Научно-практический журнал
Scientific and Practical Journal

ISSN 2307-9266
e-ISSN 2413-2241

ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

PHARMACY & PHARMACOLOGY

2019 Том / Volume VII

№ 3

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Scientific and Practical Journal

PHARMACY & PHARMACOLOGY

Scientific and practical journal
Volume VII, Issue 3, 2019
The mass media registration certificate:
ПИ №ФС77–67428 от 13.10.2016
ISSN 2307-9266 e-ISSN 2413-2241

Editors office address: 357532, Pyatigorsk, Kalinina, 11.
Pyatigorsk Medical and Pharmaceutical Institute – branch of Volgograd State Medical University
Phone number: +7(8793) 32-44-74. E-mail: pharmjournal@mail.ru
www.pharmpharm.ru
Union catalogue. Russian Press / Newspapers an journals. Code 94183
A4 size, 1000 issues circulation.

Journal "Pharmacy & Pharmacology' is recommended International Comittee Of Medical Journal Editors and included in Scopus, Higher Attestation Commission, Russian citation database, eLibrary, ARISTI (All-Russian Institute of Scientific and Technical Information), RSL (Russian State Library), CyberLeninka, Socionet, EMBASE, Chemical Abstracts (CAS), Directory of Open Access Journals (DOAJ), EBSCO Discovery Service, RNMJ, University of CAMBRIDGE, Ulrich'sWeb, Google Scholar, Biefeld Academic Search Engine (BASE), Directory of Open Access Scholarly Resources (ROAD), Research Bible, Open Archives Initiative, Academic Keys, JournalTOCs, WorldCat, OpenAIRE, University of Oxford, The British Library, Universitait Gent, Université de Montréal, University of Saskatchewan.

Printed in the LLC "Amirit" in accord with provided materials, 410004, Saratov, 88, Chernishevsky Str.

Научно-практический журнал

ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

Периодичность 6 номеров в год

Том 7, Выпуск 3, 2019

Свидетельство регистрации СМИ:
ПИ №ФС77–67428 от 13.10.2016 г.

ISSN 2307-9266 e-ISSN 2413-2241

Адрес редакции: 357532, г. Пятигорск, пр-т Калинина, 11.
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www.pharmpharm.ru
Объединенный каталог. Пресса России. Газеты и журналы. Индекс 94183
Формат А4, тираж 1000 экз.

Журнал «Фармация и фармакология» включен в перечень рецензируемых научных изданий, входящих в международные реферативные базы данных и системы цитирования, и в соответствии с пунктом 5 правил формирования перечня рецензируемых научных изданий, в которых должны быть опубликованы основные научные результаты диссертаций на соискание ученой степени кандидата наук, на соискание ученой степени доктора наук (Перечень ВАК), Scopus, РИНЦ, eLibrary, ВИНИТИ, РГБ, Киберленинка, Соционет, EMBASE, Chemical Abstracts (CAS), Directory of Open Access Journals (DOAJ), EBSCO Discovery Service, RNMJ, University of CAMBRIDGE, Ulrich'sWeb, Google Scholar, Biefeld Academic Search Engine (BASE), Directory of Open Access Scholarly Resources (ROAD), Research Bible, Open Archives Initiative, Academic Keys, JournalTOCs, WorldCat, OpenAIRE, University of Oxford, The British Library, Universitait Gent, Université de Montréal, University of Saskatchewan.

Отпечатано в соответствии с предоставленными материалами в ООО «Амирит», 410004, г. Саратов, ул. Чернышевского, 88.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

CONTENS / СОДЕРЖАНИЕ

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

STUDY OF *NEPETAE CATARIAE* HERBA FRUITS AS PROMISING MEDICINAL PLANT RAW MATERIAL

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Received 21.11.2018 Review 1 09.04.2019 Review 2 06.05.2019 Accepted for publication: 22.05.2019

Nepetae catariae herba is used in the Russian Federation as spice. The chemical compounds of herb have been studied. This plant has been introduced into the culture. There are its domestic varieties. The fruits of the plant accumulate up to 25% of fatty oil and contain specific laballenic acid, which has a wide range of antimicrobial and fungicidal actions. The yield of fruit crops is 5–6 c/ha.

The aim is to study some criteria for the standardization of the quality of *Nepetae catariae* herb as a new medicinal plant raw material.

Materials and methods. The studied fruits are from the biological collection of Federal State Budgetary Institution of All – Russia Research Institute of Medicinal and Aromatic Plants. Binocular magnifier brand MBS-10 and Axioplan 2 imaging microscope by Carl Zeiss were used. The sample preparation was carried out according to the State Pharmacopoeia of the Russian Federation (XIV edition). Anatomical diagnostic features were studied in powder and cross section with staining reagents for the presence of some biologically active substances. Qualitative reactions were carried out with water and alcohol-water extracts from the fruits. The content of the lipid complex according to pharmacopoeia monograph 2.5.0035.15 has been determined.

Results. A description of the external and diagnostic anatomical features is given. The main groups of biologically active substances and the content of the lipid complex in a possible new material – the fruits of *Nepeta cataria* – have been identified.

Conclusion. The description of the external features of the fruit has been specified. For the first time, crushed fruits have been characterized. It has been established that the morphology of endocarp cells and seed embryo cells are best preserved in a mellow fruit. Physico-optical properties of cellular structures and the ability for basic microchemical reactions are preserved in all zones of pericarp and seeds. Qualitative reactions showed the presence of the following components in the fruits: saponins, flavonoids and a lipid complex. A dispersion composition has been studied. The yield of the lipid complex and its appearance have been determined. Fruits can be used as promising fat-oil raw materials. The results of the study can be used in drafting Pharmacovigilance Reference Document considering a promising type of medicinal plant raw material on the basis of *Nepetae catariae* herba fruits.

Keywords: *Nepeta catariae*, fruits, external features, fatty oil, dispersion analysis

For citation: A.G. Devyatov, G.S. Lapshin, E.Yu. Babaeva, E.A. Motina, E.V. Zvezdina, V.V. Vandishev. Study of nepetae catariae herba fruits as promising medicinal plant raw material. *Pharmacy & Pharmacology*. 2019;7(3): 120-128. **DOI:** 10.19163/2307-9266-2019-7-3-120-128

Для цитирования: А.Г. Девятов, Г.С. Лапшин, Е.Ю. Бабаева, Е.А. Мотина, Е.В. Звездина, В.В. Вандышев. Изучение плодов котовника кошачьего (fructus nepetae catariae) как перспективного лекарственного растительного сырья. *Фармация и фармакология.* 2019;7(3): 120-128. **DOI:** 10.19163/2307-9266-2019-7-3-120-128

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2019-7-3-120-128

ИЗУЧЕНИЕ ПЛОДОВ КОТОВНИКА КОШАЧЬЕГО (FRUCTUS NEPETAE CATARIAE) КАК ПЕРСПЕКТИВНОГО ЛЕКАРСТВЕННОГО РАСТИТЕЛЬНОГО СЫРЬЯ

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Получено 21.11.2018 Рецензия 1 09.04.2019 Рецензия 2 06.05.2019 Принята к печати 22.05.2019

Траву котовника кошачьего (Herba *Nepetae catariae*) в РФ используют как пряность. Изучен ее химический состав. Растение введено в культуру, имеются отечественные сорта. Плоды растения накапливают до 25% жирного масла и содержат специфичную лабалленовую кислоту, обладающую широким спектром антимикробного и фунгицидного действия. Урожайность плодов 5–6 ц/га.

Цель исследования – изучение некоторых критериев для стандартизации качества плодов котовника кошачьего как перспективного лекарственного сырья.

Материалы и методы. Плоды биоколлекции ФГБНУ ВИЛАР. Использовали бинокулярную лупу марки МБС-10, микроскоп Axioplan 2 imaging Carl Zeiss. Пробоподготовка согласно ГФ РФ XIV издания. Анатомические диагностические признаки изучали в порошке и на поперечном срезе с окрашиванием реактивами на присутствие некоторых БАВ. Качественные реакции проводили с водным и спиртоводным извлечениями из плодов. Определение содержания липидного комплекса согласно ФС 2.5.0035.15.

Результаты. Дано описание внешних и диагностических анатомических признаков, определены основные группы БАВ и содержание липидного комплекса в возможном новом сырье – плоды котовника кошачьего.

Заключение. Уточнено описание внешних признаков плодов, впервые охарактеризованы измельченные плоды. Установлено, что в зрелом плоде лучше всего сохраняется морфология клеток эндокарпия и зародыша семени. Физико-оптические свойства клеточных структур и способность к основным микрохимическим реакциям сохраняются во всех зонах перикарпия и семени. Качественными реакциями показано наличие в плодах: сапонинов, флавоноидов и липидного комплекса. Изучен дисперсный состав. Определен выход липидного комплекса и его внешний вид. Плоды могут служить перспективным сырьем для получения жирного масла и препаратов на его основе. Результаты исследования могут быть использованы при составлении проекта НД на перспективный вид сырья «Fructus Nepetae catariae – Плоды котовника кошачьего».

Ключевые слова. *Nepeta cataria*, плоды, внешние признаки, липидный комплекс, микроскопия, дисперсный анализ

Список сокращений: ЛРС – лекарственное растительное сырье, НД – нормативная документация

INTRODUCTION

Nepeta cataria L. is a plant of the *Lamiaceae* family [1]. The Nepeta genus L. was referred to the Nepetoideae subfamily, the *Nepetae* tribe, by A.L. Budantsev [2]. *Nepeta cataria* occurs in the flora of the Commonwealth of Independent States [3, 4]. The plant is also registered in Central and Southern Europe up to Central Asia; North and South America [5].

The herb of *Nepeta cataria* contains essential oil, iridoids, which include nepetolactone; triterpene saponins, phenols and their derivatives, phenolcarboxylic, hydroxycinnamic acids and their derivatives, flavonoids [6–8]. *Herba Nepetae catariae* is found in teas with antipyretic and sedative effects [9].

Infusion of *Herba Nepetae catariae* reduces the temperature and decreases skin rashes with measles and chicken pox. It is considered an effective and harmless sedative remedy [10]. The essential oil and extracts from *Herba Nepetae catariae* obtained by non-polar and polar organic solvents, exhibit cytotoxic activity and affect the apoptosis of PC3, DU-145 and MCF-7 cell lines [11]. Researchers from Bulgaria showed that *Herba Nepetae catariae* extract in 70% ethanol exhibits antioxidant activity [12].

Herba *Nepetae catariae* used to be harvested from wild plants. However, the demand for essential oil and the herb for the production of spice has increased. This plant has been introduced into the culture. As a result of selection work, varieties of *Nepeta cataria* "Kentavr", "Basilio", "Goluboy ineyi", "Barkhatt" with a pronounced lemon scent are zoned in Russia [13].

According to the literature data, the fruits of *Nepeta cataria* are rich in fatty oil, the content of which reaches 25%. The characteristic feature of the fruits of the *Lamiaceae* family is the presence of fatty acids with an allenic group in them. Most often such acids are represented by labullenic acid (Fig. 1).

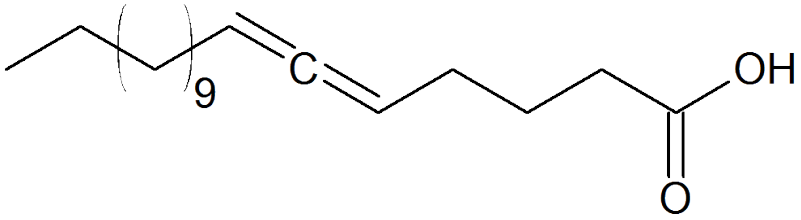

Figure 1. Labullenic acid

Allenic acids have a wide range of antimicrobial and fungicidal actions [15]. Labullenic acid is also founds in fatty oil of *Nepeta cataria* fruits [14]. The yield of *Nepeta cataria* fruit crops is 5–6 c/ha, and a standard quantity of seed per hectare in herb cultivated is 6–7 kg. [16]. The seed material is fruits (eremas). It is necessary to have a seed insurance fund when growing crops.

The fruits unused for sowing, in case of an excess of insurance material, or substandard low-germination fruits, can be recycled to obtain fatty oil. Thus, study of *Nepeta cataria* fruits as a promising kind of medicinal raw material, is relevant.

THE AIM of the work is to study some criteria for the standardization of the quality of *Nepeta cataria* fruit as a new promising type of medicinal plant raw material.

To achieve the aim of the work, the following tasks were set:

to establish diagnostic features in the morphology and anatomical structure of *Nepeta cataria* fruits;

to carry out qualitative reactions;

to determine the content of the lipid complex in *Nepeta cataria* fruits.

MATERIALS AND METHODS

Medicinal plant raw material

Nepeta cataria fruits were obtained from the plants raised in the All Russia Research Institute of Medicinal and Aromatic Plants Botanic Garden (Central district, nonchernozem belt), during the period of 2016–2018.

Microscopic study

A binocular magnifier brand MBS-10 and a Axioplan 2 imaging microscope by Carl Zeiss were use for conducting the research. The harvested fruits were prepared for the anatomical research, according to the methods described in the State Pharmacopoeia of the Russian Federation (XIV edition) and the reference book on botanical microtechnology [17–19].

Histochemical study

For carrying out an appropriate investigation of the optical properties of cell membranes and intracellular structures, the microchemical study of microscope slides was undertaken in transmitted polarized light with crossed axes of the polarizer and analyzer.

To evaluate the distribution of flobaphenes, the sections were not stained, since flobaphenes have their own brown color;

To determine the localization of lignin, the lignification reaction with a 1% solution of phloroglucinol and concentrated hydrochloric acid was carried out;

To elicit the presence of starch, the sample was processed with a solution of iodine in potassium iodide;

To identify the distribution of proteins, the xanthoproteic reaction was carried out;

To determine the localization of lipids and other non-polar substances the sample was processed with a Sudan III solution [17].

Qualitative reactions were carried out with water- and alcohol-water extracts [20]. Fruit crushing was produced using a mechanical mill. The used reagents were: 5% aqueous solution of NaOH, 2% alcohol solution of $AlCl_3$, HCl conc. plus powder Mg.

Quantitative determination

The study of the lipid complex level was carried out according to the method described in monograph 2.5.0035.15 (*"Sílybum mariánum fruits")* of the Russian State Pharmacopoeia (XIV edition). [21]. A precisely weighed quantity of the crushed medicinal plant raw material for the analysis was about 10.0 g. The extractant was removed from the extract using a vacuum rotary evaporator in the water bath at the temperature of +50° C and a residual pressure of 0.25 mm Hg.

RESULTS AND DISCUSSION

Morphological examination

A general review of the external features of the *Lamiaceae* family fruits is given in "Comparative Seeds Anatomy" [18]. The morphological features of *Nepeta cataria* fruits are listed in the directory by V. Brouwer and A. Shtelin [5]. The external features of the fruits

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DOI: 10.19163/2307-9266-2019-7-3-120-128

have been adjusted in the article by A.L. Budantsev and T.A. Lobova: “Erem ovoid, 1.2–1.5 mm long, 0.8–1 mm wide, 0.7–0.9 mm thick”.

The abaxial side is convex, with a round edge, the adaxial side is obtuse roof-like. The hilum forms two white “eyes” in the shape of the letter V (areola) at the base of the adaxial side. The surface is slightly rough, matte. The color is from red-brown to black [19]. The fruits studied, were completely consistent with the descriptions in the mentioned above literature. The appearance of *Nepeta cataria* fruits from the adaxial side, where the areola is visible, is in fig. 2. The appearance of *Nepeta cataria* fruits from the abaxial side with the rounded edge is in Fig. 3.

The description of the fruit features, have been supplemented with characteristics of the areola. Each part of the white areola has a size of 0.25–0.30 mm, there is a dark brown gap of 0.15–0.20 mm between them.

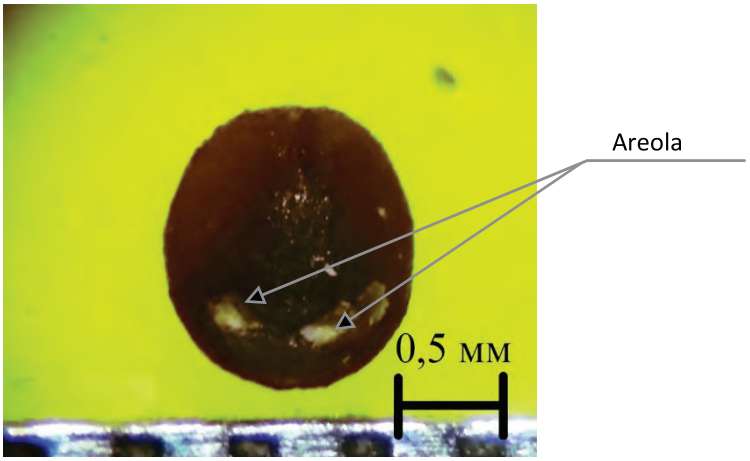

Figure 2. The adaxial side of Nepeta cataria fruit

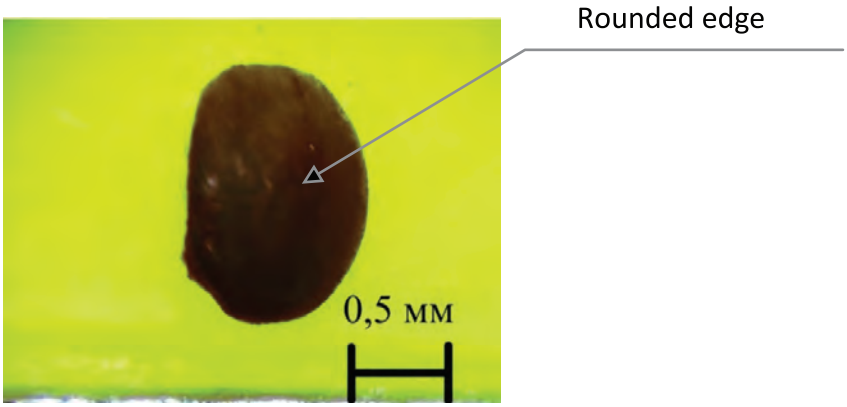

Figure 3. The abaxial side of Nepeta cataria fruit

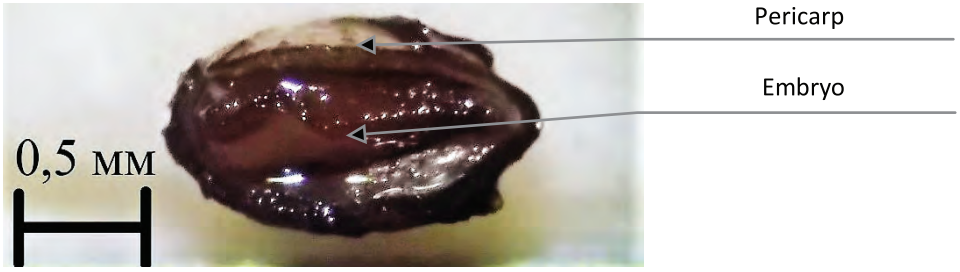

Figure 4. Lengthwise section of the erem

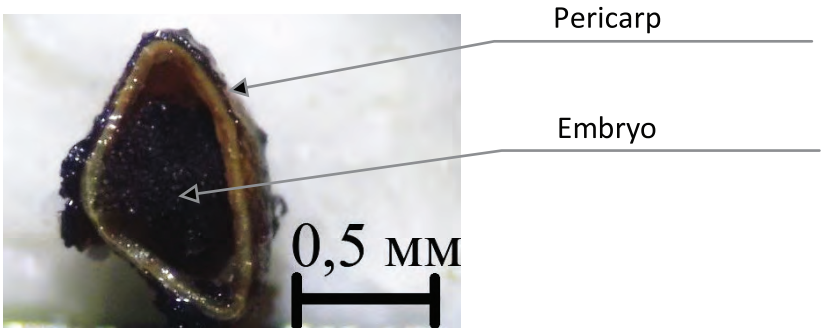

Figure 5. Cross section of the erem

The authors also suggest characterizing the shape of the cross and lengthwise sections of the fruit at half the length / width of the erem when describing the external features (Fig. 4, 5).

The cross section contour is a smoothed triangle, where the convex part corresponds to the rounded edge. The lengthwise section of the fruit is elliptical. In the middle, cotyledons of the embryo are visible. The shape of the contour of the sections can be used for diagnosis and in establishing the authenticity of the studied raw materials by the method of macroscopy.

The ultrastructure of the fruits exocarp has been studied by Romanian researchers using a scanning electron microscope. The results are presented in the work [24].

Microscopic examination. At the cross section of the fruit there is single-layer exocarp. It consists on radially flattened cells with thickened outer non-ligated walls (Fig. 6). The mesocarp is two- or three-layered with thin-walled obliterated cells, between which the boundaries are invisible. The cell shells of exo- and mesocarp are optically isotropic with a slightly alkaline reaction. The cell content is colored dark brown or black by flobaphenes.

The endocarp is two-layered. The outer layer consists of radially elongated cells with thickened walls and a small rounded cavity. Numerous pore channels are noticeable in the outer walls of the endocarp. The shells of endocarp cells are acidic, optically anisotropic, non-ligated and give a positive protein response. The cells contain a light-brown substance. The inner layer of the endocarp consists of isodiametric empty cells with unthickened, optically anisotropic shells.

An important diagnostic feature is the lack of lignin response of the outer layer membranes of the endocarp.

Spermoderma is two-layered. The outer spermoderma layer consists of obliterated cells with light brown content. The inner layer of the spermoderma consists of elongate in the periclinal direction cells with slightly thickened, optically anisotropic shells. Their reaction is slightly alkaline.

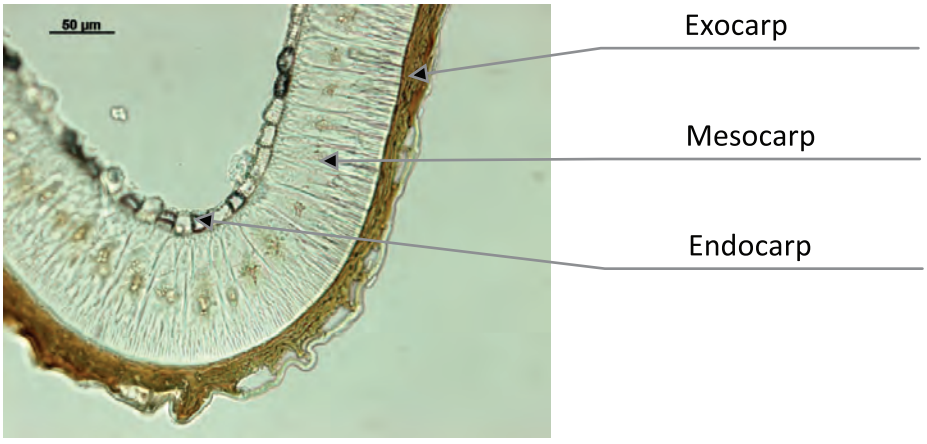

Figure 6. Fragment of the cross section of Nepeta cataria fruit

The embryo is composed of isodiametric thin-walled cells. The shells and walls of these cells are optically isotropic. There are structures with double refraction in the cell content of the embryo (Fig. 7).

The cell content gives a positive xanthoproteic reaction. The germ cells absorbed iodine. Their color became dark blue. This indicates the presence of starch. As a result of histochemical reaction with Sudan III, the vestige of the cuticle, the cell membrane of the outer layer of the mesocarp, numerous drops of fatty oil in the endosperm and the embryo turned orange.

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DOI: 10.19163/2307-9266-2019-7-3-120-128

Figure 7. Fragment of cotyledon cross section of Nepeta cataria embryo

The fruits were crush before receiving the lipid complex. It was a mixture of pericarp fragments and cotyledons (Fig. 8).

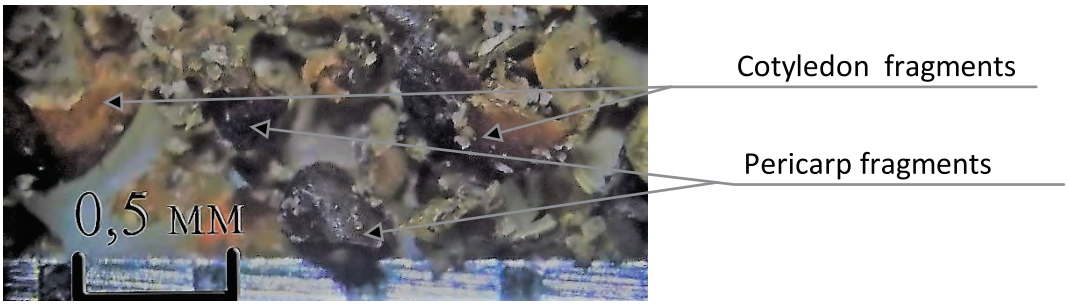

Figure 8. Crushed Nepeta cataria fruits

We noted the presence of numerous drops of fatty oil in the study of micropreparations of crushed fruits was noted (Fig. 9).

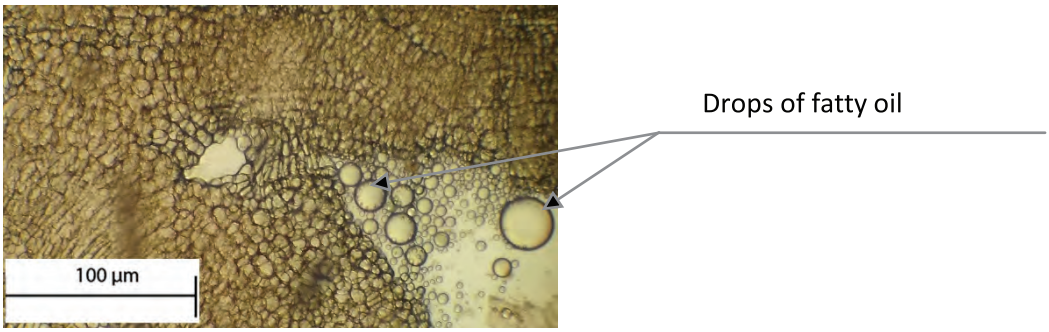

Figure 9. Fragment of cotyledon of *Nepeta cataria* embryo viewed from the surface

When adding reagent Sudan III, the drop of fatty oil turned orange (Fig. 10).

ISSN 2307-9266 e-ISSN 2413-2241

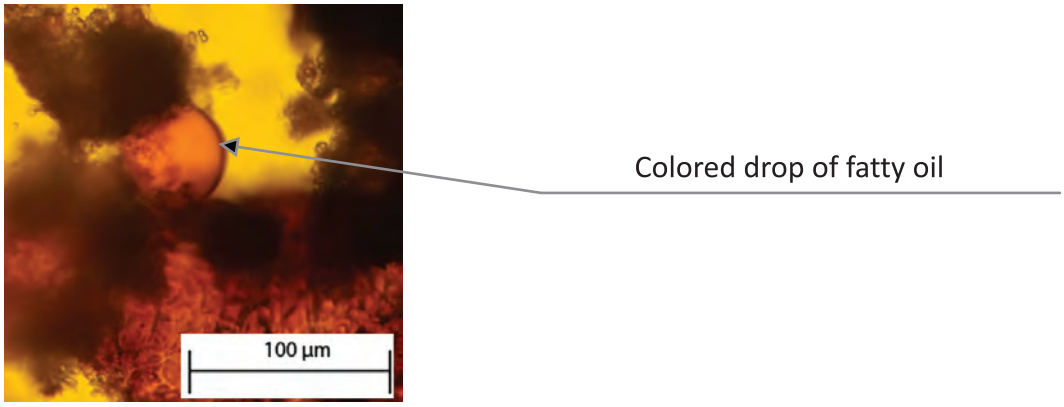

Figure 10. Fragment of Nepeta cataria fruit powder

Dispersed analysis

The dispersed analysis showed that the composition of whole fruits could be established using a set of sieves. The fruits passed through a 2 mm sieve, and the main fraction was localized on a sieve with 1 mm openings in diameter. After crushing, fruit powder was obtained, hereby, the main fraction of the particles was located on a sieve with 0.25 mm openings in diameter.

The micropreparations of the fruit powder were fragments of cotyledons and pericarp. On the exocarp fragment there are polygonal cells with evident thickenings of the walls (Fig. 11).

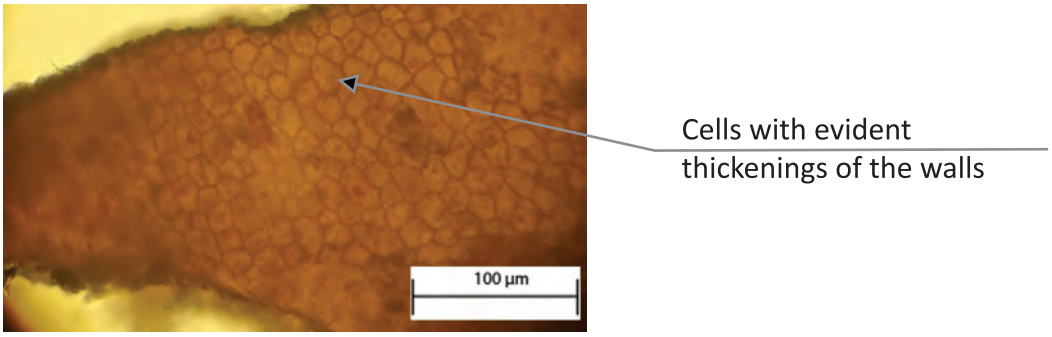

Figure 11. Fragment of Nepeta cataria fruit exocarp

Fragments of exocarp epidermis were observed the among the particles of the crushed fruits (Fig. 12). These are polygonal isodiametric cells. They do not contain pigment.

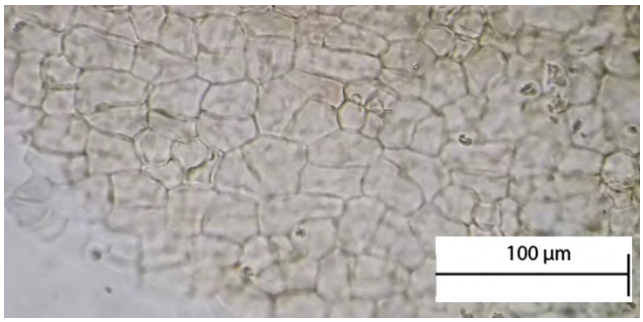

Figure 12. Fragment of exocarp epidermis of Nepeta cataria fruit

On the fruit epidermis the presence of 4-cell trichomes has been established. They are similar to the essential oil glands on the epidermis of *Nepeta cataria* leaf blade (Fig. 13) [25].

