Federal Technical Regulation and Metrology Agency (Rosstandart)

Technical
Committee TC 450
"Pharmaceuticals"

PHARMACEUTICALS QUALITY ASSURANCE ISSUES

ISSN: 2309-6039
Journal On-line version: **www.humanhealth.ru**

№4 (26) 2019

Dear friends and colleagues!

In 2018 we turned 5 years old. This is the first major milestone for a new scientific and practical journal, which we passed together with our Editors and Partners. During the reporting period, we were able to enter the list of periodicals recommended by the Higher Attestation Commission of the Ministry of Science and Higher Education of the Russian Federation. We are reflected in the RSCI, as well as scrupulously working for future indexing in well-known international databases. Starting in 2019, it is scheduled to release an English-language version and to involve more European specialists. Weare looking to cooperate with all interested parties for productive interaction and filling the publication with content.

Best regards,

Chief Editor, Professor

A.A. Markaryan

Registered with the Federal service for supervision of communications, information technology and public communications (Roskomnadzor)

ISSN №2309-6039

PI № FS 77-53661
dated April 10, 2013

The Journal is included in the List of peer – reviewed scientific publications, where the main scientific results of thesis for Candidate's Degree, Doctor's Degree should be published according to the letter from the Ministry of Education and Science dated 01.12.2015 № 13-6518.

Address of the editors office: 9, Sharikopodshipnokovskaya str., Moscow, 115088 ROOI "Human Health"
Proof-reader:
Didevich A.V.
Page Make-up:
Ermakova E.V.

Tel.: 8 (495) 674–65–22
8 (926) 917 61 71
E-mail: journal@humanhealth.ru
www.humanhealth.ru

Index in the catalog of Federal Agency on Press and Mass Communications (Rospechat) (Publications of scientific and technical information bodies) in Russia: 57958

Publishing company
ROOI "Human Health"
E-mail: izdat@humanhealth.ru

Printing support:типография
Printing company
"Moscow Print Yard"
Tel.: (495) 7816411, (495) 5455812,
www.printyard.ru

Circulation 3000 copies
Order №1718

ISSN 2309-6039 №4 (26) 2019

PHARMACEUTICALS QUALITY ASSURANCE ISSUES

JOURNAL OF PHARMACEUTICALS QUALITY ASSURANCE ISSUES

Journal of Research and Practice
Central peer-reviewed publication
A Quarterly Edition. Published since August 2013

CONTENTS

UDK 582.681:581.2:57.082

MORPHOLOGICAL AND ANATOMICAL STUDY OF MOLDAVIAN DRAGONHEAD HERB (DRACOCEPHALUM MOLDAVICA L.), INTRODUCED IN VILAR FSBSI

E.A. Konyaeva, *Senior Researcher of the Department of Phytochemistry and Standardization of "All-Russian Research Scientific Institute of Medicinal and Aromatic Plants" FSBI (VILAR FSBI), Moscow*
O.G. Alentyeva, *Senior Researcher of the Department of Phytochemistry and Standardization of "All-Russian Research Scientific Institute of Medicinal and Aromatic Plants" FSBI (VILAR FSBI), Moscow, oksal60@mail.ru*

Morphological and anatomical study of a new promising domestic type of medicinal plant raw materials – Moldavian Dragonhead herb – was made according to the thematical schedule of the R&D of VILAR FSBI. As a result of the research, morphological and anatomical features were identified that have diagnostic value and allow to establish the authenticity of this type of medicinal plant raw materials. The obtained data will be used in development of the draft regulatory documentation for a new type of medicinal plant raw materials – Moldavian Dragonhead herb.

Key words: Moldavian Dragonhead, herb, morphological and anatomical features

Moldavian Dragonhead has been known in Europe since the second half of the XVI century under the name "Turkish Melissa". For a long time, the Moldavian Dragonhead has been used in folk medicine in many countries of the world as an anti-inflammatory, wound-healing, expectorant and tranquilizing agent [1,2].

Moldavian Dragonhead – *Dracocephalum moldavica* L., family of Lamiaceae – annual herbaceous plant of 15–50 cm height with a straight branched stem which is puberulent near the root. Leaves on short petioles are pubescent with short hairs, oblong-ovate or oblong-lanceolate, blunt-toothed or deep-serrate at the edges. Flowers are on short pedicels in six-flowered false whorls. It blooms in June – September. Moldavian Dragonhead in nature does not form thickets, is cultivated as an essential oil and honey plant [2,3].

Moldavian Dragonhead in the Russian Federation is not a Pharmacopoeia plant and is not used by official medicine.

Currently, employees of VILAR FSBI are studying the possibility of using the aerial part of the Moldavian Dragonhead as a raw material for development of a new medicinal drug. In this regard, a need to standardize this type of raw material occurred. One of the stages of standardization of medicinal plant raw materials is the establishment of morphological and anatomical characteristics of raw materials as one of the features of its authenticity.

The purpose of this work was to study the morphological and anatomical structure of aerial organs of Moldavian Dragonhead (leaves, stems, flowers) and identify their diagnostic markers, which will be used to develop a pharmacopoeial monograph on new medicinal raw materials.

MATERIALS AND METHODS

A study object is Moldavian Dragonhead herb, grown in the North-Caucasian branch of the

VILAR FSBI and harvested during the flowering phase of the plant in 2018.

Morphological and anatomical study of raw materials was made according to the general pharmacopoeial monographs of the State Pharmacopoeia of the Russian Federation, XIV edition: OFS.1.5.1.0002.15 "Herbs" [4] and OFS.1.5.3.0003.15 "Technique of microscopic and microchemical studies of medicinal plant raw materials and herbal medicinal products" [5]. Microscope slides were studied using a biological microscope "Altami BIO 2 LED" with a digital ocular USB camera 3.1 Mpix (Russia). The photos were processed on a computer in Adobe Photoshop 7.0.

RESULTS AND DISCUSSION

Morphological study. Macroscopic examination revealed morphological characters of the Moldavian Dragonhead herb. It is established that the medicinal raw materials are whole or partially crushed frondose stems with buds or flowers, pieces of stems, some leaves, buds, flowers and immature seeds. The stems are branched, four-sided, longitudinally grooved, slightly pubescent, with a loose white core, hollow in the lower part. The leaves are opposite, on short petioles, thin, oblong-ovate or oblong-lanceolate, toothed at the edges, truncated at the base or wedge-narrowed, pubescent with short hairs. Buds and flowers on short pedicels are collected in false whorled inflorescences, located in the axils of the upper leaves, which are connivent in the upper part of the stem and spaced in the lower part; bracts are oblong-wedgy, with thin awned points along the edges in the lower half. The flower calyx is bilabiate; the upper lip is incised into three broadly ovate awl-pointed teeth, the lower one is with two oblong-lanceolate awl-pointed teeth. Corolla is bilabiate; upper lip is unifoliate, lower lip is trilobate. There are four stamens, two of which are shorter than the others. Seeds are ovate, three-edged. Leaf color is gray-green or green; color of stems is grayish-green, yellowish-green, greenish-violet or violet; color of flower cups is grayish – green, green or greenish- violet; color of corollas is medium blue or blue-violet. The smell is faint, lemon. The taste of water extraction is slightly bitter.

Anatomic study. Microscopic examination revealed anatomical characters of the Moldavian Dragonhead herb. When viewing a leaf from the surface (Fig. 1–6) the cells of upper epidermis with slightly -flexuose walls and the smaller cells of lower epidermis with highly- flexuose walls can be seen. Stomatas on both sides of the leaf are numerous, oval, surrounded by two cells of the epidermis, the adjacent walls of which are perpendicular to the stomatal slit (diacytic type), there are stomatas of the anomocytic type. The leaf is pubescent on both sides with simple and glandular hairs. Simple straight and curved hairs with warty surface are made of 1–3-cells. The glandular hairs are small, consisting of a double-celled head and a very short one-celled leg. Around the point of attachment of the hairs and above the veins the cuticle folding is sometimes noticeable. On the lower side of the leaf, there are essential oil glands with 8–16 excretory cells located radially; in the center, a round leg of the gland is visible.

The epidermis of the stem (Fig. 7, 8) consists of quadrangular – hexangular cells with straight, often with beaded- thickened walls. Stomatas of diacytic type are with a stomatal cleft oriented along the length of the stem. In some places, near the stomata, the radiant folding of the cuticle is visible. The hairs are simple and glandular of the same structure as on the leaf. After breaking off the simple hairs, on the stem epidermis the clearly visible rollers remains often with a serrated edge of the shell; glandular hairs leave small rollers. Essential oil glands are rare.

When viewing the calyx from the surface (Fig. 9–13) the epidermis cells with highly-

flexuose walls, cuticle folding, especially at the base of the calyx, and stomata of diacytic type are visible. Along the edge of the calyx, as well as at the base of the teeth and along the veins there are 1–3-cell simple hairs, also sometimes there are glandular hairs, the same as on the leaf and stem. In addition, there are simple single-celled thick-walled retort-like hairs and single-celled thin-walled hairs with smooth and slightly bumpy surface. The essential oil glands are numerous.

The corolla epidermis (Fig. 14–18) on the inner side and along the edge is covered with papillary outgrowths, the cells of the corolla epidermis on the outer side are highly- flexuose. Corolla is pubescent with long narrow simple thin-walled subtly-warty 1–5-celled hairs, sometimes with some collapsed cells, there are also hairs with a rough warty surface; glandular hairs and essential oil glands which are characteristic of this raw material. In addition, there are glandular hairs with a long 2–3-celled leg and a slightly elongated 1–2-celled head.

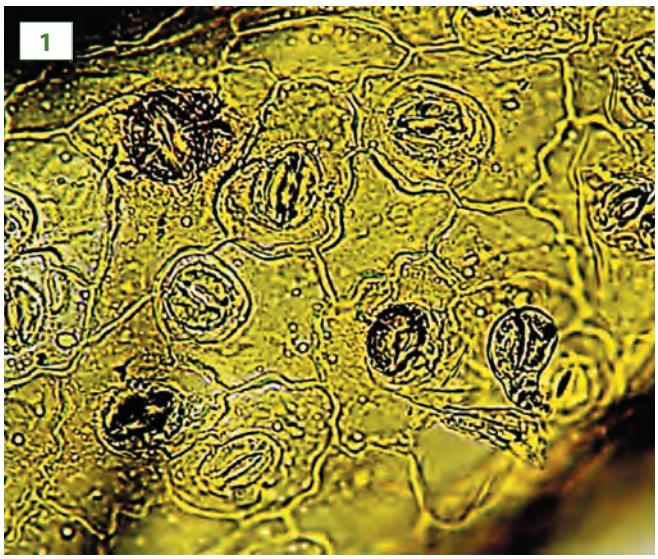
CONCLUSION

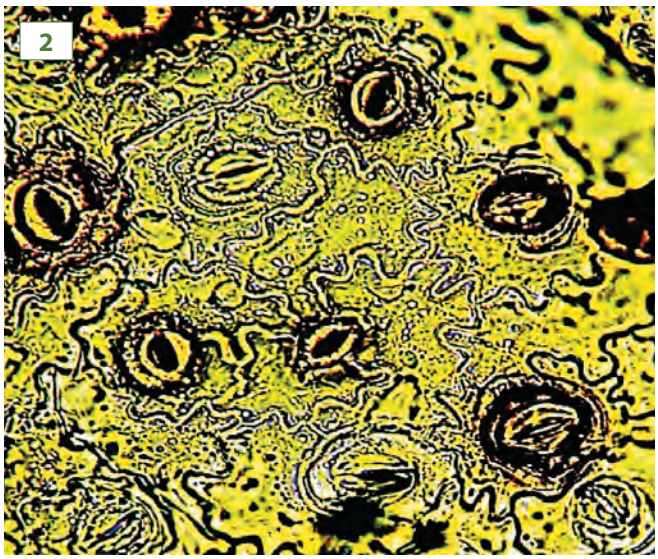
On the basis of the study of Moldavian Dragonhead herb the morphological and anatomical features were identified that have diagnostic value and allow to establish the authenticity of the medicinal plant raw materials which will be included in regulatory documentation on a new and promising type of domestic medicinal plant raw material – Moldavian Dragonhead herb.

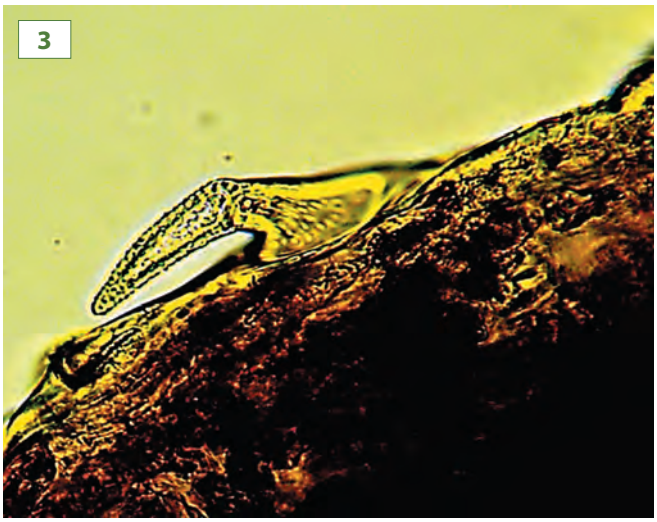
REFERENCES

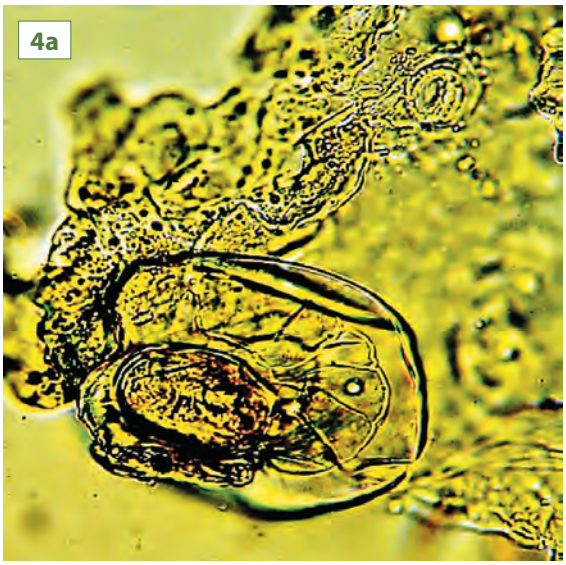
1. *Kubiak M. Pszczelmic moldavski (Dracocephalum moldavica L.) jako roslina cutralova // Act. Pol. Pharm., 1959, 16, s. 144–156.*
2. *Nikitina A. S., Popova O. I. Polysaccharides of Moldavian dragonhead, cultivated in the conditions of Stavropol territory // Journal of VSU. Series "Chemistry, biology, pharmacy". – 2006. – № 2. – pp. 327–329.*

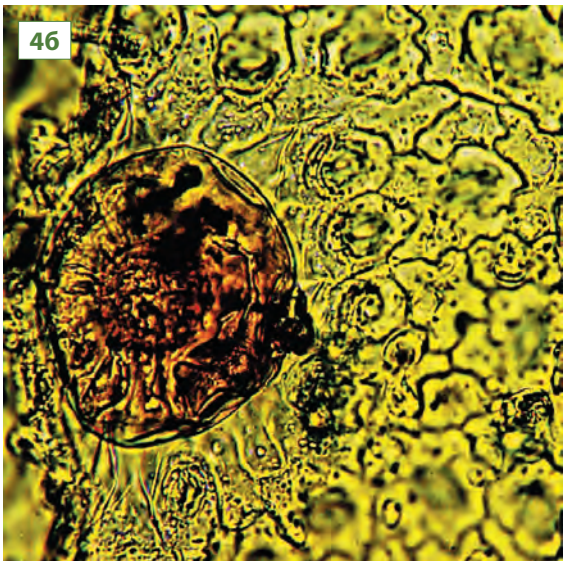
FIG. *Diagnostic signs of the anatomical structure of the Moldavian Dragonhead herb (1–9, 12–18 – X400; 10, 11 – X200): 1 – upper leaf epidermis; 2 – lower leaf epidermis; 3 – simple hair on the edge of the leaf; 4a, 4b – essential oil glands; 5a, 5b – simple hairs on the leaf vein; 6 – hairs on the leaf epidermis; 7 – stem epidermis; 8 – hairs on the stem epidermis; 9 – calyx epidermis; 10 – hairs on the vein of the calyx; 11 – glands; 12 – hair on the edge of serration of the calyx; 13 – hairs on epidermis of the calyx: a – single – celled thin-walled, b-retort-shaped; 14- epidermis of the corolla on the outside; 15 – epidermis of the corolla on the inside; 16 – glandular hair; 17 – essential oil gland; 18 – simple hairs: a – with subtly-warty surface, b – with coarse-warty surface*





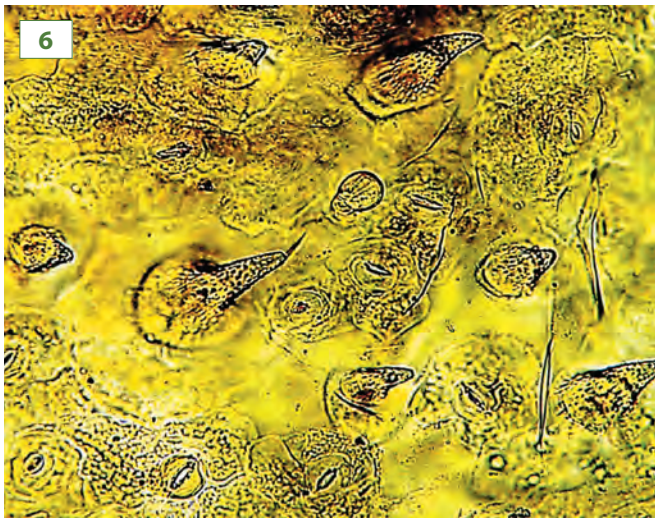

3

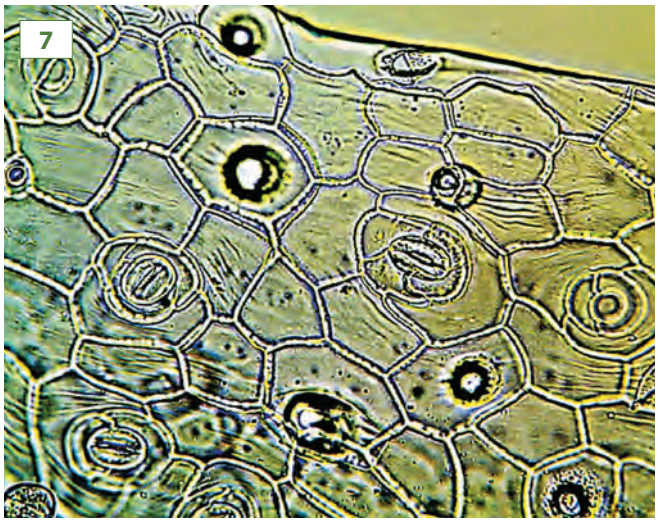

4а

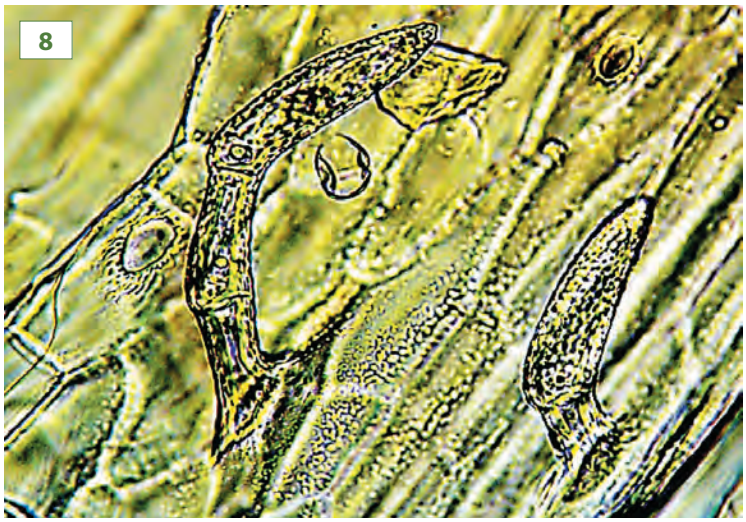

4б

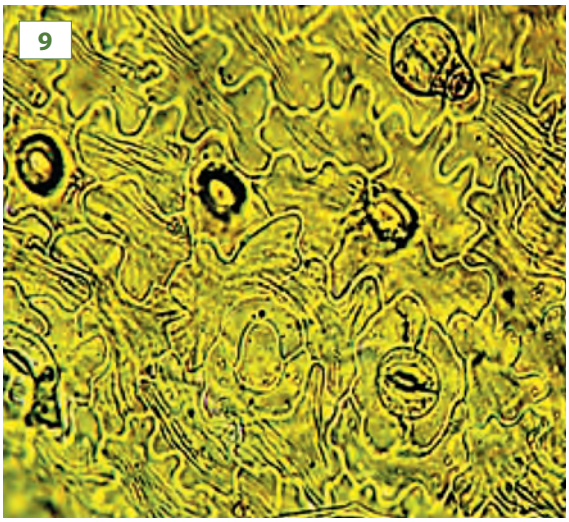

5а

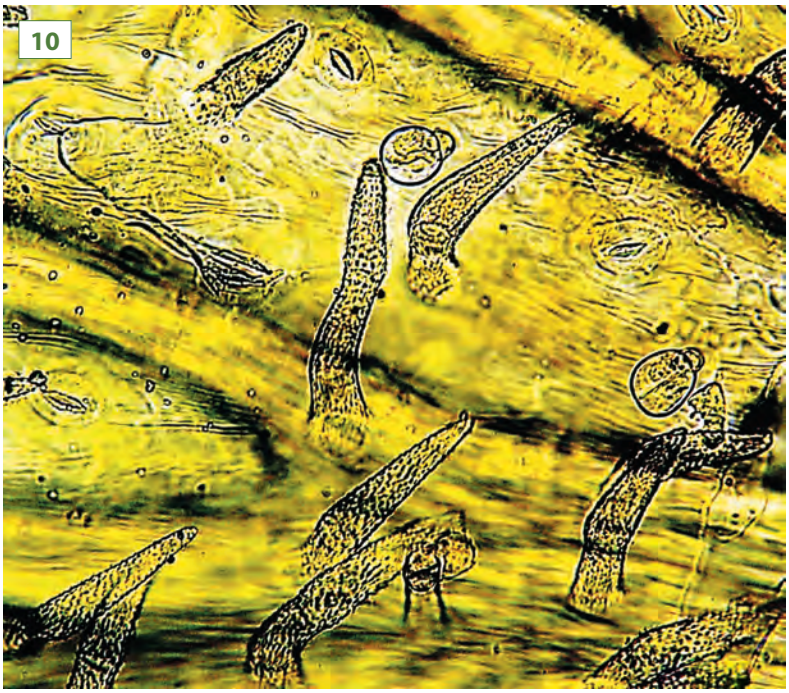

5б

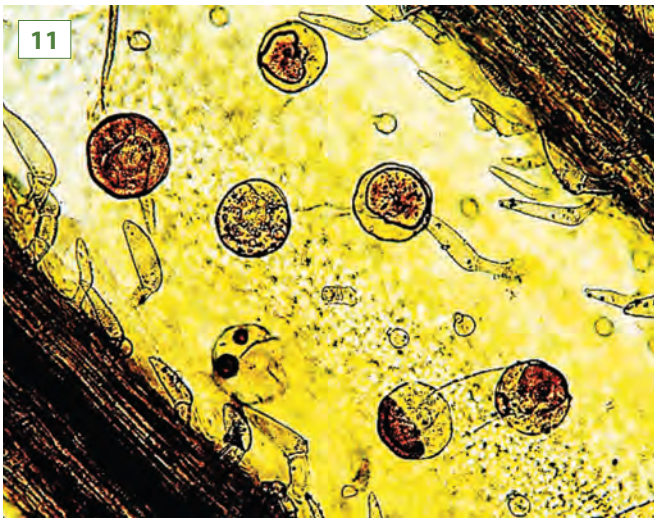

6

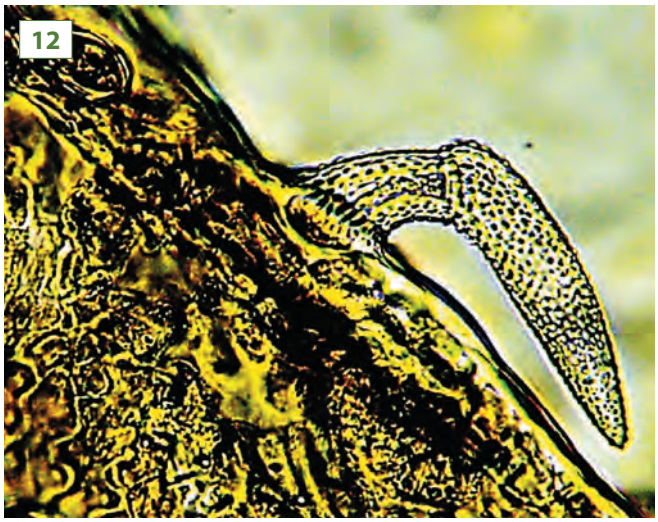

7

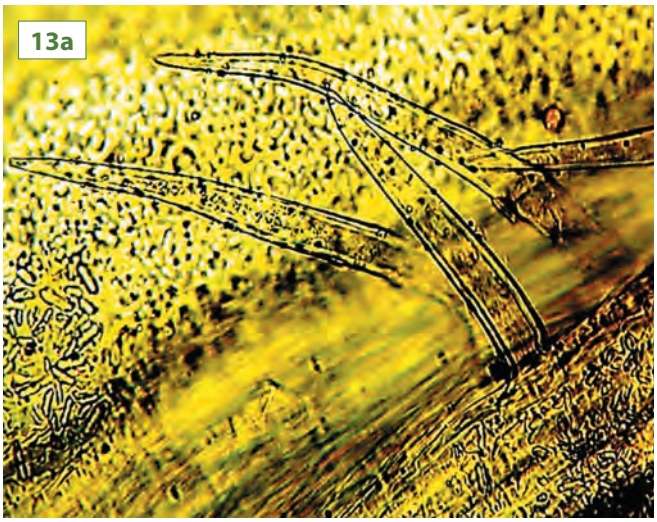

8


9

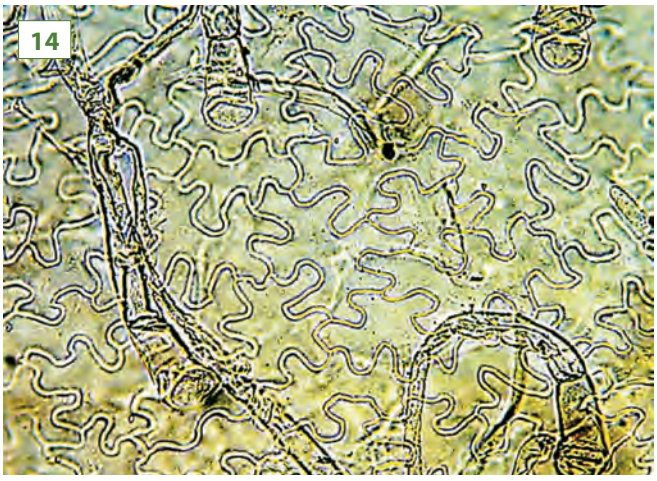

10

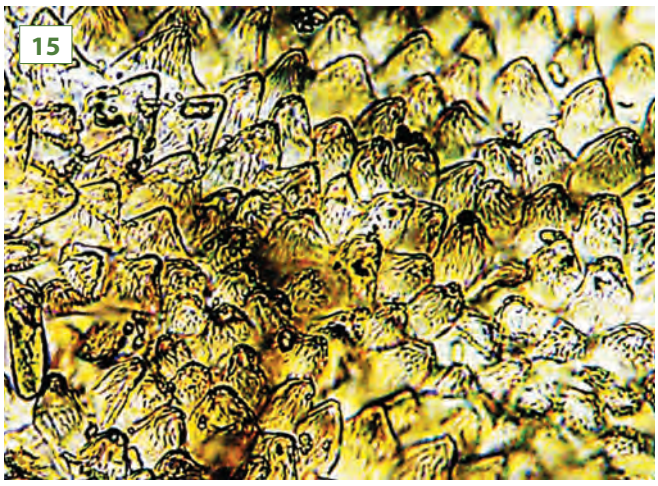

11

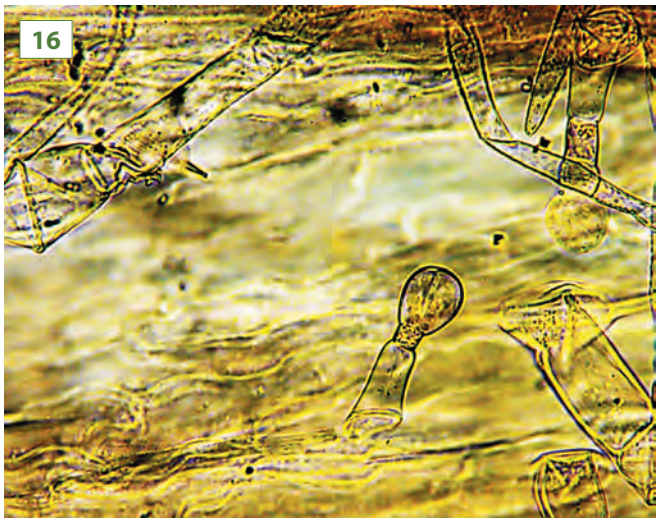

12

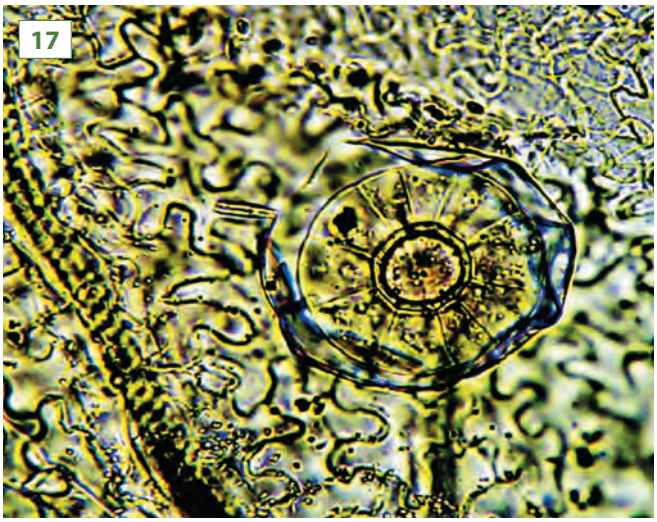

13а

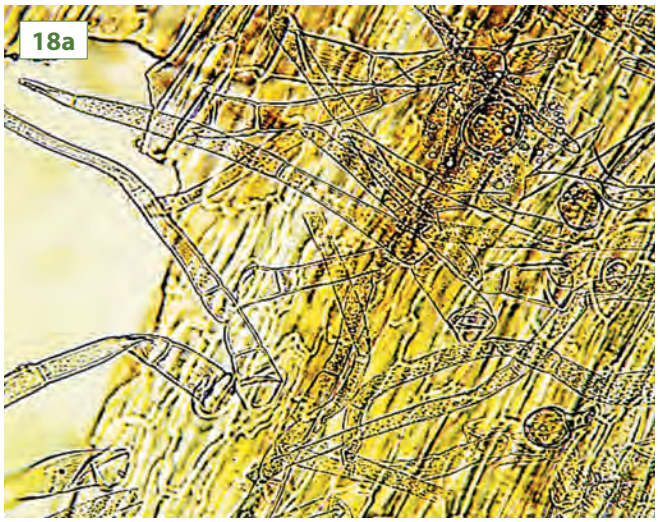

13б

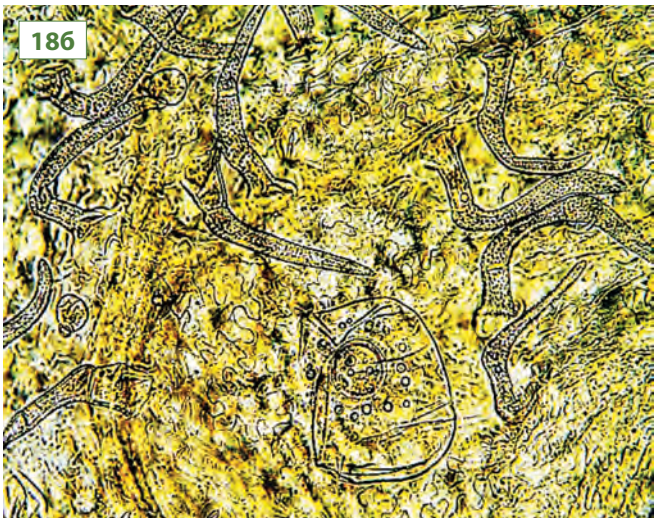

14


15









3. *Flora of the USSR. – M. – L., Publishing house of the USSR Academy of Sciences, 1954, V. XX. – pp. 439–474.*
4. *OFS 1.5.1.0002.15 "Herbs".*
5. *OFS 1.5.3.0003.15 "Technique of microscopic and microchemical studies of medicinal plant raw materials and herbal medicinal products".*

UDC 634.75 581.45 582.6/.9

COMPARATIVE ANALYSIS OF SOME FEATURES OF LEAVES OF COMMON GARDEN STRAWBERRY (FRAGÁRIA ANANÁSSA) CULTIVATED IN THE RUSSIAN FEDERATION AND UZBEKISTAN

I.K. Azizov, *PharmDr., professor, Tashkent Pharmaceutical Institute, Tashkent, Republic of Uzbekistan*
N.A. Musaeva, *PharmDr., Head of Department of Pharmacy 2 – Republican Medical College of Tashkent Pharmaceutical Institute, Tashkent, Republic of Uzbekistan*
V.Yu. Reshetnyak, *PharmDr., professor, Professor of the Department of Chemistry of the Institute of Pharmacy, Federal State Autonomous Educational Institution of Higher Education I.M. Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation (Sechenov University), Moscow*
I.N. Avertseva, *Candidate of chemical sciences, Associate Professor of the Department of Chemistry of Institute of pharmacy, Federal State Autonomous Educational Institution of Higher Education I.M. Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation (Sechenov University), Moscow*
D.A. Dobrokhotov, *Associate Professor of Pharmaceutical Sciences, Associate Professor of the Department of Chemistry of the Institute of Pharmacy, Federal State Autonomous Educational Institution of Higher Education I.M. Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation (Sechenov University), Moscow, Russia*

The analysis of the quantitative content of extractive substances extracted with water and 70% ethyl alcohol, as well as flavonoids in terms of rutin in such raw materials as leaves of common garden strawberries (Fragaria ananassa) of cultivars, widely represented in Russia and in Uzbekistan, in comparison with leaves of the wild strawberry (Fragaria vesca L.), which are pharmacopoeial raw materials. The study revealed that the leaves of garden strawberry contain comparable amounts of extractives and flavonoids and can be considered as an alternative raw material to the leaves of wild strawberry, which are harvested from wild plants. The study of influence of conservation methods on the content of extractive substances extracted with water and 70% ethyl alcohol, and flavonoids in terms of rutin, in the leaves of wild strawberries and garden strawberries cultivated in the territory of the Russian Federation and the Republic of Uzbekistan, as a result of which it was established the best persistence of the studied substances was in the raw material, which was preserved by freezing.

