DOI: 10.5281/zenodo.3958557
UDC: 615.451.3.014.4:616.28-002

Stability studies of combined ear drops for the treatment of otitis

[*]Eugeniu Nicolai, Oxana Vislouh, Vladimir Valica, Sergiu Parii, Livia Uncu

Scientific Center for Drug Research, *Nicolae Testemitanu* State University of Medicine and Pharmacy
Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

[*]Corresponding author: eugeniu.nicolai@usmf.md
Manuscript received 30 July, 2020; revised manuscript August 14, 2020; published online August 26, 2020

Abstract

Background: The stability of a drug is an important factor in ensuring its quality.The studied combined ear drops have an increased tendency of degradation, which requires an extensive stability study and obtaining data to determine the shelf life and establish the storage conditions. The purpose of the work: Stability studies of ear drops containing ciprofloxacin, dexamethasone, loratadine and volatile basil oil.
Material and methods: International Harmonized Guideline ICH Q1A (R2) stability testing methodology; 3 series of ear drops; reference standards for the active substances (Sigma Aldrich, USA); Shimadzu LC-20AD liquid chromatograph with UV-VIS detector; Fungilab Smart R viscometer; pH meter inoLab 7110; solvents, reagents in accordance with the European Pharmacopoeia.
Results: Ciprofloxacin is stable in acid medium, degrades in alkaline medium after 3 hours (approximately 10.0%), under oxidation (19.7%) and light action (17.1%). Dexamethasone degrades in acid medium (by 7.7%) and under oxidation (by 19.9%), it is stable in alkaline medium and under the action of light. Loratadine degrades in acid medium (by 3.0%), is stable in alkaline medium, under oxidation and action of light. In real-time storage conditions (25°C±2°C and RH 60%±5%), it was found that the pharmaceutical form did not change its quality parameters for 24 months.
Conclusions: The stability studies under stress and in real time conditions allowed us to select the packaging, the optimal storage conditions and to establish the provisional shelf life for the combined auricular pharmaceutical form during 2 years.
Key words: stability, combined ear drops, otitis, shelf life.

Cite this article
Nicolai E, Vislouh O, Valica V, Parii S, Uncu L. Stability studies of combined ear drops for the treatment of otitis. *Mold Med J.* 2020;63(3):43-50. doi: 10.5281/zenodo.3958557.

Introduction

Stability study is one of the most important areas, in relation to the registration of pharmaceutical products, as it predicts shelf life and storage instructions for batches. It also determines degradation of products, mechanism of breakdown and conditions under which the breakdown occurs. With the help of stability studies, any parameter subject to change within the ear drop during storage can be measured, such as appearance, pH, viscosity and density (where relevant), solubility time (reconstitution and appearance thereof) sterility, preserving ability and preservative content (where relevant). Tests are also performed to ensure compatibility between the container-closure system and the product. Stability testing is the cornerstone of drug development or formulation [1]. In addition to the degradation of the unstable product into toxic decomposition products, loss of activity up to 85% level of the concentration indicated on the label may lead to the failure of therapy. For this reason, data on stability studies are absolutely necessary [2].

Stability testing and study is a mandatory step in drug development [3]. The need for stability studies has a major impact in order to obtain the following information: providing evidence on how product quality changes and degrades; data on optimal storage conditions; establishing a validity period with determination of shelf life; choosing of a justified packaging for the pharmaceutical product storage; obtaining an inoffensive drug for the patient's life; setting high quality and safety conditions [4]. Depending on the mode, stages and conditions of the stability research, two study methods are used: real-time stability and stress stabi-lity studies [5].

For real-time stability studies, the dosage form, at the preformulation stage, is stored for an indefinite period (long time), under normal conditions, which are typical for the dosage form and each component in particular. This method is performed in order to obtain the data that will provide information about the degradation and change of characteristics (properties) of the product under the normal conditions recommended for storage. The test period does not have a normal time, depending on the properties and the actual stability of the product, which should be as long as possible. During testing, the data are collected with a regular frequency of time in order to observe a change scale characteristic for the dosage form [5]. This type of research can value the performance of the dosage form based on its physico-chemical characteristics, the product must maintain its good quality for a pre-designed period [6].

Accelerated stability studies provide extremely valuable information for finding alternative dosage forms, which is the best way to obtain data depending on optimal storage

conditions, packaging materials, manufacturing process etc. As a rule, accelerated stability testing is performed at the same time as the beginning of the real-time test [5].

Forced degradation studies allow the evaluation of the main degradation pathways of the active principles: the delimitation of the degradation products of the active substances from those formed by the auxiliary compounds; obtaining data about the structure of degradation products, their toxic impact on the drug; determining the intrinsic stability of all substances used in the formulation; obtaining more data about the physicochemical properties of the substances; prevention of additional costs during the formulation and production process, by introducing some changes at early stages [7].

Data on the behavior of each substance exposed to different stress conditions are extremely valuable, especially for the choice of excipients and packaging methods [8]. The stress condition that has the greatest influence on the stability of the preparation is the temperature increase, it is important to have a higher temperature than the one possibly considered normal for the given form [9]. In addition to temperature, it is also necessary to test the drug under stress conditions, such as humidity, light, pH change, the action of oxidants and reducing agents [10].

Stability is one of the key problems in development of the combined pharmaceuticals, as it can be influenced by chemical interaction between the combined active principles in a formulation [11].

Within the Scientific Centre for Drug Research, there are in the process of ongoing the research of elaboration and development of combined ear drops for the treatment of otitis with the following composition: ciprofloxacin hydrochloride (antibiotic), dexamethasone (corticosteroid, anti-inflammatory), loratadine (antihistamine, desensitizing) and volatile basil oil (calming, regenerating and natural antiseptic) [12, 13]. This pharmaceutical form has undergone the stability testing process in order to obtain data that would highlight any physico-chemical changes in the components and to establish the shelf life and storage conditions.

