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# ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

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# CLUSTER ANALYSIS OF INTEGRATED "DRUG SUPPLY" CONCEPT

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**The aim** of the article is the structure and content specification of the subject area professional term "drug supply" in order to deepen and concretize the conceptual apparatus in the field of the pharmaceutical activity.

**Materials and methods.** The review presents the analysis of 389 titles of scientific pharmaceutical publications for the periods of 1995–1998 and 2010–2019. The selection of publications was carried out by the random sampling based on the phrases: «drug provision», «medical care», «provision of medicines», «pharmaceutical care», «medicinal services», «drug supply» and their English-language counterparts in Russian and foreign electronic information sources. A methodological base of the study was a cluster analysis of the subject area concept of "drug supply" according to the methodology proposed by E.A. Korzhavykh and I.V. Voronovich. In this study, a cluster was interpreted as a set of publications in which a certain pair of terms was simultaneously cited with a preset frequency of co-citation.

**Results.** When analyzing the publications, two clusters of the "drug supply" concept were formed for the time periods indicated above. The comparison of the clusters showed an increase in the activity of the scientific research to expand the structure and content of the subject area of the "drug supply" concept. For the modern cluster, 124 co – cited pairs of lexical units were selected. They were grouped into 9 hierarchy levels according to the strength of relationship between an interval of 10 units and mentioning frequencies of the "drug supply" term. At the final stage, a graphical model of the "drug supply" concept cluster was formed. It was established that the lexical units included from the first to the fifth level, are the cluster nucleus of the "drug supply" concept as the most stable part of the lexical array, which it is advisable to rely on when developing a definition for the concept under study.

**Conclusion.** Thus, the subject field of the "drug supply" concept is characterized by the scientific research, reflecting the socio-economic significance of the objects under study in the field of pharmaceutical activities, and its content is characterized by a high level of pharmaceutical knowledge which describes the most stable elements that make it possible to formulate an adequate definition of the "drug supply" definition.

**Keywords:** drug supply; pharmaceutical research; cluster analysis; cluster; vocabulary; lexical unit

**Abbreviations:** DS – drug supply; MEPh – Management and Economics of Pharmacy; LU – lexical unit.

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# КЛАСТЕРНЫЙ АНАЛИЗ КОМПЛЕКСНОГО ПОНЯТИЯ «ЛЕКАРСТВЕННОЕ ОБЕСПЕЧЕНИЕ»

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**Цель.** Уточнение структуры и содержания предметной области профессионального термина «лекарственное обеспечение» для углубления и конкретизации понятийного аппарата в сфере фармацевтической деятельности.

**Материалы и методы.** В обзоре представлен анализ 389 заголовков научных фармацевтических публикаций за периоды 1995–1998 гг. и 2010–2019 гг. Выбор публикаций осуществлялся методом случайной выборки при помощи словосочетаний: «лекарственное обеспечение», «лекарственная помощь», «обеспечение лекарственными препаратами», «фармацевтическая помощь», «лекарственное обслуживание», «лекарственное снабжение» и их англоязычных аналогов в российских и зарубежных электронных источниках информации. Методической базой исследования выбрали кластерный анализ предметной области понятия «лекарственное обеспечение» по методике, предложенной Э.А. Коржавых и И.В. Вороновичем. Под кластером в настоящем исследовании понимали совокупность публикаций, в которых одновременно цитировалась определенная пара терминов с установленной частотой социтирования.

**Результаты.** При анализе публикаций сформировали два кластера понятия «лекарственное обеспечение» по указанным временным периодам. Сравнение кластеров показало рост активности научных исследований по расширению структуры и содержания предметной области понятия «лекарственное обеспечение». Для современного кластера отобрали 124 социтируемые пары лексических единиц, которые сгруппировали по 9-ти уровням иерархии по силе связи с интервалом 10 единиц и частотой упоминания термина «лекарственное обеспечение». На заключительной стадии сформировали графическую модель кластера понятия «лекарственное обеспечение». Установили, что лексические единицы, входящие с первого по пятый уровень, являются ядром кластера понятия «лекарственное обеспечение» как наиболее стабильная часть лексического массива, на которую целесообразно опираться при разработке определения для исследуемого понятия.

**Заключение.** Таким образом, предметное поле понятия «лекарственное обеспечение» характеризуется научными исследованиями, отражающими социально-экономическую значимость изучаемых объектов сферы фармацевтической деятельности, а его содержание – высоким уровнем фармацевтического знания, которым описываются наиболее устойчивые элементы, позволяющие сформулировать адекватное определение дефиниции «лекарственное обеспечение».

**Ключевые слова:** лекарственное обеспечение; фармацевтическое исследование; кластерный анализ; кластер; лексика; лексическая единица

**Список сокращений:** ЛО – лекарственное обеспечение; УЭФ – управление и экономика фармации; ЛЕ – лексическая единица.

## INTRODUCTION

In modern science, a unity of views on objects, processes and phenomena is ensured by a generally accepted conceptual (terminological) apparatus. However, it is not always possible to obtain consensus in the development of terms due to the presence of subjective approaches to the subject area analysis of scientific knowledge and the description of the changes dynamics taking place in it. Pharmaceutical scientific, practical and educational knowledge exists in the form of a set of interrelated conceptual systems. It should be notified that in the leading profile academic discipline “Management and economics of pharmacy” (MEPh) and a scientific specialty “Organization of pharmaceutical business”, as

their names imply, the concepts and ideas borrowed from management and economic sciences, play a significant role. In modern research, the provisions of these sciences are widely used and developed in relation to scientific tasks and problems of pharmacy as an application area of knowledge.

As a rule, when applying the classical methods of general economics and management, the terms and concepts that have an established interpretation are used, and therefore, no additional interpretations are developed by pharmaceutical specialists. The exception is usually large concepts marked with the word "pharmaceutical" and taking their place in the system of concepts and terminology of the MEPh discipline (i.e., pharmaceutical management, pharmaceutical marketing, and pharmaceutical informing).

The earlier study of the pharmacy fundamental foundations showed that in pharmaceutical knowledge, there are different types of concepts: inherent to only one of the disciplines, interdisciplinary, and so-called concepts-categories. Concepts-categories give an interpretation not only to objects, but also to processes, as well as to phenomena. They are used in almost all pharmaceutical disciplines. This terminology has clear scientific definitions, and can be also institutionalized in legislative and regulatory legal acts that regulate various types of activities. These are such concepts as "drug"; "medicinal preparation"; "dosage form"; "drug circulation"; "drug production"; "pharmaceutical business", etc[1]. At the same time, in the professional pharmaceutical and medical vocabulary, the term "drug supply" (DS) known not only to healthcare professionals, but also to the majority of the population, is widely used. However, this interdisciplinary term has not got an adequate definition yet. In the modern legal field of Russian pharmacy, this concept implies only supplying decreed (preferential) categories of citizens with drugs. Hence it appears that the subject area of the term "drug supply" has not been fully identified and agreed between healthcare professionals.

In the way of formulating the development and substantiation of the DS concept, one of the obstacles, in the authors' opinion, can be its significance not only for pharmaceutical and medical specialists, but also for the general population who are in close contact with the healthcare system. The experience of professional communication shows that the terminological problems of pharmaceutical science and practice go beyond purely linguistic problems. That is due to the high social significance of this terms and concepts group. According to Doctor of Philology, Professor of Tver State University I.P. Susov, "... the formal system becomes a social system, a language in this capacity acts as an activity system. The nomination (name, naming) of elements and processes of human activities is carried out by the entire language system in the unity of its levels"[2]. Therefore, when developing the DS definition, it is necessary to take into account the actual pharmaceutical activities to fulfill the entire range of tasks for the complete, uninterrupted and high quality supplying the population with drugs in order to maintain a socially necessary level of their consumption.

Peculiarities of pharmaceutical terminology, an abundance of terms and nomenclature names that medical and pharmaceutical specialists should master during training in educational institutions and then use in everyday activities, require urgent updating of the existing terminology and developing the modern optimal professional one in order to ensure the unity of approaches to understanding objects, processes and phenomena in the field of pharmaceutical activities, and training the specialists involved in supplying the population with medicines. The expediency of such training is also pointed out by lecturers of leading Russian pharmaceutical universities, who consider the formation of terminological competencies in their students among the priorities of modern and promising pharmaceutical education [1, 2]. For this reason, the authors implemented a review of the subject field to identify and analyze literary sources that define the multidimensional nature of the DS complex concept to clarify its key definition.

The knowledge degree analysis of the scientific problem did not reveal Russian and foreign publications that open up the views of the professional pharmaceutical community on the justification and development of the DS concept.

**THE AIM** to clarify the structure and content specification of the subject area professional term "drug supply" in order to deepen and concretize the conceptual apparatus in the field of the pharmaceutical activity.

The research objectives were as follows:

1. To identify and explore trends and statistical characteristics of lexical arrays of the structure and content specification of the subject area professional concept term "drug supply" in the main time periods of the formation, development and functioning of the Russian system for providing the population with drugs;

2. To analyze the features of the vocabulary associated with the use of the term "DS" in professional activities;

3. To form and characterize the cluster of the DS concept.

## MATERIALS AND METHODS

### Study design

At the first stage of the work (February 2020–March 2021), the formation of the initial lexical array of

[1] Federal Law of the Russian Federation on April 12, 2010 No. 61-FZ "On Circulation of Medicines". Available from: http://www.consultant.ru/cons/cgi/online.cgi.

[2] Susov IP. Yazykovaya nominaciya v svete predstavleniya o yazyke kak kodiruyushchej sisteme [Language nomination in the light of the idea of language as a coding system]. Actual problems of language nomination: abstracts of reports of a regional scientific seminar. Saratov: Saratov State Pedagogical Institute. 1988: 14-5. Russian

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scientific papers titles in the MEPh discipline, affecting the subject area of the DS concept (articles, reports, monographs, theses, materials of scientific and practical conferences, congresses) was carried out. At the second stage (April – November 2021), the statistical characteristics of the DS concept lexical arrays were analyzed according to the selected time periods (1995–1998 and 2010–2019). At the third stage (December 2021 – March 2022), the results were discussed and the results of the work done were summed up. The data collection and processing were carried out by the working group of four people: two Doctors of Sciences (Pharmacy), one Doctor of Sciences (Medicine), and one Candidate of Sciences (Pharmacy).

### Study time frame

The design of the study included an analysis of trends and qualitative and quantitative changes in the professional pharmaceutical vocabulary in the modern period compared to the trends of the 1990s of the last century, and not a parallel study of two proportional time periods, so the lexical array was formed for the periods of 1995–1998 and 2010–2019. The choice of the non-equivalent time periods used in this study was justified by the fact that in 1995–1998, there was a transition to new market mechanisms for managing pharmaceutical activities, accompanied by a radical transformation of the management system and, in particular, the active formation of a new conceptual apparatus. In the Russian Federation, the end of this period was marked by the adoption of the Federal Law dated June 5, 1998, No.86-FL "On Medicines"[3]. For the first time, it institutionally fixed the basic concepts used in the pharmaceutical science and practice. During 1999–2009, there was practically no scientific activities to improve the terminological apparatus in the field of the Russian pharmacy organization and management. At that time, the industry was adapting to the newly established requirements and accumulating new knowledge, during which some contradictions arose between the institutional order of its regulation and practical development. These contradictions also affected the conceptual apparatus. The second time period (2010–2019) attracted the authors' attention due to the need to review and improve the approaches to the management of the pharmaceutical activities. They were: compiling lists of decreed medicines, introducing labeling of medicines, using good practices in various areas of activity), as well as the widespread use of new terms (for example, "drug care", "pharmaceutical care", "drug service"). On the one hand, the adoption of the Russian Federation Law on Medicinal Products dated April 12, 2010[4] was accompanied by a clarification of professional terminology. On the other hand, it opened a new field for scientific discussions on the problems of forming uniform approaches to defining the conceptual apparatus for the most significant phenomena, processes, objects and subjects that make up the content of pharmaceutical activities.

### Information resources

A continuous screening method made it was possible to select 3540 publications in the MEPh discipline and form the initial lexical array on their basis. It consisted of 389 titles of scientific pharmaceutical publications devoted to the problem of forming the conceptual apparatus of modern pharmaceutical science and practice.

The publications choice was carried out with the help of the Russian-language phrases: «лекарственное обеспечение»; «лекарственная помощь»; «обеспечение лекарственными препаратами»; «фармацевтическая помощь»; «лекарственное обслуживание»; «лекарственное снабжение». The English-language analogues were also used: "drug provision", "medical care", "provision of medicines", "pharmaceutical care", "medicinal services", "drug supply". They were used in Russian and foreign electronic sources of information: scientific electronic libraries (e-LIBRARY, CyberLeninka, ational Electronic Library, State Public Scientific and Technical Library, Russian State Library); scientific works archives of the State Commission for Academic Degrees and Titles of the Ministry of Science and Higher Education of the Russian Federation, international databases (Scopus, Web of Science, MEDLINE, Pubmed).

### Methodological study base

The main criterion determining the preference of the methodological approach in this work, in comparison with others, was the possibility of decomposing the sample into groups of similar objects with non-numeric features, followed by their processing by standard statistical procedures. As a methodological basis for the study, the cluster analysis was chosen as the most appropriate for the subject area analysis of the term "DS". That includes the processes of selecting objects, generalization and analysis of reliable information about their properties and subsequent clustering, i.e. the distribution of the objects of the obtained sample into separate levels (subgroups), based on their relative identity of properties. The main aim of clustering is to categorize the data into clusters, where the objects are grouped into a certain category according to the principles of a greater similarity between themselves than with the objects from other clusters [3, 4]. The basis for the choice of this cluster analysis was, first, the structure of knowledge in the scientific field, which is a set of cognitive units, the so-called concepts, united by a certain hierarchy and having their own names – terms. A concept is a set of interrelated, simple (elementary) notions and auxiliary lexical units that describe a specific subject area of sci-

[3] Federal Law of the Russian Federation of 05.06.1998 No. 86-FL "On Medicines. Available from: http://www.consultant.ru/document/cons_doc_LAW_19106/.

[4] Federal Law No. 61-FL dated April 12, 2010 "On the Circulation of Medicines" (last edition). Available from: http://www.consultant.ru/document/cons_doc_LAW_99350/.

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ence. Second, the presence of links between elementary notions in the concept makes it possible for us to classify it as a kind of clusters.

### Analysis methodology for study subject area

The cluster analysis of the DS subject area was carried out according to the methodology proposed by the specialists of Peoples' Friendship University of Russia – E.A. Korzhavykh and I.V. Voronovich. The essence of the technique lies in the following fact: to calculate strength of relationship between the term DS and another lexical unit (LU), the indicator of the absolute frequency of simultaneous mentioning (co-citation) of the pair "the term "DS → LU" in the analyzed array was used. As a threshold value, the mentioning frequency equal to 3 was taken; therefore, the pairs of co-citations with a link strength value of less than 3 were not included in the cluster. Next, the co-cited pairs were grouped according to the strength of relationship between an interval of 10 LUs and a simultaneous mentioning frequency with the term "DS" equal to from 3 to 10, from 11 to 20, from 21 to 30, "…", from 81 to 90. At the final stage, they formed a graphical model of the DS cluster indicating the frequency of co-citation for its elements and the overall mentioning frequency for the title term. The lexical arrays were characterized using quantitative indicators in absolute and relative (%) terms.

## RESULTS AND DISCUSSION

In the pharmaceutical studies devoted to the development of the conceptual apparatus, a cluster was understood as a set of publications in which a certain pair of terms was simultaneously cited (mentioned) with a frequency of joint citations above the accepted threshold value [5, 6]. When analyzing the array of scientific papers, it was revealed that Russian scientists performed a number of works to identify the subject areas of scientific clusters of the MEPh discipline. These are such clusters as "pharmaceutical safety" [7], "management of pharmaceutical activities" [8, 9], "consumers of pharmaceutical products and services" [10], "management of the pharmaceutical products range" [11], "pharmaceutical market" [12, 13] and others. A similar methodological approach was applied by Russian scientists in order to identify the directions and dynamics of the research development in the scientific and practical field of "the drug supply organization" for 1991–2020. [14]. A statistical research tool based on the cluster approach was used by Russian scientists to solve traditional problems when grouping the sets of objects in pharmaceutical marketing [15–17]. In the foreign literature, the publications using the cluster analysis ideology as one of the tools for science researching by quantitative methods, are presented in almost all the areas of health care: in management [18]; in psychiatry [19, 20]; when studying the spread of the COVID-19 pandemic [21]; in pharmacognosy [22, 23]; in pharmacology [24, 25].

### General characteristics of lexical arrays

A comparative statistical characteristics analysis of the lexical arrays distributed over periods, made it possible to draw several conclusions about the state of the DS term in the subject area in the analyzed time periods (Table 1).

First, by 2020, the average annual number of papers on the study of the DS system had increased by 3 times: from 11.25 publications per year in the 1990s up to 34.4 publications in 2010s. This fact objectively reflects a difficult economic situation in the country in the 1990s. It prevailed in all areas of activity, including healthcare in general, as well as in medical and pharmaceutical research, which led to a significant decrease in the publication activity of scientists and practitioners.

Second, by 2020, the structure of the subject area of the DS concept had noticeably expanded, which, in the authors' opinion, had been due to the involvement of new ideas, approaches, and resources in the processes of improving the DS system as the Russian healthcare system is being restored and reformed.

Third, by 2020, the subject area of the DS concept had become denser and more concentrated in its content, as indicated by the values of the fourth indicator – "Percentage of LUs with a mentioning frequency equal to 1 and 2". This percentage decreased by 1.3 times (from 80.19% to 59.67%). The fact of the percentage decreasing of such a vocabulary by 2020 may mean its transition to a more stable part of the array. This opinion has been also confirmed by the authors' calculations: 23 positions (21.7%) of the LUs were transferred from the vocabulary array of 1995-1998 in the array of 2010-2019; 7 LUs (6.6%) were entrenched in it with a mentioning frequency of 3 or more times. Therefore, a rarely mentioned vocabulary can be considered as a potential resource for the development of the DS concept cluster.

Fourth, the statistical distribution nature of the mentioning frequency ($F_k$) has noticeably changed: if in 1995-1998 the first quartile (25% of all LUs positions) accounted for about 60% of the total frequency for these LUs, by 2020 the value of the indicator had reached almost 80%. The fact that in the both arrays the first quartile included more than 50% of the most frequently mentioned LUs allows us to consider such LUs as a "core set" of the vocabulary, the most important for the subject area of the DS term (a cluster core).

### Identification and analysis of vocabulary features associated with DS term

The data obtained, primarily at the level of the vocabulary, reflect the formation of quantitative and qualitative changes in the subject area of the DS concept in the studied periods (Table 2).

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**Table 1 – Main indicators of vocabulary arrays related to the "drug supply" term in periods of 1995–1998 and 2010–2019**

| Indicator | Indicator value in periods | |
|---|---|---|
| | 1995–1998 | 2010-2019 |
| 1. Total number of publications titles with term "DS" | 45 (average 11.25 per year) | 344 (average 34.4 per year) |
| 2. Total number of LU (without repetitions) mentioned simultaneously with term "DS" (*N*) | 106 (average 26.5 per year) | 305 (average 30.5 per year) |
| 3. Overall mentioning frequency identified by LUs (*F*) | 213 (average 53.3 per year) | 1813 (average 181.3 per year) |
| 4. Percentage of LUs positions with mentioning frequency equal to1 and 2 times | 80.19% | 59.67% |
| 5. First 25% quartile of LUs array:<br>– number of LUs positions ($N_k$)<br>– total frequency of LUs included in the quartile ($F_k$) | <br>27 LUs<br>27 (59.62% of total frequency) | <br>76 LUs<br>414 (78% of total frequency) |

**Table 2 – Composition of vocabulary most often mentioned simultaneously with "drug supply" term in periods of 1995–1998 and 2010–2019**

| Ser. No. | Vocabulary | Percentage in the of LU, % | |
|---|---|---|---|
| | | 1995–1998 | 2010–2020 |
| 1. | Common-literary | 35.85 | 40.57 |
| 2. | General scientific | 16.98 | 22.64 |
| 3. | Pharmaceutical | 3.77 | 4.72 |
| 4. | Medical | 17.92 | 10.38 |
| 5. | Other areas of science and technology (economics, finance, sociology, technology, etc.) | 25.47 | 21.69 |
| Total: | | 100% (106 LUs positions) | 100% (LUs positions) |

**Table 3 – Variants of the term "drug supply" and its synonyms in the array of publications titles for 1995–1998**

| Ser. No | Variants of the term "drug provision" (identified synonyms) | Number of titles*, units | Percentage of titles, % |
|---|---|---|---|
| 1. | Drug provision (including preferential) | 32 | 64.0 |
| 2. | Medicinal assistance | 9 | 18.0 |
| 3. | Supply by medicinal preparations | 4 | 8.0 |
| 4. | Pharmaceutical assistance | 2 | 4.0 |
| 5. | Medicinal service | 1 | 2.0 |
| 6. | Provision by medicines | 1 | 2.0 |
| 7. | Drug supply | 1 | 2.0 |
| Total: | | 50 | 100.0 |

Note: * – 2 LUs could be present in the title at once.

**Table 4 – Variants of the term "drug supply" and its synonyms in the array of publications titles for 2010–2019**

| Ser. No | Variants of the term "drug provision" (identified synonyms) | Number of titles*, units | Percentage of titles, % |
|---|---|---|---|
| 1. | Drug provision (including: guaranteed, additional, additional preferential, preferential, preferential and free, software) | 255 | 88.9 |
| 2. | Supply by medicinal preparations (including: gratuitous, guaranteed, necessary) | 26 | 9.1 |
| 3. | Supply by medicinal preparations | 4 | 1.4 |
| 4. | Additional drug provision | 1 | 0.3 |
| 5. | Provision by medicinal preparations | 1 | 0.3 |
| Total: | | 287 | 100.0 |

Note: * – 2 LUs could be present in the title at once.

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**Table 5 – Rating fragments of LUs mentioned simultaneously with the term "drug supply" in publications titles in 1995–1998 and 2010–2019**

| Ser. No | LU of 1995–1998 period (in descending order of mentioning frequency) | Absolute frequency | LU of 2010–2019 period (in descending order of mentioning frequency) | Absolute frequency |
|---|---|---|---|---|
| 1. | The sick | 15 | Population | 74 |
| 2. | Population | 14 | The sick | 69 |
| 3. | System | 9 | System | 69 |
| 4. | Conditions | 9 | Region (*geographic*) | 58 |
| 5. | City | 6 | Analysis | 57 |
| 6. | Organization *(process)* | 6 | Optimization | 41 |
| 7. | Improvement | 6 | Improvement | 39 |
| 8. | Insurance | 6 | Citizens | 38 |
| 9. | Russia | 5 | Category | 34 |
| 10. | Optimization | 4 | Program | 32 |
| 11. | Problem | 4 | Assistance | 30 |
| 12. | State | 4 | Organization *(process)* | 29 |
| 13. | Analysis | 3 | Patients | 27 |
| 14. | Group | 3 | Implementation | 27 |
| 15. | Period | 3 | Level | 27 |
| | … | … | … | … |
| | Total: *N* = 106 | *F* = 213 | Total: *N* = 305 | *F* = 1813 |

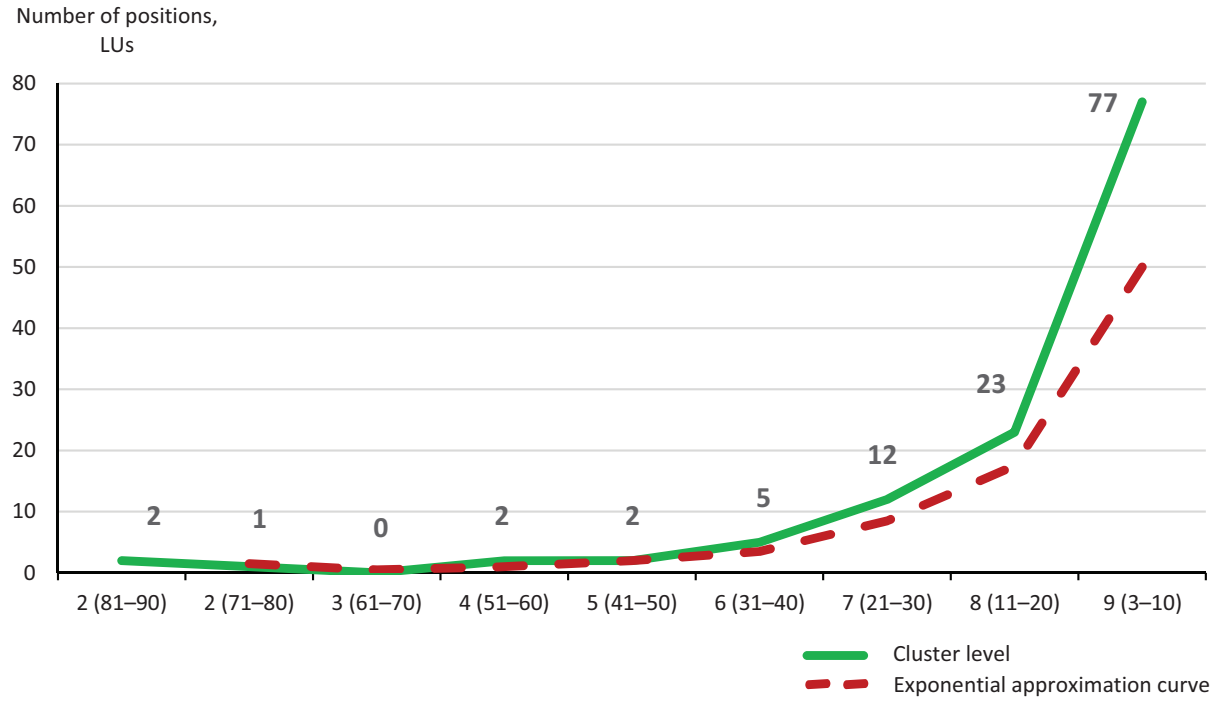


**Figure 1 – Distribution of LU by levels in the united cluster of "Medicinal provision" concept for period of 1995–2019**

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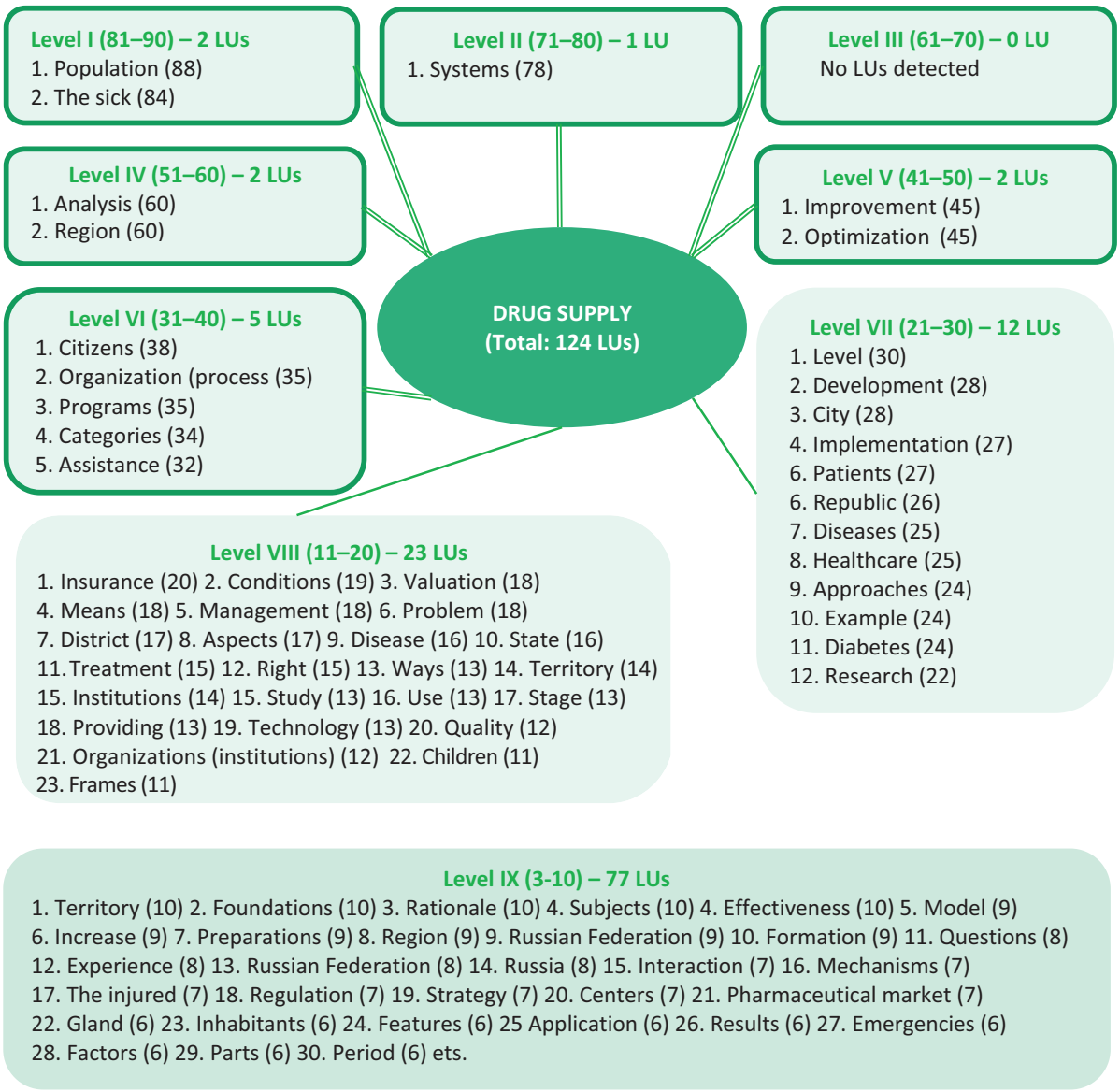


**Figure 2 – Modern cluster of the concept of "drug supply"**

Note: total number of non-recurring LUs (124) is named DS in the center of the model; for each level of the cluster hierarchy, the number of LUs included in it is indicated; after each LU, in parentheses, the individual strength of relationship is given – this LU named DS, which determined its place at the corresponding level of the cluster.

For a greater accuracy, the comparison was carried out on the same arrays of LUs – 106 positions each with the highest mentioning frequency. It was established that by 1999, general scientific and pharmaceutical vocabularies had begun to prevail in the subject area of DS. Besides, a reduction in the influx of the medical vocabulary and the vocabularies from other fields of science can serve as a sign of the final phase of its formation and the progressive development of the system for providing the population with medicines.

Among the general scientific vocabulary of the array developed by 2020, such conditionally new terms as "mechanism", "modelling", "components", "forecast", etc. have been detected. The modern pharmaceutical vocabulary has included such well-known terms as "pharmacy", "pharmacist", "pharmacy organizations", "pharmacy chains", which had been absent from the array by the beginning of 1999. This circumstance suggests an expansion of the scientific research range, updated by an increase in the role of the distribution network of the pharmaceutical market.

A somewhat different situation is observed with the vocabulary denoting the key functions of pharmaceutical specialists in the system of providing the population with medicines – organization and management. If in 1995-1998 the frequency percentage attributable to the term "organization" (process) was 2.82%, and to the term "management" – 1.41%, then, by 2020, this indicator had decreased to 1.60% and 0.83%, i. e. by 1.8 and 1.7 times, respectively. Consequently, to date, the acti-

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vity of specialists in the field of pharmacy in the study of organizational and managerial functions has decreased. In the authors' opinion, this phenomenon is due to the influence of the external environment, primarily the development and legislative consolidation of the institutional norms that regulate pharmaceutical activities and socio-economic relations that arise between the subjects of the pharmaceutical market.

