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*Best regards,*

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# THREE-DIMENSIONAL ADDITIVE PRINTING IN DOSAGE FORM TECHNOLOGY

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*Additive technologies (three-dimensional printing) are currently the most developing area in various fields and industries. In pharmaceutical technology, this type of production is closely related to the concept of personalized medicine, where a dosage form with individual characteristics is created on the basis of the patient's personal data. This literature review presents various 3D printing technologies with physical classification of different types of 3D printers. The principle of operation, characteristics, advantages and disadvantages of such types of 3D printing as extrusion printing, powder printing (TheriForm™) and stereolithography are described. Features of the use of three-dimensional printing in such dosage forms as: implants, tablets and transdermal delivery systems (patches with microneedles) are demonstrated. The conclusion is made about the acceptability of three-dimensional printing in pharmaceutical technology, the development prospects and the necessary conditions for formation of a regulatory framework and the implementation of the principles of additive production of dosage forms in everyday practice.*

**Keywords:** 3D printing, additive printing, dosage forms, printers

Currently, the development of pharmaceutical technology is provided in several directions, one of which is the production of personalized dosage forms (DF). This direction is associated with the need for individual pharmacotherapy for patients taking medicines that have a narrow therapeutic window, as well as active pharmaceutical ingredienta (API) that have a proven direct proportional "dose – effect" relationship [2]. In connection with development of the presented direction, it is necessary to create a universal technological mechanism for production of medicines in an individual dosage that corresponds to the metabolism, age, and genetic characteristics of the patient. 3D printing, or additive printing in other words, allows you to produce individual medicines for patients in a wide range of dosages, shapes and sizes, so it is the main technology for creating personalized medicines.

Three-dimensional printing is a form of additive production in which an object is created by successive layer-by-layer deposition or binding of materials [1,3]. Use of additive technologies in the field of the dosage form development was studied and implemented in the form of a 3D-printed orodispersible tablet Spritam® (levetiracetam, USA), which was registered and went on sale in 2016.

The advantages of using additive methods for development and production of dosage forms include the ability to accurately control the spatial distribution of API in the dosage form, create a variety of complex geometric forms of medicines, control the dosage of the minimum number of medicines, and reduce the amount of waste.

**Purpose of** the review – to highlight the 3D printing methods that are being developed for production of dosage forms, as well as to show the advantages of manufacturing the personalized dosage forms.

## CLASSIFICATION OF 3D-PRINTING TECHNOLOGIES

A large number of technologies have been developed for industrial production of three-dimensional structures, but several basic methods are used for pharmaceutical 3D printing. The technologies used can be classified (Fig. 1) based on the origin and running of the main physical processes: extrusion (melting of the filament), dripping (using binding solutions) and laser systems (sintering/curing) [22].

Each method is characterized by the structure of the printed object and the use of material for 3D-printing. In addition, each of them shows certain process characteristics that are preferred for different APIs. The advantages and disadvantages associated with each of the existing approaches can be demonstrated by comparing the dimensional accuracy, mechanical properties, surface roughness, assembly speed, and material cost on several 3D printing platforms [5].

## EXTRUSION PRINTING TECHNOLOGY

3D printing by polymer filament extrusion is a semi-liquid material deposition technology known under the brand name Fused Deposition

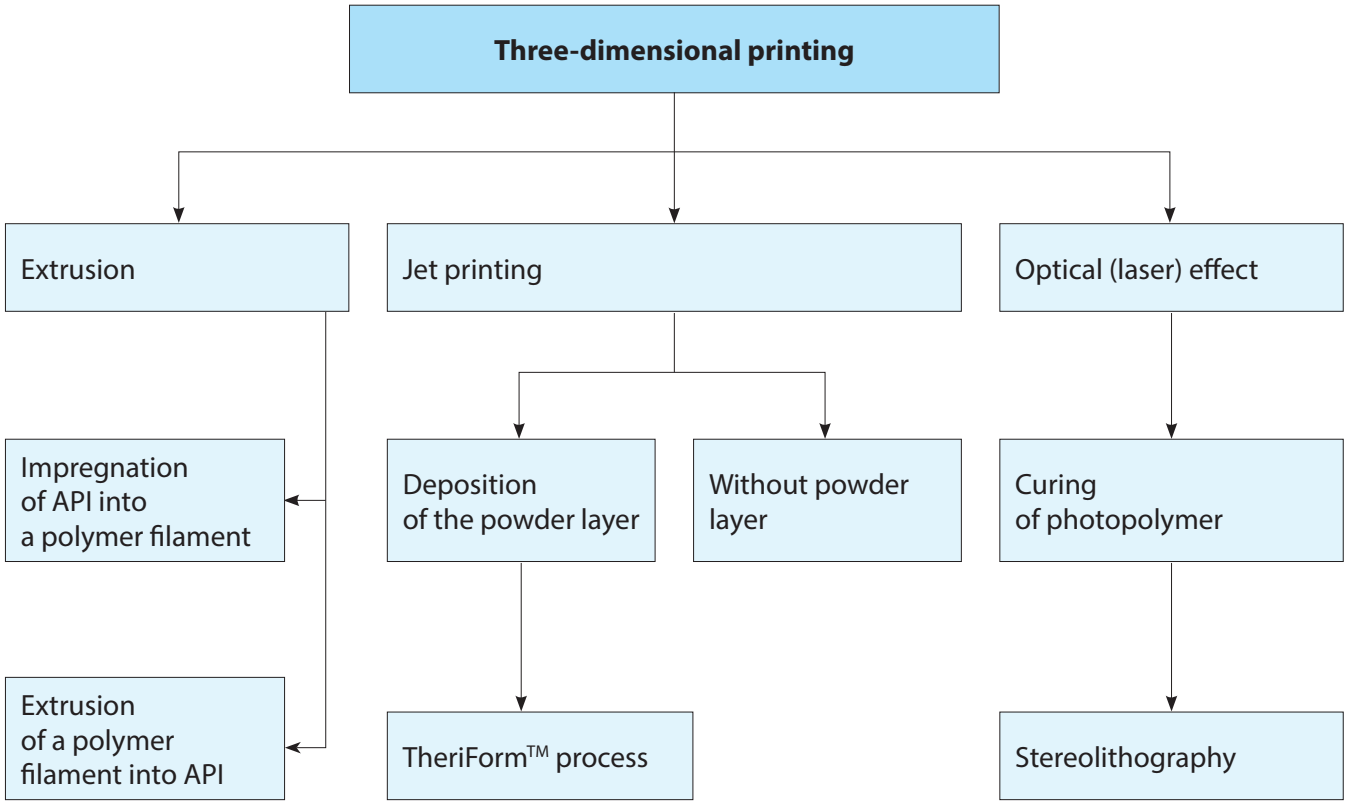


**FIG. 1.** *Classification of 3D printing methods*

ModelingTM (FDM) [6]. In extrusion printing systems, solid filaments are used that are heated above the material melting point, and then the extrudate is deposited in a continuous flow in layers by means of a movable guidance head. After deposition of one layer in the XY plane, the platform (z axis) is lowered and the procedure is repeated. The presented process continues until the dosage form is created. During thermal melting, the material binds to the underlying layer and solidifies, forming a strong connection between the two layers (Fig. 2).

To improve the interlayer connection, the entire process is performed in a closed chamber that maintains a constant temperature [7].

In 3D extrusion devices, it is possible to place multiple print heads for the use of various excipients and API in a single 3D printed dosage form. If necessary, a second printing head is used to provide a temporary supporting substrate for the manufacture of complex structures with a protrusion, offset, or cavity.

The method of extrusion printing allows you to produce structures with controlled pore size and porosity by changing the amount of deposition of the material, the distance between the filaments and the height interval. The most important criteria for selecting a material for extrusion printing are heat transfer characteristics and rheology, so this method requires certain polymers as filaments.

The main advantage of extrusion printing is that polymer filaments are produced with the specified characteristics by hot melt extrusion.

The extrusion printing process usually requires strict specifications for filaments, for example, the requirements for manufacturing the hydroxypropyl cellulose filaments (Klucel ™ dosage form, Ashland): obtaining uniform filaments with minimum length of 25 cm, round cross-section and the same diameter (1.75±0.05 mm). A smaller diameter of the filament leads to formation of air bubbles inside the 3D-printed dosage form, and too large diameter leads to clogging of the tip [4,12]. The diameter of the extruded filaments depends not only on the diameter of the extrusion head, but also on the process of stress relaxation (swelling)

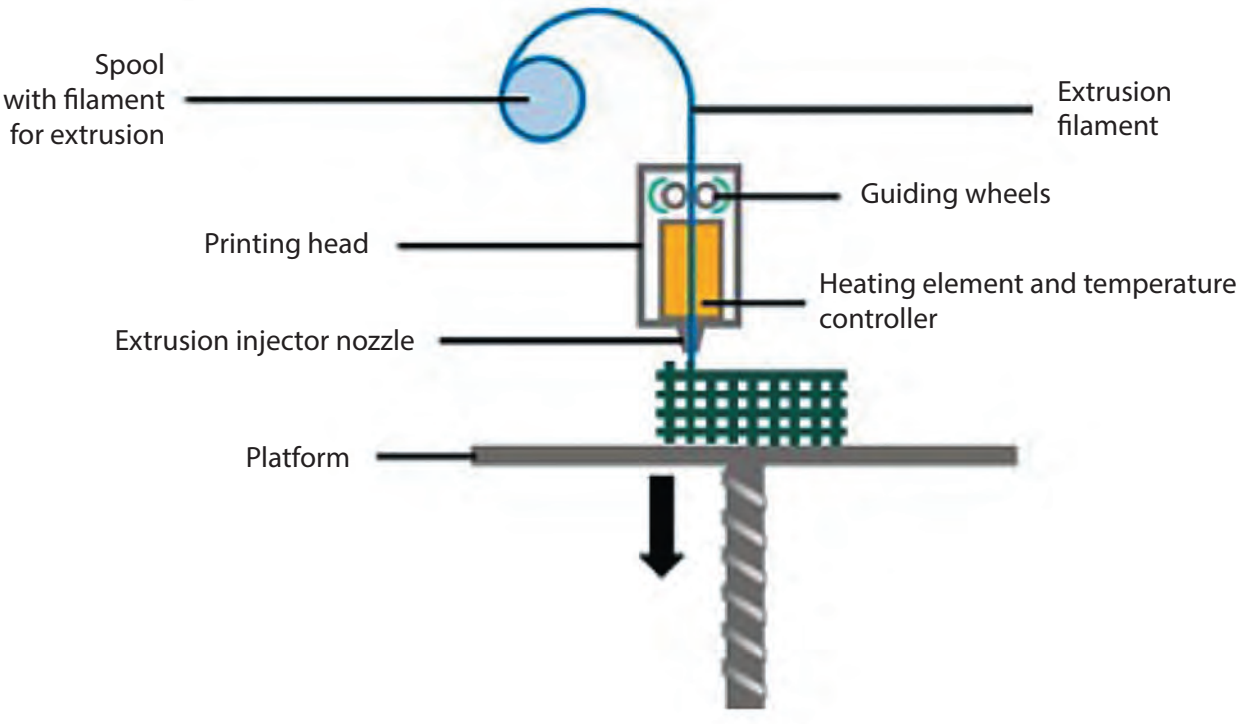


**FIG. 2.** *3D extrusion printing process diagram*

of polymer and speed of the conveyor belt (Fig. 3). Despite the value of the diameter and dispersion of the filament along the length, the constancy of the diameter is more important than the exact achievement of this value, equal to 1.75 mm.

Suitable polymer materials for an extrusion printer are thermoplastic and melt at sufficiently low heating temperatures (usually below 250°C). They are characterized by rapid solidification due to the high glass transition temperature and retain their shape well.

Despite the influence of the technological process on the physical and chemical properties of the polymer, the main parameters remain unchanged and depend on the nature of the raw material used in extrusion. There are various technological parameters of material processing, such as layer thickness, assembly orientation, 3D object raster projection angle, and raster width, which significantly affect the mechanical properties and characteristics of the polymer thread [12]. Table 1 shows the variables that affect 3D-printed dosage forms.

Initially, acrylonitrile-butadiene-styrene was used as a starting material for creating polymer filaments in extrusion 3D printing, and then such polymers and their mixtures as: polycarbonate, polypropylene, polyphenylsulfone, polyglycolic acid, poly (L-lactic-L-glutamic acid), polycaprolactone, and polydioxanone [10].

The main advantages of the extrusion 3D printing process are that it does not require toxic or organic solvents, and the use of polymer filaments provides continuous low-cost production with high flexibility (variability) of material processing. Despite these advantages, the extrusion printing process is limited by thermolability of the active substance and excipient of filaments used for extrusion and feeding them through the rollers and nozzle. There are also difficulties in calibration of the extrusion filament feed when replacing the polymer material. In addition, dosage forms produced by extrusion printing, due to the variety of interacting technological parameters of the process, show less accuracy in size compared to other methods of additive manufacturing.

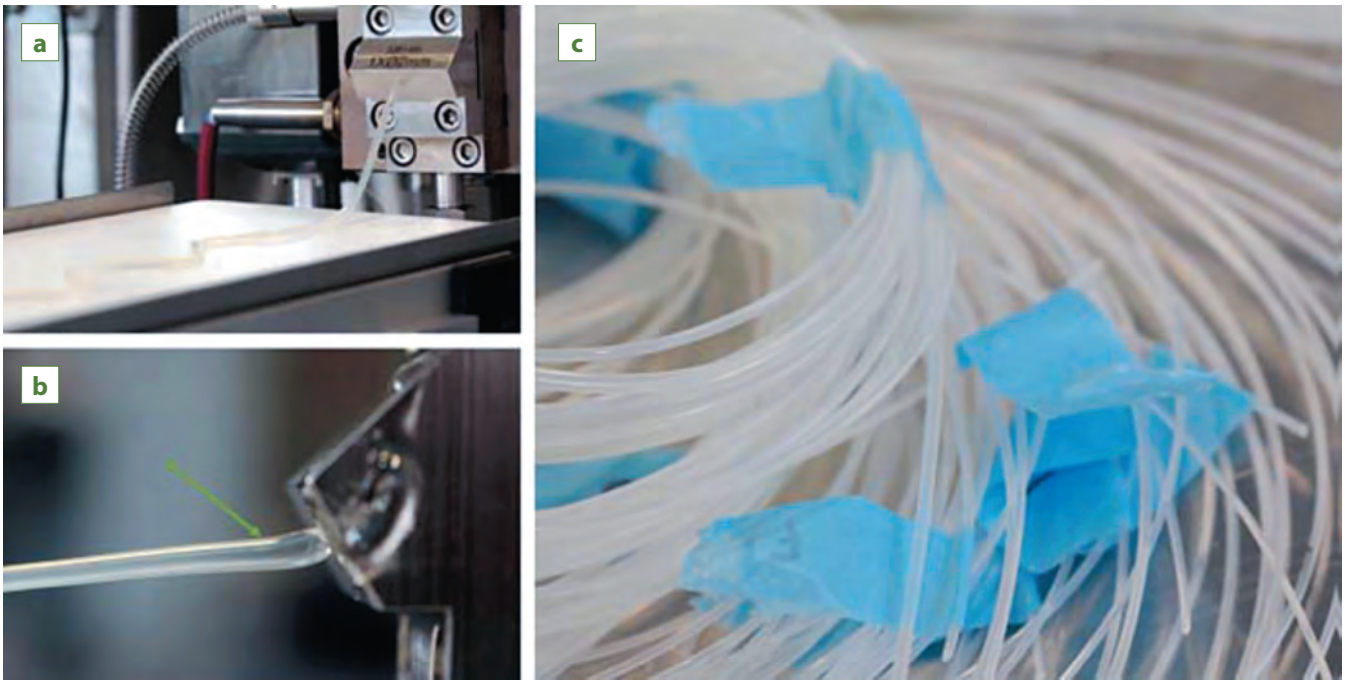


**FIG. 3:** *a – hot melt extrusion of hydroxypropylcellulose (HPC) filaments; b – polymer relaxation (swelling) of HPC after exiting the extrusion head; c – HPC filaments of different diameters (<1.8 mm)*

*Table 1*

**VARIABLES THAT AFFECT THE MECHANICAL PROPERTIES OF 3D-PRINTED DOSAGE FORMS**

| Process parameters | Equipment parameters | Properties of excipients |
|---|---|---|
| Orientation of a dosage form during assembling | Model of a printer | Rheologic properties |
| Thickness of a layer | | Density of raw material |
| Screen angle of an object projection | | |
| Width of the projection raster | Type of a process | Temperature properties of polymers and other materials |
| The gap between the projection rasters | | |
| Fill projection | Diameter of extrusion-type injector nozzle | Composition, solubility and concentration of components |
| Speed of material filling; amount of material | | |
| Extrusion temperature | Software | Diameter of extrusion filaments |
| Chamber temperature | | |
| Environmental conditions (humidity, etc.) | | Uniformity of a filament diameter |

## POWDER PRINTING – APPLICATION OF DROPS ON THE POWDER (THERIFORM™)

Powder printing under the commercial name TheriForm ™ was developed at the Massachusetts Institute of technology [11]. A special feature of the technology is the use of a liquid binder delivered by an inkjet printhead for layer-by-layer creation of objects from powder. The process starts with uniform distribution of the powder layer on the binding plate (the layer consists of powder with a particle size in the range of 50–100 microns and usually has a thickness of 200 microns) [13]. The inkjet printhead then precipitates drops of liquid binder solution onto the surface of the powder. Two-dimensional cross-section of the wetted powder layer is bonded with binder solution in the required form. Then the support platform is lowered to the depth of the next layer and another even layer of powder is distributed over the surface to print a new cross-section. The process continues until the entire object is completed in the required form in the powder layer. Overhanging structures and pores inside the dosage form are supported by unbound powder during the printing process. After the process is completed, the object is released from the surrounding unbound powder, which is removed from the cavities and pores of the finished structure. This method of 3D printing tends to form porous structures, which may require additional processing to provide the required mechanical strength. The process of powder printing is illustrated in Fig. 4.

Powder printing is characterized by the use of jet heads for distribution of the binder, which are classified according to the principle of ejection of drops of binder. Inkjet printing devices are generally divided into two groups: continuous inkjet printing and on-demand printing. The continuous inkjet printing process creates a continuous stream of charged droplets

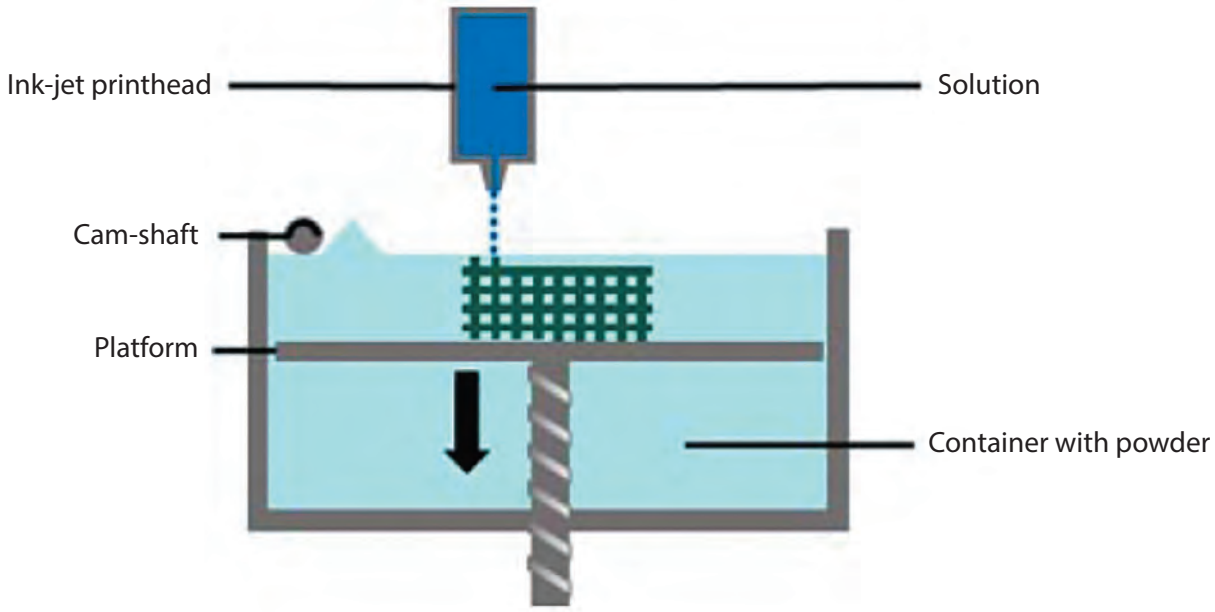


**FIG. 4.** *Schematic illustration of 3D powder printing*

that are directed by electrostatic plates into the powder layer or deflected into the waste recycling line. When printing on demand, drops of liquid binder are distributed only when the process requires it, which is more economical. In addition, this method is more accurate than continuous inkjet printing, since it is possible to control the volume of droplets in the range of 1–300 µl at their release frequencies up to 10,000 Hz [14]. The production of drops in print heads on demand is achieved using a thermal or piezoelectric method. Thermal printing heads consist of a thin-film resistor that heats up when an electrical pulse is applied. A bubble of superheated steam is formed, which expands and throws a drop out of the print nozzle. The subsequent collapse of the bubble creates partial vacuum into which the fresh binder solution is drawn [17]. Temperatures up to 300°C can be reached on the surface of the resistor, but the exposure time is only a few milliseconds. At the same time, only a small part of the liquid, about 0.5% of the volume, is heated, which minimizes the potential destructive effect on thermolabile components [16]. The formation of drops in a piezoelectric printhead is the result of pressure waves induced by deformation of the piezoelectric transducer. The liquid reservoir is then filled when the piezoelectric material is re-shaped. In contrast to thermal jet print heads, the piezoelectric process is thermally constant and can be performed at any temperature.

As a powdery layer in such systems, pharmaceutical-grade excipients can be used that have sufficient flowability for even distribution across the thickness required for each layer. They should also be easily removed from the cavities and pores of the finished product. In the printing process, combination of powdered excipients is used with a binder that has a sufficiently low viscosity for formation of drops, but at the same time forms strong bonds in the dosage form. For effective and uniform drop flowing out of the print heads, the viscosity of the binder solution should be in the range of 5–20 Pa, and the surface tension should be in the range of 35–40 MJ-1. A number of synthetic polymers, including polycaprolactone, poly (lactic acid – co-glycolide), and polylactic acid, were used with organic solvent-based binders. Natural polymers such as starch, dextran, and gelatin were used in combination with water as a binder. It is also possible to include additional excipients into the

powder layer or binder solution to extend the versatility of the powder printing process.

## STEREOLITHOGRAPHIC (LASER) PRINTING – PHOTOPOLYMER- BASED SYSTEMS

Stereolithography is an established 3D printing technology that uses UV or visible light to cure liquid photopolymer resins. Products are created by sequential lighting and curing of thin layers of photopolymers using a laser beam or by projecting a drawing from a digital projector. The product can be built from top to bottom by highlighting the drawing on the top layer of the surface of the bath with polymer resin. As soon as the layer is completed, the platform descends a little deeper and the blade passes over the surface to apply another layer of photopolymer. The new layer of photopolymer is then highlighted with the next layer's drawing. However, you can also build a product from the bottom up. In this case, the resin is placed in a bath made of material that is transparent to ultraviolet radiation, such as polyethylene terephthalate (PET) [14]. The layer drawing is displayed through a transparent window on the lower surface of the resin. The cured layer then rises up by one layer thickness, the liquid resin fills the space under the solid layer and is illuminated by a beam to form the next layer. A schematic representation of these two approaches is shown in Fig. 5

In both cases, the depth of curing of each layer is slightly greater than the vertical step motion. Then unreacted functional groups in the solid layer can be polymerized in a new layer, thus ensuring good adhesion between all the layers of the 3D printing product. At the end of the process, when all the layers are completed, the excess resin is drained and washed away. The finished product is often subjected to the final stage of curing using ultraviolet radiation to ensure complete conversion of unreacted functional groups in the polymer.

Advantages of preparing the dosage form using stereolithography: high accuracy of product construction, ease and speed of 3D printing. However, there are also limitations of the presented method, which consist in a small number of biocompatible photopolymers and

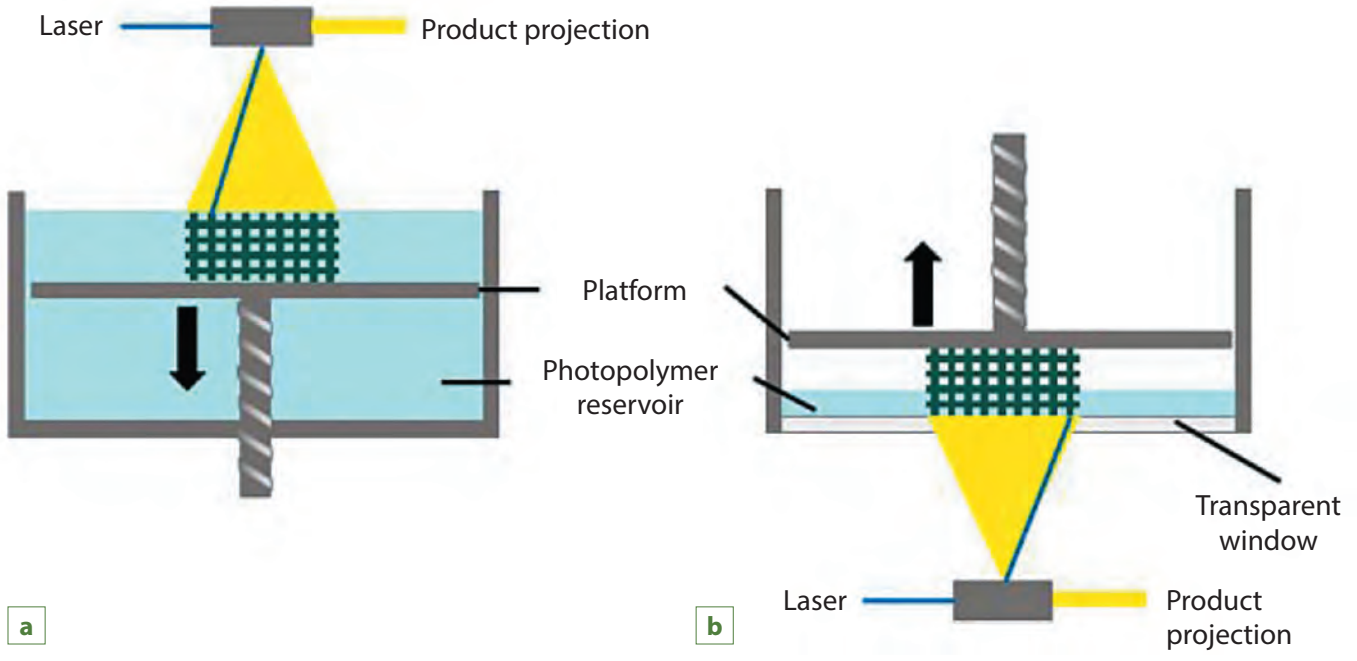


**FIG. 5.** *Schematic representation of the stereolithography process: a-descending process; b-ascending process*

the impact of physical processes on the active substance. For stereolithography, poly (propylene fumarate), poly (caprolactone-trimethylenecarbonate), poly (D, L-lactic acid), polycaprolactide and polyethylene glycol are used. It is also difficult to include more than one polymer in the dosage form to create modified release [19].

