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RESEARCH ARTICLE

The influence of stress factors on dioxoindolinone stability

Tatiana Ştefaneţ

Department of Pharmaceutical Chemistry and Toxicology, Drug Development Center; *Nicolae Testemiţanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

ABSTRACT

Introduction. Stability studies for pharmaceutical products represent a primary stage in the development and manufacture of a new medicinal product, being a fundamental condition that guarantees its quality and efficacy. The research was initiated with the aim of the determining the stability of Dioxoindolinone under stress conditions in order to find out the factors that can induce possible changes in the molecular structure of the Dioxoindolinone, which consequently can lead to a partial or total diminution of the therapeutic effect.

Materials and methods. In the experimental research, Dioxoindolinone was used, synthesized in the Organic Synthesis Laboratory of the Institute of Chemistry, at USM, (purity 99.9%). The following apparatus was used: analytical balance (*OHAUS DV215 CD*, Switzerland); spectrophotometer (*Shimadzu UV-1800*, Japan); a pair of quartz cuvettes with a layer thickness of 10 mm; ultraviolet lamp chamber (UV with CN-6 filter, France) for exposure to 254 nm and 365 nm radiation; thermostat (*TC-80M-2*, Ukraine) set at 60 ± 1°C; ultrasonic bath (*Sapfir*, St. Petersburg); reagents: analytical grade reagents – 0.1 M hydrochloric acid (HCl) (ChemLab, Belgium); 0.1 M sodium hydroxide (NaOH) (ChemLab, Belgium); 3% hydrogen peroxide solution (H_2O_2) (CentroChem, Poland); ethanol (96%) (CentroChem, Poland).

Results. The influence of stress factors, such as oxidants, acids, bases, humidity, high temperatures and UV irradiation on the stability of Dioxoindolinone was studied. Under conditions of oxidative, hydrolytic, thermal, acid-base, photolytic stress by the UV-Vis spectrophotometric method it was determined that the substance is stable to humidity and in the acidic environment. Dioxoindolinone degrades under the influence of oxidant, it was found to be unstable in the basic environment (a change in concentration was observed). The insignificant influence of UV light and high temperature was demonstrated.

Conclusions. The influence of stress factors on the stability of Dioxoindolinone was studied. The results obtained will be used to establish optimal storage conditions that will be introduced in the Quality Standardization Specification for Dioxoindolinone.

Keywords: stability, Dioxoindolinone, forced degradation.

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***Corresponding author: Tatiana *Ştefaneţ,* assistant professor
Department of Pharmaceutical Chemistry and Toxicology, Drug Development Center
Nicolae Testemiţanu State University of Medicine and Pharmacy
165 Ştefan cel Mare şi Sfânt blvd., Chişinău, Republic of Moldova, MD-2004
e-mail: tatiana.stefanet@usmf.md

Author's ORCID ID
Tatiana Ştefaneţ – https://orcid.org/0000-0003-2060-8382

Key messages

What is not yet known about the issue addressed in the submitted manuscript

Dioxoindolinone has significant pharmacological potential as an MAO inhibitor. This substance is being investigated experimentally in order to develop the Quality Specification. To date, no stability study of Dioxoindolinone has been performed.

The research hypothesis

The stability of medicinal substances is an important and mandatory factor in determining the quality of the medicine, and for establishing the optimal storage conditions that will be included in the Quality Specification for Dioxoindolinone.

The novelty added by the manuscript to the already published scientific literature

For the first time, a study of the stability of Dioxoindolinone to the action of stress factors was carried out.

Introduction

Neurodegenerative and mental disorders, such as Parkinson's disease and major depressive disorder, present a considerable health issue, affecting hundreds of millions globally [1]. Monoamine oxidase (MAO) inhibitors remain important therapeutic agents in the treatment of these diseases [2]. The development of new drugs with improved safety and stability characteristics is thus of notable pharmaceutical interest. At the Organic Synthesis Laboratory, Institute of Chemistry, Moldova State University, a newly synthesized compound Dioxoindolinone (1-(2-oxo-propyl)-spiro[[1,3]dioxolane-2,3'-indolin]-2'-one) was obtained [3]. The substance has demonstrated significant pharmacological potential as an MAO inhibitor [4]. Therefore, establishing its stability profile represents a critical step for further pharmaceutical development and rational dosage form design.