The frequency dynamics analysis of using LUs "problems" and "approaches" gave paradoxical results: by 2020, with a decrease in the frequency of consideration of "problems" in the publications from 1.88% to 0.77%, the percentage of scientific papers with LUs concerning "approaches" (methodological, organizational-pharmaceutical, organizational-economic, etc.), had increased by almost 3 times. Consequently, the activity of pharmaceutical scientists in relation to the search for ways to improve the system for providing the population with medicines had increased.

An example of a management decision implemented in the healthcare system, adopted at the highest level of the legislative and executive power, is insurance (for example, such LUs as "insurance medicine", "compulsory medical insurance", "voluntary medical insurance"). All these LUs were present in the analyzed arrays. It has been established that in 1995–1998, the above-mentioned terms were used more often than by 2020: with a 3.29% share vs. a 1.05% share of the total frequency (according to the rating of the most frequently mentioned LUs).

One of the most important management functions is related to the possibility of reliable control of process parameters and indicators. A comparative analysis of the vocabulary in the area of the DS population found out that in the array of 1995-1998, there were only 2 such LUs (1.89% of the total number of positions) with a mentioning frequency equal to 2 (0.94% of the total frequency); and these are LUs "quality" and "monitoring". The vocabulary of 2010-2019, on the contrary, is distinguished by a wider set of estimated DS parameters. These are such properties as "quality", "efficiency", "accessibility", "satisfaction", as well as the vocabulary of the evaluation process itself – "monitoring", "indicators", "indices" and others. In total, 9 positions (2.95%) of LUs were identified with a total frequency of 41 mentions (2.26% of the total frequency in the array). The most frequently mentioned LUs were "quality" (12 times) and "efficiency" (10 times). As a rule, in the context of relevant publications, the evaluation aspects of preferential provision of the population with medicines were discussed.

A special area of the studied lexical arrays is formed by a medical vocabulary. This area includes such LUs as "patients", "medical technologies", "treatment", "diagnostics", etc.; the second part of LEs are the terms denoting nosologies, for example, "asthma", "diabetes", "pneumonia", etc. In general, a significant reduction in the percentage of medical LUs and the frequency of their use in the titles of publications was found, respectively, from 16.47% to 8.53% in terms of the number of LUs, and from 15.96% to 10.26% in terms of frequency. In absolute terms, these figures had increased by 2020.

The phenomenon of synonymy in the both studied vocabulary arrays are of particular interest (Tables 3 and 4). The analysis of the publications contexts in which, e.g., the terms "pharmaceutical care", "drug care" are used, showed that it was about providing the population with medicines.

When comparing the data in Tables 3 and 4, it was established that over time the number of synonyms had decreased from 7 to 5, and the use of the term "DS" had increased in absolute and relative terms – from 32 (71.11%) over the period 1995-1998 up to 255 (74.13%) in 2010–2019. This means that the term "DS" is gradually being recognized by an increasing number of researchers as the only one correct.

A noticeable increase in the use of the term "DS" is due to its formation and development within this cluster in 2010–2019. This LU subgroup is associated with the guaranteed provision by medicines to certain categories of citizens who are entitled to state social assistance, including free drug provision. In this array, that is confirmed by the appearance of the following characteristic LUs: "additional drug provision", "citizens", "financing", "beneficiaries", "persons", etc.

Thus, a comparison of the structure and composition of lexical arrays for two periods separated by more than ten years, made it possible to establish the directions the system of providing the population with medicines had developed, and what vocabulary had been consistently mentioned in these years along with the term "DS". The identified features of the vocabulary, form the basis for constructing a schema of the subject area of the term DS in the form of a concepts cluster.

### Formation and characteristics of concept "DS" modern cluster

Arranging the analyzed arrays according to the mentioning frequency of the LUs made it possible to form two corresponding ratings. Grouping of LUs by a mentioning frequency, together with the term "DS" and an interval of 10, made it possible to identify the structure of clusters of the DS subject areas by 1999 and 2020. Due to a large volume of ratings, Table 5 shows only 15 positions with the highest frequency.

It was established that by 1999, the cluster of the DS concept had two hierarchical levels: at the first level, there were 2 LUs – "sick" (a mentioning frequency of 15) and "population" (14); at the second level, there were 19 LUs with a mentioning frequency from 3 to 10 – "system(s)", "conditions" (9 of each), "city(cities)", "organization" (process), "improvement", "insurance" (6 of each) and other LUs.

A small number of cluster levels is most likely due to a limited selection of publications and, consequently, relat-

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ed vocabularies. The second reason may be the decrease in the number of the studies on the functioning of the drug supply system for the population in the mid-1990s.

By 2010–2019, the cluster of the DS concept had developed to a significantly larger number of levels – 8, while the numerical values of the mentioning frequency had increased notably. So, at level 1, there was one word – "population" – with a frequency of 74; at level 2, there were two LUs, "sick" and "systems" (69 of each); at level 3, there were also two LUs – "region(s)" as a territorial unit and "analysis" (the frequency of 58 and 57, respectively). As the hierarchical level increased, so did the number of LUs at it. In particular, at level 7, there were 24 LUs with a mentioning frequency from 11 to 20, and at the highest level, 8, there were already 74 LUs with a mentioning frequency from 3 to 10.

Thus, the clusters of the DS concept subject area based on the vocabulary of the two periods differ significantly in structure and in composition; as evidenced by the data shown in Table 5, they have many identical LUs. In the vocabulary of 2010–2019, which is a part of the first 25% quartile, there are 21 LUs (28%) from the array of 1995-1998. Taking into account this fact it was considered expedient to combine these two quartiles and make up a modern cluster of the DS concept based on a new vocabulary array. It is also noteworthy that both 25% quartiles contain more than 60% of LUs positions.

The third (combined) set of the analytical data included 124 LUs with a mentioning frequency from 3 to 88. When grouping LUs by frequency with an interval of 10, 9 hierarchical levels were formed. Fig. 1 shows a statistical distribution of LUs by the established levels (a blue curve), which is described by an exponential curve using the formula (1):

$$y = 02443e^{0.5672x} \quad (1)$$

Herewith, the accuracy of the approximation is high ($R^2 = 0.8628$).

Fig. 2 shows a graphical model of the DS concept united cluster as of 01/01/2020, built at hierarchical levels, taking into account the strength of relationship, i.e. the joint mentioning frequency of LUs with the title term.

Fig. 2 shows the closest relationship of the DS term with the following LUs: "population" (the strength of relationship, i.e. the frequency of the joint mentioning, is 88) and "sick" (84), located at level I. This fact takes place in all two arrays of the vocabulary, retaining its significance for almost 25 years, and, therefore, the LUs "population" and "sick" serve as keys, cluster-forming elements of the DS concept subject area. In this regard, in the authors' opinion, it is incorrect to use the term DS to refer exclusively to the processes of providing reimbursement medicines to the population or certain citizens categories. As this study showed, a preferential provision is only one of the concepts that form the DS concept, and, in turn, is not an elementary (simple) concept either.

The numerical values of the strength of relationship close to the first level of the cluster hierarchy, were revealed for the LUs "system" (78), which was a part of the second level. The third cluster level remained unfilled, while level IV included such LUs very close to it as "region" (an administrative-territorial unit) and "analysis", which have frequency co-citation indicators equal to 60.

The vocabulary denoting processes, activities, is at level V. These are the words "optimization" and "improvement" with the strength of relationship each equal to 45. The LUs occupying level VI – "citizens", "programs", "organization" (process), "categories", "assistance" with the strength of relationship in the range of 31–40, complete the formation of the concepts zone most closely related to the "DS" term (this is confirmed by the horizontal section of the curve in Fig. 1). Due to such a limitation of levels, it is advisable to speak of the "core of the cluster", which includes hierarchy levels from I to V, as the most stable part of the lexical array in terms of composition, which it is advisable to rely on when developing the definition of the DS concept.

Based on the foregoing, a mechanism for the formation of a "cluster core" was developed. A new vocabulary first appears at level IX and can quickly gain a foothold there or quickly go to level VIII (the area of a sharp rise on the exponential curve in Fig. 1); this is the vocabulary operational part of the system for providing the population with medicines. "Borderline" level IX is characterized by the vocabulary that actively penetrates the cluster as it is necessary to promptly solve new organizational and managerial tasks, set by the state for the healthcare system or arising from the practical need for such measures, in the field of providing the population with medicines.

The vocabulary composition at levels VI and VII is also characterized by a variability (the zone of the rise beginning in Fig. 1), representing a kind of "filter" for new words and concepts that come from levels IX and VIII and migrate over time to the higher cluster levels.

Thus, it can be concluded that the DS concept cluster has a dual structure. On the one hand, this is a hierarchical multi-level structure, which is based on the grouping of LUs according to the strength of relationship (a mentioning frequency) with a given interval, and makes it possible to assess the development degree of the subject area under study, based on the number of levels. On the other hand, there are 3 zones in the cluster, conventionally named as "core", "filter" and "border". This type of structure takes into account the distribution nature of the number of LUs by levels and the correspondence of the identified conditional zones to the sections of the exponential curve.

## Study Limitation

The presented review limitation of the subject field is in the absence of the quality evaluation of the scientific articles, reports and other materials used in the study.

Besides, the evidence base was limited only by the contexts of these publications titles. Thus, the conclusion drawn from the results of the cluster analysis is based on the available scientific writings, and not on their intrinsic quality or strength of evidence.

## CONCLUSION

The results of the study indicate the effectiveness of the applied methodological approach to the quantitative assessment, analysis of the state and dynamics of the subject area development of a large complex pharmaceutical DS concept.

Taking into account the aim and objectives of this study, it should be concluded that the multi-level structure of the DS concept subject area is to a more extent characterized by scientific research reflecting a high degree of socio-economic significance of the objects under study, as well as practical interest in specific problems in the field of pharmaceutical activities and proposals for their decision.

The subject area content of the studied concept is characterized by the methodological conceptual level of accumulated pharmaceutical knowledge, which describes the most stable elements that allow us to formulate an adequate DS definition.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHORS' CONTRIBUTION

BGS – development of the study concept and design, data collection, analysis and interpretation of the results, preparation of a draft manuscript; KTI – planning and management of the study, processing of the results, participation in the description and analysis of the resultswriting the manuscript; GAB – literature search and analysis, the obtained data analysis and interpretation, writing and design of the final manuscript version.

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# PHYTOCHEMICAL AND MICROBIOLOGICAL ASPECTS OF THE *NIGELLA SATIVA* L. HERBS STUDY

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*Nigella sativa* L. is a promising plant object, herbal medicinal raw materials of which can be comprehensively used for the development of drugs with an antimicrobial activity.

**The aim** of the study was to screen and compare the antimicrobial activity of water-ethanolic extractions from the *Nigella sativa* L. herbs with a eucalyptus tincture of as a reference preparation.

**Materials and methods.** Chromatograms of the extracts were obtained by thin layer chromatography in the system of chloroform – ethanol – water (26:16:3). The detection of adsorption zones was carried out in daylight, in the UV light at λ=254 nm and λ=365 nm, as well as by treatment with reagents – a 3% alcohol solution of aluminum chloride and a solution of diazobenzosulfonic acid in a 20% sodium carbonate solution. The next step was to determine the minimum inhibitory concentration by the method of double serial dilutions in Mueller-Hinton nutrient broth (Bio-Rad, USA). As test cultures, the strains of the American Type Culture Collection (ATCC) microorganisms were used: *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), as well as *Candida albicans* (a clinical strain). Simultaneously, an experiment to establish a "negative" control was carried out. For the comparative evaluation of the studied samples activity, its activity was compared with the reference preparation with a proven antimicrobial activity – a eucalyptus tincture.

**Results.** For all water-ethanolic extractions and the *Nigella sativa* L. herb tincture, the adsorption zones characteristic of flavonoids with $Rf_1$ = 0.28, $Rf_2$ = 0.15, $Rf_3$ = 0.11 were revealed, and under the action of an alcoholic solution of aluminum chloride, the fluorescence of the adsorption zones was also enhanced, which indicates the phenolic nature of these compounds. In the course of the study, it was found out that all water-ethanolic extractions from the *Nigella sativa* L. herbs have the greatest antimicrobial effect against the *Pseudomonas aeruginosa* strain. When compared with the reference preparation – a eucalyptus tincture, it was notified that the specified tincture of the *Nigella sativa* L. herbs has an advantage in the antimicrobial activity over the strain of *Pseudomonas aeruginosa* – the action at the 16-fold dilution vs the 4-fold dilution. The action on the *Escherichia coli* and *Candida albicans* strains is comparable for the both tinctures.

**Conclusion.** The obtained results of phytochemical and microbiological analyses will be used as a rationale for the introduction of antimicrobial preparations based on the *Nigella sativa* herbs in medical and pharmaceutical practice.

**Keywords:** *Nigella sativa* L.; herbs; water-ethanolic extractions; tincture; minimum inhibitory concentration; antimicrobial activity

**Abbreviations:** ATCC – American Type Culture Collection; CLSI – Clinical and Laboratory Standards Institute; MRS-strains – methicillin-resistant *Staphylococcus*; MRSA – methicillin-resistant *Staphylococcus aureus*; SP RF XIV ed. – State Pharmacopoeia of the Russian Federation XIV edition; CFUs/ml – Colony forming units/ml; MIC – minimum inhibitory concentration; Gs – Guidelines; *N.* – *Nigella* L. (eg. *N. sativa*).

# ФИТОХИМИЧЕСКИЕ И МИКРОБИОЛОГИЧЕСКИЕ АСПЕКТЫ ИЗУЧЕНИЯ ТРАВЫ ЧЕРНУШКИ ПОСЕВНОЙ (*NIGELLA SATIVA* L.)

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Чернушка посевная – *Nigella sativa* L. является перспективным растительным объектом, лекарственное растительное сырье которой может быть комплексно использовано для разработки препаратов с антимикробной активностью.

**Цель.** Проведение фитохимического скрининга и сравнения антимикробной активности водно-спиртовых извлечений травы чернушки посевной (*Nigella sativa* L.) с препаратом сравнения – настойкой эвкалипта.

**Материалы и методы.** Методом тонкослойной хроматографии в системе хлороформ – этанол – вода (26:16:3) были получены хроматограммы извлечений. Детектирование зон адсорбции проводилось при дневном свете, в УФ-свете при λ=254 нм и λ=365 нм, а также обработкой реактивами – спиртовым раствором алюминия (III) хлорида 3% и раствором диазобензосульфокислоты в 20% растворе натрия карбоната. Далее проводилось определение минимальной ингибирующей концентрации методом двойных серийных разведений в питательном бульоне Мюллера-Хинтона (Bio-Rad, США). В качестве тестовых культур были использованы штаммы микроорганизмов Американской коллекции типовых культур (АТСС): *Staphylococcus aureus (ATCC 29213*), *Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), а также Candida albicans* (клинический штамм). Параллельно проводили опыт для постановки «отрицательного» контроля. Для сравнительной оценки активности изучаемых проб сопоставляли активность с препаратом сравнения – настойкой эвкалипта с доказанной антимикробной активностью.

**Результаты.** Для всех водно-спиртовых извлечений и настойки травы чернушки посевной выявлены характерные для флавоноидов зоны адсорбции с $Rf_1$ = 0,28, $Rf_2$ = 0,15, $Rf_3$ = 0,11, также под действием спиртового раствора алюминия (III) хлорида 3% происходит усиление флуоресценции зон адсорбции, что говорит о фенольной природе данных соединений.

В ходе проведенного микробиологического исследования установлено, что все водно-спиртовые извлечения травы чернушки посевной оказывают наибольший антимикробный эффект в отношении штамма *Pseudomonas aeruginosa*. Отмечено, что препарат – настойка травы чернушки посевной на 70% спирте этиловом имеет преимущество по антимикробной активности к штамму *Pseudomonas aeruginosa* – *действие* при 16-ти кратном разведении против 4-кратного у настойки эвкалипта. Действие на штаммы *Escherichia coli* и *Candida albicans* сравнимо для обеих настоек.

**Заключение.** Полученные результаты фитохимического и микробиологического анализа будут использованы в качестве обоснования для внедрения антимикробных препаратов на основе травы чернушки посевной в медицинскую и фармацевтическую практику.

**Ключевые слова:** Чернушка посевная; *Nigella sativa* L.; трава; водно-спиртовые извлечения; настойка; минимальная ингибирующая концентрация; антимикробная активность

**Список сокращений:** АТСС – Американская коллекция типовых культур (American Type Culture Collection); CLSI – Институт клинических и лабораторных стандартов (Clinical and Laboratory Standards Institute); MRS-штаммы – метициллин-резистентные стафилококки (Methicillin-resistant *Staphylococcus*); MRSA – метициллинрезистентный золотистый стафилококк (Methicillin-resistant *Staphylococcus aureus*); ГФ РФ XIV изд. – Государственная фармакопея Российской Федерации XIV издания; КОЕ/мл – колониеобразующие единицы; МИК – минимальная ингибирующая концентрация; МУК – методические указания; *N.* – *Nigella* L. (н-р, *N. sativa*).

## INTRODUCTION

The creation of new antimicrobial drugs based on herbal raw materials, has always been and remains an urgent task of modern pharmacy. The proliferation of various new viral and microbial infections does not make possible a stable improvement in the quality of life and health of people and in many ways aggravates the course of other associated diseases in patients. Currently, the increase in antimicrobial resistance poses a serious danger, which consists in the reduction of the effectiveness of measures for the prevention and treatment of human infectious diseases [1–3].

The species of *Nigella* L genus have been used in folk medicine as promising medicinal and food plants since ancient times and are of great interest [4–6]. The genus *Nigella* L. consists of 24 species growing in the Mediterranean, southern and southeastern Europe, the Caucasus, Asia Minor and Central Asia, North Africa; about 11 species are distributed in the Commonwealth of Independent States [7, 8].

In modern medical practice, the *N. damascena* L. seeds and *N. sativa* L are mainly used. In the world publication stream, there is quite a lot of information about the pharmacological activity of various groups of biologically active compounds contained in the seeds and, respectively, in the *Nigella saliva* L. seeds oil. There is a high content of unsaturated fatty acids in the fatty oil, a high thymoquinone content and the presence of nigellone as components of the essential oil, carbohydrates, as well as lipolytic enzymes [6, 8–10].

The most studied are the *Nigella* L. seeds as well as the fatty oil obtained from them by cold pressing. It is reported that the *Nigella* L. essential oil has a strong antibacterial activity against Gram-positive (*Staphylococcus aureus)* and Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria [11, 12]. It has also a synergistic effect in combination with streptomycin and gentamicin, while its additive effect is manifested in combination with spectinomycin, erythromycin, tobramycin, doxycycline, chloramphenicol, nalidixic acid, ampicillin, lincomycin and co-trimoxazole, and is similar to topical mupirocin [11]. The activity against many multiresistant resistant Gram-positive and Gram-negative bacteria has also been described [11–14]. The researchers believe that these antimicrobial properties are due to the high

content of thymoquinone, particularly, thymohydroquinone [12–15].

The extractions from the seeds of *Nigella* L. can be used as an antimalarial and antiviral agents [16]. The recent studies of a number of scientists showing a great promising character of *Nigella* L. in the prevention and treatment of COVID-19, are of particular interest. That confirms the uniqueness of this plant once again [17, 18].

In view of the proven spectrum of antimicrobial activity for seeds and, accordingly, for the *N. sativa* L. oil, from the complex processing perspective of raw materials, it seems appropriate to study the antimicrobial properties of water-ethanolic extractions and preparations based on the *N. sativa* L herb. These studies will expand a spectrum of representations about the *N. sativa* L. pharmacological activity and evaluate the possibility of using this object in the creation of domestic drugs used in the antibacterial therapy.

For the objective assessment of the antimicrobial activity of the studied raw materials, it is necessary to conduct a screening analysis of water-ethanolic extractions and determine the minimum inhibitory concentration (MIC) in relation to the main clinically significant strains of the microorganisms. Comparing the activity with the reference preparation – a eucalyptus tincture[1,2], for which antimicrobial properties, including the ones against the MRS strains, have been proven, should be also carried out. [19, 20].

**THE AIM** of the study was to screen and compare the antimicrobial activity of water-ethanolic extractions from the *Nigella sativa* L. herbs with a eucalyptus tincture as a reference preparation.

## MATERIALS AND METHODS

The objects of the study were experimental *N. sativa* L. water-ethanolic extractions at various concentrations of a chemically pure ethanol grade (40%, 70%, 96%) (alcohol 96%, CJSC "Hippocrat", Russia, Samara, series: 360919) in the raw materials. For the selection of the ethyl alcohol concentration, the raw materials-to-the extractant ratio was 1:30. The *N. sativa* L. tincture was also obtained with 70% ethyl alcohol in the materials-to-the extractant ratio of 1:5 by fractional maceration with the inclusion of the final thermal stage – 0.5 hours at the temperature of 70°C.

The required alcohol concentrations of were obtained by diluting 96% alcohol according to Table No. 5 of the appendix to the State Pharmacopoeia of the Russian Federation XIV edition (SP RF XIV ed.)[3]. For most flavonoid-containing plants, the optimal extractant is 70% ethanol, since this concentration of ethyl alcohol allows the maximum amount of flavonoids in the plant to be extracted and has a better penetrating ability into the deep layers of the epidermis compared to higher concentrations [21]. In addition, compounds of the terpenoid nature also pass into the liquid phase, it was found in the course of the preliminary phytochemical studies.

### Analyzed samples of raw materials

The *N. sativa* L. herb was harvested from July to August 2021 in the Ulyanovsk region, the Cherdakly district, Cherdakly). *N. sativa* L. commercial seeds («Nora zdorov'ya», the habitat is Egypt) were used for cultivation. From the position of the phytochemical analysis results of various samples by their origin and places of harvesting, this raw material was chosen as the most valuable relative to the content of phenolic and terpenoid compounds, and also as perspective for the industrial cultivation in the European part and the south of Russia. The species specificity of the analyzed objects was also confirmed using determinants and a plant atlas[4].

### Reference preparation

The reference preparation with the established antimicrobial activity was a eucalyptus tincture in 70% ethyl alcohol of a commercial production in a bottle of 25 ml (LLC "Hippocrat", series: 010620, the date of production: 01.06.2020, the expiration date: 5 years).

### Test cultures

The following strains of the American Type Cultures Collection (ATCC) were used as test cultures: *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), as well as *Candida albicans* (clinical strain). A clinical strain of *Candida albicans* was isolated from a patient's sputum with bronchopulmonary pathology at "Samara State Medical University Clinics", the structural subdivision of Samara State Medical University. The study was conducted in accordance with the approval of the Bioethics Committee at Samara State Medical University (protocol No.196 dated October 31, 2018).

### Research methods

For the primary phytochemical analysis of the stud-

[1] State Register of Medicines. Available from: https://www.rlsnet.ru/tn_index_id_5478.htm.
[2] State Pharmacopoeia of the Russian Federation, XIV edition. Ministry of Health of the Russian Federation. Vol. 1–4, 2018. Available from: https://femb.ru/record/pharmacopea14.
[3] Ibid.
[4] *Nigella sativa* L. Plantarium. Plants and lichens of Russia and neighboring countries: open online galleries and plant identification guide. Available from: https://www.plantarium.ru/lang/en/page/view/item/25118.html

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ied extracts composition, the thin-layer chromatography (TLC) method was used, which was carried out in accordance with GM.1.2.1.2.0003.15 "Thin-layer chromatography" of the SP RF XIV ed.[5].

The TLC was carried out using Sorbfil PTSKh-AF-A-UF chromatographic plates; 0.02 ml of water-ethanolic extractions and the *Nigella sativa* herbs tincture were applied with a micropipette. Nearby, a witness solution was applied with a micropipette – a standard sample (SS) of rutin, which met the requirements of the SP RF XIV ed., and was provided for research by the Center for Collective Use of the Institute of Pharmacy of Samara State Medical University. The determination was carried out in the chloroform – ethanol – water (26:16:3) system. The resulting chromatogram was viewed in the UV light at λ=254 nm and λ=365 nm, it was also treated with a 3% alcohol solution of aluminum chloride ($AlCl_3$) and a solution of diazobenzosulfonic acid in a 20% sodium carbonate solution (DSA).

The minimum inhibitory concentration (MIC) was determined by the method of double serial dilutions in broth (a tube test, a macro method) in accordance with the methods described in "Guidelines" (G) 4.2.1890-04[6]. The method of double serial dilutions in comparison with a diffusion method allows a qualitative assessment of the antimicrobial effect presence by a visual assessment in comparison with the standard, and the determination of the minimum inhibitory concentration of the studied sample, which slows down the growth of the microorganisms strains under study. Mueller-Hinton nutrient broth (Bio-Rad, USA) was used as a nutrient medium [22].

### Methods

Testing of the samples under study was carried out in the volume of 1 ml of each sample dilution in water-ethanolic extractions.

### Preparation of working solution

To determine the sensitivity, 0.5 ml of the nutrient broth was poured into each tube. In addition to the number of tubes required to dilute the sample, one tube was used to set up a "negative" control. A working solution of a test sample was prepared from a stock solution using a liquid nutrient medium (Mueller-Hinton nutrient broth). The concentration of the working solution was calculated based on the required maximum concentration in a series of dilutions, taking into account the dilution factor of the drug during the subsequent inoculation.

Using a micropipette with a sterile tip, the working solution in the amount of 0.5 ml was introduced into the first tube containing 0.5 ml of broth. Then it was thoroughly mixed up, and with a new sterile tip, 0.5 ml of the test solution in the broth was transferred into the second tube containing initially 0.5 ml of broth. The procedure had been repeated until the entire required dilution series was prepared. 0.5 ml of the broth was removed from the last test tube. Thus, a number of test tubes with the solutions of the tested samples of the water-ethanolic extractions were obtained, the concentrations of which differed in the adjacent test tubes by a factor of 2. Simultaneously, additional series of serial sample dilutions were prepared for testing control strains.

### Inoculum preparation

For the inoculation, a standard microbial suspension was used. According to McFarland's standard, it was equivalent to 0.5 units diluted 100 times in the nutrient broth. After that, the concentration of the microorganism in it would be approximately $10^6$ CFUs/ml. 0.5 ml of inoculum was introduced into each tube containing 0.5 ml of the corresponding dilution of the test sample, and into one tube with 0.5 ml of nutrient broth without a sample (a "negative" control). The final concentration of the microorganism in each tube reached the required concentration of about $5\times10^5$ CFUs/ml. The inoculum was introduced into the test tubes with the sample dilutions not later than 15–30 min from the moment of its preparation.

The tubes were closed with sterile gauze and cotton stoppers, and all the tubes with the tested strains, except the tube with the "negative" control, were incubated at 35°C for 20–24 hours. The tube with the "negative" control was placed in a refrigerator at 4°C, and stored until the results were taken into account.

### Assessment of microorganisms growth

To determine the presence of the microorganisms growth, the test tubes with inoculations were viewed in the transmitted light. The growth of the culture in the presence of the test sample was carried out by the comparison with the tube of the "negative" control containing the original inoculum and stored in the refrigerator. The MIC was determined by the lowest concentration of the test sample, which suppresses the visible growth of the microorganisms.

[5] State Pharmacopoeia of the Russian Federation. Ministry of Health of the Russian Federation. XIV edition. Vol. 1–4, 2018.

[6] Determination of the sensitivity of microorganisms to antibacterial drugs. Guidelines. Guidelines 4.2.1890-04. Clinical microbiology and antimicrobial chemotherapy. 2004; 6 (4): 306–359. (The guidelines were approved and put into effect by the Chief State Sanitary Doctor of the Russian Federation – First Deputy Minister of Health of the Russian Federation G.G. Onishchenko, March 4, 2004.)

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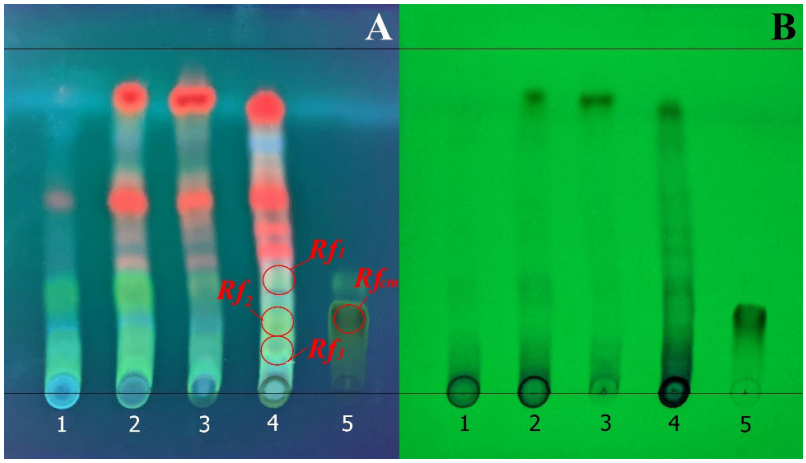


**Figure 1 – Chromatogram of the analysis of water-ethanolic extractions and *N. sativa* L. herb tincture**

Note: A – detection in UV light at 365 nm; B – detection in UV light at 254 nm. 1 – 40% water-ethanolic extraction; 2 – 70% water-ethanolic extraction; 3 – 96% water-ethanolic extraction; 4 – *N. sativa* L. herb tincture; 5 – SS rutin.

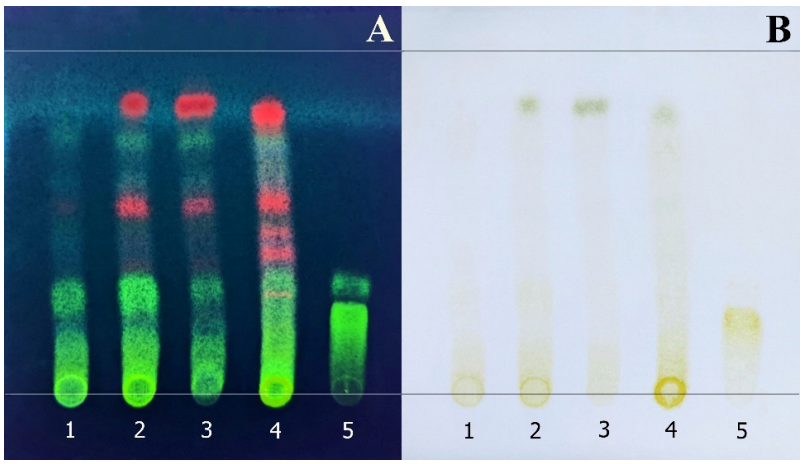


**Figure 2 – Chromatogram of the analysis of water-ethanolic extractions and *N. sativa* L. herb tincture**

Note: A – detection in UV light at 365 nm after $AlCl_3$ treatment; B – detection after DS**A** treatment. 1 – 40% water-ethanolic extraction; 2 – 70% water-ethanolic extraction; 3 – 96% water-ethanolic extraction; 4 – *N. sativa* L. herb tincture; 5 – SS rutin.

