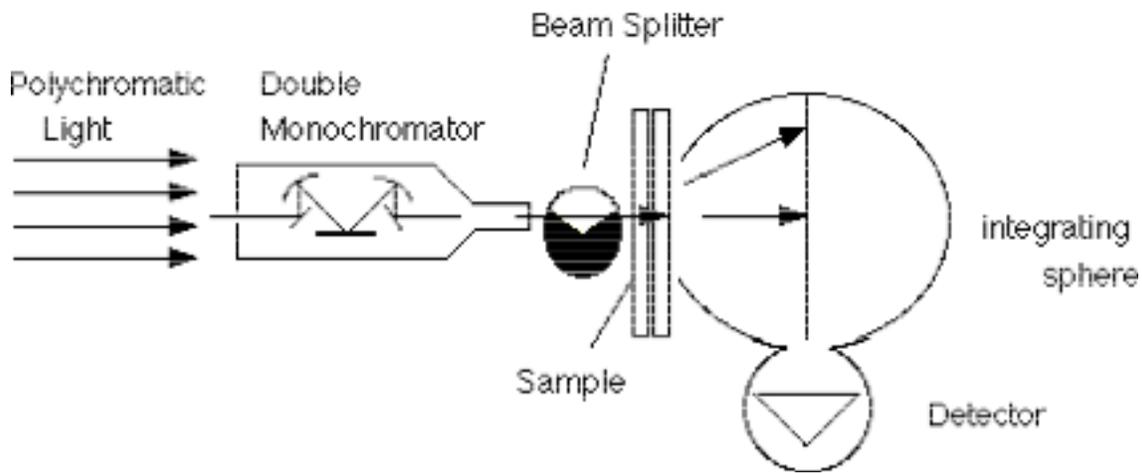
Polychromatic Light Source



This instrument reverses the configuration of the optical paths, such that the light source is contained within the sphere and the scattered light is presented through the sample. The light source is a flash Xenon lamp and exposure process time is around 5 seconds. Fibre optics provide the reference beam.

Fig 5 Integration Sphere with UV/Vis Spectrophotometer

This configuration is that of a typical UV visible spectrophotometer, but with the addition of an integrating sphere. Deuterium and Halogen lamps form the light source, and a beam splitter is used to provide a reference beam.



Method Reproducibility

In proposing suitable instruments capable of providing method reproducibility, the following studies provide support. A collaborative study completed by an NDMA task force, in preparation for a submission to the FDA Final Monograph as data in support of critical wavelength has been extrapolated by Dr Nearn (4). in order to arrive at the following chart.

Fig 5 Calculation of UVA/UVB Ratio from Inter-laboratory Results

Lab	5% Oxybenzon	e ZnO Oint	CTFA Control	Mak
*A	0.47	0.86	0.44	0.68
** B	0.52	0.85	0.39	0.84
** C	0.50	0.88	0.38	0.84
*E	0.49	0.87	0.31	0.65
Mean	0.495	0.865	0.38	0.75
S.D.	2.08	1.29	5.35	10.18

In all cases, pre-irradiation was completed. Some participants evaluated both with and without integrating sphere. It should be noted that several film thicknesses were evaluated. Whilst there is good correlation of results of single active products, variability shows with more complex formulations. The participants in the study also had the option of choice of the product substrate. It would seem that methodology must be carefully specified in order to reduce this risk of variability. It is also evident that film thickness variation does not provide linearity.

Results of a more comprehensive collaborative study (6) were recently presented at the IFSCC Congress in Berlin. Figure 6 summarises the results of the interlab round robin, which evaluated both in-vitro methods for both SPF and UVA. Again, integrating sphere devices were present in all instruments used.

The authors, from the Sunscreen Task force of the German Society for Scientific and Applied Cosmetics concluded that, whilst there was lack of reproducibility of SPF measurement, the critical wavelength as well as the UVA/UVB ratio was found to be reproducible and comparable.

7

Fig 6 UVA/UVB Ratio of 9 products as determined in 8 laboratories.

Prod A B C D E F G H I

										K
Lab 1	0.65	0.64	0.36	0.48	0.53	0.19	0.55	0.27	0.32	0.45
Lab 2	0.62	0.60	0.33	0.43	0.50	0.16	0.51	0.25	0.31	0.42
Lab 3	0.67	0.60	0.40	0.48	0.58	0.23	0.60	0.34	0.35	0.42
Lab 4	0.63	0.63	0.39	0.43	0.55	0.18	0.52	0.30	0.33	0.37
Lab 5	-	0.64	0.33			0.17	0.30	0.27		
Lab 6	0.63	0.64	0.36	0.47	0.54	0.21	0.53	0.29	0.32	0.39
Lab 7	0.66	0.64	0.36	0.46	0.55	0.20	0.51	0.29	0.36	0.40
Mean	0.64	0.63	0.36	0.46	0.54	0.19	0.50	0.29	0.33	0.41
S.D.	3.10	2.40	7.60	5.10	4.80	12.20	18.80	9.70	6.00	6.60

The laboratories in the above study utilised the following instrumental systems.

Instrument

Optometrics (3 participants) VarianPerkin ElmerDr. Kockott

Instrumental Validation

Light Source

Xenon Arc Deut/Halogen Deut/Halogen Xenon Arc

Beam

polychromatic mono-chrom mono-chrom polychromatic

For the selected instrument, it is important to ensure validation and calibration. Licenced sunscreen manufacturers in Australia are required to comply with TGA Requirements under the Code of GMP - Appendix D - Guidelines for Laboratory Instrumentation; For spectrophotometers, these guidelines set out requirements for range, wavelength accuracy, photometric accuracy, resolution, stray light and equipment maintenance and provide specifications for the quality and performance of quartz cells, which can easily be interpreted to the quartz plates used in UVA instruments.

8

In conclusion, it can be said that the most supportable instrumental method would appear to be that which can accurately and reproducibly provide the most information with the least cost. The instrument envisaged as being suitable for the ASMI supported

UVA method first proposed by Dr. Malcolm Nearn is ideal in that the data obtained can also be used to determine the values for compliance with the current AS/NZS 2604 Method 3, Boots Star, Critical Wavelength and UVA/UVB Ratio. Not only does it provide an improvement over the current Australian methodology, but it also supports compliance with data requirements for other markets.

(1) Ref : B. Diffey, J. Robson. A new substrate to measure sunscreen protection factors throughout the ultraviolet spectrum. J.Soc.Cosmet. Chem, 40, 127-133 May/June 1989.

(2) The Guide to Practical Measurement of UVA/UVB Ratios

(3) Ref : Diffey, B.L. A method for broad spectrum classification of Sunscreens Int. J.Cosmetic Sci.16, 47-52 (1994)

(4) Ref: M. Nearn - unpublished extrapolation from : CTFA/NDMA task force report on Critical Wavelength Determination for the Evaluation of the UVA Efficacy of Sunscreen Products, April 1996 .

(5) Ref : H. Gers-Barlag, R. Bimczok, H. Driller, P. Finkel, H.U. Gonzelbach, U. Heinrich, W. Johncock, K. Juhkason, E. Klette, D. Kockott, R. Langner, F.PFlucker, T., Rudolph, P., Schneider, C.Springob,H.Tronnier. Multicenter comparison of sunscreen by in-vitro determination of relative parameters. XX1 IFSCC International Congress, Berlin Sept 2000