Original Article

e-ISSN: 2581-0545 - https://journal.itera.ac.id/index.php/jsat/

Received 16th March 2025
Accepted 04th August 2025
Published 26th January 2026

Open Access

DOI: 10.35472/jsat.v9i2.2172

Analysis Interaction of Nicotinamide and Ascorbic Acid and Its Derivatives as Active Substances in Skin Lightening Cosmetic Products

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Abstract: Ascorbic acid and its derivatives with nicotinamide are often used as skin lightening, anti-aging, and anti-pigmentation agents. Combining these two active substances may increase the risk of instability due to degradation. This research was conducted to determine the effect of nicotinamide concentration on the degradation process of ascorbic acid and its derivatives, and to determine the degradation products formed using liquid chromatography-mass spectrometry (LC-MS). The study was conducted by combining nicotinamide concentrations ranging from 5 to 15 micrograms per milliliter with incubation times of 1, 2, 3, and 24 hours to react with ascorbic acid and its derivatives. The spectral shape and maximum wavelength were analyzed using a UV-Vis spectrophotometer. Samples that showed changes in chemical structure were subsequently analyzed by LC-MS to identify degradation products. The results showed that only the combination of ascorbic acid and nicotinamide shifted the maximum wavelength and generated a new peak at 310 nm after 24 hours of incubation. Nicotinamide can accelerate the degradation process of ascorbic acid. The LC-MS analysis results show that the degradation products formed from the combination of ascorbic acid and nicotinamide samples are dehydroascorbic acid and 3-hydroxy-2-pyrone. These findings highlight the importance of incorporating stabilizing agents into cosmetic formulations containing unstable active ingredients, such as ascorbic acid, to ensure product stability during storage and use.

Keywords: Ascorbic acid, Nicotinamide, Interactions, Degradation, LC-MS

Introduction

Ascorbic acid and nicotinamide are active substances known to have benefits as skin-lightening agents. Generally, these two active substances are combined in a single formulation, which is expected to increase the potential for skin brightening. However, when combining these two active substances in a single formulation, it is essential to analyze their stability. There is a possibility of interaction between the two active substances, which could affect the product's stability and the efficacy or biological activity of each.

According to 2022 statistics [1], ascorbic acid and nicotinamide are the first and second most widely used skincare ingredients among Indonesian consumers. Ascorbic acid, or the chemical formula for vitamin C, is $C_6H_8O_6$, and its molecular weight is 176.12 grams/mol. It is also known as L-Ascorbic Acid [2]. Ascorbic acid is an active substance that is known to have low stability against exposure to light, high temperatures, alkaline pH, the presence of oxygen, and contact with metal ions [3][4]. Because of ascorbic acid's poor stability, ascorbic acid derivatives exist that, in terms of their physicochemical characteristics, are more stable than ascorbic acid in its pure form. Several ascorbic acid derivatives commonly used as alternatives in cosmetic formulations include ascorbyl 2- glucoside and 3-O-ethyl-l-ascorbic acid [5][6]. Nicotinamide is a derivative of vitamin B3, which is relatively stable to exposure to light, elevated temperatures, pH, and oxygen content [7]. Nicotinamide has the chemical name Pyridine-3-carboxamide, with a molecular weight of 122.12 g/mol and the chemical formula $C_6H_6N_2O$ [7].

Previous research demonstrates that nicotinamide and ascorbic acid form a 1:1 complex. In addition, it is known that increasing the concentration of nicotinamide and exposing ascorbic acid to light at pH 2–12 will accelerate its degradation. However, when combining the two active substances into a single formula, it is necessary to analyse their stability [8]. Other research shows that both ascorbic acid and its derivatives, and nicotinamide, exhibit a shift in

maximum wavelength when analyzed by UV-Vis spectrophotometry [10].

With a shift in the maximum wavelength of ascorbic acid or ascorbic acid derivatives with nicotinamide, it is possible for degradation to occur, which affects the stability of the active substance, thereby impacting its quality and biological activity [11]. This research was also supported by findings showing that one of the active substances, ascorbic acid or nicotinamide, was not detected in cosmetic products that claimed to contain these two active substances [12]. This research was conducted to determine the interaction of nicotinamide with ascorbic acid or its derivatives. In addition, this research aims to identify possible degradation products using liquid chromatography-mass spectrometry (LC-MS).

