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Stability studies of isohydrafural and fluocinolone acetonide combined ointment

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Abstract

Background: The isohydrafural and fluocinolone acetonide combined ointment contributes to the diversification of the treatment of dermatitis and psoriasis associated with bacterial infections. Complex stability studies were performed to ensure the quality of it during the shelf life. The objective of this study was to determine the stability and shelf life of the combined ointment containing isohydrafural and fluocinolone acetonide.
Material and methods: Three series of ointment were tested by the real-time method (temperature 25± 2°C; relative humidity 60 ± 5%) over a period of 30 months, periodically determining the appearance, homogeneity, pH, viscosity, identity, purity and assay. OHAUS DV215 CD electronic balance, Shimadzu LC-20 A HPLC, Consort C861 pH meter and Fungilab rheometer were used to fulfil the study.
Results: At 24 months after storage, the three series of the ointment proved to be homogeneous, with a pH between 5.76 and 5.53. The rheograms showed a pseudoplastic behavior, with a slight thixotropy, with a viscosity close to 70 cP. The active substances were detected at the characteristic retention times: isohydrafural – 3 minutes, fluocinolone acetonide – 5.9 minutes and also no additional peaks occurred. The content of active substances was within the permitted limits: 0.098-0.11% (m/m) for isohydrafural and 0.0248-0.025% (m/m) for fluocinolone acetonide.
Conclusions: The combined ointment containing isohydrafural and fluocinolone acetonide was found to be stable under the storage conditions stipulated in the quality specification. The established shelf life is 24 months.
Key words: combined ointment, stability, shelf life.

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Introduction

Currently, the combination of several active substances with different mechanisms of action in a single drug presented interest in medical practice [1-3]. In recent years, combinations of medicinal substances have been developed for different purposes, such as achieving synergism, reducing antibiotic resistance, reducing side effects, lower prices and increasing the treatment compliance [2, 3]. Currently, there is an interest in local combination therapy in dermatology [4]. There are already multiple combinations of antibiotics, topical corticosteroids, local anesthetics for external use [4-6]. However, the biggest challenge of dermatological drug combinations remains the simultaneous treatment of antibiotic-resistant bacterial infections and skin diseases [7].

In recent years, the development of antibiotic resistance has led to use antibacterial drugs, such as nitrofuran derivatives [8]. Researchers have also focused on modernizing the chemical structure of existing antibacterial substances to obtain new, less toxic molecules [9]. A similar problem was resolved by obtaining isohydrafural (isonicotinoyl hydrazone of 5-nitro-2-furan aldehyde) by a group of researchers from the Republic for Moldova [9, 10]. It has significant bactericidal activity in concentrations between 1.25-5.0 μg/ml, of all investigated strains of *Staphylococcus* genus [9, 10]. Isohydrafural is also less toxic (LD_{50}=990 mg/kg) than nitrofural (LD_{50}=166.7 mg/kg) and effective for topical application [9, 10]. Fluocinolone acetonide is a topical glucocorticoid, used in various dermatological diseases, such as dermatitis, psoriasis, lupus erythematosus etc. It has already existed in combined dosage forms, mixed with neomycin sulphate [11]. The combination of isohydrafural and fluocinolone acetonide in the same ointment dosage form, contributes to the diversification of the treatment of dermatitis and psoriasis associated with bacterial infections.

Stability of a drug is an important indicator of physical, chemical, therapeutical and microbiological safety. For the semisolid dosage forms the most important causes of instability are the chemical interactions and also the conferring by the excipients of a favorable environment for bacteria growth [12-14]. Stability studies are performed to prevent or eliminate these undesired causes. Stability studies are required in the process of developing a new drug before the elaboration of Analytical and Standardization Documents in order to establish its shelf life [12-14]. For semisolid dermatological dosage forms, such as ointment, the ICH guideline recommends determining the stability and shelf life by real-time (long-term) method, which consists in analysis of the quality parameters corresponding to the dosage form at

certain time intervals, by storage at temperature 25±2°C and relative humidity 60±5% [12]. According to the ICH guideline, the shelf life is considered to be established when there has been a decrease in the concentration of active substances of 90% [12-14].

