Blonde Hair Toning Assay

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Tradename: AC Violet'Blond Toner

Code: 21020

CAS #: 90131-47-8 & 84012-42-0 & 68333-16-4 (or) 92128-79-5 & 90082-41-0

Test Request Form #: 10622

Lot #: N231002A

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Drumwright*
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Test Performed:
Blonde Hair Toning Assay

Introduction

Temporary hair dyes, such as blue- or purple-pigmented shampoos, act as a toner to brighten the original hair shade to a lighter and more vibrant color. Blue shampoos use ingredients that are designed to counteract orange tones in brunette hair, while purple shampoos are more often used to rid brass in lighter blonde shades. These hair care products typically use large blue or purple dye molecules to extrude brassy tones from hair and return it to a cooler and more salon-fresh shade. Consistent use of these pigmented shampoos aid bleached or color-treated hair to appear more vibrant. Specifically, these blue- and purple-pigmented shampoos camouflage dull yellow and orange tones in permanently dyed or bleached hair after prolonged exposure to tarnishing agents (sebum, sweat, pollutants, etc.). However, increased consumer demand for more natural alternatives has led to a gap in the formulation of these blue or purple shampoos that traditionally use textile acid dyes. To achieve a similar toning or brightening effect on hair historically obtained with pigmented shampoos, **AC Violet'Blond Toner** was designed as a natural and pigment-free alternative.

Accordingly, a qualitative and quantitative assay was performed to determine the *ex vivo* color toning ability of **AC Violet'Blond Toner** on bleached hair.

Assay Principle

Brunette human hair tresses were bleached blonde as this shade is prone to exhibiting brassy tones. Tresses were imaged before and after treatment with test materials and analyzed for color shifts.

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Materials

A. **Hair Sample:** Brunette Human Hair Tresses Bleached Blonde
B. **Product:** 30 Volume Bleach; Base Shampoo (Table 1), amika: Bust Your Brass Blonde Purple Shampoo
C. **Software:** ImageJ (NIH); Excel Analysis ToolPak (Microsoft)

Table 1. Base Shampoo Compositional Breakdown.

Base Shampoo Formulation	
INCI	**%**
Water	41.0
Guar Hydroxypropyltrimonium Chloride	1.0
Sodium Methyl 2-Sulfolaurate (and) Disodium 2-Sulfolaurate	35.0
Cocamidopropyl Betaine	15.0
Lactobacillus Ferment & Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract	4.0
Polysorbate 20	2.0
Fragrance	2.0

Methods

Virgin brunette hair tresses were bleached twice using 30 volume bleach to obtain a brassy blonde hair color. Images were taken before and after washing five bleach blonde hair tresses with the following conditions: Untreated (no shampoo, water rinse), Base Shampoo, amika: Bust Your Brass Blonde Purple Shampoo, 2.0% **AC Violet'Blond Toner** in a Base Shampoo, and 5.0% **AC Violet'Blond Toner** in a Base Shampoo. The amika: Purple Shampoo was implemented for comparative purposes as a market standard, purple-pigmented shampoo. Each hair tress was evenly washed in its designated treatment and products were allowed to sit on the hair for four minutes. Tresses were then rinsed thoroughly and blown dry for one minute. ImageJ histogram analysis was performed on the acquired data to evaluate the red, green, and blue (R+G+B) color distribution present in each hair tress. The R+G+B color spectrum ranges from 0 (left) to 255 (right), where the left side of each histogram reflects exclusively red pixels, and the right side of each histogram reflects exclusively blue pixels. Percent change was calculated using the formula below:

$$Percent\ Change\ (\%) = \frac{Mean_{Treatment\ Step} - Mean_{Before\ Treatment}}{Mean_{Before\ Treatment}} \times 100$$

Three separate experiments were performed, and average values were recorded. Data was analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$.

Results

The data obtained from this study met criteria for a valid assay and the controls performed as anticipated. **AC Violet'Blond Toner** in the base shampoo reduced brassy tones in bleach blonde hair.

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Figure 1. Representative images of bleached blonde hair tresses before and after washing with corresponding R+G+B histograms. The green arrows indicate a shift towards the blue end of threshold indicating a reduction in orange tones.