Thus, it is hoped that the results of this research will increase the usefulness of science and MIPA, especially in the cosmetics sector, to support ITERA for Sumatra. The research results of this study provide a preliminary assessment of possible interactions among nicotinamide, ascorbic acid, and ascorbic acid derivatives that may affect the stability of the active substance. In addition, the results of this research can help formulators design and develop more optimal formulas.

Method

Materials and tools

The ingredients required are ascorbic acid (Sigma-Aldrich), nicotinamide (JUBILANT INGREVIA), ascorbyl 2-glucoside (Shaanxi Fruiterco Biotechnology Co., Ltd.), 3-O-ethyl-l-ascorbic acid (Shanghai JAKA Biotech Co., Ltd.), methanol pro analysis (MERCK), and distilled water (OneMed).

The tools required include a UV-Vis spectrophotometer (Thermo Scientific), Liquid Chromatography-mass spectrometry, micro pipettes, quartz cuvettes, brown glass bottles, and glassware.

Optimization of Solvent Type and Determination of Maximum Wavelength

The solvents used are distilled water and methanol, which are used separately. Each standard powder was weighed into a stock solution by dissolving 2.5 mg in 25 ml of solvent to obtain a concentration of 100 µg/ml for each standard. Then, the concentration of each standard was diluted to 5, 10, 15, and 20 µg/ml of the stock solution. After that, a UV-Vis spectrophotometer with a wavelength range of 200–400 nm was used to measure the absorption of each individual sample.

Single Sample Stability Analysis Using Incubation Time Variation Function

Each sample was prepared as a stock solution by weighing 2.5 mg of powder, dissolving it in the appropriate solvent, and adding 25 ml to obtain a final concentration of 100 µg/mL. Then, each sample was diluted to 5, 10, and 15 µg/mL from the stock solution. The sample was then incubated for 1, 2, 3, and 24 hours. A UV-Vis spectrophotometer with a wavelength range of 200–400 nm was used to measure absorbance after incubation. To ensure test results and reduce errors in testing, each test is carried out in duplicate and tested on different days

Combination Sample Interaction Analysis

Table 1. Combination sample design

No	Ascorbic Acid and Its Derivatives (µg/mL)	Nicotinamide (µg/mL)
1	5	5
2	5	10
3	5	15

A combination sample was prepared at the concentration specified in **Table 1** and dissolved in an optimized solvent. Then, each combination sample will be incubated for 1, 2, 3, and 24 hours. After incubation, the absorbance was measured using a UV-Vis spectrophotometer over 200–400 nm.

Sample Analysis Using Liquid Chromatography-Mass Spectrometry (LC-MS)

Single and combination samples that show signs of interaction and the potential formation of degradation products will be analysed by liquid chromatography-mass spectrometry using the Thermo HPLC-DIONEX ULTIMATE-TSQ Quantum Access MAX Triple Quadrupole Mass Spectrometer.

1. Stationary Phase: Thermo Scientific™ Accucore™ Phenyl Hexyl
2. Mobile phase (**Table 2**):
    a. Water Fischer Scientific MS Grade (Formic Acid 0.1%)
    b. Methanol Fischer Scientific MS Grade (Formic Acid 0.1%)

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Table 2. Retention time and mobile phase

Minutes	Flow Rate (mL/min)	composition	
		A	B
0	0.3	50	50
1	0.3	50	50
5	0.3	5	95
8	0.3	5	95
10	0.3	50	50

Preparation of the LC-MS analysis was started by filtering the material with a 0.22 um syringe filter after dissolving it in MS-grade methanol. The sample injection volume was 2 µL. The reading method used is Screening using Quadrupole 1 (Q1) with MS+ and MS- and fragmentation using Quadrupole 3 with MS+ and MS- modes using 30 electron volts in the Collision Cell (Q2).

Results And Discussion

Optimization of Solvent Type and Determination of Maximum Wavelength Using a UV-Vis Spectrophotometer

Two types of solvents were used in this study: distilled water and methanol. The optimization results showed that distilled water interacts with ascorbic acid, as evidenced by a bathochromic shift in its absorbance maximum (**Figure 1**).

