

**STUDY OF STABILITY AND ESTABLISHMENT OF THE LIFE DURATION OF  
BIOLOGICALLY ACTIVE CONNECTION OF GLIGISCINS****\*A. D. Tashpulatova and A. N. Yunuskhodjaev**

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**ABSTRACT**

The results of experimental studies on the stability and the expiration dates of the biologically active compound Gligiscin with hepatoprotective and lipid-lowering properties are presented. The expiry date is after 3 years.

**KEYWORDS:** Stability, expiry date, shelf life, packaging, storage conditions, biologically active compound Gligiscin.

**INTRODUCTION**

Providing the population with effective and safe medicines is one of the main tasks of the public health of the Republic of Uzbekistan. Therefore, the national drug policy of the development of the pharmaceutical and medical industry is aimed that reducing the dependence of Uzbekistan's health care on the import of medicines through the most complete use of its own production facilities, raw materials and scientific and technical potential. Currently, given the rich raw material resources of Uzbekistan, the main efforts are focused on the production of substances and finished dosage forms from raw materials of plant origin.

Licorice, among medicinal plants at the present time, occupies a leading place in the number of proposed and used drugs. Licorice root preparations are used in medicine for the treatment of a number of autoimmune, allergic, immunodeficient, viral, tumor, inflammatory, infectious and other diseases.<sup>[1]</sup> High biological activity of licorice root is mainly due to the presence of triterpene glycoside – glycyrrhizic acid (GA).

It should be noted that although the medicinal plant raw materials do not show high toxicity, but, as a rule, in most cases they are not distinguished by high biological activity. In this regard, research aimed at a comprehensive study of the active components of licorice root and the products of their chemical modifications with the aim of creating new highly effective drugs is becoming more important.

Taking this into account, as well as to create a new hepatoprotective drug based on local plant materials, in particular glycyrrhizic acid, we synthesized a new coordination compound of zinc with glycyrrhizic acid

and histidine, which has hepatoprotective and hypolipidemic properties and tentatively called Gligiscin.<sup>[2,3]</sup>

In the course of the research, we studied the physicochemical properties, composition, structure, quality control and standardization of the obtained compound.

The study of a new biologically active compound as a potential drug in addition to the development of effective methods for assessing quality at the preclinical stage includes the establishment of stability and shelf life. The study of the above regulatory requirements is necessary to establish the time during which the substance remains unchanged physical, chemical, biological properties, that is, it meets all the requirements of regulatory documentation. Justification of the established shelf life of the substance is included in the section of the registration dossier on the methods of quality assessment.<sup>[4,5]</sup>

**Purpose of the study:** The purpose of this study is to establish the shelf life and stability of the biologically active compound Gligiscin.

**MATERIALS AND METHODS**

As test objects, 5 experimental batches of laboratory samples of the substance Gligiscin were used. Solvents, reagents and consumables produced by MERCK (Germany), as well as prepared nutrient media from HIMEDIA Laboratories Pvt. Ltd (India). The following measuring and auxiliary equipment was also used in the tests: Atomic-Absorption spectrometr (AAS) model 3030B, (manufactured by Perkin-Elmer, USA); spectrophotometer UV-spectrophotometr 8453 (by Agilent Technologies, Germany); device for

determining the melting point ("Electrothermal" IA 9100, Germany); IR spectrophotometer Protege 460 (manufactured by Nicolet Instruments Corporation, USA); magnetic mixers, electronic analytical scales BP-310S (manufactured by Sartorius Germany), moisture meter MV 35 Halogen OHAUS R (Switzerland), air sterilizer HS 32 AC with automation, pH meters Seven Easy (manufactured by Mettler Toledo, Switzerland) and 766 Calimatic Knisk (Germany).

## RESULTS AND DISCUSSION

The following qualitative and quantitative properties were determined: description, solubility, authenticity, melting point, impurities, sulfate ash and heavy metals, weight loss on drying, microbiological purity, quantitative determination.

The studies used the techniques given in the GF XI and in the draft regulatory document on the study drug. The results of the study of the quality indicators of the biologically active compound Gligiscin are presented in table 1.