## APPLICATION OF 3D PRINTING IN PHARMACEUTICAL TECHNOLOGY. 3D PRINTING OF IMPLANTS

The first dosage form produced with a 3D printer after patenting and publishing a powder-based inkjet printing device were biodegradable implants. Researchers from the Massachusetts Institute of Technology used polyethylene oxide as a polymer matrix and polycaprolactone as a component that restricts the rate of release, and prepared various systems with yellow dyes of the methylene blue and alizarin series as substitutes for the API. Studies have shown the ability to accurately place small amounts of dye solution drops and the ability to create a microstructure with controlled wall thickness depending on the rate of binder deposition and printing speed. Also, three-dimensional printing has shown the ability to control the geometry, surface area, spatial deposition and other technological parameters that affect the kinetics of medicine release. These studies have demonstrated that 3D-printed implants show a more controlled zero-order release than conventional implant manufacturing techniques, such as pressing or injection molding [18].

In 2007, Huang et al. made monolithic levofloxacin implants for comparison with pressed implants, as well as implants with complex structures for immediate and double pulsating release. 3D-printed implants showed a more porous structure than those obtained by pressing, and the part of the implant intended for immediate release of API showed a relatively fast and slightly more complete release than the pressed dosage form. The implants printed with an internal reservoir for active substance, internal and external layers of the dosage form, could, respectively, show an immediate and pulsating release of the medicine. In the study demonstrated, implants achieved the immediate release of up to 400 mg of API and the steady release of 120 mg or less for 90 days. Later, the same group of researchers developed pulse-release implants to deliver two APIs, levofloxacin and rifampicin, with immediate release of levofloxacin on day 1 and delayed release of the internal rifampicin reservoir on day 8 with prolonged release of both APIs up to 6 weeks. This work demonstrated the possibility of creating 3D-printed combined implants with different types of release [19].

## 3D PRINTING OF TABLETS

The earliest work on obtaining 3D-printed tablets was carried out using three-dimensional printing of the powder layer. In the Massachusetts Institute of Technology the research has been carried out to produce tablets that demonstrate hardness and friability which are comparable with pressed dosage forms by increasing the concentration of polymer/binder, but three-dimensional inkjet printing usually produced more porous and loose tablets. The increased porosity of 3D printed dosage forms is due to incomplete interaction of the binder solution with the powder layer, which leads to the appearance of areas of unbound particles. Aprecia Pharmaceuticals took advantage of the increased porosity of the obtained dosage forms to produce tablets dispersed in the oral cavity. They created a patented ZipDose ® technology based on three-dimensional printing of the powder layer, which makes it possible to dose APIs in a tablet up to 1000 mg. According to the presented technology,

the LP Spritam ® (levetiracetam) for the treatment of epilepsy was put on the market [20].

Despite the existing examples of 3D printing in the production of tablets dispersed in the oral cavity, the main potential of this method lies in the creation of combined dosage forms with modified release. With the help of three-dimensional printing technology, it is possible to produce tablets with immediate, prolonged, delayed and pulsating-released components in the same dosage form by applying different layers or sections containing one or more APIs. In addition, it is possible to use the advantages of creating the tablets that have different geometric shapes and, accordingly, releases. For example, in some studies, to create a specific release profile, tablets were made in a flat-cylindrical shape with a concentric round hole inside, as shown in Fig. 6.

In the presented tablet structure, the upper and lower layers, as well as the side surfaces, contained ethylcellulose to obtain impermeable barriers, while the inner core was made using API of acetaminophen and a binder consisting of 2% ethylcellulose and creating a slower release from the outer surface. Three-dimensional inkjet printing allows you to produce very thin functional barrier layers at the top and bottom, as well as an external surface containing ethylcellulose or other excipients. Theoretically, this technology made it possible to create a dosage form with zero-order release kinetics, since the surface area through which the API passed practically did not change.

Extrusion printing as a method of manufacturing tablets is often used using PVA fibers as a filament. There are known developments with impregnated solutions of aminosalicylates in PVA filaments. At that, non-aqueous (ethanol) solvents were used, since PVA is soluble in aqueous solutions. The solubility of API in the solvent affected the final inclusion of pharmaceutical product into the PVA filaments. Thus, 4-aminosalicylates with lower solubility were impregnated into the filament at 0.06% concentration, and 5-aminosalicylates with higher solubility – respectively, at 0.25% concentration. The presented process was used with prednisone, which demonstrated high thermal stability: it was possible to successfully produce tablets with API content of 88.7–107% of the theoretical value [11].

The inclusion of API into the filament for extrusion printing is also possible at the stage

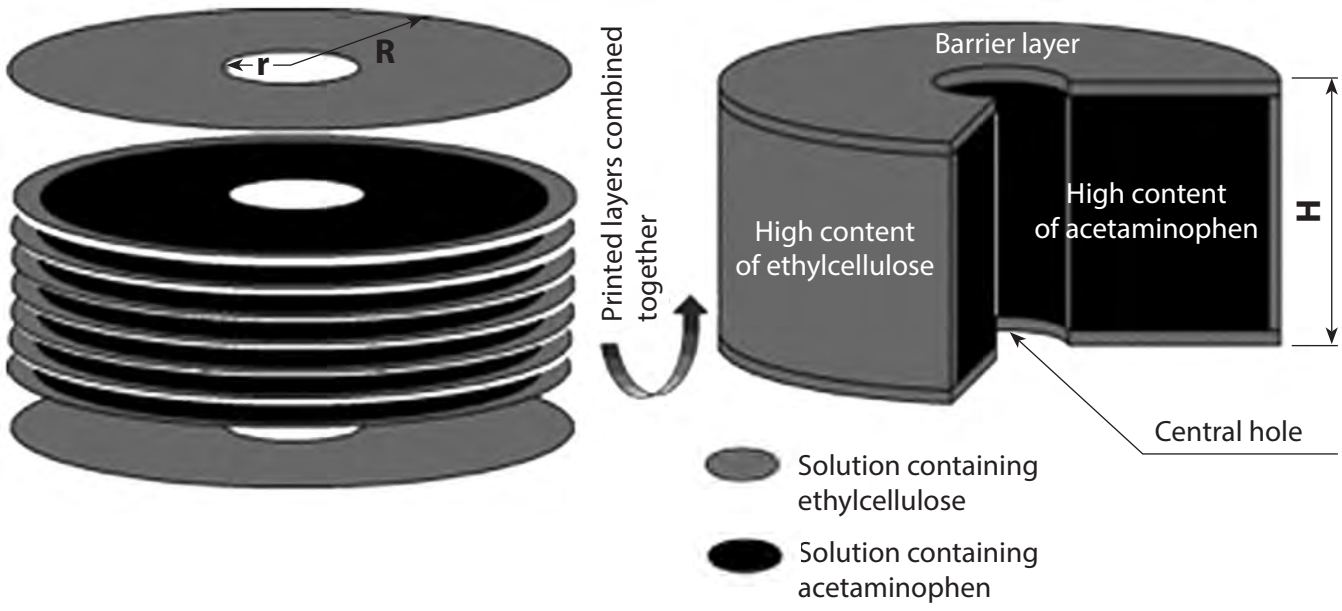


**FIG. 6.** *A flat cylindrical tablet with a concentric round hole for release with zero order kinetics*

of hot melt extrusion, which allows to increase the content of API in the filament. The literature describes the production of PVA filaments with acetaminophen and caffeine by hot melt extrusion. Filaments with API were used to make multilayer tablets containing acetaminophen in the inner layer and caffeine in the outer layer. The tablet showed the 135 minutes-delayed release of the internal component of acetaminophen and the 50 minutes -extended release of the outer layer. During the hot melt extrusion process, acetaminophen was in an amorphous state, while caffeine remained crystalline. This study showed not only the ability to produce highly concentrated dosage forms with fixed API content, but also the ability to use amorphous solid dispersion to obtain a filament and corresponding increase in the solubility of the medicine [21].

## 3D PRINTING OF TRANSDERMAL DELIVERY SYSTEMS

Transdermal delivery systems are used to avoid the effect of first passing through the liver or pH- mediated degradation, as well as to ensure compliance with the treatment of chronic diseases such as diabetes. 3D printing methods can be used for manufacturing the multi-layer transdermal patches, in particular, approaches are being developed for printing microneedles filled with API for percutaneous administration. Microneedles usually have height of less than 500 microns and are designed to penetrate the corneal layer (10–15 hours) for API deliver (Fig. 7).

Microneedles should be strong enough to penetrate the epidermis, but not have a hardness that will cause pain or irritation. In addition, biodegradable polymers are preferred for manufacturing microneedles with the tip remaining in the epidermis. Since microneedles have a complex morphology, micro-size, and sufficiently high density per unit of the dosage form, they are difficult to be obtained by traditional methods of coating, but 3D printing technology makes it possible to effectively and relatively simply create patches with microneedles [8].

Researchers from (Boehm et al.) applied additive processes to make microneedles with API. Stereolithography technology was used to create microneedles using poly (methylvinyl ether-alt-maleic anhydride) coated with amphotericin B and filled with miconazole. For the resulting patches,

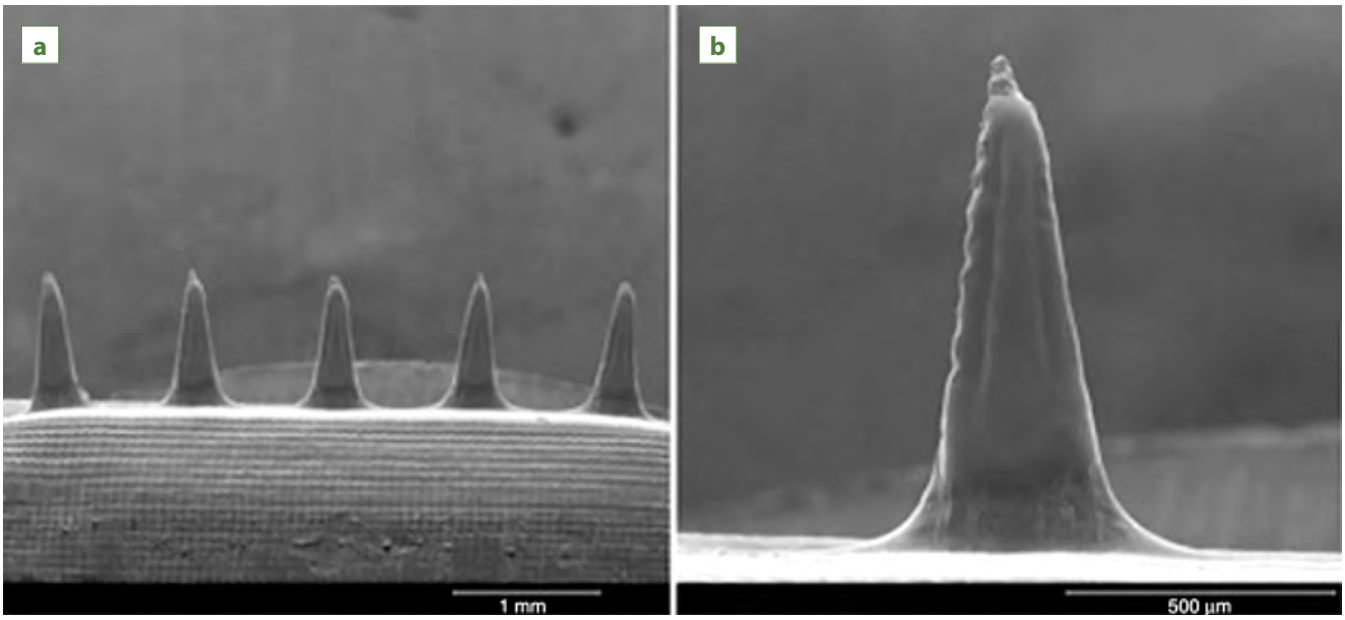


**FIG. 7.** *Micrographs of (a) an array and (b) a single miconazole-containing needle*

it was demonstrated that the microneedles had good mechanical strength for transdermal administration. However, microparticles coated with solutions of amphotericin B showed some changes in the geometry of the microneedles, since their surface was wetted with amphotericin and it was not possible to apply excipients evenly. Studies with miconazole demonstrated a smaller effect on the structure of the microneedle, since API deposition was concentrated in the upper part of the needle and coated the surface, as shown in Fig. 7 [19,21].

## CONCLUSION

Three-dimensional printing is becoming the main tool for manufacturing the dosage form in pharmaceutical technology for personalized medicine, because it allows you to quickly create a wide range of medications with individual dosage. The advantages of 3D printing include a variety of ways to prepare the dosage forms and, consequently, a wide range of technological processes that are suitable for certain properties of API. In addition, certain methods of 3D printing have their own advantages and disadvantages, which allows you to vary the production methods of personalized medications to achieve your goals. The main prospects for the development of 3D printing are observed in production of implantable, solid and transdermal dosage forms. However, despite the development of three-dimensional printing, it is necessary to solve a large number of regulatory, technological and normative tasks that are of paramount importance for implementation of these methods in everyday practice.

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# SOME QUESTIONS OF PHARMACEUTICAL DEVELOPMENT OF INTRAVAGINAL DOSAGE FORM CONTAINING HUMULUS LUPULUS EXTRACT

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*The paper presents the results of the studies on development of the process flow scheme of production and study of antioxidant activity of an intravaginal dosage form. Research by the chemiluminescence method has demonstrated direct antioxidant effect, reduction of free radical formation processes in model systems.*

**Keywords:** biodegradable collagen repair patches containing Humulus lupulus extract, pharmaceutical development, antioxidant properties

The development of formulations for dosage forms of vaginal use is an urgent direction in pharmaceutical technology due to the fact that the wide spread of inflammatory diseases of the female sexual sphere affects the microecobiocenosis of the vagina, women's reproductive functions and, in general, demographic profile in the Russian Federation.

To reduce the manifestations of possible adverse reactions of local antibacterial therapy and to increase the time of remission of inflammatory gynecological diseases, we recommend a comprehensive, multi-level approach and a rationally selected dosage form [2].

A promising dosage form for prevention and treatment of gynecological diseases is intravaginal biodegradable collagen repair patches (BCP). A successful combination of technological and consumer properties, such as simplicity of composition and technology, stability during storage, combination of adhesion and moisture absorption, uniform distribution in the vaginal fluid, natural excretion without additional flushing and douching, justifies the relevance of the development of BCP.

Biodegradable collagen repair patches have osmotic activity and biopharmaceutical advantages, in particular, high bioavailability associated with significant bioadhesion, which

makes it possible to prolong the action of biologically active substances (BAS) [1,4,9].

Prospects for using herbal-based medicines in gynecological practice is obvious due to the multi-level impact on the causes of inflammatory diseases.

The source of obtaining intravaginal dosage forms for pharmaceutical development is Humulus lupulus, which is common in the territory of the Republic of Bashkortostan. Previous studies have shown the presence of a diverse complex of hydrophilic and lipophilic biologically active substances in the studied raw materials, among which polyphenolic compounds (flavonoids, catechins, phenolcarboxylic acids, oxycoric acids and phytoestrogens of particular significance), bitter glycosides (derivatives of acylfloroglucides – humulon, lupulon, etc.), essential oils and terpenoids are significant in quantitative and qualitative composition [1,3]. Such a variety of chemical compositions specifies the complex pharmacological action (anti-inflammatory, antioxidant, antimicrobial, capillaroprotective, phytoestrogenic, analgesic) of Humulus lupulus [3,5,7].

The object of our previous study was a liquid extract of Humulus lupulus multiple fruits as an active pharmaceutical ingredient (API) with identified pharmaceutical and technological properties.

**Study purpose** – development of a process flow scheme for production of BCP with a liquid extract based on Humulus lupulus multiple fruits and assessment of its antioxidant activity.

## MATERIALS AND METHODS

For experimental confirmation of the formulation and process flow scheme of the production of BCP based on 2% acetic acid solution of collagen, a liquid extract of Humulus lupulus multiple fruits was used.

Studies have shown that the optimal extractant for maximum extraction of BAS from raw materials and having the best technological properties for development of BCP was 50% ethyl alcohol with the extraction module "raw material: extractant" 1:10, the extraction method is bismaceration. Standardization of liquid extract of Humulus lupulus multiple fruits was carried out on the qualitative and quantitative content of the most significant BAS groups (the amount of flavonoids (according to rutin), the amount of APG, extractives).

The presence of phenolic and lipophilic biologically active substances in a liquid extract of Humulus lupulus multiple fruits suggests the presence of antioxidant activity

The antioxidant properties of the studied medications were evaluated by their effect on iron-induced chemiluminescence (CHL) using the domestic hardware and software complex CHLM-003, for which we used standard test systems where the process of free radical oxidation (FRO) proceeded [6]. In the model systems used, the processes of formation of reactive oxygen intermediates (ROI) and reactions of lipoperoxidation processes (LPP) take place.

## RESULTS AND DISCUSSION

The process flow scheme of production of BCP based on liquid extract of Humulus lupulus multiple fruits for vaginal use consists of several stages – production preparation, raw material preparation, the stage of obtaining a semi-product, the actual process of freeze-drying of patches, the stages of dispensing, packaging and labeling (Fig. 1).

The study of the effect on iron-induced chemiluminescence, simulating the formation of ROI, was carried out instrumentally. The results of the experiments were evaluated by the degree of change in the luminance indicators in the

addition of the studied samples and recalculated in % of the control. The test samples were diluted in saline, 1.0 ml of the test solutions were selected and added to the test systems.

As a control, the model systems were used without adding medicine solutions (with the addition of saline in the same volumes).

Methods for performing the experiment and preparing the reagents are described in the instructions for the hardware and software complex CHLM-003.

### *Study of the influence of the studied samples on the antioxidant system parameters*

As the first system where the ROI was generated, 20 ml of phosphate buffer (20 mM $KH_2PO_4$, 105 mM KCl) with adding the solution of luminol (10–5 M) and sodium citrate (50 mM). The pH value of the solution obtained was brought up to 7.45 units by titration with a saturated solution of potassium hydroxide. Into 20 ml of the model system 1 ml of each of the studied solutions was introduced: 1 – water solution of BCP with

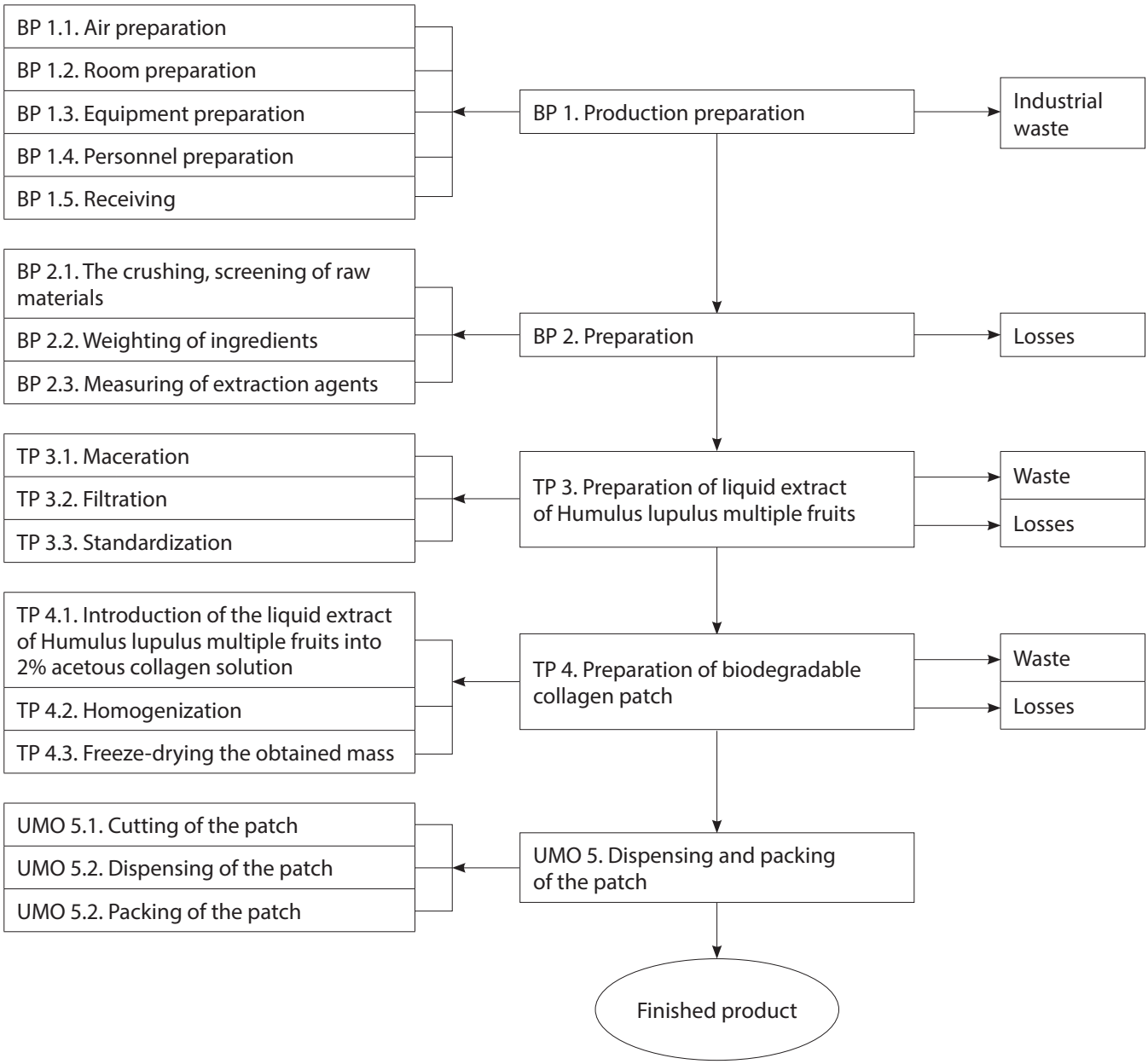


**FIG. 1.** *Technological stages of production of the BCP on the basis of liquid extract of Humulus lupulus multiple fruits*

liquid extract of Humulus lupulus multiple fruits (4.0 g; t = 40°C), 2 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (2.0 g; t = 40°C), 3 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (0,8 g; t = 40°C), 4 – liquid extract of Humulus lupulus multiple fruits freshly prepared (50% ethyl alcohol; bismaceration; 1:10). For initiation of reactions that are accompanied by generating ROI, 1 ml of 50 mM solution of $Fe^{2+}$ salts was introduced. Registration of the luminescence was continuing for 5 minutes with constant remixing.

Chemiluminescence of model systems was characterized by spontaneous luminescence, rapid flash, and then developing a slow flash. The main most informative characteristics of chemiluminescence were the amplitude of the maximum glow and the light sum of the glow, determined by the intensity of radiation.

### *Study of the influence of the studied samples on the formation of lipoperoxidation products*

For the study, a model system was used where the reactions of formation of final peroxidation products occur. The influence of the studied samples on the lipid peroxidation processes (LPP) was studied in lipids of chicken yolk, which have a composition that is similar to blood lipids. Lipids were obtained by homogenizing the chicken yolk in a phosphate buffer in ratio of 1:5 and by the following 20-fold dilution, 20 ml was taken. Into 20 ml of the model system 1 ml of each of the studied solutions were introduced: 1 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (4.0 g; t = 40°C), 2 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (2.0 g; t = 40°C), 3 – water solution of BCP with liquid

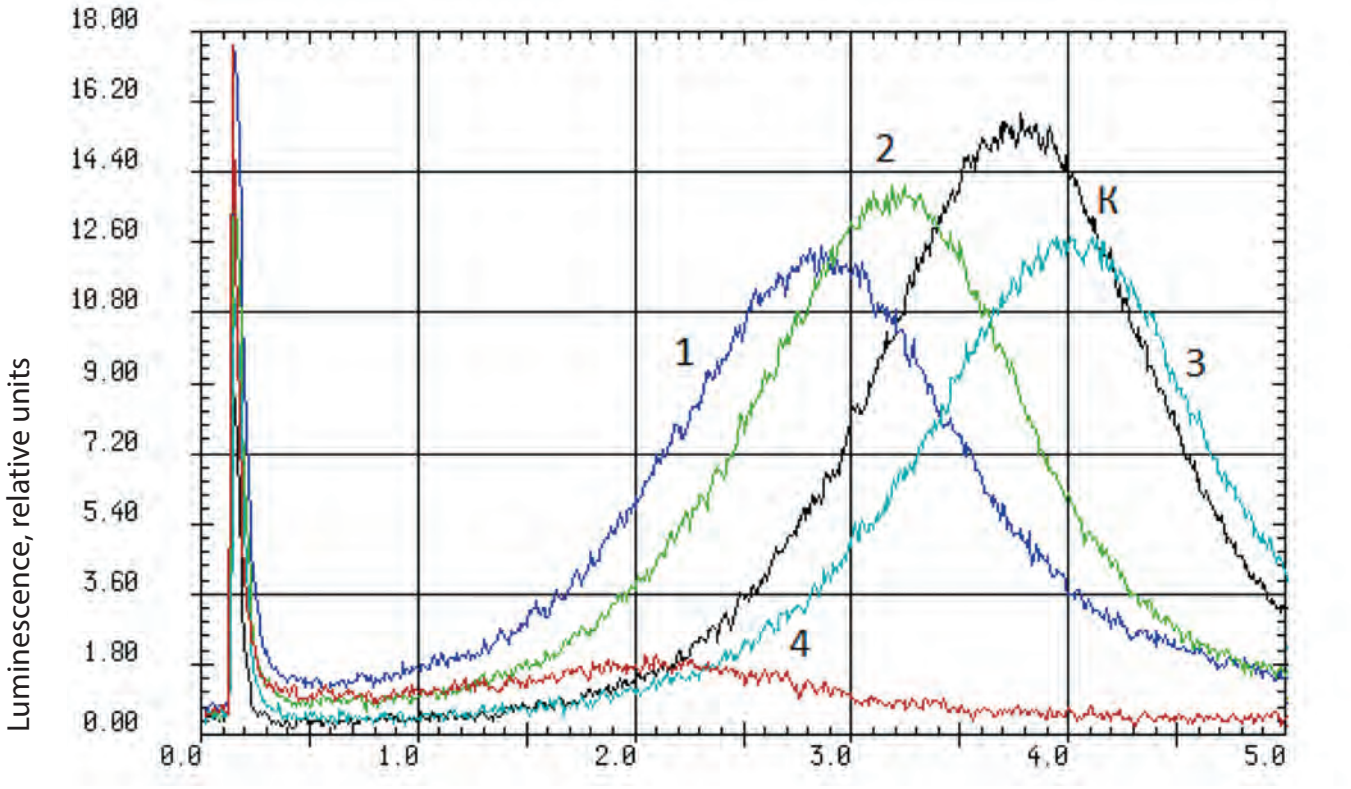


**FIG. 2.** *Influence of BCP with liquid extract of Humulus lupulus multiple fruits and liquid extract of Humulus lupulus multiple fruits on the FRO processes in the ROI model system. Designations: K – control, 1 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (4.0 g; t = 40°C), 2 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (2.0 g; t = 40°C), 3 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (0,8 g; t = 40°C), 4 – liquid extract of Humulus lupulus multiple fruits freshly prepared (50% ethyl alcohol; bismaceration; 1:10).*

extract of Humulus lupulus multiple fruits (0.8 g; t = 40°C), 4 – liquid extract of Humulus lupulus multiple fruits freshly prepared (50% ethyl alcohol; bismaceration; 1:10).

