UV Hair Protection Assay

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Tradename: AC Pina Colloida

Code: 12053

CAS #: 7732-18-5 & 68917-26-0 & 68333-16-4 (or) 1686112-36-6 (or) 9015-54-7

Test Request Form #: 10534

Lot #: N230829C

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Daniel Shill*
Principal Investigator: *Hannah Stade*

Test Performed:
UV Hair Protection Assay

Introduction

Hair proteins and lipids play a key role in the structural integrity of hair. The physical bonds between amino acids provide mechanical stability and chemical resistance to the hair fiber while lipids influence shine, feel, manageability, and strength. Both proteins and lipids within the hair are vulnerable to sunlight as it causes photo-oxidation of amino acid bonds and lipid decomposition. As a result, exposure to UV irradiation leaves hair dry, brittle, and damaged.

Tryptophan is a chromophore found in hair with a well-defined spectral range. This photo-sensitive amino acid degrades when exposed to UV irradiation and can be used as a proxy for hair photo-degradation and damage. As tryptophan oxidizes in response to UV irradiation, relative fluorescence decreases, indicating damage has occurred within the hair shaft.

Saturated and unsaturated fatty acids can also be used to understand photo-damaged hair. Exposure to UV causes lipids to oxidize forming lipid peroxides. The inverse relationship of hair lipids and lipid peroxides provides a clear interpretation of the negative impacts UV irradiation has on hair fibers. Specifically, healthy hair is characterized by high lipid content with few lipid peroxides whereas damaged hair displays reduced lipid content and increased lipid peroxides.

The deleterious effects of UV exposure in hair care are a new frontier for active ingredients given the vulnerability of hair and lack of protective mechanisms against the sun. Accordingly, a UV Hair Protection Assay was conducted to assess the *ex vivo* effect of **AC Pina Colloida** to reduce hair damage caused by UVB exposure.

Assay Principle

A multiparameter approach was used to determine the UV protection capabilities of cosmetic hair applications. Human hair tresses were treated with test materials and exposed to UVB irradiation. Extractions of amino acids and lipids were performed on each hair tress. The amino acid, tryptophan, was quantified using relative fluorescence, while lipids were quantified using the colorimetric sulfo-phospho-vanillin method. From the lipid extracts, lipid peroxides were quantified fluorometrically using a malondialdehyde-thiobarbituric acid adduct (MDA-TBA).

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Materials

A. Kits:	Lipid Peroxidation (MDA) Assay Kit (Abcam, ab118970)*; Lipid Assay Kit (Abcam, ab242305)*
B. Equipment:	Accuris UV Transilluminator (25.3 W/m^2); Synergy HT Microplate Reader; Pipettes; Warm Bead Bath; 3510 Branson Sonicator
C. Hair Samples:	Human Virgin Brunette Hair Tresses
D. Reagents:	Sodium Hydroxide (2 M); Methanol (99.8%); Sulfuric Acid (18M);
E. Microplates:	Corning 96 Well Black Side/Clear Bottom Microplate*; Flat Bottom 96-Well Microplate*
F. Product:	it's a 10 Miracle Leave-in Product*
G. Software:	Excel Analysis ToolPak (Microsoft)
H. Other:	Sterile disposable pipette tips; Microcentrifuge tubes; 15 mL test tubes; 50 mL test tubes; Filters

*Or suitable alternatives, subject to change without notice based off vendor availability

Methods

Six virgin brunette hair tresses were collected, dampened with water, and treated with 0.4 g of "it's a 10 Miracle Leave-in Product" (Positive Control), 5.0% **AC Pina Colloida** in water, or left as an Untreated Control (water rinse only). After treatment, tresses were airdried, and then subjected to 0 or 48 hours of UV exposure at 25.3 W/m2 (Table 1).