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DOI: 10.19163/2307-9266-2019-7-3-120-128

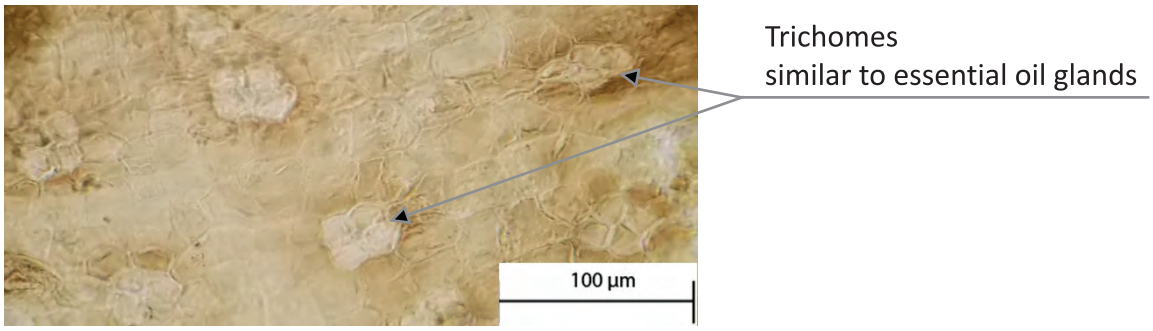

Figure 13. Fragment of exocarp epidermis of Nepeta cataria fruit with trichomes

Lipid complex was obtained from whole and crushed *Nepeta cataria* fruits. In terms of absolutely dry raw material from whole raw material, its content was 9.58%. From crushed raw material it was 26.72%. It corresponds to the literature data [14].The lipid complex looked a fluent, oily, turbid yellowish liquid.

Qualitative reactions. Qualitative reactions were conducted with the crushed fruits on the presence of some biologically active compounds. In *Nepeta cataria* fruits there are such types of biologically active substances as flavonoids and saponins. These data can be also used to establish the authenticity of *Nepeta cataria* fruits.

CONCLUSION

As a result of the investigation of whole and crushed *Nepeta cataria* fruits, a description external features and peculiarities of the anatomical structure has been given, and qualitative reactions have been carried out. It has established that morphology of endocarp and seed embryo cells are best preserved in mature fruits. Physico-optical properties of cellular structures and the ability for basic microchemical reactions are preserved in all zones of pericarp and semen. An important diagnostic feature is the lack of lignin response of the outer layer membranes of the endocarp.

The contour of the cross and lengthwise sections of the fruit has been characterized, a description of epidermis cells and trichomes on it has been given. The presence of saponins, flavonoids and lipid complex in fruits is shown by qualitative reactions. Dispersion composition has been studied. The yield of the lipid complex has been determined, characteristics of its external features have been given.

Due to the presence of laballenic acid in fatty oil of fruits and its wide antimicrobial and antifungal activity, it can be considered a valuable source of phytochemical drugs. The results of the study can be used in drafting Pharmacovigilance Reference Document considering a promising type of medicinal plant raw material on the basis of *Nepetae catariae* fruits.

REFERENCES

1. The Plant List: Nepeta cataria var. citriodora (Dumort.) Lej. [Internet]. Kew (GB): Royal Botanic Gardens, Kew; 2013 [cited 2018 Oct 14]. Available from http://www.theplantlist.org/tpl1.1/record/kew-340504/. Joint publication with the Missouri Botanical Garden.
2. Budantsev AL. Konspekt roda Nepeta (Lamiaceae) [Conspectus of the genus Nepeta (Lamiaceae)]. Bot Z. 1993;78(1):91–105. Russian.
3. Kariagin II, editor. Flora Azerbaĭdzhana. Vol. 7. Baku: Izdatel'stvo Akademii Nauk Azerbaĭdzhanskoĭ SSR; 1957. Species 2952, N. cataria L.; p. 258–9. Russian.
4. Mammadova ZA. [Studying of distribution areas of species of Catnip genus (Nepeta L.) in Azerbaijan, their morpho-biological features and estheroils]. Hortus botanicus. 2012;7:1–3. Russian.
5. Brouwer W, Shtelin A. Spravochnik po semenovedeniiu sel'skokhoziaĭstvennykh, lesnykh i dekorativnykh kul'tur s kliuchom dlia opredeleniia vazhneĭshikh semian. Leunova VI, translator. Moscow: Tovarishchestvo nauch. izd. KMK; 2010. 694 p. Russian.
6. Terninko II, Nguyen THY. [Identification of the phenolic compounds of catnip herbs (Nepeta cataria L.)]. Drug Dev Registr. 2017;18(1):120–4. Russian.
7. Budantsev AL, Shavarda AL, Medvedeva NA, Petrova NV, Leostrin AV. [Content of rosmarinic acid in leaves of some species of Lamiaceae and Boraginaceae families] Rastitelnye Resursy. 2015;51(1):105–116. Russian.
8. Süntar I, Nabavi SM, Barreca D, Fischer N, Efferth T. Pharmacological and chemical features of Nepeta L. genus: Its importance as a therapeutic agent. Phytother Res. 2018 Feb;32(2):185–198. doi: 10.1002/ptr.5946. Epub 2017 Oct 18.
9. Terninko II, Nguyen THY. [Criteria for the standardization of raw medicinal plant materials in the countries of the EAEU and the EU]. Farmatsiia 2016;65(8):5–8. Russian.
10. Mamadalieva NZ, Akramov DK, Ovidi E, Tiezzi A, Nahar L, Azimova SS, Sarker SD. Aromatic medicinal plants of the Lamiaceae family from Uzbekistan: ethnopharmacology, essential oils composition, and biological activities. Medicines (Basel). 2017 Feb 10;4(1). pii: E8. doi:10.3390/medicines4010008.
11. Emami SA, Asili J, Hossein Nia S, Yazdian-Robati R, Sahranavard M, Tayarani-Najaran Z. Growth inhibition and apoptosis induction of essential oils and extracts of Nepeta cataria L. on human prostatic and breast cancer cell lines. Asian Pac J Cancer Prev. 2016;17(S3):125–30. doi: 10.7314/apjcp.2016.17.s3.125.

12. Mihaylova D, Georgieva L, Pavlov A. In vitro antioxidant activity and phenolic composition of Nepeta cataria L. extracts. Int J Agric Sci Technol. 2013 Nov;1(4):74–9.
13. Pivovarov VF. Ovoshchi Rossii. Moscow: GNU VNIISSOK; 2006. 384 p. Russian.
14. Panekina TV, Gusakova SD, Umarov AU. Maslo semian Nepeta cataria [Nepeta cataria seed oil]. Him Prir Soedin. 1978;2:174–6. Russian.
15. Gusakova SD, Umarov AU. Masla semian sem. Labiatae [The Labiatae family seed oils]. Him Prir Soedin. 1978;1:57–63. Russian.
16. Mashanov VI, Pokrovskiĭ AA. Prianoaromaticheskie kul'tury. Moscow: Agropromizdat; 1991. 287 p. Russian.
17. Barykina RP, Veselova TD, Deviatov AG, et al. Spravochnik po botanicheskoĭ mikrotekhnike: osnovy i metody. Moscow: Izdatelstvo Moskovskogo Universiteta; 2004. 312 p. Russian.
18. Gosudarstvennaia farmakopeia Rossiĭskiĭ Federatsii [The State Pharmacopoeia of the Russian Federation] [Internet]. 14th ed. Vol. 2. Moscow: Nauchnyĭ tsentr ėkspertizy sredstv meditsinskogo primeneniia (RU); 2018. OFS.1.5.1.0007.15, Plody; [cited 2018 Dec 1]; p. 2262–9. Available from: http://resource.rucml.ru/feml/pharmacopia/14_2/HTML/447/index.html Russian.
19. Gosudarstvennaia farmakopeia Rossiĭskiĭ Federatsii [The State Pharmacopoeia of the Russian Federation] [Internet]. 14th ed. Vol. 2. Moscow: Nauchnyĭ tsentr ėkspertizy sredstv meditsinskogo primeneniia (RU); 2018. OFS.1.5.3.0003.15, Tekhnika mikroskopicheskogo i mikrokhimicheskogo issledovaniia lekarstvennogo rastitel'nogo syr'ia i lekarstvennykh rastitel'nykh preparatov; [cited 2018 Dec 1]; p. 2327–48. Available from: http://resource.rucml.ru/feml/pharmacopia/14_2/HTML/513/index.html Russian.
20. Blinova KF, editor. Fitokhimicheskiĭ analiz lekarstvennogo rastitel'nogo syr'ia: metodicheskie ukazaniia k laboratornym zaniatiiam. Saint Petersburg: Izdatel'stvo SPKhFA; 1998. 60 p.
21. Gosudarstvennaia farmakopeia Rossiĭskiĭ Federatsii [The State Pharmacopoeia of the Russian Federation] [Internet]. 14th ed. Vol. 4. Moscow: Nauchnyĭ tsentr ėkspertizy sredstv meditsinskogo primeneniia (RU); 2018. OFS.2.5.0035.15, Rastoropshi piatnistoĭ plody; [cited 2018 Dec 1]; p. 6369-75. Available from: http://resource.rucml.ru/feml/pharmacopia/14_4/HTML/1187/index.html Russian.
22. Anisimova GM, Vyshenskaia TD, Gevorkian MM, et al. Sravnitel'naia anatomiia semian. Vol. 7. Takhtadzhian AL, editor. Saint Petersburg: Nauka; 2010. 468 p. Russian.
23. Budantsev AL, Lobova TA. Fruit morphology, anatomy and taxonomy of tribe Nepeteae (Labiatae). Edinb J Bot. 1997;54(2):183–216.
24. Padure IM, Toma I. Ultrastructural features of pericarp surface in Nepeta species (Lamiaceae, Nepetoideae). Acta Hortic. 2004;32:45-50.
25. Nguyen THY, Yakovleva OV, Terninko II. Comparative morphological and anatomical studies of two herbal drugs: Nepeta cataria L. and Melissa officinalis L. J Pharm Sci Res. 2017;9(12):2463–7.

FINANCIAL SUPPORT AND SPONSORSHIP

This work has been carried out on the subject "Analysis of structural and chorological diversity of higher plants in connection with problems of phylogeny, taxonomy and stabile development" No. AAAA-A16-116021660045-2.

AUTHOR CONTRIBUTIONS

All authors had equally contributed to the research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2019-7-3-129-137

STUDY OF BAICALIN HYDROLYSIS KINETICS IN THE PROCESS OF ITS EXTRACTION FROM SCUTELLARIA BAICALENSIS GEORGI ROOTS

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Received 21.11.2018 Review 1 09.04.2019 Review 2 06.05.2019 Accepted for publication: 22.05.2019

The aim of this study was to investigate the kinetics of baicalin hydrolysis in the process of its extraction from *Scutellaria baicalensis* Georgi roots.

Materials and methods. For the studies, *Scutellaria baicalensis* Georgi roots with a particle range of 0.1–0.5 mm were used. The method of extraction was a simple maceration during a specified period of time, the ratio of plant raw material : extractant was 1:10 w/v at the temperature of 24±1°C. Baicalin and baicalein contents were analyzed by reverse phase high performance liquid chromatography (RP HPLC) at the analytical wavelength of 275 nm. The extractant was a water solution of ethanol 26, 43, 59, 72, 81, 97±1% v/v. The time of the extraction was from 1 to 24 hours.

Results. The experimental points of dependency of baicalin concentration in the extract on the time of extraction for ethanol solutions with a concentration of 43 and 72% v/v are closely approximated by a linear equation in coordinates ln$C$=f(t). The value of determination coefficient is more than R^2>0,99. Half lifetime for baicalin has been calculated: for ethanol with the concentration of 43% v/v it is 4.3±0.7 hours, and for ethanol with the concentration of 72% v/v it is 42.3±1.8 hours.

Conclusion. Baicalin hydrolysis kinetics in the process of its extraction from *Scutellaria baicalensis* Georgi roots with 43 and 72% v/v ethanol concentration. has been studied. It has been established that the process of baicalin hydrolysis is well described by the first order kinetic equation. The constants of baicalin hydrolysis during its extraction from *Scutelaria baicalensis* roots with ethanol having different concentrations have been calculated. Recommendations on technology optimization for baicalin or baicalein extraction from *Scutellaria baicalensis* Georgi roots have been given.

Keywords: *Scutellaria baicalensis* Georgi roots, baicalin, baicalein, hydrolysis, first order reaction, half lifetime

For citation: N.N. Boyko, D.I. Pisarev, E.T. Zhilyakova, A.Yu. Maljutina, O.O. Novikov, M.A. Bocharnikova. Study of baicalin hydrolysis kinetics in the process of its extraction from scutellaria baicalensis georgi roots. *Pharmacy & Pharmacology*. 2019;7(3):129-137. **DOI:** 10.19163/2307-9266-2019-7-3-129-137

Для цитирования: Н.Н. Бойко, Д.И. Писарев, Е.Т. Жилякова, А.Ю. Малютина, О.О. Новиков, М.А. Бочарникова. Изучение кинетики гидролиза байкалина при его экстракции из корней шлемника байкальского. *Фармация и фармакология.* 2019;7(3): 129-137. **DOI:** 10.19163/2307-9266-2019-7-3-129-137

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

ИЗУЧЕНИЕ КИНЕТИКИ ГИДРОЛИЗА БАЙКАЛИНА ПРИ ЕГО ЭКСТРАКЦИИ ИЗ КОРНЕЙ ШЛЕМНИКА БАЙКАЛЬСКОГО

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Получено 21.11.2018 Рецензия 1 09.04.2019 Рецензия 2 06.05.2019 Принята к печати 22.05.2019

Цель. Изучить кинетику гидролиза байкалина при его экстракции из корней шлемника байкальского.
Материалы и методы. Корни шлемника байкальского с размером частиц 0,1-0,5 мм. Используемый метод экстракции – простая мацерация в течение заданного промежутка времени, при соотношении сырье: экстрагент 1:10 м/о и температуре 24±1°С. Содержание байкалина и байкалеина анализировали с помощью обратно-фазовой высокоэффективной жидкостной хроматографии (ОФ ВЭЖХ) при длине волны 275 нм. Экстрагент: водные растворы этанола 26, 43, 59, 72, 81, 97±1% об. Время настаивания от 1 до 24 часов.
Результаты. Экспериментальные точки зависимости концентрации байкалина в извлечении от времени настаивания для этанола с концентрацией 43 и 72% об., хорошо аппроксимируются линейным уравнением в координатах lnC=f(t). Коэффициент детерминации боле R^2>0,99. Рассчитано время полураспада байкалина в этаноле с концентрацией 43% об., которое составило 4,6±0,5 часа, в этаноле с концентрацией 72% об., данный показатель равен 42,3±1,8 часа.
Заключение. Изучена кинетика гидролиза байкалина при его экстракции из корней шлемника байкальского с помощью этанола с концентрацией 43 и 72% об. Установлено, что процесс гидролиза байкалина хорошо описывается кинетическим уравнением первого порядка. Найдены константы процесса гидролиза байкалина во время его экстракции из корней шлемника байкальского с помощью этанола различной концентрации. Даны рекомендации по оптимизации технологии выделения байкалина или байкалеина из корней шлемника байкальского.
Ключевые слова: корень шлемника байкальского, байкалин, байкалеин, гидролиз, реакция первого порядка, время полураспада

INTRODUCTION

Scutellaria baicalensis Georgi is a plant of *Lamiaceae* family that grows in the Russian Federation in Transbaikal, Amur River and Primorye regions, as well as in Mongolia and China. The plant raw material used for medicinal purposes is the root.

The root contains flavonoids (baicalin – more than 9%, baicalein – up to 5%, wogonoside – up to 4%, wogonin – up to 0.7%, scutelarein, etc.), steroids (beta-sitosterol, stigmasterol, etc.), coumarins and some other compounds [1, 2].

Biologically active compounds (BACs) from *Scutellaria baicalensis* Georgi roots have different useful pharmacological effects. They have effects on the central nervous system (sedative, hypotension, and anticonvulsant). BAC from *Scutellaria baicalensis* Georgi roots are useful for liver as they demonstrate hepatoprotective and antioxidant activities; besides, they decrease inflammation processes, inhibit the growth of pathogenic microorganisms (bacteria and viruses), have a cytotoxic effect on different cancer cell lines, etc. [3–18].

Therefore, BACs from this type of plant raw material (PRM) have useful pharmacological properties, and all kinds of research in the field of technology of their extraction is important.

According to the known scientific literature data, the type of extractant, the temperature and time of the extraction have a great influence on the qualitative and quantitative composition of the extract obtained [19, 20].

These kinds of influence are determined by the presence of the active form of beta-glucuronidase enzyme in the cells of *Scutellaria baicalensis* Georgi roots. After wetting the raw material with the extractant containing water, this enzyme starts hydrolyzing baicalin actively to its aglicone (baicalein) and glucuronic acid [21].

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DOI: 10.19163/2307-9266-2019-7-3-129-137

This fact should be taken into account during the development of quality control methods and extraction technology of BAC from this PRM.

Therefore, the decision to study the process of baicalin hydrolysis during its extraction from *Scutellaria baicalensis* Georgi roots was made up. Moreover, this information may be used as a starting point for optimization of BAC extraction technology from this type of PRM in further researches.

The aim of this study was to investigate the kinetic process of baicalin hydrolysis during its extraction from *Scutellaria baicalensis* Georgi roots.

MATERIALS AND METHODS

Object of investigation

Scutellaria baicalensis Georgi roots were purchased from LLC Pharmaceutical shop "Medicinal plants", Kharkiv, Ukraine, lot No. 921217, best before IX/2020. For the studies, the roots were ground to the particle fraction of 0.1 to 0.5 mm using high-speed multifunction grinder HC-500Y, China.

Extraction method

For the studies, a simple maceration method for a certain period of time was used. Hereby, the PRM:extractant ratio was 1:10 w/v at the temperature of 24±1°C. For that, a precisely weighed amount of 1.0 g of ground PRM was put into an airtight flask, 10.0 ml of the extractant was added, then the flask was sealed and left for a specified period of time. After that the extract was decanted, centrifuged at 3,000 rpm for 5 min. and delivered to the analysis for the contents of baicalin and baicalein.

Before the analysis, the extract was additionally centrifuged at 13,000 rpm for 5 min. Hydroethanolic solutions 26, 43, 59, 72, 81, 97±1% v/v were used as extractants.

Sample preparation

The analysis of the initial content of baicalin and baicalein in the plant raw material was carried out by a simple maceration method under the following conditions: ethanol 43% v/v was used as an extractant, the ratio of plant raw material : extractant was approximately 1:50 m/v, the extraction time was 30 min at the temperature of 95±5°C (water bath). A precisely weighed amount of 1.0 g of ground PRM was put into a flask, 50.0 ml of extractant (ethanol 43% vol.) was added; the flask was connected to a backflow condenser and the process of extraction took place in water bath for 30 min.

Then the flask was cooled, the extract was decanted and the plant raw material was rinsed out with an additional portion of the extractant (5.0 ml). The obtained extract was added to the great bulk of the extract and weighed. The ultimate extract was analyzed by the method of reverse phase high performance liquid chromatography (RP HPLC). The density of the extract was determined by method 1 according to general pharmacopoeia monograph 1.2.1.0014.15 [22].

The contents of baicalin and baicalein in the plant raw material ($X_{1,2}$, %) was calculated by the following equation (1):

$$X_{1,2} = \frac{C \cdot M \cdot 100}{m \cdot \rho} \quad (1)$$

where

C – baicalin or baicalein concentration, g/ml;

M – mass of the extract, g;

m – plant raw material, g;

ρ – density of the extract, g/ml.

Method of analysis

Qualitative contents of baicalin and baicalein in the extracts were analyzed by reverse phase high performance liquid chromatography (RP HPLC). The analyses were carried out with Agilent Technologies equipment, Agilent 1200 Infinity series, the USA. More details on RP HPLC analysis conditions are described in this work [23].

As standards, baicalin and baicalein of the State Pharmacopoeia of Ukraine with the content ≥95.0 were used. The analytical wavelength was 275 nm.

The main parameters for the validation method of the analysis and suitability of RP HPLC system for the determination of baicalin and baicalein are presented in Table 1.

Table 1 – Main parameters for validation method of the analysis and suitability of RP HPLC system for baicalin and baicalein determination

Parameter	Pharmacopoeia limitation [22]	Baicalin	Baicalein
Retention time (t_R), min.*	–	22,6±0,5	29,4±0,5
Asymmetry coefficient (T)	0,8-1,5	1,35	0,94
Separation coefficient (R_s)	≥1,5	1,58	1,62
RSD of peak's area, %	≤2,0	1,6	1,5
LOD, g/ml	–	$2,9 \cdot 10^{-5}$	$3,9 \cdot 10^{-6}$
LOQ, g/ml	–	$8,8 \cdot 10^{-5}$	$1,2 \cdot 10^{-5}$
Determination coefficient, r^2	≥0,98	0,9992	0,9999
Calibration linear equation, C(g/ml)=f(S(mAU·s))	–	$C=(2,52±0,10) \cdot 10^{-7} \cdot S$	$C=(1,78±0,01) \cdot 10^{-7} \cdot S$

* Note. The mean value and its confidence interval (Mean±SEM) are calculated with repeat counts n=3 and significance level P=0.95.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
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RESULTS AND DISCUSSION

Fig.1 presents a chromatogram of the extract during the determination of baicalin and baicalein contents in the plant raw material according to the subsection "Sample preparation" in the section "Materials and methods".

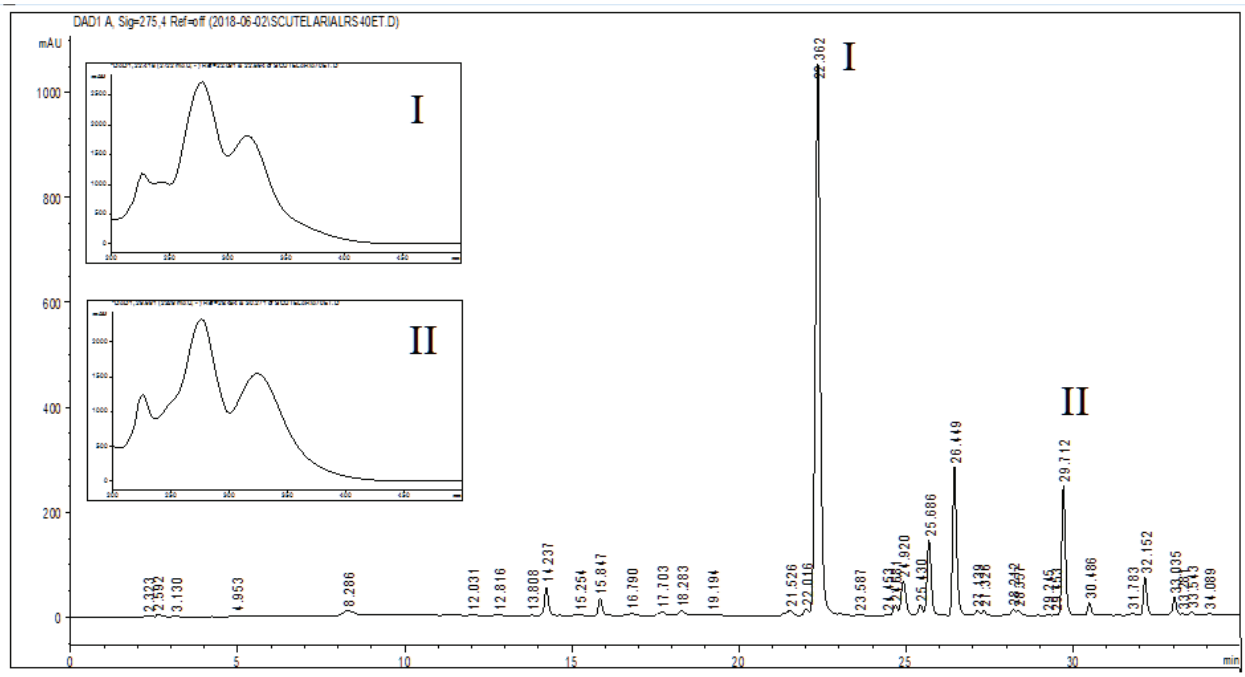

Figure 1 – Chromatogram of the extract obtained during determination of baicalin and baicalein contents in the plant raw material

Note: the analytical wavelength was 275 nm; I – baicalin; II – baicalein.

As Fig.1 shows, baicalin (I) dominates in the extract obtained (the retention time was 22.4 min). After the substitution of the experimental values of baicalin/baicalein peak area into the regression equation (see Table 1), the concentration of these substances in the ultimate extract was calculated and then the calculation of their contents in the plant raw material was carried out using the equation (1). The initial content of baicalin in the plant raw material was 14.8% m/m, and the one for baicalein was 1.89% m/m.

Fig. 2 presents a chromatogram of the extract at the analytical wavelength of 275 nm obtained under the following conditions: ethanol 72% v/v was used as an extractant, the time of maceration was 13.3±0.2 h, the temperature was 24±1°C, and the ratio of the plant raw material to the extractant was 1:10 m/v.

As Fig. 2 shows, the two substances, baicalin (I) and baicalein (II), dominate in the extract, while in the PRM it was only baicalin that was dominating. It means that for 13.3 ± 0.2 hours of infusion, hydrolysis of baikalin occurred with the formation of a significant amount of baikalein.

The results of RP HPLC analysis of baicalin and baicalein yield into the extracts at different concentrations of ethanol under the above-mentioned conditions (the time of maceration – 13.3±0.2 h, the temperature – 24±1°C, and the ratio of the plant raw material to the extractant – 1:10 m/v) are presented in Fig. 3.

The yield of baicalin was calculated compared to its initial value in PRM. The yield of baicalein (X_3), was calculated equivalent to its hypothetical content in PRM given that the total amount of baicalin (X_1), transforms into it ($X_3=X_2+X_1 \cdot Mr_2/Mr_1=1,89+14,8 \cdot 270,2/446,4=10,9\%$ mass.). The repeat count is n=3, and the significance level is P=0.95.

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DOI: 10.19163/2307-9266-2019-7-3-129-137

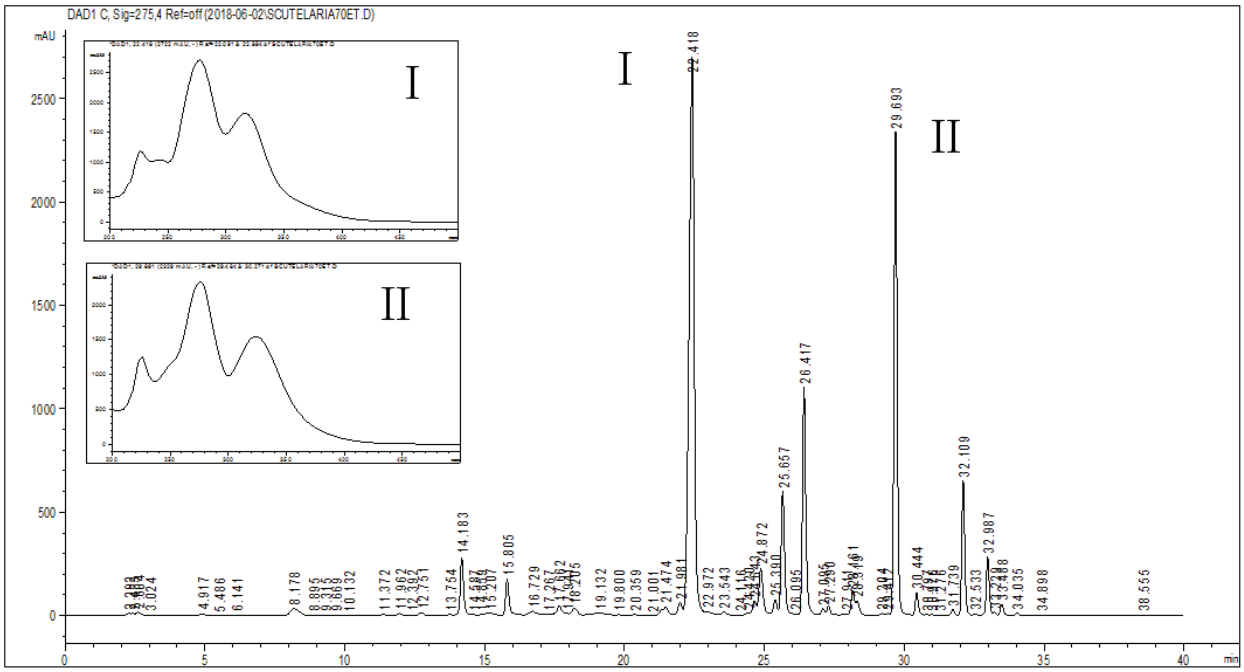

Figure 2 – Chromatogram of the extract from Scutellaria baicalensis Georgi roots
Note: analytical wavelength was 275 nm; I – baicalin; II – baicalein

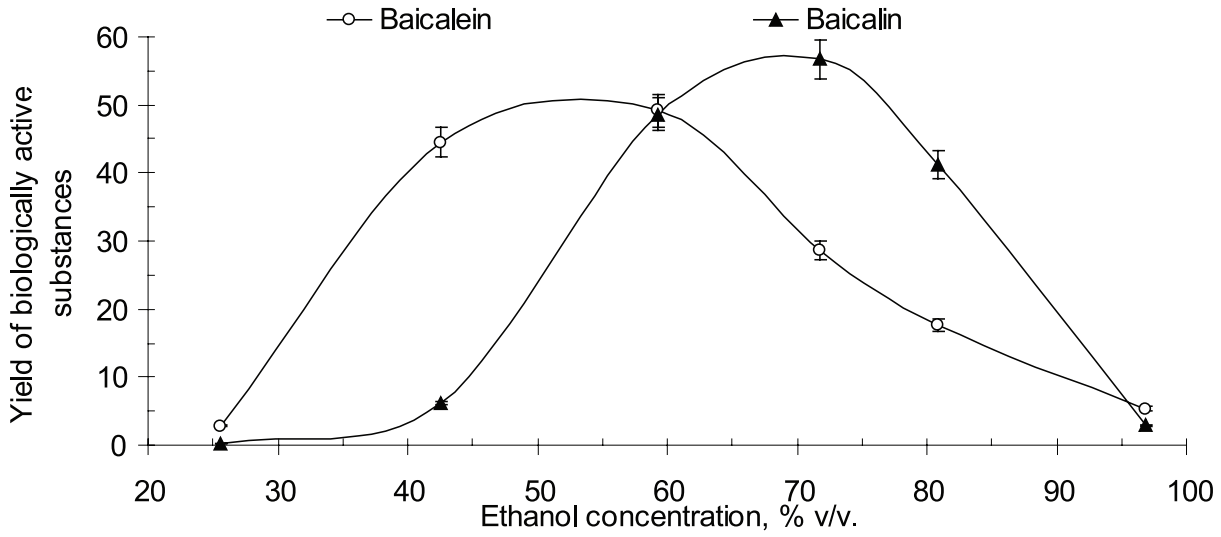

Figure 3 – Dependency of baicalin and baicalein yield on ethanol concentration

Empirical graphs presented in Fig. 3 show that under the experimental conditions (the time of maceration of 13.3±0.2 h, the temperature of 24±1°C, the ethanol concentration of 43% v/v, the PRM / extractant ratio of 1:10 m/v), a considerable part of baicalin disintegrates up to baicalein. Herewith, the yield of baicalin into this extractant was 6.2% of its initial content in the PRM, and the yield of baicalein was 44.5% of its hypothetic content in the PRM. These values make it possible to calculate the percentage of converted baicalin, which was 44.8%={100·[(10.9-1.89)·44.5/100]·446.4/(270.2·14.8)}, i.e. almost a half of baicalin of its initial content in the plant raw material disintegrated. Its residual part (49%=100–44.8–6.2) did not possibly dissolve in the ethanol of this concentration and remained in the PRM. That fact requires additional studies.