Material and methods

For stability studies in stress and real-time conditions were used 3 series of ear drops prepared in laboratory conditions (Laboratory Analysis, Standardization and Drug Control of the Scientific Center for Drug Research within *Nicolae Testemitanu* State University of Medicine and Pharmacy) where all experimental research was performed.

Materials: Medicinal substances (ciprofloxacin hydrochloride, loratadine, dexamethasone and volatile basil oil), excipients (Methyl paraben (Methyl-4-hydroxybenzoate, nipagen), Polysorbate 20, KOLLISILV ™ PEG E 400) and former procured standards at SIGMA-ALDRICH, USA, meet all the requirements of the European Pharmacopoeia.

Apparatus: The HPLC method, previously developed, was used for the assay of the active principles [14]. Shimadzu LC-20AD HPLC chromatographic system was used, analytical column NUCLEOSIL® 100-5 C18, 5 µm, 4.0 x 150 mm; UV-VIS detector, wavelength 280 nm. The pH on the pH meter inoLab 7110, and the viscosity on the Fungilab Smart R viscometer were determined.

Chemicals: Acetonitrile (ACN) and methanol (MeOH) were used in purity grade "pro HPLC analysis" (Sigma Aldrich), orthophosphoric acid and triethylamine purity grade "pro analysis" (Merck), bidistilled purified water.

Chromatography conditions: 0.1% orthophosphoric acid was mixed with ACN in 65:45 proportions by volume (sol. A). Solution A was mixed with MeOH in 80:20 proportions, homogenized and adjusted with triethylamine to pH 3.0; filtered under vacuum through a Millipore XF 5423050 capron filter (0.2-0.45 µm), degassed in the DONAU-LAB SONIC DLS 660-T / H. The temperature of the chromatographic column was 400°C; injection volume 20 µl; mobile phase flow – 1 ml / min.

Preparation of *standard solutions:* 0.01 g (exact mass) of each standard substances ciprofloxacin hydrochloride, loratadine and dexamethasone were accurately weighed and transferred into a 25 ml volumetric flask. Then it was dissolved in the mobile phase and made up to the level with the same solvent.

Preparation of *the sample solution:* 5.0 ml of pharmaceutical form was placed in a 25 ml volumetric flask and diluted with the mobile phase to the mark.

Stress degradation methods.

Preparation of the solution *for acid degradation:* 2.5 ml of pharmaceutical form was mixed with 2.5 ml of 0.1 mol / l hydrochloric acid, then it was homogenized and left for 24 hours at room temperature (25^0C). 4.0 ml of solution was diluted with mobile phase to 10 ml in a volumetric flask.

Preparation of the solution *for basic degradation:* 5.0 ml of pharmaceutical form was mixed with 0.5 ml of 0.1 mol / l sodium hydroxide, then it was homogenized and left for 24 hours at room temperature (25^0C). 5.0 ml of solution was diluted with mobile phase to 25 ml in a volumetric flask.

Preparation of the solution *for oxidative degradation:* 2.5 ml of pharmaceutical form was mixed with 2.5 ml of 3% hydrogen peroxide, then it was homogenized and left for 24 hours at room temperature (25^0C). 4.0 ml of solution was diluted with mobile phase to 10 ml in a volumetric flask.

Preparation of the solution *for photolytic degradation:* 5.0 ml of pharmaceutical form was irradiated with UV light (300 nm) for 48 hours. 5.0 ml of UV treated solution was diluted with mobile phase to 25 ml in a volumetric flask.

Preparation of the solution *for thermal degradation:* 5.0 ml of pharmaceutical form was stored in a thermostat at 600 °C for 48 hours. 5.0 ml of solution under thermal stress was diluted with mobile phase to 25 ml in a volumetric flask.

Chromatography technique: In the chromatographic column, 20 µl of standard and sample solutions (for all stress conditions) were injected alternatively, preventively filtered through a 0.45 µm membrane filter, obtaining at least 3-5 chromatograms for each solution.

Real-time stability study.

The pharmaceutical form, packed in dark glass vials with plastic stoppers, was stored for a long time, under normal conditions, at a temperature of 25 °C ± 2 °C; relative humidity 60% ± 5%. The periodicity of the real-time test was performed at equal time intervals: 0, 3, 6, 9, 12, 18, 24 months.

During this period, the main quality parameters were monitored: appearance, identification of active ingredients (ciprofloxacin hydrochloride, dexamethasone and loratadine), pH, viscosity and assay.

Statistical analysis.

Statistical analysis was done by using the Statistical Package for the Social Sciences (IBM SPSS Statistics) 10.5 software.

Results and discussion

To evaluate the degradation processes under stress, the assay variations of the active principles in pharmaceutical form were followed. For the assay of the active ingredients, the chromatograms of the standard solutions of drug substances and of the sample solution were recorded before the application of the stress conditions (fig. 1).

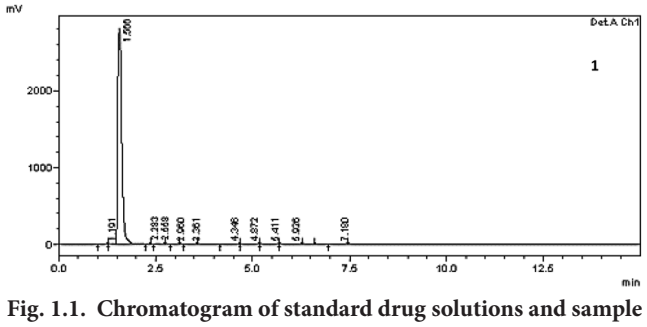

Fig. 1.1. Chromatogram of standard drug solutions and sample solution before application of stress conditions: 1 – standard solution of ciprofloxacine hydrochloride