Table 1. Descriptions of the Conditions and Treatments for each Hair Tress

Condition	Treatment Description
Untreated Control	Dampened, 0 Hours UV Exposure
	Dampened, 48 Hours UV Exposure
Positive Control	Dampened + it's a 10 Miracle Leave-In Product, 0 Hours UV Exposure
	Dampened + it's a 10 Miracle Leave-In Product, 48 Hours UV Exposure
5.0% **AC Pina Colloida**	Dampened + 5.0% **AC Pina Colloida** in water, 0 Hours UV Exposure
	Dampened + 5.0% **AC Pina Colloida** in water, 48 Hours UV Exposure

Three separate experiments were performed as described below and average values were recorded. Data are displayed as averages and analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$.

A. Tryptophan Degradation

Tryptophan was extracted from each hair tress by dissolving the hair in a 2 M sodium hydroxide (NaOH) solution (1:1 Hair:NaOH). After sitting for 24 hours, the solutions were filtered and 200 µL of each extract was added in duplicate to a 96 well black side/clear bottom plate. Fluorescence was measured at an excitation wavelength of 290 nm and an emission wavelength of 355 nm. Tryptophan degradation was evaluated based on changes in relative fluorescence over time within each treatment group. Percent change in tryptophan for each treatment group was calculated as follows:

$$Percent\ Change\ (\%) = \left(\frac{(Fluorescence_{Time} - Fluorescence_{0\ Hours})}{Fluorescence_{0\ Hours}}\right) \times 100$$

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B. Quantification of Lipids

Concurrently, lipids were extracted from each hair tress in a 100% methanol (MeOH) solution (125 mg hair: 2.5 mL MeOH) and sonicated for 1 hour. The extracts were aspirated from the hair, placed in clean microcentrifuge tubes and allowed to evaporate completely. Dried extracts were diluted again in 375 µL MeOH.

Lipids were quantified utilizing the Lipid Assay Kit (ab242305) according to the manufacturer's instructions. Briefly, 15 µL of standards and samples were added to each well and incubated at 80˚C for 30 minutes until the solvent evaporated. The plate was transferred to 4˚C for 5 minutes, then 150 µL of 18 M Sulfuric Acid was added to each well. Next, the plate was incubated at 80˚C for 10 minutes, followed by 5 minutes at 4˚C. 100 µL of each standard and sample was transferred in duplicate to a clean 96 well plate and absorbance was read at 540 nm to determine background. Afterwards, 100 µL of Vanillin Reagent was added to each well, mixed, and incubated at 37˚C for 15 minutes. The optical density of each well was read at 540 nm again. Actual absorbance was calculated by subtracting the background from the second reading.

Lipid concentrations from the standards were plotted and a regression equation was determined from the standard curve. The lipid concentration of each hair sample was extrapolated from the standard curve and expressed in mg/dL. Percent change in lipids for each treatment group was calculated as follows:

$$Percent\ Change\ (\%) = \left(\frac{(Lipids_{Time} - Lipids_{0\ Hours})}{Lipids_{0\ Hours}}\right) \times 100$$

C. Quantification of Lipid Peroxides

Following lipid quantification, lipid peroxidation was quantified utilizing the Lipid Peroxidation (MDA) Assay Kit (ab118970) according to the manufacturer's instructions. Briefly, 200 µL of standards and samples were mixed with 600 µL Developer and incubated at 95˚C for 1 hour. Standards and samples were cooled to room temperature in an ice bath and 200 µL of each was added in duplicate to a black side/clear bottom 96 well plate. Fluorescence was measured at an excitation wavelength of 532 nm and an emission wavelength of 553 nm.

The MDA standards were plotted, and a regression equation was determined from the standard curve. The MDA amount in each hair sample well was extrapolated from the standard curve and expressed in nmol. MDA concentration for each sample extract was calculated as follows:

$$[MDA\ Concentration]\left(\frac{nmol}{mg}\right) = \left(\frac{MDA_{sample\ well}}{125\ mg\ Hair}\right) \times 4 \times 6.7$$

Percent change in MDA concentration for each treatment group was calculated as follows:

$$Percent\ Change\ (\%) = \left(\frac{([MDA]_{Time} - [MDA]_{0\ Hours})}{[MDA]_{0\ Hours}}\right) \times 100$$

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Results

The data obtained from this study met criteria for a valid assay as the controls performed as anticipated. The Untreated Control had reductions in tryptophan and lipid content as well as increased lipid peroxides after 48 hours of UV exposure. The Positive Control protected hair from UV exposure as demonstrated by minimal changes in tryptophan, lipid content, and lipid peroxides after 48 hours of UV exposure. Similarly, **AC Pina Colloida** at 5.0% demonstrated minimal changes in all three parameters after the same amount of UV exposure.

