A new in vitro screening method to assess water resistancy

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Abstract

Background: Recently, an increased number of methods for determining the ultraviolet protection factor have been proposed. But *in vitro* water resistance is yet not validated.
Here, the authors describe a new method for determining the *in vitro* water-resistance of sunscreen products based on the SPF *in vitro* determination as recommended by Cosmetics Europe for the sunscreen application and spreading and on a previous publication for the immersion aspect and the absorption of sunscreens in dilute solution.
Methods: This protocol in 4 steps consists in studying the absorbance of sunscreens extracted from a product before and after rinsing to determine a water resistance index correlated to the *in vivo* ISO 18861 & ISO 16217.
Results: The statistical analysis of the obtained results demonstrated that the method is repeatable and reproducible. The correlation with the results of the *in vivo* water-resistance determination was satisfactory whatever the SPF level or the % of water-resistance of the product.
Conclusion: The *in vitro* water resistance percentages obtained for the 11 products tested were then found to be reproducible and in line with the results obtained *in vivo*. This new method seems to provide a more accurate water resistance percentage from the product tested, since they are not/less influenced by the redistribution of the thin film. We concluded that this new technical approach seems promising and might become a good candidate for validation in a ring test.

Keywords: Water resistance - *in vitro* – ISO - film-forming - spectroscopy

Introduction. Recently, an increased number of methods for determining the ultraviolet (UV) protection factor have been proposed. But *in vitro* water resistance is yet not validated. In previous studies, the authors demonstrated that the absorbance value of sunscreen depends on the uniformity/homogeneity of spreading on the test substrate [1]. This uniformity of spreading can be modified by different factors such as heat or ventilation as it has already been demonstrated elsewhere [2-3], but also by the interaction with water which can modify the film homogeneity of the sunscreen. This is one reason why spectroscopic methods based only on UV transmission to measure the water-resistance *in vitro* can lead to reproducibility issues.

Here, the authors describe a new method for determining the water-resistance *in vitro* of sunscreen products based on

- the SPF *in vitro* determination as recommended by Cosmetics Europe for the sunscreen application and spreading [4-6]
- and on a previous publication for the immersion aspect and the absorption of sunscreens in dilute solution [7].

Materials and Methods. This 4 steps protocol consists in studying the absorbance of sunscreens extracted from a product before and after rinsing to determine a water resistance index correlated to the in vivo ISO 18861 & ISO 16217 [8-9].

The extracted solutions obtained must be compared to sunscreen dilutions to evaluate extraction efficiency. Figure 1

