Analytical Chemistry: A Journal (2025)
Clausius Scientific Press, Canada

DOI: 10.23977/analc.2025.040106
ISSN 2616-1958 Vol. 4 Num. 1

Comparative Evaluation of UV Spectrophotometry for Sun Protection Factor (SPF) Determination in a Reproducible in Vitro Method

Hanxuan Kong

Shanghai Shangde Experimental School, Shanghai, China

Keywords: Ultraviolet Radiation, Sunscreen, SPF, UV–Vis Spectrophotometry, Photostability, Labeling Accuracy

Abstract: Ultraviolet (UV) radiation is a recognized carcinogen, with UVA (320–400 nm) and UVB (290–320 nm) contributing to photoaging, skin damage, and cancer. Sunscreens mitigate these risks, but there are discrepancies between labeled and actual Sun Protection Factor (SPF) values. This study employed UV–Vis spectrophotometry to assess SPF accuracy and photostability of commercial formulations under dark storage and natural sunlight over three weeks. Most products showed measured SPF values consistent with labels, though SkinBoard and L'Oréal were significantly lower, and Vaseline Brightening Skin Isolation fell within an acceptable deviation. SPF values declined across all samples, with slower reductions under dark storage. Dabao Watery Multi-action displayed the highest photostability, while SkinBoard declined most rapidly. These results prove UV–Vis spectrophotometry as a reliable, ethical alternative to in vivo testing and emphasize the need for improved quality control and photostability in sunscreen development.

1. Introduction

1.1 Ultraviolet Radiation and Its Biological Impact

The sun serves as the primary source of energy and radiation reaching Earth, emitting a wide spectrum of radiation, including a significant portion in the ultraviolet (UV) range [1]. Although UV radiation is invisible to the human eye and cannot be directly sensed, exposure to solar UV radiation is universal and unavoidable. The existence of UV light was first identified by a German physicist Johann Wilhelm Ritter in 1801, and its electromagnetic spectrum spans wavelengths from 100 to 400 nanometers [2]. Traditionally, UV radiation is divided into three categories: UVA (320–400 nm), UVB (280–320 nm), and UVC (100–280 nm) [3]. In recent years, this classification has been refined to include subdivisions such as narrowband UVB (311–313 nm), UVA2 (320–340 nm), and UVA1 (340–400 nm), based on their distinct biological and photophysical effects. Due to its ability to induce mutations, UV radiation was officially recognized as a human carcinogen in 2002 by the WHO International Agency for Research on Cancer (IARC) [4]. Additionally, numerous studies have demonstrated that prolonged exposure to UVB (290–320 nm) and UVA (320–400 nm) radiation is directly correlated to skin damage, photoaging, and carcinogenesis [5].

Mechanically, UVB have shorter wavelengths and higher energy levels than UVA. Because of these higher energy levels, UVB has better penetrating abilities and contributes to photoaging, pigmentation, and skin cancer [6]. The effects of UVB radiation on target cells and tissues are triggered by molecular and cellular damage pathways that are induced when UVB radiation is absorbed by chromophores present in the skin [7]. The skin is abundant in UV chromophores, which are molecules or molecular regions that absorb UV energy to facilitate biochemical reactions [8]. These chromophores include DNA, urocanic acid, aromatic amino acids, retinoids, carotenoids, bilirubin, flavins, and hemoglobin.

