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Optimization of technological parameters for the preparation of a lyophilized hydroxycobalamin dosage form

Abdullaeva N.K., Pshenichnov E.A., Kondrasheva K.V.

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Abstract

Preparation of the composition of excipients and modes for the production of the drug allows the use of hydroxocobalamin chloride, in the form of a freeze-dried level. The main physicochemical characteristics of the lyophilisate are investigated.

Keywords: hydroxocobalamin chloride, D-mannitol, lyophilisate, freezing modes.

INTRODUCTION

Currently, drugs based on cyanocobalamin metabolites: methylcobalamin and hydroxocobalamin are widely used in medical practice [1, 2]. They are chemically active substances that enter into biochemical processes.

However, the search for the optimal dosage form with a minimum number of stabilizers and preservatives, which will preserve the therapeutic effect of the vitamin B12 metabolite.

The purpose of the study: selection of the composition of excipients, modes for the treatment of lyophilization, obtaining gyroxocobalamin and studying the properties of the obtained lyophilisate.

MATERIALS AND METHODS

Production "ApexMedichem Ltd." (India), D-mannitol production "ShandongTianliPharm. Production of industrial substances hydroxocobalaminachloride (GKB). ”Co. Ltd. "(China) and polyvinylpyrrolidone (PVP) manufactured by Merck (Germany), as well as chemicals from Sigma-Aldrich (USA) and Hi-Media (India).

Sample preparation for isolation: 1 ml solutions are dispensed in 5 ml vials (glass containers class: HC-1) for the production of Neo-Tech-Plast (Republic of Uzbekistan). Lyophilization of samples and corking of bottles with a lyophilizate of bromobutyl caps with combined aluminum caps are carried out on automated Tofflon conveyor equipment (China).

Conditions for lyophilization: shelves of lyophilic drying were kept at –50 ° C and pressure in ¬mere 0.01 mbar for 5 hours. Heated shelves in modes:
from –50 ° C to –10 ° C with a speed of 10 ° C / h;
from –10 ° C to 0 ° C with a speed of 3 ° C / h;
from 0 ° C to +25 ° C with a speed of 5 ° C / h.

After achieving a temperature of + 25 ° C (pressure in a chamber of 0.01 mbar), it kept at this temperature for 4 hours. Lyophilization lasted 22 hours.

Tests of the physicochemical properties of the finished product and quantitative determination of the active substance were determined in the chemical laboratory of the Quality Control Department of the pharmaceutical plant of the Jurabek Laboratories JV (Republic of Uzbekistan) in accordance with the requirements of the European Pharmacopoeia [4].

The appearance of the lyophilisate was determined visually. The solubility of the substance and lyophilisate was determined visually, fixing the time of dissolution with a stopwatch. A sufficient amount of water for injection was

was added to a sample of the substance (1 g) or to the contents of the vial, and it was shaken for 3 min.Determination of mass loss during drying was carried out by drying the preparation in a vacuum oven over P2O5 at room temperature and a residual pressure of 5 mm Hg. to constant weight. The mass loss in all the samples studied did not exceed 3.0%.The transparency of the solution was determined by comparing the test fluid with the solvent visually.The pH of the aqueous solution of the drug was determined potentiometrically using a Mettler Toledo instrument (USA). Quantitative determination of hydroxocobalamin was performed by HPLC on an Agilent Technologies (Germany) instrument.

RESULTS

Hydroxocobalaminachloride is a water-soluble metabolite of vitamin B 12. According to EurPh. 9.0hydroxocobalamin is sparingly soluble in water (1: 10-30) [4] and practically insoluble in a number of organic solvents [5] used for lyophilization. Taking into account the above, as well as the fact that the developed product is planned to be produced in the form of a lyophilisate for the preparation of a solution for injection, the only suitable solvent is water for injection.

In order to optimize the composition, increase the solubility rate and preserve the properties of the drug, it was necessary to choose the most suitable cryoprotectant. D-mannitol and polyvinylpyrrolidone (PVP), both highly soluble in water, have been proposed as auxiliary substances [4].

To select the optimal composition of the lyophilisate, 6 variants of mixtures with different contents of auxiliary components were prepared in accordance with table 2. A mixture of hydroxocobalamin chloride and an auxiliary substance was dissolved in water for injection (30 ° C) with stirring using an IKA RST basic magnetic stirrer (IKA, Germany) (500 rpm for 35 min).Selection of the freezing mode of a solution of hydroxocobalamin chloride. To obtain a stable form of the preparation, the resulting mixtures were dissolved in water and lyophilized. Given that the speed of freezing can affect the quality of the lyophilisate, it was first necessary to determine the most appropriate mode of freezing the solution (fast or slow).

For each freezing regime, 5 series of each hydroxocobalamin composition with auxiliary components of 5 samples in each series were used. Samples were poured into vials, loaded onto freeze-dried shelves at a temperature of + 22 ... + 25 ° C, and they were frozen to –50 ° C using technology ¬Check schemes shown in Table 1. Freezing diagrams are presented in Figure 1.