Adding 1 ml of 50 mM $Fe^{2+}$ solution to the system led to initiation of processes of oxidation of unsaturated fatty acids that are part of lipids, and to formation of end products of lipoperoxidation, which was accompanied by luminescence. The level of spontaneous luminescence characterized the intensity of lipid peroxidation processes before the introduction of the catalyst; the amplitude of the rapid flash reflected the rate of oxidation of $Fe^{2+}$ ions and formation of ROI and lipid hydroperoxides; the duration of the latency period correlated with the antioxidant activity of the studied medication. The value of the light sum of the luminescence determined the ability of lipids to undergo oxidation.

Data on the influence of the studied samples on CHL in the model system generating ROI are shown in Fig. 2.

Quantitative characteristics of the influence of the studied samples on FRO are presented in Table 1.

Adding of aqueous solutions of BCP with liquid extract of Humulus lupulus multiple fruits of different concentrations into the model system

*Table 1*

**INFLUENCE OF BCP WITH LIQUID EXTRACT OF HUMULUS LUPULUS MULTIPLE FRUITS AND LIQUID EXTRACT OF HUMULUS LUPULUS MULTIPLE FRUITS ON THE LIGHT SUM OF CHL IN THE MODEL SYSTEM GENERATING ROI**

| № | Sample of the studied products | Light sum | | Spontaneous luminescence, abs. | Flash abs. | Maximal luminescence | |
|---|---|---|---|---|---|---|---|
| | | abs. | % in relation to control | | | abs. | % in relation to control |
| 1. | Control | 26.63 | 100 | 0.45 | 8.96 | 15.91 | 100 |
| 2. | Water solution of BCP with liquid extract of Humulus lupulus multiple fruits (4.0 g; t = 40°C) | 25.64 | 96.2 | 0.70 | 17.47 | 12.49 | 78.5 |
| 3. | Water solution of BCP with liquid extract of Humulus lupulus multiple fruits (2.0 g; t = 40°C) | 25.98 | 97.6 | 0.52 | 13.55 | 14.09 | 88.6 |
| 4. | Water solution of BCP with liquid extract of Humulus lupulus multiple fruits (0,8 g; t = 40°C) | 22.35 | 83.9 | 0.51 | 11.16 | 12.77 | 80.2 |
| 5. | Liquid extract of Humulus lupulus multiple fruits freshly prepared (50% ethyl alcohol; bismaceration; 1:10) | 6.45 | 24.2 | 1.21 | 16.53 | 2.25 | 14.1 |

*Note. The intensity of the luminescence of model systems without addition of the studied substances is assumed to be 100%. The average data of 6 measurements is вызушашув. * – significant differences ($p < 0.05$) are marked.*

shortened the latent period, for BCP with liquid extract of Humulus lupulus multiple fruits at concentrations of 4.0 and 2.0 and fresh liquid extract of Humulus lupulus multiple fruits the slow flash started and went out early. All samples of solutions of BCP with liquid extract of Humulus lupulus multiple fruits of different concentrations reduced the parameters of the light sum of the luminescence of CHL in the model system depending of a dose, the most effective solution was BCP with liquid extract of Humulus lupulus multiple fruits at a concentration of 0.8 g.

Freshly prepared liquid extract of Humulus lupulus multiple fruits, which is the basis for obtaining BCP, almost 3.5–4 times reduced the most significant factor of CHL in comparison with solutions of BCP with liquid extract of Humulus lupulus multiple fruits and control.

Further, a model system was used for the study, where the processes of final lipoperoxidation occur. Data on the influence of the studied samples on CHL in this model system are presented in Fig. 3.

Introduction of samples into the egg yolk lipoprotein model system was also accompanied by decrease in the parameters of the light sum and amplitude of maximum luminescence (Fig. 2).

Quantitative characteristics of studied parameters are presented in table. 2.

The results of the study showed that all samples of solutions of BCP with liquid extract of Humulus lupulus multiple fruits of different concentrations reduced the light sum of the luminescence and maximal luminescence of CHL in the model system depending of a dose, the most effective solution was BCP with liquid

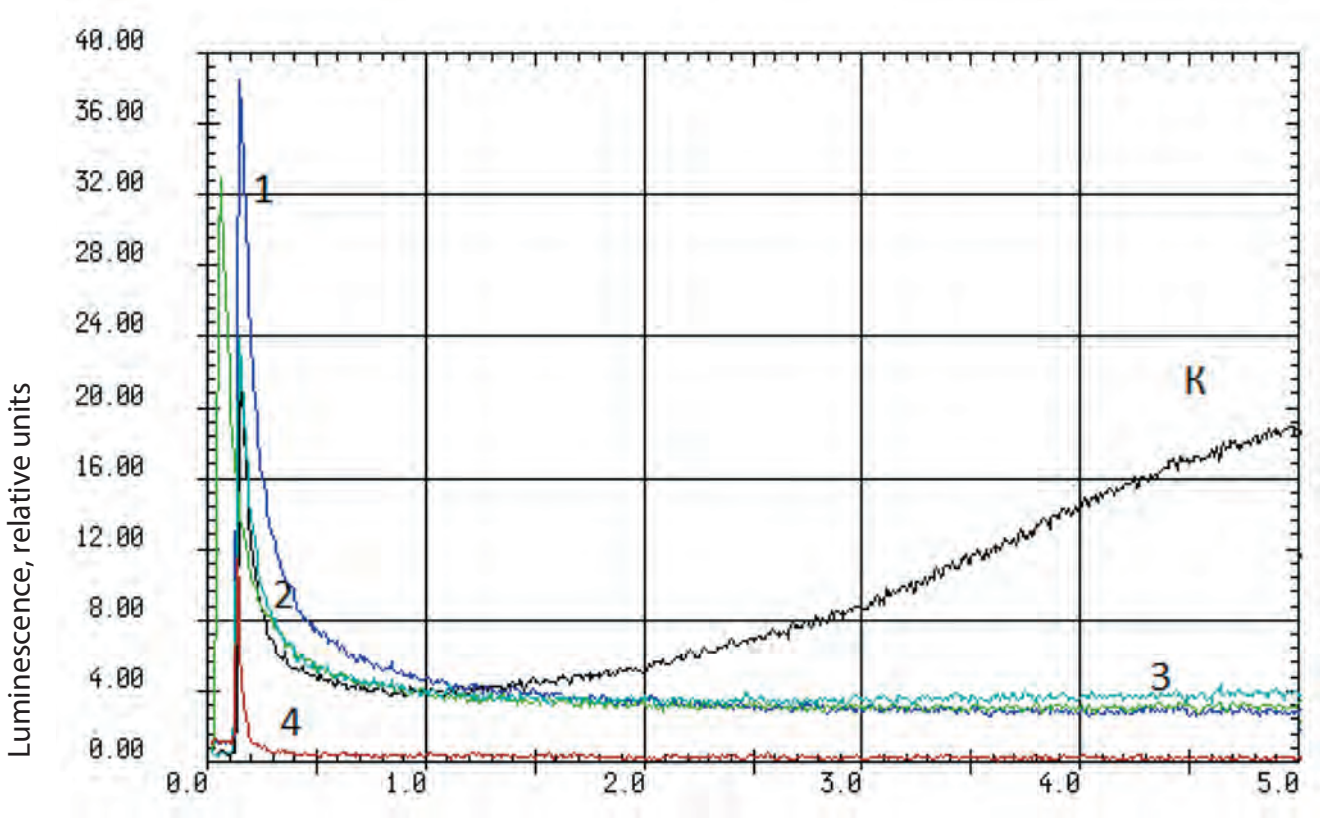


**FIG. 3.** *Influence of BCP with liquid extract of Humulus lupulus multiple fruits and liquid extract of Humulus lupulus multiple fruits on the FRO processes in the model system of the end lipid peroxidation. Designations: K – control, 1 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (4.0 g; t = 40°C), 2 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (2.0 g; t = 40°C), 3 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (0,8 g; t = 40°C), 4 – liquid extract of Humulus lupulus multiple fruits freshly prepared (50% ethyl alcohol; bismaceration; 1:10).*

*Table 2*

**INFLUENCE OF BCP WITH LIQUID EXTRACT OF HUMULUS LUPULUS MULTIPLE FRUITS AND LIQUID EXTRACT OF HUMULUS LUPULUS MULTIPLE FRUITS ON THE LIGHT SUM OF CHL IN THE MODEL SYSTEM WHERE THE END LIPID PEROXIDATION PROCESSES TAKE PLACE**

| № | Sample of the studied products | Light sum | | Spontaneous luminescence, abs. | Flash abs. | Maximal luminescence | |
|---|---|---|---|---|---|---|---|
| | | abs. | % in relation to control | | | abs. | % in relation to control |
| 1. | Control | 45.32 | 100 | 0.53 | 22.77 | 18.96 | 100 |
| 2. | Water solution of BCP with liquid extract of Humulus lupulus multiple fruits (4.0 g; t = 40°C) | 21.56 | 47.6 | 1.13 | 38.48 | 10.60 | 55.9 |
| 3. | Water solution of BCP with liquid extract of Humulus lupulus multiple fruits (2.0 g; t = 40°C) | 20.28 | 44.7 | 18.84 | 32.89 | 7.22 | 38.1 |
| 4. | Water solution of BCP with liquid extract of Humulus lupulus multiple fruits (0,8 g; t = 40°C) | 20.48 | 45.1 | 0.48 | 23.94 | 6.96 | 36.7 |
| 5. | Liquid extract of Humulus lupulus multiple fruits freshly prepared (50% ethyl alcohol; bismaceration; 1:10) | 1.86 | 4.1 | 1.16 | 11.39 | 0.68 | 3.6 |

extract of Humulus lupulus multiple fruits at concentration of 0.8 g.

The model system luminescence level was suppressed to almost zero when adding freshly prepared liquid extract of Humulus lupulus multiple fruits, which is the basis for obtaining BCP.

## CONCLUSION

The process flow scheme of production of BCP with liquid extract of Humulus lupulus multiple fruits was developed.

Studies have shown that in both model systems, the studied samples acted in the same direction: they reduced formation of free radicals and showed a direct antioxidant effect.

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# COMPARATIVE MORPHOLOGICAL AND ANATOMICAL ANALYSIS OF CHAGA AND TINDER FUNGUS AND FALSE T.F.

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*A comparative morphological and anatomical study of the chaga and the tinder fungus and the false tinder fungus was conducted. Diagnostic distinctive morphological and anatomical features are established. Identified signs form the basis of the sections "External signs" and "Microscopy" of the Russian Federation State Pharmacopoeia, the XIV edition of FS.2.5.0103.18 "Chaga – Inonotus obliquus".*

**Keywords:** chaga, tinder fungus, false tinder fungus, morphology, anatomy, macroscopic analysis, microscopic analysis

Chaga (birch fungus, Inonotus obliquus, timber fungus oblique) – Inonotus obliquus (Pers.) Pil., Hymenochaetaceae family – perennial sterile growths up to 40–50 cm in diameter that occur on living trees (usually birch), formed by a parasitic fungus [3,8]. In medical practice, birch fungus is used as a process aid in the comprehensive treatment of chronic gastritis, gastrointestinal dyskinesia of the hypokinetic type, as well as a non-specific (symptomatic) agent for oncological diseases that improves the general condition of cancer patients [5,7]. In appearance, the true tinder fungus – Fomes fomentarius (L.: Fr.) Fr. – Poriaceae family and false tinder fungus – Phellinus igniarius (L.: Fr.) Quel., Hymenochaetaceae family are similar to the chaga, impurities to chaga and can be harvested instead of chaga. [3]. In this regard, it is of interest to conduct a comparative study of chaga and tinder fungus by morphological and anatomical features in order to identify distinctive macroscopic and microscopic diagnostic signs.

**Study purpose** – study of the morphological and anatomical structure of chaga and impurities (true and false tinder fungi) and identification of distinctive diagnostic signs.

## MATERIALS AND METHODS

The study material was samples of chaga, true tinder fungus and false tinder fungus, harvested in the Perm region in the autumn-winter period of 2016–2017. Macroscopic and microscopic analysis was performed according to the generally accepted methods of the State Pharmacopoeia, XIV edition [4].

Chaga pieces are very dense, so to prepare slices, the raw material was pre-soaked in a mixture of "96% ethyl alcohol – 1 part: glycerine – 1 part" for 3–7 days, after which longitudinal and cross sections were made by 30 microslides from each sample.

To prepare powder specimens, pieces of raw material with a diameter of 1–2 mm were boiled in a 5% sodium hydroxide solution, then washed with water and placed on a slide in a drop of chloral hydrate solution.

The study of anatomical features was performed using a Biomed-6 microscope, magnification 640x, 400x, 160x. Microphotographs were performed using a DCM 510 digital camera in the Scope Photo program. Cell sizes were measured using a Motic microscope in the Motic Educator program. The results were processed in Microsoft Excel.

## RESULTS AND DISCUSSION

Chaga and tinder fungus are parasitic fungi that infect various types of trees, and are capable of long-term existence.

Chaga is a stenotrophic plant, shows a high degree of specialization in relation to birch wood [1,2,9], is less common on alder, rowan-tree, beech and some other hardwoods, develops on living trees [2,10].

Tinder fungus is a poorly specialized eurythrof that inhabits coniferous (fir) or deciduous (oak, maple, linden, birch, alder, poplar, aspen, willow) trees [6,9], and develops on dead trunks, stumps, and trees [3,8].

According to the literature data, the false tinder fungus does not have a strict substrate specialization [9]. In Europe, the preferred substrate for Phellinus igniarius is birch wood, the species was observed somewhat less frequently on alder and willow [6, 9]. However, sometimes along with birch, hornbeam, willow, and maple are considered as preferred substrates in the Urals, while aspen, willow, and rowan-trees are considered in Western Siberia [2]. False tinder fungus develops on live trees, fallen trunks, stumps [2,8].

Thus, along with the chaga both the true tinder fungus and false tinder fungus can be seen on the birch.

Inonotus obliquus (chaga) has an annual fruit body, widely spread, developing under the bark, reaching 3–4 meters long along the trunk and 10–50 cm wide (Fig. 1a), depending on the thickness of the affected trunk. In the fresh state,the fruit body is soft-skinned, later fibrous and cracking, in the dry state, it is hard and brittle, easily separated by parts from the substrate. The fruit body of the chaga under the bark is pale brown at first, as it matures, it destroys the bark of the tree and becomes dark brown, gradually drying out.

The development of the fruit body on the trunk of a living tree is usually preceded by the formation of sterile growths (forma sterilis (Van.) Nikol), called "chaga" (Fig. 1b). The size of the growths, depending on their age, can range from the size of a walnut to 40–50 cm in diameter. Chaga has a nodulous shape, very hard, woody body. The outer surface is uneven, cracking, black, and hard. The inner tissue is rusty-brown with white spots, somewhat softer than the outer surface. The weight of the nodule can range from a few grams to ten kilograms [2,6].

Fruit bodies of tinder fungus are perennial, sessile, of characteristic hoof-shaped. The fruit body is attached to the tree trunk only by its upper central part. Stipe is missing. The fruit body is large, in old fungi – up to 40 cm wide and up to 20 cm high, covered with a hard crust. The crust is matt, uneven, wavy, with concentric rollers, darker in the depressions, at first gently velvety-napy, then naked, almost smooth. Small cracks may occur on the surface. The color of the pileus varies from light gray to dark gray in old fungi, sometimes light beige tones. The flesh is dense, soft, resembles a cork, occasionally woody. The cut is velvety-suede. Color is brown, rich reddish-brown, less often a nutty shade. The hymenophore is tubular with fairly large rounded pores, light in color, in which basidiospores are formed. When pressed, it darkens. The pores are rounded with entire pubescent edges 3–4 per1 mm [3,6,8].

Fruit bodies of false tinder fungi are perennial (they can grow for decades), sessile, rounded


**FIG. 1.** *Inonotus obliquus: a – fruit body beneath the bark of birch, b – asexual form*

when young, then they get a characteristic hoof-like appearance, sometimes they are pillow-shaped or flat, prostrate. The fruit body is very firmly attached to the trunk of the affected tree, by this way false tinder fungus differs from the true tinder fungus. Stipe is missing. In fruit bodies, cracking is quite common, and the presence of radial cracks is characteristic. The tissue of the fruit body is very hard, woody, reddish-brown, rust-colored or chestnut-brown. The fruit body is medium-sized, reaches 20–26 cm wide in old fungus, covered with a hard crust. The crust is matt, uneven, with concentric rollers. The color of the pileus varies from dark gray to almost black or brownish-black in old fungi. The outer (growing) roller sometimes has a more noticeable brownish tint. The hymenophore is tubular, inside the same color as the tissue. Every year a new layer of hymenophore grows, and the old layers eventually overgrown with white hypha. Outside, the surface of the hymenophore is rusty brown to rich chestnut color. The pores are all-edged, rounded, often with grayish pubescence along the edge, 2–6 per 1 mm [2,6,8].

Based on the literature data and morphological characteristics of the raw materials we have harvested, we have compiled a comparative table of the distinctive characteristics of chaga and tinder fungi species (see table).

According to morphological characteristics, the chaga differs from the true and false tinder fungi by the absence of tubular hymenophore and the shape of the fungus. The nature of the outer surface and color of the chaga and true and false tinder fungi are close.

According to the generally accepted structure of the pharmacopoeial monograph, microscopic analysis is mandatory when determining the identity of raw materials, as well as performing microphotographs.

When studying the anatomical structure of the cross and longitudinal section of the chaga, we considered the outer, middle and inner layers, which differ in consistency and

*Table*

**DISTINCTIVE MORPHOLOGICAL FEATURES OF CHAGA AND TRUE AND FALSE TINDER FUNGI**

| Feature | Chaga | True tinder fungus | False tinder fungus |
|---|---|---|---|
| Form | nodulous | hoof-shaped | hoof-shaped |
| Outer surface color | Black | from light gray to dark gray, sometimes light beige tones | from dark grey to almost black or brownish black |
| Outer surface character | uneven, dehiscing | uneven, wavy, with concentric rollers, small cracks may take place | uneven, with concentric rollers, cracking in the form of radial cracks often takes place |
| Hymenophore | not available | hymenophore tubular, light color | hymenophore tubular, from rusty brown to rich chestnut color |

color. In the outer layer, a dense interweaving of hyphae is found, in the middle and in the inner layer, the hyphae are located more loosely (Fig. 2). There is no tubular hymenophore. The inner layer may contain holes of different diameters in the range from 73 to 240 microns, located randomly (Fig. 3).

In contrast to the chaga, the true and false tinder fungi have a tubular hymenophore, which on the longitudinal section has the form of longitudinal strands consisting of a dense intertwining of hyphae (Fig. 4). On the cross section of the hymenophore, there are ordered rounded pores of size from 128 to 148 microns (Fig.5). In the raw material harvested in the autumn period, basidia with basidiospores can be found in the hymenophore (Fig. 6).

When studying the chaga powder specimens, a branched cell mycelium is visible along the edges and on the surface of the pieces, spores are absent (Fig. 7). In the specimens of true tinder fungus and false tinder fungus, there are hyphae often with spores (Fig. 8).

Since the powder specimens have a particle size of 1–2 mm, it is not possible to detect the presence of hymenophore, so you should pay attention to the mycelium and the presence or absence of spores.

Thus, it is established that the common anatomical feature of chaga and true and false tinder fungi is the presence of branched cell mycelium. A distinctive feature of chaga is the absence of tubular hymenophore and spores, which are found in tinder fungus specimens.

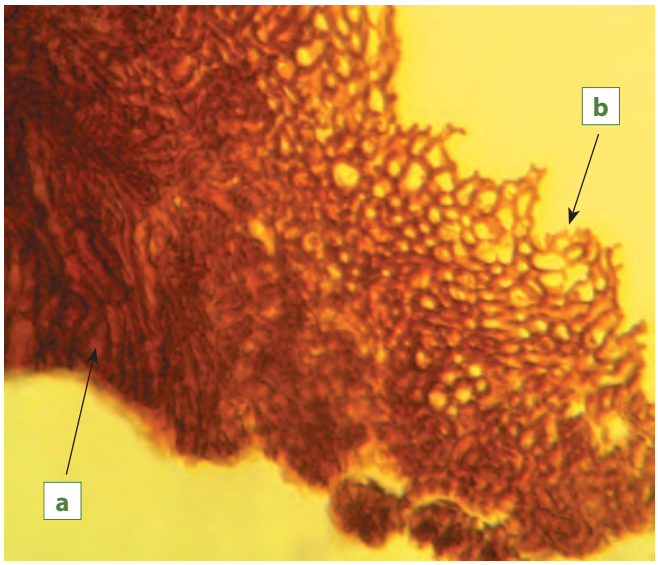


**FIG. 2.** *A fragment of a cross-section of the fungus (microphotography): a – outer layer (dense interweaving of hyphae); b – inner layer (hyphae are located loosely) (640x)*

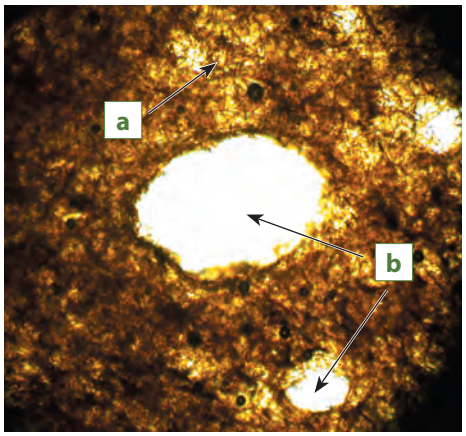


**FIG. 3.** *A fragment of a cross-section of the inner layer of chaga (microphotography): a -mycelium; b – holes (640x)*

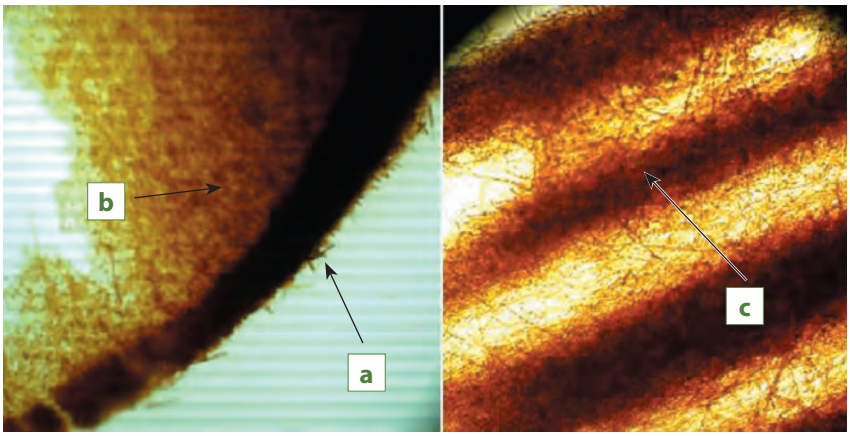


**FIG. 4.** *A fragment of a longitudinal section of tinder fungus (microphotography): a – an outer layer (dense interweaving of hyphae), b – a middle layer (hyphae are located loosely), c – a tubular hymenophore (160x)*

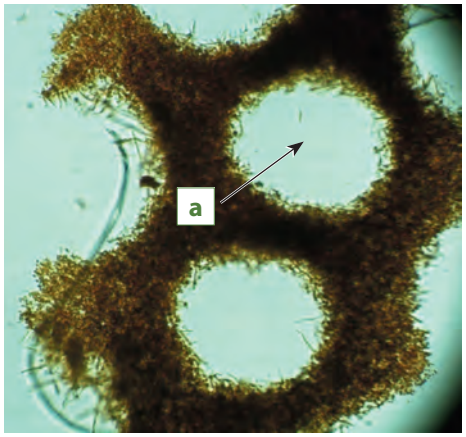


**FIG. 5.** *A fragment of a tinder fungus cross-section (microphotography): a – pores of the tubular hymenophore (160x)*

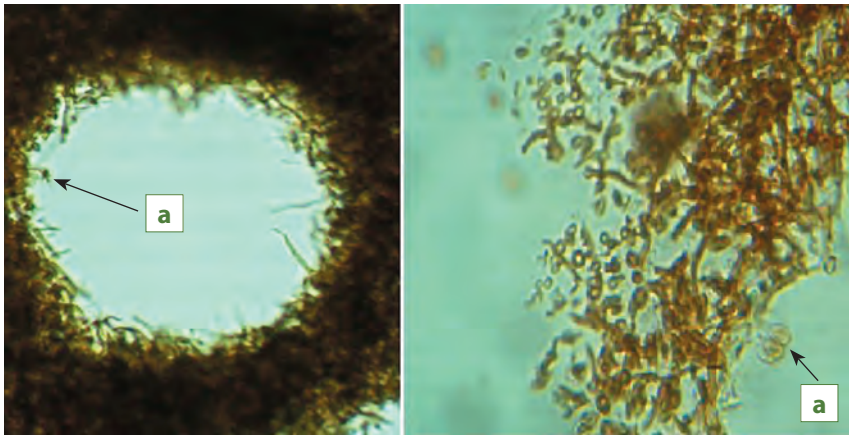


**FIG. 6.** *A fragment of a tinder fungus cross-section (microphotography): a – basidia with basidiospores (400x, 800x)*

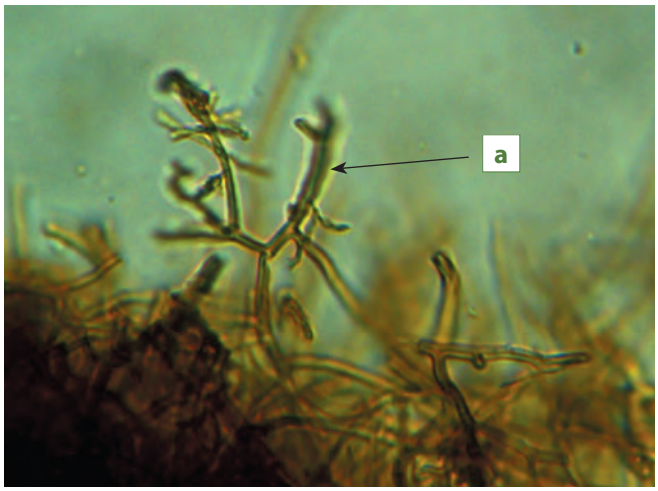


**FIG. 7.** *A fragment of the fungus powder (microphotography): a – branched cell mycelium (800x)*

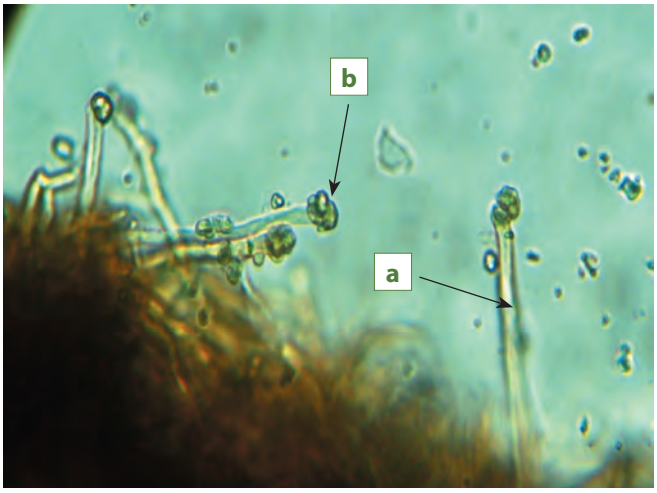


**FIG. 8.** *Fragment of tinder fungus powder (microphotography): a – hyphae, b – spores (640x; 160x)*

## CONCLUSION

1. Comparative morphological and anatomical study of chaga and true and false tinder fungi was carried out.

2. Morphological and anatomical diagnostic signs have been established to distinguish chaga from true and false tinder fungi

3. The study results are included in the State Pharmacopoeia, XIV edition of FS. 2. 5. 0103. 18 ''Chaga – Inonotus obliquus", section ''External features" and ''Microscopy".

