ORIGINAL ARTICLE

Selection of technological parameters for the preparation of the combined oral solution Maglycimet™

Výběr technologických parametrů pro přípravu kombinovaného perorálního roztoku Maglycimet™

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Summary

The problem of magnesium deficiency is an urgent issue of modern humanity. The population of many countries has a tendency to reduce the intake of this element in the daily diet. Its level decreases due to stress, inappropriate lifestyle, increased excretion from the body because of influence of medicines, etc. Therefore, creation of magnesium-containing medicines is a perspective scientific area. Currently at the pharmaceutical market, combination drugs with a multi-therapeutic effect are preferred. We have developed a technology for the preparation of the combined oral solution named Maglycimet™, which consists of the following active pharmaceutical ingredients: magnesium aspartate, magnesium glutamate, glycine, methylcobalamin. Technological parameters of the solution preparation were established. Temperature modes and the loading order of main and auxiliary substances were studied and determined. The valuable point of the preparation of this medicine was to obtain the salts magnesium aspartate and magnesium glutamate directly in the reaction mixture. Based on the theoretical and experimental studies, technological parameters for the preparation of these salts were chosen. In this experimental study, the main indicators of the quality of the solution at the preparation stage were evaluated: organoleptic properties, pH, density, quantitative content of magnesium, glycine, methylcobalamin. The results indicated the significance of further research for subsequent industrial production of the developed medicine by Ukrainian manufacturers.

Key words: magnesium deficiency • magnesium salts • technology • oral solution

Souhrn

Problém nedostatku hořčíku je naléhavou otázkou moderního lidstva. Populace mnoha zemí má tendenci snižovat jeho příjem v denní stravě. Pokles jeho hladiny je způsoben stresem, špatným životním stylem, zvýšeným vylučováním z těla v důsledku užívání některých léků atd. Proto je vývoj přípravků obsahujících hořčík perspektivní vědeckou oblastí. V současné době se dává přednost kombinovaným přípravkům s více terapeutickými účinky. Vyvinuli jsme technologii pro přípravu kombinovaného perorálního roztoku pod názvem Maglycimet™, který se skládá z následujících účinných farmaceutických složek: magnesium-aspartát, magnesium-glutamát, glycin, methylkobalamin. Byly stanoveny technologické parametry přípravy roztoku. Byly studovány teplotní režimy a pořadí zapracování účinných a pomocných látek. Důležitým bodem přípravy tohoto léku byl zisk solí magnesium-aspartátu a magnesium-glutamátu přímo z reakční směsi. Na základě teoretických a experimentálních studií byly zvoleny technologické parametry pro jejich přípravy. V této experimentální studii byly hodnoceny hlavní ukazatele kvality roztoku v přípravném stádiu: vzhled, pH, hustota, kvantitativní obsah hořčíku, glycinu a methylkobalaminu. Výsledky naznačují nezbytnost dalšího výzkumu pro následné zavedení přípravku do průmyslové výroby ukrajinskými výrobci.

Klíčová slova: nedostatek hořčíku • hořečnaté soli • technologie • perorální roztok

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Introduction

Magnesium is one of the most important macroelement of the human body. It regulates the state of

cell membranes and participates in trans-membrane transport of sodium and calcium ions. Also magnesium ions take part in many metabolic reactions connected with the formation, accumulation, transfer and utilization of energy, free radicals and their oxidation products, neurochemical transmission and other functions[1]. However, nowadays the population of many countries has an insufficient magnesium intake and content in the body[2–5]. As a result, well-known symptoms of this element deficiency occur: irritability, fatigue, insomnia, apathy, muscle spasms and cramps, dizziness, nausea, numbness. Further magnesium deficiency can lead to more serious health problems such as cardiovascular diseases, diabetes, osteoporosis, infertility. That is why it is necessary to take magnesium-containing medicines in order to return the amount of magnesium back to normal.

