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PHARMACEUTICAL AND PHARMACOLOGICAL ASPECTS OF CREATING A NEW COMBINATION DRUG WITH ANTIVIRAL ACTIVITY

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Annotation

This article describes the pharmaceutical aspects of creating a combination drug: pharmaceutical compatibility of active and inactive ingredients, selection of dosage form, its composition and production technology, quality control, standardization, validation of developed methods, and stability during storage. Experimental studies of the specific activity of the combination drug in vitro are presented.

Keywords: glycyrrhizic acid, acyclovir, herpes, synergism, pharmaceutical compatibility, production technology, analysis methods, standardization, validation characteristics, antiviral activity, efficacy, safety

Аннотация

В статье описаны фармацевтические аспекты создания комбинированного лекарственного препарата: фармацевтическая совместимость активных и неактивных ингредиентов, выбор лекарственной формы, ее состава и технологии получения, контроль качества, стандартизация, валидация разработанных методик, стабильность в процессе хранения. Представлены экспериментальные исследования специфической активности комбинированного лекарственного препарата в опытах in vitro.

Ключевые слова: глицирризиновая кислота, ацикловир, герпес, синергизм, фармацевтическая совместимость, технология получения, методы анализа, стандартизация, валидационные характеристики, противовирусная активность, эффективность, безопасность

Introduction. Viral diseases represent one of the most significant public health threats of the 21st century. Every year, viral infections affect millions of people worldwide, causing not only acute but also chronic pathological conditions [1]. Respiratory viruses (influenza, parainfluenza, adenoviruses, and coronaviruses), as well as herpes viruses, hepatitis, and the human immunodeficiency virus (HIV) [2] predominate in the structure of infectious diseases. Large-scale epidemics and pandemics, such as COVID-19, have clearly demonstrated the potential of viral diseases to destabilize healthcare systems and economies on a global scale [3].

Despite significant advances in pharmaceutical science, the problem of effective treatment of viral infections remains unresolved. Highly effective and safe etiotropic drugs are still lacking for many viruses. For example, antiviral therapy for influenza is limited by a short therapeutic window and the risk of developing resistance to oseltamivir and zanamivir [4]. Difficulties persist in the treatment of chronic viral hepatitis, HIV infection, and herpesvirus diseases, including the need for long-term drug administration, the development of side effects, and decreased viral sensitivity [5].

Respiratory viruses, including the SARS-CoV-2 coronavirus that caused the

COVID-19 pandemic, are attracting particular attention. This disease has highlighted the need for agents with not only direct antiviral properties but also pronounced anti-inflammatory, antioxidant, and immunomodulatory effects. Such drugs can reduce the severity of the inflammatory response, decrease viral load, and prevent the development of complications [6].

In modern conditions, a priority area of pharmaceutical research is the development of combination drugs based on natural substances with a multifaceted mechanism of action [7]. One such promising compound is glycyrrhizic acid (GA), a triterpene saponin isolated from licorice root (Glycyrrhiza glabra). GA exhibits a broad spectrum of pharmacological activity: antiviral, anti-inflammatory, immunomodulatory, antioxidant, and hepatoprotective [8]. Its effectiveness has been proven against influenza, herpes, hepatitis, HIV, and SARS-CoV-2 viruses [9].

Due to its combination of direct antiviral effects and ability to modulate the immune response, GA is considered a versatile component for the development of new combination drugs. Such drugs could improve the effectiveness of viral infection therapy, reduce the toxicity of existing antiviral agents, and minimize the risk of developing drug resistance [10].

The Department of Pharmaceutical Disciplines at Astana Medical University has developed a new combination drug (CD) based on the natural adaptogen GA, the biological antioxidant ascorbic acid (AA), and a synthetic analogue of cyclic nucleosides, acyclovir (ACV). This drug can enhance antiviral activity due to molecular synergy, reduce side effects by lowering the ACV dosage, and enhance the body's resistance due to the adaptogenic properties of GA and the immunomodulatory properties of GA and AA.

Methods. The following methods were used to analyze the new CD: chemical (qualitative reactions to active ingredients), physical (to determine the stability of the drug and to study physical constants), physicochemical (optical and chromatographic methods to control related impurities, quantitative determination and study of pharmaceutical compatibility of ingredients), biological (in vitro study of specific activity) and statistical (to analyze data obtained during validation of analytical methods, as well as to process the values obtained in in vitro experiments).