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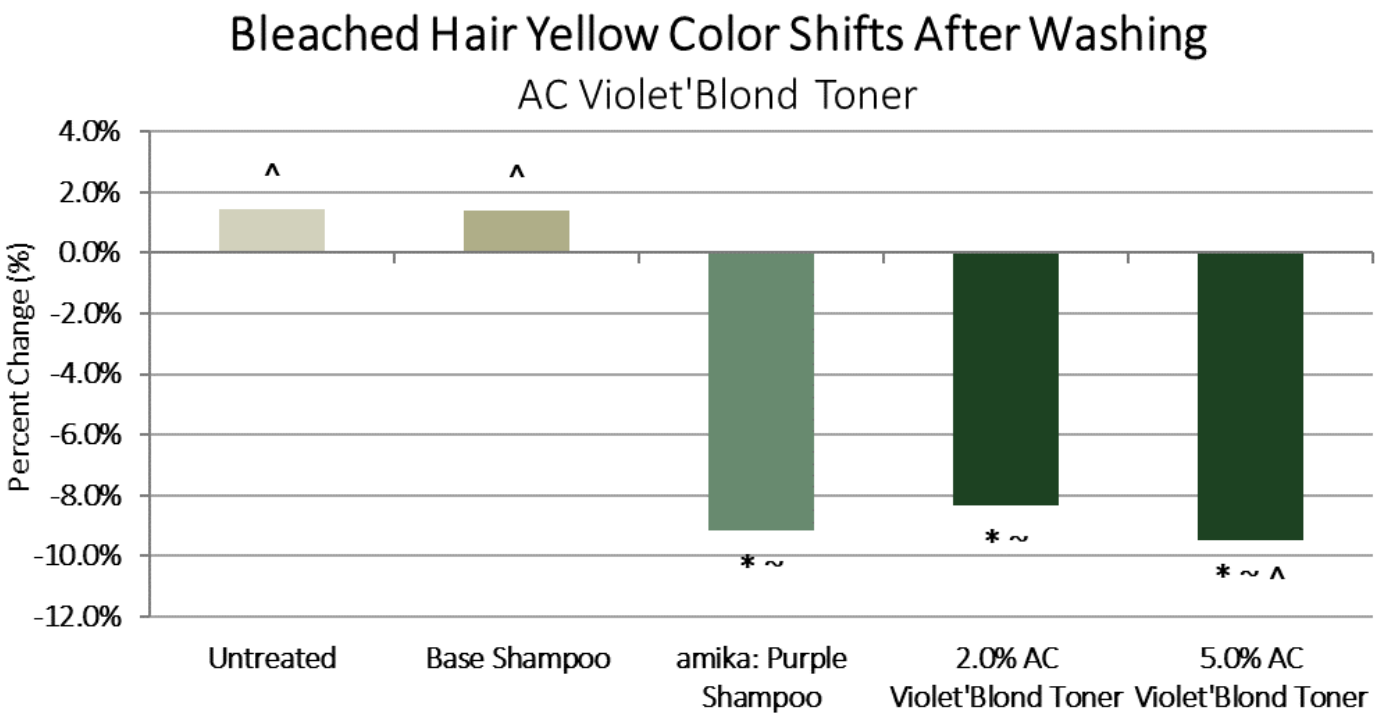

Figure 2. Bleached brunette hair shifts in yellow color (R+G+B intensity) after washing. A positive percent change indicates a shift towards more yellow tones (indicating an increase in orange tones), whereas a negative percent change indicates a shift away from yellow tones towards bluer tones (indicating a reduction in orange tones). * indicates significance ($p \leq 0.05$) compared to Untreated. ~ indicates significance ($p \leq 0.05$) compared to Base Shampoo. ^ indicates significance ($p \leq 0.05$) compared to amika: Purple Shampoo.

Table 2. Results from one-way ANOVA between Conditions Compared for Bleached Brunette Hair Shifts in Red Color. * indicates significance ($p \leq 0.05$) compared to Untreated. ~ indicates significance ($p \leq 0.05$) compared to Base Shampoo. ^ indicates significance ($p \leq 0.05$) compared to amika: Purple Shampoo.

	Untreated	Base Shampoo	amika: Purple Shampoo	2.0% AC Violet'Blond Toner	5.0% AC Violet'Blond Toner
Untreated	-----	> 0.05	0.004*	0.019*	< 0.001*
Base Shampoo	> 0.05	-----	0.011~	0.024*	0.001~
amika: Purple Shampoo	0.004^	0.011^	-----	> 0.05	0.044^

Discussion

As demonstrated in Figure 1, the untreated bleached blonde hair tress visibly retained brassy tones after washing. Quantitatively, R+G+B histogram analysis after washing without shampoo confirmed the presence of brassy tones which increased slightly by 1.4% compared to before washing (shift towards the yellow side of the spectrum) (Figure 2). Similarly, washing the bleached hair tress with the base shampoo exhibited a 1.4% increase in brassy tones compared to before washing (Figure 2). This data demonstrates brassy tones in both bleach blonde hair tresses were not removed after washing with just water (no shampoo) or the base shampoo.

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Alternatively, the bleached blonde hair tress washed with amika: Purple Shampoo had visibly reduced brassy tones (Figure 1). Histogram analysis confirmed the market standard, amika: Purple Shampoo significantly reduced brassy tones by 9.2% in bleached hair tresses compared to before washing (Figures 1 & 2, Table 2). This data demonstrates market standard, purple-pigmented shampoos effectively reduce brassy tones in bleach blonde hair.

The 2.0% and 5.0% **AC Violet'Blond Toner** shampoos visibly removed brassy tones present in the bleached blonde hair tresses. Regarding histogram analysis, brassy tones were significantly reduced by 8.3% and 9.5% on the bleached hair tresses washed with 2.0% and 5.0% **AC Violet'Blond Toner**, respectively, compared to before washing (Figures 1 & 2, Table 2). This data demonstrates **AC Violet'Blond Toner** achieves a comparable toning effect in bleached blonde hair to a market standard, purple-pigmented shampoo.

Collectively, these results indicate **AC Violet'Blond Toner** achieves a similar qualitative and quantitative toning effect on bleached blonde hair to a market standard, purple-pigmented shampoo when utilized at the recommended use-levels. In summary, **AC Violet'Blond Toner** extrudes brassy tones from bleached blonde hair and is a natural, pigment-free color toning alternative to products with large and acidic dye molecules.