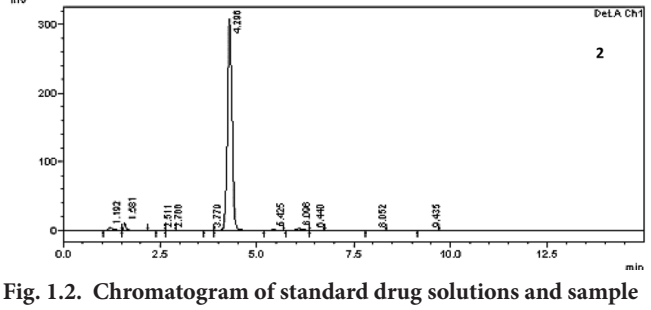

Fig. 1.2. Chromatogram of standard drug solutions and sample solution before application of stress conditions: 2 – standard solution of dexamethasone

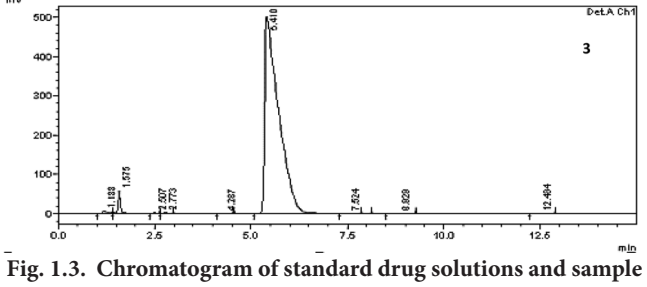

Fig. 1.3. Chromatogram of standard drug solutions and sample solution before application of stress conditions: 3 – standard solution of loratadine

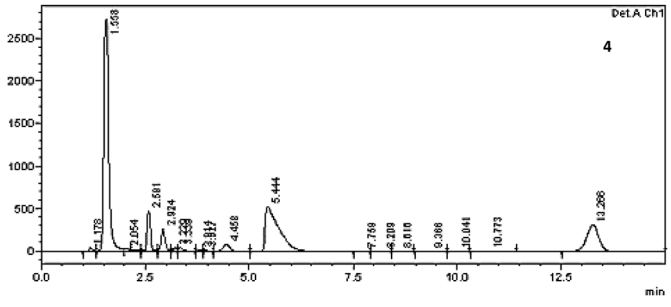

Fig. 1.4. Chromatogram of standard drug solutions and sample solution before application of stress conditions: 4 – sample solution

Degradation under stress conditions

Hydrolytic stress

Hydrolysis is one of the most common chemical degradation pathway over a wide range of pH variations. The hydrolytic study in acid and basic conditions involves the catalysis of the ionizable functional groups present in the molecule. Testing by acid or basic stress involves the forced degradation of a drug substance [15, 16].

Chromatograms of stressed solutions in acid (fig. 2.1) and basic medium (fig. 2.2) were recorded. The chromatograms show the appearance of additional peaks, which denotes the onset of degradation processes.

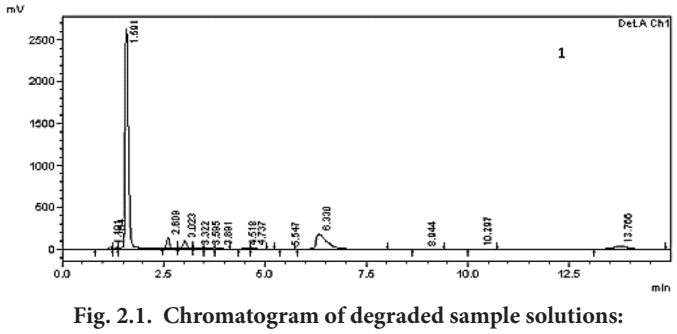

Fig. 2.1. Chromatogram of degraded sample solutions: 1 – in acid medium

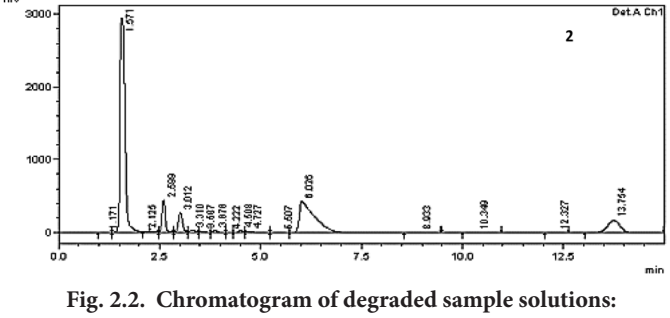

Fig. 2.2. Chromatogram of degraded sample solutions: 2 – in alkaline medium

The results of the quantitative determinations of the active principles were statistically evaluated (table 1). The standard relative deviation of assay results after acid and basic hydrolysis does not exceed 1% for ciprofloxacin hydrochloride and loratadine, and constitutes 3.646 and 2.279 for dexamethasone, respectively.

As can be seen from table 1 and figure 3.1., in acid medium, ciprofloxacin degraded practically insignificantly, while

Table 1

The results of stability studies of ear drops following acid and alkaline hydrolysis

	Test period hours	Ciprofloxacin hydrochloride				Dexamethasone				Loratadine			
		#$t_{R, min}$	#S	#C, g	RSD	#$t_{R, min}$	#S	#C, g	RSD	#$t_{R, min}$	#S	#C, g	RSD
Acid degradation	**0**	1.566	30561193.83	0.29914	0.333	4.294	259125.85	0.01926	3.646	5.402	1118775.74	0.01876	0.741
	3	1.556	30459876.57	0.29814		4.294	249173.46	0.01852		5.403	1102373.41	0.01848	
	24	1.564	30358432.48	0.29715		4.294	239098.89	0.01777		5.410	1091954.81	0.01831	
Basic degradation	**0**	1.566	30161093.87	0.29522	0.208	4.294	256125.85	0.01903	2.279	5.410	1148875.57	0.01926	0.302
	3	1.556	30059876.57	0.29423		4.294	249173.46	0.01852		5.403	1146373.47	0.01922	
	24	1.566	30161193.83	0.29522		4.294	241098.89	0.01792		5.410	1141954.79	0.01915	

