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FINAL REPORT FOR THE ISO 24443 *IN VITRO* METHOD FOR THE DETERMINATION OF THE UVA PROTECTION FACTOR AND "CRITICAL WAVELENGTH" VALUES OF TEST PRODUCTS

Report Date: March 2, 2021	**FSTI's Study #:** 21-208
Sponsor: [redacted] **Sample Description:** Sea Clearly SPF 50	**Sponsor's Formula #:** 210012-01/338-075

Summary:
The Sponsor's test product was tested, as per the attached Florida Suncare Testing (FSTI) test protocol, Appendix I. Adjusted mean graphs of before and after UV exposure scans are included in Appendix 2. Below is a summary of the results.

- The Critical Wavelength passes with a value over 370nm with an average of **374.00nm**
- The mean UVAPF value of **19.30**, with a ratio of **2.59,** based on label SPF 50 is **38.61%.**
- Therefore, satisfying the 3:1 ratio being equal to or greater than 33.3% of the label SPF value.

ISO 24443 Determination of UVA photoprotection *in vitro*

21-208 UVA-PF	Plate 1	Plate 2	Plate 3	Plate 4	**Mean**	STDV	CoV (%)	95% CI
Critical Wavelength	374.20	374.10	373.90	373.80	**374.00**	0.18	0.05%	
ISO 24443 *in vitro* UVAPF	18.19	20.16	19.49	19.36	**19.30**	1.00	5.18%	8.24%

Ratio = SPF_{label} / UVAPF = (50/19.30) =**2.59** Percentage=100/2.59=**38.61%**

S2 Reference	
Critical Wavelength	387.40
ISO 24443 *in vitro* UVAPF	11.88

Ratio = SPF_{label} / UVAPF = (16/11.88) = **1.34** Percentage=100/1.34=**74.62%**

Approvals: [redacted] 3/2/2021 **Date** [redacted] 3/2/2021 **Date**

(Clinical Manager) **(Clinical Director)**

FSTI STUDY NUMBER: 21-208

SPONSOR: [redacted]
SPONSOR'S FORMULA NUMBER: 210012-01/338-075
SPONSOR'S FORMULA DESCRIPTION: Sea Clearly SPF 50
DATE RECEIVED: February 9, 2021 **DATE COMPLETED:** March 2, 2021

FSTI'S PROTOCOL NUMBER: Attached (2012-12)

I. OBJECTIVE

To measure the UVA protection factor and critical wavelength for a sunscreen formula, *in vitro*, in accordance with the ISO 24443 test method. The test will be performed utilizing an Optometrics LLC, Model SPF-290S UV spectrometer Analyzer System, equipped with the WinSPF Software 4.2.

II. SAMPLE DESCRIPTION

FSTI's Study Number	Sponsor's Formula Number	Sponsor's Formula Description
21-208	210012-01/338-075	Sea Clearly SPF 50

III. TEST MATERIAL HANDLING

FSTI received the test product as described above from Allure Labs, Inc.

IV. ARCHIVING

All original protocols, raw data sheets, and copies of final reports are maintained on the premises of Florida Suncare Testing, Inc., in limited access storage files in accordance with FSTI SOP# 2008-10. A duplicate copy of all final reports is kept on a secured, password-protected hard drive

V. EXPERIMENTAL DESIGN

The design was as described in the protocol, Appendix 1.

VI. GENERAL REPORTING

A. The instrumentation used in the experiment was as described in the attached protocol. Including the Optometrics SPF290 and the Oriel. A cooling tray was used in conjunction with the Oriel in order to maintain the sample plate temperature below 40°C.

B. Molded PMMA plates with a surface area of 25 cm^2 (5cm X 5cm) were used. The plates were supplied by Solar Light (Lot 8-5105).

C. To achieve an application quantity of 1.3 mg/cm^2, ≈ 32.5 mg of the product was added to the test plate and applied with a pre-saturated fingertip.

D. Appendix 2 provides an absorbance graph of the results for the test product.

E. The labeled *in vivo* sun protection factor used for the test product was 50. The labeled *in vivo* sun protection factor used for the S2 reference was 16.

