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STUDY OF THE PHYSICOCHEMICAL COMPATIBILITY OF ACTIVE SUBSTANCES AND EXCIPIENTS AT THE PHARMACEUTICAL DEVELOPMENT PHASE OF THE ORIGINAL DRUG IN THE FORM OF CAPSULES "NEURONUCLEOS"

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Abstract

Physical-chemical properties of active pharmaceutical ingredients that developed a new drug composition, as well as auxiliary substances, were studied and their compatibility with each other, as well as with auxiliary components in the drug in the form of capsules was predicted. With the use of methods for determining the accompanying impurities of uride-5 monophosphate of dynatrium salt, citidine-5 monophosphate of dynatrium salt and pyridoxine hydrochloride by TCH method and the impurity of α-lipoic acid by LC method showed no chemical modification of API in their joint presence. Compatibility of API and auxiliaries is estimated by changes in the physical and chemical characteristics by samples of model mixtures. The evaluation was carried out taking into account the complex analysis using visual and chromatographic methods of analysis. None of the mixtures found changes in physical characteristics and the accumulation of by-products that can adversely affect the quality and efficiency of the drug. Auxiliaries selected to produce the mass for capsule - sorbitol, aerosil, magnesium stearate - are inert for the components studied by API. The LC method ar studied the dynamics of impurities growth - citidine, uridine, and pyridoxine impurities as the main critical parameters of finely products under accelerated aging conditions. The increase in the content of admixtures of citidine and uridine with the increase in the storage temperature was established. The process of hydrolytic destruction most strongly depends on temperature and is most critical for citidine-5 dynatrium salt monophosphate. Based on the results of the work carried out, a rational composition of the preparation was chosen, critical factors of influence were determined and the storage temperature of final product was chosen.

Keywords: *uridine-5-monophosphate disodium salt, related impurities, liquid chromatography, compatibility, excipients.*

Introduction

Today, due to the high prevalence of neuropathic pain and the difficulty of achieving the therapeutic effect, the problem of treatment of this pathology is becoming increasingly important for clinicians of various profiles. It causes suffering and different degrees of disability for a significant part of the population [1, 2].

The most promising direction in the treatment of neuropathy is the need to conduct not only symptomatic but also pathogenic therapy aimed at correcting metabolic disorders. For this purpose, combined treatment is prescribed, which includes medicines with metabolic effects. Metabolic therapy involves the use of drugs containing substances that are inherent in the internal environment of the body and have a primary metabolic effect [3, 4]. At present, it is considered expedient to use medicines of neurotrophic action (containing pyrimidine nucleotides), preparations of α-lipoic (thiocta) acid, group of vitamins B (thiamine, pyridoxine, cyanocobalamin), magnesium preparations, etc [1, 5-7]. However, the choice of an effective and safe drug for long-term therapy is quite complicated.

Literature [8, 9] describes a number of medicines based on uridine-5 monophosphate (UMPh) of dynatrium salt, citidine-5 monophosphate (CMPh) of dynatrium salt, vitamin B6 (pyridoxine hydrochloride), thioct (α-lipoic acid) and magnesium lactate dihydrate in various combinations:

- UMPh and CMPh dynatrium salts: "Keltican N" by Trommsdorff-Gmbh, Germany, "Nucleo C.M.F. Forte" by Ferrer International, S.A., Spain;

- pyridoxine hydrochloride and magnesium lactate dihydrate: "Magvit™", "GlaxoSmithKline", England, "Magne-V6", "Sanofi", France, "Magnesium", Kiev vitamin plant, Ukraine;

- thioctic acid: "Alpha-lipon", Kiev Vitamin Plant, Ukraine, "Berlithion", "Menarini Group/Berlin-Chemie", Italy/Germany, "Thioctacid", "MEDA Pharmaceuticals Switzerland", Sweden, "Espa-lipon", "Esparma", Germany.

Today in Ukraine, combined medicines containing a combination of active pharmaceutical ingredients (API) - uridine-5 monophosphate (UMPh) dynatrium salt, citidine-5 monophosphate (CMPh) dynatrium salt, vitamin B6 (pyridoxine hydrochloride), thioctic (α-lipoic acid) and magnesium lactate dihydrate are not registered and not produced. Results of studies showed that the specified combined composition has a more significant neuropathic activity compared with drugs containing one or two of the active substances - "Celtican", "Magne-B6" and "Thioctacid".

The data obtained justified the unconditional prospect of creating a combined drug based on the salts of pyrimidine nucleotides, vitamin B6, thioctic acid and magnesium lactate dihydrate in the form of capsules for oral administration.

The choice of a dosage form in the form of capsules is due to its advantages: the ability to achieve high accuracy medicinal substances (MS) dosage, the ability to mask the unpleasant taste and smell of MS, the sufficient ease of administration by patients, the ability to dissolve and absorb in the gastrointestinal tract in a short period of time, the pharmacological effects of MS are fairly fast substances that are released from the capsule [10, 11].

Thus, given the steady increase in neuropathy morbidity, its high prevalence worldwide, its progressive deterioration leading to loss of work capacity and disability, significant financial expenses for treatment testify to the relevance of creating a new original combined drug based on uridine-5 monophosphate (UMPh) of dynatrium salt, citidine-5 monophosphate (CMPh) of dynatrium salt, vitamin B6 (pyridoxine hydrochloride), thioctic (α-lipoic) acid and magnesium lactate dihydrate. It should be noted that the combined prescription of specific MS and vitamins in a single drug will significantly improve the ease of treatment.

Pharmaceutical drug development research begins with the selection of active substances. Therapeutic concentrations of active ingredients are proposed by pharmacologists based on the results of pharmacological studies of neuropathic activity of mixtures containing different ratios of these ingredients, in oral administration. The maximum neuropathic effect was given by the composition containing all the selected ingredients in the following quantities (per one dose): uridine-5 monophosphate dynatrium salt 2.0 mg, cytidine -5

monophosphate dynatrium salt 5.0 mg, pyridoxine hydrochloride 50.0 mg, thioctic acid (alpha-lipoic acid) 100.00 mg, magnesium lactate dihydrate 393.00 mg, which corresponds to 40 mg of magnesium.

According to the requirements of ICH Q 812 at the stage of pharmaceutical development of medicines should be evaluated physicochemical compatibility of active pharmaceutical ingredients with auxiliary substanexcipients in the proposed dosage form. In the case of combined medicines, it is necessary to investigate the compatibility of active ingredients with each other. These studies are a necessary part of the materials of Module 3 "Quality" of the registration dossier in CTD format (item 3.2.R. 2.1.1 - Active substances).

