CENTRE FOR CRUELTY–FREE TESTING (CCFT)
(MIET Meerut)

FIST Centre Sponsored by DST, Government of India
(ISO 9001:2015; 14001:2015, OECD Compliant)

INVITRO ESTIMATION FOR
SUN PROTECTION FACTOR AND PA RATING OF NIACINAMIDE SUNSCREEN

(Batch No.-SY24)

SPONSOR

PUREPLAY SKIN SCIENCES INDIA PVT. LTD.

CLINICAL RESEARCH ORGANIZATION

MITTAL GLOBAL CLINICAL TRIAL SERVICES (MGCTS)

TEST LABORATORY

Centre for Cruelty-Free Testing, MIET Meerut
NH-58, Delhi-Roorkee Highway, Baghpat Bypass Road Crossing, Meerut, U.P. – 250005

PROJECT NO	**: 2024-08-464H**
REPORT NO.	**: MIET/CCFT/2024-08/464H/SPFPA**
DATE	**: 05-08-2024**

Test Compound : NIACINAMIDE SUNSCREEN
SPONSOR : PUREPLAY SKIN SCIENCES INDIA PVT. LTD.
CRO : MITTAL GLOBAL CLINICAL TRIAL SERVICES (MGCTS)
STUDY : INVITRO SPF AND PA RATING
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1. STATEMENT OF COMPLIANCE

I, the undersigned hereby declare that **Project No. CCFT-2024-08-464H/ Report No. MIET/CCFT/2024-08/464H/SPFPA**; entitled "***Invitro Estimation for Sun Protection Factor and PA Rating of NIACINAMIDE SUNSCREEN*** " was performed by the standard procedure of Centre for Cruelty-free Testing, MIET Meerut, UP, as well as the approved study plan.

I hereby attest to the authenticity of the study and guarantee that this report is a true and accurate record of the results obtained and shall not be reproduced except in full, without the written approval of the Sponsor.

All original raw data including documentation, the draft report, a copy of the final report, and the representative test sample are archived at CCFT, MIET, NH-58, Delhi-Roorkee Highway, Baghpat Bypass Road Crossing, Meerut – 250005, India for a period of nine years. There were no circumstances that may have affected the quality and integrity of the study.

The sponsor of the study is responsible for the necessary evaluation of the test sample concerning the chemicals, purity, identity, stability, and other required data.

Anurag

Principal Investigator **05-08-2024**
Dr. Anurag Chaudhary (M.Pharm PhD) **Date**

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2. STATEMENT BY TEST FACILITY MANAGEMENT

Management of the test facility has made available all the resources to the Principal Investigator necessary for the conduct of the present study in compliance with the principles of GLP.

I, the undersigned, take overall responsibility for the reliability of the work described in the report in accordance to GLP.

Laboratory Scientist **05-08-2024**
Dr. Prabhash Nath Tripathi (M.Pharm Ph.D.) **Date**

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3. QUALITY ASSURANCE REPORT

This **Project No. CCFT-2024-08-464H/ Report No. MIET/CCFT/2024-08/464H/SPFPA**; entitled "***Invitro Estimation for Sun Protection Factor and PA Rating of NIACINAMIDE SUNSCREEN***" was subjected to inspection by the Quality Assurance Unit.

This report has been audited by the Quality Assurance Unit and is considered to be an accurate account of the data generated and of the procedures followed. In each case, the outcome of the QA evaluation is reported to the Principal Investigator and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

Standard Test Method Compliance Audit	: 26-07-2024
Test Material Preparation	: 31-07-2024
Date of Testing	: 01-08-2024
Draft Report Audit	: 05-08-2024
Final Report Date	: 05-08-2024

QUALITY ASSURANCE OFFICER **05-08-2024**
Ms. Garima Agarwal (M.Pharm) **Date**

4. STUDY PERSONNEL

Principle Investigator : Dr. Anurag Chaudhary (M.Pharm, PhD)

