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OPTIMIZATION OF THE TECHNOLOGY OF "PHOSFARGININE SUCCINATE"INFUSION SOLUTION

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ABSTRACT

In recent years, much attention has been paid to providing the population with domestically produced pharmaceuticals, the production of new import-substituting drugs that are affordable from an economic point of view, important tasks have been identified for "the development of the pharmaceutical industry, as well as improving the provision of the population and medical institutions with cheap, high-quality drugs". In this regard, the search for new drugs, their introduction into the pharmaceutical industry is being carried out. One of these drugs is "Phosfarginine succinate" (consisting of D-fructose-1,6-diphosphate trisodium salt, L-arginine hydrochloride, succinic acid) infusion solution - a drug that stimulates and regulates the metabolic process. The infusion solution technology was developed on the basis of Temur Med Farm LLC (Republic of Uzbekistan). In order to select a scientifically based composition, develop and optimize the technological process, the method of mathematical planning of the experiment was used - Latin plans. The application of the method of mathematical planning of the experiment made it possible to select the optimal composition and technology of the infusion solution "Phosfarginine succinate".

KEYWORDS: cardiovascular diseases, Phosfarginine succinate, metabolic agent, antihypoxic agent, solution for infusion, composition, D-fructose-1,6-diphosphate trisodium salt, L-arginine hydrochloride, succinic acid, sodium metabisulfite, stabilizer, water for injections, technology, method of mathematical planning of experiment, optimization of composition and technology.

INTRODUCTION

According to the statistics of diseases of the World Health Organization (WHO), over the past 20 years, the statistics of mortality due to cardiovascular diseases has consistently remained in first place and this figure is growing every year.[1] Among the population of the Republic of Uzbekistan aged 30-70 years, 53% of the causes of death are cardiovascular diseases and this number has increased by 20% over the past 5 years.To solve the problem that has arisen in the Republic of Uzbekistan, they are switching to a new treatment system: identifying cardiovascular diseases at an early stage and conducting effective treatment. For this purpose citizens of our republic after 40 years of age undergo annual examinations, and if morbidity is detected, low-income sections of the population will be provided with medical assistance free of charge. For this purpose, more than 580 billion soums were allocated from the state budget.[2]

In the treatment of cardiovascular diseases, a number of additional drugs are used to enhance metabolic processes:

D-fructose-1,6-diphosphate - is a natural cellular metabolite, has a powerful anti-shock effect, in conditions of myocardial ischemia and hypoxia, due to the activation of phosphofructokinase and pyruvate kinase in cells, it increases the concentration of adenosine triphosphate, creatine phosphate and the flow of potassium ions, as a result of which energy metabolism in myocardial cells, glucose is utilized in the tissues and their damage is reduced. Fructose-1,6-diphosphate is used as an auxiliary therapeutic agent for myocardial ischemia, angina pectoris and ischemic stroke of the brain.

With atherosclerosis, hypertension, diabetes mellitus and renal failure in the patient's body, due to a lack of nitric oxide, the function of blood vessels is disrupted. L-arginine is one of the essential amino acids necessary for the body; this amino acid is synthesized in the body from

glutamic acid and proline. L-arginine in the body stimulates the synthesis of nitric oxide, which in turn maintains the function of blood vessels at the same level and stimulates the metabolism of fats, stops platelet aggregation, and stimulates the process of fibrinolysis.

Succinic acid - is used as a biologically active additive that enhances the effect of the main drug, in order to reduce the dose and shorten the period of use of the main drug, it is introduced into the composition of drugs for vascular ischemia, spasm, physical activity, in stressful situations and diseases of the cardiovascular system.[3,4]

In the treatment of cardiovascular diseases, a number of infusion solutions are used that have a metabolic effect on metabolic processes and antihypoxic properties: Tivortin (L-arginine hydrochloride 42 mg/ml, "Yuria-Farm" LLC, Ukraine), Neoton (1.0 g lyophilized Phosphocreatine powder for infusion solution, Alfasigma S.p.A., Italy), Esophosphine (D-fructose-1,6-diphosphate trisodium salt trihydrate, Biomedica Foscama Group S.p.A., Italy).[3,5,6]

The given drugs do not fully satisfy the needs of our republic, therefore it is important to expand the range of drugs that stimulate metabolism in cardiovascular diseases and introduce them into domestic pharmaceutical production.

