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STUDY OF THE ANTIMICROBIAL ACTIVITY OF CAPTOPRIL PHARMACEUTICAL PRODUCTS PRODUCED IN KAZAKHSTAN

According to the requirements of the State Pharmacopoeia of the Republic of Kazakhstan for the control of medicinal products, one of the essential components in ensuring the safety, efficacy, and high quality of pharmaceutical products manufactured in the country is the assessment of their antimicrobial activity. Compliance with microbiological purity standards enables a comprehensive evaluation of drug quality, strengthens the alignment of domestic pharmaceutical production with international standards, and contributes to maintaining competitiveness in the pharmaceutical market.

This study experimentally investigated the antimicrobial activity of the pharmaceutical substance and finished dosage form of Captopril produced in Kazakhstan against pharmacopoeial test microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus brasiliensis*. It is known that certain medicinal products may possess intrinsic inhibitory activity, which can distort microbiological test results and lead to false-negative outcomes. Therefore, preliminary assessment of a drug's impact on microbial growth is a critical step for accurate microbiological evaluation.

Three batches of Captopril substance and three batches of the tablet dosage form were examined. The results demonstrated that dilutions of 1:10 and 1:20 exhibited a pronounced inhibitory effect against *E. coli*, *S. aureus* and *B. subtilis*. No activity was observed against *Candida albicans*, *Aspergillus brasiliensis* or *P. aeruginosa*, while the 1:50 dilution completely lacked antimicrobial activity.

The findings confirm the importance of employing methodologies fully compliant with pharmacopoeial requirements when assessing the quality of Captopril substance and its finished dosage form. Moreover, the results justify the necessity of including information on the drug's intrinsic antimicrobial properties in the manufacturer's specification. This study holds practical significance for ensuring the quality and safety of domestically produced pharmaceutical products.

Keywords: drug, substance, Captopril, antimicrobial activity, test strain, microorganisms.

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Қазақстанда өндірілетін каптоприл дәрілік препаратының антимикробтық белсенділігін бағалау

Қазақстан Республикасының Мемлекеттік Фармакопеясымен дәрілік заттарды бақылауға қойыған талаптарына сәйкес, елімізде өндірілетін дәрілік өнімдердің қауіпсіздігін, тиімділігін және жоғары сапасын қамтамасыз ету үшін жүргізілетін негізгі зерттеулердің бірі – олардың антимикробтық белсенділігін бағалау болып табылады. Микробиологиялық тазалық талаптарының сәйкестігі жалпы дәрілік заттардың сапасын кешенді бағалау мүмкіндік береді және отандық өндірістің халықаралық стандарттарға сәйкестігін арттырып, фармацевтикалық нарықтағы бәсекеге қабілеттілігін қамтамасыз ететін негізгі факторлардың бірі болып табылады.

Бұл ғылыми жұмыста Қазақстанда өндірілетін Каптоприл дәрілік препаратының фармацевтикалық субстанциясы мен дайын дәрілік формасының фармакопеялық тест-микроорганизмдері *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans* және *Aspergillus brasiliensis* қатысты антимикробтық белсенділігі тәжірибе

Көптеген дәрілік заттардың құрамында табиғи түрде белгілі бір деңгейде ингибиторлық белсенділік болуы мүмкін, ал бұл қасиет микробиологиялық талдау нәтижелерін бұрмалап, жалған теріс көрсеткіштерге алып келуі ықтимал. Сондықтан микробиологиялық тазалықты дұрыс бағалау үшін зерттелетін препараттың микроорганизмдерге әсерін алдын ала анықтау ерекше маңызды.

Біздің зерттеуде Каптоприл субстанциясының және таблетка түріндегі дәрілік өнімнің үш сериясы қолданылды тест штаммдарына әсері бағаланып, нәтижесінде *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*,1:10 және 1:20 сұйылтуларда препараттың айқын тежегіш әсер көрсететіні анықталды. *Candida albicans* және *Aspergillus brasiliensis,* сондай-ақ *P. aeruginosa* штаммдарына қатысты белсенділік байқалған жоқ, ал 1:50 қатынастағы тәжірибеде антимикробтық активтілік ингибирленді.