F. The chart below provides the constant C for each product. C is the coefficient of adjustment, iteratively determined to adjust the calculated *in vitro* SPF value to the labeled (*in vivo*) SPF value.

Product	C
S2 Reference	1.182
21-208-1	0.939
21-208-2	0.948
21-208-3	0.922
21-208-4	0.917

G. The chart below provides the UVA dose (in W/cm^2) administered to each plate.

Product	UVA Dose (.0171W/cm2)
S2 Reference	16mins 32sec
21-208-1	22mins 25sec
21-208-2	21mins 35sec
21-208-3	23mins 16sec
21-208-4	23mins 23sec

H. The measured temperature of the plate level during exposures was 27°C

Appendix 1

FLORIDA SUNCARE TESTING, INC.

PROTOCOL FOR THE ISO 24443 *IN VITRO* METHOD FOR THE DETERMINATION OF THE UVA PROTECTION FACTOR AND "CRITICAL WAVELENGTH" VALUES OF TEST PRODUCTS

Study 21-208

I. OBJECTIVE

To measure the UVA protection factor and critical wavelength for a sunscreen formula, *in vitro*, in accordance with the ISO 24443 Guideline. The test will be performed utilizing an Optometrics LLC, Model SPF-290S UV spectrometer Analyzer System, equipped with the WinSPF Software 4.2.

II. STUDY TYPE

In vitro study, with the UVAPF, UVA/UVB ratio, and critical wavelength determined of the absorbance curve obtained by measuring the UV transmittance of the test sunscreen formula.

III. TEST PRODUCT

FSTI's Study Number	Sponsor's Formula Number	Sponsor's Formula Description
21-208	210012-01/338-075	Sea Clearly SPF 50

IV. MATERIALS AND INSTRUMENTATION

A. Spectrophotometer (specifications)

The spectrophotometer that will be used in this test is an Optometrics LLC, Model SPF-290S UV spectrophotometer Analyzer system equipped with the WinSPF software 4.2. The wavelength range measured by the spectrophotometer is 290 to 400 nm with an increment wavelength step of 1nm. The SPF-290S used in this test is equipped with a horizontal x-y stage that holds the PMMA plate in a horizontal position. It is mounted as close as possible to the input optics of the spectrophotometer to maximize capture of forward scattered irradiation. It has a suitable aperture through which UV radiation can pass. The PMMA plate will be placed on the upper surface of the sample holder with the

roughened side up. The SPF-290 has a xenon light source that produces a continuous spectral distribution of UV radiation from 290 to 400 nanometers.
The SPF-290 is equipped with an integrating sphere. The dynamic range of the SPF-290 is sufficient to measure transmittance accurately through all terrestrial solar UV wavelengths (290 to 400 nm). Its irradiance is sufficiently low so that photostability of the product is not unduly challenged. The performance of the spectrophotometer is checked at regular intervals by the measurement of defined reference materials.

B. UV source for irradiation of the test product

The device used for irradiation in this test will be an Oriel solar simulator. A black matte material will be placed under the PMMA plates to produce a non-reflecting surface during irradiation. The emission of the UV source is checked annually for compliance by a suitably qualified expert.

C. Substrate / Plate

The plates to be used for this test are PMMA plates (polymethylmethacrylate) with one side of the substrate molded. They have an area of 25 cm^2 (5 cm x 5 cm) with a 5u roughness. The plates were manufactured by Solar Light.

V. METHOD

A. Transmission measurements through the untreated plate

A 100% transmission reference sample will be prepared by spreading approximately 15 mg of glycerin to the molded side of a PMMA plate. The transmission of UV radiation through the reference plate will then be determined.

B. Sample Application

The test product will be applied to the molded side of the PMMA plate at a rate of 1.3 mg/cm^2 (actual quantity applied to the plate). The test product will be applied as a large number of small droplets close to the same size, distributed evenly over the whole surface of the plate. This will be done quickly to limit product evaporation. After the test product is deposited on the surface of the plate, it shall be spread immediately over the whole surface using light stokes with a fingertip (without finger cot). Spreading should be completed in a two-phase process. First, the test product should be distributed over the whole area as quickly as possible (less than 30 seconds) using small circular motions with minimal pressure. Then the test product should be rubbed on the plate surface using alternating horizontal and vertical strokes with increased moderate pressure. The second phase should take 20 to 30s. The test product shall be allowed to dry for at least 30 minutes in the dark at the same temperature that will be experienced under the UV exposure conditions.