Table 1: Scheme for freezing solutions of hydroxocobalamin chloride with a cryoprotectant

Slow freeze			Quick freeze		
Temperature, °C	**Time, h**	**Excerpt**	**Temperature, °C**	**Time, h**	**Excerpt**
to –25	1 hour	2 hours–25°C	to –25	1 hour	Absent
to −35	1 hour	2 hours –35°C	to −35	1 hour	Absent
to −45	1 hour	2 hours –45°C	to −45	1 hour	Absent
to –50	1 hour	5 hours –50°C	to –50	1 hour	5 hours –50°C

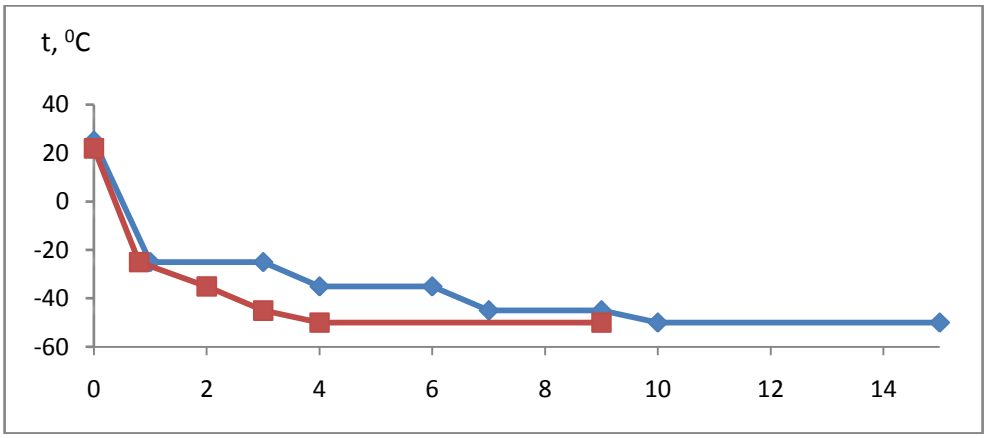

Figure 1: Freeze Charts

Blue line – Slow freeze
Red line – Quick freeze

Frozen solutions were lyophilized. At the end of the lyophilization process, the effect of the freezing regimen was evaluated according to the following indicators (Table 2, 3):

1. The first definition - appearance, lyophilization, solubility in water for injection, the transparency of the solution;
2. The second definition is the loss in mass upon drying, pH, quantitative content.

Table 2: Compositions and quality indicators of lyophilisates (with quick and slow freezing)

Bap.	Composition per 1 vial, in mg	Appearance	Solution transparency and solubility in water for injection
№1	HCB – 10 PVP – 80	Pink porous mass	Opaque solution, contains insoluble particles after vigorous shaking with water for injection for 3 minutes
№2	HCB – 10 ПВП – 100		
№3	HCB – 10 PVP – 120		
№4	HCB – 10 D-mannitol-80		Clear solution after vigorous shaking with water for injection for 1 min
№5	HCB – 10 D-mannitol-100		Clear solution after vigorous shaking with water for injection for 3 min
№6	HCB – 10 D-mannitol-120		Clear solution after vigorous shaking with water for injection for 3 min

Table 3: Quality indicators of lyophilisates containing D-mannitol (with quick and slow freezing)

Indicators	Freeze Mode / Options					
	Slow			**Quick**		
	№4	**№5**	**№6**	**№4**	**№5**	**№6**
Lost in mass with drought,%	2,5±0,2	2,4±0,2	2,6±0,2	2,7±0,2	2,5±0,2	2,4±0,2
pH	6,5±0,05	6,8±0,05	6,2±0,05	6,3±0,05	6,6±0,05	6,7±0,05
Quantity of HCB, mg / fl	9,96±0,2	9,96±0,2	9,94±0,2	9,92±0,2	9,94±0,2	9,93±0,2

The lyophilizate obtained in all six variants of the solutions represented a mass of pink in both modes of freezing. After recovery of the lyophilisates of options No. 1-3 (with polyvinylpyrrolidone) were opaque solutions containing insoluble particles. These formulations do not meet the EurPh 9.0 [4] requirements for injectable dosage forms in terms of “Transparency” and “Solubility”. Compositions with D-mannitol as a cryoprotectant when reconstituted with a solvent form a clear solution, and the minimum concentration of the component reduces the solubility time of the lyophilisate. In this regard, a further quality assessment was carried out with options No. 4-No. 6 (Table 3).

Analyzing the data of table 3, it should be noted that the quality indicators of lyophilisates obtained from a mixture of hydroxocobalamin chloride and D-mannitol are practically independent of the solution freezing scheme.

CONCLUSIONS

1. The obtained data of the five irrelevant experiments for each and every one and the mode of fasting was done, which, as a matter of fact, was possible right now, it’s time to use D-mannitol.
2. Lyophilisates using D-mannitol as a cryoprotectant at concentrations of 0.080; 0,100; 0.120 g per 1 vial corresponded to the required quality indicators. The recovery time of the lyophilisate containing 0.080 g / fl excipient was the shortest. In this regard, for further research, a composition of hydroxocobalamin chloride with D-mannitol at a concentration of 0.080 g / fl.
3. The freezing regimen does not significantly affect the quality of the lyophilisate. A quick freeze mode is most preferred due to the saving of the production cycle time.

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