1.2 Sunscreens and Mechanisms of UV Protection

Extended exposure to ultraviolet B (UV-B, 290–320 nm) and ultraviolet A (UV-A, 320–400 nm) radiation has been shown to contribute significantly to skin damage, premature aging, and the development of skin cancer [9]. The increasing awareness of these harmful effects of UV radiation on human skin has driven extensive research on skin products that reduce UV radiation [10]. Sunscreens, which contain compounds that either absorb or reflect UV rays, play a critical role in protecting the skin from these harmful effects. Until today, sunscreen is the most commercially applied product in reducing risks of UV skin damage [11]. Organic, or chemical, sunscreens function by absorbing ultraviolet (UV) radiation through specific molecular structures such as aromatic rings linked to carbonyl groups. These configurations enable the compounds to absorb high-energy UV photons, enter an excited state, and then dissipate the energy as lower-energy light or heat as they return to their ground state [12]. The absorption spectrum of each compound varies, allowing them to target specific UV wavelengths. Chemical sunscreens generally contain a combination of agents that absorb UVB (290–320 nm) and portions of UVA radiation. While UVB filters effectively cover the full UVB range, most UVA filters do not span the entire UVA spectrum. UVA rays are further categorized into UVA II (320–340 nm) and UVA I (340–400 nm) [13]. Broad-spectrum formulations are designed to offer protection across both UVA and UVB ranges.

1.3 SPF Measurement through UV–Vis Spectrophotometry

The effectiveness of a sunscreen product is typically evaluated using the Sun Protection Factor (SPF), a metric that reflects its capacity to shield the skin from UV-B–induced redness or sunburn [14]. Traditional SPF determination relies on in vivo testing, a method constrained by ethical concerns, high costs, and inter-subject variability [15]. To address these limitations, in vitro approaches such as UV-Vis spectrophotometry have emerged as promising alternatives. This technique measures the transmittance of UV radiation through a sunscreen film applied to an artificial substrate, enabling rapid and reproducible SPF calculations [16]. Recent studies validate the utility of UV-Vis spectrophotometry in SPF assessment. For instance, Cao and Xiao (2013) systematically analyzed the correlation between sunscreen transmittance in the UV range (290–400 nm) and its protective efficacy. Their experimental protocol, which involved measuring absorbance spectra of various sunscreen formulations, provides a foundational framework for standardizing substrate selection and film thickness. Similarly, Mansur et al. (2007) demonstrated that spectrophotometric SPF values support in vivo results across diverse formulations, including organic and inorganic UV filters. Recent advancements include a simplified spectrophotometric protocol using a single-layer substrate and derived SPF values through integration of absorbance data across the UV-B range by Tsai and Chen (2005).

Despite these innovations, challenges persist in standardizing in vitro methodologies. Berardesca et al. (2003) identified discrepancies in SPF values due to variations in substrate texture and sunscreen application techniques, diminishing the need for stringent experimental controls. Moreover,

commercial manufacturers employ varying methodologies for determining sun protection factor (SPF) values. In particular, the presence of non-active excipients capable of absorbing distinct regions of the light spectrum may result in discrepancies between the labeled and actual SPF values. Variability introduced by advertising practices and differences in acceptable margins of error may further contribute to these inconsistencies. Based on these recent approaches, our study aims to apply standardized UV–Vis spectrophotometric techniques to determine SPF values. We will first compare experimentally measured SPF with the corresponding labeled values to assess labeling accuracy. Subsequently, we will evaluate the effects of time and light exposure on sunscreen products by quantifying changes in SPF, with an emphasis on improving reproducibility across heterogeneous formulations. Ultimately, this work seeks to improve regulatory standards and support the development of high-efficacy sun protection products.

2. Method

2.1 Sample Preparation

To prepare sunscreen samples for UV absorbance analysis, 1.00 ± 0.01 grams of each formulation was accurately weighed and diluted with 12.7 mL of anhydrous ethanol (200 proof, ≥99.5%) to achieve a 1:10 weight-to-volume (w/v) solution. This dilution ratio was selected to ensure optimal transmittance for spectrophotometric analysis within the UVB range. The mixture was then subjected to ultrasonic treatment in a water bath for five minutes to eliminate entrapped air bubbles and to ensure thorough homogenization of the active and inactive components within the ethanol solvent [17]. For formulations with emulsion bases, the solutions were further filtered through 0.45 μm nylon membrane filters to remove any residual particulate matter that could cause light scattering or interfere with absorbance readings during UV-Vis spectrophotometry [18].