Results. *Technology for obtaining a dosage form.*

Effervescent tablets produced by direct compression were chosen as the most rational dosage form for the CD. This choice is based on the physicochemical properties of the active ingredients—GA, AA, and ACV. To enhance the bioavailability of the developed CD, polyvinylpyrrolidone K30 (PVP K30) and carbon dioxide were added to the excipients. PVP K30 acts as a solubilizer, increasing the contact area between the active ingredients and the solvent or biological fluid, which improves solubility and accelerates absorption of the active ingredients. Carbon dioxide, formed as a result of the acid-base reaction, acts as a super-disintegrant, ensuring rapid disintegration and dissolution of the effervescent tablet, which is especially important for GA, which has a viscous structure.

The use of direct compression with a new-generation lubricant, sodium stearyl fumarate PRUV®, significantly simplified the process and reduced its energy consumption by eliminating the granulation step. The inherent stickiness of GA, due to the presence of sugar residues in its structure, ensures the formation of a stable dosage form without the need for large quantities of excipients.

The use of sodium stearyl fumarate in PRUV® not only prevents the pressed mass from sticking to the punches and die but also ensures high hydrophilicity, a distinct advantage over traditional magnesium stearate. Unlike magnesium stearate, PRUV® is completely water-soluble, has a low electrostatic charge, and does not form insoluble compounds, ensuring its compatibility with most pharmaceutical ingredients. An additional advantage of the developed formulation is the

natural sweetness and aroma of GA, eliminating the need for synthetic sweeteners and flavorings (such as acesulfame potassium), reducing chemical load and increasing drug safety.

Study of compatibility of active ingredients in CD.

The pharmaceutical compatibility of medicinal products and excipients is determined by the nature of their interactions. Therefore, early in the development of dosage forms, it is essential to evaluate potential interactions between components to predict their compatibility, optimize their composition, and select rational process parameters, including temperature conditions for drug production and storage. Currently, differential thermal analysis (DTA) and differential scanning calorimetry (DSC) are widely used to assess and predict pharmaceutical compatibility. The use of thermoanalytical methods allows one to obtain information on thermal effects in the studied temperature range, which is essential for the development and optimization of technological processes [11].

During the study, a thermal analysis of the individual components and their mixture was conducted. The heating results for the samples are shown in Figure 1. According to the data obtained, the thermograms do not exhibit peaks characteristic of exothermic or endothermic processes within the temperature range corresponding to the process conditions. This indicates the absence of chemical interaction between the components of the composition under study.

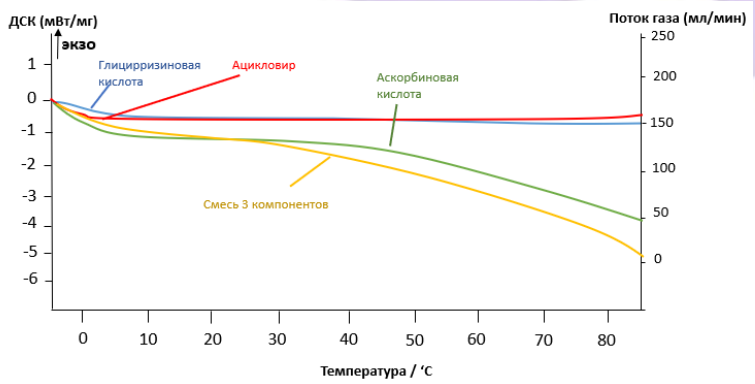

Figure 1. DSC curves of the test samples

Study of stability and establishment of shelf life of CD.

In order to predict the shelf life of the CD, its stability was studied using the "accelerated aging" method at elevated temperatures.

Five batches of laboratory samples of CD were stored in a thermostat at 60°C for 69 days. The test samples were placed in dark glass vials with ground-glass stoppers. Quality control was performed at intervals (11.5 days) equivalent to 6 months of storage under natural conditions. The key quality parameters during storage, according to the manufacturer's guidelines for stability studies, shelf life determination, and repeat testing of medicinal products, were:

1. Description
2. Average weight of one tablet
3. Homogeneity of mass
4. Impurities
5. Quantitative content

The results of the experiment (Table 1) showed that the drug remains stable for 69 days of experimental storage, which corresponds to 1104 days of storage under natural conditions, calculated according to the Van't Hoff rule: C = conformity coefficient.

$$A^{\frac{tэ-txp}{10}} = 2^{\frac{60-20}{10}} = 16$$

Hence the shelf life is 16x69=1104 days, which is 3 years.