On the empirical curves obtained, the maximum for baicalin yield in ethanol with the concentration range of 70±5% v/v is clearly seen, and for baicalein it takes place in ethanol with the concentration range of 53±10% v/v. It is interesting to notify the existence of the interception (isobestic) point of empirical curves for ethanol with

ISSN 2307-9266 e-ISSN 2413-2241

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the concentration of 62±3% v/v, at which 50% value of each component yield is observed.

Moreover, the curves also show that baicalin and baicalein are practically not extracted by ethanol with the concentration of less than 30% v/v and more than 90% v/v.

In ethanol with the concentration from 40 to 60% v/v, the maximum yield of baicalein up to 45–50% from its hypothetic content in PRM is seen. It can probably be explained by its partial solubility in ethanol at this concentration, as it has already been mentioned above.

From the abovementioned data we can suppose that the activity of a beta-glucuronidase enzyme in *Scutellaria baicalensis* Georgi roots is not inhibited by ethanol and has a high level of activity in ethanol with the concentration from 30 to 90% v/v.

The next stage of the work was connected with the study of baicalin hydrolysis kinetics in *Scutellaria baicalensis* Georgi roots under the same conditions of the extraction process.

For this purpose, ethanol with the concentration of 43 and 72% v/v was used. Hereby, an assumption that hydrolysis of baicalin should occur in the first order reaction was made, thus the experimental data in coordinates ln*C*=*f*(*t*) should be closely approximated by the linear regression equation.

The results of processing of the obtained experimental data are presented in Fig. 4.

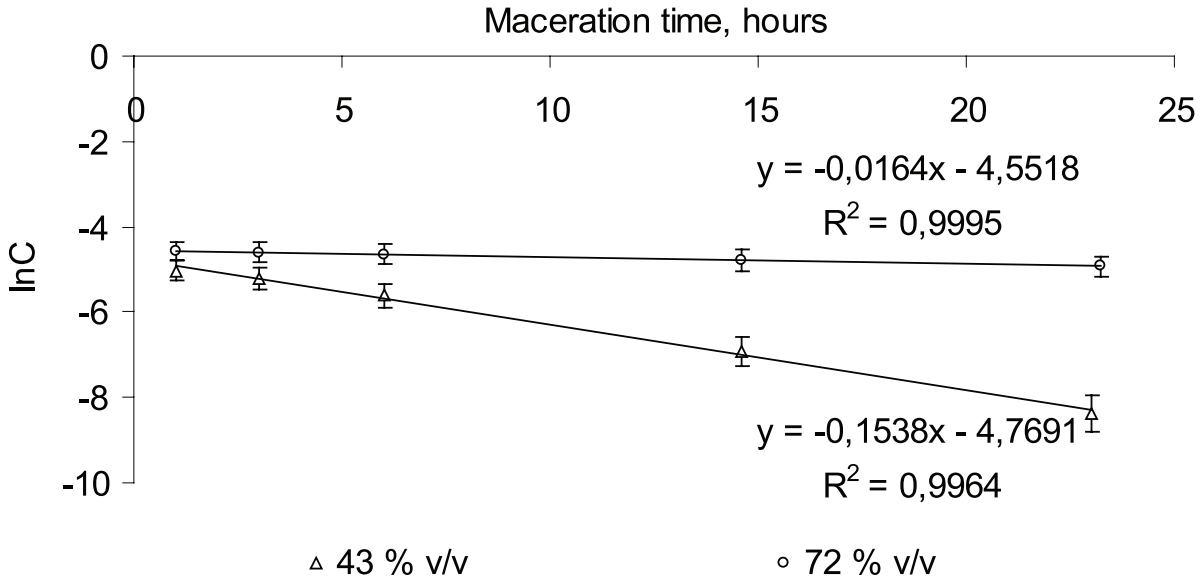

Figure 4 – Dependency of change in baicalin concentration in the extract on the maceration time with ethanol 43 and 72% v/v in coordinates lnC=f(t)

As Fig. 4 shows, the experimental points for the dependency of baicalin concentration in the extract on the maceration time are closely approximated in the predicted coordinates by the linear equation (the determination coefficient is more than R^2>0.99, which indicates the functional dependency between the parameters).

Therefore, this experiment confirms our assumption about the mechanism of baicalin hydrolysis in *Scutellaria baicalensis* Georgi roots. It follows the first order kinetic equation. It should be pointed out that hereby, ethanol affects the energy of the enzymatic hydrolysis process and slows it down with an increase in the concentration of ethanol in the extraction mixture.

The constants obtained, as well as some other derivative parameters that can be calculated from them in the view of chemical kinetic laws for the first order reactions, are presented in Table 2.

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DOI: 10.19163/2307-9266-2019-7-3-129-137

Table 2 – Values of experimentally obtained constants and some other derivative parameters

Constant/parameter	Ethanol 43% v/v	Ethanol 72% v/v
Slope of regressive curve, 1/h (k)	0.15±0.02	0.0164±0.0007
Intercept, b	–4.8±0.2	–4.6±0.1
Initial concentration of baicalin, g/ml (C_0=exp[b])	0.0082±0.0004	0.0101±0.0002
Half lifetime of baicalin, h ($t_{½}$=ln2/k)	4.6±0.5	42.3±1.8

As Table 2 shows, the values of the derivative parameter of the initial baicalin concentration in ethanol, 43 and 72% v/v, are close but statistically different (0.0082±0.0004<0.0101±0.0002, g/ml). Moreover, the values calculated are less than the ones determined experimentally for baicalin in PRM by 1.8 and 1.4 times, respectively (0.0148±0.0007 g/ml). These inconsistencies require additional experiments and theoretical interpretation.

It should be pointed out that such a derivative parameter as half lifetime of baicalin in ethanol with the concentration of 43% v/v is by about one order lower than the one in ethanol with the concentration of 72% v/v (4.6±0.5<<42.3±1.8, h).

The half lifetime baicalin values calculated show that to obtain the extract with a maximum baicalin content and a minimum baicalein content, it is reasonable to use the technology of rapid extraction (for 1–2 h) and ethanol with the concentration of 70–80% v/v.

And if baicalein extraction is necessary, it is recommended to use maceration for not less than 12 h and ethanol with the concentration of 30–60% v/v.

In general, the results obtained give the possibility to describe baicalin kinetic hydrolysis within a framework of the laws of chemical kinetics and catalysis.

Moreover, to study the influence of the type and composition of the extractant on the baicalin yield and its hydrolysis kinetics, we should also use laws of physical chemistry and additional studies that provide means for development of an advanced type of the mathematical model with the introduction of energy activation of the hydrolysis process.

These results can be used for further development of the extraction technology of baicalin and baicalein from *Scutellaria baicalensis* Georgi roots.

CONCLUSION

Baicalin hydrolysis kinetics at its extraction from *Scutellaria baicalensis* Georgi roots with ethanol concentration of 43 and 72% v/v has been studied. It has been found out that the process of baicalin hydrolysis is well described by the first order kinetic equation. The constants of baicalin hydrolysis process during its extraction from *Scutellaria baicalensis* roots with ethanol having different concentrations have also been found out.

REFERENCES

1. Popova NV, Litvinenko VI, Kutsanyan AS. Lekarstvennyye rasteniya mirovoy flory: entsiklopedicheskiy spravochnik [Medicinal plants of the world flora: encyclopedic reference]. Kharkiv: Disa Plus; 2016:540 p. Russian.
2. WHO monographs on selected medicinal plants. Vol. 3. Geneva: World Health Organization. 2007:390 p.
3. Li C, Lin G, Zuo Z. Pharmacological effects and pharmacokinetics properties of Radix Scutellariae and its bioactive flavones. Biopharmaceutics & Drug Disposition. 2011;32(8):427–445. DOI: 10.1002/bdd.771.
4. Bie B, Sun J, Li J, Guo Y, Jiang W, Huang C, Yang J, Li Z. Baicalein, a Natural Anti-Cancer Compound, Alters MicroRNA Expression Profiles in Bel-7402 Human Hepatocellular Carcinoma Cells. Cell Physiol Biochem. 2017;41(4):1519–1531. DOI: 10.1159/000470815. Epub 2017 Mar 24.
5. Sowndhararajan K, Deepa P, Kim M, Park SJ, Kim S. Neuroprotective and Cognitive Enhancement Potentials of Baicalin: A Review. Brain Sciences. 2018;8(6):104. DOI: https://doi.org/10.3390/brainsci8060104
6. Shi H, Ren K, Lv B, Zhang W, Zhao Y, Tan RX, Li E. Baicalin from *Scutellaria baicalensis* blocks respiratory syncytial virus (RSV) infection and reduces inflammatory cell infiltration and lung injury in mice. Scientific Reports. 2016 Oct 21;6:35851. DOI: 10.1038/srep35851.
7. Zhang XW, Li WF, Li WW, Ren KH, Fan CM, Chen YY, Shen YL. Protective effects of the aqueous extract of Scutellaria baicalensis against acrolein-induced oxidative stress in cultured human umbilical vein endothelial cells. Pharm Biol. 2011;49:256–261. DOI: https://doi.org/10.3109/13880209.2010.501803
8. Gasiorowski K, Lamer-Zarawska E, Leszek J, Parvathaneni K, Yendluri BB, Blach-Olszewska Z, Aliev G. Flavones from root of Scutellaria baicalensis Georgi: drugs of the future in neurodegeneration? CNS Neurol Disord Drug Targets. 2011;10:184–191. DOI: https://doi.org/10.2174/187152711794480384
9. Li-Weber M. New therapeutic aspects of flavones: the anticancer properties of Scutellaria and its main active constituents Wogonin, Baicalein and Baicalin. Cancer Treat Rev. 2009;35:57–68. DOI: https://doi.org/10.1016/j.ctrv.2008.09.005

10. Kumagai T, Muller CI, Desmond JC, Imai Y, Heber D, Koeffler HP. Scutellaria baicalensis, a herbal medicine: anti-proliferative and apoptotic activity against acute lymphocytic leukemia, lymphoma and myeloma cell lines. Leuk Res. 2007;31:523–530. DOI: https://doi.org/10.1016/j.leukres.2006.08.019
11. Himeji M, Ohtsuki T, Fukazawa H, Tanaka M, Yazaki S, Ui S, Nishio K, Yamamoto H, Tasaka K, Mimura A. Difference of growth-inhibitory effect of Scutellaria baicalensis-producing flavonoid wogonin among human cancer cells and normal diploid cell. Cancer Lett. 2007;245:269–274. DOI: https://doi.org/10.1016/j.canlet.2006.01.011
12. Huang ST, Wang CY, Yang RC, Chu CJ, Wu HT, Pang JH. Wogonin, an active compound in Scutellaria baicalensis, induces apoptosis and reduces telomerase activity in the HL-60 leukemia cells. Phytomedicine. 2010;17:47–54. DOI: https://doi.org/10.1016/j.phymed.2009.06.005
13. Ozmen A, Madlener S, Bauer S, Krasteva S, Vonach C, Giessrigl B, Gridling M, Viola K, Stark N, Saiko P, Michel B, Fritzer-Szekeres M, Szekeres T, Askin-Celik T, Krenn L, Krupitza G. In vitro anti-leukemic activity of the ethno-pharmacological plant Scutellaria orientalis ssp. carica endemic to western Turkey. Phytomedicine. 2010;17:55–62. DOI: https://doi.org/10.1016/j.phymed.2009.06.001
14. Ikezoe T, Chen SS, Heber D, Taguchi H, Koeffler HP. Baicalin is a major component of PC-SPES which inhibits the proliferation of human cancer cells via apoptosis and cell cycle arrest. Prostate. 2001;49:285–292. DOI: https://doi.org/10.1002/pros.10024
15. Ciesielska E, Gwardys A, Metodiewa D. Anticancer, antiradical and antioxidative actions of novel Antoksyd S and its major components, baicalin and baicalein. Anticancer Res. 2002;22(5):2885–2891.
16. Ciesielska E, Wolszczak M, Gulanowski B, Szulawska A, Kochman A, Metodiewa D. In vitro antileukemic, antioxidant and prooxidant activities of Antoksyd S (C/E/XXI): a comparison with baicalin and baicalein. In Vivo.2004;18(4):497–503.
17. Lin XC, Liu XG, Chen XW, Chen WZ, Liang NC. Inhibitory effect and its kinetic analysis of baicalein on recombinant human protein kinase CK2 holoenzyme. Ai Zheng. 2004;23(8):874–878.
18. Chen YJ, Wu CS, Shieh JJ, Wu JH, Chen HY, Chung TW, Chen YK, Lin CC. Baicalein Triggers Mitochondria-Mediated Apoptosis and Enhances the Antileukemic Effect of Vincristine in Childhood Acute Lymphoblastic Leukemia CCRF-CEM Cells. Evid Based Complement Alternat Med. 2013;2013:124747. DOI: http://dx.doi.org/10.1155/2013/124747
19. Yu C, Qu F, Mao Y, Li D, Zhen Z, Nass R, Calway T, Wang Y, Yuan CS, Wang CZ. Different extraction pretreatments significantly change the flavonoid contents of *Scutellaria baicalensis*. Pharm Biol. 2013 October;51(10):1228–1235. DOI:10.3109/13880209.2013.784922
20. Li JH, Wang LS, Zou JM. Study on degradation of baicalin by endogenous enzymes in water extraction of *Scutellaria baicalensis*. Chin Trad Herb Drugs. 2009;40:397–400.
21. Sasaki K, Taura F, Shoyama Y, Morimoto S. Molecular characterization of a novel beta-glucuronidase from *Scutellaria baicalensis* Georgi. J Biol Chem. 2000;275:27466–27472.
22. Gosudarstvennaya farmakopeya Rossiyskoy Federatsii. XIV izdaniye. Tom I [State Pharmacopoeia of the Russian Federation. XIV edition. Volume I]. Moscow: Ministerstvo zdravookhraneniya Rossiyskoy Federatsii; 2018. Russian.
23. Zhilyakova ET, Novikov OO, Pisarev DI, Malyutina AY, Boyko NN. Studying the polyphenolic structure of *Laurus Nobilis* L. leaves. Indo Am. J. Pharm. Sci. 2017;4(9):3066–74. DOI: http://doi.org/10.5281/zenodo.910685

ACKNOWLEDGMENT

The authors express their highest esteem and thanks to Prof. Litvinenko Vasiliy Ivanovich, Doctor of Chemistry, the Head of the Laboratory of Chemistry and Technology of Phytochemical Drugs of the State Enterprise “State Scientific Center of Drugs and Medicinal Products”, Kharkiv, Ukraine, for his help with reference substances.

FINANCIAL SUPPORT AND SPONSORSHIP

The results were obtained within the frame of Federal grant No. 12.6429.2017 / BCh “Complex researches of plant-origin objects in the process of creating targeted dosage forms for proctology”.

AUTHOR CONTRIBUTIONS

All authors had equally contributed to the research work.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2019-7-3-129-137

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

OPTIMIZATION OF THE METHOD FOR OBTAINING NANOCAPSULES, DEVELOPMENT OF THE METHODS OF DETERMINING THE DEGREE OF CINNARIZINE INCLUSION IN A PROLONGED DOSAGE FORM BASED ON POLY-D, L-LACTID-CO-GLICOLIDE, AND ITS VALIDATION

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Received 26.01.2019 Review 1 29.03.2019 Review 2 15.05.2019 Accepted for publication: 10.06.2019

Common pathologies of the cardiovascular system are cerebrovascular disorders, for which cinnarizine is prescribed. An innovative, prolonged nanocapsule dosage form based on poly-D, L-lactide-co-glycolide (PLGA) has been developed.

The aim of the research was improvement of the technology and development of the methods for determining the level of cinnarizine inclusion in nanocapsules. The research problem consisted of a great number of drug encapsulation peculiarities, as well as various physicochemical properties of the substances that cannot be taken into account in the existing methods of determination.

Materials and methods. In the study, the following substances were used: cinnarizine, PLGA (50:50), polyvinyl alcohol (PVA). The remaining reagents and solvents fitted into the category of chemically pure. For development of the methods for quantitative determination of cinnarizine and its validation, a spectrophotometric method of analysis was used. Model mixtures used as objects of the study, had been prepared. Validation assessment of the methods was carried out upon such indicators as specificity, linearity, detection limit, repeatability, reproducibility.

Results. Methods for spectrophotometric determination of the degree of cinnarizine inclusion has been developed. It has been established that encapsulation reaches 63.74%. Validation testing methods has been carried out. The results of such tests as specificity, linearity, detection limit, repeatability, reproducibility correspond to the safe range of values regulated by Product specification file. Due to the impossibility of determining the degree of cinnarizine inclusion on the basis of standard methods of preparation, the technology of producing nanocapsules has been adjusted.

Conclusion. The technology has been optimized and new techniques have been developed. Taking into account the characteristics of production and the physicochemical properties of the components, they make a reliable analysis of the degree of cinnarizine inclusion in nanocapsules possible. The relative error of the developed methods of determination does not exceed ± 2.67%. Based on the results of the validation assessment, this methods is valid for all indicators.

Keywords: cinnarizine, poly-D,L-lactide-co-glycolide, microparticles, degree of incorporation, UV spectrometry

For citation: M.V. Sorokoumova, N.V. Blagorazumnaya, V.A. Kompantsev, L.I. Shcherbakova, N.S. Zyablitseva, Y.G. Medvetskaya, T.M. Vasina, K.A. Miroshnichenko. Optimization of the method for obtaining nanocapsules, development of the methods of determining the degree of cinnarizine inclusion in a prolonged dosage form based on poly-d, l-lactid-co-glicolide, and its validation. *Pharmacy & Pharmacology*. 2019;7(3): 138-147. **DOI:** 10.19163/2307-9266-2019-7-3-138-147

Для цитирования: М.В. Сорокоумова, Н.В. Благоразумная, В.А. Компанцев, Л.И. Щербакова, Н.С. Зяблицева, Ю.Г. Медвецкая, Т.М. Васина, К.А. Мирошниченко. Оптимизация способа получения нанокапсул, разработка методики определения степени включения циннаризина в пролонгированную лекарственную форму на основе поли-d,l-лактид-ко-гликолида и ее валидация. *Фармация и фармакология.* 2019;7(3): 138-147. **DOI:** 10.19163/2307-9266-2019-7-3-138-147

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2019-7-3-138-147

ОПТИМИЗАЦИЯ СПОСОБА ПОЛУЧЕНИЯ НАНОКАПСУЛ, РАЗРАБОТКА МЕТОДИКИ ОПРЕДЕЛЕНИЯ СТЕПЕНИ ВКЛЮЧЕНИЯ ЦИННАРИЗИНА В ПРОЛОНГИРОВАННУЮ ЛЕКАРСТВЕННУЮ ФОРМУНА ОСНОВЕ ПОЛИ-D,L-ЛАКТИД-КО-ГЛИКОЛИДА И ЕЕ ВАЛИДАЦИЯ

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Получено 26.01.2019 Рецензия 1 29.03.2019 Рецензия 2 15.05.2019 Принята к печати 10.06.2019

Распространенными патологиями сердечно-сосудистой системы являются цереброваскулярные расстройства, для лечения которых назначают препарат циннаризин. Разработана инновационная пролонгированная лекарственная форма в виде нанокапсул на основе поли-D,L-лактид-ко-гликолид (PLGA).

Целью исследования явилось совершенствование технологии и разработка методики определения уровня включения циннаризина в нанокапсулы. Исследовательская проблема заключалась в наличии множества особенностей инкапсулирования препарата, а также различных физико-химических свойств веществ, которые невозможно учесть в имеющихся методиках определения.

Материалы и методы. В ходе исследования были использованы субстанция циннаризина, PLGA (50:50), поливиниловый спирт (ПВС). Остальные реактивы и растворители соответствовали категории х.ч. Для разработки методики количественного определения циннаризина и ее валидации, использован спектрофотометрический метод анализа. Были приготовлены модельные смеси, которые использовались как объекты исследования. Валидационную оценку методики проводили по таким показателям, как специфичность, линейность, предел обнаружения, повторяемость, воспроизводимость.

Результаты. Разработана методика спектрофотометрического определения степени включения циннаризина. Установлено, что инкапсуляция составляет 63,74%. Осуществлено валидационное тестирование методики. Результаты таких испытаний, как специфичность, линейность, предел обнаружения, повторяемость, воспроизводимость соответствуют допустимому интервалу значений, регламентированных нормативной документацией. Скорректирована технология получения нанокапсул, в связи с невозможностью определения степени включения циннаризина при использовании стандартной методики получения.

Заключение. Оптимизирована технология и разработана методика, позволяющая провести достоверный анализ степени включения циннаризина в нанокапсулы, с учетом особенностей производства и физико-химических свойств компонентов. Относительная погрешность разработанной методики определения не превышает ±2,67%. Исходя из результатов валидационой оценки, по всем показателям методика валидна.

Ключевые слова: циннаризин, поли-D,L-лактид-ко-гликолид, микрочастицы, степень включения, УФ-спектрометрия

INTRODUCTION

In cerebrovascular disorders, progressive neuronal damage is observed, followed by their apoptosis due to metabolic disorders, nutritional deficiencies and oxygen. These are hemorrhagic and ischemic kinds of stroke, encephalopathy, migraine, dementia, cognitive impairment. One of the leading pathogenetic factors in the development of these disorders is a change in the properties of cerebral vessels under the influence of such factors as atherosclerosis, arterial hypertension, leading to impaired cerebral circulation [1–4]. The main group of drugs prescribed for this pathology, are calcium channel blockers, in particular cinnarizine, the effectiveness of which has been proven by numerous clinical studies [5]. Existing dosage forms of cinnarizine have several disadvantages, namely: frequency of administration and adverse effects [6, 7]. The solution to leveling these undesirable factors is developing a prolonged form of cinnarizine, allowing to reduce the frequency of administration, and thus avoid abrupt changes in the concentration of the drug in the body, and hereby to maintain the concentration of the drug in the body relatively constant and therapeutically effective. Modern pharmaceutical technologies make it possible to create prolonged dosage forms of nano- and microsizes with controlled release of active substances based on polymeric carriers using the encapsulation method [8, 9]. One of the promising polymers is a copolymer of lactic and glycolic acids (50:50) – poly-D, L-lactide-co-glycolide (PLGA). Nanocapsules based on this biopolymer provide a high level of

sorption, prolonged and programmed release of drugs. The polymer is also biocompatible and biodegradable, it is metabolized in the body to endogenous compounds: lactic acid (decomposes to carbon dioxide and water) and glycolic acid (excreted unchanged). An innovative prolonged dosage form of cinnarizine in the form of micro- and nanocapsules has been developed. It is based on biodegradable polymer PLGA, supposedly capable of leveling the adverse effects of the drug.

THE AIM of the investigation was the adjustment of the standard technology, the development and validation of the methods for determining the degree of cinnarizine inclusion in the resulting microparticles, taking into account the features of encapsulation and the physicochemical properties of the compounds used in their production.

MATERIALS AND METHODS

In this study, the substance of cinnarizine, PLGA (50:50) and PVA have been used. The rest of the reagents and solvents fitted into the category of chemically pure.

1. Methods for determining the degree of cinnarizine inclusion in nanocapsules

Methods 1

The object of the study was an aqueous solution of the supernatant obtained after centrifugation [10–14]. The supernatant was placed in a 100 ml volumetric flask, adjusted to the mark with purified water (solution A). Considering the solubility of cinnarizine in ethyl alcohol and the literature data on the determination of the degree of incorporation of medicinal substances in PLGA-based microcapsules, the following methods was proposed.

The degree of cinnarizine inclusion was established spectrophotometrically [15–21]. To do this, the content of free cinnarizine, and then the content of cinnarizine included in the microparticles were determined, A 0.4 ml aliquot was taken from solution A and placed in a 10 ml volumetric flask, made up to the mark with ethyl alcohol 95% [22–24].

Methods 2

A 20 ml aliquot was taken from solution A, placed in a separatory funnel, then 5 ml of chloroform was added and extracted. The spectrum of chloroform extraction was measured in the range of 220–360 nm on an SF-56 spectrophotometer in cuvettes with a layer thickness of 1 cm; chloroform was used as a reference solution. The spectrum of a standard sample of cinnarizine in chloroform had been preliminarily measured.

The completeness of cinnarizine extraction from solution A was monitored by a repeated addition of 5 ml of chloroform to the aqueous solution obtained after the first extraction. The spectrum of the second chloroform extraction was measured in the range of 220–360 nm on an SF-56 spectrophotometer in cuvettes with a layer thickness of 1 cm; chloroform was used as a reference solution.

Method 3

In this case, the object of the study was the solution of the supernatant in hydrochloric acid. The entire volume was transferred into a volumetric flask with a capacity of 50 ml, adjusted to the mark with a 0.1 M solution of hydrochloric acid. Previously, the spectrum of a standard sample of cinnarizine in a 0.1 M solution of hydrochloric acid was measured; an absorption maximum A = 0.576 at the wavelength of 253 nm was observed.

The evaluation of the degree of cinnarizine inclusion in nanocapsules was performed by measuring the absorption spectrum in the rage of 220–360 nm on an SF-56 spectrophotometer in cuvettes with a layer thickness of 1 cm; a solution of hydrochloric acid 0.1M was used as a reference solution.

2. Optimization of the method of obtaining nanocapsules

It is known from the literature data, that cinnarizine is soluble in a 0.1 M solution of hydrochloric acid. Consequently, a study on the solubility of PLGA in a 0.1 M solution of hydrochloric acid was carried out. For this purpose, the PLGA sample was placed in 50 ml of a 0.1 M solution of hydrochloric acid. While stirring for three hours, the polymer did not dissolve, and therefore, it was proposed to change the standard technology of nanocapsules.

Standard nanocapsules technology

To obtain PLGA-based cinnarizine microparticles, the coprecipitation method was used. Precise weights of cinnarizine and PLGA were dissolved in a small volume of chloroform, and then added dropwise to the aqueous solution of PVA, using an Ultra-Turrax T-18 apparatus (IKA, Germany) for homogenization. The resulting solution containing microparticles, was centrifuged at the rotation speed of 10,000 rp/m for 20 minutes. The submicrocapsule fluid – supernatant – was decanted.

Adjusted nanocapsules technology

Precise weights of cinnarizine and PLGA were dissolved in a small volume of chloroform, and then added dropwise to a 0.1 M hydrochloric solution of PVA, using an Ultra-Turrax T-18 apparatus (IKA, FRG) for homogenization. The resulting solution containing microparticles, was centrifuged at the rotation speed of 10,000 rpm for 20 minutes. The submicrocapsule fluid – supernatant – was decanted.

3. Methods for quantitative determination of cinnarizine in nanocapsules and its validation

3.1. Quantitative determination

Based on the data obtained in determining the level

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of cinnarizine inclusion in nanocapsules, for the quantitative analysis, the conditions corresponding to Method 3 were selected.

The measurements were carried out six times at the room temperature, the data were averaged.

The calculation of the content of cinnarizine in the supernatant produced, is shown by formula 1:

$$X = \frac{A_x \times C_{cm} \times W_1 \times W_2}{A_{cm} \times a \times V_a} \quad (1),$$

where

A_x – optical density of the supernatant;
C_{cm} – CO concentration of cinnarizine, g/ml;
W_1, W_2 – volume of volumetric flasks, ml;
A_{cm} – optical density CO cinnarizine;
a – amount of cinnarizine, g;
V_a – aliquot volume, ml.

3.2 Validation of the methods of cinnarizine quantitative determination in nanocapsules

The validation estimation of the developed methods for the quantitative determination of cinnarizine in microparticles has been carried out. The developed methodology is evaluated according to the following indicators: specificity, linearity, detection limit, repeatability, reproducibility.

Determination of specificity

When testing a technique for specificity, the influence of a solvent and excipients on the optical density of a solution of a model mixture is determined.

The solvent used is a 0.1 M hydrochloric acid solution. Two solutions are prepared: the first is a solution of a model mixture of microparticles with cinnarizine, the second is a solvent and excipients.

Determination of linearity methods

The determination of the linearity of the methods of cinnarizine quantitative analysis is carried out by a spectrophotometric method. To do this, 6 parallel determinations in the weights of a model mixture of microparticles are conducted.

Preparation of model mixtures

Samples of cinnarizine microparticles equal to 0.01 g, 0.02 g, 0.03 g, 0.04 g, 0.05 g and 0.06 g, respectively, are placed in 100 ml volumetric flasks, dissolved in a 0.1 M hydrochloric solution of chlorohydric acid, brought to the mark with the same solvent, then mixed. The optical density of the obtained solutions is measure on spectrophotometer SF-56 at the maximum absorption at the wavelength of 253 nm in a cuvette with a layer thickness of 10 mm.

Determination of the detection limit

When determining the detection limit, a model mixture is prepared: 0.03 g of cinnarizine microparticles are placed in a 100 ml volumetric flask, dissolved in a 0.1 M hydrochloric acid solution and made up to the mark with the same solvent (solution A).

From solution A, model solutions were prepared with a cinnarizine concentration of $1 \cdot 10^{-3}$ g/ml, $1 \cdot 10^{-4}$ g/ml, $1 \cdot 10^{-5}$ g/ml. The *determination* was started with the solution with the highest concentration, setting the concentration at which there would be no cinnarizine absorption maximum at the wavelength of 253 nm.

Determination of convergence

The technique of convergence is characterized by such indicators as repeatability and reproducibility.

To determine convergence, a spectrophotometric method of analysis in the wavelength range of 220–360 nm was used.

Repeatability is examined on a model sample of a mixture of cinnarizine microparticles in 10 replications.

Reproducibility of the methods is estimated on five samples of model mixtures in two replications.

RESULTS AND DISCUSSION

Using methods of determination No 1, it was found out that when ethyl alcohol is added to the aqueous solution of the supernatant, white opalescence and turbidity are observed. The reason for these phenomena is the interaction of PVA with ethyl alcohol, in connection with which this method cannot be used for the spectrophotometric determination of the degree of cinnarizine inclusion.

Using methods No 2, the quantitative content of cinnarizine (1% of the introduced cinnarizine sample) was detected in the supernatant, which is confirmed by solubility of cinnarizine in water [25].