Following sample preparation, the solutions were divided into two treatment groups to assess photostability profiles. Group 1 consisted of samples A and B, which were transferred into quartz vials and stored in a dark environment at controlled room temperature (~25 ℃) to assess thermal stability in the absence of light-induced degradation. The average solar irradiance during the exposure period was approximately 3.62 kWh/m ²per day, simulating realistic environmental conditions [19]. Group 2 included Samples C through J, which were sealed in identical quartz vials and exposed to natural sunlight in Shanghai, China. All samples were stored under their respective conditions for a total duration of 3 weeks, and aliquots were collected at three key time points (week 1, week 2, and week 3) for analytical evaluation.

2.2 Measurement of UV Absorption

The UV absorbance of each sample was recorded using a UV-Visible spectrophotometer equipped with an integrating sphere (UV-IS) to ensure accurate and diffuse light detection. Measurements were performed using 1 cm path-length quartz cuvette. Anhydrous ethanol was used as the reference blank for baseline correction to eliminate solvent background and standardize optical density readings. The absorbance spectra were collected across the ultraviolet B (UVB) range of 290 to 320 nm, which is clinically relevant for erythema and sunburn protection. This method allowed for the quantitative assessment of each formulation's UV-filtering efficacy over time and under different environmental conditions, while retaining the stability and performance of chemical sunscreen agents.

2.3 SPF Value Determination

The in vitro Sun Protection Factor (SPF) of each sunscreen formulation was calculated using a

spectrophotometric method based on the absorbance of UVB radiation. Measurements were conducted across the 290–320 nm wavelength range, which is most relevant for erythema induction. The SPF values were determined using the equation (1):

$$\mathrm{SPF}_{\mathrm{spectrophotometric}} = \mathrm{CF} \times \sum_{\lambda=290}^{320} EE(\lambda) \times I(\lambda) \times \mathrm{Abs}(\lambda) \tag{1}$$

Where EE(λ) represents the erythemal effect spectrum, I(λ) denotes the solar intensity spectrum specific to Shanghai, China, and Abs(λ) is the measured absorbance of the sunscreen sample at each wavelength λ. A correction factor (CF) of 10 was applied in accordance with standard methodology to normalize the calculated SPF values for comparison with empirical in vivo data. Absorbance readings were obtained at 5 nm intervals using a UV-Vis spectrophotometer, as described in Section 2.2. The product of EE(λ), I(λ), and Abs(λ) was summed across the entire wavelength range to generate a representative SPF value for each sample.

3. Results

3.1 Determination of Labeled SPF Accuracy

Our findings suggest that, for most samples, the measured SPF values closely matched the labeled SPF values, either being identical or slightly lower. However, SkinBoard and L'Oréal exhibited significantly lower measured SPF values compared to their labeled claims. Vaseline Brightening Skin Isolation also demonstrated lower measured SPF values relative to the labeled values, though the difference remained within an acceptable range (Figure 1 & 2).

3.2 Evaluation of Light-induced Degradation

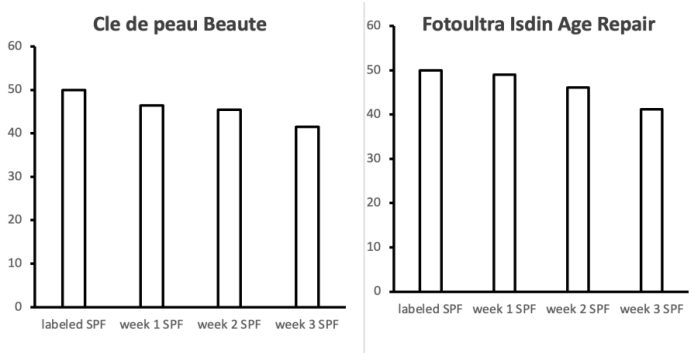

Figure 1 Comparison of labeled SPF values and the average spectrophotometrically determined SPF values at three time points (week 1, week 2, and week 3) for Group 1 sunscreens in absence of sunlight. A minimal decline in SPF effectiveness is observed over time, likely due to thermal instability which reduced UV absorption efficiency.