Note: The average results for three series of ear drops are shown

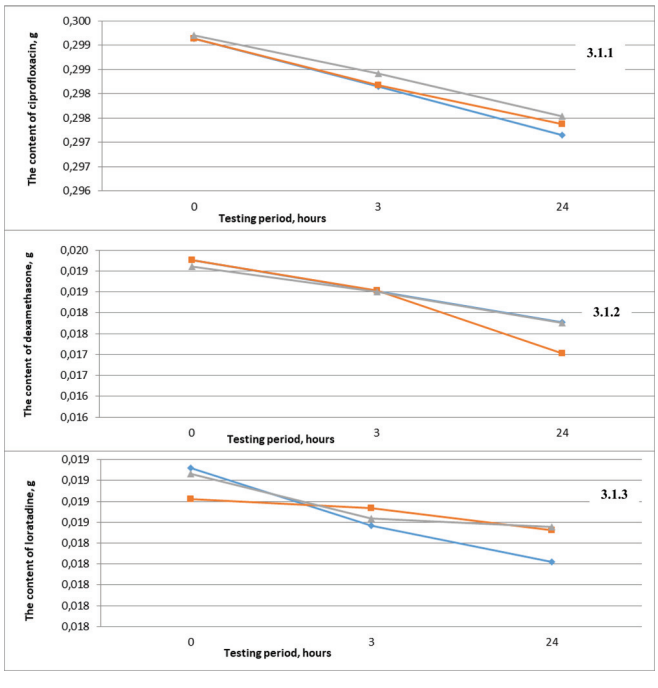

Fig. 3.1. Modification of the concentration of active principles following acid hydrolysis (for 3 series of pharmaceutical form): 3.1.1. – ciprofloxacine hydrochloride; 3.1.2. – dexamethasone; 3.1.3. – loratadine

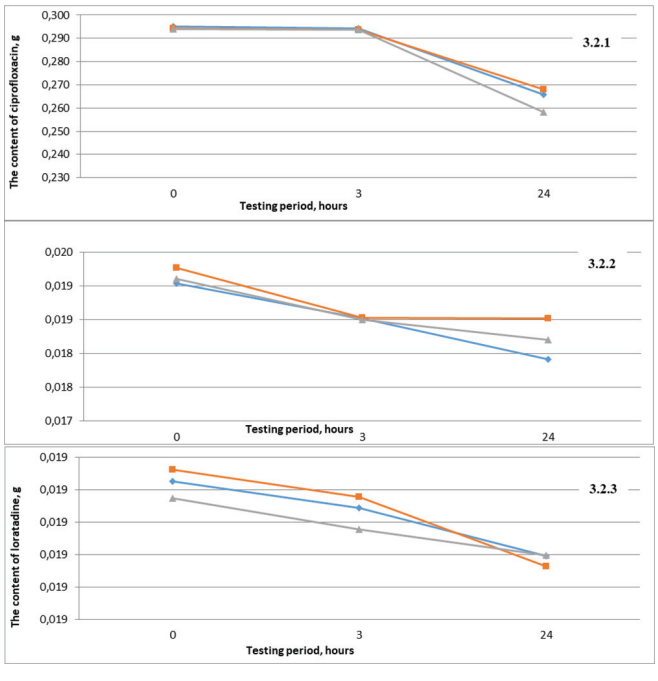

Fig. 3.2. Modification of the concentration of active principles following alkaline hydrolysis (for 3 series of pharmaceutical form): 3.2.1. – ciprofloxacine hydrochloride; 3.2.2. – dexamethasone; 3.2.3. – loratadine

dexamethasone began to degrade after the first 3 hours, with the decrease of the substance concentration after about 24 h. Loratadine, showed its degradation as well as dexamethasone, after 24 h from exposure to acid medium.

In the alkaline medium (fig. 3.2.), ciprofloxacin shows considerable degradation, which begins in about 3 hours, with the formation of the precipitate. Dexamethasone and loratadine in alkaline medium, have a greater tendency of stability, the relative degradation begins only after 24 h.

Oxidative stress

Oxidative degradation of the drug substance involves an electron transfer mechanism to form reactive anions and cations [17]. Molecular oxygen is a key element that affects the physico-chemical stability of the drug system. Strong oxidizers, such as 3% hydrogen peroxide, can trigger irreversible processes in the investigated medicinal substances, which, when studied, allow to establish the nature of the toxic degradation products and the methods of prevention of these processes [15, 18, 19].

The chromatogram of the pharmaceutical form subjected to oxidative stress was recorded (fig. 4).

The results of the quantitative determination of the active principles were statistically evaluated (table 2). The standard deviation does not exceed 1% for loratadine, and is high for ciprofloxacin hydrochloride (12.173) and dexamethasone (13.254).