On the basis on the obtained results it was proposed to change the standard technology of microparticles by using a hydrochloric solution of PVA instead of an aqueous solution, which would make it possible to transfer non-included cinnarizine into the solution. Then it would be possible to determine the degree of its inclusion [26, 27].

The use of methods No 3 allowed us to determine the quantitative content of cinnarizine in the hydrochloric solution of the supernatant. Accordingly, it became possible to calculate the degree of encapsulation of the drug in microparticles from the difference in concentrations.

Having made a series of determinations, the following data were obtained.

Fig. 1 shows the UV absorption spectrum of the solution of the supernatant obtained by the adjusted technology. This UV absorption spectrum coincides with the results of the previous studies.

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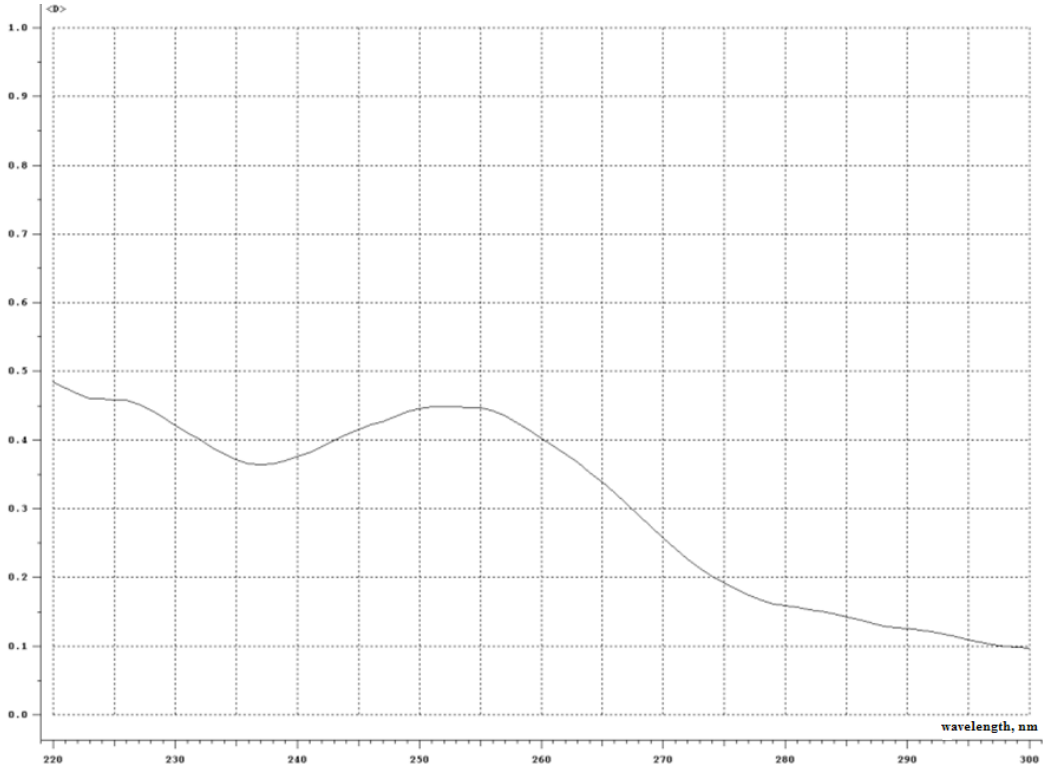

Figure 1 – UV spectrum of the supernatant

According to the results of the study, the average optical density at the wavelength of 253 nm was 0.447.

The content of cinnarizine in the supernatant was calculated by formula 1.

$$X = \frac{0,447 \cdot 0,001 \cdot 50 \cdot 10}{0,576 \cdot 0,0107 \cdot 1} = 36,26\%$$

By differences, the degree of cinnarizine inclusion can be calculated:

100% – x = 100 – 36,26=63,74% (Tab. 1)

Table 1 – Data on the degree of encapsulation

Sample No	Optical density of solution	Amount of unbound cinnarizine, %	Amount of bound cinnarizine, %	Metrological data
1	0.425	34.48	65.52	
2	0.482	39.10	60.90	
3	0.447	36.26	63.74	
4	0.450	36.51	63.49	S=1.62
5	0.430	34.88	65.12	S_x =0.6635
6	0.448	36.34	63.66	ε=±2,67
$\overline{X}$	0.447	36.26	63.74	

Validation assessment of the methods

Determination of specificity

The obtained solutions were analysed by a spectrophotometric method. Fig. 2 shows the UV spectrum of a model mixture of cinnarizine microparticles. In the rage of 230–270 nm, an absorption maximum characteristic of the preparation is observed. Fig. 3 shows the absorption spectrum of the second solution, consisting of a solvent and excipients. It is clearly seen that the absorption in the rage of 230–270 nm is practically absent.

Judging by the obtained definition data, one can see the specificity of the developed methodology. [28]

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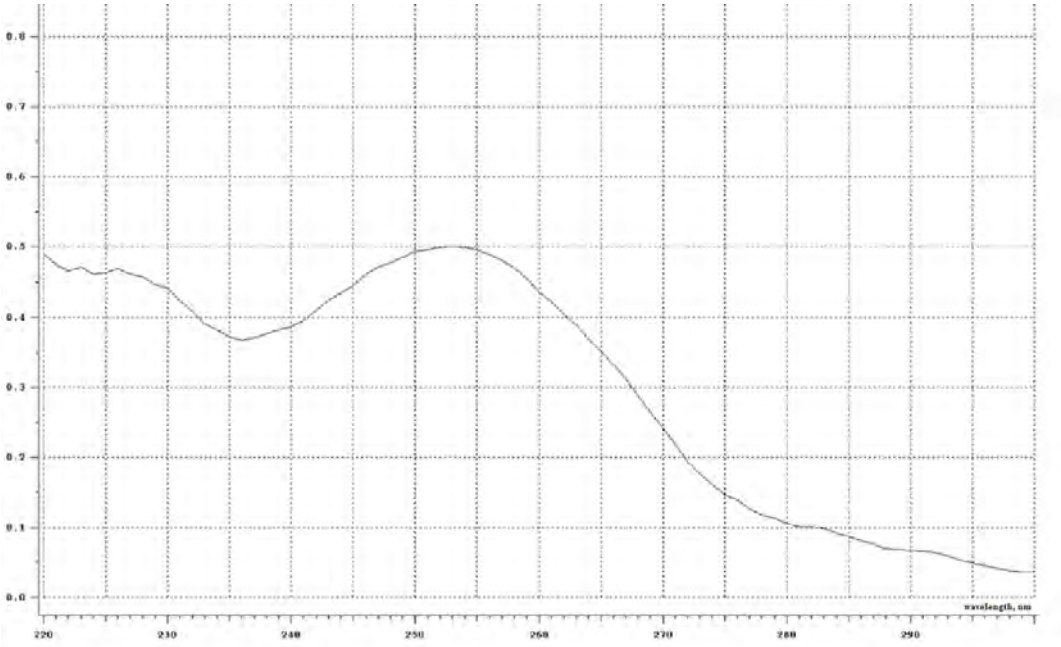

Figure 2 – UV spectrum of a model mixture of microparticles

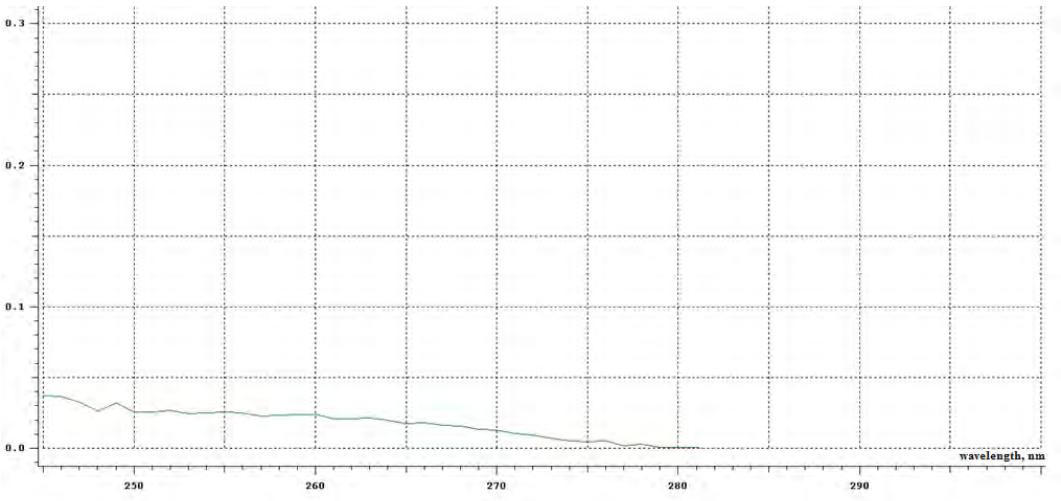

Figure 3 – UV spectrum of the solvent with excipients

Determination of linearity methods

According to the test results, a calibration graph for the dependence of the optical density value on the mixture sample was constructed; the calibration curve equation and the correlation coefficient were calculated. The graph is presented in Fig. 4.

The linearity index is characterized by a correlation coefficient, its value was 0.998875, which meets the requirement of not less than 0.995.

The calibration curve is subject to linear dependence. The correlation coefficient meets the specified requirements ND [28]. Therefore, it can be argued that a linear dependence is observed in the concentration range of cinnarizine, in a sample of a model mixture of microparticles 0,000345–0,002%.

ISSN 2307-9266 e-ISSN 2413-2241

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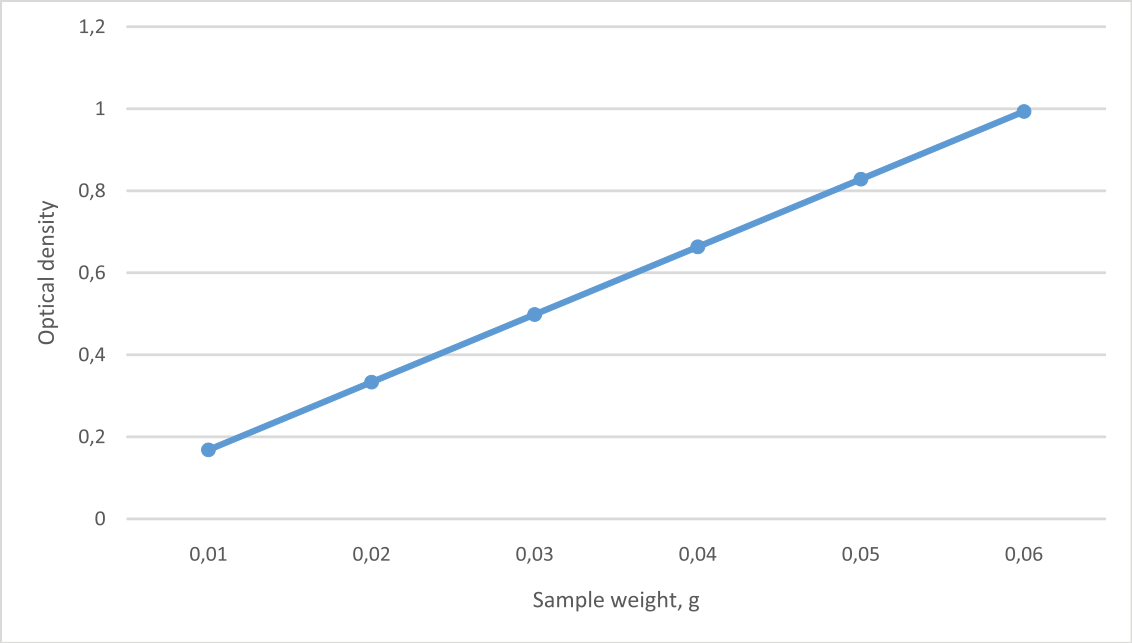

Figure 4 – Calibration graph of the dependence of cinnarizine optical density on a sample of a model mixture of microparticles

Determination of detection limit

The data obtained during the study are reflected in Table 2. The developed method allows determining the detection limit, the concentration at which the characteristic absorption for cinnarizine will not be observed at the wavelength of 253 nm. The detection limit of cinnarizine in microparticles has been established. It is $1 \cdot 10^{-4}$ g/ml.

Table 2 – Data on detection limit

Concentration of cinnarizine in solution, g/ml	Optical density of solution
$1 \cdot 10^{-3}$	0.4980
$1 \cdot 10^{-4}$	0.0508
$1 \cdot 10^{-5}$	0.0048

Determination of convergence

Convergence is characterized by such parameters as repeatability and reproducibility of the methods.

Repeatability is estimated by the indicator – coefficient of variation, hereby its value at the request of ND should not exceed 2.0% [28]. The data on repeatability of the methodology are given in Table 3.

The established coefficient of variation was 1.4%, which is within the range of acceptable values.

The test results, in terms of reproducibility, are shown in Table 4.

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Table 3 – Data on repeatability of the methods

No. of measurement	Optical density
1	0.498
2	0.505
3	0.504
4	0.497
5	0.496
6	0.507
7	0.503
8	0.500
9	0.494
10	0.496
Average value	0.500
Degree of variation, %	1.4

Table 4 – Data on the reproducibility of the methods

First measurement series					
Sample number	1	2	3	4	5
Researcher No.1	0.502	0.500	0.503	0.498	0.496
Researcher No.2	0.504	0.503	0.505	0.499	0.502
Second measurement series					
Sample number	1	2	3	4	5
Researcher No.1	0.506	0.507	0.500	0.503	0.501
Researcher No.2	0.504	0.507	0.507	0.501	0.503

The acceptability indicator was the coefficient of variation; its value, according to the requirements of ND, should not exceed 3% [28]. For 10 parallel measurements in two series, it was 0.60%, which does not go beyond the acceptable indicator of the acceptance criterion.

CONCLUSION

In the course of the research, the standard microparticle technology based on biodegradable polymer PLGA was adjusted by using a 0.1M hydrochloric acid solution as a solvent. A method for determining the degree of cinnarizine inclusion in microcapsules by the UV spectrophotometry method has been developed.

The content of cinnarizine in microcapsules was 63.7% of the introduced cinnarizine sample. The relative error does not exceed ±2,67%. The validation of the above-developed methods has been performed according to the following indicators: specificity, linearity, detection limit, repeatability, reproducibility. According to the test results, the data obtained can be used to judge the validity of the methods.

REFERENCES

1. Shchukin IA, Lebedeva AV, Burd SG, Fidler MS, Shikhkerimov RK, Ismailov AM, Bolotov AV, Belgusheva MKh. Hronicheskie cerebrovaskulyarnye zabolevaniya voprosy diagnostiki I lecheniya [Chronic cerebrovascular disease: diagnosis and treatment questions]. Consilium Medicum. 2016;18(2):85–94. Russian.
2. Smith EE, Saposnik G, Biessels GJ, Doubal FN, Fornage M, Gorelick PB, Greenberg SM, Higashida RT, Kasner SE, Seshadri S; American Heart Association Stroke Council; Council on Cardiovascular Radiology and Intervention; Council on Functional Genomics and Translational Biology; and Council on Hypertension. Prevention of Stroke in Patients With Silent Cerebrovascular Disease: A Scientific Statement for Healthcare Professionals From the American Heart Association/American Stroke Association. Stroke. 2017 Feb;48(2):e44–e71. DOI: 10.1161/STR.0000000000000116. Epub 2016 Dec 15.
3. Thrift AG, Howard G, Cadilhac DA, Howard VJ, Rothwell PM, Thayabaranathan T, Feigin VL, Norrving B, Donnan GA. Global stroke statistics: An update of mortality data from countries using a broad code of «cerebrovascular diseases» Int J Stroke. 2017 Oct;12(8):796–801. DOI: 10.1177/1747493017730782. Epub 2017 Sep 12.
4. Patel RAG, McMullen PW. Neuroprotection in the Treatment of Acute Ischemic Stroke. Prog Cardiovasc Dis. 2017 May – Jun;59(6):542–48. DOI: 10.1016/j.pcad.2017.04.005. Epub 2017 Apr 30.
5. Ashrafi MR, Salehi S, Malamiri RA, Heidari M, Hosseini SA, Samiei M, Tavasoli AR, Togha M. Efficacy and safety of cinnarizine in the prophylaxis of migraine

in children: a double-blind placebo-controlled randomized trial. Pediatr Neurol. 2014 Oct;51(4):503–8. DOI: 10.1016/j.pediatrneurol.2014.05.031. Epub 2014 Jun 6.

6. Sorokoumova MV., Komantsev VA., Shcherbakova LI., Miroshnichenko, KA., Platonova, AYu.Obshchaya kharakteristika, primeneniye v meditsine, metody kachestvennogo i kolichestvennogo analiza tsinnarizina [General characteristics, application in medicine, methods for qualitative and quantitative analysis of cinnarizine]. Proceeding of Voronezh State University. Series: Chemistry. Biology. Pharmacy. 2017;4: 135–40. Russian.
7. Raghuvanshi S, Pathak K. Recent advances in delivery systems and therapeutics of cinnarizine: a poorly water soluble drug with absorption window in stomach. J Drug Deliv. 2014; 2014:479246. DOI: 10.1155/2014/479246. Epub 2014 Nov 13.
8. Shahba AA, Mohsin K, Alanazi FK. Novel self-nanoemulsifying drug delivery systems (SNEDDS) for oral delivery of cinnarizine: design, optimization, and in-vitro assessment. AAPS PharmSciTech; 13(3):967–977. DOI:10.1208/s12249-012-9821-4
9. Nguyen TH, Hanley T, Porter CJ, Boyd BJ. Nanostructured liquid crystalline particles provide long duration sustained-release effect for a poorly water soluble drug after oral administration. J Control Release. 2011 Jul 30;153(2):180–6. DOI: 10.1016/j.jconrel.2011.03.033. Epub 2011 Apr 8.
10. Sorokoumova MV, Kompancev VA, Miroshnichenko KA. Razrabotka I issledovanie prolongirovannogo lekarstvennogo sredstva cinnarizina na osnove poli-D-L-laktid-ko-glikolida [Development and research of prolonged drug cinnarizine based on poly-D, L-lactide-co-glycolide]. Eurasian Union of Scientists. 2017;10-1(43):75–9. Russian.
11. Verma S, Nagpal K, Singh SK, Mishra DN. Unfolding type gastroretentive film of Cinnarizine based on ethyl cellulose and hydroxypropylmethyl cellulose. Int J Biol Macromol. 2014 Mar;64:347–52. DOI: 10.1016/j.ijbiomac.2013.12.030. Epub 2013 Dec 24.
12. Shahba AA, Ahmed AR, Alanazi FK, Mohsin K, Abdel-Rahman SI. Multi-Layer Self-Nanoemulsifying Pellets: an Innovative Drug Delivery System for the Poorly Water-Soluble Drug Cinnarizine. AAPS PharmSciTech. 2018 Jul;19(5):2087–102. DOI: 10.1208/s12249-018-0990-7. Epub 2018 Apr 25.
13. Sassene PJ, Mosgaard MD, Löbmann K, Mu H, Larsen FH, Rades T, Müllertz A. Elucidating the Molecular Interactions Occurring during Drug Precipitation of Weak Bases from Lipid-Based Formulations: A Case Study with Cinnarizine and a Long Chain Self-Nanoemulsifying Drug Delivery System. Mol Pharm. 2015 Nov 2;12(11):4067–76. DOI: 10.1021/acs.molpharmaceut.5b00498. Epub 2015 Sep 30.
14. Morrison JS, Nophsker MJ, Haskell RJ. A combination turbidity and supernatant microplate assay to rank-order the supersaturation limits of early drug candidates. J Pharm Sci. 2014 Oct;103(10):3022–32. DOI: 10.1002/jps.24090. Epub 2014 Jul 28.
15. Ammar HO, Ghorab M, Kamel R, Salama AH. Design and optimization of gastro-retentive microballoons for enhanced bioavailability of cinnarizine. Drug Deliv Transl Res. 2016 Jun;6(3):210–24. DOI: 10.1007/s13346-016-0280-4.
16. Haress NG. Cinnarizine: Comprehensive Profile. Profiles Drug Subst Excip Relat Methodol. 2015;40:1–41. DOI: 10.1016/bs.podrm.2015.01.001. Epub 2015 Apr 1.
17. Ciftci Z, Deniz M, Yilmaz I, Ciftci HG, Sirin DY, Gultekin E. In vitro analysis of a novel controlled release system designed for intratympanic administration of N-acetylcysteine: a preliminary report. Am J Otolaryngol. 2015 Nov–Dec;36(6):786–93. DOI: 10.1016/j.amjoto.2015.08.004. Epub 2015 Aug 12.
18. Portnova S.V., Mtsariashvili M.R. Validatsiya metodiki kolichestvennogo opredeleniya tsinnarizina v smyvakh.[Validation of the method of quantitative determination of cinnarizine in flushes]. Bulletin of the Technological University 2016; 19(12):75–8. Russian
19. Morrison J, Nophsker M, Elzinga P, Donoso M, Park H, Haskell R. A polychromatic turbidity microplate assay to distinguish discovery stage drug molecules with beneficial precipitation properties. Int J Pharm. 2017 Oct 5;531(1):24–34. DOI: 10.1016/j.ijpharm.2017.07.086. Epub 2017 Aug 7.
20. Abdelrahman MM. Simultaneous determination of Cinnarizine and Domperidone by area under curve and dual wavelength spectrophotometric methods. Spectrochim Acta A Mol Biomol Spectrosc. 2013 Sep;113:291-6. DOI: 10.1016/j.saa.2013.04.120. Epub 2013 May 21
21. Choudhari VP, Ingale S, Gite SR, Tajane DD, Modak VG, Ambekar A. Spectrophotometric simultaneous determination of Tenofovir disoproxil fumarate and Emtricitabine in combined tablet dosage form by ratio derivative, first order derivative and absorbance corrected methods and its application to dissolution study. Pharm Methods. 2011;2(1):47–52. DOI:10.4103/2229-4708.81096
22. Lamie NT. Comparative study of spectrophotometric methods manipulating ratio spectra: an application on pharmaceutical binary mixture of cinnarizine and dimenhydrinate. Spectrochim Acta A Mol Biomol Spectrosc. 2015 Apr 15;141:193–201. DOI: 10.1016/j.saa.2015.01.033. Epub 2015 Jan 28.
23. Blagorazumnaya YeYu, Dukkardt LN, Blagorazumnaya NV. Kachestvennyy i kolichestvennyy analiz trimetiloktadetsil ammoniya bromida v lekarstvennom sredstve bakteritsid. [Qualitative and quantitative analysis of trimethyloctadecyl ammonium bromide in a bactericide drug]. Izvestiya Vuzov. Severo-Kavkazskii Region. Natural Science. 2006;S23:60–1.Russian

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2019-7-3-138-147

24. Beregovykh VV, Pyatigorskaya NV, Belyayev VV, Aladysheva ZHI, Meshkovskiy AP. Validatsiya v proizvodstve lekarstvennykh sredstv. Uchebnoye posobiye [Validation in the production of medicines. Tutorial.]. M.:Publishing House Russian Doctor, 2010. – 286p. Russian. https://www.twirpx.com/file/1570313/
25. Mishra B, Sahoo J, Dixit PK. Enhanced bioavailability of cinnarizine nanosuspensions by particle size engineering: Optimization and physicochemical investigations. Mater Sci Eng C Mater Biol Appl. 2016 Jun;63:62–9. DOI: 10.1016/j.msec.2016.02.046. Epub 2016 Feb 19.
26. Chmelevskaya N.V., Illarionova Ye.A. Analiticheskiy kontrol' odnorodnosti dozirovaniya tabletok tsinnarizina[Analytical control of dosage uniformity of cinnarizine tablets]. Siberian Medical Journal. 2009;3:50–2. Russian
27. Illarionova YeA, Chmelevskaya NV, Murav'yeva GM. Razrabotka metodik obnaruzheniya pikamilona i tsinnarizina v kombinirovannykh sochetaniyakh s psikhotropnymi lekarstvennymi sredstvami [Development of detection methods for picamilone and cinnarizine in combination with psychotropic drugs]. Siberian Medical Journal.2015;(4):68–71. Russian
28. Obshchaya farmakopeynaya stat'ya OFS 1.1.0013.15 «Statisticheskaya obrabotka rezul'tatov khimicheskogo eksperimenta» [Pharmacopoeial item (PIA) 1.1.0013.15"Statistical processing of the results of a chemical experiment"]. State Pharmacopoeua of Russian Federatoin XIV-th edition. Moscow; 2018. Russian

CONFLICTS OF INTEREST

The authors and peer reviewers of this paper report no conflicts of interest.

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RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

EFFECTS OF VARIOUS AVERSIVE ENVIRONMENTS ON OXYGEN CONSUMPTION OF MUSCLE AND BLOOD IN MICE UNDER CONDITIONS OF THE "FORCED SWIMMING" TEST

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Received 14.05.2019 Review 1 28.05.2019 Review 2 10.06.2019 Accepted for publication: 17.06.2019

The aim of the study is to assess the effect of various aversive environments on the oxygen consumption in muscles and blood in mice Under conditions of the "forced swimming" test.

Materials and methods. The study was performed on outbred male mice. Exhausting physical activity was modeled in the "forced swimming" test in various aversive environments. The oxygen consumption by the muscle tissue, as well as the oxygen capacity of the blood, were estimated using the respirometry method (AKPM1-01L ("Alfa Bassens", Russia)).

Results. In the course of the study it was found out that in the group of the animals swimming in hot water (at the temperature of 41°C) as an aversive environment, there was no significant change in the oxygen consumption by mitochondria of striated muscle and by red blood cells in comparison with the intact group of the animals. At the same time, in the group of the mice, where cold water (at the temperature of 15°C) as an aversive environment was used, a statistically significant (by the end of the experiment) decrease in the swimming time was observed in relation to the intact group of the animals. It was accompanied by a decrease in the oxygen consumption by muscle mitochondria, with a constant level of the blood oxygenation. Under conditions of exhausting physical exertion, in the group of the animals that received Metaprot®, an increase in working capacity was noted in both hot and cold water. After peak days of working capacity, a slight decrease in physical activity was observed in both experimental groups. At the same time, it should be noted that oxygenation of blood and muscle tissue against the background of exhausting physical exertion in the group that received Metaprot®, did not differ from the group of intact animals in various aversive environments.

Conclusion. Thus, based on the obtained data, it can be assumed that under conditions of "forced swimming" with loading, the most profound changes in the structure and functions of the striated muscles are observed in animals in cold (15°C) water That is reflected in a decrease in the physical strain and in reducing the oxygen consumption by muscle tissue. The use of the drug Metaprot® promoted correcting the changes in the physical performance of the animals, which was reflected in its increase by 144.8% ($p < 0.05$), compared with the initial swimming time of this group, without the oxygen consumption by erythrocytes and mitochondria of striated muscles.

Keywords: exhausting physical overload, forced swimming with loading, oxygen consumption, blood oxygen capacity, respirometry, Metaprot®

For citation: A.V. Voronkov, A.D. Gerashchenko, D.I. Pozdnyakov, D.V. Khusainov. Effects of various aversive environments on oxygen consumption of muscle and blood in mice under conditions of the "forced swimming" test. *Pharmacy & Pharmacology*. 2019;7(3): 148-157. **DOI:** 10.19163/2307-9266-2019-7-3-148-157

Для цитирования: А.В. Воронков, А.Д. Геращенко, Д.И. Поздняков, Д.В. Хусаинов. Влияние различной аверсивной среды на потребление кислорода в мышцах и крови у мышей в условиях теста «принудительного плавания». *Фармация и фармакология.* 2019;7(3): 148-157. **DOI:** 10.19163/2307-9266-2019-7-3-148-157

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2019-7-3-148-157

ВЛИЯНИЕ РАЗЛИЧНОЙ АВЕРСИВНОЙ СРЕДЫ НА ПОТРЕБЛЕНИЕ КИСЛОРОДА В МЫШЦАХ И КРОВИ У МЫШЕЙ В УСЛОВИЯХ ТЕСТА «ПРИНУДИТЕЛЬНОГО ПЛАВАНИЯ»

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Получено 14.05.2019 Рецензия 1 28.05.2019 Рецензия 2 10.06.2019 Принята к печати 17.06.2019

Цель исследования – оценить влияние различной аверсивной среды на потребление кислорода в мышцах и крови у мышей в условиях теста «Принудительного плавания».

Материалы и методы. Исследование было выполнено на беспородных мышах-самцах. Истощающие физические нагрузки моделировали в тесте «Принудительного плавания» в различных аверсивных средах. Потребление кислорода мышечной тканью, а также кислородную емкость крови оценивали с помощью метода респирометрии (АКПМ1-01Л (Альфа Бассенс, Россия)).

Результаты. В ходе проведенного экспериментального исследования было установлено, что в группе животных, у которых в качестве аверсивной среды использовалась горячая вода (температура 41°С) существенного отличия потребления кислорода митохондриями поперечно-полосатой мускулатуры и эритроцитами в сравнении с интактной группой животных отмечено не было. В тоже время у группы мышей, где в качестве аверсивной среды использовали холодную воду (температура 15°С) продолжительность плавания (к концу эксперимента) была статистически ниже по отношению к интактной группе животных, сопровождаемое уменьшением потребления кислорода митохондриями мышц, при неизменном уровне оксигенации крови. В условиях истощающих физических нагрузок, в группе животных, получавшая Метапрот®, было отмечено нарастание работоспособности, как в горячей, так и в холодной воде. После пиковых дней работоспособности, в обеих экспериментальных группах наблюдалось незначительное падение физической активности. При этом, необходимо отметить, что оксигенация крови и мышечной ткани на фоне истощающих нагрузок в группе, получавшей Метапрот®, не отличалась от группы интактных животных в различных аверсивных средах.

Заключение. Таким образом, на основании полученных данных можно предположить, что в условиях принудительного плавания с отягощением у животных наиболее глубокие изменения функций поперечно-полосатой мускулатуры отмечаются в холодной воде (15°С), выступающей в роли стрессора, что отражается в снижении физической работоспособности, а также в снижении потребления кислорода мышечной тканью. Применение препарата Метапрот® способствовало корректировке возникших изменений физической работоспособности животных, что нашло свое отражение в ее повышении на 144,8% ($p<0,05$), в сравнении с исходным временем плавания данной группы, без изменения потребления кислорода эритроцитами и митохондриями поперечно-полосатых мышц.

Ключевые слова: истощающие физические нагрузки, потребление кислорода, кислородная емкость крови, респирометрия, Метапрот®

INTRODUCTION

Currently, a large number of people are exposed to exhausting physical overload. This category includes highly skilled athletes [1], as well as individuals whose professional activity is associated with a number of negative environmental factors affecting the body [2–4]. These include: reduced partial pressure of O_2 and CO_2, high and low temperatures, dustiness, desynchronosis leading to a breakdown of the adaptive capabilities of a person and the development of disadaptation [5].

