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# Correlation of *in vitro* SPF values from two laboratories

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**Keywords:** Correlation, R – Squared, Sunscreen Products, SPF

**Objectives:** To compare and correlate *in vitro* SPF values obtained from two different laboratories
**Methods:** Six sunscreen products in markets label with SPF in a range of 7 - 30 (7, 15, 20, 25, 30 and 30 on label), in container closer systems proposed for marketing were evaluated for their SPF values. The *in vitro* SPF values of each sample were determined in two different laboratories; i.e. Chulalongkorn University Drug and Health Products Innovation Promotion Center (CU-D-HIP) and a company laboratory. Both laboratories utilized the same equipment, the Optometric SPF 290 S Analyzer, and the same substrate, Transpore® tape. The only different step in these two laboratory procedures was product weight per square centimeter that applied on the substrate; i.e. around 2 mg/cm$^2$ vs. 13 mg/cm$^2$ of sample on the substrate according to CU-D-HIP vs the company laboratory procedure, respectively. The obtained SPF values were correlated using the linear regression. Two COLIPA reference sunscreen formulations were prepared and employed in this study
**Results:** The SPF values from the company laboratory were about 1.5 times higher than that from CU-D-HIP. The SPF values from the same products obtained from two laboratories, showed linear correlation with high deviation ($R^2$ ≈ 0.60 – 0.65). Sunscreen products containing Diethylamino Hydroxy Benzolyhexyl Benzoate (DHHB) caused high deviation from linear regression since these products were either non-homogeneous or viscous. Product with non-homogeneity or with high viscosity directly affects surface smoothness and weight uniformity of sample when applied on the substrate. Therefore, three sunscreen products were withdrawn from this correlation study. Two COLIPA reference sunscreen formulations were evaluated by the company laboratory under a hypothesis that COLIPA standard sunscreen formulation should give the SPF values equivalent to their specified values. The remaining three sunscreen products and two COLIPA reference sunscreen formulation showed good linear correlation with $R^2$ around 0.95 - 0.96.
**Conclusion:** Small differences in *in vitro* SPF evaluation procedures led to obvious different in results. However, results from different procedures can be correlated using simple linear regression technique.

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## Introduction

Several methods were developed to test efficiency of sunscreen products. The most preferred method is an *"in vitro"* measurement of Sun Protection Factor (SPF) using a spectrophotometer i.e. Optometrics SPF 290 S Analyzer. The principle of measuring the SPF values with a spectrophotometer[1] is based on UV absorption by layer of sunscreen product. Although *in vitro* SPF evaluation method is preferred, such method reports SPF values with high variability due to several reasons; for examples, type of substrate[2] (a material which the sunscreen products are applied on) and measurement procedures. In this study, *in vitro* SPF values obtained from the Optometrics SPF 290 by two different laboratories were compared and correlated using linear regression technique.

## Materials

Selected sunscreen products with SPF values in a range 7 - 30 (7, 15, 20, 25, 30 and 30 on label), were less than 6 months of age from their corresponding manufactured dates. Transpore® Tape (3M Co., Ltd., Thailand) was used as received. Two COLIPA standard sunscreens (SPF 4 and 15) were prepared according to the published formulations[3].

## Methods

Two units of selected sunscreen products with SPF 7, 15, 20, 25, 30 and 30 were kept in their original packages. The first unit of each product was submitted to Chulalongkorn University Drug and Health Products Innovation Promotion Center (CU-D-HIP) laboratory for evaluation of *in vitro* values by the Optometrics SPF 290 S Analyzer. The SPF values of the second unit were evaluated by a company laboratory using the same equipment. The CU-D-HIP laboratory employed the COLIPA method by applying about 2 mg/cm$^2$ of test samples were applied on the substrate.[4-5] The sample plates were left to dry in dark place for 15 minutes prior to evaluate the SPF values. The test were done in triplicate. A rough procedure for SPF measurement which was performed in the company laboratory was as followed. Transpore®

Tape was placed on a plate. About 13 mg/cm$^2$ of sunscreen product was evenly spread over 7.5 cm$^2$ of the Transpore® Tape surface. The actual weight of the applied sunscreen product was recorded using a 5-digited digital scale (Mettler Toledo, AX205). The sample plate was kept in dry and dark place for 20 minutes prior to measure SPF values. Blank Transpore® Tapes were used as controlled samples. The tests were done in triplicate on each testing date. From each sample plate, the instrument was randomly estimated SPF values from 5 areas and reported the SPF values as an average number from those 5 estimated values. The SPF values of the same product obtained from two different laboratories were compared and correlated using linear regression.

