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"HS-GC-MS ANALYSIS AS A POWERFUL TOOL FOR OLFACTORY EVALUATION: LIQUID LIPSTICK STABILITY STUDY"

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1. Introduction

The global makeup market is a dynamic and expanding sector, with a valuation of USD 43.61 billion in 2024 and a projected growth to USD 70.80 billion by 2032, exhibiting a Compound Annual Growth Rate (CAGR) of 6.37%[1]. Among the various cosmetic products, liquid lipsticks have gained considerable traction among consumers. These formulations are intricate, typically comprising a base oil or emulsion, a film-forming polymer (such as acrylates or silicones), color pigments, and various additives designed to enhance texture, longevity, and other desirable properties [2].

Due to the complexity of these formulations and the incorporation of moisturizing agents like fatty compounds and emollients to enhance the user experience, maintaining product stability presents a significant challenge. In the realm of lipsticks, fragrance plays a pivotal role in the overall sensory experience and significantly influences consumer product choice. Therefore, ensuring the stability of the product's odor throughout its intended shelf life is crucial for consumer satisfaction and brand reputation. This necessitates the development of robust methodologies capable of preventing, as well as on the early detection stability alterations and providing clear direction for necessary formulation adjustments to mitigate any potential deviations.

A powerful strategy to aid in these evaluations involves the utilization of advanced analytical techniques. Headspace gas chromatography-mass spectrometry (HS-GC-MS) is a well-established technique employed for the analysis of volatile and semi-volatile organic compounds present in diverse sample matrices. The principle of HS-GC-MS involves the equilibration of a sample within a sealed container, followed by the analysis of the gaseous phase above the sample (the headspace) using GC-MS [3]. This method proves particularly advantageous for analyzing samples where the sample matrix itself is not directly amenable to injection into the gas chromatograph, as is the case with liquid lipstick formulations.

In this context, a case study was conducted to evaluate the stability of a liquid lipstick formulation, incorporating analytical assessment via HS-GC-MS to objectively identify odor alterations and potential volatile offenders. The study aimed to demonstrate the efficacy of this analytical technique in providing valuable data for stability monitoring and formulation optimization.

2. Materials and Methods

2.1 Evaluated Formulation:

For the conduction of the tests, a sample of base liquid lipstick was used, containing the following ingredients: Isododecane, Dimethicone, Caprylic/Capric Triglyceride, Trimethylsiloxysilicate, Polypropylsilsesquioxane, Paraffin, Silica Dimethyl Silylate, Dimethicone/Vinyl Dimethicone Crosspolymer, Disteardimonium Hectorite, Dimethicone Crosspolymer, Propylene Carbonate, Tocopheryl Acetate, Linoleic Acid, Parfum (Fragrance), Rosa canina Seed Oil, Rosa moschata Seed Oil, Rosa rubiginosa Seed Oil, Panthenol, Dimethiconol, Silicon Dioxide, Aluminum Hydroxide, Polysorbate 20, Aqua (Water), Sodium Saccharin, Linolenic Acid, Tocopherol, Helianthus annuus (Sunflower) Seed Oil, Rosmarinus officinalis (Rosemary) Leaf Extract, Titanium Dioxide, CI 15850 (Red 7 Lake), CI 77491 (Iron Oxides), CI 45410 (Red 28 Lake), CI 77492 (Iron Oxides), CI 77499 (Iron Oxides), CI 19140 (Yellow 5 Lake), CI 42090 (Blue 1 Lake), CI 15985 (Yellow 6 Lake), and CI 73360 (Red 30); subsequently, formulation tests were carried out through the addition of a larger amount of fragrance, the removal of inorganic pigments and the removal of the raw material Linoleic Acid.

2.2 Stability Test:

For stability evaluation, the products were kept for 90 days under different conditions – Freezer at -5°C, Refrigerator at 5°C, Ambient Condition at 25°C, Oven Condition at 40°C, and Oven Condition at 50°C – with organoleptic evaluations performed after 7, 14, 30, and 60 and 90 days of the study [4].

2.3 HS-GC/MS Analysis

The analysis of volatile compounds was performed using an Agilent 7890B gas chromatography system coupled to an Agilent 5977B mass spectrometer (GC-MS) equipped with automatic injector PAL RSI 120. Chromatographic separation was achieved on an HP-5MS column (30 m x 0.25 mm x 0.25 µm, Agilent). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature program was initiated at 40 °C (2 min) 250 °C and operated with 20:1 split mode. For headspace analysis, 3,0 g of sample were placed in 20 mL vials and incubated at 40 °C for 15 min. The transfer line was maintained at 250 °C, and the injected loop volume was 1 mL. The mass spectrometer operated in electron impact (EI) ionization mode at 70 eV, with a full scan in the m/z range of 35-500. Compound identification was based on the comparison of mass spectra with the NIST 14 library and the retention times of authentic standards.