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# STANDARDIZATION OF THE REMINERALIZING SPRAY

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*In the article the results of standardization of the remineralizing spray on parameters of "Authenticity" and "Quantitation of active ingredients", "pH level", "Viscosity" and "Microbiological purity". In the work the chemical and physicochemical methods, modified with account of specificity of dosage forms are used. All techniques, tested on lab series of spray, showed good reproducible results and are included into the normative documentation regulating technology and quality control of the remineralizing spray.*

**Keywords:** standardization, remineralization, spray, calcium chloride, potassium phosphate disubstituted, sodium fluoride

Promising for use in remineralizing therapy are polymer-based applicative dosage forms (DF) modeled using the phosphorus-calcium coefficient and the degree of supersaturation of Ca and P, developed taking into account the basic requirements of remineralizing therapy [9].

Currently, the Russian dental market is mainly represented by sprays for moistening the oral cavity in hyposialy and xerostomia. A small range of sprays based on high-molecular compounds for the prevention and treatment of caries indicates the feasibility of their development and wider implementation in dental practice [1].

Spray, having the advantages of aerosol packaging, does not have the disadvantages associated with the use of vials under high pressure and the use of propellants as a carrier gas, such as: relatively high cost, complexity, danger, the possibility of explosion of the cylinder on impact or storage in the improper temperature conditions, high flammability, fire and explosion hazard, inconvenience during transportation, the negative impact of refrigerants on the earth's ozone layer [6,7,10,12].

Features of control tests of the developed spray are associated with combination of active components that tend to interact with each other, and with the difficulty of separation due to their identical solubility in the media used in the analysis [2,3,11].

**The study purpose** was standardization of remineralizing spray.

## MATERIALS AND METHODS

As active pharmaceutical ingredients the follows are used: calcium chloride (FS 42-006-5675-04 P.003964.01, Karpov Chemical Plant

JSC, Mendeleevsk, Republic of Tatarstan, 200916, shelf life – 3 years), potassium phosphate disubstituted (FS 42-4297-79, Lenreactive JSC, Saint Petersburg, 101016, shelf life – 3 years), sodium fluoride (FS 2.2.0013.15, Reachim, Moscow, 201117, shelf life – 3 years); gel-forming agent: methylcellulose-35 (TU 2231-107-57684455-2003, UZPH JSC, Magnitogorsk, 221216, shelf life – 3 years); plasticizers: glycerine (FS 2.2.0006.15, Kupavna Reactive JSC, the town of Staraya Kupavna 082016, shelf life – 3 years), polyethylene oxide-400 (TU 2483-167-05757587-2000, VitaChim LLC, Kazan, 141116); purified water (FS 2.2.0020.18).

The study were concerned with five production samples of spray. The development of new medicinal products requires simple, selective, high-precision and objective methods of study of medicinal products, which allow to standardize them both at the time of receipt and during storage. Chemical and physico-chemical methods of analysis, modified to take into account the specifics of a dosage form were used in the work.

The research to prove the identity and quantify the active components in the spray, including the study of validation characteristics of the methods, was carried out at RTC "Farmtest" of the Ministry of Health of Russia.

Before the identity test, a sample was prepared by preparing an aqueous spray solution (solution A): 2 ml of the spray was dissolved in 10 ml of purified water.

The identity test was carried out according to the OFS.1.2.2.0001.15 "General identity reactions" [4,5].

To confirm the *calcium cation,* the reaction of ammonium oxalate precipitation with 4% solution was used, the resulting calcium oxalate is insoluble in acetic acid diluted to 30% and 10% ammonia solution, but it is soluble in dilute mineral acids. Determination of the *chloride ion* was carried out by the reaction of silver nitrate precipitation with 2% solution, the resulting low-dissociable silver chloride is soluble in 10% ammonia solution. To confirm the *potassium cation* in the spray, the reaction of tartaric acid precipitation with 20% solution was used, the resulting potassium hydrotartrate is soluble in dilute mineral acids and solutions of alkali metal hydroxides. Determination of the *phosphate ion* in the spray was carried out by the reaction of silver nitrate precipitation with 2% solution in a neutral medium, the resulting silver phosphate of yellow color is soluble in nitric acid diluted up to 16% and 10% ammonia solution. To confirm the *sodium cation* in the spray, a microcrystalloscopic reaction with potassium pyroantimonate solution was used, forming crystals of sodium pyroantimonate in the form of prisms. Determination of the *fluoride ion* was carried out by reaction with a zirconyl-alizarin complex based on its destruction due to the binding of the zirconium cation to form a stronger complex with fluoride ions, change in the color of the solution from red-purple to yellow was observed [2–5,11].

*The quantitative determination of calcium chloride* was carried out by complexometry, and 0.05 M sodium edetate solution was used as the titrant. To create the required pH value, an ammonia buffer solution was used. When testing the direct titration method, after adding an ammonia buffer solution to the analyzed solution, the abundant precipitation of calcium phosphate falls out, to avoid this reaction, a reverse titration method was proposed. A control experiment was conducted in parallel [3–5,11].

1 ml of 0.05 M sodium edetate solution corresponds to 0,01095 g $CaCl_2 \times 6H_2O$. Content of calcium chloride for 100 ml of spray is calculated by formula:

$$X = \frac{T \times (V_{K.O.} - V) \times K \times P}{a},$$

where X – content of calcium chloride in the dosage form, g; T – sodium edetate titre of 0,05 M solution for calcium chloride, g/ml; P – volume of the dosage form, ml; V – the volume of the

titrated solution used for titration, ml; $V_{к.о.}$ – the volume of the titrated solution used for titration of the control test, ml; K – coefficient of correction to the molarity of the titrated solution; a – weighted amount of the dosage form, ml.

*For the quantitative determination of potassium phosphate disubstituted* in the spray the acidimetric method, a variant of displacement, was used [11].

1 ml of 0,5 M hydrochloric acid solution corresponds to 0,1141 g $K_2HPO_4 \times 3H_2O$. The content of potassium phosphate disubstituted per 100 ml of spray is calculated by the formula:

$$X = \frac{T \times V \times K \times P}{a},$$

where X – content of potassium phosphate disubstituted in the dosage form, g; T – titre of 0,5 M hydrochloric acid solution for potassium phosphate disubstituted, g/ml; P – volume of the dosage form, ml; V – the volume of the titrated solution used for titration, ml; K – coefficient of correction to the molarity of the titrated solution; a – weighted amount of the dosage form, ml.

*For the quantitative determination of sodium fluoride,* a photoelectrocolorimetric method was used (KFC-3 photometer, ZOMZ, Russia). The method is based on reaction of destruction of the zirconyl alizarine complex with fluoride ion. The intensity of staining of the solution as a result of the reaction decreases, so in this case, the inverse dependence of the optical density on the concentration of fluoride ions is observed. The optical density of the test solution is measured at a wavelength of 520 nm, in a cuvette with layer thickness of 10 mm, the reference solution is purified water. In parallel, the optical density of the work standard solution is measured [2].

The content of sodium fluoride in the spray is calculated using the formula:

$$X = \frac{(1 - A_{AH}) \times a_{CT} \times V_{AHMK} \times P}{(1 - A_{CT}) \times a_{AH} \times V_{CTMK}},$$

where X – content of sodium fluoride in the dosage form, g; $A_{AH}$ – optical density of the test solution; $A_{CT}$ – optical density of the work standard solution; P – volume of the dosage form, ml; $a_{AH}$ – weighted amount of the dosage form, ml; $a_{CT}$ – weighted amount of the work standard (content of sodium fluoride in 2 ml of 0.02% standard solution), g; $V_{AHMK}$ – volume of the measuring flask for dilution of the spray weighted amount, ml; $V_{CTMK}$ – volume of the measuring flask for dilution of the work standard weighted amount, ml.

*The viscosity of the remineralizing spray* was determined using Ostwald capillary viscometer by the method of SP XIV ed. OFS.1.2.1.0015.15 "Viscosity" [4,5].

*The pH of the spray* was determined using a potentiometric method according to the OFS.1.2.1.0004.15 "Ionometry" with I-500 ionometric transducer (Aquilon, Russia) [4,5].

## RESULTS AND DISCUSSION

In appearance the spray is an opalescent homogeneous viscoplastic solution and has the following composition:

| | |
|---|---|
| Calcium chloride | 0.546 ($Ca^{2+}$ – 0.1 g/ion) |
| Potassium phosphate disubstituted | 0.951 ($HPO_4^{2-}$ – 0.4 g/ion) |
| Sodium fluoride | 0.014 ($F^-$ – 0.006 g/ion) |
| Methylcellulose | (35) 2.5 |
| Glycerine | 3.0 |
| Polyethylene oxide | (400) 2.5 |
| Purified water | up to 100.0 |

The spray was standardized according to the following parameters: "Identity", "Quantitative determination of active ingredients" – including validation of the proposed methods, "pH Level", "Viscosity" and "Microbiological purity" [4,5,8].

Validation of identity testing methods and quantitative determination of mineralizing

drugs was performed in accordance with OFS. 1. 1. 0012. 15 "Validation of analytical methods" [8].

The specificity of the methods was studied on model mixtures with alternating components from the declared composition.

It is found that a linear relationship is observed between the values of analytical signals and the content of the determined components in the spray in the range from 70 to 130% of the declared amount (the analytical area of the method). The correlation coefficient of the regression graph R was 0.999 for sodium fluoride, calcium chloride, and potassium phosphate disubstituted.

The correctness of the methods is confirmed by setting the "found:entered" (Zi) relationship. It is in the range of 97 to 101% for calcium chloride, 93% to 111% for potassium phosphate disubstituted, and 98% to 102% for sodium fluoride. The deviation of $Z^-$ from 100% does not exceed the confidence interval $\delta\% = |-0.48095| \leq 0.541053$, the systematic error is statistically indistinguishable from zero, which shows the satisfactory correctness of the methods.

Intra-laboratory (intermediate) precision of the methods was proved by calculating confidence intervals, which were 0.526±0.005 g (0.95%) for calcium chloride, 0.936±0.017 g (1.86%) for potassium phosphate, and 0.0139±0.0002 g (1.44%) for sodium fluoride. Based on the obtained value of the standard deviation and confidence interval, we can conclude that the precision of the methods under study is influenced by intra-laboratory variations.

Thus, the validated methods are specific, have satisfactory precision, linearity, correctness, and are used in the standardization of the spray to determine the quantitative content of calcium chloride, potassium phosphate disubstituted, and sodium fluoride. The results of quantitative determination of active ingredients are shown in Table 1.

*Table 1*

**THE RESULTS OF QUANTITATIVE DETERMINATION OF ACTIVE INGREDIENTS IN SPRAY**

| Active ingredient | Series | Metrological characteristics | | | | | |
|---|---|---|---|---|---|---|---|
| | | $X$, r | $\bar{X}$, r | S, r | $\Delta\bar{X}$ | ε, % | $\bar{\varepsilon}$, % |
| Calcium chloride | 1 | 0.550 | 0.546 | 0.0035 | 0.00435 | 1.661 | 0.80 |
| | 2 | 0.548 | | | | 1.657 | |
| | 3 | 0.541 | | | | 1.650 | |
| | 4 | 0.544 | | | | 1.645 | |
| | 5 | 0.546 | | | | 1.637 | |
| Potassium phosphate disubstituted | 1 | 0.981 | 0.967 | 0.0101 | 0.0126 | 2.86 | 1.30 |
| | 2 | 0.967 | | | | 2.90 | |
| | 3 | 0.953 | | | | 2.95 | |
| | 4 | 0.971 | | | | 2.89 | |
| | 5 | 0.965 | | | | 2.91 | |
| Sodium fluoride | 1 | 0.0141 | 0.0141 | 0.00016 | 0.0002 | 3.15 | 1.42 |
| | 2 | 0.0142 | | | | 3.13 | |
| | 3 | 0.0139 | | | | 3.20 | |
| | 4 | 0.0143 | | | | 3.11 | |
| | 5 | 0.0140 | | | | 3.18 | |

*Table 2*

## RESULTS OF STANDARDIZATION OF THE REMINERALIZING SPRAY

| Series № | Description | Identity | | | | | | Quantitative determination | | | pH potentiometric method | Capillary viscosity according to Ostwald viscometer under the SP method | Microbial purity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $Ca^{2+}$ with 4% ammonium oxalate solution | $Cl^-$ with 2% silver nitrate solution in the presence of 16% diluted nitric acid. | $K^+$ with 20% tartaric acid solution | $HPO_4^{2-}$ with 2% silver nitrate solution at pH=7.0 | $Na^+$ with potassium pyroantimonate solution | $F^-$ with zirconyl-alizarin complex | $CaCl_2$ complexometry | $K_2HPO_4$ Acidimetric method | NaF photoelectrocolorimetry method | | | |
| **Standard requirements** | | | | | | | | | | | | | |
| | opalescent homogeneous viscoplastic solution | white precipitate, HP in 30% acetic acid, diluted and 10% ammonia solution, P in diluted mineral acids | white cheesy precipitate, HP in 16% diluted nitric acid, and P in 10% ammonia solution | white crystalline precipitate P in diluted mineral acids and alkali metal hydroxides solutions | yellow precipitate, P in 16% diluted nitric acid and 10% ammonia solution | crystals intheform of prisms | changing the color of the solution from red-purple to yellow | 0.526±0.037 | 0.933±0.056 | 0.0139±0.0028 | 6.5–7.5 | 0.67–0.71 | Not higher than $10^2$ GFU per 1,0 g |
| 1 | conforming | conforming | conforming | conforming | conforming | conforming | conforming | 0.5260±0.0071 | 0.933±0.018 | 0.0139±0.0003 | 6.75±0.05 | 0.7030±0.020 | <100 |
| 2 | conforming | conforming | conforming | conforming | conforming | conforming | conforming | 0.5410±0.0066 | 0.961±0.015 | 0.0126±0.0002 | 6.86±0.05 | 0.7000±0.020 | <100 |
| 3 | conforming | conforming | conforming | conforming | conforming | conforming | conforming | 0.5460±0.0073 | 0.956±0.016 | 0.0140±0.0003 | 6.90±0.05 | 0.7012±0.020 | <100 |
| 4 | conforming | conforming | conforming | conforming | conforming | conforming | conforming | 0.5600±0.0088 | 0.951±0.018 | 0.0154±0.0003 | 6.80±0.05 | 0.7032±0.020 | <100 |
| 5 | conforming | conforming | conforming | conforming | conforming | conforming | conforming | 0.5650±0.0088 | 0.905±0.02 | 0.0165±0.0003 | 6.80±0.05 | 0.7030±0.020 | <100 |

The relative error of the average result of determining the content of calcium chloride in the spray by the complexometric method is 0.80%, potassium phosphate disubstituted in the spray by the acidimetric method – 1.30%, sodium fluoride in the spray by the photoelectrocolorimetric method – 1.42%, which indicates a good reproducibility of the proposed methods.

The pH of the developed spray was 6.75–6.90±0.05, which is within the acceptable limits for dental sprays (6.5–7.5).

The viscosity value was 0.6998–0.7032±0.02.

As of parameter "Microbiological purity" the spray meets the requirements of the OFS.1.2.4.0002.15 and can be used as a local medication.

The results of evaluating the quality of the spray in five series for appearance, qualitative and quantitative analysis, pH, viscosity and microbiological purity are presented in table. 2. The data obtained indicate that spray meets the standard requirements.

## CONCLUSION

A comprehensive standardization of the spray on 5 series in the study group was carried out according to the parameters "Identity", "Quantitative determination of active ingredients", "pH Level", "Viscosity" and "Microbiological purity", including the study of validation characteristics of testing methods for identity and quantitative determination of active ingredients.

The established parameters can be the criteria for evaluating the quality of the spray during stage-by-stage control in the production process and quality control of the final product and are the basis for the developed draft pharmacopoeial monograph for the spray.

The developed remineralizing spray can be recommended for professional oral hygiene for individual and medical use in cases of high intensity of caries, the presence of general and local cariesogenic factors (in particular, in orthodontic patients and patients with xerostomia who are subject to radiation therapy), the presence of foci of enamel demineralization and dental hyperesthesia.

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# FEATURES OF ACCUMULATION OF FLAVONOIDS BY QUINQUELOBATE MOTHERWORT (LEONURUS QUINQUELOBATUS) HERB COLLECTED IN VARIOUS URBAN BIOCENOSES AND AGROBIOCENOSES OF THE VORONEZH REGION

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*An important and little-studied aspect of the impact of human economic activity on medicinal plants is that in response to increased anthropogenic load, additional synthesis of secondary metabolites especially flavonoids, is induced, which play an important role in the adaptation of plants to changing conditions. As part of the study, the content of flavonoids in terms of rutin was determined in 51 samples of quinquelobate motherwort herb collected in various urban and agrobiogeocenoses of the Voronezh region which differ in anthropogenic impact. It has been revealed that in quinquelobate motherwort herb collected in agrobiogeocenoses, the content of flavonoids is on average 1.5 times higher than in samples collected in natural biocenoses of protected areas, and in raw materials collected in a number of urbobiogeocenoses that is 2–3.5 times higher than in samples from the controlled protected areas. This fact can be explained by the biochemical adaptation of the plant to significant oxidative stresses, in response to which the synthesis of polyphenol substances, the main representatives of which are flavonoids, is induced. At the same time, near some production enterprises and along roads with high traffic intensity, we selected samples with reduced content of flavonoids in terms of rutin in comparison with other samples of raw materials. This suggests that in case of excessive toxic influence of pollutants it is also possible to suppress the antioxidant system of plants.*

**Keywords:** Voronezh region, quinquelobate motherwort (Leonurus quinquelobatus), flavonoids, rutin

Currently, more than 6.5 thousand medicinal products prepared from medicinal plant raw materials are used in medical practice in Russia. The growing interest in herbal medicines is due to the fact that in the case of rational use, they combine a good therapeutic effect with relative harmlessness. The main part of preparations

of medicinal plant raw materials is traditionally concentrated in the European part of Russia, and in its most populated and industrially developed regions, in particular in the Central Chernozem region. At the same time, most of the exploited resources of wild medicinal plants are located in the zone of active human economic activity, on transport-accessible territories [1,2].

However, due to the growth of cities, sharp increase in the number of vehicles, and the expansion of production areas, the probability of collecting medicinal plant raw materials by the population near sources of pollutants emission increases significantly. In this regard, from a practical point of view, it is necessary to find out the influence of anthropogenic pollution on the chemical composition of medicinal plants, determine the possibility of their use for medical purposes, and identify the most environmentally unfavorable harvesting areas [3].

One of the synanthropic species, the raw material of which is harvested from wild specimens, is the quinquelobate motherwort (Leonurus quinquelobatus Gilib.) that is a perennial herb which is widely used in medicine and pharmacy as a sedative, hypotensive, antispasmodic, hemostatic, diuretic. This widespread use is due to the rich chemical composition of the quinquelobate motherwort herb, which is based on flavonoids, iridoids, alkaloids (up to 0.4%), essential oil (up to 0.9%), tannins (up to 2.5%), bitterness, vitamin C, carotene, macro – and microelements [4].

An important and little-studied aspect of the impact of human economic activity on medicinal plants is that in response to the increased anthropogenic load, the additional synthesis of secondary metabolites is induced, which play an important role in adaptation of plants to changing conditions. It is well known that amino acids, organic acids and peptides can serve as ligands for chelating toxic substances, but it has been shown that some secondary metabolites, especially phenolic compounds, can also serve as chelators and participate in detoxification of pollutants by plants. The main group of low-molecular-weight phenolic compounds consists of flavonoids. Increasing of their content is one of the non-specific reactions to environmental stress [5–7].

**Study purpose** – study of the accumulation of flavonoids in the quinquelobate motherwort herb collected in various urban and agrobiogeocenoses of the Voronezh region from the point of view of anthropogenic impact.

## MATERIALS AND METHODS

To conduct the study in the Voronezh region, points of sampling of soil and medicinal plant raw materials were selected. The choice of the studied areas is determined by the nature of the specific anthropogenic impact on it (Fig. 1, Table 1): chemical enterprises of Voronezh Giprokauchuk LLC, Minudobreniya JSC, Bormash LLC, VOGRES thermal power plant (TPP), Novovoronezh nuclear power plant (NPP), airport, city street (Leningradskaya street), high-voltage power transmission lines (HV PTL), Voronezh reservoir, cities with developed light industry (Kalach, Borisoglebsk), areas of active agricultural activity with the introduction of large amounts of fertilizers (Liskinsky, Olkhovatsky, Podgorensky, Petropavlovsky, Gribanovsky, Khokholsky, Novokhopersky, Repyevsky, Vorobyevsky, Paninsky, Verkhnekhavsky, Rossoshansky districts), zones that were exposed to radionuclide contamination as a result of the Chernobyl NPP accident (Nizhnedevitsky, Ostrogozhsky, Semiluksky districts) and as a comparison – a protected zone (Voronezh biosphere reserve, Khopersk state nature reserve). In addition, we have paid great attention to the issue of collecting medicinal plant raw materials near roads and railways. Actual reference books and manuals on medicinal plants are not unanimous:

somewhere the prohibition zone is indicated as 100 m, somewhere 200 m, and somewhere 300 m from the road. There are no instructions about railway transport at all. Therefore, we decided to analyze this problem in order to find out the proper prohibition zone for collecting medicinal plants near highways. For this study, the Voronezh region was also suitable as well as possible: it presents different natural zones – the forest zone (Ramonsky district), forest – steppe (Anninsky district), steppe (Pavlovsky district), there are major traffic interchanges – M4 "Don" highway, A144 Kursk-Saratov, as well as the non-high-speed road (Bogucharsky district) and the railway (Ramonsky district) were considered.

Natural biogeocenoses were selected for collecting samples. Each analyzed point is subject to the determining influence of one object of economic use, and cross-influence on other studied areas is practically excluded, since all territories are located at a significant distance from each other.

Determination of the amount of flavonoids in terms of rutin in selected samples of quinquelobate motherwort herb was carried out according to the standard Pharmacopoeia method [8] on the SF-2000 spectrophotometer. Each determination was performed three times. Data obtained in the course of studies of the upper layers of soils and medicinal plant raw materials for determination of the content of radionuclides were statistically processed using Microsoft Excel.

## RESULTS AND DISCUSSION

The determined parameters of the content of biologically active substances in quinquelobate motherwort herb are shown in the table.

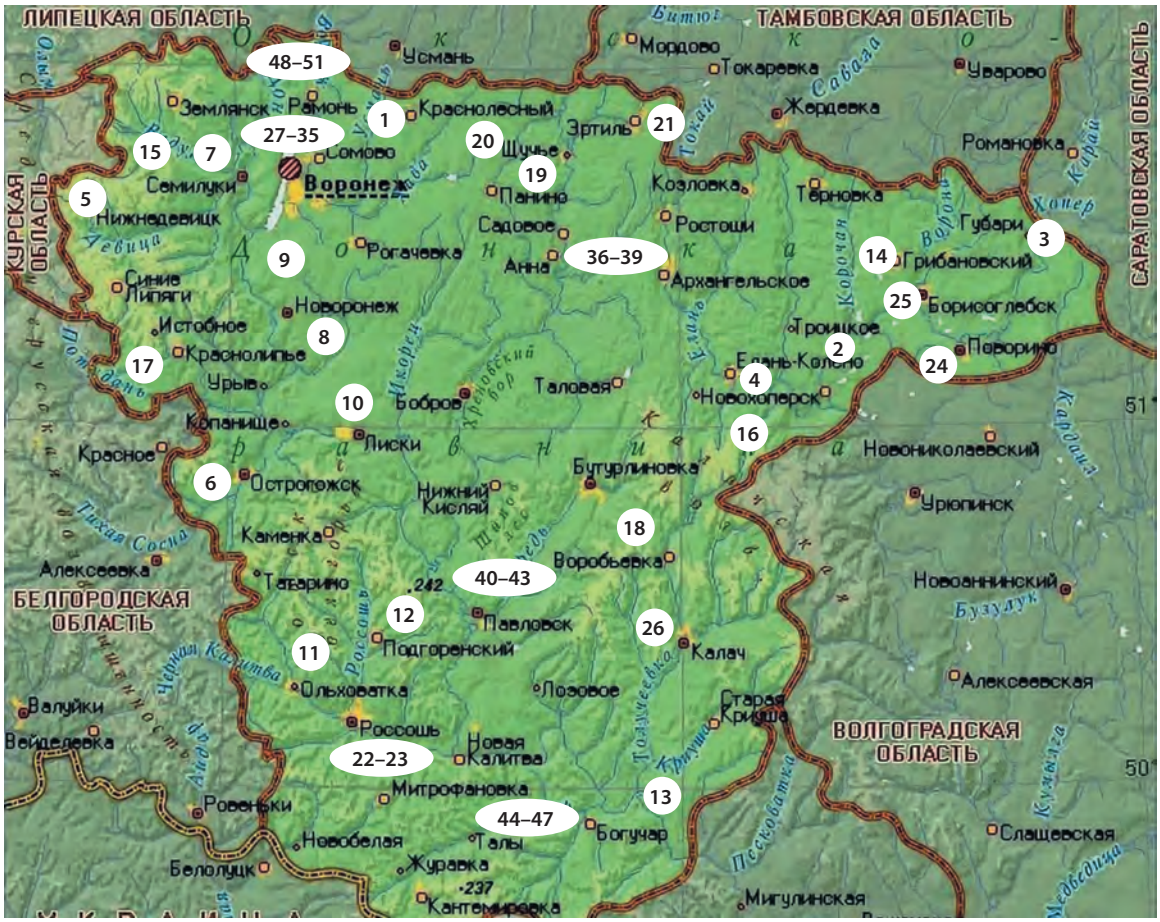


**FIG.** *Map of sampling of medicinal plant raw materials (interpretation – in the table)*

*Table*

**CONTENT OF BIOLOGICALLY ACTIVE SUBSTANCES IN SAMPLES OF QUINQUELOBATE MOTHERWORT HERB (*LEONURUS QUINQUELOBATUS* GILIB.)**

| № | Territory for collecting medicinal plant raw materials | Content of the sum of flavonoids in terms of rutin, % |
|---|---|---|
| 1. | Voronezh state natural biosphere reserve | 0,35±0,02 |
| 2. | Khoper nature reserve | 0,37±0,03 |
| 3. | Borisoglebsk district | 0,48±0,05 |
| 4. | the settlement of Elan-Koleno | 0,41±0,04 |
| 5. | the settlement of Nizhnedevitsk | 0,89±0,04 |
| 6. | the town of Ostrogozhsk | 0,95±0,05 |
| 7. | the town of Semiluky | 0,78±0,03 |
| 8. | the town of Novovoronezh | 0,85±0,05 |
| 9. | High-voltage power transmission line (Novovoronezh municipal district) | 0,84±0,03 |
| 10. | Lisky District | 0,44±0,06 |
| 11. | Olkhovatskiy District | 0,98±0,04 |
| 12. | Podgorensk District | 0,42±0,06 |
| 13. | Petropavlovsk District | 0,53±0,04 |
| 14. | Gribanovsk District | 0,65±0,05 |
| 15. | Khokholsk District | 0,50±0,06 |
| 16. | Novokhopersk District | 0,77±0,03 |
| 17. | Repjevsk District | 0,44±0,03 |
| 18. | Vorobievsk District | 0,42±0,05 |
| 19. | Paninsk District | 0,68±0,04 |
| 20. | Verkhnekhavsk District | 0,74±0,02 |
| 21. | Ertilsk District | 0,55±0,06 |
| 22. | Rossosh District | 0,51±0,04 |
| 23. | Near Minudobreniya JSC (Rossosh) | 1,11±0,03 |
| 24. | Near Bormash LLC (the town of Povorino) | 0,30±0,03 |
| 25. | the town of Borisoglebsk | 0,90±0,04 |

*Table end*

| № | Territory for collecting medicinal plant raw materials | Content of the sum of flavonoids in terms of rutin, % |
|---|---|---|
| 26. | the town of Kalach | 1,08±0,05 |
| 27. | Near VOGRES TPP (Voronezh) | 0,75±0,02 |
| 28. | Near SIBUR LLC (Voronezh) | 0,93±0,06 |
| 29. | Along the reservoir (Voronezh) | 0,67±0,05 |
| 30. | Near the Peter I airport | 0,67±0,02 |
| 31. | Street in Voronezh (Dimitrov street) | 1,04±0,03 |
| 32. | Along the M4 highway (Ramonsky District) | 0,57±0,06 |
| 33. | 100 m from the M4 (Ramonsky District) | 0,66±0,05 |
| 34. | 200 m from the M4 (Ramonsky District) | 0,87±0,05 |
| 35. | 300 m from the M4 (Ramonsky District) | 1,17±0,02 |
| 36. | Along the A144 road (Anninsk District) | 0,38±0,03 |
| 37. | 100 m from A144 (Anninsk District) | 0,64±0,04 |
| 38. | 200 m from A144 (Anninsk District) | 0,67±0,05 |
| 39. | 300 m from A144 (Anninsk District) | 0,80±0,03 |
| 40. | Along the M4 highway (Pavlovsk District) | 0,28±0,02 |
| 41. | 100 m from M4 (Pavlovsk District) | 0,51±0,04 |
| 42. | 200 m from M4 (Pavlovsk District) | 0,76±0,02 |
| 43. | 300 m from M4 (Pavlovsk District) | 0,84±0,04 |
| 44. | Along the low-speed road (Bogucharsky District) | 1,38±0,03 |
| 45. | 100 m from the low-speed road (Bogucharsky District) | 0,83±0,02 |
| 46. | 200 m from the low-speed road (Bogucharsky District) | 0,84±0,05 |
| 47. | 300 m from the low-speed road (Bogucharsky District) | 0,57±0,04 |
| 48. | Along the railway | 1,28±0,02 |
| 49. | 100 m from the railway | 0,83±0,05 |
| 50. | 200 m from the railway | 0,82±0,03 |
| 51. | 300 m from the railway | 0,64±0,04 |
| Value for API [8] | | At least 0,2 |

All selected medicinal plant raw materials of quinquelobate motherwort herb according to the results of our studies are recognized as benign in relation to flavonoid content in terms of rutin.