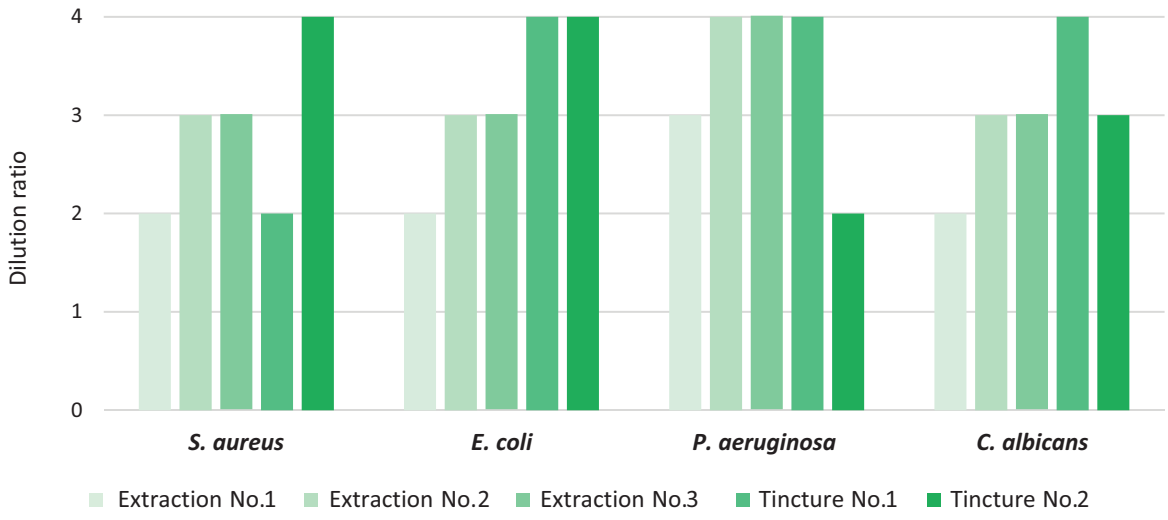


**Figure 3 – Comparative diagram of the antibacterial activity of water-ethanolic extractions of *N. sativa* L. herbs and the reference preparation**

Note: extraction 1 – 40% water-ethanolic extraction; extraction 2 – 70% water-ethanolic extraction; extraction 3 – 96% water-ethanolic extraction; tincture 1 – *N. sativa* L. herbs tincture; tincture 2 – eucalyptus tincture.

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**Table 1 – Testing results of extractions from *N. sativa* L. herbs and reference preparation**

| Object | Dilution ratio | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 |
| *Staphylococcus aureus* | | | | | | | |
| 40% *N. sativa* L. herbs | – | – | + | + | + | + | + |
| 70% *N. sativa* L. herbs | – | – | – | + | + | + | + |
| 96% *N. sativa* L. herbs | – | – | – | + | + | + | + |
| 70% *N. sativa* L. herbs tincture | – | – | + | + | + | + | + |
| 70% eucalyptus tincture (reference preparation) | – | – | – | – | + | + | + |
| *Escherichia coli* | | | | | | | |
| 40% *N. sativa* L. herbs | – | – | + | + | + | + | + |
| 70% *N. sativa* L. herbs | – | – | – | + | + | + | + |
| 96% *N. sativa* L. herbs | – | – | – | + | + | + | + |
| 70% *N. sativa* L. herbs tincture | – | – | – | – | + | + | + |
| 70% eucalyptus tincture (reference preparation) | – | – | – | – | + | + | + |
| *Pseudomonas aeruginosa* | | | | | | | |
| 40% *N. sativa* L. herbs | – | – | – | + | + | + | + |
| 70% *N. sativa* L. herbs | – | – | – | – | + | + | + |
| 96% *N. sativa* L. herbs | – | – | – | – | + | + | + |
| 70% *N. sativa* L. herbs tincture | – | – | – | – | + | + | + |
| 70% eucalyptus tincture (reference preparation) | – | – | + | + | + | + | + |
| *Candida albicans* | | | | | | | |
| 40% *N. sativa* L. herbs | – | – | + | + | + | + | + |
| 70% *N. sativa* L. herbs | – | – | – | + | + | + | + |
| 96% *N. sativa* L. herbs | – | – | – | + | + | + | + |
| 70% *N. sativa* L. herbs tincture | – | – | – | – | + | + | + |
| 70% eucalyptus tincture (reference preparation) | – | – | – | + | + | + | + |

Note: "+" – presence of microorganism growth; "–" – absence of microorganism growth.

**Table 2 – Minimum inhibitory concentrations of ethanol ("negative" control)**

| Object | Dilution ratio | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 |
| *Staphylococcus aureus* | | | | | | | |
| 40% ethanol | – | – | + | + | + | + | + |
| 70% ethanol | – | – | – | + | + | + | + |
| 96% ethanol | – | – | – | + | + | + | + |
| *Escherichia coli* | | | | | | | |
| 40% ethanol | – | – | – | + | + | + | + |
| 70% ethanol | – | – | – | + | + | + | + |
| 96% ethanol | – | – | – | + | + | + | + |
| *Pseudomonas aeruginosa* | | | | | | | |
| 40% ethanol | – | – | + | + | + | + | + |
| 70% ethanol | – | – | – | + | + | + | + |
| 96% ethanol | – | – | – | + | + | + | + |
| *Candida albicans* | | | | | | | |
| 40% ethanol | – | – | – | + | + | + | + |
| 70% ethanol | – | – | – | + | + | + | + |
| 96% ethanol | – | – | – | + | + | + | + |

Note: "+" presence of microorganism growth; "**–**" – absence of microorganism growth.

### Assessment of experimental results

The results were assessed visually by the presence/absence of the microorganisms growth in the test tubes with the appropriate dilutions of the test samples. The minimum inhibitory concentration was the lowest concentration of the studied sample, which completely suppressed the growth of the microorganisms strain. Herewith, according to the requirements of the "Guidelines" (Gs. 4.2.1890-04)[7] for determining the sensitivity of microorganisms to the antibacterial drugs, as well as the recommendations of the Performance Standard for Antimicrobial Susceptibility Tests (Clinical and Laboratory Standards Institute (CLSI)[8]. The presence of turbidity and the detection of a small number of microorganisms (one colony) were not taken into account when registering the experimental result. The experiment was repeated three times.

## RESULTS

The results of the qualitative chromatographic study revealed a number of features of the chromatographic profiles of the studied objects. For all the water-ethanolic extractions and the *N. sativa* herbs tincture, the adsorption zones typical for flavonoids of dark yellow and green color with $Rf_1$ = 0.28; $Rf_2$ = 0.15; $Rf_3$ = 0.11, were revealed (Fig. 1). It is notified that the most informative are the chromatograms viewed at the wavelength of 365 nm before and after the treatment with an alcohol solution of $AlCl_3$ and DSA (Fig. 2). Under the action of the $AlCl_3$ alcohol solution, the fluorescence of the adsorption zones increases, indicating the phenolic nature of these compounds. The DSA solution oxidizes organic compounds from yellow-orange to brick red in the visible light (Fig. 2).

The rutin adsorption zone had *Rf* = 0.20, which is close to the corresponding *Rf* values in the studied objects, especially in the *N. sativa* herbs tincture. Herewith it is noted that more adsorption zones of various kinds of nature are observed in the *N. sativa* herbs tincture. And the intensity of these zones glow is higher in 70% of the water-alcohol extract and the *N. sativa* herbs tincture, respectively.

The antimicrobial activity screening of water-ethanolic extractions from the *N. sativa* L. herb, and comparing them with the eucalyptus tincture (a reference preparation), made it possible to obtain the following data.

When testing 40% of the water-ethanolic extraction (1:30) from the *N. sativa* L herbs, the antimicrobial activity against the *S. aureus, E. coli* and *C. albicans* strains in a four-fold dilution, as well as against the *P. aeruginosa* microorganism in an eight-fold dilution was observed (Table. 1). When comparing 40% of the water-ethanolic extraction with the "negative" standard (a minimum inhibitory concentration for 40% water-ethanol), there was a slight difference in the antimicrobial activity between the test sample and the "negative" standard, which showed a slightly higher activity against *E. coli* and *C. albicans* (Table 2). This fact indicates that there is no contribution of the complex of biologically active compounds available in the extract, to the pharmacological effect at the given extraction concentration.

For a 70% water-ethanolic extraction (1:30) from the *N. sativa* L. herbs, the antimicrobial activity was expressed against the *S. aureus, E. coli* and *C. albicans* strains in the eight-fold dilution; against *P. aeruginosa* – in the 16-fold dilution (Table 1). When compared with the "negative" standard of ethanol at the 70% concentration, there was an increase in antimicrobial properties and a growth suppression of the *P. aeruginosa* microorganisms.

The 96% water-ethanolic extraction from the *N. sativa* L. herbs (1:30) showed a similar antimicrobial activity in the 70% water-ethanolic extraction: against *S. aureus*, *E. coli* and *C. albicans* strains – in an eight-fold dilution, against *P. aeruginosa* – in a 16-fold dilution.

When compared with the "negative" standard of ethyl alcohol at the concentration of 96%, a significant growth inhibition of the *P. aeruginosa* microorganism is notified. Respectively, for the concentrations of 70% and 96% water-ethanolic extraction from the *N. sativa* L. herbs (1:30), a significant suppression of the growth of the *P. aeruginosa* microorganisms is observed.

The antimicrobial activity of the reference preparation – the 70% water-alcohol eucalyptus tincture, shows its high activity against *S. aureus* and *E. coli* – reducing the growth of microorganisms in a 16-fold dilution. A similar activity in 70% and 96% extractions from the *N. sativa* L. herbs against the *C. albicans* strain in an eight-fold dilution and a weak activity against the *P. aeruginosa* strain – suppression of a microbial growth only in a 4-fold dilution (Table 1). This fact favourably allocates an action direction of the biologically active substances based on the *N. sativa* L. herbs.

The tested 70% *N. sativa* L. herbs tincture (the tincture from the *N. sativa* L. herbs -to-70% ethanol ratio of 1:5) showed the following results. Similar to the extractions in 70% and 96% ethyl alcohol, the antimicrobial effect was observed against the *P. aeruginosa* strain in a 16-fold dilution, however, besides this prevailing effect, the antimicrobial effect against the *E. coli* and *C. albicans* strains increased stopping the growth up to the 16-fold dilution. The effect against the *S. au-*

[7] Ibid.
[8] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2018.

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*reus* strain, on the contrary, slightly decreased to the 4-fold dilution (Fig. 3).

The following results were obtained in the course of the comparison analysis of the preparation based on the *N. sativa* L. herbs tincture and the reference preparation – the 70% eucalyptus tincture. A higher antimicrobial effect was observed against the *P. aeruginosa* and *C. albicans* strains in the 16-fold dilution in the *N. sativa* L. herbs tincture. A similar antimicrobial effect in the studied tinctures was observed against the *E. coli* strain; the eucalyptus tincture showed a higher antimicrobial activity against the *S. aureus* strain (Table 1).

## DISCUSSION

Currently, one of the serious factors affecting the drug therapy success of infectious diseases is the increased resistance of pathogenic microorganisms to antimicrobial agents[9]. Staphylococci or methicillin-resistant strains (MRSs) deserve special attention and are the cause of nosocomial and hospital-acquired infections.

Among the methicillin-resistant strains, *Staphylococcus aureus* (MRSA) is particularly common; its strains are resistant to many members of the β-lactam antibiotic group, including penicillins, cephalosporins, monobactams, carbapenems, etc. [21, 23, 24]. An equally dangerous strain is the Gram-negative bacterium *E. coli*, which is present in the human intestine and can cause various infectious diseases of the gastrointestinal tract and urogenital system [25].

The potential of the vegetative plant parts (leaves, branches, and stem) of *N. sativa* L. to reduce the load on the reproductive part (seeds) was also studied by researchers from Pakistan, who performed, in addition to phytochemical screening, antibacterial and antioxidant analyses, as well as the GC-MS analysis of superpotent extracts [26]. The dried plant was subjected to the extraction by the separation method in a series of concentrations ranging from 1.562 to 200 mg/ml in different solvents. The antibacterial analysis was performed on the pathogenic strains – *Clostridium difficile*, *Pasteurella sp.*, *Pseudomonas aeruginosa*, and *Xanthomonas sp*. The isolates were subcultured on the plates with nutrient agar and incubated at 37°C for 24 hrs. The analysis was performed by diffusion into the agar disk [23, 26]. The inhibition zones were measured in the agar wells containing a plant extract. The analysis was performed for methanol, chloroform, n-hexane, n-butanol, aqueous and ethyl acetate extracts at the concentrations of 1.56-200 mg/ml. The inhibition zones were measured after 24–48 hrs. The results showed that the maximum inhibition zone (40±1.73 mm) was observed for *Xanthomonas stutzeri* among all extracts, and a 100 mg/ml chloroform extract caused the maximum inhibition zone for the growth of *Xanthomonas stutzeri* bacteria (36±1.26 mm) among all the strains. The chloroform extract at the concentration of 50 mg/ml also showed a maximum inhibition of the bacterial growth, and exactly the same pattern was observed for all the concentrations, extracts and bacterial strains [26]. In addition to the above spectrum of the pharmacological activity, other authors often mention antidiabetic and antioxidant activities of the *Nigella sativa* L. herbs [27, 28].

Given the fact of previously described studies on the antimicrobial activity of the extracts from the *N. sativa* L. herb, its component composition and the dominant group of biologically active compounds (BAC) should be also discussed and clarified. Thus, many researchers refer to the work by foreign scientists of the Biotechnology Center (Tunisia), which describes the study of the *N. sativa* L. herbs by HPLC from absolute methanol extracts. These extracts identified 14 phenolic compounds with a total content of 215 mg/100 g in the shoots, among which the dominant group is phenolcarboxylic acids, represented mainly by gallic and vinyl acids [29]. In addition, the authors established the antimutagenic activity of the studied extracts from the *Nigella sativa* L. shoots using Ames test [8, 29].

In the *Nigella sativa* L. herbs, as described above, the phenolic compounds are mainly represented by phenolcarboxylic acids and a sum of flavonoids. Probably due to the free phenolic hydroxyl groups as in the gallic acid molecule, they have a potential for the antimicrobial activity [30]. The antimicrobial activity of these compounds was also discussed by the researchers from Pyatigorsk in the study on the chemical composition and antimicrobial activity of the dry extract from the flowers of (*Tagetes patula* L. The authors explain the bactericidal effect of the studied extract on the coccus and spore-forming flora, *Salmonella gallinarum* and *Pseudomonas aeruginosa*, and the bacteriostatic effect against *Escherichia coli* with a high content of gallic acid [31]. The authors' previous phytochemical study of the *Nigella sativa* herbs composition by differential and direct spectrophotometry at the wavelengths of 254 and 365 nm, confirms the dominance of phenolic substances [32]. In addition, the TLC analysis of water-ethanolic extractions and the *N. sativa* L. herbs tincture after the treatment with a specific reagent for flavonoids – an alcohol solution of $AlCl_3$ clearly demonstrates the qualitative composition of the studied samples (Fig. 1, Fig. 2). The specificity mechanism of the reagent interaction is due to the formation of bathochromic complexes with free 3- and 5-hydroxy groups of flavonoids. This fact enhances the fluorescence of the corresponding adsorption zones at the wavelength of 365 nm [33].

[9] Determination of the sensitivity of microorganisms to antibacterial drugs. Guidelines. MUK 4.2.1890-04. Russian

In the course of the screening analysis of the antimicrobial activity of water-ethanolic extractions from the *N. sativa* L. herbs, the conditions for obtaining the dosage form of the tincture were determined. As an extractant for the manufacture of the *N. sativa* L. herbs tincture from, a 70% concentration of ethyl alcohol was chosen, since this concentration is the optimal extractant for this raw material containing a complex of biologically active substances of the flavonoid group. It also contains the extractions of a valuable terpenoid gamma – together providing the observed antimicrobial effect [23, 26].

The choice in favor of the 70% alcohol concentration as an extractant for obtaining the *N. sativa* L. herbs tincture was made based on the fact that in the dosage forms with these extraction parameters, the greatest antimicrobial effect is observed against the studied strains of microorganisms, especially against the *P. aeruginosa* strain. In addition to the main directed action against the *P. aeruginosa* strain, the action against the other strains – *E. coli* and *C. albicans* – is enhanced. Also, the extracts at the given alcohol concentration have a better penetrating ability into the deep layers of the epidermis in comparison with higher and lower alcohol concentrations [21, 32].

Taking into consideration the fact that the *N. sativa* L. herb has a large phytomass in comparison with seeds and is subjected to utilization at the seed collection, its collection will help to carry out a complex and versatile use of all the raw materials of the plant and will promote new medicinal herbal preparations.

## CONCLUSION

Therefore, a phytochemical screening and a comparative study were carried out to research the antimicrobial activity of the experimental water-ethanolic extractions from the *N. sativa* L. herbs *in vitro*, as a result of which flavonoids were found as the main group of BAC. An antimicrobial effect was also established on the pathogenic strains of such microorganisms as *P. aeruginosa*, *E.* coli, *C. albicans*, *S. aureus*. It was found out that all the studied samples of the water-alcoholic extractions from the *N. sativa* L. herbs give a stable prevailing antimicrobial effect against the *P. aeruginosa* strain.

The *N. sativa* L. herbs tincture in the 70% ethyl alcohol (1:5) has a specific effect on the-*Pseudomonas aeruginosa* strain. In the proposed dosage form, the activity against the *Escherichia coli* and *Candida albicans* strains also increases, which is comparable to the eucalyptus tincture–used in medical and pharmaceutical practice. The results obtained in the study can serve as a basis for the creation of new antibacterial drugs based on the *N. sativa* L. herb, as well as for a further implementation of the preparations from the *N. sativa* L. tincture in the 70% ethyl alcohol in medical and pharmaceutical practice.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHORS' CONTRIBUTION

ARM – collecting plant materials for analysis, experiment conducting, analyzing and interpreting the data obtained, analyzing the literature, manuscript writing and finally approving of it for publication; VAK – final approval of the manuscript for publication, processing the results obtained, verification of critical intellectual content; EVA – participation in the development of the research concept and design, critical analysis of the research results; SDK – planning the study, participation in the experiment; AVZ – participation in the description and analysis of the results obtained, writing the manuscript.

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# OPTIMIZATION TECHNOLOGY OF FAT-SOLUBLE VITAMINS PRODUCTION BASED ON ALKALINE HYDROLYSIS

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In the group of fat-soluble vitamins, vitamin D is one of the most relevant objects, that is why the problem of its technology optimization is under consideration. In general, there is a number of ways to obtain this substance, although it is not produced in Russia yet.

**The aim** of the study was to select optimal process conditions to increase the efficiency of protein transformation with the isolation of a fat fraction containing fat-soluble vitamin D.

**Materials and methods.** Various types of fish and the vitamins contained in them are described as the main research models. Variants of technological solutions have been considered: the possibility of using extraction to obtain the vitamin D substance has been tested. Classical maceration and intensifying maceration have been used; the circulating extraction method and the alkaline hydrolysis method have been applied. The yield of the target product has been determined by HPLC.

**Results.** Methods for obtaining the substance of fat-soluble vitamin D from fish raw materials have been considered in detail. The optimal technological characteristics of the vitamin release by alkaline hydrolysis with a 12.5% decrease in the concentration of the hydrolyzing alkaline component – potassium hydroxide – has been established; that concentration ensured the maximum yield of the fat fraction containing vitamin D.

**Conclusion.** The use of the resulting substance makes it possible to develop domestic standard samples applicable both in the pharmaceutical field and in the field of technical regulation.

**Keywords:** fat-soluble vitamins; reference standard; alkaline hydrolysis; maceration; circulating extraction; lean manufacturin; raw material processing

**Abbreviations:** HPLC – high performance liquid chromatography; RS – reference; RF – Russian Federation; BASs – biologically active substances.

# СОВЕРШЕНСТВОВАНИЕ ТЕХНОЛОГИИ ПРОИЗВОДСТВА ЖИРОРАСТВОРИМЫХ ВИТАМИНОВ НА БАЗЕ ЩЕЛОЧНОГО ГИДРОЛИЗА

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В статье рассматривается вопрос оптимизации технологии производства жирорастворимого витамина D – одного из наиболее востребованных объектов данной группы. Показано, что в целом существует ряд способов получения этой субстанции, хотя в России она пока не производится.

**Цель.** Выбор оптимальных условий технологического процесса для повышения эффективности трансформации белков с выделением фракции жира, содержащей жирорастворимый витамин D.

**Материалы и методы.** В качестве основных моделей исследования описаны различные виды рыбы и витамины, содержащиеся в них. Рассмотрены варианты технологических решений: апробирована возможность использования экстракции для получения субстанции витамина D. Были использованы классическая мацерация и мацерация с интенсификацией, применён метод циркуляционной экстракции и метод щелочного гидролиза. Выход целевого продукта определяли методом ВЭЖХ.

**Результаты.** Подробно рассмотрены методы получения субстанции жирорастворимого витамина D из рыбного сырья. Установлена оптимальная технологическая характеристика высвобождения витамина методом щелочного гидролиза со снижением концентрации гидролизующего щелочного компонента – калия гидроксида до 12,5%, который и обеспечил максимальный выход жировой фракции, содержащей витамин D.

**Заключение.** Применение полученной субстанции дает возможность разработки отечественных стандартных образцов, применяемых как в фармацевтической сфере, так и в сфере технического регулирования.

**Ключевые слова:** жирорастворимые витамины; стандартный образец; щелочной гидролиз; мацерация; циркуляционная экстракция; бережливое производство; переработка сырья

**Список сокращений:** ВЭЖХ – высокоэффективная жидкостная хроматография; СО – стандартный образец; РФ – Российская Федерация; БАВ – биологически активные вещества.

## INTRODUCTION

The problems related to obtaining and properly using fat-soluble vitamins are constantly relevant. First of all, they concern the use of these vitamin complexes in the pharmaceutical, cosmetic and other fields, as well as the saturation of the modern pharmaceutical market with them [1, 2]. At the same time, technological directions for the production of fat-soluble vitamins including their standardization are involved; for this process, official reference samples (RSs) are necessary. Obtaining appropriate RSs requires technological optimization, especially in relation to the development of domestic technologies, which implies the possibility of their import substitution [3].

Severization of requirements for the purity of target products have increased the importance of standardization methods, both in the creation and production of medicines, and the products used in the fields related to pharmacy. Therefore, the use of RSs, the role of which in standardization is undeniable, has a wide range [4, 5].

However, at present, foreign models dominate in a pharmaceutical analysis of many pharmacologically significant objects in the Russian Federation (RF); that is a gap in the relevant regulatory framework. This is due to the lack of necessary technological solutions in the production of reference samples, which are still selective in nature in the Russian Federation [6, 7].

Despite a wide range of vitamin D dosage forms and rather a high demand for it in medical practice, vitamin D is one of such multidisciplinary objects [8, 9]. However, as a substance on the domestic market in Russia, vitamin D has not been produced yet.

Fat-soluble vitamin D is presented in the form of products for various purposes, however, its content in them is not high, and its stability is limited [10–12]. The existing technology for obtaining vitamin D can be divided into two main stages: the extraction of fat and vitamins from raw materials, and the increase in the concentration of vitamins in the resulting fraction. Herewith, the volume of vitamin concentrates production largely depends on the first stage of fat extraction, which ensures the vitamin production from the starting raw materials [9, 13]. Currently, the main source of obtaining the substance under study is fish industry [14–16].

Given that the main source of fat-soluble vitamins is fish liver, there is a problem of creating an appropriate technology with an emphasis on a lean production (waste minimization with maximum resource saving of a valuable raw material source) [17, 18].

Currently, obtaining food products from cultivated fish species is widely practiced. Such target products are marked by a high content of biologically active substances (BASs), as well as macro/microelements and vitamins [19–21].

The existing methods for obtaining fats can be divided into 4 main groups (Fig. 1). Combining these processes to maximize fat recovery remains possible. The choice of the recovery method depends on the starting raw materials and the expected target product.

The blocks presented in Fig.1 justifiably single out hydrolysis as the main production method and emphasize its diversity in the form of varieties. Hydrolysis is actually divided into three types: acidic, alkaline and enzymatic. To obtain a fat fraction with a high content of the fat-soluble vitamins group, the most effective are alkaline and enzymatic methods [22–24].

According to the literature data [25–27], the fat recovery by hydrolysis has the highest yield, since part of it is in the cells in a free form, and another part is combined with other components of the cell. The total amount of fats cannot be extracted from the cell with only solvents [19, 28, 29].

The authors decided to fix upon alkaline hydrolysis. In the framework of enzymatic hydrolysis, there are

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some limitations – for processing of all types of secondary fish production raw materials, it is impossible to isolate a single enzyme, which increases the economic costs for obtaining the substance of fat-soluble vitamin D. This fact determines the choice of alkaline hydrolysis method as the most economically favorable.

To use hydrolysis of protein catalysts, biotransformation processes can be carried out under milder conditions, and the formation of chemically active effluents can be excluded [22]. Alkaline hydrolysis can be used to even process depleted raw materials, herewith preserving natural antioxidants [25].

In raw materials, hydrolysis undoubtedly occurs under the action of their own enzymes (it is the so-called autoproteolysis) but the efficiency of this method is very low, and therefore, it is necessary to increase efficiency, which means finding optimal production conditions [22].

**THE AIM** of the study was to select optimal process conditions to increase the efficiency of protein transformation with the isolation of a fat fraction containing fat-soluble vitamin D.

To achieve this aim, it was necessary to solve the following tasks:

1. Determination of the chemical composition of primary and secondary raw materials;
2. Choosing the optimal method for extracting the fat containing a group of fat-soluble vitamins;
3. Quantitative determination of the fat-soluble vitamin D content in the resulting substance;
4. Comparative analysis of the obtained results.

## MATERIALS AND METHODS

### Objects

The main objects of obtaining the substance of fat-soluble vitamin D were fish objects purchased from the Russian fish processing factory JSC "Biserovsky Fish Processing Fish Factory" and LLC "First Rybny". The raw materials were delivered frozen at the storage temperature of –18°C. The acquisition of the research objects was carried out through the branded stores network of LLC "First Rybny", Ekaterinburg, Russia.

The following types of fish were used as a source of obtaining the target object:

Chun salmon *(Oncorhynchus keta)* is the most common member of the salmon family.

It contains vitamins PP, A, H, $B_1$ (thiamine), E, $B_2$, D, $B_5$, etc.; trace elements: primarily zinc and iron, molybdenum, fluorine, chromium, nickel; macronutrients: potassium, chlorine, phosphorus, sodium, calcium, magnesium; protein, lecithin, polyunsaturated fats [10, 30, 31].

Humpbacked salmon *(Oncorhynchus gorbuscha)* is the most common representative of the salmon family.

It contains vitamins A, C, E, D, $B_1$, $B_2$, PP, omega-3 saturated acids, phosphoric acid, pyridoxine, calcium, magnesium, phosphorus, sulfur, potassium, copper, iodine.

Coho salmon *(Oncorhynchus kisutch)* is a large salmon that lives in the North Pacific Ocean. In Russia, coho salmon is most abundant in the rivers of the Kamchatka Peninsula [10, 30, 31].

Its meat is rich in vitamins and minerals, as well as in saturated fatty acids, including Omega-3 [10, 30, 31].

Brook trout *(Salmo trutta)* is a representative of the salmon family and is in great demand. Currently, there is a tendency to increase the population. Its composition is rich in microelements and saturated fatty acids [10, 30, 31].

Flatfish *(Pleuronectidae)* is a representative of the flounders' family. It has a medium fat content, but it is low in calories. It contains a lot of lipids. By eating flatfish meat, you can replace artificial and very expensive vitamins, which are useful because omega-3 and omega-6 fatty acids have been added to their composition. It is also an excellent source of natural protein [10, 30, 32].

Mackerel *(Scomber)* is a pelagic schooling fish from the mackerel order. This is one of the most popular fishes with fatty (62%) and tender meat. The protein content in its composition is 38%. Mackerel meat is also rich in saturated, polyunsaturated and monounsaturated fats; water, ash; cholesterol; vitamins of groups A, C, E, D, $B_1$, $B_2$, PP, etc.; minerals: iodine, phosphorus, cobalt, zinc, magnesium, sulfur, chlorine, manganese, sodium, molybdenum, copper, calcium, iron, fluorine [10, 30, 32].

### Materials used

At the sample preparation stage were used:

Sterile bags – microbiological bags for sampling (Russia, LLC "LabDepo").

Inert gas – nitrogen, high purity grade, 1st grade, JSC "Linde Uraltekhgaz".

The stage of selecting the object under study:

Anhydrous sodium sulfite, chemically pure, OOO "MZHR" is in the form of colorless crystals, the salt of sodium and sulfurous acid. Sodium sulfite is dissolved in water forming crystalline hydrates. Sodium sulfite is a strong reducing agent. It is used for dehydration in the hydrolysis process.

Ascorbic acid, pure, imported from China (Shandong Luwei Pharmaceutical Co., LTD), is an organic compound, the main water-soluble antioxidant.

Potassium hydroxide, pure, imported from France, is an alkaline solution of various concentrations.

Distilled water according to GOST R 58144-2018 "Distilled water. Specifications", Russia, FBI "URALTEST".

Ethyl alcohol (95%, Russia, LLC "KONSTANTA-PHARM M") is a component of the hydrolyzing solution.

Extractants, solvents are: hexane-n (chemically pure, Russia, EKOS-1 JSC), petroleum ether grade 40-70 (high purity, Russia, JSC "EKOS-1"); isopropyl alcohol (chemically pure, Russia, LLC "KhromLab").

Analytical research stage:

State Standard Samples (SSSs) RS 11779-2021 for the composition of vitamin D3 (cholecalciferol), Russia, FBI "URALTEST".

Cholecalciferol ≥98% (HPLC), batch number SLCJ0796, manufactured by Sigma-Aldrich, USA.

Isopropyl alcohol (chemically pure, Russia, LLC "KhromLab").

The mobile phase is presented by bacetonitrile:-methanol (20:80). Acetonitrile, chemically pure, Russia, JSC "ECOS-1"; methanol, chemically pure, Russia, JSC "VEKTON".

### Equipment

Electric pharmacy water distiller DE-4-02 "EMO", model 737, factory No. 2506, manufactured by CJSC "Electrooborudovanie". Country of manufacture: Russia.

Laboratory straight blade knife 150 mm made of stainless steel. Country of manufacture – China.

Rotary evaporator IKA RV 10 auto pro V-C. Country of manufacture – Germany, IKA®-Werke GmbH & Co., with an automatic evaporating temperature detection, 1500 $cm^2$ cooling surface condenser.

Dispersant IKA T 10 basic Ultra. Country of manufacture – Germany, IKA®-Werke GmbH & Co., with a speed range from 8000 to 30000 rpm.

Ultrasonic machine, model 1510E-DTH. Country of manufacture – Switzerland, Branson Ultrasonics Corporation, with an industrial transducer at 40 kHz with a frequency regulator for optimizing ultrasonic exposure and a temperature heating regulator with an accuracy of ± 4°C.

Soxhlet fat extraction apparatus is used for the extraction of non-volatile substances from solid samples with the help of volatile solvents. Country of manufacture: Russia, the company is OOO "EcogeosProm".

Chromatographic experiments were carried out on a "Stayer M" liquid chromatograph, Russia, OOO "NPO Akvilon", Manufactory No. 0950 (Verification certificate No. C-CE/19-01-2022/126041432 dated 19.01.2022 to 18.01.2023) with a spectrophotometric detector. The calibration was carried out using standard solutions of fat-soluble vitamins prepared according to GPM.1.2.3.0017.15[1]. Fat-soluble vitamin D was detected at the wavelength of 265 nm [33, 34].

### Chromatography conditions

A mobile phase was carried out with: methanol – acetonitrile (80:20); Luna C18(2) chromatographic column 250 mm x 4.6 mm, the flow rate was 1.0 ml/min, the column temperature was 25°C. The detection was carried out with: a spectrophotometric detector with the wavelength of 265 nm, the injection volume of 20 µl; the analysis time was 12 minutes.