A. Tryptophan Degradation

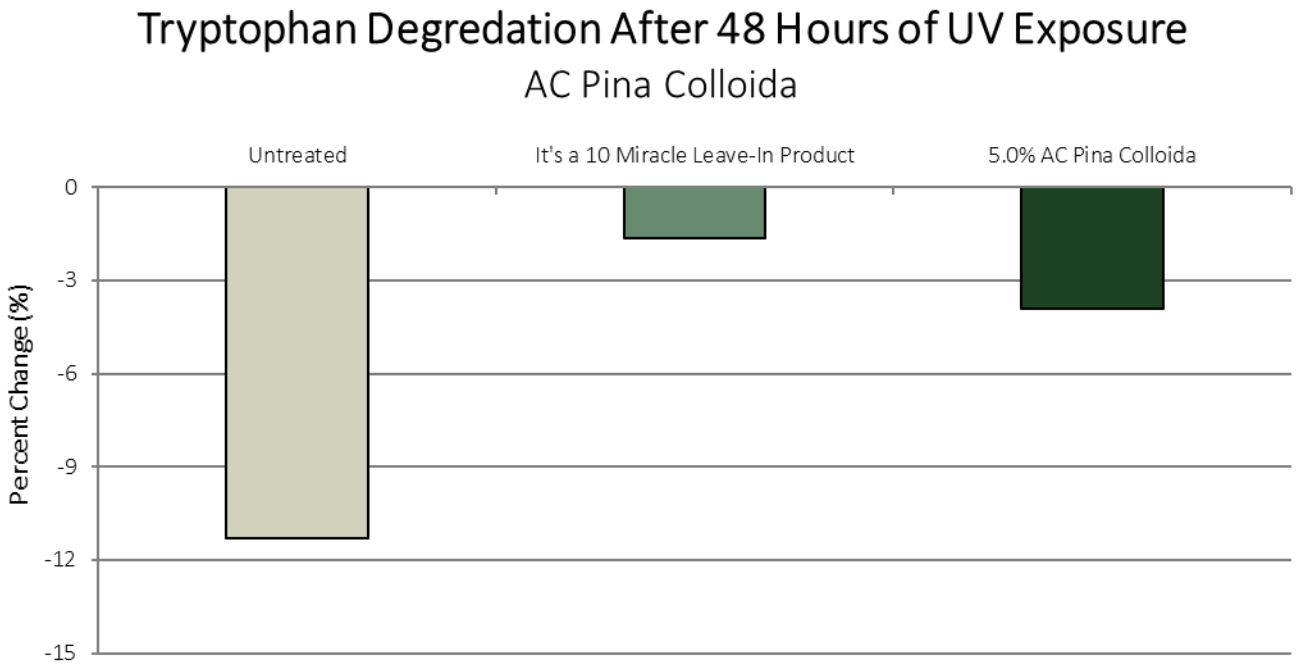

Figure 1. Percent Change in Tryptophan after 48 Hours of UV Exposure Compared to Hair without UV Exposure. Positive Control: it's a 10 Miracle Leave-In Product.

Table 2. Results from one-way ANOVA Statistical Analysis of Tryptophan Degradation between Hair Tresses with 0 and 48 Hours of UV Exposure. * indicates $p \leq 0.05$.

	Untreated Control	Positive Control	5.0% **AC Pina Colloida**
P-value	0.003*	> 0.05	> 0.05

Table 3. Results from one-way ANOVA Statistical Analysis of Tryptophan Degradation in Hair Tresses After 48 Hours of UV Exposure. * indicates $p \leq 0.05$.