The creation of drugs with high pharmacotherapeutic efficacy is possible only with a comprehensive study of the interaction of variable factors that ensure the quality of the drug. The search for a scientifically based composition of the dosage form, the development and optimization of the technological process require a significant amount of experimental research, which requires a long time and significant material costs. To successfully solve optimization problems, the method of mathematical planning of the experiment was used - Latin plans.[7] The use of the method of mathematical planning of the experiment makes it possible to significantly reduce the time of research on optimizing the composition and technology of the infusion solution "Phosfarginine succinate", as well as to quantitatively determine the significance and influence of the studied factors and reduce the experimental error.[7,8]

The objective of the research: optimization of the composition and technology of the metabolic agent for injection "Phosfarginine succinate" and assessment of its quality.

EXPERIMENTAL PART

MATERIALS AND RESEARCH METHODS. Based on the study of literary sources and pharmacological studies, the object of research was the solution for infusion "Phosfarginine succinate", containing in its composition pharmacologically active substances that contribute to the activation and normalization of the metabolic process in cardiovascular diseases: D-fructose-1,6-trisodium diphosphate salt trihydrate (Jiangsu Jingrui Pharma Tech Co., Ltd, China), L-arginine hydrochloride (Wuxi Jinghai Amino Acid Co., Ltd, China) and succinic acid (Anhui Sunsing Chemicals Co., Ltd, China).[5,9]

In preliminary studies, the compatibility of medicinal substances and excipients included in the infusion solution "Phosfarginine succinate" was studied, for which model mixtures of solutions were prepared, which were stored for 6 months in natural conditions and every month, pH values, the absence of mechanical impurities, the quantitative content active substances, in particular, the content of D-fructose-1,6-diphosphate, mg/ml), the specific antihypoxic activity of solutions on laboratory mice was also studied and satisfactory results were obtained.[9,10,11,12,13]

In these studies, in order to optimize the composition and technology of the infusion solution "Phosfarginine succinate", a set of technological factors affecting the quality of the drug and ensuring their stability were considered.[9] The stability of injectable (infusion) drugs depends on: composition, method of preparation, excipients, type of packaging, storage temperature, illumination, etc.[8,10]

As a dissolution medium in the composition of solutions for intravenous administration, water for injection, isotonic 0.9% sodium chloride solution, and 5% glucose (dextrose) monohydrate solution are used.[8]

In the process of preparation, as well as during storage of solutions for parenteral use, under the influence of many factors, it is possible to change, destroy some medicinal substances with the formation of inactive and toxic products. Obtaining stable solutions provides for the maximum elimination of factors that contribute to the decomposition of medicinal substances. Therefore, in the composition of infusion solutions, excipients are used - stabilizers, and a complex of technological methods is carried out to ensure the maximum elimination of factors that contribute to changes in the properties of medicinal substances.

Currently, more than half of parenteral solutions require the use of stabilizers, in particular antioxidants. For example, sodium hydrosulfite, sodium metabisulfite, ascorbic acid, etc. are used as antioxidants and stabilizing microbiological properties of drugs.[10]

It is known from the literature that within pH 3-4 fructose, like glucose, is most resistant to high temperatures,[9,13] therefore, to ensure the stability of the infusion solution when exposed to high temperatures (for example, when sterilizing under pressure at a temperature above 100°C) it is necessary to maintain exactly the above pH range.

The creation of new drugs with high pharmacotherapeutic efficacy requires a large number of

experiments and the study of many factors. At the first stage of the study, when the composition of the components is not yet known, the study of qualitative factors prevails. Therefore, to optimize the composition and technology of the infusion solution "Phosfarginine succinate", multifactorial plans based on Latin squares were used. Such plans are economical in terms of the number of experiments, which is important when conducting experiments in practice.[7]

Latin square 3x3 is a square table consisting of three different canonical forms, where the total number of squares is 3!(3-1)=12.[7]

To optimize the composition and technology of the infusion solution "Phosfarginine succinate", the method of mathematical planning of the experiment was used - the Latin square 3x3, where each studied factor was studied at three levels of change, without repeated observations.

The process of developing the optimal composition and rational technology of infusion solutions requires taking into account a number of variable factors, for example: the type of solvent (factor A), the type of stabilizer (factor B), the type of packaging material (factor C). Each of these factors to some extent affects the quality indicators that determine the technological properties, and the pharmacological activity of the drug of complex composition (table 1).