### Study Design

First, the basic test was carried out in accordance with GOST 7636-85 "Fish, marine mammals, marine invertebrates and products of their processing. Methods of Analysis"[2]. Thus, there was a collection of standardized baseline data on the content of fat and vitamin D in the resulting product. Further on, technological research concerned the choice of optimal process conditions.

Brook trout *(Salmo trutta trutta)* was chosen as the main object of the study. This species is cultivated, relatively inexpensive and available.

### Traditional maceration and ultrasonic intensifying maceration

Well-known extraction methods were used as preliminary methods for obtaining the target product [35, 36].

To carry out the process of extracting vitamin D, the cooled raw material was subjected to manual grinding with a laboratory knife to 1.5 × 1.5 cm, as well as machine micro-grinding in an inert gas environment to particles with the size of about 0.5 × 0.5 mm, and homogenization. The inert medium was provided during the dispersion process by a continuous nitrogen (especially pure, 1st grade, JSC "Linde Uraltekhgaz") supply to the tank. Homogenization was carried out by packing into sterile bags (LLC "LabDepo") with blowing off the test material by gas for 20-30 seconds, and subsequent sealing. Considering that a large amount of waste remains in the mass during machine processing (cleaning) at the enterprise, these products were not removed, but homogenized in the total mass. Grinding was carried out using a high-performance disperser IKA with an electronic speed control of the processed object under study. The rotational speed of the dispersing element was maintained in the range of 8000–8200 rpm for 10 minutes. Next, the resulting mass was placed in a Micro-Bio BA-400 laboratory bladed homogenizer and subjected to the homogenization process for 30 min.

The prepared minced fish was subjected to maceration with organic solvents: isopropyl alcohol (chemically pure, LLC "KhromLab"), hexane-n (chemically pure, JSC "ECOS-1"), petroleum ether (grade 40–70, pure, JSC "ECOS-1").

Maceration was carried out in two ways: intrinsic maceration and intensifying process maceration. The intensification of the process was carried out by using a mixing device at 800 rpm, and the ultrasonic influence was also used to enhance the effect on the object.

The used ultrasonic generator carried out the impact on the object under study by fluctuations of high and low pressure. Under the influence of low-pressure waves on the liquids, a cavitation effect developed; that

[1] State Pharmacopoeia of the Russian Federation XIV ed. Vol. 1–4. 2018. Available from: http://www.femb.ru/femb/pharmacopea.php.

[2] GOST 7636-85. Fish, marine mammals, marine invertebrates and their derivatives. Methods of analysis. M. Standartinform, 2010. 126 p. Russian

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contributed to an increase in the release of the fat fraction from the biological object resulting in the formation of a large number of microscopic bubbles or cavities.

During maceration, various organic solvents were used: isopropyl alcohol, hexane and petroleum ether. The solvent volume was determined by random selections. Three series of the extracts were carried out with volume fractions of each extractant: object/extractant 1:1, 1:2 and 1:3 and so on for all the abovesaid components.

### Circulating extraction

Circulating extraction was carried out using a Soxhlet apparatus. The extraction was carried out with petroleum ether until a complete depletion of the raw material. The completeness of the extraction was controlled by applying a drop of the solvent flowing from the extractor onto the watch glass until there was no greasy trace on the latter after the solvent had evaporated.

The resulting fat fraction was subjected to a preliminary assessment in accordance with GOST 8714-2014 "Fat from fish and aquatic mammals. Specifications"[3]. The obtained samples of fat fractions were within the limits established in the regulatory documentation for all used fish species. Further on, the obtained samples were tested for the content of vitamin D in them in accordance with GPM 1.2.3.0017.15 "Methods for quantitative determination of vitamins"[4].

### Isolation of vitamin D by alkaline hydrolysis

Modes of alkaline hydrolysis are determined by the following conditions: temperature, the amount of added water and alkali.

The lack of water slows down hydrolysis of the protein part, but at the same time, hydrolysis of fat is activated. An excess of water also causes an increased consumption of alkali with an increase in capacity.

With regard to the temperature regime, two-stage heating with a two-stage introduction of an alkali solution is considered the most acceptable. This is due to the fact that heating the raw mass immediately to 80-90°C coagulates the protein; as a result, the hydrolysis time is prolonged and the alkali consumption is increased to destroy the densified protein part. The introduction of the entire alkaline solution at the beginning of the process leads to a more intensive hydrolysis of the fat, and not the protein part. The fatty acids released in this process also react with alkali and form soaps. This increases the consumption of alkali and forms a stable emulsion.

With an excess of alkali, hydrolysis is obvious, which directly relates to the quantitative yield of the vitamin. However, due to the ongoing saponification, it can be reduced, since soaps are formed on the matrix, as a result of which the vitamin is sorbed. There is an adsorption of the vitamin by the formed soap and an increase in losses during the refining of the fatty fraction of the hydrolysate [37].

### Technology for vitamin D production by alkaline hydrolysis

At the first stage, the mass fraction of fat in the test object was assessed. The mass fraction of fat was determined in accordance with GOST 7636-85[5]. This indicator was necessary for further alignment of the hydrolysis process.

Before the hydrolysis beginning, 50.0 g of finely minced brook trout *(Salmo trutta)* was placed in a round-bottom flask with a capacity of 250–500 ml. The heating process was carried out in the water bath in two stages. The first heating stage continued up to 40°C, which contributed to the release of fat, most of which passed from a finely dispersed state into a coarse dispersed state, which slowed down saponification. Next, 100 ml of rectified ethyl alcohol (or methyl alcohol), 1.0 g of an antioxidant (ascorbic acid), 40 ml of a potassium hydroxide solution were added to the heated minced meat. The mix was heated for 15–45 minutes in the water bath with a reflux condenser at the temperature of 80°C, which accelerated the hydrolysis process and, as a result, destroyed the protein. The main part of the fat phase was separated from the protein and was in the upper part of the hydrolyzed mass, the lower layer was the hydrolyzate, which contained polypeptides, amino acids, minerals and soaps.

After hydrolysis, the contents of the flask were quickly cooled down to (20±5°C) and quantitatively transferred to a separating funnel. The flask was rinsed with water, the volume of which was equal to the volume of ethanol added, and then the water was poured into the same funnel. Fat-soluble vitamins were extracted with petroleum ether or n-hexane for 2 min.

The extraction was repeated 3–4 times with the portions of the extractant equal to 100 ml. The combined extract had been washed free from alkali 3–4 times with 150 ml portions of water until the alkaline reaction of the wash water disappeared.

To remove the residual amounts of water, the extract was filtered through a filter with 2–5 g of anhydrous sodium sulfate (chemically pure, LLC MZKhR). Next, the extract was evaporated to dryness using a ro-

[3] GOST 8714-2014. Edible fat from fish and aquatic mammals. Specifications. M.: Standartinform, 2019. 7 p. Russian

[4] State Pharmacopoeia of the Russian Federation XIV ed. Vol. 1–4. 2018.

[5] GOST 7636-85. Fish, marine mammals, marine invertebrates and their derivatives. Methods of analysis. 2010.

tary evaporator at the temperature not exceeding 50°C, and then treated with isopropyl alcohol. The scheme of the experimental part is shown in Fig. 2. These blocks characterize the technological experiment in detail, which can become fundamental for the development of technological regulations and transfer to manufacturing.

During the experiment, various concentrations of a potassium hydroxide solution in the concentration range of 12.5%, 25.0%, 50.0% were selected. All the abovesaid conditions were: obtaining a homogenized material for hydrolysis, as well as a stabilizer (ascorbic acid, pure, China) are identical.

## RESULTS AND DISCUSSION

According to the results of the studies, it has become quite obvious that the method of traditional maceration with intensification has not brought positive results.

In the presented chromatograms, in the detection range characteristic of vitamin D, no detector response was found out. That characterizes the absence of fat-soluble vitamin D in the substance obtained by traditional maceration (Fig. 3). Herewith, in the control determination, using the RSs, the retention time and peak area are determined in the ranges for this RS (Fig. 4).

The use of the circulating extraction method made it possible to isolate a small amount of vitamin D in the test object. When using the alkaline hydrolysis method, the quantity of the analyte is much larger. Additional alkaline hydrolysis of the resulting fat fraction slightly increased the concentration of the vitamin and did not lead to the targeted results (Table 1). During the circulating extraction, the value of the peak area varied around 0.01 AU*sec, after a combination of methods using the same raw material component, the area slightly increased up to 0.02 AU*sec.

Therefore, the problem of developing and use of a combined method for obtaining a fat-soluble substance formed from any extraction option, taking into account basic alkaline hydrolysis, is apparently not promising. Therefore, the authors had to return to alkaline hydrolysis and improve the technology for the production of vitamin D substance precisely within the framework of this reliable method.

The next, third stage of the research consisted in applying the method of alkaline hydrolysis.

The results of the experiment showed that at the 12.5% concentration of potassium hydroxide in the test samples, vitamin D was determined stably in all the series of the analysis (Table 2).

In the control analysis of SSS 11779-2021 (FBI "URALTEST"), as well as of imported cholecalciferol (Sigma-Aldrich, USA), it was determined that the release time of vitamin D corresponds to 10 minutes. The quantitative content in the samples was judged by a characteristic indicator for HPLC – the peak area. According to the results of the analysis, the peak area for the standard sample was 9.52 AU*sec, for the test sample it was 0.05 AU*sec.

The results of the chromatographic analysis of the substance, which in turn was obtained by hydrolysis with a 12.5% potassium hydroxide solution, were subjected to comparative studies. A study was carried out with samples of imported production. Herewith, it turned out that vitamin D was present in the experimental samples (Fig. 5).

The results obtained with a 25% potassium hydroxide solution, are identical to those obtained with a 12.5% alkali solution. As in the first case, vitamin D was present in the studied samples and it was in a stable form. The repeated control studies of the samples showed identical retention times and peak areas (Table 3).

During hydrolysis of the sample with a 25% potassium hydroxide solution, the quantitative content of vitamin D was also estimated by the peak area – 0.05 AU*sec.

When a 50% potassium hydroxide solution was used, vitamin D also proved to be stable, as in the first two experiments, which gives us the opportunity to assert the prospect of using lower concentrations of alkali.

During hydrolysis of the sample with a 50% potassium hydroxide solution, the peak area of vitamin D in the resulting substance was 0.05 AU*sec.

The effect obtained using a 50% potassium hydroxide solution is identical to the first two results (Table 4). Vitamin D in a stable form was determined in the studied samples. Parallel studies of the samples confirmed the result of the analysis.

For each series of the analyses, there were 6 sample tests. Tables 1-4 present the average values of the sample weight for each series of analyzes, and the arithmetic mean of 6 parallel tests was taken as the mass of the isolated fat fraction, the allowable discrepancies between which did not exceed 0.5%.

The use of different alkali concentrations in the production of fat-soluble vitamins (including vitamin D) will simplify the technological process of isolating an individual substance, which, in turn, will eliminate the negative effect and will facilitate the process of its standardization [8, 38].

Similar results were obtained with other samples of fish species: Chum salmon *(Oncorhynchus keta),* Mackerel *(Scomber),* Flatfish *(Pleuronectidae),* Humpbacked salmon *(Oncorhynchus gorbuscha).*

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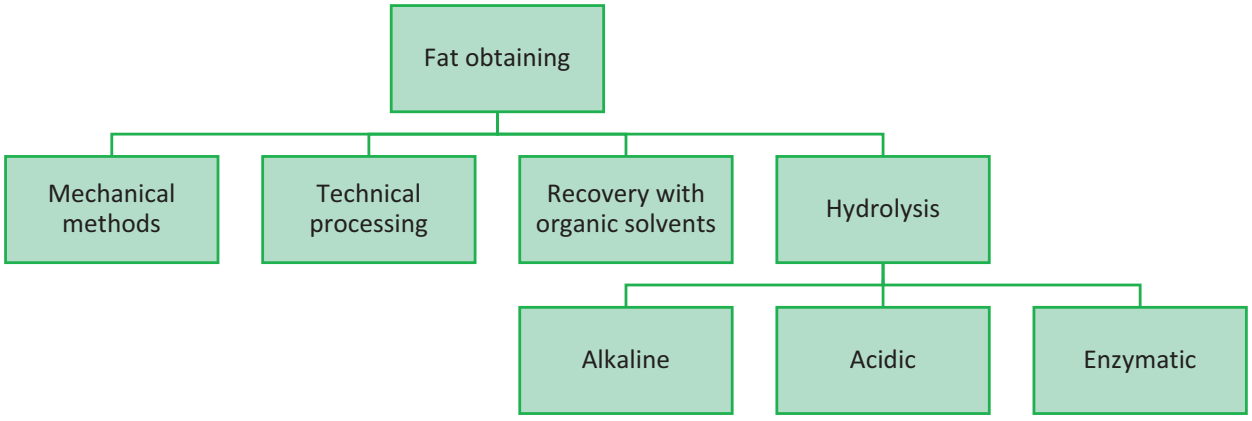


**Figure 1 – Methods of obtaining fat**

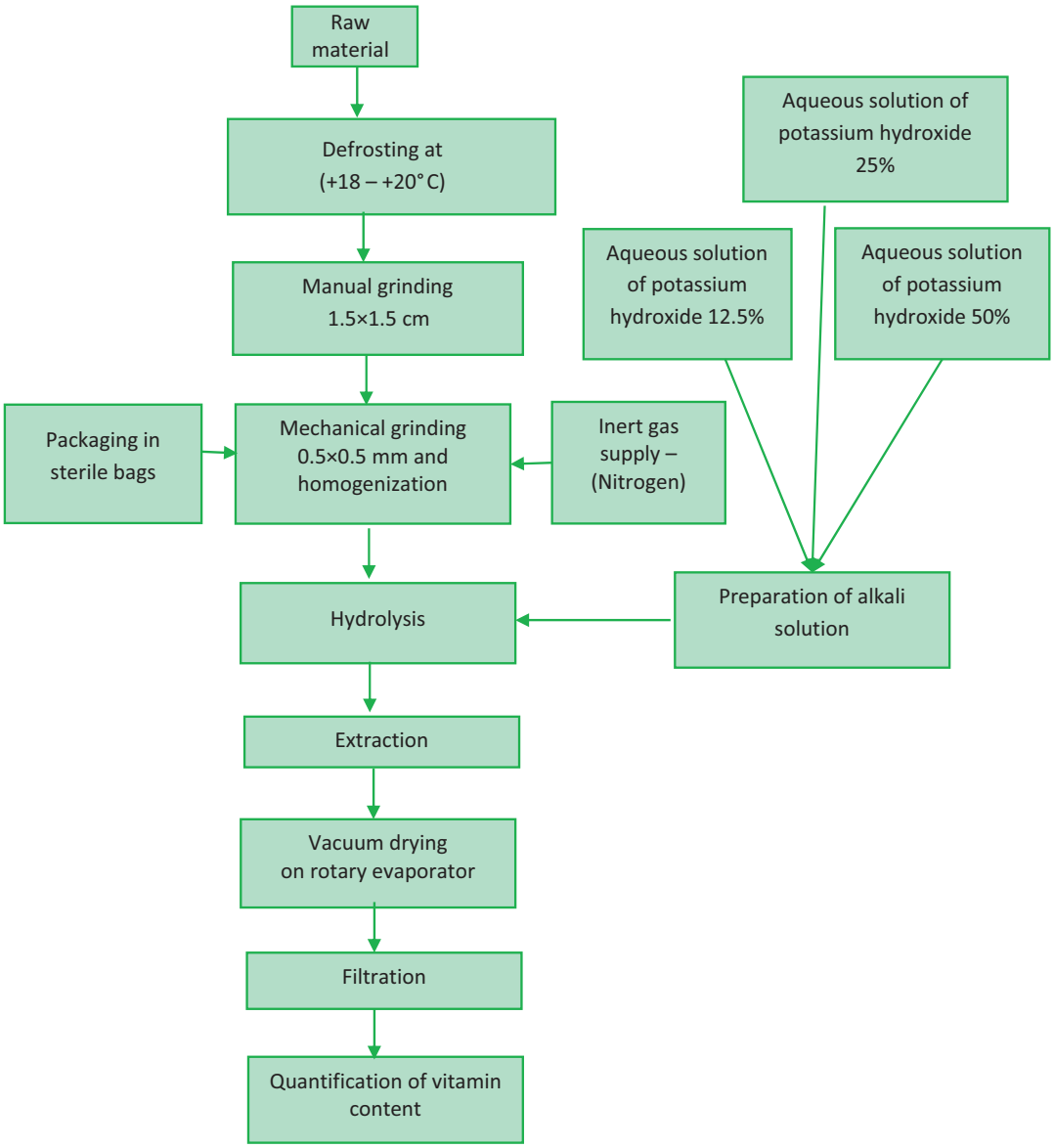


**Figure 2 – Scheme of experimental part**

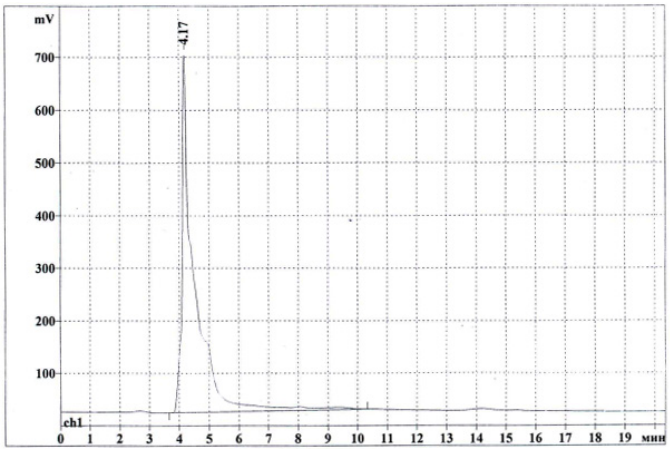


**Figure 3 – Chromatogram of direct extraction object in isopropyl alcohol when exposed to ultrasound**

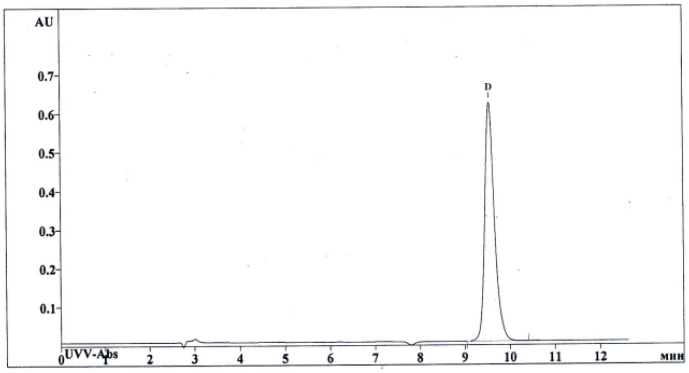


**Figure 4 – Chromatogram of vitamin D standard sample**

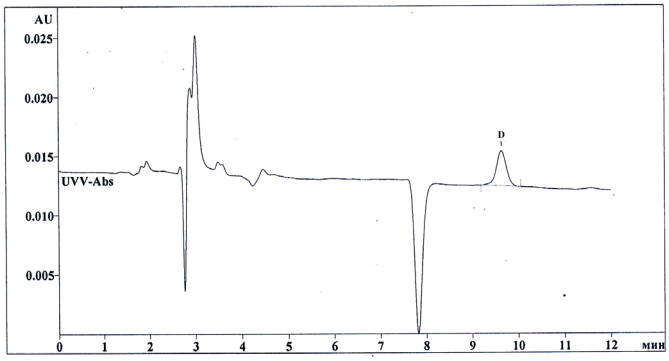


**Figure 5 – Chromatogram of vitamin D with 12.5% hydrolysis**

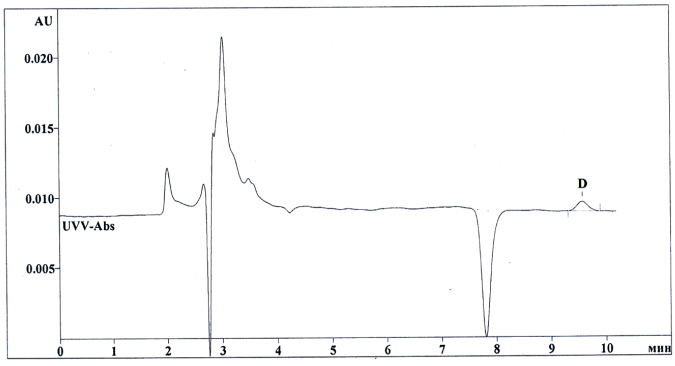


**Figure 6 – Chromatogram of vitamin D isolated by alkaline hydrolysis with 50% potassium hydroxide**

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**Table 1 – Results obtained during circulating extraction of minced brook trout *(Salmo trutta trutta)***

| S/No. | Sample weight, g | Weight of extracted fat fraction, g | Result | |
|---|---|---|---|---|
| | | | Vitamin D * | Vitamin D after alkaline hydrolysis |
| 270222 | 5.0 | 0.076 | + | + |
| 070322 | 5.1 | 0.080 | + | + |
| 140322 | 5.2 | 0.079 | + | + |
| 210322 | 5.3 | 0.081 | + | + |
| 280322 | 5.2 | 0.082 | + | + |

Note: * "+" – vitamin presence, "–" – vitamin absence.

**Table 2 – Results obtained during hydrolysis of minced brook trout *(Salmo trutta trutta)* after extraction with 12.5% potassium hydroxide**

| S/No. | Sample weight, g | Weight of extracted fat fraction, g | Result |
|---|---|---|---|
| | | | Vitamin D* |
| 051021 | 50.1 | 2.39 | + |
| 121021 | 50.4 | 2.40 | + |
| 141021 | 50.6 | 2.40 | + |
| 191021 | 50.0 | 2.39 | + |
| 261021 | 50.2 | 2.40 | + |

Note: * "+" – vitamin presence, "–" – vitamin absence.

**Table 3 – Results obtained during hydrolysis of minced brook trout *(Salmo trutta trutta)* after extraction with 25.0% potassium hydroxide**

| S/No. | Sample weight, g | Weight of extracted fat fraction, g | Result |
|---|---|---|---|
| | | | Vitamin D* |
| 051021 | 50.3 | 1.62 | + |
| 121021 | 50.1 | 1.63 | + |
| 141021 | 50.4 | 1.66 | + |
| 191021 | 50.0 | 1.61 | + |
| 261021 | 50.2 | 1.62 | + |

Note: * "+" – vitamin presence, "–" – vitamin absence.

**Table 4 – Results obtained during hydrolysis of minced brook trout *(Salmo trutta trutta)* after extraction with 50.0% potassium hydroxide**

| S/No. | Sample weight, g | Weight of extracted fat fraction, g | Result |
|---|---|---|---|
| | | | Vitamin D* |
| 051021 | 50.0 | 1.42 | + |
| 121021 | 50.1 | 1.44 | + |
| 141021 | 50.2 | 1.40 | + |
| 191021 | 50.3 | 1.41 | + |
| 261021 | 50.2 | 1.42 | + |

Note: * "+" – vitamin presence, "–" – vitamin absence.

The use of alkaline hydrolysis made it possible to state unambiguously that this method is optimal. The content of the main substance in the studied objects is in the range of 0.03–0.12%. From the experimental data obtained during the isolation of vitamin D, it follows that the optimal concentration of the hydrolyzing alkaline ingredient is 12.5% (i.e., the minimum), which makes it possible to increase the amount of the fat fraction, which in turn is a positive indicator in the implementation of the technological process.

## CONCLUSION

An integrated approach to the research on the production of vitamin D by methods of alkaline hydrolysis,

intensive maceration, circulating extraction made it possible to choose the optimal technological scheme based on hydrolysis of the protein part of the test object and using a scheme with a reduced amount of potassium hydroxide.

The reduction of technological costs and consumables in the production of fat-soluble vitamin D substance will make it possible to implement the principles of lean production and, as a result, increase the economic benefit of the enterprise producing state-owned RSs and medicinal substances, as well as reduce the financial burden on the end consumer.

## FUNDING

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHORS' CONTRIBUTION

EBS – performing a study, obtaining a substance of fat-soluble vitamin D, comparative evaluation, data analysis; EFS – collection of literature data, review editing; VDN – performing a study, obtaining a substance of fat-soluble vitamin D, comparative evaluation, data analysis.

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# POLYPHARMACY IN MANAGMENT OF IN-PATIENTS WITH NOVEL CORONAVIRUS DISEASE (COVID-19)

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**The aim.** To identify polypharmacy cases and develop the ways to optimize pharmacotherapy of patients with COVID-19 hospitalized in infectious disease facilities.
**Materials and methods.** ATC/DDD analysis with calculation of DDDs/100 bed days and a sample analysis of 500 patients' prescriptions were performed for presenting drug utilization statistics in the infectious disease facilities of Volgograd region, which had been reassigned to treat patients with COVID-19 in 2020 and 2021.
**Results.** Five or more drugs were administered simultaneously in 96.8% of patients. Antibacterial drugs were in 74.3% of the analyzed prescriptions in 2020 and in 73.5% in 2021. The total consumption of antibiotics was 102.9 DDDs/100 bed-days in 2020 and 95.7 DDDs/100 bed-days in 2021. The cases of multiple administrations of biological disease modifying antirheumatic drugs and the use of cyclophosphamide have been identified. In 73.6% of prescriptions in 2020 and 85.4% of 2021, omeprazole at the dose of 40 mg per day was used (77.3 and 84.6 DDDs/100 bed-days, respectively). In 2021, there were cases of concomitant intravenous prescribing of acetylcysteine under the trade name of Fluimucil® with tableted forms of ambroxol and acetylcysteine under the name of ACC®. The cumulative consumption of hepatotoxic drugs was 269.2 DDDs/100 bed-days in 2020 and 401.5 DDDs/100 bed-days in 2021.
**Conclusion.** Lack of drugs with proven effectiveness for treatment of COVID-19, worked-out treatment algorithms, a high mortality of patients in the hospitals led to polypragmasy, excessive prescribing of drugs in the hospitals. The prescription of antibacterial drugs, omeprazole, mucolytics, hepatotoxic drugs, immunosuppressors in infectious hospitals should be monitored by clinical pharmacologist.
**Keywords**: polypharmacy; COVID-19; ATC/DDD analysis; antibiotics; immunosuppressors; mucolytics; omeprazole; drug-induced liver injury
**Abbreviations:** INN – international nonproprietary name; GEBP – genetically engineered biological; GCS – glucocorticosteroid; NSAIDs – nonsteroidal anti-inflammatory drugs; PPIs – Proton Pump Inhibitors; ACE – angeotensin converting enzyme; ARDS – acute respiratory distress syndrome; MP(s) – medicinal preparations; RF – Russian Federation; WHO – World Health Organization; SD – standard dose; SCD – standard course dose; DDD – defined daily dose; PDD – prescribed daily dose; NDDDs – Number of Defined Daily Doses; NPDDs – Number of prescribed Daily Doses; CI – confidence interval; ICU – intensive care unit; ADR – adverse drug reactions; DILI- drug-induced liver injury.

# ПОЛИПРАГМАЗИЯ ПРИ ЛЕЧЕНИИ СТАЦИОНАРНЫХ БОЛЬНЫХ С НОВОЙ КОРОНАВИРУСНОЙ ИНФЕКЦИЕЙ COVID-19

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**Цель.** Выявить случаи полипрагмазии и разработать пути оптимизации фармакотерапии пациентов с COVID-19, госпитализированных в инфекционные отделения.
**Материалы и методы.** Для оценки объёма и структуры потребления ЛС в инфекционных отделениях Волгоградской области, перепрофилированных для лечения больных COVID-19 в 2020 и 2021 гг., был проведен ATC/DDD-анализ с расчётом показателей DDD/100 койко-дней и выборочный анализ 500 листов назначений.
**Результаты.** Одновременно 5 и более ЛС принимали 96,8% пациентов. Антибактериальные ЛС встречались в 74,3% проанализированных врачебных назначений в 2020 г. и в 73,5% в 2021 г., суммарный объём потребления составил 102,9 DDD/100 койко-дней в 2020 г. и 95,7 DDD/100 койко-дней в 2021 г. Были выявлены случаи множественных введений ГИБП и применение циклофосфамида. В 73,6% врачебных назначений 2020 г. и 85,4% в 2021 г. применялся омепразол в дозе 40 мг в сутки (77,3 и 84,6 PDD/100 койко-дней соответственно). В 2021 г. были выявлены случаи одновременного назначения ацетилцистеина под торговым наименованием «Флуимуцил®» внутривенно с таблетированными формами амброксола и ацетилцистеина под наименованием «АЦЦ®». Суммарное потребление гепатотоксичных ЛС составило 269,2 DDD/100 койко-дней в 2020 г. и 401,5DDD/100 койко-дней в 2021 г.
**Заключение.** Отсутствие препаратов с доказанной эффективностью для лечения COVID-19, отработанных алгоритмов лечения, наличие высокой летальности пациентов в стационаре привели к полипрагмазии, избыточному назначению ЛС в стационаре. Назначение антибактериальных ЛС, омепразола, муколитиков, гепатотоксичных препаратов, иммуносупрессоров в инфекционных стационарах должно быть ограничено и проводиться под контролем клинического фармаколога.
**Ключевые слова:** полипрагмазия; COVID-19; ATC/DDD анализ; антибиотики; иммуносупрессоры; муколитики; омепразол; лекарственно-индуцированное повреждение печени
**Список сокращений:** МНН – международное непатентованное название; ГИБП – генно-инженерные биологические препараты; ГКС – глюкокортикостероиды; НПВС – нестероидные противовоспалительные средства; ИПП – ингибиторы протоновой помпы; АПФ2 – ангиотензтин превращающий фермент 2; ОРДС – острый респираторный дистресс синдром; ЛС – лекарственные средства; РФ – Российская Федерация; ВОЗ – Всемирная организация здравоохранения; СД – стандартная доза; СК – стандартная курсовая доза; DDD – средняя поддерживающая суточная доза; PDD – средняя назначенная суточная доза; NDDD – число средних поддерживающих доз; NPDD – число средних назначенных суточных доз; ДИ – доверительный интервал; ПИТ – палата интенсивной терапии; НЛР – нежелательные лекарственные реакции; ЛИПП – лекарственно-индуцированное поражение печени.

## INTRODUCTION

In the context of the COVID-19 pandemic, there has been a rapid increase in the use of both new and old "off-label" drugs, the effectiveness and safety of which have not been sufficiently studied. High lethality, deficiency of drugs with proven efficacy, a desire to help patients can lead to the prescription of a great number of medicinal preparations (MPs) – polypharmacy.

The term "polypragmasia" (from the Greek "poly" – a lot, "pragmasia" – an object, thing) or, in non-russian literature, "polypharmacy" (from the Greek poly – a lot and pharmacy – medicine), first appeared in the medical literature more than 150 years ago. It was defined as "mixing together a lot of drugs in one prescription", "the use of a lot of drugs to treat one or more diseases." A consistent consensus in the concept of "polypharmacy" has not been reached [1, 2].

In 2017, Masnoon N. et al. published an analysis of 1156 English-language articles on the problem of defining "polypharmacy/polypragmasia". 138 definitions were identified, 111 of which were only quantitative, 15 took into account the duration of the simultaneous use of drugs and 12 were qualitative definitions. In quantitative definitions, "polypharmacy" was most often understood as the prescription of 5 or more drugs (45.4% of the articles included in the analysis). Some authors distinguished small (simultaneous prescription of 2–4 drugs), large (simultaneous prescription of 5–9 drugs) and excessive (simultaneous prescription of 10 or more drugs) polypharmacy. In a qualitative definition, polypharmacy has been described as "prescription of more drugs to a patient than it is required by a clinical situation"; "a simultaneous prescription of several drugs"; "a simultaneous and prolonged use of different drugs by the same person." At the same time, a number of authors distinguished between appropriate (rational) and inappropriate (unreasonable) kinds of polypharmacy [3].