The storage temperature that allows to ensure the established shelf life is:

$t_{xp} = tэ + x \lg = 60 + x \lg = 20$ oC$\frac{10}{lgA}\frac{Cэ}{C}\frac{10}{lg2}\frac{69}{1104}$

The maximum permissible storage temperature is:

$t_{max.add.} = 20\ o + x = 20 + x \lg = 26\ o\ C$

$\frac{10}{lgA} \quad \frac{C_э}{2 \times 365} \quad \frac{10}{lg2} \quad \frac{1104}{730}$

Thus, as a result of the studies conducted using the "accelerated aging" method, the expiration date and storage temperature regime for the drug were established.

Table 1 -***Quality indicators of the drug during storage using the "Accelerated aging" method***

Indicators quality	According to specification	*Series*				
		010363	020363	030363	040363	050363
Description of effervescent tablets	pills creamy colors	pills creamy colors	pills creamy colors	pills creamy colors	pills creamy colors	pills creamy colors
Average weight of one tablet	1,5032	1,5054	1,5067	1,5101	1,5034	1,4991
Mass homogeneity, %	no more ± 7.5%	+3.2 - 4.8	+3.3 -4.2	+ 2.0 - 4.3	+3.5 -4.7	+3.7 -5.1
Impurities, %	no more than 1%	resp.	resp.	resp.	resp.	resp.
Quantitative content, g ± %						
GA	0.0390-0.0401	0.0401±1.6	0.0390±1.5	0.0399±1.6	0.0401±1.5	0.0401±1.6
AA	0.0995-0.102	0.0989 ±2.1	0.0997 ± 2.1	0.0995 ± 2.1	0.1017±2.1	0.1019±2.1
ACV	0.3995-0.4023	0.3998 ±1.1	0.3997 ± 1.2	0.4022 ± 2.1	0.4017±2.2	0.3999±2.1

Quality control of CD.

A) Qualitative reactions.

To confirm the authenticity of the active ingredients (GA, AA), chemical reactions were used in accordance with the State Pharmacopoeia of the Russian Federation (monograph on the substance glycyram) and the State Pharmacopoeia of the Republic of Kazakhstan, Volume 2 (monograph on the substance ascorbic acid). According to regulatory documents, there are no chemical reactions for the ACV molecule; authenticity is confirmed only by physicochemical methods [12].

To determine the authenticity of the licorice root glycerin in the combined preparation, specific reactions for the triterpenoid structure (the Liebermann-Burchard reaction and foaming) were used. For qualitative analysis of the licorice root, identification methods using color and chromatographic reactions, described in the relevant section, were applied (reaction with silver nitrate and with 2,6-dichlorophenolindophenol sodium salt).

B) Methodology for identification of active ingredients and their related impurities using thin-layer chromatography.

As a stationary phase plates coated with reversed-phase silica gel were used, since the molecules of the active ingredients of the studied combination drug are polar in nature, which improves their separation when present together with the optimal R_f value of each of the analytes.

In the study and search for the optimal composition of the eluent, alcohols (methanol, ethanol and isopropanol), water and concentrated ammonia solution were used as polar solvents, which at different ratios increased or decreased the R_f height of highly polar molecules of GA and AA. Methylene chloride was used as a non-polar component of the mobile phase, the fractional part of which in the eluent significantly affected the retention coefficient of ACV, and to a lesser extent GA and AA. Thus, chromatography was carried out in the following solvent systems of neutral and basic nature: phosphate buffer-acetonitrile (7:3), (8:2), (6:4); chloroform-methanol-water (25:20:5), (30:17:3); isopropanol methylene chloride-water (6:3:1); isopropanol-methylene chloride-concentrated ammonia (6:3:1), ethanol-methylene chloride-concentrated ammonia solution (6:3:1); n-butanol-methylene chloride-concentrated ammonia solution (7:2:5); concentrated ammonia solution-water 96% ethanol-ethyl acetate (1:9:25:65).

Solvents with medium and low polarity were also used: acetonitrile and chloroform. Manipulating their volumes resulted in a lower percentage impact on the R_f and R_s (separation coefficient) of substances both in the model drug mixture and in the individual pathways of the reference substances. The most optimal mobile phase was ethanol-methylene chloride-concentrated ammonia solution (6:3:1), which ensured the selective separation of the three active substances and impurity A of acyclovir. Adsorption zones, visible under UV light, are formed as round, clearly defined violet spots on a greenish background. The detection limit for GA is 1 µg, for AA – 0.5 µg, and for ACV – 0.1 µg, impurity A – 0.1 µg. The R_f values were 0.33 for GA at the level of the SS GA, 0.66 for AA, 0.54 for ACV, and 0.75 for impurity A.