Зерттеу нәтижелері Каптоприл субстанциясы мен дәрілік затының сапасын бақылау кезінде фармакопеялық талаптарға толық сәйкес келетін әдістемені қолданудың маңыздылығын дәлелдеп, өндіруші спецификациясына препараттың антимикробтық белсенділігі туралы ақпаратты енгізу қажеттілігін негіздеді. Бұл жұмыстың нәтижелері отандық фармацевтикалық өндірісте сапа мен қауіпсіздікті қамтамасыз етуге бағытталған тәжірибелік құндылыққа ие.

Түйін сөздер: дәрілік препарат, субстанция, Каптоприл, антимикробтық белсенділік, тест-штамм микроағзалары.

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Оценка антимикробной активности лекарственного препарата Каптоприл, производимого в Казахстане

В соответствии с требованиями Государственной Фармакопеи Республики Казахстан к контролю лекарственных средств, одним из ключевых этапов обеспечения безопасности, эффективности и высокого качества отечественных фармацевтических продуктов является оценка их антимикробной активности. Определение соответствия показателям микробиологической чистоты позволяет комплексно оценить качество препарата, повысить соответствие национального производства международным стандартам и обеспечить его конкурентоспособность на фармацевтическом рынке.

В данном исследовании экспериментально изучена антимикробная активность фармацевтической субстанции и готовой лекарственной формы Каптоприла, производимого в Казахстане, по отношению к фармакопейным тест-микроорганизмам *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans* и *Aspergillus brasiliensis*. Известно, что некоторые лекарственные средства обладают собственной ингибирующей активностью, которая может искажать результаты микробиологических испытаний и приводить к ложноотрицательным показателям. Поэтому предварительная оценка влияния препарата на рост микроорганизмов является необходимым условием корректного анализа.

В рамках исследования оценено действие трёх серий субстанции и таблетированной формы Каптоприла. Установлено, что в разведениях 1:10 и 1:20 препарат проявляет выраженный ингибирующий эффект в отношении *E. coli*, *S. aureus* и *B. subtilis*. Активность в отношении *Candida albicans*, *Aspergillus brasiliensis* и *P. aeruginosa* не наблюдалась, а при разведении 1:50 антимикробный эффект полностью отсутствовал.

Полученные результаты подтверждают необходимость применения методик, полностью соответствующих фармакопейным требованиям, при контроле качества субстанции и готового лекарственного средства Каптоприл. Кроме того, показано, что сведения о собственной антимикробной активности препарата должны быть отражены в спецификации производителя. Проведенное исследование имеет практическую значимость для обеспечения качества и безопасности отечественных фармацевтических продуктов.

Ключевые слова: лекарственные препараты, субстанция, Каптоприл, антимикробная активность, тест-штаммы, микроорганизмы.

Introduction

The modern arsenal of medicines includes a wide range of drugs. However, the technology of their production does not always guarantee complete microbiological purity of the drugs. Injectable and ophthalmic drugs must be absolutely sterile, which is taken into account during their production. For other categories of drugs, contamination with microorganisms is undesirable, but possible, since the rules for their production do not require strict sterility. The main sources of contamination of medicinal products (DPs) are: the substance (most often of plant and animal origin), process water, production equipment, industrial air, workers, containers and finished DP packaging [1,2].

In order to implement the goal of the Address of the President of the Republic of Kazakhstan to the people – to enter the list of the 50 most developed and competitive countries in the world, leading enterprises of the domestic pharmaceutical industry, carrying out restructuring in the production sector, have been introducing the principles of GMP (Good Manufacturing Practice) since January 2008 and are moving to new requirements for controlling medicinal products established by the State Pharmacopoeia of the Republic of Kazakhstan (MPK RK), which ensure the safety, effectiveness, and high quality of the product, which in turn is the main condition for its competitiveness in the domestic and global pharmaceutical markets [3].