In addition to efficacy, purity, and safety, stability is an important factor in ensuring the quality of a drug [5].

The quality, therapeutic efficacy and safety of the drug during storage directly depend on its ability to maintain its properties within the limits established by regulatory/normative documentation over a certain period of time under appropriate storage and transportation conditions, i.e., stability. Based on the results of the stability study, the shelf life is determined, the materials used for primary and secondary packaging and its type are selected, and the storage conditions of the drug are determined, which are indicated in regulatory/normative documents [6]. The main method for establishing and confirming the shelf life is considered to be real-time testing, which is carried out under conditions as close as possible to the expected storage conditions of commercial products. To determine the stability of new drug substances, forced degradation methods are usually applied. The main objective of these studies is to find out some fundamental properties of the substances, such as the nature and direction of degradation reactions, the identification (based on the data obtained) of the most important degraded products and the selection of the most appropriate analytical techniques to determine the active substance and its degradation products in the presence of each other. At the same time, the results of these tests can highlight how not only the substances but also, sometimes, the pharmaceutical forms, can cope with short-term, but extremely critical conditions, such as those during transportation [7-9].

However, data concerning the stability and degradation behavior of newly synthesized Dioxoindolinone are presently missing. The absence of such information constrains further pharmaceutical development and formulations studies.

The objective of the present study was to investigate the stability profile of Dioxoindolinone under stress conditions (acidic, alkaline, thermal, oxidative, humid and photolytic exposure) to identify possible degradation pathways and degradation products, and to formulate a stability-indicating analytical procedure appropriate for future quality monitoring of the substance.

Material and methods

The study was conducted at the Drug Development Center within *Nicolae Testemițanu* State University of Medicine and Pharmacy. The stability evaluation of Dioxoindolinone was performed in accordance with ICH regulations and guidelines [10, 11].

For the stability studies under stress conditions, three experimental synthesis batches (01, 02, 03) of Dioxoindolinone, obtained at the Organic Synthesis Laboratory, Institute of Chemistry, Moldova State University, were used. An internal reference standard of 1-(2-oxo-propyl)-spiro[[1,3] dioxolane-2,3'-indolin]-2'-one-substance, purified by recrystallization with a purity of 99.98%, was also employed.

The following apparatus was used: analytical balance (*OHAUS DV215 CD*, Switzerland); spectrophotometer (*Shimadzu UV-1800*, Japan) and 10 mm quartz cuvettes; ultraviolet lamp chamber (UV with CN-6 filter, France) for exposure to 254 nm and 365 nm radiation; thermostat (*TC-80M-2*, Ukraine) set at 60 ± 1°C; ultrasonic bath (*Sapfir*, St. Petersburg). The following analytical grade reagents were used: 0.1 M hydrochloric acid (HCl) (ChemLab, Belgium); 0.1 M sodium hydroxide (NaOH) (ChemLab, Belgium); 3% hydrogen peroxide solution (H_2O_2) (CentroChem, Poland); ethanol (96%) (CentroChem, Poland).

To assess the stability of Dioxoindolinone, the spectrophotometric method, previously developed and validated [12], was used.

Preparation of the standard solution: 0.05 g Dioxoindolinone internal standard (exact mass) was dissolved in 20 ml of ethanol (96%) in an ultrasonic bath for 1 min and diluted to 50 ml with the same solvent in a volumetric flask. 1.5 ml of this solution was further diluted to 50 ml in a volumetric flask with the same solvent, and absorbance was measured at 257 ± 1 nm, using ethanol (96%) as the reference solution.

Preparation of the sample solution. 0.05 g of the Dioxoindolinone substance (exact mass) was dissolved in 20 ml of ethanol (96%) in an ultrasonic bath for 1 min and diluted to 50 ml with the same solvent in a volumetric flask (solution A).

The study of forced degradation.

The sample solution was subjected to oxidative degradation and hydrolysis in acidic and basic environments for 24 hours, with three UV-Vis spectrophotometric analyses: at 0, 3 and 24 hours. The absorbances of the test solutions were measured at 257 ± 1 nm, using ethanol (96%) as the reference solution. The concentration was calculated in relation to the absorbance of the standard solution.