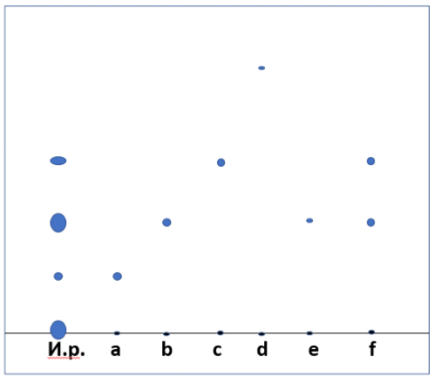

Figure 2. Chromatogram of the test solution and comparison solutions

I.R. – Test solution (model tablet mixture)

a – SS GA

b – SS AA

c – SS АЦК

d – SS impurity A

e – ACV solution for standardization of impurities

f – solution for determining the suitability of the chromatographic system

Validation was carried out in accordance with recommendations, taking into account the specifics of TLC methods [13,14], determining the following analytical characteristics: specificity, precision (intermediate) and robustness. The values of the validation characteristics of the developed TLC method are presented below (Table 2-6, Figure 3).

Table 2 –Resolution and selectivity characteristics in the determination of active ingredients in CD

Ingredient	R_f	Spot volume, cm	Sensitivity	Specificity
Impurity A	0.75	0.06	-	-
ACV	0.66	0.35	PrA-ACV: 4	PrA-ACV: 1.1
AA	0.54	0.93	PrA-AA: 3.6	PrA-AA: 1.2
GA	0.33	0.14	PrA -GA: 8	PrA-GA: 1.6

Table 3 –Retention coefficient values taking into account the standard deviation

Analyte	s_r (%)	$R_{f\ avg}$ (n=5)	Open rate (recovery) (%)
Impurity A	0.02	0.75	99.9
ACV	0.02	0.66	101
AA	0.05	0.54	99.8
GA	0.01	0.33	100

Figure 3. Probability distribution graph of R_f values for GA, AK and ACV

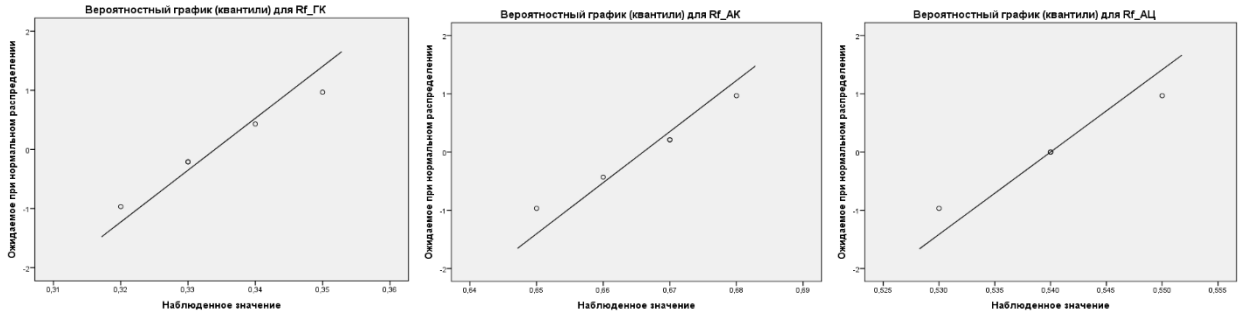

Table 4- Precision of R_f values corresponding to GA on the model mixture of CD

Lab	**Laboratory of the Department of Pharmaceutical Disciplines**		
Date	October 5, 2023	October 5, 2023	October 6, 2023
Type of precision	On one record	On different records	Intralaboratory precision
dimension 1, R_f	0.323	0.333	0.345
dimension 2, R_f	0.344	0.356	0.356
dimension 3, R_f	0.333	0.375	0.311

Average value R_f X mid	0.32	0.35	0.34
Standard deviation of repeatability	0.01	0.02	0.02
Relative Standard Error, % (CV, RSD)	3.15	5.93	6.95
R_f max-R_f min	0.02	0.04	0.05

Table 5 -Precision of R_f values corresponding to the AA on the model mixture of CD

Laboratory	**Laboratory of the Department of Pharmaceutical Disciplines**		
Date	October 5, 2023	October 5, 2023	October 6, 2023
Type of precision	On one record	On different records	Intralaboratory precision
dimension 1, R_f	0.66	0.68	0.66
dimension 2, R_f	0.65	0.66	0.67
dimension 3, R_f	0.66	0.67	0.66
Average value of R_f, Xmid	0.55	0.58	0.66
Standard deviation of repeatability	0.01	0.03	0.05
Relative Standard Error, % (CV, RSD)	2.09	4.31	9.09
R_f max-R_f min	0.01	0.02	0.01