Therefore, when creating an original medicines "Neuronucleos" in the form of capsules, which represents a composition of several active ingredients, i.e. is a combined medicines, in addition to the pharmacological compatibility of the active components of the medicinal composition it is necessary to consider their physicochemical compatibility in the composition of the drugs.

The purpose of this work is to study the physicochemical compatibility of active pharmaceutical ingredients (API) with each other, as well as with exipients in the process of pharmaceutical development of the drug in the form of capsules.

Methods

The following active substances were used as research objects: dynatrium salt uridine-5-monophosphate, dynatrium salt cytidine-5-monophosphate, alpha-lipoic acid, pyridoxine hydrochloride and magnesium lactate; auxiliary substances (AS): sorbitol ("EVONIC Degussa GmbH", Germany); magnesium stearate ("Calmags GmbH", Germany); silicon dioxide colloidal anhydrous ("EVONIC Degussa GmbH", Germany), model mixtures on their basis and capsules.

Substances uridin-5-monophosphate dynatrium salt and citidin-5-monophosphate dynatrium salt produced by the company "Shanghai Orifarm Co. Ltd., China is not described in foreign pharmacopoeias. Their quality meets the DMF requirements of the firm-manufacturer.

Alpha lipoic acid, pyridoxine hydrochloride and magnesium lactate are described in European Pharmacopoeia 9.0 (Eur.Ph. 9.0) in the monographs "Thioctic acid", "Pyridoxine hydrochloride", "Magnesium lactate dihydrate" [13]. Alpha lipoic acid substance manufactured by Shanghai Modern Pharmaceutical Co., Ltd., China, pyridoxine hydrochloride produced by DSM Nutritional Products GmbH, Germany and magnesium lactate produced by Moes Cantabra S.L., Spain were used to develop the drug. The level of requirements of these manufacturers for substances corresponds to the level of requirements of Eur.Ph. 9.0.

Requirements for dosage forms containing a combination of these APIs have not been established in leading pharmacopoeias (EP, BP, USP).

Analytical studies were carried out by liquid chromatography (LC) (SPhU, 2.2.29) [14] - determination of the impurities of uridine-5-monophosphate disodium salt, cytidine-5-monophosphate disodium salt and pyridoxine hydrochloride on a liquid chromatograph with a spectrophotometric two-wave or diode-matrix detector the following conditions: column Waters Atlantis T3 measuring 150x4.6 mm, 3 microns with a pre-column measuring 20x4.6 mm; column temperature - 25 ℃; flow rate - 1.3 ml / min; detection wavelength 270 nm; the volume of the injected sample is 20 µl; integration time - 59 min; by thin layer chromatography - determination of impurities of α-lipoic acid (SPhU, 2.2.27). The quantitative content of uridine-5-monophosphate disodium salt, cytidine-5-monophosphate disodium salt, pyridoxine hydrochloride and thioctic acid was determined by LC according to SPhU, 2.2.2914, the magnesium content of lactate by complexometric titration according to the method of SPhU, 2.5.11 [14]. Analytical methods for the determination of concomitant impurities and the quantitative content of APIs, as well as the results of validation of these methods will be given in our next publications.

Standard samples were used for analysis: uridine-5-monophosphate disodium salt (Sigma Aldrich, Cat. No. U6375), uracil (Sigma Aldrich, Cat. No. U0750),

uridine (Sigma Aldrich, Cat. No. U3750), uridine-5-diphosphate disodium salt hydrate (Sigma Aldrich, Cat. No. 94300), uridine-5-triphosphate trisodium salt hydrate (Sigma Aldrich, Cat. No. U6750), cytidine-5-monophosphate disodium salt (Sigma Aldrich, Cat. No. C1006), cytosine (Sigma Aldrich, Cat. No. C3506), cytidine (Sigma Aldrich, Cat. No. C122106), cytidine-2,3-cyclic phosphate (Sigma Aldrich, Cat. No. C9630), cytidine-5-diphosphate sodium salt hydrate (Sigma Aldrich, Cat. No. C9755), cytidine-3,5-cyclic phosphate (Sigma Aldrich, Cat. No. C0985), citidina-5-triphosphate sodium salt (Sigma Aldrich, Cat. No. C1506), pyridoxine hydrochloride impurity B (Sigma Aldrich, Cat. No. D0501), pyridoxine hydrochloride impurity A (EP CRS, p.2), thioctic acid (EP CRS) and impurity A (EP CRS).

To determine the water content in the substances of uridine-5-disodium salt monophosphate and cytidine-5-disodium salt monophosphate, the Fisher method was used: titration (semi-micro method) with K. Fisher reagent with electrometric indication of the end point (SPhU, 2.5.12); in substances of pyridoxine hydrochloride and magnesium lactate was used by drying in an oven at 105 ° C, according to SPhU 2.2.32, α-lipoic acid was used by drying in vacuum at 40 °C for 3 hours (SPhU, 2.2.32) [14] .

The determination of hygroscopicity was carried out in accordance with the recommendations of SPhU, 5.1114.

Pharmacological and technological properties of the mass for encapsulation are studied according to the methods of SPhU [14].

Results

Thus, the compatibility of some APIs with each other and the use of selected substances in the form of encapsulated dosage forms allow us to predict the possibility of their joint presence in the form of a single combined drug. Since all the main active substances are complex organic compounds, the analysis of their compatibility, carried out on a theoretical basis, was continued in the process of experimental studies.

At the initial stage of research, we determined one of the main physicochemical parameters for obtaining the mass for encapsulation - the moisture content in all active substances that make up the drugs. Research results are presented in Table 1.

The data of Tab. 1 show the compliance of all tested substances with the established criteria of acceptability for the "Water" indicator. It should be noted that the moisture content in the substance of thioctic acid is at the upper limit. When developing a technological process, one of the critical points at the stage of "Getting mass for encapsulation" is the technological control of the accuracy of weighing. When calculating the quantities of active substances to obtain the mass for encapsulation, it is necessary to take into account the quantitative content of substances and the value of "Water" indicator. Incorrect calculation of their technical mass can affect the quantitative content of API in the intermediate product.