To test the significance of these factors, according to the experimental plan, 9 experiments were carried out under the conditions provided for by the planning matrix.

The optimization criteria were the following factors - pH solution for infusion, Y_1; quantitative content in the preparation of D-fructose-1,6-diphosphate, (mg/ml), Y_2; antihypoxic effect of the infusion solution "Phosfarginine succinate", (% effect), Y_3.[7]

Table 1: Characterization of variable factors affecting the quality indicators of the "Phosfarginine succinate" infusion solution.

Levels	Factors		
	Solvent type (A)	**Type of stabilizer (B)**	**Type of packaging material (C)**
1	Water for injection	Sodium hydrosulfite	Bottle made of glass brand NS-1 (I type)
2	Isotonic sodium chloride solution 0.9%	Sodium metabisulphite	Bottle made of glass brand NS-2 (II type)
3	Dextrose monohydrate solution 5%	Ascorbic acid	Polymer bottle (Type III)

The planning matrix and the results of studies on optimizing the composition and technology of the "Phosfarginine Succinate" infusion solution are shown in Table 2. In Table 2, all three factors vary at three levels.

Table 2: Experiment planning matrix and results of studies on optimizing the technology of the "Phosfarginine succinate" infusion solution.

Experiment number	Factors			Optimization Criteria			D
	A	B	C	solution pH, Y_1	Content of D-fructose-1,6-diphosphate (mg/ml), Y_2	Antihypoxic effect (% effect), Y_3	
1	a_1	b_1	c_1	4.8	35.16	20.4	0.67
2	a_1	b_2	c_2	3.8	40.25	23.6	0.85
3	a_1	b_3	c_3	5.3	33.53	12.4	0.80
4	a_2	b_1	c_2	4.6	33.01	19.5	0.63
5	a_2	b_2	c_1	4.0	32.7	18.5	0.75
6	a_2	b_3	c_3	5.2	30.35	16.4	0.37
7	a_3	b_1	c_3	3.5	37.20	20.3	0.55
8	a_3	b_2	c_2	3.7	34.80	19.0	0.82
9	a_3	b_3	c_1	4.3	30.25	18.4	0.78

The experimental data were subjected to analysis of variance, the results of which are shown in Table 3.

When comparing the obtained dispersion values (Table 3) with the table value of the Fisher criterion, it was found that the pH of the infusion solution "Phosfarginine succinate" is significantly affected by factors A and B, and the quantitative content of D-fructose-1,6-diphosphate (mg/ml) and antihypoxic effect are influenced by all three factors A, B and C.

When quantifying the influence of the studied three factors on the selected optimization criteria, it is obvious that the totality of the significant interaction of factors A and B is significant, under this condition, factor C can be neglected.

Table 3: Dispersion analysis of data on optimization of the technology of the "Phosfarginine succinate" infusion solution.

Optimization criterion	Source of dispersion	Sum of squares	Number of freedom degrees	Medium square	$F_{exp.}$	F_{table}
pH value of solution for infusion	Factor A	52.32	2	28.661	0.008	19.2
	Factor B	80.79	2	40.395	0.006	19.2
	Factor C	375.77	2	187.885	0.028	19.2
	Residue	13285.22	2	6642.609	-	-
	Total amount	13799.10	8	-	-	-
Content of D-fructose-1,6-diphosphate (mg/ml)	Factor A	29.97	2	14.989	40.31	19.2
	Factor B	33.45	2	16.725	44.98	19.2
	Factor C	18.98	2	9.489	25.52	19.2
	Residue	0.744	2	0.372	-	-
	Total amount	83.14	8	-	-	-
Antihypoxic effect of the infusion solution (% effect)	Factor A	1.84	2	0.92	0.42	19.2
	Factor B	40.34	2	20.17	9.35	19.2
	Factor C	28.81	2	14.40	6.68	19.2
	Residue	4.31	2	2.155	-	-
	Total amount	75.30	8	-	-	-

In order to optimize the composition and technology of the infusion solution "Phosfarginine succinate", three criteria with different measurement values were studied. In order to identify the degree of influence of all responses on the process of preparing the infusion solution, it was necessary to generalize these measurement values into one general indicator - 2 generalized desirability function (D), defined as the geometric mean of the desirability of individual properties:

$$D= \sqrt[n]{d_1 d_2 d_3 \ldots d_n} \quad (1)$$

Harrington's desirability function scale was used to convert natural quantities with different measurement values into partial values of the desirability function.