C. Transmission Measurements Through the Plate with Test Product Applied

The test plate is placed in the path of the SPF 290's UV source and a mean value for transmission is determined from 290 to 400nm in 1 nm increments.

D. Number of determinations

At least four plates prepared with the test product will be used to establish the protection aspects. Four different measurements will be taken on each plate.

E. UV exposure using the UV source

The incident irradiance has been measured in the plane of the treated plate surface. The Oriel is air cooled and has the ability to maintain sample temperatures below 40°C. A matte dark background will be placed behind each plate to reduce the risk of any back exposure.

F. Transmission measurements after UV exposure

The transmission measurements after UV exposure will be conducted in exactly the same plate locations as those measured before.

VI. CALCULATIONS

The final UVA-PF and Critical Wavelength values will be the mean of values derived from individual plates. The necessary calculations are provided below. The spreadsheet will be employed in this test.

Calculation of the $SPF_{in\ vitro}$ for each plate

$$SPF_{in\ vitro} = \frac{\int_{\lambda=290nm}^{\lambda=400nm} E(\lambda) * I(\lambda) * d\lambda}{\int_{\lambda=290nm}^{\lambda=400nm} E(\lambda) * I(\lambda) * 10^{-A_0(\lambda)} * d\lambda} \qquad (1)$$

where:

$E(\lambda)$ = Erythema action spectrum (CIE- 1987)

$I(\lambda)$ = Spectral irradiance of the UV source

$A_0(\lambda)$ = Mean monochromatic absorbance measurements per plate of the test product layer *before* UV exposure

$d\lambda$ = Wavelength step (1 nm)

Calculation of the adjusted in vitro SPF (*in vitro, adj*) and determination of the coefficient of adjustment "C"

C is the coefficient of adjustment, iteratively determined to adjust the calculated *in vitro* SPF value to the labeled *(in vivo)* SPF value. Ring test data show that when the value of "C" falls within the range of 0.8 to 1.6, the UVAPF that is calculated is consistent and reliable. Larger or smaller values of "C" could possible distort the UV absorbance profile of the test sample and consequently may have an impact on the accuracy of the UVAPF determined. Consequently, it is recommended that the value of the "C" should fall into the range of 0.8 to 1.6. If the "C" falls outside this range, then the operator may optionally review their product application and spreading procedure, in order to establish whether a modification to their technique might help achieve this target range for the value of "C." <u>The amount of product applied to the plate, however must not be adjusted to bring C within this range.</u>

$$SPF_{in\ vitro,adj} = SPF\ label = \frac{\int_{\lambda=290nm}^{\lambda=400nm} E(\lambda) * I(\lambda) * d\lambda}{\int_{\lambda=290nm}^{\lambda=400nm} E(\lambda) * I(\lambda) * 10^{-A_0(\lambda)*C} * d\lambda} \quad (2)$$

Where:

$E(\lambda)$, $I(\lambda)$, $A_0(\lambda)$ and $d\lambda$ are defined in equation (1).

Calculation of $UVAPF_0$

$UVAPF_0$ is calculated for each plate individually.

$$UVAPF_0 = \frac{\int_{\lambda=320nm}^{\lambda=400nm} P(\lambda) * I(\lambda) * d\lambda}{\int_{\lambda=320nm}^{\lambda=400nm} P(\lambda) * I(\lambda) * 10^{-A_0(\lambda)*C} * d\lambda} \quad (3)$$

where:

$P(\lambda)$	=	PPD action spectrum
$I(\lambda)$	=	Spectral irradiance of the UV source (UVA 320-400nm for PPD testing; see Appendix I)
$A_0(\lambda)$	=	Mean monochromatic absorbance of the test product layer before UV exposure
C	=	Coefficient of adjustment previously determined in equation (2)
$d\lambda$	=	Wavelength step (1 nm)

Calculation of UVA dose "D" for sample irradiation

The single UVA dose D is derived from the $UVAPF_0$ value. To note, the sample is exposed to full-spectrum UV radiation but the dose used is defined by its UVA content.

$$D = UVAPF_0 \times D_0 \quad J\ cm^{-2} \quad (4)$$

D_0 is the unit UVA dose per unit $UVAPF_0$, to be given by the UV source (which has been determined experimentally to give a good correlation between *in vitro* UVAPF and *in vivo* PPD values). This D_0 value has been optimised using data from a multi-centre Ring Study performed by the Colipa In Vitro UV Methods group and is fixed at **1.2**J cm^{-2} UVA.