Our findings indicate a general decline in SPF values for all sunscreen samples in both Group 1(covered) and Group 2 (exposed) over the three-week observation period. In no instance did the spectrophotometrically determined SPF exceed the labeled SPF values. Notably, Samples in Group 1 have significantly slower and more consistent decline of SPF values over the three-week observation period (Figure 1). Among all samples exposed to light, Dabao Watery Multi-action has the lowest reduction rate. Skin Board had the highest rate of reduction from the first week into the second week. Dabao Facial, Vaseline, and Florasis all decrease to an SPF value close to 42 after three

weeks of observational period. The weather during the third week in Shanghai was characterized by intermittent cloud cover and light rainfall, which may have influenced the ambient UV exposure and, consequently, the apparent SPF stability in some samples. Due to this variability, data from the UV-protected control experiment were excluded from analysis. The unexpected increase in SPF values for certain samples in the third week, relative to the second week, may be attributed to inconsistencies in sample extraction concentration or possible instrument variability related to the UV-IS spectrophotometer. These factors indicate the importance of environmental control and equipment calibration in the interpretation of in vitro SPF measurements.

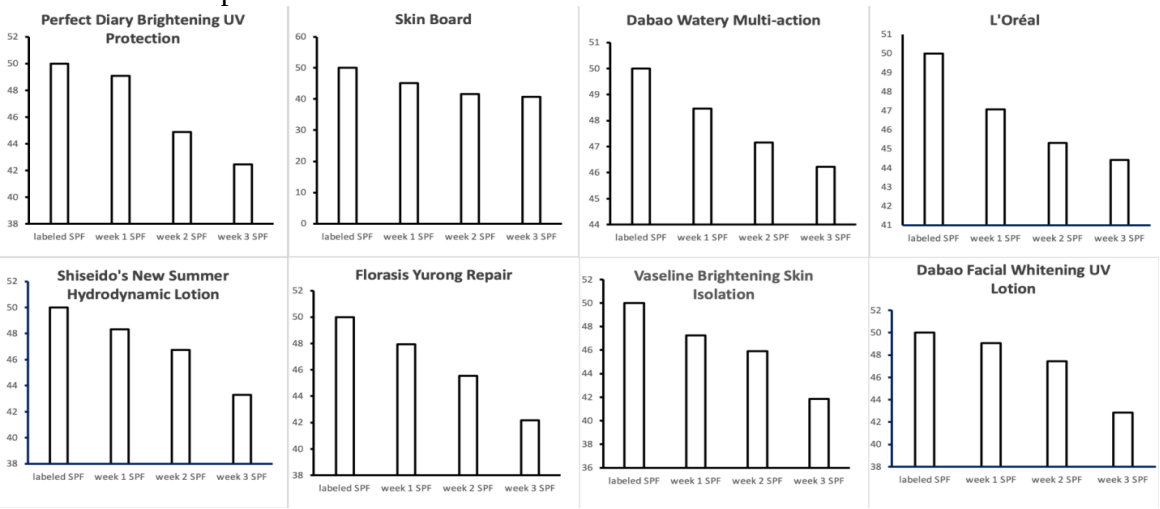

Figure 2 Comparison of labeled SPF values and the average spectrophotometrically determined SPF values at week 1, week 2, and week 3 for Group 2 sunscreens. A large decline in SPF effectiveness is observed over time, likely due to light-induced degradation.

4. Conclusion

Direct measurement of UV radiation (UVR) attenuation through spectrophotometric analysis should be prioritized in sunscreen evaluation, as it provides an objective and reproducible assessment of photoprotective efficacy across the entire UV spectrum. Unlike traditional in vivo SPF testing and persistent pigment darkening (PPD) assays, this method does not expose human participants to potentially harmful levels of radiation. Moreover, testing protocols should adopt solar simulators that more accurately reflect the spectral composition of natural sunlight, particularly the higher UVA-to-UVB ratio observed in typical outdoor environments.