Following the evaluation of the oxidation results (fig. 5), it is found that a more significant degradation suffer cipro-

Table 2

The result of stability studies of ear drops following oxidation

	Test period, hours	Ciprofloxacin hydrochloride				Dexamethasone				Loratadine			
		#$t_{R, min}$	#S	#C, g	RSD	#$t_{R, min}$	#S	#C, g	RSD	#$t_{R, min}$	#S	#C, g	RSD
Oxidative degradation	**0**	1.574	29961193.83	0.29326	12.173	4.730	261125.85	0.01941	13.254	6.014	1199214.51	0.02010	0.034
	3	1.576	29959876.57	0.29325		4.704	209173.46	0.01555		5.903	1199199.41	0.02009	
	24	1.574	24058432.48	0.23549		4.704	209098.89	0.01554		5.910	1199204.84	0.02009	

Note: The average results for three series of ear drops are shown

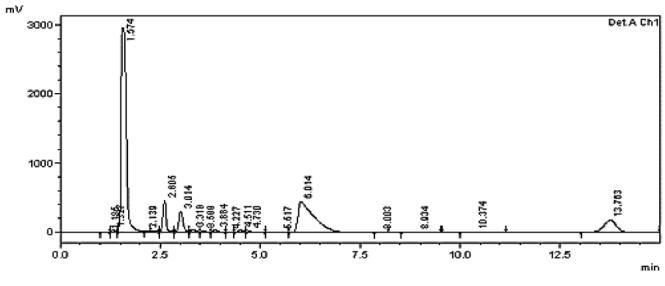

Fig. 4. Chromatogram of the sample solution subjected to oxidative degradation

floxacin and dexamethasone, starting with the first hours, and after about 24 hours it reaches its maximum point. Loratadine has been shown to be more stable to oxidants, being slightly degraded after about 3 hours.

Photolytic and thermal stress

Photostability studies are performed to generate primary degradants of drug substances by exposure to UV or fluorescent conditions. The most acceptable wavelengths are

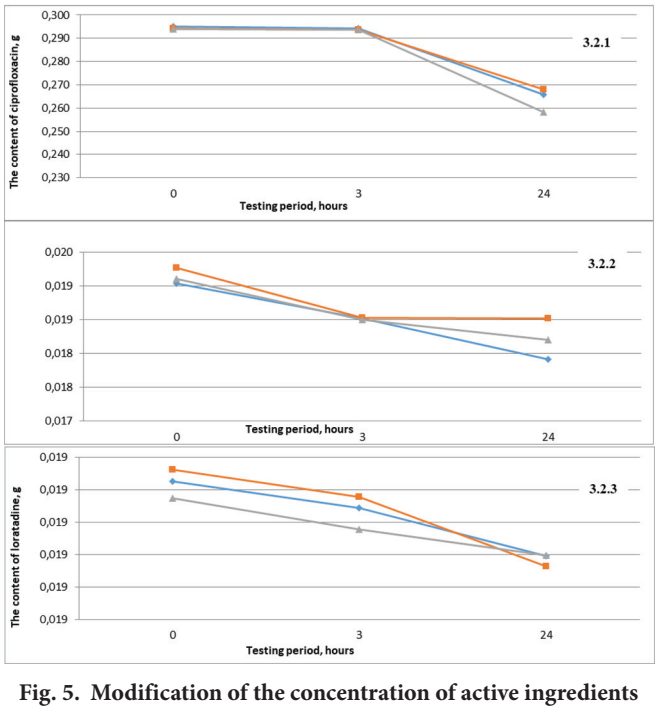

Fig. 5. Modification of the concentration of active ingredients following oxidation (for 3 series of pharmaceutical form): 5.1. – ciprofloxacine hydrochloride; 5.2. – dexamethasone; 5.3. – loratadine

usually between 300 and 800 nm to cause photolytic degradation. Mild stress conditions can induce photochemical oxidation through the mechanism of free radicals [20, 21].

Temperature changes sometimes have drastic effects on the stability of the drug. Increasing the temperature usually increases the rate of drugs hydrolysis. The effect of temperature on stability is described by Arrhenius' equation [22-25].

Chromatograms of the pharmaceutical form subjected to oxidative and thermal stress were recorded (fig. 6).

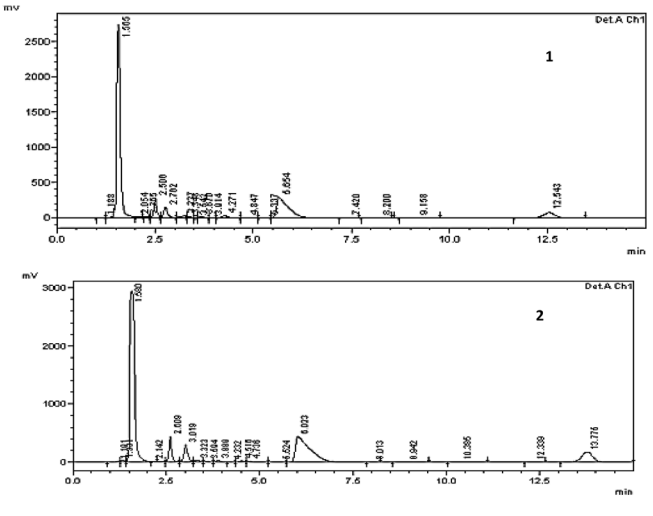

Fig. 6. Chromatograms of the sample solutions subjected to photolytic and thermal degradation: 1 – photolytic stress, 2 – thermal stress

The results of the quantitative determination of the active principles after photolytic degradation were statistically evaluated (tab.3). The standard deviation does not exceed 1% for loratadine, it is about 1.0 for dexamethasone and 10.414 for ciprofloxacin hydrochloride on photochemical degradation. In the case of heat stress, the standard deviation does not exceed 1% for ciprofloxacin hydrochloride, it is about 1.0 for dexamethasone and 3.140 for loratadine.

As can be seen from Figure 7.1, light is the decisive factor for the degradation of ciprofloxacin, the first 3 h curve being linear, and already from 3 h there is a significant decrease in concentration. Dexamethasone has a relatively slight degradation, so it is not particularly affected by the action of light. Loratadine was shown to be more stable, showing degradation after approximately 24 h.