Further studies were aimed at the determination of API impurities - uridine-5-disodium salt monophosphate, cytidine-5-disodium salt monophosphate and pyridoxine hydrochloride by HPLC. Impurities of α-lipoic acid are not determined simultaneously with other APIs, since α-lipoic acid is a lipophilic substance and requires different sample preparation and determination conditions. To determine the impurities of α-lipoic acid in the drugs, the TLC method of the European Pharmacopoeia was used to control impurities in the substance. Fig. 1 and Fig. 2 show chromatogram of test solution and chromatogram of model mixture of API and impurities obtained under the conditions for the determination of concomitant impurities of uridine-5-disodium salt monophosphate, cytidine-5-disodium monophosphate and pyridoxine hydrochloride.

The coincidence of retention times of uridine-5-disodium salt monophosphate, cytidine-5-disodium salt monophosphate and pyridoxine hydrochloride peaks in the chromatograms of test solution and model mixture of APIs and impurities indicates the absence of interaction between APIs that would lead to chemical modification of their molecules. Fig. 2 show that peaks of uridine-5-monophosphate disodium salt, cytidine-5-monophosphate disodium salt and pyridoxine hydrochloride and their impurities are well separated.

Based on the studied physicochemical properties of APIs in the developed drugs, 4 APIs are water-soluble and 1 API (α-lipoic) is not soluble in the water. A simultaneous study of the interaction of all APIs is not possible. The experiment was divided into 2 stages: determination of impurities for water-soluble substances (LC method) in model mixtures and for α-lipoic acid - TLC method. For this purpose, model samples have been developed from a mixture of three APIs - uridine-5-monophosphate disodium salt, cytidine-5-monophosphate disodium salt, pyridoxine hydrochloride, since it was found that there is no interaction between these active substances, with magnesium lactate and with α-lipoic acid.

1. A mixture of uridine-5-monophosphate disodium salt, cytidine-5-monophosphate disodium salt and pyridoxine hydrochloride in a ratio of 2: 5: 25

2. A mixture of uridine-5-monophosphate disodium salt, cytidine-5-monophosphate disodium salt and pyridoxine hydrochloride in a ratio of 2: 5: 25 + α-lipoic acid

3. A mixture of uridine-5-monophosphate disodium salt, cytidine-5-monophosphate disodium salt and pyridoxine hydrochloride in a ratio of 2: 5: 25 + magnesium lactate.

The study of the possible interaction of components in model mixtures was carried out under load exposure to temperature and humidity. Stress tests were used to understand the possible decay paths of APIs. Results of the content of impurities in samples are presented in Table 2.

As a result of the analysis (Table 2) of model mixtures by the impurity content of five APIs, there are no impurities that would characterize the interaction of APIs. Impurities of cytidine and uridine are formed, associated only with the influence factor - temperature.

Thus, when studying the compatibility of water-soluble active substances, no interaction between them was found.

The next stage of research was the study of the formation of impurities of decomposition and interaction for water-insoluble substances - α-lipoic acid by TLC.

For studies, we developed model mixtures of APIs: uridine-5-monophosphate disodium salt, cytidine-5-monophosphate disodium salt and pyridoxine hydrochloride + α-lipoic acid and α-lipoic acid + magnesium lactate. The study of the possible interaction of components in model mixtures was carried out under load exposure to temperature (keeping at 40 ° C for 6 days) and humidity (conditions for determining hygroscopicity).

As a result of studies, it was found that there is no interaction between APIs. No additional spots were found on chromatograms that could suggest the effect of other APIs on the degradation of α-lipoic acid. Temperature and humidity, as influence factors, did not show any significant increase in impurities of α-lipoic acid.

Further studies were aimed at studying the compatibility of active substances and excipients selected. For this, model mixtures were prepared containing APIs and auxiliary substances in the ratio of the approximate formulation of the composition of the drugs. In order to accelerate possible interactions, the test model powder mixtures were subjected to mechanical mixing, wet granulation. The compatibility of APIs and excipients was evaluated by changes in the physicochemical characteristics of samples. The assessment was carried out taking into account complex analysis using visual and chromatographic methods of analysis. The research results are presented in Table III.

Observations of these mixtures (Table 3) confirmed the compatibility of APIs with excipients taken in recommended concentrations. In no one mixture is not a change in physical characteristics and accumulation of by-products that can adversely affect the quality and effectiveness of the drug. So, excipients selected to obtain the mass for encapsulation - sorbitol, aerosil, magnesium stearate are inert to the components studied by the API.

To clarify the quality parameters and a preliminary forecast for the stability of the drug, studies of the laboratory series of capsules by accelerated aging were carried out under the following conditions:

aging at a temperature of 40 ° C for 6 days; keeping at a temperature of 40 ° C for 11 days; keeping at a temperature of 55 ° C for 6 days.

Since the main critical parameters of capsules in the study of stability are nucleoside impurities - cytidine and uridine, and pyridoxine impurities, the stability forecast was based on studying the dynamics of the growth of these impurities under accelerated aging.

To determine the impurities, the TLC method was used. The relative retention times of the main APIs and their impurities are shown in Table 4; chromatograms of test capsule solutions obtained under conditions for determining accompanying impurities are presented in Fig. 3-6. Results of the content of basic impurities in the drug GLF are shown in Table 5.

The data of Table V shows an increase in the content of cytidine and uridine impurities with increasing storage temperature, however, the admixture of cytidine increases more. An impurity B of pyridoxine is not detected under the experimental conditions, the total amount of pyridoxine impurities increases, but the growth rate is much lower than the increase in API impurities of cytidine-5-monophosphate disodium salt and uridine-5-monophosphate disodium salt. In the process of accelerated aging, a decomposition reaction occurs with the formation of the main impurities - cytidine and uridine. It is possible to reduce the formation of impurities to a predetermined maximum by establishing storage conditions for the drug at low temperatures.

Thus, based on conductor studies, we have obtained results that we used for the further rational development of the composition and technology of the drug, the determination of critical exposure factors, and the choice of the storage temperature of prepared capsules.

Discussion

At the initial stage of pharmaceutical development, a theoretical justification of the compatibility of the active substances with each other, as well as with excipients in the dosage form, was carried out. To this end, the physicochemical properties of active and auxiliary substances have been studied. Excipients used to develop the composition of the drug were selected as a result of theoretical screening and experimental studies. A number of excipients of various chemical nature and performing certain functions have been studied. As a binder, sorbitol was introduced into the encapsulation mass. Anhydrous colloidal silicon dioxide as a disintegrant was selected, and also to improve the flowability of the mass for encapsulation and absorption of moisture. In addition, this substance, not dissolving and not swelling in water, forms viscous gels in the body's environment, which provides the desired rate of release of active substances from the finished dosage form. Magnesium stearate was used as a glidant. Excipients were included in the composition for encapsulation in amounts recommended for such dosage forms.

