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# INGREDIENTS' TRANSPARENCY: NEEDS FOR IMPROVED ACTIVE INGREDIENT SPECIFICATIONS TO ENSURE PRODUCT SAFETY AND EFFICACY.

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## 1 INTRODUCTION

In today's world, more and more skincare cosmetic goods are available to the consumers within a mouse click reach. Online advertising and widespread data are overflowing that could easily mislead people towards complicated choices. Among all the information like product efficacy, price and sustainability, the notoriety of a cosmetic ingredient is a decisive factor driving a purchase.

Original major active ingredients innovations are the outcome of many years of scientific research. However, they could be produced by other manufacturers once their patent protection expires. At the same time, analogs are claiming their biological efficacy based on scientific publications related to the original ingredient.

In the present work, using an ingredient developed by l'Oreal's Research and identified as a skin anti-aging agent and since used in numerous skin care cosmetic applications [1] as case study. We propose to run a comparative study on an analog ingredient sharing the exact same INCI name and CAS number to highlight thorough analytical and biological experiments potential differences between the two ingredients and whether it could impact final ingredient efficacy.

## 2 MATERIALS AND METHODS

Gaz chromatography flame ionization detection GC-FID has been used to identify and quantify the isomers and diasteroisomers. Classical methods have been used to assess pH, water content, acidity, chloride content and metals.

For the biological efficacy, normal human epidermal keratinocytes or normal human dermal fibroblasts were seeded in 96-well plates and cultured for 24 hours in their respective culture medium (Keratinocytes SFM supplemented with EGF and pituitary extract for keratinocytes, DMEM supplemented with 10% fetal calf serum, 2 mM L-Glutamine and antibiotics for fibroblasts). Culture medium was then replaced by culture medium containing or not (control) the reference (10 ng/mL TGF-β for fibroblasts, 10 ng/mL 4-Nitrophenylß-D-xylopyranoside for keratinocytes) or C-Xyloside derivative at 0.2 mM and 1 mM or the analog at 0.2 mM and 1 mM. Cells were then incubated for 72 hours with adding of [35S]-sulfate or [3H]-Glucosamine during the latest 24 hours of incubation. All the experimental conditions were performed in n=3. At the end of the incubation time, glycosaminoglycans were extracted from cells using chaotropic buffer. GAGs were then purified by ion exchange chromatography: adsorption of the anionic molecules on Q-Sepharose beads and desorption of the weakly and moderately anionic molecules with a specific buffer. The radioactivity incorporated in the molecules linked to the support was then measured by liquid scintillation. The incorporation of glucosamine is representative of the production of hyaluronic acid, heparin/heparan sulfate and keratan sulfate while the incorporation of sulfate reflects the neosynthesis of all GAGs.

## 3 RESULTS & DISCUSSION

Notable analytical differences could be spotted on many aspects of the original ingredient: summarized in the table 1. The most striking difference lies in the GC profiles, especially on the isomers ratios as shown on the chromatograms (figure 1). Overall, a simple composition comparison allows to showcase major differences in terms of solvents both in nature and content (table 2).

ANALOG

| INCI NAME | CAS n° | % |
|---|---|---|
| **Hydroxypropyl Tetrahydropyrantriol** | **439685-79-7** | **25-30** |
| **1,2 Hexanediol** | 6920-22-5 | ≤ 5 |
| **Pentylene glycol** | 5343-92-0 | ≤ 5 |
| **Water / Aqua** | 7732-18-5 | ≥ 50 |

C-XYLOSIDE

| INCI NAME | CAS n° | % |
|---|---|---|
| **Hydroxypropyl Tetrahydropyrantriol** | **439685-79-7** | **35** |
| **Propylene glycol** | 57-55-6 | 25 |
| **Water / Aqua** | 7732-18-5 | 40 |

Table 2: analog and C-Xyloside composition

| | Analog | C-Xyloside |
|---|---|---|
| **pH** | 7.2 | 4.0-6.0 |
| **Sum of impurities including isomers** | 90% | ≤ 15% |
| **Water content** | 65.6% | 37.0-41.0% |
| **Acidity (%AcOH)** | ~7.5% | ≤ 0.5% |
| **Chloride content** | ~6.3% | < 2.2% |
| **Metals** | > 7000 ppm (mostly Boron) | < 30 ppm (no Boron) |