Preparation of the sample solution for oxidative degradation (acid hydrolysis, basic hydrolysis). 1 ml of solution A was transferred into three stoppered test tubes, to which 1 ml of 3% hydrogen peroxide solution (1 ml 0.1 M hydrochloric acid; 1 ml 0.1 M sodium hydroxide) was added, respectively. From each test tube, 0.75 ml of the treated solution was diluted to 25 ml in a volumetric flask with ethanol (96%), and absorbance was measured at 257 ± 1 nm, using ethanol (96%) as the reference solution. The first test tube was analyzed immediately; the second test tube after 3 hours and the third one after 24 hours.

To test the stability to physical stress factors, the substance samples were exposed to high humidity (80% RH) by keeping them for 2 weeks in a desiccator above water. Other samples were exposed to ultraviolet radiation (in the UV chamber) for 48 hours and to high temperature (60 °C in the thermostat for 2 weeks). Subsequently, quantitative analysis was performed by the UV-Vis spectrophotometric method to evaluate the variations in the stability of Dioxoindolinone under the action of these stress factors [13].

Statistical analysis. Statistical analysis was performed 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 Dioxoindolinone were followed. For the assay, the absorption spectra of the standard solution and of the sample solutions were recorded before the application of the stress conditions (Fig. 1).

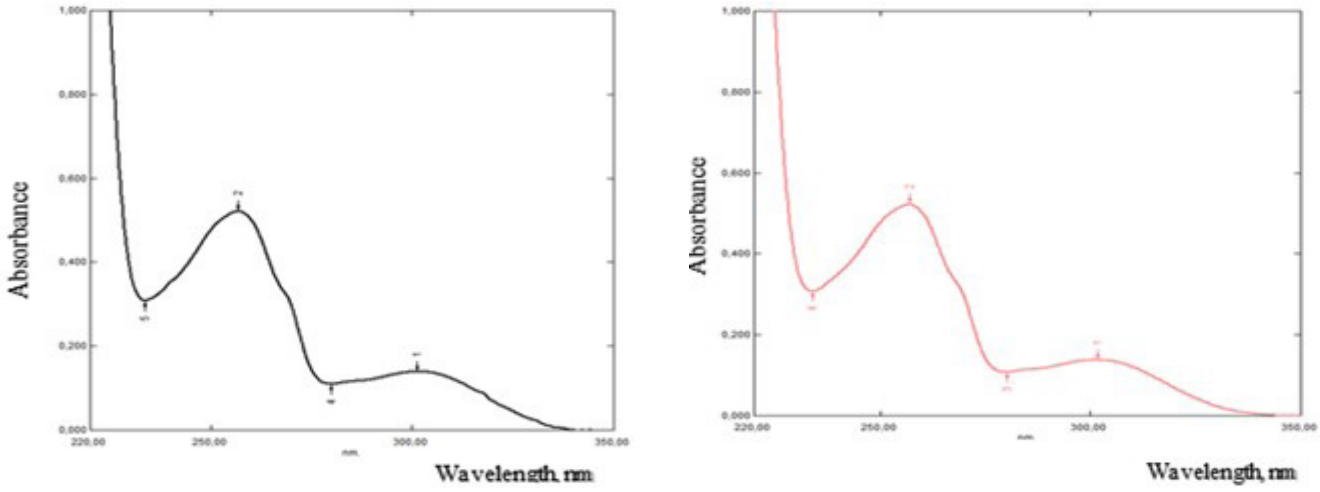

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Fig. 1 Ultraviolet absorption spectra of Dioxoindolinone standard solution (1) and Dioxoindolinone sample (2)

The samples of the substance to be analyzed were subjected to oxidative stress (3% hydrogen peroxide solution), acidic hydrolytic stress (0.1 M hydrochloric acid) and basic hydrolytic stress (0.1 M sodium hydroxide); thermal (60 °C), photolytic (UV light), the substance was investigated under the influence of humidity. The evaluation of the degree of influence of stress factors on Dioxoindolinone was carried out at time intervals: 0 minutes, 3 hours and 24 hours following exposure to the above-mentioned factors (when investigating oxidative, acidic and basic stress); 24 hours and 48 hours (photolytic stress in the UV chamber) or at time intervals: 1 week and 2 weeks (when investigating the influence of humidity and thermal stress). All experiments were conducted in triplicate, and data were expressed as the mean.