Study Managers : Ms. Garima Agarwal (M.Pharm)

: Mr. Ankit Chaudhary (M.Pharm)

5. INTRODUCTION & METHOD

The test is based on the assessment of UV transmittance through a thin film of sunscreen sample spread on a roughened substrate, before and after exposure to a controlled dose of radiation from a defined UV exposure source. Because of the several variables that cannot be controlled with typical thin film spectroscopic techniques, each set of sunscreen transmission data is mathematically adjusted so that the in vitro SPF data yields the same measured in vivo SPF value that was determined by in vivo testing.

The UV-2000S is based on the *spectral transmittance* of ultraviolet light through a sunscreen material. The term *spectral transmittance* refers to the fraction of light transmitted at a single wavelength. The UV-2000S instrument measures spectral transmittance across the 250 - 450 nm wavelength spectrum using an integrating sphere and two spectrometer instruments. The sample beam is generated inside the integrating sphere by an ultraviolet pulsed flash lamp.

MATERIAL:

- **Instrument**: UV 2000S Ultraviolet Transmittance analyzer, Solar Simulator, Balance.
- **Chemicals**: Glycerin, Distilled water.
- **Miscellaneous**: PMMA plates, Paper towel, Pipette.

METHOD:

Blank Plate Preparation

- Both sides of the PMMA plate were washed under clear, cold distilled water and the plate was blotted dry with a paper towel.
- The PMMA plate was placed onto the analytical balance with the roughened side up and the weight was recorded.

- Using a pipette, a number of small droplets of Glycerin was applied onto the clean plate distributed evenly over the roughened surface until the total weight of the Glycerin is 15 mg for a 50 x 50 mm plate.
- The plate was removed from the analytical balance and Glycerin was spread across the entire surface of the plate evenly with a finger using light strokes as quickly as possible. Stroking continued on the surface of the plate in all directions until no puddles or areas of excess agent exist. The blank plate was allowed to be set aside in a dark environment for 15 minutes.
- The PMMA plate was then scanned, and the transmittance results were compared with the following table.

Wavelength	Minimum Transmittance (%)	Maximum Transmittance (%)
290	60	70
300	69	79
320	81	91

- After achieving the transmittance limit, the blank scan was taken and the sample plate was prepared.

Sample plate preparation

- Both sides of the PMMA plate were washed under clear, cold distilled water and the plate was blotted dry with a paper towel.
- The PMMA plate was placed onto the analytical balance with the roughened side up and the weight was recorded.
- Using a pipette, a number of small droplets of the sample were applied onto the clean plate distributed evenly over the roughened surface with the application rate of 1.3 mg/cm2 i.e., 32.5 mg for a 50 x 50 mm plate.
- The plate was removed from the analytical balance and sample was spread across the entire surface of the plate evenly with a pre-saturated finger.

- Spreading was completed in two phase's process. First, the product was distributed over the whole area as quickly as possible (less than 30 seconds) using small circular motions with minimal pressure. Then the sample was rubbed on the plate surface using alternating horizontal and vertical strokes with increased moderate pressure. The second phase was completed in 20 to 30 sec.
- When the sample was spread evenly with no gaps, smears or excess product buildup, this treated sample was then allowed to equilibrate for at least 15 minutes in the dark at ambient temperature to help facilitate formation of a standard stabilized product film.

UV exposure using Solar Simulator (UV Source)

- Treated PMMA plates were placed on a non-reflecting surface during UV exposure.
- Total UV irradiance was done in between 50-140 Wm-2
- The Sample plate was exposed into the solar simulator.

Operating Procedure

- Initially blank plate was scanned using a UV2000S transmittance Analyzer.
- Then sample plate was scanned for determination of Pre-irradiation SPF.
- The dose of UV delivered during one measurement cycle did not exceed by 0.2 J/cm2.
- This was followed by irradiation of the plate in the solar simulator.
- Further sample plate was scanned for determination of Post-irradiation SPF.