The establishment of the MF of the Republic of Kazakhstan was carried out with state support since 2005. The first edition of the Pharmacopoeia was published in three volumes in the state and Russian languages. Volumes I and II were approved and put into effect in 2008, and Volume III in 2015. The legislative status of the MF of the Republic of Kazakhstan is established by the Code of the Republic of Kazakhstan "On Public Health and the Healthcare System"[4].

Contamination of DP with microorganisms can be the cause of infectious processes in the person receiving it. In clinical practice, cases of diseases caused by enterobacteria, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, spore-forming anaerobes, mold fungi, etc. are known [5].

Contamination of DP with microorganisms violates its stability. Antimicrobial compounds in the composition of ointments for external use do not always guarantee their microbial purity. The multiplication of microorganisms in the composition of the ointment leads to a change in its consistency, the appearance of an unpleasant odor, etc. changes[6].

In liquid dosage forms, metabolites of microorganisms can change their chemical composition and may also lead to the formation of toxic products. Solid dosage forms are less likely to be contaminated with microorganisms, since they do not provide a suitable environment for the growth of microorganisms. Contamination of DP raw materials with microorganisms, as well as improper storage, can lead to changes in their properties [7].

The need for quality control of non-sterile medicinal products is justified by the importance of ensuring their safety and effectiveness, that is, reducing the risk when used by the consumer. Thus, it is necessary to achieve quality assurance of non-sterile medicinal products by assessing the risk of the occurrence of substandard products and creating a risk management system, starting from the development of a new pharmaceutical product, from production to the consumer. In this case, biological methods for testing pharmaceutical products related to the field of pharmacology and microbiology play a special role[8,9].

The specificity of the types of microbiological analysis is the specificity of the object of study – microorganisms with individual properties. The most important features of DP that affect the results of the analysis are: the presence of antimicrobial activity in DP themselves or their components, as well as the presence of preservatives in some of them that prevent the detection of microorganisms [4,10].

Medicinal products (Drugs) (substances, various forms of drugs – tablets, capsules, granules, solutions, suspensions, syrups, ointments, suppositories, etc., as well as excipients) can be contaminated with microorganisms. Before controlling them according to the "microbiological purity" indicator of the KR MF, volume 1, it is necessary to determine their antimicrobial activity against molds and yeasts and individual types of microorganisms, as this leads to an incorrect assessment of the results obtained [11].

The study of the antimicrobial activity of substances used in the development of drugs improves the well-known methods of studying ready-made DP, thereby preventing ways of harming human health [6,12,13].

Antimicrobial activity is a process aimed at destroying or inhibiting pathogenic microorganisms. For this, various agents are used against microor-

ganisms. Drugs against microorganisms can be antibacterial, antifungal or antiviral [14].

To eliminate antimicrobial activity, special, non-specific inactivators, special neutralizing solutions are used, the amount of normal solvent is increased, and membrane filtration is used as a test [15].

A variety of compounds involved in the treatment of diseases of non-infectious etiology show a certain antimicrobial activity in vitro against bacteria and other microorganisms. Such compounds are called non-antimicrobial drugs. At the end of the 19th century, it was known that dyes have activity against microorganisms, for example, Paul Ehrlich used methylene blue (one of the phenothiazine compounds) as an agent against microorganisms [16].

The antimicrobial activity of some non-antimicrobial drugs was first revealed on the Polish pharmaceutical market during several years of drug control at the National Institute of Medicine. Currently, about 950 drugs randomly selected from various groups of pharmaceutical products have been studied for their antimicrobial activity. During the study, it was shown that some drugs inhibit the growth of at least one of the four standard strains of microorganisms studied..

Classes of drugs such as neuroleptics, antihistamines, antidepressants, antiplatelet agents, and nonsteroidal anti-inflammatory drugs have varying degrees of activity against a broad spectrum of microorganisms [17]. These non-antimicrobial drugs affect the growth of microorganisms in a variety of ways. They may have direct activity against microorganisms, enhance the efficacy of antibiotics when coadministered (adjuvant compounds), or alter the pathogenicity or activity of microorganisms, such as by modulating macrophage activity [18].

