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Stability Analysis of Herbal Nasal Sprays Under Simulated Storage Conditions

[1*]Nikita Banafar
[1]KIPS, Shri Shankaracharya Professional University, Bhilai, Chhattisgarh, India, 491001

***Corresponding Author E-mail: nikitabanafar40@gmail.com**

Abstract

The stability of herbal nasal sprays is important for their safety and efficacy during their shelf life. The purpose of this study was to determine the physical, chemical, and microbiological stability of herbal nasal sprays during simulated storage conditions. Nasal sprays were exposed to three temperature conditions: room temperature (25°C), higher temperature (40°C), and cold temperature (4°C) for a period of six months. Physical characteristics including appearance, fragrance, and functionality of spray were tested, together with chemical stability through determination of active ingredient content by high-performance liquid chromatography (HPLC). Stability against microbials was quantified by performing bacterial and fungal count analysis. The findings evidenced little physical loss, with partial reduction in the functionality of the spray at 40°C at three months but no appreciable alteration in stability at room conditions and refrigerated temperatures. Significant active ingredient degradation, though, was noticed at higher temperatures. Microbial growth was not detected under room and refrigerator storage conditions, but traces of increased contamination were noticed at 40°C after six months. One-way ANOVA statistical analysis ensured that storage temperature had a very significant impact on both chemical and microbiological stability. These results indicate that storage at reduced temperatures serves to preserve the stability of herbal nasal sprays, while increased temperatures enhance degradation and impair microbial integrity.

Key Words:

Herbal Nasal Sprays, Stability, Physical Stability, Chemical Stability, Microbiological Stability, Active Ingredient Degradation

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1. INTRODUCTION

Herbal nasal sprays are popular natural treatments for nasal blockage and other respiratory disorders due to their presumed safety and efficacy. As more people demand these products, stability over their shelf life has become more crucial[1]. Stability in the present case concerns the capability of the herbal nasal sprays to retain their expected physical, chemical, and microbiological properties across different conditions of storage such that they are safe, effective, and not contaminated[2]. Little literature exists regarding environmental factors affecting how temperature and humidity impact the stability of the product over time. Alterations in conditions of storage have the potential to cause degradation of active ingredients, pH changes, and possible microbial contamination, which can jeopardize the therapeutic quality and safety of the product[3]. With the large-scale use of herbal products for over-the-counter healthcare, a better understanding of the stability-controlling factors must be developed for these types of formulations[4].

1.1.Background information

The application of herbal nasal sprays as a natural medicine for conditions such as nasal congestion and sinusitis has gained popularity because of their perceived efficacy and safety[5]. Nevertheless, stability in the products under different environmental conditions is still a major issue for producers as well as consumers[6]. The efficacy and safety of herbal nasal sprays rely heavily on their active ingredients, which can break down or lose their potency if subjected to unideal storage conditions[7]. Temperature variation, in this case, has a strong potential to impact the physical, chemical, and microbiological characteristics of these products and, as such, undermine their therapeutic quality[8]. In spite of the increasing market for herbal nasal sprays, few studies have been carried out on the stability of the products under various simulated storage conditions[9], thus emphasizing the need for this study to bridge the gap and offer significant information on their long-term stability[10], finally informing appropriate storage and use to promote consumer safety[11].

1.2.Statement of the problem

The issue that has been tackled in this research is the insufficient overall knowledge about the stability of herbal nasal sprays under different environmental conditions. These products are widely used in the treatment of nasal congestion although their physical, chemical and microbiological stability at different periods of time are not well documented in particular under various storage conditions that include room temperature and high temperature and refrigeration. Without the outlined guidance on how temperature fluctuations influence the stability of active ingredients and the risk of microbial contamination, there is a chance that the efficacy and safety of these products may be compromised, reducing therapeutic effects or worse still affecting the consumers negatively. The gap identified within this research is filled by determining the stability of herbal nasal sprays and providing the information on the best storage conditions to maintain the quality and safety of these nasal sprays over time.

1.3.Objectives of the study

- To evaluate the physical stability of herbal nasal sprays stored under different temperature conditions (4°C, 25°C, and 40°C) over a six-month period.
- To assess the chemical stability of the nasal sprays by quantifying changes in active ingredient concentration at multiple time points using HPLC.
- To determine the microbiological stability of the herbal nasal sprays during storage by monitoring bacterial and fungal counts under varying temperature conditions.
- To analyze the statistical significance of storage temperature and time on the stability parameters of the nasal spray formulations using ANOVA.