6. CALCULATIONS

Sunscreen Protection Factor (SPF):

SPF is a measure of the ultraviolet solar energy required to produce sunburn on skin treated with sunscreen, relative to the amount of solar energy required to produce sunburn on unprotected skin. As the SPF rating of a sunscreen product increases, sunburn protection increases. Most sunscreen manufacturers use the *in vitro* method similar to the UV light test performed by the UV-2000S to accelerate new product development, decrease in-vivo product test cycles and cost and to verify lot-to-lot manufacturing consistency. UV-2000 calculates theSPF characteristic according to the ratio.

$$SPF = \frac{\int_{290}^{400} E_\lambda S_\lambda d_\lambda}{\int_{290}^{400} E_\lambda S_\lambda T_\lambda d_\lambda}$$

Where E_λ is the erythema action spectrum, S_λ is the solar spectral irradiance, T_λ is the spectral transmittance of the sample with the integral is calculated across the 290 - 400 nm wavelengthlimits.

$UVAPF_0$:

$UVAPF_0$ is the pre-irradiation UVA Protection Factor calculated individually for each plate. First, the SPF *in vitro* sunscreen characteristic described above is adjusted to the *in vivo* SPF value determined for the same

$$UVAPF_0 = \frac{\int_{320}^{400} P(\lambda) I(\lambda) d\lambda}{\int_{320}^{400} P(\lambda) I(\lambda) 10^{-A_0(\lambda) C} d\lambda}.$$

sunscreen product, to determine the coefficient of adjustment, UV-2000 calculates the value of the Coefficient of Adjustment C automatically and appliesthe coefficient to the ratio

Where P(A) is the PPD action spectrum specified in the guidelines.

The COLIPA Method recommends that C falls within a range between 0.8 and 1.2. UVAPF0 is determined before irradiating the sample and is displayed in the Plate Data Table under the heading “UVAPF Pre-irradiation”.

UVAPF:

The UVAPF characteristic is computed in the same manner as the UVAPF0 calculation above, except that the calculation is made after the irradiance is applied to the sample plate. The value of the coefficient C is the same in both calculations. UV-2000 displays the UVAPF characteristic for each plate in the Plate Data Table under the heading "UVAPF".

Irradiation Dose:

The irradiation dose is calculated for each sample plate after the pre-irradiation scans are complete. The dose parameter is equal to the UVAPF0 parameter for a plate multiplied by thevalue 1.2 J/cm^2.

Mean UVAPF:

UV-2000 computes the average of the included plate UVAPF values and reports this characteristic at the bottom right-hand corner of the COLIPA Method Window as the "UVAPF Mean". The characteristic does not appear on the screen until a sufficient number of sample plates have been tested.

UVAPF value	2 to less than 4	4 to less than 8	8 to less than 16	More than 16
Japanese PA method	PA+	PA++	PA+++	PA++++
Level of performance	Low	Medium	High	Very High

SPF: UVAPF Ratio:

The final sunscreen characteristic under the COLIPA method is the SPF: UVAPF Ratio which is the *in vivo* SPF or SPF *label* parameter divided by the UVAPF mean.

Critical Wavelength (λc):

The critical wavelength is defined across the 290 - 400 nm spectrum by the following relation:

$$\lambda_c = \mathrm{Min}(\mathrm{A}'),$$

such that λ satisfies the relationship:

$$\frac{\sum_{\lambda=290}^{\lambda'} \mathrm{A}_\lambda}{\sum_{\lambda=290}^{400} \mathrm{A}_\lambda} \geq 0.9$$

where A(λ) is the absorbance at wavelength λ.

