**Lesson 6**

**Analysis of drugs derived from pteridine and isoalloxazine. Pharmaceutical forms of folic acid and riboflavin.**

**DESCENDANTS OF PTER**

Pterin derivatives are divided into two groups: a) vitamins with Pterin derivatives: b) folic acid derivatives.

 Vitamins with pterin derivatives

Folic acid – Acidum folicum

(Folic Acid, Vitamin B, Pteroylglutamic Acid)

Folic acid (folium means leaf and indicates the place where this vitamin is mainly collected) is found in vegetable plants, in the kidneys and livers of humans and animals. It is produced by the microflora of the intestines. Folic acid used in medicine is obtained by synthesis.

The chemical structure of folic acid was determined in 1946. The basis of the folic acid molecule is the pteridine heterocyclic system. The pteridine nucleus is a condensed system consisting of pyrimidine and pyrazine heterocycles:



Pterin (2-amine 4-oxypteridine), a derivative of pteridine, also forms the basis of the pteric acid molecule:



pterin pteric acid

Pterin is a component of the folic acid molecule. Therefore, this group of vitamins is called pterins. In addition to pteridine, the folic acid molecule also includes p-aminobenzoic acid and one or more glutamic acid residues. Pterin combines with p-aminobenzoic acid through the methylene group to form pteroic acid:



pteroic acid

Thus, the chemical structure of folic (pteroylglutamic) acid is as follows:

*p-aminobenzoylglutamic acid residue*

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*Pteroil qalığı*

N[4/-[(2-Amino-4-oxy-6-pteridyl)-methyl]-amino]-benzoyl-

-L(+)-glutamic acid

M.k. 441.4

As you can see, the folic acid molecule is made up of 3 parts: pteridine derivative, p-aminobenzoic acid and l-glutamic acid. Therefore, folic acid is also called pteroyl-l (+) glutamic acid. Other substances with folic acid activity are folic acid polypeptides containing 3 to 7 glutamic acid residues.

In the body, folic acid is converted into a coenzyme by the action of the folate reductase enzyme in the cells. Important representatives of these coenzymes include 7,8-dihydrofolic acid, 5,6,7,8-tetrahydrofolic acid, folinic acid and others.

Acquisition

To synthesize the preparation, 2,5,6-triamine-4-oxy-pyrimidine, 2,3-dibromopropion aldehyde and p-aminobenzoyl –L (+) glutamic acid are condensed and converted into 5,6-dihydrofolic acid; folic acid is obtained by dehydrogenating the last substance:



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Folic acid is a yellow or yellow-orange crystalline powder, odorless and tasteless. It decomposes under the influence of light and is hygroscopic. Practically insoluble in water, 95% alcohol, acetone, benzene, ether and chloroform. It is slightly soluble in dilute hydrochloric acid, moderately soluble in dilute sulfuric acid, and easily soluble in alkaline solutions.

Its solubility in acid and alkaline solutions is due to amphoteric nature of folic acid (due to amine and carboxyl groups).

Determination of identity

Reactions based on oxidation, complex formation, as well as amphoteric properties of folic acid solutions are used to determine the identity of the preparation.

1) 0.1 g of the drug is dissolved in 5 ml of 0.1 M NaOH solution, 5 ml of 0.1 M hydrochloric acid and 1 ml of potassium permanganate solution are added to it. The solution is placed in a water bath with a temperature of 80-850C for 3 minutes. In order to neutralize the excess amount of potassium permanganate, 0.2 ml of hydrogen peroxide solution is added drop by drop to the cooled solution and filtered. The filtrate gives blue fluorescence under ultraviolet light. The reason for this is the formation of p-aminobenzoylglutamic and pteric acids, which are the oxidation products of folic acid:

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 pterin turşusu



2) UV-spectrophotometry: a 0.001% solution of p-aminobenzoylglutamic acid in 0.1 M NaOH has 3 characteristic maxima at wavelengths of 256, 283 and 365 nm, and 3 characteristic minima at 235, 265 and 332 nm d.u. gives The ratio of optical densities (D) in the 1st and 3rd d.u., i.e. D(256 nm)/D(365 nm) = 2.8-3.0