Currently, in the health care of the Russian Federation (RF), active work is underway to reduce the incidence of polypharmacy in medical practice. In Order of Ministry of Public Health of the Russian Federation No.575n dated November 2, 2012 "On approval of the procedure for the provision of medical care in the profile of "Clinical pharmacology"[1], paragraph 6 states that in the case of a simultaneous prescription to a patient of 5 or more items of drugs or more than 10 items in course treatment (polypharmacy), the patient should be referred for a consultation with a doctor – a clinical pharmacologist.

A reason for a simultaneous prescription of several drugs may be the presence of concomitant diseases (multimorbidity), clinical recommendations, guidelines of professional medical societies and treatment

[1] Order of the Ministry of Health of the Russian Federation No. 575n dated November 2, 2012 "On approval of the Procedure for providing medical care in the profile "clinical pharmacology". Available from: http://www.rosminzdrav.ru/documents/5534-prikaz-minzdrava-rossiiot-2-noyabrya-2012-g-575n.

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standards, containing in some cases recommendations for the use of complex therapy for more than 5 drugs for only one indication, the effectiveness of which corresponds to high levels of evidence [4].

In this regard, when analyzing a patient's therapy, only a quantitative assessment of polypharmacy is insufficient and requires a more complete study of the validity of prescribing drugs, assessing the risk of developing drugs interactions and adverse drug reactions (ADRs).

**THE AIM.** To identify polypharmacy cases and develop the ways to optimize pharmacotherapy of patients with COVID-19 in infectious disease facilities.

## MATERIALS AND METHODS

To assess the volume and structure of drug consumption in infectious disease facilities of Volgograd region, converted for the treatment of patients with COVID-19 in 2020 and 2021, an ATC/DDD analysis was carried out with the calculation of the Defined Daily Doses per 100 bed-days (DDDs/100 bed-days) indicator and a sampling analysis of 500 case histories and prescriptions, including the prescription in intensive care units (ICUs).

The ATC/DDD methodology is recommended by the World Health Organization (WHO) as an international standard for the qualitative characterization of drug prescriptions, the provision of statistical data on drug consumption and comparative analyses at the international level within one framework [5].

It should be notified that the methodology under consideration, in contrast to the ABC and VEN analyses, does not depend on the cost of drugs; the data are easily comparable over time. However, the deficiency of DDD values for a number of drugs, including drugs of the domestic origin, causes some difficulties. The DDD (defined daily dose) is the estimated average maintenance daily dose of a drug used for its main indication in adults. The experts emphasized that this unit of measure does not always coincide with the PDD (a prescribed daily dose – the average prescribed daily dose, derived from a sample of prescriptions for the treatment of a particular disease). However, given the objectives of our study to identify cases of polypharmacy, as criteria for assessing the volume of consumption, in most cases, the DDDs published on the website of the WHO Center for Methodology of Drug Statistics were used[2].

Glucocorticosteroids (GCSs) are the first-choice drugs for the treatment of patients with cytokine storm. According to the Russian Guidelines[3], for the treatment of moderate and severe forms of COVID-19, various schemes for the administration of corticosteroids can be used using doses that are several times higher than the DDDs established by WHO. Thus, the DDD of dexamethasone was 1.5 mg, while most common (32.6% of medical prescriptions in 2020 and 74.3% in 2021), dexamethasone was used at the dose of 20 mg per day for 3 days with a subsequent dose reduction (the average daily dose when using this regimen is 14 mg per 1 patient for 12 bed-days). The maximum dose of dexamethasone in patients admitted to the ICU was 80 mg per day, and dexamethasone was also prescribed at the dose of 24 mg, 32 mg, and 48 mg per day.

Prednisolone (DDD/10 mg) and methylprednisolone (DDD/20 mg) have been rarely used at very high doses (prednisolone – 300–750 mg/day, methylprednisolone – 250–1000 mg/day) in short courses of up to 3 days. Thus, the doses of prednisolone and methylprednisolone used in real clinical practice exceeded the DDD by 12.5–75 times, and when calculating the DDDs/100 bed-days index, high values were obtained that do not reflect the actual consumption of these drugs.

According to the Russian Guidelines[4], the prescription of parenteral anticoagulants, at least in prophylactic doses, is indicated for all hospitalized patients. The WHO DDDs for heparin, enoxaparin, and nadroparin were 10 000 IUs, 2 000 anti-Xa IUs, and 2 850 anti-Xa IUs, respectively, while the median PDD prescriptions were consistent with the so-called median prophylactic doses reported in the guidelines (22 500 IU, 800 anti-Xa IUs and 11 400 anti-Xa IUs, respectively). Omeprazole was used in most cases at the dose of 40 mg per day (73.6% of the prescription lists), while the recommended dose of omeprazole for most indications[5] and the DDD indicated on the WHO website, is 20 mg per day. The dose of levofloxacin in most case histories was 1.0 g per day with a DDD of 0.5 g, and azithromycin was 0.5 g with a DDD of 0.3 g.

Due to the fact that dexamethasone, parenteral anticoagulants, omeprazole, levofloxacin and azithromycin were prescribed in the doses exceeding the DDDs recommended by WHO, PDDs were used to analyze the consumption volume of these drugs.

The number of the established or prescribed daily doses – NDDDs (the number of DDDs) or NPDDs (the number of PDDs) of drugs – was calculated as the ratio of the number of MPs to DDDs or PDDs. The DDDs/100 or PDDs/100 indicator was determined in relation to the consumed NDDDs or NPDDs per year, multiplied by 100, to the total bed-day for the year.

For domestic drugs olokizumab and levilimab that do not have DDDs on the WHO website, or for other

[2] WHO Collaborating Centre for Drug Statistics Methodology. Available from: http://www.whocc.no/atc_ddd_index/.

[3] Prevention, diagnosis and treatment of novel coronavirus infection (COVID-19). Interim guidelines. Version 15 (22.02.2022). Ministry of Health of the Russian Federation.; 2022.

[4] Ibid.

[5] Russian State Register of Medicines. Available from: https://grls.rosminzdrav.ru/Default.aspx.

genetically engineered biological preparations (GEBPs) either, to assess the volume of consumption, a standard dose per 100 treated patients was calculated.

A standard dose (SD) is the dose recommended for the administration in mild and moderate COVID-19. To assess the volume of the drugs consumption used in the facilities in the pulse therapy mode (the administration of high doses for 3 days), a standard course dose (SCD – a median of prescribed course doses) according to the prescription lists and the ratio of the number of SCs per 100 treated patients, was calculated.

## RESULTS AND DISCUSSION

A sampling analysis of hospitalized patients with COVID-19 case histories revealed 11 [8; 13] drugs prescriptions per patient during the period of hospitalization while taking 8 [6; 11] MPs. At the same time, 5 or more drugs were taken by 96.8% of patients.

According to the accounting and reporting documentation for the issuance of drugs to facilities for the treatment of 3,750 patients (45 315 bed-days) in 2020 and 5 130 patients (58 439 bed-days) in 2021, 117 international non-proprietary names (INNs) drugs were used in 2020 and 129 INNs drugs in 2021, a third of which did not have DDDs on the WHO website. According to the WHO Center of International Drug Statistics, it was not possible to establish an average daily maintenance dose (DDD) for topical preparations, including intranasal forms of interferon, solutions of crystalloids, colloids, parenteral nutrition, local and general anesthetics, parenteral forms of nitroglycerin and acetylcysteine. Domestic drugs olokizumab and levilimab, hepatoprotectors and metabolic drugs used in the studied facilities were not in the database of the WHO center.

The calculation of DDDs or PDDs/100 bed-days was made for 79 drugs in 2020 and 85 drugs in 2021, among which there were antibacterial drugs; agents affecting hemostasis; GCSs and other immunosuppressants; bronchodilators; mucolytics; surfactant preparations; non-steroidal anti-inflammatory drugs (NSAIDs); antihistamines; the drugs that affect the gastrointestinal tract; insulins; cardiovascular agents; iron preparations and centrally acting preparations (Fig. 1).

A further detailed analysis was made of the drugs that are not the basis of COVID-19 therapy or the drugs that are mandatory for prescribing in all cases of the disease, the volume of consumption of which, however, was high in the studied facilities.

### Antibacterial drugs

Like any other disease of viral etiology, COVID-19 is not an indication for the use of antibacterial drugs. Bacterial infections are not often complications of a novel coronavirus infection course [6, 7], so most patients with COVID-19, especially in mild and moderate cases, do not need antibiotic therapy[6]. However, antibiotics were present in 74.3% of prescriptions in 2020 and 73.5% in 2021, and in combined antibiotic therapy in 35.6%. In 2020, in facilities, ceftriaxone as monotherapy and in combination with azithromycin or levofloxacin was most often prescribed as initial therapy, and in 2021 these were levofloxacin or ceftriaxone. In all analyzed lists of intensive care units (ICUs) prescriptions, antibacterial drugs were prescribed, and the most common of them were cefoperazone-sulbactam or meropenem as monotherapy or in combination with vancomycin or linezolid.

The combination of broad-spectrum antibacterial drugs, especially *β*-lactam antibiotics with macrolides and fluoroquinolones, has long been considered irrational, including due to the weakening of an bactericidal antibiotic action while taking it with a bacteriostatic drug[7]. However, even before the COVID-19 pandemic, the growth of antibiotic-resistant strains and changes in the structure of causative agents of community-acquired pneumonia had made adjustments to clinical guidelines. Thus, in 2018, the prescription of ceftriaxone in combination with azithromycin or levofloxacin was recommended to hospitalized patients with severe community-acquired pneumonia of bacterial etiology[8].

The doses of antibacterial drugs used in facilities, in most cases coincided with the DDDs recommended by WHO (with the exception of azithromycin and levofloxacin). However, the total consumption of antibacterial drugs exceeded 100 DDDs/100 bed-days, which was the result of the combination antibiotic therapy.

An earlier ABC analysis of drug consumption in facilities [8] revealed a decrease in the share of the expenditure on antibiotics in 2021 compared to 2020. Thus, the purchase of antibacterial drugs in 2020 accounted for 52% of Segment A costs, and in 2021, it was 13.6%. However, their total consumption in terms of DDDs/100 bed-days in 2020 was only 7% higher than in 2021 (102.9 DDDs/100 bed-days in 2020 and 95.7 DDDs/100 bed-days in 2021). A decrease in the relative share of the expenditure on antibacterial drugs in 2021, based on the ABC analysis, is associa-

[6] Prevention, diagnosis and treatment of novel coronavirus infection (COVID-19). Interim guidelines. Version 15 (22.02.2022). Ministry of Health of the Russian Federation.; 2022.

[7] Strachunsky LS, Belousova YuB, Kozlov SN. Obshchie osobennosti antiinfekcionnyh himiopreparatov [General features of anti-infective chemotherapy drugs]. A practical guide to anti-infective chemotherapy. RC "Pharmedinfo", 2007. – 427 p. Russian

[8] Clinical guidelines. Community-acquired pneumonia. Russian Respiratory Society Interregional Association for Clinical Microbiology and Antimicrobial Chemotherapy. – 2018. – 97 p. Available from: https://minzdrav.midural.ru/uploads/clin_recomend%20РФ.pdf

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ted to a greater extent with an increase in the expenditure on biological disease modifying antirheumatic drugs (GEBPs) and anticoagulants. It is also caused by a restriction in the purchase of expensive antibiotics in favor of the most affordable ones, rather than a decrease in the frequency of prescribing antibacterial drugs. Thus, it was the calculation of the DDDs/100 bed-days indicator, and not the ABC analysis, which made it possible to evaluate and compare the annual consumption of antibacterial drugs, regardless of the dosing regimen, costs, the number of patients treated, and hospital stays (Fig. 2).

The prescription of antibacterial drugs for the COVID-19 treatment is a problem not only for the healthcare of the Russian Federation, but also for the whole world. Langford B.J. et al. [9] analyzed the data from 154 studies on COVID-19 antibiotic therapy (30 623 patients). The frequency of antibiotic therapy prescription was 74.6% (95% CI 68.3–80.0%), which is consistent with the authors' data. However, only 8.6% (95% CI in 4.7–15.2%) of patients in 31 studies had a laboratory-confirmed bacterial co-infection. Thus, more than half of the prescriptions of antibacterial drugs can be characterized as the prescription of more drugs than the clinical situation requires.

One of the most likely reasons for the excessive prescription of antibacterial drugs at the beginning of the COVID-19 pandemic, in the authors' opinion, may be the adherence of practitioners to previously recommended empirical therapy regimens for community-acquired pneumonia of bacterial etiology. It should be notified that in 2021, against the background of the widespread use of immunosuppressants for the treatment of cytokine storm, the risk of infectious complications, the blurring of the clinical picture of a bacterial infection and changes in the general blood test while taking GCSs, a high lethality of patients in hospitals, could serve as a reason for prescribing antibacterial drugs to patients with severe COVID-19 without a proven bacterial infection.

### Glucocorticosteroids, GEBPs, janus kinase inhibitors and cytostatics

The discovery of a hyperimmune response role, or cytokine storm as the basis for the pathogenesis of an acute respiratory distress syndrome and a multiorgan dysfunction in COVID-19 prompted the widely used in rheumatology anti-inflammatory drugs.

Dexamethasone was notified in 37.5% of physicians' prescriptions to patients with COVID-19 in 2020 and 91.3% in 2021. Fewer than 5% of prescriptions included prednisolone, methylprednisolone, baricitinib, and cyclophosphamide. GEBPs were prescribed in 3.9% of cases in 2020 (tocilizumab, olokizumab, levilimab) and in 11.6% in 2021 (tocilizumab, olokizumab, levilimab, sarilumab and secukinumab).

Prednisolone, secukinumab, and cyclophosphamide were not included in the standard COVID-19 treatment regimens recommended by the Ministry of Health of the Russian Federation and in force at the time, these drugs were prescribed[9].

According to the recommendations, janus kinase inhibitors (baricitinib) and GEBPs are indicated to patients with COVID-19 in combination with corticosteroids and were used in the studied facilities together with dexamethasone. In most prescription lists, GEBPs were administered to patients with COVID-19 in a single dose, however, in case of insufficient effectiveness, according to the recommendations, it was possible to re-administer the drug after 12–24 hours.

Despite a low frequency of GEBPs use (Table 1), the cases of multiple injections, as well as the introduction of cyclophosphamide in patients hospitalized in the ICUs after GEBPs injections have been identified. In the case history of one patient, 10 injections of 3 GEBPs were recorded. This fact is considered as polypharmacy.

The active tactics of using immunosuppressive drugs to treat patients with COVID-19 is triggered by the paradigm of "cytokine storm", a condition in which an overly strong immune response, mediated by overproduced pro-inflammatory cytokines, causes extensive lungs damage and a state of thrombosis propensity. Accordingly, it is assumed that death occurs primarily due to the inflammatory lungs disease, impaired micro- and macrocirculation and an eventually respiratory failure or vascular coagulopathy [10].

In this regard, in real clinical practice, in patients with a progressive respiratory failure in the absence of the standard therapy effect, multiple administrations of GEBPs, pulse therapy with corticosteroids, and cyclophosphamide were used as a kind of "despair" therapy.

However, there are also conflicting views, associated with a violation of the immunological defense mechanism, leading to an uncontrolled virus spread and organs damage [11]. Despite extensive research around the world, the pathophysiological processes that play a critical role in morbidity and mortality among patients, remain unknown and require further clinical studies, including retrospective ones, to assess the benefit/risk of immunosuppression in patients with COVID-19.

[9] Prevention, diagnosis and treatment of novel coronavirus infection (COVID-19). Interim guidelines. Version 14 (27.12.2021). Ministry of Health of the Russian Federation; 2021.

### Omeprazole and antisecretory therapy

According to Rambhade S. et al. [12], one of the common causes of polypharmacy is a cascade of prescriptions (prescribing cascade), when drugs are prescribed for the treatment or prevention of adverse drug reactions (ADRs) caused by other drugs. Omeprazole 40 mg daily was used in 73.6% of prescriptions in 2020 and 85.4% in 2021. A high frequency of prescribing antisecretory therapy for patients with COVID-19 against the background of the widespread use of corticosteroids, anticoagulants, NSAIDs and a high risk of gastrointestinal bleeding was most likely carried out for prophylactic rather than therapeutic purposes.

Even before the COVID-19 pandemic, retrospective cohort studies had identified an increased risk of developing community-acquired and nosocomial kinds of pneumonia with the use of proton pump inhibitors (PPIs) [13–15]. Currently, the PPIs intake is considered by many authors as an independent risk factor for more severe outcomes in patients with COVID-19 [16–22].

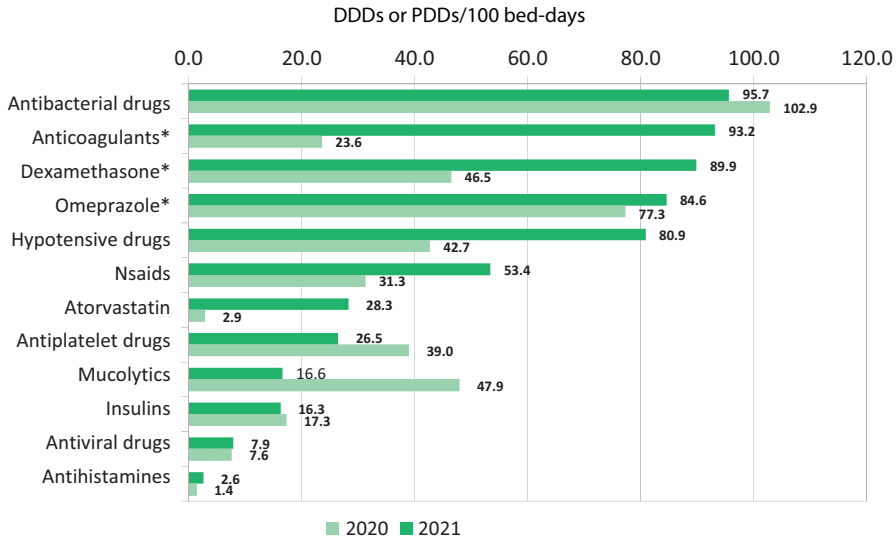


**Figure 1 – Structure and volume of consumption of the most commonly used drugs and groups of drugs in patients with COVID-19**

Note: * – PDDs were used for calculation

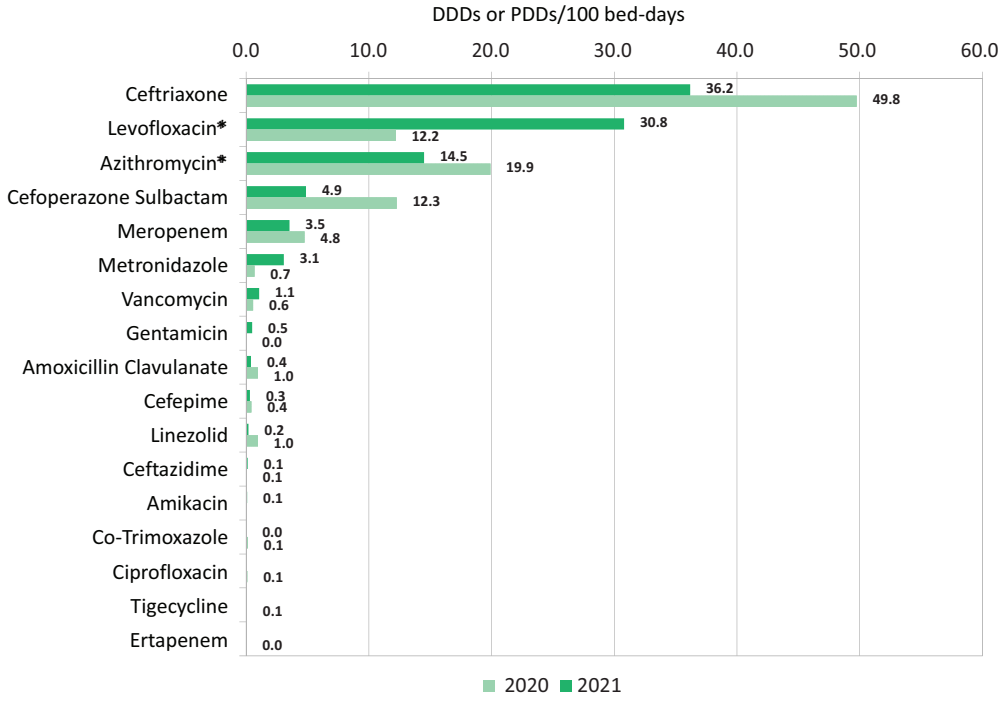


**Figure 2 – Structure and volume of antibacterial drugs consumption in patients with COVID-19**

Note: * – PDDs were used for calculation.

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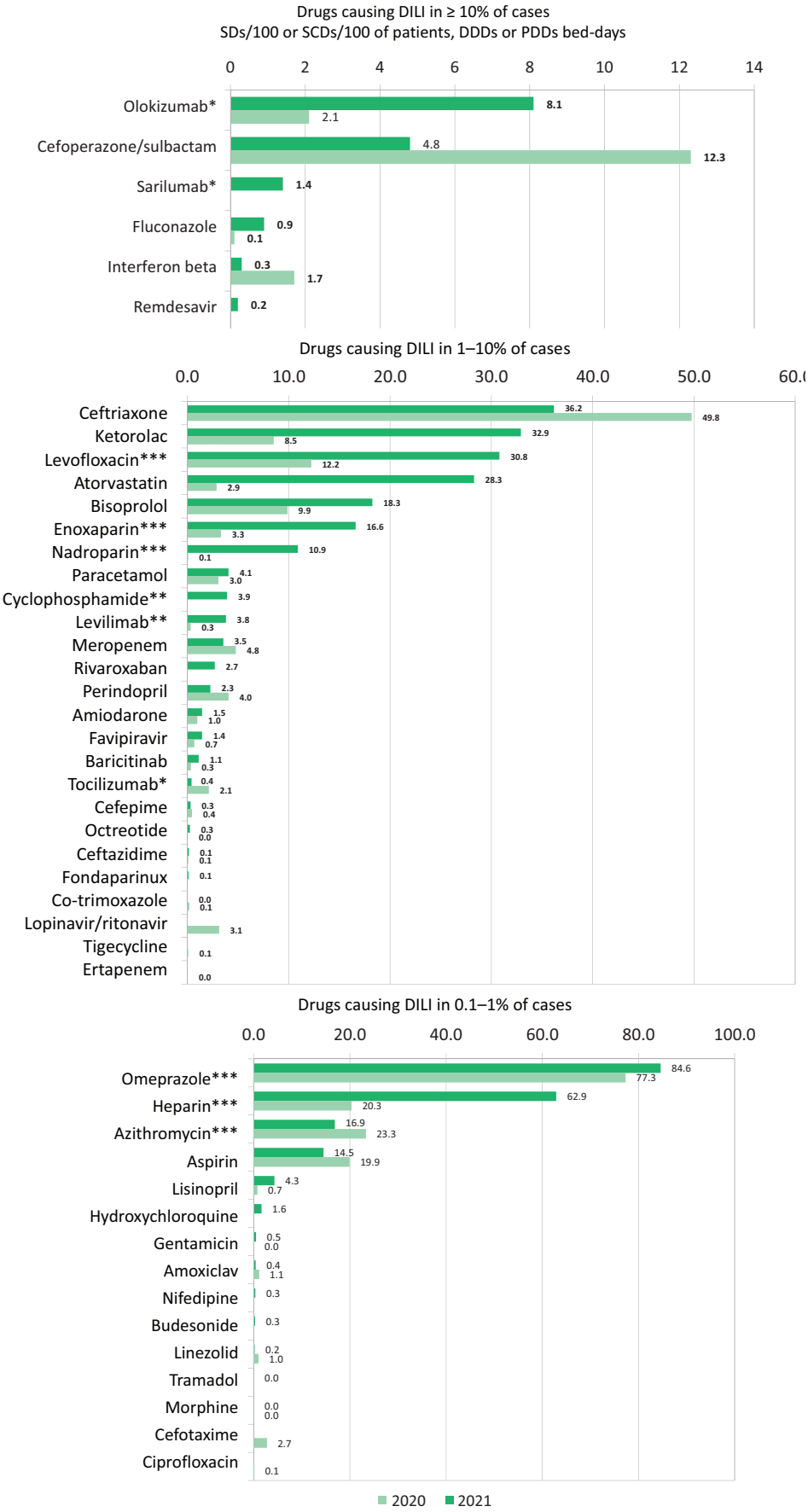


**Figure 3 – Structure and volume of hepatotoxic drugs consumption in patients with COVID-19**
Note: * – SDs were used for calculation; ** – SCDs were used for calculation; *** – PDDs were used for calculation

**Table 1 – Structure and volume of immunosuppressants consumption by patients with COVID-19**

| INN | Doses used for calculation | Calculated indicator | Volume of consumption | |
|---|---|---|---|---|
| | | | 2020<br>3 750 patients<br>(45 315 bed-days) | 2021<br>5 130 patients<br>(58 439 bed-days) |
| Dexamethasone | 14 mg | PDD/100 bed-days | 46.5 | 89.9 |
| Baricitinib | 4 mg | DDD/100 bed-days<br>(PDD coincides with DDD) | 0.31 | 1.1 |
| Tocilizumab | 320 mg | SD /100 patients | 2.1 | 0.44 |
| | **20 mg** | **DDD for RA treatment /100 bed-days** | 2.8 | **0.62** |
| Olokizumab | 64 mg | SD /100 patients | 2.1 | 8.1 |
| Levilimab | 324 mg | SD /100 patients | 0.3 | 3.8 |
| Sarilumab | 200 mg | SD /100 patients | | 1.4 |
| | **14.3 mg** | **DDD for RA treatment /100 bed-days** | | **1.7** |
| Secukinumab | 300 mg | SD /100 patients | | 0.1 |
| | **10 mg** | **DDD for RA treatment /100 bed-days** | | **0.26** |
| Prednisolone | 1800 mg | CK/100 patients | 4.4 | 13.3 |
| Methylprednisolone | 2250 mg | CK/100 patients | 2.6 | 2.5 |
| Cyclophosphamide | 800 mg | CK/100 patients | | 3.9 |

Note: PDD – prescribed daily dose; DDD – defined daily dose; SD – standard dose per 1 injection for mild and moderate COVID-19; SCD — standard course dose per 1 patient; RA – rheumatoid arthritis.

$H_2$-histamine blockers may be an alternative to omeprazole and other PPIs if antisecretory therapy is required. In small clinical trials early in the pandemic, a famotidine use was associated with improved COVID-19, reduced risks of intubation and death [23–26]. Two in silico studies suggested a direct antiviral effect of famotidine and proposed one of the two SARS-CoV-2 proteases as potential molecular targets [27, 28], but a subsequent in vitro study did not confirm this effect [29], and a meta-analysis did not reveal a significant reduction in mortality from COVID-19 against the background of famotidine use [30]. Despite the fact that famotidine was not purchased in infectious disease facilities under study in 2020 and 2021, the analysis of history cases revealed the experience of prescribing this drug to the patients who had started taking it before the admission to hospital, and a high frequency of recommendations for taking famotidine when the patient was discharged from hospital.

### Mucolytics

The Russian Guidelines on the COVID-19 case management in the "symptomatic treatment" section only indicate the possibility of prescribing mucolytics to patients with bronchitis against the background of COVID-19, without listing drugs and algorithms.

In September 2020, Olaleye O.A. et al. described the possibility of using ambroxol, an active metabolite of bromhexedine, as a blocker of the interaction between the spike protein of the SARS-CoV-2 virus and the ACE2 receptor and, thus, disrupt the penetration of the virus into the cell [31], which was subsequently confirmed in in vitro study [32]. The concentrations of ambroxol significantly exceeded the concentrations of drugs in the blood plasma of patients when it was used in standard doses, which does not allow transferring these data to the clinic. The anti-inflammatory and antioxidant activities inherent in the mucolytic acetylcysteine, also made it a good candidate for preventing the development of a cytokine storm in COVID-19 patients [33]. However, the results of a large cohort study published in early 2022, did not prove the ability of acetylcysteine to reduce the risk of developing ARDS and a severe COVID-19 course [34].

In 2020, more than a half of medical prescriptions contained ambroxol, and in 2021, acetylcysteine was added to ambroxol and used in combination with it. Moreover, cases of a simultaneous administration of acetylcysteine under the trade name Fluimucil® (intravenously) with tablet forms of ambroxol and acetylcysteine under the trade name of ACC® have been identified. Currently, there are no data in the literature on the advisability of prescribing combination therapy with mucolytics to bronchitis patients. Moreover, a number of authors believe that this may lead to an excessive sputum production [35]. A simultaneous use of parenteral and oral forms of drugs containing one active substance is also considered irrational [36].

### Hepatotoxic drugs

In the published studies, an impaired liver function has been reported in 37.2–76.3% of hospitalized patients with COVID-19. This is a result of many factors, such as a drug-induced liver injury (DILI), an acute inflammatory response, and hypoxia associated with a severe respiratory distress and ARDS, as well as a possible coronavirus liver replication [37–40].

A simultaneous intake of several drugs with a potential hepatotoxicity more often causes a liver damage as a result of a pharmacodynamic interaction. In internation-

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al[10] and Russian databases[11,12] a search was conducted on the incidence of DILI for all the drugs used in infectious in-patient hospitals in 2020 and 2021.

Among the drugs used in 2020, only 4 (cefoperazone/sulbactam, interferon beta, fluconazole, and olokizumab) are the drugs that cause DILI in 10% or more of patients. In 2021, the drugs with a severe hepatotoxicity (DILI ≥ 10%) were also joined by remdesevir and sarilumab. The volume of drugs consumption with a severe hepatotoxicity was relatively small. However, the total consumption of drugs that cause DILI in 1–10% of patients (23 drugs in 2020 and 22 drugs in 2021) and 0.1–10% of patients (12 drugs in 2020, 13 drugs in 2021) were several times higher than 100 DDDs/100 bed-days. That was the result of the combined prescription of 2 or more drugs that can cause a liver dysfunction (Fig. 3). The most commonly prescribed groups of drugs with hepatotoxic effects were PPIs, antibacterials, and anticoagulants. The volume of hepatotoxic antiviral drugs consumption was 3.8 DDDs/100 bed-days (lopinavir/ritonavir and favipiravir) in 2020 and 1.8 DDDs/100 bed-days (favipiravir and remdesavir) in 2021.

## CONCLUSION

In 2020, the healthcare system of the Russian Federation and the world faced a pandemic of a new rapidly spreading infection. The deficiency of drugs with proven efficacy, proven treatment algorithms, high mortality of patients in the hospital forced practitioners to use all available means to save patients' lives, which inevitably led to polypharmacy. It should be remembered that the prescription of a great number of drugs can lead not so much to the desired increase in the effectiveness of therapy, as to the development of ADRs and an increase in the economic burden. The prescription of antibacterial drugs, omeprazole, mucolytics, hepatotoxic drugs, immunosuppressants in the infectious disease facilities of hospitals should be limited and carried out under the supervision of a clinical pharmacologist. The Russian Guidelines on the COVID-19 case management in the "symptomatic treatment" section.