To build a desirability scale, a quantitative assessment method was used with an interval of desirability values from zero to one, intermediate desirability values correspond to points reflecting certain quality levels of the "Phosfarginine Succinate" infusion solution (Table 4).

Table 4: Desirability Standard Ratings.

Empirical system of preferences (desirability)	Numerical system of preferences (system of psychological parameters)
Very good	1.00-0.80
Good	0.80-0.63
Satisfactory	0.63-0.37
Bad	0.37-0.20
Very bad	0.20-0.00

The numerical preference system presented in Table 5 is the dimensionless desirability scale developed by Harrington. The values of this scale have an interval from 0 to 1 and are denoted by d (from fr. - desirable). The value of the i-th private optimization parameter, converted into a dimensionless desirability scale, denoted by di, is called private desirability, where i=1,2,3,…, n is the current parameter number, n is the number of private parameters. The value d_i=0 corresponds to an absolutely unacceptable level of the i-th optimization parameter. Meaning

d_i=1 – the best value of the i-th parameter.

The desirability function corresponding to the Harrington desirability scale has the following form (for a one-sided constraint):

$$d = \exp(-\exp(-y')) \quad (2)$$

When constructing the desirability scale, which sets the ratio of the response values y_1, y_2, y_3, and the corresponding partial desirability criteria d_1, d_2, d_3, we proceeded from the fact that the worst quality (d=0) corresponds to the pH value at 5.2; the quantitative content of D-fructose-1,6-diphosphate at a value of 30.35 mg / ml and the antihypoxic effect of the infusion solution is 16.4% of the effect, and the best quality indicator corresponds to the response values: y_1= 3.8, y_2=40.25 mg /ml, y_3=23.6% of the effect, respectively, intermediate responses were selected. A graphic representation of the desirability function is shown in Figure 1.

Using this scale, the response values y1, y2, y3were converted into particular desirability criteria y1, y2, y3 and found the generalized desirability function (D) presented in Table 2. Table 2 shows that the quality indicators of the infusion solution "Phosfarginine succinate" a certain influence is exerted by the type of

selected excipients: the type of solvent (A), the type of stabilizer (B), as well as the type of packaging material (C). For example, under the condition F0.05 = 4.8, Fexp.< Ftable, then this makes it possible to determine that the selected model is linear and it is possible to check the significance of the main studied factors.

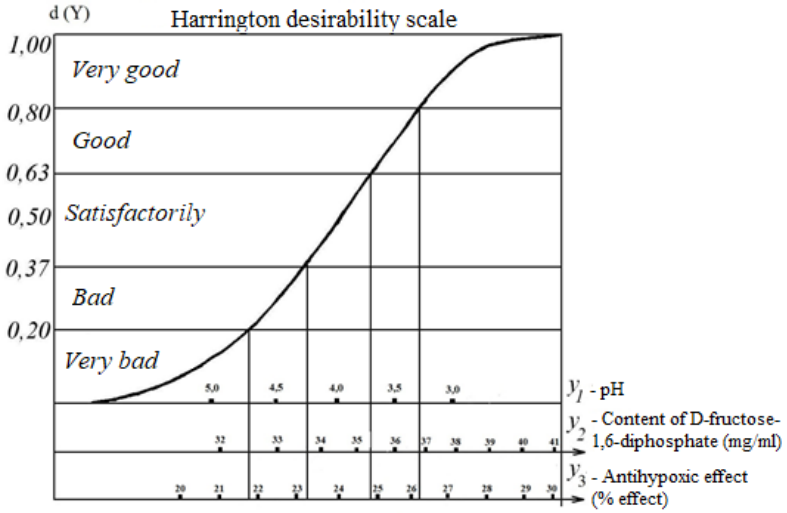

Fig. 1: Desirability function scale.

According to the experiment planning matrix, 9 experiments were carried out and, according to the optimization criteria, the significance of the selected excipients was determined, arranged in the following order: type of solvent - $a_1>a_2>a_3$; stabilizer type – $b_2>b_3>b_1$; type of packaging material – $c_1\geq c_2>c_3$. At the same time, the property of the desirability function d(x) from 0 to 1, the indicators change and $d_i\approx 0$.