3) 0.02 preparation is shaken with 2-3 ml of 0.1 M NaOH solution for 2-3 minutes and filtered. Place 2-3 drops of the obtained filtrate in a watch glass and add 1-2 drops of heavy metal salt to it. At this time, lemon color is obtained with lead-acetate, dark yellow with cobalt-nitrate, yellow-orange with silver-nitrate, green with copper 2-sulfate, and reddish-yellow with iron 3-chloride. When the alkali is added, the mixture is filtered to prevent the formation of metal hydroxides, and tests are performed using the drop method on a watch glass. The general formula of water-insoluble colored internal complex salts is:



Complex compounds are formed due to the presence of the active hydrogen atom of the hydroxyl group in the pterin residue of the molecule and the triplet nitrogen atom in the 5th position.

4) 0.01 g of the drug is dissolved in 1 ml of 0.1 M sodium hydroxide solution, 0.02-0.03 g of zinc powder is added to it, the mixture is shaken for 2-3 minutes and filtered, and then the solution is acidified with hydrochloric acid, a few drops of sodium nitrite are added they add the solution. When the obtained solution is added to the solution of β-naphthol (a) in alkali, a red azo dye is formed. N-(1-naphthyl)-ethylenediamine-dihydro-chloride (b), N-phenyl-α-naphthylamine (C) and N-phenyl β-naphthylamine (Ч) solutions can also be used to obtain azo dye.

Note. Ammonium-sulfamic acid or urea solution is taken to eliminate the excess of nitrite acid.

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Azoboya (qırmızı-bənövşəyi)

It should be noted that the first stage of this reaction - the stage of hydrolysis of the preparation - is recommended by the international Pharmacopoeia to be carried out with zinc powder in dilute hydrochloric acid.

5) It is determined by NTX. The solutions of the studied and standard sample are chromatographed in the ethanol-propanol-ammonia solution (60:20:20) solvent system. The size and position of the spots should be the same.

Determination of cleanliness

Free amine admixture in the preparation should not exceed 91%) is checked.

Quantification

It can be done in several ways:

2) Polarography method. The determination here is based on the fact that folic acid is easily reduced to 7,8 dihydrofolic acid in the presence of Na2CO3. The opposite of the process occurs even under the influence of air oxygen:



folic acid 7,8-dihydrofolic acid

3) Method of neutralization. About 0.15 g of the drug is dissolved in an excess (20 ml) of 0.1 M NaOH solution and titrated with 0.1 M hydrochloric acid. A mixture of 4 drops of phenolphthalein and 2 drops of methylene blue is taken as an indicator. The titration is carried out from a blue-violet color to a green color. 5-6 drops of thymolphthalein can also be used as an indicator. At this time, the titration is carried out from bluish-red color to yellow-green color.

In parallel, free para-aminobenzoylglutamic acid is determined. For this purpose, 0.15 g of folic acid (d.c.) per 5 ml of phenolphthalein is shaken 2 times for 5 minutes with neutralized alcohol. Alcoholic extracts are combined, filtered and titrated with 0.01 M NaOH solution (indicator-phenolphthalein). To go from 0.01 M to 0.1 M, the volume of NaOH solution used for this titration corresponds to 10 times the 0.1 M NaOH used for the titration of folic acid.

Each 1 ml of 0.1 M NaOH solution combined with the preparation corresponds to 0.01471 g of folic acid (E=M.k/3).

4) Photocolorimetry method based on the acquisition of azo dye. The essence of the appointment is that folic acid is converted into p-aminobenzoylglutamic acid under the influence of KMnO4 in a weak alkaline environment, and it is diazotized and azo dye reaction is carried out. The intensity of the obtained color is measured in a photocolorimeter (see the 4th identity determination reaction).

Since the produced folic acid contains a small amount of free p-aminobenzoylglutamic acid, a part of the solution of the drug is diazotized without treatment with KMnO4 and azo dyed. The full course of work is shown in "Leading laboratory exercises".

1) Spectrophotometry method. The determination is based on measuring the optical densities of a solution of the drug in 0.1 M NaOH at a wavelength of 365 nm or a solution of a solution in 5 ml of sulfuric acid at a wavelength of 320 nm.