Table 6 -Precision of R_f values corresponding to the ACV on the model mixture of CD

Laboratory	**Laboratory of the Department of Pharmaceutical Disciplines**		
Date	October 5, 2023	October 5, 2023	October 6, 2023
Type of precision	On one record	On different records	Intralaboratory precision
dimension 1, R_f	0.56	0.58	0.55

dimension 2, R_f	0.56	0.56	0.5
dimension 3, R_f	0.54	0.61	0.6
Average value of R_f, Xmid	0.55	0.58	0.55
Standard deviation of repeatability	0.01	0.03	0.05
Relative Standard Error, % (CV, RSD)	2.09	4.31	9.09
$R_f$max-$R_f$min	0.02	0.05	0.10

The robustness of a method is a measure of its ability to remain unchanged by small but non-random changes in the method parameters, demonstrating the suitability and reliability of the method under normal use. When determining the robustness of a method for identifying active ingredients and impurities in a drug product, the influence of chromatography conditions (sorbents of similar plates from different manufacturers, the type and saturation of the chromatographic chamber, the separation band length, and different drying conditions) on the final result was assessed.

The study of the optical characteristics of the samples in the ultraviolet range of the spectrum was carried out using an SF-2000 spectrophotometer (Russia), equipped with software from OKB Spektr (Russia).

The components of the studied combined composition are characterized by the following spectral properties in the ultraviolet region: - for GA, the maximum absorption in a 3% solution of trichloroacetic acid is (258 ± 2) nm (State Pharmacopoeia of the Russian Federation XIV, FS-42-2636);

— AA in a 0.1 M solution of hydrochloric acid has an absorption maximum at 247 nm (SP RK, Vol. 2, 2008);

— ACV in a 0.1 M hydrochloric acid solution is characterized by an absorption maximum at 254 nm with a shoulder at 272 nm (State Pharmacopoeia of the Republic of Kazakhstan, Vol. 2, 2008, “Acyclovir Tablets”).

It was established that chromatograms obtained on Sorbfil and FilterBio plates, with different chamber saturation times - 20, 30, 40, 60 min, under different drying conditions of the developed plate - at room temperature and in a drying cabinet, give comparable results and can be used in analysis.

The obtained validation characteristics demonstrate the suitability and reliability of the developed method, which was tested on laboratory samples of effervescent tablets.

C) UV spectrophotometry for quantitative determination of active ingredients in CD.

Differences in the solubility of the composition's components were used to separate the active ingredient—GA—from the inactive compounds. The latter, unlike GA, are highly soluble in water, allowing for their effective separation from the active substance.

Quantitative analysis also required preliminary separation of the active ingredients, as the electronic absorption spectra of GA and AA partially overlap, precluding their simultaneous determination. It should be noted that AA is prone to oxidation in neutral and alkaline environments. Therefore, precipitation with a 0.1 M hydrochloric acid solution was used to isolate the active components from the CD, followed by dissolution of AA and ACV. The resulting filtrate was used for quantitative determination of ACV and AA, while the precipitate, freed from inactive components by dissolution in water, was used to analyze the

GA content. The spectra of the active ingredients are presented in Figures 4-6.

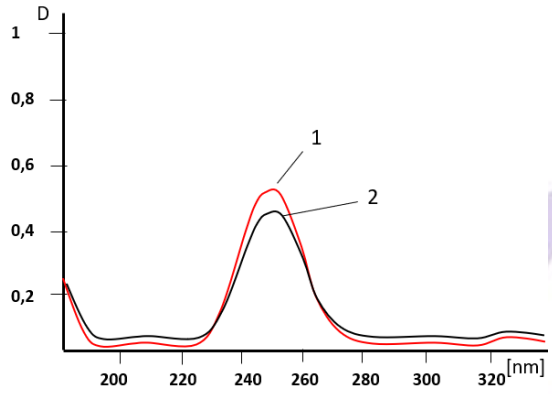

Figure 4. UV spectra of the solution of the SS GA (1) and the test solution of the medicinal product in the form of effervescent tablets (2)