The main aspects of the quality of DP are physicochemical and microbiological tests [19]. An important aspect of conducting microbiological tests is the study of the antimicrobial activity of the sample under study. This is necessary for the correct development of the finished DP research methodology and its inclusion in the analytical regulatory document or quality specification [20].

Currently, domestic and foreign authors have shown that non-antimicrobial drugs can have an antimicrobial effect on some test microorganisms, which justifies the need to determine this characteristic of DP during the microbiological testing of DP in order to reduce the risk of false-negative results [21,22].

If the conditions for the production and storage of medicinal products are not met, their biodegradation may occur under the influence of microbial enzymes, the speed of which is determined by the chemical composition of the medicinal product, the presence of substances in it that are easily absorbed by microorganisms or have biocidal activity, the number and species composition of contaminants, environmental conditions (humidity, temperature). Some components of drugs (starch, gelatin, kaolin, magnesium trisilicate, aluminum hydroxide, surfactants, proteins) protect the cells of microorganisms from preservatives [23]. The possibility of biodegradation processes is influenced by the type of packaging that prevents the ingress of contaminants and controls humidity. An important criterion for conducting analytical work on the appropriate microbiological purity of a drug is the study of the antimicrobial activity of the active pharmaceutical ingredients included in its composition and the drug itself during the manufacturing process [24].

Pilot-industrial batches of the non-antimicrobial active pharmaceutical ingredient Captopril and the hard tablet formulation Captopril were selected for antimicrobial activity studies.

The efficacy of the well-known angiotensin-converting enzyme inhibitor L-Captopril as an inhibitor of dapE was assessed by analyzing its binding modes and affinity for dapE, and it was demonstrated that L-Captopril inhibits the growth rate of test cultures of S. enterica and E. Coli [25].

Materials and methods

The object of the research work was the pharmaceutical active substance Captopril series 5102-17-014, 5102-17-045, 5102-18-017 and the experimental-industrial series of the drug Captopril 001, 002, 003 produced in Kazakhstan.

The antihypertensive drug Captopril is an inhibitor of angiotensin-converting enzyme (ACE). The mechanism of the antihypertensive effect of Captopril is associated with competitive inhibition of ACE activity, which leads to a decrease in the rate of conversion of angiotensin I to angiotensin II in tissues and blood plasma, as a result of which it achieves a vasodilating effect and a decrease in aldosterone secretion in the adrenal glands. As a result, Captopril reduces total peripheral vascular resistance (afterload), pulmonary capillary wedge

pressure (preload) and pulmonary vascular resistance; increases cardiac output and exercise tolerance [26,27,28].

To study the antimicrobial activity of the substance and drug Captopril, the following pharmacopoeial test cultures were used: *Escherichia coli* ATCC®8739, *Pseudomonas aeruginosa* ATCC®9027, *Staphylococcus aureus* ATCC®6538, *Bacillus subtilis* ATCC®6633 *Candida albicans* ATCC®10231, *Aspergillus brasiliensis* ATCC®16404, produced by the Italian company Liofilchem®, which produces standard reference cultures of microorganisms from the American Type Culture Collection (ATCC).

Each test culture is a lyophilized microorganism prepared from a reference primary culture (5 granules in 1 vial).

During the research, tryptone soy-casein agar, tryptone soy-casein broth, Sabouraud agar, produced by the German company Merck KgaA, were used.

The antimicrobial activity of the substance and drug captopril was studied using the method described in the first volume of the National Pharmacopoeia of the Republic of Belarus – "Determination of antimicrobial activity under microbiological purity testing conditions (for water-soluble drugs)" [29].