Samples collected in control territories contain this group of biologically active substances on average 2 times more than the lower numerical value specified in the Pharmacopoeial monograph.

In agrocenoses of the Voronezh region the content of flavonoids in terms of rutin in the quinquelobate motherwort herb varies in the range of 0.42% to 0.98% (in the Lisky, Olkhovatskiy, Podgorensk, Petropavlovsk, Gribanovsk, the Khokholsk, Novokhopersk, Repjevsk, Vorobievsk, Paninsk, Verkhnekhavsk, Rossosh Districts), but the average is 0.59%, which is almost 3 values specified by the statutory regulatory documentation and 1.5 times higher than the content of flavonoids in the samples from the control conservation areas. Collection of raw materials in these areas was carried out in places of active agricultural activity. They are characterized by the introduction of a large amount of fertilizers, under the influence of which, apparently, phenylalanine amiacliase is activated, which is a key enzyme in the phenylpropanoid pathway of flavonoid biosynthesis [5,9,10].

Samples of quinquelobate motherwort herb collected in the conditions of urban-biocenoses of the Voronezh region are characterized by highly varying results. Thus, in samples collected near Minudobreniya JSC in the Rossosh District, near SIBUR LLC in the city of Voronezh, on the streets of Borisoglebsk, Kalach, Voronezh, along the low-speed highway and along the railway, as well as at distance of 300 m from highways with high traffic intensity (M4 in Ramonsk and Pavlovsk Districts and A144 in Anninsk District), there are very high concentrations of flavonoids in terms of rutin (from 0.80% to 1.38%), which is 2–3.5 times higher than the content of flavonoids in samples of control protected areas. This fact can be explained by the biochemical adaptation of the plant to significant oxidative stress, in response to which there is an induction of synthesis of polyphenolic substances, the main representatives of which are flavonoids. Flavonoids make a corresponding contribution to the mechanisms of higher plants' response to a variety of abiotic stresses. In addition to the long-known function of shielding from damage by short-wave solar radiation, flavonoids in stressed plants play a key antioxidant function by inhibiting the generation and reduction of reactive oxygen intermediateы as soon as they are formed. These properties are mainly inherent in flavones and flavonols, especially quercetin derivatives, in particular6 rutin. At the biochemical level, the results can be explained by the fact that the key enzyme of flavonoid synthesis – phenylalanine-ammonia-lyase – has a pronounced stress-inducibility [5,7,9,10]. Therefore, the synthesis of phenolic compounds is always enhanced in conditions of anthropogenic impact and pollution of the habitat of the species with toxic substances which are stress for the plant organism.

However, it is possible to distinguish samples also collected under conditions of significant anthropogenic impact, characterized by significantly lower content of flavonoid compounds, for example, near the machine-building enterprise Bormash LLC in the Povorinsk District, along the M4 highway in the Pavlovsk District, along the A144 highway in the Anninsk district, where the content of flavonoids in terms of rutin is noted at the level of 0.28–0.38%. At the same time, at distance of 100 m from the highway, there is sharp, almost twofold increase in the content of flavonoids. Thus, this example implements the ecological law of interaction of factors, which states that the optimal zone and tolerance limits of organisms can be shifted in relation to any environmental factor

depending on the strength and combination of other factors acting simultaneously [1]. That is, for example, near major roads and industrial enterprises, the habitat conditions of the species differ not only in increased concentrations of toxic substances in the soil, but also in high gas content, dustiness of territories, which also affects both the physiological characteristics of the plant organism and the strength of the toxic effects of pollutants. In this case, it seems that instead of stimulation, the enzyme system of flavonol biosynthesis is suppressed. Thus, it is likely that the excessive toxic effect of pollutants may also inhibit the antioxidant system of plants, which is manifested by reduced content of flavonoids.

## CONCLUSION

1. More than 50 samples of quinquelobate motherwort herb collected in various urban and agrobiogeocenoses of the Voronezh region were analyzed, where the content of flavonoids in terms of rutin was determined. All selected medicinal plant raw materials according to the results of our study are considered to be of good quality for this factor.

2. It was found that the content of flavonoids in quinquelobate motherwort herb collected in agrobiogeocenoses is 1.5 times higher on average than in samples collected in natural biocenoses of protected areas. The raw material of quinquelobate motherwort herb collected in a number of urban-biogeocenoses of the Voronezh region also has a significant content of flavonoids in terms of rutin, which is 2–3,5 times higher than the content of this group of biologically active substances in samples of control protected areas. This can be explained by the fact that the key enzyme of flavonoid synthesis – phenylalanine-ammonia-lyase – has a pronounced stress-inducibility. Therefore, the synthesis of antioxidant substances, among which flavonoids play a crucial role, is always enhanced under conditions of toxic stress.

3. The study has revealed that the anthropogenic impact, characterized by a significant release of various toxic substances into the environment, is not always accompanied by the induction of flavonoid synthesis in the plant body. Thus, near some manufacturing plants and along highways with high traffic intensity, we selected samples with reduced content of flavonoids in terms of rutin in comparison with other raw materials. This suggests that the excessive toxic effect of pollutants may also inhibit the antioxidant system of plants.

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# DETERMINATION OF ISOFLAVONE CONTENT IN DRY HERBAL EXTRACT OF MEADOW CLOVER (TRIFOLIUM PRATENSE L.) BY HPLC

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*Meadow clover, also known as red clover (Trifolium pratense L.), is a natural source of phytoestrogenic isoflavonoids. The purpose of the study is to study the qualitative and quantitative content of individual isoflavones in the dry herbal extract of meadow clover by HPLC in order to expand the range of medicines based on this phytosubstance. In the dry herbal extract of meadow clover, daidzein, genistein, formononetin, prunetin and biochanin A were identified, their content was determined as 0.36±0.01%, 0.84±0.01%, 3.02±0.05%, 0.22±0.01% and 1.62±0.03% respectively. The established significant content in the dry extract of formononetin and biochanin A allows us to consider the dry herbal extract of meadow clover as a promising substance for development of medicines.*

**Keywords:** isoflavone content, dry extract, meadow clover herb (Trifolium pratense L.), HPLC, identification, quantitative determination

Flavonoids are one of the most widely distributed classes of polyphenolic substances in nature. Numerous studies show that preparations based on flavonoids are highly effective antitumor agents, have antioxidant properties, and reduce the risk of cardiovascular diseases.

Plants of the clover species (Trifolium L.), which belong to the legume family (Fabaceae) are of particular interest as sources of flavonoid compounds. In particular, representatives of meadow clover species (Trifolium pratense L.) rich in flavonoids and isoflavonoids of various groups attract interest [1].

In connection with the above, it is relevant to study the qualitative and quantitative determination of individual isoflavones in a dry extract of meadow clover herb by HPLC in order to expand the range of medicinal products based on this phytosubstantiation.

## MATERIALS AND METHODS

To obtain a dry extract, the meadow clover herb produced by Company Horst LLC, Barnaul and purchased from a pharmacy chain, was used.

The technology for obtaining a dry extract of meadow clover herb was developed earlier [2]. Content of individual substances of isoflavone groups was determined on the base of the Department of Analytical Chemistry of Saint Petersburg State Chemical and Pharmaceutical University by HPLC method using a HPLC liquid chromatograph Shimadzu SCL – 10A (Japan), software – "MultiChrom" for Windows.

Chromatographic conditions: Selectra C18 column (250×4.6 mm) filled with 5 μm sorbent particles; mobile phase A is 0.1% aqueous solution of formic acid, mobile phase B is acetonitrile; gradient elution mode: linear change of mobile phase B from 20% to 70% within 40 minutes. The speed of the mobile phase is 1 ml/min. Volume of the injected sample is 20 μl. Detection was performed using a UV detector at a wavelength of 270 nm. The temperature of the column was 40°C.

Identification of individual substances was performed by comparing the retention times of peaks obtained on chromatograms of the tested and standard solutions. The content of isoflavones was determined using the standard method.

Standard solutions of daidzein (CAS #486-66-8, 97.8%, HWI group), genistein (CAS #446-72-0, 99%, SIAL), formononetin (CAS #485-72-3, 98.6%, Sigma-Aldrich), prunetin (CAS #552-59-0, 98.2%, Sigma), biochanin A (CAS #491-80-5, 97.4%, Sigma). The initial standard solutions of isoflavones were prepared in 80% ethyl alcohol. Reference Standards of daidzein, genistein, formononetin, prunetin and biochanin A are weighed by 0.005 g each (exact weight), placed in a measuring flask with capacity of 5 ml and brought to the mark with 80% ethyl alcohol, the solutions were mixed. At the same time, solutions with the content of standard substances of 0.001 g/ml (initial solutions) were obtained. Standard solutions of isoflavones were prepared immediately before analysis by diluting the initial solutions with 80% ethyl alcohol up to concentrations of 0.0002 g/ml and stored in a dark place at 4°C during study.

*Preparation of a sample of the test sample.* 0.125 g of dry extract (exact weight) was placed in a measuring flask with capacity of 25 ml, then, 10 ml of 40% ethyl alcohol was added and the flask was placed in an ultrasonic bath at 35°C for 10 minutes. Then the volume of the solution was brought up to the mark with 40% ethyl alcohol, after that, the solution was mixed and filtered (solution A). From solution A, 5 ml was transferred to a 25 ml measuring flask, brought to the mark with 40% ethyl alcohol and thoroughly mixed (the test solution).

Solutions were passed through a filter with a pore size of 0.45 microns and chromatograms were recorded.

The content of individual isoflavones in the dry extract in terms of reference standard in percents (X) was calculated using the formula:

$$X = \frac{C_{st} \times S_x \times 25 \times 25 \times P \times 100}{5 \times m \times S_{st} \times (100 - W)},$$

where $C_{st}$ – reference standard concentration, g/ml;

$S_{st}$ – area of a standard peak, mV/c;

$S_x$ – area of a peak of substance in a dry extract, mV/c;

P – content of a substance in a reference standard, %;

m – weight of a dry extract, g;

W – weight loss during drying of dry extract, %.

## RESULTS AND DISCUSSION

The proposed HPLC technique was applied to identify and quantify five isoflavones in a

dry extract of meadow clover herb. In Figures 1 and 2 the typical chromatograms of reference standards of isoflavones are shown, and the table shows the results of their identification and determination.

The retention time of the reference standards of daidzein, genistein, formononetin, prunetin, and biochanin A in the conditional analysis was 14.56 minutes, 19.45 minutes, 23.47 minutes, 29.10 minutes, and 29.34 minutes, respectively (Fig. 1). Based on the retention time of standard references in the studied sample of a dry meadow clover extract, the following were identified: daidzein (14.59 min.), genistein (19.47 min.), formonetin (23.46 min.), prunetin (29.11 min.) and biochanin A (29.34 min.) (Fig. 2). As a result of the study, the content of individual isoflavones in the dry extract of meadow clover was determined, which was: daidzein (0.36±0.01%), genistein (0.84±0.01%), formononetin (3.02±0.05%), prunetin (0.22±0.01%) and biochanin A (1.62±0.03%) (see table). The proposed method

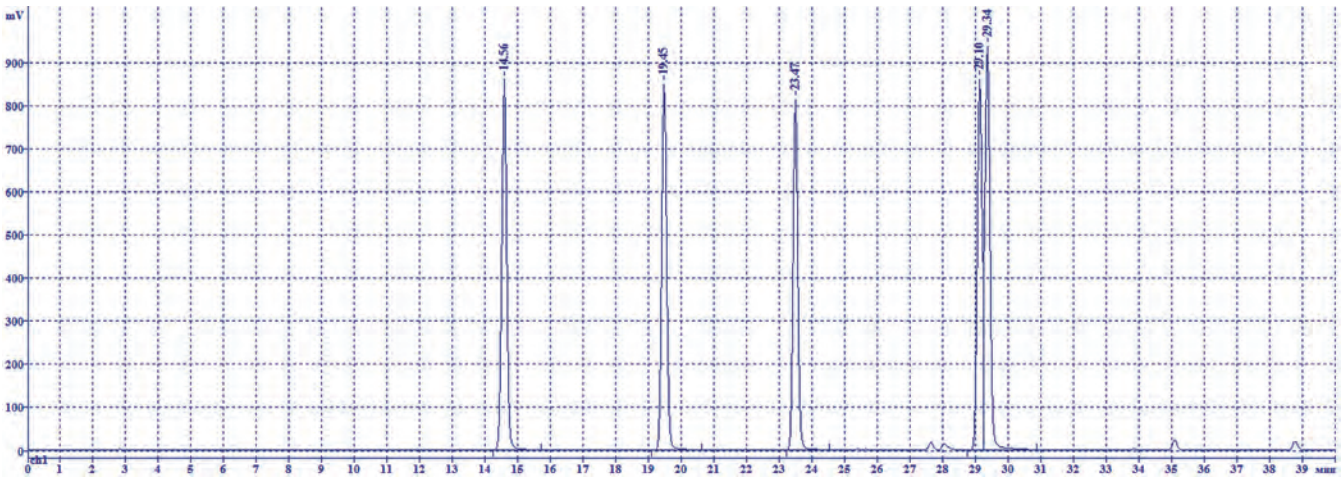


**FIG. 1.** *Chromatogram of mixture of reference standards of daidzen (0.0002 g/ml), genistein (0.0002 g/ml), formononetin (0.0002 g/ml), prunetin (0.0002 g/ml) and biochanin A (0.0002 g/ml)*

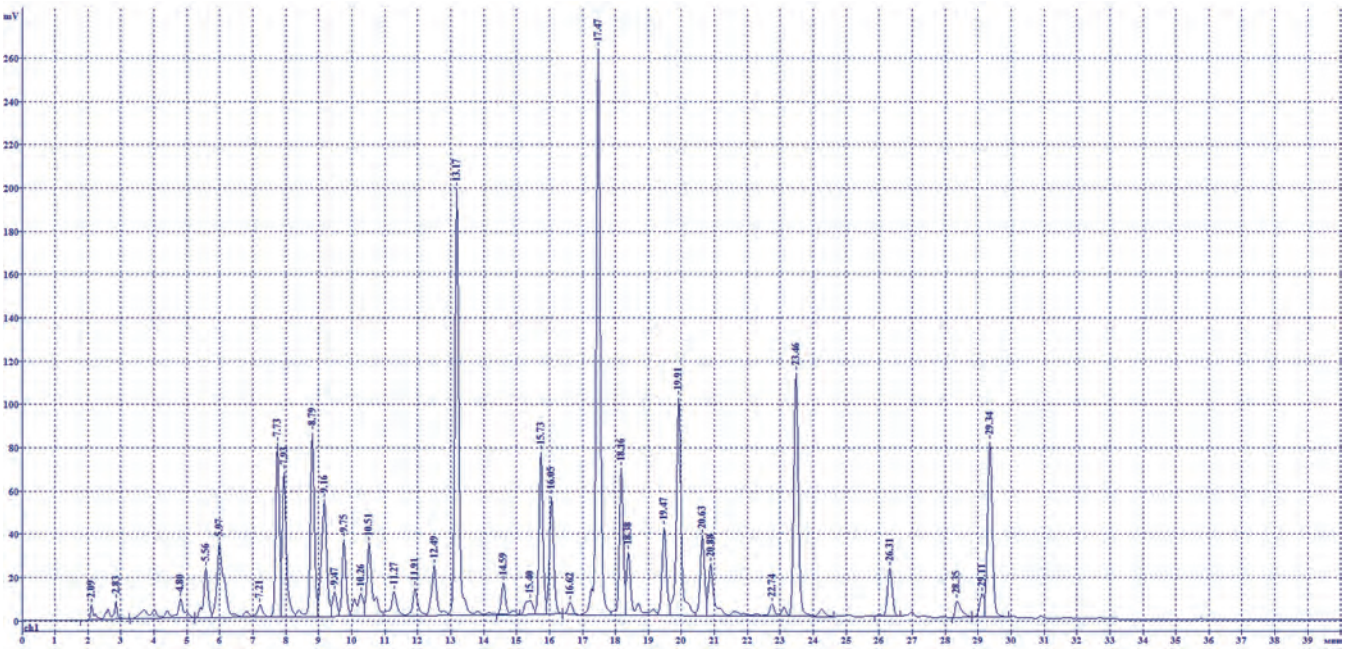


**FIG. 2.** *HPLC- chromatogram of a dry extract of the meadow clover herb*

*Table*

**IDENTIFICATION AND DETERMINATION OF INDIVIDUAL ISOFLAVONES IN DRY MEADOW CLOVER EXTRACT BY HPLC, N=3, F=2, P=95%, T (P, F) =4,30**

| № | Name of a substance | $t_{st}$, min | $t_x$, min | $S_{st}$, mV/s | $S_x$, mV/s | Substances content in dry extract, % | Metrological characteristics of results |
|---|---|---|---|---|---|---|---|
| 1 | Daidzen | 14.56 | 14.59 | 9197.614 | 145.397<br>149.710<br>140.837 | 0.3505<br>0.3601<br>0.3601<br>$\bar{X}$ = 0.36±0.01 | $S^2$ = 0.000031<br>S = 0.0055<br>$S\bar{x}$ = 0.0032<br>ε, % = 2.8 |
| 2 | Genistein | 19.45 | 19.47 | 9153.454 | 388.630<br>387.539<br>380.939 | 0.8506<br>0.8462<br>0.8353<br>$\bar{X}$ = 0.84±0.01 | $S^2$ = 0.00015<br>S = 0.012<br>$S\bar{x}$ = 0.0069<br>ε, % = 1.19 |
| 3 | Formononetin | 23.47 | 23.46 | 8210.147 | 1145.592<br>1172.919<br>1135.778 | 3.0074<br>3.0716<br>2.9863<br>$\bar{X}$ = 3.02±0.05 | $S^2$ = 0.0017<br>S = 0.041<br>$S\bar{x}$ = 0.024<br>ε, % = 1.7 |
| 4 | Prunetin | 29.10 | 29.11 | 8878.792 | 92.041<br>95.113<br>87.596 | 0.2266<br>0.2335<br>0.2123<br>$\bar{X}$ = 0.22±0.01 | $S^2$ = 0.00014<br>S = 0.012<br>S = 0.0069<br>ε, % = 4.6 |
| 5 | Biochanin A | 29.34 | 29.34 | 10426.282 | 835.150<br>833.634<br>806.617 | 1.6422<br>1.6353<br>1.5875<br>$\bar{X}$ = 1.62±0.03 | $S^2$ = 0.0012<br>S = 0.035<br>$S\bar{x}$ = 0.020<br>ε, % = 1.85 |

*Note: $t_x$ – retention time of the substance in the dry extract, minutes; $t_{st}$ – retention time of the reference standard, minutes; $S_{st}$ – peak area of the reference standard, mV/s; $S_x$ – peak area of the substance in the dry extract, mV/s.*

is reproducible, the results obtained are reliable, and metrological characteristics are given.

Scientific studies have shown that biochanin A has significant lipid-lowering effect [3]. It was shown that in mammalian cell culture, biochanin A reduced the amount of binding of [3H] benzopyrene to DNA by 37–50% in a dose of 25 μg/ml and reduced the metabolism of [3H] benzopyrene by 54% compared to control cultures [4]. Biochanin A has cardioprotective, antitumor activity, antioxidant properties, and anti-inflammatory effects [5].

Formononetin has a potential anti-cancer effect in vitro and in vivo. When using formononetin in combination with other chemotherapeutic medicines such as bortezomib, LY2940002, U0126, sunitinib, epirubicin, doxorubicin, temozolomide and metformin, the anti-cancer potential of both formononetin and the corresponding pharmaceuticals is enhanced due

to the synergistic effect [6]. The neuroprotective effect of formononetin has been demonstrated in patients with Alzheimer's disease in both in vivo and in vitro studies [7]. In addition, it has been shown that formononetin exhibits vasorelaxant, antiapoptotic, cardioprotective, proliferative antioxidant, antimicrobial, and anti-inflammatory activities [8].

## CONCLUSIONS

In a dry extract of meadow clover, five isoflavone compounds such as daidzein, genistein, formononetin, proetin, biochanin A were detected by HPLC. It was found that the highest content is characteristic for isoflavones – formononetin (3,02±0,05%) and biochanin A (1,62±0,03%), and that fact allows to consider the dry extract of meadow clover herb as a promising substance for medicine development

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# DYNAMICS OF MINERAL COMPOSITION OF RAW MATERIALS OF BIRTHWORT DUTCHMAN'S-PIPE (*ARISTOLOCHIA CLEMATITIS* L.) AND SOIL FROM ITS HABITAT

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*The ultimate composition of the Birthwort Dutchman's pipe (Aristolochia clematitis L.) herb and soil samples from its habitat in the dynamics of 2017–2019 was analyzed by inductively coupled plasma-atomic emission method. The majority macro- and microelements are potassium, magnesium and calcium, iron, manganese and zinc, respectively. The content of heavy metals and the coefficient of biological absorption of Birthwort Dutchman's pipe herb were determined.*

**Keywords:** Birthwort Dutchman's pipe (*Aristolochia clematitis* L.), mineral composition, heavy metals, maximum permissible concentration

One of the important elements of a comprehensive study of the chemical composition of medicinal plant raw materials (MPRM) is the study of the element profile. Macro- and microelements have a significant impact on the biosynthesis of various groups of biologically active substances (BAS): they affect the processes of plant growth, act as components of enzymes. Thus, potassium, sodium and calcium are key elements in respiration and many other biochemical processes of plants [1], phosphorus is a part of ATP, and magnesium is a main component of chlorophyll.

In the process of obtaining the plant extracts, the mineral components, along with the rest of the BAS groups, are isolated from the MPRM and, in turn, affect the manifestation and severity of the pharmacological action of the medicine, as well as show their own activity. It was noted [2] that microelements of plant origin are better absorbed by the human body, since they are in "biological" concentrations in physiologically balanced complexes.

The chemical composition of plants is influenced by many factors: environmental, genetic, and physiological [3,4]. Phytochemical composition of MPRM depends on the growing environment, namely, the elemental

composition of the soil and the ability of the plant to concentrate elements [5]. Therefore, it is advisable to study the elemental composition of medicinal plants in conjunction with soil research. The literature [6] shows that chemical elements contained in the soil significantly affect the biosynthesis of BAS in plants and are capable of selective accumulation. The identified natural concentrators of micro-elements from plants can be successfully used in practical medicine for corrective therapy. At the same time, an important issue is the environmental aspect, namely, environment pollution with heavy metals. Coming through the soil, they change the biochemical processes of plants, disrupt the normal processes of life [7] and, as a result, can be in herbal medicinal products. Therefore, it is necessary to determine and compare with the maximum permissible concentrations (MPC) the content of heavy metals in the harvested raw materials.

Plants that are considered as poisonous have always attracted the attention of researchers due to their high activity [8]. Therefore, interest in the possible pharmacotherapeutic profile of toxic compounds remains high. One of these plants is the Birthwort Dutchman's pipe (*Aristolochia clematis* L.), which contains toxic aristolochiic acids [9,10]. Previously, scientists studied the accumulation of individual elements in the A. clematitis herb growing in the Stavropol territory [11,12]. However, the geochemical aspects of vegetation have a significant impact on the elemental profile of the plant, and in addition, there is no data in the literature on the dynamics of accumulation of macro – and microelements. Therefore, the **purpose** of our work was to study the macro – and microelement composition of Birthwort Dutchman's pipe (*Aristolochia clematis* L.) herb, in comparison with soil samples from its habitat in the dynamics of the periods of harvesting the raw materials and highlight the maximum concentration.

## MATERIALS AND METHODS

As the study object, we used the Birthwort Dutchman's pipe (*Aristolochia clematis* L.) herb and soil from its habitat to form a conclusion about the selective accumulative capacity of the plant. Raw materials were harvested in early July 2017, 2018, 2019 in the city of Novy Oskol, Belgorod region, according to the rules for harvesting medical herbs in the forest phytocenosis (in dry weather at noon in the afternoon from unpolluted, well-developed, healthy plants at a distance of at least 10 km from major industrial cities and no closer than 500 m from the roadside with heavy traffic). Raw material is dried using natural shade drying. The degree of grinding is 3–5 mm. The soil was prepared in early July 2017 and 2019 in the city of Novy Oskol, Belgorod region. To determine the ultimate composition of the soil, samples were taken at 3 points around the plant in a diameter of 30 cm and from a depth of 25 cm, thoroughly mixed and formed a single average sample. Then the soil was sifted and large particles were removed. For a more objective picture of changes in the concentration capacity of Birthwort Dutchman's pipe (*Aristolochia clematis* L.), the soil was collected in two years (in 2017 and 2019).

The analysis was performed by inductively coupled plasma-atomic emission method using the Optima 8000 spectrometer (Perkin Elmer, USA) in accordance with the recommendations of the RF SP XIII OFS.1.5.3.0009.15 [13] based on CCU "Analytical center".

