Original Article

ABOUT RESEARCHES OF TECHNOLOGY OF PREPARATION OF INFUSION SOLUTION "PHOSPHARGININE SUCCINATE"

Yusupova Shakhlo Bokhodirovna[1], Fayzullaeva Nodira Sultanovna[2], Maksudova Firuza Khurshidovna[3], Khaydarov Vosil Rasulovich[4]

[1]Assistant Of The Department Of Industrial Technology Of Medicines Tashkent Pharmaceutical Institute, Uzbekistan.
[2]Candidate Of Pharmaceutical Sciences, Associate Professor Of The Department Of Technology Of Dosage Forms Tashkent Pharmaceutical Institute, Uzbekistan.
[3]Doctor Of Pharmacy (Dsc), Associate Professor Of The Department Of Industrial Technology Of Medicines Tashkent Pharmaceutical Institute, Uzbekistan.
[4]Candidate Of Pharmaceutical Sciences, Professor Of The Department Of Industrial Technology Of Medicines Tashkent Pharmaceutical Institute, Uzbekistan.
*e-mail: yusupova.shahloxon@bk.ru

DOI: 10.47750/pnr.2022.13.S06.529

Abstract

To date, metabolic drugs are widely used in the treatment of cardiovascular diseases, and there is a great demand for these drugs in our republic. Model compositions and technology of solutions for infusions of Phospharginine succinate containing fructose-1,6-diphosphate, L-arginine hydrochloride and succinic acid have been developed, which are subsequently recommended for industrial production at the local enterprise “TEMUR MED PHARM” LLC. Theoretical calculations were carried out and the current osmotic concentrations (osmolarity, milliosmol (mosm)) and pH values of infusion solutions were established.

Key words: cardiovascular diseases, Phospharginine succinate, metabolic agent, anti-hypoxic agent, solution for infusions, composition, D-fructose - 1,6-diphosphate sodium salt trihydrate, L-arginine, succinic acid, sodium metabisulfite, stabilizer, water for injection, osmomolarity, pH.

Introduction:

According to statistics from the World Health Organization (WHO), in the last 20 years, cardiovascular diseases are still the leading cause of death, and this indicator is increasing every year [1]. Today, despite the improvement in the quality of medical care, even in socially developed countries, the growth of diseases such as hypertension, coronary heart disease, stroke is a great threat. Yesterday, under the leadership of the President of the Republic of Uzbekistan Sh.M Mirziyoyev at the medical council noted that 53% of deaths of the population of our republic aged 30-70 years are associated with cardiovascular diseases, and over the past 5 years this number has increased by 20%. Introduction of a new treatment system in solving problems related to heart diseases: early detection and effective treatment of the disease. To this end, citizens of our republic over 40 years of age annually undergo targeted examinations, and free medicines are distributed to low-income patients. 70 billion soums will be allocated from the state budget for the implementation of these goals, including 580 billion soums for the provision of basic medicines, which is 3 times more than the amount allocated for the current year [2]. In the treatment of cardiovascular diseases, a number of additional therapeutic agents are used to increase metabolism:

D-fructose-1,6-diphosphate is a metabolite in natural cells, and in myocardial ischemia and hypoxia, as a result of activation of phosphofructokinase and pyruvate kinase, increases the concentration of adenosine triphosphate and creatine phosphate in cells and activates the intake of potassium ions, resulting in increased energy metabolism in myocardial cells, tissue glucose is released into waste and tissue damage is reduced. Fructose diphosphate is used as an additional therapeutic agent for myocardial ischemia, angina pectoris and cerebral ischemic stroke.

With atherosclerosis, hypertension, diabetes mellitus and renal insufficiency, vascular activity is disrupted due to a lack of nitric oxide in the patient's body. L-arginine belongs to a number of interchangeable amino acids necessary for the body, this amino acid is synthesized in the body from glutamic acid and proline, the resulting L-arginine activates the synthesis of nitric oxide in the body, which supports vascular activity and fat metabolism at a normal level, stops platelet aggregation, stimulates the process of fibrinolysis.

As a biologically active additive, succinic acid is used for tissue ischemia, spasms, physical overstrain, stress, cardiovascular diseases in order to enhance the effect of the main drugs, reduce the dose and shorten the duration of treatment [3].