Key words: wild strawberry, garden strawberry, leaves of strawberry, extractive substances, flavonoids, conservation methods of medicinal plant raw materials

In recent years, the interest of researchers is increasingly attracted by the study of raw materials such as leaves (folia), as pharmacopoeial or food raw materials harvested from plants from which fruits are used, and the content of biologically active substances in these products often exceeds content of such substances in traditional raw materials [1–6].

As it is known, in the Russian Federation, the official raw material is the leaves of wild strawberry (FS 2.5.0016.15 "Wild strawberry leaves-Fragariae vescae folia"). They are collected, according to the recommendations of SPh [7],

from wild strawberry (Fragaria vesca L.) that is a wild perennial herbaceous plant, for which the presence of wound healing, analgesic properties, antihypoxic effect on the model of acute tissue hypoxia, gastroprotective action on different models of ulceration, as well as gastroprotective action [8] is established.

It is known that one of the most common small-fruit crops in the Russian Federation is garden strawberry (Fragaria ananassa), the fruits of which are standardized in accordance with the requirements of GOST 33953–2016 "Fresh strawberries", GOST R 53884–2010 (UNECE FFV-35: 2002) "Strawberries for retail. Specifications".

According to Petukhova O.V., the density of the stock of air-dry raw materials for garden strawberry leaves is 565 to 3849 g/m2 with the corresponding values for wild strawberries of 45 to 118 g/m2. Biological stock of the strawberries on comparable areas is 12 times as much as the biological stock of wild strawberries, and the yield of air-dry raw material of garden strawberry is 2–3 times as much as the same parameter for leaves of the wild strawberry [9,10].

As we can see from the data in Diagram 1, providing a specified volume of collection of the strawberries is possible when there is significant acreage that actualizes the study of the potential use of garden strawberry leaves with a wide range of pharmacological actions, for expanding the raw material base consisting of "the wild strawberry leaves" permitted in the Russian Federation for medical use. Keeping in mind the increased interest in cultivation of garden strawberries on the territory of Uzbekistan, it is expedient and promising to compare the quality factors of leaves of garden strawberries of different varieties cultivated in the Russian Federation and Uzbekistan.

The purpose of the work is a comparative study of the content of extractive substances and the amount of flavonoids in terms of rutin in accordance with the requirements of FS 2.5.0016.15 "Wild Strawberry leaves – Fragariae vescae folia" for raw materials harvested from varietal samples in Moscow region and Uzbekistan, as well as an assessment of the prospects of using strawberry leaves in medical practice

MATERIALS AND METHODS

Study subjects were leaves of garden strawberry of such cultivars as "Albion", "Isabella", "Charlie", collected in July 2018 in garden nurseries

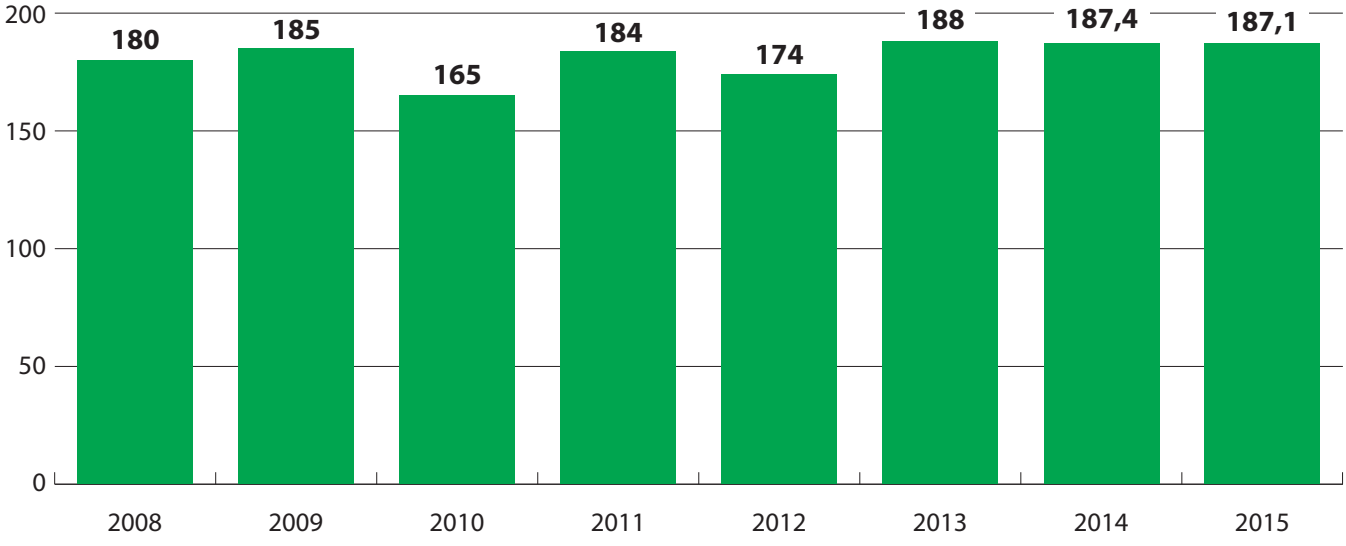


DIAGRAM 1. *Gross yield (in tons) of strawberries in the Russian Federation, according to the Expert-analytical center of agribusiness*

of the city of Tashkent, as well as the leaves of the cultivars of "Sudarushka", "Darselect", harvested in the summers of 2018–2019 in Moscow suburbs, and the leaves of forest strawberries, collected in the undergrowth of mixed forests in Chekhov, Istra districts of Moscow region.

The studied raw materials were subjected to air-shadow drying for the purpose of preservation, and then they were frozen using dry ice-air freezing technology at temperature not higher than –18 °C with additional placement of granular dry ice as a refrigerant agent in the raw material mass [11].

Analysis of the total content of extractives in the studied raw materials was carried out in accordance with the requirements of OFS 1.5.3.0006.15 "Determination of extractives in medicinal plant raw materials and herbal medicinal products", as well as FS 2.5.0016.15 "Wild Strawberry leaves – Fragariae vescae folia". The content of flavonoids in terms of rutin was determined by spectrophotometry under the conditions specified in FS 2.5.0016.15 "Wild Strawberry leaves – Fragariae vescae folia". Antimicrobial activity of water extracts from a mixture of varietal leaves was evaluated by the "cylinder method" (SPh XI part 2, pp. 210–213). The degree of antimicrobial properties was studied in relation to strains of test microorganisms of Staphylococcus aureus 209P, Escherichia coli ATCC 1257, Pseudomonas aeruginosa NCTC2134, Streptococcus mutans.

RESULTS AND DISCUSSION

Taking into account the available literature data proving the prospects of using raw materials that have been preserved by freezing along with dried plant raw materials [12–17], the analysis of content of extractive substances extracted by water (FS 2.5.0016.15 "Wild Strawberry leaves – Fragariae vescae folia"), as well as 70% ethyl alcohol, and the total content of flavonoids in

Table 1

TOTAL CONTENT OF EXTRACTIVE SUBSTANCES

Raw materials analyzed	Total content of extractive substances. %			
	Extracted by water		Extracted by alcohol	
	Frozen raw material	Dried raw material	Frozen raw material	Dried raw material
Wild Strawberry leaves	26.82±0.02	25.31±0.01	28.08±0.03	27.09±0.02
Garden Strawberry leaves. "Sudarushka" cultivar	25.14±0.04	21.42±0.02	26.32±0.04	24.11±0.04
Garden Strawberry leaves. "Darselect" cultivar	24.83±0.02	21.01±0.02	25.92±0.03	21.84±0.01
Garden Strawberry leaves. "Albion" cultivar	24.34±0.02	20.81±0.06	26.31±0.04	22.41±0.05
Garden Strawberry leaves. "Isabella" cultivar	21.44±0.02	19.35±0.06	24.12±0.05	20.82±0.06
Garden Strawberry leaves. "Charlie" cultivar	22.65±0.06	19.36±0.05	25.60±0.06	23.41±0.05

Table 2

TOTAL CONTENT OF FLAVONOIDS IN TERMS OF RUTIN

Raw materials analyzed	Total content of flavonoids in terms of rutin. %	
	Frozen raw material	Dried raw material
Wild Strawberry leaves	2.89±0.02	2.27±0.01
Garden Strawberry leaves. "Sudarushka" cultivar	2.45±0.04	2.08±0.03
Garden Strawberry leaves. "Darselect" cultivar	2.24±0.01	1.96±0.01
Garden Strawberry leaves. "Albion" cultivar	2.62±0.06	2.13±0.04
Garden Strawberry leaves. "Isabella" cultivar	2.38±0.02	1.8±0.04
Garden Strawberry leaves. "Charlie" cultivar	2.51±0.02	2.05±0.03

terms of rutin was provided for selected varietal samples of dried and frozen raw materials.

The results of the study are presented in tables and diagrams.

An infusion of a varietal mixture of strawberry leaves cultivated in Moscow region and Uzbekistan revealed antimicrobial activity in relation to strains of Escherichia coli (1:30), Staphylococcus aureus (1:70), Pseudomonas aeruginosa (1:50), Streptococcus mutans (1:30).

Thus, the data of antimicrobial activity obtained in the study of water extracts of leaves of wild and garden strawberries growing in Ural, in relation to strains of Escherichia coli and Staphylococcus aureus were confirmed [10]. In the data obtained by us the particular interest is attracted by the moderate antimicrobial action in relation to Streptococcus mutans, which is the main pathogenic microorganism of the oral cavity and has a significant impact

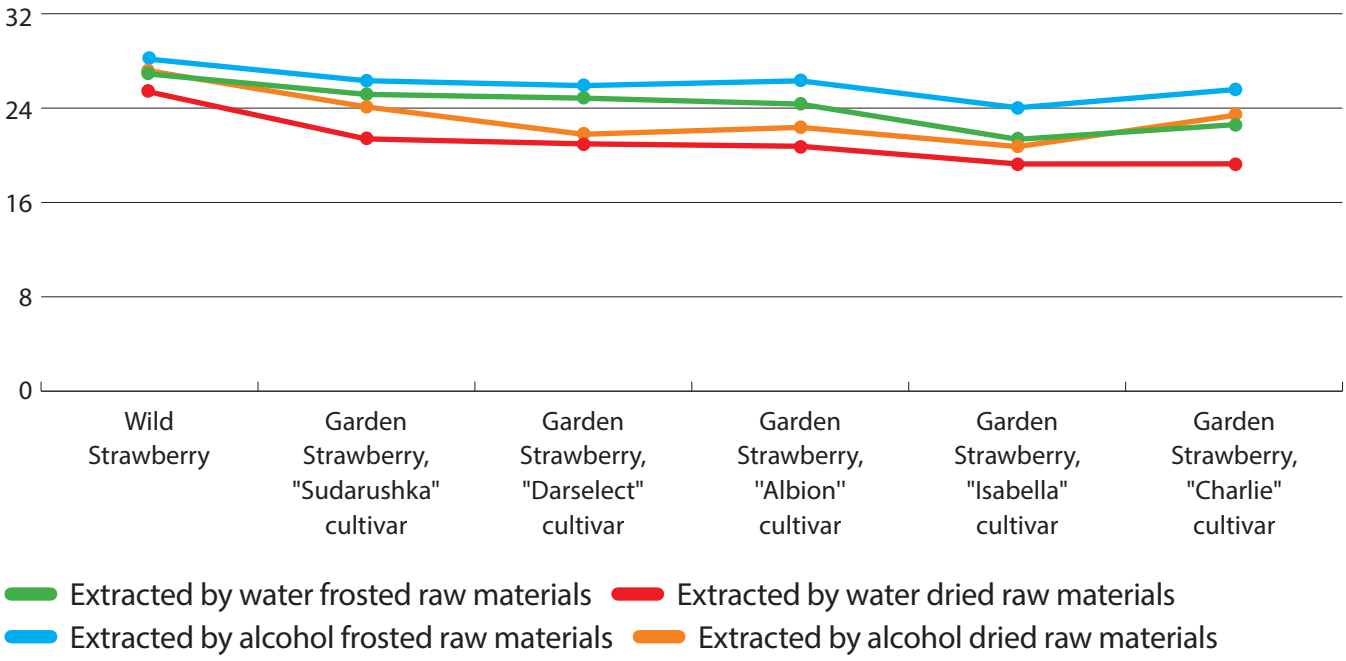


DIAGRAM 2. *Content of extractive substances in varietal garden strawberries leaves for different ways of preservation*

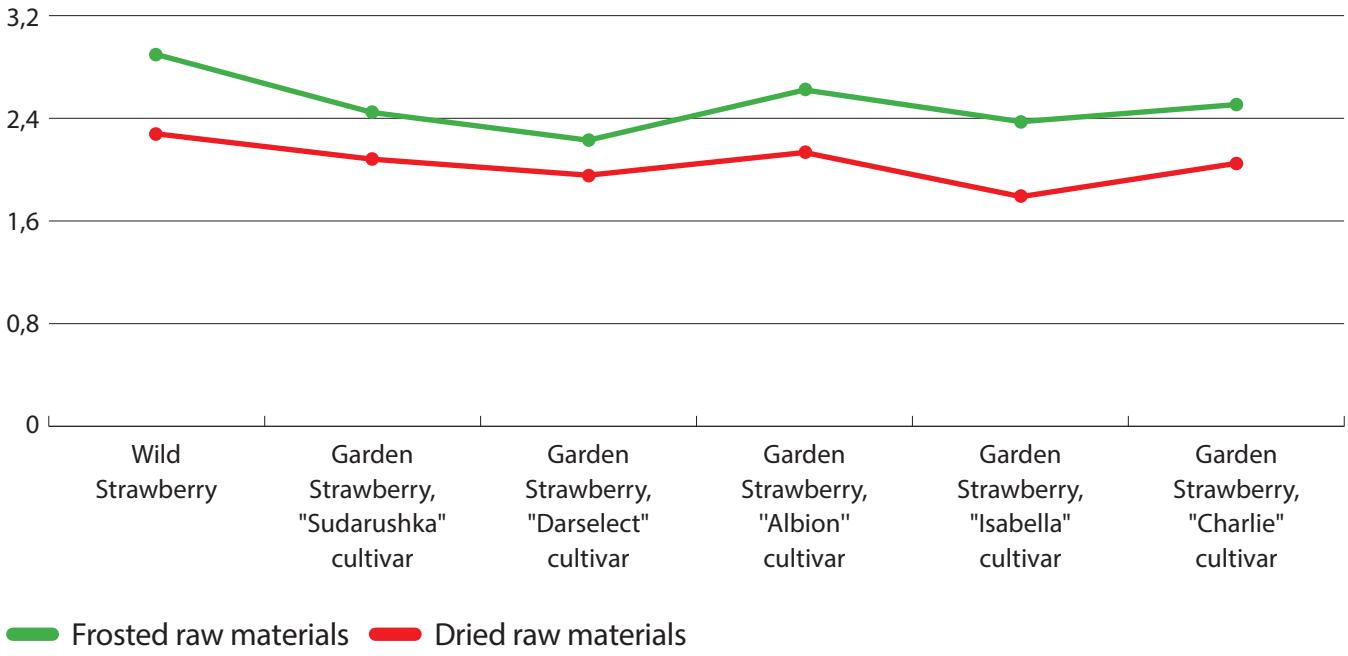


DIAGRAM 3. *Content of flavonoids in terms of rutin in varietal garden strawberries leaves for different ways of preservation*

on formation of periodontal disease. The presence of antimicrobial activity in relation to this microorganism allows us to consider the leaves of wild and garden strawberry as a promising source of medicines for treatment and prevention of inflammatory diseases of the oral cavity.

CONCLUSION

As a part of the study the quantitative content of extractive substances extracted with water and 70% ethyl alcohol, as well as flavonoids in terms of rutin in raw material "Garden Strawberry leaves" of cultivars which are widely grown in Moscow region and the Republic of Uzbekistan was analyzed in comparison with the Pharmacopoeia raw material "Wild Strawberry leaves". It was found that the leaves of garden strawberry contain comparable amounts of extractives (19.35 to 26.82% extracted with water and 20.82 to 28.08% extracted with alcohol) and flavonoids (2.08 to 2.62% in terms of rutin) and can be considered as an alternative raw material to leaves of wild strawberry, which are collected from wild plants.

The influence of preservation methods on the content of extractive substances extracted with water and 70% ethyl alcohol and flavonoids in terms of rutin in wild and garden strawberry leaves cultivated in the territory of the Russian Federation and the Republic of Uzbekistan was studied. As a result, the best persistence of the studied substances was established in raw materials, which were preserved by freezing.

REFERENCES

1. *Nesterova O. V., Sankova M. V. Rationale for the use of garden gooseberry leaves Grossularia reclinata (L.) Mill.) in medicine / Collection of articles of the IV International scientific-research competition. – Penza: Publishing house "Science and education" (IP Gulyaev G. Yu.). 2018. – pp. 195–199.*
2. *Nesterova N. V., Samylina I. A. Studying the qualitative composition and quality factors of leaves of wild and cultivated apple trees // Health*

and education in the XXI century. – 2015. – No. 4. – Vol. 17. – pp. 251–258.

3. *Trofimova S. V. Pharmacognostic study of leaves of blood-red hawthorn (Crataégus sanguínea Pall.) from the flora of Bashkortostan / Author's abstract of thesis for a Candidate Degree in Pharmaceutical Sciences – Perm, 2014. – 24 p.*
4. *Nesterova N. V., Harina A. S. Analysis of prospects for the use of leaves of grape vine (Vitis vinifera L.) / In collection book: "Scientific and educational space: prospects for development". Proceedings of the VII International research and practice conference. Editorial Board: O. N. Shirokov [et al.]. 2018. – pp. 26–27.*
5. *Nesterova O. V., Tabachenko E. S. Study of macro – and microdiagnostics characteristics of sweet-berry honeysuckle leaves (Lonicera caerulea L.) / In collection book: "Modern technologies: major aspects, achievements and innovations" Proceedings of the XX International research and practice conference. 2018. pp. 94–98.*
6. *Sankova M. V., Nesterova O. V. Index of expediency of use of leaves of plants in comparison with their fruits and its value in medicine // Health and education in the XXI century. 2018. Vol. 20. No. 12. – pp. 144–148.*
7. *State Pharmacopoeia XIII Edition.*
8. *Klimentova D. A. Pharmacological properties of complex extracts from wild strawberries / Author's abstract of thesis for a Candidate Degree in Medicine – Tomsk, 2005. – 24 p.*
9. *Petukhova O. V., Ivanova G. A., Olezhko G. I. Comparative resource studies of strawberry leaves/Actual problems of pharmaceutical science and education: results and prospects. Materials of Inter-University research and practice conference "Universities and regions". – Perm. 2003. – pp. 111–112.*
10. *Petukhova O. V. Pharmacognostic study of wild and garden strawberry leaves of Urals region/ Author's abstract of thesis for a Candidate Degree in Pharmaceutical sciences. – Perm, 2003. – 24 p.*
11. *Shishkina N. S., Karastoyanova O. V. Effective trends of development of technologies for production of quick-frozen vagetable products // World of ice cream and quick-frozen foods. 2013, No. 1. – pp. 7–74.*
12. *Sergunova E. V. Influence of preservation method on quality of fruits and water extracts of cranberry tree (viburnum opulus) / E. V. Sergunova, I. A. Samylina // Pharmacy. 2009. – No.5. – pp. 16–18.*
13. *Sergunova E. V. Study of hawthorn berries of various methods of preservation and water extraction / E. V. Sergunova, A. S. Avrach, A. A. Sorokina // Pharmacy. – 2010. – No.5. – pp. 16–18.*
14. *Sergunova E. V. Study of composition of biologically active substances in medicinal plant raw materials of various methods of preservation / E. V. Sergunova, A. A. Sorokina // Main aspects of development of megalopolises and pharmaceutical clusters, training and improvement of pharmaceutical specialists. Proceedings of the XX Russian national congress "Man and medicine" (April 15–19, 2013). – M.: EkOOnis, 2013. – pp. 184–190.*
15. *Nesterova N. V. Study of the dependence of quantitative content of biologically active substances of the leaves of wild and domesticated apple trees on the methods of preservation // Health and education in the XXI century, 2017. – Vol. 19, No. 8. – pp. 206–209.*
16. *Pogosyan R. A. Nesterova O. V., Dobrokhotov D. A. Determining the influence of preservation methods on safe keeping of pomegranate fruit pulp // Health and education in the XXI century, 2017, No. 4. Vol. 19. – pp. 136–141.*
17. *Nesterova N. V. Study of qualitative composition and quality factors of leaves of wild and domesticated apple trees /N. V. Nesterova, I. A. Samylina // Health and education in the XXI century. – 2016. – No.5. – Vol. 18. – pp. 251–258.*

UDK 543.89:612

DEVELOPMENT OF A NORMATIVE DOCUMENT FOR A PHARMACOPOEIA REFERENCE STANDARD FOR A NEW ANTIFUNGAL AGENT BASED ON THE 1,3,4-THIADIAZOLE DERIVATIVE

N.Yu. Sipkina, *a research scientist of the testing laboratory of the Center of medicine quality control of FSBEI of Higher Education "Saint-Petersburg State Chemical-pharmaceutical University" of Ministry of Health of the Russian Federation (FSBEI HE RF Sp.SChPhU MOH), Saint-Petersburg, nadya.sipkina@pharminnotech.com.*

I.P. Yakovlev, *Doctor of Chemistry, Professor of the Organic Chemistry Faculty of FSBEI of Higher Education "Saint-Petersburg State Chemical-pharmaceutical University" of Ministry of Health of the Russian Federation (FSBEI HE SpSChPhU RF MOH), Saint-Petersburg*

This paper presents a normative document on the Pharmacopoeia reference standard for a new antifungal agent based on the 1,3,4-thiadiazole derivative. The normative document includes both Pharmacopoeia analysis methods for the specified quality factors, and newly developed methods using such methods as high-performance liquid chromatography, gas chromatography, spectrophotometry and potentiometric titration. The document was developed and issued in accordance with the requirements of the current edition of the State Pharmacopoeia of the Russian Federation.

Key words: normative document, antifungal agent, Pharmacopoeia reference standard

1,3,4-thiadiazole derivatives are widely known as compounds with various types of biological activity that exhibit antitumor, anti-inflammatory, antibacterial, antifungal, antiviral, anticonvulsant and antiparasitic activity [1–4]. Change in the spectrum of pathogens and the high frequency of adverse events when using systemic antimycotic agents cause the need to create new antifungal agents. In view of this, in Saint-Petersburg State Chemical-pharmaceutical University a new compound – chloride 2-[(Z)-1-(3,5-diphenyl-1,3,4-thiadiazole-2(3H)-ilidene) methyl]-3,5-diphenyl-1,3,4-thiadiazole-3-iya (TDZ, Fig. 1) [5] was synthesized, which showed antifungal activity comparable to available antimycotic agents on the market [6,7]. To achieve the proper quality of the pharmaceutical substance and the finished dosage form (FDF) of the new antifungal agent, it was necessary to create a Pharmacopoeia reference standard (PRS) and a normative document (ND) for it. PRS with a necessary degree of purity, intended for qualitative and quantitative determination, was obtained by direct synthesis. Its quality was evaluated by Pharmacopoeia analysis methods, as well as newly developed techniques based on such methods as high-performance liquid chromatography (HPLC), gas chromatography (GC), spectrophotometry and potentiometric titration. The obtained TDZ PRS can be used in testing and research laboratories for qualitative and quantitative determination of TDZ in the pharmaceutical substance of TDZ, in the finished dosage form containing TDZ as a main active substance, as well as for analysis of biological fluids in the study of the pharmacokinetics of TDZ.

The purpose of this work is development of ND for PRS for a new antifungal agent intended

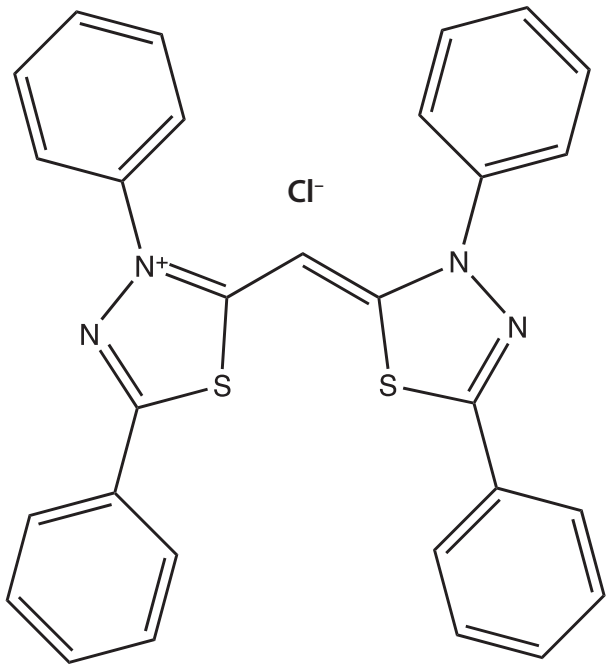


FIG. 1. *Structural formula of chloride 2-[(Z)-1-(3,5-diphenyl-1,3,4-thiadiazole-2(3H)-ilidene)methyl]-3,5-diphenyl-1,3,4-thiadiazole-3-iay (TDZ)*

for quantitative and qualitative analysis of a pharmaceutical drug (PD) and FDF.

MATERIALS AND METHODS

Study subject – chloride 2-[(Z)-1-(3,5-diphenyl-1,3,4-thiadiazole-2(3H)-ilidene) methyl]-3,5-diphenyl-1,3,4-thiadiazole-3-iya, series 010718–130,0 g (expiration date 07.2020), series 020718–130,0 g (expiration date 07.2020), series 030818–130,0 g (expiration date 08.2020), manufacturer – FSBEI of HE SpSChPhU of Russia.

Research methods – Table 1 presents the selected quality factors and standards, as well as a list of pharmacopoeial methods and developed analytical techniques for analysis of PRS according to the requirements [8,9].

RESULTS AND DISCUSSION

In the course of the tests we have established quality standards, as well as selected pharmacopoeial methods and developed new methods of analysis to prove the quality and identity of the PRS of a new antifungal agent. The obtained data were systematized and registered as ND for PRS in accordance with the requirements of the current edition of the State Pharmacopoeia (SP).

Chemical name according to IUPAC: chloride 2-[(Z)-1-(3,5-diphenyl-1,3,4-thiadiazole-2(3H)-ilidene)methyl]-3,5-diphenyl-1,3,4-thiadiazole-3-iay.

Empirical formula: $C_{29}H_{21}ClN_4S_2$

Molecular weight: 525.1

Description: Yellow fine crystalline powder, odorless.

Solubility: Moderately soluble in alcohol, poorly soluble in acetonitrile, almost insoluble in water.

Identity.

1. PRS infrared spectrum, measured in potassium bromide tablet in the region of 4000–400 cm-1 should match the attached spectrum pattern according to the position of absorption bands (Fig. 2).

Table 1

LIST OF QUALITY FACTORS, STANDARDS AND METHODS FOR ANALYSIS OF PRS

Factors	Methods	Standards
Description	Organoleptic	Yellow fine crystalline powder, odorless
Solubility	SP XIV	Moderately soluble in 96% ethanol, very slightly soluble in acetonitrile, almost insoluble in water
Identity	IR spectrophotometry Spectrophotometry	IR spectrum of the substance according to the position of absorption bands should correspond to the pattern of the spectrum of the thiadiazole derivative. The absorption spectrum of 0.0005% solution of the substance in the range from 200 to 600 nm should have maxima at (435 ± 2) nm and (250 ± 2) nm
Melting point	SP XIV	269 to 271°C
Specific absorbance	SP XIV	1600 to 1640 at 435 nm
Related substances	HPLC	A single unidentified impurity – no more than 0.1%; the amount of impurities – no more than 0.5%
Sulphated ash	SP XIV	no more than 0.1%
Heavy metals	SP XIV, method 1	no more than 0.001%
Weight loss on drying	SP XIV	no more than 0.5%
Residual organic solvents	SP XIV	Acetonitrile – no more than 0.040%
Microbiological quality	SP XIV	Category 2.2
Assay	Photometric titration	99.0% to 101.0% $C_{29}H_{21}ClN_4S_2$ on a dry substance basis
Storage	SP XIV	In a dry, protected from light place at a temperature not higher than 20±0.5°C
Shelf life	SP XIV	2 years
Packing	SP XIV	5 g each in sealed plastic bags

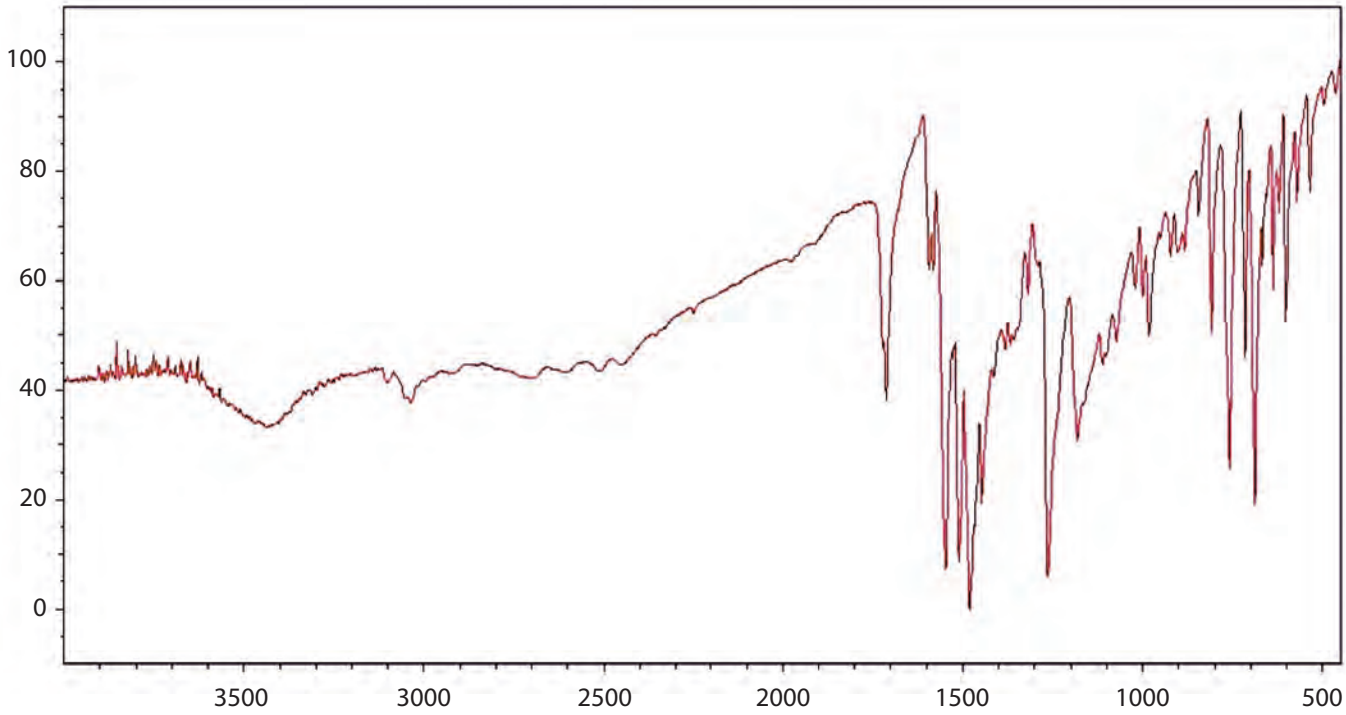


FIG. 2. *TDZ PRS infrared spectrum*

2. The ultraviolet absorption spectrum of 0.0005% PRS solution in alcohol, measured in the range from 200 to 600 nm, should have maximum absorption at 435±2 nm and 250±2 nm.