Determination of antimicrobial activity under microbiological purity testing conditions (for water-soluble drugs)For this purpose, a suspension was prepared using pharmacopoeial test culture microorganisms (*Escherichia coli* ATCC®8739, *Pseudomonas aeruginosa* ATCC®9027, *Staphylococcus aureus* ATCC®6538, *Bacillus subtilis* ATCC®6633, *Candida albicans* ATCC®10231, *Aspergillus brasiliensis* ATCC®16404) [30,31,32].

Suspensions of bacterial test cultures are inoculated onto tryptone soy-casein agar slants and incubated for 48 hours at 30-35◦C.

Cultures of yeasts are inoculated onto Sabouraud agar slants in test tubes and incubated for 5 days at 20-25◦C [6,11]..

After the incubation period, bacterial cultures and *Candida albicans* cultures are washed from the slants with 0.9% sodium chloride solution, and *Aspergillus brasiliensis* cultures are washed with 0.9% sodium chloride solution with 0.05% polysorbate 80, and suspensions are made[11,12].

The resulting bacterial suspensions are diluted to a concentration of 103 CFU/ml, and fungal suspensions to a concentration of 103-104 CFU/ml with phosphate buffer solution.

The Captopril substance and the drug substance for the study were diluted in three different concentrations in standard phosphate buffer solution: 1:10, 1:20 and 1:50.

To determine the antimicrobial activity against the test microorganisms *Candida albicans*, *Aspergillus brasiliensis*, *Bacillus subtilis*, 1 ml of each prepared concentration (1:10, 1:20, 1:50) of the sample was inoculated into a sterile Petri dish for the purpose of deep inoculation. In the Petri dishes with the sample, suspensions of the test microorganisms were added in the volume of: 0.2 ml *Bacillus subtilis*; 0.2 ml *Candida albicans*; 0.2 ml *Aspergillus brasiliensis* (for each concentration of the sample under study). Inoculation was carried out in 3 replicates for each sample.

Petri dishes contaminated with *Bacillus subtilis* test cultures are poured with 15-20 ml of tryptone soy-casein agar cooled to 45-50◦C and mixed.

Petri dishes contaminated with *Candida albicans*, *Aspergillus brasiliensis* test strains are poured with 15-20 ml of Sabouraud agar cooled to 45-50◦C and mixed.

To determine the antimicrobial activity of the sample under study against the test culture microorganisms *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, 1 ml of each prepared concentration of the sample (1:10, 1:20, 1:50) is dropped into 9 test tubes containing 10 ml of tryptone soy-casein broth. The test-strain suspensions are added to the test tubes (for each concentration of the test sample) in the following volumes: 1 ml of *Escherichia coli* culture suspension to 3 tubes; 1 ml of *Pseudomonas aeruginosa* culture suspension to 3 tubes; 1 ml of *Staphylococcus aureus* test-strain suspension to 3 tubes.

In parallel, the growth rate of the test-culture microorganisms is monitored. For this, 0.2 ml of *Bacillus subtilis* (at a concentration of 103 CFU/ml) of each test-culture suspension prepared is added to tryptone soy-casein agar; 0.2 ml of *Candida albicans*; 0.2 ml of *Aspergillus brasiliensis* Sabouraud agar medium is added and mixed.

Escherichia coli ATCC®8739, *Pseudomonas aeruginosa* ATCC®9027, *Staphylococcus aureus*

ATCC®6538 test culture microorganisms (at a concentration of 103 CFU/ml) were inoculated in 1 ml of 10 ml test tubes. The control was performed in 3 replicates.

Samples contaminated with test culture microorganisms *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* are incubated in a thermostat for 48 hours at a temperature of 35-37◦C.

Samples contaminated with test culture microorganisms *Candida albicans*, *Aspergillus brasiliensis* are incubated in a thermostat for 5 days at a temperature of 22-25◦C.

After the incubation period, the presence or absence of a characteristic and normal growth rate of microorganisms is determined in Petri dishes and test tubes containing the tested sample and test culture microorganisms and in controls [6,11].

The applied methods are consistent with the fundamental principles of biomedical statistics described by Altman (1991), including the use of mean values, standard deviation, and variability analysis [33].