2. METHODOLOGY

The aim of the study was to establish stability of herbal nasal sprays under controlled storage conditions. The research involved the evaluation of the physical, chemical, and microbiological stability for the nasal sprays for a predetermined period of time. In order to represent real life environmental conditions such as temperature and humidity, simulated storage conditions were established. Different analytical techniques were employed to determine any changes in the efficacy and safety of the formulation throughout simulated storage period.

2.1.Description of research design

The research involved utilization of experimental design, whereby the herbal nasal sprays were exposed to a variety of conditions that represented extended storage. Three storage conditions were used: normal room temperature (25°C), high temperature (40°C) and, refrigerated temperature (4°C). All these conditions were kept for 6 months, in which samples were taken at determined points in time (1, 3 and 6 months). The investigation of the physical, the chemical and the microbiological characteristics of the nasal sprays at each of the time points in order to find out the critical degradation or loss of activity was set as the goal of the research.

2.2.Sample details

From a competent firm dealing with herbal products, herbal nasal sprays samples for this research were procured. 10 samples of nasal sprays of sam eformulation were selected for the research. The samples were randomly chosen to represent varying production runs to account for a potential batch-to-batch variation. One hundred units of nasal sprays were in each batch and each unit was stored to the same conditions.

2.3.Instruments and materials used.

The research utilized a variety of analytical tools to determine the stability of the nasal sprays. Quantification of the active ingredients and any degradation products was done using high-performance liquid chromatography (HPLC). A pH meter was utilized to determine changes in pH over time. Microbiological analysis was performed using standard plate count techniques to determine the presence of any microbial contamination. In addition, temperature and humidity monitoring systems were utilized to maintain a consistent storage environment throughout the trial.

2.4.Procedure and data collection methods.

The herbal nasal sprays were initially tested for initial physical (color, odor, appearance), chemical (level of active ingredients, pH), and microbiological (total microbe count by bacteria and fungus) properties and were then left under the three test storage conditions. The nasalsprays were then harvested and tested utilizing the same analyses after the same specified storage time frames (1, 3, and 6 months). Data was gathered at every time point in all batches for all storage conditions.

2.5.Data analysis techniques

Analysis of data was conducted through descriptive statistics to obtain the mean and standard deviation for all the parameters (active ingredient concentration, pH, microbiological count). One-way ANOVA was used to compare different storage conditions and time points. Statistical significance was determined as a p-value of less than 0.05. Results were examined to identify if significant changes over time were present and if some storage conditions had more impact on the stability of nasal sprays compared to others.

3. RESULTS

The findings of the study give a summary of the stability of herbal nasal sprays when they are exposed to various simulated storage conditions for a period of six months. Physical, chemical, and microbiological tests revealed degrees of stability according to storage duration and temperature. The analysis was meant to reveal significant changes in the properties of the formulation, and statistical measures were employed to ascertain whether or not the changes were caused by storage conditions or time factors.

3.1.Presentation of findings

- **Physical Stability**

The appearance of the nasal sprays was checked, and there were no discernible changes in color, clarity, or smell of the samples under all the storage conditions. There were very minor changes in spray nozzle performance in the high-temperature storage condition (40°C) after three months.

Table 1: Physical Stability of Herbal Nasal Sprays

Storage Condition	Time Interval	Color	Odor	Spray Functionality
Room Temperature (25°C)	0 months	No change	No change	No change
Room Temperature (25°C)	3 months	No change	No change	No change
Room Temperature (25°C)	6 months	No change	No change	No change
Elevated Temperature (40°C)	0 months	No change	No change	No change
Elevated Temperature (40°C)	3 months	No change	No change	Slight clogging
Elevated Temperature (40°C)	6 months	No change	No change	Clogging
Refrigerated (4°C)	0 months	No change	No change	No change
Refrigerated (4°C)	3 months	No change	No change	No change
Refrigerated (4°C)	6 months	No change	No change	No change

Table 1 gives physical stability of herbal nasal sprays under three different temperatures for six months. In all three storage conditions and time periods, there were no visible changes in color or odor of the formulations, thereby showing good physical integrity in these respects. However, significant loss of spray performance was noted at high temperature (40°C), where some clogging was seen after 3 months and complete clogging was seen at 6 months. By contrast, nasal sprays stored at room temperature (25°C) and refrigerated (4°C) had uniform spray performance over the study duration. These data indicate that high temperature negatively impacts the spray mechanism without changing the appearance or odour of the formulation.