Degradation under stress conditions

Oxidative stress

Oxidative degradation can be initiated by three factors: UV light, heat or physical strain in the presence of an oxygen-containing atmosphere, and may occur via two mechanisms: photo-oxidation and thermal oxidation. The selection of an oxidizing agent, its concentration and conditions depend on the drug substance, the most frequently used standard agent is hydrogen peroxide [9].

Under the action of the oxidant (3% hydrogen peroxide solution), complete degradation of Dioxoindolinone was observed, with a change in the absorption spectrum, which made it impossible to determine the absorbance and content (Fig. 2).

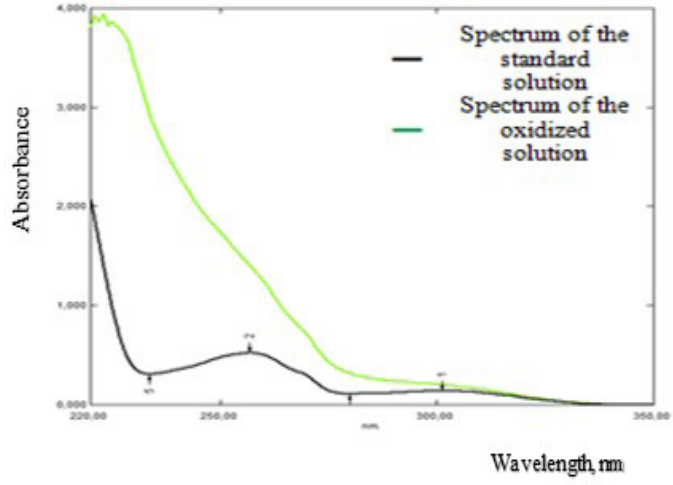

Fig. 2 Modification in the absorbtion spectrum of Dioxoindolinone following oxidative stress

Note: *oxidant – 3% hydrogen peroxide solution,*

Hydrolytic stress

Hydrolysis is one of the most prevalent chemical degradation reactions, which includes the splitting of a chemical compound by reaction with water. The ionizable functional groups present in the molecule are catalyzed during hy-

drolytic investigation in acidic and alkaline environment. Acid and base stress testing involve the forced degradation of drug substances by exposure to acidic and basic environments that produce primary degradants in the desired range. The choice of the type and strength of the acid or base depends on how stable the drug substance is. As suitable hydrolysis agents, hydrochloric acid or sulfuric acid for acid hydrolysis and sodium hydroxide or potassium hydroxide for alkaline hydrolysis are proposed [5, 6, 8, 14].

To highlight the influence of acids on Dioxoindolinone, 0.1 M hydrochloric acid was used, and for creation of the basic medium – 0.1 M sodium hydroxide. Absorption spectra of stressed solutions in acidic and basic media were recorded.

Acidic hydrolytic stress caused insignificant degradation of Dioxoindolinone, by 2.45% at 3 hours and 2.04% at 24 hours (Fig. 3).