UV-2000 displays the computed SPF and critical wavelength in the data window for the scan selected in Scan Sets Window. The mean statistics are calculated for the entire set that applies to the scan or set selected in the Scan Sets Window:

$$\text{SPF Mean} = \sum_{i=1}^{n} \frac{SPF(i)}{n}, \qquad \text{Lambda Critical Mean} = \sum_{i=1}^{n} \frac{\lambda_c(i)}{n}$$

The standard deviation statistics are calculated for the entire set that applies to the selected scanor set in the Scan Sets Window:

$$\text{SPF STD} = \sqrt{\sum_{i=1}^{n} \frac{(SPF(i) - SPF\ mean)^2}{n-1}}.$$

$$\text{Lambda Critical STD} = \sqrt{\sum_{i=1}^{n} \frac{(\lambda c(i) - Lambda\ Critical\ Mean)^2}{n-1}}$$

The coefficient of variation terms are defined for each set as:

$$\text{COV} = \frac{\text{SPF}}{\text{Mean}} 100\%$$

UVA:UVB Ratio:

The UVA:UVB Ratio sunscreen characteristic is calculated for pre-exposure and post-exposureconditions:

$$\text{UVA:UVB Ratio} = \frac{\text{UVA}}{\text{UVB}}$$

UV-2000 displays a UVA:UVB Ratio for each sample plate before and after the ultravioletradiation exposure.

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		INITIAL Mean UVA:UVB RATIO			
		0.0 to 0.59	0.6 to 0.79	0.8 to 0.89	0.9 and over
Post Exposure Mean UVA:UVB RATIO	0.0 to 0.56	No Rating	No Rating	No Rating	No Rating
	0.57 to 0.75	No Rating	***	***	***
	0.76 to 0.85	No Rating	***	****	****
	0.86 to Over	No Rating	***	****	*****

7. STATISTICS

Sunscreen Sample Plate 1 Pre-irradiation

Parameters	SPF	UVAPF	SPF/UVAPF	UVA/UVB Ratio	Lambda Critical
No. of Scans	3	3	3	3	3
Mean	34.07	12.94	2.632	0.636	382.2
STD	1.44	0.05	0.11	0.01	0.45
COV	4.22%	0.35%	4.11%	1.37%	0.12%

Sunscreen Sample Plate 1 Post-irradiation

Parameters	SPF	UVAPF	SPF/UVAPF	UVA/UVB Ratio	Lambda Critical
No. of Scans	3	3	3	3	3
Mean	53.62	15.92	3.234	0.605	382
STD	22.84	2.91	0.93	0.07	1.22
COV	42.60%	18.26%	28.83%	12.09%	0.32%

Sunscreen Sample Plate 2 Pre-irradiation

Parameters	SPF	UVAPF	SPF/UVAPF	UVA/UVB Ratio	Lambda Critical
No. of Scans	3	3	3	3	3
Mean	32.77	13.46	2.435	0.635	382.6

STD	1.72	0.04	0.13	0.01	0.55
COV	5.25%	0.27%	5.37%	1.95%	0.14%

Sunscreen Sample Plate 2 Post-irradiation

Parameters	SPF	UVAPF	SPF/UVAPF	UVA/UVB Ratio	Lambda Critical
No. of Scans	3	3	3	3	3
Mean	49.81	17.24	2.889	0.599	382
STD	4.56	0.16	0.27	0.02	0
COV	9.15%	0.91%	9.29%	3.29%	0.00%

Sunscreen Sample Plate 3 Pre-irradiation

Parameters	SPF	UVAPF	SPF/UVAPF	UVA/UVB Ratio	Lambda Critical
No. of Scans	3	3	3	3	3
Mean	33.56	13.04	2.574	0.629	382.6
STD	2.14	0.06	0.17	0.02	0.55
COV	6.37%	0.47%	6.51%	3.28%	0.14%