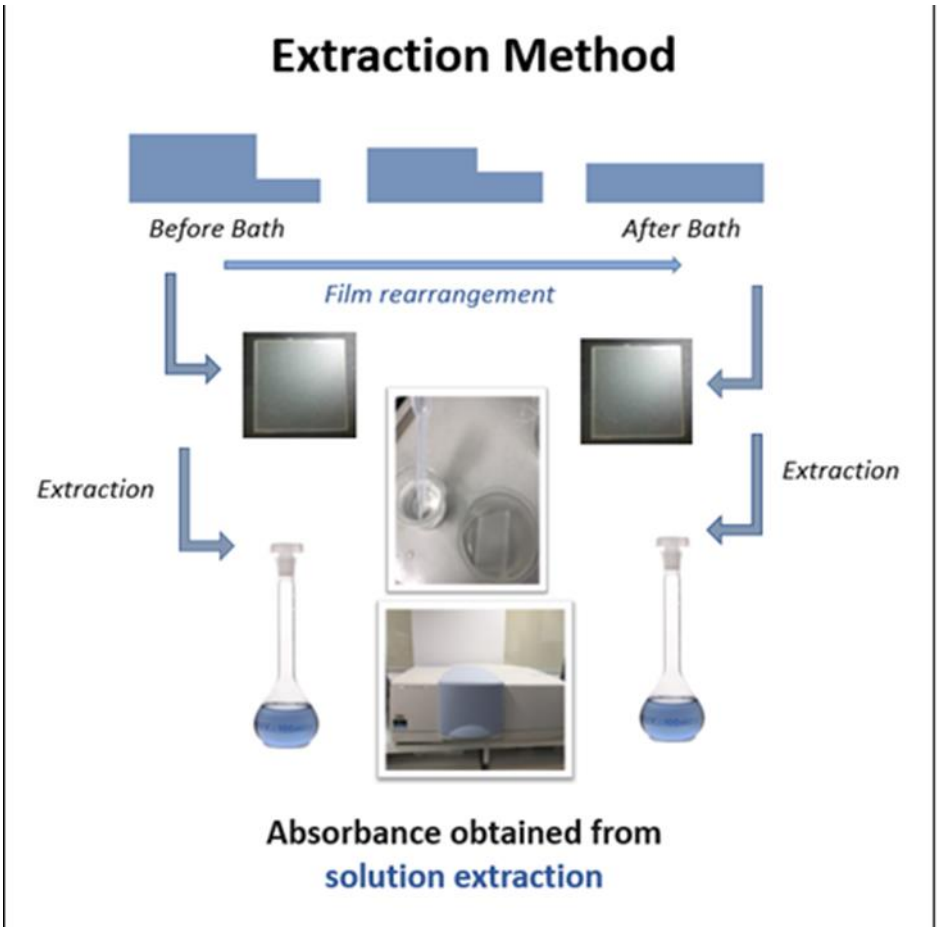

Figure 1: Extraction method principle

Dilution: Weigh directly in a 50mL volumetric flask 26.5mg ± 0.5 of sunscreen, add 50 mL of isopropanol.

Extraction Step 1: Sunscreen application: Weigh 26,5 mg of studied sunscreen on SB6 PMMA plates and spread it thanks to a spreading protocol used to determine the SPF recommended by Cosmetics Europe. Spread 3 plates for the "Before Bath" (BB) measurement and 3 plates for the "After Bath" (AB) measurement.

Extraction Step 2: Plates rinsing: Rinse the 3 plates spread for the "After Bath" measurement with a pipette over its entire surface turning it methodically with 40mL of demineralized water then let dry it for 30 minutes in an oven at 30°C (figure 2).

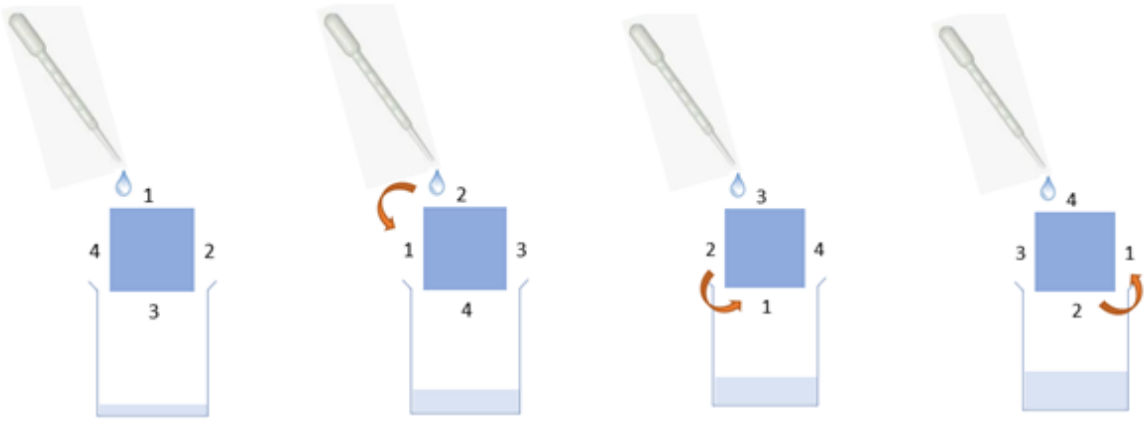

Figure 2: Plates rinsing process

Extraction Step 3: Sunscreen filters extraction: Immerse each plate in a 250ml beaker using approximately 45mL of Isopronol and extract the filters from the sunscreen thanks to 15 minutes of ultrasound divided into 3 stages to avoid a rise in the temperature of the ultrasonic bath. Recover the beaker solution into a 50ml volumetric flask then make up to the mark after few minutes.

Extraction Step 4: Absorbance measurements of the extracted solutions: Blank with Isopropanol then analyze each solution from the less concentrated to the more concentrated thanks to a UV spectrophotometer, from 290 to 400nm using a 1mm cuvette.