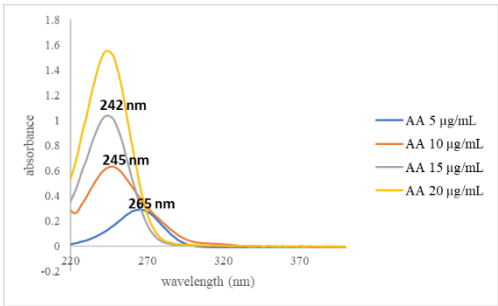

Figure 1. UV Spectrum of Ascorbic Acid at Various Concentrations with Aquadest Solvent.

In contrast, no wavelength shift was observed for ascorbic acid, ascorbyl 2-glucoside, 3-O-ethyl-L-ascorbic acid, and nicotinamide when dissolved in methanol, as shown in **Figure 2**. The maximum wavelengths obtained were: ascorbic acid at 245 nm, nicotinamide at 262 nm, 3-O-ethyl-L-ascorbic acid at 246 nm, and ascorbyl 2-glucoside at 234 nm [13],[14],[15],[16]. Therefore, methanol was selected as the most suitable solvent for this study.

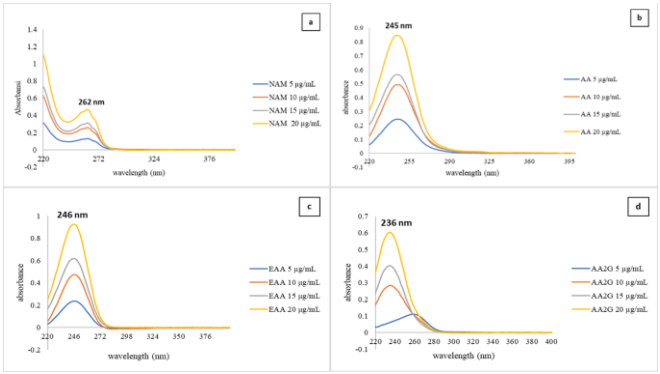

Figure 2. UV Spectrum of Analytes of Various Concentrations with Methanol Solvent: Nicotinamide/NAM (a), Ascorbic Acid/AA (b), 3-O-Ethyl-L-Ascorbic Acid/EAA (c), and Ascorbyl 2- Glucoside/AA2G (d)

Stability Analysis of a Single Analyte with Variation in Incubation Time Using a UV-Vis Spectrophotometer

The analysis's findings demonstrated that, in comparison, one ascorbic acid has the lowest stability to nicotinamide, ascorbyl 2-glucoside, and 3-O-ethyl-l-ascorbic acid. Ascorbic acid showed a shift in the maximum wavelength in **Figure 3** across all incubation conditions at room temperature. Therefore, this ascorbic acid will be subjected to further analysis using LC-MS.

Combination Analyte Interaction Analysis Using a UV-Vis Spectrophotometer

At this stage, the combination analytes are divided into two treatments: combination samples without incubation time and with varying incubation times. The results of this interaction analysis showed that only combination samples treated with variations in incubation time are likely to have interactions.

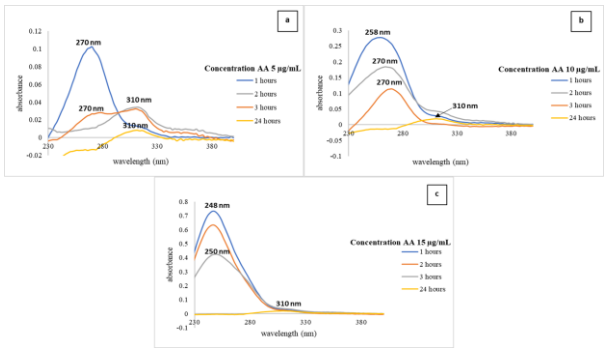

Figure 3. UV Spectrum of Ascorbic Acid (AA) Against Incubation Time: AA Concentration 5 µg/mL (a), AA Concentration 10 µg/mL (b), and AA Concentration 15 µg/mL (c)

Only the combination of pure ascorbic acid and nicotinamide shows an interaction, as shown in **Figure 4**. The image shows a new peak at 310 nm after 24 hours of incubation. This new peak showed a degradation product, which will be analyzed further by LC-MS.