In the treatment of cardiovascular diseases, infusion solutions are used that have a metabolic and antihypoxic effect on a number of metabolic processes: Tivortin (L-arginine hydrochloride 42 mg/ml, “Yuria-Pharm” LLC, Ukraine), Neoton (Phosphocreatin for the preparation of an infusion solution of 1.0 g of lyophilized powder, Alfasigma S.p.A., Italy), Esaphosphine (fructose-1,6-diphosphate sodium salt, "Biomedica Foscama Group S.p.A.", Italy) [3, 4, 5].

To date, these drugs do not fully cover the needs of our republic, therefore, the expansion of the range of drugs that stimulate metabolism used in the treatment of cardiovascular diseases and their introduction into production are urgent issues.

The purpose of the work:

To develop the composition and technology of preparation of a solution for infusions of the metabolic preparation "Phospharginine succinate".

Experimental part

Materials and test methods.

As an object of research, taking into account the therapeutic effectiveness of drugs that metabolically activate and regulate metabolic processes in the treatment of cardiovascular diseases, in order to create the composition of an infusion solution of complex action "Phospharginine succinate", in accordance with the composition of the model mixture of substances is presented in a number of scientific literature [6, 7] (Table 1).

Water for injection, isotonic sodium chloride solution and 5% glucose solution were used as a solvent in the infusion solution of “Phospharginine succinate”. All solvents used for the infusion solution meet the requirements of the articles of the pharmacopoeia "Bacterial endotoxins" and "Pyrogenicity" [8, 9].

To ensure the stability of the infusion solution, auxiliary substances such as ascorbic acid, sodium hydrosulfite and sodium metabisulfite were used. The amount of sodium hydrosulfite and sodium metabisulfite stabilizers used did not exceed 0,2% [10].

The drugs and excipients included in the infusion solution "Phospharginine succinate" were dissolved in the solvents indicated in Table 1, taking into account their solubility. Infusion solutions prepared on water for injection were prepared by the mass-volume method, and infusion solutions obtained on isotonic 0,9% sodium chloride solution and 5% glucose monohydrate solution, the density of which differs from the density of water, were prepared only by weight. Infusion solutions of all model formulations were prepared under aseptic conditions on the basis of “TEMUR MED PHARM” LLC.

In addition to the general requirements for the quality of infusion solutions (sterility, absence of visible mechanical additives, pyrogenicity and stability). A number of additional requirements are also established: isotonicity, isohydricity, isoosmolarity and isoplasticity [8, 9]. In the course of the preliminary studies carried out, the theoretical and practical concentrations of the osmotic infusion solution "Phospharginine succinate" were studied [8]. The theoretical osmotic concentration in these model compositions was calculated by the equation:

$$C_{osm} = \frac{m}{M} \cdot n \cdot 1000 \qquad (1)$$

Here: C_{osm} – osmolality of the solution, milliosmol per liter (mOsm/l);

m – is the amount of substance in solution, g/l;

M – is the molar mass of the substance, g;

n – is the total number of ions formed from one dissolved molecule as a result of dissociation (n = 1 for substances not undergoing dissociation, n = 2, 3 for substances forming the corresponding number of ions during dissolution) [8, 11].

Effective osmotic concentration of the infusion solution "Phospharginine succinate", prepared according to the selected composition, was carried out cryoscopically using a laboratory osmometer and a Beckman thermometer, which allows monitoring temperature changes [8, 12].

The effective osmotic concentration of the infusion solution "Phospharginine succinate" was evaluated by cryoscopic determination of the depression of the freezing point of infusion solutions compared with the freezing point of a pure solvent. At the same time, 1 osmol per kilogram of water reduces the freezing point by 1,86°C.

This relation can be expressed by the following equation:

$$C_{osm} = \frac{(T_2 - T_1)}{K} \cdot 1000 \qquad (2)$$

Here: C_{osm} – osmolality of the solution, milliosmol per liter (mOsm/kg);

T_2 – is the freezing point of the powder solvent (C);

T_1– is the freezing point of the test solution (C);

K – is the cryoscopic constant of the solvent (for water: 1,86) [8].

1,858 - molar cryoscopic constant of distilled water - corresponds to a decrease in the freezing temperature as a result of the dissolution of 1 mole of a substance in 1 kg of water;

1000 – conversion coefficient of osm/kg to mosm/kg;

The pH values of solutions of model mixtures prepared according to the compositions given in Table 1 were determined by the potentiometric method [9].

Results and discussion:

Infusion solutions of “Phospharginine succinate” prepared according to model formulations were prepared under homogeneous conditions in the factory of “TEMUR MED PHARM” LLC, and the technological process consisted of the following stages:

• Auxiliary work 1. Preparation of industrial premises: cleanliness class B and local area A; Cleanliness in rooms of class C, D.