Table 3

The result of stability studies of ear drops following exposure to light and temperature

	Test period, hours	Ciprofloxacin hydrochloride				Dexamethasone				Loratadine			
		#$t_{R, min}$	#S	#C, g	RSD	#$t_{R, min}$	#S	#C, g	RSD	#$t_{R, min}$	#S	#C, g	RSD
Photolytic degradation	0	1.674	29161193.83	0.28543	10.414	4.289	276135.67	0.02052	1.122	6.014	1196775.34	0.02010	0.289
	24	1.656	29059876.57	0.28444		4.291	272273.64	0.02023		5.903	1196473.37	0.02006	
	48	1.664	24158432.48	0.23647		4.291	270188.89	0.02007		5.910	1192954.67	0.01999	
Termal degradation	0	1.580	30299973.67	0.29658	0.036	4.124	276589.27	0.02056	1.023	5.92	1198775.74	0.0201	3.140
	24	1.588	30289876.24	0.29648		4.125	274192.38	0.02038		5.911	1192393.27	0.01999	
	48	1.588	30278432.61	0.29637		4.127	271001.37	0.02014		6.033	1131959.28	0.01898	

Note: The average results for three series of ear drops are shown

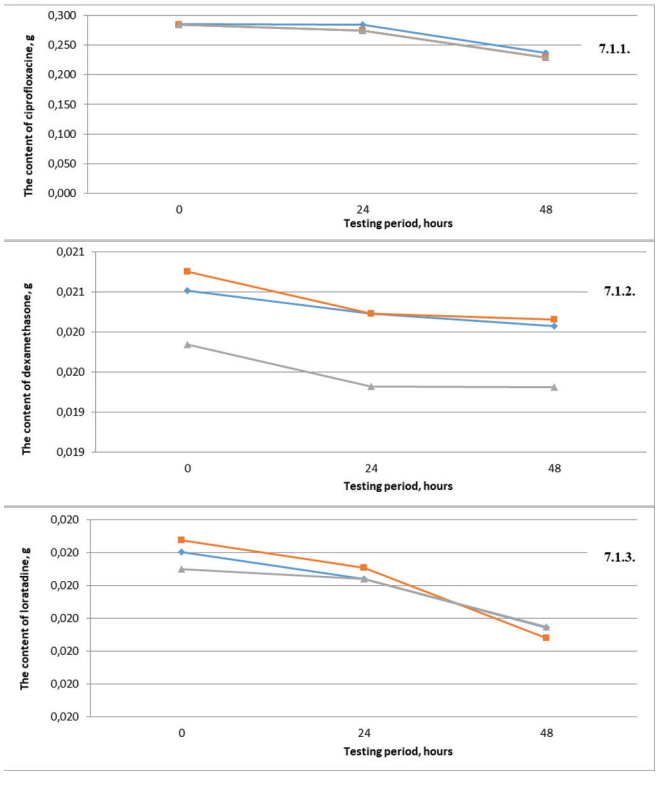

Fig. 7.1. Modification of the concentration of active principles following photolytic degradation (for 3 series of pharmaceutical form): 7.1.1. – ciprofloxacine hydrochloride; 7.1.2. – dexamethasone; 7.1.3. – loratadine

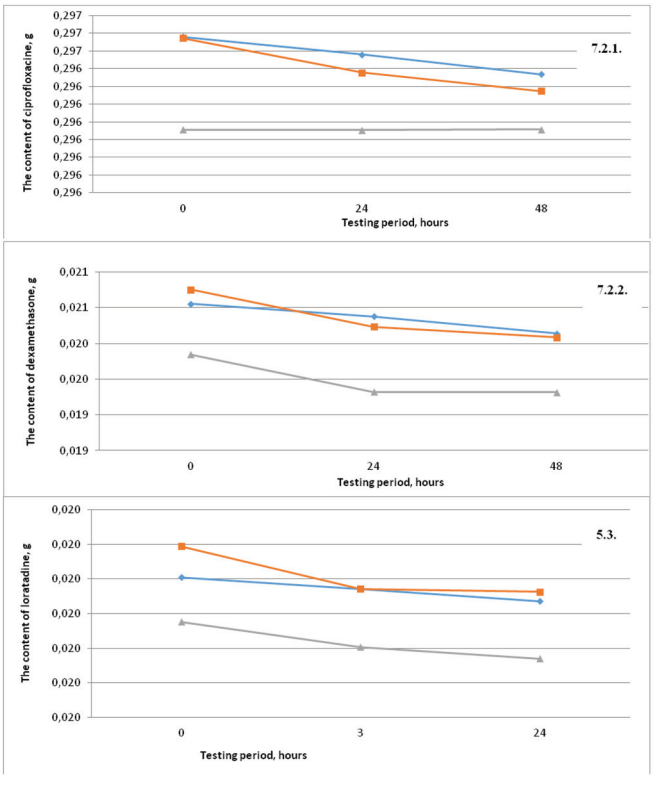

Fig. 7.2. Modification of the concentration of active principles following thermal degradation (for 3 series of pharmaceutical form): 7.2.1. – ciprofloxacine hydrochloride; 7.2.2. – dexamethasone; 7.2.3. – loratadine

Thermal stress practically does not influence ciprofloxacin and dexamethasone, which are stable during the test period (48 hours). Loratadine is temperature labile, degrading by about 5% for 48 hours of exposure to 600°C (fig. 7.2).

Real-time stability study

In the stability study process, it is very important to establish the shelf life of the product, during which the drug can be used according to medical prescriptions. The International Harmonized Guideline ICH Q1E recommends that a shelf life test period be established. For this purpose, the regression analysis of the stability data is used, the degradation being the primary factor in the analysis of duration (long term) [5].

The main quality parameter, which denotes the presence or absence of degradation processes of the multicomponent pharmaceutical form, is the medicinal product assay. Thus, for the entire storage period (24 months), the dosing of the active principles by HPLC method was performed, along with the other parameters. At the same time, the parameters were monitored: appearance, identity of active principles, viscosity, pH of the pharmaceutical form (tab. 4).