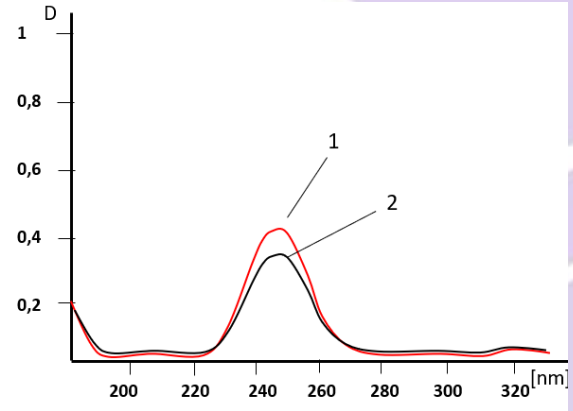

Figure 5. UV absorption spectra of the test solution (2) and SS AK (1) in 0.1 M hydrochloric acid solution

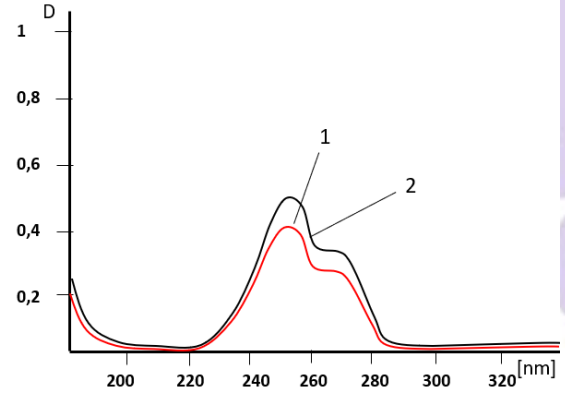

Figure 6. UV absorption spectra of the test solution (2) and ACV SS (1) in 0.1 M hydrochloric acid solution

The content of active ingredients was determined by the formula:

$$X = \frac{D_1 \text{ x } m_0 \text{ x } 50 \text{ x } 1 \text{ x } 50\text{x } b}{D_0\text{x } m_1 \text{ x } 50 \text{ x } 1\text{x } 50}$$

Where is

D_1 - the optical density of the test solution;

D_0– optical density (OD);

m_1- weight of the drug sample, in g;

m_0– sample weight (SW), in g;

b– average weight of 1 tablet, in g.

Tables 7-10 below show the results of testing parameters such as identity, linearity, accuracy and precision.

Table 7– Characteristics of the linearity of the method

Ingredient	D	E	Y_{esr}	Openability
GA				
35	0.4050	134.8		
35	0.4312	135.2		
34	0.4645	135.2		
33	0.4901	135.3	133.5	0.9997
42	0.5412	135.4		
41	0.5702	135.6		
46	0.5981	135.7		
48	0.6276	135.8		
39	0.6542	136.4		
AA				
13	0.2845	212.7		
13	0.3312	212.6		
15	0.3775	211.4		
20	0.4325	211.9	215.2	0.9996
21	0.4826	212.0		
25	0.5402	211.8		
25	0.5841	210.9		
29	0.6312	212.6		
28	0.6875	212.6		
ACV				
400	0.5455	323.5		
402	0.5503	324.1		
410	0.6234	323.6		
415	0.6901	324.0	324.0	0.9998
420	0.7434	324.5		
432	0.7753	323.5		
435	0.8123	324.9		
445	0.8509	325.1		
450	0.9207	323.6		

Table 8- characteristics of the correctness of the method on tablets

Quantity of sub-stance found in the table, %	Tablet, composition,G			Found,G			Open rate, %		
	GA	AA	ACV	GA	AA	ACV	GA	AA	ACV
70	0.0280	0.070	280	0.0281	0.0693	0.2767	99.6	99.5	99.3
80	0.0320	0.080	320	0.0323	0.0792	0.3182	99.8	99.4	99.5
90	0.0360	0.090	360	0.0367	0.089	0.3583	99.9	99.0	99.2
100	0.0410	0.100	400	0.0419	0.099	0.3989	99.3	99.3	99.0
110	0.0440	0.110	440	0.0442	0.1089	0.4378	99.7	99.5	99.6
120	0.0470	0.120	480	0.0479	0.1188	0.4776	99.6	99.2	99.4
130	0.0540	0.130	520	0.0547	0.1287	0.5174	99.8	99.7	99.3

Table 9– characteristic of the ACVuracy of the method on a model mixture

The amount detected, mg	m	Y mid	R	Δ Y mid	R f	Y mid±Δ Y mid	rcp%
According to the GA 40.27 40.84 41.12 41.54 41.92 42.62 42.79 43.02 43.19	9	35.92	1.04	0.82	2.36	35.92±0.82	1.96
According to AA 100.34 99.11 99.45 100.23 99.60 99.84 100.31 99.54 100.92	9	99.70	1.73	1.36	2.36	99.70±1.36	2.74
According to the ACV 399.34 400.11 399.45 400.23 399.60 399.84 401.31 399.54 399.92	9	399.53	1.52	1.24	2.23	399.53±1.24	2.53