Combined medicines contain two or more active pharmaceutical ingredients. Their advantages include: polycomponent pharmacological action, rational combination of composition and dose, ease of usage, and economic benefits. Examples of combined magnesium-containing medicines at the Ukrainian pharmaceutical market are as follows: Magne-B_6® in the form of an oral solution (magnesium lactate dihydrate, magnesium pidolate, pyridoxine hydrochloride), Asparkam® in the form of tablets and injection solution (potassium aspartate, magnesium aspartate), Cardioargin-Zdorovye® in the form of syrup and injection solution (arginine aspartate, diarginine succinate, magnesium aspartate, potassium aspartate) and others[6]. Also multivitamin medicines belong to this group, for example, Vitacap®, Supradin®, Vitrum®. The largest part of the combined magnesium-containing medicines, proposed for the Ukrainian consumer, contain in their composition only one magnesium compound with other active ingredients[6–7].

Nowadays at the pharmaceutical market of Ukraine, magnesium-containing medicines are presented as the following dosage forms: nearly 60% – tablets, 24% – injection and infusion solutions, 15% – other forms (powders, suspensions, solution for internal use)[7]. Due to the fact that there is no domestic magnesium-containing combined medicine in the form of an oral solution with stress-protective activity, and only one is imported, we have developed a technology for producing a combined oral solution with magnesium salts, glycine and methylcobalamin. The composition of the medicine includes magnesium aspartate and magnesium glutamate, which were selected based on the scientific literature and exploratory pharmacological studies[8, 9]. According to the structure they are chelated complexes of magnesium and amino acids and provide high bioavailability of magnesium. Elements in chelates often possess thousand-fold higher activity compared to the activity of the metal in the ionic state. Also, chelate complexes are practically non-toxic, well dissolved in water, they are not destroyed by microorganisms, and show stability in a wide range of pH values.

The aim of the research was to select the technological parameters for the preparation of a combined oral solution based on magnesium salts with glycine and methylcobalamin with stress-protective activity named Maglycimet™.

Experimental part

Materials

Following initial substances and active ingredients were used in the process of the composition and technology development: for the magnesium aspartate preparation – magnesium oxide (Wuqiang Liche Opto Co., Ltd., China) and L-aspartic acid (Shanghai Synnad Fine Chemical Co., Ltd., China); for magnesium glutamate – magnesium oxide (Wuqiang Liche Opto Co., Ltd., China) and L-glutamic acid (Hezhong Biochemical Manufacture Co., Ltd., China); glycine (Tessenderlo Chemie NV, Belgium); methylcobalamin (Ningxia Kingvit Pharmaceutical Co., Ltd., China). All the initial components met the requirements of the State Pharmacopoeia of Ukraine (SPhU)[10] and/or the European Pharmacopoeia (EP)[11]. Methylcobalamin met the requirements of the Japanese Pharmacopoeia[12]. Sodium metabisulfite (AppliChem, Germany) was added as an antioxidant. Sodium saccharinate (Cuzhou Chemicals Co., Ltd., China) was used as a sweetener and potassium sorbate (Makrohim, Ukraine) as a preservative. Their quality corresponds to the requirements of the EP[11]. Food flavoring agents «cherry», (Aroma-Gold, Ukraine) corresponded to TU U 15.8-23788752-001-2001 (Tehnicheskie Usloviya Ukrainyi – Technical Conditions of Ukraine). Purified water obtaining according to the requirements of EP served as solvent[13].

Methods

The object of the investigation is the process of the preparation of the combined oral solution Maglycimet™.

In the pharmaceutical development of oral solution organoleptic, physicochemical methods were used according to the requirements of SPhU[10] and EP[11]. Using the potentiometric method (SPhU, EP, 2.2.3.), the pH of the solution was measured and the completeness of the reaction was monitored. For this, a pH-meter Mi-180 («MARTINI instruments», Romania) was used. The density of the solution (SPhU, EP, 2.2.5.) was determined using a pycnometer. Quantitative determination of glycine was performed by HPLC method (SPhU, EP 2.2.29.). 2,4-dinitrofluorobenzene was used as a modifier. Chromatography was performed on a liquid chromatograph «Waters» (USA) with a UV detector, a column Symmetry C18 (3.9 × 150) mm, size of particles 5 μm. Quantitative determination of methylcobalamin was performed by HPLC method (SPhU, EP 2.2.29.). Chromatography was performed on a liquid chromatograph brand «Waters» (USA) with a UV detector under the following conditions: a chromatographic column (250 × 4.6) mm in size with octadecylsilyl silica gel (C18), size of particles 5 μm. Quantitative determination of magnesium was performed by the complexometric method (SPhU, EP, 2.5.11.). We used an electronic scales PA 194C («OHAUS», China) and measuring dishes of class A. During the research, all

laboratory and analytical equipment, which have passed metrological certification, were used.

