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RESEARCH ARTICLE

TO DETERMINE THE SUN PROTECTION FACTOR BY USING ULTRA VIOLET VISIBLESPECTROPHOTOMETER FOR TOPICAL HERBAL FORMULATIONS

K. Sunil[1], T.P. Prasanna kumara[1] and B. Arun kumar[2]

1. Department of Chemistry, Sri Siddhartha Academy of Higher Education, Tumkur, Karnataka, India.
2. R&D, The Himalaya drug company, Bengaluru, Karnataka, India.

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Abstract

This research was done to determine the sun protection factor (SPF) of sunscreens available at commercial market, in-house developed samples involving chemical and physical sunscreens and in-house developed samples involving Schiff base compounds using ultraviolet spectrophotometry. 10 in-house topical herbal sunscreen emulsions formulation were evaluated based on the MED equation for UVB sunscreen substances.The repeatability of the method was tested. It is observed that the proposed spectrophotometric method is simple, rapid and repeatable for the in vitro determination of SPF values of sunscreen products. The proposed spectrophotometric method is simple and rapid.

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Introduction:-

The fast growth of commercially accessible products containing sunscreens indicates that even though a suntan is still desired, people are very aware of the possible dangers of photo aging and skin cancer, occurring as a result of sun over exposure (Erika, 2004). Around 10,000 die from malignant melanoma every year. Most frequently skin cancer occurs on the areas of the body that are exposed to the sun (Mbanga et.al, 2014; Sax, 2000). The injurious effects of solar radiation are caused mainly by the ultraviolet (UV) region of the electromagnetic spectrum, which can be separated into three regions: UVA, from 320 to 400 nm; UVB, from 290 to 320 nm and UVC, from 200 to 290 nm. UVC radiation is filtered by the atmosphere before reaching earth. UVB radiation is not completely filtered out by the ozone layer and is responsible for the damage due to sunburn. UVA radiation reaches the deeper layers of the epidermis and dermis and provokes the premature aging of the skin. Ultraviolet radiations have been involved as a causative factor of skin cancer (Malsawmtluangi et.al, 2013). Due to these facts, sunscreen substances are now integrated into routine products such as moisturizers, creams, lotions, shampoos, and other hair and skin preparations. The regular use of sunscreen products may help to reduce the chance of the harmful effects of ultraviolet radiation. This is possible only when very efficient sunscreen substance is used in the cosmetic formulation.

The efficiency of a sunscreen is usually expressed by the sun protection factor (SPF), which is defined as the UV energy required producing a minimal erythema dose (MED) on protected skin, divided by the UV energy required to produce a MED on unprotected skin.

(Equation 1):

$$SPF = \frac{\text{Minimal erythema dose in sunscreen – Protected skin}}{\text{Minimal erythema dose in non-sunscreen – Protected skin}}$$

Corresponding Author:- K. Sunil
Address:- Department of Chemistry, Sri Siddhartha Academy of Higher Education, Tumkur, Karnataka, India.

The minimal erythemal dose (MED) is defined as the lowest time interval or dosage of UV light irradiation sufficient to produce a minimal, perceptible erythema on unprotected skin (Wolf et.al, 2001; wood et.al, 2000).

The photo protection afforded by topical sunscreens against solar ultraviolet radiation exposure can be determined in vivo or in vitro, and it is ideally determined by photo testing in human volunteers. This type of determination has been used for many years and although useful and accurate, it is a time-consuming process, very complex and more expensive, particularly when information regarding to the protection against long wavelength (UVA) is required (Azevedo et.al, 1999; Gasparro et.al, 1998). As significance, much effort has been devoted to the development of in vitro methods for evaluating the photo protection of sunscreen compounds.

The methods in vitro are in general of two types. Methods which involve the measurement of absorption rays or the transmission rays of UV radiation through sunscreen product films in quartz plates or bio membranes, and methods in which the absorption characteristics of the sunscreens agents are determined based on spectrophotometric analysis of dilute solutions (Fourneronet.al, 1999; Mansuret.al, 1986; Pissavini et.al, 2003; Walters et.al, 1997; Gordon, 1993).

Mansur et al.1986, developed a very simple mathematical equation which substitutes the in vitro.

Method proposed by Sayre et al., 1979, utilizing UV spectrophotometry and the following equation-2:

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

Table I:- normalizes the product function used in the calculation of SPF.

Wave length (λ nm)	EE x I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

EE- erythemal effect spectrum; I – Solar intensity spectrum, nm- nano meter.