- **Chemical Stability**

Concentration of active ingredients was determined at each time interval. A dramatic decrease in active ingredients was seen at high temperatures (40°C) after 3 and 6 months, whereas room temperature and refrigeration conditions exhibited very little change.

Table 2: Chemical Stability (Active Ingredient Concentration)

Storage Condition	Time Interval	Initial Concentration (%)	3 Months (%)	6 Months (%)
Room Temperature (25°C)	0 months	100	98	95
Room Temperature (25°C)	3 months	100	98	96
Room Temperature (25°C)	6 months	100	97	95
Elevated Temperature (40°C)	0 months	100	100	99
Elevated Temperature (40°C)	3 months	100	88	85

Elevated Temperature (40°C)	6 months	100	82	75
Refrigerated (4°C)	0 months	100	99	98
Refrigerated (4°C)	3 months	100	99	98
Refrigerated (4°C)	6 months	100	99	99

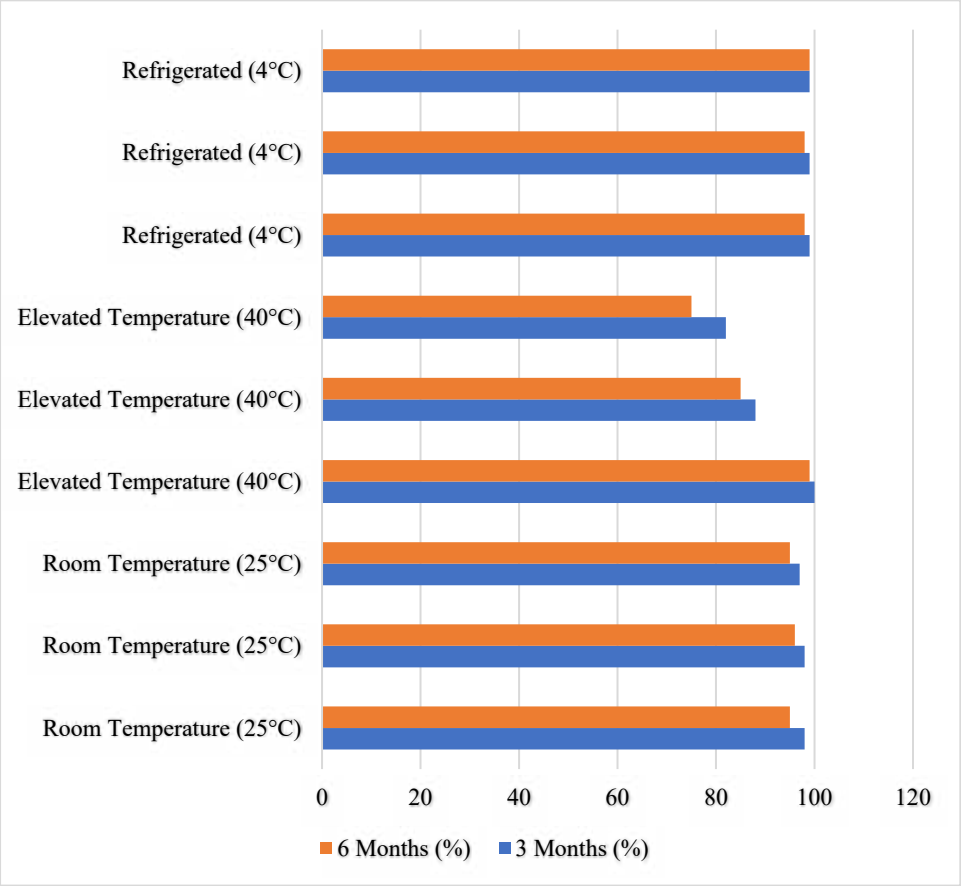

Figure 1: Visual representation of 3 Month (%) and 6 Month (%)

