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 (30) 2020

Dear friends and colleagues!

Scientific and practical Journal of Pharmaceutical Quality Assurance Issue has been published since 2013 with the publication frequency of 4 issues per year. Throughout the years the publication demonstrated a qualitative approach to selection and publishing the works that represent all areas of modern pharmaceutical science. For the reporting period we managed to be included into the list of periodicals, recommended by the HAC of the Ministry of Science and Higher Education of the Russian Federation. We are reflected in the RSCI, as well as we work scrupulously for the future indexing in well-known international databases. Since 2019 the English language version of the Journal has been published to attract European specialists. We invite all concerned parties to cooperate for efficient 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 №2602-2/21

ISSN 2309-6039 №4 (30) 2020

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

UDC 615.15
https://www.doi.org/110.34907/JPQAI.2020.40.49.001

ADMISSION OF EMPLOYEES TO THE CIRCULATION OF NARCOTIC DRUGS AND PSYCHOTROPIC SUBSTANCES

T. A. Barankina, *Director for Insurance Medicine and Off-budget Activities, Federal Siberian Research Clinical Centre of the Federal Medical-Biological Agency of the Russian Federation, Krasnoyarsk State Medical University named after Prof. V. F. Voino-Yasenetsky, Krasnoyarsk, Russia, dmytry238@rambler.ru*
I.V. Krasnopeeva, *Pharmaceutical Inspector, Federal Siberian Research Clinical Centre of the Federal Medical-Biological Agency of the Russian Federation, Krasnoyarsk State Medical University named after Prof. V. F. Voino-Yasenetsky, Krasnoyarsk, Russia, krasnopeeva_iv@skc-fmba.ru*
E.A. Kozlova, *Deputy Executive Director for Legal Affairs, Federal Siberian Research Clinical Centre of the Federal Medical-Biological Agency of the Russian Federation, Krasnoyarsk, Russia, kozlova_ea@skc-fmba.ru*
O.N. Yakimenko, *Deputy Director of the Training and Educational Center, Federal Siberian Research Clinical Centre of the Federal Medical-Biological Agency of the Russian Federation, Krasnoyarsk State Medical University named after Prof. V. F. Voino-Yasenetsky, Krasnoyarsk, Russia, yakimenko_on@skc-fmba.ru*
N.B. Barankina, *Pharmaceutical Inspector, Federal Siberian Research Clinical Centre of the Federal Medical-Biological Agency of the Russian Federation, Krasnoyarsk State Medical University named after Prof. V.F. Voino-Yasenetsky, Krasnoyarsk, Russia, barankina_nb@skc-fmba.ru*

When organizing the work on the circulation of narcotic drugs and psychotropic substances (ND and PS), one of the conditions for the implementation of the activity is the registration of the admission of employees to work with ND and PS. For the past three years, this subject has become relevant in terms of the frequency of registration of one of the main documents such as a Certificate of absence of drug addiction, substance abuse and chronic alcoholism. Unfortunately, up to date, the validity period of this document has not been clarified by the regulatory authorities and is not regulated by law. When conducting control and supervisory activities, there is no unified and clear understanding of the specified requirements. In the practical activities of medical and pharmacy organizations, when meeting the requirements for annual registration of the certificate, additional financial and organizational difficulties arise, which can lead to a limitation in the number of employees engaged in activities with ND and PS, and thereby, to decrease in the availability of pain – relieving medical care to the population.

Keywords: narcotic drugs, psychotropic substances, medical examination, medical clearance

When organizing the work on the circulation of narcotic and psychotropic drugs in medical and pharmacy organizations, one of the conditions for the implementation of activities is the registration of the admission of employees to work with narcotic drugs and psychotropic substances.

The procedure for obtaining the admission for employees to work with ND and PS is closely related to Article 10 of the Federal Law No. 3 dated 8.01.1998 "On Narcotic Drugs and Psychotropic

Substances" [1], which specifies the special requirements for the conditions for carrying out activities related to the circulation of ND and PS and their precursors included in List I, and the cultivation of narcotic-containing plants. Sub-paragraphs 4 and 5 of paragraph 3 of this Article provide for the mandatory availability of the following documents for legal entities:

- Certificates of absence of drug addiction, substance abuse and chronic alcoholism issued by the medical institutions of the public health system or municipal health system in the manner prescribed by the Federal Executive authority responsible for formulation and implementation of state policy and normative-legal regulation in the sphere of health, in coordination with the Federal Executive authority in the sphere of internal affairs, to the employees, who in accordance with their work responsibilities should have access to the ND and PS, List I precursors or cultivated drug-containing plants;
- the reports of conclusion of the Internal Affairs Bodies that the employees, who in accordance with their duties shall have access to ND and PS, List I precursors or cultivated drug-containing plants, have no unexpunged or unspent conviction for misdemeanor, serious crime, very serious crime or illegal trafficking of the ND and PS, their precursors or illicit cultivation of narcotic-containing plants, including the crime committed outside the Russian Federation.

According to the requirements of p. 4 of the Decree of the Government of the Russian Federation dated 06.08.1998 No. 892 "On approval of the Rules for admission of persons to work with the narcotic drugs and psychotropic substances as well as to activities connected with circulation of precursors of narcotic drugs and psychotropic substances", [2], the presence of the above certificates and conclusion reports is a prerequisite for registration of admission of employees to work with ND and PS: "The persons under 18 years of age, and the persons, who have no certificates and conclusions provided for in sub-paragraphs 4 and 5 of paragraph 3 of Article 10, respectively, are not admitted to work with the narcotic drugs and psychotropic substances, as well as activities related to circulation of precursors."

In accordance with p. 6 of abovementioned Decree, the head of the organization issues to the person to be hired to work with the ND and PS, as well as for the implementation of activities related to circulation of precursors, the referrals to healthcare organizations for preliminary (periodic) **medical examination** and mandatory psychiatric **evaluation** in a prescribed manner for **obtaining certificates** stipulated in pp. 4, p. 3 of Article 10 of the Federal Law № 3-FZ. This paragraph, according to some lawyers, indicates the relationship between medical examinations and psychiatric examinations to obtain a certificate of absence of drug addiction, substance abuse and chronic alcoholism [3].

Previously (until 2017), the form of the certificate and the procedure for obtaining it were not specified, the absence of drug addiction, substance abuse and chronic alcoholism was confirmed only by the absence of information from the drug treatment and (or) psychiatric dispensary on employees (that are not registered), issued in any form by the relevant organizations, as well as by the medical conclusion of a professional pathologist on the state of health of the employee (after a preliminary or periodic examination), which indirectly confirmed the absence of these diseases. The validity period of such certificates was not specified, it was enough to issue (receive) them to the employee once when the employee obtained admission to work with the ND and PS.

However, since February 2017, the Order of the Ministry of Health of the Russian Federation No. 988н dated 22.12.2016 "On the procedure for issuing a certificate of absence of drug addiction, substance abuse, and chronic alcoholism to employees who, in accordance with their employment duties, must have access to ND

and PS included in List I and Table I of List IV of NDs and PSs and their precursors subject to control in the Russian Federation, precursors or cultivated narcotic-containing plants" [4], which regulates the procedure for obtaining such a certificate and does not provide for the need to undergo a preliminary (periodic) medical examination and a mandatory psychiatric examination in accordance with the specified procedure.

The specified procedure for issuing a certificate applies to all employees whose activities are related to the circulation of **narcotic drugs and psychotropic substances subject to control in the Russian Federation,** including in medical and pharmacy organizations, which is directly regulated by sub-paragraph 4 of paragraph 3 of Article 10 of the Federal Law "On Narcotic Drugs and Psychotropic Substances", while the Decree of the Government of the Russian Federation No. 892 dated 06.08.1998 reminds that "the persons in respect of whom there are no certificates" are not allowed to work with Narcotic Drugs (ND) and Psychotropic Substances (PS). The Order of the Ministry of Health of the Russian Federation dated 22.12.2016 No. 988н, regulating the procedure for obtaining a certificate of absence of drug addiction, substance abuse, and chronic alcoholism, is closely related to the normative legal acts specifying the rules for admission to work with ND and PS.

Taking into account the current regulatory legal acts, to obtain an admission for each employee of a medical or pharmacy organization whose activities are related to the treatment of ND and PS, it is necessary have the following documents (see Table).

On the basis of the documents received for each employee, the HR department prepares an order on admission for the head of the organization.

It should be noted that, in accordance with paragraphs 7 and 9 of the Decree of the RF Government dated 06.08.1998 No. 892 it was specified that the validity of the admission is limited to the duration of the employment contract. Also, the admission of a person to work with ND and PS, to activities related to the circulation of precursors, is terminated when circumstances are identified that prevent the issuance of relevant certificates and conclusions provided for in sub-paragraphs 4 and 5 of paragraph 3 of Article 10.

The main documents required for registration of admission are a Criminal record certificate and a Certificate on the absence of drug addiction, substance abuse and chronic alcoholism.

A special feature of a Criminal record certificate for an employee whose activities are related to the circulation of ND and PS (see Table) is that it is issued for the organization where the employee works, and is valid for the period of performance of his duties related to the circulation of ND and PS.