Folic acid together with vitamin B12 accelerates erythropoiesis (the process of formation of erythrocytes), participates in the synthesis of amino acids, nucleic acids, purine and pyrimidine derivatives and choline metabolism. Folic acid in tablets of 0.001 g is used internally as an increaser of the activity of blood-clotting organs, in anemias and chronic gastrointestinal diseases. Folic acid (0.0008 g), together with ascorbic acid (0.1 g), is also released in tablets and multivitamin preparations.

The drug is stored in tightly closed containers, in a dry, dark place, away from light. If the storage conditions are not observed, the drug rapidly decomposes under the influence of light, especially under the influence of UV rays at 365 nm d.u. in an acidic environment. As a result of decomposition, p-aminobenzoylglutamic acid and 6-formylpterin are formed, and the latter is oxidized to pteric acid under the influence of air oxygen:



6-formylpteric pteric acid

As a result of this process, folic acid loses its activity.

Folic acid derivatives

The chemical structure of folic acid is characteristic for the biological activity against anemia. Minor changes in the molecular structure of the substance lead to the loss of its vitamin activity, or even to the emergence of an anti-vitamin effect.

Methotrexate, one of the folate metabolites, is used as an antitumor agent. The principle of searching for antitumor substances similar to natural metabolites was used in the creation of methotrexate, which is structurally similar to folic acid and its antagonist.

**Methotrexate – Methotrexate**

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4-amino-4-deoxy-10-methylpteroyl L-glutamic acid or 4-deoxy-

-4-amino-N10-methylfolic acid

Methotrexate is a mixture of 4-deoxy-4-amino-N-10-methylfolic acid and other pterin compounds.

Acquisition

The synthesis of the main ingredient of methotrexate is similar to the synthesis of folic acid. Tetraaminepyrimidine, trichloroacetone and N-[4-(N-methylaminobenzoyl)] barium-glutamate are condensed for synthesis:



tetraaminepyrimidine N-[4-(Nmethylaminobenzoyl)]- trichloroacetone

 barium-glutamate



4-deoxy-4-amino-N-10-methylfolic acid

Yellow or orange-yellow garnet is a crystalline powder. Practically insoluble in water, dichloroethane and alcohol. Easily soluble in alkalis and dilute solutions of carbonates.

Definition of personality

1) UV-spectrophotometry: a 0.001% solution of the drug in 0.1 M NaOH gives three absorption maxima at wavelengths of 258±1 nm, 303±1 nm and 370±2 nm. The ratio of optical densities determined at 303 nm and 370 nm should be 2.8-3.3.

2) Chromatography is performed on paper. A spot of a 1% solution of the drug in 0.05 M Na2CO3 is applied to the paper for chromatography and chromatography is carried out by the ascending method. Phosphate buffer with pH 5.8 was used as a solvent. On the chromatogram there should be a methotrexate spot with Rf 0.63-0.75 and 3 other spots (pterin) that give fluorescence.

3) IR-spectroscopy: the IR-spectrum of methotrexate should correspond to the spectra shown in HC.

4) Oxidizing with potassium permanganate, it gives blue fluorescence in UV light (see the 1st reaction of identity in folic acid). Potassium chlorate can also be used as an oxidizer.

Quantitative assessment

It is determined spectrophotometrically. For this, before taking folic acid

methotrexate is chromatographed on paper (see definition of identity 2); Their spots are washed with 0.1 M NaOH solution and their optical density is measured. The amount of methotrexate is calculated based on the optical density.

According to the International Pharmacopoeia, it is carried out by the YEMX method. Moving

The acetonitrile-phosphate-citrate buffer (8:92) system is used as a phase. Calculations are made based on the area of the peak of the standard and test solutions.

This is one of the antimetabolites, which are analogues of folic acid.

Stops the development of malignant tumors, is used in the treatment of leukemia (leukocytes). Drug 0.0025; 0.05; In tablets of 0.01 g sodium salt 0.02; 0.05; 1 for infusions in vials in the amount of 0.1 g; 2; 4; 8; 10; 20; 40; Released 200 ml.