**Table 1: The results of the study of the quality indicators of the biologically active compound Gligiscin.**

Description	Method of analyses	Norm
Description	Visually	Amorphous powder from light yellow to yellow.
Solubility	Visually GF XI, issue 1, p.175.	It is very soluble in water, insoluble in alcohol, acetone and ether, soluble in alkali solutions.
Authenticity	According to ND: Qualitative reactions for zinc (GF XI, issue 1, p. 165), glycyrrhizic acid, histidine.  IR spectroscopy  Spectrophotometry	1. Zinc - white precipitate; 2. Glycyrrhizic acid - violet color; 3. Histidine - blue-violet staining; The infrared absorption spectrum of the drug, obtained in the form of a disk with potassium bromide, in the range from 4000 to 400 cm-1 should have complete coincidence of the absorption bands with the absorption bands of the attached spectrum. Glycyrrhizic acid (SF) - absorption maximum at 251 + 2nm; Histidine - absorption maximum at 568 + 2nm.
Melting temperature	According to ND	The drug decomposes at temperatures above 235°C.
Impurities	Thin layer chromatography (free glycyrrhizic acid, histidine).	Glycyrrhizic acid - Less than 0.5%; Histidine - Less than 0.5%.
Sulphated ash and heavy metals	Visually GF XI, issue 1, p.172.	Must withstand the requirements of the GF XI, issue 1, p.172.
Loss on drying	Gravimetric method.	Less than 10.5%
Microbiological purity	European Pharmacopoeia (9th edition, 2016)	The drug must withstand the requirements of the European Pharmacopoeia.
Quantitation	1. Zinc content - complexometric titration; 2. The content of glycyrrhizic acid - spectrophotometric method in the UV region of the absorption spectrum; 3. Histidine content - spectrophotometric method in the visible region of the absorption spectrum.	1. The zinc content in the preparation should be from 4.45% to 5.45%; 2. The content of glycyrrhizic acid in the preparation should be from 59.10% to 62.30%; 3. The content of histidine in the preparation should be from 22.3% to 24.3%.

To establish the ability of the substance Gligiscina maintain their properties during the storage period, experiments to assess the stability were conducted. The stability of a substance depends, on the one hand, on environmental factors, such as temperature, humidity and lighting, and, on the other hand, on the physicochemical properties directly related to the preparation itself. Those in the Gligiscin compound include the possibility of the presence of certain impurities, the particle size, the water content and its composition.

Considering that for the registration of a substance, reports of stability assessment under real-time conditions are necessary, the experiments were conducted by the method of natural storage at a temperature of 20+ 20C in a place protected from light. The relative humidity during the test was fixed at 65 + 5%.

When conducting research, samples of the drug were analyzed at regular intervals with an interval of 6 months according to the following indicators: description, solubility, authenticity, weight loss during

drying, melting point, quantitative determination of the constituent components (using the developed SF, FEC and complexometric titration methods), Microbiological purity.

At the same time, stability studies were carried out comparatively and by the method of "accelerated aging" at a temperature of 400°C, a storage period of 10 months and similarly analyzed at regular intervals (45 days) using the above parameters.

When working, they were guided by the Temporary Instruction on the work to determine the shelf life of medicines based on the method of "accelerated aging" at elevated temperatures (I-42-2-82).

The samples were stored in dark glass glass containers with screw-on plastic caps.

The report on the results of the stability assessment tests is presented in the form of tables 2, 3.

Table 2: The results of determining the stability of the compound Gligiscin by the method of natural storage at a temperature of 200 °C.