• Auxiliary work 2. Preparation of technological equipment, inventory and tools.

• Auxiliary work 3. Training of production workers.

• Auxiliary work 4. Preparation of raw materials and materials.

• Auxiliary work 4.1. Weighing of raw materials.

• Auxiliary work 4.2. Preparation of sterile containers.

• Auxiliary work 4.3. Preparation of sterile rubber stoppers

• Auxiliary work 4.4. Preparation of aluminum caps

• Auxiliary work 5. Water preparation.

• Technological process 1. Preparation of the infusion solution "Phospharginine succinate".

• Technological process 2. Filtration of the infusion solution "Phospharginine succinate".

• Technological process 3. Filling the infusion solution into vials, closing with rubber stoppers and capping with aluminum caps.

• Technological process 4. Visual control (control 1).

• Technological process 5. Sterilization.

• Technological process 6. Visual control (control 2).

• Packaging and labeling operations 1. Labeling.

• Packaging and labeling operations 2. Packaging.

Preparation of infusion solutions of "Phospharginine succinate" was carried out in hermetically sealed double-walled reactors equipped with a stirrer.

Model solutions of "Phospharginine succinate" were prepared according to the compositions given in Table 1, taking into account the solubility of the medicinal and auxiliary substances included in the composition. The prepared solutions were filtered and poured into 50 ml vials, then sterilized in a steam sterilizer at a temperature of 110 ± 1 °C with a pressure of 10 kPa for 45 minutes.

Table 1 The amount of medicinal and auxiliary substances included in the solution for infusions "Phospharginine succinate"

Name of the active substances and excipients	**Molar mass of the substance, g/mol**	**Osmolarity of the susbstance, mOsm/l**	**The amount of substances included in the composition, g**					
			1	2	3	4	5	6
D-Fructose-1,6-Diphosphate sodium salt	550,18	90,88	5,0	-	5,0	-	5,0	-
D-fructose-1,6-diphosphoric acid								

	340,12	110,26	-	3,75	-	3,75	-	3,75
L-Arginine hydrochloride	210,70	99,67	2,1	-	2,1	-	2,1	-
L-Arginine Glutamate	321,33	124,48	-	4,0	-	4,0	-	4,0
Succinic acid	118,09	16,94	0,2	0,2	0,2	0,2	0,2	0,2
Glucose monohydrate 5% solution	180,16	277,53	up to 100 ml	-	-	up to 100 ml	-	-
Sodium chloride 0.9% isotonic solution	58,44	308,01	-	up to 100 ml	-	-	up to 100 ml	-
Water for injection	18,01	-	-	-	up to 100 ml	-	-	up to 100 ml
Sodium metabisulfite	190.11	1,052	-	-	0,01	-	-	0,01
Sodium hydrosulfite	104.06	1,922	-	0,01	-	-	0,01	-
Ascorbic acid	176,13	5,68	0,1	-	-	0,1	-	-
Theoretical osmotic concentration of ingredients, mOsm/l		∑ SM OSM, norm > 285-310	490,7	561,6	208,5	534,9	517,4	252,7
pH indicators of model mixed solutions		Norm 3,5-4,0	4,5	5,0	3,5	3,0	5,0	4,5

Calculation of theoretical osmotic concentration (osmolarity, milliosmol (mOsm)) the substances included in the infusion solutions of the model mixture were carried out as follows:

$C_{mosm\ D\text{-}Fructose\text{-}1,6\text{-}salt\ of\ sodium\ phosphate}$ = (50,0:550,1834) x 1 x 1000 = 90,88

$C_{mosm\ D\text{-}fructose\text{-}1,6\text{-}diphosphoric\ acid}$ = (37,5:340,116) x 1 x 1000 = 110,256

$C_{mosm\ L\text{-}Arginine\ hydrochloride}$ = (21,0: 210,70) x 1 x 1000 = 99,67

$C_{mosm\ L\text{-}Arginine\ Glutamate}$ = (40,0: 321,33) x 1 x 1000 = 124,48

$C_{mosm\ Succinic\ acid}$ = (2,0: 118,09) x 1 x 1000 = 16,94

$C_{mosm\ Sodium\ chloride}$ = (9,0: 58,44277) x 2 x 1000 = 308,008

$C_{mosm\ Glucose\ monohydrate}$ = (50,0: 180,16) x 1 x 1000 = 277,53

$C_{mosm\ Sodium\ metabisulfite}$ = (0,1: 190,11) x 2 x 1000 =1,052

$C_{mosm\ Sodium\ hydrosulfite}$ = (0,1: 104.061) x 2 x 1000 = 1,922

$C_{mosm\ Ascorbic\ acid}$ = (1: 176,13)x 1 x 1000 = 5,68

The results of calculating the theoretical osmotic concentration of model mixtures of the infusion solution "Phospharginine succinate" showed that when using 5% glucose monohydrate and 0.9% isotonic sodium chloride solutions as solvents, infusion solutions created a hyperosmolar concentration (content 1, 2, 4, 5), it was also found that when using water for injection as a solvent a hypoosmolar solution was formed (compositions 3 and 6).

