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A simple and rapid method developed to determine the Sun protection factor (SPF) by using UV-visible spectrophotometer for topical formulations.

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Abstract: *The new and an innovative method was developed to quantify the sun protection factor (SPF) in sunscreen formulations. This method developed based on the MED equation for UVB sunscreen substances, the method validated as per ICH guidelines and found robust, accurate and linear. Method optimized by using UV-VIS spectrophotometry concentration level of 2 mg/ml for clear formulations/solutions. The proposed spectrophotometric method is simple and rapid.*

Key words: *UV-Visible spectroscopy, sunscreen substance, sun protection factor, Erythema dose, UV radiation,*

I. Introduction:

To determine the SPF in topical formulations many methods are available. Majorly the in-vivo and in-vitro are well known which were approved by colipa guidelines (1). Both the methods are time consuming and more expensive. The new innovative UV-VIS spectrophotometric method was developed and validated based on the in-vivo and in-vitro SPF by using MED equation. The test substances were prepared and subjected for photo protective activity study by UV spectrophotometer in the range of 290-320 nm. It was observed that the SPF values of topical applications were validated up to 30 SPF. [2-3].

Sunscreens and sun blocks are chemicals that absorb or block UV rays and show a variety of harmful immunosuppressive effects of sunlight. The use of skin care products especially sunscreens may be an effective approach for reducing UV-B generated ROS (Reactive oxygen species) mediated photo aging [4-5]. Solar ultra violet radiations (UVR) are divided into three categories: UVC (200-280 nm), UVB (280-320 nm) and UVA (320-400 nm). UVC is the most biologically damaging radiation, but it is filtered out by ozone layer. Currently UVB radiation and to a lesser extent (having energy of 30 to 40 times greater than UVA) UVB radiations is involved in 65% of all skin cancers and UVB radiation is not completely filtered out by ozone layer and is responsible for the damage due to sunburn, UVA radiation has been further subdivided into short wave (UVA II, 320 to 340 nm) and long wave (UVA I, 340 to 400 nm). UVA radiation reaches the deeper layer of the epidermis and dermis and provokes the premature aging of the skin. Among these radiations, UVA and UVB radiations are mainly responsible for skin pathologies such as sunburns, cutaneous, degeneration, photosensitivity, phototoxicity, actinicelastosis, photo aging, immunosuppression and Ultraviolet radiations have been implicated as a causative factor of skin cancer [6, 7-8].

Due to these facts, sunscreen substance are now incorporated into everyday products such as moisturizers, creams, lotions, shampoos and other hair and skin preparations. The regular use of these products may help to reduce the chance of the harmful effects of ultraviolet radiations. However, it is necessary that a very efficient sunscreen substance is used in the topical formulations.

The efficacy of sunscreens are usually expressed as sun protection factor (SPF), which is defined as the UV energy required to produce a minimal erythemal dose (MED) in protected skin, divided by the UV energy required producing an MED in unprotected skin.

The in-vitro SPF were determined according to the method described. The observed absorbance values at 5 nm intervals (290-320 nm) were calculated by using the formula.

$$SPF = C \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Here, CF = correction factor (10), EE (λ) = erythmogenic effect of radiation with wavelength λ, Abs (λ) = spectro photometric absorbance values at wavelength λ. The values of EE (λ) x I are constants. They were determined by Sayre et al and are given in Table 1.

Table 1. Values of EE (λ) x I at different wavelength

Wavelength	Value of EE x I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0837
320	0.0180

However, there are many factors affecting the determination of SPF values, like the use of different solvents in which the sunscreens are dissolved, the concentration of the sunscreens, the type of emulsion, type of cuvettes, the effects and interactions of vehicle components, the interaction of the vehicle with the dilute solution, the addition of other active ingredients, which can increase or decrease UV absorption of each sunscreen. The effect that different solvents and emollients have upon the wavelength of maximum absorbance, alone or in combination, is reported in several studies [9, 10, 11, 12-13].

Vehicles used for sunscreens formulations, water-in-oil or oil-in-water emulsions and oily lotions. The sunscreen preparation must spread on the skin, should remain in place as a continuous film, should closely adhere to the surface and should resist washing off by perspiration. Standard techniques for spectrophotometric evaluation of sunscreens preparations involve solution of a known weight of the screen or preparation in an ultraviolet clear solvent. [14-15].

II. Materials And Methods:

The achieved method was performed using Shimadzu UV-VIS-1700 equipped with 1cm quartz cell cuvettes, D_2 detector and powdered by (UV probe) software UV-VIS spectrophotometer 1700 analyzer.

2.1 Material: Green tea extract obtained from plant Camellia sinensis, procured form local vendor.

2.2 Solvents: Absolute alcohol 99. 9 % (Analytical grade) was procured from Hyman.

2.3 Optimization studies: The method was optimized based on the concentration and acceptance criteria as per ICH guidelines (16).