As the results of the analysis of variance showed, that the obtained value for the optimization criterion, the quantitative content of D-fructose-1,6-diphosphate (mg/ml) –$F_{exp.}>F_{table}$, therefore, we conclude that they have a significant effect on stability during sterilization and storage, so how to check the significance of this effect becomes impossible. The physical meaning of the significance of the interaction is that when the value of the level of one factor changes, the value of the level for another factor changes.

The value of such optimization criteria as the pH index and antihypoxic effect, where $F_{exp}<F_{table.}$. Therefore, the linear model is suitable for analysis and it is possible to check the significance of the main effects. This fact mainly predetermined the composition and technology of the infusion solution "Phosfarginine succinate".

As a result of the research, to ensure a satisfactory technological process for the preparation of the infusion solution "Phosfarginine succinate", the following excipients were selected: water for injection (solvent -a_1); sodium metabisulfite (stabilizer - b_2); bottle made of glass brand NS-2 (type II), (type of packaging material - c_2) [7].

Based on the results of the research using the method of mathematical planning of the experiment, the following composition of the infusion solution “Phosfarginine succinate” was developed (composition per 100 ml):

Active substances
D-fructose-1,6-diphosphate trisodium salt trihydrate 5.0 g
(equivalent to D-fructose-1,6-diphosphoric acid - 3.75 g)
(Manufacturer specification)
L-Arginine hydrochloride 2.1 g
(Eu.P., Br.P., US.P)
Succinic acid 0.2 g
(Eu.P., Br.P., US.P)
Excipients:
Sodium Metabisulphite 0.01 g
(Eu.P., Br.P., US.P)
Water for injection up to 100 ml
(PA 42 Uz-0512-2017)

Technology: the infusion solution "Phosfarginine succinate" was prepared on the basis of "Temur Med Farm" LLC for filling into neutral glass bottles (type II) of 100 ml. The process flow diagram is shown in Figure 2.

The technological process consists of the following stages: preparatory operations of the technological process; preparation of water for injection; weighing medicinal substances and excipients; dissolving D-fructose-1,6-diphosphate trisodium salt trihydrate in water for injection at a temperature of 60°C; the finished solution is passed through a special filter column, then L-arginine hydrochloride and succinic acid are added to the solution; determine the pH of the solution 3.0-3.2 (potentiometrically); fructose is dissolved separately in chilled water for injection; the quality of the finished solution is evaluated, to stabilize the solution sodium metabisulfite at 3.3-3.5 is added; the solution is filtered

and packaged in 50 and 100 ml. Infusion solutions were sterilized at 110°C under a pressure of 10 kPa for 45 minutes.