3. Results

To initiate the determination of olfactory stability, the primary potential contributors to oxidation and subsequent odor alteration within the formulation were considered. Among the components, linoleic acid, a polyunsaturated fatty acid known for its inherent instability and susceptibility to oxidation and breakdown upon exposure to environmental factors such as heat, light, and air, was identified as a key potential offender [5]. This vulnerability to oxidation can compromise the storage stability of various matrices, including foods and oils containing this fatty acid. Furthermore, the potential catalytic effect of iron, originating from the inorganic pigments present, on oxidative processes was also acknowledged. Consequently, a stability study was conducted on five distinct formulations designed to elucidate the role of these potential offenders: a complete liquid lipstick formulation; a formulation devoid of inorganic pigments; a formulation excluding linoleic acid; a fragrance-free formulation; and a formulation with a 50% increased fragrance concentration. Following the selection of these study formulations, a sensory evaluation was performed using a trained olfactory assessor to systematically compare and describe any olfactory changes observed across the different formulations (TABLE 1)

Table 1. Olfactory evaluation of the different formulations after the stability study

Test Formulation	Results
Complete Liquid Lipstick Formulation	The oxidized odor in oven conditions
Formulation without inorganic pigments	Slight oxidized odor that can be masked by the fragrance without olfactory alteration.
Formulation without linoleic acid	Slight oxidized odor that can be masked by the fragrance without olfactory alteration.
Formulation with 50% more fragrance	It is still possible to observe the oxidation odor, however it is lighter than in the standard formula
Formulation without fragrance	Oxidized odor developed in all storage conditions.

With the obtained data, the analytical evaluation phase of the aforementioned samples was carried out, aiming to objectively verify the recorded sensory perceptions and to identify, through chemical methods, a specific marker for the observed alteration. The analyses were conducted using gas chromatography coupled with mass spectrometry (GC-MS), employing the Headspace (HS) technique. This methodology enabled the analysis of volatile compounds directly from the sample container, dispensing with preparation and solubilization steps, which would have posed a challenge given the complexity of the matrix under study.

The chromatographic results revealed a similar profile across the various samples analyzed (FIGURE 1), with the exception of a signal detected at a retention time of 7.9 minutes (FIGURE 2), which was identified by the NIST library as Hexanal (FIGURE 3). It was observed that this compound was absent in the samples that exhibited a slight olfactory alteration – specifically, the formulation without inorganic pigments and without linoleic acid. In contrast, the concentration of Hexanal progressively increased in samples with a greater intensity of the oxidation odor – including the formula with 50% more fragrance, the complete formula, and the fragrance-free formulation. These findings indicate a direct correlation between the presence and intensity of Hexanal and the odor alteration identified by sensory analysis.

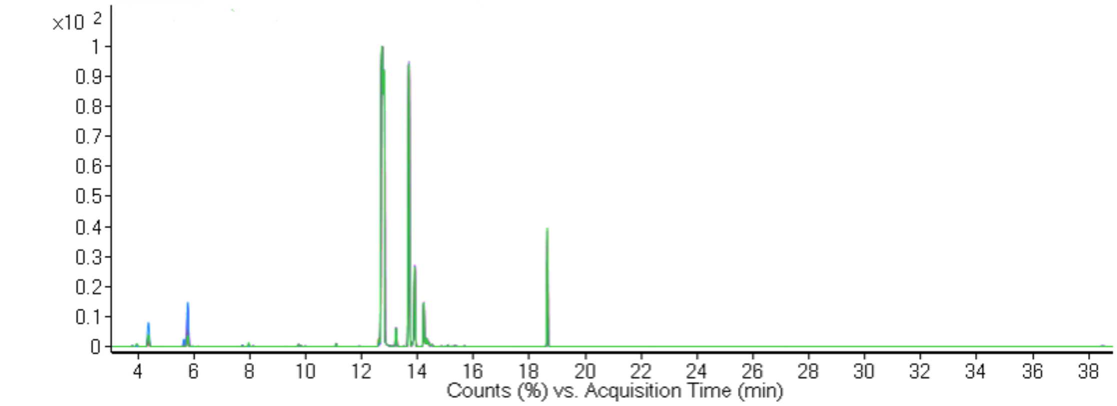

Figure 1. Chromatograms obtained after sample evaluation by HS-GC/MS - Complete Liquid Lipstick Formulation (purple line); Formulation without inorganic pigments (blue line); Formulation without linoleic acid (red line); Formulation with 50% more fragrance (pink line); Formulation without fragrance (green line).