Melting point 269 to 271°C.

Specific absorbance 1600 to 1640 at 435 nm.

About 0.05 g (precisely weighed amount) of PRS, pre-dried at a temperature of 100 to 105°C, is placed into a measuring flask with capacity of 100 ml and dissolved in 50 ml of alcohol, then the volume of the solution is brought to the mark using the same solvent and the solution is stirred. 1 ml of the resulting solution is placed into a measuring flask with capacity of 100 ml, the volume of the solution is brought to the mark with alcohol and the resulting solution is stirred.

The optical density of the resulting solution is measured by spectrophotometer at 435 nm in a cuvette with layer thickness of 10 mm, using alcohol as the comparison solution

Weight loss on drying. About 1.0 g (precisely weighed amount) of PRS is dried at temperature of 100–105°C to constant mass. The weight loss should not exceed 0.5%.

Sulphated ash. Sulphated ash obtained from 1,0 g (precisely weighed amount) of PRS should not be higher than 0,1%.

Heavy metals. Sulphated ash obtained from 1,0 g of PRS shall pass the test for heavy metals.

Related substances. A single unidentified impurity – no more than 0.1%; the amount of impurities – no more than 0.5%.

Reagents: acetonitrile for chromatography or of similar quality, trifluoroacetic acid ("optic standard") or of similar quality, water for chromatography or of similar quality, sample solvent – acetonitrile solution and trifluoroacetic acid solution in ratio of 1:1.

Trifluoroacetic acid solution. 500 ml of water is placed in a measuring flask with capacity of 1.0 l, then, 0.5 ml of trifluoroacetic acid is added, the solution is mixed and the volume of the solution is brought to the mark with the same solvent. The freshly prepared solution is only used.

Test solution. Weighed amount of 0.025 g (precisely weighed amount) of PRS is placed into a measuring flask with capacity of 25 ml and dissolved in 20 ml of the sample solvent,

the volume of the solution is brought to the mark with the same solvent and the resulting solution is stirred. The resulting solution is filtered through a filter with pore diameter of 0.45 microns.

Comparison solution. 1 ml of the test solution is placed into a measuring flask with capacity of 100 ml, the volume of the solution is brought to the mark with the sample solvent and the resulting solution is stirred. 1 ml of the resulting solution is placed into a measuring flask with capacity of 10 ml, the volume of the solution is brought to the mark with the sample solvent, the resulting solution is stirred and filtered through a filter with pore diameter of 0.45 microns. The shelf life of the solution is 72 hours at temperature of 25°C.

Chromatographic conditions: column Zorbax Eclipse Plus C8 250 mm x 4,6 mm x 5 µm, the use of alternative column that satisfies the requirements of chromatographic system suitability is allowed; the mobile phase is a mixture of acetonitrile and a solution of trifluoroacetic acid, flow rate is 1.0 ml/min, column temperature is 40°C, the wavelength of the detector is 250 nm, the sample volume is 20 µl, the elution mode is gradient one: increase in the content of acetonitrile in the system from 40 to 80% for 20 min.

The comparison solution is chromatographed to obtain at least 5 chromatograms under specified conditions. The retention time of the TDZ peak should be about 16 minutes.

A chromatographic system is considered as a suitable one if the following conditions are met:

- the efficiency of the chromatographic column, calculated under the peak of the TDZ on the chromatograms of the comparison solution, is not less than 3000 theoretical plates;
- the asymmetry factor of the peak of TDZ on the chromatograms of the comparison solution is 0.8 to 1.8;
- relative standard deviations of retention times and peak areas of TDZ calculated by using 5 consecutive chromatograms of the comparison solution are no more than 2.0%.

The test solution is chromatographed to obtain at least 5 chromatograms under specified conditions.

On the chromatogram of the PRS test solution, the peak area of any impurity shall be no more than the peak area of the TDZ on the chromatogram of the comparison solution (no more than 0.1%); the total area of the impurity peaks shall not exceed 5 times the peak area of the TDZ on the chromatogram of the comparison solution (no more than 0.5%). The peaks whose area is less than 0.5 of the TDZ peak area on the chromatogram of the comparison solution, and the peaks with retention time less than 2.5 min, and the peaks related to the peaks of solvent and mobile phase are not considered. A typical chromatogram of the PRS comparison solution is shown in Fig. 3.

Residual organic solvents. The content of acetonitrile in the PRS should not be more than 0.040%.

Reagents and reference standards. DMSO for chromatography or of similar quality; state reference standard (SRS) consisting of 99.9% acetonitrile.

Test solution. About 0.10 g (precisely weighed amount) of PRS is placed into a flask for headspace analysis, 2.0 ml of dimethylsulfoxide is added, then, the flask is closed with a plug and sealed.

Reference standard. About 1,00 g (precisely weighed amount) of the reference standard of acetonitrile is placed into a measuring flask with capacity of 50 ml, the solution volume is brought to the mark with dimethyl sulfoxide and the solution is stirred; 5.0 ml of the resulting solution is placed into a measuring flask with capacity of 50 ml, the solution volume is brought to the mark with dimethyl sulfoxide and the solution is stirred; 1.0 ml of the resulting solution is placed into a measuring flask with 100 ml capacity, the solution volume is brought to the mark with dimethyl sulfoxide and the

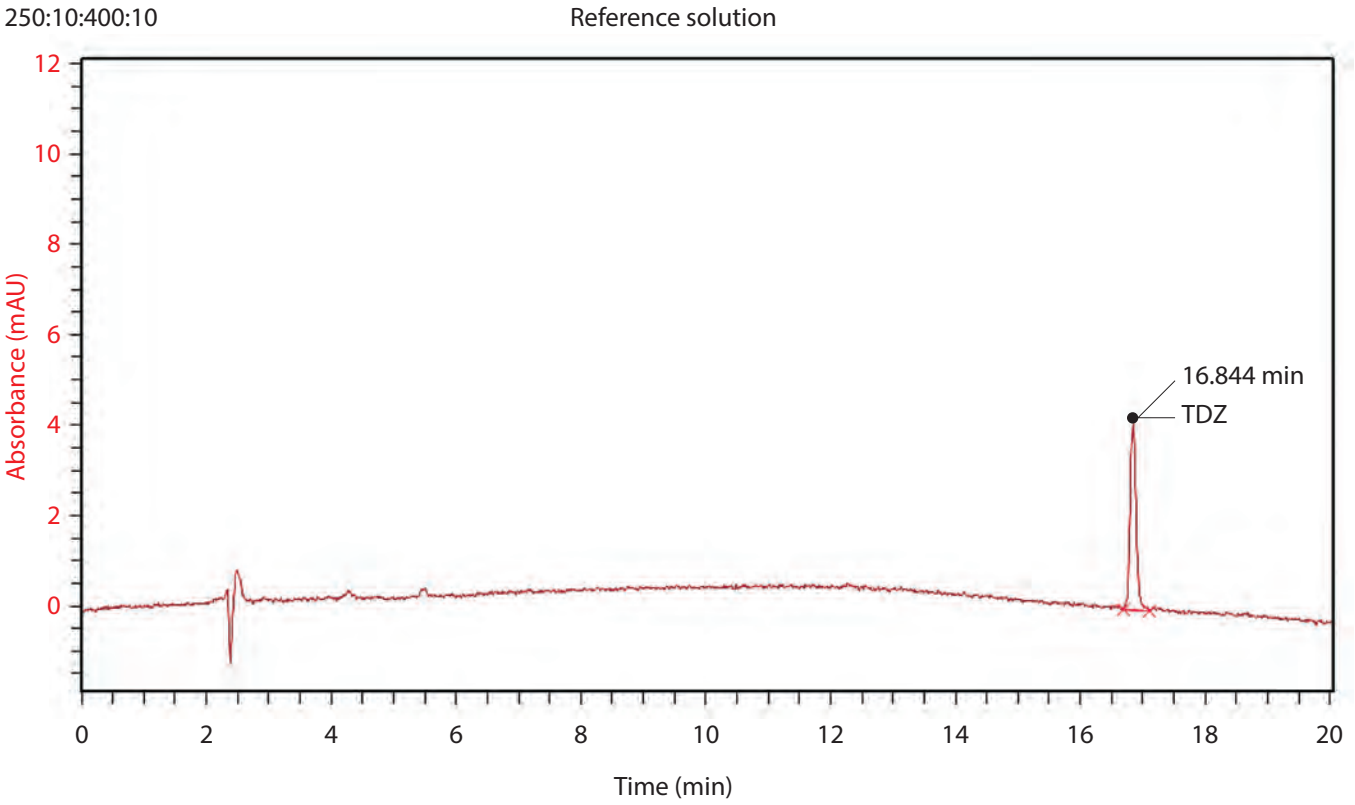


FIG. 3. *Chromatogram of the PRS comparison solution*

solution is stirred. The shelf life of the solution is 72 hours.

2.0 ml of the reference solution is placed into a flask for headspace analysis, the flask is closed with a plug and sealed. At least three samples of the test reference standard and five samples of the standard solution are prepared.

Chromatographic conditions: capillary column – Rtx 1301 60 m x 0,32 мкм x 1,8 µm, the use of an alternative column that satisfies the requirements of chromatographic system suitability is allowed; the carrier gas is nitrogen, flow rate is 2.0 ml/min, split ratio is 10 ml/min; column thermostat temperature is 60°C for 8 min, temperature increase up to 180°C with rate of 15°C/min, then for 4 minutes at the injector end temperature that is 120°C, a flame-ionization detector is used, hydrogen flow rate is 40 ml/min, air flow rate is 400 ml/min, detector temperature is 220°C, chromatography time is 20 min.

Conditions for headspace injector: thermostat control temperature of the standard is 80°C, temperature control time is 40 min., needle temperature is100°C, transfer line temperature is 120°C, discharge time is 2 min., sample injection time is 0.06 min. (1.7 ml), pressure is 26 psi.

The vapor phase of the reference standard solution is chromatographed, with obtaining at least 5 chromatograms. The sequence of the output components: acetonitrile, dimethyl sulfoxide.

The results of the analysis are considered as reliable if the requirements of the test "Checking the suitability of the chromatographic system" are met.

A chromatographic system is considered as suitable if the following conditions are met:

- efficiency of the chromatographic column, calculated from the peak of acetonitrile on the chromatogram of the standard solution, is not less than 10,000 theoretical plates;
- the asymmetry factor of the acetonitrile peak on the chromatogram of the standard solution is no more than 2,0;

- relative standard deviations of retention times and areas calculated from the acetonitrile peak on the chromatogram of the standard solution is no more than 5%.

The vapor phase of the reference standard solution is chromatographed with obtaining at least 3 chromatograms.

The content of acetonitrile in PRS as a percentage (X) is calculated by the formula:

$$X = \frac{S \cdot a_0 \cdot P \cdot 5 \cdot 2}{S_0 \cdot a \cdot 50 \cdot 50 \cdot 100} = \frac{S \cdot a_0 \cdot P}{S_0 \cdot a \cdot 25\,000},$$

where S is an average value of the areas of the acetonitrile peaks on the chromatograms of the test solution, μV·sec; S_0 is an average value of the areas of the peaks on the chromatograms of the acetonitrile reference standard solution, μV·sec; a_0 is weighted amount of the reference standard of acetonitrile, g; a is weighted amount of the test PRS, g; P is the content of the basic substance in the reference standard, %. A typical chromatogram of the reference standard solution is shown in Fig. 4.

Assay. 99,0 to 101,0% C29H21ClN4S2 on a dry substance basis.

About 0.05 g (precisely weighed amount) is dissolved in 50 ml of 50% alcohol solution (test solution). The resulting solution is titrated potentiometrically with 0.01 M silver nitrate solution using a combined platinum electrode.

1 ml of 0.01 M silver nitrate solution corresponds to 5.252 mg of the thiadiazole derivative ($C_{29}H_{21}ClN_4S_2$).

Microbiological quality. The tests are carried out in accordance with the requirements of SP XIV. Category 2.2.

Analytical method validation. Validation tests of analytical methods for such quality factors as "Assay", "Related substances" and "Residual organic solvents" were carried out in accordance with the requirements of SP XIV. The list of validation parameters and test results are presented in Table. 2.

In the course of validation of analytical methods, we obtained experimental evidence confirming the suitability of the developed methods for quality control of TDZ PRS and the possibility of including these methods into the ND on PRS.

Analysis of control samples of TDZ PRS. The obtained series of TDZ control samples were analyzed according to the developed ND. The test results are presented in Table. 3, from which it can be seen that the control samples of three series of TDZ meet the quality standards of ND.

CONCLUSION

On the basis of the developed analytical methods using such techniques as spectrophotometry,

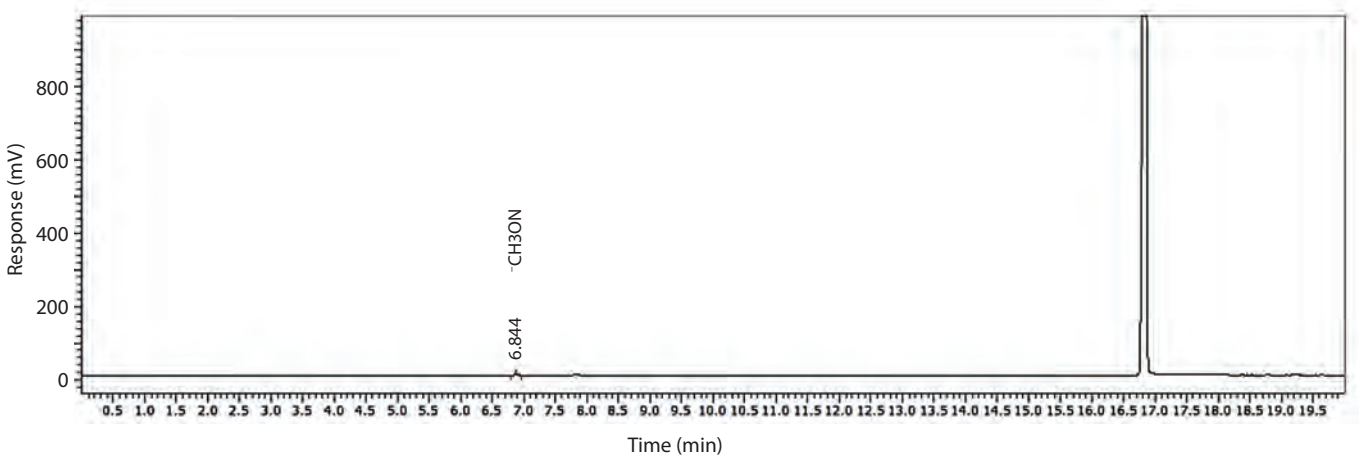


FIG. 4. *Chromatogram of a reference standard solution of acetonitrile*

Table 2

THE RESULTS OF ANALYTICAL METHOD VALIDATION TESTS "ASSAY", "RELATED SUBSTANCES" AND "RESIDUAL ORGANIC SOLVENTS"

Validation parameter	Analytical method		
	Assay	Related substances	Residual organic solvents
Specificity	The test results confirm the ability of the potentiometric titration technique to reliably determine the equivalence point in the studied solutions in the presence of foreign impurities.	On the chromatograms of the mobile phase and the sample solvent, there are no systemic peaks with retention times of the peaks of the thiadiazole derivative and N' – phenylthiobenzhydrazide. On the chromatogram of a specific solution, the peaks of the thiadiazole derivative and N' – phenylthiobenzhydrazide are well separated (R=6.1) between them and the systemic peaks (vapour phase (VP)).	On the chromatograms of the blank solution there are no systemic peaks with the retention time of the acetonitrile peak. On the chromatogram of a specific solution, the peaks of acetonitrile and dimethylsulfoxide are well separated (R=44) between them and the systemic peaks.
Quantitation limit	–	0,0001 mg/ml	0,0010 mg/ml
Linearity: $y = bx + a$, r	$y = 0.194x - 0.11$ $r = 0.9996$	$y = (2.0 \cdot 10^7)x + 55$ $r = 0.9996$	$y = (9.07 \cdot 10^5)x+156$ $r = 0.9997$
Accuracy: recovery, R%	99.3–100.4	97.20–99.33	96.1–109.3
Repeatability: $RSD_{(n=6)}$	0.38%	0.90%	2.31%
Intermediate precision: $RSD_{(n=6)}$, t(95%,5), F(95%,5)	0.14% t (95%.5) = 0.663, F (95%.5) = 0.127	0.87% t (95%.5) = 0.9102, F (95%.5) = 1.0559	3.40% t (95%.5) =0.6544, F (95%.5) =2.2156
Analytical range, mg/ml	0.80 to 1.2	0.0001 to 0.0014	0.001 to 0.050
Chromatographic system suitability	–	As specified	As specified

Table 3

RESULTS OF TESTS OF CONTROL SERIES OF TDZ PRS

Quality factor	Results of tests		
	Series 010718	Series 020718	Series 030818
Description	Yellow fine crystalline powder, odorless	Yellow fine crystalline powder, odorless	Yellow fine crystalline powder, odorlessa
Solubility	Moderately soluble in 96% ethanol, very slightly soluble in acetonitrile, almost insoluble in water	Moderately soluble in 96% ethanol, very slightly soluble in acetonitrile, almost insoluble in water	Moderately soluble in 96% ethanol, very slightly soluble in acetonitrile, almost insoluble in water
Identity	As specified	As specified	As specified
Melting point, °C	270–271	270–271	269–270
Specific absorbance, l/g·cm	1629	1632	1610
Related substances, %	A single impurity – no more than 0.1; the amount of impurities – no more than 0.5	A single impurity – no more than 0.1; the amount of impurities – no more than 0.55	A single impurity – no more than 0.1; the amount of impurities – no more than 0.5
Sulphated ash, %	0.02	0.01	0.01
Heavy metals, %	Less than 0.001	Less than0.001	Less than 0.001
Weight loss on drying, %	0.2	0.2	0.2
Residual organic solvents, %	0.021	0.020	0.019
Microbiological quality	As specified	As specified	As specified
Assay, %	99.5	99.7	99.3

titrimetry, HPLC and GC, as well as pharmacopoeial methods, we developed a ND on the PRS for a new antifungal agent based on 1,3,4-thiadiazole according to the requirements of SP XIV.

REFERENCES

1. *Hu Y., Li C.Y., Wang X.M., Yang Y.H., Zhu H.L. 1,3,4-Thiadiazole: Synthesis, Reactions, and*

Applications in Medicinal, Agricultural and Materials Chemistry // Chem. Rev. – 2014. – №114 (10). – pp. 5572–5610.

2. *Matysiak J. Biological and pharmacological activities of 1,3,4-thiadiazole based compounds // Mini-Rev. Med. Chem. – 2015. – №15 (9). – pp. 762–775.*
3. *Li Y., Geng J., Liu Y., Yu S., Zhao G. Thiadiazole – a Promising Structure in Medicinal Chemistry // Chem. Med. Chem. – 2012. – № 8 (1). – pp. 27–41.*
4. *Jain A. K., Sharma S., Vaidya A., Ravichandran V., Agrawal R. K. 1,3,4-Thiadiazole and its Derivatives: A Review on Recent Progress in Biological Activities // Chemical Biology & Drug Design. – 2013. – №81 (5). – pp. 557–576.*
5. *Yuskovets V. N., Koshevenko A. S., Yakovlev I. P., Ananieva E. P., Semakova T. L. Substituted chlorides 2-[(1Z)-1-(3,5-diaryl-1,3,4-thiadiazole-2(3H)-ilidene)methyl]-3,5-diaryl-1,3,4-thiadiazole-3-iya and methods of their preparation / Patent No. 2014140482/04. – 2015. – No.35.*
6. *Koshevenko A. S., Ananieva E. P., Yakovlev I. P., Yuskovets V. N. Antifungal activity of new 1,3,4-thiadiazole derivatives // Problems of medical mycology. – 2016. – Vol. 18. – No. 3. – pp. 42–44.*
7. *Koshevenko A. S., Yakovlev I. P., Yuskovets V. N., Ananieva E. P., Kuzmich N. N., Ksenofontova G. V. Synthesis and antifungal activity of new chlorides 2-[(Z)-1-(3,5-diaryl-1,3,4-thiadiazole-2(3H)-ilidene)methyl]-3,5-diaryl-1,3,4-thiadiazole-3-iya // Journal of Pharmacy and Chemistry. – 2017. – Vol. 51. – No. 6. – pp. 18–20.*
8. *State Pharmacopoeia (Russia). XIV Edition – M.: Federal Electronic Medical Library (FEML), 2018.*
9. *Mironov A. N. Guidline for Expertise of Medicines. Vol.II – Moscow: Grif and K., 2013. – 280 p.*

UDK 615.322

DETECTION OF QUERCETIN POLYPHENOL COMPOUND IN PROPOLIS TINCTURE BY THE METHOD OF MATRIX-ACTIVATED LASER DESORPTION/IONIZATION

E.A. Sitnikova, *Master of Chemistry, Head of NGO ECOlab Moscow Branch, Moscow region, Elektrogorsk, lalobai@yandex.ru*
S.G. Mardanly, *Doctor of Medicine, Research Director of ECOlab CJSC, Professor of the Department of pharmacology and pharmaceutical disciplines of the State University of Humanities and Technology (MR HE SEI SHTU), Moscow region, Orekhovo-Zuyevo, ekolab-president@mail.ru*
S.A. Bagatelia, *Process engineer of NGO ECOlab Moscow Branch, Moscow region, Elektrogorsk, ekolab-bagateliya@mail.ru*

This article presents the results of detection of the Quercitin polyphenolic compound in bee products by the example of propolis tincture by matrix-activated laser desorption/ionization in combination with thin-layer chromatography (MALDI/TLC). MALDI mass spectra were recorded from TLC metal targets and plates on a mass spectrometer equipped with a solid-state UV laser and a reflectron. Mass spectra were obtained in the mode of registration of positive ions. The maximum laser energy was 8 kJ/m^2. To visualize the spots on thin-layer chromatograms, UV lamp illumination was used in the UV room. In general, the MALDI/TLC data allowed us to conclude that Quercitin polyphenolic compounds and dihydroquercitin are available in propolis tincture

Keywords: quercetin, dihydroquercetin, MALDI, TLC, polyphenolic compounds, propolis tincture

Scientific research on the composition and properties of propolis is carried out all over the world. Many researchers draw attention to noticeable differences in its composition depending on the place of origin and time of collection. A possible reason for these differences is the sources of sticky substances and pollen: trees and flowers from which the bees collect. Thus, the article [1] shows that there are significant differences in the content of flavonoids in propolis samples from Brazil and South Africa. In the article [2] diterpene acids, triterpene alcohols and ketones are indicated as major components of Brazilian propolis. In [3], prenylated benzophenone was found in propolis samples from Venezuela during the study of phenolic compounds. According to the results of numerous studies, in [4] several types of propolis were identified, for which the typical components are pinosembrine, rinobaskin, rinobaskin-3-O-acetate, chrysin, galangin and derivatives of caffeic acid (benzyl, prenyl and phenylethyl).

It is known from the literature that propolis samples, regardless of the region of origin, contain phenolic acids and flavonoids [5].

The following methods of qualitative and quantitative determination of substances of the specified structure are known: UV-spectrophotometric, GC and HPLC with mass detection, galvanostatic coulometry [6–12].

One of the flavonoids class representatives with polyphenolic structure, is Quercitin, which is commonly found in plant extracts and apitherapy medicinal products (Fig. 1).

The above methods for determining polyphenols are quite expensive and time-

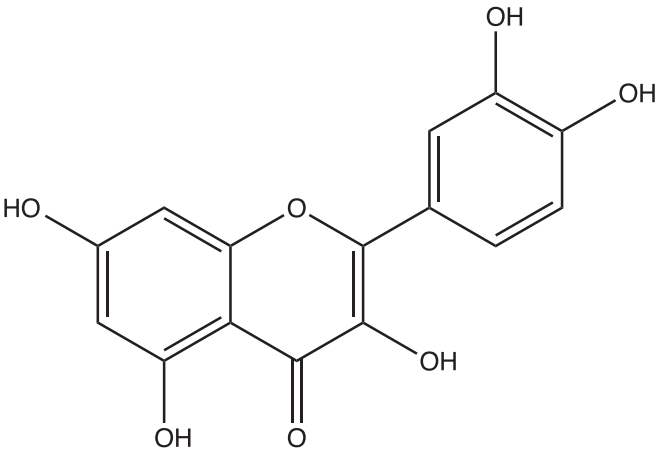


FIG. 1. *Structural formula of 3,3',4',5,7-pentahydroxyflavone (Quercitin)*

consuming, so the development of new methods of determination is undoubtedly interesting. There is no work on a direct combination of MALDI/TLC methods for quality control of honey products.

Purpose of this reserach was to control the quality according to factor "authenticity of honey products" on the example of propolis tincture, to study the possibility of determining Quercitin in honey products by the MALDI/TLC method.

MATERIALS AND METHODS

In this work, the tincture of propolis – series 70715, shelf life until 07.2018, Tula farmfabrika LLC was used.

MALDI mass spectra were recorded from TLC metal targets and plates on a Bruker autoflex speed mass spectrometer equipped with a solid-state UV laser with λ=355 nm and a reflectron. Mass spectra were obtained in the mode of positive ions registration. The maximum laser energy was 8 kJ/ m^2. To visualize the spots on thin-layer chromatograms the UV lamp illumination was used in the UV room of CAMAG (Switzerland). To separate the components of the analyzed samples, a mixture of solvents "chloroform – methyl alcohol – water" was used. Further, the spots on the TLC plates were visualized, covered with a matrix composition "glycerin-graphite-ditranol" and the elution zones corresponding to the Quercitin Rf were irradiated with laser.

RESULTS AND DISCUSSION

The presence of several weakly acidic phenolic hydroxyl groups in Quercitin and dihydroquercitin suggests that the compounds should have a relatively high desorption/ionization efficiency under MALDI conditions.

To derivatize Quercitin, 15 µl of alcohol and 20 µl of triethylamine were dissolved in 200 µl of tetrahydrofuran. To the resulting mixture 15 µl of bromacetyl chloride (or other haloacyl chloride: chloroacetyl chloride, 3-bromopropionyl chloride, 4-chlorobutyril chloride) was added after which the mixture was intensively stirred in a shaker at room temperature for 30 seconds. The resulting reaction mixture was mixed with the matrix solution without further purification.

For derivatization, using a microliter pipette, each spot of the analyte and sequentially 3-bromopropionyl chloride and pyridine, 2 µl each were applied on the contour of the visualized spots (after the TLC method). After drying, combined matrices were applied to the TLC plates with a soft brush and mass spectra were recorded.

The matrix composition was chosen based on the results of optimizing the sample preparation of the standard substance.

Since at the first stage of work it was found that the use of ditranol allows to register the MALDI mass spectra of Quercitin, dihydroquercitin compounds, a composite matrix with the addition of ditranol was used to obtain their mass spectra from TLC plates. The application of this approach made it possible to obtain the mass spectra of these target analytes (Fig. 2–3). Decrease in the signal-to-noise ratio in the case of target compounds is explained by a decrease in the analyte concentration in the near-surface layer of the sorbent.