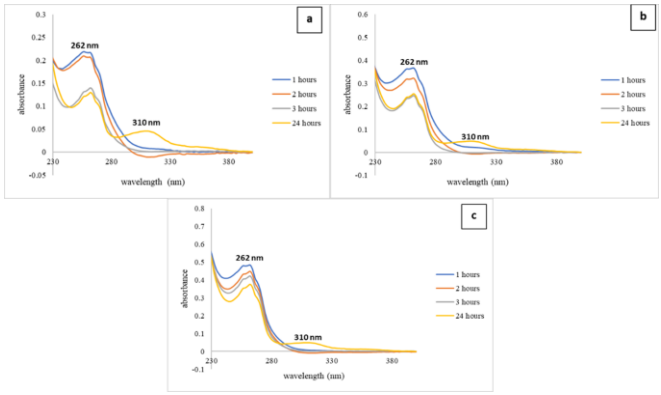

Figure 4. UV Spectrum of Combination Samples of Ascorbic Acid (AA) and Nicotinamide (NAM) Against Incubation Time: 5 µg/mL AA and 5 µg/mL NAM (a), 5 µg/mL AA and 10 µg/mL NAM (b), and 5 µg/mL AA and 15 µg/mL NAM (c).

Additionally, this analysis demonstrates that the nicotinamide sample combination and ascorbic acid decrease in absorbance intensity, which is proportional to the increase in nicotinamide concentration, as shown in **Table 3**. Previous research has proposed hypotheses regarding interactions between ascorbic acid and nicotinamide, namely redox interactions and possible free radical reactions, as shown in **Figure 5** [8].

$$NA \rightarrow [^1NA]$$
$$[^1NA] \rightarrow [^3NA]$$
$$[^3NA] + AH_2 \rightarrow NAH + AH^-$$
$$2NAH + O_2 \rightarrow 2\,NA + H_2O_2$$
$$2AH^- \rightarrow AH_2 + A2^-(DHA)$$

Figure 5. Scheme of interaction of nicotinamide and ascorbic acid [8]

Based on the interaction scheme in **Figure 5**, nicotinamide acts as an electron acceptor and ascorbic acid as an electron donor, leading to a redox interaction (oxidation-reduction). The products of the redox reaction are ascorbyl radical (AH-) and NAH. Then, NAH, which is the reduced form of NA (nicotinamide), will interact with oxygen to become H_2O_2, and NA is formed again. Next, $2AH^-$ dissociates into dehydroascorbic acid (DHA). The formation of H_2O_2 is likely to speed up the degradation process because hydrogen peroxide (H_2O_2) is a strong oxidizer. Therefore, according to this study, nicotinamide may accelerate ascorbic acid's breakdown, leading to the production of dehydroascorbic acid (DHA), a degradation product [8].

Table 3. The difference in reducing the absorbance value of the combination of ascorbic acid and nicotinamide samples.

Concentration Variations µg/mL		Absorbance at 262 nm		Decrease Difference	Absorbance at 310 nm
AA	NAM	1 hour	24 hours		
5	5	0.216	0.129	0,087	0,04
5	10	0.367	0.255	0,112	0,04
5	15	0.483	0.355	0,128	0,04

Sample Analysis Using Liquid Chromatography-Mass Spectrometry (LC-MS).

This assay was performed using a combination of pure ascorbic acid samples with nicotinamide and a single analyte of ascorbic acid only, both incubated for approximately 24 hours. Considering the combined sample's LC-MS test findings, it detected nicotinamide at a retention time of 0.59 minutes and degradation products of ascorbic acid, namely dehydroascorbic acid at 0.47 and 3-hydroxy-2-pyrone at 5.97, as shown in **Figure 6**. Meanwhile, the pure form of ascorbic acid in this combination sample was not detected. The LC-MS method is Selected Ion Monitoring (SIM), which targets specific compounds. In reading mode, this analysis uses Ion M+, so the m/z values will increase by at least 1 proton. The m/z value of nicotinamide is 123, dehydroascorbic acid 177, and, as shown in **Figure 7**, 3-hydroxy-2-pyrone 113. The results of the m/z value are in accordance with existing literature [16][17][18]. Previous research indicates that 3-hydroxy-2-pyrone is an ascorbic acid degradation product in aerobic conditions, whereas dehydroascorbic acid is an ascorbic acid degradation product in anaerobic conditions [10].