Number of series	Description	Solubility	Authenticity	Melting temperature	Impurities	Sulphated ash and heavy metals	Loss on drying	Microbiological purity	quantitation			
									Zinc	Glycyrrhizinic acid	Histidine	
0 month												
№1	Complies	Complies	Confirmed	235,4	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	60,5%	23,5%	
№2	Complies	Complies	Confirmed	235,8	Less than 0,5%	Endures	Less than 10,5%	Complies	5,1%	61,2%	23,4%	
№3	Complies	Complies	Confirmed	235,6	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	61,5%	23,8%	
№4	Complies	Complies	Confirmed	235,7	Less than 0,5%	Endures	Less than 10,5%	Complies	5,4%	60,8%	23,6%	
№5	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,4%	61,8%	24,0%	
6 months												
№1	Complies	Complies	Confirmed	235,4	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	60,5%	23,5%	
№2	Complies	Complies	Confirmed	235,7	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	61,2%	23,4%	
№3	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	61,5%	23,8%	
№4	Complies	Complies	Confirmed	235,6	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	60,3%	23,6%	
№5	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,4%	61,7%	24,1%	
12 months												
№1	Complies	Complies	Confirmed	235,4	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	60,5%	23,5%	
№2	Complies	Complies	Confirmed	235,7	Less than 0,5%	Endures	Less than 10,5%	Complies	5,1%	61,2%	23,4%	
№3	Complies	Complies	Confirmed	235,7	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	61,5%	23,8%	
№4	Complies	Complies	Confirmed	235,7	Less than 0,5%	Endures	Less than 10,5%	Complies	5,4%	60,7%	23,5%	
№5	Complies	Complies	Confirmed	235,4	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	61,6%	24,0%	
18 months												
№1	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	60,5%	23,4%	
№2	Complies	Complies	Confirmed	235,6	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	61,0%	23,4%	
№3	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	61,5%	23,5%	
№4	Complies	Complies	Confirmed	235,6	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	60,8%	23,4%	
№5	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,4%	61,9%	24,0%	
24 months												
№1	Complies	Complies	Confirmed	235,6	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	60,4%	23,4%	
№2	Complies	Complies	Confirmed	235,6	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	61,2%	23,4%	
№3	Complies	Complies	Confirmed	235,6	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	61,4%	23,5%	
№4	Complies	Complies	Confirmed	235,4	Less than 0,5%	Endures	Less than 10,5%	Complies	5,4%	60,8%	23,5%	
№5	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	61,5%	24,0%	
30 months												
№1	Complies	Complies	Confirmed	235,4	Less than 0,5%	Endures	Less than 10,5%	Complies	5,0%	60,5%	23,4%	
№2	Complies	Complies	Confirmed	235,7	Less than 0,5%	Endures	Less than 10,5%	Complies	5,1%	61,2%	23,4%	
№3	Complies	Complies	Confirmed	235,4	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	61,4%	23,5%	
№4	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	60,7%	23,2%	
№5	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	61,5%	24,0%	
36 months												
№1	Complies	Complies	Confirmed	235,3	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	60,4%	23,4%	
№2	Complies	Complies	Confirmed	235,6	Less than 0,5%	Endures	Less than 10,5%	Complies	5,0%	61,2%	23,4%	
№3	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	61,4%	23,4%	
№4	Complies	Complies	Confirmed	235,4	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	60,8%	23,4%	
№5	Complies	Complies	Confirmed	235,4	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	61,6%	24,0%	

Table 3: The results of determining the stability of the compound Gligiscin by the method of "accelerated aging" at a temperature of 400 ° C.