The combined ointment containing isohydrafural and fluocinolone acetonide was elaborated within the research laboratories of *Nicolae Testemitanu* State University of Medicine and Pharmacy. Complex stability studies are necessary to ensure the quality of the combined ointment containing isohydrafural and fluocinolone acetonide during its shelf life. As a consequence, the objective of this study was to determine the stability and shelf life of the combined ointment containing isohydrafural and fluocinolone acetonide.

Material and methods

Ointment preparation. The pure substances, isohydrafural, (synthesized at the Department of Organic Chemistry, Chisinau State University of Moldova, concentration 99.9%) and fluocinolone acetonide (Sigma Aldrich, 99.9% concentration) were used. The excipients: propylene glycol, cetostearyl alcohol and petroleum jelly were used according to specifications of pharmacopoeia. All other materials were of analytical grade. Extemporaneous preparation of ointment was done by heating 3.0 g cetostearyl alcohol at 50-55^{0}C, cooling at 35^{0}C and then adding 91.875 g of petroleum jelly with stirring (lipophilic phase). Then 0.025 g of fluocinolone acetonide was dissolved in 5.0 g of propylene glycol and the obtained solution with 0.1 g of isohydrafural was added to lipophilic phase with stirring. The ointment was transferred to a suitable dark glass container.

Stability studies and shelf life determination. According to ICH Harmonised Tripartite Guideline Topic *Q1C*, 3 series of ointment were tested by the real-time method (temperature 25± 2°C; relative humidity 60±5%) over a period of 30 months, periodically determining the appearance, homogeneity, pH, viscosity, identity, purity and assay [12-14].

Appearance and homogeneity were tested by visual observation. *pH measurement* was determined by using the pH meter Consort C861 and the solution prepared by dissolving 1.0 g of ointment in 25 ml of deionized water and heating at 37^{0}C, cooling at room temperature and then filtering. The measurements were carried out in triplicate.

Rheological behavior was tested by using Rotational Viscometer Multi Visc Rheometer, Fungilab (Spain). The viscosity was analyzed at a fix shear rate of 3 rpm at 20±2^{0}C, which is a typical determination for ointments [12, 14]. The rheograms of shear stress as a function of shear rate during the period of 30 months of storage at 20±2^{0}C were recorded in order to determine the rheological behavior of the combined ointment.

Identity, purity and assay of isohydrafural and fluocinolone acetonide from the ointment were performed by HPLC method, which has been previously developed and validated. For chromatographic separation, the Shimadzu HIGH Performance liquid chromatograph LC-20 A and Nucleosil C18 chromatographic column, with dimensions 5x300 mm, particle size 2.6 µm were used. The mixture of acetonitrile and purified water in the ratio 40:60 was selected as the mobile phase. UV-VIS detection was performed at a wavelength of 260 nm. The flow rate of the mobile phase was 0.4 ml/minute. The temperature of the chromatographic column was maintained at 30°C.

Preparation of the isohydrafural standard solution: 0.05 g (exact mass) of isohydrafural standard was transferred into a 50 ml volumetric flask, then 20 ml of mobile phase was added and stirred until the substance dissolved. Then it was made up to the mark with mobile phase. Further 1 ml was transferred into a 10 ml volumetric flask and was made up to the mark with mobile phase.

Preparation of the fluocinolone acetonide standard solution: 0.05 g (exact mass) of fluocinolone acetonide standard was transferred into a 100 ml volumetric flask, then 30 ml of mobile phase was added and stirred until the substance dissolved. Then it was made up to the mark with mobile phase. Further 0.5 ml was transferred into a 10 ml volumetric flask and was made up to the mark with mobile phase.