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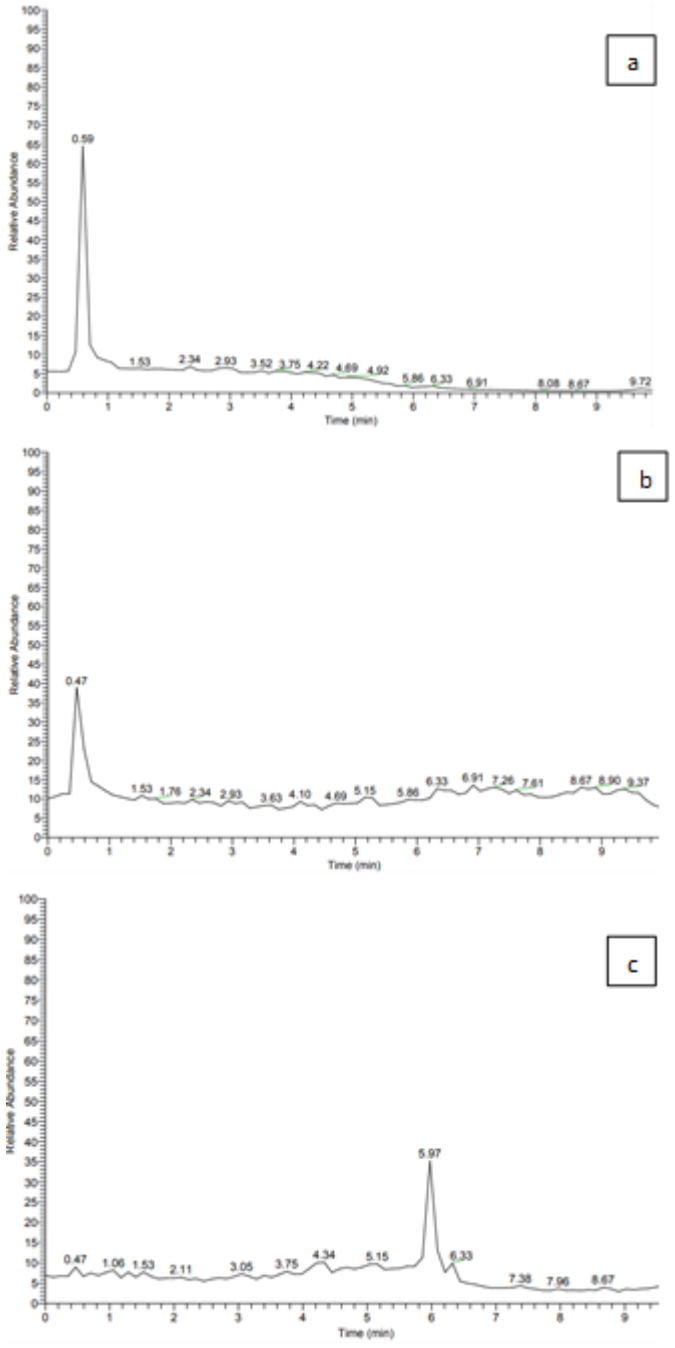

Figure 6. Chromatograms of (a) Nicotinamide, (b) dehydroascorbic acid, (c) 3-hydroxy-2- pyrone.

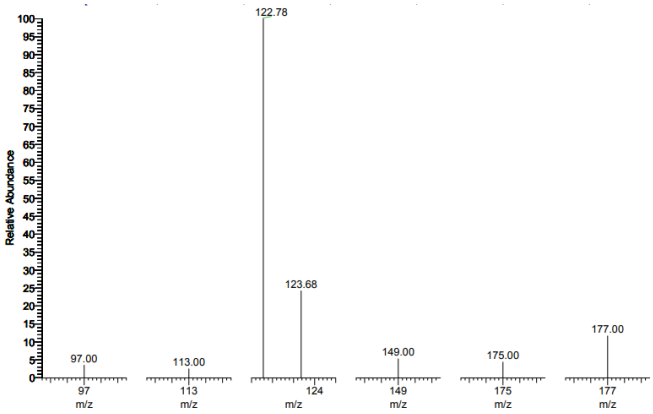

Figure 7. Mass spectrum of combined samples.