	Untreated Control vs Positive Control	Untreated Control vs 5.0% **AC Pina Colloida**	Positive Control vs 5.0% **AC Pina Colloida**
P-value	< 0.001*	0.027*	> 0.05

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B. Quantification of Lipids

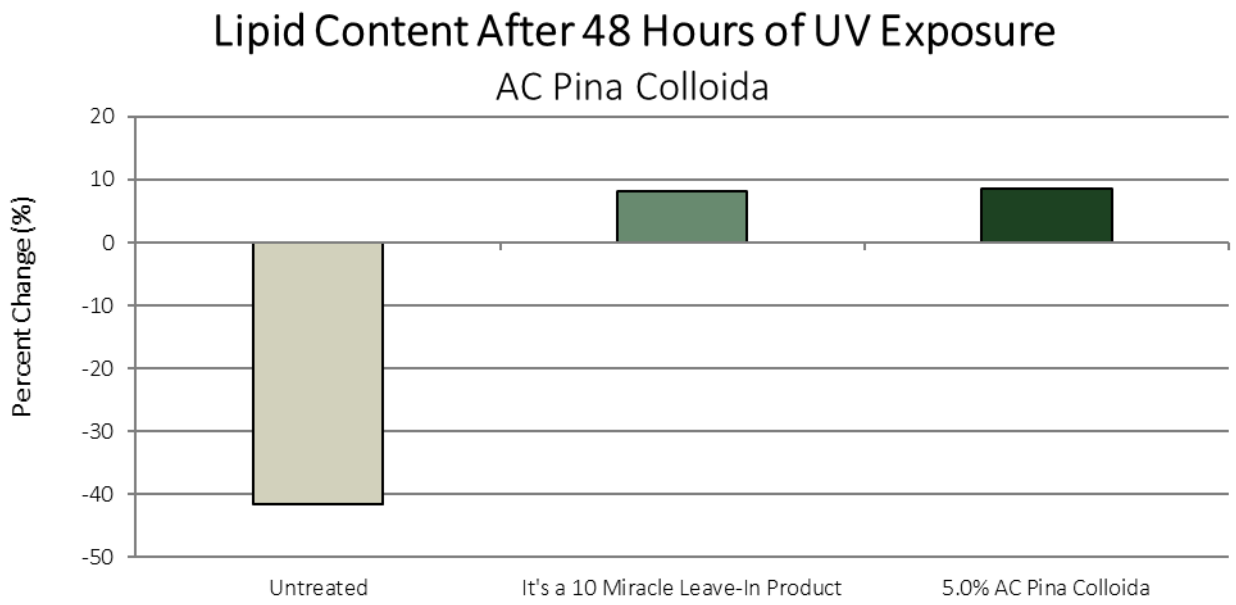

Figure 2. Percent Change in Lipid Content after 48 Hours of UV Exposure Compared to Hair without UV Exposure. Positive Control: it's a 10 Miracle Leave-In Product.

Table 4. Results from one-way ANOVA Statistical Analysis of Lipid Content between Hair Tresses with 0 and 48 Hours of UV Exposure. * indicates $p \leq 0.05$.

	Untreated Control	Positive Control	5.0% **AC Pina Colloida**
P-value	0.022*	> 0.05	> 0.05

Table 5. Results from one-way ANOVA Statistical Analysis of Lipid Content in Hair Tresses After 48 Hours of UV Exposure. * indicates $p \leq 0.05$.

	Untreated Control vs Positive Control	Untreated Control vs 5.0% **AC Pina Colloida**	Positive Control vs 5.0% **AC Pina Colloida**
P-value	0.015*	0.043*	> 0.05

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C. Quantification of Lipid Peroxides

