

**Tradename:** AC Curezyme

**Code:** 20562

**CAS #:** 7732-18-5 & 100209-45-8 & 1686112-10-6 (or) 84775-94-0 (or) 9015-54-7 & 80146-85-6 (or) 300711-04-0

**Test Request Form #:** 13786

**Lot #:** N250926E

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**Test Performed:**

Hair Substantivity via Gravimetric Analysis

**Introduction**

Haircare substantivity is the ability of a product to deposit onto the hair during use. Ideally a product will be resistant to rinsing out, but not so substantive that it cannot be washed out. Products lacking substantivity do not impart significant benefits to the hair as they wash out too easily. Alternatively, products that are too substantive are resistant to washing out and build up on the hair causing hair damage over time.

Gravimetric Analysis is a precise technique used to determine amounts on analytes in a sample based on mass measurements. Changes in the weight of a sample can be calculated to give information about the material that was applied. This method can be applied to hair care products that claim substantive abilities for the hair. By applying the cosmetic material to a tress of hair, information about product deposition can be quantified.

Accordingly, a Gravimetric Analysis was performed to determine the substantive properties of **AC Curezyme**.

**Assay Principle**

Human hair tresses were tested to understand the substantive capability of a cosmetic product. Tresses are weighed before and after application of the testing materials then subjected to a rinse treatment to determine the substantive ability of the product. After rinsing, tresses are reweighed and product loss is calculated for each tress.

## Materials

- A. **Hair Samples:** Human Virgin Brunette Hair Tresses
- B. **Incubation Conditions:** Ambient Temperature (25°C)
- C. **Equipment:** Mettler Toledo Precision Balance ME103TE; Medium Size Weigh Trays
- D. **Software:** Excel Analysis ToolPak (Microsoft)

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

## Methods

Three hair tresses were collected, weighed, and then treated with DI water, 100% **AC Curezyme**, or left as an Untreated Control. After treatment, hair tresses were weighed again while wet and then allowed to air dry overnight in a humidity-controlled chamber. Tresses were reweighed after drying, then the DI water and 100% **AC Curezyme** tresses were both rinsed with tap water for 30 seconds before hanging to dry for 24 hours. Final weights were collected after fully drying. Hair substantivity was determined by calculating the changes in hair weight. Assays were repeated three separate times and averages from all three experiments are displayed. Data was analyzed using a one-way ANOVA with statistical significance accepted at  $p \leq 0.05$ .

## Results

The data obtained met criteria for a valid assay and the Untreated Control and DI water performed as anticipated. Compared to the Untreated Control and DI water, hair treated with **AC Curezyme** deposited on the hair and demonstrated substantive properties.

**Table 1.** Hair Substantivity via Gravimetric Analysis of Virgin Brunette Hair Tresses. Data represents change in hair weight compared to untreated virgin hair tresses.

	Untreated Control	DI water	100% <b>AC Curezyme</b>
After Air Drying Overnight	0%	0%	9%
After Airdrying Overnight and Rinse Out	0%	-1%	3%

**Table 2.** Results from one-way ANOVA Statistical Analysis for Virgin Brunette Hair Substantivity. Results represent p-values compared to untreated virgin hair tresses. \* indicates significance ( $p \leq 0.05$ ) compared to untreated virgin hair tresses.

	Change in Hair Weight after Drying Overnight	Change in Hair Weight after Airdrying Overnight and Rinse Out
<b>P-value</b>	< 0.05*	< 0.05*

## Discussion

As demonstrated in Table 1, the Untreated Control did not experience any changes in weight indicating there were no environmental impacts influencing hair weight over the duration of the assay. The DI Water control did not experience any change in weight after airdrying indicating full evaporation of water from the tresses.

Conversely, tresses treated with 100% **AC Curezyme** demonstrated a measurable increase in mass following application and drying, indicating product deposition onto the hair fiber. After air drying overnight, **AC Curezyme**-treated tresses exhibited a 9% increase in weight relative to untreated, fully dried hair, representing the total amount of material deposited during application.

Following rinsing and drying, the hair tresses retained a 3% increase in weight compared to the untreated controls, indicating that a portion of the initially deposited material remained bound to the fiber. Retention was calculated by comparing the post-rinse weight increase to the initial deposited mass, demonstrating that approximately 31% of the deposited **AC Curezyme** remained on the hair after rinsing, while the remaining 69% was removed during the rinse step.

This retained fraction corresponds to the observed net 3% increase in hair weight following rinsing and drying, confirming that **AC Curezyme** exhibits meaningful hair substantivity and resistance to rinse-off under aqueous conditions (Tables 1 and 2).

Taken together, these results indicate **AC Curezyme** deposits on hair and is resistant to rinse off. Collectively, **AC Curezyme** demonstrates hair substantivity properties which improves protective functions and contributes to the appearance of healthier looking hair.