Number of series	Description	Solubility	Authenticity	Melting temperature	Impurities	Sulphated ash and heavy metals	Loss on drying	Microbiological purity	Quantitation		
									Zinc	Glycyrrhizic acid	Histidine
1,5 months											
№1	Complies	Complies	Confirmed	235,4	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	60,8%	23,5%
№2	Complies	Complies	Confirmed	235,8	Less than 0,5%	Endures	Less than 10,5%	Complies	5,1%	61,3%	23,4%
№3	Complies	Complies	Confirmed	235,6	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	61,5%	23,8%
№4	Complies	Complies	Confirmed	235,7	Less than 0,5%	Endures	Less than 10,5%	Complies	5,4%	60,8%	23,6%
№5	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,0%	62,8%	24,0%
3 месеца											
№1	Complies	Complies	Confirmed	235,4	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	60,5%	23,5%
№2	Complies	Complies	Confirmed	235,7	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	61,2%	23,4%
№3	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	61,5%	23,8%
№4	Complies	Complies	Confirmed	235,6	Less than 0,5%	Endures	Less than 10,5%	Complies	5,4%	60,8%	23,8%
№5	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,1%	61,8%	24,0%
4,5 месеца											
№1	Complies	Complies	Confirmed	235,4	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	60,5%	23,5%
№2	Complies	Complies	Confirmed	235,7	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	61,2%	23,5%
№3	Complies	Complies	Confirmed	235,7	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	61,4%	23,8%
№4	Complies	Complies	Confirmed	235,7	Less than 0,5%	Endures	Less than 10,5%	Complies	5,4%	60,7%	23,6%
№5	Complies	Complies	Confirmed	235,4	Less than 0,5%	Endures	Less than 10,5%	Complies	5,0%	61,6%	24,0%
6 месяцев											
№1	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,0%	60,5%	23,5%
№2	Complies	Complies	Confirmed	235,6	Less than 0,5%	Endures	Less than 10,5%	Complies	5,1%	61,4%	23,4%
№3	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	61,4%	23,8%
№4	Complies	Complies	Confirmed	235,6	Less than 0,5%	Endures	Less than 10,5%	Complies	5,4%	60,8%	23,5%
№5	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	61,7%	24,1%
7,5 месеца											
№1	Complies	Complies	Confirmed	235,6	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	60,5%	23,5%
№2	Complies	Complies	Confirmed	235,6	Less than 0,5%	Endures	Less than 10,5%	Complies	5,1%	61,2%	23,5%
№3	Complies	Complies	Confirmed	235,6	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	61,4%	23,8%
№4	Complies	Complies	Confirmed	235,4	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	60,8%	23,8%
№5	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,0%	61,7%	24,0%
9 месяцев											
№1	Complies	Complies	Confirmed	235,4	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	60,5%	23,5%
№2	Complies	Complies	Confirmed	235,7	Less than 0,5%	Endures	Less than 10,5%	Complies	5,1%	61,2%	23,4%
№3	Complies	Complies	Confirmed	235,4	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	61,5%	23,7%
№4	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	60,5%	23,5%
№5	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,0%	61,5%	24,0%
10 месяцев											
№1	Complies	Complies	Confirmed	235,3	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	60,5%	23,6%
№2	Complies	Complies	Confirmed	235,6	Less than 0,5%	Endures	Less than 10,5%	Complies	5,0%	61,0%	23,4%
№3	Complies	Complies	Confirmed	235,5	Less than 0,5%	Endures	Less than 10,5%	Complies	5,2%	61,5%	23,7%
№4	Complies	Complies	Confirmed	235,4	Less than 0,5%	Endures	Less than 10,5%	Complies	5,3%	60,4%	23,5 %
№5	Complies	Complies	Confirmed	235,4	Less than 0,5%	Endures	Less than 10,5%	Complies	5,0%	61,4%	24,1%

During the period of observations in the conditions of natural storage, there were no significant changes in the physical, chemical and microbiological properties of the samples studied. The data of qualitative and quantitative indicators corresponded to the limits of the established norms of the developed regulatory document.

The results of the analysis of five experimental series of Gligiscin compound, which were stored under the conditions of “accelerated aging”, show that the active substance undergoes slight changes. All tested parameters under the specified storage conditions remained within the normal range.

### CONCLUSION

Thus, according to research results, the biologically active compound Gligiscin is stable during storage. The expiry date of the drug is 3 years.

### REFERENCES

1. Irismetov MP, Dzhiembaev B.Zh., Arystanova T.A., Baramysova G.T. Chemistry and application of natural glycyrrhizic acid and its derivatives // Almaty-2002., - C. 50-51.
2. Tashpulatova A.D., Yunuskhodzhaev A.N., Akbarov A.B. Complex compound of zinc with glycyrrhizic acid and histidine // Pharmaceutical journal.-2004., - №2.- P. 38-40.
3. Tashpulatova A.D., Aliyev Kh.U., Mukhitova F.T. Influence of mixed ligand complex compound of zinc on biliary excretion and bile formation in rats // Pharmaceutical Journal.-2004., - No. 3.- S. 65-67.
4. Meshkovsky A.P. Recommendations of the World Health Organization to study the stability of replicated pharmaceutical products // Farmateca. - 2002.-№6.-C. 12-15.
5. Mashkovsky M.D. The problem of stability and shelf life of drugs is relevant for Russia // Farmateca.- 2000. - 2000.-№1.-C. 38
6. European Pharmacopoeia, 9th edition, 2016.
7. State Pharmacopoeia of the USSR, XI edition. USSR Ministry of Health.-11th ed., -M.: Medicine, 1990. –Vyp.1,2.