As can be seen from table 4, after storage in real-time

Table 4

The result of real-time ear drops stability studies

Periodicity of testing, months	Analyzed parameters and admissibility conditions						
	Appearance milky suspension, with a yellowish shade, with a specific smell, bitter taste	**Identity HPLC (Ret. time)** Ciprofloxacine hydrochloride – 1.53-1.57 Dexamethasone - 4.10-4.45 Loratadine - 5.40-5.43	**pH** 4.5 – 6.0	**Viscosity** 10 - 30 P 10^2	**Assay, HPLC**		
					Cipro-floxacine hydrochloride 0.29-0.31g	Dexa-methasone 0.019-0.021g	Loratadine 0.019-0.021g
Batch 1							
0	Corresponds	Corresponds	5.5	25.7	0.30012	0.02000	0.02010
3	Corresponds	Corresponds	5.5	25.8	0.30010	0.02001	0.02009
6	Corresponds	Corresponds	5.4	25.8	0.30009	0.02000	0.02000
9	Corresponds	Corresponds	5.5	25.8	0.29999	0.02000	0.01995
12	Corresponds	Corresponds	5.6	25.7	0.29996	0.01999	0.01979
18	Corresponds	Corresponds	5.7	25.9	0.29950	0.01998	0.01977
24	Corresponds	Corresponds	5.7	25.9	0.29949	0.01997	0.01976
Batch 2							
0	Corresponds	Corresponds	5.6	27.4	0.30012	0.02000	0.01958
3	Corresponds	Corresponds	5.6	27.4	0.30013	0.02001	0.01957
6	Corresponds	Corresponds	5.4	27.5	0.30010	0.02000	0.01958
9	Corresponds	Corresponds	5.5	27.5	0.29952	0.02000	0.01958
12	Corresponds	Corresponds	5.7	27.5	0.29951	0.01999	0.01957
18	Corresponds	Corresponds	5.6	27.6	0.29951	0.01987	0.01955
24	Corresponds	Corresponds	5.7	27.7	0.29951	0.01987	0.01954
Batch 3							
0	Corresponds	Corresponds	5.4	26.1	0.29949	0.01999	0.01998
3	Corresponds	Corresponds	5.4	26.2	0.29939	0.01999	0.01997
6	Corresponds	Corresponds	5.5	26.2	0.29950	0.01999	0.01999
9	Corresponds	Corresponds	5.5	26.1	0.29939	0.01999	0.01995
12	Corresponds	Corresponds	5.6	26.2	0.29921	0.01999	0.01993
18	Corresponds	Corresponds	5.6	26.2	0.29926	0.01989	0.01993
24	Corresponds	Corresponds	5.6	26.1	0.29926	0.01989	0.01992

conditions for 24 months, the drug substances underwent no significant changes regarding the concentration, pH and viscosity, the values being within the admisible limits. Storage at 25°C ± 2°C and relative humidity 60% ± 5% continues, to establish the maximum shelf life. Currently, it can be said with certainty that the pharmaceutical form *Combination ear drops containing ciprofloxacin, dexamethasone, loratadine and volatile basil oil* is valid for 2 years.

Conclusions

The stress factors degree of influence on the pharmaceutical form was established by evaluating the degradation process under temperature, acid and alkaline hydrolysis, light, oxidative stress. Ciprofloxacin is stable under acid medium and temperature action, degrades in alkaline medium after 3 hours (approximately by 10.0%), under oxidation (by 19.7%) and light action (by 17.1%).

Dexamethasone degrades in acid medium (by 7.7%) and under oxidation (by 19.9%), but it is stable in alkaline medium and under light and temperature action. Loratadine degrades in acid medium (by 3.0%) and under temperature action (by 5%), but is stable in alkaline medium, under oxidation and light action. Stability studies under stress storage conditions have allowed the selection of optimal packaging and storage conditions for the investigated pharmaceutical form: dark colored containers (protected from light), tightly closed (inaccessible to oxygen from the air and humidity), at temperatures not more than 25 °C ± 2 °C and 60% RH ± 5%. These results will be included in the analytical quality standardization documentation for the researched product.

In real-time storage conditions (it was found that the pharmaceutical form did not change its quality parameters for 24 months (by April 2020). Real-time stability studies for this product continue.