The drug is stored in a tightly closed container, in a dry place protected from light, at a temperature of up to +5℃.

**ISOALLOXAZINE DERIVATIVES**

The isoalloxazine heterocyclic system is chemically similar to pteridine and consists of two heterocycles: pyrazine and pyrimidine, as well as an additional benzene nucleus, in other words, it is a partially hydrogenated derivative of benzpteridine. The pyrimidine core in the isoalloxazine molecule is similar to lactam:



Isoalloxazine Benzpteridine

Isoalloxazine derivatives are natural compounds with vitamin B2 (riboflavin) activity. B2 vitamins are also called flavin ("flavum"-"yellow") vitamins. Lactoflavin from milk, ovoflavin from egg white, citroflavin from lemon have the same chemical structure, riboflavin consists of isoalloxazine heterocycline and ribose residues.

**Riboflavin**

**(Vitamin B2)**

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**6,7-Dimethyl-9-(D-1-ribityl-isoalloxazine)**

**M. k. 376,37**

Vitamin B2 is widely distributed in the plant and animal world. A person receives this vitamin with milk and meat products. Some food products are colored with riboflavin, such as dairy products, egg yolks, liver, kidneys, germ and husk of cereals (barley, wheat), peas, vegetables (spinach, tomato), etc.

Riboflavin is part of flavin enzymes in the body and ensures the performance of dehydrogenation reactions. Lack of riboflavin in the body (hypovitaminosis) and deficiency (avitaminosis) causes ariboflavinosis, which damages the skin around the mouth and, in severe cases, the eyes. In addition, the weight of the body decreases, loss of appetite occurs, the vision process is disturbed. Riboflavin converts visible UV and blue rays into long-wave rays when the eye is more sensitive. Thus, it expresses the role of a sensitizer (increases sensitivity to light) by creating a bathochromic effect in vision. The retina of some animals (fish, monkey) contains riboflavin. Riboflavin entering the body is phosphated under the influence of ATF (at the expense of the monoalcohol –CH2OH group in the ribityl residue) and two coenzymes are formed - flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The vitamin activity of riboflavin is related to the presence of labile azadein group and ribityl radical in its molecule. The azadein group gives it oxidation-reduction properties. Due to these properties, flavins participate in metabolic reactions of sugars, lipids and proteins and perform various biological functions in the body. Riboflavin is reduced and loses its yellow color and becomes colorless leucoriboflavin:



 riboflavin (sarı) leykoriboflavin (rəngsiz)

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The reason for the color of isoalloxazine derivatives is the chromophore-azomethine group (=C=N–). One of the characteristic properties of riboflavin is that it is sensitive to light. Under the influence of light, the chemical structure of riboflavin changes, and this change depends on the pH of the medium, as well as the intensity of the light. Under the action of light, in a neutral or weakly acidic medium, the ribose residue is separated, resulting in lumichrome, which does not emit yellow fluorescence. When riboflavin is irradiated in an alkaline medium, lumiflavin is obtained. Lumiflavin solutions are yellow in color and give yellow-green fluorescence:

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Lumichrome and Lumiflavin do not show vitamin activity.

Unlike riboflavin, lumiflavin is soluble in chloroform. Using this property, they determine the presence of lumiflavin as a mixture in riboflavin and riboflavin mononucleotide:

Acquisition

Riboflavin can be obtained from plant or animal raw materials. However, this process requires a lot of labor and the yield of the obtained product is very low. So, to get 1 gram of riboflavin, it is necessary to process 5.4 tons of milk whey.

Industrially, riboflavin is obtained by synthesis method. To synthesize riboflavin, 3,4-dimethylaniline is condensed with D-ribose, the resulting imine is hydrogenated, and the arylribamine formed as a result of the azo coupling reaction (reduction of the azo group) is condensed with alloxan and converted to riboflavin:





At present, the most convenient way to obtain riboflavin is microbiological synthesis. Here, the physiology of microorganisms and genetic engineering are taken as a basis, as a result of which the yield of riboflavin increases 4-5 thousand times.

Riboflavin is a yellow-orange crystalline powder with a faint characteristic odor and bitter taste. It is resistant to light. It is slightly soluble in water, in alkalis, practically insoluble in 95% alcohol, ether, acetone, benzene and chloroform.