**Sample preparation:** a sample weight of about 0.4 g (exact weight) was placed in a Teflon vessel ("bomb"). 5 ml of concentrated nitric acid for inductively coupled plasma-atomic emission (65%, Sigma Aldrich), 3 ml of hydrogen peroxide were added to the weighted sample, carefully mixed, and left for 10 minutes to remove vapors. Then the vessel was placed in the BERGHOF SpeedWave Entry Two microwave system, and the temperature conditions were set for mineralization of plant objects. After cooling,

the mineralizate was transferred to polymer measuring flasks with capacity of 50 ml, brought to the mark with purified water of type 1 (solution No.1). 250 ml of solution No.1 was placed in a polymer measuring flask with a capacity of 25 ml with a mechanical dispenser and brought to the mark with solvent (15.4 ml of concentrated nitric acid and 30 ml of hydrogen peroxide were brought in a polymer measuring flask with purified water of type 1 to 500 ml) – solution No.2. The resulting solution No. 2 was used for quantitative determination of macro-elements in the solution

As standard samples, we used Multi-Element Calibration Standard-3 with certified element values of 10 mg /l and Pure Plus Mercury 10 mg /l (Perkin Elmer, USA).

**Test conditions:** plasma feed rate – 10 l/min, auxiliary – 0.2 l/min, spray – 0.7 l/min. Power 1300 W, the viewing position is axial. The mud flow rate (peristaltic pump) – 1.5 ml/min, for washing the rapid feed of solvent – 2.5 ml/min, the delay time – 25 sec., read time – 0.1–1.0 sec., repetitions (replicas) – 3.

The choice of analytical waves is based on the minimum value of sensitivity and the maximum intensity. Several analytical wavelengths (2–3 lengths) are pre-viewed, the wavelengths, where the overlapping spectra of other elements (for example, iron), are noticeable, are removed, the position of the peak and the baseline is adjusted, and the calculation is provided relative to the solvent by the peak area. The monochromatic spectrometer registers the signal intensity and gives the value of the calibration concentration according to the calibration curve. The reliability of the results is evaluated by RSD (the acceptance criterion of RSD is 2%, it is acceptable for micro-concentrations of RSD up to 30%). The content of elements (X, mg/kg) in the sample was calculated using the formula:

$$\mathbf{X, mg/kg} = \frac{C_x \times V_1 \times V_2}{m \times V_3} \times 1000 \times 1000,$$

where $C_x$ – element concentration according to the calibration curve, mg/l; $V_1$ – solution volume, l; $V_2$ – dilution volume, ml; $V_3$ – solution volume assumed for dilution, ml; $m_1$ – sample weight, mg; 1000 – conversion of mg into g; 1000 – conversion of g into kg.

## RESULTS AND DISCUSSION

According to the results of the study of macro- and microelements in the Birthwort Dutchman's pipe (*Aristolochia clematis* L.) herb and soil from its habitat, the content of 17 elements was found in 2017, 11 elements in 2018 and 2019 (see Table 1).

Comparing the results of the ultimate composition in dynamics over the years of harvesting, we can say that the majority macro-and microelements in the Birthwort Dutchman's pipe (*Aristolochia clematis* L.) herb do not change, they are potassium, magnesium and calcium, iron, manganese and zinc, which is a normal physiological element profile of plants. The content of magnesium in the raw materials harvested in 2017 and 2019 significantly exceeds its content in the soil (7361.3 mg /kg – 42.04 mg/kg; 2188.5 mg/kg – 979.2 mg/kg, respectively). Considering that magnesium is a pigment-forming element, its quantitative variability over the years in a significant range is normal. The increase in the content of calcium in herb in 2019 (12629.5 mg/kg) by 25% compared to the soil can be explainedby unevenness of the harvested herb elements (the predominance of stems compared to leaves) and accumulation of physiological inclusions of calcium oxalate.

The data from the analysis of birthwort Dutchman's-pipe herb harvested in different years correlate by the majority components, but differ in the content of individual elements, especially, normalized heavy metals. Thus, it is possible to trace a gradual decrease in the

*Table 1*

**RESULTS OF THE STUDY OF MACRO-AND MICROELEMENT COMPOSITION OF THE BIRTHWORT DUTCHMAN'S PIPE (*ARISTOLOCHIA CLEMATIS* L.) HERB AND SOIL FROM ITS HABITAT**

| Element | Content of elements, mg/kg (n=5) | | | | |
|---|---|---|---|---|---|
| | Raw material, 2017 | Soil, 2017 | Raw material, 2018 | Raw material, 2019 | Soil, 2019 |
| Al | 230.12 | 19903.23 | 83.65 | 63.4 | 3838.5 |
| **As (MPC 0,5 mg/kg)** | **–** | **–** | **0.07** | **–** | **–** |
| Ba | 83.29 | 107.31 | not determined | *20.4* | 17.0 |
| Ca | 7112.76 | 13838.71 | not determined | *12629.5* | 10051.0 |
| **Cd (MPC 1,0 mg/kg)** | **0.58** | **0.86** | **0.34** | **–** | **–** |
| Co | – | – | – | – | – |
| Cr | 2.39 | 42.58 | 0.75 | – | – |
| Cu | *49.14* | 16.45 | *27.95* | *6.6* | 2.6 |
| Fe | 374.81 | 5707.53 | 119.2 | 82.0 | 3085.0 |
| K | *16016.46* | 13591.40 | not determined | *25730.0* | 1546.5 |
| Li | – | 200.00 | not determined | – | – |
| Mg | *7361.32* | 42.04 | not determined | *2188.5* | 979.2 |
| Mn | 110.86 | 142.90 | 40.70 | 35.4 | 82.8 |
| Na | *91.19* | 17.44 | not determined | *137.3* | 69.7 |
| Ni | 3.13 | 18.71 | 1.48 | – | – |
| **Pb (MPC 6,0 mg/kg)** | ***2.39*** | **–** | ***1.34*** | **–** | **–** |
| Se | *6.42* | – | not determined | – | – |
| Sr | – | – | – | 27.7 | 20.8 |
| Zn | *109.05* | 0.91 | *54.21* | *22.3* | 15.2 |
| **Hg (MPC 0,1 mg/kg)** | ***4.61*** | **0.22** | **0.03** | **–** | **–** |
| Bi | *0.082* | – | not determined | – | – |

*Note: bold font – elements whose content is normalized by the RF SP; italics with underscores – elements whose content in raw materials exceeds the same indicator in soil*

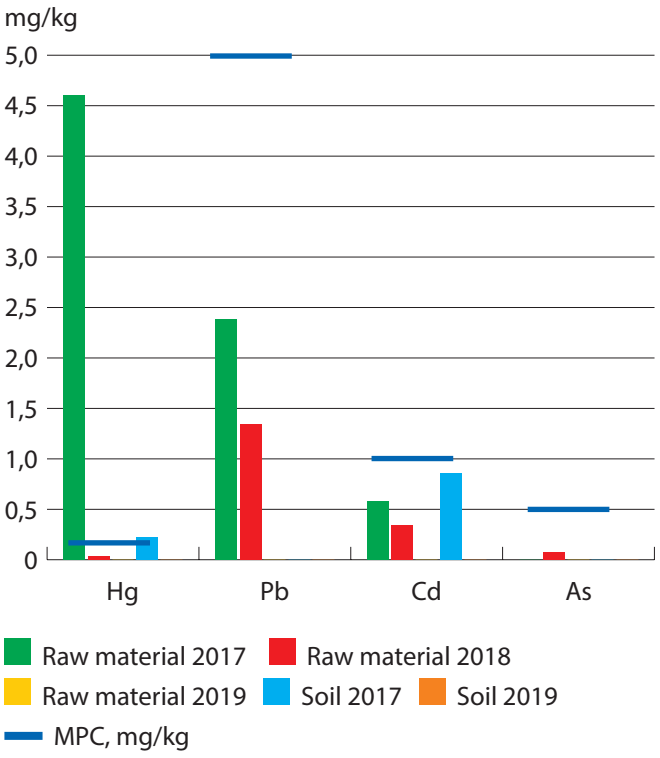


**FIG.** *Dynamics of the content of heavy metals and arsenic (mg /kg) in the Birthwort Dutchman's pipe (Aristolochia clematis L.) herb and soil from the Belgorod region in different years*

content of mercury, lead and cadmium in the birthwort Dutchman's-pipe herb 3 years until the disappearance. The content of mercury in the raw material harvested in 2017 is 4.61 mg/kg and exceeds the MPC by 40 times. The content of this heavy metal in the soil is 0.22 mg/kg, which allows us to conclude that the raw material of birthwort Dutchman's-pipe is able to concentrate mercury. The mercury content in the herb harvested in 2018 is much lower (0.03 mg/kg) and is within normal limits, and in raw materialand soil in 2019 the mercury was not identified. The content of other heavy metals is normal and does not exceed the MPC. However, lead and arsenic were identified in raw materials (2.39/1.34 mg/kg (2017 and 2018) and 0.07 mg/kg (2018), respectively) and were not identified in the soil. This proves that the birthwort Dutchman's-pipe herb concentrates heavy metals of any origin. In comparison with 2017, in 2019 the content of all identified heavy metals does not exceed the MPC. Thus, we can assume that the plant is the most representative indicator of the degree of environmental pollution in a particular region, since it is able to accumulate certain toxic elements. Based on the research data, we can assume that the environmental situation in the Belgorod region deteriorated in 2016–2017. The results of the content of heavy metals and arsenic in the birthwort Dutchman's-pipe herb and soil from the habitat and MPC according to the RF SP OFS.1.5.3.0009.15 "Determination of the content of heavy metals and arsenic in medicinal plant raw materials and medicinal plant medicines" are shown in the figure [13].

The coefficients of biological absorption (CBA – the ratio of the element content in the aboveground part to its content in the soil) were

*Table 2*

**CBA FOR THE BIRTHWORT DUTCHMAN'S PIPE (*ARISTOLOCHIA CLEMATIS* L.) HERB IN DYNAMICS OF 2017 AND 2019**

| CBA | 2017 | 2019 |
|---|---|---|
| Elements of energetic and strong accumulation (CBA>1) | **Cu, K, Mg, Na, Zn, Hg** | **Cu, K, Mg, Na, Sr, Zn, Ba, Ca** |
| Elements of weak accumulation and average capture (1>CBA>0,1) | **Ba, Ca, Cd, Mn, Ni** | **Mn** |
| Elements of weak capture (CBA<0,1) | **Al, Cr, Fe** | **Al, Fe** |

calculated by the N.F. Ganzhar scale [14] for the Birthwort Dutchman's pipe (*Aristolochia clematis* L.) herb harvested in different years (Table 2).

It is established that regardless of the year of harvesting, the Birthwort Dutchman's pipe (*Aristolochia clematis* L.) herb is Cu, K, Mg, Na, and Zn accumulator. These elements are distributed in descending order as follows: raw materials harvested in 2017 – Mg, Zn, Na, Cu, K; raw materials harvested in 2019 – K, Cu, Mg, Na, Zn. This allows us to consider the accumulation of these elements as an additional chemical marker for the Birthwort Dutchman's pipe (Aristolochia clematis L.) herb.

## CONCLUSION

The macro-and microelement composition of the Birthwort Dutchman's pipe (*Aristolochia clematis* L.) herb and the soil on which it grows in the dynamics of 2017–2019 was determined. The content of 17 elements was determined in 2017 and 11 elements in 2018 and 2019, respectively. Moreover, the data from the analysis of the Birthwort Dutchman's pipe (*Aristolochia clematis* L.) herb harvested in different years correlate by the majority components (potassium, magnesium, calcium, iron, manganese and zinc) and accumulation elements (copper, potassium, magnesium, sodium and zinc), but differ in content. It was found that the Birthwort Dutchman's pipe (*Aristolochia clematis* L.) herb is able to concentrate heavy metals (mercury and lead), the content of which in the raw material harvested in 2017 is significantly higher than in the soil. This fact makes it possible to consider the raw material of the Birthwort Dutchman's pipe (*Aristolochia clematis* L.) as a biological marker of mercury contamination of the environment and to assume the detoxification properties of the raw material.

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# CURRENT STATE OF THE PHARMACEUTICAL MARKET OF MEDICINES FOR VETERINARY USE IN THE EEU COUNTRIES

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*The paper presents a statistical analysis of the pharmaceutical market of medicines for veterinary use in the EEU countries (the Russian Federation, the Republic of Belarus, the Republic of Kazakhstan, the Republic of Armenia). Based on the state registers of medicines for veterinary use in these countries, the dynamics of registration of medicines, the number and ratio of pharmacotherapeutic groups of veterinary medicines are shown. The shares of groups of medicines intended for different types of animals in the registers of medicinal products are specified.*

**Keywords:** pharmacy, veterinary medicine, pharmaceuticals, pharmaceutical market

In recent years, there has been development of the domestic market of medicines for veterinary use, increase in the market capacity, the number of registered medicines, development of medicine manufacturers both at the expense of domestic organizations and due to the arrival of foreign companies. Thus, according to the Russian Veterinary Association, the market capacity of veterinary medicines in 2018 amounted to about 39.0 billion rubles, and in 2016–36.5 billion rubles [6,7].

The Russian Federation, as a member state of the Eurasian Economic Union (EEU), in accordance with the Treaty on the Eurasian Economic Union (signed in Astana on 29.05.2014), forms a common market for medicines with other member states of the Union

**The purpose** of our work was to study the state of the pharmaceutical market of medicines for veterinary use in the EEU countries – the Russian Federation (RF), the Republic of Belarus (RB), the Republic of Kazakhstan (RK), the Republic of Armenia (RA).

## MATERIALS AND METHODS

In the course of the study, the statistical analysis and content analysis methods were used. Based on data from the state registers of medicines for veterinary use in Russia [URL: https://galen.vetrf.ru], Belarus [URL: http://www.dvpn.gov.by], Kazakhstan [URL: https://moa.gov.kz] and Armenia [URL: http://www.pharm.am] we have analyzed the types, quantity, pharmacotherapeutic groups, groups of medicines depending on the type of animals represented on the pharmaceutical market of medicines for veterinary use in the EEU countries.

## RESULTS AND DISCUSSION

It is established that as of 01.09.2019 in the Russian Federation for veterinary use in accordance with the established procedure, 2196 medicines for veterinary use were registered and entered into the State register, including 630 immunobiological drugs (Fig. 1).

In the Republic of Belarus, 2013 medicines for veterinary use were registered and entered into the State register, including 197 immunobiological drugs.

In Kazakhstan, the pharmaceutical market is represented by a smaller number and includes 1111 medicines for veterinary use, 89 of which are immunobiological drugs.

330 animal medicines have been registered and entered into the State register in Armenia, including 213 immunobiological drugs.

The number of registered medicines for veterinary use in the EEU countries is shown in Fig. 1.

As can be seen from the presented data, the most extensive market of medicines for veterinary use is represented in the Russian Federation, only 8.3% less than veterinary medicines in the Republic of Belarus. In Kazakhstan, the pharmaceutical market of medicines for veterinary use is almost half (49.0%) less than in the Russian Federation. However, the market of veterinary medicines is significantly less represented in Armenia (15.0% of the Russian market).

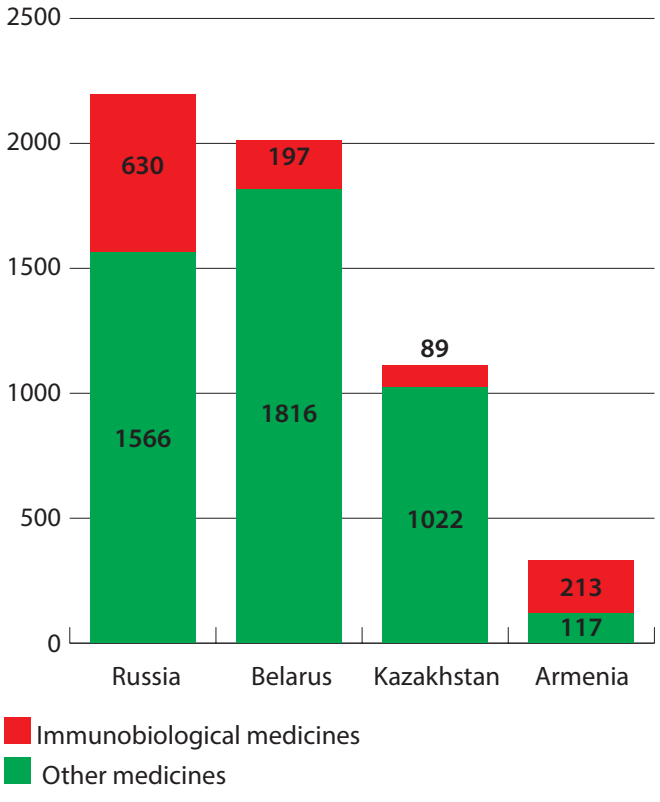



**FIG. 1.** *Number of medicinal products for veterinary use in the EEU countries*

Next, we analyzed the dynamics of state registration of medicinal products for veterinary use. As it can be seen from the presented data (Fig. 2), in the Russian Federation for the past 5 years (2014-2018), at average 352 medicines are registered annually. The largest number of registered medicines is in 2016, and the smallest number is in 2015.

In the Republic of Belarus, at average 380 medicines have been registered annually for the past 5 years. The smallest number of medicines was registered in 2014. The largest number was registered for 2016–2015, which in total is 40.3% of the total volume of medicines entered into the register. As you can see on the graph, there have been no strong jumps in the number of registered medicines for the past 4 years. The number of registered medicines in 2018 increased by 1.2 times compared to 2014, which indicates a positive trend in the RB market.

In Kazakhstan, for the past 5 years, about 120 medicines have been registered annually. The smallest number of medicines was registered in 2016–2015. The largest number was registered in 2017–2018, which in total is 45% of the total volume of medicines entered into the register.

In Armenia, at average 55 medicines are registered annually. The smallest number of medicines was registered in 2018. The largest number was registered in 2016–2017, which in total is almost 67.0% of the total volume of medicines entered into the register. As you can see on the graph, until 2016 there was a positive trend in the development of the veterinary pharmaceutical market, after 2016 the number of registered medicines began to decline sharply and reached its minimum last year.

Based on the data on the dynamics of registration of medicines for veterinary use, it can be said that for the past 5 years, the most intensively medicines were registered in the Republic of Belarus (at average, 380 medicines

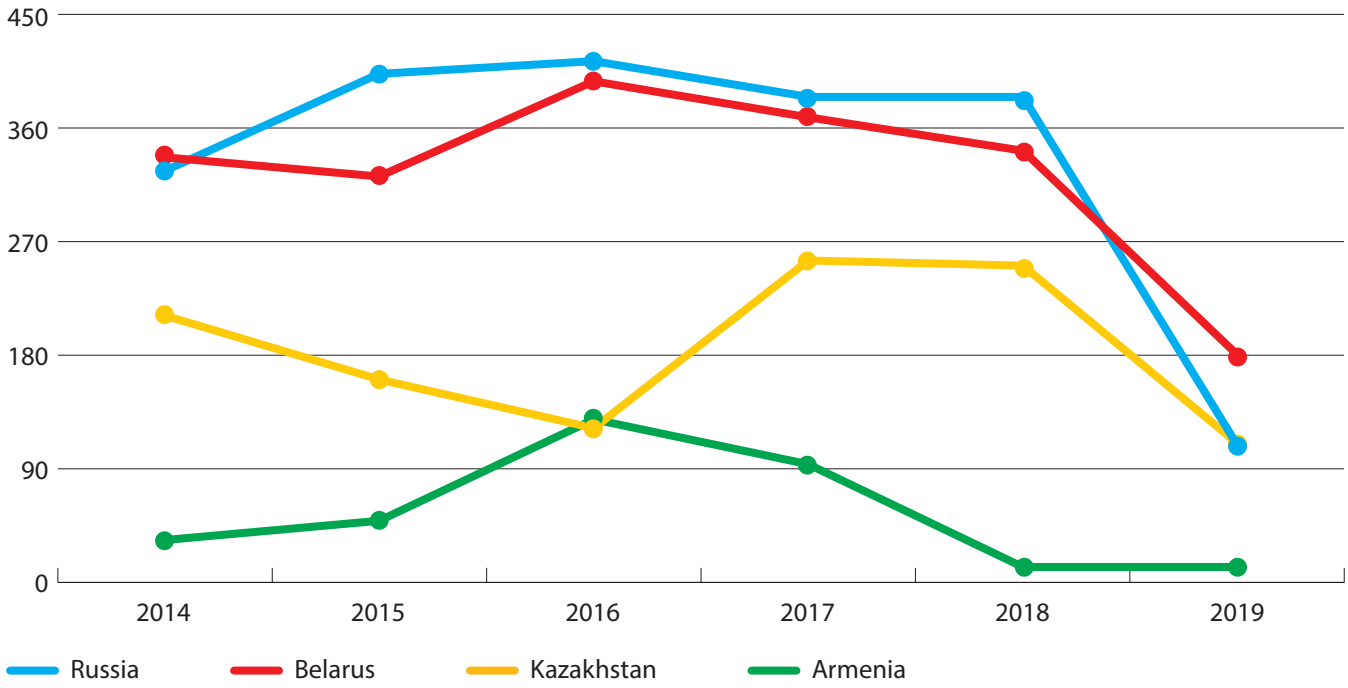



**FIG. 2.** *Dynamics of registration of medicines for veterinary use in the EEU countries*

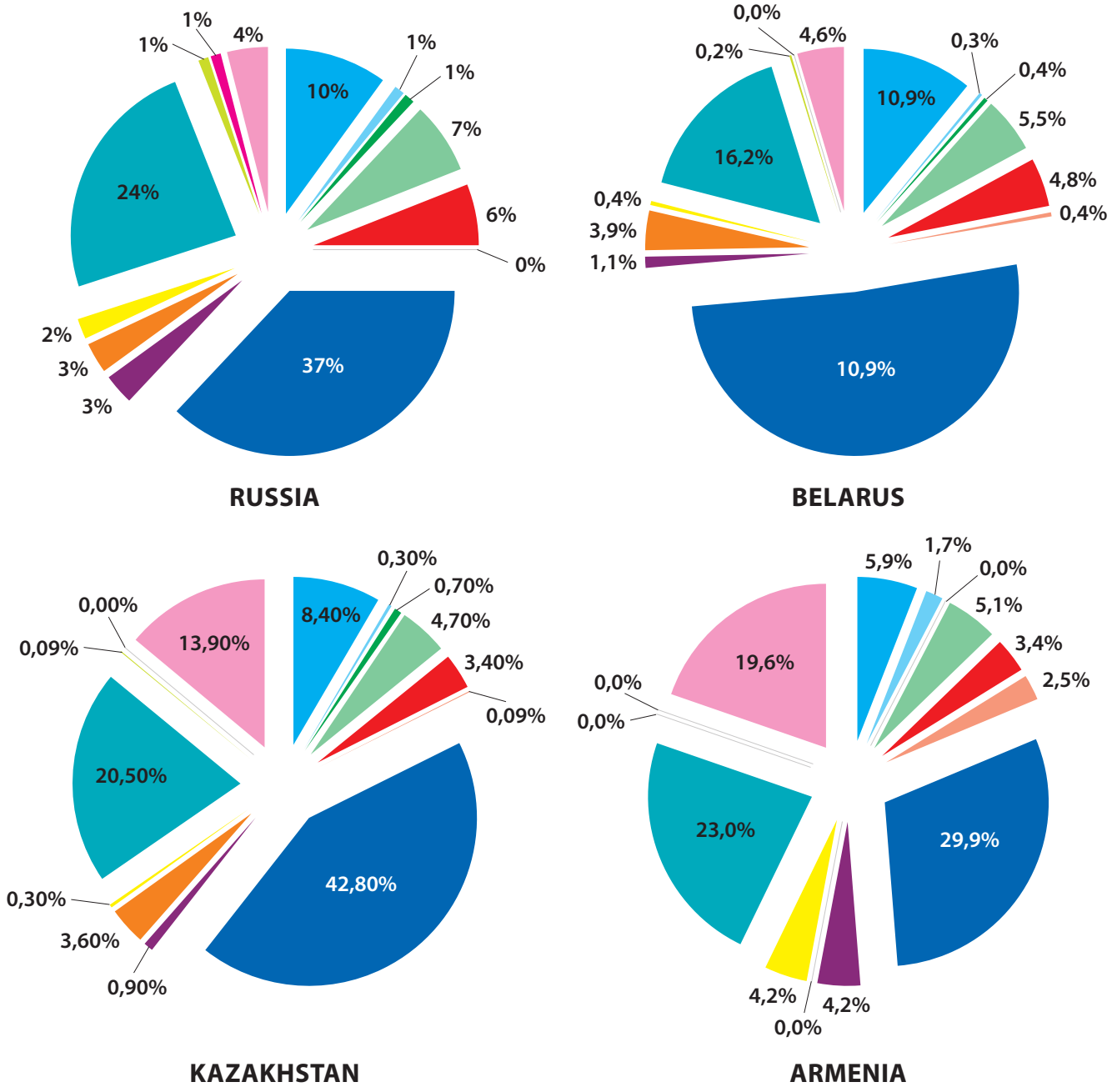
per year), 7.0% lower than the dynamics of registration in the Russian Federation (352 medicines per year). In the third place in terms of registration dynamics is Kazakhstan (120 medicines per year), which is 68.0% lower than in the Republic of Belarus, and 65.0% lower than in the Russian Federation. In the last place there is the Republic of Armenia, which registers at average 55 medicines per year, which is 85.0% lower than in the Republic of Belarus, and 84.0% lower than in the Russian Federation.

Further, based on the composition and indications for the use of medicinal products available in the registers, we have classified veterinary medicinal products in accordance with the anatomic-therapeutic-chemical classification (Fig. 3).

As it can be seen from the presented data, not all pharmacotherapeutic groups of medicines are currently represented in the State register of the Russian Federation in full, some groups (antimicrobials for systemic use – 37.0%) are represented in excess. Hormones for systemic use (0.54%), medicines for respiratory system (0.68%) and sensory organs (0.82%) are not represented in sufficient volume. The pharmaceutical market is mainly represented by the three largest groups: antimicrobials for systemic use (37.0%), antiparasitic agents, insecticides and repellents (24.0%), as well as medicines that affect the digestive tract and metabolism (10.0%), which in total is 71.0% of the total number of medicines.

In Belarus the situation was the same: there are no almost completely the medicines for treatment of sensory processing disorders, there are catastrophically small numbers of medicines for treatment of respiratory system (0.2%) and medicines for hematosis treatment (0,3%). Antimicrobials for systemic use (51.0%) are present in excess.

In the pharmaceutical market of Kazakhstan, there are no medicines for treatment of sensory processing disorders; only one name is represented in the group of medicines for treatment of respiratory diseases and one name is among hormonal drugs, excluding reproductive hormones. The largest number of registered medicines also refers to antimicrobial (42.0%) and antiparasitic (20.0%) agents.




- A – Medicines effecting on intestinal track and metabolism
- B – Medicines effecting on hematosis and blood
- C – Medicines for treatment of cardiovascular diseases
- D – Medicines for treatment of skin diseases
- G – Medicines for treatment of urogenital disorders and reproductive hormones
- H – Hormonal medicines for system use (without reproductive hormones)
- J – Antimicrobial medicinal products for system use
- L – Anitumourals and immunomodulatory medicines
- M – Medicines for treatment of musculo-skeletal system diseases
- N – Medicines for treatment of nervous system diseases
- P – Antiparasitic agents
- R – Medicines for treatment of respiratory diseases
- S – Medicines for treatment of sensory processing disorders
- V – Other medicines

**FIG. 3.** *Anatomic-therapeutic-chemical classification of medicinal products for veterinary use in the EEU countries*

In the Republic of Armenia, there are no medicines for the treatment of sensory processing disorders, respiratory diseases, as well as diseases of musculoskeletal and cardiovascular systems. The largest number of registered medicines are medicines belonging to antimicrobial (29.0%) and antiparasitic (23.0%) groups.