Calculation of UVAPF of plates after UV irradiation of the sample

$$UVAPF = \frac{\int_{\lambda=320nm}^{\lambda=400nm} P(\lambda) * I(\lambda) * d\lambda}{\int_{\lambda=320nm}^{\lambda=400nm} P(\lambda) * I(\lambda) * 10^{-A(\lambda)*C} * d\lambda} \quad (5)$$

Where:
$P(\lambda)$,$I(\lambda)$,C and $d\lambda$ are defined in equation (3).
$A(\lambda)$ is the mean monochromatic absorbance of the test product layer *after* UV exposure.

The UVAPF of an individual plate is calculated from the mean absorbance value from all individual spots. If the coefficient of variation of absorbance between spots exceeds 50% then the plate should be rejected and a new plate prepared.

Calculation of UVAPF

At least 4 plates prepared with the test product will be used to establish the protection aspects of the test sample. Additional plates will be added to the sampling if the 95% confidence interval (CI) is greater than 17% of the mean value of the UVAPF value, until the 95% CI is less than 17% of the mean UVAPF value.

Ratio SPF / UVAPF Calculation

Using the *in vivo* sun protection factor (labelled SPF) and the *in vitro* UVA protection factor UVAPF, calculate the ratio below.

$$Ratio = \frac{SPF_{label}}{UVAPF}$$

Calculation of Critical Wavelength Value

The Critical Wavelength λ_c value for the test product is defined as that wavelength where the area under the absorbance spectrum for the irradiated product (obtained using the method described above) from 290nm to λ_c is 90% of the integral of the absorbance spectrum from 290nm to 400nm, and is calculated in the following way:

A series of absorbance values (dependent on the wavelength increment) are calculated for each of the four separate plates to which the test product has been applied. Absorbance at each wavelength increment (A_λ) is calculated thus:

$$A_\lambda = \log (C_\lambda / P_\lambda) \quad (6)$$

where

$$C_\lambda = \sqrt[n]{(c_\lambda[1] \times c_\lambda[2] \times \ldots \times c_\lambda[n]} \quad (7)$$

$$P_\lambda = \sqrt[n]{(p_\lambda[1] \times p_\lambda[2] \times \ldots \times p_\lambda[n]}$$

$c_\lambda[n]$ = arithmetical mean of transmission measurements taken at measurement point n and at wavelength λ for the reference sample (glycerine-treated roughened PMMA plate)

$p_\lambda[n]$ = arithmetical mean of transmission measurements taken at measurement point n and at wavelength λ for irradiated, sunscreen product treated sample (roughened PMMA plate)

Critical Wavelength λ_c is calculated for each irradiated plate as follows:

$$\int_{290}^{\lambda c} A_\lambda . d\lambda = 0.9 \int_{290}^{400} A_\lambda . d\lambda \quad (8)$$

The final Critical Wavelength value for each tested sunscreen product is the mean of values recorded for each measured, irradiated, product-treated PMMA plate.

VII. REFERENCE SUNSCREEN

To ensure the validity of this method a reference sunscreen, formula S2, will be tested along with the test product. A value of 16 will be used as the S2 *in vivo* SPF value for computation purposes. The results of the reference S2 UVAPF must be between 10.7 and 14.7 for the test to be valid.

VIII. REPORTING

At the completion of the study, the Technician will provide the Sponsor with a final report on the determination of the UVA protection factor and the Critical Wavelength.