Results and discussion

A medicinal preparation is a product of pharmaceutical science that has a certain composition, dosage form, packaging, expiration date. It is intended for administration into the human or animal body for the purpose of diagnosing, treating or alleviating the symptoms of the disease or changing the state of the physiological functions of the body, as well as for diseases prevention[14]. A medicine should correspond to a number of requirements: it must be effective, safe, non-toxic, must have an accuracy of dosing of active substances, and maintains its stability during storage.

We have developed the composition of the combined oral solution Maglycimet™ based on the magnesium aspartate and magnesium glutamate. Organic derivatives of magnesium and amino acids from the chemical point of view are special compounds, which are called chelate complexes. They exhibit low toxicity and mostly good solubility in water. These complexes are not destroyed by microorganisms and stay stable in a wide range of pH values. Moreover, they effectively increase the bioavailability of metal captured inside[15]. Amino acid glycine and vitamin B_{12} – methylcobalamin – were additionally introduced into the composition. Glycine readily penetrates inside body tissues (including the brain tissue) and is well metabolized. It acts as a neurotransmitter in the brain and spinal cord, participates in reflex coordination, sensory signal processing and pain, normalizes and activates the inhibition processes in the central nervous system, improves the metabolic processes in tissues. It exhibits an antioxidant, anti-toxic effect, reduces psycho-emotional stress, improves mood, etc. In the developed medicine, methylcobalamin was chosen as an active pharmaceutical ingredient due to its valuable properties and the ability to regenerate and protect the nervous tissue. The justification for the selection of active pharmaceutical and auxiliary ingredients is described in the article[16]. The qualitative and quantitative composition of the solution is given in Table 1.

To obtain magnesium salts (aspartate and glutamate) directly during the process of solution production is an important advantage of this medicine preparation. This fact allows avoiding several steps in its manufacture and subsequently reduces its production costs[17, 18].

Aspartic and glutamic acids are dicarboxylic aliphatic amino acids. Therefore, the reaction of obtaining salts with bivalent magnesium can proceed through one or two carboxyl groups, depending on the selected reaction conditions[19]. The salt formation reaction of magnesium aspartate, which was carried out for the developed drug, is presented in Figure 1.

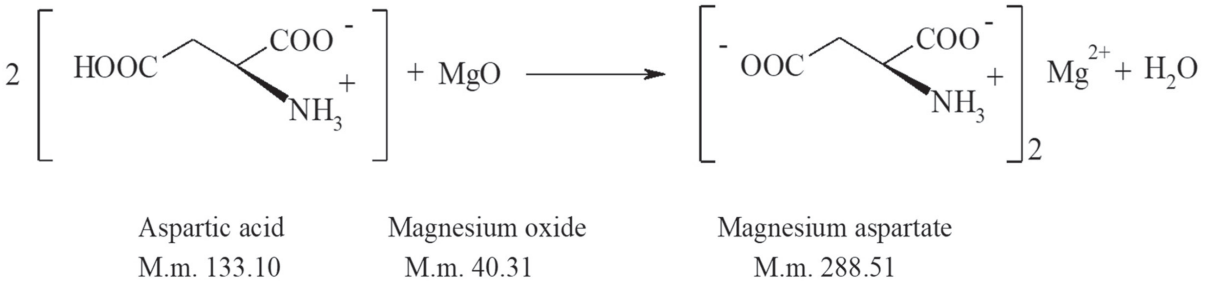

Fig. 1. The reaction of magnesium aspartate obtaining

Table 1. Composition of the oral solution

Substance	Quantity, g/100 ml	Role
Magnesium aspartate	8.313	Active ingredient
Magnesium glutamate	3.932	Active ingredient
Glycine	1.000	Active ingredient
Methylcobalamin	0.00025	Active ingredient
Sodium metabisulphite	0.150	Antioxidant
Sodium saccharinate	0.100	Sweetener
Potassium sorbate	0.150	Preservative
Flavoring agent «cherry»	0.600	Flavoring agent
Purified water	Up to 100 ml	Solvent

According to the equation, aspartic acid reacts with magnesium oxide in a ratio 2 : 1. The formation of magnesium glutamate proceeds similarly. We took these data into account when calculating the quantitative composition of the developed medicine.

