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

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

IN-VITRO WATER RESISTANT CAPACITY OF THE PLUM RICE WATER & NIACINAMIDE 2% HYBRID SUNSCREEN– 4 HOURS

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	**: 2024-08-496H**
REPORT NO.	**: MIET/CCFT/2024-08/496H/WRR**
DATE	**: 16-08-2024**

Test Compound : **PLUM RICE WATER & NIACINAMIDE 2% HYBRID SUNSCREEN**
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1. STATEMENT OF COMPLIANCE

I, the undersigned hereby declare that **Project No. CCFT-2024-08-496H/ Report No. MIET/CCFT/2024-08/496H/WRR**; entitled "***In-Vitro Estimation of water-resistant capacity of the PLUM RICE WATER & NIACINAMIDE 2% HYBRID SUNSCREEN***" was performed in accordance to the standard procedure of Centre for Cruelty-free Testing, MIET Meerut, UP, as well as the approved study plan.

I hereby attest 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 year. 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 **16-08-2024**
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 with accordance to GLP.

Laboratory Scientist
Dr. Prabhash Nath Tripathi (M.Pharm PhD)

16-08-2024
Date

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3. QUALITY ASSURANCE REPORT

This **Project No. CCFT-2024-08-496H/ Report No. MIET/CCFT/2024-08/496H/WRR**; entitled "***In-Vitro Estimation of water-resistant capacity of the PLUM RICE WATER & NIACINAMIDE 2% HYBRID SUNSCREEN***" was subjected to inspection by Quality Assurance Unit.

This report had 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	: 18-07-2024
Test Material Preparation	: 13-08-2024
Date of Testing	: 14-08-2024
Draft Report Audit	: 16-08-2024
Final Report Date	: 16-08-2024

QUALITY ASSURANCE OFFICER **16-08-2024**
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)

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5. INTRODUCTION & METHOD

The test is based on the assessment of UV-transmittance through a thin film of PLUM RICE WATER & NIACINAMIDE 2% HYBRID SUNSCREEN spread on a roughened substrate, before and after exposure to a water bath. Because of the several variables that cannot becontrolled with typical thin film spectroscopic techniques, each set of sunscreen transmission data is mathematically adjusted so that the in vitro SPF data yield 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, Water bath.
- **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, 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 was 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 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, blank scan was taken and the sample plate was prepared.

Sample plate preparation

- Both sides of the PMMA plate was 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 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.

Operating Procedure

- Initially blank plate was scanned using UV2000S transmittance Analyzer.
- Then sample plate was scanned for determination of SPF Before water immersion.
- Plate was then immersed in water bath for 20 mins and then allowed to dry for 20 minutes.
- Then sample plate was scanned for determination of SPF after water immersion.

Formula

Water resistant = SPF obtained after water immersion/ SPF obtained before water immersion*100

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, the 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.

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:

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

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:

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

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7. STATISTICS

PLUM RICE WATER & NIACINAMIDE 2% HYBRID SUNSCREEN Plate 1 Before

Parameters	SPF	Lambda Critical
No. of Scans	3	3
Mean	49.73	376.67
STD	0.53	0.58
COV	1.07%	0.15%

PLUM RICE WATER & NIACINAMIDE 2% HYBRID SUNSCREEN Plate 1 After

Parameters	SPF	Lambda Critical
No. of Scans	3	3
Mean	46.67	376
STD	4.69	0
COV	10.04%	0.00%

PLUM RICE WATER & NIACINAMIDE 2% HYBRID SUNSCREEN Plate 2 Before

Parameters	SPF	Lambda Critical
No. of Scans	3	3
Mean	46.64	376.33
STD	1.6	0.58
COV	3.44%	0.15%

PLUM RICE WATER & NIACINAMIDE 2% HYBRID SUNSCREENPlate 2 After

Parameters	SPF	Lambda Critical
No. of Scans	3	3
Mean	42.46	376
STD	1.23	0
COV	2.90%	0.00%

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PLUM RICE WATER & NIACINAMIDE 2% HYBRID SUNSCREEN Plate 3 before

Parameters	SPF	Lambda Critical
No. of Scans	3	3
Mean	53.16	377
STD	13.98	0
COV	26.29%	0.00%

PLUM RICE WATER & NIACINAMIDE 2% HYBRID SUNSCREEN Plate 3 After

Parameters	SPF	Lambda Critical
No. of Scans	3	3
Mean	40.02	376
STD	0.87	0
COV	2.16%	0.00%

Final Results

% water resistant after 04hrs = SPF obtained after water immersion/ SPF obtained before water immersion*100.

Plate 1 = 46.67/49.73*100 = 93.84**%**

Plate 2 = 42.46/46.64*100 = 91.03**%**

Plate 3 = 40.02/53.16*100 = 75.28**%**

Mean water-resistant capacity after 04 hrs is **86.71%**.

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8. RESULTS

The water resistance of the Plum Rice Water & Niacin amide 2% Hybrid Sunscreen is measured at 86.71%, and it remains water-resistant for up to 5 hours.

9. ARCHIVE

On completion of the study, study plan, experiment data, raw data, documentation and a copy of the final report (MIET/CCFT/2024-08/496H/WRR) 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 ***In-Vitro Estimation of water-resistant capacity of the PLUM RICE WATER & NIACINAMIDE 2% HYBRID SUNSCREEN*** supplied by **Mittal Global Clinical Trial Services** was performed. **The Water-resistant capacity at 4 hours obtained for PLUM RICE WATER & NIACINAMIDE 2% HYBRID SUNSCREEN is 86.71%.**

The report of the ***In-Vitro Estimation of water-resistant capacity of the PLUM RICE WATER & NIACINAMIDE 2% HYBRID SUNSCREEN*** conducted has been submitted through **Project No: CCFT-2024-08-496H.**

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

ARCHIVE: Study plan, Experiment data, raw data, documentation, and a copy of the final report (MIET/CCFT/2024-08/496H/WRR) 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 year.

PRINCIPLE INVESTIGATOR	SIGNATURE	DATE
Dr. Anurag Chaudhary (MPharm PhD)		**16-08-2024**

11. ANNEXURE – I: REFERENCES

1. COLIPA Guidelines, 2011. *In vitro* method for the determination of the UVA protection factor and "critical wavelength" values of sunscreen products.