Thus, we can conclude that the overall picture is observed in the pharmaceutical markets in the EEU countries. Namely, there is disparity between the pharmacotherapeutic groups of medicines, the overwhelming predominance of antimicrobial and antiparasitic agents.

The next stage of the study was the analysis of medicines for veterinary use, depending on the species of animals for which they are intended. The results revealed that among all the medicines presented in the State register of the Russian Federation, 17.0% of medicines are used regardless of the animal species; 23,4% of medicines are intended for large cattle and sheep and goats; 11.3% are used for pigs; 21.4% are used for poultry; 20.1% are for dogs and cats; 2,4% are for horses; and 1.7% are for fur animals; 1,6% are for the bees; 0,5% are for fish and 0.6% – for reptiles (Fig. 3).

In the Republic of Belarus, in the majority the medicines for use for several types of animals are also represented (53.0%) if we consider only the species, the number of medical products for cattle/goats and sheep (21.0%) and pigs (9.0%) prevails.

In Kazakhstan, the medicines has been registered for many animal species, including cattle/goats and sheep (12.0%), farm birds (8.0%) and pigs (7.0%).

In Armenia, the market for medicinal products is also represented for many animal species, with the predominant shares of medicinal products for cattle/goats and sheep (27.0%) and pigs (10.0%).

Thus, the range of medicines for veterinary use in the EEU countries is mainly focused on farm animals (large cattle, goats and sheep, pigs, horses,

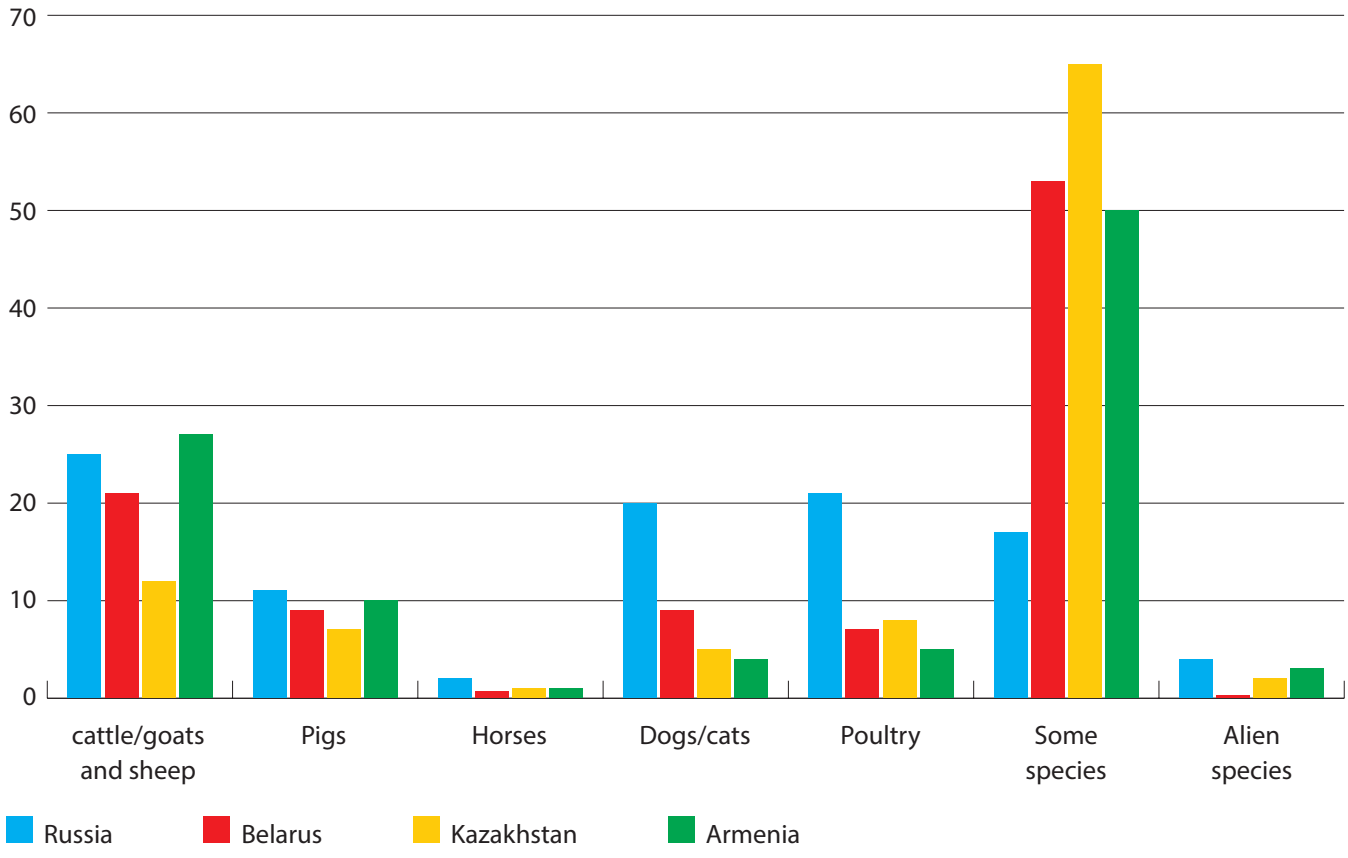



**FIG. 4.** *Shares of medicines for different types of animals in the EEU countries*

poultry). The total share of medicinal products for farm animals in the Russian Federation is just over 75.0%, in Belarus, Kazakhstan and Armenia this share is more than 90.0%.

The analysis found that the share of medicines for domestic and exotic animals in the Russian Federation is about 25.0%, and in other countries – members of the Union such share is less than 10.0%, which, from our point of view, is insufficient with increase in the number of small pets in cities.

## CONCLUSION

Thus, the pharmaceutical market of medicines for veterinary use in the EEU countries has been steadily and actively developing in recent years, being attractive to many domestic and foreign companies. The pharmaceutical market is most widely represented in the Russian Federation and the Republic of Belarus, which is confirmed by a significant number of registered medicines and stable annual dynamics of their registration.

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# EVALUATION OF PROCUREMENT OF MEDICINES NOT INCLUDED IN VED LIST BY DISTRICT HOSPITALS OF THE KRASNOYARSK TERRITORY

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*An analysis of the top-10 medicines purchased annually by regional hospitals in the Krasnoyarsk territory in addition to the list of VED showed that 8 out of 10 medicines are included in various standards of medical care and clinical recommendations approved by the Ministry of Health of the Russian Federation. The acquisition of medicines to ensure the implementation of standards of care is a priority in the medicine provision of medical organizations, despite the legal contradictions. In order to optimize the procurement of medicines by medical organizations, it is necessary to harmonize the standards of medical care, clinical recommendations and the list of VED.*

**Keywords:** organization of provision of medicines, district hospitals, VED-list, cost optimization

On the territory of the Russian Federation, in accordance with Federal Law No. 61-FZ of 12.04.2010 "On circulation of medicines" and Government decree No. 865 of 29.10.2010 "On state regulation of prices for medicines included in the list of vital and essential drugs", the state regulation covers prices for medicines included in the list of vital and essential drugs (VED), annually approved by the order of the government of the Russian Federation.

State regulation of prices for VED is carried out by registering the producers' maximum selling prices at the Federal level and setting the maximum wholesale and retail markups at the regional level. The registered producers' maximum selling prices are entered in the state register of prices. The register is published on the official website of the Ministry of Health of the Russian Federation on the Internet (http://grls.rosminzdrav.ru). Fixed prices for VED ensure the implementation of Federal law No. 61-FZ "On circulation of medicines", No. 323-FZ "On the basics of public health protection in the Russian Federation" and No. 178-FZ "On state social assistance".

Prices for other medicines are not subject to state regulation, prices for medicines that are not included in the VED – list are determined by pharmacy organizations independently, based on the costs associated with the purchase, storage, sale of medicines, etc.

The VED-list serves as the basis for providing medical care in a day or round-the-clock hospital. Prescription of medicines in the provision of medical care in stationary conditions can be carried out by the medical officer alone only in case of prescription of medicine, which is included in the VED-list. The medicines that are not included in the VED-list, if they are replaced due to individual intolerance, for life reasons, should be prescribed only by the decision of the Medical Commission, which is recorded in the patient's medical documentation and the log of the Medical Commission [1]. This method of prescription of the medicine is aimed at improving the availability of the medicine for medical organizations due to the lack of risk of excess prices for medicines included in the VED-list above the regulated level in the bidding process.

But the medicines that are not included in the VED-list can be included in the standards of medical care in the absence the duly approved documents regulating the development of a unified methodology of forming the list of medicines of standard [2]. There are quite a few such medicines: the VED – list for 2019 includes 735 medicines [3], which is significantly less than the total number of registered medicines in the Russian Federation – more than 32,000 [4].

Assessment of the cost structure of regional hospitals in the Krasnoyarsk territory has shown that the purchase of medicines that are not included in the VED-list accounts for about 16% of the funds [5]. The purpose of our study was to assess the feasibility of purchasing them.

**The purpose** of the study: to assess the feasibility of purchasing the medicines, not included into the VED-list.

## MATERIALS AND METHODS

The analysis of consolidated applications for medicines for 40 regional hospitals (all health organizations – 1 level) of the Krasnoyarsk territory, formed for the purpose of conducting joint bidding in 2015–2018, to identify medicines that are not included in the existing VED-lists purchased annually by these health organizations. The appropriateness of purchases was based on standards and clinical recommendations for the treatment of diseases published on the website of the Ministry of Health of the Russian Federation.

## RESULTS AND DISCUSSION

Analysis of the range of medicines purchased annually in excess of the VED-list has shown that the largest share in both monetary and physical terms is accounted for by 10 medicines (see table).

In 2015, the largest share of costs was for Metamizole sodium (9.9%), in 2016, 2017, and 2018, the share of costs was 10.6%, 11.3%, and 9.1%, respectively. Despite the fact that the world health organization (WHO) does not recommend its use in medical practice either for fever control or for the purpose of analgesia, Metamizole sodium is widely distributed in Russia and the post-Soviet countries, both due to the traditional prejudice against opioid analgesics, and its low cost compared to other non-narcotic analgesics [6]. An essential argument for purchasing Metamizole is its inclusion into the list of medicines required for equipping the emergency medical teams [7].

Among other listed medicines that are not included in the VED-list, most medicines are included in various standards of medical care and/or clinical recommendations. According to the Federal law of 21.11.2011 №323-FZ "About basics of health protection of citizens of the Russian Federation", medical care is arranged and provided in accordance with the procedures of medical care, which are obligatory for execution in territory of the Russian Federation by all the health organizations on the basis of clinical guidelines and standards of medical care, except medical care provided within the frames of clinical testing [8].

*Table*

**TOP-10 OF MEDICINES PURCHASED ANNUALLY BY REGIONAL HOSPITALS IN THE KRASNOYARSK TERRITORY IN EXCESS OF THE VED-LIST**

| Purchase date | 2015 | | 2016 | | 2017 | | 2018 | | 2018 by 2015 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Purchased over the VED-list | 22.2 mln. RUB | 124.5 ths. pack. | 24.9 mln. RUB | 143.9 ths. pack. | 21.5 mln. RUB | 117.5 ths. pack. | 22.0 mln. RUB | 93.3 ths. pack. | | |
| Medicine INN | % RUB | % pack. | % RUB | % pack. | % RUB | % pack. | % RUB | % pack. | growth. RUB | growth. RUB. |
| Metamizole sodium | 9.9 | 18.4 | 10.6 | 15.1 | 11.3 | 18.7 | 9.1 | 22.4 | -8.4 | 21.8 |
| Articaine + Epinephrine | 7.7 | 2.2 | 3.9 | 1.2 | 6.4 | 1.7 | 13.7 | 3.2 | 76.4 | 43.7 |
| Nadroparin calcium | 4.3 | 0.2 | 4.7 | 0.3 | 4.6 | 0.4 | 7.0 | 0.7 | 61.9 | 254.3 |
| Deproteinized hemoderivative calves' blood (Actovegin) | 4.1 | 1.0 | 4.0 | 0.9 | 3.1 | 0.7 | 1.1 | 0.2 | -73.6 | -81.6 |
| Mifepristone | 4.1 | 1.6 | 11.6 | 1.2 | 12.9 | 1.3 | 13.9 | 1.8 | 240.1 | 9.3 |
| Papaverine | 3.4 | 7.7 | 2.8 | 6.4 | 1.8 | 5.3 | 1.7 | 5.2 | -49.4 | -32.6 |
| Nikethamide | 3.2 | 1.5 | 2.5 | 1.3 | 1.8 | 1.2 | 1.4 | 1.4 | -57.2 | -7.2 |
| Hydroxymethyl-khinoxalindi -oxide | 2.8 | 1.0 | 2.1 | 0.8 | 2.0 | 0.8 | 2.1 | 1.1 | -25.9 | 5.6 |
| Ferrous sulfate + ascorbic acid | 2.5 | 1.1 | 2.1 | 0.8 | 3.2 | 1.1 | 3.2 | 1.5 | 31.7 | 38.2 |
| Enalaprilat | 1.2 | 0.5 | 5.2 | 2.2 | 7.6 | 2.8 | 7.2 | 3.3 | 486.0 | 520.6 |

Articaine + Epinephrine (trade names – Ultracaine D–C, Ultracaine D–C Forte, Ubistesin, etc.) is a combined medicine, the effect of which is based on its components, and which has a local anesthetic effect. The medicine is indicated for infiltration and conduction anesthesia, including in dentistry [9]. Calcium nadroparin (trade name – Fraxiparin) is a direct-acting anticoagulant, indicated for treatment of deep vein thrombosis, pulmonary embolism, acute coronary syndrome, prevention of thrombosis in patients with orthopedic, oncological and general surgical operations, as well as for hemodialysis and hemofiltration in patients with chronic renal failure [10,11]. Deproteinized hemoderivate of calves' blood (Actovegin) is registered in the Russian Federation for treatment of vascular and metabolic disorders of the brain, circulatory

disorders and their consequences (trophic ulcers), burns and wounds. Despite the fact that Actovegin is not used in a number of countries in America and Europe and its effectiveness is questionable [12], in the Russian Federation it is included in the standards of medical care for strokes, diabetic polyneuropathy, and treatment of meningococcal infection [13, 14]. Mifepristone (trade names – Genale, Miropristone, Ginestril, etc.) is a synthetic steroid antihestagenic agent that is used mainly for the medical termination of uterine pregnancy in the early stages and the preparation and induction of labor in full-term pregnancy [15]. Papaverine (trade names – Papaverine hydrochloride solution for 2% injection, Papaverine bufus) is a medicine of spasmolytic and hypotensive action, known in medical practice since 1848 [16], used for alleviation of spasms of smooth muscles of the abdominal cavity (cholecystitis, pylorospasm, spastic colitis, renal colic), peripheral vessels (endarteritis), as an adjuvant for premedication and as a part of complex therapy of pain syndromes in oncology [17]. The combination medication of "iron sulfate + ascorbic acid" (trade names – Sorbifer Durules, Ferroplex) is an effective medicine for treatment of iron-deficiency anemia (IDA), including in pregnant women. It not only quickly eliminates clinical symptoms, normalizes hemoglobin and is generally well tolerated by patients, but is also the most pharmacoeconomically beneficial. Sorbifer Durules has an additional advantage, as it is represented in the largest number of pharmacies among all iron preparations for oral administration [18, 19]. Enalaprilat (trade name- Enap P) is an active metabolite of enalapril, that is a widely known medicine included into the group of angiotensin-converting enzyme (ACE) inhibitors. This allows you to get a pronounced hypotensive effect with intravenous administration of the medicine in the treatment of arterial hypertension, hypertensive crisis not only in the hospital, but also at the pre-hospital stage, where enalaprilat is also included into the list of medicines required for emergency medical teams supplies [7, 20]. The inclusion of sodium metamizole and enalaprilate into the mandatory range of medicines for units and kits of emergency medical teams requires not only the mandatory purchase of these medicines, but also regular replenishment of the stock. It is known that the content of the kits is regularly checked by various regulatory authorities (health insurance company, territorial compulsory medical insurance fund, Prosecutor's office, etc.), and the availability of more effective and safe analgesics on the market (compared to metamizol) does not allow modifying the content of kits.

2 of 10 medicines, namely, niketamide (trade name – Cordiamine), hydroxymethylquinoxalin-dioxide (trade name – Dioxin) are not included into the VED – list and into the standards and clinical recommendations.

Niketamide is a respiratory stimulant, indicated for hypotonic conditions, as an additional mtdicine for collapse, shock, asphyxia, including in newborns [21]. Respiratory stimulants also include bemegrid (trade name – 0.5% Bemegrid solution for injection), methylamide ethylimidazoldicarbonate (trade name – 1.5% Etymizole solution in ampoules) and caffeine (trade name – Caffeine-sodium benzoate solution for injection). Among them, bemegride, etimizol as well as nikethamide, are not included in the VED- list. Caffeine, on the contrary, is included into the VED-list and has the registered indications for use similar with niketamide, which makes its purchase for the needs of budget hospitals more rational.

Hydroxymethylquinoxalindioxide (trad e name – Dioxydine) is the only antimicrobial medicine registered for intravenous infusions and external use (5 mg/ml solution) and for intra-cavity and external use (10 mg/ml solution), which allows it to be used not only systemically, but also for the treatment of wounds, cavities as

an antiseptic. The medicine is characterized by a narrow therapeutic margin, so it is necessary to strictly observe the recommended doses, not allowing them to be exceeded. Experimental studies have shown that Dioxydine has mutagenic, teratogenic, embryotoxic and damaging effects on the adrenal cortex. The Toxicological properties of Dioxydine in systemic use define the limitations to its use in the clinic. At the same time, such a well-known antiseptic as chlorhexidine, included in the VED- list in the form of a solution for local and external use, is not allowed for washing the cavities [22]. In addition to Dioxydine, the antiseptic of Miramistin, which does not have the ability to be absorbed through the skin and mucous membranes, is not included in the VED – list. In total, the VED- list includes 5 antiseptics: chlorhexidine, povidone-iodine, hydrogen peroxide, potassium permanganate, ethanol [23]. The absence of the medicine for registered intravital use in this list leads to the need to purchase the medicine in addition to the VED – list.

## CONCLUSION

Analysis of the top 10 medicines purchased annually by regional hospitals in the Krasnoyarsk territory in addition to the list of VED showed that 8 of 10 medicines are included into various standards of medical care and clinical recommendations approved by the Ministry of Health of the Russian Federation.

The acquisition of medicines in order to ensure compliance with the standards of medical care is a priority in the provision of medicines to a health organization, despite the existing organizational and legal contradictions.

3. In order to optimize the procurement of medicines by health organizations, it is necessary to harmonize the standards of medical care, clinical recommendations and the VED -list.

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# GELS AS A PHARMACEUTICAL FORM IN THE STATE PHARMACOPOEIA XIV EDITION, REGULATORY LEGAL ACTS OF THE MINISTRY OF HEALTH OF RUSSIA AND THE STATE REGISTER OF MEDICINES

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*The content analysis method was used to study the regulatory documentation and data of the State register of medicines for the nomenclature and classification of gels. The analysis of the range of medicines represented by the gel dosage form is carried out. The results of the analysis of the domestic pharmaceutical market of gels by trade names, pharmacotherapy groups, manufacturing countries, referentiality and interchangeability are presented. The current direction is the development of domestic gels from the point of view of import substitution of foreign medicines.*

**Keywords:** dosage forms, gels, regulatory documents, State Register of Medicines

Currently, a large number of gel medicines are registered in Russia, and their further development, registration, production and use are very promising areas in medicine and pharmacy.

For external and local use, gels have an impressive set of advantages in comparison with ointments. For example, when spread on the skin, they form finest smooth films, providing, in some cases, rapid and complete absorption of active substances, on the other hand, they form a resilient protective film with prolonged action; provide comfort (support normal heat-, moisture – and gas exchange in the skin, have a pH close to pH of human skin, do not have irritating effects, etc.); both hydrophilic and hydrophobic substances can be entered into gels; the high viscosity of the dispersion medium of gels prevents the interaction of chemically incompatible substances. Gels have good thixotropic properties, which specifies their optimal spreading ability, good squeezability out of the tube. When spread on the mucous membranes, the gels are well retained and provide long-term contact with the treated surface

The physical and chemical properties of gels allow us to consider them as a promising form for oral use. Oral gels combine the advantages of solid and liquid oral dosage forms. One of their advantages is the large bioavailability compared to solid dosage forms. The convenience of administration and the possibility of taste correction allow using medicines in the form of gels for oral administration in children's and geriatric practice, as well as for patients suffering

from chronic diseases, including those associated with swallowing disorders [3,4].

**The purpose** of the study: study of the current state of the nomenclature of medicines in the form of gels.

## MATERIALS AND METHODS

The study was conducted using content analysis of data from the State Pharmacopoeia of the Russian Federation, edition XIV (SP XIV), normative legal acts of the Ministry of Health of the Russian Federation and the State Register of medicines.

## RESULTS AND DISCUSSION

At the first stage of the study, the General Monographs (GM) included in the SP XIV, and normative legal acts of the Ministry of Health of the Russian Federation were reviewed. General Monographs and Pharmacopoeial monographs included in SP XIV were approved by the Order of Ministry of Health of the Russian Federation on October 31, 2018 №749 "On approval of General Monographs and Pharmacopoeial monographs and invalidation of certain orders of the Ministry of Health and Medical Industry of Russia, Ministry of Public Health and Social Development and Ministry of Health of Russia".

SP XIV, released in 4 volumes, came into force on December 1, 2018. Volume 2 begins with the section "Dosage forms of medicines and methods of their analysis", which includes the subsections "Dosage forms of medicines" and "Pharmaceutical and technological tests for dosage forms".

The list of General Monographs (GM) for various dosage forms has been significantly expanded – from 21 to 42. For example, along with traditional General Monographs (GM), such as "Aerosols and sprays", "Eye dosage forms", "Granules", "Capsules", "Inhalation dosage forms", "Parenteral dosage forms", "Ointments", "Medical patches", "Powders", "Solutions", "Syrups", "Suppositories", "Suspensions", "Tablets", "Transdermal patches", "Emulsions", "Infusions and decoctions", "Tinctures", "Teas", "Extracts", "Cut and pressed pellets", the following General Monographs (GM) were introduced for the first time: "Medical gases", "Medicinal sponges", "Implants", "Drops", "Medicinal pencils", "Concentrates", "Lozenges", "Lyophilisates", "Pastilles", "Foams", "Films", "Bars", "Medical chewing gums", "Therapeutic Systems", "Juices", "Medical swabs", "Medical Shampoos", "Elixirs".

According to SP XIV, all dosage forms can be hierarchically classified: by aggregate state, type of a dispersed system, route of administration and type of release (Table 1).

For the first time in SP XIV, the concepts of a medicine "for external use" and "for topical use" are clearly specified. Medicines for external use are dosage forms applied on intact and/or damaged skin, including wound and/or burn surfaces, and/or hair, and/or nails: ointments, solutions, aerosols. Medicines for topical use are dosage forms applied on the mucous membranes, including eye, nasal, rectal, vaginal application, application on the gums, on the oral mucosa, etc., as well as introduction to the external auditory canal: ointments, solutions, aerosols.

The term "for topical use" is used if the dosage form is intended for three or more methods/routes of administration and application related to topical use; if methods/routes are less than three, specify a specific method/route of administration/application (for example, urethral gel).

If the dosage form for topical use in the oral cavity is intended for three or more methods of application (for example, for applying on the oral mucosa, teeth, gums, etc.), use the term "dental"; if there are one or two methods of application, then specify a specific method of application (for example, for applying on the oral mucosa).

*Table 1*

**CLASSIFICATION OF DOSAGE FORMS**

| Level | Criterion of classification | | | | |
|---|---|---|---|---|---|
| **1** | ***Dosage forms by aggregate state*** | | | | |
| | solid | liquid | soft | gas | |
| **2** | ***Dosage forms by type of a dispersed system*** | | | | |
| | homogeneous | heterogeneous | | combined | |
| **3** | ***Dosage forms by a route of administration*** | | | | |
| | for oral use | for external use | for topical use | for parenteral use | for inhalational use |
| **4** | ***Dosage forms by a type of release*** | | | | |
| | immediate release | | modified release | | |

Gels as medicines are designated in three General Monographs: OFS.1.4.1.0003.15 "Eye dosage forms", OFS.1.4.1.0007.15 "Dosage forms for parenteral use", OFS.1.4.1.0008.15 "Ointments".

In accordance with the OFS.1.4.1.0008.15 "Ointments", gels are classified as ointments. Ointments is a soft dosage form intended for application on the skin, wounds and mucous membranes. According to the consistency, ointments are divided into proper ointments, creams, gels, pastes and liniments.

Gels is a soft dosage form as a colloidal dispersion obtained by gelation with the use of special substances. The main applications of gels are indicated, for example, hydrophilic gels for application on the oral mucosa, periodontal gels, gels for application on gums, dental and stomatologic gels are referred to as dosage forms for use in the oral cavity. Gels (usually hydrophilic) can be for oral administration, and can also be used to prepare oral suspensions by dispersing in the appropriate solvent.

There are also gels for administration into the body cavity using appropriate applicators: endocervical gels are intended for administration into the cervical canal, and urethral gels are intended for administration into the urethra. Intestinal gels are gels intended for administration into the intestine (duodenum, small intestine, ileum, colon). Transdermal gels are gels intended to be applied on the skin in order to provide a systemic effect due to the penetration of active substances into the bloodstream through the skin barrier.

Dosage forms for parenteral use include gels for injection and gels for subcutaneous administration, which are hydrophilic gels intended for injection into certain tissues and organs or for injection directly under the skin. Eye dosage forms, for example, include eye gels, which are a sterile dosage form, intended, as a rule, for application on the mucous membrane of the eye (conjunctiva).

Gels of high-molecular carbohydrates (cellulose esters, starch, agar) and proteins (gelatin, collagen, etc.), gels of inorganic substances (bentonite), gels of synthetic high-molecular compounds (polyethylene oxide, polyvinylpyrrolidone, polyacrylamide), etc. are used as bases for production of gels. There are oleogels i.e. gels prepared on bases consisting of a hydrophobic solvent (vaseline or vegetable oil, etc.) and a lipophilic gelling agent (polyethylene wax, colloidal silicon dioxide, aluminum or zinc

soap, etc.); hydrogels i.e. gels prepared on bases consisting of water, a hydrophilic mixed or non-aqueous solvent (glycerol, propylene glycol, ethanol, isopropanol) and a hydrophilic gelling agent (urea, cellulose derivatives, tragacanth, etc.) [5,7].

In accordance with the OFS.1.4.1.0003.15 "Eye dosage forms", soft eye dosage forms for topical use include eye gels. This is a dosage form containing one or more active substances, dissolved or dispersed in a suitable base, intended for application on the eye mucous membrane (conjunctiva), on the eyelids and cornea.

In OFS.1.4.1.0007.15 "Dosage forms for parenteral use" soft dosage forms for parenteral use include gels for injection and gels for subcutaneous administration. Their definition is also provided in the OFS.1.4.1.0008.15 "Ointments".