III. Procedure:

Take 100 ml volumetric flask weigh accurately 1.0 gm of sample add 50 ml of ethanol and sonicate for 15 minutes then make up to the mark with ethanol (solution-A) then filter through whatman filter paper, rejecting the first 10 ml. Take 5.0 ml aliquot (solution-A) was transferred to 25 ml volumetric flask and diluted to volume with ethanol (Solution-B). Then transfer a 2.5 ml of Solution-B into a 25 ml of volumetric flask and make up the volume with ethanol (Solution-C).

Transfer Solution-C into 1 cm cuvettes and subject to test solution are exposed with UV Light and measure of the spectrum absorbance of the test solution.

Take the sample absorbance for UVB range 290 nm to 320 nm every 5 nm wavelength interval, and same was performed in triplicate for each sample determinations were made at each point, followed by the application of Mansur equation(2).

IV. Method Validation:

The proposed method for the simultaneous determination SPF number of all sunscreen formulation is validated by the following parameters and guidelines mentioned in ICH guidelines (16).

4.1 Limit of Detection and Limit of Quantification:

The linearity of the UV source response for the prepared test solution was assessed by means of linear regression regarding the amounts of each test solution, measured in mg/ml and the absorbance of the corresponding concentration on the spectrum. Limits of detection and quantification were determined by calculation of the signal to noise ratio. Signal-to-noise ratios of approximately 3:1 and 10:1 were used for estimating the detection limit and quantification limit respectively of the method (Table.01).

Table.01: Limit of detection and Limit of quantification.

LIMIT OF DETECTION - 1 ppm		LIMIT OF QUANTIFICATION - 10 ppm	
Test trial	SPF values	Test trial	SPF values
1	0.36	1	1.4
2	0.36	2	1.4
3	0.36	3	1.41
Average	0.36	Average	1.403333333
SDEV	6.7987E-17	SDEV	0.005773503
% RSD	1.88853E-14	% RSD	0.411413493

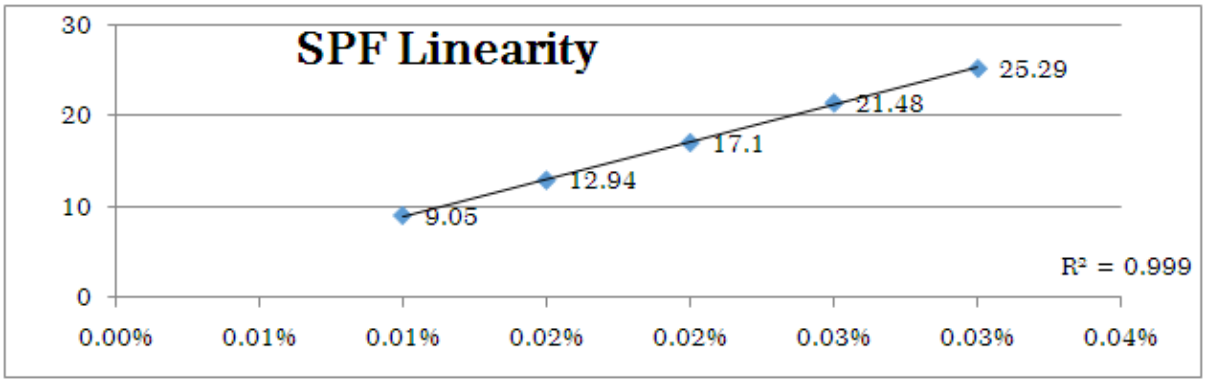
4.2 Linearity:

Linearity was carried out by test solution are irradiated with UV light and measuring the absorbance of the concentration ranging from 0.01 % to 0.030 % of sunscreen substance test solution respectively. The calibration curve was linear for green tea extract (Table.02 & graph-1).

Table.02: Linearity, 0.01 % to 0.03 %.

Linearity – SPF values					
Trials	0.01 %	0.015 %	0.02 %	0.025 %	0.03 %
1	9.03	12.97	17.12	21.41	26.02
2	9.05	12.88	17	21.48	24.44
3	9.07	12.96	17.14	21.55	25.43
Average	**9.05**	**12.9366667**	**17.0866667**	**21.48**	**25.29666667**

Graph-1: Linearity.



4.3 Accuracy:

The recovery method was carried out by spiking developed compounds over sample ranging from 0.015 % to 0.025 % of Green tea extract respectively each samples were prepared in triplicate and each samples were subjected in duplicate recovery and % RSD were calculated and reported with % RSD < 2 % and mean recovery in each level was 96.79 % to 100.52 % (Table.03).

Table.03: Accuracy.