Preparation of the sample solution: about 2.5 g of ointment was accurately weighed and transferred into a porcelain cup, to which 10 ml of mobile phase was added and heated in a water bath at 30°C until the ointment was melted. The sample was allowed to cool at room temperature and was filtered and collected into a 25 ml volumetric flask. The extraction was repeated twice with each 5 ml mobile phase. The obtained samples were added to the first extraction solution and were made up to the mark with mobile phase.

The concentration of isohydrafural in the combined ointment was calculated by the formula 1.

$$X = \frac{A_x \cdot 25 \cdot m_{st}}{A_{st} \cdot m_x \cdot 50 \cdot 10} \cdot P, \quad \text{in which:} \quad (1)$$

X – the content of isohydrafural in the ointment, g;

A_x – the area of the isohydrafural peak from the chromatogram of the sample solution;

A_{st} – the area of the isohydrafural peak from the chromatogram of the standard solution;

m_x – the amount of ointment taken for analysis, g;

m_{st} – the amount of isohydrafural standard, g;

P – total ointment mass, g.

The concentration of fluocinolone acetonide in the combined ointment was calculated by the formula 2.

$$X = \frac{A_x \cdot 25 \cdot m_{st} \cdot 0.5}{A_{st} \cdot m_x \cdot 100 \cdot 10} \cdot P, \quad \text{in which:} \quad (2)$$

X – the content of fluocinolone acetonide in the ointment, g;

A_x – the area of the fluocinolone acetonide peak from the chromatogram of the sample solution;

A_{st} – the area of the fluocinolone acetonide peak from the chromatogram of the standard solution;

m_x – the amount of ointment taken for analysis, g;

m_{st} – the amount of fluocinolone acetonide standard, g;

P – total ointment mass, g.

For measurements was used OHAUS DV215 CD electronic balance.

The shelf life of the combined ointment was determined in months and was based on a limit of 10% degradation of active substances in accordance with the recommendations of ICH guideline [12, 13, 14].

Statistical analysis. All values were reported as mean ± standard deviation. Statistical measurements were carried out by using the Statistical Package for the Social Sciences (IBM SPSS Statistics) 10.5 software.

Results and discussion

The stability studies of the combined ointment containing isohydrafural and fluocinolone acetonide were investigated to improve its quality and to predict the period of its stable storage [14].

Appearance and homogeneity. Appearance and homogeneity are very important parameters for the stability studies, the appropriate characteristics contributing to easier packaging and application on the skin, while any noticeable change shows the degradation of the drug [12-14].

Determinations made on the day of preparation showed that the combined ointment containing isohydrafural and fluocinolone acetonide was smooth, yellow, odorless and homogeneous. During 24 months, the combined ointment showed no change in appearance. At 30 months after storage, an intensification of the color and loss of homogeneity were observed.

pH measurement. pH is a quality parameter necessary to be studied in determining the stability of a dermatological drug, indicating its compatibility with the pH of the skin. The deviation from the limits (between 4.0 and 6.0) indicates the degradation of the drug [12-14].

For the combined ointment, pH values were recorded compatible with skin application over the period of 30 months, although a slight decrease in values was observed (fig. 1).

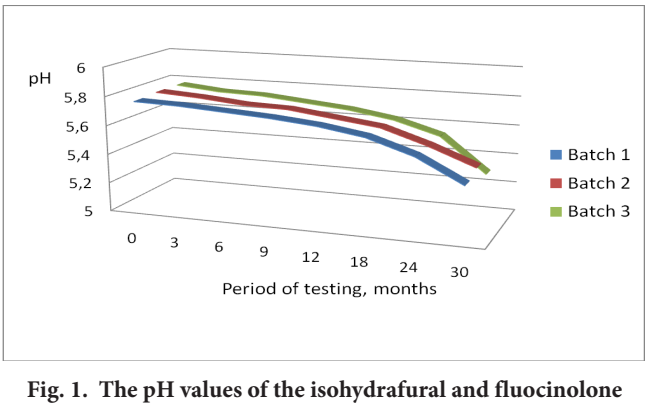

Fig. 1. The pH values of the isohydrafural and fluocinolone acetonide combined ointment

Rheological behavior. In the process of developing a new ointment it is essential to create an optimal viscosity by determining the amounts of viscosity enhancer at the preformulation and formulation stages of the drug, in order to prevent further instability. For the combined ointment containing isohydrafural and fluocinolone acetonide, the cetostearyl alcohol excipient was used as an enhancer, its amount being determined to provide an optimal viscosity close to 70 cP [12, 14]. A significant change of the ointment viscosity value in the stability study process is a reliable indication of drug degradation [12-14]. During the period of 30 months the viscosity values of all three batches of the combined ointment were close to 70 cP, being compatible for application on the skin (tab. 1).