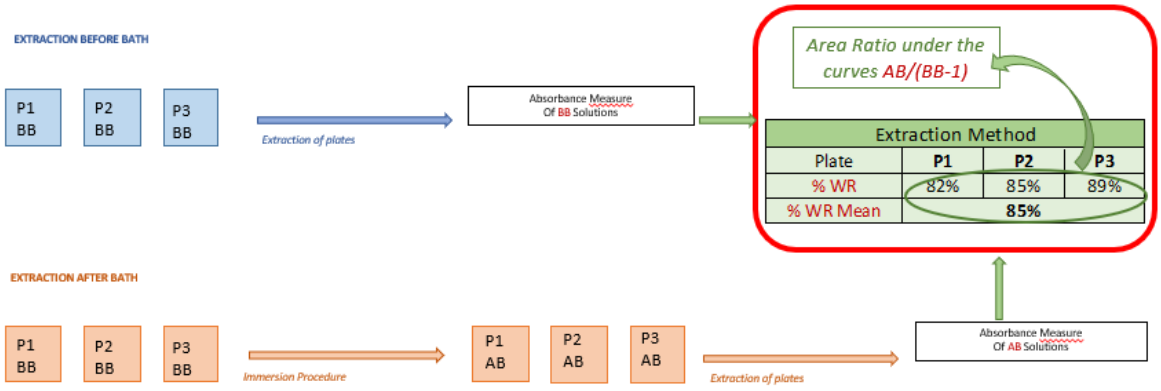

Figure 3: Water resistance % calculation

The area under the absorbance curve before bath (BB) and after bath (AB) are calculated (figure 3).

The percentage of water resistance is then calculated using the following formula 1:

%WR = (ABx100)/(BB-1)

Results. Extracted solution absorbance is used to calculate the *in vitro* percentage of water resistance (Table 1).

SPF Claim	Base	%WR Vivo	%WR Extraction
30	O/W	**53**	**35**
50+	O/W	**51,8**	**48**
30	W/O	**64,5**	**71**
30	W/O	**71**	**77**
30	O/W	**50,3**	**31**
11,5	O/W	**68**	**85**
50	O/W	**30,7**	**40**
30	O/W	**63,3**	**65**
50+	O/W	**24**	**34**
30	O/W	**51,5**	**50**
30	O/W	**44,7**	**23**

Table 1: % of water resistance *in vivo* and *in vitro*

The statistical analysis of the obtained results demonstrated that the method is repeatable and reproducible (figure 4).

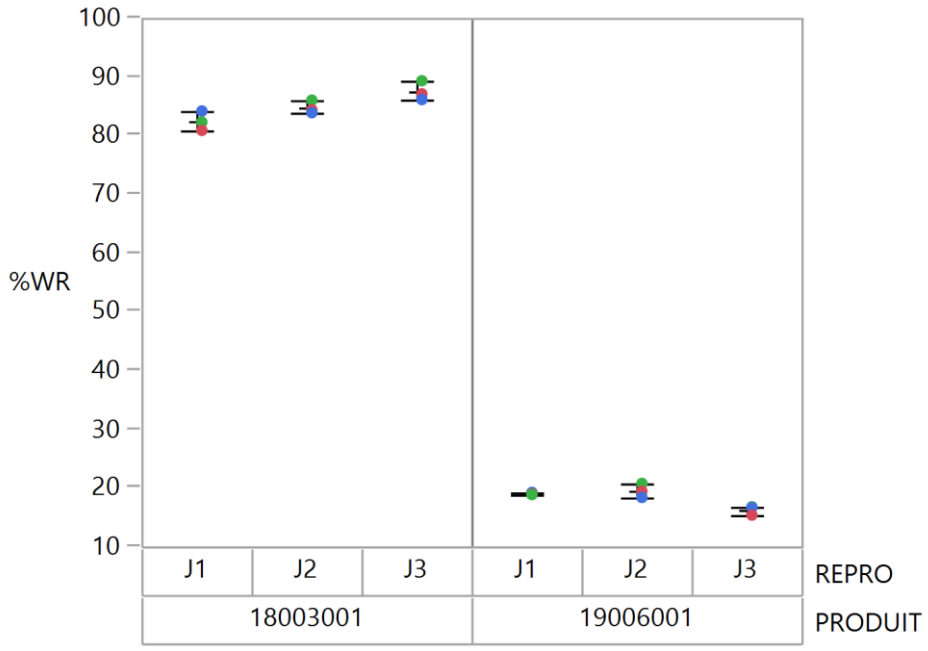

Figure 4: Statistical analysis.

The correlation with the results of the *in vivo* water-resistance determination (ISO methods) was satisfactory whatever the SPF level or the % of water-resistance of the product (figure 5).