IX. PRIVACY POLICY

Each study will be issued an individual study number. All final reports and any supporting documents will always be held in the strictest confidence and filed using the individual study number only. No one other than the Study Sponsor and the Staff of Florida Suncare Testing, Inc. will have access to the results from this study. Test products are identified by a study number. At no time will the Sponsor or Manufacturer's identity, product name, or any other information be shared unless requested by a regulatory government agency and then only on a need to know basis.

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X. PROTOCOL APPROVAL

For: [redacted]

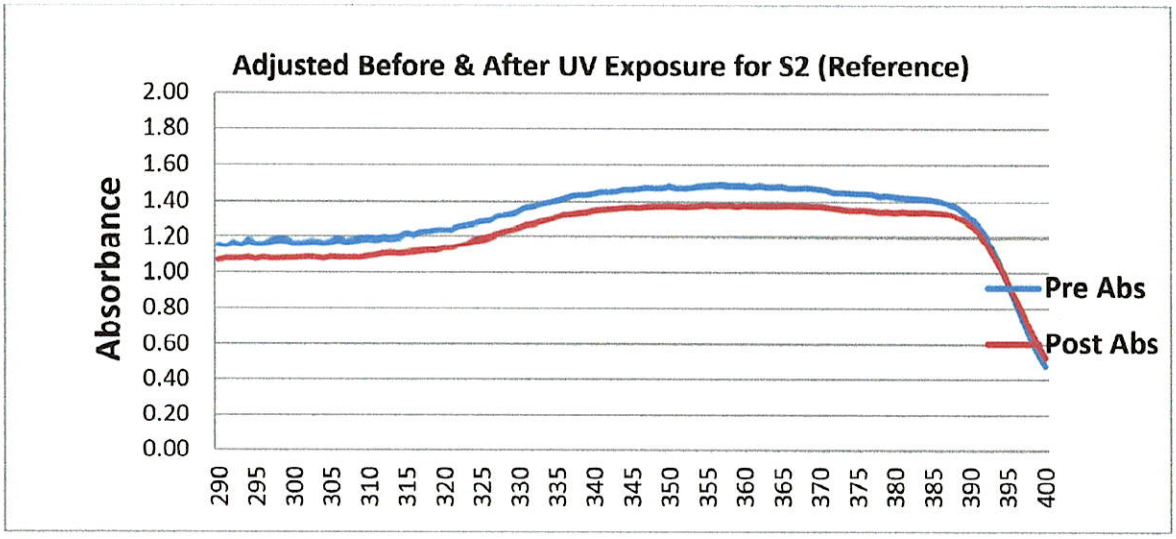
Approved By: Sample Submission Form on File Date: February 9, 2021

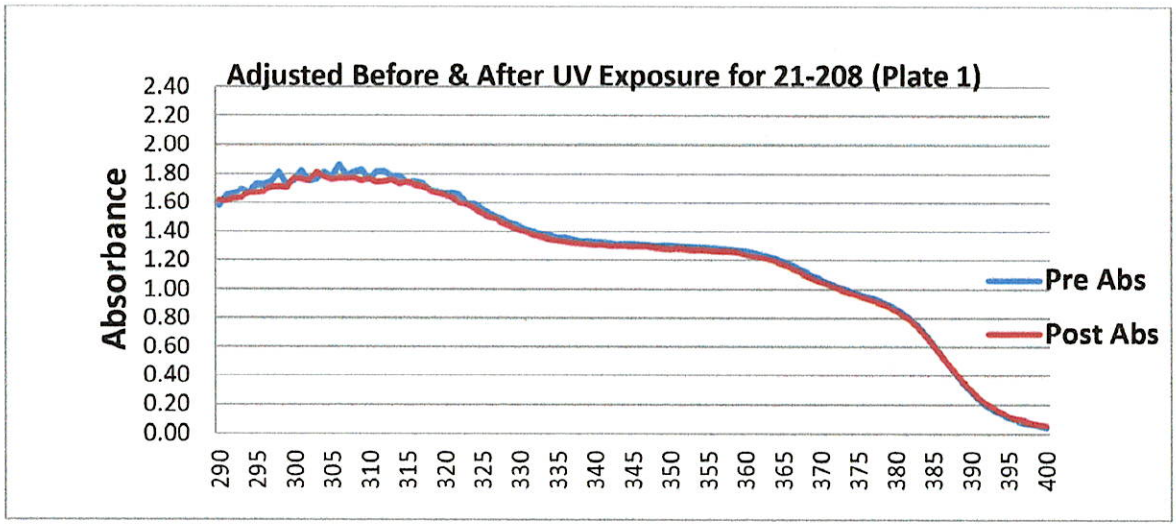
For: Florida Suncare Testing, Inc.