Table 1

The viscosity values of the isohydrafural and fluocinolone acetonide combined ointment

Period of testing, months	Viscosity, cP (mean±standard deviation, 3 determinations)		
	Batch 1	Batch 2	Batch 3
0	73.5±0.6	73.8±0.5	73.1±0.1
3	71.0±0.1	71.0±0.2	71.0±0.1
6	70.5±0.4	70.2±0.1	70.2±0.1
9	69.7±0.1	69.9±0.1	69.9±0.1
12	69.1±0.1	69.1±0.1	69.1±0.1
18	68.9±0.1	68.9±0.1	68.9±0.1
24	68.2±0.1	68.2±0.1	68.2±0.1
30	69.4±0.2	71.1±0.1	71.1±0.1

The rheograms of shear stress as a function of shear rate during the period of 30 months of storage at 20±2^{0}C, indicate that the combined ointment showed a non-Newtonian, pseudoplastic, characteristic for ointments behavior, although a slight decrease in values was observed (fig. 2.1, 2.2., 2.3).

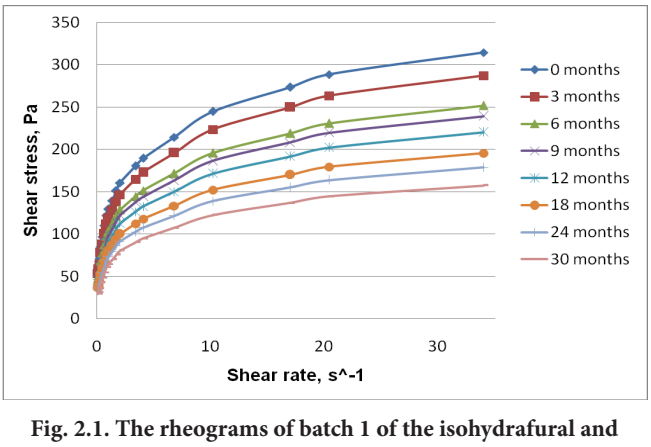

Fig. 2.1. The rheograms of batch 1 of the isohydrafural and fluocinolone acetonide combined ointment

Identity and purity. Identity and purity quality parameters demonstrate that no degradation products have appeared as a result of hydrolysis, oxidation, reduction, decomposition etc. of active substances and excipients [12-14].

The chromatograms of the three batches of the combined ointment corresponded to the quality parameters identity and purity during the period of 24 months, both active substances being detected, while the retention time values were included within the permissible limits: isohydrafural

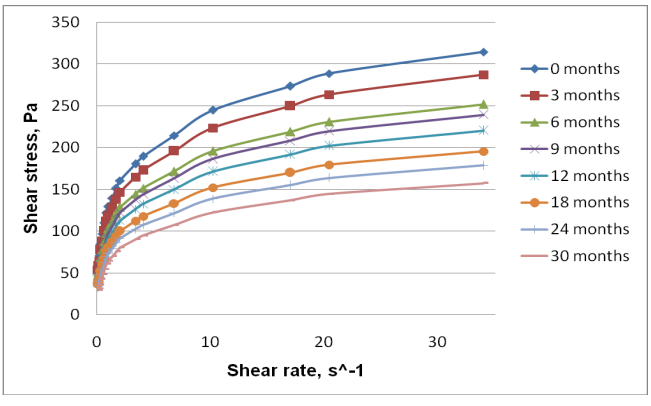

Fig. 2.2. The rheograms of batch 2 of the isohydrafural and fluocinoloneacetonide combined ointment