Disodium salt cytidine-5-monophosphate and uridine-5-monophosphate are inorganic salts of organic acids with similar chemical structures, similar physicochemical properties and a close pH range from 7.0-9.5 (1 % solution). Melting point of disodium salt of UMPh is 208-210 ° C; disodium salt of CMPh is 300 ° C. The content of the basic substance in the substances determined by the manufacturer not less than 98,0 %, based on anhydrous substance. Permitted a fairly high water content: not more than 26,0 % (disodium salt of UMPh) and not more than 25 % (disodium salt of CMPh). The particle size of the powder of disodium salt of the UMPh are regulated thus: 60 mesh (250 μm), not less than 98,0 %. As noted earlier, well-known drugs in the form of capsules containing substances in such concentrations ("Celtica N", "Nukleo C. M. F. Forte"). Therefore, these APIs are compatible with each other within a single dosage form.

Pyridoxine hydrochloride (vitamin B6) refers to a group of oximethylpyrydin. It is a white or almost white crystalline substance, easily soluble in water, slightly soluble in 96% ethanol. pH of 5% aqueous solution: 2.4 up to 3.0. In solutions of a substance capable of forming colorless crystals in the form of flakes, melting point (with decomposition) is 206-208 °C7. The loss in weight when drying is not more than 0.5 %. Pyridoxine is not stable in solutions with pH greater than 4.8. Under the influence of light

pyridoxine rapidly degraded in alkaline and neutral solution 7. The characteristic of vitamin B6 is its ability under the action of oxygen, interconvert of pyridoxine to pyridoxal and pyridoxamine.

Magnesium lactate is a white or almost white crystalline powder, slightly soluble in water. The loss in weight when dried is: not less than 14.0 % and not more than 17.0 percent. pH is: 6,5 - 8,5. Melting point (with decomposition) is 175 °C. The particle size of the powder of magnesium lactate determined by the manufacturer thus: less than 0.4 mm - 100,0 %, less than 0.2 mm 89 %. The flowability is 16.1 g/sec. Bulk density: before the seal is – 0.77 g/cm3, after the seal is 0.86 g/cm3. The content of the basic substance in the substance is regulated not less than 98,0 %, based on anhydrous substance. Drugs in the form of tablets or capsules ("Maguid", "Magne-B6", "MINICOM") containing pyridoxine hydrochloride and magnesium lactate dihydrate in similar concentrations (5 mg and 470 mg, respectively) are known. Therefore, these APIs are compatible with a single dosage form.

Alpha-lipoic acid is a light yellow to yellow crystalline powder, with a characteristic odour. Very little or practically insoluble in water, soluble in methanol, very slightly soluble in N,N-dimethylformamide. Melting point is 60 to 62 °C. The loss in weight when drying is: not more than 0.5 %. The content of the basic substance in substance determined by the manufacturer isnot less than 97.0 % calculated on the anhydrous substance. drugs containing thioctic acid (alpha-lipoic acid) is produced in the form of capsules or tablets: "alpha lion", "Berlition", "Thioctacid", etc are known.

Sorbitol is a white or almost white crystalline powder. It is soluble in water, practically insoluble in ethanol (96 %). Has polymorphism. Optical rotation is between -1.5 and -2.5. Water content is: not more than 1.5 %. Quantitative content regulated from 97,0 % to 102,0 % (anhydrous substance).

Silicon dioxide colloidal anhydrous (Aerosil-200) is white or almost white, light, thin, amorphous powder, with a particle size of not more than 15 nm. Easily forms agglomerates of spherical shape. It is practically insoluble in water and mineral acids, with the exception of hydrofluoric. It is soluble in hot solutions of alkali metal hydroxides. pH is 3.5-5.5. The particle size of Aerosil-200 powder, the predominant fraction is 12 nm. Bulk density is 50 g/L. Aerosil-200 is pure silicon dioxide with a content of not less than 99.0% and not more than 100.5%, calculated on the substance after burning.

Magnesium stearate is a white, very thin and light powder, slippery when touched, practically insoluble in water and anhydrous alcohol, melting point is 120-150 °C. Magnesium stearate is a mixture of magnesium salts of various fatty acids, which consists mainly of stearic and palmitic acids with a small portion of other fatty acids. The quantitative content of magnesium is from 4.0 to 5.0% (on a dry matter basis); stearic acid is: not less than 40.0%; stearic and palmitic acid: not less than 90.0%. Loss on drying is: not more than 6.0%. The particle size of the magnesium stearate powder is regulated as follows: less than 1.0% (325 mesh); the predominant fraction is 80-150 microns. The bulk density is 0.33 ± 0.02 g / ml.

Conclusions

1. The physicochemical properties of active pharmaceutical ingredients that make up the new drug composition, as well as excipients, were studied, and their compatibility in the capsule-shaped drug was predicted.

2. Using developed methods for determining concomitant impurities of uridine-5-monophosphate disodium salt, cytidine-5-monophosphate disodium salt and pyridoxine hydrochloride by LC and α-lipoic acid impurities by TLC, the absence of chemical modification of API with their joint presence was shown. LC has proved the compatibility of APIs with excipients.

3. To clarify quality parameters and preliminary prediction of the stability of the drug, studies using accelerated aging were conducted. It is established that the use of heat treatment at all stages of the technological process contributes to the growth of impurities. The process of hydrolytic destruction is most dependent on the temperature and is most critical for cytidine-5-monophosphate disodium salt.

4. It was found that a reduction to a specified maximum of the formation of impurities is possible due to the establishment of storage conditions for the drug at low temperatures.

5. Further studies will be aimed at confirming the chemical compatibility of the active pharmaceutical ingredients with excipients by conducting long-term observations of the stability of the drug.