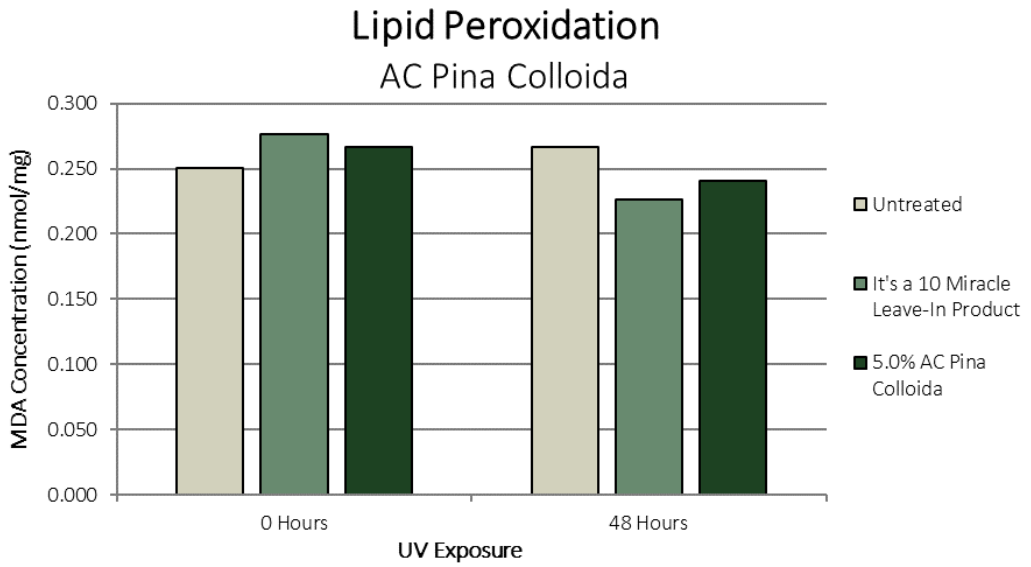

Figure 3. MDA Concentration in Hair Tresses without UV Exposure and After 48 Hours of UV Exposure. Positive Control: it's a 10 Miracle Leave-In Product.

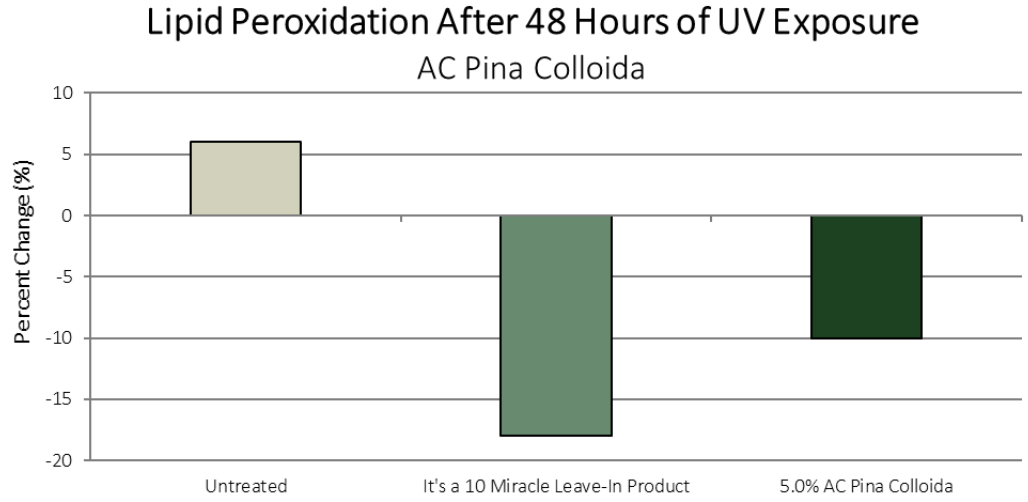

Figure 4. Percent Change in MDA Concentration after 48 Hours of UV Exposure Compared to Hair without UV Exposure. Positive Control: it's a 10 Miracle Leave-In Product.

Table 6. Results from one-way ANOVA Statistical Analysis of Lipid Peroxidation between Hair Tresses with 0 and 48 Hours of UV Exposure. * indicates $p \leq 0.05$.

	Untreated Control	Positive Control	5.0% **AC Pina Colloida**
P-value	0.046	0.045	0.044

Table 7. Results from one-way ANOVA Statistical Analysis of Lipid Peroxidation in Hair Tresses After 48 Hours of UV Exposure. * indicates $p \leq 0.05$.