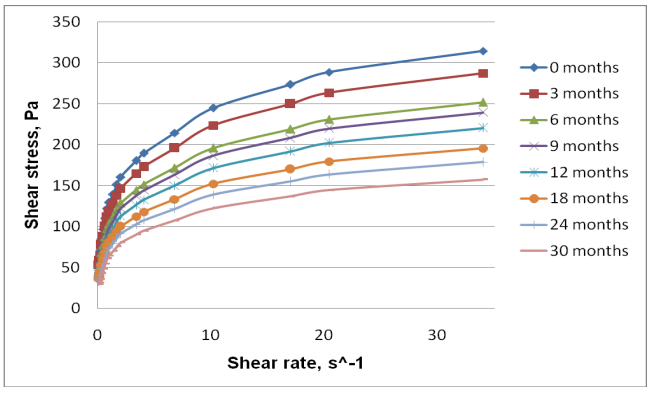

Fig. 2.3. The rheograms of batch 3 of the isohydrafural and fluocinolone acetonide combined ointment

– 3 minutes, fluocinolone acetonide – 5.9 minutes. At 30 months after storage, there were changes of the retention time values specific to fluocinolone acetonide, indicating its degradation (tab. 2).

Table 2

The retention time values of active substances from the isohydrafural and fluocinolone acetonide combined ointment

Period of testing, months	Retention time, minutes					
	Batch 1		Batch 2		Batch 3	
	IHF	FLAc	IHF	FLAc	IHF	FLAc
0	3.112	5.997	3.112	5.996	3.112	5.997
3	3.110	5.995	3.112	5.998	3.112	5.996
6	3.112	5.997	3.113	5.997	3.111	5.998
9	3.113	5.997	3.112	5.998	3.112	5.994
12	3.112	5.996	3.110	5.997	3.112	5.997
18	3.111	5.998	3.112	5.997	3.111	5.998
24	3.110	6.011	3.111	6.014	3.110	5.998
30	2.897	6.277	2.877	6.401	2.884	6.371

Note: IHF – isohydrafural; FLAc – fluocinolone acetonide.

Assay. Assay analysis of the dosage form is essential, because a change of the concentration of active substance can influence the efficacy of the drug. Also, the drug can be considered unstable if it retained less than 90% of in initial concentration of active substance [12-14].

During the period of 24 months, both active substances were detected on the chromatograms of all three series of the combined ointment in concentrations within the permitted limits: 0.098 – 0.110 g/100 g ointment for isohydrafural and 0.0248 – 0.0250 g/100 g ointment for fluocinolone acetonide. At 30 months after storage, the concentration of fluocinolone acetonide was below 0.0248 g/100 g ointment, indicating its degradation (tab. 3).

The stability profile curves of all three batches of the combined ointment, obtained by the real-time method, indicate a reducing up to 90% of fluocinolone acetonide concentration at 30 months of testing, that undergoes faster degradation than isohydrafural (fig. 3.1, 3.2, 3.3).

Table 3

The content of active substances of the isohydrafural and fluocinolone acetonide combined ointment

Period of testing, months	Content, g/100 g ointment					
	Batch 1		Batch 2		Batch 3	
	IHF	FLAc	IHF	FLAc	IHF	FLAc
0	0.10013	0.02504	0.10001	0.02501	0.09996	0.02500
3	0.09996	0.02500	0.09992	0.02499	0.09988	0.02498
6	0.09984	0.02498	0.09985	0.02498	0.09978	0.02497
9	0.09976	0.02495	0.09975	0.02496	0.09965	0.02494
12	0.09960	0.02493	0.09960	0.02493	0.09965	0.02492
18	0.09946	0.02492	0.09949	0.02491	0.09954	0.02491
24	0.09932	0.02490	0.09936	0.02488	0.09943	0.02490
30	0.09872	0.02268	0.09905	0.02267	0.09915	0.02262

Note: IHF – isohydrafural; FLAc – fluocinolone acetonide.