Table 10– study of the accuracy of the method for quantitative determination of active substances in several batches of a medicinal product

Series No.	GA	AA	ACV
010363	40.27	99.34	399.34
020363	40.84	100.11	400.11
030363	41.12	99.45	399.45
040363	41.54	100.23	400.23
050363	41.92	99.61	399.60
060363	42.62	99.84	399.84
Average value, mg	41,385	99,763	399.54
Differences, mg	2.35	2.5	2.4
Relative difference, %	5,678	5,127	5,341
Average square Off, mg	0.83	0.964	0.765
Rel. Average square Off, %	2,006	1,977	1,234

Table 11– Study of the accuracy of analysis in the quantitative determination of active ingredients in several batches of CD

Series No.	GA	AA	ACV
010363	40.27	99.34	399.34
020363	40.84	100.11	400.11
030363	41.12	99.45	399.45
040363	41.54	100.23	400.23
050363	41.92	99.61	399.60
060363	42.62	99.84	399.84
Average value, mg	41,385	99,763	399.54
Differences, mg	2.35	2.5	2.4
Relative difference, %	5,678	5,127	5,341
Average square Off, mg	0.83	0.964	0.765
Rel. Average square Off, %	2,006	1,977	1,234

The identity of the active ingredients of the effervescent tablets GA, AA, and ACV was confirmed by matching the absorption maxima and minima of the analyzed samples with the corresponding RSO preparations under optimally selected spectrophotometric conditions. Statistical processing of the experimental results was performed in accordance with the State Pharmacopoeia of the Republic of Kazakhstan, Volume 1.

The linear dependence of the method characterizes the possibility of obtaining an analytical signal in the form of optical density proportional to the content of the analyte in the sample being studied.

The linearity of the test method was studied using model mixtures in the range of 70-130% of the declared GA, AA, and ACV content in effervescent tablets. Statistical anal-

ysis of the model mixture results revealed a linear relationship between the active ingredient concentration and optical density.

The percentage levels of regeneration were observed within the range of 99.6-99.9% for GA, 99.0-99.7% for AA, 99.2-101.8% for ACV.

As shown in Table 9, the relative error of the average results for active substances is within the range of 1.90-2.74%, which indicates good reproducibility of the developed method.

Thus, it was established that the developed method for determining GA, AA and ACV in pharmaceutical preparations has the correct accuracy and reproducibility in relation to the declared content of the active substance in the dispersion, with a linear correlation of ±30% in the analytical range of the tablet, which makes them useful for a reliable assessment of the quality of drugs.

Study of the specific activity of CD in in vitro experiments

The antiviral activity of four compounds, both individually and in triple combinations, was studied against two strains of herpes simplex virus type 1 (HSV-1)—sensitive and resistant to acyclovir. Activity was assessed using microtiter assays to determine the inhibition of virus-induced cytopathic effect (CPE) in Vero E6 cell culture. The combinations studied included GA, AA, αIFN and ACV. The main criterion for the antiviral activity of individual substances and their combinations was the ability of the drugs to prevent the death of infected cells by inhibiting the development of virus-induced CPE.

The experiment was conducted using a monolayer culture of Vero E6 cells grown in 96-well polymer plates. Infection was performed at a multiplicity of infection of 0.1 PFU/cell. Incubation was carried out for 48 hours at 37°C. In control samples, the CPE development rate was 95–100%, corresponding to complete destruction of the cell monolayer.

The efficacy of the studied compounds was expressed as ID_{50} and ID_{95}—the concentrations of the substances that inhibit the development of virus-induced CPE by 50% and 95%, respectively. When studying the combined effect of the drugs, the concentrations of the components that provide these levels of inhibition when used together were determined.

The antiherpetic activity of the combinations was assessed by the fractional inhibitory concentration (FIC) index, calculated using the formula:

FIC = ID_{50} of compound A in combination/ID_{50} of compound A + ID_{50} of compound B in combination/ID_{50} of compound B

Table 12- Antiherpetic activity of triple combinations of a number of compounds in the model of HSV-1 with different drug sensitivities in Vero E6 cell culture

№	Compoun	CD_{50} mcg/ml	ID_{50} μg/ml	ID_{95} μg/ml	FIC	Effect
1	ACV+AA + αIFN	>15.6+31.25+7.8	7.8+15.6+3.91/7.8+31.25+7.82	15.6+31.2+7.81/15.6+62.5+7.82	0.681/0.682	Synergistic / Synergetic
2	ACV+AA+GA	>125+1000+125	7.8+15.6+1251/15.6+31.25+125	15.6+15.6+2501/31.2+15.6+250	0.871/1.02	Synergistic/ additive
3	ACV+GA + αIFN	>31.2+250+15.6	7.8+62.5+1.951/5.6+62.5+3.92	15.6+125+3.91/31.25+125+3.92	0.871/0.872	Synergistic / Synergetic