References

1. Waterman KC, Adami RC. Accelerated aging: prediction of chemical stability of pharmaceuticals. Int J Pharm. 2005;293:101-125. doi: 10.1016/j.ijpharm.2004.12.013.
2. Jamrógiewicz, M. Consequences of new approach of chemical stability tests of active pharmaceutical ingredients (APIs). Front Pharmacol. 2016;7:17. doi: 10.3389/fphar.2016.00017.
3. World Health Organization. Stability testing of active pharmaceutical ingredients and finished pharmaceutical products. WHO Technical Report Series, No. 953 [Internet]. Geneva: WHO; 2009 [cited 2020 June 12]. Available from: https://extranet.who.int/prequal/sites/default/files/documents/TRS1010_Annex10.pdf.
4. Colgan ST, Watson TJ, Whipple RD, Nosal R, Beaman JV, De Antonis DM. The application of science- and risk-based concepts to drug substance stability strategies. J Pharm Innov. 2012;7(3-4):205-13. doi:10.1007/s12247-012-9135-9.
5. European Medicines Agency. ICH Topic Q1A (R2): Stability testing of new drug substances and products [Internet]. Amsterdam: EMA; 2003 [cited 2020 Apr 12]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-1-r2-stability-testing-new-drug-substances-products-step-5_en.pdf.
6. Singh R, Rehman Z. Current trends in forced degradation study for pharmaceutical product development. J Pharm Educ Res. 2012;3(1):54-63.
7. Singh A, Singh P, Shukla D. Technical considerations of forced degradation studies of new drug substances and product: regulatory perspectives. J Drug Deliv Ther. 2018;8(2):163-168. doi: 10.22270/jddt.v8i2.1681.
8. Naveed S, Basheer S, Qamar F. Stability of a dosage form and forced degradation studies. J Bioequivalence Bioavailab. 2016;8:191-3. doi: 10.4172/jbb.1000292.
9. Singh S, Junwal M, Modhe G, Tiwari H, Kurmi M, et al. Forced degradation studies to assess the stability of drugs and products. TrAC - Trends Analyt Chem. 2013; 49:71-88. doi: 10.1016/j.trac.2013.05.006.
10. Maggio RM, Vignaduzzo SE, Kaufman TS. Practical and regulatory considerations for stability-indicating methods for the assay of bulk drugs and drug formulations. TrAC - Trends Anal Chem. 2013;49:57-70. doi: 10.1016/j.trac.2013.05.008.
11. Savkare AD, Wakhare RK. Review on the fixed dose combination drugs and their incompatibilities. Int J Pharm Sci Res. 2017;8(7):2798-2807. doi: 10.13040/IJPSR.0975-8232.8(7).2798-07.
12. Maniuc M, Valica V, Parii S, Uncu L, Ababii Polina, Nicolai E, Stefarta R, inventors. Medicinal preparation for the treatment of otitis. The Republic of Moldova patent (MD) no. 4291. 2013 Dec 27. BOPI [Off Bull Intellect Prop] (Chisinau). 2014;(7):22-23.
13. Uncu L, Donici E, Valica V, Vîslouh O, Gonciar V, Parii S. Development and validation of an assay method for ciprofloxacin hydrochloride determination in combination ear drops. Chem J Mold. 2019;14(2):56-61. doi: http://dx.doi.org/10.19261/cjm.2019.607.
14. Nicolai E, Parii S, Valica V, Uncu L. Evaluarea conținutului de impurități de etilendiaminoderivat de ciprofloxacină în studiul stabilității picăturilor auriculare combinate [Evaluation of the impurity content of ethylenediamine derivative of ciprofloxacin in the study of the stability of combined ear drops]. In: [Challenges of contemporary pharmacotherapy: National Conference of Clinical Pharmacy, 2nd edition; 2017 June 15-17; Bucharest, Romania]. p. 195-198. ISBN 978-973-0-24609-4. Romanian.
15. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs: a review. J Pharm Anal. 2014;4(3):159-165. doi: 10.1016/j.jpha.2013.09.003.
16. Wall GM, Stroman DW, Roland PS, Dohar J. Ciprofloxacin 0.3 %/dexamethasone 0.1 % sterile otic suspension for the topical treatment of ear infections: a review of the literature. Pediatr Infect Dis J. 2009;28:141-144. doi: 10.1097/inf.0b013e31818b0c9c.
17. Alsante KM, Ando A, Brown R, et al. The role of degradant profiling in active pharmaceutical ingredients and drug products. Adv Drug Deliv Rev. 2007;59(1):29-37. doi: 10.1016/j.addr.2006.10.006.
18. Sharma MK, Murugesan M. Forced degradation study an essential approach to develop stability indicating method. J Chromatogr Sep Tech. 2017;8(1):349. doi: 10.4172/2157-7064.1000349.
19. Pyka A, Babuska-Roczniak M, Bochenska P. Determination of hydrocortisone in pharmaceutical drug by TLC with densitometric detection in UV. J Liq Chromatogr Relat Technol. 2011;34(9):753-769. doi: 10.1080/10826076.2011.563891.
20. Baertschi SW, Thatcher SR. Sample presentation for photo stability studies: problems and solutions. In: Piechocki J, Thoma K, editors. Pharmaceutical photostability and stabilization technology. New York: Taylor & Francis; 2006. p. 445-450.
21. Glass BD, Novák C, Brown ME. The thermal and photostability of solid pharmaceuticals. J Therm Anal Calorim. 2004;77(3):1013. doi: 10.1023/B:JTAN.0000041677.48299.25.
22. Tollefson AE, Hermiston TW, Lichtenstein DL, Colle CF, Tripp RA, Dimitrov T, Toth K, Wells CE, Doherty PC, Wold WS. Forced degradation of Fas inhibits apoptosis in adenovirus-infected cells. Nature. 1998;392(6677):726-30. doi: 10.1038/33712.
23. Naveed S, Naseem Y, Samie S, Khan S, Ramzan S. Degradation study of five different brands of ciprofloxacin using UV visible spectrophotometer and their comparative study. Int Res J Pharm. 2014;5(3):189-190. doi: 10.7897/2230-8407.050339.
24. Aksoy B, Kucukguzel I, Rollas S. Development and validation of a stability-indicating HPLC method for determination of ciprofloxacin hydrochloride and its related compounds in film-coated tablets. Chromatographia. 2007;66:57-63. doi: 10.1365/s10337-007-0287-6.
25. Sharma EA, Shah NJ. Stability indicating RP-HPLC method for combination of pseudoephedrine sulphate and loratadine hydrochloride in pharmaceutical formulation. Int J Pharm Sci Res. 2018;9(2):599-607. doi: 10.13040/IJPSR.0975-8232.9(2).599-07.

Authors' ORCID iDs and academic degrees

Eugeniu Nicolai, PharmD, PhD Applcant – https://orcid.org/0000-0003-4666-9335.
Oxana Vislouh, PharmD, – https://orcid.org/0000-0001-7537-3758.
Vladimir Valica, PharmD, Pharm PhD, Professor – https://orcid.org/0000-0002-1068-5504.
Sergiu Parii, MD, PhD, Associate Professor – https://orcid.org/0000-0003-2229-4444.
Livia Uncu, PharmD, Pharm PhD, Associate Professor – https://orcid.org/0000-0003-3453-2243.

Authors' contribution

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

Funding

This study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The trial was the authors' initiative. The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate

No approval was required for this study.

Conflict of Interests

No competing interests were disclosed.