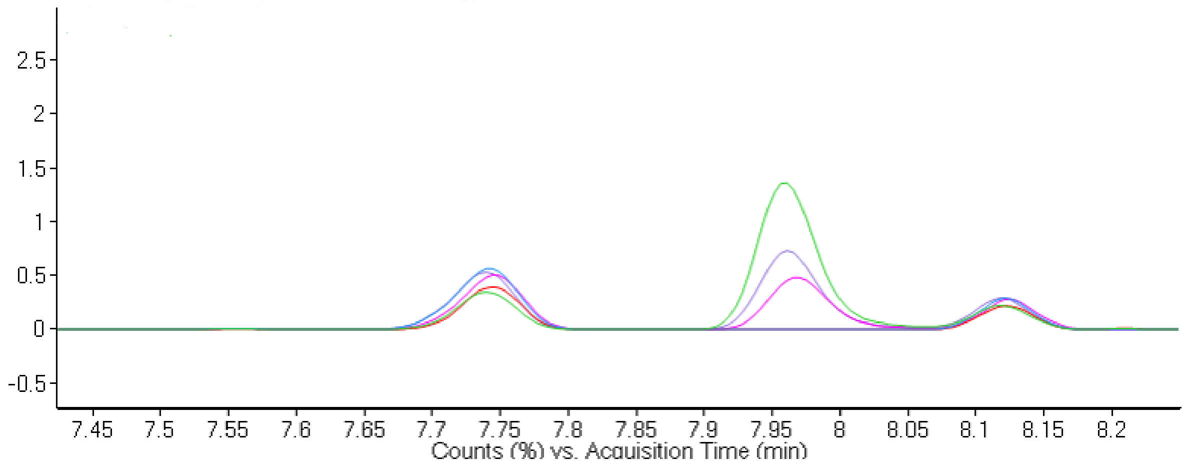

Figure 2. Chromatogram obtained through HS-GC-MS analysis of liquid lipstick samples with a zoom on compounds with retention times between 7.5 min and 8.5 min - Complete Liquid Lipstick Formulation (purple line); Formulation without inorganic pigments (blue line); Formulation without linoleic acid (red line); Formulation with 50% more fragrance (pink line); Formulation without fragrance (green line).

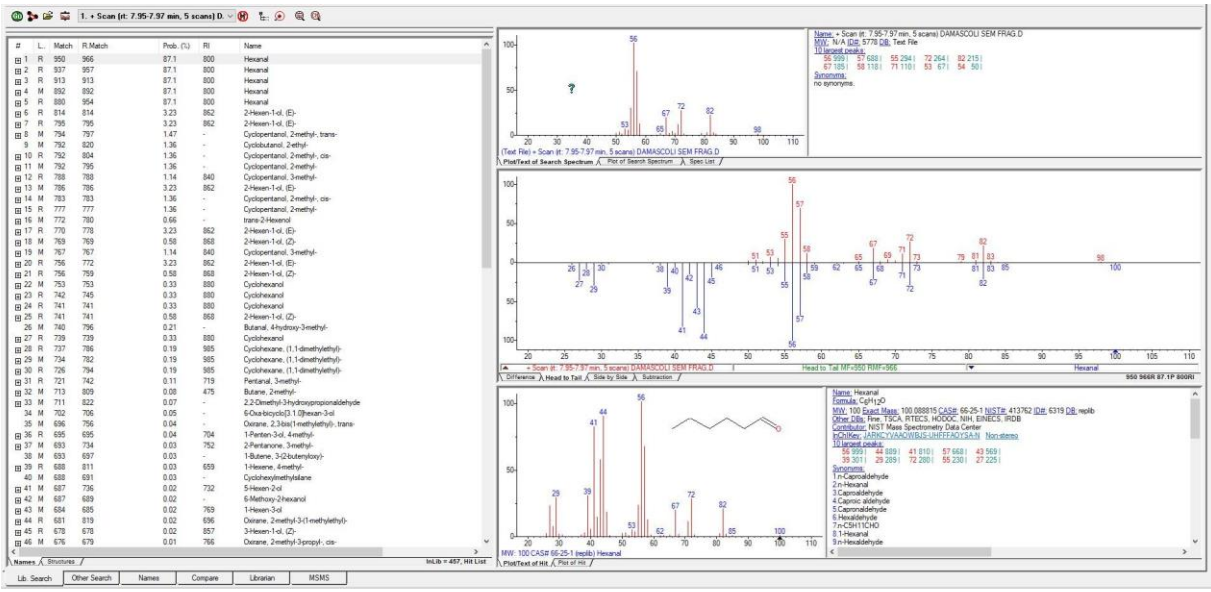

Figure 3. Chemical identification via NIST library of a compound with a retention time of 7.9 min.