Results and discussion

In the experimental work, the antimicrobial activity of three batches of Captopril (substance: 5102-17-014, 5102-17-045, 5102-18-017; finished dosage form: batches 001, 002, 003) was evaluated against the pharmacopeial test strains Escherichia coli ATCC®8739, Pseudomonas aeruginosa ATCC®9027, Staphylococcus aureus ATCC®6538, Bacillus subtilis ATCC®6633, Candida albicans ATCC®10231, and Aspergillus brasiliensis ATCC®16404. For each concentration/strain combination, three replicates were performed (x1, x2, x3), and the mean values ($\bar{x}$) and standard deviations were calculated. The results are summarized in Tables 1–3.

Table 1 – Results of the Study of the Antimicrobial Activity of Captopril Substance (Batches 5102-17-014, 5102-17-045, 5102-18-017)

Test cultures		Sample conc.	Unit of measure	Captopril substance series											
				5102-17-014				5102-18-017				5102-17-045			
Bacillus subtilis	With medication	1:10	CFU	x_1	x_2	x_3	$\underline{x_i}$	x_1	x_2	x_3	$\underline{x_i}$	x_1	x_2	x_3	$\underline{x_i}$
				0	0	0	0	0	0	0	0	0	0	0	0
		1:20		0	0	0	0	0	0	0	0	0	0	0	0
		1:50		94	95	95	95	90	89	90	90	95	91	93	93
	Without medication	-	CFU	96	94	95	95	89	92	91	91	93	94	94	94
	Common standard deviation														
	With medication	1:50	%	x_i=95				x_i=90				x_i=93			
				2.52											
	Without medication	-	%	x_i=95				x_i=91				x_i=94			
				2.08											

Continuation of the table

Test cultures		Sample conc.	Unit of measure	Captopril substance series											
				5102-17-014				5102-18-017				5102-17-045			
Candida albicans	With medication	1:10	CFU	x_1	x_2	x_3	$\underline{x_i}$	x_1	x_2	x_3	$\underline{x_i}$	x_1	x_2	x_3	$\underline{x_i}$
				87	86	86	87	94	95	92	94	90	92	92	91
		1:20		92	87	90	90	94	96	95	95	89	91	92	91
		1:50		93	94	94	94	96	93	95	95	89	94	92	92
	Without medication	-	CFU	89	95	92	92	91	95	93	93	90	94	92	92
	Common standard deviation														
	With medication	1:10	%	x_i=87				x_i=94				x_i=91			
				3.51											
		1:20		x_i=90				x_i=95				x_i=91			
				2.64											
		1:50		x_i=94				x_i=95				x_i=92			
				1.52											
	Without medication		%	x_i=92				x_i=93				x_i=92			
				0.57											
Aspergillus brasiliensis	With medication	1:10	CFU	x_1	x_2	x_3	$\underline{x_i}$	x_1	x_2	x_3	$\underline{x_i}$	x_1	x_2	x_3	$\underline{x_i}$
				48	45	40	44	44	39	35	39	39	37	40	39
		1:20		47	42	40	43	41	40	38	40	40	38	35	38
		1:50		39	40	41	40	38	31	39	36	36	38	36	37
	Without medication		CFU	42	40	43	42	39	42	40	40	39	36	38	38
	Common standard deviation														
	With medication	1:10	%	x_i=44				x_i=39				x_i=39			
				2.88											
		1:20		x_i=43				x_i=40				x_i=38			
				2.51											
		1:50		x_i=40				x_i=36				x_i=37			
				2.08											
	Without medication		%	x_i=42				x_i=40				x_i=38			
				2											

At concentrations 1:10 and 1:20, the substance demonstrated pronounced inhibition of B. subtilis, manifested by the absence of microbial growth, whereas at 1:50 growth was present and the CFU counts were close to the control. For *Candida albicans* and *Aspergillus brasiliensis*, the substance did not show significant fungicidal activity at the tested concentrations; at 1:10–1:50, numerical values and mean counts differed insignificantly from the control. Standard deviations across the three replicates were small, indicating reproducibility of the measurements.