The second mandatory document for registration of admission is a Certificate of absence of drug addiction, substance abuse and chronic alcoholism (hereinafter referred to as the Certificate). Ministry of Health Order No. 988н allocated a **separate medical examination** for obtaining the Certificate of absence of drug addiction, substance abuse and chronic alcoholism, which includes the following steps:

- examination by an addiction psychiatrist;
- determination of the presence of psychoactive substances in the urine;
- qualitative and quantitative determination of carbohydrate-deficient transferrin (CDT) in blood serum by capillary electrophoresis;
- analysis of the information contained in the employee's medical records (if available).

This examination is more accurate and indicative for detection of drug addiction, substance abuse and chronic alcoholism, but it becomes an independent procedure, regardless of the preliminary (periodic) examination and psychiatric evaluations of employees, which are also provided by the RF Government Decree No. 892 and binding in the organization.

Table

LIST OF DOCUMENTS REQUIRED TO OBTAIN ADMISSION TO THE ND AND PS

Normative legal act regulating the availability of the document	Document	Validity period of a document
1. Paragraph 3 of Article 10 of the Federal Law "On Narcotic Drugs and Psychotropic Substances" 2. Paragraphs 4, 6 and 9 of Decree of the RF Government dated 06.08.1998 No. 892 "On approval of the Admission Rules…" 3. Paragraph 7 "d" of Decree of the RF Government dated 22.12.2011 No. 1085 "About licensing activities…" [5] 4. Order of Ministry of Internal Affairs of the Russian Federation dated 17.07.2017 No. 470 "On the approval of the Administrative Regulations…" [6]	The report of conclusion of the Internal Affairs Bodies that the employees, who in accordance with their duties shall have access to ND and PS, List I precursors or cultivated drug-containing plants, have no unexpunged or unspent conviction for misdemeanor, serious crime, very serious crime or illegal trafficking of the ND and PS, their precursors or illicit cultivation of narcotic-containing plants, including the crime committed outside the Russian Federation.	NA An employee may be excluded from the conclusion at the request of the organization in cas of: • changes in the employee's work obligations, • employee termination; • availability of information about the new criminal record. The deadline for submitting an application for the exclusion of an employee from the conclusion report is not specified.
1. Paragraph 3 of Article 10 of the Federal Law "On Narcotic Drugs and Psychotropic Substances" 2. Paragraphs 4, 6 and 9 of Decree of the RF Government dated 06.08.1998 No. 892 "On approval of the Admission Rules…" 3. Paragraph 7 "d" of Decree of the RF Government dated 22.12.2011 No. 1085 "About licensing activities…" 4. Order of the Ministry of Health of the Russian Federation dated 22.12.2016 No. 988н "On the procedure of issuing the Certificate of absence of drug addiction, substance abuse, and chronic alcoholism…"	Certificates of absence of drug addiction, substance abuse, and chronic alcoholism issued to employees who, in accordance with their employment duties, must have access to ND and PS, precursors included in List I or cultivated narcotic-containing plants.	Not applicable

End of the table

Normative legal act regulating the availability of the document	Document	Validity period of a document
1. Paragraphs 6 of Decree of the RF Government dated 06.08.1998 No. 892 "On approval of the Admission Rules…" 2. Order of MHSD dated 12.04.2011 No. 302н "On the approval of the lists of harmful and (or) dangerous production factors and works for performing of which the mandatory preliminary and periodic medical examinations are provided, and the Procedure for conducting the mandatory preliminary and periodic medical examinations of employees engaged in heavy work and work with harmful and (or) dangerous working conditions" [7]	Medical conclusion according to the results of a preliminary (periodic) examination of employees.	1 year
1. Paragraphs 6 of Decree of the RF Government dated 06.08.1998 No. 892 "On approval of the Admission Rules…" 2. Article 6 of the Law of the Russian Federation dated 02.07.1992 No. 3185-1 "On psychiatric care and guarantees of the rights of citizens in delivery of such care" [8] 3. Decree of the RF Government dated 28.04.1993 No. 377 "On the implementation of the Law of the Russian Federation "On psychiatric care and guarantees of the rights of citizens in delivery of such care"[9] 4. Decree of the RF Government dated 23.09.2002 No. 695 "On the mandatory psychiatric evaluation of employees who carry out certain types of activities, including activities related to sources of increased risk (with the influence of harmful substances and unfavorable production factors), as well as who work in conditions of increased risk" [10]	The decision of the medical commission (in writing) that conducted the psychiatric examination.	5 years

Unfortunately, the regulatory document ***does not specify the frequency*** of receipt of this Certificate. In other documents regulating the need to obtain a Certificate (see Table), there is also no data on the period of its validity.

An important factor affecting the execution of a Certificate of absence of drug addiction, substance abuse and chronic alcoholism is the difficulty of obtaining it [11], because the required studies are carried out using the special equipment, which is only available in large clinics, as well as due to the duration of the tests and the high cost. All of that create significant difficulties not only for medical and pharmacy organizations located in rural areas, but also for large multidisciplinary medical organizations, where hundreds of employees are allowed to work with ND and PS. At the same time, the financial expenses of these studies are borne by the employer.

The following two documents – the Conclusion Report on the results of the preliminary (periodic) medical examination of employees and the Decision of the medical commission that conducted the psychiatric evaluation – do not directly affect the admission of employees to the ND and PS. According to the RF Government Decree No. 892, their execution is required only to "obtain the certificates provided under p. 3 of Article 10," but in the Order of the Ministry of Health of the Russian Federation No. 988н they are not required.

Some experts refer to the direct dependence of the Order of the MHSD of Russia dated 12.04.2011 №302н and Order Ministry of Health of Russia dated 22.12.2016 №988н [12]. However, from the point of view of the health care organizers, there is no hierarchy of these documents in circulation of the ND and the PS.

Let's consider the procedure of execution of the Order of the MHSD of Russia dated 12.04.2011 No. 302н in relation to the circulation of ND and PS. The Conclusion Report on the results of preliminary (periodic) medical examination shall be executed in accordance with Order No. 302н, according to which the **use of the ND and PS** in medical activities is not harmful and (or) dangerous production factor, as well as the activity for circulation of ND and PS is not specified in the list of works, for performing of which the mandatory preliminary and periodic medical examinations of employees shall be conducted. The order provides for mandatory preliminary and annual periodic examination only **in the production of ND and PS** (p. 1.3.9.6 of Appendix No. 1), as well as for employees of medical and pharmacy organizations in accordance with paragraphs 17 and 24 of Appendix No. 2, regardless of whether their activities are related to the circulation of ND and PS or not. When conducting a medical examination of these categories of employees, the laboratory and functional studies do not provide for mandatory tests (toxicology screening, CDT) required to obtain a Certificate of absence of drug addiction, substance abuse and chronic alcoholism, but when conducting PME (periodic medical examinations), the participation of a psychiatrist and a narcologist is always mandatory, who, in accordance with paragraph 38 of Order No. 302н, must **in case of identification** of persons with suspected medical contraindications (drug addiction, substance abuse, alcoholism) send them "for examination by the medical commission authorized by the health body".

Definitions and types of **"medical evaluation"** and **"medical examination"** are specified in Federal Law No. 323 dated 21.11.2011 "On the Basics of Public Health Protection in the Russian Federation" [13]. In accordance with p. 1 of Article 46 of this law, "a medical examination is a complex of medical interventions aimed at identifying pathological conditions, diseases and risk factors for their development." In accordance with p. 1 of Article 65 of this law, a medical evaluation of a person is a set of methods of medical examination and medical investigation aimed at

confirming such health status of a person that results in the legal implications.

From these definitions, it can be concluded that only **the evaluation of the patient, carried out in case of suspected** medical contraindications, involves an in-depth study of health parameters (including the urine psychoactive substances tests and determination of the carbohydrate-deficient transferrin in the blood serum) with obtaining a certificate of the absence of drug addiction, substance abuse, chronic alcoholism.

Psychiatric evaluation is an independent procedure, which is regulated by the Law of the Russian Federation dated 02.07.1992 No. 3185-1 "On psychiatric care and guarantees of the rights of citizens in its provision". Thus, according to the Decree of the RF Government dated 28.04.1993 No. 377, the activities for circulation of ND and PS is included in the List of medical psychiatric contraindications for the implementation of certain types of professional activities related to the source of increased danger. The frequency and timing of psychiatric evaluation of employees are regulated, the procedure is available in many medical organizations that have the appropriate license, which does not cause difficulties and disagreements when obtaining the decision of the medical commission that conducted the psychiatric evaluation.

CONCLUSIONS

In relation to circulation of ND and PS, it is necessary to clearly separate the concepts of **"medical examination"** and **"medical evaluation",** in accordance with Federal Law No. 323-FZ dated 21.11.2011. Based on the comparison, it can be concluded that the annual evaluation should be carried out **only in case of suspected** medical contraindications, which involves an in-depth study of health parameters (including the urine psychoactive substances tests and determination of the carbohydrate-deficient transferrin in the blood serum) with obtaining a Certificate of the absence of drug addiction, substance abuse, chronic alcoholism.

It should be noted that the Certificate under discussion is only an integral part of the materials issued to persons whose activities are related to the circulation of ND and PS.