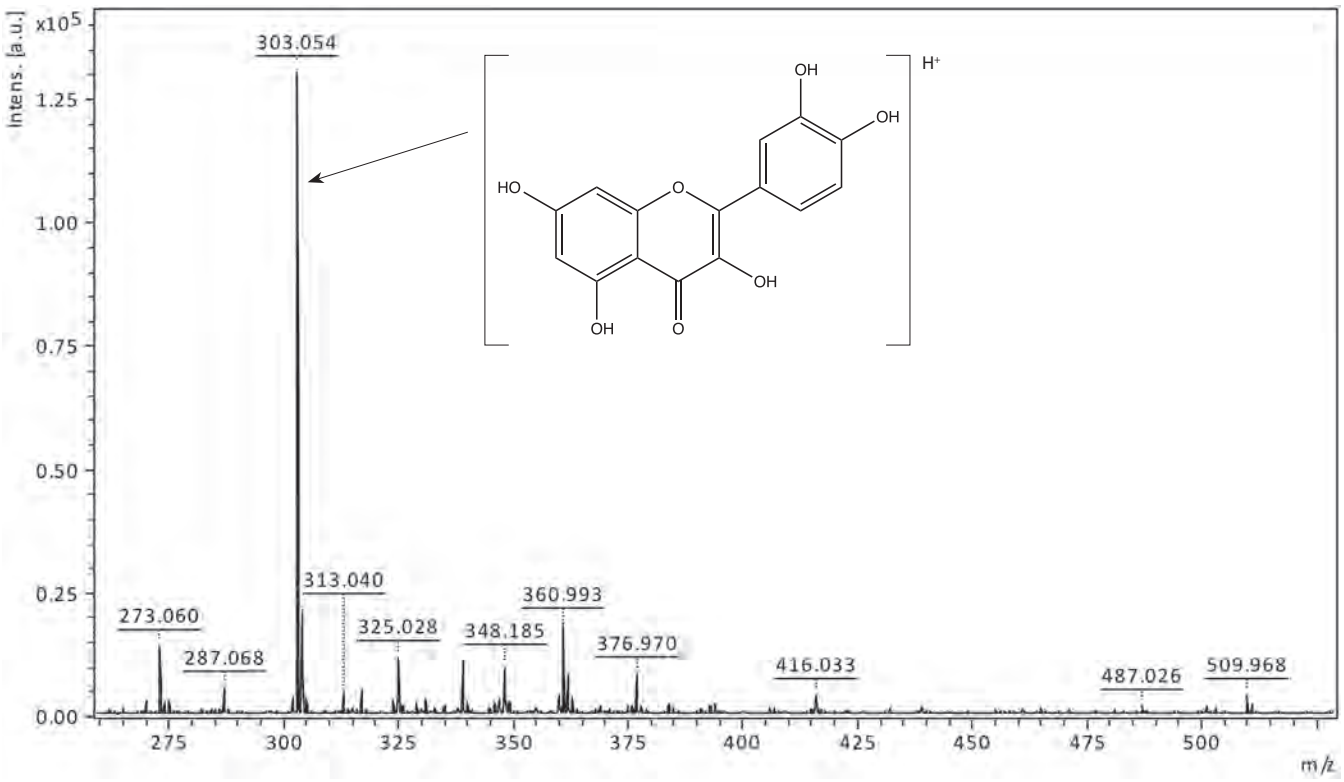


FIG. 2. *MALDI mass spectrum of Quercitin obtained from the TLC plate using a composite matrix "glycerol-graphite-ditranol"*

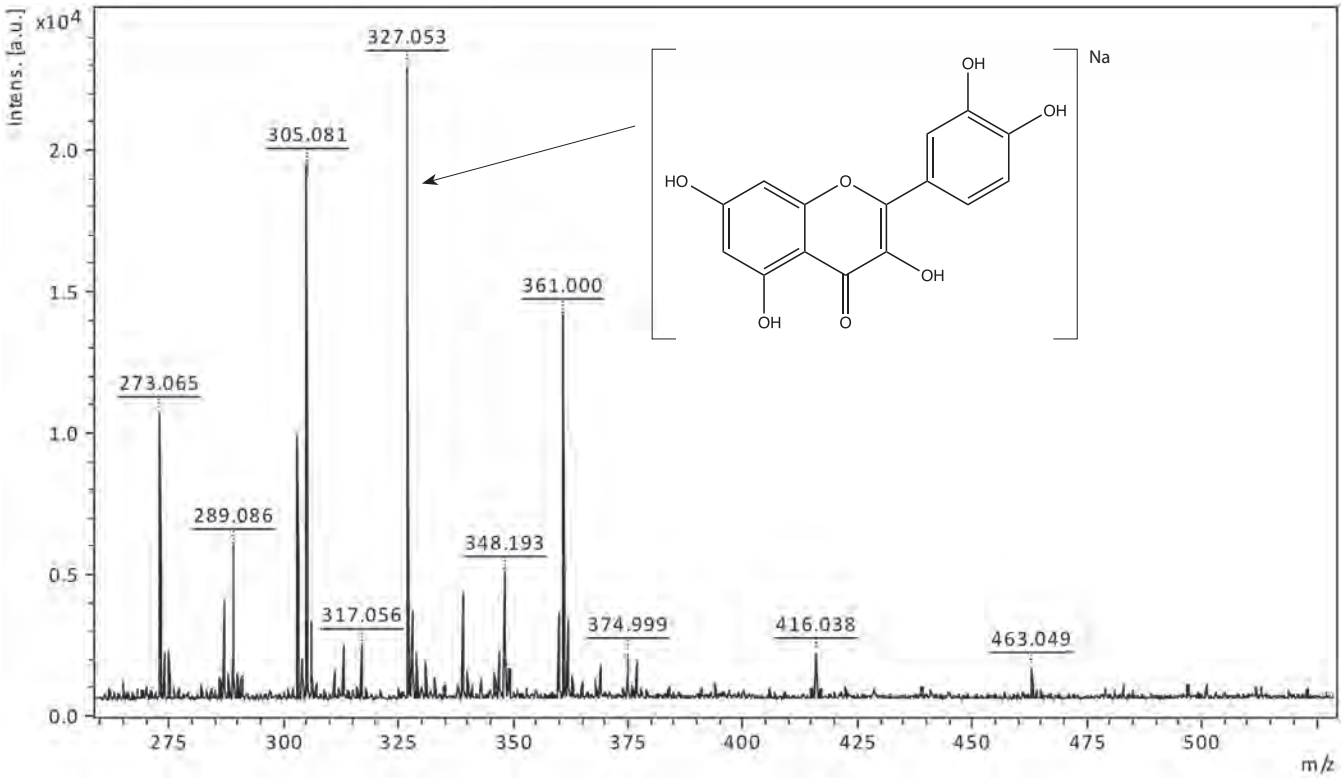


FIG. 3. *MALDI mass spectrum of dihydroquercitin obtained from the TLC plate using a composite matrix "glycerol-graphite-ditranol"*

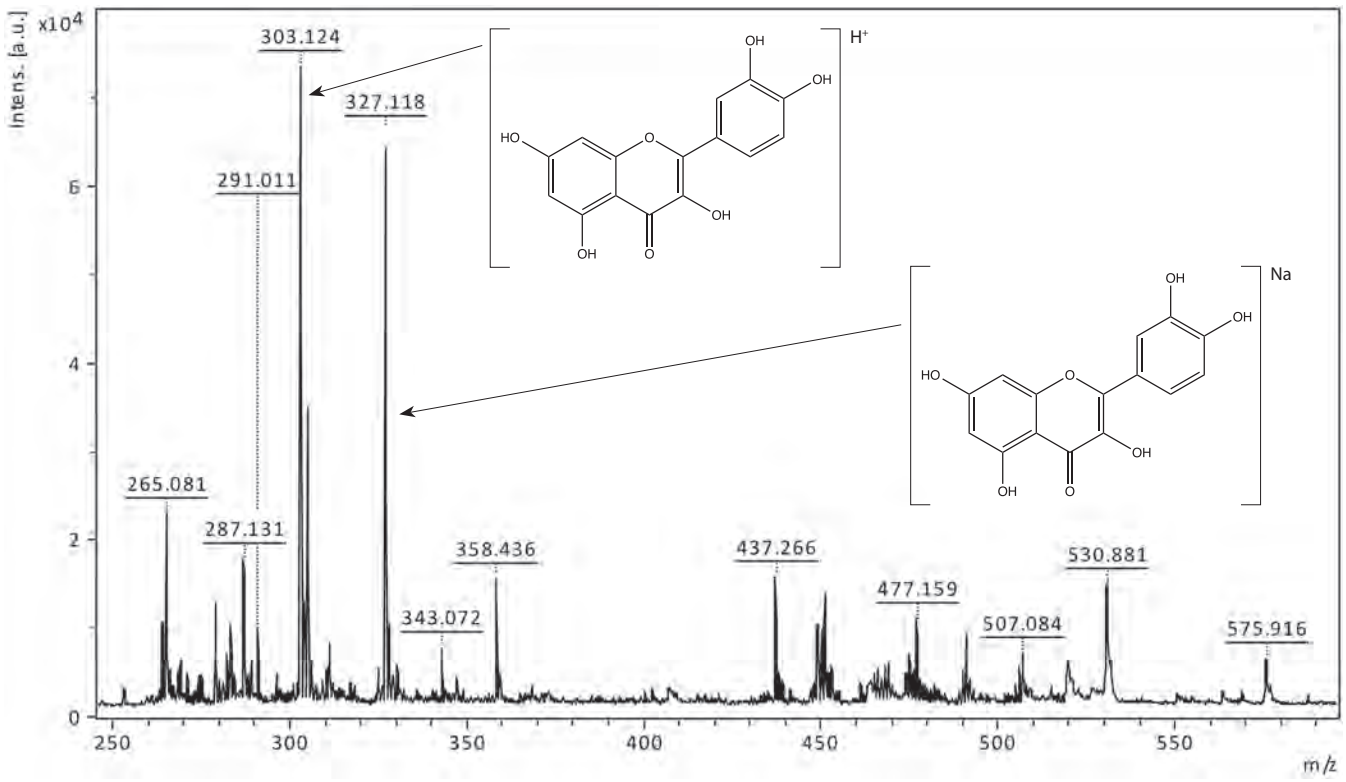


FIG. 4. *MALDI mass spectrum obtained from the surface of the TLC plate with separated propolis extract components*

The obtained mass-spectral data from the study of propolis tincture showed that the elution zones of Quercitin and dihydroquercitin compounds are overlapped and the registered mass spectra simultaneously contain corresponding ion peaks, which confirms their presence in the sample (Fig. 4).

CONCLUSION

In the result of the study:

1. An innovative method of qualitative confirmation of the presence of quercitin in propolis tincture by the MALDI/TLC method has been developed.

2. The quality control of propolis tincture for identification factor was carried out by the MALDI/TLC method.

3. The presence of quercitin and dihydroquercitin in propolis tincture is confirmed.

REFERENCES

1. *Rasote D., Suleman N., Chen W., Sandasi M., Viljoen A., Vuuren S. van. Chemical profiling and chemometric analysis of South African, Propolis // Biochemical Systematics and Ecology, 55 (2014). – P. 156–163.*
2. *Bankova V., Christov R., Marcucci C., Popov S. Constituents of Brazilian geopropolis // Z. Naturforsch. (1998). 402–406. – P. 53.*
3. *Tomas-Barberan F. A., Garcia-Viguera C., Vit-Olivier P., Ferreres F., Tomas-Lorente F. Phytochemical evidence for the botanical origin of tropical propolis from Venezuela // Phytochem. 34 (1993). – P. 191–196.*
4. *Bankova V. S., de Castro S. L., Marcucci M. C. Propolis: recent advances in chemistry and plant origin // Apidologie. 2000. 31. – P. 10–11.*
5. *Orlandini D. G., Pinetti D., Benvenuti S., HPLC-DAD and HPLC-ESI-MC/MC methods for metabolite profiling of propolis extracts // Journal of*

Pharmaceutical and Biomedical Analysis. 55, 920110. – P. 934–348.

6. *Cheng H., Qinc Z.H., Guo X.F., Huc X.S., Wu J.H. Geographical origin identification of propolis using GC-MS and electronic nose combined with principal component analysis // Food Research International. 51 (2013). 813–822.*
7. *Lowry O.H., Rosebrought N.J., Farr A.G., Randall R.J. // J. Biol. Chem. 1951, vol. 193, №1, p. 265–275.*
8. *Lupina E.V., Pisarev D.I., Novikov O.O., Malyutina A.Yu., Vasiliev G.V., Vasilieva Yu.G. Chemical study of the composition of flavones and flavonols in propolis // Pharmacy and pharmacology. 2018, 6 (3):241–254.*
9. *GOST R 55488–2013 Propolis. Method for determination of polyphenols.*
10. *Xiao P., Zhao F., Zeng B. // Microchem. J. 2007. V. 85. №2. – P. 244.*
11. *Halket J.M., Zaikin V.G. Derivatization in mass spectrometry-1. Silylation // Eur.J. Mass Spectrom. 9. 1, 2003.*
12. *Zaikin V.G., Halket J.M., Derivatization in mass spectrometry-2. Acylation // Eur.J. Mass Spectrom. 9, 42, 2003.*

UDK 615.15

A COMPREHENSIVE ASSESSMENT OF QUALITY OF PHARMACEUTICAL COUNSELING AT PHARMACIES IN VIETNAM

V.V. Dorofeeva, *PharmDr., Professor of the Department of Management and Economics of Pharmacy, FSAEI HE "Peoples' Friendship University of Russia" (FSAEI HE PFUR), Moscow, Russia wwd.pro@gmail.com*
V.D. Chan, *PG student of the Department of Management and Economics of Pharmacy, FSAEI HE "Peoples' Friendship University of Russia" (FSAEI HE PFUR), Moscow; Lecturer of the Department of Traditional Medicine "Can Tho University of Medicine and Pharmacy", Van Tho, Vietnam, vandepro@gmail.com*

According to the International Pharmaceutical Federation (FIP), pharmaceutical counseling is an approach aimed at improving the skills of pharmaceutical professionals in solving patient problems related to the use of medicines (drugs), in order to improve or maintain the quality of health and life of the patient. The scientific significance of this study is connected with the fact that for the first time a methodological approach to a comprehensive assessment of the quality of pharmaceutical counseling in pharmacies is proposed. To achieve the goals of this study, a questionnaire was developed and its quality was checked using the Rasch model. Quantitative data analysis was performed using jMetrik 4,0,6 and SPSS 22,0 software. The results of the study showed that the quality of pharmaceutical counseling in pharmacies in Vietnam is at an average level according to the proposed rating scale. To improve pharmaceutical counseling in pharmacies in Vietnam, it is necessary to organize additional professional education aimed at improving skills in this area.

Keywords: comprehensive assessment, pharmaceutical counseling, quality of a questionnaire, construct validity, reliability, Rasch model, Rasch analysis, pharmacy, Vietnam

As a basic element of the concept of good pharmacy practice, pharmaceutical counseling plays an important role in ensuring the effective and safe use of medicines. It consists in providing the patient with oral or written information about the use of medicines, side effects, precautions, as well as advices on storage, diet and lifestyle changes [1]. Therefore, in practice, pharmaceutical counseling is a process of interaction between a consumer and a pharmaceutical specialist, whose goal is to help the patient make the right decisions on the use of medicines and improving the health [2].

According to R. Abdel-Tawab, Kurtz S.M., and others, the process of pharmaceutical counseling can be divided into the following four stages: (1) start of counseling – building the therapeutic relationship with the patient; (2) collecting data about the patient – identifying needs and problems related to medicines and the patient's health; (3) content of the counseling – solving the patient's problems and consulting on the use of medicines and the patient's health; (4) the end of counseling – summarizing key information and establishing a plan for subsequent actions of the patient [3,4]. In addition, the behavior of the staff during the counseling process plays an important role.

Analysis of world (including Vietnamese) scientific publications on pharmaceutical counseling showed that the main areas of research in this area were: development of a model and algorithm for pharmaceutical

counseling [5]; study of individual stages of counseling (collection of patient data, content of the counseling, the end of the counseling) and study of the behavior of pharmaceutical personnel in the process of counseling [6,7]. Thus, comprehensive study of the entire counseling process with simultaneous assessment of behavior of pharmaceutical personnel was not carried out.

The purpose of this study was a comprehensive assessment of the quality of pharmaceutical counseling in pharmacies in Vietnam. To achieve this goal, the following tasks were solved: (1) development of a comprehensive assessment questionnaire for pharmaceutical counseling, (2) control of quality of the developed questionnaire based on the Rasch model, and (3) comprehensive assessment of pharmaceutical counseling in pharmacies in Vietnam.

MATERIALS AND METHODS

Questionnaire development. In this study, the questionnaire is based on conceptual models for developing pharmaceutical counseling skills: *Calgary-Cambridge Guide* [4], *United States Pharmacopeia (USP) Medication Counselling Behavior Guidelines* [8] and *Medication-Related Consultation Framework* [3]. The structure of the questionnaire includes 31 items containing the most important aspects of professional activity in the field of counseling and behavior of a pharmaceutical professional. These items were grouped into 5 blocks: block A – start of counseling (6 items), block B – collection of information (6 items), block C – content of the counseling (7 items), block D – end of the counseling (6 items), block E – behaviour during counselling (6 items) (see table. 1). Then,

Table 1

QUESTIONNAIRE FOR COMPREHENSIVE ASSESSMENT OF QUALITY OF PHARMACEUTICAL COUNSELING

№	Description of an item	1	2	3	4	5
	Block A – Beginning of counseling					
1	Welcome the patient					
2	Introduce yourself to the patient					
3	Verify the patient's identity					
4	Explain the purpose and content of counseling					
5	Express respect to and interest in the patient					
6	Pay attention to comfort and privacy					
	Block B – Information acquisition					
1	Determine the reasons for visiting the patient					
2	Identify the problems that the patient wants to solve					
3	Check and confirm the patient's problems and examine other problems					
4	Evaluate all current/potential problems, as well as problems which are important for the patient					

End of table 1

№	Description of an item	1	2	3	4	5
5	Collect information related to the history of medicine use (allergies, adverse reactions, self-medication, additional or alternative therapies)					
6	Identify other medical conditions that may affect the effects of the medicine. Identify social diseases (alcohol use, smoking, lifestyle, profession, diet, etc.)					
	Block C–Content of counseling					
1	Discuss the name and indication of the medicine					
2	Counsel on how and when to take the medicine, the duration of treatment					
3	Explain the timing of taking the medicines for the desired effect					
4	Discuss recommendations for storage, auxiliary actions (shake…)					
5	Explain the risks of side effects, how to prevent and manage side effects if they occur					
6	Discuss possible drug interactions (between drugs and drugs, drugs and food, drugs and diseases)					
7	Send them to other healthcare professionals accordingly					
	Block D – End of counseling					
1	Help the patient plan the following steps and actions					
2	Explain what to do if the patient has difficulties with following the prescribed therapy, and who to contact					
3	Summarize and emphasize the key points of the information received					
4	Use feedback to check the patient's understanding					
5	Check whether the patient agrees with the proposed action plan					
6	Allow to find out the final problems or questions					
	Block E – Behavior during counseling					
1	Actively listen and allow the patient to express his/her demand without any interruptions					
2	Avoid medical terms. Use language that is understandable to the patient					
3	Demonstrate empathy and support for the patient					
4	Share the patient's views to encourage their participation in the dialogue					
5	Manage time effectively					
6	Demonstrate effective non-verbal behavior					

Grade: 1 – undone, 2 – badly, 3 – satisfactory, 4 – good, 5 – excellent

according to this questionnaire, pharmaceutical professionals conducted a self-assessment of the quality of counseling. A five-point Likert scale was used in the range from 1 (undone) to 5 (excellent).

Collection of quantitative data. 422 pharmaceutical professionals were randomly selected from pharmacies in four major cities in Vietnam: Hanoi, Da Nang, Ho Chi Minh and Can Tho. The questionnaire was provided from June 10 to October 30, 2017. This data set served as the material for the following two stages: checking the quality of the questionnaire and analyzing the current state of counseling.

Check of the questionnaire quality. In this study, to check the quality of the questionnaire, the main stages of the Rasch-analysis were consistently carried out: (1) evaluation of the validity and (2) evaluation of the reliability of the questionnaire. The collected data was analyzed using the jMetrik software, version 4,0,6.

At the first stage, when analyzing the construct validity of the questionnaire, we used the following tests: item severity, item polarity *(PTMEA Corr),* item fit-statistics *(Infit and Outfit MNSQ).*

According to Wright B.D. et al., items whose severity value varies from –2 to +2 are considered as appropriate [9]. A value less than –2 represents an extremely simple item, while a value greater than +2 represents an extremely difficult item.

The item polarity was estimated using the general correlations coefficient of correlated items *(PTMEA Corr).* The range of acceptable *PTMEA Corr* values is 0.3–0.8, under this condition the items work in the same direction to measure the same basic construct [10].

In accordance with the rules of the Rasch model, an important index of the item quality is fit statistics *(Infit and Outfit MNSQ),* which can be used to determine how well or poorly this item fits into the general continuum of all items, maintaining or violating the uniformity of the questionnaire [11]. *Infit and Outfit MNSQ* values in the range 0.5–2.0 are considered as acceptable. A value greater than 2.0 indicates that the item has too much deviation (noise), and a value less than 0.5 indicates too high consistency [12]. Items that do not meet the requirements of these tests must be removed from the questionnaire.

At the second stage, the questionnaire was estimated based on reliability and separation statistics. The questionnaire has a high level of reliability if the reliability factor is higher than 0.8 [13], and the value of the separation statistics is greater than 3.0 [14].

Assessment of the counseling quality. For a comprehensive assessment of the quality of pharmaceutical counseling, we have proposed the following formula:

$$O = O_A + O_B + O_C + O_D + O_E,$$

where O_A – start of counseling; O_B – information acquisition; O_C – content of counseling; O_D – end of counseling; O_E – behavior during counseling.

O_A is calculated from the average value of all items in the block A. Calculation for other values – O_B, O_C, O_D, O_E – is analogical. As a result, the possible maximum value of O is 25 points. The scale for evaluating the quality of counseling is presented in table 2.

The data obtained at this stage was analyzed using the SPSS computer application, version 22.0.

Table 2

SCALE FOR EVALUATING THE QUALITY OF COUNSELING

Evaluation. points	The level of development of ccounseling
5.0–9.9 and lower	low
10.0–14.9	lower than average
15.0–19.9	average level
20.0–25.0	high

RESULTS AND DISCUSSION

The socio-demographic profile of the respondents who took part in this study is presented in the table. 3: 68.2% were female pharmacists, 67.8% of respondents have secondary pharmaceutical education. The majority of respondents are aged from 26 to 35 years (45.7%) and have 2 to 5 years of professional experience (43.4%).

Table 3

SOCIO-DEMOGRAPHIC PROFILE OF PHARMACEUTICAL PROFESSIONALS IN PHARMACIES IN VIETNAM (N = 422)

Socio-demographic characteristics	Relative share. %
Gender	
male	31.8
female	68.2
Pharmaceutical education	
high	32.2
Secondary level	67.8
Age (years)	
Up to 25	20.1
26–35	45.7
36–45	23.7
46–55	5.7
56–60	2.6
Higher than 60	2.1
Work experience (years)	
Less than 2	13.5
2–5	43.4
6–10	23.9
11–20	12.6
21–30	5.0
More than 30	1.7

As mentioned earlier, a Rasch analysis was used to assess the quality of the questionnaire developed by us, the results of which are presented in the tables 4 and 5.

Analysis of validity of the questionnaire showed the following:

- the values of the "severity of items" parameter in all blocks of the questionnaire were in an acceptable range (from –2 to 2), i.e. all items of the questionnaire have an average level of severity. In this case, item A5 is the simplest (–0.89), and item A2 is the most severe (1.40);
- the values of the "item polarity" parameter had *PTMEA Corr* from 0.47 to 0.77, i.e. they were in an acceptable range (from 0.3 to 0.8);
- the values of the *Infit and Outfit MNSQ* fit-statistics were in the range corresponding to the Rasch model – from 0.5 to 2.0.

Thus, at this stage, all items in the questionnaire meet the requirements of the Rasch model and, therefore, cannot be deleted.

In table. 5 the results of calculating reliability factors and the separation index are presented. It is established that the questionnaire has high reliability level (the reliability factor of the questionnaire as a whole, 0.97 and for each block – in the range 0,94–0,99; a separation statistics factor of the questionnaire as a whole – 6,11 and for each block – in the range of 3.99–11,83). Thus, based on the conducted Rasch analysis, we can conclude that the questionnaire developed by us has good metric properties and can be used for the next stage of the study i.e. for a comprehensive assessment of the quality of pharmaceutical counselling.

At this stage the analysis of the four components of the counseling process (start of counseling, information acquisition, content of counseling, end of counselling) and behavior of pharmaceutical personnel during performance of their employment functions (Fig. 1).

According to the results of the analysis of block A – "start of counseling" – it was found that this activity reaches an average level (O_A = 3.55).

Table 4

RESULTS OF THE QUESTIONNAIRE VALIDITY ANALYSIS

Block	Item	Severity	PTMEA Corr	Infit MNSQ	Outfit MNSQ
A	1	–0.60	0.68	1.23	1.19
	2	1.40	0.47	1.38	1.36
	3	0.33	0.55	0.94	0.93
	4	0.09	0.68	0.65	0.66
	5	–0.89	0.69	0.71	0.74
	6	–0.34	0.53	1.05	1.13
B	1	–0.03	0.74	1.08	1.05
	2	–0.32	0.77	0.70	0.71
	3	0.50	0.64	1.06	1.07
	4	0.86	0.67	1.11	1.16
	5	–0.72	0.73	0.94	0.90
	6	–0.28	0.71	1.11	1.13
C	1	0.45	0.61	1.17	1.17
	2	–0.58	0.72	0.85	0.82
	3	0.06	0.69	0.74	0.77
	4	–0.20	0.69	0.86	0.92
	5	–0.13	0.63	0.81	0.81
	6	–0.02	0.62	1.14	1.13
	7	0.42	0.49	1.37	1.37
D	1	0.07	0.77	1.11	1.09
	2	0.58	0.76	0.98	0.99
	3	–0.27	0.71	1.09	1.11
	4	0.01	0.72	0.87	0.86
	5	0.01	0.69	0.85	0.84
	6	–0.41	0.67	1.04	1.02
E	1	–0.26	0.69	0.86	0.86
	2	–0.44	0.68	0.68	0.71
	3	–0.44	0.74	0.56	0.56
	4	0.35	0.67	0.88	0.88
	5	0.79	0.47	1.80	1.79
	6	0.00	0.52	1.14	1.14

Table 5

RESULTS OF ANALYSIS OF RELIABILITY FACTORS AND SEPARATION STATISTICS

Statistics	Blocks					Questionnaire as a whole
	A	B	C	D	E	
Reliability factor	0.99	0.97	0.94	0.96	0.98	0.97
Separation index	11.83	5.72	3.99	4.85	6.89	6.11

At the same time, pharmacy personnel fulfill well the criteria such as A_5 "express respect to and interest in patients" (A_5 = 4.00) and A_1 "welcome the patient" (A_1 = 3.86), but lower than average value – A_2 "introduce yourself to the patient" (A_2 = 2.77) and A_3 "check the patient's identity" (A_3 = 3.39).

Analysis of block B – "Information acquisition" – showed that pharmacy employees best comply with the criterion B_5 "collect information related to the history of medicine use" (B_5 = 3.82). Criteria such as B_2 "identify the problems that the patient wants to solve" and B6 "study social history" were also met fairly well (3.69 and 3.68, respectively). Worst of all the following criteria are fulfilled: B3 "check and confirm the patient's problems and examine other problems" and B4 "evaluate all current/potential problems, as well as problems which are important for the patient" (3.41 and 3.28, respectively). The average value of the "information acquisition" block is O_B = 3.58, which reflects the average level of quality of this stage of counseling.

In the analysis of block C – "content of counseling" – it was found that pharmaceutical professionals well comply with such criteria as C_2 "Counsel on how and when to take the medicine, the duration of treatment" (C_2 = 3.77) and C_4 "discuss recommendations for storage, auxiliary instructions" (C_4 = 3.63). But C_7 "send them to other healthcare professionals accordingly" (C_7 = 3.40) and C_1 "discuss the name and indications of drugs"

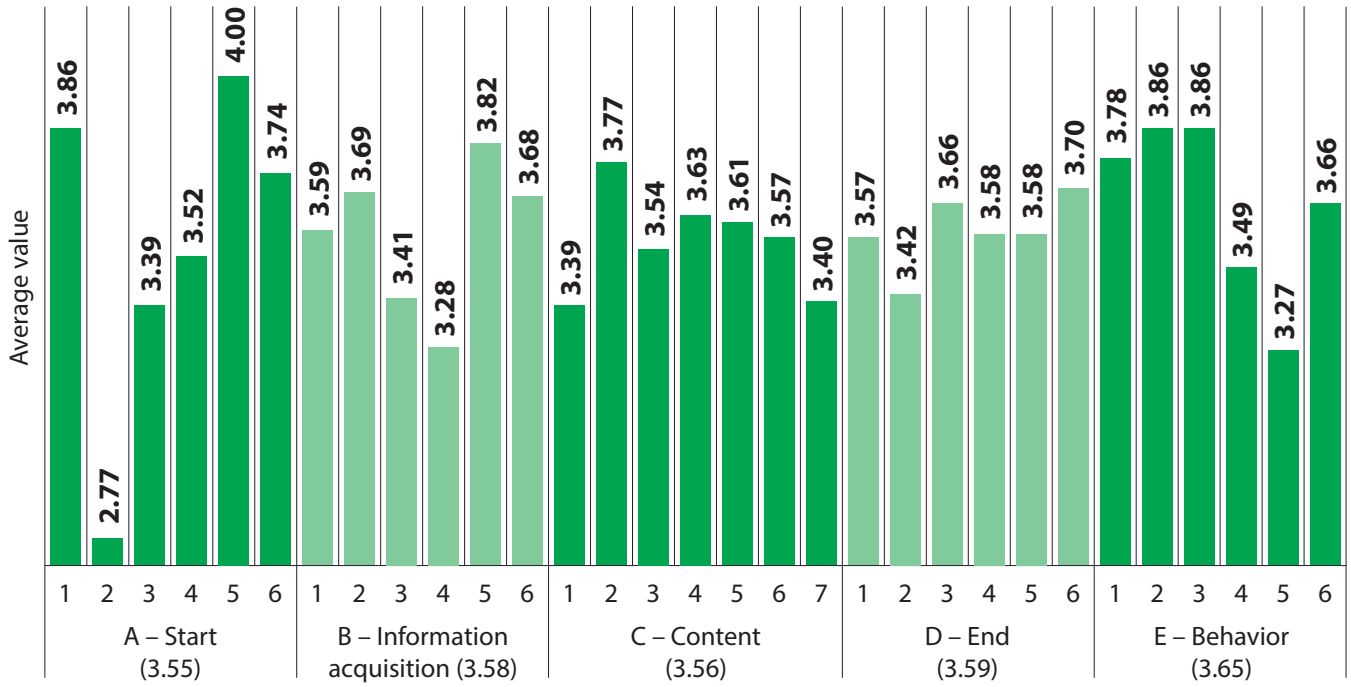


FIG. 1. *Comprehensive assessment of the quality of pharmaceutical counselling (n = 422)*

are worse to be fulfilled (C_1 = 3.39). The average value of block C – "content of counseling" – is O_C = 3.56, which corresponds to the average level of quality of pharmaceutical counseling.

The results of the analysis of block D – "end of counseling" – also showed an average level of quality of the counseling process (O_D = 3.59). At the same time, the pharmacy personnel best met the criteria D_6 "allow to find out the final problems or questions" (D_6 = 3.70) and D_3 "summarize and emphasize the key points of the information received" (D_3 = 3.66). The level of fulfillment of the D_2 criterion "explain what to do if the patient has difficulties with following the prescribed therapy, and who to contact" was lower than the average value (D_2 = 3.42).

Analysis of the behavior of pharmaceutical personnel showed that during counseling, pharmacy professionals avoid jargon and use language that is understandable to the patient (E_2 = 3.86), as well as demonstrate empathy and support for the patient (E_3 = 3.86). However, they much less often share the views of the patient in order to encourage their participation in the dialogue (E_4 = 3.49), and have poor control over the time allotted for consultation (E_5 = 3.27). The average value of this block is O_E = 3.65, which is a satisfactory level of behavior during counseling.

Thus, the overall assessment of quality of pharmaceutical counseling in the studied pharmacies in Vietnam was:

$$O = O_A + O_B + O_C + O_D + O_E =$$
$$= 3.55 + 3.58 + 3.56 + 3.65 + 3.59 = 17.92.$$

According to our proposed scale (see table. 2), this corresponds to the average level. Therefore, in order to improve the quality of counseling in pharmacies in Vietnam, it is necessary to constantly organize additional professional education programs aimed at improving skills in this area, as well as to develop practical tools for providing consulting support to pharmaceutical personnel.

CONCLUSION

In this study, we developed a questionnaire to conduct a comprehensive assessment of the quality of pharmaceutical counseling. Based on the results of the analysis of psychometric properties of the questionnaire using the Rasch model, it was found that this questionnaire has satisfactory construct validity, reliability and can be used by pharmaceutical professionals as a tool for self-assessment of the quality of counseling.

According to the developed questionnaire, a comprehensive assessment of the quality of pharmaceutical counseling by professionals of pharmacies in the largest cities of Vietnam was carried out. Based on the results obtained, it was found that this parameter is at an average level. To improve the skills of pharmaceutical counseling, a working program and practical recommendations have been developed for students within the framework of the professional qualifications system.

REFERENCES

1. *ASHP Reports. ASHP guidelines on pharmacist-conducted patient education and counseling / ASHP Reports // Am.J. Health. Syst. Pharm. – 1997. – Vol. 54 (4). – P. 431–434.*
2. *Da-Costa F.A. The pharmacist guide to implementing pharmaceutical care / F. A. da Costa, J.W. F. van Mil, A. Alvarez-Risco. – Cham, Switzerland: Springer, 2019. – 506 p.*
3. *Abdel-Tawab R., James D.H., Fichtinger A., Clatworthy J., Horne R., Davies G. Development and validation of the Medication-Related Consultation Framework (MRCF) // Patient Educ. Couns. – 2011. – Vol. 83 (3). – P. 451–457.*
4. *Kurtz S.M. Teaching and learning communication skills in medicine / S.M. Kurtz, J. Silverman, J. Draper. – UK: Radcliffe Medical Press, 1998. – 245 c.*

5. *Kugach V.V., Tarasova E.N., Kunitsky V.S. Pharmaceutical counseling of pharmacy visitors for rhinitis and sore throat // Bulletin of pharmacy. – 2014. – No. 3. – pp. 15–30.*
6. *Alfadl A.A., Alrasheedy A.A., Alhassun M.S. Evaluation of medication counseling practice at community pharmacies in Qassim region, Saudi Arabia // Saudi Pharm. J. – 2018. – Vol. 26 (2). – P. 258–262.*
7. *Hoang T.N. V., Hoang T.N. V., Tran V.H., Cu T.T. Survey on medicine for the flu retail skills of the staff in some drug stores at Ho Chi Minh city // Ho Chi Minh City J.Med. – 2018. – Vol. 22 (1). – P. 267–272.*
8. *Puumalainen I., Halonen P., Enlund H., Johnson K., Airaksinen M. Validation of the United States Pharmacopeia (USP) medication counselling behaviour guidelines // Pharm. Educ. – 2005. – Vol. 00 (0). – P. 1–10.*
9. *Wright B.D. Measurement essentials. 2nd. / B.D. Wright, M.H. Stone. – Wilmington: Wide Range, Inc. 1999. – 221 p.*
10. *Allen M.J. Introduction to Measurement Theory / M.J. Allen, W.M. Yen. – USA: Waveland Press, 2001. – 310 p.*
11. *Bond T.G. Applying the Rasch model. Fundamental measurement in the human sciences / T.G. Bond, C.M. Fox. – New York, London: Routledge Taylor & Francis Group, 2007. – 340 p.*
12. *Meyer J.P. Applied measurement with jMetrik. – New York: Taylor & Francis, 2014. – 149 p.*
13. *Fisher W.P. Rasch Measurement Transaction: Transaction of the Rasch Measurement SIG // Am. Educ. Res. Assoc. – 2007. Vol. 21 (1). – P. 1095.*
14. *Boone W.J., Noltemeyer A. Rasch analysis: A primer for school psychology researchers and practitioners // Cogent. Educ. – 2017. Vol. 4 (1). – P. 1416898.*

UDK 615.014:615.453.8:578.81

BIOPHARMACEUTICAL ASSESSMENT OF ANTIBACTERIAL MEDICATED FILMS

N.A. Kovyazina, *Candidate of Pharmaceutical Sciences, Associate Professor of the Department of Industrial Technology of Drugs with the course of biotechnology, FSBEI of Higher Education "Perm State Pharmaceutical Academy", Ministry of Health of the Russian Federation (FSBEI HE PSPA RF MoH), Perm, natanat.k@mail.ru*

A.M. Nikolaeva, *Doctor of Biological Sciences, Professor of the Department of Industrial Technology of Drugs with the course of biotechnology, FSBEI of Higher Education "Perm State Pharmaceutical Academy", Ministry of Health of the Russian Federation (FSBEI HE PSPA RF MoH), Perm, nikolaeva.alla@gmail.com*

The article is devoted to biopharmaceutical research of Sextaphag® medicated films. A comparative assessment of the matrix nature influence on release of immobilized combined bacteriophage was carried out for the following parameters: swelling kinetics, release of bacteriophages from medicated films by diffusion into agar, wound healing effect. Sextaphag® medicated films are prolonged-release systems. The release is carried out by a diffusion mechanism with gradual biodegradation of the polymer system with an immobilized combined bacteriophage. Methylcellulose-based films release the bacteriophage with its active release at the beginning of the swelling process, and then with a gradual decrease in the release rate and complete biodegradation of the polymer. Gelatin-based films are able to release the bacteriophage slowly and continuously. The study of effect of the film composition on release of immobilized bacteriophage from the matrix system by diffusion into agar showed that the rational release-active dosage form is Sextaphag® films based on gelatin, which have better bioavailability compared to the film composition based on methylcellulose. Sextaphag® medicated films have wound-healing activity comparable to the activity of the comparator drug (Levomecol ointment), and a pronounced antibacterial action.