	Untreated Control vs Positive Control	Untreated Control vs 5.0% **AC Pina Colloida**	Positive Control vs 5.0% **AC Pina Colloida**
P-value	< 0.001	0.003	> 0.05

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Discussion

The ability of **AC Pina Colloida** to protect hair from UV irradiation was assessed via determination of amino acid and lipid degradation. The results from this study indicate 5.0% **AC Pina Colloida** provides UV protection capabilities when applied to hair.

A. Tryptophan Degradation

As shown in Figure 1 & Table 2, tryptophan was significantly degraded by 11% in the Untreated Control tresses after 48 hours of UV exposure compared to the Untreated Control tresses without UV exposure. Conversely, hair treated with the Positive Control exhibited an insignificant decrease of 2% in tryptophan after 48 hours of UV exposure, indicating the product protected the hair from UV irradiation.

Similarly, hair tresses treated with 5.0% **AC Pina Colloida** demonstrated an insignificant decrease of 4% in tryptophan after 48 hours of UV exposure compared to hair without UV exposure (Figure 1, Table 2). The hair tresses treated with 5.0% **AC Pina Colloida** and the Positive Control lost significantly less tryptophan compared to the Untreated Control hair tresses after 48 hours of UV exposure. Additionally, there was no difference in the slight tryptophan degradations observed in hair tresses treated with the Positive Control and 5.0% **AC Pina Colloida** after 48 hours of UV exposure tryptophan (Table 3).

In summary, these results demonstrate **AC Pina Colloida** helps maintain hair shaft structural integrity by protecting amino acids like tryptophan from UV exposure, resulting in healthier hair.

B. Quantification of Lipids

As shown in Figure 2 & Table 4, lipid content was significantly decreased by 42% in the Untreated Control tresses after 48 hours of UV exposure compared to the Untreated Control tresses without UV exposure. Conversely, hair treated with the Positive Control increased lipids by 8% after 48 hours of UV exposure, indicating the product protected existing lipids from UV irradiation and provided additional lipids.

Similarly, hair tresses treated with 5.0% **AC Pina Colloida** exhibited an 8% increase in lipids after 48 hours of UV exposure compared to hair without UV exposure (Figure 1, Table 4). The hair tresses treated with 5.0% **AC Pina Colloida** and the Positive control had significantly higher lipid contents compared to the Untreated Control hair tresses after 48 hours of UV exposure. Additionally, there was no significant difference in the lipid content of hair tresses treated with the Positive Control and 5.0% **AC Pina Colloida** after 48 hours of UV exposure (Table 5).

In summary, these results demonstrate **AC Pina Colloida** protects lipids in hair from UV irradiation resulting in healthier, shiny hair.

C. Quantification of Lipid Peroxides

As demonstrated in Figures 3 & 4, lipid peroxides significantly increased by 6% in Untreated Control tresses after 48 hours of UV exposure compared to the Untreated Control tresses without UV exposure (Table 6). Conversely, hair treated with the Positive Control exhibited a significant decrease of 18% in lipid peroxides after 48 hours of UV exposure, indicating the product prevented lipid peroxidation in hair after UV irradiation.

Similarly, hair tresses treated with 5.0% **AC Pina Colloida** demonstrated a 10% decrease in lipid peroxides after 48 hours of UV exposure compared to hair without UV exposure (Figures 3-4, Table 6). The hair tresses treated with 5.0% **AC Pina Colloida** and the Positive control had significantly lower lipid peroxide content compared to the Untreated Control hair tresses after 48 hours of UV exposure. Additionally, there was no significant difference in

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lipid peroxidation of the hair tresses treated with the Positive Control and 5.0% **AC Pina Colloida** after 48 hours of UV exposure (Table 7).

In summary, these results demonstrate **AC Pina Colloida** prevents lipid peroxidation in hair caused by UV exposure, enabling hair to maintain a robust lipid content necessary for a healthy appearance.

Taken together, these results indicate **AC Pina Colloida** maintains tryptophan and lipid levels, while reducing lipid peroxidation in hair after 48 hours of UV exposure when utilized at recommended use levels. Collectively, **AC Pina Colloida** enhances the structural integrity of hair by preventing the harmful effects of UV irradiation resulting in healthier hair.