To obtain the Certificate in question, the head of the organization issues referrals to medical organizations to the person being hired in order to undergo a preliminary (periodic) medical examination and a mandatory psychiatric evaluation (once every 5 years) [14].

Thus, as a result of the **medical examination,** it is necessary to evaluate not the general mental health of the candidate, but to determine whether he can engage in a specific activity – participation in the handling of ND and PS.

The employee applies to a medical organization and undergoes a medical examination, which includes:

- examination by an addiction psychiatrist;
- determination of the presence of psychoactive substances in the urine;
- qualitative and quantitative determination of carbohydrate-deficient transferrin (CDT) in blood serum by capillary electrophoresis;
- analysis of the information contained in the employee's medical records (if available).

If an employee doesn't suffer from drug addiction, substance abuse and chronic alcoholism, a corresponding Certificate is issued. The term of its validity is not specified by legal acts, there is only a requirement to have such a Certificate when applying for a job.

Taking into account the above, the validity period of a person's admission to work with narcotic drugs, psychotropic substances, as well as to activities related to the circulation of precursors, is limited by the validity period of the employment contract.

There is an opinion of some lawyers that as part of the mandatory annual periodic

medical examinations of employees, it is necessary to receive a new Certificate each time. We believe that this opinion should be considered critically, since, as mentioned above, the Certificate is only an integral part of the materials for admission to work with ND and PS, as well as to activities related to the circulation of precursors. In accordance with part 2 of Art. 213 of the Labor code [15], the employees, including medical organizations, undergo regular medical examinations in order to protect public health, prevent the occurrence and spread of diseases, and therefore, the employers or medical institutions have no legal grounds to send annually the employee for a medical evaluation to obtain a new Certificate about the absence of drug addiction, substance abuse, chronic alcoholism.

Currently, the Ministry of Health of the Russian Federation is actively working to improve the legal regulation of medical care. The amendments are aimed at increasing the availability of pain-relieving therapy and ensuring the necessary control over the circulation of ND and PS. However, in the practical work of medical and pharmacy organizations, sometimes there is a different understanding of the specified requirements, which needs additional explanations from the federal executive authorities.

REFERENCES

1. *Federal Law No. 3-FZ dated 08.01.1998 "On Narcotic Drugs and Psychotropic substances".*
2. *Decree of the Government of the Russian Federation dated 06.08.1998 No. 892 "On Approval of the Rules for the admission of persons to work with narcotic drugs and psychotropic substances, as well as to activities related to the circulation of precursors of narcotic drugs and psychotropic substances".*
3. *A. Yu. Abramov. Regulation of the admission of persons to work with narcotic drugs and psychotropic substances as a licensing requirement // Roszdravnadzor Newsletter. – 2014. – No. 2. – p. 47.*
4. *Order of the Ministry of Health of the Russian Federation dated 22.12.2016 No. 988н "On the procedure for issuing a Certificate of absence of drug addiction, substance abuse, chronic alcoholism to the employees who, in accordance with their work duties, must have access to ND and PS included in List I and Table I of List IV of ND and PS and their precursors subject to control in the Russian Federation, precursors or cultivated narcotic-containing plants".*
5. *Decree of the Government of the Russian Federation No. 1085 dated 22.12.2011 (as amended on 04.04.2020) "On Licensing Activities for the Circulation of Narcotic Drugs, Psychotropic Substances and their Precursors, Cultivation of narcotic-containing plants" (together with the "Regulations on Licensing Activities for the Circulation of Narcotic Drugs, Psychotropic Substances and Their Precursors, Cultivation of Narcotic-containing Plants").*
6. *Order of the Ministry of Internal Affairs of Russia dated 17.07.2017 №470 "On approval of Administrative Regulations of the Ministry of Internal Affairs of the Russian Federation on provision of the state service for issuance of the conclusion reports on the absence of unexpunged or unspent conviction for misdemeanor, serious crime, very serious crime or illegal trafficking of the ND and PS, their precursors or illicit cultivation of narcotic-containing plants, to employees, who in accordance with their duties shall have access to ND and PS, List I precursors or cultivated drug-containing plants…"*
7. *Order of MHSD dated 12.04.2011 No. 302н "On the approval of the lists of harmful and (or) dangerous production factors and works for performing of which the mandatory preliminary and periodic medical examinations are provided, and the Procedure for conducting the mandatory preliminary and periodic medical examinations of employees engaged*

in heavy work and work with harmful and (or) dangerous working conditions"

8. *Law of the Russian Federation dated 02.07.1992 No. 3185-1 "On psychiatric care and guarantees of the rights of citizens in delivery of such care".*
9. *Decree of the RF Government dated 28.04.1993 No. 377 "On the implementation of the Law of the Russian Federation "On psychiatric care and guarantees of the rights of citizens in delivery of such care"*
10. *Decree of the RF Government dated 23.09.2002 No. 695 "On the mandatory psychiatric evaluation of employees who carry out certain types of activities, including activities related to sources of increased risk (with the influence of harmful substances and unfavorable production factors), as well as who work in conditions of increased risk"*
11. *The Order of the MHSD of Russia dated January 27, 2006, No. 40 "On organization of Toxicology screening with analytical diagnosis of the presence of alcohol, narcotic drugs, psychotropic and other toxic substances in the human body".*
12. *M.F. Mikaelyan, M.M. Khachatryan, I.N. Ayro. Debatable issues on the circulation of narcotic drugs and psychotropic substances // Health and education in the XXI century. – 2018. – Vol. 20, – №11, – p. 76–80*
13. *Federal Law No. 323-FZ dated 21.11.2011 "On the Basics of Public Health Protection in the Russian Federation".*
14. *"Information materials on the medical use of narcotic drugs, psychotropic substances and other medicines containing controlled drugs": for managers and administrative staff of medical and pharmacy organizations, medical and pharmaceutical professionals, distribution organizations: In 2 Parts. Part 2. – M.: ABV-press, – 2017. – pp. 121–122.*
15. *The Labor Code of the Russian Federation dated 30.12.2001 №197-FZ (Rev. dated 24.04.2020) [Electronic resource Consultant.ru›document/cons_doc_LAW_34683, access 15.06.2020].*

UDC 614.27+615.12
https://www.doi.org/210.34907/JPQAI.2020.25.17.002

STUDY OF CONSUMER PREFERENCES WHEN BUYING BIOLOGICALLY ACTIVE ADDITIVES IN PHARMACY ORGANIZATIONS OF THE REPUBLIC OF BASHKORTOSTAN

S.N. Ivakina, *Candidate of Pharmaceutical Sciences, Associate Professor of the Department of Management and Economics of Pharmacy with a course of Medical and Pharmaceutical Commodity Science, Bashkir State Medical University, Ufa, Russia, ivakinasn@mail.ru*
T.V. Shaikhatarova, *Extramural Postgraduate Student of the Department of Management and Economics of Pharmacy with a course of Medical and Pharmaceutical Commodity Science, Bashkir State Medical University, Ufa, Russia, Tanya.barkova.93@mail.ru*

Based on the opinion poll findings of 261 consumers of biologically active additives (BAAs), including BAAs of Vita pharmacy chain private label, the BAA consumer profile in the Republic of Bashkortostan was created. It was found that 35.4% of consumers are ready to spend no more than 200 rubles per month for purchase of BAAs, preferring the domestically produced BAAs. The consumers evaluated BAAs of the Vita pharmacy chain private label taken by them in terms of efficiency and intensity of side effects. The consumers chose 14 most efficient BAAs of the Vita pharmacy chain private label (average weighted score varied from 3.3 to 3.8). The majority of respondents pointed to occurrence of side effects along with the efficiency with regard to four BAAs chain private label taken.

Keywords: biologically active additives, private label, consumer preferences

In the modern pharmaceutical market, among retail sales, a sufficient capacious part is occupied by biologically active additives (BAAs), which are very popular with the population. According to the All-Russian Public Opinion Research Center, the majority of Russian residents think the BAAs are additions to food (60%), about 16% of respondents believe that BAAs are vitamins, and 5% of respondents takes BAAs for medicines [1,8]. However, in the context of the economic crisis, there is a decrease in real incomes, solvency of the population and volume of the commercial pharmaceutical market. As a result, in 2014, there was a decrease in sales of medicines and biologically active additives in natural values by 5.8% and 7.2% compared to 2013 [1,2,3]. One of the areas of adaptation to changes in environmental conditions that have been developed in the pharmacy business is the promotion of biologically active additives under the private label brand (PLB) of the pharmacy chain, which are characterized by price availability. Therefore, it is relevant to study consumer behavior when choosing and buying biologically active additives, including biologically active additives under the private label brand, which allows us to characterize the trends that have developed in the Republic of Bashkortostan (RB).

Purpose of the study is study of consumer preferences when buying biologically active additives in pharmacy organizations of the Republic of Bashkortostan.