Cosmetics 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 (Sayre et.al, 1979; Dutra et.al, 2004)

Materials and Methods:-

The achieved method was performed using Shimadzu UV-VIS-1700 equipped with 1cm quartz cell cuvettes, D2 detector and powdered by (UV probe) software UV-VIS spectrophotometer 1700 analyzer.

Material:

Green tea extract obtained from plant Camellia sinensis, procured form local vendor, N-Benzylideneaniline,N-(4-methoxybenzylidineaniline) and N-Salicylideneaniline, obtained from Schiff base reaction by using Berry fruit juice freeze dry powder.

Solvents:

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

Optimization studies:
The method was optimized based on the concentration and acceptance criteria as per ICH guidelines (ICH Q2, R1).

Procedure:-
Standard Green tea extract & Sunscreen products:
Accurately 1.0 gm of sample was weighed into 100mL volumetric flask and 50 ml ethanol was added and sonicated for 15 minutes. Then volume was made up to the mark with ethanol (solution-A) and filtered through Whatman filter paper, rejecting the first 10 ml. 5.0 ml aliquot (solution-A) was transferred to 25 ml volumetric flask and diluted to volume with ethanol (Solution-B). 2.5 ml of Solution-B was further diluted to 25 ml with ethanol (Solution-C).

Solution-C was transferred into 1 cm cuvettes and absorbance at UVB range i.e., 290 nm to 320 nm at every 5 nm wavelength interval was taken in triplicate.This was repeated for each sample and Mansur equation(2) was applied on the resultant data.

Standard Schiff base compounds:
Accurately, 10 mg of N-Benzylideneaniline and N-Salicylideneaniline were weighed in 25ml volumetric flask; 10 ml of ethanol was added and sonicated for 15 minutes. Volume was made up to the mark with ethanol (solution-A) and filtered through Whatman filter paper, rejecting the first 5 ml. 0.5 ml aliquot (solution-A) was transferred to 25ml volumetric flask and diluted to volume with ethanol (Solution-B). Accurately, 10 mg of N-(4-methoxybenzylidine aniline) was weighed in 25ml volumetric flask; 10 ml of ethanol was added and sonicated for 15 minutes. Volume was made up to the mark with ethanol (solution-A) and filtered through Whatman filter paper, rejecting the first 5 ml. 1.25 ml aliquot (solution-A) was transferred to 25ml volumetric flask and diluted to volume with ethanol (Solution-B).

Solution-B was transferred into 1 cm cuvettes and absorbance at UVB range i.e., 290 nm to 320 nm at every 5 nm wavelength interval was taken in triplicate. This was repeated for each sample and Mansur equation-2 was applied on the resultant data.

Topical in-house herbal formulations by using Schiff base compounds:
Accurately, 2.0 gm of sample was weighed and 50 ml of ethanol was added and sonicated for 15 minutes. Volume was made up to the mark with ethanol (solution-A), then filtered through Whatman filter paper, rejecting the first 10 ml.

Solution-A was transferred into 1 cm cuvettes and absorbance at UVB range i.e., 290 nm to 320 nm at every 5 nm wavelength interval was taken in triplicate. This was repeated for each sample and Mansur equation-2 was applied on the resultant data.

Method Validation:-
The proposed method for the simultaneous determination SPF number of all sunscreen formulation was validated by the following parameters and guidelines mentioned in ICH guidelines (ICH Q2, R1).

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.II).

Table.II:- 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
Avg	0.36	Avg	1.403
SDEV	6.7987E-17	SDEV	0.005
% RSD	1.88853E-14	% RSD	0.411

SPF-Sun protection factor

Linearity:

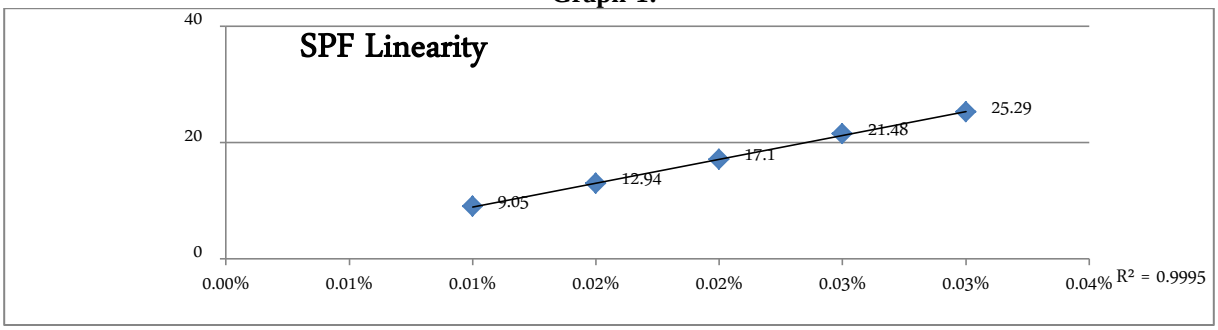
Linearity was carried out by test solution are irradiated with UV light and measuring the absorbance of the concentration ranging from 0.01% to 0.030 % of sunscreen substance test solution respectively. The calibration curve was linear for green tea extract (Table.III and Figure-1).