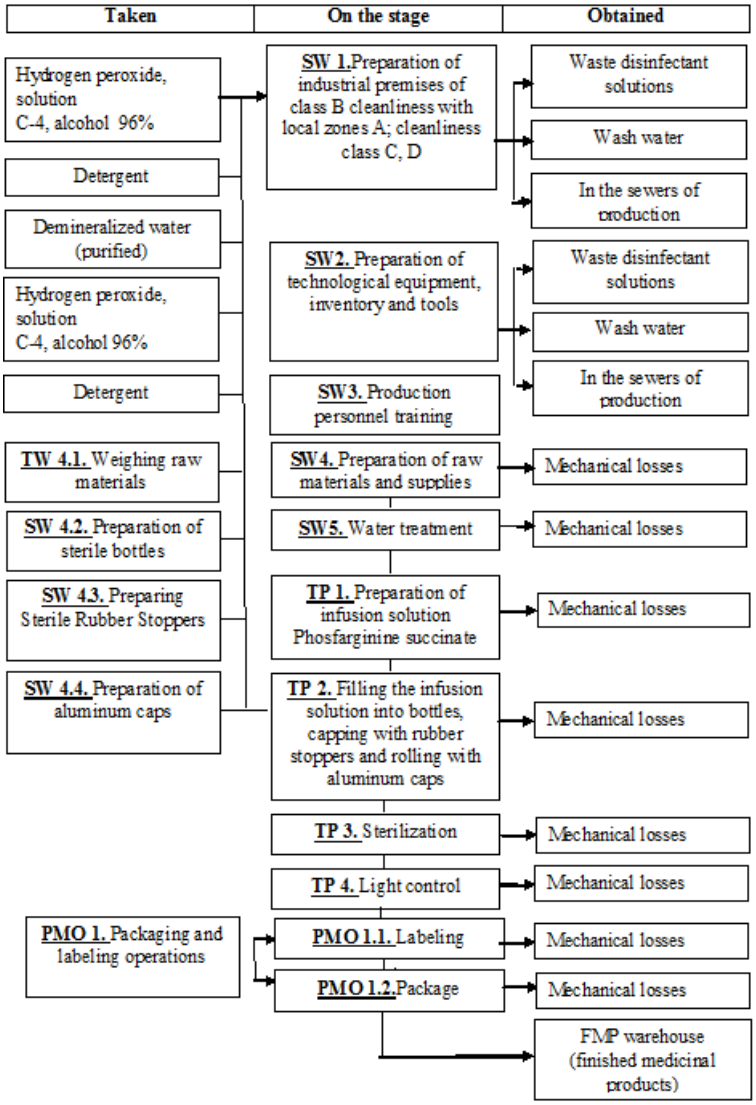

Fig. 2: Scheme of the technological process of the "Phosfarginine succinate" infusion solution.

Next, we studied the quality indicators of the infusion solution "Phosfarginine succinate" according to the following indicators:

Description. Clear, colorless or slightly yellowish solution.

Authenticity. 1 ml of the prescribed solution is mixed with 2 ml of the molybdovandenic acid reagent. Yellow color develops. (D-fructose - 1,6 - diphosphoric acid) (Br.P., Eur.P.).

To 5 ml of the drug 15 ml of water is added. To 2 ml of the resulting solution, acidify 1 ml of α-naphthol solution and 2 ml of a mixture of equal volumes of 3% sodium hypochlorite solution and water; a red color is formed (arginine hydrochloride).

20 ml of the tested preparation are evaporated to dryness in a boiling water bath. The dry residue with 0.1 g of resorcinol and concentrated sulfuric acid upon heating of the crystals appears a brick-red color, upon cooling, adding water and sodium hydroxide to an alkaline reaction, an orange solution with green fluorescence in UV (succinic acid) is formed.

Note: Reagent molybdovanadenic-acid. In a 150 ml beaker, 4.0 g (accurately weighed) of powdered ammonium molybdate and 0.1 g (accurately weighed) of powdered ammonium vanadate are mixed. 70 ml of water are added and grind the particles using a glass rod. A clear solution is obtained within a few minutes. 20 ml of diluted nitric acid and 100 ml of water are added.

Transparency. The drug should be transparent compared to water for injection or opalescence should not exceed the standard I. (SP XI, ed. 1, p. 198).

Chromaticity. The color of the preparation should not be more intense than the standard $У_6$. (Eu.P., Br.P).

pH. From 3.5 to 4.0 (Potentiometrically; SP XI, ed. 1, p. 113).[11]

Packing volume. The determination is carried out in each of the 5 vials (bottles). Separately the contents of each vial (bottle) is placed into a dried calibrated graduated cylinder. In this case, a cylinder of such a size is used so that the solution is 40% filled and the measurement is carried out. The volume of the solution should not be less than the nominal volume indicated on the label, the maximum volume should be 51 ml, 102 ml, respectively, for volumes of 50 ml, 100 ml (SP XI, ed. 2, p. 141).[12]

Mechanical inclusions.
1. Visible mechanical particles. The test is carried out in accordance with Eu.P. 2.9.20, BPA 42 Uz-0006-3341-2018. On visual inspection, the preparation should contain practically no visible particles.
2. Invisible particles. The analysis is carried out by a counting-photometric method, in accordance with Eu.P. 2.9.19, BPA 42 Uz-0005-3340-2018. On an electronic counter, the number of particles in a 50 ml and 100 ml vial should be:
- For particles ≥ 25 microns there should be no more than 600 pieces;
- For particles ≥10 microns, there should be no more than 6000 pieces.[8,9]

Sterility. The drug must be sterile. The test is carried out by the membrane filtration method in accordance with the requirements of the SP XI, ed. 2, p. 187 and Amendment No. 2 of October 12, 2005. Category 1.[12]

Pyrogenicity. The drug must be non-pyrogenic. Test dose 5 ml of the drug per 1 kg of animal weight. It is injected intravenously slowly, for 1 min. (BPA 42 Uz-0003-2338-2016).[11,12]

Quantitation
Fructose-1,6-diphosphoric acid.
The test is carried out by the spectrophotometric method.

Test solution: About 10 ml of the drug is placed in a 100 ml volumetric flask, dissolved in purified water, adjusted to the mark with the same solvent and mixed.