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API	Result
Uridine-5- disodium salt monophosphate	23.3%
Cytidine-5- disodium salt monophosphate	23.5%
Pyridoxine hydrochloride	0.07%
α-lipoic acid	0.5%
Magnesium Lactate	15.2%

Table 1. Values of the "Water" indicator in substances

Model mix	***Impact factors / result***		
	General conditions	Heating 55°C - 6 days	Humidity (in hygroscopies determination conditions)
Acceptance criterion (Cytidine - ≤ 0.4%; uridine - ≤ 0.3%; impurities of pyridoxine: impurity A - ≤ 0.3%, impurity B (deoxypyridoxine) - ≤0.3%)			
UMPh disodium salt, CMPh disodium salt and pyridoxine hydrochloride	Cytidine - up to 0.4% Uridine - up to 0.2% Impurities of pyridoxine - up to 0.2%	Cytidine - up to 2.8% Uridine - up to 1.6% Impurities of pyridoxine - approx. 0.5% No new impurities were detected	Cytidine - up to 0.4% Uridine - up to 0.2% Impurities of pyridoxine - up to 0.2%
UMPh disodium salt, CMPh disodium salt and pyridoxine hydrochloride + α-lipoic ac	Cytidine - up to 0.4% Uridine - up to 0.2% Impurities of pyridoxine - up to 0.2% No new impurities were detected	Cytidine - up to 2.8% Uridine - up to 1.6% Impurities of pyridoxine - approx. 0.5% No new impurities were detected	Cytidine - up to 0.4% Uridine - up to 0.2% Impurities of pyridoxine - up to 0.2% No new impurities were detected
UMPh disodium salt, CMPh disodium salt and pyridoxine hydrochloride + magnesium lactate	Cytidine - up to 0.4% Uridine - up to 0.2% Impurities of pyridoxine - up to 0.2%. No new impurities were detected	Cytidine - up to 2.8% Uridine - up to 1.6% Impurities of pyridoxine - approx. 0.5% No new impurities were detected	Cytidine - up to 0.4% Uridine - up to 0.2% Impurities of pyridoxine - up to 0.2%. No new impurities were detected

Table 2. Impurity content in model samples from API mixtur

Composition / Model Mix		*Name of the technological parameter and / or indicator*	*Actual values*
Name of the component	*% for 1 capsule*		
1	2	3	4
Model mix 1			
UMPh disodium salt	0,35	Bulk density, g/cm3	0,512
CMPh disodium salt	0,87	Density after shrinkage, g/cm3	0,576
pyridoxine hydrochloride	8,65	Fluidity, g/s	10,0
α-lipoic acid	17,3	Gausner's Coefficient	1,13
magnesium lactate +	67,99	Carra Index,%	11
sorbitol	3,0		
Pharmacological and technological indicators			
Appearance (Fine granular powder, light yellow)		Fine granular powder, light yellow	
Associated impurities (Cytidine - ≤ 0.4%; uridine - ≤ 0.3%; impurities of pyridoxine: impurity A - ≤ 0.3%, impurity B (deoxypyridoxine) - ≤0.3%)		Cytidine - up to 0.4% Uridine - up to 0.2% Impurities of pyridoxine - up to 0.2%	
Quantification, based on the average weight of 1 capsule			
UMPh disodium salt (from 0.0019 g to 0.0022 g)		0,00195	
CMPh disodium salt (0.0048 g to 0.0055 g)		0,0050	
Pyridoxine hydrochloride (from 0.0480 g to 0.0550 g)		0,0496	
α-lipoic acid (from 0.0925 g to 0.1075 g)		0,1010	
magnesium lactate (from 0.0383 g to 0.0417 g)		0,0400	
Model mix 2			
UMPh disodium salt	0,35	Bulk density, g/cm3	0,595
CMPh disodium salt	0,87	Density after shrinkage, g/cm3	0,675
pyridoxine hydrochloride	8,65	Fluidity, g/s	10,0
α-lipoic acid	17,3	Gausner's Coefficient	1,13
magnesium lactate +	67,99	Carra Index,%	11,8
sorbitol	3,0		
magnesium stearate	1,0		
Pharmacological and technological indicators			
Appearance (Fine granular powder, light yellow)		Fine granular powder, light yellow	

Associated impurities (Cytidine - ≤ 0.4%; uridine - ≤ 0.3%; impurities of pyridoxine: impurity A - ≤ 0.3%, impurity B (deoxypyridoxine) - ≤0.3%)	Cytidine - up to 0.4% Uridine - up to 0.2% Impurities of pyridoxine - up to 0.2%
Quantification, based on the average weight of 1 capsule	
UMPh disodium salt (from 0.0019 g to 0.0022 g)	0,0020
CMPh disodium salt (0.0048 g to 0.0055 g)	0,0049
Pyridoxine hydrochloride (from 0.0480 g to 0.0550 g)	0,0495
α-lipoic acid (from 0.0925 g to 0.1075 g)	0,0995
magnesium lactate (from 0.0383 g to 0.0417 g)	0,0398

1	2	3	4
Model mix 3			
UMF disodium salt	0,35	Bulk density, g/cm3	0,605
CMF disodium salt	0,87	Density after shrinkage, g/cm3	0,688
pyridoxine hydrochloride	8,65	Fluidity, g/s	10,0
α-lipoic acid	17,3	Gausner's Coefficient	1,14
magnesium lactate +	67,99	Carra Index,%	12
sorbitol	3,0		
magnesium stearate	1,0		
aerosil-200	1,0		

Pharmacological and technological indicators	
Appearance (Fine granular powder, light yellow)	Fine granular powder, light yellow
Associated impurities (Cytidine - ≤ 0.4%; uridine - ≤ 0.3%; impurities of pyridoxine: impurity A - ≤ 0.3%, impurity B (deoxypyridoxine) - ≤0.3%)	Cytidine - up to 0.4% Uridine - up to 0.2% Impurities of pyridoxine - up to 0.2%
Quantification, based on the average weight of 1 capsule	
UMPh disodium salt (from 0.0019 g to 0.0022 g)	0,0021
CMPh disodium salt (0.0048 g to 0.0055 g)	0,0052
Pyridoxine hydrochloride (from 0.0480 g to 0.0550 g)	0,0497
α-lipoic acid (from 0.0925 g to 0.1075 g)	0,1000
magnesium lactate (from 0.0383 g to 0.0417 g)	0,0410

Table 3. Quality indicators of model mixtures of APIs with excipients

Cytidine	Pyridoxine	Uridine	Imp	CMP	Imp	UMP	Imp	Imp	Imp
3,2	4,6	5,4	5,8	6,53	9,8	11,4	16,6	17,7	20,2

Table 4. The retention time of main APIs and their impurities

Test conditions	Impurity content,%		
	Cytidine	Uridine	Pyridoxine
t = 40°C, time - 6 day	1,0	1,0	0,34
t = 40°C, time - 11 days	1,2	1,0	0,34
t = 55°C, time - 6 days	2,8	1,6	0,53

Table 5. Impurity content in capsules under various conditions of accelerated aging