Determination of identity

In order to determine the identity of isoalloxazine derivatives, including riboflavin, chemical reactions based on oxidation-reduction based on double bonds, oxidation and esterification of the ribitol part of the molecule, complex formation and hydrolysis, presence of a triple nitrogen atom, sodium ion and phosphate residue located in the molecule are used.

1) Fluorescence: 0.01 g of the drug is dissolved in 100 ml of water, the solution is light greenish-yellow in color. When viewed under UV rays, green fluorescence is observed. Fluorescence is lost when hydrochloric acid and alkaline solution are added, and both fluorescence and color are lost when sodium hyposulfite is added. This is due to the conversion of riboflavin into leucoriboflavin (see p. 22).

2) red color is formed when 2-3 drops of sulfuric acid are added to 0.001 g of the preparation; adding a few drops of water changes the color to yellow.

3) When 3-4 drops of AgNO3 solution are added to the preparation, an orange-red-red complex compound is formed (riboflavin combines with silver ions in a 1:1 ratio; the compound is in the imide group in the 3rd position).

4) When riboflavin solution is treated with Denije reagent (0.5 g of yellow mercury oxide + 2 ml of solid H2SO4 + 10 ml of water), an orange color is obtained:

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5) 0.001 g of the drug is dissolved in water, 1-2 drops of 0.1 M NaOH solution and 3-4 drops of 0.25% ninhydrin solution are added to the obtained solution and heated until it boils; a green color is formed.

6) Riboflavin, as a nitrogen-containing organic compound, reacts with Dragendorf's reagent and other precipitating general alkaloid reagents.

7) The special rotation of the preparation should be from -1100 to -1300. 0.125 g of drug (d.k.) is dissolved in 5 ml of 0.1 M solution of potassium hydroxide in alcohol in a volumetric flask with a volume of 25 ml. They measure the volume of the solution with water and determine the angle of rotation. Then they calculate the specific rotation based on the formula 〖[α]〗\_D^20=(α ∙100)/(l ∙c).

8) IR-spectroscopy: the IR-spectrum of riboflavin should be the same as the spectra given in NS.

9) UV-spectrophotometry: the aqueous solution of riboflavin should give 4 absorption maxima at 223, 267, 370 and 445 nm d.u.

Determination of cleanliness

It should not contain lumiflavin. To determine this, 0.025 g of the preparation is shaken for 5 minutes with 10 ml of pure chloroform and filtered. The color of the filtrate should not be darker than the color of the ethanol solution of potassium bichromate. To prepare standard solution, 3 ml of 0.0167 M potassium-bichromate solution is diluted with water to 1 l.

Quantification

It is done in several ways.

1) UV-spectrophotometry method. 0.06 g (d.c.) of the drug is dissolved in a mixture of 2 ml of glacial acetic acid and 500 ml of water by heating it on a water bath in a 1000 ml flask. The solution is cooled and brought to volume with water. Take 10 ml of that solution, place it in a volumetric flask with a volume of 100 ml, add 3.5 ml of 0.1 M sodium-acetate solution and bring the volume up to volume with water. The optical density of the obtained solution is measured in a spectrophotometer at a wavelength of 267 nm in a cuvette with a layer thickness of 1 cm. The percentage amount of riboflavin (x) is calculated according to the following formula:

$$x=\frac{D ·10000}{a ·850}$$

Here,

D – optical density of the tested solution;

a - gr-la etc.;

850 – pure riboflavin 267 nm d.u. is a specific absorption index.

The preparation should contain 98.0-102% riboflavin based on dry matter.

2) The method based on the neutralization of HNO3 formed by reaction with silver nitrate (alkalimetry).

5-10 drops of 0.02 M AgNO3 solution, 3-4 drops of bromothymol solution are added to 10 ml (d.h.) 0.02% solution of the preparation and titrated with 0.01 M NaOH solution until brick-red color (T=0 ,0037637 g/ml).

In parallel, a control experiment is carried out.

3) It is carried out by the method of fluorimetry.

4) Determination according to the Ribitil residue.