**Results**

The SPF values of sunscreen products obtained from two different laboratories were presented in table 1. According to the SPF values reported by CU-D-HIP, product C and D possessed the SPF values lower than the values indicated on the labels. The SPF values and standard deviation obtained from the company laboratory were higher than those obtained from CU-D-HIP. Picture 1 shows a linear relationship between the SPF values obtained from two laboratories with $R^2$ around 0.60 – 0.65.

**Table 1:** SPF value obtained by CU-D-HIP and company laboratory (n = 3)

| Sample | Labeled SPF | SPF values reported by CU-D-HIP (mean ± SD ) | SPF values from company lab 250614 (mean ± SD ) | SPF values from company lab 260614 (mean ± SD ) |
|---|---|---|---|---|
| A | 7 | 7.04 ± 0.52 | 11.04 ± 1.54 | 12.09 ± 1.92 |
| B | 15 | 28.98 ± 2.15 | 34.55 ± 14.89 | 37.67 ± 11.24 |
| C | 20 | 15.24 ± 1.11 | 40.17± 5.28 | 43.17 ± 6.38 |
| D | 25 | 21.08 ± 1.36 | 39.03 ± 7.34 | 41.45 ±11.55 |
| E | 30 | 36.75 ± 4.06 | 77.37 ± 20.37 | 79.44 ± 33.76 |
| F | 30 | 31.01 ± 9.30 | 35.52 ± 11.54 | 41.84 ± 23.26 |

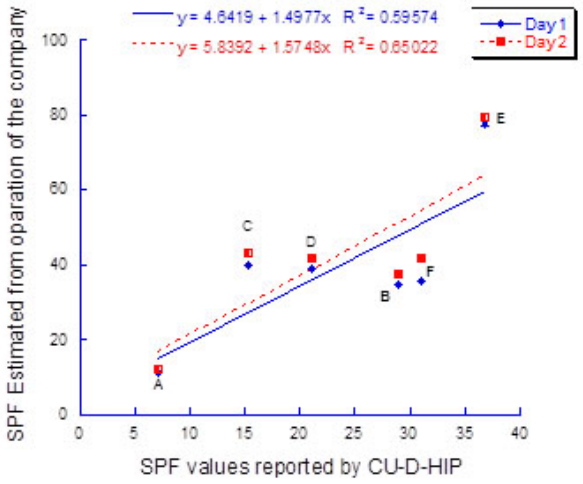


**Figure 1** A relationship between the SPF values obtained from CU-D-HIP and company laboratory

Product C, E, F which showed high deviation from the linear regression line contains Diethylamino Hydroxy Benzolyhexyl Benzoate (DHHB), a highly stable inorganic UVA absorber filter. Even though DHHB doesn't possess UVB filter property[6-7], it shows synergistic effect when combines with other UV filters[8]. In addition, product C and E are non-homogeneous giving rise to rough sample surface after application on the Transpore® Tape[9]. The non-homogeneity of product C and E may lead to the observed deviation from the linear regression line. Product F has the highest viscosity (140,000 – 250,000 cps.) when compare with other formulations which have viscosity about 80,000 cps. An uneven surface may be obtained after high viscosity product is applied on the substrate and result in inaccurate SPF values. Thus, products C, E, F were left out from the correlation study. Figure 2 shows a linear relationship between the SPF values obtained from two laboratories after the mentioned adjustment.