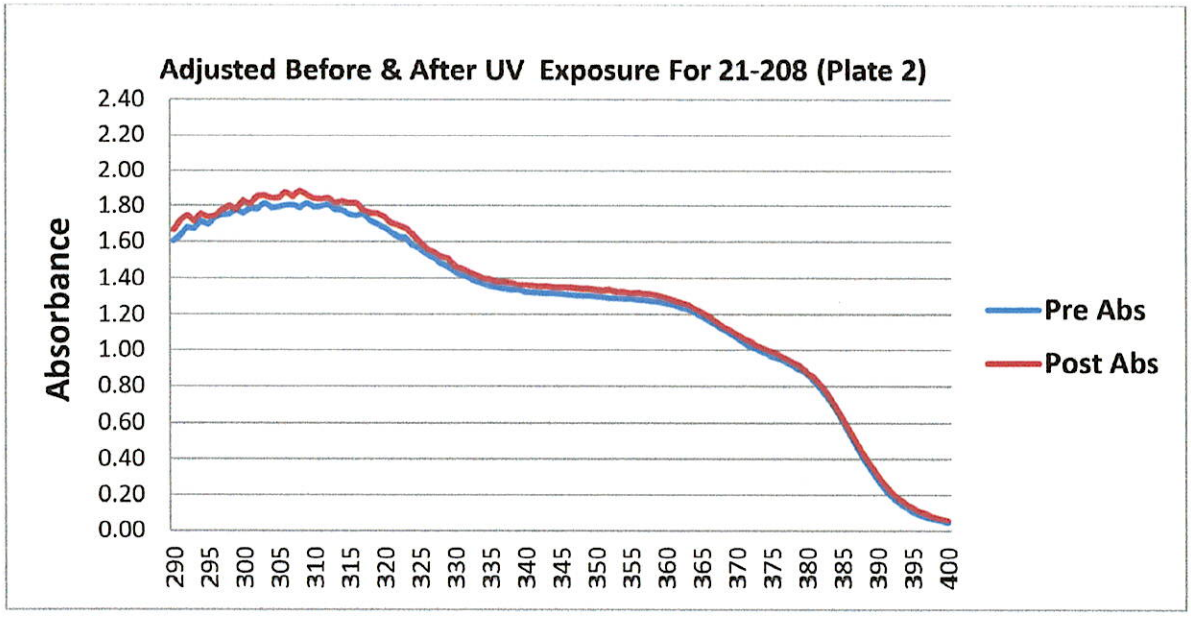
Approved By: [redacted] Date: 2/9/2021

[redacted] Clinical Director

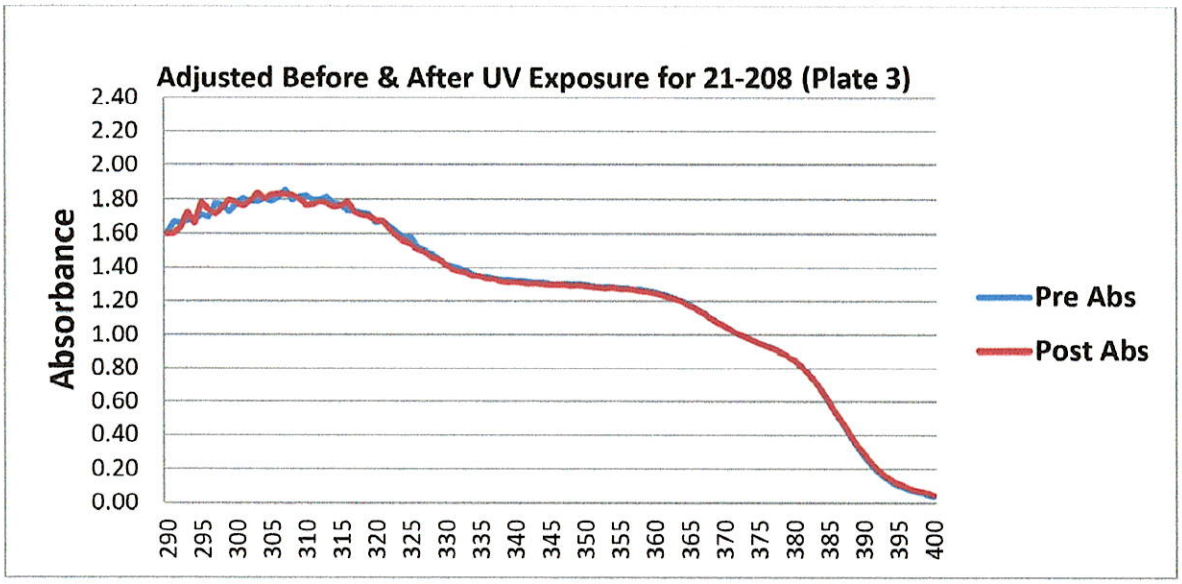
Appendix 2

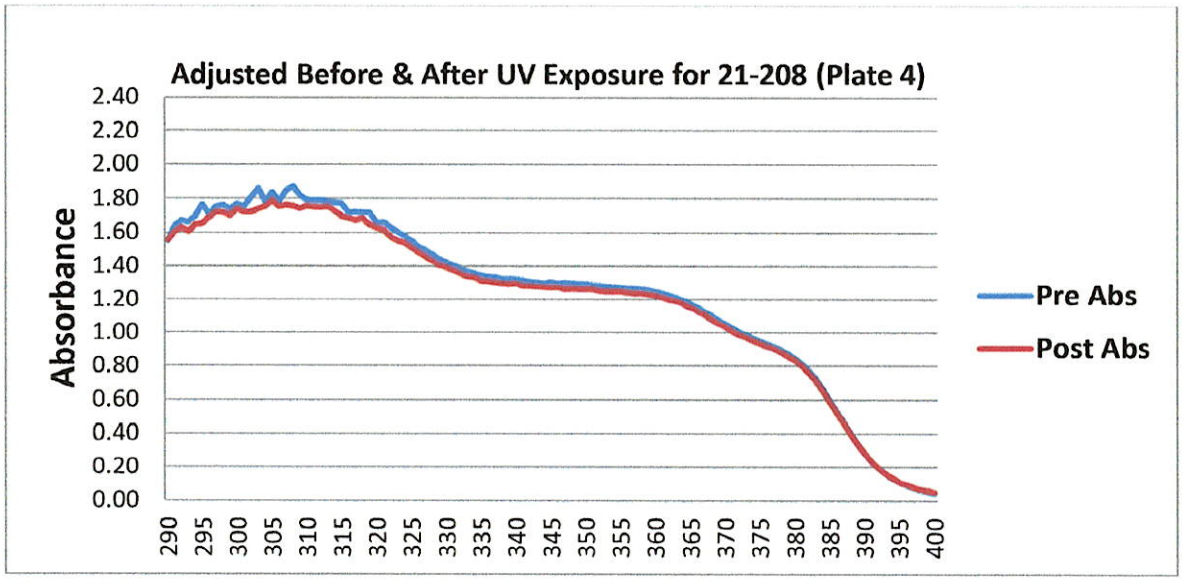

Adjusted Before & After UV Exposure for S2 (Reference)
Absorbance
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
290 295 300 305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395 400
Pre Abs
Post Abs


Adjusted Before & After UV Exposure for 21-208 (Plate 1)
Absorbance
2.40
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
290 295 300 305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395 400
Pre Abs
Post Abs


Adjusted Before & After UV Exposure For 21-208 (Plate 2)
Absorbance
2.40
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
290 295 300 305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395 400
Pre Abs
Post Abs

Appendix 2


Adjusted Before & After UV Exposure for 21-208 (Plate 3)
Absorbance
2.40
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
290 295 300 305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395 400
Pre Abs
Post Abs


Adjusted Before & After UV Exposure for 21-208 (Plate 4)
Absorbance
2.40
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
290 295 300 305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395 400
Pre Abs
Post Abs