Key words: bacteriophage, bioavailability, medicated films, swelling, wound, release

Long-term use of bactericides for repair of pyoinflammatory wounds leads to the emergence of antibacterial resistance of microbial flora [5]. In this regard, it is necessary to expand the range of antibacterial agents for infectious diseases of various etiologies. One of the promising pharmaceutical products is combined bacteriophages, which have proven themselves in surgery, dentistry, obstetrics and gynecology [1,2,6,7].

The development of scientific research in the field of biopharmaceuticals has convincingly shown that the right choice of a dosage form which provides ease of use and aimed action of the pharmacologically active medicine contained in it is important for the effective treatment of diseases. Rational application dosage form is films that provide stability of bacteriophages, local action, prolonged release, as well as a complex effect of the phage and the carrier matrix on the pathogenesis of the wound process.

The purpose of work – study of biological availability of bacteriophages from antibacterial medicated films.

MATERIALS AND METHODS

Medicated films, composition № 1 (gelatine – 5,0; glycerine – 2,0; Sextaphag® – 40,0; purified water – 53,0), obtained by air drying. *Medicated films, composition No. 2* (methylcellulose – 3,0; glycerine – 1,5; calcium carbonate – 1,5; Sextaphag® – 40.0; purified water – 54,0), obtained by vacuum drying at vacuum depth of –0.05 MPa. Films of compositions 1 and 2 have specific (lytic) activity according to the Appelman method 10^{-3} and higher in relation to *Staphylococcus (aureus, epidermidis), Streptococcus (pneumoniae, agalactiae, pyogenes), Proteus (mirabilis, vulgaris), Pseudomonas aeruginosa, Klebsiella pneumoniae, энтеропатогенных Escherichia coli.*

The kinetics of swelling of medicated films was determined by gravimetric method [4,8] and evaluated by the following parameters:

a) the degree of swelling during the period of τ was evaluated by the amount of water absorbed and calculated by the formula:

$$\alpha = \frac{m - m_o}{m_o} \times 100\%,$$

where α – degree of swelling, %; m – film weight after swelling, g; m_o – initial weight of film, g.

b) The swelling rate was determined by the rate of diffusion of solvent molecules into polymer and calculated by formula:

$$v = \frac{d\alpha}{d\tau} = K_н(\alpha_\infty - \alpha_\tau),$$

where Кн – swelling rate constant; α_∞ – maximum degree of swelling, %; α_τ – degree of swelling by time τ, %.

c) Swelling rate constant, characterizing the kinetics of the swelling process by time τ, calculated by formula:

$$K_н\,\tau = \frac{1}{\tau} \ln \frac{\alpha_\infty}{\alpha_\infty - \alpha_\tau},$$

where $K_н\,\tau$ – swelling rate constant by time τ; α_∞ – maximum degree of swelling, %; α_τ – degree of swelling by time τ, %.

d) To calculate the swelling rate constant that characterizes the kinetics of the swelling process of polymer matrix systems, a graph was plotted in coordinates of $\ln\alpha_\infty / (\alpha_\infty - \alpha_\tau)$ vs τ, by tangent of the angle of inclination of the straight line leaving the origin of the coordinates, the swelling rate constant kN = Tgθ was found.

Release of bacteriophages from medicated films was determined *in vitro* by diffusion into agar on dense substrate. The bacterial culture of test strains of the following microorganisms isolated in the laboratories of medical institutions of Perm and Perm region was inoculated on Petri dishes with dense substrate according to General Monograph OFS.1.7.1.0002.15 "Therapeutic and prophylactic bacteriophages": *Staphylococcus (aureus, epidermidis), Streptococcus (pneumoniae, agalactiae, pyogenes), Proteus (mirabilis, vulgaris), Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli.* Samples of medicated films with immobilized Sextaphage® with area of 1 cm^2 were applied to the surface of the test system and thermostated for 24 hours at a temperature of 37°C. Results were evaluated by measuring the diameter of the test strain inhibition zone.

Wound-healthing action of Sextaphage® was studied *in vivo* by tensiometric method on the model of linear aseptic wound [5]. Studies were carried out on white male Wistar rats weighing 180–220 g in total 48 individuals. The conditions of quarantine and keeping of experimental animals corresponded to the current sanitary rules of Federal Law FZ-I-BP-03–308.3-B1–2584 "Quarantine of laboratory animals". Laboratory animals were divided into groups: group 1 – without treatment; group 2 – treatment using a comparator agent (Levomekol ointment); group 3 – treatment using Sextaphag® films based on gelatin; group 4 – treatment using Sextaphag® films based on methylcellulose. Rats under short-term ether anesthesia were subject to a skin linear

incision of 5 cm in length to the subcutaneous tissue, then, the wound was sewed with a number of interrupted stitches. In group 1, no treatment was performed. Wounds of animals of the 2nd – 4th groups were treated daily with the studied samples. Animals were killed on the 5th and 7th day after surgery, and the scar-breaking strength was tested by wound- tensiometric way. The strength of the scar was calculated by the formula:

$$F = m \times g,$$

where F – breaking strength of a scar, N; m – load weight, required for scar breaking; g – gravity acceleration, m^2/s.

Statistical processing of the study results were carried out with Microsoft Excel in accordance with General Monograph OFS.1.1.0013.15 "Statistical processing of chemical experiment results".

RESULTS AND DISCUSSIONS

Quality factor of Sextaphag® medicated films is ability to provide optimal bioavailability of immobilized bacteriophages. A certain contribution to kinetics of release of pharmaceutical products is made by process of swelling of polymer matrices. The duration and effectiveness of the pharmacotherapeutic action of medicated films depend on the amount of water absorbed and the time of swelling. Kinetic curves of swelling of films based on gelatine and methylcellulose are shown in Fig. 1.

From the presented data it can be seen that with increasing the process duration, the degree of swelling increases, but with different intensity. Gelatin-based films have high ability to swell, but less swelling is observed in methylcellulose-based films. The kinetic curve for methylcellulose-based medicated films has the form of an unlimited swelling curve and passes through a maximum of 305% swelling degree. In gelatin-based films, the degree of swelling within three hours was 410%, and, according to the kinetic curve, the polymer matrix system belongs to the limited swelling polymers, since when swelling this system is not transformed into colloidal solution and does not dissolve when interacting with purified water.

Swelling rate constants of Sextaphag® films based on gelatin and methylcellulose were determined using the curves presented in Fig. 2.

Swelling rate constants were for gelatin -based medicated films – 0,4877 min^{-1}, for methylcellulose-based medicated films – 1,0724 min^{-1}. Thus, the swelling rate constant of polymer systems based on methylcellulose is 2.2 times greater than that for gelatin-based systems.

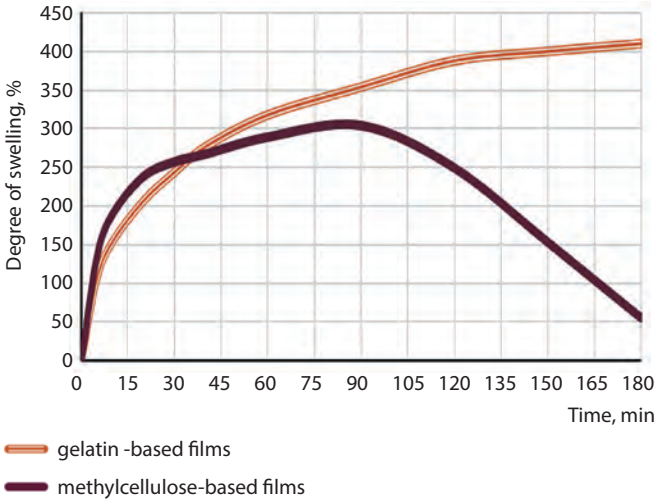


FIG. 1. *Kinetic curves of swelling of Sextaphag® films*

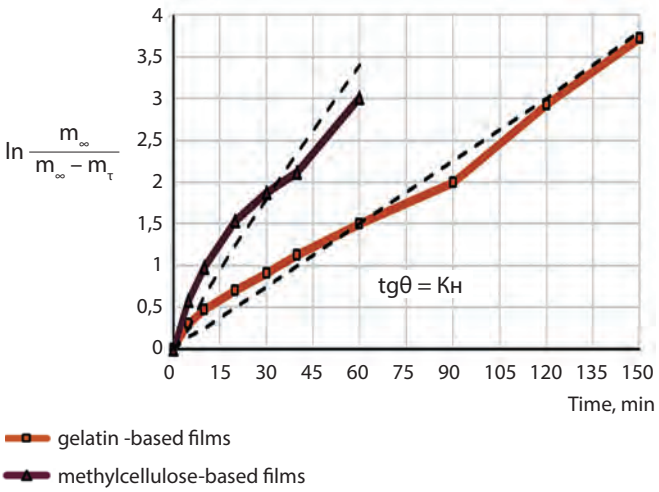


FIG. 2. *Curves for determination of swelling rate constant*

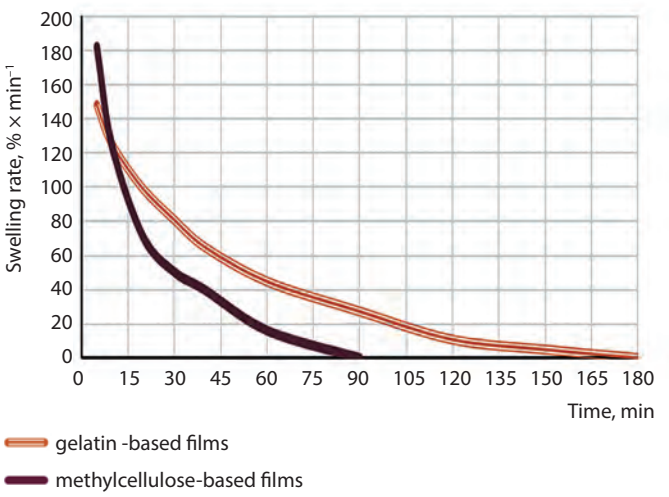


FIG. 3. *Profile of swelling rate of polymer matrix systems*

The profile of the swelling rate of polymer matrices was determined according to the graph shown in Fig. 3.

The swelling rate for the studied polymer systems decreases exponentially with increasing the process duration.

Thus, Sextaphag® medicated films are prolonged-release systems. The release is carried out by a diffusion mechanism with gradual biodegradation of the polymer system with an immobilized combined bacteriophage. Methylcellulose-based films release the bacteriophage with its active release at the beginning of the swelling process, and then with gradual decrease in the release rate and complete biodegradation of the polymer. Gelatin-based films are capable of slowly and continuously releasing the bacteriophage.

A further stage of research was to study the effect of the film composition on the release of the immobilized bacteriophage from the matrix system by diffusion into agar. Fig. 4 shows a diagram of degree of release of the bacteriophage from the film compositions. From the presented data, it can be seen that the matrix system based on gelatin more effectively releases the immobilized combined bacteriophage.

Thus, the conducted research has shown that the efficient release-active dosage form is Sextaphag® medicated films based on gelatin.

At the next stage of work, the effect of medicated films with immobilized bacteriophage on the healing of linear skin wounds was studied on a model of laboratory animals. As a comparison standard, Levomekol ointment under FS 42-2922-98, widely used for treatment of local purulent infection, was used. The results of the study of wound healing activity of Sextaphag® medicated films on the model of a linear aseptic wound are presented in the table.

From the presented data it can be seen that the Sextafag® medicated films have wound-

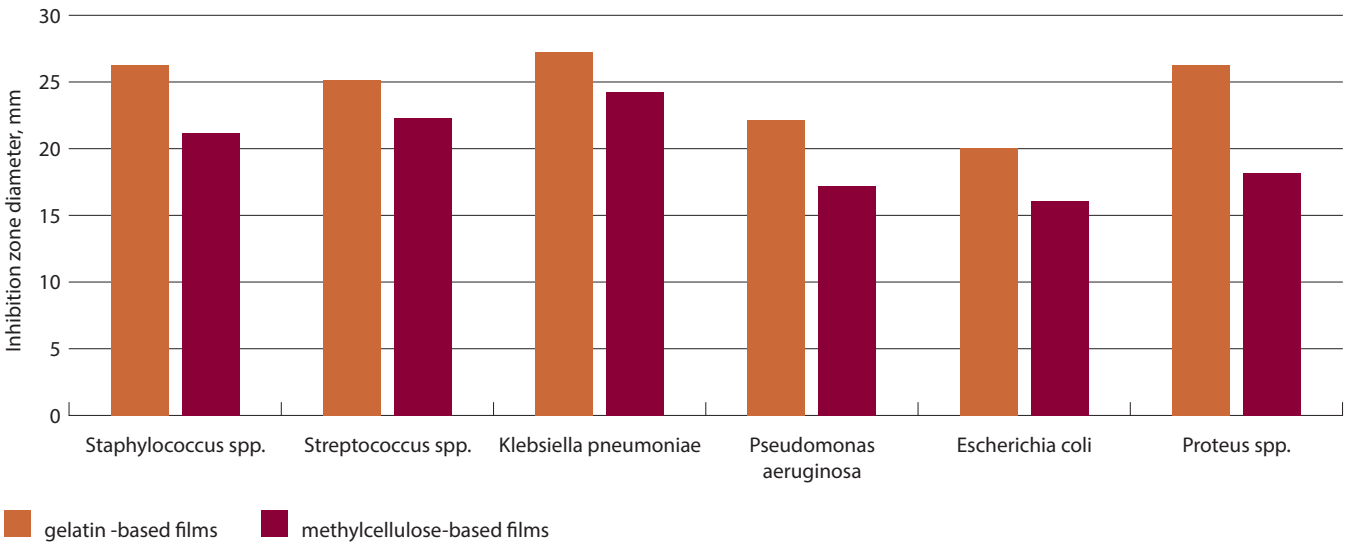


FIG 4. *Diagram of degree of release of the bacteriophage from the film compositions*

Table

WOUND HEALING ACTIVITY OF SEXTAPHAG® MEDICATED FILMS

Group of animals, n = 6	Scar breaking force, (M ± m), N		Number of infectious complications, %
	5th day	7th day	
Without treatment	229.0 ± 29.8	487.5 ± 36.5	75
Levomekol ointment	346.7 ± 34.1	672.0 ± 46.7	37.5
Methylcellulose-based Sextaphag® films	357.0 ± 45.1	682.0 ± 43.2	0
Gelatin -based Sextaphag® films	372.0 ± 42.2	665.0 ± 38.7	0

healing activity comparable to the activity of the comparison agent – Levomekol ointment, while having a more pronounced antibacterial effect.

CONCLUSION

1. The rational release-active dosage form is Sextaphag® gelatin-based films, which have better bioavailability compared to the methylcellulose-based film composition.

2. Medicated Sextafag® Films have wound-healing activity comparable to the activity of the comparison agent (Levomekol ointment), and a pronounced antibacterial effect.

REFERENCES

1. *Akimkin V.G., Alimov A.V., Polyakov V.S. Epidemiological effectiveness of the use of bacteriophages for the prevention of acute respiratory infections of bacterial etiology in organized groups // Bacteriology, 2016, Vol. 1, No. 1. – pp. 80–87.*
2. *Gileva O.S., Bondarenko E.A., Gibadullina N.V. and others. Topical phagotherapy in the complex treatment of gingivitis and periodontitis // Perm medical journal. – 2011. – Vol. XXVIII, No. 2. – pp. 87–92.*
3. *Gorbunov S.M., Zaikonnikova I.V., Abdrakhmanova N.G. Device for determining the breaking strength of wounds being healed// Pharmacological regulation of regenerative processes in the experiment and clinic. – Yoshkar-Ola, 1979. – pp. 100–104.*
4. *Lipatov, S.M. Physics and chemistry of colloids. – Moscow-Leningrad: Goskhimizdat, 1948. – 375 p.*
5. *Mamchik N.P., Batishcheva G.A., Gabbasova N.V., Belichenko N.S., Kolesnik V.V., Kosinova N.M. Microbial landscape and the level of antibiotic resistance of wound fluid of patients of the city emergency hospital // Medical anthology, No. 3 (43), 2016. – pp. 11–14.*
6. *Khadiyatov I.I., Adiev R.F., Nasibullin I.M. and others. Experimental application of PermaFoam bandage and Sextaphag polyvalent piobacteriophage in the treatment of chronic anal fissure // Medical Bulletin of Bashkortostan. – 2014. Vol. 9. No. 3. – pp. 78–81.*
7. *Stepanova N.R., Gevorkyan M.A. Bacteriophages: aspects of application in obstetrics and gynecology // Medical Council. – 2015. No. 9. – pp. 10–14.*
8. *Legonkova O.A., Vasiliev V.G., Asanova L.Yu. Research of operational properties of polymeric dressings // Wounds and wound infections. Journal named after Professor B.M. Kostyuchenko. – 2015. Vol. 2. – pp. 32–39.*

UDK 543.4:544.2

ORGANIZATION OF THE ANNUAL MEDICINES QUALITY REVIEW PROCESS AT THE PHARMACEUTICAL ENTERPRISE

M.R. Mtsariashvili, *Leading validation engineer of the Quality Assurance Department of Tatkhimfarmpreparaty JSC, Kazan, m.r.mtsariashvili@yandex.ru*
S. V. Ilina, *Head of Validation Bureau of the Quality Assurance Department of Tatkhimfarmpreparaty JSC, Kazan, silina@tatpharm.ru*
S.Yu. Garmonov, *Dr. Chem. Professor, Department of Analytical Chemistry, Certification and Quality management, Kazan National Research Technological University, HE FSBEI, Kazan. serggar@mail.ru*
S.N. Egorova, *PharmDr., Professor, Deputy Director for educational activities of the Institute of pharmacy, Kazan National Research Technological University, HE FSBEI, Kazan, Svetlana.egorova@kazangmu.ru*

The organizational structure for ensuring the review of the quality of medicines is presented. A pharmaceutical quality system has been formed that includes the following elements (subsystems): monitoring of processes and product quality, corrective and preventive actions, change management, and management analysis. An algorithm for conducting a quality review at Tatkhimpharmpreparaty JSC and measures to improve production based on it is presented. To regulate the quality review taking into account the specifics of production, a matrix of responsibility of departments for compiling an annual review of the quality of medicines has been developed.

Keywords: medicines, quality review, pharmaceutical quality system, quality assurance and improvement

Continuous improvement of the quality system is a fundamental principle of GMP. To identify opportunities for continuous improvement of products, processes, and the system itself, the management should provide periodic reviews of the functioning of the pharmaceutical quality system. According to the order of the Ministry of Industry and Trade of the Russian Federation dated June 14, 2013 No. 916 «Rules of good manufacturing practice», the quality review (QR) is the main analysis tool for the management to monitor the stability and continuous improvement of the production processes and quality of medicines.

Quality review is a regular periodic assessment of the quality of each medicinal product carried out by the manufacturer to document the stability of production processes, the suitability of the applied specifications and to identify possible improvements [1]. The procedure for Quality review, the methods used, and the format of registration depend on the characteristics of pharmaceutical companies: the specifics of the products and technological equipment, the organization of the pharmaceutical quality system, the age of development and production of medicines (before the introduction of GMP requirements), and others.

Currently, Tatkhimpharmpreparaty JSC works in accordance with GMP standards and produces solid (tablets), soft (ointments, gels), liquid (tinctures, syrups, solutions), eye dosage forms of 30 pharmacological groups of medicines, such as antibiotics, anti-inflammatory medicines, antivirals, analgesics, neuroleptics, tranquilizers, antitumor, anti-migraine, cardiovascular agents, sedatives

and agents that regulate metabolic processes [2].The purpose of work is improvement of organization of quality reviews of medicines at Tatkhimpharmpreparaty JSC.

MATERIALS AND METHODS

The object of the study was the pharmaceutical quality system of Tatkhimpharmpreparaty JSC, the subject of the study is the organizational structure of ensuring the quality review of medicines. The study uses methods of structural and logical analysis.

RESULTS AND DISCUSSIONS

In accordance with the requirements of ICH Q10 [3], Tatkhimpharmpreparaty JSC has formed a pharmaceutical quality system that includes the following elements (subsystems): monitoring of production processes and product quality, corrective and preventive actions, change management and analysis by the management.

The management analysis tool is the annual review of medicines. The algorithm of the quality review of Tatkhimpharmpreparaty JSC and activities for improvement of production on its basis is presented in Fig. 1.

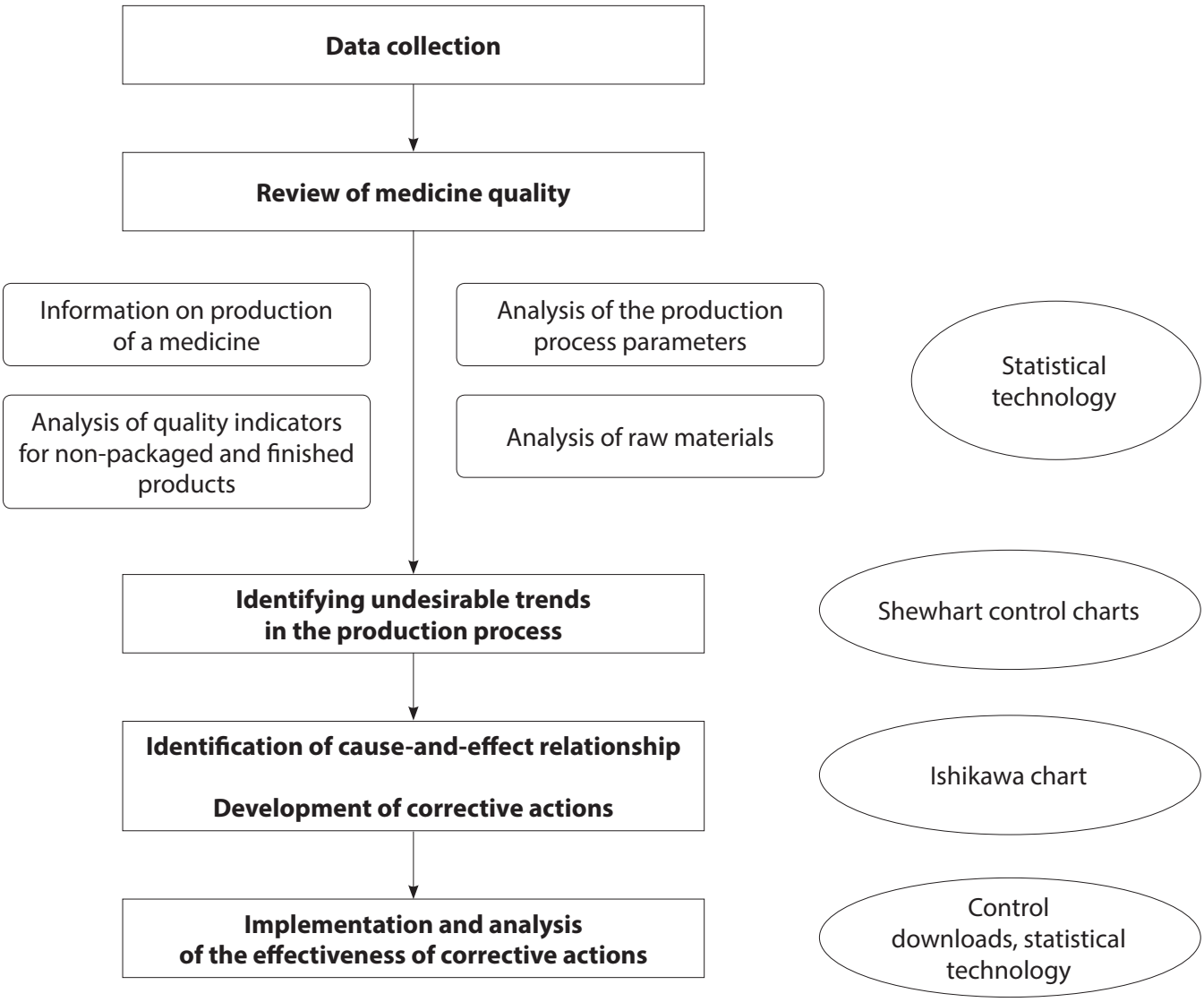


FIG. 1. *Algorithm for improving the production of medicines based on the methodology of their quality review*

At the preparatory stage, the necessary data is collected from the production protocols for the batch record.

The QR stage includes 4 subsections. Medicine release information contains information about the medicine, sales dynamic, claims, and returns. Based on the results of the analysis of the parameters of the production process, the analysis of quality factors of non-packaged and finished products, the analysis of raw materials using statistical methods, the undesirable trends in the production process are identified. To assess the variability and manageability of processes at Tatkhimpharmpreparaty JSC, we used Shewhart control charts (X - charts of individual values and Rm - charts of rolling ranges) [4], indicators of reproducibility and suitability of the process [5].

The next step is to identify cause-and-effect relationships using Ishikawa diagrams and develop the corrective actions to improve the process.

At the final stage, the implementation and analysis of the effectiveness of corrective actions is carried out using statistical methods and control downloads.

During the QR, special attention was paid to the medicines that were put into production before the company switched to GMP standards and developed without taking into account modern requirements for risk analysis of pharmaceutical development. For routine production of this group of medicines, the primary annual quality review was provided. If the results of the analysis of the stability of production processes by statistical methods established the presence of a process in statistically controlled condition, the obtained data was the source for the subsequent analysis and the basis for identifying trends in routine production process when conducting the periodic repeated quality review. In case of detection of statistically uncontrolled process the quality review data was the rationale for comprehensive assessment, change implementation and process improvement, at that, developing of the corrective actions was accompanied by a risk analysis.

Thus, the quality review serves as a link between the provisions reflected in the documents of the International Council for harmonization of technical requirements for pharmaceutical products intended for human use: ICH Q8 «Pharmaceutical development», prescribing continuous improvement of processes during the product life cycle [6], and ICH Q10 «Pharmaceutical quality system», aimed at stabilizing the processes of serial production of medicines (Fig. 2). According to the

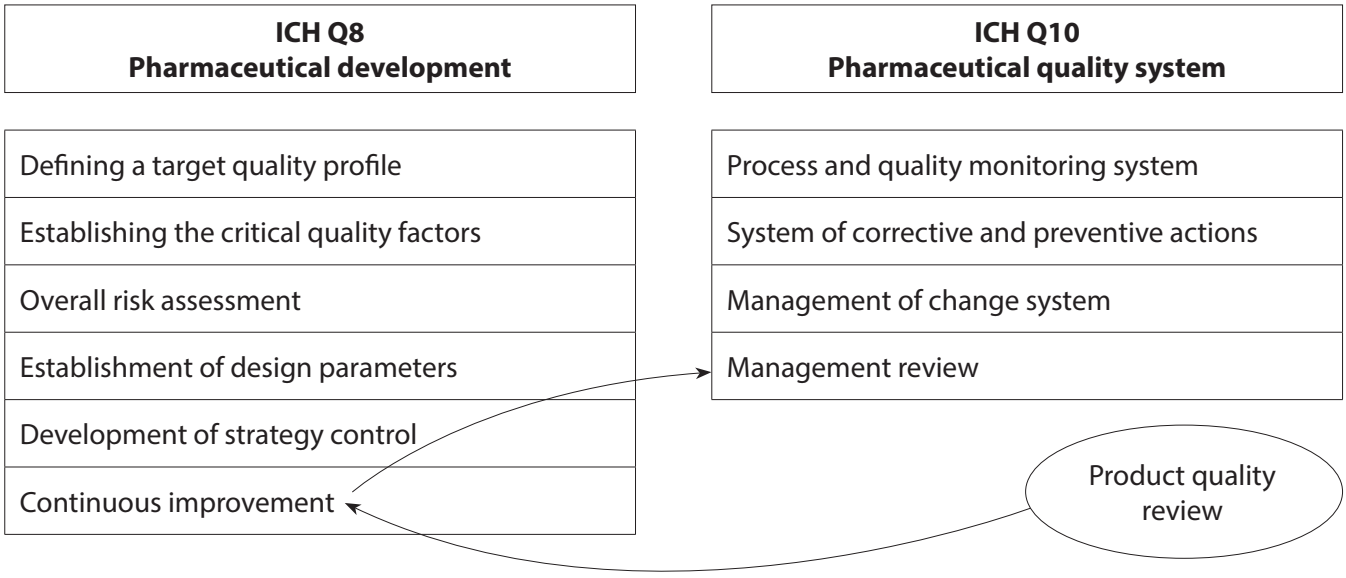


FIG. 2. *Scheme for integrating the quality review into pharmaceutical development*

presented scheme, the introduction of the main management analysis tool that is quality review, into pharmaceutical development, provides the basis for continuous improvement of the routine production process.

To regulate the QR process, taking into account the specifics of production, a matrix of responsibility of the departments of Tatkhimpharmpreparaty JSC for preparation of the annual QR of medicines was developed (see Table).

Table

MATRIX OF RESPONSIBILITY FOR DATA COLLECTION, PREPARATION OF AN ANNUAL REVIEW OF MEDICINE QUALITY AND DEVELOPMENT OF CORRECTIVE ACTIONS

Section	Production	Chief technologist Department	Registration Department	Sale and shipping Department	Procurement Department	QCD	QAD	Research Department	Deputy General Director for quality
Information on a product			R, C	R, C					
Assessment of the quality of raw and packaging materials					R, C	R, C			
Quality control of the medicine and process parameters	R, C	R, C				R, C			
Assessment of nonconforming of product batches	R, C	I				I	I		
Assessment of deviations and nonconformities identified in the production process	R, C	I				I	I		
Assessment of changes		R, C	R, C			I			
Assessment of registration changes			R, C						
Analysis of the results of the stability monitoring program						R, C			
Assessment of claims, returns, and comments on product						R, C			
Assessment of corrective and preventive actions						I	R, C		

The end table

Section	Production	Chief technologist Department	Registration Department	Sale and shipping Department	Procurement Department	QCD	QAD	Research Department	Deputy General Director for quality
Assessment of post-registration obligations			R, C						
Assessment of the results of certification (testing) of equipment, processes and technological environments	I					I	R, C		
Assessment of contract agreements	R, C					I	I		
Interpreting the results of the quality review	I	I				I	R, C		
Risk analysis, development of corrective actions	C	C				C	R, C	C	
CONCLUSION							C		R

C – Contractors, I – Interaction, R – Responsible Person

Responsibility for drawing up the annual quality review (QR) is assigned to the Deputy Director for Quality, the executor is the Quality Assurance Department (QAD).

General information about the product in the QAD is provided by the registration and sales and shipping departments.

Quality control of the product and process parameters is performed by the Quality Control Department (QCD) in cooperation with the production and the Chief Technologist Department.

Responsibility for evaluating of non-relevant product batches, as well as for evaluating of deviations and non-conformities identified during the production of medicines, is assigned to the production in cooperation with the Chief Technologist Departme, QCD and QAD.

Changes are evaluated by the Registration Department in collaboration with the Chief Technologist Department and the QCD, and the registration changes and post-registration obligations are evaluated by this Department independently.

The QCD is responsible for analyzing the results of the stability monitoring program and assessment of claims, returns, and product feedback.

Assessment of corrective and preventive actions, results of certification (testing) of equipment, processes and technological environments is carried out by the QAD in cooperation with the CCD and production.

The evaluation of contract agreements is the responsibility of the production in cooperation with the QCD and the CCD.

The key place in the responsibility distribution matrix is the interpretation of the results of the quality report. As a basis for optimizing the production and quality control of medicines, this function is assigned to the QAD in cooperation with the Research Department, the QCD, the Department of the chief technologist and production. The risk analysis is carried out by the QAD in conjunction with the production and Research Department, thereby ensuring the relationship between ICH Q8 «Pharmaceutical development» and ICH Q10 «Pharmaceutical quality system» in the aspect of continuous improvement of production processes.

CONCLUSION

The implementation of the above approaches makes it possible to effectively identify technological problems in the production process and take appropriate corrective actions aimed at continuous improvement of the organization of the medicine production.