It is worth noting that the influence of an aversive environment on the body under conditions of exhausting physical overload is realized through changes in the activity of skeletal muscles, while the main compensatory mechanisms in muscle tissue, in response to the stressor, are: increased oxygen consumption, activation of protein-synthetic processes, intensification of metabolic reactions, etc. [6, 7].

Thus, the assessment of changes in oxygen consumption by muscles and blood in various aversive environments can become the main strategy for developing means of preventing muscle fatigue.

MATERIALS AND METHODS

Laboratory work

The experimental study was performed on 80 outbred male mice weighing 23–25 g, obtained from the laboratory animal's nursery «Rappolovo» (St. Petersburg), and kept in the vivarium of Pyatigorsk Medical and Pharmaceutical Institute – branch of Volgograd State Medical University. All reproducible manipulations

with animals complied with the rules of the «European Convention for the Protection of Vertebrate Animals» (Strasbourg, 1986). The mice were kept in the natural lighting mode of the vivarium; the air temperature and moderate humidity were 22–24°C and 60 ± 5%, respectively. The animals were kept in macrolone boxes with sawdust with free access to feed and water.

Initially, all the male mice were randomized by swimming time, and distributed into the following experimental groups: an intact group of mice underwent swimming with rest days (IN_c, the water temperature of 15° C) (n = 30), a negative control group (NC_c, the water temperature of 15°C) (n = 10) [8]; an intact group (IN_h, the water temperature of 41° C) (n = 30) and a negative control group (NC_h, the water temperature of 41°C) (n = 10) [9]. Two groups of mice (10 animals in each) were treated by «Metaprot» at the dosage of 25 mg/kg («Pharmproekt», Russia) [10].

Muscular Dysfunction Model

Validation method**s** for assessing the physical stress of the animals in an aversive environment (15°C) with a load of 20% of the body weight of the animal has already been carried out before [8]. Along with this method**s**, it could be of interest to assess physical performance in hot water [9]. On the 10th day of the experiment, the animals were decapitated with biological material collected. It was followed by an assessment of oxygen consumption by striated muscles and blood in various aversive environments.

Biomaterial sample preparation

In the work, skeletal muscles and blood were used as biological materials. The skeletal muscles were homogenized in Potter mechanical homogenizer with the addition of HEPES buffer. The obtained cell population was subjected to differential centrifugation: first in the mode of 3,500g for 5 min, then the supernatant was transferred to other tubes, and centrifugation was repeated in the second mode: 13000 g for 10 min. For the further analysis, the supernatant liquid was selected [11].

Respirometric analysis

Oxygen consumption in cell cultures was performed using AKPM1-01L laboratory respirometer (“Alfa Bassens”, Russia). The course of work was fully consistent with the manufacturer’s instructions attached to the device.

Methods of statistical analisys

Statistical processing was performed using the program “STATISTICA 6.0”. Intergroup differences were analyzed by parametric or non-parametric methods, depending on the type of distribution. T-Students test was used as a parametric criterion; Mann-Whitney U-test was used for a nonparametric one. The differences were considered significant at $p < 0.05$.

RESULTS

As a result of the conducted experimental study in various aversive environments, it was noted that in the group of the intact animals, the duration of swimming was unchanged throughout all the days of the experiment (Fig. 1).

The swimming time of the initial day of the experiment (the 1st day) in the group of NC_c mice was 128.7±9.5 seconds. Then, starting from the second day of the experiment, the duration of swimming of animals increased linearly, while the peak of physical performance was noted on the fifth day of swimming of mice with loading (161.3±11.0 sec.). On the peak day, the duration of swimming was longer by 25.3% ($p < 0.05$) relative to the initial day of swimming in this group. However, from the sixth day of the experiment in the group of the mice subjected to daily physical overload, there was a slight decrease in their swimming time, which was shorter in comparison with the day of maximum swimming time of this group by 23.1% ($p < 0.05$). It should be pointed out that the tendency to a noticeable decrease in swimming was noted beginning with the 7th day and until the end of the experiment (Fig. 1). The performance of mice in the “forced swimming” test with loading in the NC_c group was lower by 51.4% ($p < 0.05$) relative to the initial swimming of the animals in this group and by 56% ($p < 0.05$) in comparison with the initial time of the performance in the IN_c. group.

Against the background of the daily administration of Metaprot® to the mice, a linear increase in their performance was noted. The day of maximal swimming of the animals was recorded on the 7th experimental day. It was higher relative to the baseline data of this group by 144.8% ($p < 0.05$) and 140.3% ($p < 0.05$) and 165.1% ($p<0.05$) relative to the intact and negative control groups. Subsequently, a slight smooth drop in working capacity was observed, while on the 10th experimental day, the swimming time of the animals was longer than that of the intact group and the negative control group by 54.7% ($p < 0.05$) and 251.5% ($p < 0.05$), respectively.

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DOI: 10.19163/2307-9266-2019-7-3-148-157

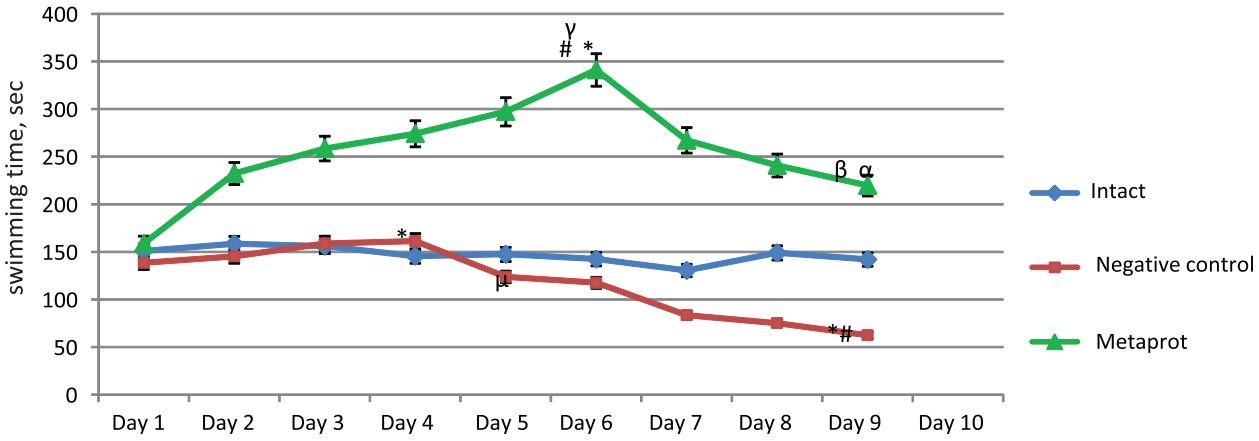

Figure 1 - Duration of mice's swimming time in the test "forced swimming with a load" in cold water

*Note: * – statistically significant relative to the initial swimming time of this group (Student's t-test, (p <0.05)); μ – statistically significant relative to the peak day of swimming for this group (Student's t-test, (p <0.05)); # – statistically significant relative to the initial swimming time of the animals' intact group of (Student's t-test, (p <0.05)); γ – statistically significant relative to the initial swimming time of the animals' negative control group (Student's t-test, (p <0.05)); в – statistically significant relative to the final swimming time of the animals' negative control group of (Student's t-test, (p <0.05)); α – statistically significant relative to the end swimming time of an intact group of animals (Student's t-test, (p <0.05)).*

When estimating forced swimming of the mice in hot water, a linear increase in the performance of animals was noted. Thus, the highest working capaity was noted on the 4th day of the experiment. It was higher by 73% (p<0.05) in comparison with the initial swimming time and by 140.7% (p <0.05) in comparison with the initial swimming day of the group of intact animals (Fig. 2). Starting from the 4th experimental day, the performance of the animals began to decline significantly. So, on the 7th day of the experiment, the swimming time of the mice was shorter by 56.4% (p <0.05) compared to the peak day of the mice's working capacity in the negative control group and shorter by 33.5% (p <0.05) in comparison with the IN_h group of the animals. It should be noted that by the 10th experimental day, the working capacity of the animals subjected to daily physical overload in hot water had got lower by 2.8 times (p <0.05) in comparison with the initial data of this group. Besides, in comparison with the swimming time of the IN_h group, the mice's working capacity of the negative control group was 2.0 times lower (p<0.05) because of hot water.

Under conditions of exhausting physical overload in the group of the mice treated with Metaprot®, an increase in their working capacity from the 1st to the 6th experimental days (the reak of swimming) was observed. It was found out that the peak of physical performance was higher by 129.2% (p<0.05), relative to the initial swimming time of the group receiving Metaprot®. It should be noted that the duration of swimming on the peak day in the group that received Metaprot® was longer compared to the intact and negative control groups, by 193% (p<0.05) and 110.6% (p<0.05), respectively. It is worth noting that by the end of the experiment there had not been sharp drops in efficiency. The duration of swimming of the mice treated with Metaprot®, on the final experimental day was higher by 119% (p<0.05) and 330.2% (p<0.05), relative to the 10th day of the intact and negative control groups.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

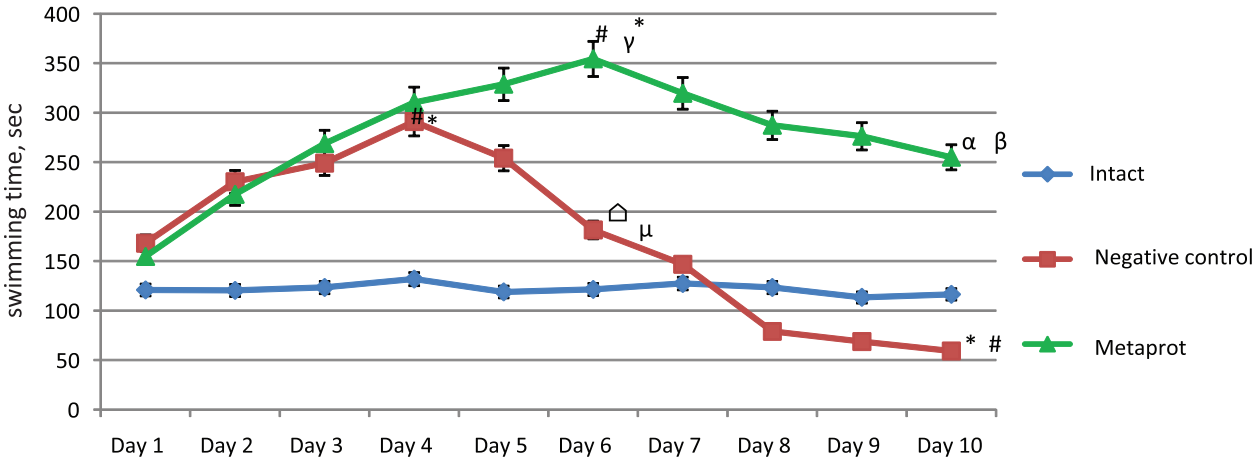

Figure 2 – Duration of mice's swimming time in the "Forced swimming test with loading" in hot water

*Note: * – statistically significant relative to the initial swimming time of this group (Student's t-test, (p <0.05)); # – statistically significant relative to the initial swimming time of the animals' intact group (Student's t-test, (p <0.05)); μ – statistically significant relative to the peak day of swimming for this group (Student's t-test, (p <0.05)); ⌂ – statistically significant relative to the peak day of swimming of the animals' intact group (Student's t-test, (p <0.05)); γ – statistically significant relative to the initial swimming time of the animals' negative control group (Student's t-test, (p <0.05)); β – statistically significant relative to the final swimming time of the animals' negative control group (Student's t-test, (p <0.05)); α – statistically significant relative to the final swimming time of the animals' intact group (Student's t-test, (p <0.05))*

As Fig. 3 shows, in the *animals' negative control* group, the oxygen consumption by erythrocytes compared to the intact group, was not statistically and significantly different in hot water.

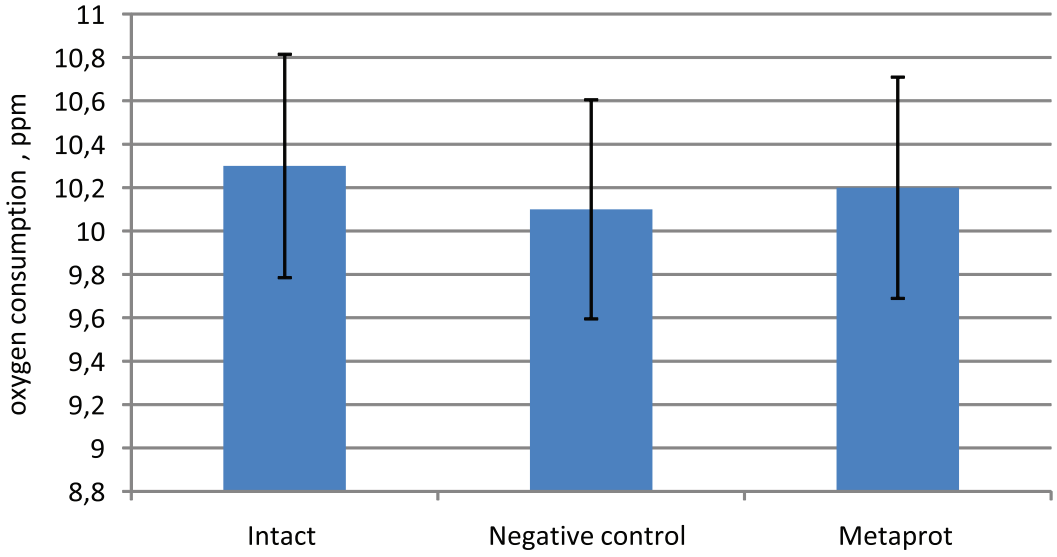

Figure 3 – Estimation of oxygen consumption by red blood cells against the background of the "forced swimming test with loading" in hot water

In the group of the intact animals and rats receiving Metaprot®, no changes in blood oxygenation were detected.

While the oxygen consumption by mitochondria of striated muscles in the animals' negative control group was 1.8 times lower (p <0.05) in comparison with the IN_h group of mice,there were no changes established in the oxygen consumption by the muscles in the group that received Metaprot®, relative to the animals' intact group. However, relative to the negative control group, this parameter was higher by 2.6 times (p<0.05).

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DOI: 10.19163/2307-9266-2019-7-3-148-157

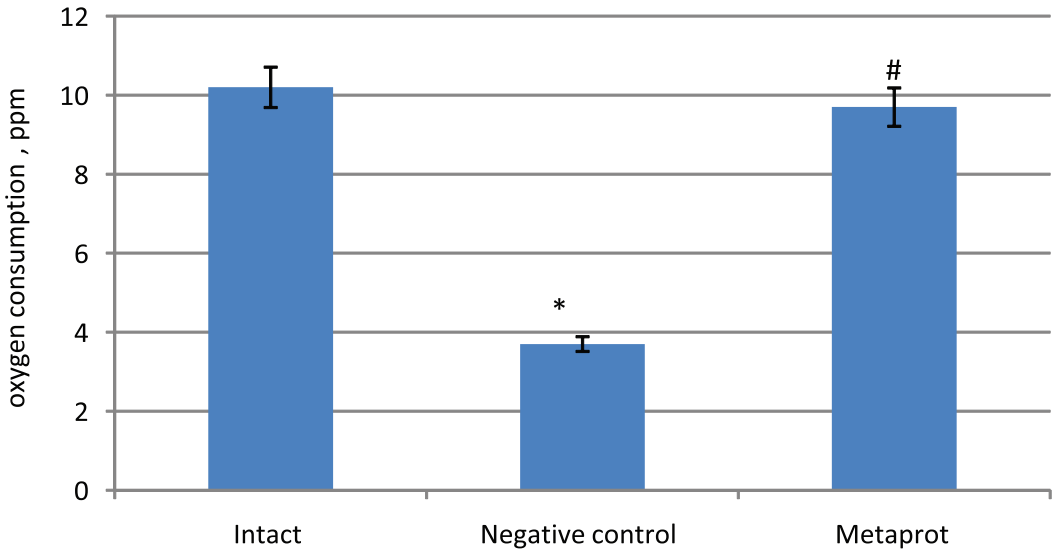

Figure 4 – Evaluation of the oxygen consumption by mitochondria of striated muscles against the background of the "forced swimming test with loading" in hot water

*Note: * – statistically significant relative to the animals' intact group (Student's t-test, (p <0.05)); # – statistically significant relative to the animals' negative control group (Student's t-test, (p <0.05))*

In the group of negative control mice, where cold water was used as an aversive environment, there was a statistically significant decrease in the oxygen consumption by muscle mitochondria by 2.4 times (p<0.05), relative to the intact group of the animals (Fig. 5, 6). Hereby, the blood oxygenation level remained unaffected.

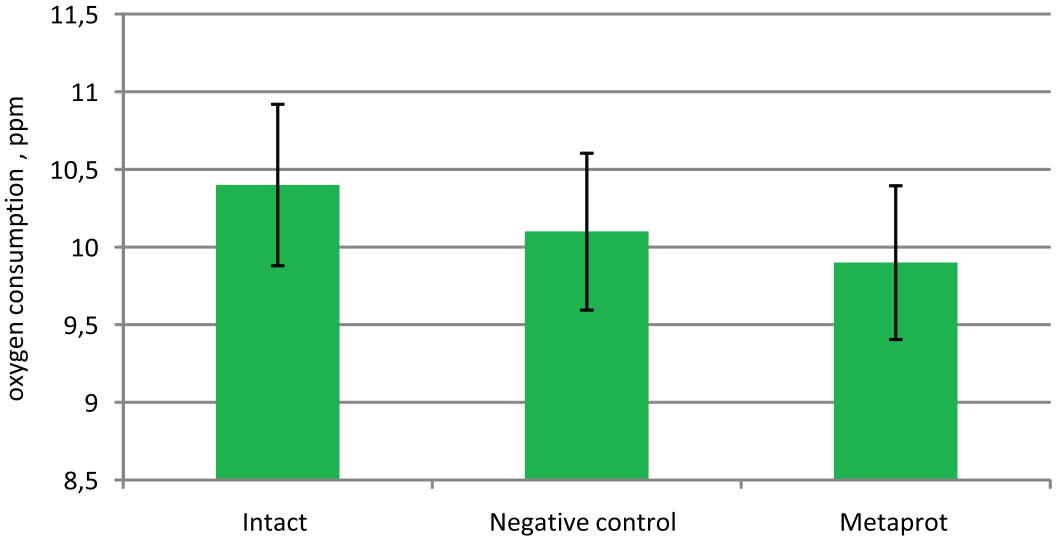

Figure 5 – Evaluation of oxygen consumption by red blood cells against the background of the "forced swimming test with loading" in cold water

It should be noted that the oxygen consumption by the mitochondria of the striated muscles in the group that received Metaprot® was higher, compared to the group of the animals of the negative control, by 4.3 times (p<0.05). As for the comparison with the group of intact animals, there were no statistically significant changes detected. It was also noted that against the background of "forced swimming" in cold water, in the group of the mice that received Metaprot® as the reference drug,, their blood oxygenation level remained unaffected.

ISSN 2307-9266 e-ISSN 2413-2241
Scientific and Practical Journal
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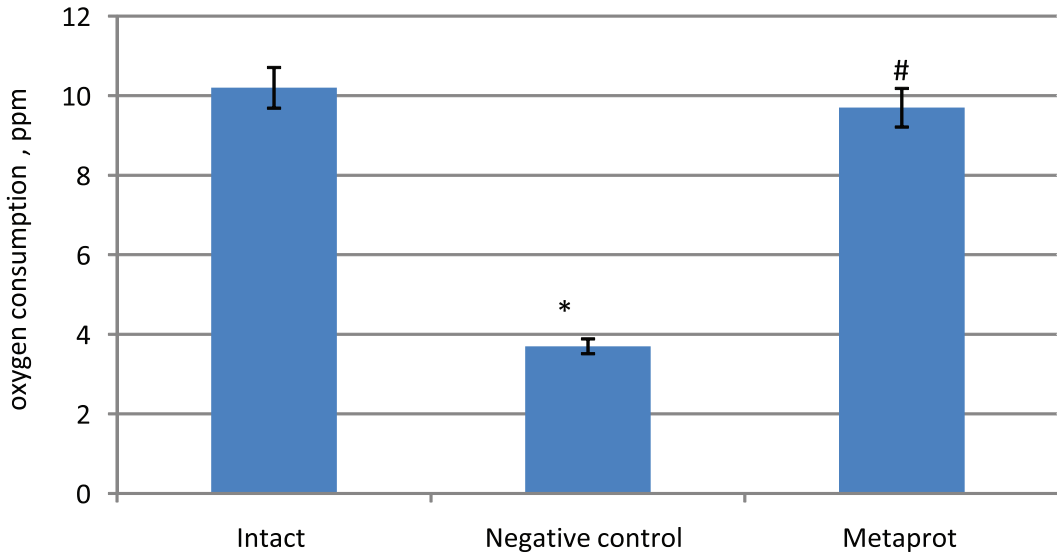

Figure 6 – Estimation of oxygen consumption by mitochondria of striated muscles against the background of the "forced swimming test with loading" in cold water

*Note: * – statistically significant relative to the animals' intact group (Student's t-test, (p <0.05)); # – statistically significant relative to the animals' negative control group (Student's t-test, (p<0.05)).*

DISCUSSION

The optimal level of physical activity is one of the main components of a healthy lifestyle, providing the necessary quality of life of the population [12]. It has been found out that individuals suffering from physical inactivity have an increased risk of developing diabetes, hypertension, cancer, obesity, and mental disorders (anxiety, panic attacks, depression, cognitive deficiency) [13]. However, excessive, depleting physical exertion, on the contrary, are a powerful stress factor and can contribute to a significant deterioration in the quality of life of the population, cause the development of cardiovascular pathology, and a decrease in the intensity of immune responses [14]. In many ways, the stress on the human body is determined by the nature of the unfavorable (aversive) environment, which aggravates the effect of the stressor on the body [15]. Under conditions of depleting physical overload, the effect of an aversive environment on the body is largely realized through a change in the activity of skeletal muscles – the main component of optimal physical activity [7]. Hereby, in response to the action of an unfavorable environmental factor the main compensatory mechanisms in the muscle tissue are: increased oxygen consumption, activation protein-synthetic processes, the intensification of metabolic reactions and the activation of metabotropic transport systems [6]. However, with progressive muscle fatigue, these compensatory mechanisms "turn off", and an increase in the oxygen consumption by tissues is not accompanied by an increase in the energy output, which, in turn, reduces the activity of striated muscles and the overall level of physical performance [16].

In the carried out study on the impact of the nature of an aversive environment on the level of oxygen consumption in muscle tissue and blood oxygen capacity, it was found out that in the animals' negative control group, in which hot water (the temperature of 41^0C) was used as an aversive environment, a change in the working capacity was observed: in mice, the peak of swimming was the 4^{th} day – 70% ($p<0.05$), relative to the initial day of swimming in this group. A similar pattern of change in physical activity in the mice's negative control group can be presumably mediated by the action of the aversive environment (hot water) on the skeletal muscle activity of the experimental animals. Under current conditions (hot water), dilatation of blood vessels of striated muscles is likely to be noted, which, despite the increased muscle need for oxygen, increases the delivery of oxygen and nutrients to skeletal muscle myocytes [17]. In addition, in the literature data it is reported that thermal effects on skeletal muscles activate metabolic processes and prevent muscle fatigue [17, 18]. In the future, a decrease in the level of performance was observed, which was lower by 2.8 times ($p<0.05$) and 2.0 times ($p<0.05$) relative to the initial swimming time value of the negative control group of mice, and the intact group, respectively. There were changes in the oxygen consumption only by the mitochondria of the striated muscles. It was lower by 1.8 times ($p<0.05$) in comparison with the intact group of animals. This fact of impairment can probably be attributed to the fact that an unfavorable environment (hot water) can lead to structural changes in the muscles, namely, the unfolding and denaturation of the protein, which, in turn, can contribute to the violation of the contractile function of the muscles [19].

At the same time, a group of mice (negative control),

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DOI: 10.19163/2307-9266-2019-7-3-148-157

where cold water (the temperature of 15°C), was statistically significant (by the end of the experiment) relative to the intact group of animals, the decrease in swimming duration, accompanied by a decrease in the oxygen consumption by muscle mitochondria, with a constant level of blood oxygenation. At the same time, on the 5th day of the experiment, the duration of swimming of mice in cold water increased by 25.3% ($p<0.05$) relative to the intact group. The results may be associated with the peculiarities of the effect of low temperatures on the body. Thus, *Naresh C., et al., 2017,* showed that the effect of low temperatures on muscle tissue causes significant metabolic changes in muscles, followed by activation of the mitochondrial uncoupling protein,Type 1 (UCP-1) [20]. Activation of UCP-1 in striated myocytes helps to reduce the proton gradient in the mitochondrial matrix, which, in turn, leads to the intensification of glycolysis with reduced ATP production, accompanied by increased thermogenesis. That can contribute to the preservation of muscle activity and, consequently, physical performance [21]. However, this compensatory mechanism has a short-term effect**.** Later, a significant decrease in physical activity was noted (a decrease in the duration of swimming of mice in cold water in comparison with the intact group of animals by 105.9% ($p<0.05$) by the 10th day of the experiment). However, later, with sufficient oxygenation of the blood (in cold water in mice, no decrease in the oxygen transport function of the blood was observed), hyperfunction of the UCP-1 protein contributes to the separation of oxidation and phosphorylation due to the practical loss of the proton gradient of the mitochondrial matrix [22]. That leads to a decrease in the oxygen utilization by skeletal muscles and, as a result, the synthesis of macroergic compounds deteriorates, the activation of oxidative stress takes place, which negatively affects the level of physical activity [23].

It can be assumed, that in addition to the activation of thermogenin UCP-1 under conditions of the effect of cold temperatures on the muscle tissue, there is a dysfunction of metabotropic transport systems GLUT4 [24]. The activity of AMP-activated kinase also decreases [25], which impairs the entry of cross-striped muscle glucose into myocytes and aggravates energy shortages.

Under conditions of exhaustive physical overload, in the group of the animals that received Metaprot®, an increase in their working capacity was noted, both in hot (from the 1st to the 6th experimental day (swimming peak) and in cold water - the maximum day of the animals' swimming was the 7th one. Further on, in both experimental groups there was a slight smooth drop in performance. At the same time, it should be noted, that the oxygenation of the blood and the muscle tissue against the background of debilitating loads in the group receiving Metaprot® remained unchanged in comparison with the group of intact animals in various aversive environments. An increase in the physical performance of the animals against the background of Metaprot® can probably be attributed to the inhibition of glycogen disintegration, a decrease in heat production, and also the activation of the synthesis of mitochondrial proteins (gluconeogenesis enzymes) [26, 27].

CONCLUSION

Thus, based on the obtained data, it can be assumed that under conditions of forced swimming with loading in animals, the most profound changes in the structure and functions of the striated muscles are observed in cold water (15^0C) acting as a stressor. That is reflected in a decrease in physical performance and in reducing the oxygen consumption by the muscle tissue. The use of the drug Metaprot® helped to correct the changes in the physical performance of the animals, which was reflected in its increase by 144.8% ($p<0.05$) compared to the initial swimming time of this group, without an increase of the oxygen consumption by red blood cells and mitochondria of striated muscles.