Accuracy SPF values								
Std,Spike as %	Sample SPF	% STD Assay	Sample SPF	Average SPF	Recovery	% Recovery	STDV	% RSD
0.015%	12.97	100%	12.45	12.46666667	0.967908903	96.7908903	0.037859	0.303684938
0.015%	12.88	100%	12.44					
0.015%	12.96	100%	12.51					
0.02%	17.12	100%	17	17.09	1.005294118	100.529412	0.095394	0.558185606
0.02%	17	100%	17.08					
0.02%	17.14	100%	17.19					
0.025%	21.41	100%	21.08	21.13666667	0.984016139	98.4016139	0.066583	0.315013158
0.025%	21.48	100%	21.21					
0.025%	21.55	100%	21.12					

4.5 Robustness:

For the determination of the method robustness a number of spectrophotometric parameters, such as test solution temperature, time and instrument were varied to determine their influence in the quantitative analysis (Table.04).

Table. 04: Robustness.

Robustness							
Temperature 25 ±2 ^{0}C		**Time-30 minutes**		**Time-60 minutes**		**Different instrument**	
0.02 %	SPF VALUES	0.02 %	SPF VALUES	0.02 %	SPF VALUES	0.02 %	SPF VALUES
1	17.28	1	17.3	1	17.06	1	17.68
2	17.4	2	17.38	2	17.17	2	17.33
3	17.05	3	17.34	3	17.28	3	17.32
Average	17.24333333	Average	17.34	Average	17.17	Average	17.44333333
SDEV	0.177857621	SDEV	0.04	SDEV	0.11	SDEV	0.205020324
% RSD	1.031457303	% RSD	0.230680507	% RSD	0.640652301	% RSD	1.175350607

4.6 Precision and Intermediate Precision:

Method was validated for Precision and Intermediate Precision the assay and % RSD was calculated and reported the % RSD was < than 2.0 % (Table 05).

Table. 05: Precision 0.02 %.

Precision					
Method precision		System suitability		System precision	
0.02 %	SPF VALUES	0.02 %	SPF VALUES	0.02 %	SPF VALUES
1	16.79	1	17.05	1	16.85
2	17.03	2	16.89	2	16.93
3	16.83	3	16.98	3	17.03
4	16.89	4	16.92	4	17.36
5	16.94	5	16.98	5	16.92
6	17.27	6	17.11	6	17.15
Average	16.95833333	Average	16.98833333	Average	17.04
SDEV	0.174403746	SDEV	0.081342896	SDEV	0.188255146
% RSD	1.028425039	% RSD	0.478816221	% RSD	1.104783721

V. Results And Discussion:

The validated SPF method is a quantitative measurement of the effectiveness of a sunscreen formulation. The innovative and validated spectrophotometric methodology being used for determination of the absorption characteristics of the sunscreens agents of clear dilution. However, there are many factors affecting the determination of SPF values, as for example, the use of different solvents in which the sunscreens are dissolved, age solution, the combination and concentration of the sunscreens, the type of emulsion, the interaction of the concentration of solution v/s absorbance properties, among other factors, which can increase or decrease UV absorption of each sunscreen. The effect that different solvents and emollients have upon the wavelength of maximum absorbance and upon the UV absorbance of several sunscreens chemical, alone or in combination is well known and documented (9-14). Excipients and other active ingredients can also produce UV absorption and, thus interfering with those of UVA and UVB sunscreen. This effect is reflected in a finished product formulation, especially for lotions, creams with an SPF greater than 15. The effect of a solvent is only realized at high percentages, so our method is optimized at SPF rang up to 30.0, by applying a simple formula developed by Mansur, in vitro method (3).

The developed and validated spectrophotometric method SPF value co-related with in-vitro SPF values (1), the in vitro SPF value very close argument with in-house spectrophotometric method
(Table 06).

Table.06. SPF values co-related as per In-Vitro and Spectrophotometry.

formulation	SPF by In-vitro	SPF by spectrophotometry
A	18	17.39
B	15	14.65
C	30	30.02

The spectrometric method is very use full and support to in-house development all different sunscreen formulations, like creams, lotions and actives, for the sum of commercially available sunscreen substance and in-house development different sunscreen products was performed SPF value, the SPF value of these sunscreen substance and sunscreen development products very close argument with BOM composition of accepted SPF values (Table.07).

Table.07: BOM composition accepted SPF values.

Development samples (function)	Accepted SPF as per BOM composition	Found SPF value by UV spectrophotometry
Formula A	15.O	14.23
Formula B	15.0	14.60
Formula C	15.0	15.52
Formula D	15.O	15.62

VI. Conclusions:

The developed UV spectrophotometric method is simple, rapid, employs low cost. The liability of the proposed method has been established by evaluating validation parameters as per ICH guidelines (16). The results were in good agreement with acceptable limits of in vitro determination of SPF values. Therefore the method has been proven to be linear, precise, accurate, specific and robust. This method can be adopted for the estimation of SPF value of sunscreen cosmetic formulation. The analytical method can be taken up for further research for other analytical marker compounds as this traditional formulation comprises of other sunscreen substances.

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