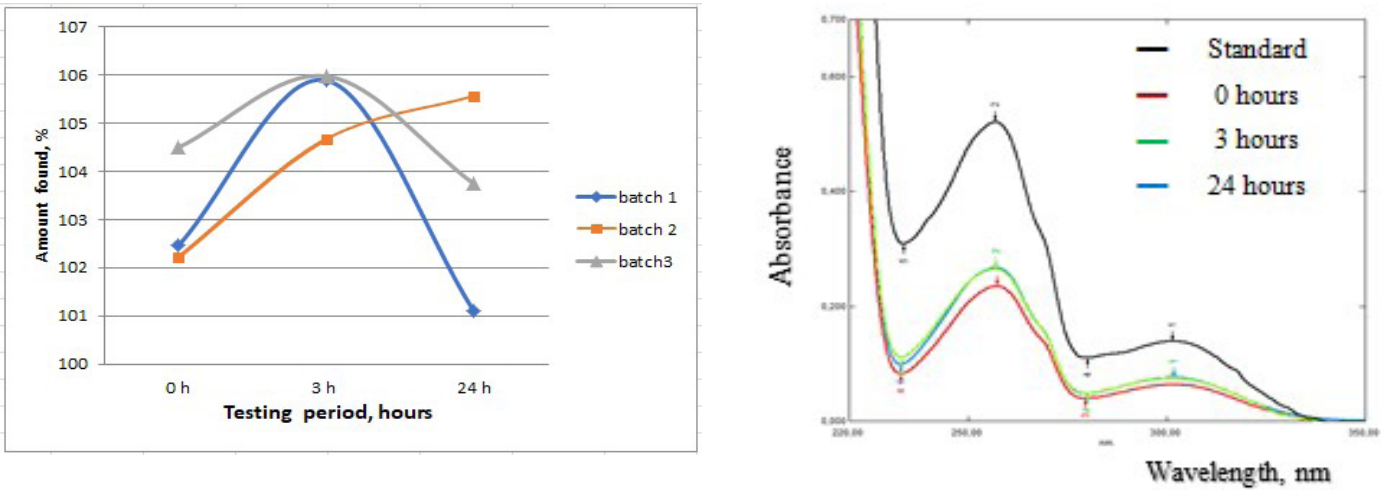

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Fig. 3. Modification of the concentration of Dioxoindolinone following acid hydrolysis (1) and the absorption spectra of Dioxoindolinone under the action of acid hydrolytic stress (2)

Note: *The average results for three batches of substance are shown*

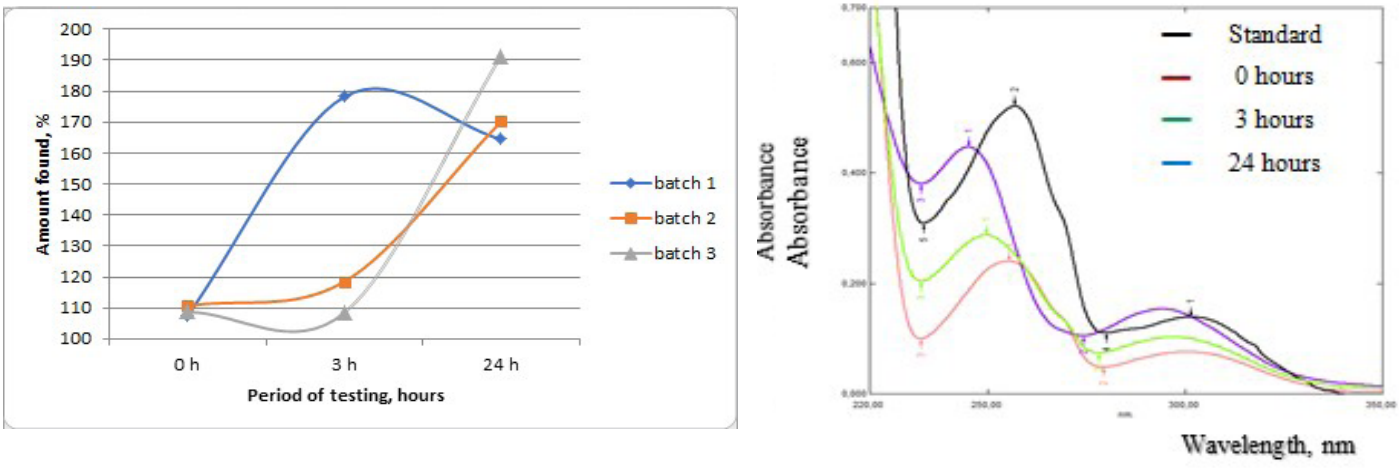

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Fig. 4. Modification of the concentration of Dioxoindolinone following basic hydrolysis (1) and the absorption spectra of Dioxoindolinone under the action of basic hydrolytic stress (2)

Note: *The average results for three batches of substance are shown*

The results obtained from basic hydrolysis indicate degradation processes with the shift of the absorption maximum from 258 nm to 255 nm (0 min), to 250 nm (3 hours) and to 243 nm (24 hours), and the increase in measured concentration due to the formation of degradation products (0 min – 108.99%, 3 hours – 135.04%, 24 hours – 175.33%) (Fig. 4.).