MATERIALS AND METHODS

To study the consumer preferences for biologically active additives, the opinion poll method in the form of a questionnaire was used, in which the problem is studied by developing a questionnaire containing a certain set of questions and by questioning each of the participants of the selected group [5,6]. The opinion poll involved 261 respondents who were the buyers of BAAs and BAAs of Vita pharmacy chain private label on the territory of the Republic of Bashkortostan (RB), which meets the conditions of the representativeness of the target sampling with probability p=95% [7]. The opinion poll was conducted from January to March 2017. The data from the biologically active additives consumer questionnaires was processed using a package of computer applications (Microsoft Excel). The developed questionnaire included two sections. In the first section, the questions required for drawing up a profile of the consumer of biologically active additives and for identifying the influence of various factors on consumer preferences in relation to biologically active additives by a correlation analysis were presented. The reliability of the obtained values of the correlation coefficient (C) was evaluated by comparison with the standard values for the corresponding number of degrees of freedom p=95% (according to L.S. Kaminsky) [8].

The second section of the questionnaire provided a list of BAAs of the pharmacy chain private label for evaluation. Consumers (buyers) were proposed to choose and evaluate BAAs under the private label, which they used for treatment and prevention of diseases. A total of 43 trade names (TN) of biologically active additives under the private labels were proposed. To evaluate the effectiveness and presence of side effects of biologically active additives under the private labels used by consumers, a 4-point scale is proposed, the characteristics of which are presented in Table 1.

The degree of consistency of consumer opinions was evaluated by calculating the coefficient of variation. The calculated values of the coefficient of variation did not exceed 25%, which indicates the consistency of the opinions of the respondents [8]. To assess the effectiveness and severity of side effects of biologically active additives under the private labels, we calculated the average weighted ratings of respondents for each name of biologically active additives under the private labels and proposed the intervals (in points) and their characteristics (Table 2).

Table 1

CHARACTERISTICS OF THE RATING SCALE FOR EVALUATING THE BIOLOGICALLY ACTIVE ADDITIVES UNDER THE PRIVATE LABELS FOR VARIOUS PARAMETERS

Rating scale	Characteristics
4	Very effective biologically active additives under the private labels (improvement was immediately felt after administration)
3	Effective biologically active additives under the private labels (the effect appeared after a while)
2	Effective biologically active additives under the private labels, but there are side effects (allergies, nausea, stomach pain, etc.).
1	Low-effective biologically active additives under the private labels (no particular difference was felt from the use of this biologically active additives under the private labels)

Table 2

INTERVALS (IN POINTS) FOR EVALUATING THE EFFECTIVENESS AND SEVERITY OF SIDE EFFECTS OF BIOLOGICALLY ACTIVE ADDITIVES UNDER THE PRIVATE LABELS

Interval values (in points)*	Characteristic of biologically active additives under the private labels
3.5–4.0	Very effective biologically active additives under the private labels
2.5–3.0	Effective biologically active additives under the private labels with rare side effects
1.5–2.0	Effective biologically active additives under the private labels with frequent side effects
Less than 1.5	Low-effective biologically active additives under the private labels

** Note: the calculated weighted average estimates of respondents were grouped by value in the following boundaries: from 3.7 to 3.99 rounded to 4,0; 3,3–3,69 – to 3,5; 2,8–3,29 – to 3,0; 2,3–2,79 – to 2,5; 1,8–2,29 – to 2,0; 1,3–1,69 – to 1,5; 0,8–1,29 – to 1,0.*

RESULTS AND DISCUSSION

The opinion poll showed that among the consumers of biologically active additives in pharmacy organizations of the Republic of Bashkortostan, 76% of respondents were women. The distribution of respondents by age and social status is shown in Table 3.

The analysis of respondents' income per a family member showed that 42% of respondents had income of up to 10 thousand rubles per month, 33% – 10–15 thousand rubles, 25% – more than 15 thousand rubles. At the same time, 46.8% of consumers are ready to spend from 200 to 400 rubles a month for biologically active additives, 35.4% are ready to spend no more than 200 rubles. 17.8% of respondents are willing to spend more than 600 rubles to buy biologically active additives. The opinion poll showed that 67.7% of respondents purchase biologically active additives in pharmacy organizations. Respondents believe that biologically active additives should be taken both for the prevention of diseases (41.7% of consumers surveyed) and for prevention and treatment (39.6%).

As a result of the opinion poll, it became known that the majority of consumers of biologically active additives know about their effectiveness from their own experience (51%). Also, 85.4% of respondents answered that regular use of biologically active additives in combination with traditional methods of treatment contributes to a faster recovery and facilitates the patient's condition. Figure 1 shows the use of biologically

Table 3

DISTRIBUTION OF RESPONDENTS BY AGE AND SOCIAL STATUS

No.	Age group	Relative share, %	Social status	Relative share, %
1	Up to 20 years	5	Student	11
2	from 21 to 30 years	19	Householder	8
3	from 31 to 40 years	25	Worker	36
4	from 41 to 50 years	29	Office employee	21
5	Older than 50 years	22	Pensioner	24

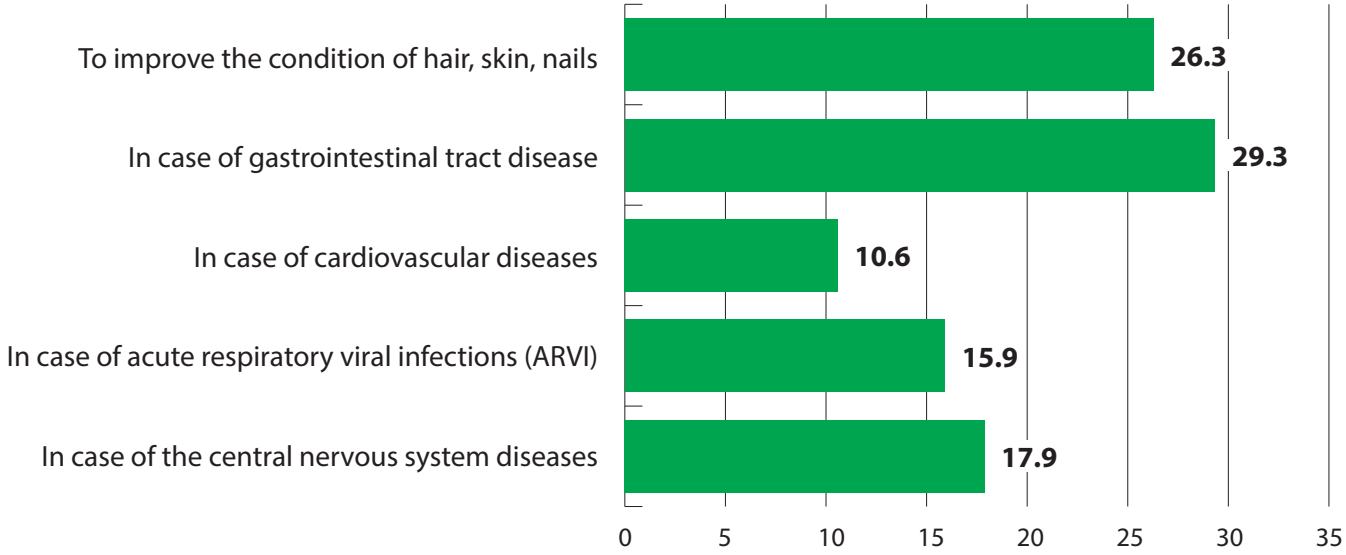


FIG. 1. *Use of biologically active additives in the personal experience of consumers, %*

active additives in the personal experience of respondents, namely: in diseases of the gastrointestinal tract (GI), in diseases of the central nervous system (CNS), in acute respiratory viral infections (ARVI) and others.

The opinion poll showed that 31.2% of consumers prefer the Evalar brand, 28.2% prefer imported brands and 17.7% – biologically active additives under the private labels. The majority of respondents (36%) learned about them from relatives and friends, 28% – from pharmacists and chemists in the pharmacy. The majority of respondents – 45.8% – said that they had not experienced any side effects after taking biologically active additives, and 40.6% could not associate them with taking biologically active additives.

The correlation analysis of the factors influencing the choice and purchase of biologically active additives revealed that a significant relationship (C=0.68) is observed between such factors as the average monthly income per a family member and the cost of biologically active additives. In addition, it was found that consumers knew about the effectiveness of biologically active additives in the prevention and treatment of diseases from their own experience (C=0.67 and 0.65, respectively). Regular use of biologically active additives purchased in a pharmacy organization contributes to a faster recovery and facilitates the patient's condition, especially in the complex treatment of gastrointestinal diseases with medications and biologically active additives, as well as improves the condition and beauty of hair, nails and skin (the values of the correlation coefficient vary from 0.51 to 0.62). In addition, the consumers who prefer biologically active additives of the Evalar brand learned about them from relatives and friends (C=0.51).

Based on the results obtained, we formed two profiles of consumers with an income of up to 10,000 rubles and more than 10,000 rubles per person, each of which has its own characteristics.