Sunscreen Sample Plate 3 Post-irradiation

Parameters	SPF	UVAPF	SPF/UVAPF	UVA/UVB Ratio	Lambda Critical
No. of Scans	3	3	3	3	3
Mean	53.74	18.2	2.952	0.601	382
STD	3.78	0.13	0.2	0.02	0
COV	7.04%	0.73%	6.61%	2.76%	0.00%

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Sunscreen Sample Plate 4 Pre-irradiation

Parameters	SPF	UVAPF	SPF/UVAPF	UVA/UVB Ratio	Lambda Critical
No. of Scans	3	3	3	3	3
Mean	34.75	12.89	2.696	0.633	382.4
STD	1.61	0.05	0.13	0.01	0.55
COV	4.64%	0.42%	4.99%	1.23%	0.14%

Sunscreen Sample Plate 4 Post-irradiation

Parameters	SPF	UVAPF	SPF/UVAPF	UVA/UVB Ratio	Lambda Critical
No. of Scans	3	3	3	3	3
Mean	50.77	18.69	2.717	0.628	382
STD	1.37	0.3	0.08	0.02	0
COV	2.70%	1.59%	2.95%	2.68%	0.00%

Mean Pre-Irradiation Statistics

Parameters	SPF	UVAPF	SPF/UVAPF
No. of Plates	4	4	4
Mean	33.7875	13.0825	2.58425
STD	1.7275	0.05	0.135
COV	5.12%	0.38%	5.25%

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Mean Post-Irradiation Statistics

Parameters	SPF	UVAPF	SPF/UVAPF
No. of Plates	4	4	4
Mean	51.985	17.5125	2.948
STD	8.1375	0.875	0.37
COV	15.37%	5.37%	11.92%

Pre Irradiation UVA/UVB	0.63325
Post Irradiation UVA/UVB	0.60825
Boots Star Rating	***

Final SPF Result

Invitro SPF	51.985
PA Rating	PA++++
Critical Wavelength	382
Boots Star	***
Broad Spectrum	YES

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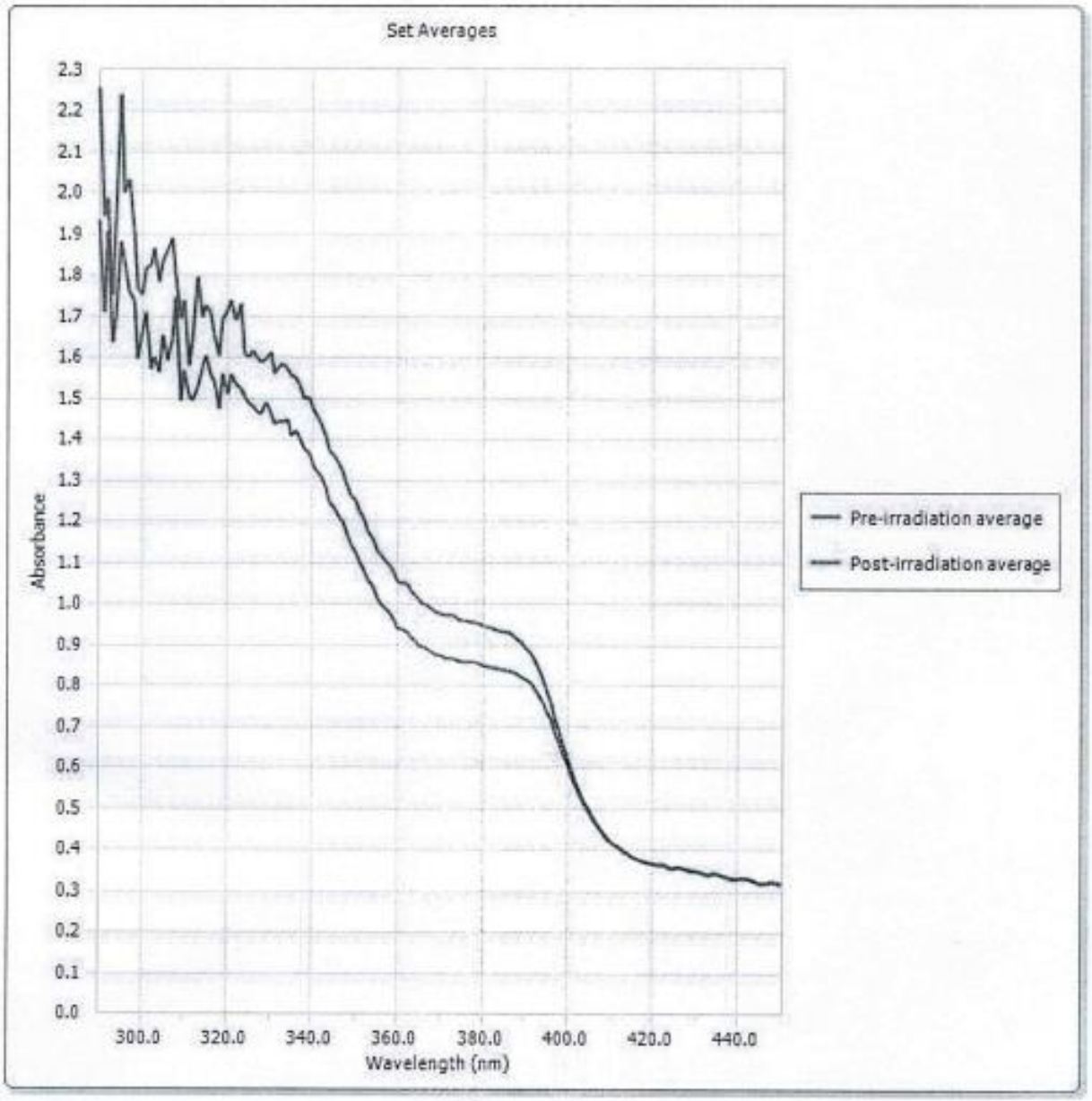