Influence of humidity, photolytic, and thermal stress

Moisture, as an atmospheric factor, creates favorable conditions for oxidation processes, hydrolysis, as well as for microbial growth. At the same time, light and temperature represent activating factors of drug degradation reactions; an increase in temperature leads to an increase in the rate

of the degradation reaction. Therefore, these factors must be studied in the process of analyzing of the substance [8, 9, 14, 15].

Dioxoindolinone exhibited a high degree of stability under humid conditions (in a desiccator above water for 2 weeks). The results obtained indicate that Dioxoindolinone is not hygroscopic and does not undergo degradation, the quantitative content being practically unchanged (0 min – 99.87%, 1 week – 99.51%, 2 weeks – 99.71%) (Fig. 5.).

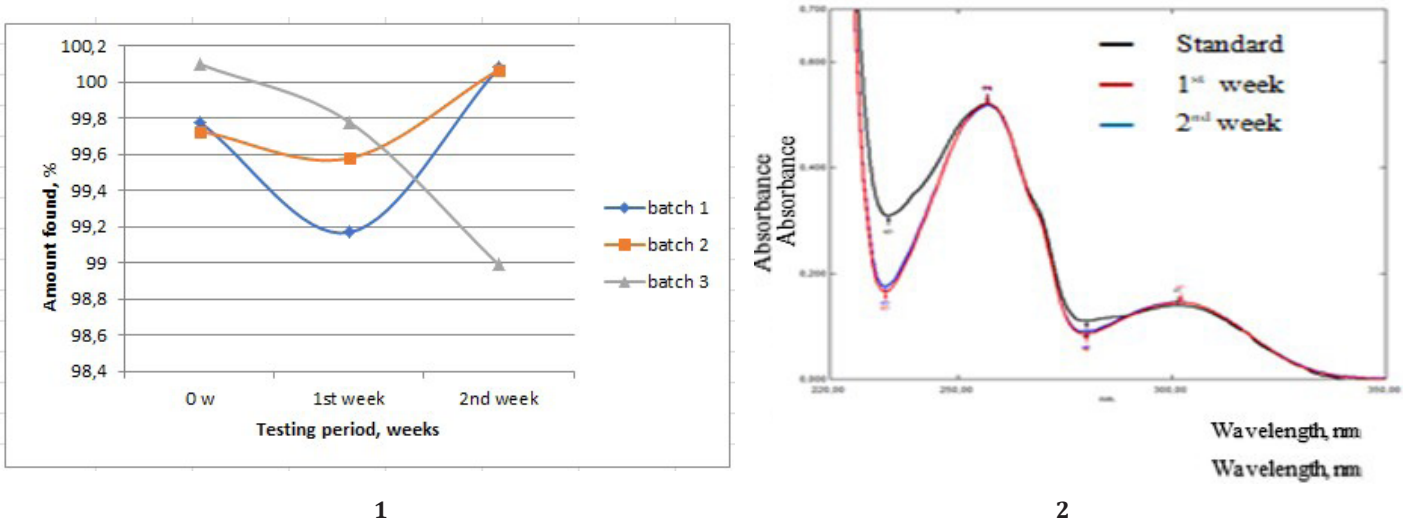

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Fig. 5. Modification of the concentration of Dioxoindolinone following influence of humidity (1) and the absorption spectra of Dioxoindolinone under the influence of humidity (2)/