The first profile is of **a woman with income of up to 10,000 rubles per person.** She believes that biologically active additives should be used for prevention of diseases, and knows from her own experience that regular use of biologically active additives contributes to a faster recovery. When choosing biologically active additives, she is guided by the reviews of friends, relatives and pharmaceutical professionals, buying biologically active additives in pharmacy organizations. For the purchase of biologically active additives, she is ready to spend no more than 200 rubles a month. She uses biologically active additives of a private label and Evalar brand for prevention

of diseases of the central nervous system (Motherwort P, Eleutherococcus TM, Glycine VIS, Glycine MS, the cost of which is within 100 rubles.) in combination with other traditional methods of treatment. After taking biologically active additives, no side effects are noted

The second profile is of **a woman with income of over 10,000 rubles per person.** She believes that biologically active additives are necessary for both the treatment and prevention of diseases. In this case, biologically active additives can be used both separately and in combination with other methods of treatment. She knows from her own experience that regular use of biologically active additives contributes to faster recovery, in particular in the treatment and prevention of diseases of the gastrointestinal tract and acute respiratory viral infections. She receives the information about biologically active additives from doctors. When choosing biologically active additives, she focuses on the information received from the doctor, as well as on the reviews of friends, relatives and pharmaceutical professionals. She purchases biologically active additives in pharmacy organizations, as a rule, at a cost of 200 to 600 rubles. Side effects are encountered, but she may not always connect them with taking biologically active additives.

The results of the point assessment of the private label biologically active additives used by consumers, according to the effectiveness and severity of side effects in the treatment

Table 4

DISTRIBUTION OF THE PRIVATE LABEL BIOLOGICALLY ACTIVE ADDITIVES ACCORDING TO THE INTERVALS FOR EVALUATING THE EFFECTIVENESS AND SEVERITY OF SIDE EFFECTS

Intervals (in points)	Characteristics of efficiency of the private label BAAs	Trade names of the private label BAAs (average weighted score)
3.5–4.0	Very effective	Cimi-klimin (3,8), Hawthorn Premium (3,7), Hepalux (3,4), White sorbent (3,3), Altai Mumiyo (3,3), Aevit (3,5), vitamin and mineral complex (VMC) for children of 3–7 years (3,4), VMC for children of 7–14 years (3,8), Glycine VIS (3,3), Glycine MS (3,8), Solisept (3,4), Motherwort P (3,5), Eleutherococcus TM (3,3), Bronchial tea (3,3), Plantain and Farfara syrup (3,7)
2.5–3.0	Effective with rare side effects	Ovelux (2,7), Lactosorbicum (2,4), Frutolax (2,4), Livecil/ Milk Thistle (2,9), Benegast Redugas (2,6), Gastric tea (2,4), Milk Thistle extra (3,1), Cimi-klimin (3,8), Glucosamine-chondroitin complex (2,6), GCC Ultra (2,9), Superum (2,5), Tutti-frutti Omega-3 (2,6), Unic Omega (2,3), Bilberry forte with lutein (2,9), B vitamins (2,6), Beauty complex (3,2), Brewer's yeast TM (2,4), VMC for men (2,9), Motherwort premium (3,2), Lozenges TM Sage and eucalyptus (3,2), Lozenges TM Alpine honey (3,2), Lozenges TM Sage and honey (3,2), Echinacea extra (2,6), Real Ginseng extract (2.5), Echinacea syrup with vitamins (2,9)
1.5–2.0	Effective with frequent side effects	Senna-D (1,9), Artichoke premium (2,2), Phytocomplex Lux (1,5), Ginkobil (2,1)
Less than 1.5	Low-effective	–

and prevention of various diseases are presented in Table 4.

In Table 4 it can be seen that 14 private label biologically active additives, such as Glycine MS, VMC for children from 7 to 14 years, Hawthorn premium, Hepalux, Plantain Syrup with Farfara, Aevit, Motherwort P and others are very effective. The weighted average score varies from 3.3 to 3.8 points. 25 private label biologically active additives are assigned by consumers to effective additives with rare side effects. And for four private label biologically active additives (Artichoke premium, Ginkobil, Senna-D and Phytocomplex Lax), the majority of respondents (more than 50% of respondents who took these private label biologically active additives) indicated side effects along with the effectiveness.

CONCLUSIONS

1. Consumer behavior when choosing and buying biologically active additives in pharmacy organizations of the Republic of Bashkortostan was studied. Based on the results of the opinion poll, two consumer profiles were formed depending on income, reflecting the amount of expenses for the purchase of biologically active additives, the use in personal experience, preferred brands, as well as other factors.

2. Consumer opinion on the effectiveness and severity of side effects of the biologically active additives of the Vita pharmacy chain private label among consumers was studied. It was revealed that 14 private label biologically active additives of this pharmacy chain were noted by consumers as very effective (the average rating ranges from 3.3 to 3.8 points). 25 private label biologically active additives were noted by consumers as effective with rare side effects, but four biologically active additives (Senna-D, Artichoke premium, Phytocomplex Lux, Ginkobil) of the Vita pharmacy chain private label were characterized by often side effects according to consumers.

REFERENCES

1. *T.V. Barkova, S.N. Ivakina. Comparative analysis of the Russian market of biologically active additives // Bulletin of the Bashkir State Medical University (online edition), No.4, 2016, pp. 469–473.*
2. *S.N. Ivakina, T.V. Barkova, G.M. Nagimova, G.F. Lozovaya. Revealing the significance of medicine costs in the structure of consumption expenditures // Medical Bulletin of Bashkortostan. Vol. 11, No. 5 (65), 2016 – pp. 39–43.*
3. *S.N. Ivakina, L.A. Zotova. Cost-frequency analysis of the assortment of antihistamines in pharmacy organizations // Issues of ensuring the quality of medicines, No. 2 (12), 2016, pp. 14–19.*
4. *S.N. Ivakina, G.M. Nagimova, B.A. Bakirov. Assessment of the current state of the Russian pharmaceutical market in the context of the socio-economic crisis // Medical Bulletin of Bashkortostan, No. 4, 2016, pp. 1–8.*
5. *F.K. Usmanova, M.S. Ashin. Questioning as an element of students' design and research activities. Fundamental Research, 2014, No.12, pp. 1762–1765.*
6. *S.N. Ivakina, G.M. Nagimova, B.A. Bakirov, Zh.V. Mironenkova. Consumer evaluation of iron-containing medicinal products in the Republic of Bashkortostan // Issues of ensuring the quality of medicines, No.3 (21), 2018, pp. 16–23.*
7. *N.B. Dremova. Marketing in the pharmacy: step by step: practical guidance. – M.: MCFR, 2008. – 198 p.*
8. *A. V Kirillov. Statistics. Part 1. General theory of statistics: Educational Guidance – Samara: Publishing House of Samara State Agriculture University, 2012. – 112 p.*
9. *Pharmaceutical market of Russia 2012/electronic resource. http://www.dsm.ru/docs/analytics/godovoy_othet_cd_2012.pdf.*

UDC 615.072
https://www.doi.org/310.34907/JPQAI.2020.63.56.003

QUANTITATIVE SPECTROPHOTOMETRIC ANALYSIS OF THE MEDICINAL PRODUCT "METROKETOCONAZOLE"

A.I. Zamaraeva, *Postgraduate Student of the Chair of Chemistry, Tyumen State Medical University, Tyumen, Russia, anyuta.zamaraeva@yandex.ru*
N.S. Bessonova, *Candidate of Biological Sciences, Assistant Professor of the Chair of Chemistry, Tyumen State Medical University, Tyumen, Russia, bessonov21@mail.ru*
T.A. Kobeleva, *Doctor of Pharmacy, Professor, Head of the Chair of Chemistry, Tyumen State Medical University, Tyumen, Russia, kobeleva57@yandex.ru, kobeleva@tyumsmu.ru*
A.I. Sichko, *Doctor of Pharmacy, Professor of the Chair of Chemistry, Tyumen State Medical University, Tyumen, Russia, sichko@tyumsmu.ru*

The results of experiments on the analysis of metronidazole and ketoconazole in a new medicinal product "Metroketoconazole" made on a titanium-containing basis ("Tizol" gel) are presented. Ultraviolet absorption spectra of ethanol solutions of ointment ingredients are presented. It is established that for quantitative determination it is necessary to use the wavelengths of 241 nm and 312 nm. Optimal conditions were selected and a method was developed for analyzing compounds in a model mixture with a relative error of ±1.47–1.73%. A procedure for quantitative spectrophotometric determination of metronidazole and ketoconazole in the studied soft dosage form with an error not exceeding the limits of permissible levels and deviations is proposed.

Keywords: ketoconazole, metronidazole, Tizol gel, quantitative analysis, spectrophotometry

Currently, the need for compounded ointments is increasing due to the growth of the nomenclature of finished pharmaceutical products and the development of factory pharmacy [1, 8]. In addition, new low-toxic ointment bases with pharmacological properties have appeared in practice. Such bases include the titanium-containing Tizol gel. We have proposed a soft dosage form consisting of 0.1 g of metronidazole, 0.1 g of ketoconazole and 9.8 g of Tizol gel, conventionally called Metroketoconazole. This medicine can be used as a promising chemotherapeutic and antifungal agent [6,7]. Due to the presence of the Tizol gel, the ointment will also have anti-inflammatory, local analgesic, antiseptic and antipruritic actions [3,5]. The gel, which has excellent intratissual conductivity, will bring metronidazole and ketoconazole to the affected area. Therefore, the Metroketoconazole ointment is of interest for physicians and patients. The introduction of new medicines into medical practice must be accompanied by the development of methods of analysis that allow us to determine the quality of their manufacture.

The purpose of the study is development of a method for the quantitative determination of metronidazole and ketoconazole in a soft dosage form on a titanium-containing base.