In order to obtain a high-quality and stable solution, we needed to choose optimal temperature conditions and reaction time for the production of the salts. We investigated the temperature regimes for magnesium aspartate and magnesium glutamate creation in an aqueous medium. Experimental data are presented in Table 2.

It was experimentally established that maintaining the temperature regime of the reaction significantly affected the process of salts formation. Therefore, the reaction mixture had to be maintained at a temperature of 100 °C for 10–15 minutes. At lower temperatures, the reaction course was not completed. The solution pH reaching in the range between 5.0–6.0 was considered as the criterion for the reaction termination. If necessary, pH correction with amino acid is possible.

Similar results were obtained in the study of magnesium glutamate: the complete dissolving of the input ingredients occurred at 100 °C after an interval of 10–15 minutes. The pH of the resulting transparent solution of magnesium salts of aspartic and glutamic acid reached the value of 5.3 ± 0.3.

One of the possible reasons of amino acids instability is their oxidation by atmospheric oxygen[20, 21]. In this case an oxidative deamination reaction probably undergoes[22, 23]. As a result of this reaction, the amino acid is converted to the corresponding keto acid and ammonia is released. To prevent such processes, the antioxidant sodium metabisulphite has been chosen and added to the solution immediately after obtaining the magnesium salts of amino acids. Since, at a temperature above 60 °C, the antioxidant can decompose with the release of sulfur dioxide[24], it was loaded after cooling the solution to a temperature 30 ± 5 °C.

Glycine is the simplest aliphatic amino acid[25, 26]. Like all amino acids, glycine is an amphoteric compound with a bipolar ionic structure. It exhibits strong pH dependence of the charge. In a strongly acidic medium it exists predominantly in the cationic form, and in the strongly alkaline form, in the anionic form. Glycine isoelectric point derived from the inflection point of its titration curve is 5.97. This point determines the conditions (acidity of the solution) under which almost all amino acid molecules exist in the form of zwitterions[27]. These simultaneously have both ionic states (ammonium a positively charged cationic group, a negatively charged anionic carboxyl group) and their net static charge is zero.

The amino acid glycine was added to the prepared solution of magnesium salts with sodium metabisulphite at pH 5.2 ± 0.5 and a temperature of 30 ± 5 °C.

Methylcobalamin is a bioavailable and metabolically active coenzyme form of B_{12} group vitamins. An important factor of the vitamin stability is the optimal pH, which ranges in an interval from 4 to 6[28]. This information was taken into account for technology developing. Vitamin was added to the solution after all other active pharmaceutical ingredients and sodium metabisulphite, eliminating a sharp shift in pH values. The pH value before adding B_{12} was 5.2 ± 0.5, which is in the range ensuring the substance stability. Separately, it should be noted that methylcobalamin is a photosensitive compound[28]. This should be taken into account when choosing packaging (primary, secondary) and determining the storage conditions of the medicine.

In order to produce a solution with pleasant organoleptic characteristics, saccharin sodium as a sweetener and «cherry» flavoring agent were added. Saccharin sodium is a thermally stable and hydrolysis-resistant compound. However, if the sweetener is heated for a long period of time, it is possible that its imide ring will split, and sweetness will be decreased[29]. This was taken into account when introducing a sweetener into the medicine. The «cherry» flavoring agent is a thermo-stable substance with good solubility in water.

One of the main factors of the drug quality is the preservation of its microbiological stability during storage[30]. Since our medicine is an oral solution in a multi-dose vial of 100 ml, a preservative should be added to the solution. As a result of microbiological research, potassium sorbate was chosen as a preservative. On the one hand, the range of stability of this preservative is quite wide – pH from 2 to 6.5[31]. On the other hand, potassium sorbate is a thermally unstable compound[32]. Therefore, it was introduced into the solution at a temperature of 25 ± 5 °C.

