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Vitamin C Skincare Serum: A multivariate approach of evaluation

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Introduction:

Vitamin C (Ascorbic Acid) is a potent free radical scavenger. When applied topically on skin, vitamin C stimulates collagen synthesis and inhibits melanin production, becoming a very interesting molecule for use to develop anti aging, antioxidant and blemish treatment products. Although vitamin C is an important player for anti-aging skincare products, its topical application remains a challenge due to its instability and aqueous solubility. In the past few years, several chemically modified derivatives of vitamin C have been developed in an attempt to increase the molecule stability, percutaneous absorption and overall activity of this ingredient in topical formulations. 3-O-Ethyl-L-Ascorbic Acid (EAC) is one of the ascorbic acid-derived molecules and it is relatively new. Although EAC is becoming more and more used for the cosmetic industry, its benefits for the skin are still poorly documented scientifically. During this work, a series of in vitro assays and clinical trials were conducted in order to evaluate the safety and efficacy of a 10% EAC aqueous serum.

Materials & Methods:

For the present study, a 10% 3-O-Ethyl-L-Ascorbic Acid (EAC) aqueous serum with pH 4.0 - 4.5 was developed and used during all the experiments. EAC was obtained from Sarfam, São Paulo, Brazil.

For stability evaluation, the 10% EAC was stored for 91 days in four conditions: 23°C, 5°C, 40°C and fluorescent light. The EAC content was measured by high performance liquid chromatography at 0, 28, 63 and 91 days. The chromatographic analysis was carried out using HPLC Agilent 1260 integrated system equipped with an automated injector, pump and multiwavelength diode-array detector (Agilent, Santa Clara, CA, USA).

The clinical study was conducted with a group of 60 female participants, from Brazil and all skin types according to Fitzpatrick, aged 40 to 68 years. Subjects were advised to apply 3 to 4 drops of the treatment serum with 10% of EAC, once a day on the whole face for 90 days.

Skin parameters were measured at baseline (D0) and after 30 (D30), 60 (D60) and 90 days (D90) of the use of the product. During the visits, after answering a self questionnaire, subjects were required to rinse their face thoroughly with a neutral lotion and acclimatize to the ambient environment for at least 20 minutes before measurements.

Instrumental skin parameters was evaluated as following methods:

- VISIA CR® (Canfield) thrown the skin analysis program Complexion Analysis, determined some superficial parameters: darkened areas and apparent pores;
- Oblique Lighting Technique uses a digital camera (Canon T3i) full-face frontal photographs to analyze skin relief, the software Image Pro-Media Cybernetics evaluates the amount, width and length of shadows that translate the depth of wrinkles and expression lines.

Statistical analysis was performed to all instrumental results, Anderson-Darling test was used to determine the normality of the data distribution followed by Student's because all distributions were normal. Differences between parameters were considered biologically significant for P-values of .05 or less, when compared to the baseline measurement (D0).

An in vitro technique also was performed to evaluate the protection of the product against the action of visible light and blue light, human fibroblasts were treated with 3 non-cytotoxic concentrations of the investigational product for 48 hours, then exposed to a dose of 100J/cm2 of the visible light spectrum (400 -700 nm) or the dose of 50J/cm2 of blue light (478 nm). After photo exposure the cells were kept in culture for an additional 24 hours for further quantification of MMP-1 using the enzyme immunoassay method (Elisa).

Results & Discussion:

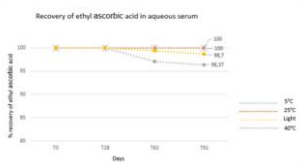

Figure 1. Recovery of ethyl ascorbic acid in aqueous serum in 5°C, 23°C, 40°C and fluorescent light at 0, 28, 63 and 91 days.

The results from stability studies showed that after 91 days, even at 40°C conditions, the maximum decay observed for ethyl ascorbic acid was 5%. This result indicates that the product presents vitamin C in a very stable form, maintaining its properties and characteristics.

Table 1. Results of instrumental skin parameters (N=25).

Evaluation Parameters	Time variation (days)	Parameter variation	Participants with improvement (%)	P value
Pores	After 30 days	-5,24%	50%	0,187
	After 60 days	-9,80%	70%	0,034*
	After 90 days	-11,94%	60%	0,031*
Dark areas	After 30 days	-3,29%	65%	0,047*
	After 60 days	-4,35%	70%	0,030*
	After 90 days	-5,32%	65%	0,014*
Depth of wrinkles and lines	After 30 days	-0,52%	60%	0,200
	After 60 days	-2,93%	90%	<0,001*
	After 90 days	-3,43%	100%	<0,001*

Table 2. Perceptions of participants after 90 days of use (N=60).

Perception from the product	Participants who agree (%)
Light and pleasant texture	93%
Fast absorption	96%
Leaves a pleasant feeling on the skin	92%
Does not leave skin oily	92%
Leaves skin more radiant	88%
Leaves skin soft	88%
Leaves skin silky	90%

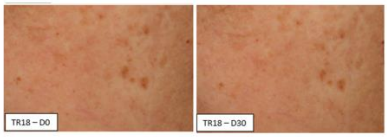

Figure 2. Result before and after 30 days of use in skin uniformity.

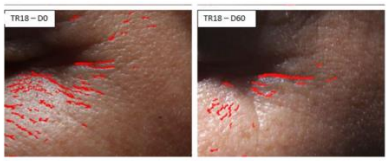

Figure 3. Result before and after 60 days of use in depth of wrinkles.

The clinical study, using instrumental parameters, shows the reduction of pores, lines, wrinkles and hyperpigmentation in dark areas during the use of the product, which corroborates with the expected effects of vitamin C already described in literature.

The product was well tolerated by the skin and the aqueous characteristic was approved by 93% of the participants, besides the pleasant experience in the use of the product and the perception of improvement of the skin in general. Sensory attributes and the satisfaction experience during the use are very important to consumers' decision to buy.

In the exposure to visible light, the product reduced the production of MMP-1 in 32%, 28%, 29%, in the evaluations of 0.0316; 0.0100 and 0.00316 mg/mL, primarily, respectively, when compared to the group irradiated with visible light. When exposure to blue light, the product reduces the production of MMP-1 by 27%, 38%, 43%, under conditions of 0.0316; 0.0100 and 0.00316 mg/mL, respectively, when compared to the group irradiated with blue light. In vitro analysis indicates that EAC serum also acts inhibiting metalloproteinase-1 (MMP-1), the enzyme responsible for the degradation of collagen and others biological effects attached to skin aging, during the exposition against visible and blue light.

Conclusions:

The present study demonstrated that EAC is a very stable molecule that can be easily used as a potent substitute for pure vitamin C (ascorbic acid) in cosmetic formulations. All results demonstrated that a serum containing 10% of EAC can visually improve the signs of skin aging: mainly skin darkening, radiance, smoothness, pores and wrinkles. In addition, the serum was well tolerated and well evaluated by the participants.

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