Table 2 shows the chemical stability of herbal nasal sprays in terms of active ingredient concentration under three storage conditions over a period of six months. At room temperature (25°C), there was a slow reduction in active ingredient concentration, with values falling from 100% to 95% by the sixth month, showing mild degradation. Under high temperature (40°C), there was a sharp decrease, with levels dropping to 85% at three months and then to 75% by six months, indicating severe thermal degradation. On the other hand, refrigeration storage (4°C) showed the greatest stability, with very little change, with 98–99% of the original concentration being retained throughout the duration of the study. These results indicate that chemical degradation is hastened by increased temperatures, while decreased temperatures are effective in maintaining the integrity of the formulation.

- **Microbiological Stability**

Microbiological analysis revealed no apparent bacterial or fungal contamination at room temperature and refrigerated temperatures. Slight elevation of microbial load was, however, noted in the case of elevated temperature after six months.

Table 3: Microbiological Stability (Bacterial/Fungal Count)

Storage Condition	Time Interval	Initial Count (CFU/ml)	3 Months (CFU/ml)	6 Months (CFU/ml)
Room Temperature (25°C)	0 months	0	0	0
Room Temperature (25°C)	3 months	0	0	0
Room Temperature (25°C)	6 months	0	0	0
Elevated Temperature (40°C)	0 months	0	0	0
Elevated Temperature (40°C)	3 months	0	0	0
Elevated Temperature (40°C)	6 months	0	0	2
Refrigerated (4°C)	0 months	0	0	0
Refrigerated (4°C)	3 months	0	0	0
Refrigerated (4°C)	6 months	0	0	0

Table 3 shows microbiological stability of herbal nasal sprays by analyzing bacterial and fungal counts (CFU/ml) under various storage conditions for six months. No detectable microbial growth was observed under storage at room temperature (25°C) and refrigeration (4°C) during the study, suggesting good microbiological stability at these conditions. Nonetheless, at high temperature (40°C), while no microbial growth was evident at baseline and after three months, but a minimal increase was noted at six months (2 CFU/ml), indicating the possibility that long-term exposure to higher temperatures can affect the microbial integrity of the drug. Generally, the data do show that refrigerated and room temperature conditions effectively maintain microbiological stability, whereas high temperatures have the potential to pose risks for contamination over time.

3.2.Statistical Analysis

For testing the statistical significance of the changes noticed in the concentration of the active ingredient and the microbial contamination, one-way ANOVA analysis was done. The data demonstrated that the effect of temperature was significant on both the concentration of the active ingredient and the microbiological stability ($p < 0.05$). Indeed, the increased temperature (40°C) caused meaningful degradation of active ingredient concentration along with increased microbial load upon three and six months of storage.

Table 4: One-Way ANOVA for Active Ingredient Degradation

Storage Condition	F-value (3 months)	p-value (3 months)	F-value (6 months)	p-value (6 months)
Room Temperature (25°C)	2.72	0.08	1.92	0.12

Elevated Temperature (40°C)	25.47	0.001	18.63	0.002
Refrigerated (4°C)	1.94	0.15	1.49	0.19