MATERIALS AND METHODS

Metronidazole substances (China, FS-000349, 2012), ketoconazole substances (India, FS-000507,

2013) were used in the work, the quality of which correspond to the regulatory documentation. As an ointment base, Tizol gel, produced by Olymp LLC (FSP 42-3157-06, Yekaterinburg), was used. The object of the study is Metroketoconazole in soft dosage form, containing 1.0% of metronidazole and ketoconazole in the Tizol gel. The experimental work was carried out by the method of spectrophotometry, which is a highly-demanded method in pharmaceutical analysis [2, 4], using a Russian-made spectrophotometer SF-2000 (OKB Spektr LLC, St. Petersburg) in quartz cells.

RESULTS AND DISCUSSION

As shown by experimental data, ethanol solutions of metronidazole and ketoconazole obey the basic absorption law, and their ultraviolet spectra (Fig. 1) are overlapped (λ=215–320 nm). It is difficult to quantify each medication, so we used the well-known method of K. Firordt, applied for the analysis of two-component mixtures.

According to this method, a system of equations is derived for the thickness of the working layer of 1 cm. The optical density in a mixture of the two compounds under study is written by the following equations:

$$A(\lambda_1) = \varepsilon_1(\lambda_1) \cdot C_1 + \varepsilon_2(\lambda_1) \cdot C_2,$$

$$A(\lambda_2) = \varepsilon_1(\lambda_2) \cdot C_1 + \varepsilon_2(\lambda_2) \cdot C_2,$$

where C_1 and C_2 – component concentrations, mol/l; $\varepsilon_1(\lambda_1)$, $\varepsilon_1(\lambda_2)$, $\varepsilon_2(\lambda_1)$, $\varepsilon_2(\lambda_2)$ – molar absorption coefficients at the wavelengths λ_1 and λ_2.

From the system of equations, the concentration of each component in the mixture was found according to the following formulas:

$$C_1 = \frac{\varepsilon_2(\lambda_2) \cdot A(\lambda_1) - \varepsilon_2(\lambda_1) \cdot A(\lambda_2)}{\varepsilon_1(\lambda_1) \cdot \varepsilon_2(\lambda_2) - \varepsilon_1(\lambda_2) \cdot \varepsilon_2(\lambda_1)},$$

$$C_2 = \frac{\varepsilon_1(\lambda_1) \cdot A(\lambda_2) - \varepsilon_1(\lambda_2) \cdot A(\lambda_1)}{\varepsilon_1(\lambda_1) \cdot \varepsilon_2(\lambda_2) - \varepsilon_1(\lambda_2) \cdot \varepsilon_2(\lambda_1)},$$

When developing a method for quantitative spectrophotometric analysis of metronidazole and ketoconazole in the ointment, the optimal wavelengths were selected and the molar absorption coefficients were calculated. To do this, a curve was constructed for the dependence of ε (ket) – ε (met) on the wavelength (Fig. 2). The extreme point on the curve is observed at a wavelength of 243 nm and located near the maximum absorption of ketoconazole (λ=241 nm) in an ethanol solution. In addition, the curve has a pronounced minimum at a wavelength of 312 nm, which is similar to the second maximum absorption of metronidazole. The results obtained give grounds to take the values of 241 nm and 312 nm as the optimal wavelengths.

To confirm the above data, a curve was constructed for the dependence of ε (ket)/ε (met) on the wavelength at which there is a maximum at λ=243 nm (Fig. 3). Therefore, λ=241 nm and λ=312 nm corresponding to the maxima in the absorption spectra of medicinal product were taken as the analytical wavelengths for

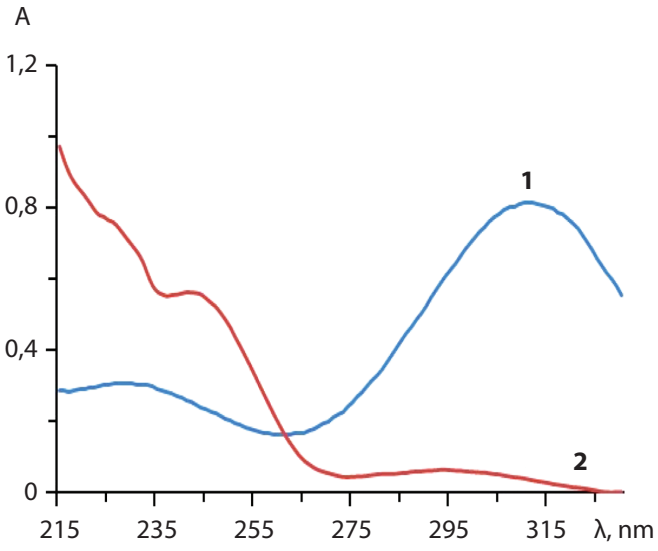


FIG. 1. *Absorption curves of metronidazole (1 – C=1,0 · 10⁻⁴ mol/L) and ketoconazole (2 – C=3,0 · 10⁻⁴ mol/L) in ethanol as a function of wavelength*

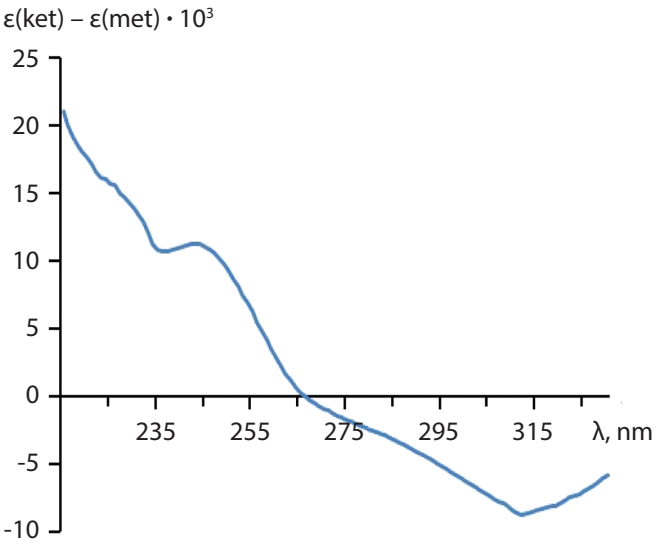


FIG. 2. *The curve of the dependence of ε(ket) – ε(met) on λ, nm*

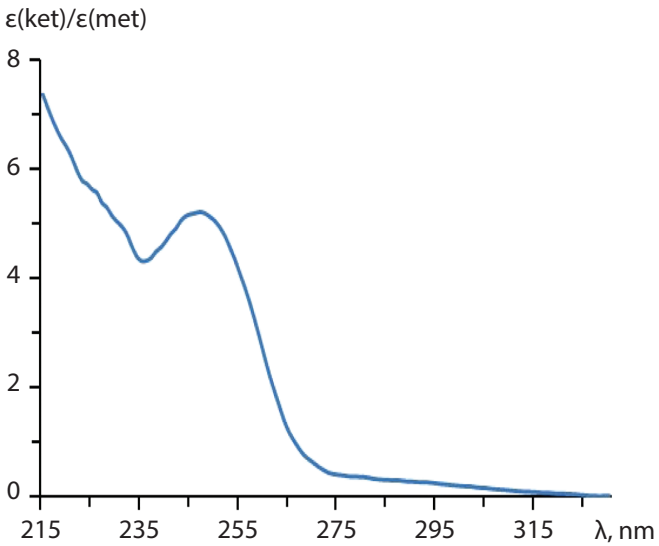


FIG. 3. *The curve of the dependence of ε(ket)/ε(met) on λ, nm*

the spectrophotometric analysis of metronidazole and ketoconazole in the mixture.

The quantitative determination of the ingredients of the soft dosage form by the spectrophotometric method was carried out at the selected wavelengths. For this purpose, the molar concentrations of ketoconazole were specified as C_1, the molar absorption coefficients – as $\varepsilon_1(241)$, $\varepsilon_1(312)$, and these parameters of metronidazole were specified as C_2, $\varepsilon_2(241)$, $\varepsilon_2(312)$. The system of K. Firordt's equations and the calculation of concentrations were expressed in the following form:

$$A(241) = \varepsilon_1(241) \cdot C_1 + \varepsilon_2(241) \cdot C_2,$$

$$A(312) = \varepsilon_1(312) \cdot C1 + \varepsilon_2(312) \cdot C_2,$$

$$C = \frac{\varepsilon_2(312) \cdot A(241) - \varepsilon_2(241) \cdot A(312)}{\varepsilon_1(241) \cdot \varepsilon_2(312) - \varepsilon_1(312) \cdot \varepsilon_2(241)},$$

$$C = \frac{\varepsilon_1(241) \cdot A(312) - \varepsilon_1(312) \cdot A(241)}{\varepsilon_1(241) \cdot \varepsilon_2(312) - \varepsilon_1(312) \cdot \varepsilon_2(241)}.$$

The calculated absorption coefficients of medicines at analytical wavelengths are shown in Table 1.

To develop a method for the spectrophotometric analysis of the components in the ointment, a model mixture containing 1.0% metronidazole and ketoconazole in ethyl alcohol was prepared. The analysis was carried out in the following way: 1 ml of the model mixture was introduced into a 50 ml volumetric flask and the volume of liquid in the flask was brought with ethanol up to the mark. Further, ethanol was added to 2 ml of the resulting mixture to obtain total volume of 25 ml and optical densities were measured at wavelengths of 241 nm and 312 nm using a spectrophotometer in a cell with a working layer thickness of 1 cm. The reference solution was ethanol.