Two COLIPA standard sunscreen formulations with SPF 4 and 15 were prepared[10]. This experiment had an assumption that COLIPA standard sunscreen formulations should give the SPF values equivalent to their specified values. The standard formulations were evaluated by the company laboratory which showing the SPF values of 6.62 and 18.41 from the standard formulation with SPF 4 and 15, respectively. . However, these 2 COLIPA standard sunscreen formulations were not evaluated for the SPF values from the CU-D-HIP laboratory. The obtained SPF values by the company

laboratory were also about 1.5 times higher than their specified values. Therefore, these additional data fell on the same linear trend (Figure 3).

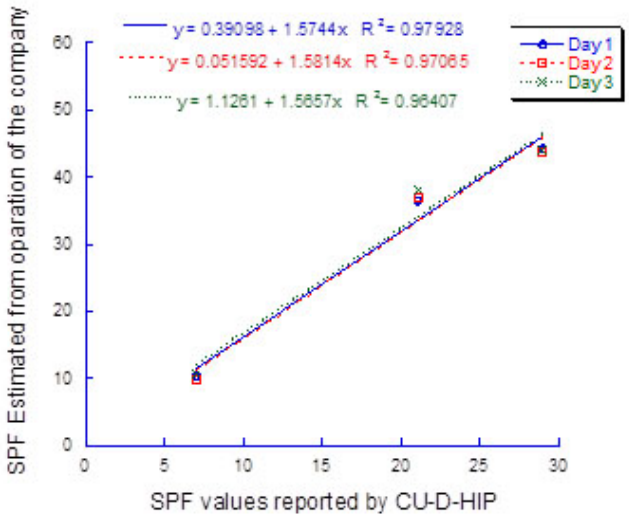


**Figure 2** A linear relationship between SPF obtained from CU-D-HIP and company laboratory

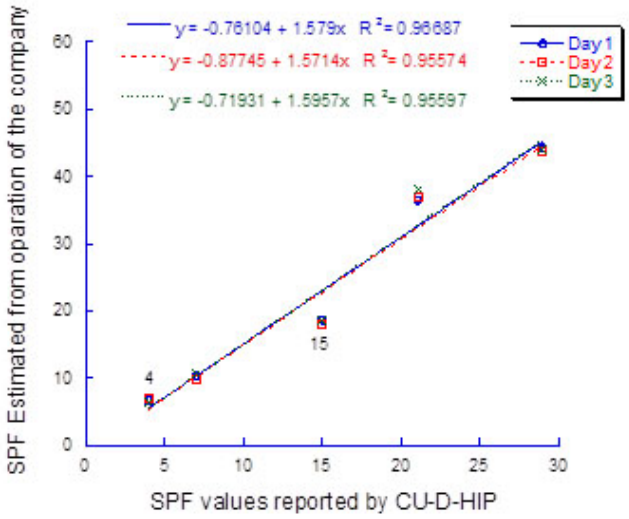


**Figure 3** A linear relationship between SPF obtained from CU-D-HIP and company laboratory

**Discussion**

Variation of SPF values obtained from the Optometrics SPF 290 S Analyzer came from several factors. Mixture of sunscreen substances, such as DHHB with other sunscreens, causes high SPF values than expected values. Since DHHB was known to possess synergistic sunscreen effect when combine with other sunscreens. Sample texture such as homogeneity and viscosity directly affected the *in vitro* SPF values. The Optometrics SPF 290 S Analyzer estimated SPF values based on intensity of the reflected UV light; therefore, uneven sample surface could refract the UV light resulting in inaccurate SPF values[12]. Sample weight per area affected thickness of sample layer and the SPF values. Since, the more amount of the sunscreen or the thicker of the product layer was applied the higher SPF values were estimated.

**Conclusion**

The *In vitro* SPF values were affected by several parameters. Effect of substrate type, i.e. Transpore® Tape and PMMA on *in vitro* SPF value was reported.[2] This study showed that non-homogeneity of products containing DHHB and product with high viscosity led to inaccurate *in vitro* SPF values. In order to obtain good correlation of SPF values between 2 laboratories, the sunscreen products should be homogeneous and have viscosity less than 80,000 cps. Amount of sunscreen (or weight), which was applied on the substrate per area, is another important parameter. However, this study was able to show a good correlation of SPF value obtained from the same equipment by two different laboratories

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