Table.III :- 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	17.00	21.48	24.44
3	9.07	12.96	17.14	21.55	25.43
Avg	9.05	12.94	17.09	21.48	25.29

SPF-Sun protection factor

Graph-1:-



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.IV).

Table.IV:- Accuracy.

Accuracy SPF values								
Std spike as %	Sample SPF	% STD Assay	Sample SPF	Avg SPF	Recovery	% Recovery	STDV	%RSD
0.015%	12.97	100%	12.45	12.466	0.967	96.790	0.037	0.303
0.015%	12.88	100%	12.44					
0.015%	12.96	100%	12.51					
0.02%	17.12	100%	17.00	17.090	1.005	100.529	0.095	0.558
0.02%	17.00	100%	17.08					
0.02%	17.14	100%	17.19					
0.025%	21.41	100%	21.08	21.136	0.984	98.401	0.066	0.315
0.025%	21.48	100%	21.21					
0.025%	21.55	100%	21.12					

SPF-Sun protection factor, STD-Standard, STDV-Standard deviation

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.V).

Table. V:- 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	17.30	1	17.06	1	17.68
2	17.40	2	17.38	2	17.17	2	17.33
3	17.05	3	17.34	3	17.28	3	17.32
Avg	17.243	Avg	17.34	Avg	17.17	Avg	17.443
SDEV	0.177	SDEV	0.040	SDEV	0.110	SDEV	0.205
% RSD	1.031	% RSD	0.230	% RSD	0.640	% RSD	1.175

SPF-Sun protection factor, SDEV-Standard deviation, %RSD-Relative standard deviation

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 VI).

Table. VI:- 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
Avg	16.958	Avg	16.988	Avg	17.040
SDEV	0.174	SDEV	0.081	SDEV	0.188
% RSD	1.028	% RSD	0.478	% RSD	1.104

SPF-Sun protection factor, SDEV-Standard deviation, %RSD-Relative standard deviation

Experimental Results & Discussion:-

The SPF is assessable measurement of the efficiency of sunscreen products. A sunscreen product to be more effective in preventing sunburn and other skin damage, it should process a wide range of absorbance between 290 and 400 nm. Different methods are available, to test the efficiency of a sunscreen formulation, for a long time in-vivo test was used for evaluation of the efficacy of a sunscreen which is performed with human volunteers. The in-vivo test is very time consuming and is normally subject to certain degree of variability, not mention the appropriate problems of testing with human. The in vitro SPF is useful for screening test during product development, as a supplement of the in vivo SPF measure.

In this research five different commercially available sunscreen products, five in-house development products and ten in-house developed Herbal products by using Schiff base compounds were evaluated by UV spectrophotometry applying Mansur mathematical equation (Mansur et.al.1986). The SPF labeled values was in the range of 2 to 30. These products and SPF values of samples obtained using the UV spectrophotometric method, were shown in Table 06. It can be observed that the SPF values found for commercial samples A, B, E (Table-VII) and SPF values found for in-house development samples from A to O (Table –VIII) are in close agreement with the excepted SPF. Commercial sample D presented SPF values above the labeled amount and commercial sample C presented SPF values below the labeled amount and ten in-house developed Herbal products by using Schiff base compounds were evaluated by UV spectrophotometry applying Mansur mathematical equation (Mansur et.al.1986), the observed SPF

values are as per table VIII, product F to O, SPF values are found 0.1 % to 0.2 % of compounds are enough to control and protect the sunburn.

Among commercial samples (Table-VII) analyzed, sample B exhibits a maximal absorbance higher than all samples with known amounts of sunscreens, as it can be observed in Table-VII. This is probably since sample B has a total amount of sunscreen substance higher than the other samples, presenting thus, a SPF higher than the calculated.

Commercial samples A and B (Table-VII) present the same SPF values labeled (SPF= 15.0 and 30.0). They present the same sunscreens, but at different concentrations. Sample A has less total amount of sunscreens than sample B, which was reflected in the obtained SPF values. Sample A presented a calculated SPF value smaller than the one of sample B.