Table 1

THE RESULTS OF CALCULATION OF MOLAR ABSORPTION COEFFICIENTS OF MEDICINES

Medical products	C, mol/L	A(241nm)	ε(241nm)	A(312nm)	ε(312nm)
Ketoconazole	$4.0 \cdot 10^{-5}$	0.563	14075	0.033	825
Metronidazole	$1.0 \cdot 10^{-4}$	0.259	2590	0.813	8130

Using the above formulas and the obtained values of optical densities, the concentration of ketoconazole (C_1) and metronidazole (C_2) was calculated and the mass fraction and mass of medicines in the model mixture were found:

$$m(med.) = \frac{C(med.) \cdot M(med.) \cdot V(total) \cdot V_2 \cdot V_3}{V \cdot V_1 \cdot 1000},$$

где W(med.) – mass fraction of a medicine, %; m(med.) – mass of a medicine, g; C(med.) – concentration of a medicine, mol/L; M(med.) – molar mass of metronidazole (171,16 g/mol) and ketoconazole (521,43 g/mol); V(total) – volume of a volumetric flask, 50 ml; V – volume of the mixture taken for analysis, 1 mL; V_1, V_2-dilution factor, 2 mL и 25 mL correspondingly; V_3 – total volume of the mixture, 10 mL; a(med.) – sample weight of medicinal product, g.

For the reliability of the experimental data, eight parallel determinations were performed, and the results were statistically processed (Table 2). The results of the studies showed that the relative error of the analysis of ketoconazole and metronidazole by the proposed spectrophotometric method is ±1.47% and 1.73%, respectively.

As experimental data have shown, ethanol solutions of ketoconazole practically do not absorb light at a wavelength of 312 nm at concentrations less than $4.0 \cdot 10^{-5}$ mol/L. This makes it possible to quantify metronidazole in the presence of ketoconazole, and the analysis of the two-component mixture is simplified. Therefore, the system of equations proposed above was expressed for $\varepsilon_1(312) = 0$ in the following form:

$$A(241) = \varepsilon_1(241) \cdot C_1 + \varepsilon_2(241) \cdot C_2,$$

$$A(312) = \varepsilon_2(312) \cdot C_2.$$

Molar concentrations of medical products were calculated using the following formulas:

$$C_1 = \frac{A(241) - \varepsilon_2(241) \cdot C_2}{\varepsilon_1(241)},$$

$$C_2 = \frac{A(312)}{\varepsilon_2(312)}.$$

Table 2

RESULTS OF STATISTICAL PROCESSING OF MEDICINE ANALYSIS DATA IN THE MODEL MIXTURE

No.	Obtained values				Metrological characteristics	
	Ketoconazole		Metronidazole			
	C, mol/L	W, %	C, mol/L	W, %		
1	$3.02 \cdot 10^{-5}$	98.42	$9.53 \cdot 10^{-5}$	101.95	Ketoconazole $\bar{\bar{w}} = 100.54\%$ $S = 1.766$ $S_{\bar{\bar{w}}} = 0.624$ $\varepsilon_\alpha = 1.48$ $A = \pm 1.47\%$ $\Delta = \bar{\bar{w}} \pm \varepsilon\alpha =$ $= 100.54 \pm 1.48\%$	Metronidazole $\bar{\bar{w}} = 100.26\%$ $S = 2.065$ $S_{\bar{\bar{w}}} = 0.730$ $\varepsilon_\alpha = 1.73$ $A = \pm 1.73\%$ $\Delta = \bar{\bar{w}} \pm \varepsilon\alpha =$ $= 100.26 \pm 1.73\%$
2	$3.13 \cdot 10^{-5}$	102.00	$9.19 \cdot 10^{-5}$	98.33		
3	$3.11 \cdot 10^{-5}$	101.35	$9.57 \cdot 10^{-5}$	102.41		
4	$3.02 \cdot 10^{-5}$	98.42	$9.19 \cdot 10^{-5}$	99.33		
5	$3.12 \cdot 10^{-5}$	101.68	$9.53 \cdot 10^{-5}$	101.95		
6	$3.13 \cdot 10^{-5}$	102.00	$9.19 \cdot 10^{-5}$	98.33		
7	$3.02 \cdot 10^{-5}$	98.42	$9.19 \cdot 10^{-5}$	98.33		
8	$3.13 \cdot 10^{-5}$	102.00	$9.57 \cdot 10^{-5}$	102.41		

Table 3

DATA FROM THE ANALYSIS OF MEDICINES IN THE MODEL MIXTURE

A(241)	A(312)	Concentration, mol/L		m_1(ket), r	m_2(met), r
		C_1(ket)	C_2(met)		
Firordt method					
0.68	0.80	$3.08 \cdot 10^{-5}$	$9.53 \cdot 10^{-5}$	0.1004	0.1019
0.70	0.80	$3.22 \cdot 10^{-5}$	$9.51 \cdot 10^{-5}$	0.1049	0.1017
0.70	0.85	$3.11 \cdot 10^{-5}$	$10.14 \cdot 10^{-5}$	0.1014	0.1085
0.69	0.80	$3.15 \cdot 10^{-5}$	$9.52 \cdot 10^{-5}$	0.1027	0.1018
0.70	0.84	$3.13 \cdot 10^{-5}$	$10.01 \cdot 10^{-5}$	0.1020	0.1071
Simplified Firordt method					
0.70	0.82	$3.12 \cdot 10^{-5}$	$10.09 \cdot 10^{-5}$	0.1017	0.1079
0.68	0.80	$3.02 \cdot 10^{-5}$	$9.84 \cdot 10^{-5}$	0.0984	0.1053
0.72	0.82	$3.26 \cdot 10^{-5}$	$10.09 \cdot 10^{-5}$	0.1062	0.1079
0.70	0.80	$3.16 \cdot 10^{-5}$	$9.84 \cdot 10^{-5}$	0.1030	0.1092
0.71	0.83	$3.17 \cdot 10^{-5}$	$10.21 \cdot 10^{-5}$	0.1033	0.1053

The masses of the study objects found in the model mixture are shown in Table 3. According to the results of parallel experiments, it was found that the content of ketoconazole (m_1), calculated by the Firordt method and a simplified system of equations, is in the range of 0.0984–0.1062 g, metronidazole (m_2) – 0.1017–0.1092 g, which corresponds to the permissible deviations in the mass of individual doses, presented in the Order of the Ministry of Health of the Russian Federation dated 26.10.2015 No. 751н "On approval of the rules for the manufacture and dispensing of medicines for medical use by pharmacy organizations, individual entrepreneurs licensed for pharmaceutical activities"

The method of analysis described above is proposed to be used for the quantitative determination of medicines in an ointment prepared on the basis of the Tizol gel. Procedure: in a glass chemical cup, place a sample of ointment of about 0.1 g (exact weight), add 25 ml of 95% ethanol solution, mix the mixture and filter it through a paper folded filter. After that, ethanol is added to 4 ml of the filtrate to obtain a total volume of 10 ml and the optical density of the solution is measured at wavelengths of 241 nm and 312 nm with respect to the reference solution (ethanol extract of the Tizol gel prepared in a similar way). According to the values of optical densities and molar absorption coefficients (Table. 1), calculate the molar concentrations of medicines using the above formulas. The content of metronidazole and ketoconazole in soft dosage form is calculated by the following formula:

$$m(med.) = \frac{C(med.) \cdot M(med.) \cdot V(total) \cdot V_2 \cdot P}{10^3 \cdot a(ointment) \cdot V_1},$$

where V(total) – the volume of ethanol in which the ointment weighed sample is dissolved, 25 ml; a (ointment) – the dosage form weighed sample, g; V_1, V_2 – the dilution factor, 4 ml and 10 ml, respectively; P – the mass of the dosage form, 10 g.

Error in the analysis of the studied medicines in the ointment (Table. 4) regardless of

Table 4

DATA FROM THE SPECTROPHOTOMETRIC ANALYSIS OF MEDICINES IN THE OINTMENT

Weighed sample		Obtained results				Permissible levels	
m(ointment), g	m(Tizol), g	C_1(ket), mol/L	C_2(met), mol/L	m_1(ket), g	m_2(met), g	g	%
Firordt method							
0.1039	0.1033	$3.11 \cdot 10^{-5}$	$10.14 \cdot 10^{-5}$	0.0975	0.1044	0.085–0.115	±15.0
0.1039	0.1033	$3.18 \cdot 10^{-5}$	$9.76 \cdot 10^{-5}$	0.997	0.1005		
0.1039	0.1033	$3.32 \cdot 10^{-5}$	$19.75 \cdot 10^{-5}$	0.1041	0.1004		
0.1039	0.1033	$3.23 \cdot 10^{-5}$	$9.88 \cdot 10^{-5}$	0.1013	0.1017		
Simplified Firordt method							
0.1042	0.1033	$3.12 \cdot 10^{-5}$	$10.09 \cdot 10^{-5}$	0.0976	0.1036	0.085–0.115	±15.0
0.1042	0.1033	$3.26 \cdot 10^{-5}$	$10.09 \cdot 10^{-5}$	0.1020	0.1036		
0.1042	0.1033	$3.09 \cdot 10^{-5}$	$10.21 \cdot 10^{-5}$	0.0966	0.1048		
0.1042	0.1033	$3.19 \cdot 10^{-5}$	$10.09 \cdot 10^{-5}$	0.0998	0.1036		

the method of calculation is within the permissible standard values in grams and deviations in percentages provided for in the regulatory documentation.