Table 1: Main analytic differences between analog and C-Xyloside

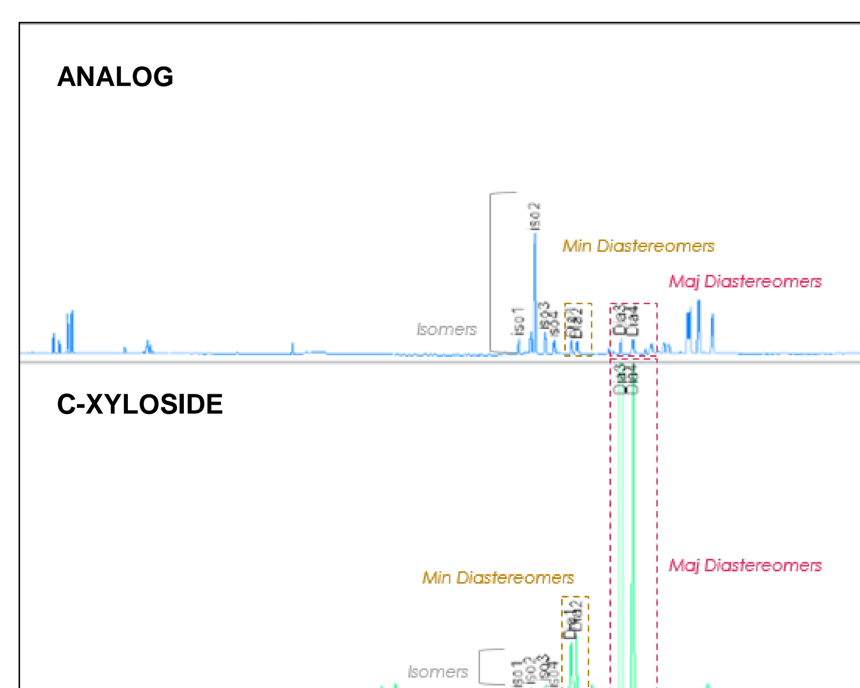


Figure 1: GC Chromatogram profiles

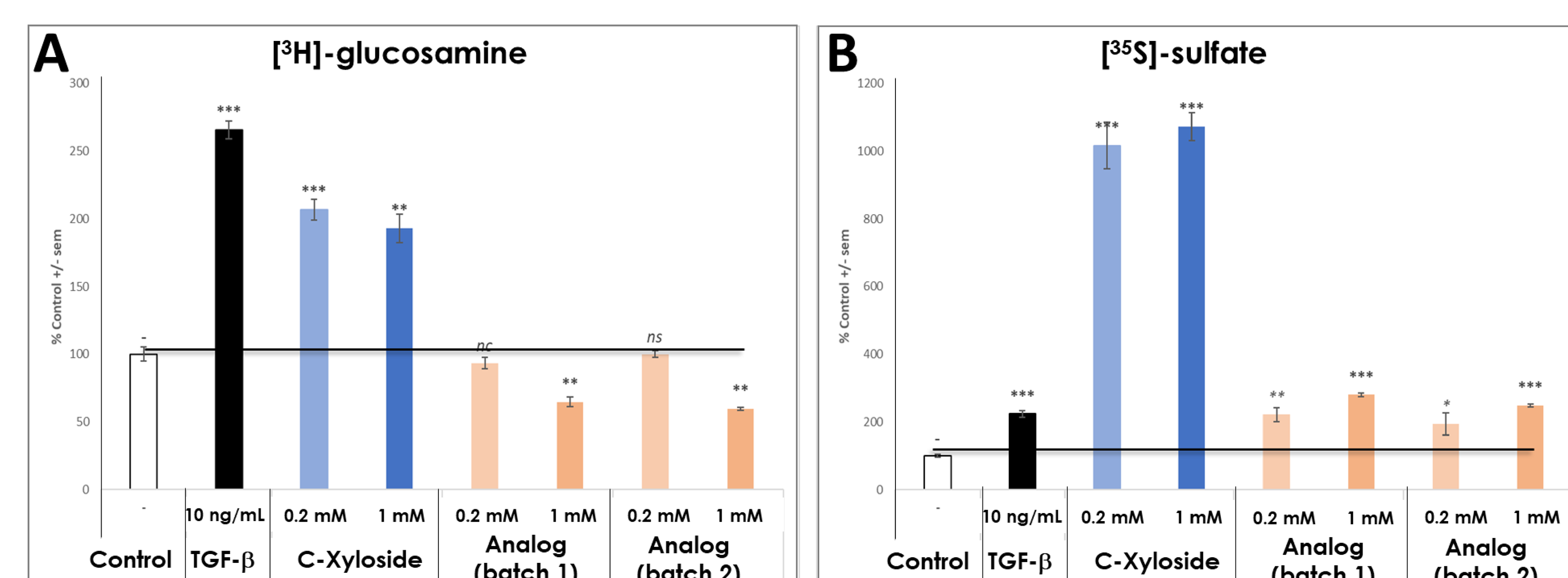


Figure 2: The effects of C-Xyloside derivative and the analog (two different batches) on the total GAG neosynthesis (A) and sulfated one (B) by normal human dermal fibroblasts