REFERENCES

1. Baron DA, Martin DM, Abol Magd S. Doping in sports and its spread to at-risk populations: an international review. World Psychiatry.2007; 6: 118–123.
2. Kupko EN, Gusova BA, Molchanov MV, Semuhin AN. Analiz farmakologicheskix podxodov k povy`sheniyu fizicheskoj rabotosposobnosti spasatelej v usloviyax chrezvy`chajny`x situacij [Analysis of pharmacological approaches to improving the physical performance of rescuers in emergency situations]. Pharmacy and pharmacology. 2014; 6 (7): 88–91. Russian.
3. Savilov ED. Tekhnogennoe zagryaznenie okruzhayushchej sredy - novyj faktor riska infekcionnoj patologii [Technogenic pollution of the environment – a new risk factor for infectious diseases]. Epidemiology and infectious diseases. 2011; 2: 4–8. Russian.
4. Yakovlev AA. E`kologicheskoe napravlenie v e`pidemiologii [Environmental direction in epidemiology]. Epidemiology and infectious diseases. 2011; 3: 33–37. Russian.
5. Kundashev UK, Zurdinov AZ, Morozov IS, Barchukov VG. Farmakologicheskaya korrekciya adaptivnyh reakcij serdechno-sosudistoj i central'noj nervnoj sistem u rabochih vysokogornogo rudnika pri vahtovom metode organizacii truda. Mediko-biologicheskie i social'no-psihologicheskie problemy bezopasnosti v chrezvychajnyh situaciyah [Pharmacological correction of adaptive reactions of the cardiovascular and central nervous systems in workers of a high-mountainous mine with the shift method of work organization]. Medical-biological and socio-psychological problems of security in emergency situations. 2013; 4:76–81. Russian.
6. Ferraro E, Giammarioli AM, Chiandotto S, Spoletini I, Rosano G. Exercise-induced skeletal muscle remod-

eling and metabolic adaptation: redox signaling and role of autophagy. Antioxid Redox Signal.2014; 21 (1): 154–176. Doi:10.1089/ars.2013.5773

7. Murach KA, White SH, Wen Y, et al. Differential requirement for satellite cells during overload-induced muscle hypertrophy in growing versus mature mice. Skelet Muscle. 2017; 7 (1):14. DOI:10.1186/s13395-017-0132-z
8. Voronkov AV, Pozdnyakov DI, Voronkova MP. Kompleksnaya validacionnaya ocenka novogo metodicheskogo podhoda k izucheniyu fizicheskogo i psihoehmocionalnogo perenapryazheniya v ehksperimente [Comprehensive validation assessment of a new methodological approach to the study of physical and mental strain in the experiment]. Fundamental research. 2015; 1-5:915-9.
9. Abdelhamid RE, Kovács KJ, Nunez MG, Larson AA. Depressive behavior in the forced swim test can be induced by TRPV1 receptor activity and is dependent on NMDA receptors. Pharmacol Res. 2013; 79: 21–27. Doi:10.1016/j.phrs.2013.10.006
10. Voronkov AV, Efremova MP, Gerashchenko A. D, Voronkova MP. Vliyanie novyh perspektivnyh aktoprotektorov na razvitie kognitivnogo deficita u krys na fone istoshchayushchih fizicheskih nagruzok [The impact of new promising actoprotectors on the development of cognitive deficits in rats on the background of debilitating physical exertion] Bulletin of the Volgograd State Medical University. 2018; 66(2): 107–11.
11. Patel SP, Sullivan PG, Pandya JD, et al. N-acetylcysteine amide preserves mitochondrial bioenergetics and improves functional recovery following spinal trauma. Exp Neurol.2014; 257: 95–105. DOI: 10.1016/j. expneurol.2014.04.026.
12. Ohno Y, Goto K, Yamada S, et al. Effects of heat stress on muscle mass and the expression levels of heat shock proteins and lysosomal cathepsin L in soleus muscle of young and aged mice. Molecular and cellular biochemistry.2012;369:45-53. DOI:10.1007/s11010-012-1367-y
13. Stults-Kolehmainen MA, Sinha R. The effects of stress on physical activity and exercise. Sports Med. 2014;44(1):81–121. DOI:10.1007/s40279-013-0090-5
14. Koolhaas JM, Bartolomucci A, Buwalda B, et al. Stress revisited: a critical evaluation of the stress concept. Neurosci Biobehav Rev. 2011;35(5):1291–301.
15. Zhang S, Wei Z, Liu W, et al. Indicators for Environment Health Risk Assessment in the Jiangsu Province of China. Int J Environ Res Public Health. 2015;12(9):11012-24. DOI:10.3390/ijerph120911012
16. Kjøbsted R, Hingst JR, Fentz J, et al. AMPK in skeletal muscle function and metabolism. FASEB J. 2018;32(4):1741–77. DOI:10.1096/fj.201700442R
17. Ohira T, Higashibata A, Seki M, et al. The effects of heat stress on morphological properties and intracellular signaling of denervated and intact soleus muscles in rats. Physiol Rep.2017; 5(15): e13350. DOI:10.14814/phy2.13350
18. Wei M, Gibbons LW, Kampert JB, et al. Low cardiorespiratory fitness and physical inactivity as predictors of mortality in men with type 2 diabetes. Ann Intern Med. 2000;132(8): 605–11.
19. Locke M, Celotti C. The effect of heat stress on skeletal muscle contractile properties. Cell Stress Chaperones. 2013; 19(4):519–527. DOI:10.1007/s12192-013-0478-z
20. Bal NC, Singh S, Reis FCG, et al. Both brown adipose tissue and skeletal muscle thermogenesis processes are activated during mild to severe cold adaptation in mice. J Biol Chem. 2017; 292(40):16616–25. DOI:10.1074/jbc.M117.790451
21. Gorski T, Mathes S, Krützfeldt J. Uncoupling protein 1 expression in adipocytes derived from skeletal muscle fibro/adipogenic progenitors is under genetic and hormonal control. J Cachexia Sarcopenia Muscle. 2018;9:384–99. DOI:10.1002/jcsm.12277
22. Chung N, Park J, Lim K. The effects of exercise and cold exposure on mitochondrial biogenesis in skeletal muscle and white adipose tissue. J Exerc Nutrition Biochem.2017; 21(2):39–47. DOI:10.20463/jenb.2017.0020
23. Wakabayashi H, Nishimura T, Wijayanto T, et al. Effect of repeated forearm muscle cooling on the adaptation of skeletal muscle metabolism in humans. International journal of biometeorology. 2017;61(7):1261–7. DOI 10.1007/s00484-016-1303-z
24. Reynolds TH, Brozinick JT, Larkin L.M, et al. Transient enhancement of GLUT-4 levels in rat epitrochlearis muscle after exercise training. J Appl Physiol (1985). 2000;88(6): 2240–5. DOI:10.1152/jappl.2000.88.6.2240
25. Kang C, Li Ji L. Role of PGC-1α signaling in skeletal muscle health and disease. Ann N Y Acad Sci. 2012;1271(1):110–7. DOI:10.1111/j.1749-6632.2012.06738.x
26. Vorob'eva VV, Zarubina IV, Shabanov PD. Zashchitnye effekty metaprota i etomerzola v eksperimental'nyh modelyah otravlenij bytovymi yadami [The protective effects of methaprot and etommerzol in experimental models of household poisoning poisoning]. Reviews of clinical pharmacology and drug therapy. 2012;10(1):3–21. Russian.
27. Sviryaeva IV, Mercalova AS, Ruuge EK. Obrazovanie superoksidnyh radikalov v izolirovannyh mitohondriyah serdca pri maloj koncentracii kisloroda [Formation of superoxide radicals in isolated heart mitochondria with low oxygen concentration]. Biophysics. 2010;55(2):271–6. Russian.

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DOI: 10.19163/2307-9266-2019-7-3-148-157

FINANCIAL SUPPORT AND SPONSORSHIP

This study did not have any financial support from outside organizations.

AUTHOR CONTRIBUTIONS

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CONFLICTS OF INTEREST

The authors and peer reviewers of this paper report no conflicts of interest.

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RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

MODERN MODEL FORMATION OF DRUG PROVISION OF PATIENTS WITH COMMUNITY-ACQUIRED PNEUMONIA IN KABARDINO-BALKARIA

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Received 06.05.2019 Review 1 29.05.2019 Review 2 15.06.2019 Accepted for publication: 27.06.2019

The increase in the incidence of community-acquired pneumonia (CAP) is a major problem of national and regional importance. Its pharmacotherapy is based on the active use of antibacterial drugs. That requires a special attention of both, a doctor and a patient, the search for all new and advanced medicines (drugs).

The aim of the study is to justify and develop a regional model of drug provision of pharmacotherapy in community-acquired pneumonia.

Materials and methods. The use of the resource approach and the methodology of pharmaceutical care (PhC) for the formation of a modern model of drug supply have been tested. Logical, retrospective, sociological, pharmacoeconomic, marketing, statistical and other analytical methods have been used. The materials were official statistics, literature data and the results of the research carried out by the authors themselves.

Results. The state and tendencies of development of organizational, personnel, financial (population) and drug resources as the external factors affecting PhC to patients with CAP in the Kabardino-Balkarian Republic (KBR) have been identified. The following internal factors of PhC have been defined: medical prescriptions, consumer demands and the cost of pharmacotherapy. A model of drug provision for patients with CAP in the region has been formed. It is aimed at increasing the effectiveness of treatment, taking into account the stage of treatment and the price factor.

Conclusion. Under the conditions of a particular region, the use of the resource approach and the PhC methodology allows to more accurately identify the problems and risks of both drug provision and pharmacotherapy of the patients' population under study, and form a model adequate to reality, develop recommendations for all participants of PhC – a doctor, a patient and a pharmaceutist (a pharmacist).

Keywords: community-acquired pneumonia (CAP), patients, drug provision, model, pharmaceutical care (PhC), resource approach, Kabardino-Balkaria

For citation: A.A. Umirova, V.L. Adzhienko, T.I. Kabakova, E.A. Korzhavykh, V.V. Gatsan. Modern model formation of drug provision of patients with community-acquired pneumonia in Kabardino-Balkaria. *Pharmacy & Pharmacology*. 2019;7(3): 158-169. **DOI:** 10.19163/2307-9266-2019-7-3-158-169

Для цитирования: А.А. Умирова, В.Л. Аджиенко, Т.И. Кабакова, Э.А. Коржавых, В.В. Гацан. Формирование современной модели лекарственного обеспечения пациентов с внебольничной пневмонией в Кабардино-Балкарии. *Фармация и фармакология.* 2019;7(3): 158-169. **DOI:** 10.19163/2307-9266-2019-7-3-158-169

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2019-7-3-158-169

ФОРМИРОВАНИЕ СОВРЕМЕННОЙ МОДЕЛИ ЛЕКАРСТВЕННОГО ОБЕСПЕЧЕНИЯ ПАЦИЕНТОВ С ВНЕБОЛЬНИЧНОЙ ПНЕВМОНИЕЙ В КАБАРДИНО-БАЛКАРИИ

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Получено 06.05.2019 Рецензия 1 29.05.2019 Рецензия 2 15.06.2019 Принята к печати 27.06.2019

Рост заболеваемости внебольничной пневмонией (ВП) является крупной проблемой государственного и регионального значения. Ее фармакотерапия базируется на активном применении антибактериальных препаратов, что требует особого внимания врача и пациента, поиска всё новых и совершенных лекарственных средств (ЛС).

Цель исследования – обоснование и разработка региональной модели лекарственного обеспечения фармакотерапии при ВП.

Материалы и методы. Проверялась применимость ресурсного подхода и методологии фармацевтической помощи (ФП) для формирования современной модели лекарственного обеспечения. Использованы логический, ретроспективный, социологические, фармакоэкономические, маркетинговые, статистические и другие аналитические методы. **Материалами** служили данные официальной статистики, литературные сведения и результаты собственных исследований.

Результаты. Выявлено состояние и тенденции развития организационного, кадрового, финансового (население) и лекарственного ресурсов как внешних факторов, влияющих на ФП пациентам с ВП в Кабардино-Балкарской Республике (КБР). Определены внутренние факторы ФП – назначения врачей, потребительский спрос и стоимость фармакотерапии. Сформирована модель лекарственного обеспечения пациентов с ВП в регионе, направленная на повышение эффективности лечения с учетом этапа лечения и ценового фактора.

Заключение. В условиях конкретного региона применение ресурсного подхода и методологии ФП позволяет более точно определить проблемы и риски как лекарственного обеспечения, так и фармакотерапии исследуемой популяции пациентов и сформировать адекватную реальности модель, выработать рекомендации для всех участников ФП – врача, пациента и провизора (фармацевта).

Ключевые слова: внебольничная пневмония, пациенты, лекарственное обеспечение, модель, фармацевтическая помощь, ресурсный подход, Кабардино-Балкария

INTRODUCTION

For many years, pneumonia has remained one of the most significant diseases for the population and health systems around the world. High mortality and disability rates, a particular vulnerability of children and elderly and old people, high financial costs of patients' treatment and elimination of severe consequences of the disease that have occurred – all these factors require close attention to finding the best strategy for pneumonia pharmacotherapy [1, 2].

In Russia, pneumonia is also a serious public health problem. According to the data of Russian Federal State Statistics Service, in the Russian Federation in the period from 2006 to the present, the incidence for every 100 thousand people of the adult population varies from 344.0 to 404.0.

In these circumstances, according to a number of authoritative scientists [3–7], the real incidence may be significantly higher. Currently, CAP is the most common type of infection. The highest morbidity rates are recorded among the elderly people and children aged 4–5 years [2, 8–11].

The result of the wrong treatment regime, late diagnosis of patients, incomplete elimination of the inflammatory process at discharge from hospital in patients with pneumonia is the transition from an acute form to chronic. In addition, uncontrolled therapy causes the emergence of antibiotic resistance. 30% of pneumococcal strains today are known to have resistance to penicillin; the number of strains insensitive to cephalosporin antibiotics is growing progressively [12].

Such medical factors as diagnostic errors (approximately in 20% of cases), delays with diagnosis (on average for 3 days) and others, lead to the appearance of pharmacotherapy problems. With uncontrolled therapy,

antibiotic resistance occurs more often. The economic damage from the elimination of these problems annually reaches 15 billion rubles. [13–15].

The experience of the developed countries of the world shows that in medical and pharmaceutical practice, overcoming the potential problems of pharmacotherapy is greatly facilitated by the application of the principles and techniques of specialized pharmaceutical care (PhC), focused primarily on optimizing medical and informational support of a doctor and a patient [16–22].

Over the past 20 years, PhCstudies in patients with various diseases have been carried out by Dremova N.B., Ovod A.I., Korzhavikh E.A. [23–26], Spichak I.V., Glembotsky G.T., Zakharova O.V., Alexeev I.V., Varenykh G.V., Trubina L.V., Petrov A.G. and others [27–33].

In pharmacy, only a few works are devoted to the improvement of drug supply for patients with pneumonia: by Z. Zakaryaeva [34], Wolfram N.A. [35].

No research has been carried out on the subject matter of PhC in relation to the situation in the Kabardino-Balkarian Republic. At the same time, as the study of profile literature shows, a regional approach to improving drug provision for patients with various nosological forms of diseases is increasingly used in modern pharmaceutical research, both in foreign [36–39] and in domestic kinds of research [40–42].

It is noted that in the conditions of modern Russia, due to its large extension, climatic-geographical, economic, socio-demographic differences between the constituent territories of the Russian Federation, the regional approach makes it possible to justify the problem and arrive at the most correct decisions in the sphere of drug provision.

The researchers emphasize the fact that the relevance of finding rational schemes for the treatment of pneumonia is due to the significant costs associated with not only drugs but also medical consumables. In addition, foreign scientists also include the cost of consulting a specialist, laboratory and diagnostic studies in the cost of treatment [1, 8, 21, 22, 35, 39].

All of theabove-listed indicates the relevance of a comprehensive analysis of drug supply issues for the subsequent rationalization of costs, as well as the achievement of the highest efficiency of drug treatment.

THE AIM of the study is to justify and develop a regional model of pharmacotherapy drug provision for patients with CAP.

MATERIALS AND METHODS

The objects of the study were statistical data on the Russian Federation and the KBR for 2008–2018, statistical reports of health care organizations, lists of drugs (State Register of Medicinal Remedies, vital essential and necessary medicines, treatment standards, etc.); the results of sociological surveys of doctors (30 questionnaires; a set sample), pharmacy workers (41 questionnaires), patients with CAP (70 questionnaires); price lists of wholesale and retail pharmaceutical organizations within the territory of the KBR (the actual range of drugs).

The leading research methods were: systematic, resource and regional approaches; methods of historical, logical, scientometric (content analysis), marketing (assortment structure, price), pharmacoeconomic (cost of disease) and economic and statistical analysis (grouping, variation statistics), sociological (questionnaire survey, expert assessments). The results were processed using standard computer programs.

On the basis of the preliminary research, a working hypothesis of the study was formulated: considering the problem from the standpoint of the resource approach and the ideology of specialized pharmaceutical care will allow the most complete and reasonable improvement of drug supply to patients with CAP within the territory of the KBR.

In accordance with this hypothesis, the following tasks were to be solved:

1. to assess the status and development trends of regional resources – organizational, personnel, financial (population), and drugs – as external factors of PhC for patients with CAP;
2. to characterize the internal factors of CAP pharmacotherapy in the KBR – doctors' prescriptions (stationary stage of medical care), consumer demand (outpatient stage), the cost of pharmacotherapy (inpatient and outpatient stages);
3. to form a regional conceptual model of drug provision for patients with CAP.

To ensure the theoretical unity of the study, the following terms and definitions were used:

- drug provision – an organizational system (a set of measures) to bring drugs from the manufacturer to the consumer (Shukil LV, 2018) ;
- drug care – a type of professional health care, aimed at the provision, prescription and use of drugs (Fomina AV, 2006) [43];
- pharmaceutical care – the system of drug, information and organizational-methodological support of individualized pharmacotherapy of specific diseases (Dremova N. B. et al., 2005) [26].

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DOI: 10.19163/2307-9266-2019-7-3-158-169

RESULTS AND DISCUSSION

At the first stage of the work, it was found out that in terms of socio-economic indicators, the KBR usually occupies middle positions of the seven subjects of the North Caucasus Federal District (NCFD; Table 1). This confirms the validity of the choice of the KBR as a model region in our study.

Table 1 – Some socio-economic indicators of Kabardino-Balkaria (according to Federal State Statistics Service data, 2018 [3, 4])

Indicators	KBR
Area, thousandsquarekm	12,5
Populationon 01.01.18, thousandpeople	865,8
Average annual number of employed, thousand people	362,6
Average per capita cash income (per month), rub.	20385,0
Gross regional product in 2016, mln. rub.	132706,9

The following indicators of socio-demographic resource, compared with the average for the Russian Federation, refer to the features of the KBR region:

- lower proportion of the average annual number of the employed in the total population of the KBR;
- higher rate of population ageing;
- lower standard of living (subsistence minimum and per capita income);
- outstripping growth of the General morbidity.

In addition, the increase in the incidence of CAP per 100 thousand population for the period of 2013–2017 has been identified: 1) in the RF – by 1.06 times (in 2018 – already by 1.3 times), 2) in accordance with the resource approach, the pharmaceutical, organizational, human and medicinal resources of the model region have been evaluated, as they are most dependent on the state of the drug supply system.

Due to the lack of official statistics of pharmaceutical organizations to assess the pharmaceutical organizational resource in the country, we have analyzed the composition and structure of the collection of licensing entities (licensees) doing pharmaceutical activities in the region

It has been established that in 2018, there were 382 holders of 547 licenses for pharmaceutical activities in the KBR. By legal status, the majority of licensees – 211 (55.2%) – were legal entities; the number of individuals was 171 (44.8%). The distribution of licensees according to the form of ownership is presented in Table 2.

Table 2 – Distribution of licensees for pharmaceutical activities in the Kabardino-Balkarian Republic according to the form of ownership (the data by the end of 2018)

Formofownership	Type of owner	Quntity oflicensees	
		Absolute	Relative, %
Publicownership, including:		49	12.83
– state	State autonomousInstitution (SAI), State Budgetary Institution (StateBudgetaryHealthcareInstitution) State Public Healthcare Institution (SPHI)	37	9.69
– federal	Federal Public Institution, Federal PublicHealthcare Institution	7	1.83
– municipal	Municipal Unitary Enterprise (MUE), Municipal Unitary Pharmaceutic Enterprise (MUPhE)	5	1.31
Private, including:		333	87.17
– individual	self-employedentrepreneur (SEE)	171	44.76
– collective	Closed Joint-Stock Company (CJSC), Open Joint-Stock Corporation (OJSC)	162	42.41

The results, shown in Table 2, indicate a clear predominance of private owners among pharmaceutical license holders – 87.17%; these are mainly self-employed entrepreneurs, who account for almost half of all licensees and 40.77% of all licenses for pharmaceutical activities.

The second important result is that medical, rather than pharmaceutical, organizations clearly dominate among publicly owned licensees. The latter include the autonomous public health care institution "Pharmacy Warehouse" of the Ministry of Health of the KBR and five pharmacy enterprises with municipal property; in total, they make up only 1.57% of all licensees, possessing seven licenses (1.28%).

The third parameter that deserves attention is the ratio of urban and rural licensees. In our study, the corresponding number was 3.66 : 1, i.e. 300 licensees operating in urban areas (78.5%) with 436 (79.7%) licenses accounted for 82 (21.5%) rural licensees with 111 (20.3%) licenses.

Thus, an excessive concentration of a pharmaceutical organizational resource, including individual entrepreneurs, most often owners of pharmacies, in cities (79.7% of licensees), is a problem, while almost half of the population of the republic lives in rural areas. This problem significantly reduces the availability of drug care to the rural population of the KBR.

In the process of evaluation of personnel pharmaceutical resource, it was found out that the statistical records of pharmaceutical personnel (pharmaceutists and pharmacists) at the Federal level are conducted only in respect of their training – the number of applicants and the number of graduates. Information on the number of pharmaceutists and pharmacists employed in the drug supply system at the country or regional level could not be identified from available sources.

In this regard, the estimated number of pharmaceutical professionals in Russia as a whole was determined using two different methods: 1) on the basis of extrapolation of official data, as of 1998, and 2) taking into account trends in the development of the pharmacy network of the country and the number of available pharmaceutists and pharmacists (Fig. 1 and 2 are based on the data of Federal State Statistics Service).

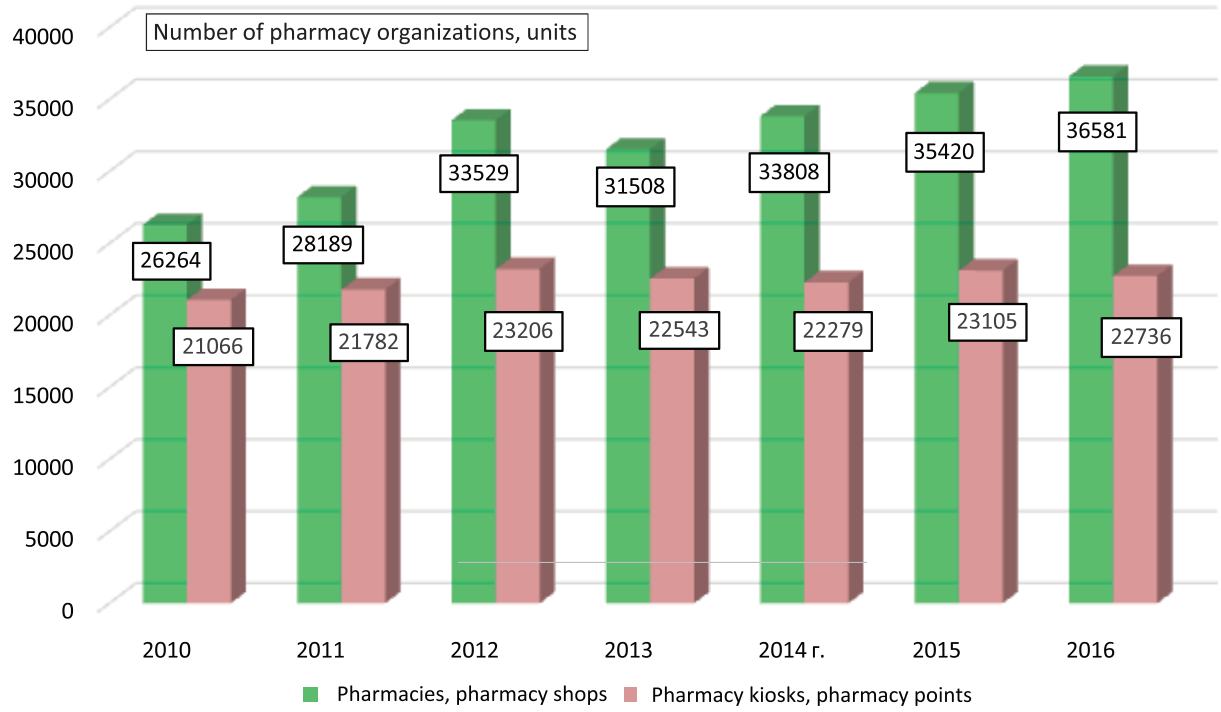

Figure 1 – Dynamics of the number of pharmacy organizations in Russia (2010–2016)

As a result, an estimated number of pharmaceutical workers has been determined: as of 2016, there can be at least 184.46 specialists with higher and secondary pharmaceutical education in the Russian Federation. Considering the personnel trained before 1995 and who have not yet reached the retirement age, as well as pensioners who continue to work in pharmacy organizations, this number can be increased to 200 thousand people or more.

The foregoing leads to the conclusion about the intensive development of the pharmaceutical human resources in the Russian Federation and the high availability of pharmaceutical personnel to the country's population. At the same time, the lack of official statistical records of pharmaceutical personnel, primarily in the regions, does not allow assessing the analyzed resource properly from the point of view of the actual state, the region's need for such personnel and the satisfaction of this need.

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Figure 2 – Dynamics of the number of graduates in the specialty "Pharmacy" in Russia (1995–2016)

The methodical approach used further for the assessment of regional medicinal resource for CAP treatment, provided marketing characteristics of the resource in the qualitative and quantitative (assortment) and price aspects at the following levels:

- lists of drugs for CAP pharmacotherapy, which are the starting point for the assessment of the Russian market for this target segment and, accordingly,
- the wholesale pharmaceutical market of the KBR;
- the retail pharmaceutical market of the KBR.

The sources of baseline information are the following: standards for providing medical care to patients with pneumonia in 2007 (severe forms with complications) and in 2012 (forms of moderate severity); State Register of Medicinal Products; Directory of Synonyms of Medicinal Products (Pharmaceutical Center, 2015); lists of vital and essential drugs, the minimum range of drugs needed for medical care, KBR Territorial programmeon State Guarantees, as well as price lists of distributors and pharmacy organizations.

Federal standards for pneumonia treatment have been established to include drugs under 18 international nonproprietary names (INN). The comparative analysis of the lists revealed the following facts:

- 14 INNs (78%) are included in all 4 lists; 1 INN – Cefotaxime – is not included in the list of KBR Territorial programmeon State Guarantees, 3 INNs – a combination of amoxicillin+sulbactam, ketoconazole and netilmicin – are not included in the VED and Provision of Necessary Medicinal Products; five INNs (22%) are included in the Minimum assortment list for pharmacy organizations;
- 72% of each INN is included in the regulative lists of vital essential and necessary medicines and Provision of Necessary Medicinal Products, on the basis of which preferential and free supply of medicines is carried out;
- 411 medicines in 42 dosage forms (non-recurring names) were registered under 322 trade names as 18 INNs mentioned in the standard of 2007;
- the total number of dosage forms was 392 for 411 drugs. At the same time, the widest range of dosage forms was noted for such drugs as Ciprofloxacin (44), Ambroxol and Azithromycin (41 each), the smallest – for the combination of Ipratropium bromide + Fenoterol and Netilmicin (3); the total number of the countries producing drugs of this target segment was 39 non-recurring names;
- the largest number of dosage forms accounted for tablets was 148 (37.76%);
- the number of oral drugs (61.74%) was twice as large as the number of parenteral ones (Table 3).

Table 3 – Range of drugs used in pneumonia, according to the standard of 2007 (adopted as the base)

International Nonproprietary Name (INN)	Number of trade names (TN)	Number of dosage forms (DF)	Number of producing countries
Drugs affecting respiratory system			
Other drugs for the treatment of respiratory organs diseases, not designated in other headings			
Acetylcysteine	12	17	6 countries
Anti-asthmatics			
Ipratropium bromide + Fenoterol	3	3	3 countries
Aminophylline	6	6	3 countries
Ambroxol	33	41	18 countries
Agents for prevention and treatment of infections			
Antibacterial agents			
Ampicillin	5	7	3 countries
Amoxicillin	12	14	7 countries
Amoxicillin + clavulanic acid	23	28	11 countries
Amoxicillin + sulbactam	2	3	1 country
Azithromycin	29	41	9 countries
Clarithromycin	29	31	14 countries
Moxifloxacin	12	14	4 countries
Netilmicin	3	3	2 countries
Cefuroxime	17	21	10 countries
Levofloxacin	34	41	14 countries
Cefotaxime	23	21	9 countries
Ciprofloxacin	34	44	8 countries
Antifungal agents			
Fluconazole	30	37	13 countries
Ketoconazole	15	20	8 countries
Total: 18 INNs	**322 TNs (100%)**	**392 (100%); 42 non-recurring names of forms**	**39 non-recurring names of countries; 411 medicinal preparations (100%)**

The obtained data were used for comparison with the actual state of the medicinal resource in the KBR, primarily in pharmaceutical wholesale organizations that meet the needs of hospital and retail sectors of the regional market in the target segment medicinal preparations for pneumonia treatment.

The assortment profiles made up for the target drug segment in the federal and regional (wholesale) markets, are presented in Fig. 3.

As Fig. 3 shows, the regional range is significantly poorer than that in the Russian Federation on the whole – in the number of INNs, the number of trade names and the number of dosage forms. However, in the KBR the proportion of oral drugs and tablets is significantly higher.

The revealed facts make it possible to draw a conclusion about the lack of completeness and breadth of the range of this target segment in the regional pharmaceutical market.

Thus, marketing studies have shown that the development of the regional medicinal resource for the CAP treatment is characterized by satisfying the need for the rangerecommended by the CAP treatment standards, by about 78%, while oral drugs make up about 91%. In the region, there is also a significant differentiation in drugs prices for the CAP treatment.

At the next stage, the internal factors of PhC affecting pharmacotherapy and its consequences for CAP patients were studied. The following factors have been established:

– pharmaceutists and pharmacists of pharmacy organizations are practically not considered by medical specialists as informants and consultants on drug issues. Consequently, the interaction in the process of pharmacotherapy as a mandatory element of PhC is not developed, and a high qualification of pharmaceutical workers is not used by doctors to the proper extent;

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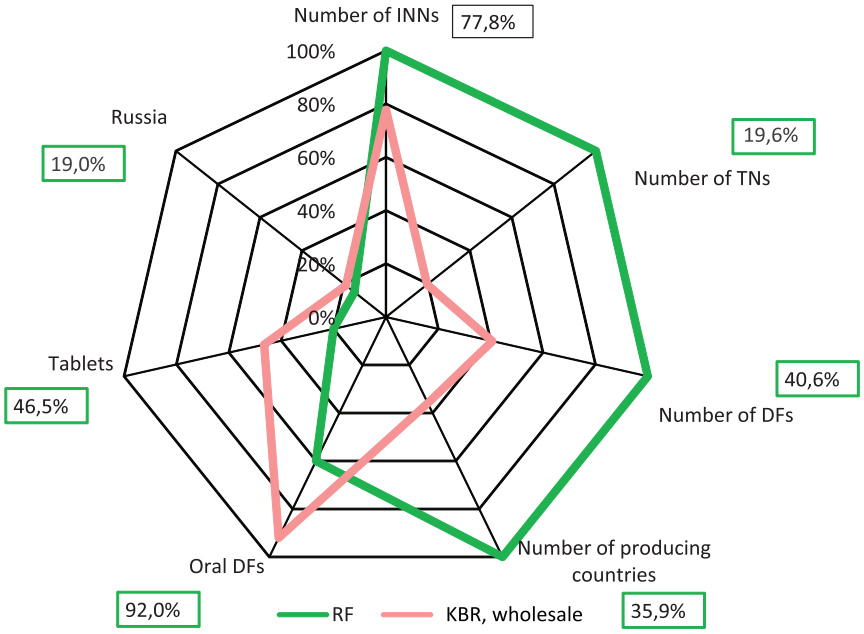

Figure 3 – Assortment profile of funds for pneumonia treatment

– the group of the highest medical trust (preferences) includes only one drug – Moxifloxacin; above the average confidence there is a combination of Ipratropium bromide + Fenoterol, Fluconazole, Acetylcysteine;
– according to the survey of pharmacists, the key factors determining a patient's choice when buying a drug for flu and cold treatment, are the price and recommendations of a pharmacists and a pharmaceutists (60% and 43%, respectively);
– only a quarter of patients (23%) respond the doctor's recommendations. The data obtained are consistent with the results of an expert survey of doctors: all experts agree that self-treatment exacerbates the course of the disease;
– the highest consumer demand is typical of a group of remedies for symptom control (87%);
– patients tend to change the prescribed therapy or make up an independent decision when choosing a drug therapy.

The revealed differences between pharmacy consumer demand and the preferred prescriptions of medical experts in relation to the required drugs indicates high risks in the self-selection of antibacterial drugs by patients; the consequences of these risks may be the inefficiency of the chosen pharmacotherapy, complications and side effects, increased resistance of microorganisms.

In the study of CAP in-patients, such negative behavioral characteristics of CAP patients as a high tendency to self-treatment and the use of antibiotics without a doctor's prescription were revealed. As a rule, the duration of the use of antibacterial drug is often not familiar to the patient.

These circumstances are consistent with the data obtained in the survey of medical experts and specialists of pharmacy organizations, and serve as an undoubted basis for purposeful informing patients about the properties and risks of the use of antibacterial agents without medical supervision.

Among the most influential factors of pharmacotherapy is the factor of its cost, dependent on the price of a medicinal preparation, so we have analyzed the prices of medicinal preparations under the trade names, registered for INNs, and referred to in the standard of care for pneumonia patients.

It has been established that the minimum differences in price are characteristic of Ampicillin preparations (5.2 rubles), the maximum ones are for Ambroxol (1061.2 rubles), Levofloxacin (1452.2 rubles) and Moxifloxacin (3158.6). The obtained data correspond to the global practice and are the reasons for the emergence of stepwise antibiotic therapy, in which the ways of drug administration are combined. This increases efficiency and reduces the cost of treatment.