Riboflavin is oxidized with 0.02 M potassium periodate solution at room temperature, in a neutral environment (Malaprad reaction); as a result, formic acid is obtained, which is also determined by alkalimetry:



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2HCOOH + 2NaOH → 2HCOONa + 2H2O

Riboflavin ribityl residue can be determined by esterification reaction with solid sulfuric acid. Excess sulfuric acid is titrated with potassium hydroxide.

Riboflavin participates in the metabolism of sugar, proteins and fats, and in the synthesis of hemoglobin. For therapeutic purposes, riboflavin is used in hypo- and ariboflavinosis, eye diseases (0.01% solution). Internally, it is prescribed in the form of powder, tablets (0.002, 0.005 and 0.01 g). It is included in some multivitamin preparations.

Riboflavin preparations are stored in tightly closed containers, protected from light.

**Riboflavin-mononucleotide - Riboflavin mononucleotide**

**(Riboflavin Phosphate)**



Riboflavin-5'-monophosphate-sodium

It is a yellow-orange crystalline powder, odorless and bitter in taste. Moderately soluble in water, practically insoluble in alcohol. Decomposes on exposure to light. Aqueous solutions fluoresce under the influence of UV rays.

Determination of identity

1) Yellow-green fluorescence is observed when looking at 10 ml of a solution of the preparation prepared in a ratio of 0.02:20 under UV rays. Loses fluorescence due to alkali and acid (riboflavin).

2) When a glass rod is immersed in a solution of the drug and exposed to a colorless flame, it turns yellow (sodium).

3) Phosphate ion reaction: add 3 ml of solid HNO3 to 5 ml of the solution in step 1, boil for 5 minutes, cool, add 10 ml of water, 0.5 g of ammonium nitrate and 2 ml of ammonium molybdate solution they heat up; the solution turns yellow, and then a yellow crystalline precipitate forms:

H3PO4 + 12(NH4)2MoO4 + 21HNO3 → 21NH4NO3 + 12H2O +

+ (NH4)3PO4 · 12MoO3↓

4) UV-spectrophotometry: the optical density of the solution prepared for the determination of the amount of the drug is determined at the wavelength of 266, 373 and 445 nm, in a cuvette with a layer thickness of 1 cm. The ratio of optical densities (D) should be within the following limits:

$$\frac{D\left(373 nm\right)}{D\left(445 nm\right)}=0,83-0,86; \frac{D\left(266 nm\right)}{D\left(445 nm\right)}=2,3-2,75$$

Determination of cleanliness

It should not contain lumiflavin (see riboflavin).

Quantification

It is carried out by the spectrophotometry method with a wavelength of 445 nm. (Given for 1% injection solution of the drug). 1 ml (d.h.) of the drug is placed in a 250 ml volumetric flask, 0.5 ml of glacial acetic acid is added and the volume is brought to volume with water. They take 10 ml of this solution and place it in a volumetric flask with a volume of 25 ml. 9 ml of 0.1 M sodium acetate solution a.e. and bring the volume to measure with water. The optical density of the obtained solution is measured in a spectrophotometer at a wavelength of 445 nm with a layer thickness of 1 cm. The amount of riboflavin mononucleotide in 1 ml of the preparation is calculated according to the following formula:

$$x=\frac{D ∙250 ∙25 ∙1,2709}{1 ∙10 ∙323 ∙100}=D ∙ 0,02459;$$

Here,

D – optical density of the tested solution;

$E\_{1sm}^{1\%}$323;

1.2709 is the coefficient for conversion to riboflavin-mononucleotide.

The amount of riboflavin mononucleotide should be 0.0095-0.0105 g.

Riboflavin-mononucleotide is a coenzyme drug, it is formed from riboflavin in the body. When combined with protein, it is included in the composition of enzymes involved in oxidation-reduction processes. Along with riboflavin, it is used in hypo- and avitaminosis, dermatosis, chronic eczema, neurodermatitis, keratitis, etc. It is used in cases. The drug is injected subcutaneously and into the muscle. A 1% solution is released for injection in the amount of 1 ml. It is included in the composition of "Komplivit" which is a polyvitamin preparation.

The drug is stored in a dry place, protected from light.