Also, according to the data presented in the literature, the environment should be in the pH range of 3-4 in order to ensure the stability of the infusion solution "Phosphargine succinate" under the influence of high temperatures during the sterilization of D-fructose-1,6-diphosphate sodium salt and D-fructose-1,6-diphosphoric acid[9]. Therefore, in further studies, the infusion solution "Phospharginine succinate" was chosen, since, despite the fact that the theoretical osmolar concentration of composition number-3 is hypoosmolar – 208,5 mOsm/l, it has an optimal pH value of 3,5 and ensures the stability of medicinal substances in solution during sterilization.

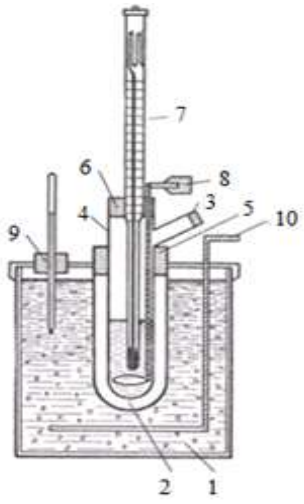
In further studies, the effective osmotic concentration of the infusion solution "Phospharginine succinate" prepared according to the selected composition was determined by the cryoscopic method.

The essence of the definition:

Using a laboratory cryoscope, the freezing point of the studied infusion solution was determined (Fig. 1).

The laboratory cryoscope is a thick-walled Bunsen glass (1), in which a cooling mixture is placed, and an empty glass (2) with an average volume of air. Between them there must be an air gap for uniform cooling of the test solution. A tube (3) and a test tube (4) are placed inside the cup-case in a small side part, which is the main part of the device. A rubber gasket (5) attaches the cups to each other, this device is called a cryoscope. A sample of 28-30 g of the investigated infusion solution is placed through a tube on the side of the tube. The test tube for test tubes is tightly closed with a cork stopper of the upper part (6), and from the opening of this stopper, the Beckman thermometer (7) and part of the label are lowered into the melt using a stirrer (8) with an annular loop (the lower part of the thermometer is inserted into the ring of this stirrer). During the freezing of the solution, the mixer moves from top to bottom, which allows you to accurately measure the temperature of the test solution.

Figure 1. Laboratory cryoscope.



Beckman's metastatic thermometer differs from the usual one in that the upper part of its capillary is connected to an additional reservoir in which part of the mercury can be transferred from the lower reservoir to the upper one. The less mercury remains in the lower container, the higher the temperature in the capillary will be. Another positive feature of this thermometer is the large volume of mercury, as a result of which the large scale is divided into 5-6 degrees, which allows measurements to be made with an accuracy of 0,002 degrees.

The assembled cryoscope device is placed in a thick-walled Bunsen cup filled with a freezing mixture, in which a thermometer (9) and a stirrer (10) are additionally placed. (Figure 1). The temperature of the freezing mixture (a mixture of ice and sodium chloride) is constantly maintained at a temperature level 3-4 °C below the freezing point of the analyzed solution, and the accuracy of temperature measurement should be at least 0.01 degrees [8, 11, 12]. The point "0" of the device was set using water for injection.

The freezing point of the infusion solution was calibrated using standard reference solutions of sodium chloride (Table 2).

When determining the freezing point of the infusion solution, the mercury column in the thermometer begins to fall as the liquid cools. Usually, before freezing, the solution is supercooled and the temperature of the liquid drops below the freezing point. When the crystallization process begins, the temperature of the solution rises above the freezing point. The temperature increase occurs due to the release of the open heat of solidification.