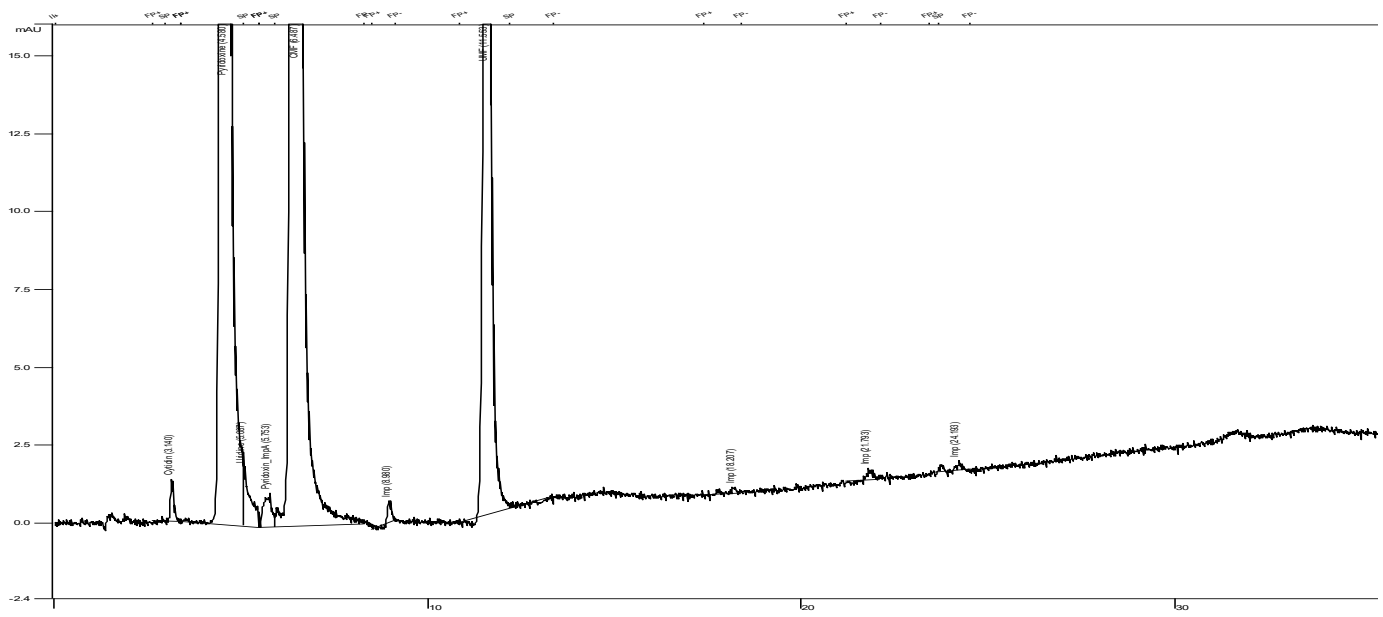

Figure 1. Chromatogram of test solution obtained under the conditions for the determination of concomitant impurities of uridine-5-monophosphate disodium salt, cytidine-5-monophosphate disodium salt, and pyridoxine hydrochloride.

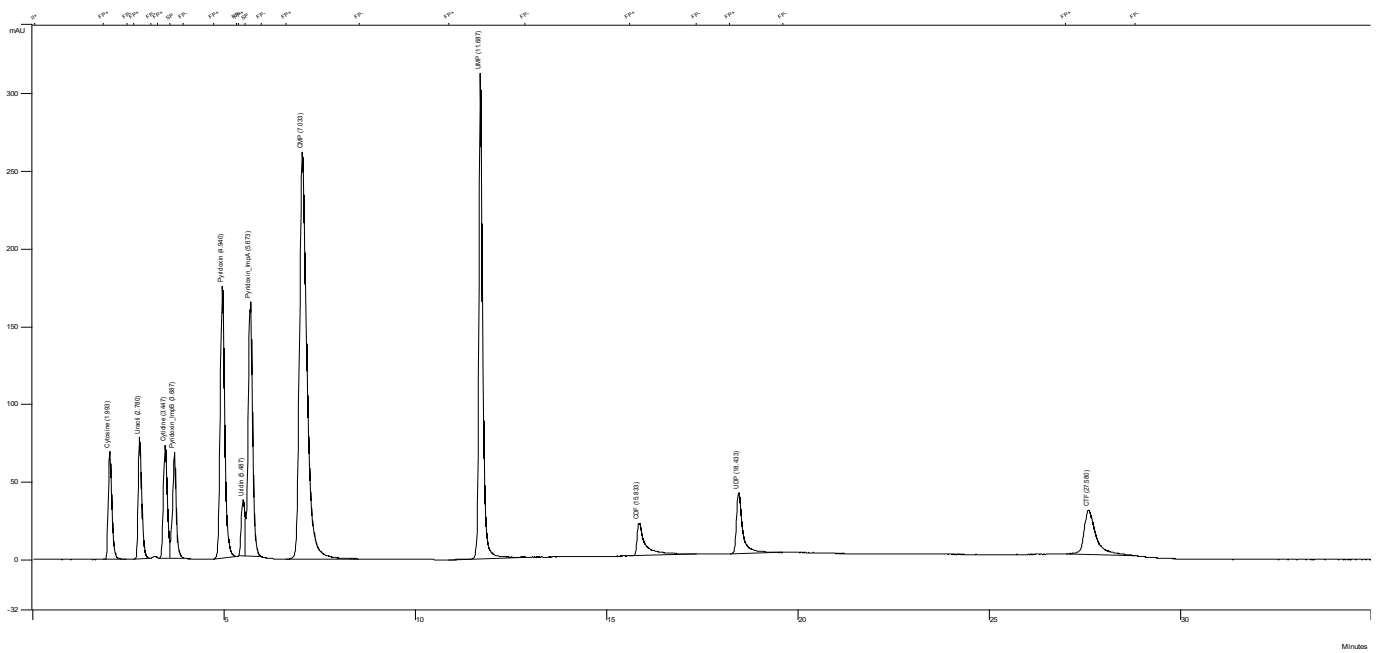

Figure 2. Chromatogram of model mixture of APIs and impurities, obtained under conditions of determination of concomitant impurities of uridine-5-disodium salt monophosphate, cytidine-5-disodium monophosphate and pyridoxine hydrochloride.