The latest versions of the Russian Guidelines of the Ministry of Health of the Russian Federation on the COVID-19 case management contain clear criteria for prescribing antibiotic therapy, compliance with which will reduce the unreasonable prescription of this group of drugs. If antisecretory therapy is needed, considering famotidine, a drug that does not adversely affect the course of COVID-19, unlike omeprazole, and causes DILI with a lower incidence (<0.1%), is recommended. The routine use of mucolytics in patients with COVID-19, especially combined ones, should be used only in case of sputum that is difficult to separate. The prescription of combined immunosuppressive therapy with the use of multiple GEBPs injections, pulse therapy with corticosteroids, cyclophosphamide is currently unreasonable, and further retrospective clinical studies are required.

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## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

## AUTHORS' CONTRIBUTION

VIP – development of research design, editing and final approval of the article;
AYuR – data processing, article writing and final approval of the article; NSP – collection of material, data processing and final approval of the article; DAN – collection of material and final approval of the article.

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# CLINICAL AND ECONOMIC EVALUATION OF ATEZOLIZUMAB + VEMURAFENIB + COBIMETINIB COMBINATION AND NIVOLUMAB + IPILIMUMAB COMBINATION: ADMINISTRATION IN METASTATIC MELANOMA TREATMENT WITH BRAF-CONFIRMED MUTATION IN ADULT PATIENTS

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**The aim** of the study was to conduct a pharmacoeconomic evaluation of the atezolizumab, vemurofenib and cobimetinib (ATZ+VM+COB) combination and the nivolumab and ipilimumab (NIVO+IPI) combination for the treatment of BRAF-confirmed metastatic melanoma in adult patients.

**Materials and methods.** With the help of mathematical modeling methods, a pharmacoeconomic "cost-effectiveness" analysis; a "budget impact" analysis; a sensitivity analysis to the changes in the initial parameters of the model, were carried out.

**Results.** The analysis of literature sources made it possible to conclude that the combination of ATZ+VM+COB compared with the combination of NIVO+IPI (15.1 and 11.2 months, respectively) has a greater clinical efficacy in terms of a progression-free survival (PFS) in patients with metastatic melanoma. When choosing the ATZ+VM+COB combination, the total cost of treatment for one adult patient with metastatic melanoma per course was lower, compared to the NIVO+IPI combination (RUB 8 326 864.89 vs RUB 7 172 751.68); the difference amounted to 1 154 113.21 rubles. When calculating the "cost-effectiveness" ratio for a year of a progression-free survival, the advantage of the ATZ + VM + COB combination in comparison with the NIVO + IPI combination, remained (5 700 200.01 rubles vs 8 942 400.10 rubles); the difference amounted to 3 242 200.09 rubles. The sensitivity analysis demonstrated the developed model stability to an increase in the cost of the ATZ + VM + COB course up to + 16%, a decrease in the cost of the NIVO + IPI course to –13%, and a reduction in the PFS to –37% against the background of the ATZ + VM + COB course. The "budget impact" analysis showed the possibility of reducing costs by 8 655 849.11 rubles with an increase from 5% to 20% in the proportion of the patients administrated with the ATZ+VM+COB combination, and with a decrease from 95% to 80% in the proportion of the patients administrated with the NIVO+IPI combination.

**Conclusion.** The results of the work have shown that within the healthcare system of the Russian Federation, the triple combination of ATZ+VM+COB is a clinically cost-effective option for the treatment of adult metastatic melanoma patients with a confirmed BRAF mutation.

**Keywords:** metastatic melanoma; BRAF mutations; melanoma treatment; "cost-effectiveness" analysis; "budget impact" analysis; atezolizumab; vemurafenib; cobimetinib

**Abbreviations:** ATZ – atezolizumab; COB – cobimetinib; IPI – ipilimumab; NIVO – nivolumab; VM – vemurafenib; PFS – progression-free survival; OS – overall survival; RR – relative risk; RCT – randomized clinical trial; AE – adverse event; CEA – "cost-effectiveness" analysis; BIA – "budget impact" analysis; OS – overall survival.

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# КЛИНИКО-ЭКОНОМИЧЕСКАЯ ОЦЕНКА ПРИМЕНЕНИЯ КОМБИНАЦИИ АТЕЗОЛИЗУМАБ, ВЕМУРАФЕНИБ И КОБИМЕТИНИБ С КОМБИНАЦИЕЙ НИВОЛУМАБ И ИПИЛИМУМАБ В ТЕРАПИИ МЕТАСТАТИЧЕСКОЙ МЕЛАНОМЫ С ПОДТВЕРЖДЕННОЙ BRAF-МУТАЦИЕЙ У ВЗРОСЛЫХ ПАЦИЕНТОВ

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**Цель.** Провести фармакоэкономическую оценку применения комбинации препаратов атезолизумаб, вемурофениб и кобиметиниб (ATZ+VM+COB) с препаратами ниволумаб и ипилимумаб (NIVO+IPI) для терапии метастатической меланомы с подтвержденной BRAF-мутацией у взрослых пациентов.

**Материалы и методы.** С помощью методов математического моделирования был проведен фармакоэкономический анализ «затраты-эффективность»; анализ «влияния на бюджет»; анализ чувствительности к изменениям исходных параметров модели.

**Результаты.** Проведенный анализ литературных источников позволил сделать вывод о большей клинической эффективности в отношении выживаемости без прогрессирования (ВБП) у пациентов с метастатической меланомой для комбинации ATZ+VM+COB по сравнению с комбинацией NIVO+IPI (15,1 и 11,2 мес. соответственно). Общие затраты на курс лечения одного взрослого пациента с метастатической меланомой при выборе комбинации ATZ+VM+COB были ниже в сравнении с комбинацией NIVO+IPI (8 326 864,89 руб. против 7 172 751,68 руб.); разница составила 1 154 113,21 руб. При расчете коэффициента «затраты-эффективность» на год жизни без прогрессирования сохранялось преимущество комбинации ATZ+VM+COB в сравнении с комбинацией NIVO+IPI (5 700 200,01 руб. против 8 942 400,10 руб.); разница составила 3 242 200,09 руб. Анализ чувствительности продемонстрировал устойчивость разработанной модели к увеличению стоимости курса ATZ+VM+COB до +16%, снижению стоимости курса NIVO+IPI до –13%, сокращению ВБП на фоне курса ATZ+VM+COB до –37%. Анализ «влияние на бюджет» показал возможность снижения затрат на 8 655 849,11 руб. при увеличении доли пациентов, получающих комбинацию ATZ+VM+COB, с 5 до 20%, и при снижении доли пациентов, получающих комбинацию NIVO+IPI, с 95 до 80%.

**Заключение.** Результаты проведенной нами работы показали, что тройная комбинация ATZ+VM+COB является клинически эффективным и экономически предпочтительным вариантом терапии пациентов с метастатической меланомой с подтвержденной BRAF-мутацией у взрослых пациентов в рамках системы здравоохранения Российской Федерации.

**Ключевые слова:** метастатическая меланома; BRAF-мутации; лечение меланомы; анализ «затраты-эффективность»; анализ «влияния на бюджет»; атезолизумаб; вемурафениб; кобиметиниб

**Список сокращений:** ATZ – атезолизумаб; COB – Кобиметиниб; IPI – ипилимумаб; NIVO – ниволумаб; VM – вемурафениб; ВБП – выживаемость без прогрессирования; ОВ – общая выживаемость; ОР – относительный риск; РКИ – рандомизированное клиническое исследование; НЯ – нежелательные явления; CEA – анализ «затраты-эффективность»; BIA – анализа «влияния на бюджет»; ОВ – общая выживаемость.

## INTRODUCTION

Melanoma is a malignant tumor originating from melanocytes, pigment skin cells that produce melanin [1]. The treatment of melanoma requires an interdisciplinary approach that includes surgery, radiation therapy, chemotherapy, immunotherapy, and targeted therapy[1] [2]. There is a distinct increase in the incidence of melanoma. According to the data of Herzen Moscow Scientific and Research Oncological Institute, since 2010, the prevalence rate has increased from 46.6:100 000 to 69.1:100 000 of the population (at the beginning of 2021)[2].

Melanoma is one of the most aggressive oncological diseases, which quickly and often metastasizes. The rate of neglect at the time of the disease detection in skin melanoma is quite high and amounts to 19.6% (i.e. the detection in late III-IV stages). The mortality within a year after this disease registration is 8.2% [3, 4].

In some cases, only distant metastases can be detected, and the primary lesion on the skin (or in other organs) cannot be detected (for example, due to the spontaneous regression of the primary tumor or removal of the lesion during medical or cosmetic manipulations without morphological examinations) [5, 6].

While surgery and adjuvant cytokine therapy are the mainstay of the treatment for resectable melanoma, the treatment for unresectable or metastatic melanoma is based on the use of the drug therapy [7–10]. The agents available for the unresectable/metastatic malignant melanoma treatment, can be divided into three classes: immunotherapy (e.g., nivolumab, pembrolizumab, ipilimumab, cytokines); BRAF/MEK inhibitors (e.g., vemurafenib, dabrafenib, cobimetinib, trametinib); chemotherapy (temozolomide) [2, 11, 12].

The development of immunotherapeutic agents over the past decade has dramatically changed the prognosis for melanoma patients, significantly increasing survival and improving their quality of life [13]. However, BRAF/MEK inhibitors, like immunotherapy, are expensive kinds of treatment that carry a large financial burden on the healthcare budget, so it is important to conduct a pharmacoeconomic evaluation of the therapy for melanoma patients with a confirmed BRAF mutation in the Russian Federation [3].

At the moment, the combination of immunodrugs nivolumab and ipilimumab NIVO+IPI has shown its high efficacy in the treatment of melanoma [1, 2]. However, a randomized clinical trial (RCT) showed that this therapy regimen had to be canceled in 42% due to the development of drug-related adverse events (AEs) [14].

The search for new therapeutic options led to the development of the atezolizumab, vemurofenib, and cobimetinib (ATZ+VM+COB) combination, the only triple treatment regimen included in international and Russian clinical guidelines for the treatment of melanoma [1, 2] and available on the Russian market since 2020. The triple combination has proven its high effectiveness in RCTs, as well as a better tolerability [15].

**THE AIM** of the study was to conduct a pharmacoeconomic evaluation of the atezolizumab, vemurofenib and cobimetinib (ATZ+VM+COB) combination and the nivolumab and ipilimumab (NIVO+IPI) combination for the treatment of BRAF-confirmed metastatic melanoma in adult patients.

## MATERIALS AND METHODS

The design of the study consisted of a pharmacoeconomic "cost-effectiveness" analysis (CEA), a "budget impact" analysis (BIA), a sensitivity analysis of the changes in the initial parameters of the model. A hypothesis that the triple combination of ATZ+VM+COB is clinically and cost-effective for the treatment of metastatic melanoma with a confirmed BRAF mutation in adult patients within the healthcare system of the Russian Federation was formulated.

The ATZ+VM+COB combination under study includes: 1) Atezolizumab – Tecentriq® (F.Hoffmann-La Roche, Ltd, Switzerland) concentrate for the infusion solution, 60 mg/ml in 1200 mg/20 ml vials and 840 mg/14 ml vials; 2) Vemurafenib – Zelboraf® (F. Hoffmann-La Roche, Ltd, Switzerland) film-coated tablets, 240 mg; 3) Cobimetinib – Cotellic® (F. Hoffmann-La Roche, Ltd, Switzerland) film-coated tablets, 20 mg.

The NIVO+IPI comparison combination includes 1) Ipilimumab – Yervoy® (Bristol-Myers Squibb, USA) concentrate for the infusion solutions, 5 mg/ml, in 10.7 ml vials; 2) Nivolumab – Opdivo® (Bristol-Myers Squibb, USA) concentrate for the infusion solutions, 10 mg/ml, in 4 ml and 10 ml vials.

### Description of research methodology

At the preliminary stage of the investigation, an information search for studies on the efficacy and safety of ATZ + VM + COB and NIVO + IPI combinations in adults with metastatic melanoma according to the PICOS and PRISMA criteria in the Cochrane, Pubmed and eLIBRARY databases, was carried out [16, 17].

The analysis included 7 publications: five – on the clinical efficacy and safety of the NIVO+IPI combination, two – on the ATZ+VM+COB combination. The following works were included in the analysis: 1) RCT IMspire150, Gutzmer R. et al., 2020 [15]; 2) RCT CheckMate 067, Wolchok J.D. et al., 2017 [18]; 3) RCT CheckMate 067, Hodi F.S. et al., 2018 [19]; 4) RCT CheckMate 067, Larkin J. et al., 2019 [14]; 5) RCT CheckMate 069, Hodi F.S. et al., 2016 [20]; 6) RCT CheckMate 511, Lebbe C. et al., 2019 [21]; 7) Network meta-analysis by Lee J. et al., 2022 [22].

[1] The Association of Russian Oncologists (AOR), Russian melanoma professional association the Russian Society of Clinical Oncology (RUSSCO). Melanoma kozhi i slizistyh obolochek [Melanoma of the skin and mucous membranes]. Clinical guidelines. 2022: 136 p. Russian

[2] Kaprin AD, Starinsky VV, Shakhzadova AO. Sostoyanie onkologicheskoj pomoshchi naseleniyu Rossii v 2020 godu [The state of oncological care for Russian population in 2020]. M.: MNIOI im. P.A. Herzen – branch of "NMITs Radiology". 2021: 239 p. Russian

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**Table 1 – Model of patient treatment according to ATZ + VM + COB protocol**

| Preparations | Pharmaceutical form | Dosing regimen |
|---|---|---|
| Introductory period | | |
| VM | 240 mg, 56 tab. per pack | 960 mg twice a day for 21 days, then – 720 mg twice a day for 7 days |
| COB | 20 mg, 63 tab. per pack | 60 mg per day for 21 days, 7 days off |
| Maintenance period (from the 29th day on) for 9.2 months | | |
| ATZ | 60 mg/ml, 1200 mg/20 ml or 850 mg/14 ml, 1 pc. in pack | 1200 mg once every 21-st day or 840 mg once every 14-th day |
| VM | 240 mg, 56 tab. per pack | 720 mg twice a day |
| COB | 20 mg, 63 tab. per pack | 60 mg a day for 21 days, 7 days off |

Note: ATZ – atezolizumab; COB – cobimetinib; IPI – ipilimumab; NIVO – nivolumab; VM – vemurafenib.

**Table 2 – Model of patient treatment according to NIVO + IPI protocol**

| Preparations | Pharmaceutical form | Dosing regimen |
|---|---|---|
| Introductory period | | |
| NIVO | 10 mg/ml, 4 ml vials, 1 pc. per pack | 1 mg/kg – 80 mg once every 21-st day |
| IPI | 5 mg/ml, 10.7 ml, 1 pc. per pack | 3 mg/kg – 240 mg once every 21-st day |
| Maintenance period (from the 22-nd day) for 7.5 months | | |
| NIVO | NIVO Opdivo® 10 mg/ml, 10 ml and 4 ml vials, 1 pc. per pack | 3 mg/kg – 240 mg once every 14-th day |

Note: ATZ – atezolizumab; COB – cobimetinib; IPI – ipilimumab; NIVO – nivolumab; VM – vemurafenib.

**Table 3 – Prices for individual drugs included in combinations under study**

| MP (INN) | Pharmaceutical form (mg) | Cost per pack (rub.) | Trade mark-ups and VAT (rub.) |
|---|---|---|---|
| Tecentriq® (ATZ) | 1200 mg/20 ml per vial, No.1 | 215 930.09 | 265 657.71 |
| Tecentriq® (ATZ) | 840 mg/14 ml per vial, No.1 | 151 151.06 | 185 960.39 |
| Zelboraf ® (VM) | 960 mg, 56 tab. | 43 185.94 | 53 131.44 |
| Kotellik® (COB) | 20 mg, 63 tab. | 141 335.82 | 173 884.75 |
| Opdivo® (NIVO) | 10mg/1ml, 4 ml per vial, No.1 | 31 076.23 | 38 232.93 |
| Opdivo® (NIVO) | 10mg/1ml, 10 ml per vial, No.1 | 77 691.35 | 95 583.27 |
| Yervoy® (IPI) | 5mg/1ml, 10.7 ml per vial, No.1 | 186 134.59 | 229 00.46 |

Note: the prices are indicated in rubles, including trade mark-ups and VAT.

**Table 4 – Calculation of treatment costs according to ATZ + VM + COB protocol per metastatic melanoma patient**

| Preparation | Dosing regimen | Requirement (pcs/pack) | Cost per pack (rub.) | Cost per course (rub.) |
|---|---|---|---|---|
| Introductory period 1–28 days | | | | |
| Zelboraf®, 240 mg | 960 mg twice a day for 21 days. | 168 / 3 | 53 131.4461 | 159 394.34 |
| | Then 720 mg twice a day | 42 / 1 | 53 131.4461 | 53 131.45 |
| Kotellic®, 20 mg | 60 mg/day for 21 days. Then a 7-day break | 63 / 1 | 173 884.753 | 173 884.75 |
| Maintenance phase up to 9.2 months | | | | |
| Tecentriq®, 1200 mg/20 ml | 1200 mg once every 21-st day | 13 / 13 | 265 657.71 | 3 453 550.23 |
| Zelboraf®, 240 mg | 720 mg twice a day | 1,656 / 30 | 53 131.4461 | 1 593 943.38 |
| Kotellic®, 20 mg | 60 mg per day for 21 days. Then a 7-day break | 630 / 10 | 173 884.753 | 1 738 847.53 |
| Course costs | | 7, 172, 751.68 | | |

Note: the prices are indicated in rubles, including trade mark-ups and VAT.

**Table 5 – Calculation of treatment costs according to NIVO + IPI protocol per metastatic melanoma patient**

| Preparation | Dosing regimen | Requirement (pcs/pack) | Cost per pack (rub.) | Cost per course (rub.) |
|---|---|---|---|---|
| Introductory period | | | | |
| Opdivo®, 10 mg/ml – 4 ml | 1mg/kg – 80 mg once every 21 days | 8 | 38 232.93 | 305 863.44 |
| Yervoy®, 5 mg/ml – 10.7 ml | 60 mg once a day, 21 days, 7 days break | 20 | 229 000.45 | 4 580 009.11 |
| Maintenance phase: | | | | |
| Opdivo®, 10 mg/ml – 10 ml | 3 mg/kg – 240 mg once every 14 days | 10 ml 30 vials | 95 583.27 | 2 867 498.38 |
| Opdivo®, 10 mg/ml – 4 ml | | 4 ml 15 vials | 38 232.93 | 573 493.96 |
| Course costs | | 8 326 864.89 | | |

Note: the prices are indicated in rubles, including trade mark-ups and VAT.

**Table 6 – "Cost-effectiveness" ratios**

| Index number | ATZ+VM+COB | NIVO+IPI |
|---|---|---|
| Cost analysis | | |
| Costs for a treatment course (rubles) | 7 172 751.68 | 8 326 864.89 |
| Therapy costs per month (rubles/patient) | 788 214.47 | 1 110 248.65 |
| Effectiveness analysis | | |
| Progression-free survival (months) | 15.1 | 11.2 |
| "Cost-effectiveness" ratio, rub/year progression-free survival | 5 700 200.01 | 8 942 400.10 |
| Difference (rub.) | 3 242 200.09 | |

**Table 7 – Sensitivity analysis**

| Value | ATZ+VM+COB (rub.) | NIVO+IPI (rub.) | Economic benefit (rub.) |
|---|---|---|---|
| Initial | 7 172 751.68 | 8 326 864.89 | 1 154 113,21 |
| Sensitivity to price increases for ATZ+VM+COB rate | | | |
| Value +10% | 7 890 026.84 | 8 326 864.89 | 436 838.05 |
| Value +15% | 8 248 664.42 | 8 326 864.89 | 78 200.47 |
| Value +20% | 8 607 302.01 | 8 326 864.89 | –280 437.12 |
| Sensitivity to price reduction for NIVO+IPI course | | | |
| Value –5% | 7 172 751.68 | 7 910 521.64 | 737769.96 |
| Value –10% | 7 172 751.68 | 7 494 178.40 | 321 426.72 |
| Value –15% | 7 172 751.68 | 7 077 835.15 | –94 916.53 |

**Table 8 – Results of "budget impact" analysis**

| Region: Russian Federation | | Number of patients: 50 | |
|---|---|---|---|
| Distribution | ATZ + VM + COB | NIVO +IPI | Total |
| Share 1 (%) | 5.00% | 95.00% | 100.00% |
| Share 2 (%) | 20.00% | 80.00% | 100.00% |
| Expenses | | | |
| Budget 1 (rub.) | 17 931 879.19 | 395 526 082.30 | 413 457 961.49 |
| Budget 2 (rub.) | 71 727 516.76 | 333 074 595.62 | 404 802 112.38 |
| Savings (rub.) | | | 8 655 849.11 |

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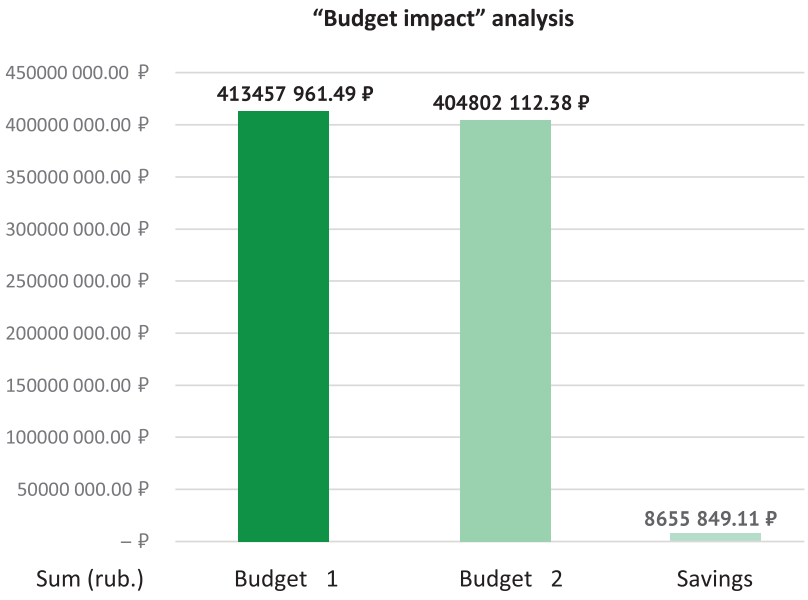


**Figure 1 – Savings in administration of ATZ + VM + COB combination**

Next, the data on marginal prices for the drugs from the Russian State Register[3] included in the ATZ + VM + COB and NIVO + IPI combinations, were copied.

Based on the current clinical guidelines[4], a model for the treatment of a metastatic melanoma patient with a confirmed BRAF mutation for the triple ATZ+VM+COB (Table 1) and dual NIVO+IPI (Table 2) combinations was developed. The duration of the treatment was calculated based on the clinical studies in real practice (IMspire 150 [16] and Checkmate 511 [21]), where the average treatment period was 9.2 months for the triple combination and 7.5 months for the double one.

At the next stage of the study, a comparative "cost-effectiveness"[5] analysis of the triple ATZ + VM + COB combination and the dual NIVO + IPI combination was carried out. A progression-free survival (PFS) derived from a systematic meta-analysis was selected as an efficacy criterion. The cost-effectiveness ratio was calculated using the formula[6]:

$$CER = DC/Ef,$$

where: CER (cost-effectiveness ratio) is the ratio of costs and effectiveness; DC – direct costs; Ef (effectiveness) – an indicator of the effectiveness which the drugs are compared by.

Next, a sensitivity analysis to determine the sensitivity of the model (cost-effectiveness ratio) to the changes in the main initial parameters – the costs of a therapy course per patient with metastatic melanoma and PFS[7], was performed.

At the final stage of the study, a "budget impact" analysis (BIA)[8] was carried out.

## RESULTS

### Effectiveness evaluation according to the literature data

At the first stage of the investigation, an information search for the studies on the efficacy and safety of the ATZ + VM + COB and NIVO + IPI combinations in adults with metastatic melanoma was conducted.

The efficacy of atezolizumab in combination with cobimetinib and vemurafenib was evaluated in the double blind, randomized (1:1), placebo-controlled, multicenter trial (IMspire150) of phase 3 [15]. The group consisted of 514 IIIC-IV melanoma patients at the unresectable stage with a positive BRAF V600 mutation. The treatment was carried out in 28-day cycles. After the first cycle of COB+VM (prescribed in the both groups), the study participants, while on-going the COB+VM therapy, were administrated with either ATZ or placebo. The duration of the course was determined on the basis of the data from the IMspire 150 [15] and CheckMate 511 [21] studies.

In the both cases, the treatment was carried out until progression or unacceptable toxicity. For the triple com-

[3] Russian State Register of Maximum Selling Prices for Medicines, 2021. Available from: http://www.grls.rosminzdrav.ru/Default.aspx.

[4] The Association of Russian Oncologists (AOR), Russian melanoma professional association the Russian Society of Clinical Oncology (RUSSCO). Melanoma kozhi i slizistyh obolochek [Melanoma of the skin and mucous membranes]. Clinical guidelines, 2022.

[5] Omelyanovsky VV, Avksentieva MV, Sura MV. et al. Metodicheskie rekomendacii po provedeniyu sravnitel'noj kliniko-ekonomicheskoj ocenki lekarstvennogo preparata [Methodological recommendations for comparative clinical and economic evaluation of the drug]. Approved by the order of the "Center for expertise and quality control of medical care" of the Ministry of Health of Russia dated December 29, 2018 No. 242. Moscow. 2018: 46 p. Russian

[6] Khabriev RU, Kulikov AYu, Arinina EE. Metodologicheskie osnovy farmakoekonomicheskogo analiza [Methodological bases of pharmacoeconomic analysis]. M.: Medicine. 2011: 352 p. Russian

[7] Omelyanovsky VV, Avksentieva MV, Sura MV. et al. [Metodicheskie rekomendacii po provedeniyu sravnitel'noj kliniko-ekonomicheskoj ocenki lekarstvennogo preparata] Methodological recommendations for comparative clinical and economic evaluation of the drug, 2018. Russian

[8] Khabriev RU, Kulikov AYu, Arinina EE. Metodologicheskie osnovy farmakoekonomicheskogo analiza [Methodological bases of pharmacoeconomic analysis], 2011. Russian

bination, the duration of the maintenance phase was 9.2 months, for the double combination –7.5 months (the median of 15 injections).

The primary evaluation standard of efficacy in the IMspire 150 protocol was the PFS, measured as the time from randomization to the first occurrence of the disease progression or death from any cause. Secondary endpoints included an objective response, a duration of response, an overall survival (OS), the time to the global health status deterioration, and the time to the deterioration in physical functions. In the ATZ group, the median PFS was 15.1 months (95% CI: 11.4, 18.4) and in the placebo group it was 10.6 months (95% CI: 9.3, 12.7) (the relative risk (RR) was 0.78; 95% CI: 0.63, 0.97; p=0.0249). At the time of this interim OS analysis, 205 patients died: 93 (36%) of 256 patients in the atezolizumab group and 112 (43%) of 258 patients in the control group (the hazard ratio was 0.85; 95% CI: 0.64–1 .11; p = 0.23 in the Logrank test) [15].

Common treatment-related adverse events (AEs) (the incidence >30%) in the ATZ group were: elevated blood creatine phosphokinase (51.3%), diarrhea (42.2%), rash (40.9%), arthralgia (39.1%), pyrexia (38.7%), increased alanine aminotransferase (33.9%) and increased lipase (32.2%). The discontinuation of the therapy due to AEs was observed in 13% of patients in the ATZ group and in 16% of patients in the placebo group [13]. According to the search results, the IMspire150 trial is currently the only protocol that has examined the combination of ATZ+VM+COB in patients with metastatic melanoma. The NIVO+IPI combination has been used for a relatively longer period and therefore has a broader evidence base, which is founded on the CheckMate study cycle.

CheckMate 067 is a phase 3 double-blind RCT [14, 18, 19]. The protocol included patients with previously untreated advanced melanoma, with a confirmed BRAF V600 mutation, aged 18 and older. The primary endpoints were defined as PFSs and OSs. Randomization occurred in three groups in the ratio of 1:1:1. The main group patients were administrated with a combination of NIVO + IPI (NIVO at the dose of 1 mg/kg body weight, once in 21 days, 4 infusions + IPI 3 mg/kg body weight, once in 21 days, 4 infusions; then NIVO at the dose of 3 mg/kg body weight every 14 days). The comparison groups' patients were administrated with either NIVO (at the dose of 3 mg/kg body weight every 14 days) + placebo or IPI (at the dose of 3 mg/kg body weight, once in 21 days, 4 infusions) + placebo. The treatment continued until the progression, unacceptable toxic effects, or withdrawal of the consent.

In the work by Larkin J. et al. (2019) the results of the CheckMate 067 study after a five-year follow-up (60 months) have been presented. In the NIVO+IPI group, the OS median was not reached for 38.2 months, in the NIVO group it was 36.9 months, in the IPI group – 19.9 months. The RR of death with NIVO+IPI compared with IPI, was 0.52. No persistent deterioration in the health-related quality of life was observed during or after the treatment with NIVO+IPI or NIVO. No new late toxic effects were notified. The authors concluded that among the advanced melanoma patients, a sustained long-term survival of 5 years was observed in a large percentage of patients treated with the NIVO+IPI combination [14].

In a multicenter, double-blind, phase 2 RCT CheckMate 069 (Hodi F.S. et al., 2016) [20], which also included adult patients with previously untreated, unresectable stage III or IV melanoma, the primary endpoint was the proportion of patients with wild-type melanoma BRAF V600 who had achieved an objective response assessed by the investigator. The main group patients were administrated with a combination of NIVO + IPI (NIVO at the dose of 1 mg/kg body weight, once in 21 days, 4 infusions + IPI 3 at the dose of mg/kg body weight, once in 21 days, 4 infusions; then NIVO at the dose of 3 mg/kg body weight once in 14 days). The comparison group patients were administrated with IPI + placebo (IPI 3 at the dose of mg/kg m body, once in 21 days, 4 infusions, then placebo once in 14 days). The protocol included 142 patients (95 patients in the NIVO+IPI group and 47 patients in the IPI group). At the median follow-up of 24.5 months (the interquartile interval of 9.1-25.7), a 2-year OS was 63.8% (95% CI 53.3–72.6) in the NIVO+IPI group and 53.6% (95% CI 38.1–66.8) in the IPI group.

The aim of the phase IIIb/IV CheckMate 511 study (Lebbe C. et al., 2019) [21] was to determine the safety of the NIVO+IPI combination at different dosages. A complete response was observed in 15.0% and 13.5% of patients, respectively. The median PFS was 9.9 months in the NIVO3+IPI1 group and 8.9 months in the NIVO1+IPI3 group. The median OS was not reached in any of the groups [21].

A network meta-analysis using a Bayesian model by Lee J. et al., 2022 [22] compared the efficacy and safety of the ATZ+VM+COB combination vs other therapies for unresectable or metastatic melanoma in adults. The endpoints included PFS, an objective response, the frequency and proportion of patients who had discontinued the treatment due to AEs.

The meta-analysis included 11 studies (not only ATZ+VM+COB and NIVO+IPI combinations, but also other approved regimens). The result was that in the general population, the use of the ATZ + VM + COB combination significantly increases the PFS compared with all comparators. This result was statistically significant for most comparators, including NIVO+IPI [RR 95% CI: 0.75 (0.58–0.97)]. An indirect comparison of IMspire-150 and CheckMate 067 study data showed a better PFS with ATZ+VM+COB (15.1 months) compared to NIVO+IPI (11.147 months). The ATZ+VM+COB combination was rated as the best treatment option in terms of the PFS and an objective response to the treatment.