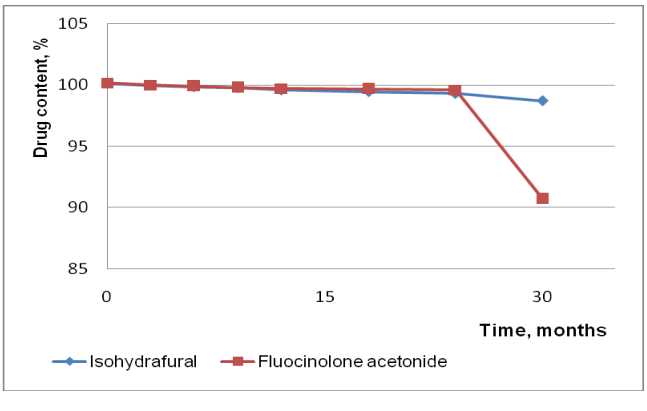

Fig. 3.1. Stability profile curves of batch 1 of the isohydrafural and fluocinolone acetonide combined ointment

Shelf life. The results of stability testing of the combined ointment containing isohydrafural and fluocinolone acetonide indicate a shelf life of 24 months at temperature 25±2°C and relative humidity 60±5%, time required for fluocinolone acetonide concentration to reduce up to 90%. During 24 months of real-time testing, the combined ointment presented no physical instability and underwent no significant changes of pH, viscosity, retention time and concentration of the active substances, the values being within the admissible limits (tab. 4).

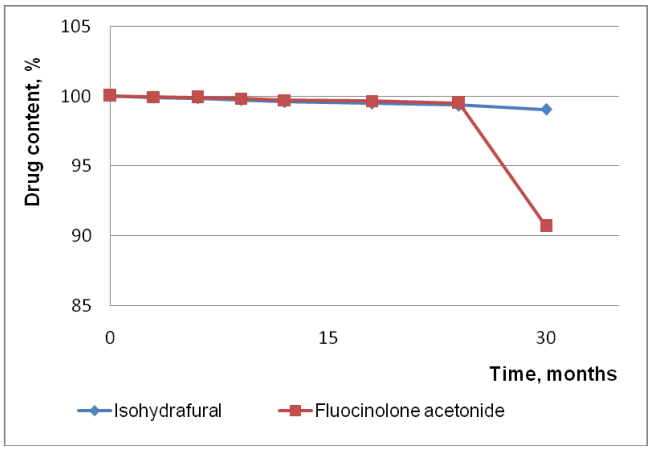

Fig. 3.2. Stability profile curves of batch 2 of the isohydrafural and fluocinolone acetonide combined ointment

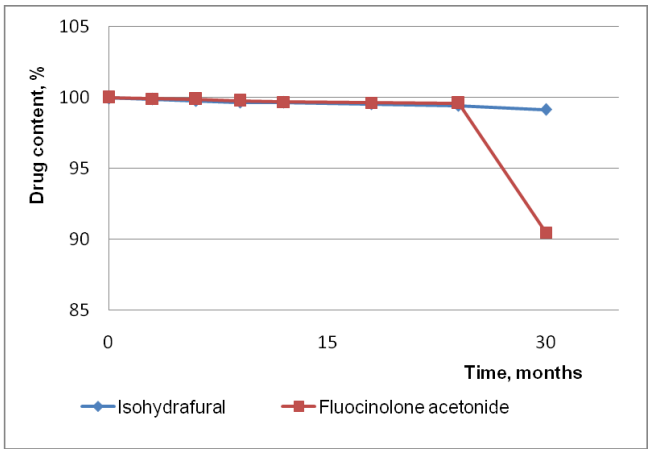

Fig. 3.3. Stability profile curves of batch 3 of the isohydrafural and fluocinolone acetonide combined ointment

Table 4

The result of real-time stability studies of the isohydrafural and fluocinolone acetonide combined ointment