According to the classification presented in SP XIV (Table 1), gels are classified as soft dosage forms and can be homogeneous, heterogeneous and combined, intended for oral, external, topical and parenteral use with immediate and modified release [1].

The analysis of regulatory legal acts of the Ministry of Health of Russia has shown that the main regulatory document on the classification and nomenclature of dosage forms is the Order of the Ministry of Health of the Russian Federation of July 27, 2016 No. 538н "On approval of the List of dosage forms of medicines for medical use" (hereinafter – Order No. 538н).

Order No. 538H includes [6]:

- 32 basic dosage forms (aerosol, medical gas, gel, granules, dispersion, liquid, implant, drops, capsules, concentrate, cream, liniment, lyophilizate, ointment, oil, tincture, sticks, paste, foam, patches, films, powder, solution, syrup, system, spray, suppositories, suspension, tablets, medical swabs, extract, emulsion);
- 17 other dosage forms (medicinal sponge, pills, medicinal pencil, pharmaceutical nail polish, lozenges, pastilles, medicinal plates, bars, medical chewing gum, medicinal absorbable wipes, medicinal shampoo, elixir, cut-pressed granules, infusions and decoctions, tablets, teas, juice);
- 6 special forms of homeopathic medicines (homeopathic opodeldok, homeopathic impregnated pills, homeopathic mixtures, homeopathic triturations, homeopathic solutions and liquid dilutions, homeopathic matrix tinctures);
- 2 types of solvents (solvent for preparation of dosage forms for injection, solvent for preparation of vaccines for injection).

Order No.538n not only describes the dosage forms, but also specifies their variants. For example, a gel is a soft dosage form represented by colloid dispersion obtained by gelation using special substances, which fully coincides with the definition presented in SP XIV. The order specifies 20 types of gels depending on the place of application with their description (Table 2).

At the second stage of the study, the nomenclature of gels included in the State register of medicines was reviewed. Content analysis of the product range of the pharmaceutical market of medicines represented by the gel dosage form showed that the product range is represented by 205 trade names (as of 30.11.2019) [2].

Analysis of data from the State register of medicines under the section "Form of presentation" showed that the range is represented by 16 varieties of gels, among which the largest share is taken by the dosage form of gels for external use – 77% (158 TN out of 205 TN) (Table 3).

Study of the range of gels on the basis of "Pharmacotherapeutic group" has shown that, according to the State register of medicines of the Russian Federation, gels are represented by 33 pharmacotherapeutic groups, among which the following groups have the largest relative share: "Non-steroidal anti-inflammatory medicines" (34%, 70/205 TN), "Anticoagulants of indirect action" (10%, 21/205 TN), "Venotonic

*Table 2*

## CLASSIFICATION OF GELS DEPENDING ON THE PLACE OF APPLICATION

| № | Name of a dosage form | Description of of a dosage form |
|---|---|---|
| 1. | Vaginal gel | Gel intended for injection into the vagina to provide local action |
| 2. | Ophthalmic gel | Sterile gel intended for application on the eye mucous membrane (conjunctiva) |
| 3. | Injection gel | Sterile hydrophilic gel intended for injection into certain tissues and organs |
| 4. | Topically-applied gel | Gel intended for topical use |
| 5. | Gum paint-on gel | Gel intended for application on the gums in order to provide local action |
| 6. | Gel for application on the oral mucosa (gel for the oral mucosa) | Hydrophilic gel intended for application on the oral mucosa in order to provide local action |
| 7. | Gel for cutaneous use | Gel intended for cutaneous use |
| 8. | Gel subcutaneous administration | Sterile hydrophilic gel intended to be injected directly under the skin |
| 9. | Gel for preparation of suspension for oral use (gel for suspension for oral administration) | Gel intended for preparation of a suspension for oral administration by dispersion in an appropriate solvent |
| 10. | Oral gel | Gel (usually hydrophilic) intended for oral administration |
| 11. | Dental gel | Hydrophilic gel intended for application on the teeth and/or gums by rubbing in |
| 12. | Intestinal gel | Gel intended for injection into the intestine (duodenum, small intestine, ileum, colon) using an appropriate device |
| 13. | Nasal gel | Gel intended for injection into the nasal cavity or application on the nasal mucosa |
| 14. | Periodontal gel | Gel intended for injection into the pocket between the tooth and gum |
| 15. | Rectal gel | Gel intended for injection into the rectum to provide local action |
| 16. | Stomatological gel | Gel intended for three or more routes of administration (for example, for periodontal application on the teeth, gums, and oral mucosa) |
| 17. | Transdermal gel | Gel intended for application on the skin in order to provide a systemic effect due to the penetration of active substances into the bloodstream through the skin barrier |
| 18. | Urethral gel | Gel intended for injection into the urethra using an appropriate applicator |
| 19. | Ear gel | Gel intended for introduction into the external auditory canal, if necessary with a tampon impregnated with it |
| 20. | Endocervical gel | Gel intended for injection into the cervical canal using an appropriate applicator |

and angioprotective agents" (8%, 16/205 TN) (Fig. 1). Group 'Others" combined the gels of the following pharmacotherapeutic groups: "Decongestants", "Rosacea treatment agent", "Anti-Inflammatory agent", "Homeopathic agent", "Antiparkinsonian agent", "Antifoaming agent", "Antiviral agent", "Psoriasis treatment agent", "Local irritants", "Keratoprotective agent", "Hypercicatrizant", "Glucocorticoid for topical use" and others.

The analysis of gels on the basis of "Country-manufacturer" showed that the State register of medicines of the Russian Federation contains a range of gels from 25 manufacturing countries, among which the leaders in the number of trade names produced in this country, namely: Russia (40%, 83/205 TN), Germany (11%, 23/205 TN), India (10%, 21/205 TN) (Fig. 2). The "Others" group includes the following countries-manufacturers: Finland, Hungary, Austria, Estonia, Latvia, Slovenia, Turkey, Spain, Norway, Ireland, Denmark, Bosnia and Herzegovina, and the United States.

Study of the range of gels on the basis of *"Referentiality"* has shown that the number of trade names of gels registered in the State register of medicines of the Russian Federation with the attribute *"Reference"* is 46% of the total number of trade names, *"Interchangeable"* – 53% (Fig. 3.). For 3 TN this attribute is not specified in the State register of medicines of the Russian Federation.

*Table 3*

**DISTRIBUTION OF THE RANGE OF GELS IN ACCORDANCE WITH THE STATE REGISTER OF MEDICINES**

| № | Varieties of gels | Number of trade names | Example |
|---|---|---|---|
| 1. | For external use | 162 | Androgel® |
| 2. | Intestinal | 1 | Duodopa® |
| 3. | For application on gums (paediatric) | 1 | Lidocain + Cetylpyridinium chloride |
| 4. | Dental | 8 | Cholisal® |
| 5. | Ophthalmic | 4 | Oftagel® |
| 6. | Nasal | 3 | Rinorus® |
| 7. | Vaginal | 7 | Krinon® |
| 8. | Transdermal | 1 | Oestrogel® |
| 9. | For oral use | 3 | Pepsan-R® |
| 10. | Intracervical | 2 | Prostenongel® |
| 11. | Topical | 9 | Kamistad® |
| 12. | For external use homeopathic | 1 | Arnigel® |
| 13. | For topical administration | 5 | Panavir® |
| 14. | For external and topical use | 1 | Viferon® |
| 15. | For rectal and external use | 1 | Fissario |
| 16. | For preparation of suspension for oral use | 1 | Enterosgel® |

## CONCLUSION

As a result of the content analysis of data from the RF State Pharmacopoeia XIV edition (SP XIV), regulatory legal acts of the Ministry of Health of the Russian Federation and the State register of medicines, it was found that gels are a popular dosage form in the development and production of modern medicines, the range of the domestic market of gels includes 205 trade names of medicines, which are represented by 16 varieties.

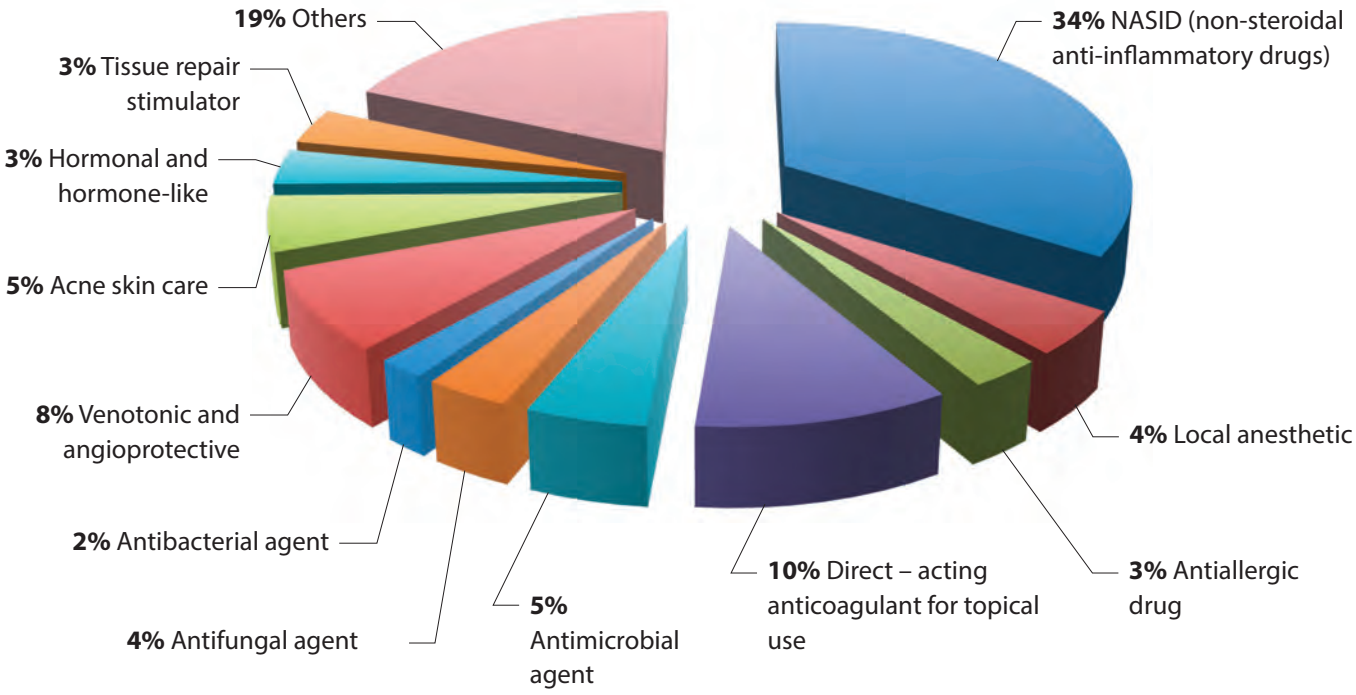


**FIG. 1.** *Percentage of registered gels by "Pharmacotherapeutic group" in the total number of trade names*

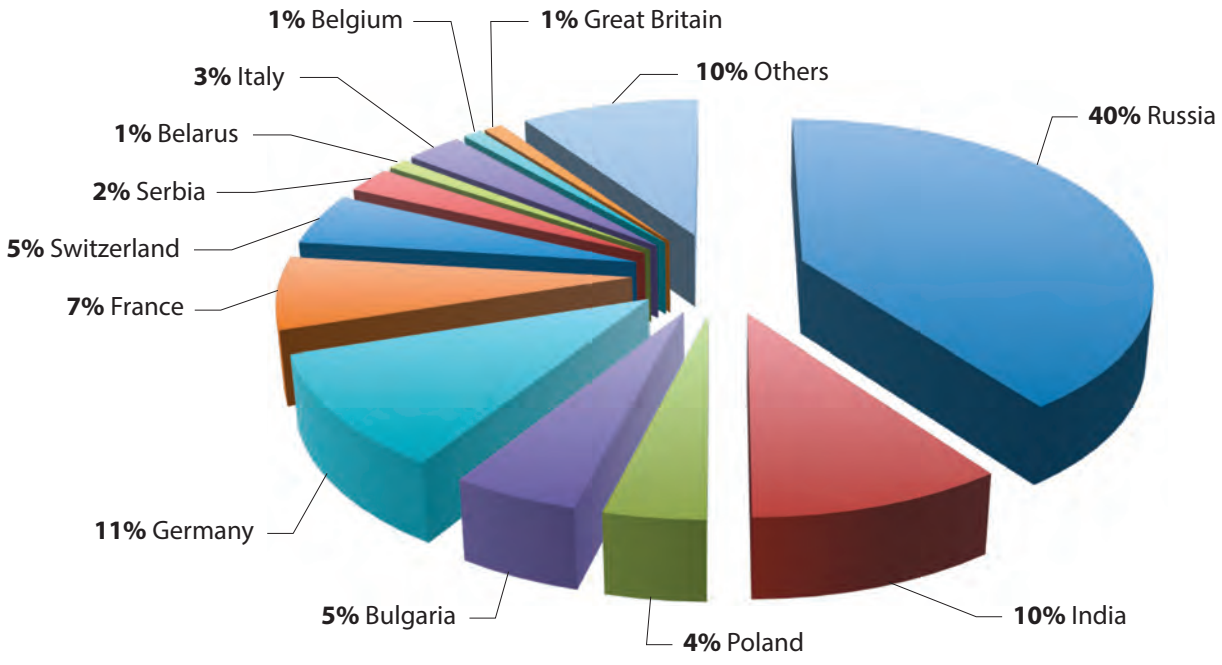


**FIG. 2.** *Percentage of registered gels by "Country-manufacturer" in the total number of trade names*

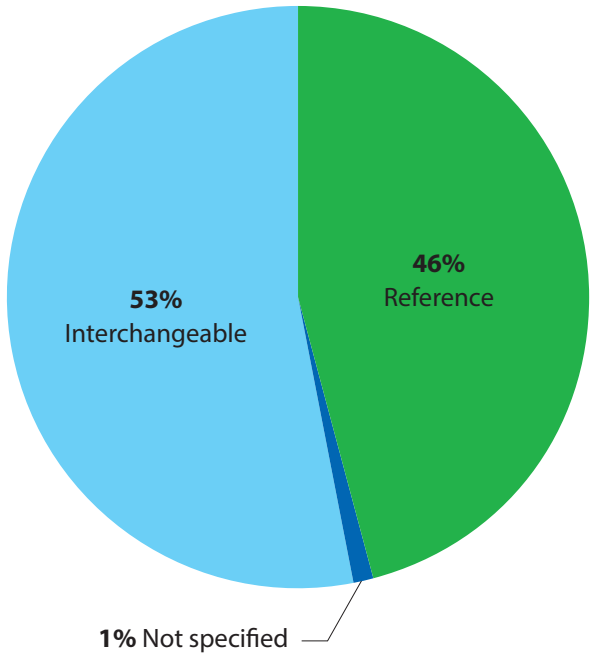


**FIG. 3.** *Percentage of registered gels by "Referentiality" in the total number of trade names*

It was found that the range of gels is diversified by pharmacological groups (33), among which the largest relative share is occupied by the following groups: "Non-steroidal anti-inflammatory medicines", "Anticoagulants", as well as "Venotonic and angioprotective agents".

A study of the range of gels according to countries of origin showed that the largest number of registered gels was produced in Russia, Germany and India. Most of the reference products in the form of gels are represented by imported products, and in Russia, to a greater extent, the interchangeable medicines are produced. Some gels are presented only by foreign manufacturers.

Based on the above, one of the most relevant areas of the pharmaceutical industry is the development of gels for domestic production for the purpose of import substitution, as well as the development of unique and highly effective medicines in the form of gels that have no analogues abroad.

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# REGIONAL FEATURES OF PRICING IN THE IN COMPULSORY HEALTH INSURANSE ON THE EXAMPLE OF USING THE MEDICAL AND ECONOMIC STANDARD OF FEMORAL NECK FRACTURE (WITH THE INSTALATION OF AN ENDOPROSTHESIS)

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*Based on the analysis of normative legal documents, scientific publications and research, the methodology for constructing a tariff based on a medical and economic standard for accounting and payment for specialized medical care provided in a round-the-clock hospital on the profile "traumatology and orthopedics" is considered. In the framework of the completed case of treatment of femoral neck fracture (ICD-10 code: S72.0) in the compulsory health insurance system, a comparative analysis of the cost of the tariff of the medical and economic standard was made with the cost of a similar diagnosis-related group (st 29.013) used for accounting and payment for endoprosthetics for femoral neck fractures.*

**Keywords:** medical and economic standard, diagnosis-related group, tariff calculation, compulsory health insurance

"Diagnosis-related group" (hereinafter as DRG) and "Medical and Economic Standard" (hereinafter as MES) as concepts used in the Russian system of organization and financing of health care are differentiated as follows: DRG is a group of diseases that is separated in each class of the International classification of diseases and united by a similar level of medical care costs.

MES is a standard that defines the scope of diagnostic and therapeutic procedures, medical treatment, a list of medical devices, as well as the amount of appropriate financial support [1–3].

There are still many unresolved problems in implementing the payment models [3,4]

## MATERIALS AND METHODS

In a number of regions of the Russian Federation (e.g. Moscow) the accounting and payment of special medical care provided in the round-the-clock and day hospitals, are conducted in the framework of completed or aborted treatment cases in accordance with the Programme of state guarantees, as well as procedures and standards of healthcare delivery, which, together with the medico-economic standards are used to calculate the tariffs for such complex medical services. The tariffs for medical services in the CHI system are calculated in accordance with the Methodology for calculating the tariffs for medical care under

compulsory health insurance, specified by section XI of the Rules of compulsory health insurance, approved by Order of the Ministry of Health of the Russian Federation dated 28.02.2019 No.108N [5].

In accordance with clause 7 of art. 35 of Federal law No. 326-FZ of 29.11.2010, tariffs for payment of medical care include expenses for wages, charges on payroll, other payments, purchase of medicines, consumables, food, hospital linen, armamentarium, reagents and chemicals, other inventory, expenses for payment for laboratory tests and instrumental methods of diagnosis conducted in other institutions (if the medical organization does not have laboratory and diagnostic equipment), catering (if there is no organized food in a healthcare organization), expenses for communication services, transport services, utilities, works and services for the maintenance of property, expenses for rent for the use of property, payment for software and other services, social security of employees of medical organizations specified by the legislation of the Russian Federation, other expenses, expenses for purchase of fixed assets (equipment, production and household inventory) at cost up to one hundred thousand rubles per unit [6].

In accordance with p. 5.1.1 of the Tariff agreement for payment of medical care provided under the territorial program of the CHI of the city of Moscow, approved annually by the Government of Moscow, the proposals for making amendments to the list of simple, complex and comprehensive medical services by the types and profiles of medical care and indexation of tariffs for payment of medical care can be made by the parties to the Tariff agreement to the Commission for development of the CHI territorial program in accordance with the Procedure of sending the proposals for accounting of medical care to the working group on calculation and adjustment of tariffs in the compulsory health insurance system [7].

The calculation of tariffs for medical care includes the costs of medical organizations directly related to the provision of the medical service and consumed in the process of providing it, as well as the costs necessary to ensure the activities of medical organizations as a whole, but not consumed directly in the process of providing the medical service:

- the cost of labor and charges on payroll are determined proceeding from the demand for the number of personnel taking direct part in providing of a medical service, and the average staff time for provision of the services (in the calculation of the tariff for a simple service) or services (when calculating the tariff for complex or comprehensive service) (section 1 of the process flow diagram), in accordance with the current system of remuneration [9];
- the cost of inventories (medications, necessarily used in the provision of a medical service – Section 2 of the process flow diagram; medical devices and supplies necessarily used in the provision of a medical service – Section 3 of the process flow diagram) are calculated based on the average multiplicity factor of application and the average application frequency on the basis of normative consumption volumes and actual volumes of consumption of inventories for last years;
- costs necessary to support the activities of the medical organization as a whole, but not consumed directly in the provision of a medical service, are calculated in proportion to the amount of time spent on providing the medical service (outpatient care) or in proportion to the number of bed-days (patient-days) provided in the process flow diagram for the comprehensive medical service (inpatient and in a day hospital), based on the data of the reporting form 14-F (CHI) "Data on receipt and expenditure of CHI funds by health organizations" [10].

Tariffs for payment for high-tech medical care in the CHI system of the city of Moscow for all medical organizations that provide the high-tech

medical care within the framework of the basic compulsory health insurance program, regardless of their organizational and legal form and departmental affiliation, are specified:

- in accordance with the standards of financial costs per unit of the high-tech medical care volume approved by the decree of the Government of the Russian Federation under the State Guarantee of Free Medical Care to the Citizens of the Russian Federation;
- based on Appendix No. 10 to the letter of the Ministry of Health of Russia [11];
- taking into account the application of the coefficient of differentiation for the city of Moscow for 2019, calculated in accordance with the decree of the Government of the Russian Federation [12].

According to the method of forming the tariffs for medical care in the CHI, the structure of tariffs under the basic program of compulsory health insurance includes expenses for wages and charges on payroll, the purchase of medicines and dressings, consumables, hospital linen, other inventory, food costs [13].

As an illustration, we present a scheme for calculating the costs in the tariff structure:

1. Calculation of personnel labour costs.

2. Medications = Maximum price with wholesale surcharge and VAT, RUB / Quantity in consumer package / Dosage form, dosage × Frequency of delivery × Multiplicity of delivery × Qty.

3. Consumables = Price per package, RUB / Qty per package × Frequency of use × Multiplicity of use × Qty per a course.

4. Catering = Average frequency of use × Number of bed days × Cost of 1 bed day according to the diet.

5. Hospital linen. The cost of hospital linen is reduced to a standard unit based on the service life of this linen.

6. Other expenses = Amount of other expenses (according to form no. 14-F (CHI) for the 1st half year of 20___), RUB / Number of bed days for the 1st half year of 20___ (according to the personalized database of CHI AP AIS) × Number of days of treatment.

## RESULTS AND DISCUSSIONS

Here is one of the examples of successful implementation in the Russian Federation (Moscow) of the medical and economic standard (MES) for endoprosthesis replacement in case of femoral neck fracture (ICD-10 code: S72.0), developed by the Department of Health of the city of Moscow (with the involvement of the chief specialist – traumatologist-orthopedist). This medical and economic

*Table 1*

**METHODOLOGY FOR CALCULATING THE PERSONNEL LABOUR COSTS**

| Position | Average official salary per month, including charges on payroll (rubles) | Month working time fund (min) | Standard time for providing the medical service (min) | Personnel labour cost (rubles) (5) = (2)/ (3) × (4) |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| 1. | | | | |
| 2. | | | | |
| – | | | | |
| Total | x | x | x | |

standard, developed on the basis of the standard of medical care, took into account current trends in the provision of medical care, detailing by items allowed for more accurate accounting of medical care costs and, accordingly, optimally calculating the tariff for medical services for endoprosthesis replacement in case of femoral neck fracture, taking into account the costs of medical personnel labor directly involved in providing medical care within a specific comprehensive medical service, as well as provide for the costs of new medicines, supplies and depreciation of medical equipment based on new technologies.

The MES process flow diagram developed by the chief specialist of the Moscow Department of Health, based on the standard of medical care approved by the Ministry of Health of the Russian Federation is used in the system of compulsory health insurance of the city of Moscow to calculate the tariff for a comprehensive medical service: "Femoral neck fracture (with the installation of an endoprosthesis)" for accounting and payment for specialized medical care provided in a round-the-clock hospital in the profile "Traumatology and orthopedics" as part of a completed case of treatment of femoral neck fractures (ICD-10 code: S72.0). From 01.01.2019, this service was introduced into the Tariff agreement by the decision of the Commission for development of the territorial program of compulsory health insurance of the city of Moscow.

The cost of the tariff for the developed medical and economic standard is higher than the cost of a similar diagnosis-related group (st 29.013). In this case, the development of medical and economic standard for the level of the region of the Russian Federation allowed to accurately calculate the tariff for this service using the process flow diagram and considering the cost and modern implants, as well as to provide phase I of rehabilitation of the patient included in the structure of the finished case of treatment.

According to the Tariff agreement for the payment of medical care provided under the Territorial compulsory health insurance program of the Moscow region for 2020, the cost of endoprosthesis for femoral neck fractures (S72.0) was 74,962.94 rubles using the base rate of 23,797. 76 rubles and the cost-intensity coefficient of 3.15, without taking into account

*Table 2*

**EXAMPLE OF THE STRUCTURE OF MES TARIFF "FEMORAL NECK FRACTURE (WITH THE INSTALLATION OF AN ENDOPROSTHESIS). ICD-10 CODE: S72. 0**

| No. | Name of item | Value, ruble |
|---|---|---|
| 1 | Labour costs | (20.48%) N1 |
| 2 | Charges (30,2%) | (6.18%) N2 |
| 3 | Medications | (2.83%) N3 |
| 4 | Consumables | (63.47%) N4 |
| 5 | Hospital linen wear | (0.10%) N5 |
| 6 | Catering | (1.17%) N6 |
| 7 | Other expenses | (5.78%) N7 |
| 8 | Total | N1+N2+N3+N4+N5+N6+N7=N8 |
| | Number of bed-days | 10x |
| 9 | Average cost of a bed-day, rubles | N8/x |

the management coefficient recommended by the Federal Compulsory Health Insurance Fund. Studies of site: www.zakupki.gov.ru have shown that currently the average cost of an endoprosthesis is 86,355 rubles for purchases [14].

An example of the structure of the MES tariff "Femoral neck fracture (with the installation of an endoprosthesis). ICD-10 code: S72. 0

Common points in the development of the MES (hip replacement) and a similar DRG for hip replacement are that "When forming the diagnosis-related groups within the framework of methodological recommendations for formation of methods of payment for medical care under the State guarantees program (SGP) based on groups of diseases, including diagnosis-related groups of diseases, the draft orders on Approval of medical care standards were used. Subsequently, when developing "Recommendations on ways to pay for specialized medical care in inpatient settings and in day hospitals based on groups of diseases, including diagnosis-related groups (DRG) and clinical-profile groups (CPG) at the expense of the compulsory health insurance system", all standards of medical care developed by the Ministry of Health of the Russian Federation were taken into account. The recommendations indicate that the calculation of the coefficient of relative cost intensity of DRG and CPG was carried out taking into account the cost of medical care standards specified by the Ministry of Health of the Russian Federation, as well as the cost of a set of medical services provided for diseases for which standards were not set. When calculating coefficients for diseases that do not have standards, the actual expenses of medical organizations for providing medical care for these diseases were taken into account" [15].

## CONCLUSION

The common points in MES and DRG are that the structure and content of medical and economic standards and DRG groups is based on standards of medical care, taking into account the codes of diagnoses of diseases in the International classification of diseases (ICD-10).

Calculations of the tariff using the medical and economic standard for the example of hip replacement in case of the femoral neck fractures allow you to most accurately assess the working hours of medical personnel and the costs of providing the medical care taking into account the cost of modern implants, as well as to consider phase I of rehabilitation of the patient included in the structure of the finished case of treatment (phase I of rehabilitation in DRGs is not included).

It should be noted that the difference in the increase in the MES tariff relative to the corresponding DRG group for hip replacement in case of the femoral neck fractures (ICD-10 code: S72. 0) allows you to compensate for the expenses of the medical organization for medical care, taking into account the constantly growing cost of implants (endoprostheses), selected individually (models: single-pole, double-pole, metal, metal-ceramic) under the clinical indications and depending on the age of the patient, as well as the use of other consumables and medications, inclusion of the first phase of necessary medical rehabilitation (this is the beginning of movements of the operated limb, physical therapy, massage, training to walk on crutches in the first days after surgery).

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