In the current regulatory landscape of sunscreen products, many commercially available sunscreen products advertise SPF values that are not substantiated by laboratory-based measurements of UVR attenuation. This disconnect between in vivo erythema-based SPF values and spectrophotometrically measured UV absorption contributes to inadequate UVA protection in formulations—a shortcoming that places them behind international standards, particularly those in Europe and Asia. Transitioning away from erythema-dependent in vivo SPF testing toward validated in vitro methods, coupled with the approval and availability of a broader range of modern UVA filters, would significantly enhance the effectiveness of sunscreen products. Such reforms are essential for improving consumer protection and advancing public health efforts aimed at mitigating the risks associated with chronic and cumulative UV exposure.

Variability in SPF data can often be attributed to the use of non-validated spectrophotometric methods for assessing the UV absorption characteristics of sunscreen agents. While spectrophotometry offers a valuable in vitro approach, the reliability of results is influenced by

numerous formulation and methodological factors. One major source of variability is the choice of solvent used to dissolve sunscreen activities; different solvents can alter the solubility, stability, and absorbance behavior of UV filters. Additionally, the specific combination and concentration of active ingredients, as well as the overall formulation type (e.g., oil-in-water or water-in-oil emulsions), play a critical role in modulating UV attenuation. Interactions among vehicle components—including esters, emollients, and emulsifiers—can further influence the bioavailability and spectral properties of UV filters by altering their distribution or microenvironment within the formulation.

Other contributing factors include the formulation's rheological properties, pH, presence of additional active ingredients, and the potential for interaction between the vehicle and the skin surface, all of which can enhance or diminish UV absorption. Notably, excipients themselves may exhibit absorbance in the UV range, potentially overlapping with or masking the absorption bands of the UVA and UVB filters. This is especially relevant in high-SPF formulations (e.g., SPF ≥15), where the influence of excipients and vehicle composition becomes more pronounced. Prior research has shown that solvents and emollients can shift the wavelength of maximum absorbance and alter the intensity of absorption for individual or combined sunscreen agents [20],[21]. However, such solvent effects typically become significant only at high solvent concentrations. Collectively, these factors highlight the need for standardized, validated protocols in spectrophotometric SPF determination to ensure reproducibility and meaningful comparison across sunscreen products.

As noted by Pissavini et al. (2003), accurately determining high SPF values presents significant methodological challenges. Higher SPF ratings are typically associated with greater variability and uncertainty in in vivo measurements, largely due to inter-individual biological differences among test subjects. These variations complicate the reproducibility and reliability of high-SPF evaluations. Consequently, the development of sunscreen formulations that are both effective and safe at high SPF levels requires a comprehensive understanding of not only the UV absorption properties of the active ingredients but also the physicochemical interactions within the vehicle system. Components such as esters, emollients, and emulsifiers can influence the solubility, distribution, and stability of UV filters. These interactions may enhance or diminish the overall photoprotective performance of the formulation. Therefore, the formulator must consider the holistic behavior of the entire system, including excipients and their potential to alter the functional properties of actives, to ensure consistent and reliable SPF performance.

References

[1] International Agency for Research on Cancer (2012). Solar and Ultraviolet Radiation. [online] www.ncbi.nlm.nih.gov. International Agency for Research on Cancer.

[2] Meyers, R. (2001). Encyclopedia of Physical Science and Technology. ScienceDirect.

[3] Gray, N.F. (2014). Ultraviolet Disinfection. Microbiology of Waterborne Diseases, pp.617–630.

[4] Aislyn Oulee, Ahn, G.S., Javadi, S.S. and Wu, J.J. (2023). Phototherapy and DNA Damage: A Systematic Review. The Journal of Clinical and Aesthetic Dermatology, [online] 16(6), p.55.