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### Cost analysis results

When copying the data on marginal prices for medicines from the Russian State Register of Maximum Selling Prices for Medicines, the prices for the medicines included in the ATZ+VM+COB and NIVO+IPI combinations, which are the subject of interest of this study, were included in the analyzes. Since there is only one price for each pharmaceutical and dosage form in the SRMRs, the median was not calculated. In the analysis, trade mark-ups and VAT were taken into account (Table 3).

Based on clinical guidelines the treatment models for a single patient with a BRAF-confirmed metastatic melanoma were constructed for the triple ATZ+VM+COB combination and the dual NIVO+IPI combination. The cost analysis results for the ATZ+VM+COB treatment course are presented in Table 4. The cost analysis results for the NIVO+IPI treatment course are presented in Table 5.

In the calculation shown in Table 4, the dosing regimen of atezolizumab 1200 mg once every three weeks was used. According to the Clinical guidelines[9], within the ATZ+VM+COB protocol, atezolizumab can be also administered at the dose of 840 mg once every two weeks.

According to this dosing regimen, one patient undergoing the ATZ + VM + COB protocol will require 19 packs per course (atezolizumab 840 mg / 14 ml), which increases the cost of ATZ up to 3 533 247.47 rubles and the entire course up to 7 252 448.92 rubles. Thus, the use of atezolizumab (840 mg every 14 days) increases the cost of the therapy by 79 697.24 rubles compared with atezolizumab (1200 mg once every 21 days).

With the choice of the ATZ+VM+COB combination, the total course treatment costs for one adult patient with metastatic melanoma was lower compared to the NIVO+IPI combination (8 326 864.89 rubles vs 7 172 751.68 rubles); the difference was 1 154 113.21 rubles.

### Results of “cost-effectiveness” analysis

At the next stage of the study, a comparative “cost-effectiveness” analysis on the use of the triple ATZ + VM + COB combination and the dual NIVO + IPI combination was carried out. The PFS indicators obtained from an indirect comparison were chosen as an efficiency criterion.

When calculating the “cost-effectiveness” ratio for a year of a progression-free life, the advantage of the ATZ + VM + COB combination in comparison with the NIVO + IPI combination remained (5 700 200.01 rubles vs 8 942 400.10 rubles). The difference was significant and amounted to 3 242 200.09 rubles (Table 6). Thus, the combination of ATZ+VM+COB has shown an economic advantage.

### Sensitivity analysis results

At the next stage of the study, a sensitivity analysis was carried out. Its aim was to determine the sensitivity of the model ( “cost-effectiveness” ratio) to the changes in the main initial parameters – the cost of a therapy course per patient with metastatic melanoma and the PFS indicator (Table 7).

The sensitivity analysis demonstrated the stability of the developed model to an increase in the cost of the ATZ + VM + COB course up to + 16%; the reduction in the cost of the NIVO + IPI course up to –13%; the reduction in the PFS against the backdrop of the ATZ + VM + COB rate up to –37% (Table 7).

### "Budget impact" analysis

At the final stage of the study, the budget impact analysis of 50 patients with a possible cohort of melanoma was carried out. The analysis showed a potential opportunity to reduce the budget costs by 8 655 849.11 rubles, with an increase in the proportion of the patients administrated with the ATZ + VM + COB combination from 5% to 20% and a decrease in the proportion of the patients administrated with the NIVO + IPI combination from 95% to 80% in public procurement. The data are presented in Table 8 and Fig. 1.

## DISCUSSION

The Russian epidemiological data of the State Register of Medicinal Remedies[10] (SRMRs) on the prevalence of metastatic melanoma raise certain concerns – there is an increase in the incidence and pathology often detected at late stages, and associated with high mortality.

The implementation of new treatment regimens into practice, in particular, combinations of modern classes of drugs – BRAF/MEK inhibitors and an anti-PD-L1 immune preparation – can significantly improve the prognosis of such patients, increasing the relapse-free period compared to the previously existing therapy regimens.

Herewith, immunological drugs are highly likely to cause the development of immune-mediated AEs and are characterized by high costs, which emphasize a great importance of identifying the most clinically effective, safe and cost-effective combinations.

The NIVO+IPI combination has been shown in clinical studies to achieve good results in adult patients with metastatic melanoma, providing the longest overall survival among existing therapy regimens. However, a recent study has found out the benefits of the ATZ+VM+COB combination in this group due to the lower complication rate [22].

According to the results of the CheckMate 067 study [14], when prescribing an NIVO+IPI course, in 42% of cases the patients had to interrupt the therapy due to the drug-related AEs. For comparison, in the coBRIM study [10], against the background of the

[9] Kaprin AD, Starinsky VV, Shakhzadova AO. Sostoyanie onkologicheskoj pomoshchi naseleniyu Rossii v 2020 godu [The state of oncological care for Russian population in 2020], 2021. Russian

[10] Russian State Register of Maximum Selling Prices for Medicines, 2021.

VM+COB course, the treatment was interrupted in only 11% of subjects, which is comparable to the data of the IMspire150 study (16% of cases) [15]. When the ATZ component was added to the dual VM+COB combination, the number of withdrawals amounting to 13%, did not increase [15]. The difficulties in using the NIVO+IPI combination, in addition, are due to the high costs for the healthcare system, as noted by foreign authors [19, 25]. For 2017-2018, the costs of the NIVO + IPI course per patient per year reached the amount equal to 226 thousand pounds in the UK, 258 thousand euros in Germany, 234 thousand dollars in America [25].

To date, there are no direct comparative studies on the benefits of one or another combination; in this regard, in the study, indirect comparison data were used and the costs analysis of these treatment regimens were performed. When comparing the endpoints of large clinical trials with a similar methodology, the ATZ + VM + COB combination was found out superior to the NIV + IPI combination in terms of the PFS median, lower costs per therapy course and, as a result, a lower "cost-effectiveness" ratio was notified.

The results of the work have shown that the triple ATZ+VM+COB combination is a clinically cost-effective option for the treatment of metastatic melanoma with a confirmed BRAF mutation in adult patients within the healthcare system of the Russian Federation.

## CONCLUSION

The analysis of the literature sources made it possible to conclude that the ATZ+VM+COB combination was more clinically effective than the NIVO+IPI combination in relation to the PFS, which was 15.1 and 11.2 months, respectively, in the patients with metastatic melanoma.

With the choice of the ATZ+VM+COB combination, the total course treatment costs for one adult patient with metastatic melanoma was lower compared to the NIVO+IPI combination (8 326 864.89 rubles vs 7 172 751.68 rubles); the difference was 1 154 113.21 rubles.

The calculation of the "cost-effectiveness" ratio for a year of progression-free life showed the advantage of the ATZ + VM + COB combination in comparison with the NIVO + IPI combination (5 700 200.01 rubles vs 8 942 400.10 rubles); the difference was 3 242 200.09 rub.

The sensitivity analysis demonstrated the stability of the developed model to increase the costs of the ATZ+VM+COB course to +16%, to reduce the costs of the NIVO+IPI course to –13%, and to reduce the PFS against the backdrop of the ATZ+VM+COB course to –37%.

The "budget impact" analysis showed the possibility of reducing costs by 8 655 849.11 rubles with an increase in the proportion of the patients administrated with the ATZ+VM+COB combination from 5% to 20%, and with a decrease in the proportion of the patients administrated with the NIVO+IPI combination from 95% to 80%.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHORS' CONTRIBUTION

ISK – concept development and study design, carrying out calculations; EVM – development of research models, carrying out calculations, text writing; VYuE – information search and analysis, editing of article.

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# EVALUATION OF WEIGHT REDUCTION EFFICACY AND SAFETY OF SIBUTRAMIN-CONTAINING DRUGS IN PATIENTS WITH ALIMENTARY OBESITY

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The article presents clinical study results of the efficacy and safety of combination therapy with sibutramine and metformin (fixed combination) in comparison with sibutramine therapy with microcrystalline cellulose in patients with alimentary obesity.
**The aim** is to evaluate the efficacy and safety of using the sibutramine+metformin fixed dose combination (Reduxin® Forte) and compare it with the sibutramine + microcrystalline cellulose combination (Reduxin®) in patients with alimentary obesity in the course of the obesity therapy.
**Materials and methods.** Male and female patients (240 people) aged 18 to 65 years inclusive with alimentary obesity, meeting the inclusion criteria and not meeting the non-inclusion criteria, were randomized into 2 groups in a 1:1 ratio. One group (n=120) received sibutramine+ metformin p. o., 1 tablet (850 mg + 10 mg) once per day, the second group (n=120) received sibutramine+ microcrystalline cellulose (MCC) p. o., 1 capsule (10 mg + 158.5 mg) once per day in the morning. On day 30 ± 1, in the absence of a 2 kg weight loss compared to the first visit, the dose was increased in accordance with the medical instruction. The therapy period was 180 days. The randomization list was generated by the factory method of random numbers. The efficacy and safety were assessed by anthropometric, clinical and laboratory parameters and the SF-36 questionnaire. The proportion of patients who achieved a decrease in body weight by more than 5% in 6 months, the magnitude and dynamics of changes in body weight and body mass index, waist and hip measurements, their ratios, changes in lipid profile, blood pressure, as well as the total number of adverse events, their frequency and nature of occurrence were analyzed.
**Results**. The both drugs have demonstrated efficacy in all parameters of the obesity therapy. At the same time, in a comparative analysis, a statistically significant advantage of therapy with sibutramine + metformin was demonstrated in relation to the proportion of patients who had achieved more than 5% weight loss (body weight dynamics). Significant benefits were shown in terms of the magnitude of the change in body mass index (BMI); there was a statistically significant increase in the proportion of the patients who had switched from one category of BMI to another. By the end of the study, the vast majority of patients had no longer met the criteria for the diagnosis of “Obesity”. There was also a statistically significant benefit of

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sibutramine + metformin in terms of lowering triglycerides and low-density lipoprotein levels. The analysis of the safety parameters of sibutramine + metformin confirms a high safety profile of the drug, a comparative statistical analysis of adverse events in terms of their presence, severity, causal relationship with therapy and outcome have not revealed intergroup differences. Adverse events were transient and did not require discontinuation of therapy.

**Conclusion.** The results of the study showed that therapy with Reduxin® and Reduxin® Forte provides a pronounced decrease in body weight. However, the use of a fixed combination has a more effective positive effect on the lipid profile and patients' quality of life, which, combined with a high safety profile, proves the possibility and expediency of using Reduxin® Forte for the treatment of obesity and restoring metabolic health, even in patients without additional carbohydrate metabolism disorders.

**Keywords:** obesity; pharmacotherapy; sibutramine; metformin; Reduxin®; Reduxin® Forte

**Abbreviations:** AH – arterial hypertension; BP – blood pressure; BAS – biologically active supplement; WHO – World Health Organization; GLP-1 – glucagon-like peptide-1; BMI – body mass index; MS – metabolic syndrome; BW – body weight; MCC – microcrystalline cellulose; LDL – low density lipoproteins; HDL – high density lipoproteins; AE – adverse events; HW – hip width; WM – waist measurement; DM2 – type 2 diabetes mellitus; CVDs – cardiovascular diseases; NAFLD – non-alcoholic fatty liver disease; TGs – triglycerides; PhA – physical activity; CS – cholesterin; HR – heart rate.

# ОЦЕНКА ЭФФЕКТИВНОСТИ СНИЖЕНИЯ ВЕСА И БЕЗОПАСНОСТИ ПРИМЕНЕНИЯ СИБУТРАМИНСОДЕРЖАЩИХ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ У ПАЦИЕНТОВ С АЛИМЕНТАРНЫМ ОЖИРЕНИЕМ

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*ОРИГИНАЛЬНАЯ СТАТЬЯ*

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В статье представлены результаты клинического исследования эффективности и безопасности фиксированной комбинации сибутрамином с метформином в сравнении с терапией сибутрамином с микрокристаллической целлюлозой у пациентов с алиментарным ожирением

**Цель.** Оценить эффективность и безопасность применения препарата сибутрамин в комбинации с метформином (Редуксин® Форте) в сравнении с терапией препаратом сибутрамин с микрокристаллической целлюлозой (Редуксин®) у пациентов с алиментарным ожирением.

**Материалы и методы.** Пациенты мужского и женского пола (240 человек) в возрасте от 18 до 65 лет включительно с алиментарным ожирением, соответствующие критериям включения и не соответствующие критериям невключения, рандомизировались в 2 группы в соотношении 1:1. Одна группа (n=120) получала препарат сибутрамин+метформин (фиксированная комбинация), перорально по 1 таблетке (850 мг + 10 мг) 1 раз в день, вторая группа (n=120) получала препарат сибутрамин+микрокристаллическая целлюлоза (МКЦ) перорально по 1 капсуле (10 мг + 158,5 мг) 1 раз в день утром. На 30±1 день при отсутствии снижения массы тела на 2 кг по сравнению с первым визитом, доза увеличивалась в соответствии с инструкцией по медицинскому применению. Период терапии составил 180 дней. Рандомизационный список был сгенерирован методом генерации случайных чисел. Эффективность и безопасность оценивались по антропометрическим, клинико-лабораторным показателям и опроснику SF-36. Анализировалась доля пациентов, достигших снижения массы тела более, чем на 5% за 6 месяцев терапии, величина и динамика изменения массы тела и индекса массы тела, окружности талии и бёдер, их соотношения, изменения показателей липидного профиля, артериального давления, а также общее количество нежелательных явлений, их частота и характер возникновения.

**Результаты.** Оба лекарственных препарата продемонстрировали эффективность в отношении всех параметров терапии ожирения. При этом, при сравнительном анализе было продемонстрировано статистически значимое преимущество терапии препаратом сибутрамин+метформин в форме фиксированной комбинации в отношении доли пациентов, достигших более 5% снижения массы тела, динамики массы тела. Значимые преимущества были показаны в отношении величины изменения индекса массы тела (ИМТ), отмечалось статистически значимое увеличение доли пациентов, перешедших из одной категории ИМТ в другую. К концу исследования абсолютное большинство пациентов перестали соответствовать критерию диагноза «Ожирение». Было также выявлено статистически значимое преимущество препарата сибутрамин+метформин в отношении снижения уровня триглицеридов и уровня липопротеидов низкой плотности. Анализ параметров оценки безопасности сибутрамин+метформин подтверждает высокий профиль безопасности препарата, сравнительный статистический анализ нежелательных явлений по их наличию, тяжести, причинно-следственной связи с терапией и исходу не выявил межгрупповых различий. Нежелательные явления носили транзиторный характер и не требовали отмены терапии.

**Заключение.** Результаты исследования показали, что терапия лекарственными препаратами Редуксин® и Редуксин® Форте обеспечивает выраженное снижение массы тела. Однако применение фиксированной комбинации оказывает более эффективное положительное влияние на показатели липидного профиля и качества жизни пациентов, что, в сочетании с высоким профилем безопасности, доказывает возможность и целесообразность применения лекарственного препарата Редуксин® Форте для лечения ожирения и восстановления метаболического здоровья даже у пациентов без дополнительных нарушений углеводного обмена.

**Ключевые слова:** ожирение; фармакотерапия; сибутрамин; метформин; Редуксин®; Редуксин® Форте

**Список сокращений:** АГ – артериальная гипертензия; АД – артериальное давление; БАД – биологически активная добавка; ВОЗ – Всемирная организация здравоохранения; ГПП-1 – глюкагоноподобный пептид-1; ИМТ – индекс массы тела; МС – метаболический синдром; МТ – масса тела; МКЦ – микрокристаллическая целлюлоза; ЛПНП – липопротеиды низкой плотности; ЛПВП – липопротеиды высокой плотности; НЯ – нежелательные явления; НАЖБП – неалкогольная жировая болезнь печени ОБ – окружность бёдер; ОТ – окружность талии; СД2 – сахарный диабет 2 типа; ССЗ – сердечно-сосудистые заболевания; ТГ – триглицериды; ФА – физическая активность; ХС – холестерин; ЧСС – частота сердечных сокращений.

## INTRODUCTION

Obesity is a chronic disease characterized by an excessive accumulation of adipose tissue in the body, which poses a threat to health, and is also a major risk factor for several other chronic diseases, including type 2 diabetes mellitus (DM2) and cardiovascular diseases (CVDs) [1].

In May 2022, World Health Organization (WHO) experts compiled a report on a new pandemic of modern humanity, which states that 60% of citizens in Europe are either overweight or obese. The prevalence of obesity worldwide has nearly tripled since 1975, largely due to a gradual shift towards sedentary lifestyles and less healthy diets. According to WHO estimates, by 2025, one in five adults in the world will suffer from this pathology. The most depressing fact is the widespread increase in obesity among children and adolescents [2-4].

The relevance of obesity control is due not only to its high prevalence, but also to the negative impact on the quality of people's life and a particularly high risk of developing various diseases that lead to an early disability and a significant decrease in life expectancy. Overweight and obesity are major risk factors for cardiovascular diseases, diabetes mellitus and its complications, including blindness, limb amputation and a chronic kidney disease (CKD), a musculoskeletal disease (including osteoarthritis), gastrointestinal and respiratory diseases. Obesity is also associated with certain types of cancer, i. e. endometrial, breast, ovarian, prostate, liver, gallbladder, kidney and colon [5].

Many researchers emphasize the priority of fundamental changes in an obese patient's lifestyle, corrections of his diet and an increase in his physical activity (PhA). However, not all patients manage to achieve and/or maintain the target anthropometric parameters with the help of diet and PhA correction. Thus, according to the US National Institutes of Health, in 30-60% of patients treated with diets and exercises, body weight

(BW) returns to its baseline within one year, and after 5 years – in almost 100% patients. Thus, the feasibility and importance of developing additional approaches to weight loss is beyond doubt. To date, the main developments are carried out in two areas: pharmacotherapy and surgical treatment [6-8].

According to current guidelines, patients with body mass index (BMI) ≥ 27 kg/m$^2$ and 2 or more risk factors (smoking, AH > 140/90 mmHg, low density lipoproteins (LDLs) above 160 mg/dL, high density lipoproteins (HDLs) below 35 mg/dL, fasting hyperglycemia or impaired carbohydrate tolerance, a family history of an early cardiovascular disease (age < 45 years for men and < 55 years for women) or with a BMI ≥ 30 kg/m$^2$) require pharmacological intervention as an adjunct to the correction of the regime of physical activity and diet [9]. Based on this, the vast majority of patients with obesity and overweight who come to the attention of practitioners need medical treatment [10]. And taking into account the changes in metabolic processes related to age and an increased risk of developing diseases associated with weight gain (CVDs, T2DM, NAFLD, etc.), it is advisable to include the aid aimed at weight loss and its control in the program of preventive medical examination and medical recommendations, regardless of specialties.

For the treatment of obesity, pharmacological agents are divided into drugs of central (phentermine + topiromate, sibutramine, fluoxetine), peripheral (orlistat) and mixed (central and peripheral) actions (thermogenic sympathomimetics, a growth hormone, androgens, glucagon-like peptide-1 (GLP-1) agonists, the sibutramine + metformin combination [7, 8]. According to the National Clinical Guidelines, the following drugs for the treatment of obesity are currently registered in the Russian Federation (RF): orlistat, liraglutide, sibutramine + microcrystalline cellulose (MCC), sibutramine + metformin (fixed dose combination). At the same time, the broadest evidence base is currently available for the preparations containing sibutramine [8].

Sibutramine is a substance that has an anorexigenic effect by increasing satiety and reducing appetite. It inhibits the reuptake of neurotransmitters (serotonin and noradrenaline), and also increases thermogenesis, affecting brown adipose tissue [11, 12]. However, insulin resistance, as a key link in almost all pathogenetic "ways" of obesity with its metabolic, energy, hemodynamic and inflammatory links, further draws attention to metformin. Traditionally, one of the main mechanisms of a metformin action is its effect on the insulin resistance by suppressing gluconeogenesis in the liver (an endogenous glucose production). The drug also reduces the absorption of glucose in the intestine and possibly improves the absorption and utilization of glucose by peripheral tissues: skeletal muscle and adipose tissue. New evidence suggests that metformin-associated weight loss is due to the modulation of hypothalamic appetite control centers, changes in the gut microbiota, and effects on the aging process. In addition, metformin has a beneficial effect on lipid metabolism: it reduces the content of total cholesterol (CS), LDLs and triglycerides (TGs). Thus, metformin has a number of pleiotropic effects, which make it possible to use the drug in metabolic syndrome (MS), obesity, steatohepatosis, and a number of other diseases. It is important that many of the putative new targets are closely associated with obesity [13]. The prescription of metformin is clinically significant for metabolic health, but the minimal dynamics of weight loss against its background (1–5 kg per year) does not allow the use of the drug as a monotherapy for the obesity treatment [14]. In the medical correction of alimentary obesity, the sibutramine + metformin combination is mainly used [9]. Positive results of the fixed sibutramine + metformin combination in patients with obesity and other carbohydrate metabolism disorders (impaired glucose tolerance, type 2 diabetes) have been shown in numerous clinical studies [15–44]. It has been shown that the combined use of sibutramine with metformin increases the therapeutic efficacy of the combination used in patients with overweight and carbohydrate metabolism disorders.

It seems promising to study metformin protective effects and the action synergism of the sibutramine + metformin combination in a single dosage form, in relation to the restoration of metabolic health in obese patients without additional disorders of carbohydrate metabolism, as well as the assessment of such therapy safety.

**THE AIM** is to evaluate the efficacy and safety of using the sibutramine+metformin combination (Reduxin® Forte) and compare it with the sibutramine + microcrystalline cellulose combination (Reduxin®) in patients with alimentary obesity in the course of the obesity therapy.

## MATERIALS AND METHODS

This "Open multicenter randomized study to evaluate the efficacy and safety of the drug Reduxin® Forte, film-coated tablets, in comparison with the drug Reduxin®, capsules, in patients with alimentary obesity", was conducted from 07/03/2020 to 05/21/2021 in 5 cities of the Russian Federation (St. Petersburg, Ivanovo, Kirov, Samara, Rostov-on-Don) on the basis of 8 research centers. The study was conducted in accordance with the principles of good clinical practice and was authorized by the Ministry of Health of the Russian Federation (No. 304 dated July 3, 2020), approved by the Ethics Council of the Ministry of Health of the Russian Federation, as well as by independent ethics committees of all clinical centers participating in the study.

### Study Design

Male and female patients (240 people) aged 18 to 65 years inclusive with alimentary obesity with a body mass index of more than 30 kg/m$^2$, meeting the inclu-

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sion criteria and not meeting the exclusion criteria, were randomized into 2 groups in a 1:1 ratio. The randomization list was generated by the factory method of random numbers.

Group 1 (n=120) received sibutramine + metformin, film-coated tablets, p. o., 1 tablet (850 mg + 10 mg) once per day in the morning, without chewing and followed with a glass of water, during meals, for 180 days.

Group 2 (n=120) received sibutramine + MCC capsules p. o., 1 capsule (10 mg + 158.5 mg) once per day in the morning, without chewing and followed with a glass of water, during meals for, 180 days.

A patient's condition was monitored at visits (V) at the research center in accordance with the Protocol. At V3 (Day 30 ± 1), in the absence of a 2 kg weight loss compared to V1 (Day 1), the zero dose was increased. Patients in the sibutramine+metformin group received 1 tablet (850 mg + 15 mg), and patients in the sibutramine + MCC group received 1 capsule (15 mg + 153.5 mg).

The total duration of the study for each patient was no more than 191 days: screening – no more than 10 days; randomization – no more than 1 day; therapy – no more than 180 days (6 months); the study completion – no more than 3 days. The graphic scheme of the study is shown in Figure 1.

### Criteria for patients' inclusion in the study

Every patient had given written informed consent prior to the participation in the study. The study could include men and women aged 18 to 65 years with a diagnosis of "Alimentary obesity", BMI> 30 kg/m$^2$ and ineffective non-drug treatment at the time of screening (weight loss < 5% within 3 months of treatment). The consent from each patient was obtained for changes in diet, eating behavior, an increased physical activity, and adherence to the study physician's recommendations throughout the study participation. The patients were warned to use reliable methods of contraception throughout the study and for 3 weeks after its completion.

### Main criteria for patients' non-inclusion in the study

These criteria are as follows: hypersensitivity to the components of the study / reference drug, secondary (symptomatic) obesity, type I or II diabetes mellitus in history and/or at the time of screening, a low-calorie (<1600 kcal/day) diet within 3 months before screening, a previous administration of sibutramine-based drugs, the use of drugs, herbal remedies or dietary supplements for the obesity treatment less than 3 months before screening, as well as a number of other criteria, including impaired liver and / or kidney function, uncontrolled arterial hypertension (AH), etc.

The decision to exclude a patient from the study was made by the investigator. The patient was withdrawn from the study in case of a treatment failure (weight loss <5% to V7 relative to V1); an increase in resting heart rate ≥10 bpm or systolic/diastolic pressure ≥10 mmHg at two visits in a row; an increase in blood pressure over 145/90 mmHg twice when remeasured; if any diseases or conditions appear during the study that worsen the patient's prognosis, and also make it impossible for the patient to continue participating in the clinical trial; if it is necessary to prescribe prohibited concomitant therapy (glucocorticoids, antidepressants, lipid-lowering, hypoglycemic drugs, macrolides, etc.) or if the study protocol is violated; if the patient refuses to participate in the study, and there can be a number of other reasons.

The choice of dosage, dosing regimen, route of administration and duration of therapy for the study drugs, was based on these medical instructions for sibutramine + metformin[1], film-coated tablets, and sibutramine + MCC[2] capsules; on the standard of specialized medical care for obesity (Order of the Ministry of Health of the Russian Federation dated November 9, 2012 No. 850n "On approval of the standard for specialized medical care for obesity")[3], National guidelines for the diagnosis, treatment, prevention of obesity and associated diseases[4], as well as modern clinical studies to research the efficacy and safety of sibutramine, including its combination with metformin [14–30].

The use of the study / reference drug was carried out in combination with diet therapy, changes in eating behavior and an increase in PhA. The patients were recommended a diet with a deficit of 600 kcal per day of the total caloric content calculated for the patient or a diet with a restriction of fat intake. Recommendations were given on proper food intake (frequent and fractional meals in small portions; careful chewing of food; the last meal – not later than 3 hours before bedtime, etc.). It was also recommended to have 225-300 minutes of moderate intensity PhA per week or 150 minutes of high intensity aerobic PhA per week, which is equivalent to spending 1,800–2,500 kcal per week.

### Estimated indicators of effectiveness and safety

The primary efficacy endpoint was the rate of achieving >5% weight loss at the last visit (V13, 6 months

[1] Instructions for the medical use of the drug Reduxin® Forte. Available from: https://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=c-7cab986-bfca-49f5-8b22-61387351e80a. Russian

[2] Instructions for the medical use of the drug Reduxin®. Available from: https://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=108c4981-0dbb-4bdc-a3fe-08395333313f. Russian

[3] Ministry of Health Order of the Russian Federation dated 2012 Nov 9, No. 850n "Ob utverzhdenii standarta specializirovannoj medicinskoj pomoshchi pri ozhirenii" [On approval of the standard for specialized medical care for obesity]. Available from: https://minzdrav.gov.ru. Russian

[4] Russian Society of Cardiology; Russian Scientific Medical Society of Therapists; Antihypertensive League; Organization to promote the development of prehospital medicine "Outpatient Doctor"; Association of Clinical Pharmacologists. Diagnostika, lechenie, profilaktika ozhireniya i associirovannyh s nim zabolevanij [Diagnosis, treatment, prevention of obesity and associated diseases] (National Clinical Guidelines) St. Petersburg; 2017: 164 p. Available from: https://scardio.ru/content/Guidelines/project/Ozhirenie_klin_rek_proekt.pdf. Russian

of therapy) compared with the first visit (V1, starting of therapy).

In addition, a few important factors were additionally assessed. They are: the proportion of the patients requiring an increase in the study / reference dose by V3; the magnitude of the change in body weight to V13 (6 months of therapy) relative to V1; change in BMI (%) to V13 relative to V1; dynamics of body weight (kg), BMI (kg/m$^2$), waist measurement, hip width, waist / hip measurements, dynamics of lipid profile indicators (TG, total cholesterol, LDL-cholesterol, HDL-cholesterol) and quality of life (according to the SF-36 questionnaire). The efficacy of treatment with the study drug was estimated at V1–7, 9, 11, 13. Anthropometric data (height, body weight, waist measurement, hip width) were collected by measurements. BMI was determined by the calculation method (body weight (kg) / height (m$^2$) based on the data obtained.

To determine the safety of the therapy, the dynamics of blood pressure, heart rate, ECG, as well as the total number and frequency of adverse events (AEs) and serious adverse events (SAEs) associated with the use of the study / reference drug, were estimated. The proportion of the patients with at least one AE and the proportion of the patients who had interrupted the treatment due to AEs, was taken into account. The changes in laboratory parameters were the AEs only if they were clinically significant and/or required therapeutic intervention.

Adverse events were assessed in all patients who received at least one dose of the drug.

### Statistical processing of results

A statistical analysis was performed in accordance with the requirements of ICH9, the Rules of Good Clinical Practice approved by the Eurasian Economic Commission and other applicable requirements and laws. For the statistical analysis, certified statistical software with validated algorithms for performing statistical analyzes and proper documentation was used (StatSoft Statistica 10.0., IBM SPSS Statistics 22). Descriptive statistics was presented for all efficacy and safety measures collected during the study. Continuous (quantitative) data were presented using the number of observations, arithmetic mean, 95% confidence interval (CI) for the mean, standard deviation, median, interquartile range (25$^{th}$ and 75$^{th}$ percentiles), minimum and maximum. Ordinal, categorical, and qualitative data were presented as absolute frequencies (number of observations), relative frequencies (percentage), and 95% CI. The normality checking of the distribution was carried out by one of the generally accepted methods (Shapiro-Wilk test, Kolmogorov-Smirnov test). In the case of a non-Gaussian distribution, non-parametric evaluation methods could be used to compare the efficacy and safety performance. Significance levels and CIs were calculated as two-tailed: if the statistical significance of differences is two-tailed by default, then it was referred to a significance level of 0.05.

## RESULTS

The study was completed by 228 patients (110 patients in the sibutramine + MCC group and 118 patients in the sibutramine + metformin group). The estimation of the study drugs effectiveness, based on the statistical analysis, showed that in the sibutramine + metformin group, the proportion of patients who had achieved more than 5% weight loss by the end of therapy (V13 or 6 months) was 99.15% (117/118), in the sibutramine + MCC group – 93.64% (103/110). As a result of a comparative frequency analysis of achieving more than 5% weight loss to V13, statistically significant differences were detected between the study groups. The difference in proportions was 0.0552 (5.52%), the 95% CI for the difference in proportions was [-0.0019; 0.1233] ([-0.19%; 12.33%]). Thus, it was demonstrated that both study drugs provide a clinically significant decrease in body weight, however, during the therapy with sibutramine + metformin, the effect was more pronounced.

As a result of the additional comparative analysis of the patients' frequency who had achieved a 5% decrease in body weight by V7 (90±1 days of therapy), statistically significant differences were found out between the study groups (p=0.0016). In the sibutramine + metformin group, the proportion of patients who had achieved a 5% decrease in body weight to V7 was 100.0%, in the sibutramine + MCC group – 91.67%, which indicates a high effectiveness of the therapy in terms of achieving an early response to it. Moreover, against the background of taking a fixed combination, the proportion of early responders was higher.

Moreover, the additional analysis showed that in the sibutramine + metformin group, the proportion of the patients who had achieved a weight loss of 10% or more by V13 (180±1 days), was 93.22% (110/118), in the sibutramine group + MCC – 80.00% (88/110). The differences between the study groups were statistically significant (p=0.0032).