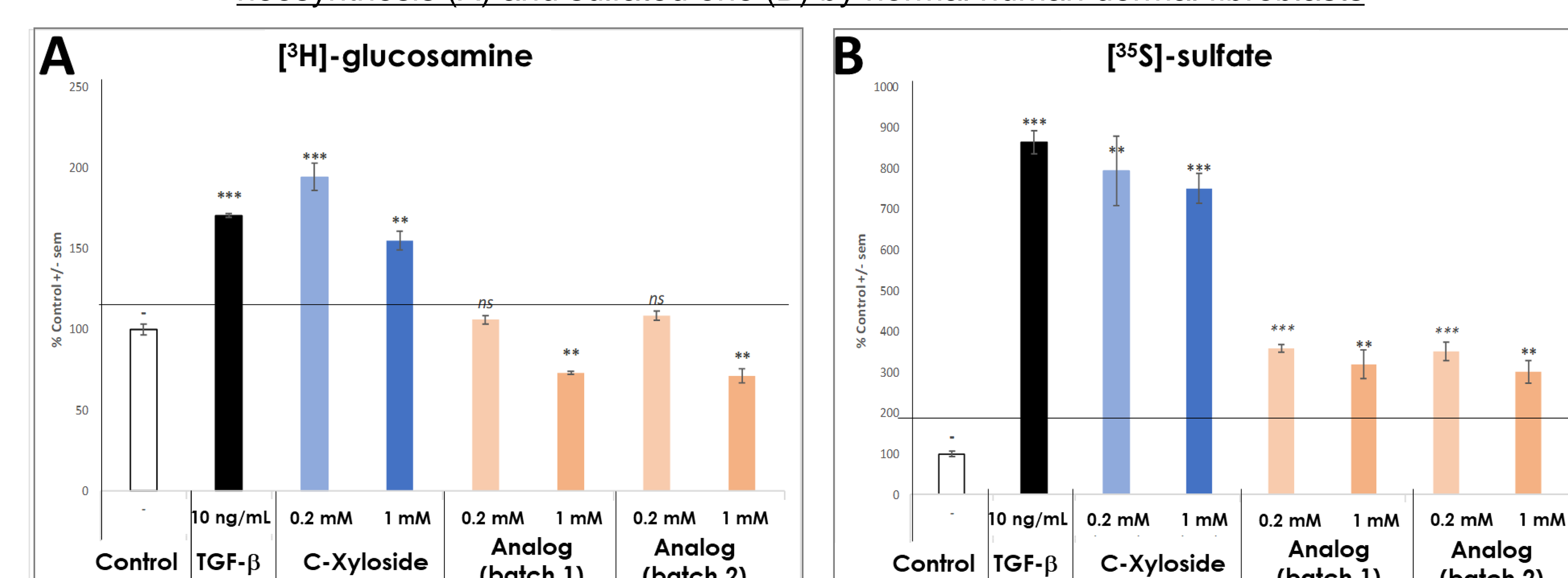


Figure 3: The effects of C-Xyloside derivative and the analog (two different batches) on the total GAG neosynthesis (A) and sulfated one (B) by normal human epidermal keratinocytes

C-Xyloside derivative tested at 0.2 mM and 1 mM dramatically increased sulfated-GAGs neosynthesis in dermal fibroblasts (fig. 2B). The same effect was observed on keratinocytes monolayers (fig. 3B). The analog, tested also at 0.2 mM and 1 mM, did not show the same activity profile compared to the original cosmetic ingredient. If the analog induces slightly sulfated GAGs neosynthesis by both fibroblasts and keratinocytes, its effect was clearly lower than that observed with the C-Xyloside derivative. In addition, unlike what is observed with the original ingredient, the analog significantly inhibits the neosynthesis of GAGs using glucosamine as basal hexosamine (fig. 2A and 3A).

All the above-mentioned analytical differences tend to highlight the fact that the C-Xyloside derivative and the analog ingredients are chemically inequivalent. Beside, we chose to rely on the largely described effect on the C-Xyloside derivative on the GAGs neosynthesis [2] and we showed that the C-Xyloside derivative clearly stimulated the total GAGs neosynthesis and the sulfated ones in both cutaneous cell types. On the contrary the analog did not succeed in increasing the total GAGs neosynthesis and even, inhibited it at the highest concentration. For the specific sulfated GAGs, the analog shows less efficacy than the C-Xyloside derivative. GAGs but also proteoglycans play an important role in skin homeostasis due to their ability to participate in a wide variety of functions [3, 4] and in various pathophysiological events [5]. They are an original target in the treatment of skin aging as structural components of the extra cellular matrix, in the orientation and structural arrangement of other ECM constituents but also they are functional components capable of modifying signals from soluble and cell surface molecules, enzymes and/or growth factors. They are also of interest during the wound healing process to influence growth factor functions [6, 7].

Therefore, even we did not perform the *in vivo* comparison of both actives, showing a lesser efficacy in inducing both total GAGs neosynthesis and the sulfated ones, the analog should have a lower efficacy than the C-Xyloside derivative on the global skin quality improvement.

## 4 CONCLUSIONS

In conclusion, we demonstrated by these analytical and biological comparisons, with the C-Xyloside derivative as a case study that under a same INCI name and even CAS number, we have no bioequivalence between the original cosmetic ingredient and its analog. In this comparative study, these differences led to a different biological efficacy that could be perceived in vivo by the consumer. Moreover, the presence of some unexpected impurities might compromise final product safety. This highlights the needs for more transparency regarding the characterization of cosmetic active ingredient in order to ensure safety and efficacy to the consumer.

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