Table 2 Indicators of standard sodium chloride solutions prepared to determine the degree of freezing of the infusion solution "Phospharginine succinate"

Mass of sodium chloride in 1 kg of water, g	Osmolarity determined by measurement, mosm / kg	Theoretical osmolarity, mosm / kg	Molar osmotic coefficient, F, mOsm/kg	Decreasing freezing temperature, degrees, C
3,087	100	105,67	0,9463	0,186
6,260	200	214,20	0,9337	0,372
9,463	300	323,83	0,9264	0,558
12,684	400	437,07	0,9215	0,744
15,916	500	544,66	0,9180	0,930
19,147	600	655,24	0,9157	1,116
22,380	700	765,86	0,9140	1,302

After that, the test tube is removed from the liquid, the crystals are melted by heating the test tube with your hand, and the determination is repeated again. The experiment is carried out three times. The difference between the definitions should not exceed 0,01 °C. In case of hypothermia, a solvent crystal must be added to the liquid. The detection accuracy in this technique is +5%. Before each measurement, the test tube is washed with the infusion solution obtained for the study, and the test samples are also measured. If the value obtained for calibration solutions is within two values of the calibration scale, the device is ready to measure readiness for the test. To reduce the error and verify reproducibility, it is recommended to repeat measurements with several samples from the same sample, averaging the results. The measurement error should not exceed 2%. The actual osmolarity of the phosphate arginine succinate infusion solution is calculated according to equation 2, and the results are presented in Table 3.

Table 3 Results of determination of the effective osmotic concentration of the infusion solution "Phospharginine succinate"

mOsm/l	C %	Metrological description	
204,5	98,08	Xcp=97,41	ΔX=3,7262
207,2	99,38	S^2=1,7966	ΔXcp=1,6664
200,0	95,94	S=1,3404	E,%=3,87253
202,0	96,90	Sx=0,5994	E,%cp=1,7107
201,7	96,75	t(95%,4)=2,78	

Conclusion:

As a result of the experiments conducted on the basis of “TEMUR MED PHARM” LLC, a model composition and technology of an infusion solution of “Phospharginine succinate” was developed, which is recommended as a stimulating and regulating metabolism in vascular diseases, and also the theoretical (208.5 mOsm) and actual osmotic concentration of solutions (osmolarity, milliosmoles (mOsm) and pH indices) were evaluated.

Literature:

1. Bezverkhov A.A., Ishchenko O.Yu. Dynamics of mortality from cardiovascular diseases for the period 2015-2019//Norwegian Journal of development of the International Science.- №54.-2021.- pp. 35-38.
2. Meeting on medicine on mortality of the population of the Republic of Uzbekistan from cardiovascular diseases. - Tashkent, March 2022. [upl.uz›obshestvo/22347-news.html] (accessed 10.12.2022).
3. Electronic reference book of medicines VIDAL 2021.- M: Vidal Rus.- 2021.- 1120 p. ISBN 9785604443828. Access mode: http://www.vidal.ru (accessed 17.12.2022).
4. Mashkovsky, M D. Medicines. In two volumes. Ed. 15th / M. D. Mashkovsky. - M. "New Wave". - 2005. - 1200 p.
5. State Register. Medicines, medical devices and medical equipment approved for use in medical practice.- Ed. No. 25, revised and supplemented, as of January 2021 – Tashkent, 2021.- 1067 p.
6. Kovalskaya G.N. Drug assistance: combined infusion pharmacotherapy. / G.N. Kovalskaya / New Pharmacy. - 2004. - No. 10. - pp. 25-36.
7. Osmolovsky A.N. Infusion therapy Textbook / Osmolovsky A.N. - Vitebsk: VSMU, 2010. - 106 p. ISBN 978-985-466-444-6.
8. State Pharmacopoeia 13 edition volume 1. OFS.1.2.1.0003.15. Osmolarity. Access mode: https://nevacert.ru/files/pharmacopoeia_v2/216.pdf (accessed 12/16/2022).
9. 9. State Pharmacopoeia of the USSR, XI issue, volume 1.- M.: "Medicine", 1987.- p. 113.
10. Yusupova Sh.B., Abdunazarov A.I., Khaidarov V.R., Kasimov E.R. Study of the specific activity of the drug “Phosfarginine succinate” solution for infusions//Infection, immunity and pharmacology.-№1.- 2022. pp. 327-331.
11. Yusupova Sh.B., Khaidarov V.R., Abdunazarov A.I. Inyektsion eritmalar. Phosforginine succinate va PC amantadin infuzion eritmalarini ishlab chikarish technologysi.- Monograph.-Tashkent: "Iqtisodiyot", 2020. - p.125.
12. Composition, technology and quality control of amantadine-based infusion solution// Qosimov A.D., Yusupova Sh.B., Haydarov V.R., Abdunazarov A.// Pharmaceutical Journal, No.4, 2018.-B. 56-60.