Based on these factors, the cost of oral and parenteral antibiotic therapy using the equivalent course dose was compared. It was revealed that the average cost of oral therapy with antibacterial drugs is 917.5 rubles, the cost of parenteral therapy is 5127.7 rubles. The minimum cost of antibiotic therapy is 219.9 rubles (the oral course of Ciprofloxacin), the maximum cost is 13913.4 rubles (parenteral course of Moxifloxacin).

Thus, the cost of a drug therapy course for CAP

varies significantly depending on the drug's dosage form and the way of its administration.

Based on the generalization of the research results and from the standpoint of the resource approach, a conceptual model for optimizing the drug supply of CAP patients in the KBR has been developed (Fig. 4). The model consists of two parts:

– results of the assessment of the external factors (resources) of drug supply for CAP patients in Kabardino-Balkaria;
– results of the study of the internal factors of CAP pharmacotherapy (physician, patient, pharmaceutical worker; drug supply – the range and price).

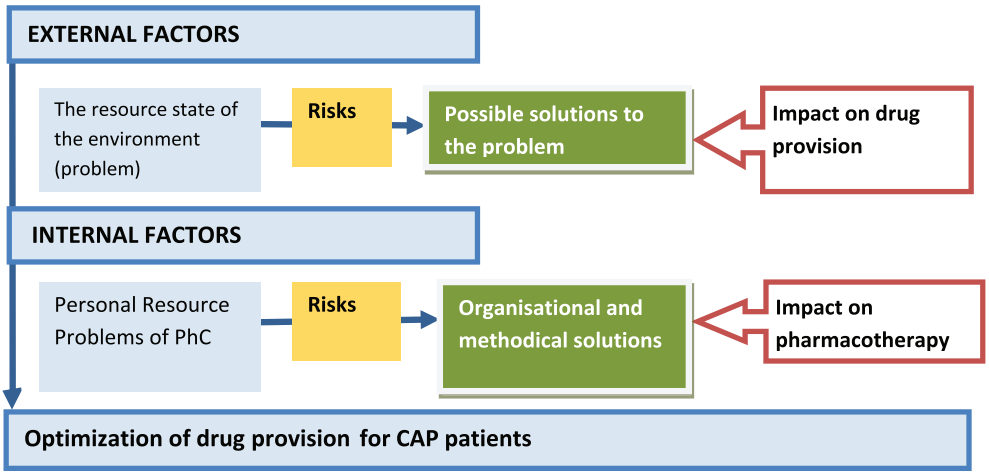

Figure 4 – Scheme of conceptual model optimization of drug provision for patients with community-acquired pneumonia in the region

The results of the factors' assessment are considered as problems causing a set of risks for drug provision and pharmacotherapy of CAP. Scientifically grounded organizational and methodological solutions aimed at reducing or eliminating manageable risks (mainly caused by internal factors) have been offered in the study.

The external factors that affect the provision of CAP treatment drugs comprise socio-demographic, organizational, personnel, financial (population) and drug resources. The main problem of these resources within the territory of the KBR is their deficit.

In particular, with regard to the organizational pharmaceutical resource, the notable problems identified in the course of the research, were the lack of accounting of pharmacy organizations and the concentration of pharmacy activities in urban areas.

The risks are:

– the difficulty of monitoring and assessing the availability of pharmaceutical organizations to the population of the KBR;
– reducing the availability of PhC for rural residents. The possible solutionis are: renewal of the previously existing system of official statistics of pharmaceutical organizations and adjustment of their location.

The main problem of the pharmaceutical human resources in the KBR is the lack of official records of pharmaceutical personnel, which leads to such risks as:

1) hiring non-core personnel to work;

2) decline in the quality of pharmaceutical services provided to the population. A possible solution, as in the previous case, is to resume the pre-existing statistics of pharmaceutists and pharmacists.

Insufficiency of the medicinal resource is in the fact that the needs of the region for CAP treatment are only satisfied by 78%. This leads to the risk of reducing the physical accessibility of drugs to the public and medical organizations. A possible solution to the problem is a scientifically based and adequate determination of the region's need for drugs to carry out CAP pharmacotherapy, which, in turn, will require further training of pharmaceutical specialists in this field.

The problem of insufficient financial resources of the Kabardino-Balkarian population is confirmed by the fact that the average per capita income is lower than in the Russian Federation. Accordingly, there is a risk of reducing the economic availability of drugs for CAP patients. The prospect of a successful solution to this problem is uncertain and poorly manageable.

The problems of the socio-demographic resource of the KBR are associated with the following trends: 1) a higher rate of population aging than in the Russian Federation; 2) lower, than in the Russian Federation, share of the average annual number of employees in the total population (impact on per capita incomes); 3) outpacing, in comparison with the Russian Federation, increase in the overall incidence and CAP incidence.

These problems cause the risk of increasing the need for preferential and free drugs, as well as geriatric medical and pharmaceutical care. The possible solutions are: a systematic maintenance of regional registers of

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persons in need of guaranteed social support of the state, and uninterrupted supplys of the required drugs.

Thus, the proposed model shows that the influence of the external factors (resources of the region) leads to the risks associated with a decrease in the physical and economic accessibility of drugs to the population of the KBR as a whole and CAP patients – in particular, against the background of a decrease in the financial security of the citizens living in the region.

The second group of factors involved in the conceptual model is internal, based on the personal, behavioral characteristics of the participants in the pharmacotherapy process and, accordingly, PhC – the team of a physician, a patient and a pharmaceutical worker. In addition to this group of factors, the "behavior" of the drug company is attributed as one of the objects of the pharmaceutical market in the region, in particular, the variability of the cost of the drug itself and its course dose.

The main problem for this group of factors is subjectivity of behavior:

- doctor prescribing a drug for CAP pharmacotherapy, taking into account his qualification, experience, standards of treatment in force, etc.;
- patient who does not have sufficient knowledge to properly take the drug in case of pneumonia and even often cannot make the right choice in favor of going to the doctor, not to a pharmacy;
- pharmaceutist, more interested in the rapid implementation of the drug, than in the preliminary direction of the patient to hospital for a detailed assessment of the patient's condition.

The risks arising from the problem of the subjectivity of the behavior of participants in PhC may, of course, be very high and serious, in terms of aggravating the disease, complications and the development of side effects.

Thus, assessing the risks of internal factors included in the model, it can be argued that all of them (a doctor, a patient and a pharmacist) are closely related to the level of their knowledge about CAP and its pharmacotherapy.

Therefore, the most important solution to the subjectivity problem is to increase the level of knowledge of each participant of PhC, as well as timely and complete informe all participants in the pharmacotherapy process about CAP and its pharmacotherapy.

Such organizational and methodological solutions as the study of medical preferences, consumer demands and the opinion of pharmacy specialists regarding drugs used for CAP, have been referred to the elements of such kind of information.

Taking into account a high need of all participants of PhC for information about the CAP disease, its pharmacotherapy and related risks, specific drugs for CAP treatment and their costs in the Kabardino-Balkarian market, a methodological guide has been developed to provide information and consulting services to doctors and patients with CAP in the framework of PhC.

CONCLUSION

Thus, the state and trends of the development of organizational, personnel, socio-demographic, financial (in relation to the population) and drug resource as external factors of the drug supply system of the KBR region have been identified. It has also been established that they are insufficient to fully provide CAP patients with the required drugs.

The main problems of pharmacotherapy of CAP patients, associated with insufficient drug and informational support of doctors and patients, as well as weak interaction between medical and pharmaceutical workers, low awareness of patients about the risks of uncontrolled antibacterial drugs have been determined. In this connection, more intensive development of PhC is needed in the region.

As a result of the comprehensive research, the working hypothesis put forward previously, has been confirmed. Its use makes it possible to form a dynamic conceptual model of improving drug supply and pharmacotherapy of patients with CAP at the regional level by monitoring external and internal factors and determining the factors that are amenable to directional managerial influences.

REFERENCES

1. Karapetyan TA. Vnebol'nichnaya pnevmoniya segodnya [Community-Acquired pneumonia today]. Bulletin of St. Petersburg University. Series 11: Medicine. 2008;1:1–14. Russian
2. Sinopal'nikov AI, Strachunskij NS. Novye rekomendacii po vedeniyu vzroslyh pacientov s vnebol'nichnymi pnevmoniyami [New guidelines for the management of adult patients with community-acquired pneumonia]. Clinical Microbiology and antimicrobial therapy. 2000; 3(1):54–58. Russian
3. Kabardino-Balkaria in numbers. 2018. Summary of statistics. OP North Caucasus stat on CBD-N. – Nalchik, 2018. – 183 p. Russian
4. Regions of Russia. Socio-economic indicators. 2018: Stat. SB. Rosstat. 2018: 1162 p. Russian
5. Russian statistical yearbook. 2018: Stat.SB. Rosstat. – M., 2018:694. Russian
6. Health in Russia. 2017: Stat.SB. Rosstat. – M., 2017:170. Russian
7. Health in Russia. 2009: Stat.SB. Rosstat. – M., 2009:365. Russian
8. Ponomareva AA, Mozgovaya NA, Zhukova OV. Ocenka effektivnosti antibakterial'noj terapii vnebol'nichnoj pnevmonii u detej na osnove farmakoekonomicheskogo analiza [Assessment of efficiency of antibacterial therapy of community-acquired pneumonia in children on the basis of pharmacoeconomic analysis]. Medical. almanac. 2011. Special issue, March 2011: 204–205. Russian
9. Yakovlev SV. Vnebol'nichnaya pnevmoniya u pozhilyh. Osobennosti etiologii, klinicheskogo techeni-

ya i antibakterial'noj terapii [Community-Acquired pneumonia in the elderly. Features of etiology, clinical course and antibacterial therapy]. Russian medical journal. 1999;7(16):763–768. Russian
10. Barlett JG, Breiman RF, Mandell LA, File TM. Community-Acquired Pneumonia in Adults: Guidelines for Management. Clinical Infectious Diseases. 1998; 28: 811–838.
11. Niederman MS, Mandell LA, Anzueto A. Guidelines for management of adults with community-acquired pneumonia. Diagnosis, assessment of severity antimicrobial therapy, and prevention. The official statement of the American Thoracic Society was approved by the ATC board of directors. Am. J. Respir. Crit. Care Med. 2001;163:1730–1754. DOI: 10.1164/ajrccm.163.7.at1010
12. Yabachinskaya EN. Vozbuditeli vnebol'nichnyh pnevmonij na poroge novogo tysyacheletiya [Pathogens of community-acquired pneumonia at the threshold of the new Millennium]. Antibiotics and chemotherapy. 2000; 45(11):21–28. Russian
13. Dvoreckij LI, Yakovlev SV. Oshibki antibakterial'noj terapii infekcij dyhatel'nyh putej v ambulatornoj praktike [Errors of antibacterial therapy of respiratory tract infections in outpatient practice]. The Attending physician. 2003;8:48–54. Russian
14. Hristolyubova EI, Volkova LI. Oshibki v diagnostike vnebol'nichnyh pnevmonij na dogospital'nom etape [Errors in the diagnosis of community-acquired pneumonia at the prehospital stage]. Therapeutic archive. 2005;3:33–36. Russian
15. Hristolyubova EI, Volkova LI. Oshibki diagnostiki i lecheniya pnevmonij v poliklinicheskih usloviyah [Errors in diagnosis and treatment of pneumonia in polyclinic conditions]. Therapeutic archive. 2005;1:8–12. Russian
16. Panfilova GL. Farmacevtichna dopomoga yak istorichna, normativno-pravova ta social'no-ekonomichna kategoriya v sistemi ohoroni zdorov'ya i farmacevtichnomu zabezpechenni naselennya [Pharmaceutical assistance as a historical, legal and socio-economic category in the system of health protection and pharmaceutical provision of the population]. Topical issues of pharmaceutical and medical science and practice. 2014;2 (15): 89–97. Russian
17. Apikoglu-Rabus S, Yesilyaprak G, IzzettinF.V Drug-related problems and pharmacist interventions in a cohort of patients with asthma and chronic obstructive pulmonary disease. Respir. Med. 2016; 120:109–115. DOI:10.1016/j.rmed.2016.10.006
18. Helper CD. The future of pharmacy pharmaceutical care. Am. J. Hosp. Pharm. 1990;30:23–29.
19. Kassam R, Collins JB, Berkowitz J. Patient satisfaction with pharmaceutical care delivery in community pharmacies.Patient Preference and Adherence. 2012;6:337-348. DOI: 10.2147/PPA.S29606.
20. Presley B, Setiabudi I, Lestiono L, Ediyono E. The Influence of Pharmaceutical Care Intervention on Inpatient Community Acquired Pneumonia: a Small Randomized Single Blind Study. J. Sains Farmasi & Klinis. 2015;2(1):84–90. DOI: https://doi.org/10.29208/jsfk.2015.2.1.54
21. KonT, MoriM, HashimotoT. Pharmaceutical Care Approach to Treating Pneumonia among the Elderly with Intravenous Azithromycin.Iryo Yakugaku (Jap. J. of Pharm. Health Care a. Sci.). 2016; 42(9):634–644. DOI.org/10.5649/jjphcs.42.634
22. Wang YH, Fang J, Zhu BD. Pharmaceutical care of a patient with severe pneumonia complicated with Parkinson's disease. Pharm. Care a. Research. 2013; 13(2);136–139. DOI: 10.5428 / pcar20130219
23. Dremova NB, Alekseev IV. Analiz medicinskoj i farmacevticheskoj informirovannosti VICH-inficirovannyh pacientov [Analysis of medical and pharmaceutical knowledge of HIV-infected patients]. Scientific. statements BSU. Ser. «Medicine. Pharmacy» .2012; 17(4): P. 39–44. Russian
24. Dremova NB, Ovod AI, Korzhavyh EA, Litvinova TM. Farmacevticheskaya pomoshch': termin i ponyatie [Pharmaceutical assistance: term and concept]. Pharmacy. 2005; 2: 37–45. Russian
25. Korzhavykh EA, Moshkova LV, SHukil' LV. Metodologicheskie aspekty rossijskih issledovanij po farmacevticheskoj pomoshchi [Methodological aspects of Russian researches on pharmaceutical care]. Pharmacy. 2016;1:39–42. DOI: https://doi.org/NoneRussian
26. Dremova NB, Ovod AI, Korzhavyh EA, Litvinova TM. Farmacevticheskaya pomoshch' – novoe napravlenie professional'noj deyatel'nosti provizora [Pharmaceutical assistance – a new direction of professional activity of pharmacist]. New pharmacy. 2005;10: 20–28. Russian
27. Mymrina AL, Geller LN, Tueva IA. Klasternyj podhod kak osnova processa okazaniya farmacevticheskoj pomoshchi na etape reanimacii i intensivnoj terapii [Cluster approach as the basis of the process of pharmaceutical care at the stage of resuscitation and intensive care]. Pharmacy. 2012;7: 24–26. Russian
28. Petrov AG, Knysh OI, Petrov GP. Farmakoekonomicheskaya ocenka farmakoterapii pri lechenii professional'nogo obstruktivnogo bronhita shahterov na stacionarnom urovne [Pharmacoeconomical evaluation of pharmacotherapy in the treatment of occupational obstructive bronchitis of miners at the stationary level]. Health and education in the XXI century.2017;19(2):113–116. Russian
29. Kabakova TI, Umirova AA, Korzhavyh EA. Vyyavlenie i analiz vektorov razvitiya sovremennyh issledovanij po farmacevticheskoj pomoshchi [Identification and analysis of vectors of development of modern research on pharmaceutical care]. Eurasian Scientific Association (ENO). 2019;4 (50):177–183. Russian
30. Spichak IV, Varenykh GV. Sovershenstvovanie farmacevticheskoj pomoshchi detyam s narusheniyami CNS v usloviyah stacionara na primere Belgorodskoj oblasti [Improvement of pharmaceutical care for children with CNS disorders in a hospital on the example of Belgorod region]. Scientific statement BSU. Ser. Medicine. Pharmacy. 2013;153(22):71–78. Russian
31. Spichak IV, Derekglazova YUS. Sovershenstvovanie farmacevticheskoj pomoshchi detyam s zabolevaniyami sustavov na territorii Belgorodskoj oblasti [Improvement of pharmaceutical care of children with diseases of the joints on the territory of Belgorod region]. Scientific statement BSU. Ser. Medicine. Pharmacy. 2013.;154(22):39–46. Russian
32. Trubina LV, Pen'evskaya NA. Sovershenstvovanie farmacevticheskoj pomoshchi klientam aptek po voprosam profilaktiki infekcij, perenosimyh iksod-

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2019-7-3-158-169

ovymi kleshchami [Improvement of pharmaceutical care to clients of pharmacies in the prevention of infections transmitted by Ixodes ticks]. Fundamental research. 2014; 11:1333–1339. Russian
33. Yachnikova MA. Sovremennye podhody k organizacii farmacevticheskogo konsul'tirovaniya klienta aptek, priobretayushchego antiseptiki dlya lecheniya boli v gorle [Modern approaches to the organization of pharmaceutical counseling of client pharmacies acquiring antiseptics for the treatment of pain in the throat]. Omsk scientific. bulletin. Sir. «Earth resources. Man.». 2012; 2 (114): 89–91. Russian
34. Zakaryaeva ZT, Andreeva IN. Osobennosti lekarstvennogo obespecheniya bol'nyh pnevmoniyami na etape stacionarnogo lecheniya v RSO-Alaniya [Peculiarities of provision of medicines of patients with pneumonia at the stage of inpatient treatment in RSO-Alania]. Modern problems of science and education. 2013; 3. URL: https://science-education.ru/ru/article/view?id=9549Russian
35. Zemskova NA, Loskutova EE. Nekotorye farmakoekonomicheskie aspekty netyazheloj vnebol'nichnoj pnevmonii. Assortiment antibakterial'nyh preparatov v aptekah [Some pharmacoeconomical aspects of mild community-acquired pneumonia. The range of antibacterial drugs in pharmacies]. Bulletin of the Russian University of friendship of peoples. 2007:62–64. Russian
36. Bell JS, Väänänen M, Ovaskainen H. Providing Patient Care in Community Pharmacies: Practice and Research in Finland. Ann. Pharmacother. 2007; 41 (6):1039–1046. DOI.org/10.1345/aph.1H638
37. Besançon L. Pharmaceutical Care. Summary of a survey from the Council of Europe. FIP.2010:32.
38. Perraudin C, Brion F, Bourdon O, Pelletier-Fleury N. The future of pharmaceutical care in France: a survey of final-year pharmacy students' opinions. BMC Clin. Pharmacol. 2011; 11:6 doi: 10.1186 / 1472-6904-11-6 DOI: 10.1186 / 1472-6904-11-6.
39. Curtiss FR. Clinical Pharmacist Interventions Associated with Appropriateness and Lengthof Inpatient Antimicrobial Therapy for Pneumonia. J. Manag. Care Spec. Pharm. 2002; 8(5):406–406. DOI: 10.18553 / jmcp.2002.8.5.406
40. Dzhuparova IA. Tipologiya farmacevticheskoj pomoshchi l'gotnym kategoriyam grazhdan v Sibirskom federal'nom okruge [Typology of pharmaceutical care preferential categories of citizens in the Siberian Federal Region]. Medicine and education in Siberia. 2010;5:10–18. Russian
41. Dzhuparova IA, Sboeva SG. Tipologiya farmacevticheskoj pomoshchi otdel'nym kategoriyam grazhdan v Sibirskom federal'nom okruge [Typology of pharmaceutical care to separate categories of citizens in the Siberian Federal district]. Bulletien of the Voronezh state University. Ser.: Chemistry. Biology. Pharmacy. 2010;2:140–143. Russian
42. Shukil' LV. Opyt okazaniya specializirovannoj farmakologicheskoj pomoshchi bol'nym saharnym diabetom v Omskoj oblasti [Experience in providing specialized pharmaceutical care for patients with diabetes in the Omsk region]. Sib. Med. journal. (Irkutsk.) 2013; 118(3): 94–96 Russian
43. Fomina AV, Moshkova LV. Dostupnost' lekarstvennoj pomoshchi naseleniyu: faktory vliyaniya [Availability of medical care to the population: factors of influence]. New pharmacy. The Director of pharmacy. 2004;3: 48–52. Russian

FINANCIAL SUPPORT

This study did not have any financial support from outside organizations.

AUTHORS' CONTRIBUTION

All authors equally contributed to the research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

QUALITY MANAGEMENT SYSTEM OF A PHARMACEUTICAL ORGANIZATION: CRITERIA AND IMPLEMENTATION

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Received 23.04.2019 Review 1 25.06.2019 Review 2 20.06.2019 Accepted for publication: 27.06.2019

The aim of the study is a theoretical justification and development of a mechanism for the implementation of quality management systems (QMS) in the activities of retail trade in pharmaceutical products.

Materials and methods. The study is based on the analysis of scientific literature data and Analytical Normative Documents regulating various aspects of QMS functioning in pharmaceutical organizations. Methods of documentary content analysis of international and national ISO standards, Series 9000, were used as the basis for the development and implementation of QMS organizations despite the type of their activities.

Results. In the course of the study, the organization of the production process has been studied and the main production (business-) operations of seven structural subdivisions of "Apteka-Alex" LLC in Angarsk have been analyzed. The basic principles of QMS functioning are set forth and the author's methodology for its development for retail trade in pharmaceutical products has been presented, taking into account the specifics of the organizational structure and production activities of pharmacy organizations. The mechanisms and successive stages of the QMS implementation have been specified and given grounds for. In accordance with the requirements of GOST R ISO 9000:2015, the definitions and classification of the main production operations (business processes) of the studied pharmacies have been made up. In addition, the list of mandatory (core) and basic standard operating procedures (SOPs), detailing the main production (business-) processes, has been made up, and an algorithm for developing and testing SOPs has been outlined.

Conclusion. The results of the study allowed us to justify and propose a step-by-step methods of QMS implementation into the work of pharmaceutical organizations in the retail sector. The first results of the approbation and use of the developed methodology show that it contributes to the rational construction and functioning of the QMS, in accordance with the requirements of the existing standards, as well as the optimization of administrative management of all production (business-) processes.

Keywords: quality management system, standard operating procedure, pharmaceutical services, pharmacy organization, the subject of retail trade in pharmaceutical products

For citation: A.A. Klimenkova, L.N. Geller, A.A. Skripko, L.A. Gravchenko, N. Fedorenko. Quality management system of a pharmaceutical organization: criteria and implementation. *Pharmacy & Pharmacology*. 2019;7(3): 170-179. **DOI:** 10.19163/2307-9266-2019-7-3-170-179

Для цитирования: А.А. Клименкова, Л.Н. Геллер, А.А. Скрипко, Л.А. Гравченко, Н.В. Федоренко. Система менеджмента качества фармацевтической организации: критерии и реализация. *Фармация и фармакология.* 2019;7(3): 170-179. **DOI:** 10.19163/2307-9266-2019-7-3-170-179

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2019-7-3-170-179

СИСТЕМА МЕНЕДЖМЕНТА КАЧЕСТВА ФАРМАЦЕВТИЧЕСКОЙ ОРГАНИЗАЦИИ: КРИТЕРИИ И РЕАЛИЗАЦИЯ

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Получено 23.04.2019 Рецензия 1 25.06.2019 Рецензия 2 20.06.2019 Принята к печати 27.06.2019

Цель. Целью исследования послужило теоретическое обоснование и разработка механизма внедрения систем менеджмента качества (СМК) в деятельности субъектов розничной торговли фармацевтическими товарами.
Материалы и методы. Исследование проведено на основе данных анализа научной литературы и нормативных документов, регламентирующих различные аспекты функционирования СМК в фармацевтических организациях. При проведении исследования применялись методы документального контент-анализа международных и национальных стандартов ISO серии 9000, положенных в основу разработки и внедрения СМК организаций независимо от вида осуществляемой деятельности.
Результаты. В ходе исследования изучена организация производственного процесса и проанализированы основные производственные (бизнес-) операции семи структурных подразделений ООО «Аптека-Алекс» г. Ангарска. Изложены основные принципы функционирования СМК и представлена авторская методика ее разработки для субъектов розничной торговли фармацевтическими товарами с учетом специфики организационной структуры и производственной деятельности аптечных организаций. Обоснованы и конкретизированы механизмы и последовательные этапы внедрения СМК. В соответствии с требованиями ГОСТ Р ИСО 9000:2015, дано определение и осуществлена классификация основных производственных операций (бизнес-процессов) исследуемых аптек. Кроме того, сформулирован перечень обязательных (основных) и базовых стандартных операционных процедур (СОП), детализирующих основные производственные (бизнес) процессы, изложен алгоритм разработки и апробации СОП.
Заключение. Результаты проведенного исследования позволили обосновать и предложить поэтапную методику внедрения СМК в работу фармацевтических организаций розничного звена. Первые итоги апробации и использования разработанной методики свидетельствуют о том, что она способствует рациональному построению и функционированию СМК, в соответствии с требованиями действующих стандартов, а также оптимизации административного управления всеми производственными (бизнес-) процессами.
Ключевые слова: система менеджмента качества, стандартная операционная процедура, фармацевтические услуги, аптечная организация, субъект розничной торговли фармацевтическими товарами

INTRODUCTION

Protection of the quality of pharmaceutical products in the process of circulation is aimed at ensuring pharmacological, environmental, technological kinds of safety, as well as the rational use of resources to preserve and promote public health [1]. Implementation of tasks of this level requires new conceptual approaches to quality management and it is possible only if there is a quality management system (QMS). It should be based on consistent keeping to and compliance with the requirements of good practices, which should function reliably at the level of all participants in the system of commodity circulation in the pharmaceutical market. It should be noted that the QMS serves as a basic mechanism to ensure strict compliance of the activities of medical and pharmaceutical organizations in the format of the requirements of existing standards and regulations. In addition, the introduction of the QMS is aimed at achieving a proper level of quality of pharmaceutical services provided and serves as a guarantor of their further improvement [2–5]. In modern conditions, traditional approaches to meeting the needs of consumers are changing dramatically. Forming the loyalty of visitors and customers to the pharmacy organization occurs not only on the basis of improving the quality of products and services provided, but also due to the continuous improvement of production activities of the organization as a whole [6]. Since the QMS is a set of interaction subsystems of management of all aspects of the organization, in full compliance with the requirements of existing standards, the use of this system is aimed at continuous improvement of the quality of services provided, and

allows each organization to achieve its highest possible level [7]. Moreover, the presence of a successfully functioning QMS in an organization gives it certain marketing advantages and contributes to the formation of a positive reputation, attracting the maximum number of new customers and increasing competitiveness in a particular market segment [2, 8, 9]. Accordingly, the financial stability of the organization improves, and the loyalty of all related counterparties, including customers, employees, suppliers, etc, increases [8].

In this regard, the aim of the study was to justify and develop methods for the phased implementation of QMS in the work of pharmaceutical organizations at the retail level, taking into account the specifics of their organizational structure and production activities.

MATERIALS AND METHODS

The content analysis of the documentary contents of the international rules for ensuring quality, efficacy and safety of medicinal preparations (MP) and the concept of standardization has made it possible to establish that, at the current stage, the leading regulator of pharmaceutical care (PhC) quality is GxP standards of drug circulation [10]. In this case, the GxP standards act as a fundamental element of the quality assurance system. Globally, good practice guidelines have established requirements for ensuring the quality of drugs at all stages of the life cycle [11, 12]. This set of good practice rules is presented by good laboratory practices (GLPs), good clinical practices (GCPs), good manufacturing practices (GMPs), good distribution practices (GDPs), good pharmacy practices (GPhPs), good purchasing practices (GPPs). According to the basic concept of GxP and the concept of comprehensive study of Medicine remedies (MRs), the concept of the "quality" category which is formed at the stage of MRs development, is further confirmed during its registration and is monitored at all production stages. In the process of providing PhC, for further use of only effective and safe MRs, the achieved production level of quality must be maintained at all stages of the "life cycle" of products, including the processes of storage, distribution, transportation and use in the wholesale and retail trade entities [12, 13].

There is no doubt that the lack of adequate control can have a negative impact on the preservation and constancy of qualitative characteristics of MRs and cause poor-quality products to enter the civil turnover [14]. In this regard, the modern regulatory system of MRs quality assurance is based on strict compliance with the requirements and rules of good practices established for each stage of the product life cycle, by all participants of the scope of their circulation. Accordingly, a drug developer must comply with GLPs and GCPs rules, the manufacturer – with GMPs rules, wholesale and retail organizations – with GDPs and GPPs standards [6].

Thus, comprehensive compliance with the requirements of the above-mentioned standards ensures the stability of the quality characteristics of pharmaceutical products, minimizes the penetration of substandard medicinal preparations and pharmacy products assortment (PhPA) into circulation, and contributes to providing pharmaceutical services (PhS) of the adequate quality, which generally corresponds to the main postulate of pharmaceutical products – meeting the needs of the population in the preservation and maintenance of their healths [15].

It should be noted that the quality assurance system of the Organization for Standardization (ISO) in the form of ISO, Series 9000, contributes to the achievement of the same aims as the GxP system, but it is more detailed. Currently, the following 4 basic national standards have been developed, approved on the basis of international ISO standards and are in force in Russia. They should be used in the construction and improvement of the QMS model:

GOST R ISO 9000: 2015 "Quality management systems. Basic Provisions and Dictionary", which establish uniform principles of terminology and basic provisions for the QMS;

GOST R ISO 9004:2010 "Management for sustainable success of the organization. The approach based on quality management", containing methodological materials to improve the already functioning QMS and the efficiency of the enterprise. In this standard, the QMS is considered in more details, taking into account the needs and expectations of all parties concerned;

GOST R ISO 19011-2012 "Guidelines for the audit of Quality Management Systems, Inc.", which describes the basic principles of the QMS audit procedure and contains guidelines for their implementation, which makes it possible to understand the essence of the audit procedures in more details, and identify opportunities for the improvement.

The study of the Quality Management methodology based on the requirements set forth in these standards, is an integral part of the knowledge in the field of management of the organization.