4. Discussion

The olfactory stability of cosmetic formulations is a critical parameter for consumer acceptance. The present study investigated odor evolution in different formulations under various storage conditions, aiming to identify the influence of specific components and environmental conditions on olfactory perception over time.

From a sensory perspective, the formulation base, even in the absence of fragrance, exhibited signs of oxidation over time, particularly under more extreme storage conditions. This finding suggests an inherent susceptibility of some base components to oxidative degradation. Conversely, increasing the fragrance concentration in the formulation revealed an improvement in olfactory performance, providing more effective coverage of the oxidized odor that develops over time. This result highlights the role of fragrance in masking the olfactory degradation of the base, maintaining product acceptability. Additionally, the presence of pigments in the formulation demonstrated a contribution to the loss of fragrance intensity throughout the study. This potential interaction between pigments and oxidation is attributed to the fact that inorganic pigments, especially metal oxides, are effective catalysts for oxidation reactions. This catalytic efficacy stems from the unique electronic structure of transition metal ions, their redox properties allowing electron gain or loss, the large surface area offering more active sites, and their ability to act as supports, promoting interactions that enhance catalytic activity and selectivity; furthermore, some oxides possess acid-base properties that also catalyze oxidation reactions [6]. Notably, the sample devoid of linoleic and linolenic fatty acids exhibited a slight oxidized odor that could be easily masked by fragrance. This result indicates that the presence of these active ingredients significantly contributes to the formulation's oxidation process under the evaluated conditions. The absence of oxidized odor under refrigeration suggests that low temperature can retard or inhibit the oxidation induced by these active ingredients.

The cosmetic industry has a significant interest in preventing, as well as early detecting, these lipid oxidation processes due to the economic benefits that can be directly achieved from increased sales, consumer acceptance, and extended shelf life. This allows for the adjustment of formulations so that these products preserve all their qualities for a longer period. Studies conducted in the food industry have demonstrated that the hexanal molecule has become a known indicator as one of the main products of fat oxidation and increases with storage time and exposure temperature [7;8]. It is formed during the oxidation of linoleic acid via 13-hydroperoxide and has an odor described as "herbaceous," which contributes to unpleasant odors, being easily detected due to its low odor threshold (in water: 4.5 µg/kg) [8; 9;10;11;12].

In recent years, hexanal has been determined in food matrices using various sample preparation methods and detection techniques. Considering the complex formulation structure of the evaluated liquid lipstick, as well as the search for volatile compound evaluation, the Gas Chromatography technique coupled with mass spectrometry and injection via HeadSpace technique was chosen, which dispenses with sample preparation and solubilization of components in organic solvents. Through the analyses performed via HS-GC-MS with the 5 different evaluated samples, it was possible to objectively assess the formation of the Hexanal compound. It was observed that its formation rate is directly related to the proportion of oxidized odor perceived in the sensory analyses. This result also confirmed the sensory findings that indicated Linoleic Acid as the primary oxidation point of the formula and that its formation was catalyzed by the presence of inorganic pigments, since Hexanal could not be detected in the samples where these two groups of substances were removed. Another important finding was the role of fragrance concentration in Hexanal formation. It is observed that the presence of fragrance, proportionally to its concentration, also protects against Hexanal formation, possibly due to an antioxidant role that some fragrance components may exert.

Furthermore, recent studies indicate that Hexanal is considered an antagonist of an important olfactory receptor, OR1G1. As these receptors are non-selective and, once bound to antagonists, can cause various olfactory variations through the same molecule, it can also be suggested that in addition to conferring a rancid odor to the sample, Hexanal can also lead to an alteration in the sensory olfactory perception of the fragrance, further contributing to the odor change identified in the samples where it is found in higher concentrations [13].

5. Conclusion

In conclusion, this study provides valuable insights into the factors influencing the olfactory stability of cosmetic formulations. The inherent susceptibility of the base to oxidation, the masking effect of fragrance, and the pro-oxidant role of pigments, particularly catalyzed by metal oxides, were clearly demonstrated. The significant contribution of linoleic and linolenic acids to oxidation under the tested conditions was also highlighted, with refrigeration effectively mitigating this process. The objective quantification of hexanal formation via HS-GC-MS corroborated the sensory findings, establishing hexanal as a relevant chemical marker for oxidative degradation in this cosmetic matrix. Furthermore, the potential interaction of hexanal with olfactory receptors suggests a complex interplay between base degradation products and fragrance perception. These findings underscore the importance of careful ingredient selection, appropriate storage conditions, and the strategic use of fragrance to ensure the long-term olfactory acceptability of cosmetic products, ultimately impacting consumer satisfaction and product shelf life.

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