Table 2. The effect of temperature and mixing time on the salt formation process (the data for reaction of magnesium aspartate creation)

Ingredients' loading order	№	Temperature (°C)	The time of mixing, minutes	Observations
Purified water L-aspartic acid Magnesium oxide	1.	40	15–20	turbid, the precipitate was present
			20–30	
	2.	60	15–20	turbid, the precipitate was present
			20–30	
	3.	80	15–20	almost transparent, there was a fine suspension
			20–30	
	4.	100	10–15	transparent solution

Considering all above-mentioned information, we proposed the following technology for the combined oral solution Maglycimet™. Purified water is poured into the solution preparation reactor and at a temperature of 70 ± 5 °C, the calculated amount of L-aspartic acid and magnesium oxide is added while stirring. The solution is gradually heated to 95 ± 5 °C and maintained at this temperature for 10–15 minutes. Then the calculated amount of glutamic acid and magnesium oxide in order to obtain the magnesium glutamate should be loaded. The mixture is stirred for 10–15 minutes at a temperature of 95 ± 5 °C. The pH of the solution should stay in the value of 5.3 ± 0.3. After the solution cooling to 30 ± 5 °C, the calculated amount of sodium metabisulphite and glycine is added while stirring, then it should be stirred for 10–15 minutes. Methylcobalamin and saccharin sodium are also added while stirring. Then the solution should be cooled to 25 ± 5 °C. At the next step potassium sorbate and the «cherry» flavoring agent are added with stirring for other 5–10 minutes. Finally, the purified water is added to the required volume, the solution is stirred for 10–15 minutes, filtered and poured into the primary packaging.

At the stage of preparation of the oral solution, organoleptic characteristics, solution pH, density, quantitative content of magnesium, glycine and methylcobalamin were studied to evaluate the pharmaceutical quality of the prepared solution. The determination was carried out according to the developed specification for the MQC project (methods of quality control). Quantitative content of magnesium was performed by the complexometric method (SPhU/Ph.Eur. 2.5.11), the content of glycine and methylcobalamin was determined by HPLC (SPhU/Ph.Eur. 2.2.29). The obtained results of three series of oral solution prepared according to the above-described technology are presented in Table 3.

Conclusion

Technological parameters of preparation of the combined oral solution named Maglycimet™ have been developed: temperature and time preparation modes, the order for loading of the main and auxiliary substances have been studied and determined. Based on the theoretical and experimental investigations, the time and temperature regimes have been selected for the preparation of active pharmaceutical ingredients of magnesium aspartate and magnesium glutamate. The salts were obtained directly in the reactor for solution preparation at a temperature of 95 ± 5 °C after 10–15 minutes. The optimum pH value of the resulting solution of magnesium salts of aspartic and glutamic acids should be in the value of 5.3 ± 0.3. The order and temperature conditions for loading of the remaining active pharmaceutical and auxiliary ingredients into the oral solution were established: sodium metabisulphite, glycine, methylcobalamin, sodium saccharinate were added separately at 30 ± 5 °C, then potassium sorbate and «cherry» flavoring agent at 25 ± 5 °C. The optimal technology for preparing of the oral solution Maglycimet™ was theoretically and experimentally substantiated. It was also confirmed by the data of quantitative determination of active substances in accordance with the specifications for the MQC project. The results of the evaluation of the three obtained series of oral solution according to the above indicated technology confirm the correctness of the chosen parameters of the technological process. Thus, at this stage of the research a number of critical factors of the technological process are determined, which will be used in the future for validation. Research revealed great prospects for a further study of the stability of the medicine and the development of scientific and technical documentation that is necessary for the introduction of such combined oral solution based on magnesium salts into industrial production.

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Conflict of interest: none.

Table 3. The quality parameters of the oral solution at the preparation stage

Indicators (MQC project)	**Series number**		
	1261118	2261118	3271118
pH (SPhU/ *Ph.*Eur.,2.2.3): **(5.0–6.0)**	5.38	5.42	5.44
Colour (visually) **(light pink)**	Complies	Complies	Complies
Density (SPhU/ *Ph.*Eur., 2.2.5): 1.060–1.080 (d (20/20))	1.073	1.074	1.074
Quantitative composition:			
Glycine (SPhU/ *Ph.*Eur., 2.2.29): 9.50–10.50 mg/ml	9.835	9.840	9.843
Methylcobalamin (SPhU/ *Ph.*Eur., 2.2.29): 0.00225–0.00275 mg/ml	0.00253	0.00254	0.00258
Magnesium: (SPhU/ *Ph.*Eur., 2.5.11): 9.00–11.00 mg/ml	10.56	10.58	10.60

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