An analysis of the data presented in Table 12 reveals that the triple combination of ACV + GA + AA is of greatest interest. The combined use of these compounds reduces the

concentration of the most toxic component, ACV, demonstrating the combination's potential to increase the therapeutic index. This combination has been shown to exhibit both synergistic and additive effects, confirming its feasibility for use in the development of a combination drug in effervescent tablet form.

DISCUSSION. THE CONDUCTED STUDIES SUBSTANTIATED THE CHOICE OF AN EFFERVESCENT FORMULATION FOR A COMBINATION DRUG CONTAINING GA, AA, AND ACV. THE USE OF DIRECT COMPRESSION ALLOWED FOR THE PRODUCTION OF A STABLE AND TECHNOLOGICALLY REPRODUCIBLE DOSAGE FORM WITH OPTIMAL QUALITY INDICATORS. THE USE OF PRUV® SODIUM STEARYL FUMARATE AS A LUBRICANT REDUCED THE LABOR AND ENERGY INTENSITY OF THE PRODUCTION PROCESS AND INCREASED THE WETTABILITY AND SOLUBILITY OF THE RESULTING TABLETS, WHICH IS ESPECIALLY IMPORTANT WHEN CREATING EFFERVESCENT DRUGS. THE NATURAL SWEETNESS AND AROMA OF GA ELIMINATED THE NEED FOR ADDITIONAL FLAVORINGS AND SWEETENERS, MAKING THE DRUG SAFER AND MORE PHARMACOLOGICALLY PURE.

The absence of exothermic and endothermic peaks in the DSC curves of the studied mixtures confirms the absence of chemical interactions between the components of the drug, demonstrating the pharmaceutical compatibility of GA, AA, and ACV in the selected composition. The obtained results are consistent with published data on the stability of these compounds under moderate temperature conditions.

The "accelerated aging" method allowed us to establish a shelf life of the drug of 3 years when stored at a temperature not exceeding 25-26°C. Throughout the entire testing period, the samples maintained their physicochemical properties, as demonstrated by the stability of their average weight, homogeneity, active ingredient content, and the absence of impurities. Thus, the selected excipient composition and direct compression technology ensure the physical and chemical stability of the dosage form.

Validation of the TLC method confirmed its selectivity, specificity, and precision. Separation of active components and impurities was achieved in the optimal solvent system of ethanol–methylene chloride–ammonia solution (6:3:1), yielding clear adsorption zones and reproducible R_f values. Low relative standard deviations (2–9%) confirm the high repeatability of the method. Robustness of the method was demonstrated with changes in non-essential analytical conditions (plate type, chamber saturation, drying conditions), demonstrating its reliability in analytical practice.

The UV spectrophotometric method demonstrated high accuracy, precision, and linearity in the range of 70–130% of the nominal active ingredient content. Correlation coefficient values (r = 0.9996–0.9998) indicate the method's high analytical sensitivity. The analysis results for tablets from different batches demonstrate stability of GA, AA, and ACV content within acceptable tolerances, confirming the reproducibility of the technological process.

An in vitro study of antiviral activity demonstrated that the combination of GA, AA, and ACV exhibits a significant synergistic or additive effect against herpes simplex virus type 1, including acyclovir-resistant strains. This demonstrates the potential of the developed composition as an effective preventative and therapeutic agent for herpesvirus infections. GA likely enhances the penetration and effectiveness of ACV due to its membrane-stabilizing and anti-inflammatory properties, while AA provides antioxidant protection and increases cellular resistance to viral aggression.

Overall, the study results confirm the rationale for the chosen composition and technology for producing effervescent tablets. The developed drug is characterized by high stability, reproducibility, safety, and potential for further preclinical and clinical trials. The pre-

sented data can serve as a scientific and practical basis for the development of a domestic combination antiviral drug with increased bioavailability and an improved profile of action.

Conclusion.

Based on the conducted research, a stable and technologically reproducible effervescent form of a combination drug containing glycyrrhizic acid, ascorbic acid and acyclovir has been developed. This form is distinguished by confirmed pharmaceutical compatibility of the components, validated quality control methods and a pronounced synergistic antiviral effect, which justifies the prospects for its further implementation in pharmaceutical practice.

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