Periodicity of testing, months	Analyzed parameters and admissibility conditions					
	Appearance and homogeneity smooth, yellow, odorless and homogeneous	pH (mean±standard deviation, 3 determinations) 4.0–6.0	Viscosity 65-75 cP	Identity and purity, HPLC Isohydrafural 2.995-3.115 Fluocinolone acetonide 5.994-5.998 minutes	Assay HPLC g/100 g ointment	
					Isohydrafural 0.09995-0.09930	Fluocinolone acetonide 0.02505-0.02485
Batch 1						
0	Corresponds	5.76±0.01	Corresponds	Corresponds	0.10013	0.02504
3	Corresponds	5.75±0.01	Corresponds	Corresponds	0.09996	0.02500
6	Corresponds	5.73±0.01	Corresponds	Corresponds	0.09984	0.02498
9	Corresponds	5.71±0.01	Corresponds	Corresponds	0.09976	0.02495
12	Corresponds	5.68±0.01	Corresponds	Corresponds	0.09960	0.02493
18	Corresponds	5.63±0.01	Corresponds	Corresponds	0.09946	0.02492
24	Corresponds	5.53±0.02	Corresponds	Corresponds	0.09932	0.02490
30	Does not correspond	5.37±0.01	Corresponds	Does not correspond	0.09872	0.02268
Batch 2						
0	Corresponds	5.77±0.02	Corresponds	Corresponds	0.10001	0.02501
3	Corresponds	5.75±0.01	Corresponds	Corresponds	0.09992	0.02499
6	Corresponds	5.72±0.01	Corresponds	Corresponds	0.09985	0.02498
9	Corresponds	5.71±0.01	Corresponds	Corresponds	0.09975	0.02496
12	Corresponds	5.67±0.01	Corresponds	Corresponds	0.09960	0.02493
18	Corresponds	5.63±0.01	Corresponds	Corresponds	0.09949	0.02491
24	Corresponds	5.53±0.01	Corresponds	Corresponds	0.09936	0.02488
30	Does not correspond	5.41±0.1	Corresponds	Does not correspond	0.09905	0.02267
Batch 3						
0	Corresponds	5.77±0.01	Corresponds	Corresponds	0.09996	0.02500
3	Corresponds	5.74±0.01	Corresponds	Corresponds	0.09988	0.02498
6	Corresponds	5.73±0.01	Corresponds	Corresponds	0.09978	0.02497
9	Corresponds	5.70±0.01	Corresponds	Corresponds	0.09965	0.02494
12	Corresponds	5.67±0.01	Corresponds	Corresponds	0.09965	0.02492
18	Corresponds	5.62±0.01	Corresponds	Corresponds	0.09954	0.02491
24	Corresponds	5.53±0.01	Corresponds	Corresponds	0.09943	0.02490
30	Does not correspond	5.29±0.01	Corresponds	Does not correspond	0.09915	0.02262

Conclusions

The results of stability testing of the combined ointment containing isohydrafural and fluocinolone acetonide, obtained by the real-time method, indicate that until 24 months the dosage form maintained its quality parameters, while at 30 months after storage, an intensification of the color, loss of homogeneity were observed and also inappropriate values of identity, purity and assay were recorded. The stability profile curves indicate that during the testing period isohydrafural is more stable in combined ointment than fluocinolone acetonide, which concentration reduced up to 90% at 30 months of storage.

The stability studies, performed by the real-time method, also allowed to determine the shelf life of the combined ointment at room temperature (25±2°C) and a relative humidity of 60±5%, which is 24 months. Therefore, it is recommended that the combined ointment containing isohydrafural and fluocinolone acetonide should be kept in cool place, protected from light in a dark container made from physically and chemically inert material that meets the pharmacopoeial standards.

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ED performed the technological part, interpreted the data, drafted the first manuscript, performed the analytical part of the laboratory work; VV interpreted the data, revised the manuscript; LU designed the study, conducted the laboratory work and revised the manuscript. All the authors revised and approved the final version of the manuscript.

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Ethics approval and consent to participate
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Conflict of Interests
No competing interests were disclosed.