Note: *The average results for three batches of substance are shown*

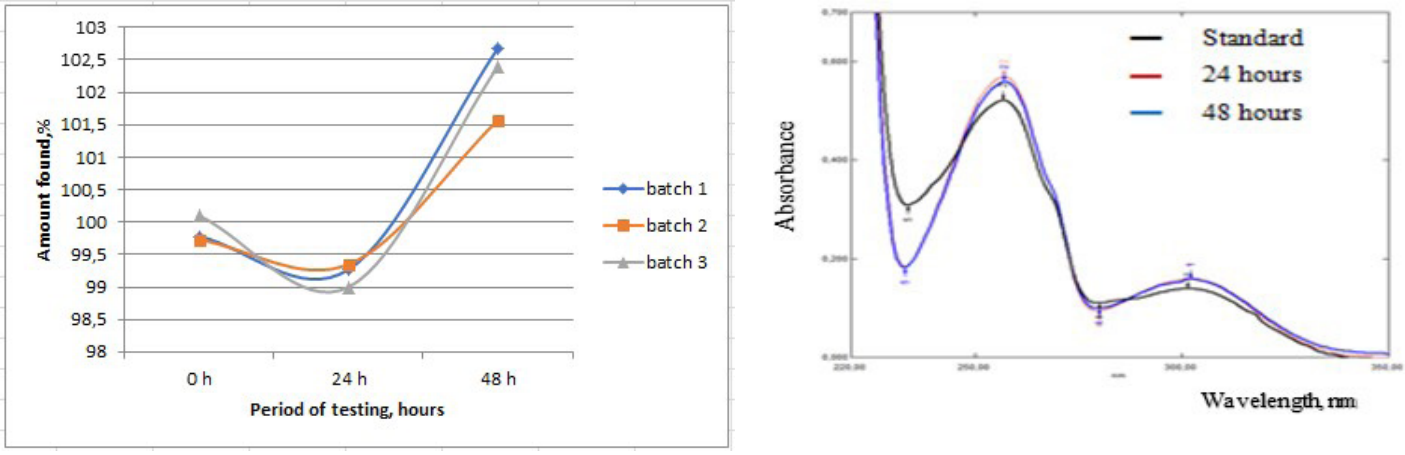

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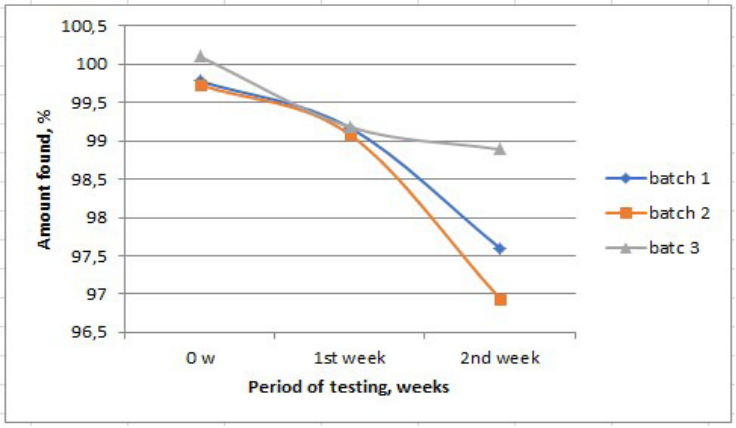
Fig. 6. Modification of the concentration of Dioxoindolinone following photolytic stress (1) and the absorption spectra of Dioxoindolinone under the action of photolytic stress (2)

Note: *The average results for three batches of substance are shown*

Dioxoindolinone was evaluated over 0-48 hours of photolytic stress. The results of the determinations are shown in Figure 6. Upon interaction with ultraviolet radiation, a change in the content of Dioxoindolinone of 2.23% was observed (0 min – 99.87%, 24 hours – 99.21%, 48 hours – 102.1%).

The influence of high temperature on Dioxoindolinone was evaluated. The substance placed in an open container was stored at high temperature (60 ^{0}C) in the thermostat for 2 weeks.

Samples subjected to thermal stress of 60 ^{o}C showed a change of 2.06% (0 min – 99.87%, 1 week – 99.14%, 2 weeks – 97.81%) (Fig. 7.).



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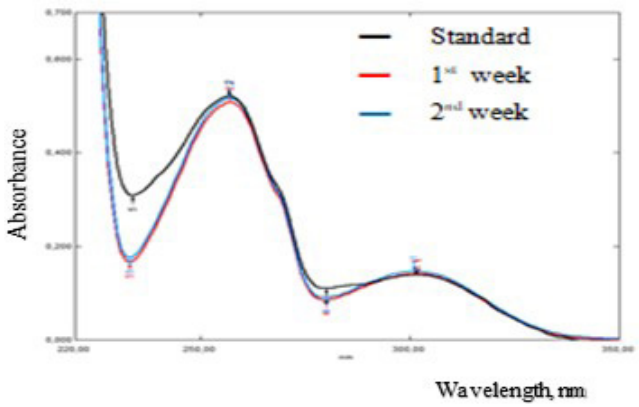