Standard solution: About 500 mg (accurately weighed) of the standard D-fructose-1,6-diphosphate trisodium salt trihydrate ($FdPNa_3H{\cdot}3H_2O$) is placed in a 100 ml volumetric flask, dissolved in purified water, adjusted to the mark with the same solvent and mixed.

2 ml of the tested solution and standard solution is placed into two 20 ml volumetric flasks, 10 ml of 8 mol/l hydrochloric acid solution, 2 ml of 2.5% diphenylamine alcohol solution are added, heated in a water bath at a temperature of 95°C ± 1°C for 30 min with shaking, then cooled, brought to the mark with 70% ethanol and mixed.

Control solution: Prepare in the same way, adding 1 ml of water with the same solvent instead of the tested solution.

Determine the optical density of the tested and standard solution on a suitable spectrophotometer, in a cuvette with a layer thickness of 10 mm, at a wavelength of 638 nm, relative to the control solution (Fig. 3.).

$$X = \frac{D_1 \times a_0 \times 340{,}1 \times 2 \times P \cdot 100}{D_0 \times 460{,}1 \times 10 \times 20 \times 1000}$$

D_1 is the optical density of the tested solution;
D_0 is the optical density of the SS solution;
a_0 is the weight of the working standard sample of D-fructose-1,6-diphosphate, in mg;
V is the volume of the preparation taken for analysis, in ml;
P is the content of D-fructose-1,6-diphosphate sodium salt of the hydrate in the working standard sample, in %;
340.1 and 460.1 - molecular weight (D-fructose-1,6-diphosphoric acid) Fd PH_4.

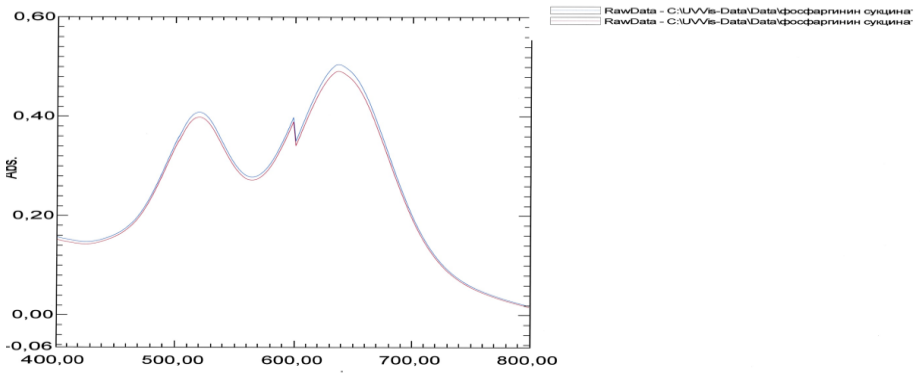

Fig. 3: Chromatogram of the "Phosfarginine succinate" infusion solution.

Table 5: The results of determining the quantitative content of fructose-1,6-diphosphoric acid in the infusion solution.

Series number	Weighed substance, g	Results		Metrological parameters	
		g	%		
0020120	0.5082 0.5087 0.5079 0.5082 0.5085	37.030 37.093 37.023 36.097 37.066	98.62 98.81 98.58 98.77 96.85	$X_{av.}$=98.3260 S^2= 0.6902 S= 0.8308 S_x= 0.3715 f= 4 P = 95%	t(P, f) = 2.78 ΔX = 2.3096 $\Delta X_{av.}$= 1.0329 Σ= 2.3489% Σ_{av}= 1.0505%
0030120	0.5081 0.5079 0.5080 0.5082 0.5081	36.036 36.029 37.030 37.044 37.037	96.48 97.12 96.76 96.15 97.19	$X_{av.}$=97.740 S^2= 0.1907 S= 0.4367 S_x=0.1953 f= 4 P = 95%	t(P, f) = 2.78 ΔX =1.214 $\Delta X_{av.}$=0.5430 Σ=1.2551% Σ_{av}=0.5613%
0040120	0.5087 0.5089 0.5087 0.5088 0.5089	37.621 37.623 37.623 36.627 37.629	99.33 98.31 98.30 99.34 99.38	$X_{av.}$=98.9320 S^2=0.3280 S=0.5727 S_x= 0.2561 f= 4 P = 95%	t(P, f) = 2.78 ΔX =1.5921 $\Delta X_{av.}$=0.7120 Σ=1.6093% Σ_{av}=0.7197%
0050120	0.5085 0.5083 0.5084 0.5082 0.5085	36.431 36.429 37.430 37.430 37.431	95.96 97.25 96.27 96.27 97.20	$X_{av.}$=96.59 S^2=0.3523 S=0.5936 S_x= 0.2655 f= 4 P = 95%	t(P, f) = 2.78 ΔX =1.6502 $\Delta X_{av.}$=0.7380 Σ=1.7084% Σ_{av}=0.7640
0060120	0.5078 0.5079 0.5080 0.5079 0.5081	36.233 36.234 37.232 37.234 37.232	95.22 96.24 97.23 95.21 94.25	$X_{av.}$=95.6280 S^2=1.3022 S=1.1411 S_x= 0.5103 f= 4 P = 95%	t(P, f) = 2.78 ΔX =3.1723 $\Delta X_{av.}$=1.4187 Σ=3.3174% Σ_{av}=1.4836%