REFERENCES

1. *Alexandrov A. V. Periodic reviews of the quality of medicines / A. V. Alexandrov // Industrial review. – 2008. – №5 (10). – pp. 28–30.*
2. *Tatkhimpharmpreparaty. Access mode http://www.tatpharm.ru [Access date: May 20, 2019].*
3. *Alexandrov A. V. Pharmaceutical quality system (ICH Q10) in algorithms and schemes. – Moscow: Vialek Group of companies, 2014. – 44 p.*
4. *GOST R ISO 7870-2-2015 Statistical technology. Control charts. Part 2. Shewhart control charts. – Moscow: Standardinform, 2016.*
5. *GOST R 50779.46-2012/ISO/TR 22514-4:2007 Statistical technology. Process management. Part 4. Evaluation of process reproducibility and suitability indicators. – Moscow: Standardinform, 2014.*
6. *Pharmaceutical development. Harmonised Tripartite Guideline [Electronic resource]. Access mode: https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q8_R1/Step4/Q8_R2_Guideline.pdf*

UDC 615.014

HARRINGTON'S DESIRABILITY FUNCTION IN DEVELOPING THE GSB-106 TABLETS FORMULATION

E.V. Blynskaya, *Candidate of Pharmaceutical Sciences, Head of the Laboratory of Finished Pharmaceutical Products (FPP) in the Experimental and Technological Department, V.V. Zakusov Research Institute of Pharmacology FSBSI, Moscow*
V.V. Bueva, *Junior Research Assistant, V.V. Zakusov Research Institute of Pharmacology FSBSI, Moscow, vikabueva@yandex.ru*
K.V. Alekseev, *PharmDr., Professor, Deputy Director for innovation, V.V. Zakusov Research Institute of Pharmacology FSBSI, Moscow*
V.K. Alekseev, *Junior Research Assistant, V.V. Zakusov Research Institute of Pharmacology FSBSI, Moscow*
S.V. Minaev, *Candidate of Pharmaceutical Sciences, Head of Experimental and Technological Department, V.V. Zakusov Research Institute of Pharmacology FSBSI, Moscow*

The optimal formulation of tablets containing the original pharmaceutical substance of GSB-106 was selected as a result of evaluating the qualitative and quantitative composition of tablets using the Harrington's desirability function. The efficacy of using this multivariate analysis method in pharmaceutics is presented.

Key words: GSB-106, generalized desirability, desirability function, individual desirability

INTRODUCTION

In the process of developing the tablet dosage forms, the selection of their qualitative and quantitative formulation is a difficult task that requires integrated knowledge and researcher's inquiry to solve it. In addition to the active pharmaceutical ingredient (API), which is part of the tablets the introduction of the following auxiliary substances (excipients) is required: filler to achieve the required size and weight of tablets, binder for improving the adhesion forces between particles, and thus, increasing the tablet strength, disintegrant to reduce the time of tablet disintegration and dissolution, as well as anti-friction substance to reduce friction and sticking of the tablet blend onto tablet tooling. The required content of each excipient depends both on the properties of the API used and on the stages of the production process, such as granulation, drying, grinding and pressing [1].

At the early stage of tablet development, it is important to minimize the number of experiments using API due to its limited availability. When a lot of parameters is studied, multivariate analysis methods are used to reduce the number of experiments performed [2]. Currently, such methods are implemented in the pharmaceutical industry [3–8].

One of the most convenient methods of multivariate analysis is the Harrington's desirability function, which is based on generalization of information by conversion of the natural values of parameters of different dimensions into a single dimensionless desirability scale [9].

Purpose of this work is to evaluate and identify the optimal qualitative and quantitative formulation of tablets containing API GSB-106 with antidepressant activity using the Harrington's desirability function.

MATERIALS AND METHODS

API GSB-106 (synthesized in the Medicine Chemistry Department of V.V. Zakusov Research Institute of Pharmacology FSBSI under the leadership of Associate Member of RAS T.A. Gudasheva) [10], excipients: lactose monohydrate (Lactochem® Fine Powder, DFE Pharma, Germany), microcrystalline cellulose (Microcel® MC 101, Blanver Farmoquimica Ltda, Brazil); polyvinyl alcohol and polyethylene glycol copolymer (Kollicoat® IR, BASF, Germany), polyvinylpyrrolidone (Kollidon® 25, BASF, Germany), partially gelatinized corn starch (Starch 1500®, Colorcon Ltd., UK), purified water (FS.2.2.0020.15), magnesium stearate (EP 01/2008:0229). All excipients are allowed for medical use.

Tablet blends with model composition were prepared by wet granulation method. Tablets weighing 0.1 g were produced using a manual hydraulic press PRG 1–50 (VNIR, Russia).

The processing characteristics of tablet blends and tablets were measured according to standard methods described in the State Pharmacopoeia of the XIV edition using the following equipment: flowability tester GDT and bulk density tester SVM-10 (Erweka, Germany), tablet disintegration tester PTZ-S and friability tester PTF 30ERA (Pharma Test, Germany), tablet strength tester TBF 1000 (Copley Scientific, UK) [11]. The Carr's compressibility index and the Hausner coefficient were calculated based on the resulted bulk density values.

Selection of qualitative and quantitative formulation was carried out using Harrington's desirability function. The essence of the method is that for each particular property yi, the individual desirability (di) is calculated, which is in the range from 0 to 1. A value of di equal to 1 represents the best value of the property, while a value of 0 is an absolutely unacceptable level of this property. In the framework of this work, one-sided restriction of individual desirabilities is adopted, so the value of di equal to 0.37 corresponds to the lower limit of acceptable values [1,7,12].

$$d = e^{-e^{-y}} \quad (1)$$

If there are several parameters with the required properties, the construction of a generalized desirability (D) shall be started, which is expressed as the geometric mean of the individual desirabilities:

$$D = \sqrt[n]{\prod_i^n d_i}, \quad (2)$$

where n – number of individual desirabilities.

D, as an individual desirability has value from 0 to 1.

The numerical values of parameters (x_i) are converted to dimensionless values (y_i) according to the following formula:

$$y = a_0 + a_1 x \quad (3)$$

where a_0 and a_1 are coefficients of the linear function.

After twice taking the logarithm of Equation (1) and substituting the y values in Equation (3), a set of equations is made for the known parameter values [1,7,12–14]:

$$\begin{cases} a_0 + a_1 x_1 = \ln\left(\dfrac{1}{\ln 1/d_1}\right) \\ a_0 + a_1 x_2 = \ln\left(\dfrac{1}{\ln 1/d_2}\right) \end{cases} \quad (4)$$

To obtain the desirability scale, we used a ready-developed Table 1.

The following parameters of tablet blends and tablets: flowability, Carr's compressibility index, Hausner coefficient, disintegration, tablet breaking force, and friability were evaluated

Table 1

STANDARD RATINGS ON THE DESIRABILITY SCALE

Rating	Numerical preference system
Very good	0.80 < d < 1.00
Good	0.63 < d < 0.80
Satisfactory	0.37 < d < 0.63
Bad	0.20 < d < 0.37
Very bad	0.00 < d < 0.20

by multivariate analysis. The rating scale for the compared model formulations is shown in Table 2.

RESULTS AND DISCUSSIONS

Model formulations of tablet blends are specified in Table 3.

The results of studying the processing characteristics of tablet blends and tablets are presented in Table 4.

"Excellent" and "satisfactory" grades for "flowability" of the tablet blend correspond to the values of 8.6 and 3. Substituting these values into formula (4), we get the following set of equations:

$$\begin{cases} a_0 + 8.6a_1 = \ln\left(\dfrac{1}{\ln 1/0.8}\right) \\ a_0 + 3a_1 = \ln\left(\dfrac{1}{\ln 1/0.37}\right) \end{cases} <> \begin{cases} a_0 + 8.6a_1 = 1.51 \\ a_0 + 3a_1 = 0{,}01 \end{cases}$$

It was found, that $a_1 = 0.27$, $a_0 = -0.79$, and the resulted values were substituted into the linear equation (3):

$$y = -0.79 + 0.27x$$

Similarly, the set of equations was solved, y and individual desirability d were calculated for all numerical values of processing characteristics of tablet blends and tablets x (Table 5, Fig. 1).

Fig. 1 shows the dependence of Harrington's individual desirability on the actual values of

Table 2

RATING SCALE

Rating	Criteria for evaluating the dimensional parameters					
	Flowability, g/s	Carr's compressibility index, %	Hausner coefficient	Disintegration, minutes	breaking force, N	Friability, %
Excellent	more than 8.6	less than 15	1.00–1.18	less than 15	more than 30	less than 3
Good	more than 6.6	less than 20	less than 1.25			
Satisfactory	more than 3.0	less than 25	less than 1.34			
Bad	more than 1.0	less than 31	less than 1.45	more than 15	less than 30	more than 3
Very bad	less than 1.0	more than 32	more than 1.45			

Table 3

MODEL FORMULATIONS OF TABLET BLENDS OF GSB-106

Formulation	GSB-106, mg	Microcrystalline cellulose 101, mg	Lactose monohydrate, mg	Kollidon 25, mg	Kollicoat IR, mg	Starch 1500, mg	Magnesium stearate, mg
1	1.0	93.0	–	5.0	–	–	1.0
2	1.0	–	93.0	5.0	–	–	1.0
3	1.0	93.0	–	–	5.0	–	1.0
4	1.0	–	93.0	–	5.0	–	1.0
5	1.0	93.0	–	–	–	5.0	1.0
6	1.0	–	93.0	–	–	5.0	1.0
7	1.0	60.0	33.0	5.0	–	–	1.0
8	1.0	60.0	33.0	–	5.0	–	1.0
9	1.0	60.0	33.0	–	–	5.0	1.0

Table 4

VALUES OF CHARACTERISTICS OF TABLET BLENDS AND TABLETS OF GSB-106

Formulation	Tablet blends			Tablets		
	Flowability, g/s x_1	Carr's compressibility index, % x_2	Hausner coefficient x_3	Disintegration, s x_4	Breaking force, N x_5	Friability, % x_6
1	1.89 ± 0.02	23.40	1.30	132.0 ± 10.5	95.2 ± 0.6	0.7 ± 0.1
2	1.84 ± 0.03	22.67	1.29	130.0 ± 8.2	68.1 ± 0.7	1.4 ± 0.1
3	2.02 ± 0.04	22.40	1.28	39.5 ± 0.6	81.2 ± 0.3	0.7 ± 0.1
4	3.74 ± 0.04	15.60	1.18	34.0 ± 0.6	34.1 ± 0.1	0.7 ± 0.1
5	3.56 ± 0.02	18.40	1.23	107.5 ± 3.1	35.2 ± 0.1	1.0 ± 0.1
6	5.14 ± 0.03	14.90	1.17	22.5 ± 0.1	27.5 ± 0.1	1.3 ± 0.1
7	2.27 ± 0.01	23.70	1.30	33.5 ± 0.2	65.1 ± 0.3	1.0 ± 0.1
8	6.90 ± 0.04	13.70	1.15	34.1 ± 0.1	78.1 ± 0.2	0.5 ± 0.1
9	2.23 ± 0.02	19.30	1.23	10.5 ± 0.2	55.3 ± 0.2	1.7 ± 0.1

Table 5

VALUES OF HARRINGTON'S INDIVIDUAL DESIRABILITY

Formulation	d_1	d_2	d_3	d_4	d_5	d_6
1	0.4584	0.4046	0.5064	0.7594	0.9915	0.7309
2	0.5462	0.4976	0.5382	0.7601	0.9675	0.6409
3	0.5620	0.5115	0.5689	0.7898	0.9829	0.7309
4	0.7572	0.7853	0.8018	0.7915	0.8345	0.7309
5	0.7469	0.6922	0.7026	0.7678	0.8419	0.6947
6	0.7789	0.8044	0.8178	0.7950	0.7785	0.6550
7	0.4943	0.3881	0.5064	0.7978	0.9623	0.6947
8	0.8556	0.8338	0.8464	0.7915	0.9803	0.7530
9	0.6592	0.6564	0.7026	0.7987	0.9387	0.5964

the compared parameters, which reflects the practical significance of the method used.

Fig. 2 illustrates the values of Table 5. It is noted that all individual desirabilities *d* fit within the normal range of acceptable values (>0.37). Indicators of the degree of flowability of tablet blends (flowability, Carr's compressibility index, Hausner coefficient) are strictly individual for each formulation and have significant differences in the final values. However, the resulting tablets

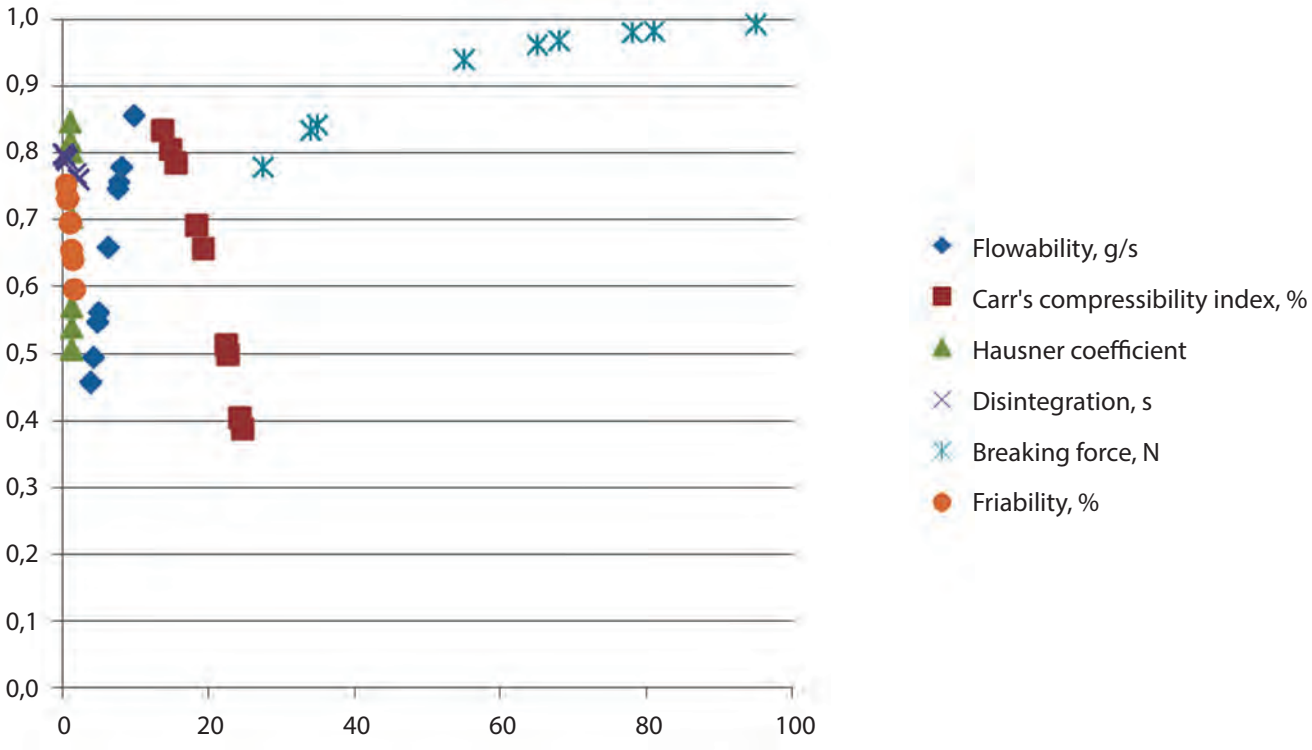


FIG. 1. *Values of Harrington's individual desirability*

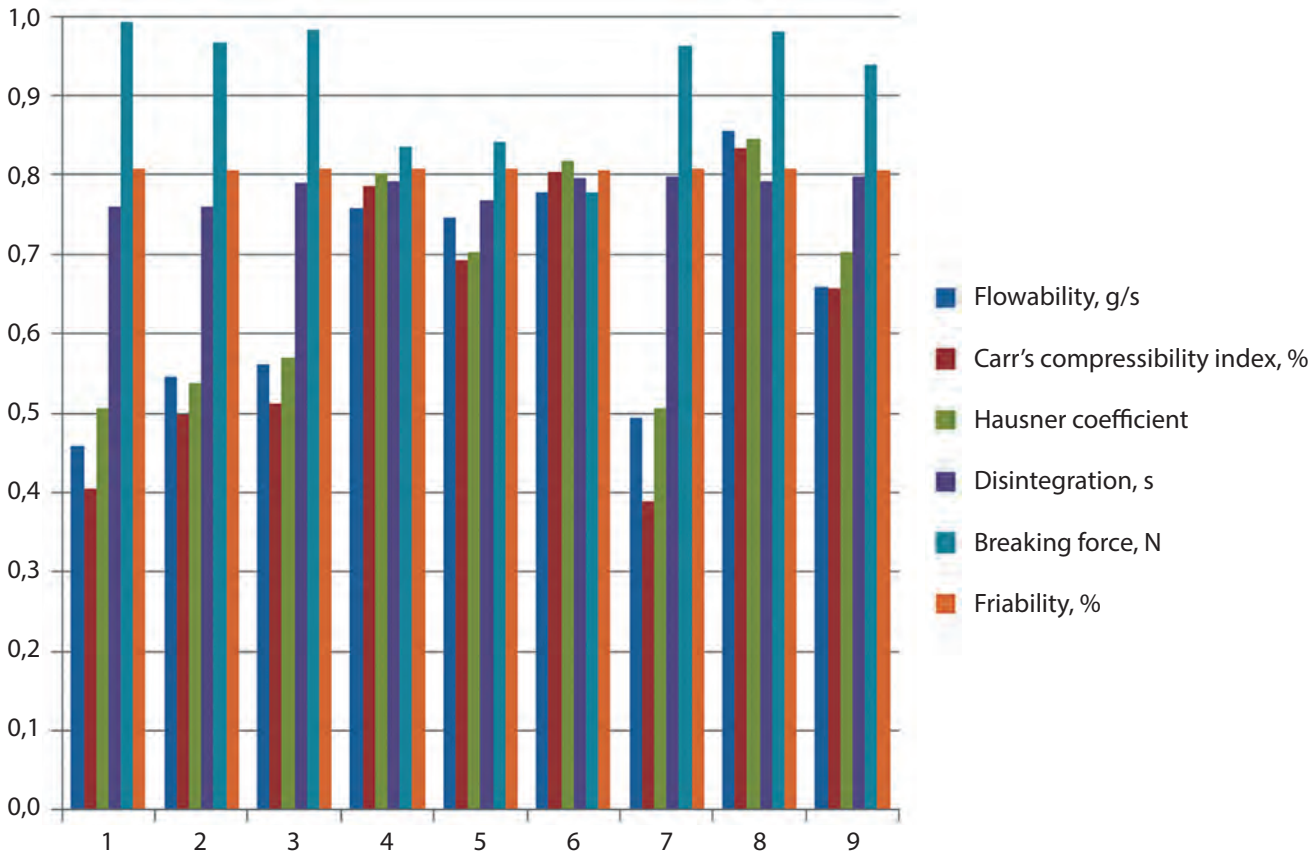


FIG. 2. *Comparative characteristics of individual desirability*

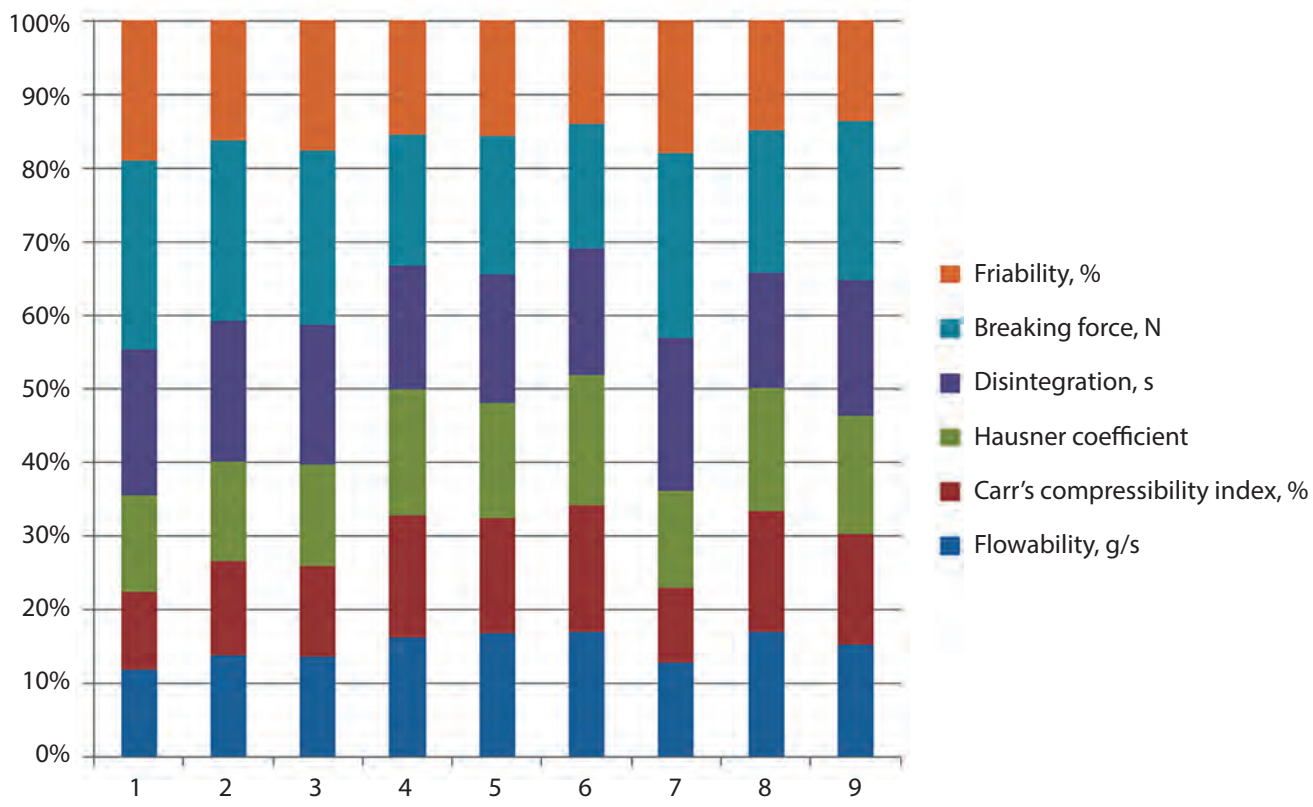


FIG. 3. *The share of individual desirabilities in generalized ones*

Table 6

VALUES OF GENERALIZED HARRINGTON'S DESIRABILITIES

Formulation	1	2	3	4	5	6	7	8	9
D	0.6103	0.6403	0.6728	0.7828	0.7392	0.7696	0.6106	0.8406	0.7170
Rating on the scale of desirability	Satis-factory	Good	Good	Good	Good	Good	Satis-factory	Excel-lent	Good

of 9 formulations are characterized by high strength (breaking strength, friability) and rapid disintegration.

The greatest contribution to the values of generalized desirability *D* is made by "breaking strength" and "friability" (Fig. 3).

The calculated generalized desirabilities *D* for all values of processing characteristics *x* are presented in Table 6, on the basis of which a graph is constructed for the values with one-sided restriction of acceptable values (Fig. 4).

Formulation No. 8 (FS GSB-106, MCC 101, lactose monohydrate, Kollicoat IR, magnesium stearate) was rated as "excellent" on the desirability scale, on the basis of which it was selected as the final formulation.

CONCLUSION

The qualitative and quantitative formulation of tablets containing FS GSB-106 with antidepressant activity was analyzed using the Harrington's desirability function. As a result, the optimal formulation of tablets was revealed: FS GSB-106, a mixture of lactose and microcrystalline cellulose in a ratio of 1:2, 10% aqueous solution of Kollicoat IR, magnesium stearate until a tablet weighing 0.1 g was obtained.

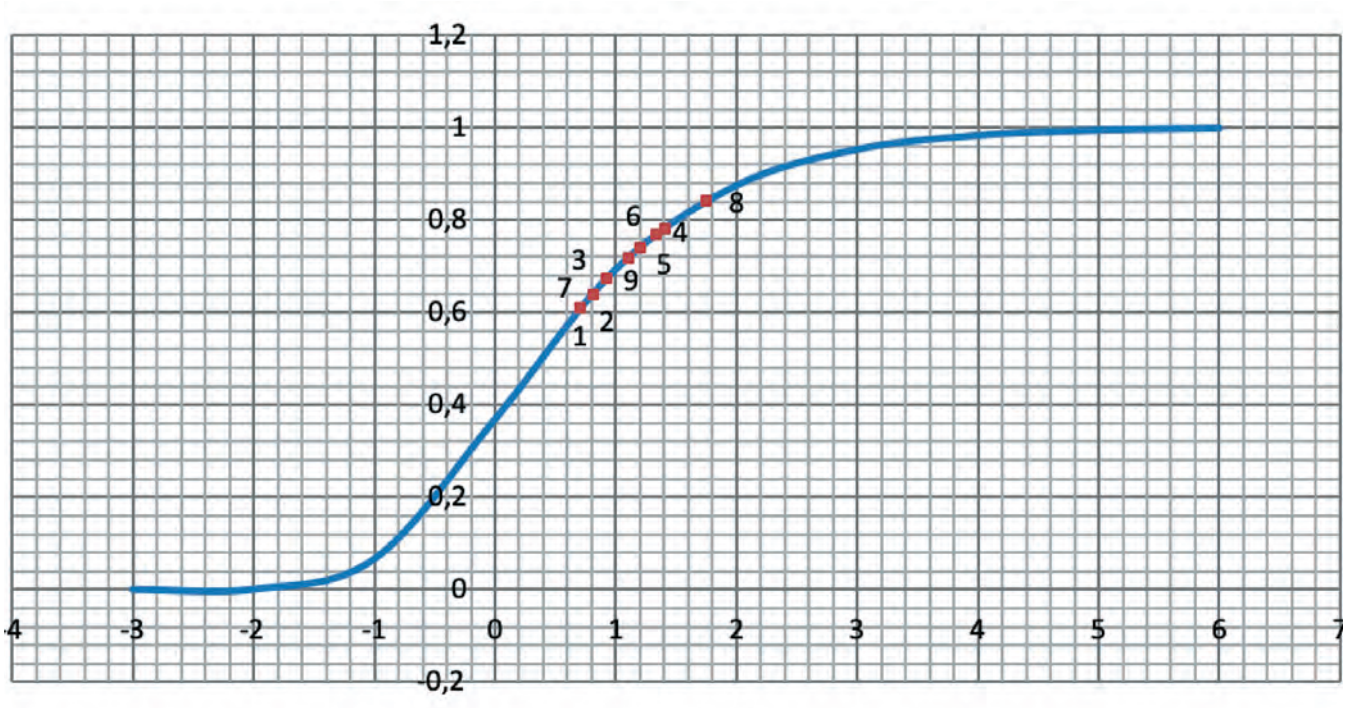


FIG. 4. *Generalized function of Harrington's desirability*

REFERENCE

1. *Andersson M., Ringberg A., Gustafsson C. Multivariate methods in tablet formulation suitable for early drug development: Predictive models from a screening design of several linked responses // Chemometrics and Intelligent Laboratory Systems. – 2007. – Vol. 87 (1). – P. 125–130.*
2. *Gabrielsson J., Lindberg N.-O., Lundstedt T. Multivariate methods in pharmaceutical applications // J. Chemom. – 2002. – Vol. – P. 141–160.*
3. *Gabrielsson J., Nyström Å., Lundstedt T. Multivariate methods in developing an evolutionary strategy for tablet formulation // Drug Dev. Ind. Pharm. – 2000. – Vol. 26 (3). P. 275–296.*
4. *Tôrres A. R., de Oliveira A. D. P., Grangeiro S., Fragoso W. D. Multivariate statistical process control in annual pharmaceutical product review // Journal of Process Control. – 2018. – Vol. 69. – P. 97–102.*
5. *Singh V. D., Daharwal S. J. Development and validation of multivariate calibration methods for simultaneous estimation of Paracetamol, Enalapril maleate and hydrochlorothiazide in pharmaceutical dosage form // Spectrochimica Acta. Part A: Molecular and Biomolecular Spectroscopy. – 2017. – Vol. 171. – P. 369–375.*
6. *Copelli D., Cavecchi A., Merusi C., Leardi R. Multivariate evaluation of the effect of the particle size distribution of an Active Pharmaceutical Ingredient on the performance of a pharmaceutical drug product: a real-case study // Chemometrics and Intelligent Laboratory Systems. – 2018. – Vol. 178. – P. 1–10.*
7. *Tishkov S. V., Blynskaya E. V., Alekseev K. V., Yudina D. V. Development of the formulation and technology of gefitinib tablets // Modern trends in the development of science and technology. – 2016. – №9 (3). – pp. 41–43.*
8. *Yudina D. V., Blynskaya E. V., Alekseev K. V., Minaev S. V., Marakhova A. I. Development of the formulation of GML-1 tablets obtained by wet granulation: choice of filler and binder // Pharmacy. – 2018. – Vol. 67. – №3. – pp. 35–40.*
9. *Harrington E. The Desirability Function // Industrial Quality Control. – 1965. – Vol. 21. – №10. – P. 494–498.*
10. *Tarasyuk A. V., Pomogaibo S. V., Kurilov D. V., Gudasheva T. A. Synthesis of dimeric dipeptide mimetic BDNF GSB-106, a potential neuroprotective drug // Chemical and pharmaceutical journal. – 2013. – Vol. 47. – №1. – pp. 21–28.*
11. *State Pharmacopoeia of the Russian Federation. XIV edition. – Moscow, 2018. – Vol. 2. – 3262 p.*
12. *Sosyukin A. E., Verveda A. B. Practical aspects of using the desirability function during psychophysiological examination of emergency rescue personnel// Medline.ru. Russian biomedical journal. – 2015. – Vol. 16. – №3. – pp. 872–884.*
13. *Bickerton G. R.,, Paolini G. V., Besnard J., Muresan S., Hopkins A. L. Quantifying the chemical beauty of drugs // Nature chemistry. – 2012. – Vol. 4. – P. 90–98.*
14. *Goethals P. L., Cho B. R. Extending the desirability function to account for variability measures in univariate and multivariate response experiments // Computers & Industrial Engineering. – 2012. – №62. – P. 457–468.*

UDK 615.015:616-08-039.57

FACTORS AND REASONS OF SELF-MEDICATION PREVALENCE AMONG THE POPULATION OF THE FAR EASTERN FEDERAL DISTRICT

M.S. Soboleva, *Cand.Sc. Biology, Associate Professor of the Department of pharmacy and pharmacology, FSBEI of Higher Education "Far-Eastern State Medical University", RF MoH, Chabarovsk, martimser@mail.ru.*

I.V. Amelina, *Candidate of Pharmaceutical Sciences, Associate Professor, chairperson of Department of organization and Economics of pharmacy, FSBEI of Higher Education "Far-Eastern State Medical University", RF MoH, Chabarovsk, amerina@bk.ru*

The article presents the literature data, as well as the data of its own sociological research on the causes of self-medication of patients in the Far -Eastern Federal district. The influence of the previous positive experience of self- medication as one of the main factors of refusal to visit a doctor, as well as the lack of money to pay for the services of a specialist which is more significant for women, is confirmed. One of the organizational aspects of the prevalence of self-medication may be sale of prescription medicines without a prescription by pharmacy organizations, as well as the lack of clear algorithms for pharmaceutical consulting when selling the medicines.

Keywords: Far Eastern Federal District, questioning, self-medication, reasons, factors

Self-medication of patients remains one of the most pressing health problems in the world, and, of course, the Russian Federation and its territorial entities are no exception [1–6]. A large number of modern international, federal and regional studies are devoted to the analysis of the causes and consequences of this medical and social phenomenon.

So, according to the results of a prospective study of FarSaR (n=3798), the frequency of self-medication in the Russian Federation on average is 63.1%. The most popular therapeutic groups were non-steroidal anti-inflammatory drugs and herbal medicine. The main symptoms that were treated by patients on their own were digestive disorders, pain of various origins, viral infections, as well as disease prevention was provided. The main sources of information about self-medication, according to the respondents, were "the opinion of relatives and friends, advices from nurses, advertising in the media, the Internet, advice and recommendations of doctors" [7].

The results of the study of the Samara State Medical University (n=1058) showed that the typological characteristics of patients who are prone to self-medication were "low-income pensioners and employees, middle-income working pensioners – men and women, as well as high-income employees or entrepreneurs – women over 40 years old". Even if there was a consultation with a medical specialist, the basis for therapy was often their own experience and knowledge. Self-medication was most relevant for diseases of the urinary tract and gastrointestinal tract [8]. A significant role in prevalence of self-medication is assigned to institutional distrust and negative emotional perception of medical personnel and organizations [9], the discrepancy between the population's income and the cost of medical services, the fear of using radical or invasive

technologies and methods, the use of the media and the Internet, and the popularization of medical knowledge [9,10].