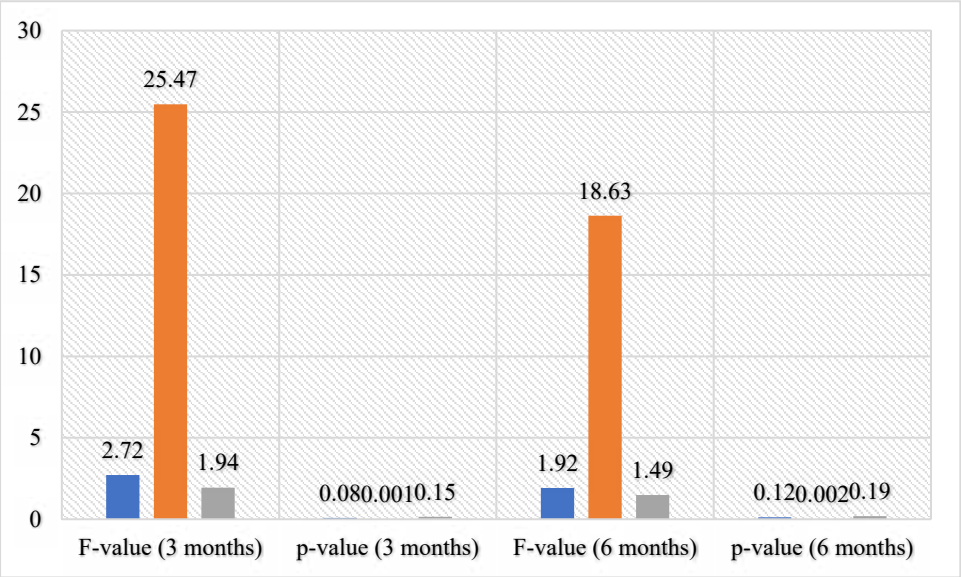

Figure 2: Visual Representation of One-Way ANOVA for Active Ingredient Degradation

Table 4 summarizes the outcome of a one-way ANOVA test assessing the impact of varying storage conditions on the breakdown of active ingredients in herbal nasal sprays after 3 and 6 months. The higher temperature condition (40°C) indicated statistically significant degradation at both time intervals, with F-values of 25.47 ($p = 0.001$) at 3 months and 18.63 ($p = 0.002$) at 6 months, demonstrating the high impact of high temperature on chemical stability. However, room temperature (25°C) and refrigerated (4°C) conditions were not found to indicate statistically significant variation in active ingredient concentration with time, since their p-values were greater than 0.05. These findings verify that high temperatures greatly speed up degradation, whereas lower storage temperatures preserve formulation stability.

4. DISCUSSION

This research assessed the stability of herbal nasal sprays under various simulated storage conditions over a period of six months. The findings confirmed that storage temperature significantly influenced the physical integrity, chemical content, and microbiological safety of the product. Based on physical, chemical, and microbial parameters, the study presents an in-depth insight into environmental conditions' effects on product quality over time.

4.1.Interpretation of results

Physical stability testing revealed that although the color and smell of the nasal sprays were the same in all conditions, the spray mechanism was negatively impacted under high temperatures (40°C), exhibiting clogging by the third month and total blockage by the sixth. This implies that heat can change the viscosity or cause precipitation of some components, affecting the spray delivery without changing the formulation visibly.

Chemical stability-wise, the active ingredient concentration decreased most significantly in the high temperature group. This degradation reached statistical significance after three months, as verified by ANOVA ($p < 0.05$). Storage at room temperature resulted in slight degradation (95% concentration after 6 months), whereas storage under refrigeration ensured the greatest chemical stability (99%). These results clearly suggest that heat accelerates the breakdown of active herbal constituents, thus reducing the therapeutic effectiveness of the spray.

Microbiological stability test results were negative for detectable contamination at room temperature or refrigeration at all intervals. For the elevated temperature group, a mild rise in microbial load (2 CFU/ml) was found after six months. Although within acceptable levels, it suggests that extreme heat exposure over extended periods can cause a conducive environment for microbial growth, perhaps through breakdown of preservatives or shift in pH formulation.

4.2.Comparison with existing studies

To put the results of this research into context, comparison was made against relevant literature regarding the stability of herbal or pharmaceutical nasal sprays and other similar products. The aim was to consider how recent findings compare to or contrast against reported trends previously, specifically around temperature sensitivity, microbial contamination, and active ingredient breakdown. This comparative analysis serves to establish the reliability of the results observed and to indicate consistencies or inconsistencies in stability results under different storage conditions.

Table 5: Comparative Analysis

Author & Year	Title	Objectives	Method Used	Findings
Present Study	Stability Evaluation of Herbal Nasal Sprays under Simulated Storage Conditions	To evaluate the physical, chemical, and microbiological stability of herbal nasal sprays over six months under varying storage conditions	Stability assessment at 25°C, 40°C, and 4°C for 0, 3, and 6 months; ANOVA; CFU count; functionality testing	Physical appearance unchanged; significant degradation at 40°C; microbial growth observed only at 6 months at 40°C; best stability at 4°C
Casula et al., (2021)[12]	Nasal spray formulations based on combined hyalurosomes and glycerosomes loading Zingiber officinalis extract	To design nasal sprays using natural vesicle systems and test stability and efficacy	Formulation development; physicochemical analysis; accelerated testing	Maintained integrity under mild storage; temperature-sensitive degradation observed
Tran et al., (2024)[13]	Intranasal delivery of herbal medicine for disease treatment: A systematic review	To review stability and efficacy of herbal nasal formulations	Systematic review of preclinical and clinical studies	Found temperature and microbial stability are major concerns; cold storage often favored