For a single sample, ascorbic acid did not show any degradation products, or in other words, ascorbic acid was not degraded. This result does not match the results of analysis using a UV-Vis spectrophotometer, which showed that ascorbic acid was degraded. Based on the LC-MS test results, ascorbic acid has a retention time of 0.53 minutes and an m/z of 175.60, as shown in **Figure 8**. According to the literature, ascorbic acid has a molecular weight of 176 g/mol [1][17]. The fragmentation results of this ascorbic acid sample have a parent peak of 175.34 and a base peak of 87.39. If we compare the fragmentation mass spectrum of ascorbic acid with the literature, which shows similarities, it can be concluded that the single sample is still pure ascorbic acid [17][19].

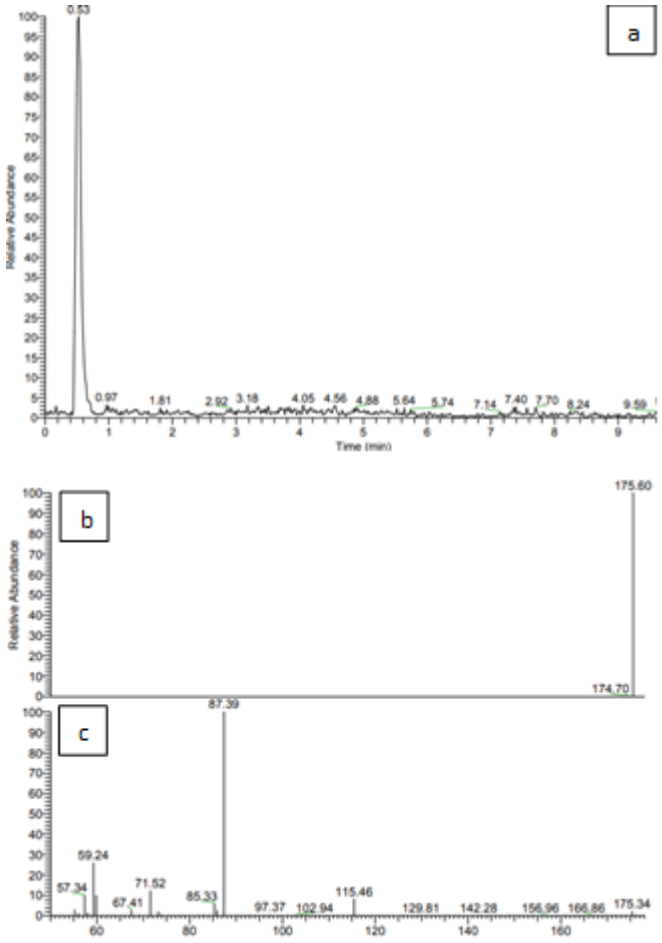

Figure 8. Results of LC-MS analysis of ascorbic acid: (a) chromatogram, (b) mass spectrum, and (c) mass fragmentation spectrum.

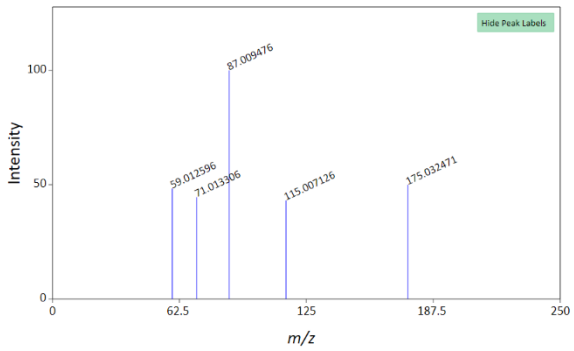

Figure 9. Mass fragmentation spectrum of ascorbic acid based on literature.

Conclusions

Only ascorbic acid shows a shift in the maximum wavelength and a new peak in the UV spectrum. Thus, ascorbic acid is the least stable. The results of this research, a combination of pure ascorbic acid and nicotinamide at 24 hours, showed a new peak in the UV spectrum at 310 nm. Dehydroascorbic acid and 3-hydroxy-2-pyrone were the breakdown products that emerged from the combination of ascorbic acid and nicotinamide samples, according to the results of the LC-MS analysis. Subsequent research should focus on the degradation and interaction analysis of niacinamide and ascorbic acid within formulated cosmetic preparations containing both active ingredients, to provide a more representative understanding of their stability under actual use conditions.

Conflicts of interest

There aren't any conflicts to disclose.

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