In this case, self-medication is used not only to correct common and mild symptoms, ailments, but also sometimes chronic non-infectious diseases (cardiovascular, pulmonological, psychiatric) [11–14].

The most successful result of self-medication is the patient's recovery, but, unfortunately, according to a study by the Volgograd regional center for monitoring the safety of medicines, adverse side effects (ASE) in every fifth case are the result of independent use of medicines. Special attention should be paid to the fact that almost half of the medicines that caused the development of adverse side effects are antibacterial drugs, that is, prescription medicines, which can also aggravate other important medical and social problems, in this case, antibiotic resistance [15–16]. Thus, a comprehensive analysis of the prevalence and causes of self-medication of patients remains one of the most urgent and significant problems of the medical and pharmaceutical communities both at the Federal level and at the level of a specific region.

The purpose of study is assessment of causes and factors of prevalence of self-medication of patients in Far-Eastern Federal District (FEFD).

MATERIALS AND METHODS

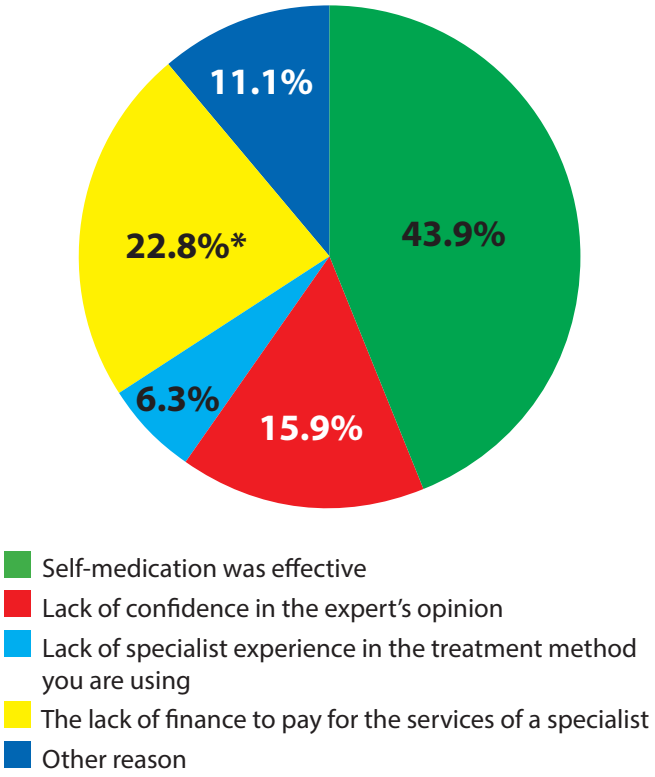
Sociological survey of patients using the questionnaire method in Khabarovsk (FSI "301st Military Clinical Hospital" of the Ministry of Defense of the Russian Federation; KGBUZ (krai government-owned publicly funded health care institution) "Regional city hospital No. 2" named after D.N. Matveev; NGHCI Railway Clinical Hospital at the Khabarovsk-1 station of JSC "Russian Railways"; OOO "Akonit" (pharmacy on Batuevskaya), Yuzhno-Sakhalinsk (SBHI "Sakhalin regional clinical hospital"), Magadan (SBHI "Magadan regional hospital"). The sample of patients was 500 people. 450 questionnaires were considered as suitable for processing (the response was 90%). Statistical processing was performed with coding of respondents 'responses. The questionnaire data was processed using Microsoft Office Excel 2015 (descriptive statistics) and IBM SPSS 25. Distribution of respondents' responses was checked for normality using the Kolmogorov – Smirnov one-factor criterion. The Spearman rank correlation coefficient was used to assess the relationship of respondents' responses to such characteristics as "gender" and "age". The correlation was considered as reliable when the two-tailed significance level was less than 0.05.

RESULTS AND DISCUSSIONS

One of the most important aspects in assessing the problem of self-medication in a particular region is the analysis of the causes. The structure of respondents' responses is shown in Fig. 1.

Thus, as the main reason for the absence of a visit to the doctor, almost half of the respondents indicated positive experience of self-medication. The correlation of the respondents' responses with the "gender" characteristic was significant when assessing the lack of finance to pay for specialist services (p=0.03), this reason was more often noted by women. Serious concerns are caused by the fact that more than 20% of respondents do not trust the opinion of the specialist and his/her experience in the used method of treatment.

The situation is aggravated by the fact that 45.5% of respondents (more often women – p=0.01) share their experience in independent treatment of diseases and/or treat their own children. The observed trend may negatively affect the state of health later and lead to the



* – p<0.05 – women

FIG. 1. *Distribution of respondents' responses about the reasons for self-medication without consulting with a medical specialist*

further prevalence of self-medication among the population.

Further, the patients specified the financial costs that are spent monthly on various means and methods of self-medication. The distribution of respondents' responses is shown in Fig. 2.

Based on the data presented in the diagram, we can conclude that almost 40% of respondents spend from 100 to 500 rubles a month for self-medication, while a quarter of respondents answered "over 1000 rubles", which may be a consequence of not always justified use of medicines, as well as the lack of correction of schemes and modes of their use by a medical specialist.

One of the factors that provoke the prevalence of self-medication is the availability of medication. On the one hand, this allows you to save time/resources/compliance of patients, on the other hand – the existing violations of the requirements of legal acts can become one of the reasons for uncontrolled use of medicines, including prescription drugs (Rx). Therefore, in the next set of questions, respondents were asked to clarify the prospects for using self-medication during hospitalization, as well as the procedure for purchasing Rx drugs in pharmacy organizations. The distribution of responses is shown in Fig. 3.

Based on the data obtained, we can conclude that almost 40% of respondents, even when hospitalized and discharged from the hospital, that is, if there are recommendations from the attending physician, are going to continue independent treatment. This situation can negatively affect the effectiveness and safety of therapy, lead to re-hospitalization, and the development of complications. At the same time, more than 30% of respondents said that they received advice from pharmaceutical specialists about the use of prescription drugs, and almost half of respondents purchased Rx-drugs without a prescription. The observed trend requires tighter control over the sale of drugs in pharmacies, as well as the introduction of algorithms and standards for consulting patients about the use of prescription drugs in pharmacies if there is an administration from a medical specialist.

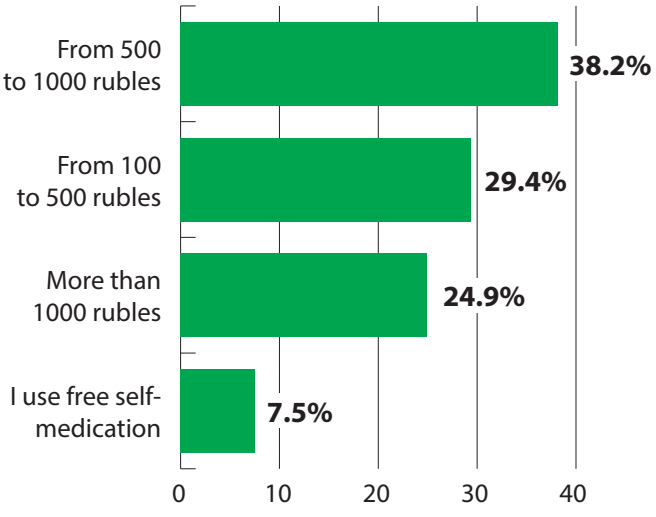
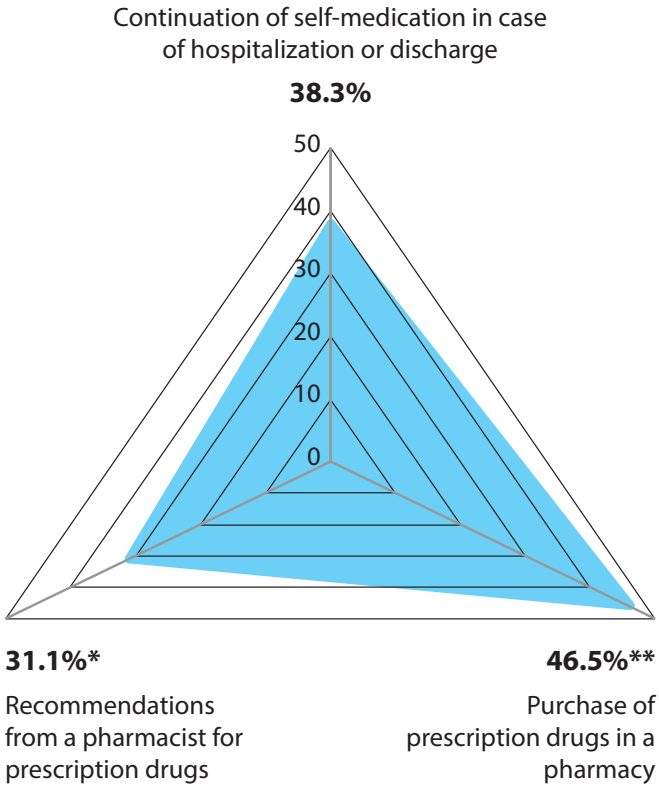


FIG. 2. *Monthly expenses of patients for self-medication*



* – p<0.05 – women, ** – p< 0.05 – the aged

FIG.3. *Positive responses from respondents about the use and purchase of prescription drugs*

CONCLUSION

The main reasons for self-medication of the population of the Far-Eastern Federal District is their own previous positive experience of effective treatment of the disease. The most frequent amount of monthly expenses for independent treatment, that is, without consulting with a medical specialist, is from 100 to 500 rubles. One of the possible factors that contribute to the problem of self-medication of patients is the violation of legislation on the procedure of sale of drugs in the pharmacy organizations of the FEFD regions and the lack of clear standards for pharmaceutical consulting about prescription medicines.

REFERENCE

1. *Karabulova Z.B., Shopabaeva A.R. Quality analysis of pharmaceutical assistance in self-medication in pharmacies of the city of Almaty // Pharmacy of Kazakhstan. – 2015. – No. 6 (169). – pp. 13–16.*
2. *Skibina K.P., Ananko S.Ya. Influence of advertising of medicines on the level of self-medication in Ukraine // Актуальні проблеми сучасної медицини: Вісник української медичної стоматологічної академії. – 2013. – Vol. 13. – №3 (43). – pp. 268–272.*
3. *Kugach V.V., Tarasova E.N. On the concept of responsible self-medication in the Republic of Belarus. Vestnik of pharmacy. – 2008. – No. 3 (41). – pp. 68–73.*
4. *Kulusheva G.A., Zurdinov A.Z. Actual state of drug self-medication in Kyrgyzstan // Kazan medical journal. – 2005. – Vol. 86. – No. 1. – pp. 72–74.*
5. *Makalkina L.G. Prospects for implementing the concept of "responsible self-medication" in the Republic of Kazakhstan // Scientific-based medicine and clinical epidemiology. – 2010. – Vol. 3. – No. 1–2. – pp. 60–63.*
6. *Klimova T.M., Kuzmina A.A., Malogulova I.Sh. Prevalence of self-medication with antibiotics in the Republic of Sakha (Yakutia) // Public health and healthcare. – 2017. – No. 2. – pp. 5–9.*
7. *Zharkova L.P., Andreeva I.V., Pasechnik E.S., Kozlov S.N. Self-medication practice in Russian cities: results of a multicenter descriptive study "Farsar" // Clinical pharmacology and therapy. – 2016. – Vol. 25. – No. 2. – pp. 13–19.*
8. *Krylova I.A., Kupaev V.I. Generalized portrait of a patient engaged in self-medication // Remedium. Journal about the Russian market of medicines and medical equipment. – 2012. – No. 5. – pp. 56–62.*
9. *Vyalykh N.A. Institutional mistrust in the system of self-medication factors in modern Russian society: a sociological view // News of higher education institutes. North Caucasus region. Series: Social Sciences. – 2015. – No. 2 (186). – pp. 24–28.*

10. *Marusiy A. A. Practice of self-medication among patients of hospital medical organizations as a medical and social problem // Bulletin of the Northern State Medical University. – 2016. – No. 1 (36). – pp. 266–267.*
11. *Arstangalieva Z. J. Trends and risks of self-medication among elderly people // Bulletin of medical Internet conferences. – 2014. – Vol. 4. – No. 3. – P. 214.*
12. *Trubnikova I. A., Konev Yu. V., Li E. D. Training in self-medication techniques for older age groups // Clinical gerontology. – 2015. – Vol. 21. – No. 3–4. – pp. 40–43.*
13. *Sergienko A. V., Ivashev M. N. Responsible self-medication – the need to teach a section for pharmacists // International journal of experimental education. – 2013. – No. 1. – p. 136.*
14. *Nesiforova O. I. The case of long-term adaptation in society for self-medication of schizophrenia // Neurological Bulletin. Journal named after V. M. Bekhterev. – 2013. – Vol. 45. – No. 2. – pp. 72–75.*
15. *Smuseva O. N., Solovkina Yu. V. Adverse drug reactions in self-medication // Saratov scientific medical journal. – 2012. – Vol. 8. No. 1. – p. 035–037.*
16. *Smuseva O. N., Gavrilov, Yu. Yu., Solovkina Yu. V. Serious adverse reactions of medicinal products as a consequence of self-medication // Astrakhan medical journal. – 2012. – Vol. 7. – No. 3. – pp. 192–195.*

UDC 615.451.16:582.998.16

A COMPARATIVE STUDY OF THE METHODS OF EXTRACTION IN THE DEVELOPMENT OF LIQUID EXTRACT FROM THE HAWKWEED OXTONGUE HERB (*PICRIS HIERACIOIDES* L.)

V.N. Bubenchikova, *PharmDr., Professor, Head of the Department of Pharmacognosy and Botany of Kursk State Medical University FSBEI HE of the RF Ministry of Health (KSMU FSBEI HE RF MoH), Kursk, bubenhikova.ksmu@yandex.ru*

L.S. Novikova, *PharmDr., Professor of the Department of pharmaceutical engineering of Kursk State Medical University FSBEI HE of the RF Ministry of Health (KSMU FSBEI HE RF MoH), Kursk*

I.V. Stepnova, *PG student of the Department of Pharmacognosy and Botany of Kursk State Medical University FSBEI HE of the RF Ministry of Health (KSMU FSBEI HE RF MoH), Kursk, General Director of the Farmoborona test center, Moscow, leader@farmoborona.ru*

I.A. Kornilova, *Student of the 5th course year of Kursk State Medical University FSBEI HE of the RF Ministry of Health (KSMU FSBEI HE RF MoH), Kursk, bubenhikova.ksmu@yandex.ru*

Hawkweed oxtongue (Picris hieracioides L.) of the sunflower family (Asteraceae) is widely distributed in the regions of the Central black earth region. Hawkweed oxtongue is used in folk medicine as a choleretic, diuretic and aperient agent. In experimental pharmacological studies, antioxidant and anti-inflammatory activities were identified. This work contains material for conducting a comparative study of extraction methods: maceration, maceration with stirring, percolation in the development of a liquid extract from Hawkweed oxtongue herb. It was found that the optimal method of extraction is a percolation method, the optimal extractant is 50% ethyl alcohol. Quality factors of liquid extract have been developed.

Keywords: Hawkweed oxtongue (Picris hieracioides L.), liquid extract, maceration, percolation

The creation of herbal medicinal products remains one of the urgent tasks of pharmaceutical science and practice. Extraction processes are widely used in the production of herbal medicines. In modern pharmaceutical practice, extracts are used as independent medicines and as a semi-product for obtaining dosage formulations (gels, liniments, ointments, suppositories, etc.) [1].

In industrial production, the most common liquid extracts (Extracta fluida) are concentrated water extracts from medical herbs. They have advantages: the same ratio of active substances contained in the finished product and medicinal plant raw materials; ease of dosing; the possibility of obtaining without the use of evaporation, which allows you to make extracts containing volatile substances (essential oils); production is simpler and cheaper than production of thick and dry extracts [1].

Wild and cultivated plants, as well as imported purchased raw materials can be used as sources for obtaining liquid extracts. Wild plants that contain a complex of biologically active substances (phenolic compounds, polysaccharides, sesquiterpene lactones, fatty and organic acids) and are of interest as a raw source of these compounds, include hawkweed oxtongue [2–4]. Hawkweed oxtongue (Picris hieracioides L.) of the

sunflower family (Asteraceae) is a polymorphic species of European flora [5].

For the first time hawkweed oxtongue (Picris hieracioides L.) was described by C. Linnaeus in 1753 [6]. Hawkweed oxtongue can be represented by a biennial or perennial herbaceous plant, height of 30–100 cm, with a taproot. The stems are erect, branched, covered with fork-like and anchor-like hairs. Leaves are oblong-elliptical in shape; basal leaves are in the rosette, long-stemmed, toothed at the edges, usually dying early; stem leaves are sessile, placed evenly on the stem, have a regular location, toothed on the edges or whole-edged, smaller than the basal leaves. Multi-flowered baskets are collected on the tops of stems and branches in a common cymose-panicled inflorescence. All flowers in the basket are ligulate, bisexual, bright yellow; marginal flowers are often with a reddish streak on the outside. Involucres are bell-shaped, three-row, pubescent ones with thin black or – less often – white hairs, not expanded at the base. Fruits are spindle-shaped wrinkled red-brown achenes with a white pappus [7].

Hawkweed oxtongue has long been used in folk medicine as a choleretic, diuretic and aperient agent. Pharmacological studies provided by foreign and domestic researchers have shown the presence of anti-oxidant and anti-inflammatory activities of hawkweed oxtongue, which is why the development of a liquid extract from the hawkweed oxtongue herb is promising [8–10].

However, the production process of liquid extracts has a number of disadvantages: low efficiency of the extraction process, incomplete depletion of medicinal plant raw materials, resulting in the loss of valuable biologically active substances. This suggests that it is necessary to develop resource-saving technologies that allow rational use of medicinal plant raw materials.

Purpose of work – influence of methods for obtaining liquid extracts from hawkweed oxtongue herb on the yield of biologically active substances.

MATERIAL AND METHODS

The object of the study was hawkweed herb, harvested in 2017–2018 in the Kursk region during the flowering period.

It was earlier established that one of the groups of active substances of hawkweed oxtongue herb are hydroxycinnamic acids [11].

In order to select the most effective method for obtaining a liquid extract of hawkweed oxtongue, the influence of the extraction method on the yield of hydroxycinnamic acids was studied. To do this, samples of the raw material under study were used to obtain liquid extracts by various methods: maceration, maceration with stirring, percolation. Extracts were prepared in 1:1 ratio using 50% and 70% ethyl alcohols as extractants [12].

When the liquid extract was obtained by maceration, the plant raw material was crushed to 2 mm, screened and sifted out from the dust. The prepared medicinal raw materials were loaded into a laboratory extractor, filled with an extractant and left for 24 hours at room temperature. After this time, the extraction was drained and the raw material was filled with fresh extractant, infused for another 6–7 hours, then the extraction was drained and added to the first one. The resulting extract was maintained in a tightly closed sediment vessel at temperature no higher than 10°C for 2 days. The clarified liquid was drained from the sediment and filtered.

Maceration with stirring was performed according to the following method: non-crushed plant raw materials were placed into a laboratory extractor, filled with calculated amount of extractant. Using a mixer with capacity of 1000 revolutions per minute, the raw material was mixed with the extractant for 5 minutes, the extraction was drained, the raw material was

pressed, and the extracts were combined. The resulting extract was maintained in a tightly closed sediment vessel at temperature no higher than 10°C for 2 days. The clarified liquid was drained from the sediment and filtered. Preparation of the liquid extract by percolation was carried out using the following method: the raw material was crushed to 2 mm, screened through a sieve with size of 2 mm, and sifted out from dust. The prepared raw material was filled with an equal amount of extractant, mixed and left to swell for 30 minutes (the duration of the process was established experimentally). The swollen raw material was transferred in small portions to the prepared percolator and carefully compacted. The filter material and a small load were placed on the compacted herb material. When the drain valve was open (to displace air), the extractant was poured on the raw material to obtain the surface of liquid. The loaded percolator was tightly closed with a lid and left to infuse for 24 hours at room temperature.

Percolation was performed at rate of 1/24 of the working volume of the percolator per hour. Concentrated extraction was collected in the collecting vessel (85 parts of the amount of loaded plant raw materials). Then percolation was continued until the raw material was completely depleted (diluted extraction), which was evaporated (up to 15 parts of the mass of the loaded plant material) in a vacuum evaporation unit. The condensed residue was added to the first extraction. The resulting extract was maintained at temperature no higher than 10°C for one day. The clarified liquid was drained from the sediment and filtered.

Liquid extracts obtained using various extraction methods were analyzed according to the following parameters in accordance with SP XIV: appearance, content of active substances, ethyl alcohol, heavy metals, and dry residue [13].

Determination of heavy metals and dry residue was carried out according to the method proposed by SP XIVedition [13].

The content of ethyl alcohol was determined according to the method of SP XIV edition by the boiling point of the drug. The alcohol content was determined using the tables of SP XIV edition [13].

For the quantitative determination of hydroxycinnamic acids, the spectrophotometric research method proposed for the herb was used. We have adapted the conditions for determining the amount of hydroxycinnamic acids in a liquid extract, taking into account the selection of the optimal dilution for a single sample [11]. The optical density was measured at a length of 328 nm, in a cuvette with a layer thickness of 1 cm.

RESULTS AND DISCUSSION

The effect of different methods of extraction on the release of hydroxycinnamic acids from hawkweed herb was studied. The content of hydroxycinnamic acids was calculated in conversion to the caffeic acid (Dr. Ehrenstorfer C No. 331-39-5) as one of the dominating acids in the hawkweed oxtongue herb.

As extractants, 50% ethyl alcohol and 70% ethyl alcohol were used, since these alcohols maximally extract hydroxycinnamic acids from the hawkweed oxtongue herb (see the table).

The highest content of hydroxycinnamic acids is observed in the liquid extract obtained by percolation. This points to the fact that this method is more effective for obtaining a liquid extract from the hawkweed oxtongue herb. Thus, we received three series of liquid extracts and evaluated their quality according to the XIV edition of the SP. It is established that liquid extract of the hawkweed oxtongue herb is a transparent greenish-brown liquid with herbal odor. The content of hydroxycinnamic acids in it was 1.15±0.12%, the normal content of at least 0.7% was established; the dry residue content was 9.16±0.36%, the normal content of

Таблица

THE EFFECT OF ALCOHOL CONCENTRATION AND METHOD OF EXTRACTION ON THE RELEASE OF HYDROXYCINNAMIC ACIDS FROM HAWKWEED OXTONGUE HERB

Method of extraction	Extractants – ethyl alcohol, %	Content of hydroxycinnamic acids, %
Maceration	50	0.69±0.03
	70	0.65±0.03
Maceration with stirring	50	0.64±0.07
	70	0.56±0.03
Percolation	50	0.77±0.02
	70	0.72±0.03

dry residue of at least 8% was established; the content of ethyl alcohol was 50.0±1.30%, it shall be at least 45%; the content of heavy metals was no more than 0.01%.

CONCLUSION

1. It is established that the optimal extraction method for obtaining a liquid extract from the hawkweed oxtongue herb is a percolation method using 50% ethyl alcohol as an extractant.

2. The analysis of the liquid extract obtained by percolation showed that it contains the largest amount of hydroxycinnamic acids (0,72±0,03% – 0,77±0,02%).

REFERENCES

1. *Dubashinskaya N. V. Characteristics of methods for obtaining extracts and their standardization (Part II)/N. V. Dubashinskaya, O. M. Hishova, O. M. Shimko // Bulletin of pharmacy. – 2007. – No. 2 (36). – pp. 70–79.*
2. *Bubenchikova V. N. Hawkweed oxtongue is a promising source of biologically active substances / V. N. Bubenchikova, I. V. Stepnova // Pharmacy and Pharmacology. – 2018. – Vol. 6, No. 1. – pp. 33–46.*
3. *Bubenchikova V. N. Fatty acid and mineral composition of the hawkweed oxtongue herb (Picris hieracioides L.) / V. N. Bubenchikova, I. V. Stepnova // Chemistry of plant raw materials. – 2018. – No. 1. – pp. 113–119*
4. *Stepnova I. V. Study of carotenoids of the hawkweed oxtongue herb (Picris hieracioides L.) / I. V. Stepnova, V. N. Bubenchikova // V scientific and practical conference "Modern aspects of the use of plant raw materials and raw materials of natural origin in medicine" (March 15, 2017, Moscow). – Moscow, 2017. – pp. 201–203.*
5. *Flora of the USSR: in 30 volumes /edited By V. L. Komarov. – Moscow -Leningrad, 1934–1964.*
6. *Slovak M. Morphological evaluation of Picris hieracioides a (Compositae) in Slovakia / M. Slovak, K. Marhold // Phyton. (Horn, Austria). – 2007. – №47. – P. 73–102.*
7. *Illustrated guide to plants in Central Russia. Vol. 3. Angiosperms (dicotyledonous: eleutheropetalous) / I. A. Gubanov [et al.]. Moscow, 2004. – 520 p.*
8. *Wild useful plants of Russia / Edited by A. L. Budantseva, S. P. Lesiovskaya. – SPb., 2001. – 663 p.*
9. *V. V. Shustov Application of flavonoids in medicine / V. V. Shustov, E. I. Terakh // XXX international scientific and practical conference "Scientific community of students of the XXI century. Natural Sciences" (April 7, 2015, Novosibirsk, Russia). – Novosibirsk, 2015. – No. 4 (29). – pp. 142–148.*
10. *Flavonoids of medicinal plants: prognosis of antioxidant activity / V. A. Kurkin, V. V. Poroikov, A. V. Kurkina, etc. // Modern problems of science and education. – 2015. – No. 2 (2). – p. 517.*

11. *V.N. Bubenchikova Development of methods for quantitative determination of the amount of phenol-carboxylic acids in the hawkweed oxtongue herb (Picris hieracioides L.) / V.N. Bubenchikova, I.V. Stepnova, N.V. Popova // Bulletin of the South Kazakh State Pharmaceutical Academy. – 2017. – Vol. 2. – No. 1 (78). – pp. 145–148.*
12. *A.V. Korochinsky Determination of optimal conditions of extraction of plant raw materials // Actual problems of pharmaceutical science and practice. – Vladikavkaz, 2014. – pp. 251–255.*
13. *State Pharmacopoeia of the Russian Federation: 14th edition, volume III. [Electronic resource]. – Access mode: http://femb.ru/felm./ (accessed 11.06.16).*

UDC 615.015.32

RESULTS OF STUDY OF TOXICITY OF HOMEOPATHIC MOTHER TINCTURE OF EUROPEAN BUGLEWEED FRESH HERB (*LYCOPUS EUROPAEUS* L.)

V.I. Zvereva, *PG student of Pharmaceutical Engineering Department, "All-Russian Research Scientific Institute of Medicinal and Aromatic Plants" FSBSI (VILAR FSBSI), Moscow, valentinca1988@mail.ru*
V.V. Bortnikova, *Cand. Sc. Biology, Senior Research Scientist of Toxicology Department, "All-Russian Research Scientific Institute of Medicinal and Aromatic Plants" FSBSI (VILAR FSBSI), Moscow, bortnikova.v@yandex.ru*
A.N. Babenko, *Cand. Sc. Biology, Senior Research Scientist of Toxicology Department, "All-Russian Research Scientific Institute of Medicinal and Aromatic Plants" FSBSI (VILAR FSBSI), Moscow, alexandra.mogileva@gmail.com*
M.V. Borovkova, *Senior scientist of Toxicology Department, "All-Russian Research Scientific Institute of Medicinal and Aromatic Plants" FSBSI (VILAR FSBSI), Moscow, borovkova_65@mail.ru*
O.A. Semkina, *Candidate of Pharmaceutical Sciences, Scientific Secretary, Head of Research Engineering Department, "All-Russian Research Scientific Institute of Medicinal and Aromatic Plants" FSBSI (VILAR FSBSI), Moscow, semkinaolga@gmail.com*

The purpose of the study was to study the acute toxicity of homeopathic mother tincture (HMT) of European bugleweed (Lycopus europaeus L.) fresh herb. The subject of study is HMT obtained from the European bugleweed (Lycopus europaeus L.) fresh herb cultivated in accordance with SP XIV, OFS 1.6.2.008.18 "Homeopathic mother tinctures" by method 2 with the use of 86% ethanol (m/m) at temperatures not higher than 20°C. The raw material of European bugleweed (Lycopus europaeus L.) gathered in 2016 on experimental field "Biocollections of VILAR FSBSI". Standardization of the resulting tincture was carried out according to the following characteristics: description, authenticity, microbiological purity, quantitative determination. Determination of toxicity was carried out by the method of probit analysis according to Litchfield and Wilcoxon. It was found that single doses of HMT of European bugleweed (Lycopus europaeus L.) fresh herb such as 1700 mg /kg and 1360 mg/kg (dry residue) introduced into the stomach of mice and rats of both sexes did not cause the death of animals. In accordance with the classification of chemical toxicity, HMT of European bugleweed (Lycopus europaeus L.) fresh herb is a low-toxic substance.

Key words: European bugleweed (Lycopus europaeus L.), homeopathic mother tincture (HMT), mice, rats, acute toxicity

European bugleweed (*Lycopus europeaus* L.) is a perennial herbaceous plant of Labiaceae (Lamiaceae) family, which is found in Russia and other European countries. This herb contains different classes of biologically active substances (BAS): ortho-dihydroxyphenils (caffeic acid, caffeic acid ethyl ester, rosmarinic acid, rosmarinic acid methyl ester, luteolin, protocatechuic aldehyde, 5, 3',4'–trihydroxytrihydroxy-6,7-dimethoxyflavone, luteolin-7- glucuronoside methyl ester, luteolin-7-glucuronoside ethyl ether); other phenols (apigenin-7-glucuronoside ethyl ether), non phenolic compounds (mesoinosit) [1–4]. Extracts

from European bugleweed (*Lycopus europeaus* L.) are actively used in traditional medicine for treatment of hyperthyroid states, as well as as a sedative, hemostatic and analgesic for neuroses, insomnia, headaches, stomach pain and uterine bleeding [5,6]. If we turn to the world experience of using the European bugleweed (*Lycopus europeaus* L.) herb, then medicines based on it can now be found on the pharmaceutical market in a number of European countries (Germany, Austria, Switzerland, France, etc.) and countries of America. Basically, these are homeopathic preparations, which are a liquid extract from the European bugleweed herb in the form of drops for oral administration (including homeopathic mother tincture), as well as a dry extract of the European bugleweed herb in the form of tablets for oral administration. The monograph on European bugleweed as a source of medicinal plant raw materials for obtaining homeopathic medicines is included in the German Homeopathic Pharmacopoeia [7].

The recognition of homeopathy as one of the most promising areas of practical health care in the Russian Federation gave rise to the development of domestic homeopathic production. Introduction of a definition of homeopathic medicinal products in Federal law №61-FZ "On circulation of medicines", appearance of section "Homeopathic medicines" within the State Pharmacopoeia XIV edition, which includes pharmacopoeial monographs on homeopathic pharmaceutical substances, as well as the convening the International scientific homoeopathic Congress in Moscow on 9–12 April 2018, organized by the International Medical Homeopathic League (LMHI) will serve as a new milestone in the development of this direction in the Russian Federation [8, 9]. However, despite the emerging trends, the nomenclature of homeopathic medicines is small and does not meet the needs of practice [10, 11]. In this regard, the development of domestic homeopathic medicines is relevant.

A homeopathic medicine in the form of mother tincture has been developed in VILAR FSBSI on the basis of European bugleweed fresh herb. According to the literature and its own phytochemical studies, the antithyroid properties of the medicine product are specified by the oxidation products of ortho-dihydroxyphenols (rosmarinic and caffeic acids and their ethers, luteolin derivatives) under the action of catechol oxidase (enzymatic oxidation) or salts of manganese, copper, and zinc (chemical oxidation) [3,4].

One of the stages of development of homeopathic medicines is experimental study of their toxicity when administered once.

Purpose of our research is the study of acute toxicity of homeopathic mother tincture (HMT) of European bugleweed (*Lycopus europaeus* L.) fresh herb for registration of the medicine product.

MATERIALS AND METHODS

The subject of study is the HMT obtained from the European bugleweed (Lycopus europaeus L.) fresh herb, gathered in July during the flowering phase of the plant (2016) and prepared according to method 2 of the State Pharmacopoeia of the XIV edition, OFS 1.6.2.008.18 "Homeopathic mother tinctures". Standardization of the resulting tincture was carried out according to the following indicators: description, identity, microbiological purity, quantitative determination, density, dry residue, heavy metals.

Description. As for appearance the HMT prepared from the European bugleweed (*Lycopus europaeus* L.) fresh herb is a clear dark-brown liquid with a specific smell.

Identity. On the chromatogram of test solution of HMT prepared from the European bugleweed fresh herb the luminous light-blue zones are observed at the level of zones of solution of the rosmarinic acid reference standard (RS) with Rf

about 0.33. The dry residue is 3.4%. The density of HMT prepared from the European bugleweed fresh herb is 0.9350 to 0.9420. The content of heavy metals is not higher than 0.001%.