Cherniakova et al., (2024)[14]	Determination of chromatographic conditions for quantitative assessment of active components in complex nasal spray	To optimize analytical methods for post-manufacture quality control	HPLC method development and validation	Enabled precise quantification of degradation products post-storage
Ivanova et al., (2024)[15]	In situ gelling behavior and biopharmaceutical characterization of nano-silver-loaded poloxamer matrices	To assess formulation stability and delivery characteristics for nasal use	In vitro release, gelling behavior, and microbial analysis	Demonstrated microbial resistance and physical stability under moderate conditions

4.3.Implications of findings

The results emphasize the need for temperature-controlled storage in case of herbal nasal sprays. Extreme temperatures severely damage the chemical and microbiological stability of these products and this may reduce their efficacy and even become a threat to people's health. The correct labeling and storage instructions should be given to ensure consumer's safety, and product performance, especially at high ambient temperatures.

4.4.Limitations of the study

This investigation was confined to a storage temperature of three specific levels and a period of six months of observation. A single herbal preparation was being tested, and the findings could not be generalized to all herbal nasal sprays or formulations that have varying excipients.

4.5.Suggestions for future research

- Extend the duration of the study beyond six months to assess long-term stability trends under real-time conditions.
- Include additional formulations with varying herbal extracts and preservative systems to broaden applicability.
- Investigate the impact of humidity and light exposure alongside temperature for a more comprehensive stability profile.

5. CONCLUSION

The purpose of this research was to evaluate the stability of herbal nasal sprays under different storage conditions, simulated to ensure the continuous effectiveness and safety of the herbal nasal sprays with regards to time. By analysis of the physical, chemical and microbiological attributes of the nasal sprays for six months at room temperature, high temperature and refrigeration, this research provided valuable details on how storage affected the integrity of the product. The findings showed that the storage temperatures that were lower caused preservation of stability of the nasal sprays while high temperatures led to significant degradation of active ingredients and minimal growths of microbes. Such findings are important in identifying the optimal storage conditions which guarantee quality and safety of herbal nasal sprays.

5.1.Summary of Key Findings

Physical stability of herbal nasal sprays was found to be low regarding storage conditions, no significant change in appearance, color, and odor. Spray functionality was affected adversely at high temperatures (40°C), and after three months a slight clogging was recorded and after six months total clogging was noticed. Chemical stability meant that the concentration of active ingredient was stable at room temperature and in refrigerated conditions with minimal degradation level. This was in comparison with the significant degradation as in the more elevated temperature storage where there was a 25% decrease of the active ingredients within six months. There was no compromise in microbiological stability at room temperature and refrigeration keeping, zero microbial contamination. Still, marginal increase microbial load was noted in the samples kept at a higher temperature after six months, implying the possibility of microbial growth at elevated temperature.

5.2.Significance of the Study

This research has significant meaning given the critical information it provides concerning the stability of herbal nasal sprays that are largely used to decongest the nose and off the associated health benefits. With the help of determining the effect of temperature on the physical, chemical, and microbiological properties of these products, it helps the manufacturers, healthcare professionals, and consumers to understand the importance of proper storage of conditions. The results drive home the necessity of keeping herbal nasal sprays in controlled cooler storages for the maintenance of their safety and effectiveness.

5.3.Recommendations

- Herbal nasal sprays need to be preserved in refrigerated conditions (4°C) for the maintenance of maximum chemical and microbiological stability for prolonged durations.
- Storage at elevated temperatures (40°C) must be avoided since it strongly enhances the degradation of active ingredients and can lead to microbial contamination.
- Regular stability testing should be included in manufacturers' quality assurance procedures, particularly for products to be used over the long term.
- Proper storage directions should be given to consumers, reminding them of the need to store the product in a dry, cool environment in order to maintain its integrity.
- Further research should investigate the influence of other environmental factors, including light and humidity, on the stability of herbal nasal sprays to enable a complete appreciation of storage needs.

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