RESULTS

As it has already been noted before, the documentary content analysis showed that the reviewed GOSTs are completely identical to the corresponding International Standards ISO, Series 9000. It should be noted that although these standards do not regulate the quality of specific products, they can be successfully used by different organizations regardless of the nature and type of their activities. At the discretion of the administration,

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DOI: 10.19163/2307-9266-2019-7-3-170-179

such standards, adapted for a specific organization, can be used to build and improve the QMS in the pharmaceutical sector. Due to the changes in current legislation and approval of regulatory legal acts on rules of good pharmacy practices (GPhPs) and good practice of storage and transportation of medicinal preparations by the Ministry of Health of the Russian Federation, the problem of developing and implementing the QMS is very relevant for medical and pharmaceutical organizations of our country [16,17]. In accordance with the requirements of the listed above documents, each entity in the field of circulation of drugs and pharmacy products assortment (PhPA) should justify, develop and implement their own QMS at the organization level, as well as provide the necessary standard operating procedures (SOP) for the implementation of basic production (business-) processes. Such a quality system that ensures the efficiency and safety of pharmaceutical products is mandatory administratively regulated and maintained in operational mode through continuous analysis and timely introduction of appropriate adjustments to it in order to continually improve its performance. When developing a QMS, it is necessary to take into account that the determining components of this system are:

1. organizational structure (a clear definition of the tasks and functions of each unit of a retail entity and the delineation of the rights and duties of employees in accordance with the assigned mission);
2. processes affecting the quality of products sold and services provided, including basic, managerial, auxiliary processes;
3. standard operating procedures that allow typing and standardization of production (business–) processes directly related to the activities of the retail entity;
4. resources necessary for the implementation of the relevant production (business-) processes, including labor, information, material.

The absence of any of these links breaks the organization's QMS and makes it defective.

In the format of the study, standardization is interpreted as an activity of establishing relevant rules, parameters and characteristics for the purpose of their voluntary multiple use to achieve arrangement in the areas of production and circulation of pharmaceutical products and improvement of their competitiveness.

The aim of standardization in the field of drug circulation is to protect the quality of pharmaceutical products at all stages of their life cycle, including the processes of production, promotion, storage, use, and, if necessary, destruction [18]. At the same time, standardization is primarily aimed at ensuring the pharmacological, environmental, technological safety of products and rational use of resources.

In general, the introduction of the QMS in organizations of the pharmaceutical profile is aimed at preserving the stability of the quality parameters and characteristics of goods, as well as at improving the level of quality of public services provided to the population [6]. It should be noted that in modern conditions the improvement of the quality of pharmaceutical services (PhSs) is the main purpose of any pharmaceutical organization [19, 20].

Achieving this goal becomes possible not only with the rational construction and implementation of the QMS, but also maintaining it at the proper functional level. In turn, the list of pharmaceutical services provided by a particular pharmacy organization is formed in the context of the functions performed. In this regard, solving the issues of optimization of the quality of pharmaceutical services performance is possible through standardizing production (business-) processes directly related to the activities of a pharmaceutical retail entity, based on the development of corresponding SOPs [21, 22]. In accordance with the research plan, in 2016–2017, the labor organization was studied and the main production (business-) operations of 7 structural divisions of the pharmacy chain of LLC "Apteka Alex" in Angarsk were analyzed.

During the study, in full compliance with the requirements of GOST R ISO 9000:2015, the main production operations (business processes) of the studied pharmacies were identified and classified. In accordance with the methodology, all production operations (business processes), repeated cyclically during a shift at least 5 times, were assigned to the main ones.

While classifying and characterizing the main production business processes of pharmacies, the process was interpreted as a set of interrelated specialists' labor activities and labor operations. These activities transformed the **input** data of such processes into **output** ones [23].

At the next stage of the study using the methodology of the PDCA (Plan-Do-Check-Act: Planning – Action – Check – Adjustment) or the Deming Cycle, which is an algorithm for a sequence of administrative actions for the proper management of a specific production process to achieve this goal, we have chosen a process approach.

The application of the process approach involves determining the system of business processes performed in the organization and further work to improve them. In turn, the PDCA cycle can be reliably applied to all processes functioning in the organization and to the QMS of the organization as a whole.

The implementation of the Deming Cycle with the established periodicity allows all the processes to be

implemented to provide the necessary resources, manage them, and look for opportunities for continuous improvement [24–27]. In accordance with the PDCA methodology, the process approach to the management of production activities of the organization includes a number of criteria and elements (Fig. 1).

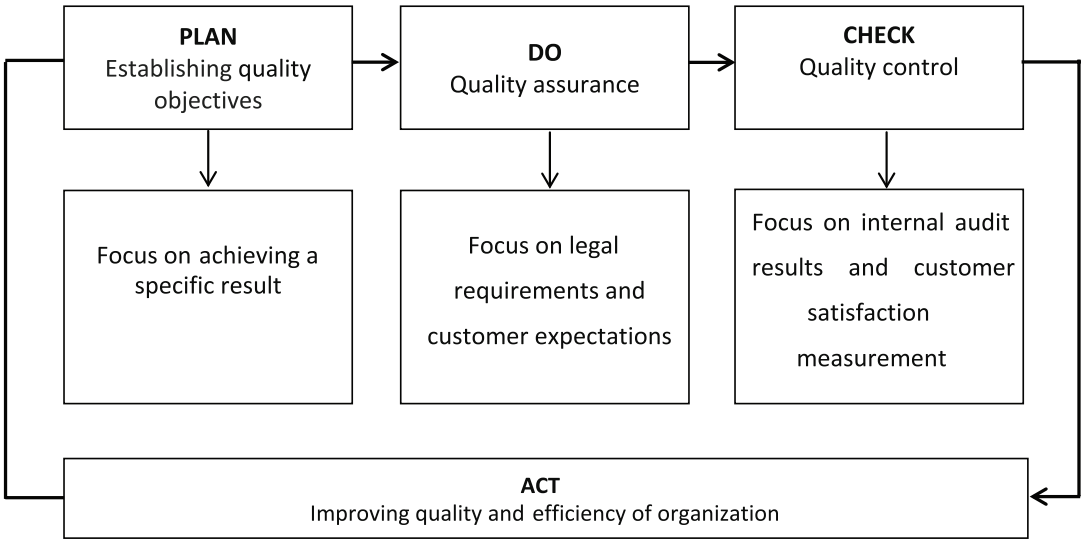

Figure 1 – Management of the pharmacy organization's production activities from the perspective of the PDCA (process approach)

In accordance with the proposed concept for the proper management of the retail entity, first of all, it is necessary to rationally manage the relevant production (business-) processes and take into account their possible interaction and impact on the work of the entity. As a result, thanks to the process approach, it is possible not only to build an optimally functioning QMS, but also to improve the efficiency of effective management of the organization.

At the final stage, using the results of the study and the data of comparative and critical analysis of ISO, Series 9000, from the standpoint of the process approach, an algorithm has been scientifically justified and proposed for the implementation of the QMS in the work of pharmaceutical organizations at the retail level. The algorithm of this technique, which includes purposeful, systematic and continuous sequential interaction of labor cyclically repeated operations of six levels, is presented in Fig. 2.

DISCUSSION

In accordance with the presented algorithm, the construction and functioning of the QMS in pharmaceutical organizations at the retail level can be considered in the form of repetitive labor (production) operations of the following 6 levels:

Labor Operations of Level I comprise awareness and establishing objectives of QMS. by the administration. As a rule, the objectives of the QMS are consistent with the objectives of the organization and are aimed at maximum satisfaction of the requirements and expectations of consumers. In this case, the objectives of the QMS are reflected in the document "Quality Policy of the Organization". The administration should foresee and plan the processes required to deliver the desired results. It is necessary to carry out continuous work to optimize functioning of the QMS to achieve the planned results.

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DOI: 10.19163/2307-9266-2019-7-3-170-179

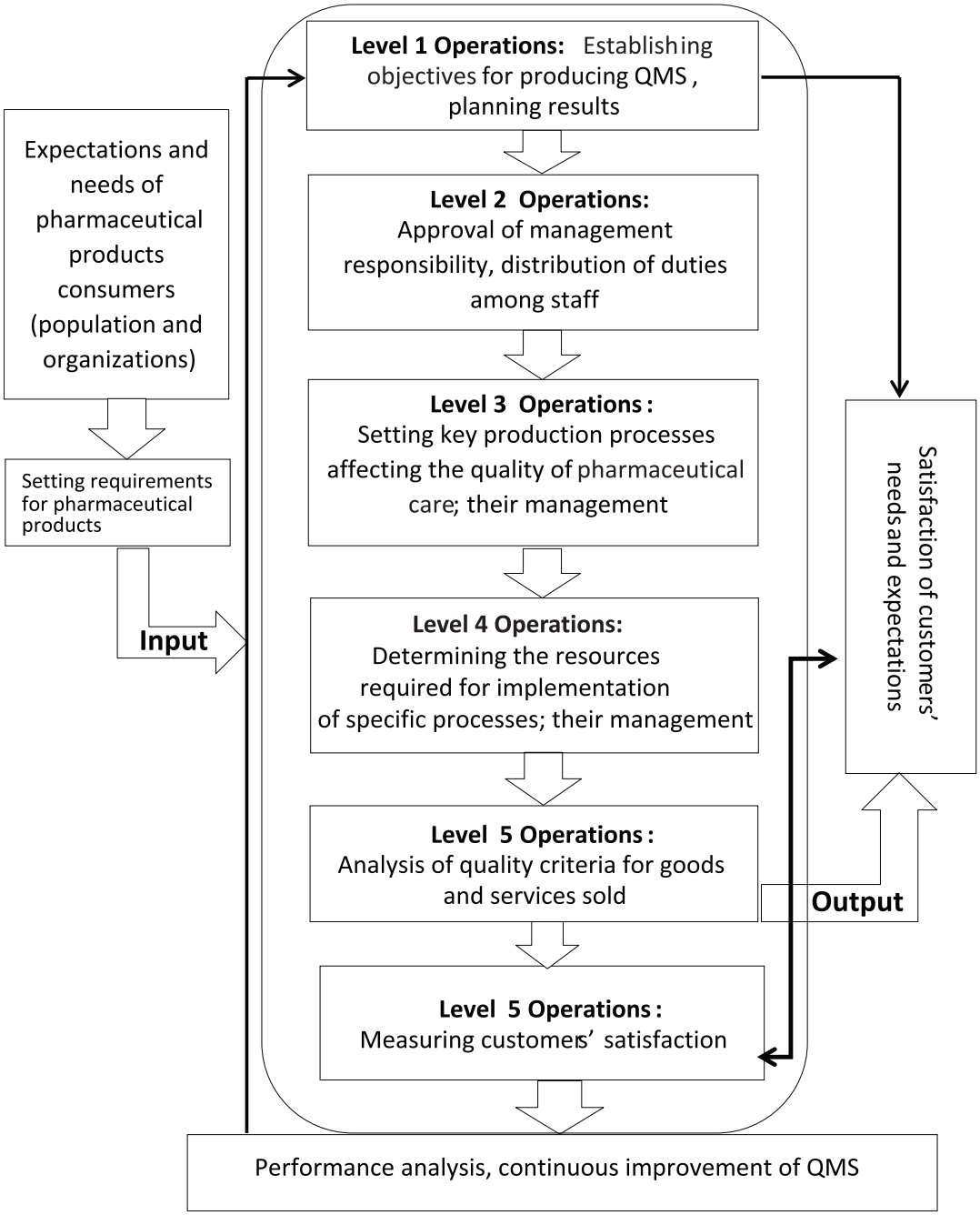

Figure 2 – Algorithm of QMS implementation methods in pharmaceutical organizations at retail level

Labor Operations of Level 2 comprise justification and establishing of management responsibility, distribution of duties. The administration should distribute all kinds of responsibility among the staff, determine the complex of their obligations, take an active part in developing and maintaining the QMS, demonstrate their leadership and commitment to the QMS, take responsibility for the effectiveness, ensure the availability of the necessary resources and promote understanding of the importance of working in the QMS among the staff.

Labor Operations of Level 3 comprise justification and setting key production processes that have a significant impact on the quality of the pharmaceutical care provided, and their management (including the step-by-step sequence of these processes, their interrelation, setting objectives of the processes, their sources and performers, the distribution of responsibility among the staff, description and documentation of the processes, the establishment of checkpoints and criteria, assessment of the possibility of the QMS implementation in real conditions). Basing on the production specifics, any pharmaceutical retail entity has the right to determine the number and contents of such processes. First, these include the processes focusing on the purchase, acceptance, storage of products and bringing them to the consumer.

Labor Operations of Level 4 comprise identification of resources required for the implementation of specific processes and functioning of the QMS as a whole, the establishment of requirements for them and maintenance in working (good) condition. Among the resources necessary to perform such procedures, the following resources should be highlighted:

labor resources – a set of employees of a retail entity. The level of staff training is determined by a set of specific requirements for them, for example, the availability of the necessary qualifications, work experience, timeliness of instruction pass, etc.;

material resources and infrastructure – the criteria necessary for a proper organization and equipping workplaces (zones) where the labor process is directly implemented (for example, equipment, technical and auxiliary aids, documentation);

information resources – a set of data on electronic and paper media necessary for the execution of the relevant processes (information systems, software, tools, allowing to obtain reference information).

Labor Operations of Level 5 comprise the analysis of product quality criteria by the administration in order to provide the parties concerned with pharmaceutical services of the adequate quality and meeting their expectations and needs. Since monitoring is one of the most important management functions and an integral part of the QMS, the analysis of the quality of pharmaceutical products and the assessment of the correct functioning of the production (business-) processes of an organization are achieved by conducting an internal audit aimed at identifying discrepancies between the current work of the organization and current regulatory requirements [28].

Labor Operations of Level 6 are measurements of customers' satisfaction. Organizations monitor and analyze the data on the requirements of the parties concerned and the extent to which their needs and expectations are satisfied (for example, consumer surveys, consumer reviews, meetings with consumers, segmentation and analysis of the pharmaceutical market share, availability of acknowledgments, analysis of claims, etc.).

Analyzing and evaluating the information obtained in the course of continuous monitoring and guided by the degree of customers' satisfaction, the administration of the organization is able to objectively and timely assess the effectiveness of the QMS of the organization, to identify existing shortcomings and weak points in the work, continuously working on further optimization of the QMS and its improvement.

Thus, a properly formed QMS allows to quickly and efficiently manage all production (business-) processes of the organization administratively at the institutional level, making decisions aimed at improving the quality of the provided pharmaceutical services.

A mandatory condition for successful implementation of the quality management system is the formation of a block of documents ensuring and accompanying functioning of the entire QMS of the organization [28]. The basis for the creation of the necessary document flow of the QMS can serve the previous documents of this retail entity, modified in accordance with the requirements of the existing standards and supplemented in the process of development and implementation of the QMS. Therefore, the organization's QMS documentation is constantly updated, improved and supplemented. The level of elaboration and quality of the documentary block largely determine the effectiveness of the QMS of the organization as a whole [29].

One of the fundamental principles of the formation of the QMS documentary block, is the development of standard operating procedures (SOPs), which are a formal algorithm of labor step by step actions of employees (a set of written instructions), the subject of retail trade to perform the relevant production (business-) process.

According to QMS requirements, all production (business-) processes of a pharmaceutical organization that affect the quality, efficiency and safety of medicinal preparations and other pharmacy products assortment must be carried out in strict accordance with the SOPs. It should be noted that the SOPs contain a strictly regulated and documented set of labor operations for the implementation of the relevant production (business-) process, which allows to establish what and in what sequence is implemented, where, when, how and by whom specific labor operations or functions are performed.

Since SOPs are a description of specific actions for the implementation of existing legal and regulatory settings in a particular pharmacy organization, their use allows to unify the entire production (business-) process, clearly define the duties and responsibilities of employees, ensure the sequence of their step-by-step actions, successfully train and evaluate employees' knowledge, reduce the number of their mistakes, eliminate duplicate and unnecessary operations and functions [26].

Development and compilation of SOPs are implemented through preliminary study, timing, documentation and description of the necessary production (business-) process. As a rule, the development of SOPs is carried out by key production (business-) processes, necessarily included in the QMS of the retail entity and subject to control. Basing on production specifics, each retail entity independently determines the number and composition of such production (business-) processes. All concretely established and fixed production (business-) processes are justified and studied.

For each process of this kind, an objective and the following data are established: the availability of the required resources, criteria for control check-points (taking into account the requirements of current legislation), the boundaries of personnel responsibilities and duties, and the procedure for acting in case of impossible performance of labor operations in the SOP format.

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In order to assess the acceptability of the requirements developed by the SOP and the compliance of the procedure with the performed labor operations, it is advisable to conduct training of employees on the rules of working with SOPs before their approval. As a result, based on the results of the training conducted (analysis and study of staff SOPs), appropriate adjustments to the SOPs are made aimed at optimizing production (business-) processes.

SOP testing is carried out after the development of their primary version (draft documents), the study and analysis of the submitted documents by all employees involved in the production process. Work on the practical application of SOP is carried out with each employee involved in the implementation of this operation.

In the process of SOP testing, the availability analysis is carried out to understand the requirements of the SOP by all employees, the detection of unrecorded elements during the production (business-) process, the validity of the practical application of the developed document. After SOP testing, employee training and making the necessary adjustments, the head of the retail entity approves the updated SOP.

Algorithm of development and SOP implementation in the practical activity of the retail trade subject is shown in Fig. 3.

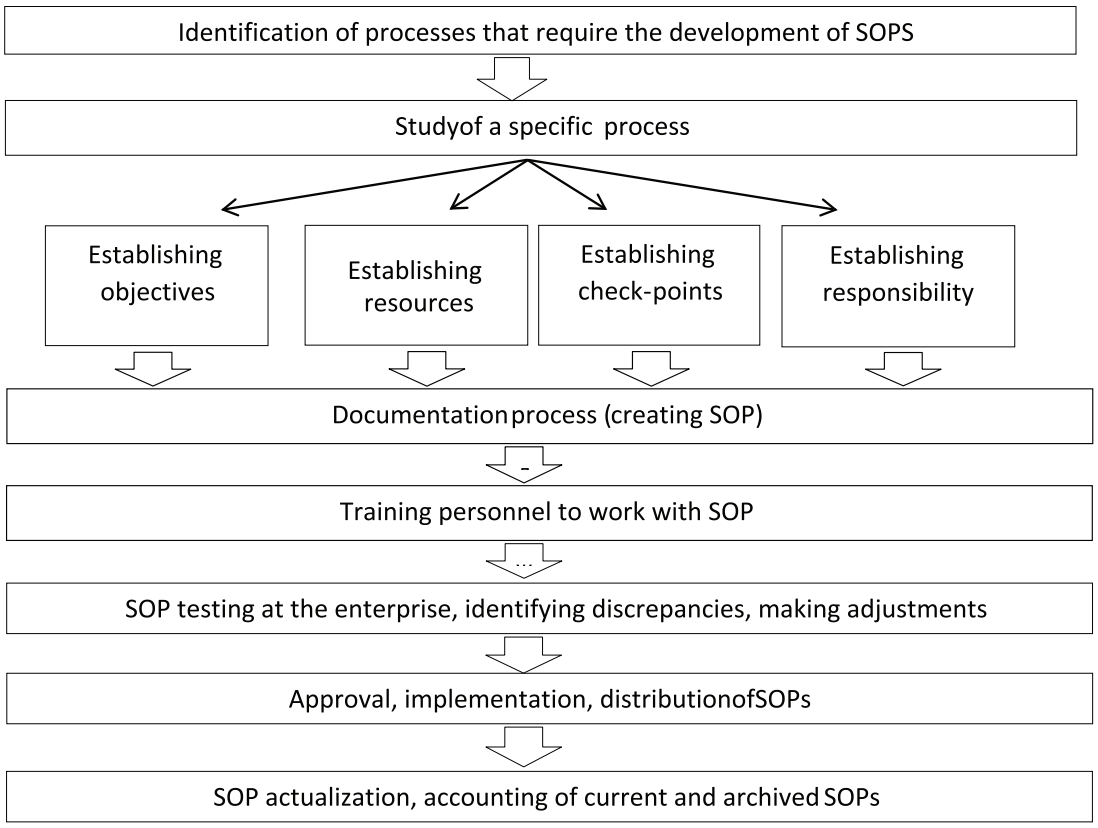

Figure 3 – Algorithm for development and implementation of a standard operating procedure in a retail entity

Since the legal acts do not regulate the requirements for a number of SOPs for each pharmacy organization, the management of the retail entity has the right to independently determine the acceptable number of production (business-) processes and describe them. However, the developed and described SOPs should cover the implementation of all necessary production (business-) processes affecting the quality of medicinal preparations and pharmacy products assortment in a particular pharmacy organization. Therefore, this block of basic production (business-) processes should be carried out in strict accordance with the approved SOPs, containing a description of specific labor actions of personnel for the implementation of existing regulatory and legal requirements in a specific pharmacy organization.

At the discretion of the management of the subject of retail trade, taking into account specifics in the specified basic production (business-) processes, additional sections of production activity which SOP and working instructions can be developed on in the same way, can be provided and allocated.

CONCLUSION

Thus, using the process approach, the results of the study made it possible to justify and propose a methodology for the justification and construction of the quality management system of the organization, as well as an algorithm for the development and implementation of SOP in the work of pharmaceutical organizations of the retail level.

The final results of approbation of the proposed methods and algorithms in the pharmacy chain of "Apteka-Alex" LLC indicate that their use is not only aimed at the rational construction and operation of the organization's QMS in accordance with the requirements of the current regulatory framework, but also contributes to the optimization of administrative management of all production (business-) processes of the pharmaceutical organization of the retail level.

REFERENCES

1. Skripko AA, Geller LN. Sotsialnaya farmatsevticheskaya pomoshch v Irkutskoy oblasti: monografiya [Social pharmaceutical assistance in the Irkutsk region]. Irkutsk: Ottisk; 2014: 119 p. Russian.
2. Klimenkova AA, Geller LN, Skripko AA. Sistema dopolnitelnogo professionalnogo obrazovaniya v formate nadlezhashchey aptechnoy praktiki [System of additional professional education in the format of good pharmacy practice]. Sistema menedzhmenta kachestva: opyt i perspektivy: sbornik materialov nauchno-prakticheskoy konferentsii. Irkutsk: IGMU; 2018:130–133. Russian.
3. Diachenko RG, Andreyeva IN, Bondareva TM, Gabriyelyan NV. Kontseptualnaya model upravleniya kachestvom farmatsevticheskoy pomoshchi na regionalnom urovne [Conceptual model of quality management of pharmaceutical assistance at the regional level]. Problemy farmatsevticheskoy nauki i praktiki sbornik materialov III Vserossiyskoy nauchno-prakticheskoy konferentsii. Vladikavkaz; 2013:35–39. Russian.
4. Semenova SV. Issledovaniye dopolnitelnykh uslug predostavlyayemykh farmatsevticheskimi organizatsiyami [Research of additional services provided by pharmaceutical organizations]. Nauka molodykh (Eruditio juvenium). 2017; 5(2):306–311.Russian. doi:10.23888/HMJ20172306-311.
5. Gergiyeva IV, Andreyeva IN, Bidarova FN, Bondareva TM. Vyyavleniye prichinno-sledstvennykh svyazey mezhdu faktorami okazyvayushchimi vliyaniye na kachestvo lekarstvennoy pomoshchi na regionalnom urovne [Identification of cause-and-effect relationships between factors influencing the quality of drug care at the regional level]. Meditsinskiy vestnik Severnogo Kavkaza. Stavropol; 2015; 10(3):269–273. Russian. doi: http://dx.doi.org/10.14300/mnnc.2015.10063.
6. Nikitin SA, Tatenko GI, Bakhtina SS, Magomedalieva OV, Kononova EE, Semenikhina AV, Morozova OI, Torgachev DN, Knyazeva VA, Laushkina NS, Kupriyanov AN, Shevtsova EA, Musatova IV, Dolgikh EL, Tronina IA, Zlobin EF. Kontseptsiya i tekhnologii menedzhmenta v otraslevom razreze: teoriya i praktika: monografiya [The concept and technology of management in the sectoral context: theory and practice]. Orel: OGU imeni Turgeneva IS; 2017:222 p. Russian.
7. Glembotskaya G. T. V labirintakh farmatsevticheskogo menedzhmenta [In the maze of pharmaceutical management]. Moscow: Litterra; 2007:225p. Russian.
8. Razdorskaya IM, Zanina IA, Plokhikh IV. Strategiya razvitiya biznes-potentsiala aptechnykh setey s uchetom sbalansirovannykh pokazateley [Development strategy for the business potential of pharmacy networks with consideration for balanced indicators]. Farmaciya, 2018; 67(2): 46–51. Russian. doi: https://doi.org/10.29296/25419218-2018-02-09.
9. Kareva NN. Perspektivnye napravleniya razvitiya sotsial'noy otvetstvennosti aptechnogo biznesa [Promising directions in the development of pharmacy business's social responsibility]. Farmaciya, 2018; 67(5): 29–34. Russian. doi: https://doi.org/10.29296/25419218-2018-05-06.
10. Balashov AI. Nenadlezhashchaya proizvodstvennaya praktika: modernizatsiya sistemy obespecheniya kachestva proizvodstva lekarstvennykh sredstv v Rossii [Good manufacturing practice: modernization of the quality assurance system for the production of medicines in Russia]. Izvestiya vysshikh uchebnykh zavedeniy (Ekonomika, finansy i upravlenie proizvodstvom), Ivanovo.2014; 1(19):72–77. Russian.
11. Morozova TE, Khoseva EN. Organizatsiya kontrolya kachestva, effektivnosti i bezopasnosti lekarstvennykh sredstv na gosudarstvennom urovne za rubezhom i v Rossii [Organization of quality control, efficiency and safety of medicines at the state level abroad and in Russia]. Kachestvennaya klinicheskaya praktika. Moscow. 2013;2:54–59. Russian.
12. Avdoshkina OI. Kachestvo i bezopasnost' lekarstvennykh sredstv: mezhdunarodnaya standartizatsiya i sistema gosudarstvennogo kontrolya [Quality and safety of medicines: international standardization and state control system]. Sistemnoe upravlenie. Saransk. 2014; 3(24):2–9. Russian.
13. Podpruzhnikov YuV, Nemchenko AS, Andryukova LN, Emshanova SV, Kozel'skaya TP, Chistyakova VV, Ishmukhametov AA. Khrestomatiya farmatsevticheskogo kachestva [Pharmaceutical quality reader]. Moscow: Gruppa Remedium.2015:415 p. Russian.
14. Chukreeva NV. Kontseptsiya upravleniya znaniem protsessov v organizatsiyakh sistemy tovarodvizheniya lekarstvennykh sredstv [The concept of knowledge management processes in organizations of the system of commodity circulation of medicines]. Vestnik voronezhskogo gosudarstvennogo universiteta. Seriya: khimiya. Biologiya. Farmatsiya. 2015;1:167–171. Russian.
15. Dremova NB, Ovod AI, Korzhavykh EA. Osnovy farmatsevticheskoy pomoshchi v zdravookhranenii [Fundamentals of pharmaceutical care in health care]. Kursk: GOU VPO KGMU Roszdrava.2009:412p. Russian.
16. Ryzhova OA, Moroz TL. Izmeneniya v roznichnoy realizatsii lekarstvennykh preparatov iz meditsinskikh organizatsiy [Changes in retail sales of medicines from medical organizations]. Innovatsionnye tekhnologii v farmatsii:sbornik materialov nauchno-prakticheskoy konferentsii. Irkutsk; 2017:206–210. Russian.
17. Moroz TL, Ryzhova OA. Kak vnedrit' sistemu obe-

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2019-7-3-170-179

specheniya kachestva khraneniya i perevozki lekarstvennykh preparatov [How to implement a system to ensure the quality of storage and transportation of medicines]. Zamestitel' glavnogo vracha. 2017; 9:62–65. Russian.
18. Nevolina EV. Osnovnye podkhody k vnedreniyu SMK v apteke [Main approaches to QMS implementation in pharmacy]. Aptechnyy biznes. 2008;1:5–8. Russian.
19. Nevolina EV. Tselesoobraznost' vnedreniya SMK v aptechnuyu praktiku [Expediency of QMS implementation in pharmacy practice]. Rossiyskie apteki. 2007;4:32–38. Russian.
20. D'yachenko RG, Andreeva IN, Bidarova FN, Bondareva TM, Gabrielyan NV. Puti sovershenstvovaniya upravleniya kachestvom aptechnykh tovarov i farmatsevticheskikh uslug v aptechnykh organizatsiyakh [Ways to improve the quality management of pharmaceutical products and pharmaceutical services in pharmacy organizations]. Sovremennye problemy nauki i obrazovaniya. 2013; 5:371–379. Russian.
21. D'yachenko, RG. Sostoyanie sistemy upravleniya kachestvom v farmatsevticheskikh organizatsiyakh Stavropol'skogo kraya [The state of the system of quality management in pharmaceutical organizations of the Stavropol territory]. Sbornik uchebno-metodicheskikh i nauchnykh trudov. Kazan'; 2014; 66–70. Russian.
22. GOST R ISO 9001-2015 Nacional'nyj standart Rossiyskoy Federatsii. Sistemy menedzhmenta kachestva. Trebovanija. Moscow:Standartinform. Russian.
23. GOST R ISO 9004-2010 Nacional'nyj standart Rossiyskoy Federatsii. Menedzhment dlja dostizhenija ustojchivogo uspeha organizacii. Podhod na osnove menedzhmenta kachestva. Moscow:Standartinform. Russian.
24. GOST R ISO 9004-2010 Natsional'nyy standart Rossiyskoy Federatsii. Menedzhment dlya dostizheniya ustoychivogo uspekha organizatsii. Podkhod na osnove menedzhmenta kachestva. Moscow: Standartinform. Russian.
25. Repin VV. Biznes-protsessy kompanii: postroenie, analiz, reglamentatsiya [Business processes of the company: construction, analysis, regulation]. Moscow: Standarty i kachestvo, 2004:398 p. Russian.
26. Kondrat'ev VV, Kuznetsov MN. Pokazyvaem biznes-protsessy ot modeli protsessov kompanii do reglamentov protsedur [We show business processes from the company's process model to the procedures regulations]. Moscow: Eksmo; 2008:256 p. Russian.
27. Rogov OA, Davidov SB. Elementy sistemy kachestva v aptechnykh organizatsiyakh [Elements of the quality management systemin pharmacies]. Sovremennaya organizatsiya lekarstvennogo obespecheniya. Moscow; 2018(2):111–113. Russian. doi: 10.30809/solo.2.2018.35.
28. Dremova NB, Korzhavykh EA. Lekarstvennoe sredstvo kak mnogomernyy ob"ekt [Medicinal product as a multidimensional object]. Farmatsevticheskaya promyshlennost'. 2005; 5:59–69. Russian.
29. Klimenkova AA, Geller LN, Skripko AA. Razrabotka, vnedrenie i realizatsiya sistemy menedzhmenta kachestva v aptechnykh i meditsinskikh organizatsiyakh, osushchestvlyayushchikh roznichnuyu torgovlyu lekarstvennymi preparatami i tovarami aptechnogo assortimenta [Development, implementation and implementation of quality management system in pharmacy and medical organizations engaged in retail trade of medicines and pharmaceutical products]. Irkutsk: Forvard, 2018:321p.

FINANCIAL SUPPORT AND SPONSORSHIP

This study did not have any financial support from outside organizations.

AUTHOR CONTRIBUTIONS

All authors had equally contributed to the research work.

CONFLICTS OF INTEREST

The authors and peer reviewers of this paper report no conflicts of interest.

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