[5] Matta, M. K., et al. (2015). Comparison of Sunscreen SPF Determination Methods: A Study on Various Approaches and Technologies. Dermatologic Therapy.

[6] Brar, G., Dhaliwal, A., Brar, A.S., Manasa Sreedevi, Ahmadi, Y., Irfan, M., Golbari, R., Zumárraga, D., Yateem, D., Lysak, Y. and Abarca-Pineda, Y.A. (2025). A Comprehensive Review of the Role of UV Radiation in Photoaging Processes Between Different Types of Skin. Cureus, [online] 17(3).

[7] Al-Sadek, T. and Yusuf, N. (2024). Ultraviolet Radiation Biological and Medical Implications. Current Issues in Molecular Biology, [online] 46(3), pp.1924–1942.

[8] Emri, G., Paragh, G. and Tósaki, A. (2018). Ultraviolet radiation-mediated development of cutaneous melanoma: An update. Journal of Photochemistry and Photobiology B: Biology, [online] 185, pp.169–175.

[9] Young, A.R. (1997). Chromophores in human skin. Physics in Medicine and Biology, [online] 42(5), pp.789–802.

[10] Amaro-Ortiz, A., Yan, B. and D'Orazio, J. (2014). Ultraviolet Radiation, Aging and the Skin: Prevention of Damage by Topical cAMP Manipulation. Molecules, [online] 19(5), pp.6202–6219.

[11] Lopes, D.M. and McMahon, S.B. (2015). Ultraviolet Radiation on the Skin: A Painful Experience? CNS

Neuroscience & Therapeutics, [online] 22(2), pp.118–126.
[12] Krull, U.J. and Thompson, Michael. (2003). Analytical Chemistry. Encyclopedia of Physical Science and Technology, pp.543–579.
[13] Wilson, B.D., Moon, S. and Armstrong, F. (2012). Comprehensive Review of Ultraviolet Radiation and the Current Status on Sunscreens. The Journal of Clinical and Aesthetic Dermatology, [online] 5(9), p.18.
[14] González, S., Aguilera, J., Berman, B., Calzavara-Pinton, P., Gilaberte, Y., Goh, C.-L., Lim, H.W., Schalka, S., Stengel, F., Wolf, P. and Xiang, F. (2022). Expert Recommendations on the Evaluation of Sunscreen Efficacy and the Beneficial Role of Non-filtering Ingredients. Frontiers in Medicine, 9.
[15] Mansur, J. A., et al. (2007). Evaluation of sun protection factor (SPF) using spectrophotometry and its application to sunscreen products. International Journal of Cosmetic Science.
[16] Tsai, Y. C., & Chen, S. Y. (2005). Determination of Sun Protection Factor (SPF) of Sunscreen Products by UV Spectrophotometry. Journal of Cosmetic Science.
[17] Shen, L., Pang, P., Zhong, M., Sun, Y., Qayum, A., Liu, Y., Rashid, A., Xu, B., Ma, H., Ma, H. and Ren, X. (2023). A comprehensive review of Ultrasonic assisted extraction (UAE) for bioactive components: principles, advantages, equipment, and combined technologies. Ultrasonics Sonochemistry, 101, pp.106646.
[18] Xu, W., Yu, H., Yang, F., Yang, F., Liu, D., Lu, K., Gao, H. and Song, Y. (2022). Second derivative UV–visible spectroscopy characterizing structural components of dissolved and particulate organic matter in an urbanized river. Environmental Sciences Europe, 34(1).
[19] Prasad, A.A. and Kay, M. (2020). Assessment of Simulated Solar Irradiance on Days of High Intermittency Using WRF-Solar. Energies, 13(2), p.385.
[20] Fitzpatrick, T. B. (1988). The validity and practicality of sun protection factor (SPF) determination in sunscreens. American Journal of Clinical Dermatology.
[21] Berardesca, E., et al. (2003). Evaluation of the effectiveness of sunscreens by skin color and sun protection factor. International Journal of Dermatology.