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8. RESULTS

In-vitro SPF obtained for NIACINAMIDE SUNSCREEN (SY24) is **51.98**

Broad Spectrum- **YES**

Critical wavelength **382**

PA rating as **PA++++**

9. ARCHIVE

On completion of the study, the study plan, experiment data, raw data, documentation, and a copy of the final report (MIET/CCFT/2024-08/464H/SPFPA) are retained in the Institute's Archives at CCFT, MIET, NH-58, Delhi-Roorkee Highway, Baghpat Bypass Road Crossing, Meerut – 250005, India for a period of nine years.

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10. CERTIFICATE

This is to certify that the ***Invitro Estimation for Sun Protection Factor and PA Rating of NIACINAMIDE SUNSCREEN*** supplied by **PUREPLAY SKIN SCIENCES INDIA PVT. LTD** was performed. During the study, it was found that the sample showed **an SPF of 51.98, a Critical wavelength of 382, and a PA rating of PA++++.**

The report of the ***Invitro Estimation for Sun Protection Factor and PA Rating of NIACINAMIDE SUNSCREEN*** conducted has been submitted through **Project No: CCFT-2024-08-464H**.

NOTE: Results and conclusions apply only to the test article tested. Any extrapolation of these data to other Samples are the supplier's responsibility.

ARCHIVE: Study plan, Experiment data, raw data, documentation, and a copy of the final report (MIET/CCFT/2024-08/464H/SPFPA) are retained in the Institute's Archives at CCFT, MIET, NH-58, Delhi-Roorkee Highway, Baghpat Bypass Road Crossing, Meerut – 250005, India for a period of nine years.

PRINCIPAL INVESTIGATOR	SIGNATURE	DATE
Dr. Anurag Chaudhary (MPharm Ph.D.)		**03.08.2024**

11.ANNEXURE – I: REFERENCES

1. ISO 24443:2021- Cosmetics- Determination of sunscreen UVA photoprotection in vitro.