The Captopril substance exhibits selective antibacterial activity against some test strains at higher concentrations, but does not demonstrate meaningful antifungal effects under the test conditions.

For the finished dosage form of Captopril (batches 001, 002, 003), inhibition of *B. subtilis* was also observed at 1:10 and 1:20, i.e., absence of growth, while at 1:50 microbial growth was restored.

Table 2 – Results of the Study of the Antimicrobial Activity of Captopril Finished Dosage Form (Batches 001, 002, 003)

Test cultures		Sample conc.	Unit of measure	Captopril drug series											
				001				002				003			
Bacillus subtilis	With medication	1:10	CFU	x_1	x_2	x_3	$\underline{x_i}$	x_1	x_2	x_3	$\underline{x_i}$	x_1	x_2	x_3	$\underline{x_i}$
				0	0	0	0	0	0	0	0	0	0	0	0
		1:20		0	0	0	0	0	0	0	0	0	0	0	0
		1:50		96	93	95	95	94	93	94	94	93	90	91	91
	Without medication	-	CFU	95	96	95	95	97	96	95	96	93	92	89	91
	Common standard deviation														
	With medication	1:50	%	x_i=95				x_i=94				x_i=91			
				2.08											
	Without medication	-	%	x_i=95				x_i=95				x_i=91			
				2.3											
Candida albicans	With medication	1:10	CFU	x_1	x_2	x_3	$\underline{x_i}$	x_1	x_2	x_3	$\underline{x_i}$	x_1	x_2	x_3	$\underline{x_i}$
				94	95	95	95	94	90	92	92	87	86	86	86
		1:20		94	96	95	95	94	92	89	92	92	87	91	90
		1:50		96	93	95	95	94	93	88	92	89	93	91	91
	With medication	-	CFU	91	95	93	93	92	94	91	92	93	89	92	91
	Common standard deviation														
	With medication	1:10	%	x_i=95				x_i=92				x_i=86			
				4.58											
		1:20		x_i=95				x_i=92				x_i=90			
				2.51											
		1:50		x_i=95				x_i=92				x_i=91			
				2.08											
	Without medication		%	x_i=93				x_i=92				x_i=91			
				1.0											

Continuation of the table

Test cultures		Sample conc.	Unit of measure	Captopril drug series											
				001				002				003			
Aspergillus brasiliensis	With medication	1:10	CFU	x_1	x_2	x_3	$\underline{x_i}$	x_1	x_2	x_3	$\underline{x_i}$	x_1	x_2	x_3	$\underline{x_i}$
				41	42	40	41	38	39	35	37	40	38	34	37
		1:20		39	40	39	39	42	40	39	40	36	34	40	40
		1:50		39	38	41	39	39	33	42	38	41	36	35	37
	Without medication		CFU	38	41	43	40	42	37	39	40	37	42	38	39
	Common standard deviation														
	With medication	1:10	%	x_i=41				x_i=37				x_i=37			
				2.3											
		1:20		x_i=39				x_i=40				x_i=40			
				0.57											
		1:50		x_i=39				x_i=38				x_i=37			
				1.0											
	Without medication		%	x_i=40				x_i=40				x_i=39			
				0.57											

For *Candida albicans* and *Aspergillus brasiliensis*, no significant deviations from the control were observed, confirming the absence of antifungal activity under the tested conditions.

Comparison of the batches shows minimal variability (similar $\bar{x}$ and SD values), indicating stability of the manufacturing process and consistency of the effect.

Thus, the finished dosage form reproduces the behavior of the substance—demonstrating a bacteriostatic effect at higher concentrations and no activity against the tested fungal strains.

In the next stage of our experiments, a qualitative assessment of growth was performed for *P. aeruginosa*. As shown in table 3, all batches and all concentrations exhibited growth ("+"), indicating no antimicrobial activity against this strain. This finding is consistent with the known high resistance of *Pseudomonas* to many substances.