Microbiological purity. 1 ml of HMT of the European bugleweed fresh herb contains less than 104 aerobic bacteria and less than 102 fungi, in the absence of Escherichia coli, Salmonella and Staphylococcus aureus (category 3. B).

Quantitative determination. The content of the sum of phenolic compounds in the HMT of the European bugleweed fresh herb in terms of rosemarinic acid at wavelength of 327 ± 2 nm is at least 0.3%.

Acute toxicity studies were performed on 30 BALB/c mice (males and females) with body weight of 20 g and 30 Wistar rats (males and females) with body weight of 200 g. Animal experiments were conducted in accordance with the rules adopted by the European Convention for the protection of vertebrates used for experimental and other scientific purposes and "Guidelines for preclinical medicines research" (2012) [12, 13]. The experiment protocol was approved by the Bioethical Commission of VILAR FSBSI.

Preparation of the drug for administration. Concentration of ethyl alcohol in HMT of the European bugleweed fresh herb was 40% (by volume), the dry residue was 3.4%. To study the acute toxicity of HMT, it was pre-dealcoholized using a rotary evaporator and brought to the initial volume with water. The work solution did not contain ethanol

Doses and method of administration. HMT of European bugleweed fresh herb was injected into the stomach of mice and rats once or twice using a metal probe in volumes of 0.5 and 1.0 ml per mouse and 5.0 and 10 ml per rat. The duration of observation of laboratory animals was 14 days. During the experiment, their behavior, appearance, motor activity, response to exogenous irritants, and death were monitored. The toxicity parameters of the test tincture were determined using the Litchfield and Wilcoxon probit analysis.

RESULTS AND DISCUSSION

In case of one-dose delivery of HMT of European bugleweed fresh herb into the stomach of male and female mice at a dose of 10 ml/kg (340 mg/kg for dry residue), 5 minutes after administration the motor activity decrease, shortness of breath, and drowsiness were observed. These symptoms persisted for 20–25 minutes, after which the animals were active, willingly ate food, and responded adequately to exogenous irritants. 5 minutes after the introduction of HMT of European bugleweed fresh herb to mice of both sexes at doses of 25 ml/kg and 50 ml/kg (850 and 1700 mg/kg for dry residue, respectively), the similar symptoms of acute intoxication described above were registered, in addition, hyperemia of the vessels of the animals ear and tail was noted. After 60 minutes, the symptoms of acute intoxication disappeared, and the animals became active. The death of mice from the administration of the tested doses was not observed for 14 days.

In case of one-and two-dose delivery of HMT of European bugleweed fresh herb into the stomach of male and female rats at a dose of 10, 20 and 40 ml/kg (340, 680 and 1360 mg/kg for dry residue, correspondingly), the motor activity decrease, shortness of breath, and drowsiness were observed. These symptoms of acute intoxication in rats of both sexes were less pronounced than in mice. After 30 minutes, the symptoms of acute intoxication disappeared. No deaths of male and female rats were recorded.

Thus, when studying the acute toxicity of HMT of European bugleweed fresh herb, LD50 indicators were not established, since the doses of the studied tincture administered into the stomach of mice and rats did not cause the death of animals. There was no difference in the sensitivity

of animals to the action of HMT depending on the species and gender. In accordance with the classification of chemical toxicity according to GOST 12.1.007-76, HMT of European bugleweed fresh herb is a low-toxic substance [14]. The obtained results allow to recommend to HMT of European bugleweed fresh herb for registration.

CONCLUSION

1. In case of one-and two-dose delivery of HMT of European bugleweed fresh herb (*Lycopus europaeus* L.) into the stomach of mice and rats of both sexes at doses up to 1700 mg/kg (on a dry residue basis) it did not cause the death of animals, and therefore no LD50 indicators were established.

2. In accordance with the classification of chemical toxicity under GOST 12.1.007-76, HMT of European bugleweed fresh herb is a low-toxic substance.

REFERENCES

1. *Winterhoff H., Gumbinger H. G., Sourgents H. // Planta medica, 1988, v. 54, №2. – P. 101.*
2. *Nahrstedt A., Albrecht M., Wray V., Gumbinger H. G., John M., Winterhoff H., Kemper F. H. // Planta medica, 1990, v. 56, №4. – P. 395.*
3. *Shelukhina H. A., Savina A. A., Sheichenko V. A., Sokolskaya T. A., Bykov V. A. Study of the chemical composition of European bugleweed (Lycopus europaeus L.) // Questions of biological, medical and pharmaceutical chemistry. – Moscow, 2010. – Vol. 11. – Pp. 7–11.*
4. *Shelukhina H. A., Savina A. A., Sheichenko V. A., Osipov V. I., Sokolskaya T. A., Bykov V. A., Laskaya O. F. Study of the chemical composition of the European bugleweed herb (Lycopus europaeus L.) // Questions of biological, medical and pharmaceutical chemistry. – Moscow, 2011. – Vol. 3. – Pp. 6–9.*
5. *Kyosev P. A. Complete Handbook of medicinal plants. – Moscow: EKSMO. 2011. – 551 p.*
6. *Zvereva V. I., Semkina O. A., Gribkova E. I., and others. Justification of the expediency of using the extract of the European bugleweed herb for the purpose of developing the oral dosage forms // Proceedings of the Second scientific and practical conference "Young scientists and pharmacy of the XXI century". – M.: VILAR FSBSI. 2014. – Pp. 256–261.*
7. *German Homoeopathic Pharmacopoeia, GHP. Volume 2. Monographs E-Z, 2000.*
8. *Federal law №61-FZ "On circulation of medicines" dated 12.04.2010 (Revised on 04.06.2018). – Available at: https://www.rosminzdrav.ru/ [Date of access: 03.02.2019].*
9. *State Pharmacopoeia of the Russian Federation, XIV edition (Vol. 1, 2, 3, 4) [Electronic media]. – Moscow, 2018. – Available at: http://www.femb.ru/feml. [Date of access: 03.02.2019].*
10. *Allen H. K. Main indications and characteristics of homeopathic medicines and nosodes with comparison of their pathogenesis. – Smolensk: Homeopathic pharmacy,1996. – P. 388.*
11. *Nazarova E. A., Tertichnaya Yu. M., Savina A. A. Prospects for creating the medicines based on plant raw materials that have thyrotropic effect // From plant to drug: traditions and modernity: Proceedings of the All-Russian conference dedicated to the 95th anniversary of the birth of Professor A. I. Schroeter. – Moscow: Shcherbinska printing house, 2014. – pp. 33–39.*
12. *European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (ETS 123). – Strasbourg. 1986.*
13. *Guidelines for preclinical studies of medicines. Part one. – Moscow: Grif and Co., 2012. – 944 p.*
14. *Berezovskaya I. V. Classification of chemical substances by parameters of acute toxicity in case of parenteral methods of administration// Journal of pharmacy and chemistry. – 2003. – Vol. 37. – No. 3. – pp. 32–34.*

UDC 616.24:616.248:615.234

EVALUATION OF ASTHMA CONTROL IN THE RUSSIAN FEDERATION

V.V. Arkhipov, *Doctor of Medicine, Professor of the Department of Clinical Pharmacology and Therapy of the Russian Medical Academy of Continuous Professional Education FSBEI of FPE (RMACPE FSBEI of FPE), Moscow, 777@startmail.com*

I.B. Zakharova, *Candidate of Medicine, Medical Adviser of the Department of Clinical Research and Post-registration Monitoring of the Russian public organization of persons with disabilities "Human Health" (ROOI "Human Health"), Moscow, zakharova@humanhealth.ru*

I.V. Tikhonovskaya, *Head of the Department of Clinical Research and Post-registration Monitoring of the Russian public organization of persons with disabilities "Human Health" (ROOI "Human Health"), obstetrics and gynecology doctor, Research Institute of Occupational Medicine named after academician N.F. Izmerov FSBSI (OMRI FSBSI) Moscow, tikhonovskaya@humanhealth.ru*

The purpose of the study was to assess the prevalence of phenotypes of bronchial asthma (BA) with small airways predominant lesion (SAWPL), to determine the proportion of patients with combined bronchial asthma and chronic obstructive pulmonary disease (COPD) and how COPD and BA phenotypes with SAWPL effect on the level of control of asthma attacks in real clinical practice of regions of the Russian Federation. The effect on the level of BA control was assessed by using alternative therapeutic options, including options for inhalation therapy combinations, the use of free combinations of inhaled glucocorticosteroids (IGCs) and long-acting β2 agonists (LABA), conventional and extra-fine inhalers, and a regimen of maintenance and reliever therapy (MART). The proportion of patients with a good level of BA control and lack of control over the clinical manifestations of asthma was determined in accordance with the criteria of the Global Initiative for Asthma (GINA), Global Strategy for Asthma Management and Prevention, 2018. In addition, when patients completed the Asthma Control Questionnaire (ACQ), the level of BA control was determined. In addition, the TAI-10 test was used to determine patients' adherence to therapy.

Keywords: asthma bronchial (BA), chronic obstructive pulmonary disease (COPD), bronchial asthma (BA) with small-airways predominant lesion (SAWPL), inhaled glucocorticosteroids (IGCs) and long-acting beta-2 agonist (LABA), maintenance and reliever therapy (MART)

Bronchial asthma (BA) is one of the most common chronic respiratory diseases. The main goal of BA therapy is to achieve and maintain disease control. In clinical studies, an acceptable level of control is achieved for more than 70% of patients with BA [5], especially with therapy based on modern fixed combinations of inhaled glucocorticosteroids (IGCs) and long-acting β2 agonists (LABA) [6]. However, in studies performed in real clinical practice conditions, about half of patients do not control or poorly control BA. For example, in the study conducted by Demoly P. [7] in five European countries in 2006–2010, only 43–47% of patients achieved control over BA, and the proportion of patients who were well in control of BA had not significantly changed for 5 years of follow-up. A study conducted in the United States in 2008–2010 also revealed an insufficient level of control, a high need for inhaled medications to relieve symptoms, and frequent exacerbations in the American patient population [8]. In Western

Europe, the proportion of patients with good asthma control was evaluated at the level of 44–55% in the current decade [9,10].

According to the researchers, poor control of BA is largely related to patients' perception of asthma therapy. Some of them take medications only when necessary, considering the feeling of symptoms as a normal phenomenon, have insufficient awareness of the specifics of asthma therapy, or are overly cautious about the risk of developing adverse reactions. Others do not notice the benefits of therapy, using the wrong inhalation technique, or due to forgetfulness, skip taking a dose of the medication. Treatment of the disease is complicated with the negative perception of IGCs therapy by patients, the high cost of treatment, or an inconvenient dosage regimen [11].

The last major Russian study to assess the level of BA control in real clinical practice (NICA), performed almost 10 years ago, involved 1,000 patients from 26 outpatient hospitals in 12 cities of the Russian Federation [12]. Since that time, such large-scale studies to assess the level of asthma control in Russia have not been conducted. The study of NICA, like the majority of similar studies abroad, had a few typical restrictions. In particular, the study did not include patients with a combination of BA and COPD [13]. Meanwhile, according to the epidemiological study conducted in the Russian Federation, approximately 13% of patients with COPD are diagnosed with BA [14]. The level of asthma control for patients with combination of BA and COPD has never been possible to assess satisfactorily, since such patients are usually not included in clinical and epidemiological studies. Along with this, the prevalence of other BA phenotypes, in particular those associated with predominant lesions of the small airways, has not been studied. At the same time, asthma with a predominant lesion of the small bronchi is an urgent clinical problem [15] and requires the use of specialized therapeutic approaches, including administration of IGCs in the form of extra-fine inhalers [16]. In published in 2013 in the Russian Federation "Concerted recommendations for justifying the choice of therapy for BA and COPD, taking into account the phenotype of the disease and the role of small airways", the authors attributed to the phenotypes of BA with primary involvement of small airways the following clinical situations: BA, which is difficult to control, with frequent exacerbations (two or more exacerbations per year); BA in smokers and former smokers; combination of BA and COPD; BA in elderly people; BA with fixed bronchial obstruction, as well as BA with functional signs of lesion of the small airways, confirmed by spirometry, bodyplethysmography or computed tomography of lungs [17]. The prevalence of BA phenotypes with a predominant lesion of the small airways (SAWPL) Russia has deliberately not yet been studied.

In recent years, new medications and therapeutic approaches have been used in clinical practice, including those that imply independent intensification of anti-inflammatory therapy by the patient when symptoms increase, for example, the regime of basic and symptomatic therapy with a single MART (maintenance and reliever therapy) inhaler. Effectiveness of this approach has not been properly evaluated in the context of actual medical practice in our country.

In scientific terms, and considering the planning and hands-on assistance to patients in accordance with the Federal clinical guidelines, a new multicenter study with as broad as possible sample of patients, including patients with combined BA and COPD and patients with predominant lesion of the small airways (SAWPL) seemed to be highly relevant objective.

MATERIALS AND METHODS

This work is a cross-sectional study in real clinical practice. The study was conducted on the basis of outpatient clinics. 124 study physicians

from 22 cities of the Russian Federation were involved in that study.

Patients were included in the study during their visits to outpatient medical institutions, regardless of the reason for contacting a doctor. The patients with BA diagnosis determined at least one year prior to inclusion in this study and confirmed by primary medical documentation were included into this study and analysis. Study participants were required to provide informed consent to participate in the study and to process their data.

During the follow-up, only non-invasive studies were performed within the standards that exist in routine clinical practice. Complaints, anamnestic data, and patient questionnaires (asthma Control Questionnaire (ACQ-5) and TAI-10) were collected at once. Information obtained during the patient survey, including data on the number of moderate and severe exacerbations for the past 12 months, was recorded in the clinical report form (CRF). Also, the CRF included the results of a spirometric study performed no earlier than one month before the date of the patient's visit and including the determination of FEV1, FVC (forced vital capacity), and increase in the value of FEV1 after a bronchodilator test, as well as the results of a general blood test performed for the last 6 months before the visit. The CRF indicated the medications taken by the patient for the BA treatment, the need for inhalation of medicines to relieve the disease symptoms.

Statistical processing of the obtained data was performed using the standard software packages Statistica (V7.0) and SPSS Statistics (V24.0) or http://medstatistic.ru/calculators/calchi.html depending on the tasks. Descriptive statistics were used for data processing. The samples were checked for normality using the Kolmogorov-Smirnov test. The samples were compared using the Student's t-criteria, the Mann-Whitney U-criteria, and the Wilcoxon T-criteria. The correlation of parameters was detected using Pearson and Spearman correlation analysis methods. The analysis of the difference in sign frequencies in independent groups was performed using the Chi-square criterion with the Yates correction.

RESULTS OF THE STUDY

Characteristics of the study participants

As part of the study, 3921 patient questionnaires were statistically processed. The average age of patients in the study population was 52.3±15.9 years, 36% of the population were men (average age – 50.3 years) and 64% were women (average age – 53.5 years). The average body mass index (BMI) in the general patient population was 27.2±5.05 kg/m2.

Most of the respondents – 2621 (68%) – have never smoked. The number of former smokers was 742 (19%), and 508 patients (13%) smoked at the time of the study. The average duration of smoking of former smokers was 19.0 pack/years. Respondents who reported about smoking at the time of the study had an average smoking experience of 25.7 pack/years.

Of all patients, 2116 patients according their social status were employees, 3413 (87%) did not have harmful working conditions (including those who had previously worked). Among the study participants, 3281 (84%) had only BA, and 610 (16%) participants had a combination of BA and COPD. The average duration of BA is 13.4 years, and the average duration of COPD is 7.7 years.

In the majority of patients – 2377 (62%) – the onset of BA occurred in the age range of 18–50 years, in 962 (25%) patients – under 18 years and in 515 (13%) patients – over 50 years. Allergen intolerance, proven by allergotests, and features of the course of BA *(allergies, aspirin intolerance, the predominance of BA nocturnal symptoms, occurrence of BA exacerbations on the background of acute respiratory infections)* were indicated by 1748 (59%) respondents.

In 1171 patients, BA was observed with a predominance of nocturnal symptoms, complaints of heartburn and/or gastroesophageal reflux were noted by 886 people, nasal polyps were diagnosed in 576 cases.

As for comorbidities, 1759 (44.9%) patients had allergic rhinitis, the remaining 2162 patients had comorbidities of various types, including CHD in 647 patients, diabetes mellitus in 287 patients, a history of cerebral circulation – in 79 cases, and arthritis of the hand joints – in 114 respondents.

The following risk factors for small airways lesions were observed in patients: smoking in 32% of patients, a combination of asthma and COPD in 16% of patients, and fixed obstruction (FEV1/FVC <0.7) in 27.2% of patients. According to the assessment of doctors, asthma control (effectiveness of treatment) was distributed as follows:

- BA under control – 725 (19%) cases;
- BA under partial control – 1951 (51%) cases;
- uncontrolled BA – 1160 (30%) cases.

The ACQ-5 survey showed that in a significant number of cases, doctors incorrectly assess asthma control in their patients, in particular, underestimate the number of patients with uncontrolled asthma (see the Table).

The proportion of patients with asthma control depended on the therapy. The maximum number of BA patients with completely controlled asthma was observed in the group where MART therapy was prescribed – 49.6% of patients with controlled asthma. Among patients whom a combination of IGCs/LABA was prescribed at medium and high doses (in the normal regimen of maintenance therapy), the controlled BA course was observed in 22.8% and 20.8%, respectively. Also, patients who received IGCs/LABA as an extra-fine inhaler had better asthma control indicators compared to conventional inhalers.

The study evaluated the main parameters related to the characteristics of disease control. It turned out that for the year prior to inclusion into the study, 25.1% of participants had hospitalizations for asthma, 37.5% missed work due to asthma exacerbations, and 37.6% were forced to call an ambulance due to BA exacerbations.

The occurrence of adverse reactions, presumably associated with IGCs therapy (pharyngitis, oral candidiasis, hoarseness of the voice), was noted in 23% of patients. Medium and high doses of IGCs caused adverse reactions (AR) in 31.3% of patients, and low doses in 26.2%. The frequency of adverse reactions was minimal (0.44%) when using finest-dispersed medicines, namely, finest-dispersed aerosol of beclomethasone dipropionate + formoterol (Foster®, "Chiesi Pharmaceuticals" S. p. A., Italy), in medium and high doses. On MART therapy with a single inhaler in the form of Foster® medicine, adverse reactions developed with the lowest frequency – 0.22% (see Fig. 1).

In 1339 (34.67%) of 3862 patients, there were no exacerbations of the disease over the past year. 2130 respondents indicated moderate exacerbations, and 176 patients indicated severe exacerbations. The average frequency of exacerbations of moderate severity for the year

Table

EVALUATION OF ASTHMA CONTROL, ACCORDING TO PHYSICIANS AND UNDER THE RESULTS OF *ASTHMA CONTROL QUESTIONNAIRE* (ACQ-5)

	Percentage of patients with different levels of control, in % of the total number	
	according to physicians	Evaluation under ACQ-5
Controlled BA	19	20
Partially controlled BA	51	19
Uncontrolled BA	30	61

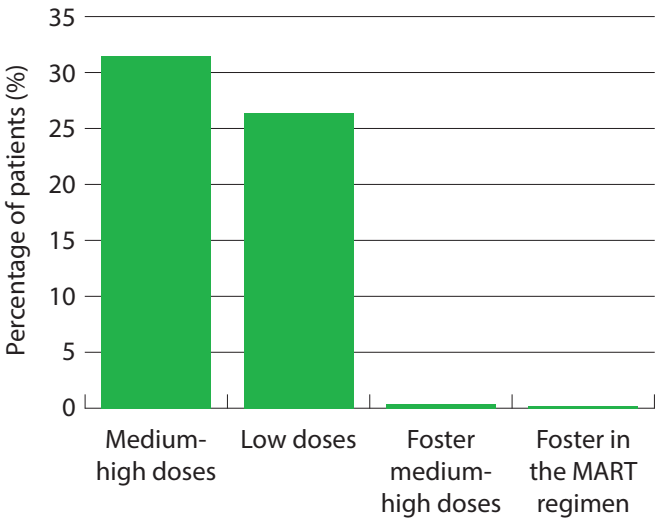


FIG. 1. *Percentage of patients with adverse reactions to IGCs therapy*

was 1.7 and severe exacerbations – 1.5 cases per year.

The test for adherence to TAI therapy, where 50 points is full adherence; 45–49 points is intermediate one and <45 points – no adherence, showed intermediate adherence to the patients therapy based on Foster® in the MART regimen. Similar test results were noted on Relvar. None of the medicines showed complete adherence to therapy (see Fig. 2).

CONCLUSION

Evaluation of patients receiving asthma therapy in real conditions of outpatient clinics in our country revealed a low proportion of patients with good disease control. In a significant number of patients, the course of asthma is complicated by comorbid diseases or risk factors for small airway lesions. In addition, most patients had BA exacerbations for the past year and / or adverse reactions associated with the therapy. At the same time, the best disease control was observed in patients who received extra-fine combinations, combinations and medicines for taking in the MART regimen.

REFERENCE

1. *Masoli M., Fabian D., Holt S., Beasley R: The global burden of asthma: executive summary of the GINA Dissemination Committee Report // Allergy. – 2004. – V. 59. – 469–478.*
2. *Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention // 2018. Available from: www.ginasthma.org*

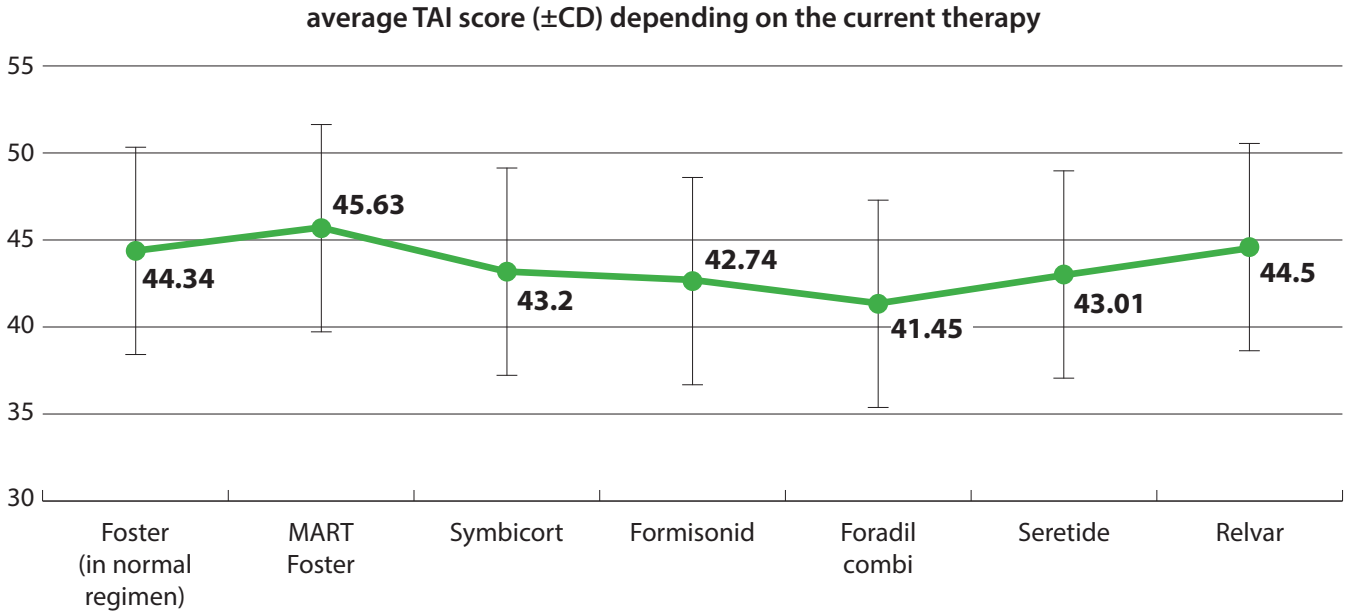


FIG. 2. *Adherence to therapy depending on the medicine used at the time of the study according to the TAI questionnaire*

3. *Martinez F.D., Vercelli D. Asthma // Lancet. – 2013. Oct. 19; 382 (9901). – P. 1360–72. doi: 10.1016/S0140–6736 (13) 61536–6.*
4. *Reddel H.K., Bateman E.D., Becker A., et al. A summary of the new GINA strategy: a roadmap to asthma control // Eur. Respir. J. – 2015. Sep. – V. 46 (3). – P. 622–39. doi: 10.1183/13993003.00853–2015.*
5. *Bateman E.D., Boushey H.A., Bousquet J., et al. Can guideline-defined asthma control be achieved? The Gaining Optimal Asthma Control study // Am. J. Respir. Crit. Care. Med. – 2004 Oct 15. – V. 170 (8). – P. 836–44 // Epub. – 2004 Jul 15.*
6. *Aalbers R., Vogelmeier C., Kuna P. Achieving asthma control with ICS/LABA: A review of strategies for asthma management and prevention // Respir. Med. – 2016. Feb; – V. 111. – P. 1–7. doi: 10.1016/j.rmed.2015.11.002.*
7. *Demoly P., Annunziata K., Gubba E., Adamek L. Repeated cross-sectional survey of patient-reported asthma control in Europe in the past 5 years // Eur. Respir. Rev. – 2012 Mar 1. – V. 21 (123). – P. 66–74. doi: 10.1183/09059180.00008111.*
8. *Slejko J.F., Ghushchyan V.H., Sucher B., et al. Asthma control in the United States, 2008–2010: indicators of poor asthma control // J. Allergy Clin. Immunol. – 2014 Jun. – V. 133 (6). – P. 1579–87. doi: 10.1016/j.jaci.2013.10.028.*
9. *Price D., Fletcher M., van der Molen T. Asthma control and management in 8000 European patients: the REcognise Asthma and LInk to Symptoms and Experience (REALISE) survey // NPJ Prim. Care. Respir. Med. – 2014 Jun 12. – V. 24. – P. 14009.*
10. *Braido F., Brusselle G., Guastalla D., et al. Determinants and impact of suboptimal asthma control in Europe: The international cross-sectional and longitudinal assessment on asthma control (LIAISON) study // Respir. Res. – 2016 May 14. – V. 17 (1). – P. 51. doi: 10.1186/s12931-016-0374-z.*
11. *Chapman K.R., Hinds D., Piazza P., et al. Physician perspectives on the burden and management of asthma in six countries: The Global Asthma Physician Survey (GAPS) // BMC Pulm. Med. – 2017. – Nov 23. – V. 17 (1). – P. 153. doi: 10.1186/s12890-017-042-5.*
12. *Arkhipov V.V., Grigorieva E.V., Gavrishina E.V. Bronchial asthma control in Russia: results of a multicenter observational study of NIKA // Pulmonology. – 2011. – № 6. – p. 87–93.*
13. *Uchida A., Sakaue K., Inoue H. Epidemiology of asthma-chronic obstructive pulmonary disease overlap (ACO) // Allergol. Int. – 2018 Mar 15. pii: S1323–8930 (18) 30006–6. doi: 10.1016/j.alit.2018.02.002.*
14. *Arkhipov V., Arkhipova D., Miravitlles M., et al. Characteristics of COPD patients according to GOLD classification and clinical phenotypes in the Russian Federation: the SUPPORT trial // Int. J. COPD. – 2017. – V. 12. – P. 1–8.*
15. *Contoli M., Santus P., Papi A. Small airway disease in asthma: pathophysiological and diagnostic considerations // Curr. Opin. Pulm. Med. – 2015 Jan. – V. 21 (1). – P. 68–73. doi: 10.1097/MCP.0000000000000122.*
16. *Usmani O.S. Small-airway disease in asthma: pharmacological considerations. // Curr. Opin. Pulm. Med. – 2015 Jan. – V. 21 (1). – P. 55–67. doi: 10.1097/MCP.0000000000000115.*
17. *Avdeev S.N., Isanov Z.R., Arkhipov V.V. et al. Concerted recommendations for justifying the choice of therapy for bronchial asthma and chronic obstructive pulmonary disease, taking into account the phenotype of the disease and the role of the small airways // Atmosphere. Pulmonology and Allergology. – 2013. – No. 2. – pp. 15–26.*

RULES FOR EXECUTION OF ARTICLES

The Editorial Board of the Journal of Pharmaceuticals Quality Assurance Issues request the authors to comply with the following rules when preparing articles for publication.

Editorial ethics. The data presented in the paper shall be original. It is not allowed to send to the editorial office the works that have already been printed in other publications or accepted for publication by other editorial offices.

The article shall be accompanied by an official referral from the institution where the work was performed, have a scientific supervisor's visa, and a seal. The article shall be signed by all authors (on the last page), which enables to publish it and place it on the publisher's website

The Editorial Board reserves the right to shorten and edit accepted works. The date of receipt of the article is the time of receipt of the final (revised) version of the article.

The article shall be carefully edited and verified by the author. The presentation should be clear, without lengthy introductions and repetitions.

1. **Structure of an article.** TITLE PAGE: 1) article heading; 2) initials and surnames of authors; 3) full name of the institution where the author works, with mandatory indication of the organization's status (abbreviation before the name) and departmental affiliation; 4) city; 5) UDC

After the title page in English the follows should be specified: article heading; initials and surnames of the authors; full name of the institution; summary (no more than 0.5 pages of typewritten text) and key words.

On a separate page the additional information about each author required for Journal processing in Russian science citation index: full name in Russian and in transliteration, e-mail, mailing address (with a postal code), contact telephone number of the authors of the article.

Further the original articles should contain the following sections: ABSTRACT; KEY WORDS; SHORT INTRODUCTION, reflecting the state of the art at the time of writing, the purpose and objectives of this study; MATERIALS AND METHODS; RESULTS AND DISCUSSION; CONCLUSIONS (item by item) or CONCLUSION; REFERENCES.

2. Size of the original article shall not exceed 10 pages of typewritten text, lecture – 8–10 pages, literature review – 18–20 pages, reviews, chronicles – 4–5 pages, personalia – 2–3 pages. When preparing review articles, please, include mainly recent publications in the references.
3. Materials shall be prepared in Microsoft Word (" DOC text" parameter); computer typing shall be performed without formatting and hyphenation in ANSII text format, 14 – font size, with 1.5 line spacing (double typewriting spacing).
4. Figures in the form of graphs and diagrams must be supplemented with digital data in the form of Excel tables.
5. Figures in the form of photos (histograms, echograms, etc.) shall be presented as separate files, not inserted into the text of the article. File format for black-and-white figures is BMP or GIF (extension *.bmp,*.gif), for color figures and gray scale – JPG format (extension *.jpg), resolution – 600 dpi (pixels per inch); it is possible to use ZIP, RAR compression,or other.
6. Figure captions are placed after the references. Each figure should have a common title, and then all the numbers and letters in it are explained. In captions to microphotographs, you must specify the colorization method, magnification.
7. Tables must be numbered (at top, right) and have a name. Abbreviations of words in tables

are not allowed. Tables can be given in the text without being placed on separate pages.

8. All physical values specified in the article must be expressed in SI units. When preparing the article, it is necessary to take into account the rules for using signs, abbreviations, symbols, etc., recommended by the Biochemical Nomenclature Commission.
9. The references shall include no more than 20 sources, for reviews – no more than 50. The references does not include unpublished works.
10. Numbering of literature sources begins with the number 1. In the text of the article, references are given in square brackets in strict accordance with the article list of references. The numbering of literature sources is determined by the order of their citation in the text, not by alphabetical order.

EXAMPLE OF CITATION OF ARTICLES FROM JOURNALS AND OTHER PERIODICALS

1. Loskutova E.E., Bazarkina O.V. Trends and structure of demand for herbal preparations // Russian pharmacies. – 2003. – No. 4. – Pp. 38–40.

EXAMPLE OF CITATION OF BOOKS AND SOME CHAPTERS

1. Anikin B.A., Rodkina T.A. Logistics. – Moscow: Welby, Prospect, 2008. – 408 p.
2. Dolgushin I.I., Bukharin O.V. Neutrophils and homeostasis/ edited by Dolgushina I.I. – Yekaterinburg, Meditsina, 2001. – 279 p.
3. Plant resources of Russia. Wild flowering plants, their component composition and biological activity. Vol. 2. St. Petersburg – Moscow: Association of scientific publications of KMC, 2009. – 513 p.

EXAMPLE OF CITATION OF THESIS AND AUTHOR'S ABSTRACTS

1. Orlov F.S. Analysis and standardization of the dosage form with modified release of dilept, a new original domestic medicine: thesis of Candidate of Pharm. Sciences. – Moscow: I.M. Sechenov the First Moscow State Medical University, 2012. – 125 p.

The author is fully responsible for the accuracy of the data provided in the article list of references.
The article is sent to the editorial office by e-mail: **journal@humanhealth.ru**

Articles executed in not compliance with the rules are returned to the authors without consideration by the Editorial Board.

ISSN 2309-6039