Data variation can be due to the use of in-house validated spectrophotometric methodology being used for determination of the absorption characteristics of the sunscreens substance. 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; the combination and concentration of the sunscreens; the type of emulsion; the effects and interactions of vehicle components, such as esters, emollients and emulsifiers used in the formulation; the interaction of the vehicle with the skin; the addition of other active ingredients; the pH system and the emulsion rheological 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 (Sayre et.al, 1979; Dutra et.al, 2004). Excipients and other active ingredients can also produce UV absorptionbands, thus interfering with those of UVA and UVB sunscreen. This effect is reflected in a finished formulation, especially for lotions and creams with an SPF greater than 15. The effect of a solvent is only realized at high percentages.

According to Pissaviniet.al.2003, a high SPF values are more difficult to measure. A high SPF normally leads to a greater uncertainty also in the final in vivo result, due to the biological variations of the volunteers.

Therefore, to develop sunscreens with better safety and high SPF, the formulator must understand the physic chemical principle, not only the UV absorbance of the actives, but also vehicle components, such as esters, emollients and emulsifiers used in the formulation, since sunscreens can interact with other components of the vehicle, and these interactions can affect sunscreens efficacy.

Table VII :- SPF labeled and found in the commercially available samples.

Commercial samples (function)	Active ingredients	Amount (%)	Labeled SPF	Found SPF
A				
(Sunscreen lotion)	Titanium dioxide	0.1%	15.00	14.65
	Extract-A	4.0%		
	Extract -B	5.5 %		
B				
(Emulsion for body)	Not specified	Not specified	30.00	30.04
C				
(Sun block lotion)	Not specified	Not specified	20.0	24.09
D				
(Emulsion for body)	Not specified	Not specified	22.0	18.42
E				
(Green tea extract)	Not specified	Not specified	18.0	17.39

SPF-Sun protection factor

Table VIII :- As per BOM composition SPF found in the in-house development samples.

Development samples (function)	Active ingredients	Amount (%)	Excepted SPF	Found SPF
A				

(Emulsion for body)	Octyl salicylate	4.0		
	Homosalate	4.0		
	OMCX	3.0	15.0	15.62
	Octocrylene	2.0		
	CHEM 1789	2.0		
B				
(Emulsion for hand)	Cinnabloc	0.1	Not specified	0.58
C				
(Emulsion for face)	Octyl salicylate	4.0		
	Homosalate	4.0		
	OMCX	3.0	15.0	14.23
	Octocrylene	2.0		
	CHEM 1789	2.0		
D				
(Emulsion for face)	Octyl salicylate	4.0		
	Homosalate	4.0		
	OMCX	3.0	15.0	15.52
	Octocrylene	2.0		
	CHEM 1789	2.0		
	Cinnabloc	0.1		
E				
(Emulsion for body)	Cinnabloc	0.3	Not specified	2.12
F	N-(4-methoxybenzylidineaniline)	0.1 %	Not specified	11.25
(Exotic berry fruit)				
G	N-(4-methoxybenzylidineaniline)	0.05 %	Not specified	4.59
(Domestic berry fruit)				
H	N-(4-methoxybenzylidineaniline)	0.05 %	Not specified	5.19
(Exotic berry fruit)				
I	N-(4-methoxybenzylidineaniline)	0.1 %	Not specified	11.78
(Exotic berry fruit)				
J	N-(4-methoxybenzylidineaniline)	0.2 %	Not specified	22.34
(Exotic berry fruit)				
K	N-(4-methoxybenzylidineaniline)	0.2 %	Not specified	22.95
(Domestic berry fruit)				
L	N-Salicylideneaniline	0.1 %	Not specified	11.04
(Domestic berry fruit)				
M	N-Salicylideneaniline	0.1 %	Not specified	12.44
(Exotic berry fruit)				
N	N-Benzylideneaniline	0.1 %	Not specified	7.24
(Domestic berry fruit)				
O	N-Benzylideneaniline	0.1 %	Not specified	6.65
(Domestic berry fruit)				

SPF-Sun protection factor.

Conclusion:-

In intensive investigation into this area, the proposed UV spectrophotometric method is simple, rapid, employs low cost reagents and can be used in the in vitro determination of SPF values in many cosmetic formulations. This method can be adopted for the estimation of SPF value of sunscreen cosmetic formulation. The analytical method can be taken up for commercial quality control analysis for the studied analytical marker compounds i.e., N-Benzyl aniline, N-Salicylide-aniline and N-(-4-Methoxy benzylidene aniline).

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