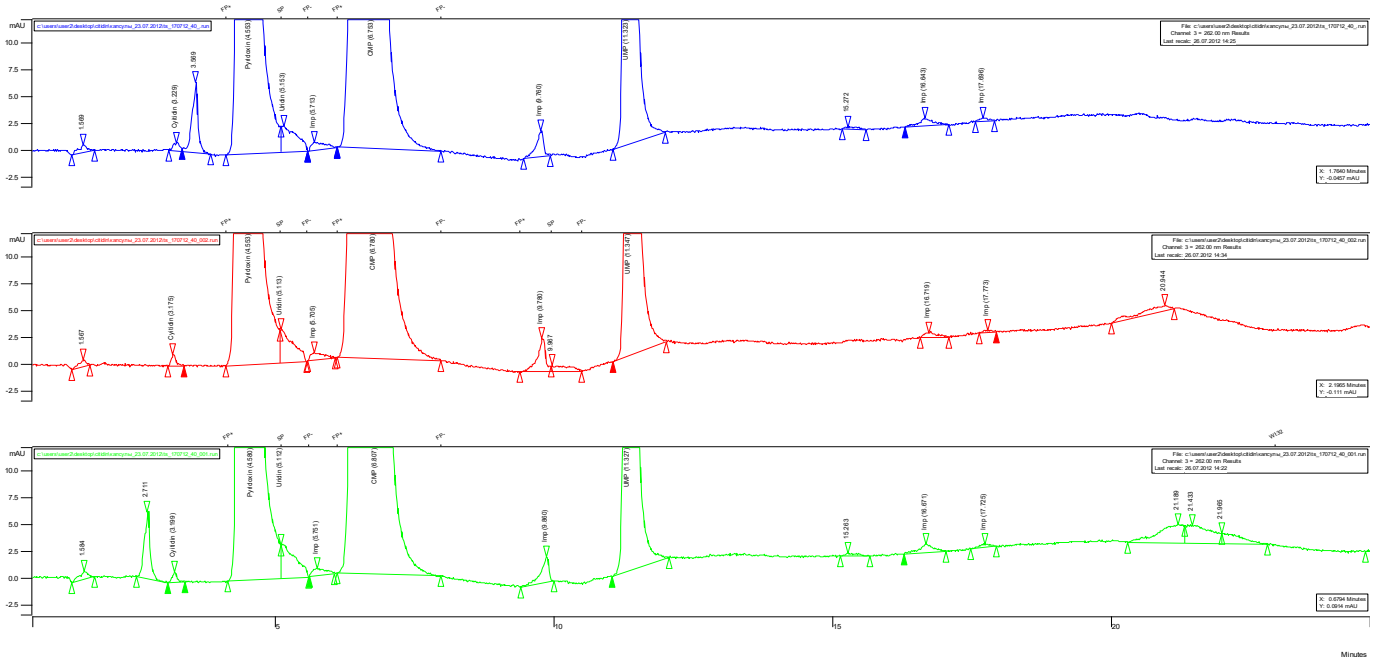

Figure 3. Chromatographms of test solution of capsules obtained under conditions of determination of concomitant impurities (t = 40 °C, 6 days).

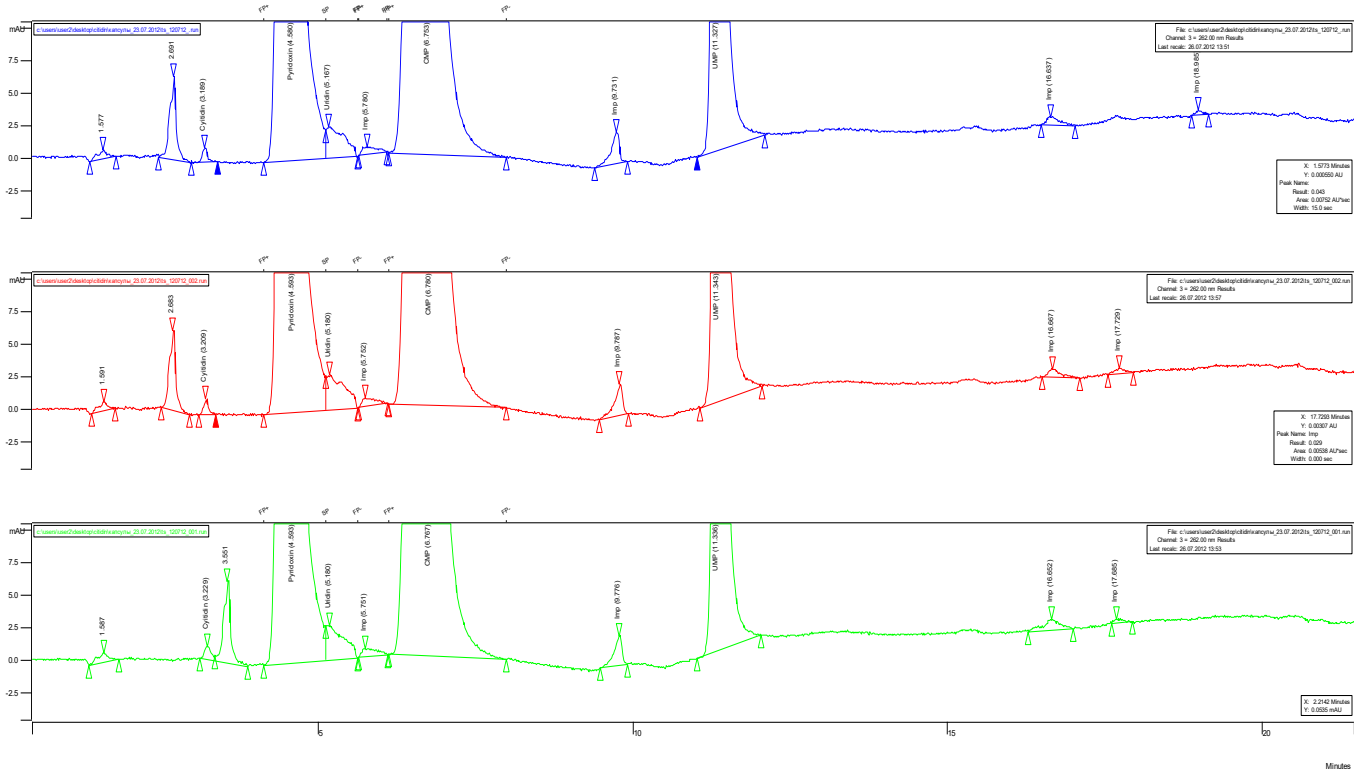

Figure 4. Chromatograms of test solution of capsules obtained under conditions of determination of concomitant impurities (t = 40 °C, 11 days).