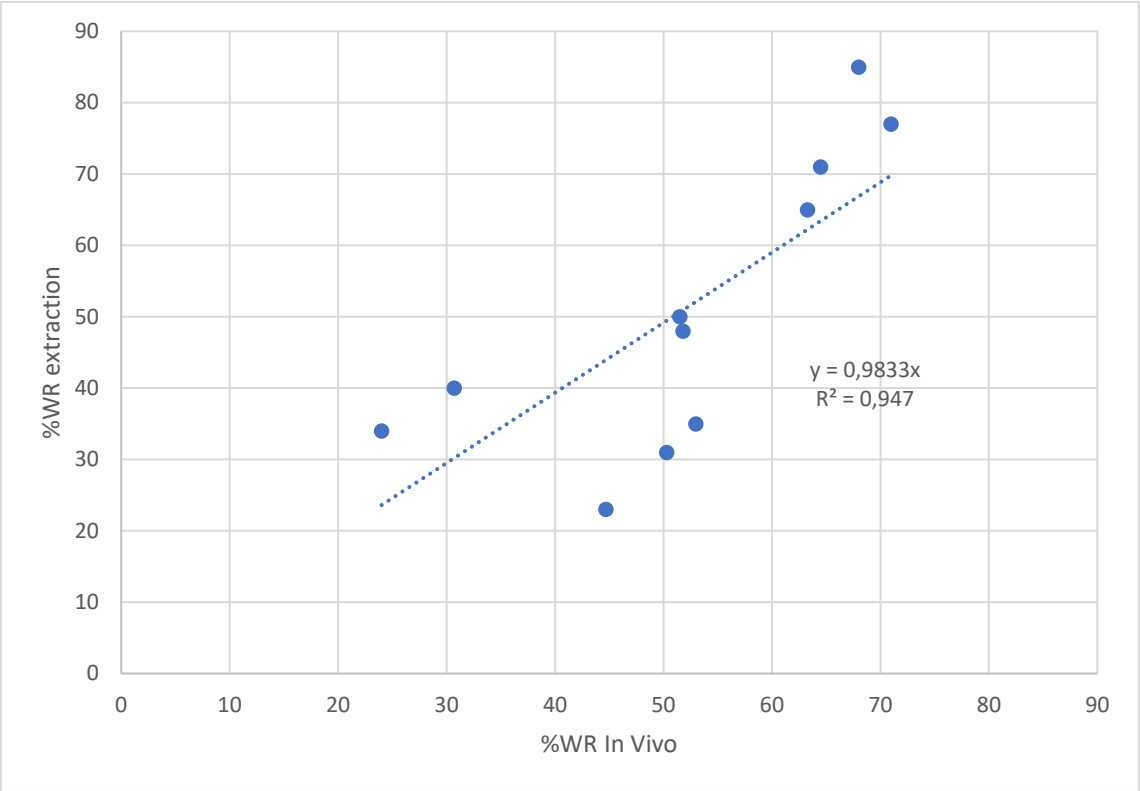

Figure 5: *in vivo* – *in vitro* correlation

Discussion. The even spreading of the sunscreen onto the substrate is key to the performance of transmission spectroscopy on a thin film [10-13]. Until this is mastered, it is not possible to obtain reproducible intra or interlaboratory results. In some cases, the rearrangement of the product can form a film distributed more evenly than before immersion, leading to an artificial increase of absorbance values. This results in a water resistance percentage that is higher than that found by *in vivo* tests, and which can be more than 100%.

In other cases, rearrangement can form a film distributed less evenly than before immersion, resulting in lower artificial absorbance values, and a water resistance percentage lower than that found *in vivo*.

The protocol was thus changed to involve a spectroscopic technique in dilute solution.

The relationship between *in vitro* and *in vivo* % of water resistance values can be seen in Figure 5. Simple regression analysis revealed a strong correlation between *in vitro* and *in vivo* values, with a r^2=0.98, a slope of 0,947.

These results encourage us to test this new *in vitro* water resistance method on a larger scale by the industry in order to validate it on a larger number of products and laboratories.

Thanks to this new method, it is possible to obtain *in vitro* water resistance percentage values which are not biased by the possible rearrangements of the thin film since the product is dissolved in a solvent. These results give the actual *in vitro* percentages of water resistance affecting the products.

Conclusion. A method for determining *in vitro* the water resistance percentage based solely on UV transmission is often inaccurate since it involves taking the absorbance measurement twice in the calculations—once before and once after immersion. The resulting ratio will therefore show significant variations.

The protocol was thus changed to involve a spectroscopic technique in dilute solution in order to avoid absorbance measurements of thin films.

The *in vitro* water resistance percentages obtained for the 11 products tested were then found to be reproducible and in line with the results obtained *in vivo*. This new *in vitro* water-resistance method seems to provide a more accurate water resistance percentage from the product tested, since they are not/less influenced by the redistribution of the thin film. We concluded that this new technical approach seems promising and might become a good candidate for validation in a ring test.

Conflict of Interest Statement. NONE.

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