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Fig. 7. Modification of the concentration of Dioxoindolinone following thermal stress (1) and the absorption spectra of Dioxoindolinone under the action of thermal stress (2)

Note: *The average results for three batches of substance are shown*

The most important changes in the samples subjected to forced degradation occurred under the action of oxidants and in a basic environment. Under the influence of the oxidizing agent the substance degraded, which was demonstrated by the complete change in the absorption spectrum, which made it impossible to determine the content of Dioxoindolinone in the sample. In a basic environment, Dioxoindolinone decomposed, and the degradation products increased the absorbance, causing concentration oscillations. In an acidic environment, the substance was stable, with concentration varying within 2%.

The results of the thermal stability evaluation indicate that Dioxoindolinone is stable under thermal stress, with concentration within 1.57%.

Although light is considered to be a destructive factor for medicinal substances, Dioxoindolinone demonstrated relative stability to UV irradiation, with a concentration increase of 2.9%.

The results of the evaluation of stability to humidity demonstrated that Dioxoindolinone is stable under high humidity, with no significant change in concentration observed.

The main quality parameter denoting the presence or absence of degradation processes in medicinal substances is Assay. In Table 1, the results of sample assay using the UV-Vis spectrophotometric method under various stress factors are presented.

Table 1. Modification of Dioxoindolinone concentrations under the action of stress factors

	Testing frequency	λ_{max}, nm	Batch 1		Batch 2		Batch 3	
			Absorbance	Amount found, %	Absorbance	Amount found, %	Absorbance	Amount found, %
H_2O_2 3 %	-	-	-	-	-	-	-	-
0.1 M HCl	0 hours	251.1	0.299	102.46	0.323	102.22	0.235	102.34
	3 hours	256.6	0.309	105.89	0.298	104.67	0.265	104.50
	24 hours	256.8	0.295	106.09	0.286	105.57	0.268	105.74
0.1 M NaOH	0 hours	256.2	0.314	107.61	0.303	110.7	0.443	108.68
	3 hours	246.3	0.521	178.2	0.321	118.49	0.441	108.43
	24 hours	241.6	0.480	164.49	0.461	170.16	0.781	191.36
t = 60 ˚C	0 hours	257	0.541	99.78	0.531	99.73	0.517	100.10
	1 week	257	0.502	99.17	0.504	99.08	0.503	99.18
	2 weeks	257	0.504	97.6	0.507	96.94	0.516	98.89
UV	0 hours	257	0.541	99.78	0.530	99.73	0.517	100.10
	24 hours	257	0.570	99.27	0.554	99.35	0.570	99.00
	48 hours	257	0.560	102.68	0.546	101.57	0.566	102.04
Moisture	0 hours	257	0.540	99.78	0.531	99.73	0.517	100.10
	1 week	257	0.522	99.17	0.524	99.58	0.524	99.78
	2 weeks	257	0.518	100.08	0.516	100.07	0.516	98.99

Note: λ_{max} *– the wavelength corresponding to maximum absorbance, t - temperature, UV - Ultra-violet,* H_2O_2 *– peroxide of hydrogen; HCl – hydrochloric acid; NaOH – sodium hydroxide;*
The average results for three batches of substance are shown

Conclusions

Forced degradation is an analytical method used to test a drug under more extreme conditions than those encountered in accelerated stability studies.

Under conditions of oxidative, hydrolytic, thermal, acid-base, photolytic stress, the UV-Vis spectrophotometric method demonstrated that the substance is stable under humid conditions humidity and in an acidic environment. Dioxoindolinone was found to degrade under the influence of oxidant, it was unstable in basic environment (a change in concentration was observed). The insignificant influence of UV light and high temperature was demonstrated.

The results obtained will be confirmed by real-time stability studies. Currently, the substance is stored under normal conditions (25°C; 65% RH) for 4 years and 10 months. So far, the drug substance meets all the quality criteria stipulated in the draft specification.

Competing interests

None declared.

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Ethics approval

No approval was required for this study.

Provenance and peer review

Not commissioned, externally peer-reviewed.

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