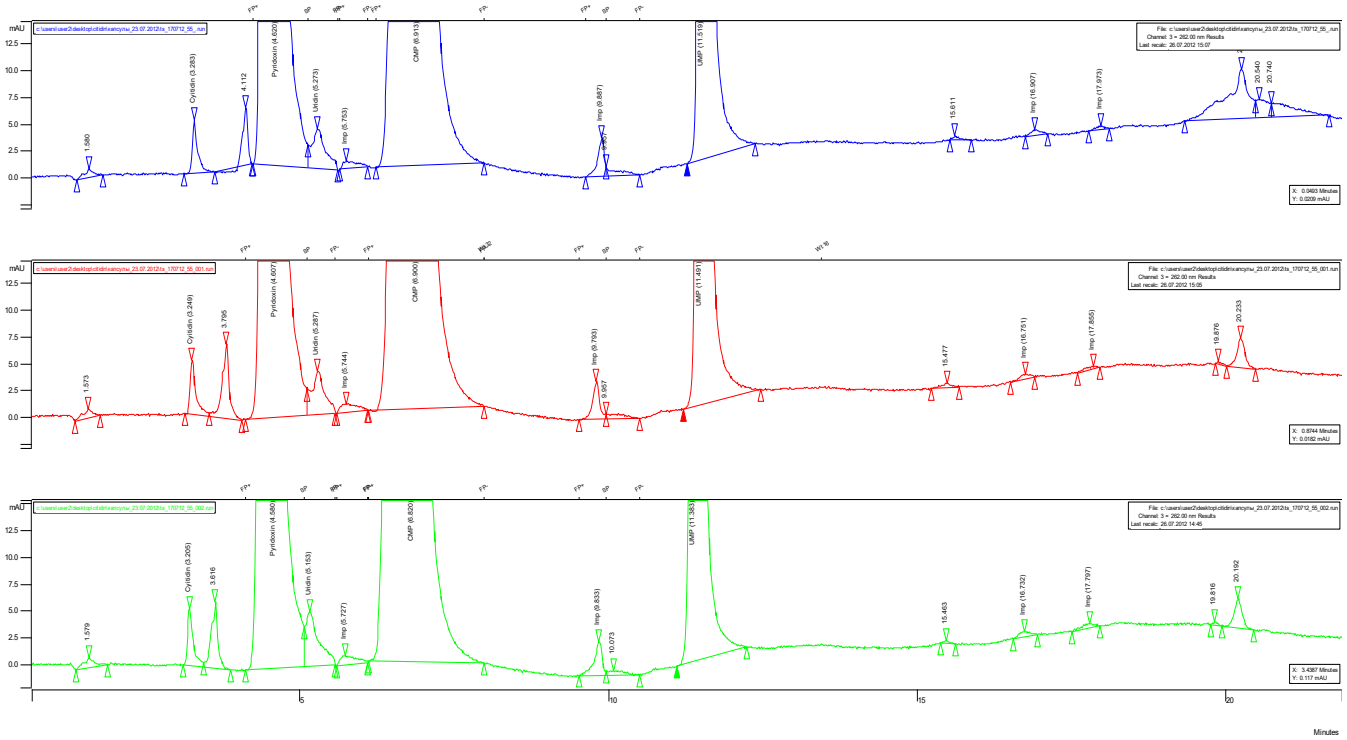

Figure 5. Chromatograms of test solution of capsules obtained under conditions of determination of concomitant impurities (t = 55 ° C, 6 days).

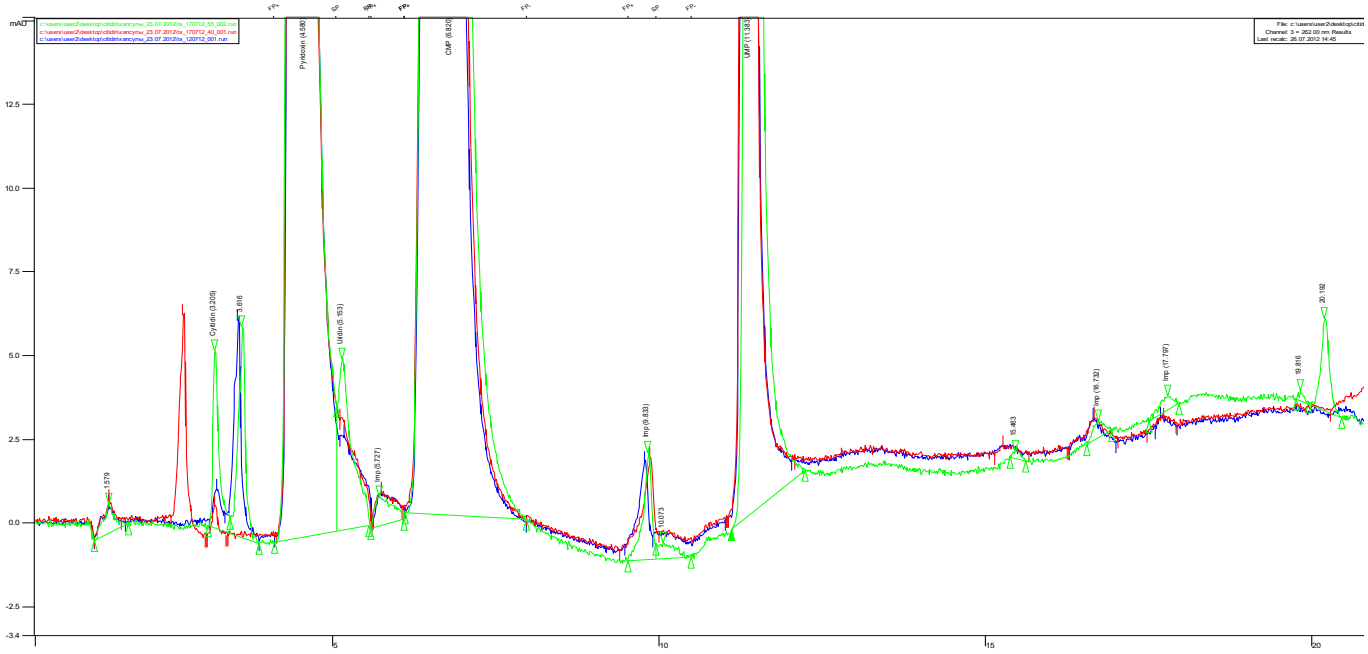

Figure 6. Comparison of chromatograms of tested capsule solutions, obtained under the conditions of determination of concomitant impurities under various conditions: blue color - t = 40 ° C, 11 days; red color - t = 40 ° C, 6 days; green color - t = 55 ° C, 6 days. Text