According to the protocol, the frequency of patients who required an increase in the dose of the study / reference drug by V3 (30±1 days), was also assessed. In the absence of a decrease in body weight of 2 kg compared with V1, the patients in the sibutramine + metformin group received 1 tablet (850 mg + 15 mg), and the patients in the sibutramine + MCC group received 1 capsule (15 mg + 153.5 mg) per day. In the sibutramine + metformin group, the proportion of patients who required an increase in the dose of the study / reference drug was 10.17% (12/118), for the sibutramine+metformine, in the sibutramine + MCC group – 14.55% (16/110) (p=0 ,2416). Thus, the absolute majority of patients showed a response to therapy when taking drugs at a minimum dose, which reduces the risk of developing AEs and preserves the possibility of increasing the dose in case of phisiological the plateau effect occurs.

Body weight significantly decreased in both groups, while statistically significant differences confirming the benefits of using the combination of sibutramine + met-

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formin, were found out (Fig. 2). The average value of the change in patients' body weight by V13 (180±1 days) relative to V1 for the entire population was (-14.29±4.97 kg), for the sibutramine + metformin group – (-14.99±4.64 kg), for the sibutramine + MCC group – (-13.54±5.22 kg). The difference in the average values of changes in patients' BW to V13 relative to V1 between the groups was 1.45 kg, 95% CI was [0.17;2.74] (p=0.0272).

Additionally, a comparative analysis of the decrease in patients' body weight by V7 (90±1 days) relative to V1 and to V13 (180±1 days) relative to V7 (90±1 days), was carried out. The average value of the changes in patients' body weight by V7 relative to V1 for the entire population was (–8.94±2.69 kg), for the sibutramine + metformin group – (–9.28±2.67 kg), for the sibutramine group + MCC – (–8.59±2.68 kg).

The average value of the changes in the patients' BW by V13 (180±1 days) relative to V7 (90±1 days) for the sibutramine + metformin group was (–5.62±3.12 kg), for the sibutramine + MCC group – (–4.54±3.70 kg) (Fig. 4).

Thus, a decrease in body weight was observed during the entire period of therapy without the occurrence of a plateau effect.

The difference in the mean values of changes in patients' BW by V7 relative to V1 between the groups was 0.99 kg, 95% CI [0.28; 1.70] and 1.09 kg, 95% CI for the difference in mean [0.21; 1.96] values of changes in the patients' BW by V13 relative to V7 (p=0.0148), respectively.

Significant dynamics of BMI was shown in the both study groups. The average value of the change in BMI (%) by V13 for the sibutramine + metformin group was (–15.67±4.31%), for the sibutramine + MCC group – (–12.90±5.36%), the difference in the average values change in BMI (%) between the groups was 1.99%, 95% CI [0.76; 3.23] and was statistically significant (p=0.0017). The changes in BMI by V7 (90±1 days) relative to V1 were also statistically significant (p=0.0052) and amounted to 0.94%, 95% CI [0.28;1.60]; the significant differences (p = 0.0129) were present at V13 relative to V7: the difference in the average values of changes in BMI (%) by V13 between the groups was 1.18%, 95% CI [0.25; 2.1

Thus, the results demonstrate a decrease in BW and BMI both in absolute terms and in percentage terms in both groups, while in the group of patients receiving a fixed combination of sibutramine + metformin, the results were more significant.

Additionally, the distribution of patients into categories in accordance with the BMI index was calculated and the frequency of patients who had moved from one category to another (i.e., reduced the degree of obesity) by V7 (90 ± 1 day) and V13 (180 ± 1 day) compared to V1, was assessed. For the distribution of patients, the following categories of BMI were distinguished (Table 1).

A frequency assessment of the patients who had moved from one BMI category to another after 3 and 6 months of therapy, was made in the both groups. At V1, the proportion of patients with category 3 in the sibutramine + metformin group was 54.24% (64/118), in the sibutramine + MCC group – 52.73% (58/110); the proportion of patients with category 4 in the sibutramine + metformin group was 32.20% (38/118), in the sibutramine + MCC group – 34.55% (38/110). The proportion of patients with category 5 at V1 in the sibutramine + metformin group was 13.56% (16/118), in the sibutramine + MCC group – 12.73% (14/110). At V7 (90±1 days), the proportion of patients with category 2 in the sibutramine + metformin group was 33.90% (40/120), in the sibutramine + MCC group it was 31.82% (35/110). Therefore, we can say that after 3 months of therapy, every third patient moved into the category of "overweight", and more than 50% of patients reduced the severity of obesity. At V13, the proportion of patients with category 1 (with normalized body weight), was notified. In the sibutramine + metformin group it was 1.69% (2/118), and in the sibutramine + MCC group it was 0.91% (1/ 110). Herewith, after 6 months of therapy, the absolute majority of patients in the sibutramine + metformin group moved from the "obesity" category to the "overweight" category. Thus, at V13, the proportion of patients with category 2 in the sibutramine + metformin group was 60.17% (71/118), in the sibutramine + MCC group – 49.09% (54/110). The distribution of the patients' proportion who took the sibutramine + metformin drug, depending on the severity of obesity according to BMI, is shown in Figure 3.

A comparative analysis of the frequency of patients who had switched from one BMI category to another, at V13 revealed statistically significant differences between the study groups (p=0.0065).

A frequency assessment of the patients who had achieved a BMI value of less than 30 kg/m$^2$ at V7 and V13 showed that after 3 months of therapy, the proportion of patients who had reached a BMI value of less than 30 kg/m$^2$ in the sibutramine + metformin group was 34.17%, and in the sibutramine + MCC group it was 30.00%. The proportion of patients who had reached a BMI value of less than 30 kg/m$^2$ in the sibutramine + metformin group after 6 months of therapy, was 61.67%, in the sibutramine + MCC group it was 46.67%. The difference between the groups was statistically significant, p = 0.0197 (Fig. 4).

Thus, the proportion of patients who had reduced the degree of obesity and even moved into the category of overweight patients was significantly higher in the sibutramine + metformin group.

When assessing the dynamics of body weight, it was shown that the change in body weight by each visit differed statistically significantly between the groups of drugs sibutramine + metformin and sibutramine + MCC (p<0.05), and in the group of the drug sibutramine + metformin, the dynamics of the decrease in BW was more pronounced.

**Table 1 – Categories of BMI for patients' distribution**

| Category No. | Description |
|---|---|
| 1 | Patients with BMI less than 25 kg/m$^2$ |
| 2 | Patients with BMI of 25 or less than 30 kg/m$^2$ |
| 3 | Patients with BMI of 30 or less than 35 kg/m$^2$ |
| 4 | Patients with BMI of 35 or less than 40 kg/m$^2$ |
| 5 | Patients with BMI of 40 kg/m$^2$ or more |

**Table 2 – Indicators of triglycerides amount in sibutramine + metformin group and in sibutramine + MCC group**

| Visit | Average value of TG, mmol/l | |
|---|---|---|
| | Sibutramine + metformin group | Sibutramine + MCC group |
| V0 | 1.71±0.76 | 1.62±0.65 |
| V7 | 1.54±0.40 | 1.50±0.44 |
| V13 | 1.47±0.40 | 1.50±0.45 |

**Table 3 – Indicators of lipid metabolism to V13 relative to V1 in sibutramine + metformin group and in sibutramine + MCC group**

| Lipid fractions, mmol/l | | Sibutramine + metformin group | Sibutramine + MCC group |
|---|---|---|---|
| Total cholesterol | V0 | 5.12±0.94 | 5.29±1.03 |
| | V13 | 4.51±0.91 | 4.73±0.94 |
| LDL | V0 | 2.44±1.42 | 2.71±1.46 |
| | V13 | 1.8±1.01 | 2.15±1.11 |
| TG | V0 | 1.71±0.76 | 1.62±0.65 |
| | V13 | 1.47±0.40 | 1.50±0.45 |
| HDL | V0 | 1.41±0.51 | 1.47±0.61 |
| | V13 | 1.48±0.74 | 1.6±0.78 |

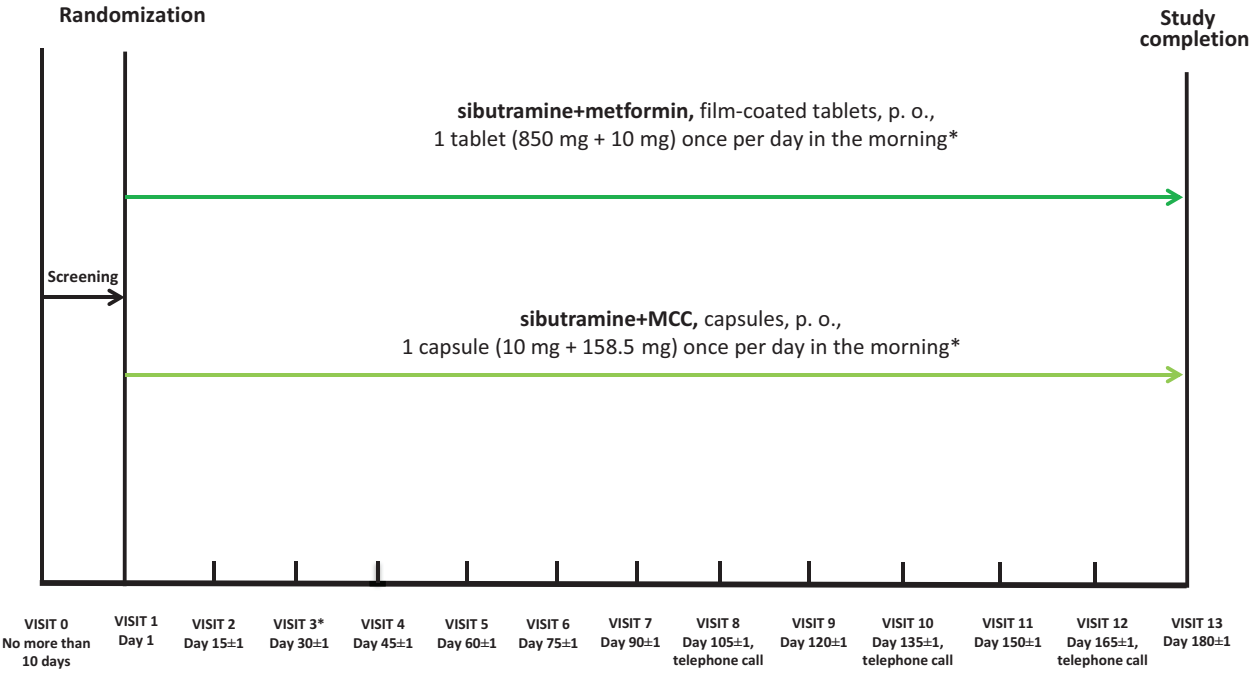


**Figure 1 – Graphic study scheme**

Note: * – at Visit 3 if there is no weight loss of 2 kg compared to Visit 1, the dose will be increased. Patients in the sibutramine+metformin group will receive 1 tablet (850 mg + 15 mg) and patients in the sibutramine+MCC group would received 1 capsule (15 mg + 158.3 mg).

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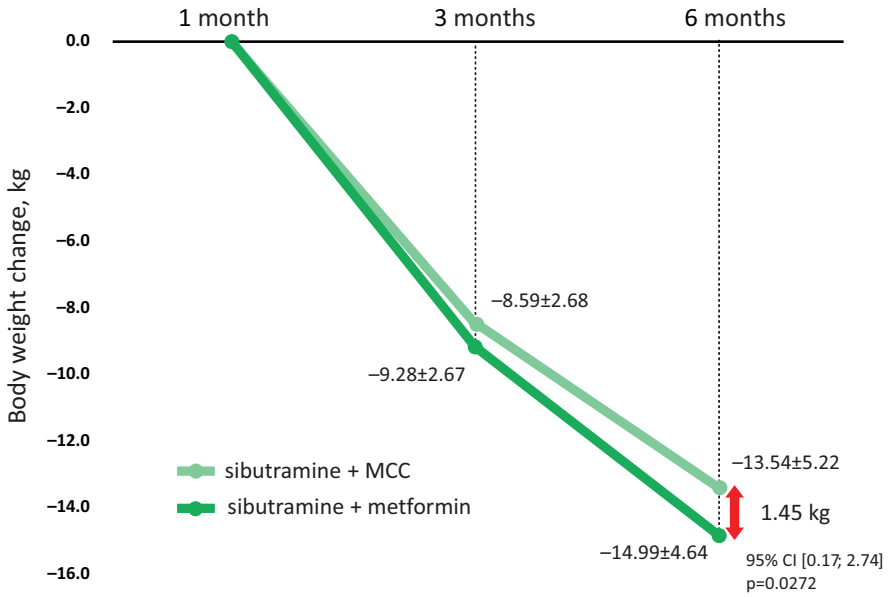


**Figure 2 – Dynamics of mean values in patients' body weight changes at V1 (1 month), V7 (3 months) and V13 (6 months) in sibutramine + MCC and sibutramine + metformin groups**

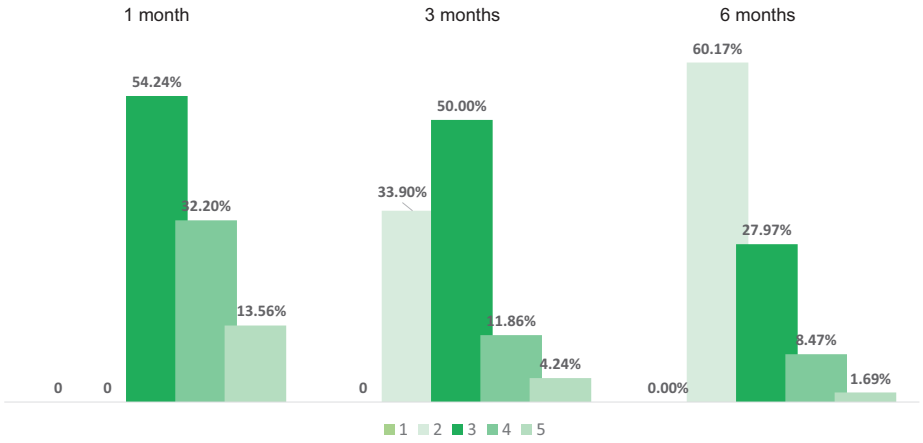


**Figure 3 – Proportion of patients who switched from one category to another after 3 and 6 months of treatment in sibutramine + metformin group**

Note: 1–5 – BMI categories in accordance with Table 1.

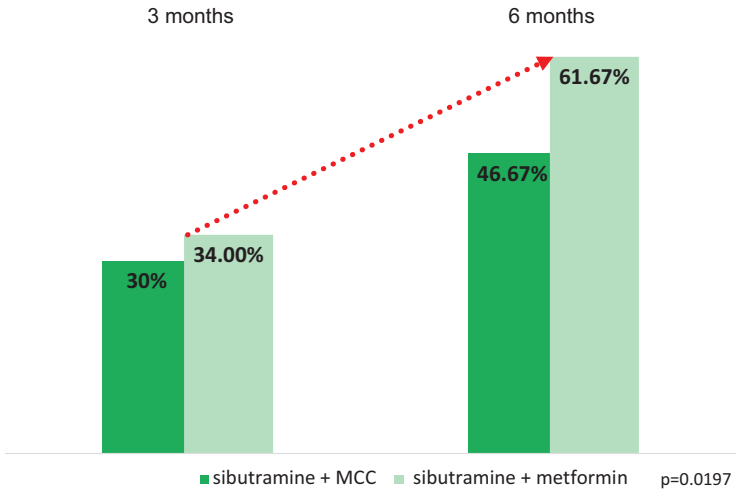


**Figure 4 – Proportion of patients who reached BMI less than 30 kg/m2 by V13 relative to V7, in sibutramine + MCC group and in sibutramine + metformin group**

The mean body weight in the sibutramine + metformin group at V0 was 99.53±13.53 kg, at V7 it was 90.37±12.77 kg, and at V13 this value was 84.74±12.23 kg. The average value of BW in the sibutramine + MCC group at V0 was 99.10±13.62 kg, at V7it was 90.83±13.04 kg, and at V13 – 86.29±13.44 kg.

Waist measurement dynamics is an important marker of a decrease in the amount of visceral fat, and hence a decrease in the risk of associated complications. The study detected that waist measurement was statistically significantly different at each visit relative to V0 ($p<0.05$) in the both sibutramine+metformin and sibutramine+MCC groups. At V0, the average waist measurement in the sibutramine + metformin group was 101.61±12.85 cm, at V7 it was 94.76±12.02 cm, at V13 – 89.24±11.51 cm. At V0, the sibutramine + MCC group showed an average waist measurement of 100.34±13.76 cm, at V7 – 93.28±12.78 cm, at V13 – 88.21±12.36 cm, respectively.

Thus, in waist measurement, the both drugs showed a reduction of about 12 cm in 6 months, which indicates their effectiveness in relation to decrease visceral obesity.

The results of the waist / hip measurements ratio were statistically significantly different for each visit relative to V0 ($p<0.05$) in the both sibutramine + metformin and sibutramine + MCC groups, which also indicates effectiveness in reducing the amount of visceral fat.

The lipid profile is an important predictor of the developing cardiovascular disease risk, atherosclerosis, and other conditions associated with obesity.

The analysis revealed a significant difference between visits in terms of lipid profile parameters in the both groups, with more pronounced dynamics observed in the group of patients taking sibutramine + metformin ($p<0.00062$), which is associated with the additional action of metformin. So, as a result of the comparative analysis of the magnitude change in TG (%) to V13 relative to V7, statistically significant differences were revealed between the studied groups ($p = 0.0240$). The difference in the mean values of the change in TG (%) between the groups was 6.44%, 95% CI for the difference in the means was [0.86; 12.02] (Table 2).

When assessing the total cholesterol in the group of patients taking sibutramine + metformin, a significant difference between visits was also found out ($p<0.00001$).

The mean value of the total cholesterol in the sibutramine + metformin group significantly decreased: at V0 it was 5.12±0.94 mmol/L; at V7 it was 4.80±0.76 mmol/L; at V13 – 4.51±0.91 mmol/L. There was also a decrease in the sibutramine + MCC group: at V0 it was 5.29±1.03 mmol/L; at V7 it was 4.90±0.95 mmol/L; at V13 – 4.73±0.94 mmol/L.

The estimation and comparative analysis of the LDL-cholesterol amount showed positive dynamics in both groups, while significant differences were established between the sibutramine + metformin and sibutramine + MCC groups at V11 ($p=0.0274$) and V13 ($p=0.0231$). This shows a more pronounced effect of the drug sibutramine + metformin on this indicator.

According to the results of the intragroup analysis, a significant difference was found out between visits in terms of the HDL cholesterol dynamics in the sibutramine + metformin group ($p<0.0088$) and in the sibutramine + MCC group ($p<0.00001$). The dynamics of lipid profile indicators is presented in Table 3. Thus, a long-term use, more than 3 months, of the drug sibutramine + metformin provides a signifikant improvent in lipid profile parameters.

According to the data obtained using the SF-36 Quality of life questionnaire, there is a pronounced positive dynamic of psychological and physical health indicators in both groups, while in the sibutramine + metformin group, the dynamics was more pronounced.

The dynamics of the average score of the physical health component in the sibutramine + metformin group was more than 10 points (from 60.99±9.27 points at V1 to 71.68±8.76 points at V13), and in the sibutramine + MCC group – from 60.74±8.58 points at V1 to 71.09±8.83 points at V13, respectively. The physical health component was also shown to be statistically significantly different at each visit relative to V1 ($p<0.05$) in both the sibutramine+metformin and sibutramine+MCC groups.

The average score of the psychological health component has also improved by 10 points: in the sibutramine + metformin group – by 58.46±8.98 points at V1 and by 68.15±10.98 points at V13. The average score dynamics of the psychological health component in the sibutramine + MCC group was 57.22±10.80 points at V1 and 67.95±10.25 points at V13, respectively. It was found out that the psychological component of health differed statistically significantly for each visit relative to V1 ($p<0.05$) in both groups.

### Safety assessment

Safety assessment was performed using a statistical analysis of safety endpoints. The condition of the study participants was assessed with respect to AEs, the data on blood pressure, heart rate throughout the study ECG and laboratory tests.

In the sibutramine + metformin group, the frequency of patients with reported cases of AEs was 33.33% (40/120), and the sibutramine + MCC group – 30.00% (36/120). In both groups, the most common AEs were dry mouth, headache, and sweating.

Among the reported AEs, 89.88% (151/168) were of mild severity, 10.12% (17/168) were of moderate severity. According to the investigators, the causal relationship with therapy based on the study / reference drug was assessed as “not related” in 41.07% (69/168) of cases, as “possible” – in 22.02% (37/168) cases, as “probable” – in 29.76% (50/168) of cases, as “certain” – in 2.38% (4/168) of cases, as “doubtful” – in 4.76% (8/168) cases. The analysis of the frequency of patients’ AEs outcomes showed that “recovery without consequences” was observed in 79.76% (134/168) of cases, “improvement” in 19.05% (32/168) of cases, the outcome “unknown” – in 1.19% (2/168) of cases.

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Thus, the majority of reported AEs were not related to the study drugs. It is important to note that the AEs were transient in nature and did not require discontinuation of therapy.

According to the results of the intragroup analysis, a significant difference between the visits was found. It indicates the presence of positive dynamics in relation to the decrease in systolic blood pressure in the sibutramine + metformin group ($p<0.00001$) and in the sibutramine + MCC group ($p<0.2623$). It also shows the presence of positive changes in diastolic blood pressure in the sibutramine + metformin group ($p<0.0138$) and in the sibutramine + MCC group ($p<0.0135$). As a result of the comparative analysis, no significant differences between the groups of the study drugs have been identified. During the study, the patients did not experience an increase in resting heart rate ≥10 bpm or systolic/diastolic pressure ≥10 mmHg at two consecutive visits, no exclusion or discontinuation of therapy was required for these criteria. There was also no need to exclude patients due to increased blood pressure.

There was no negative effect of the studied therapy on ECG parameters, as well as indicators of clinical and biochemical blood tests.

As a result of a comparative statistical analysis of AEs in terms of their presence, severity, causal relationship with therapy and its outcomes, there were no intergroup differences ($p\geq0.05$). There were no reported cases of SAEs during the study, including deaths and other significant adverse events. None of the patients required discontinuation of therapy due to the development of AEs.

Thus, the study proved a high safety profile and good tolerability of sibutramine + MCC and sibutramine + metformin, which also speaks in favor of the hypothesis of the advisability of taking sibutramine + metformin in obese patients without additional disorders of carbohydrate metabolism.

## DISCUSSION

The present study was conducted to investigate the efficacy and safety of the sibutramine+metformin fixes dose combination, as well as to evaluate the additional benefits in the treatment of overweight patients.

Sibutramine has been successfully used in clinical practice for more than 15 years and is included in all clinical guidelines and standards for the treatment of obesity.

In recent years, it has been shown that metformin, which is the drug of the first choice for the treatment of patients with carbohydrate metabolism disorders, has the potential for a wider therapeutic use, demonstrating pleiotropic effects in relation to the pathogenetic links of the metabolic syndrome and obesity, which seems to be clinically significant for metabolic health. However, the weight loss dynamics against its background has been minimal. Therefore, it is optimal to include metformin in the treatment of obesity and overweight in combinations with other drugs registered for the treatment of this pathology. The previous studies have already demonstrated the benefit of using a unique fixed metformin+sibutramine combination in patients with obesity and carbohydrate metabolism disorders [45, 46]. The combination of central and peripheral actions, the impact on the main pathogenetic links in the development of obesity and associated comorbid conditions, the effects associated with facilitating the observance and consolidation of rational eating habits in the patient and, consequently, the consolidation of the result, as well as a high safety profile, determine the place of the drug containing a fixed metformin+sibutramine combination (Reduxin® Forte), in clinical guidelines and standards [1, 8]. That makes it a kind of benchmark in the treatment of obesity.

The evaluation of this drug use in relation to weight loss and restoration of metabolic health in patients with alimentary obesity, including those without impaired carbohydrate metabolism, is of great interest.

According to the National Clinical Guidelines for the Diagnosis, Treatment, and Prevention of Obesity and Associated Diseases [8], weight loss by 5% or more is one of the main criteria for assessing response to therapy. It provides a possible reduction in health risk and improvement in the course of diseases associated with obesity; maintaining the achieved result; improving the quality of patients' life. Thus, a decrease in body weight by 5% or more improves multi-organ insulin sensitivity and the function of pancreatic β-cells, it provides a protective effect against the risks of type 2 diabetes. Further weight loss provides additional benefits in terms of predicting cardiometabolic outcomes and reducing the risks of not only T2DM, but also other obesity-associated diseases, such as cardiovascular diseases, diseases of the musculoskeletal system and the reproductive system, oncological diseases, etc. [38].

The data of the study showed that the proportion of patients who had achieved more than 5% weight loss by 6 months of therapy in the sibutramine + metformin group, was 99.15%, in the sibutramine + MCC group – 93.64%, the differences were statistically significant. This confirms the effectiveness of the complementary action of the metformin+sibutramine combination in comparison with sibutramine+MCC combination. Moreover, it is important to notify that such a high response to treatment is currently one of the most effective in comparison with the use of other pharmacotherapy options [46, 47].

Over 6 months of therapy, more than 93% of patients treated with the fixed metformin+sibutramine combination, achieved 10% or more weight loss, which confirms a high effectiveness of this therapy type in achieving the true aim of obesity therapy – reducing the risk of complications associated with obesity and improving the forecast.

Quite an important indicator for both the practitioner and the patient is the rate of weight loss in kg. Moreover, the absence of the plateau effect onset is an important motivating factor and helps to eliminate food breakdowns. Here, it is necessary to notify more pronounced values against the background of the combination ther-

apy with sibutramine + metformin, where by the 3rd month, this figure was about 9 kg, and at the end of the study, the average weight loss was almost 15 kg, which was significantly more than in the comparison group.

Achieving a clinically significant effect of therapy when using the minimum dose of the drug, is a very important point. More than 90% of patients did not require an increase in study drug/comparator dose. This makes it possible to talk about a high frequency of an early response to therapy even when taking the minimum dose of sibutramine preparations. This may be especially important for patients in whom the use of a lower dose is preferable against the background of concomitant diseases, and is also relevant from the point of view of the possibility of increasing the dose with prolonged (more than 6 months) treatment, as this may allow to overcome the plateau effect if it occurs.

BMI is the main criterion for diagnosing obesity worldwide and the change in this indicator also reflects the effectiveness of the treatment. The results of the study confirm the advantage of the fixed metformin+sibutramine combination in terms of reducing BMI both after 3 and 6 months of therapy. As a result, BMI for six months of therapy decreased by 15.67%, which was 2% more than during the therapy with the sibutramine+MCC combination. In both groups, a decrease in the degree of obesity from visit to visit was proven, and at the end of the study in the sibutramine + metformin group, more than 60% of patients reached the BMI less than 30 kg/m2 and no longer met the criteria for the diagnosis of "Obesity".

In addition to BMI, an important anthropometric indicator is waist measurement and the ratio of waist to hip measurements. A decrease in waist measurement is a marker of a decrease in the amount of visceral fat and a decrease in the risk of complications. A decrease in this indicator of about 12 cm has been demonstrated during the treatment with sibutramine preparations, along with a decrease in the ratio of waist to hip measurements.

It should be notified that an increase in the duration of the course up to 6 months increases the effectiveness of the treatment, which indicates the feasibility of obesity long-term therapy using the fixed metformin+sibutramine combination to reduce body weight and reduce the risk of complications associated with obesity. These data are consistent with Russian and international clinical guidelines [1, 8, 48].

Of course, along with anthropometric indicators, it is important to assess the effect of therapy on metabolic parameters, in particular, indicators of atherogenic blood fractions, since the normalization of the lipid profile of an obese patient improves the prognosis for cardiometabolic risks and indicates the restoration of metabolic health in general. The results of the lipid profile analysis in patients showed a decrease in cholesterol, TG, LDL and an increase in HDL, and in the group of therapy with the metformin+sibutramine combination, the dynamics of the parameters under consideration was more pronounced.

Improving the overweight patients' quality of life is an important parameter for achieving surrogate endpoints of therapy. During the study, it was found out that treatment with sibutramine preparations was accompanied by a statistically significant improvement under the condition of all assessed scales; the psychological and physical components of health differed statistically significantly by each visit.

The most important component of the algorithm for monitoring the safe pharmacotherapy of obesity is the control of the cardiovascular system state. During the study, ECG parameters, systolic and diastolic kinds of blood pressure and heart rate remained stable in both groups.

The study demonstrated good tolerability of sibutramine therapy. During the study, there were no reported cases of SAEs, including deaths and other significant adverse events. AEs were transient and did not require discontinuation of treatment.

Thus, the composition of the metformin+sibutramine fixes dose combination makes it an optimal and safe choice for achieving therapeutic goals in the treatment of obesity and the restoration of metabolic health.

## CONCLUSION

The feasibility and importance of weight loss, especially through effective and safe pharmacotherapy, is an indisputable fact. At the same time, the goals of obesity therapy include not only weight loss, but also the improvement of metabolic health parameters, as well as reducing the risk of developing comorbid diseases and complications associated with excess visceral fat.

The conducted "Open multicenter randomized study to evaluate the efficacy and safety of the use of the drug Reduxin® Forte, film-coated tablets, in comparison with the drug Reduxin®, capsules, in patients with alimentary obesity", showed a significant improvement in body weight, waist measurement and BMI, as well as the main metabolic parameters and patients' quality of life against the background of the fixed metformin+sibutramine combination. The data obtained prove the effectiveness of this therapy type in terms of normalizing metabolic health, reducing the risk of developing comorbid diseases and improving the prognosis in obese patients even without additional carbohydrate metabolism disorders. This fact was reflected in the medical instructions' changes concerning the use of the drug in question and the official approval of its use in patients with alimentary obesity without even without additional disorders of carbohydrate metabolism (impaired glucose tolerance, T2DM)[5]. It is worth considering the inclusion of weight loss aid, comprising the subscription of this type of ther-

[5] Russian State Register of Medicines. Reduxin® Forte. Available from: https://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=108c4981-0dbb-4bdc-a3fe-08395333313f. Russian

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apy, in clinical examination programs and clinical recommendations for the treatment of non-communicable diseases allied with metabolic disorders (CVD, T2DM, NAFLD, etc.) in order to prevent the development of the latter or their complications.

Taking into account the contribution of obesity to the pathogenesis of a wide range of non-communicable and even infectious diseases, and the proven positive effect of weight loss on improving the prognosis of patients, it is advisable to conduct further clinical studies to identify new possible niches for the use of this type of therapy.

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## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

## AUTHORS' CONTRIBUTION

DTYu – developing the concept of a clinical trial, analysis of the results, correction of the text; IMYa – data collection and processing, the article writing; USE – data collection and processing, the article writing; ZKYa – developing the design and concept of the study, the article writing; OAA – data collection and processing, the article writing; PVV – data collection and processing, the article writing, NME – the article writing and proofreading, BPA – text correction, consolidation of results, organization of statistical processing.

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1. Инструкция по медицинскому применению, РУ ЛП 005315 от 25.01.2019. 2. Аметов А.С. и др. Эндокринология: новости, мнения, обучение. 1, 2020, 17-26. 3. Аметов А.С., Пьяных О.П. и др. Эндокринология: новости, мнения, обучение, 2020, 2, 40-48.

ИНФОРМАЦИЯ ПРЕДНАЗНАЧЕНА ДЛЯ СПЕЦИАЛИСТОВ ЗДРАВООХРАНЕНИЯ

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