Table 3 – Antimicrobial Activity Against *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*

Tested sample/series																			
Test cultures		Captopril substance									The drug Captopril								
		Series																	
		5102-17-014			5102-17-045			5102-17-014			001			002			003		
Pseudomonas aeruginosa	1:10	x_1	x_2	x_3	x_1	x_2	x_3	x_1	x_2	x_3	x_1	x_2	x_3	x_1	x_2	x_3	x_1	x_2	x_3
		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1:20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1:50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Control (without medication)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Continuation of the table

Tested sample/series																		
Test cultures		Captopril substance									The drug Captopril							
		Series																
		5102-17-014			5102-17-045			5102-17-014			001			002			003		
Escherichia coli	1:10	x_1	x_1	x_2	x_3	x_1	x_2	x_3	x_1	x_2	x_3	x_2	x_3	x_1	x_2	x_3	x_1	x_2	x_3
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1:20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1:50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Control (without medication)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Staphylococcus aureus	1:10	x_1	x_1	x_2	x_3	x_1	x_2	x_3	x_1	x_2	x_3	x_2	x_3	x_1	x_2	x_3	x_1	x_2	x_3
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1:20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1:50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Control (without medication)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Qualitative Evaluation: "+" — growth, "—" — no growth																		

For *E. coli* and *S. aureus*, no growth ("—") was observed at concentrations 1:10 and 1:20, while at 1:50 growth was restored ("+"). Thus, the inhibitory effect is manifested only at higher concentrations.

Thus, both the substance and the finished dosage form of Captopril exhibit a pronounced inhibitory effect against *E. coli* and *S. aureus* at concentrations 1:10–1:20, whereas *P. aeruginosa* is resistant and not inhibited at the tested concentrations.

Conclusion

The obtained results on the antimicrobial activity of Captopril samples produced by a domestic manufacturer demonstrate that at concentrations 1:10 and 1:20 the preparation suppressed the growth of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*, but had no effect on *Pseudomonas aeruginosa*, *Candida albicans* or *Aspergillus brasiliensis*. At a higher dilution (1:50), antimicrobial activity disappeared. These observations apply to both the substance and the tablet form.

The observed activity against *E. coli*, *S. aureus* and *B. subtilis* is consistent with previously published data showing that L-captopril can inhibit bacterial enzymes (particularly DapE), which may slow the synthesis of cell envelope components and thereby suppress bacterial growth [15].

The absence of effect against *P. aeruginosa* can be explained by well-known resistance mechanisms of this species: reduced outer membrane permeability, active efflux systems, and biofilm formation. These features make *Pseudomonas* less susceptible to many chemical compounds that are not specifically designed for it.

The lack of activity against *Candida* and *Aspergillus* is expected, since Captopril is not positioned as an antifungal agent and its chemical structure is not typical of azole or polyene fungicides.

The use of pharmacopeial ATCC test strains and standard culture media increases the comparability of the results with other studies.

The described method for determining the "microbiological purity" indicator for the Captopril substance and finished dosage form, as well as the standard inhibition test for this drug, may be incorporated into the manufacturer's specification. The presence of intrinsic antimicrobial activity in the substance/preparation provides quality control specialists with an understanding of its potential influence on microbiological tests[1,2,16].

The obtained data confirm that Captopril exhibits concentration-dependent antimicrobial activity against several pharmacopeial bacterial strains under the test conditions. This has direct practical significance for microbiological purity testing procedures: without accounting for this property, false results may be obtained. It is recommended to implement neutralization steps or other sample preparation approaches and to document this property in the manufacturer's specification and in quality control protocols.

Information about the presence or absence of antimicrobial activity in the Captopril substance and finished dosage form is not reported in the State Pharmacopeia of the Republic of Kazakhstan. The conducted studies made it possible to improve the effectiveness of the standard pharmacopeial methodology by suppressing antimicrobial activity during sample preparation.

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