The content of D-fructose-1,6-diphosphoric acid ($FdPH_4$) should be between 33.75 mg/ml and 41.25 mg/ml.

The study of the specific - antihypoxic activity of the new combined drug "Phosfarginin succinate" solution for infusion, produced by TEMURMEDFARM LLC, Uzbekistan.

According to the requirements of Good Laboratory Practice, the assessment of specific activity is mandatory for new types of combined drugs.

The antihypoxic activity of the drug was studied on a model of normobaric hypoxic hypoxia in experiments on 12 white mice weighing 20–22 g.[13,14] For the experiment, mice were divided into 2 groups of 6 heads - experimental and control. The experimental group of mice, for 3 days, once, was injected into the tail vein with the drug "Phosfarginine succinate", manufactured by LLC "TEMUR MED FARM", Uzbekistan at a dose of 1095 mg /kg, which is 0.3 ml. The control group of mice was intravenously injected with 0.9% NaCl solution. On the third day, 30 minutes after the administration of the drug, two mice were placed in sealed containers with a volume of 250 ml. At the same time, the time from the moment of placement in the dish to the cessation of breathing and death of the animal was recorded. The results were expressed as a percentage of the control, which was taken as 100%.

The obtained data were statistically processed using the STATISTICA program for Windows 95.[14]

RESULTS AND ITS DISCUSSION

The results obtained in the study of the antihypoxic effect showed that the drug "Phosfarginin succinate", manufactured by LLC "TEMUR MED FARM" on the model of normobaric hypoxic hypoxia, increases the resistance of mice to hypoxia: at a dose of 1095 mg/kg per body weight, it significantly lengthens the lifespan of animals by 23.6 %, i.e. mice that received the drug "Phosfarginine succinate" manufactured by TEMUR MED FARM LLC, Uzbekistan lived 11.5 ± 0.54 minutes, while in the control group of mice this figure was 9.3 ± 0.45 minutes. The data obtained are presented in table 5.

Table 5: Study results of the antihypoxic effect of the Phosfarginine succinate drug, TEMUR MED FARM LLC, Uzbekistan (M ± m).

Weight, g	Dose, mg/kg	Solution volume, ml	Duration life in hypoxia, min	Effect %
Control group				
20.5 ±1.05	-	0.3 ml	9.3 ± 0.45	-
Phosfarginine succinate, TEMUR MED FARM LLC, Uzbekistan				
20.6 ± 1.1	1095	0.3 ml	11.5 ± 0.54 P < 0.005	23.6

CONCLUSIONS

In recent years, a targeted search has been carried out for new drugs that have a complex effect in the treatment of cardiovascular diseases to enhance metabolic processes. One of these drugs is "Phosfarginine succinate" (consisting of D-fructose-1,6-diphosphate trisodium salt trihydrate, L-arginine hydrochloride, succinic acid) infusion solution - a tool that stimulates and regulates the metabolic process. The infusion solution technology was developed on the basis of Temur Med Farm LLC (Republic of Uzbekistan). In order to select a scientifically based composition, develop and optimize the technological process, the method of mathematical planning of the experiment was used - Latin plans. The application of the method of mathematical planning of the experiment made it possible to select the optimal composition and technology of the infusion solution "Phosfarginine succinate". Some qualitative characteristics of the infusion solution were also studied, which showed the good quality of the developed drug. The study of the specific antihypoxic activity of the infusion solution "Phosfarginine succinate" showed that the studied drug has a significant antihypoxic effect.

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