CONCLUSIONS

As a result of studying the optical properties of ethanol solutions of metronidazole and ketoconazole, it was found that their absorption spectra are overlapped.

The optimal conditions for the analysis were determined and the analytical wavelengths of 241 nm and 312 nm were selected for the quantitative spectrophotometric determination of medicines in the prescription using the K. Firordt method.

Studies on the analysis of the model mixture were carried out and a method was developed that allows quantifying the studied compounds with a relative error of ±1.47–1.73%.

A method of spectrophotometric analysis of metronidazole and ketoconazole in a soft dosage form on a titanium-containing basis with an error not exceeding the standard deviations is proposed

The developed method for the analysis of metronidazole and ketoconazole in a soft dosage form such as Metroketoconazole can be recommended for inclusion in the regulatory documentation for establishing the quality of ointment manufacturing.

REFERENCES

1. *G.V. Ayupova. Modern aspects of the development and improvement of the composition and technology of dosage forms // Medical Bulletin of Bashkortostan. 2016. – No. 11 (5). – pp. 87–90.*
2. *V.G. Belikov. Analysis of medicinal substances by photometric methods. Experience of work*

of domestic specialists // Russian Chemical Journal. 2002. – No. 46 (4). – pp. 52–56.

3. *L.V. Dyachkova. Study of the structural and mechanical properties of ointment bases // Bulletin of pharmacy. 2012. – No.3. – pp. 23–28.*
4. *T.G. Evstafieva, N.S. Bessonova, T.A. Kobeleva, A.I. Sichko. The use of spectrophotometry in the analysis of the new drug "Metatetrazol" // Health and education in the XXI century. 2018. – No. 20 (12). – pp. 55–59.*
5. *T.G. Evstafieva, N.N. Bacheva, N.S. Bessonova, T.A. Kobeleva, A.I. Sichko. Application of spectrophotometric analysis for establishing osmotic and transcutaneous activity of new dosage forms "Metamiosol" and "Phenylbutazol" // Medical science and education of the Ural. 2018. – No. 19 (3). – pp. 56–62.*
6. *M.V. Makhotina, A.Yu. Petrov. Comparative analysis of processes of compounded preparation of Tizol- based ointments // Fundamental science and technologies – perspective developments: materials of the XIV international scientific and practical conference. – North Charleston, USA, 2018. – 2. – pp. 96–98.*
7. *N.M. Nasybullina. Creation of dosage forms of anti-inflammatory and antimycotic agents based on liposomes for external use // Health as the basis of human potential: problems and ways to solve them. 2010. – No.5 (1). – p. 370.*
8. *R.R. Sabirzhan, S.N. Egorova. Pharmacy's manufacturing of dosage forms for medical and preventive institutions: the study of modern nomenclature // Scientific Bulletin of the Belgorod State University. 2012. – No. 18/2 (10). – pp. 31–35.*

UDC 615.074
https://www.doi.org/410.34907/JPQAI.2020.86.90.004

DEVELOPMENT AND VALIDATION OF THE METHODOLOGY FOR DETERMINATION OF SIBUTRAMINE IN MEDICINES

A.M. Sukhanova, *Assistant Professor of the Department of Pharmaceutical and Toxicological Chemistry named after A.P. Arzamastsev of the Institute of Pharmacy named after A.P. Nelyubin, First Moscow State Medical University (Sechenov University); Research Assistant of the Laboratory of Metabolomic and Proteomic Analysis, Federal Research Center of Nutrition, Biotechnology and Food Safety, Moscow, Russia, annamsukhanova@gmail.com*
I.B. Perova, *Candidate of Pharmaceutical Sciences, Senior Scientist Researcher of the Laboratory of Metabolomic and Proteomic Analysis, Federal Research Center of Nutrition, Biotechnology and Food Safety, Moscow, Russia*
K.I. Eller, *Doctor of Chemistry, Head of the Laboratory of Metabolomic and Proteomic Analysis, Federal Research Center of Nutrition, Biotechnology and Food Safety, Moscow, Russia*
G.M. Rodionova, *Candidate of Pharmaceutical Sciences, Associate Professor the Department of Pharmaceutical and Toxicological Chemistry named after A.P. Arzamastsev of the Institute of Pharmacy named after A.P. Nelyubin, First Moscow State Medical University (Sechenov University), Moscow, Russia*

This study is devoted to the development and validation of a method for determination of sibutramine in medicines for their quality control in order to further develop regulatory documentation in the Russian Federation. The extraction solvent and chromatographic separation conditions were selected for the optimal determination of sibutramine in medicines by high performance liquid chromatography (HPLC) using an ultraviolet detector (UV). The specificity, linearity, correctness of the method have been proved, the relative error of a single determination does not exceed 1.5% (0.70%), the calculated value of the Student's test tcalc (2.14) is less than the tabular ttable (2.36), the coefficient of variation (RSD) does not exceed 1% (0.93%), the intermediate precision was assessed, the relative error of which is 0.16%. The detection limit was determined at the level of 0.004 mg/ml; the limit of quantitative determination is 0.1 mg/ml. The methodology is correct, there is no systematic error, therefore, it is suitable for further use and inclusion into the regulatory documents.

Keywords: sibutramine, medicines, HPLC-UV, validation, quality control, regulatory documentation

The development and validation of methods for determining active pharmaceutical ingredient (API) as a part of medicines is essential and necessary for the development and maintenance of the pharmaceutical quality system.

Currently, obesity is a fairly common disease among the population.

The number of obese patients in Russia in 2019 compared to 2018 increased by 15.8% (registered cases in 2018–446,663, in 2019–517,357), which indicates an increase in the incidence rate every year [1].

Sibutramine is a highly effective medicine for the treatment of obesity. In the Russian Federation, studies have been conducted to prove the safety and pharmacological effectiveness of this medicine. According to the results of the six-month program "Vesna" (Spring), 44% of patients got rid of the diagnosis "obesity", also a decrease in glucose, low-density lipoprotein, cholesterol and an increase in high-density lipoprotein levels were found and decrease in blood pressure was noted [2,3]. The PrimaVera study confirmed the positive effect of sibutramine on the dynamics of changes in the body weight of patients (51% of the participants got rid of the diagnosis of "obesity") and the absence of serious side effects and risks associated with taking the medicine [4].

Sibutramine is a norepinephrine and serotonin reuptake inhibitor. The pharmacological effect is due to the formation of metabolites (M1-desmethylsibutramine and M2-didesmethylsibutramine), the half – life of which is 14 h and 16 h, respectively, for sibutramine the half – life is 1.1 h [5,6].

According to IUPAC (International Union for Pure and Applied Chemistry), sibutramine hydrochloride monohydrate-cyclobutane methanamine, 1-(4-chlorophenyl)-N, N-dimethyl-α- (2-methylpropyl)-, hydrochloride, monohydrate, (±)–; (±)– 1-(p-chlorophenyl)-α-isobutyl-N, N-dimethylcyclobutanmethylamine hydrochloride monohydrate (Fig. 1) is a slightly water-soluble powder of white to cream color [7].

To this date, the pharmaceutical market of the Russian Federation presents the following medicines containing sibutramine: Reduxin (capsules: 10; 15 mg of sibutramine hydrochloride + + 158.5 mg; 153.5 mg of microcrystalline cellulose), Reduxin Met (capsules: 10; 15 mg of sibutramine hydrochloride + 158.5 mg; 153.5 mg of microcrystalline cellulose; separately attached tablets of metformin 850 mg), Reduxin Forte (tablets: 10; 15 mg of sibutramine hydrochloride + 850 mg

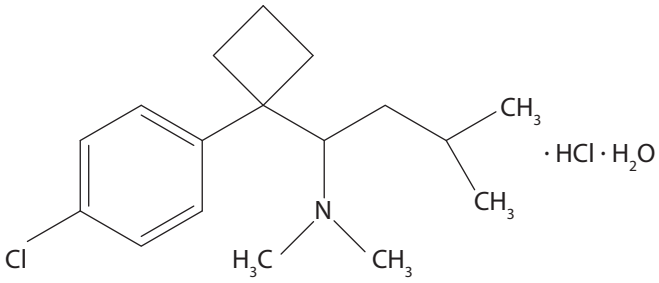


FIG. 1. *Structural formula of sibutramine*

metformin) (Promomed Rus LLC, Russia); Goldline (capsules: 10; 15 mg sibutramine hydrochloride), Goldline Plus (capsules: 10; 15 mg of sibutramine hydrochloride + 158.5 mg; 153.5 mg of microcrystalline cellulose) (Izvarino Pharma, LLC, Russia). These medicines are released strictly according to a doctor's prescription, since sibutramine hydrochloride is included in the List of potent and toxic substances and is subject to strict record keeping and storage [8].

Therefore, it is necessary to develop regulatory documentation for quality control of sibutramine hydrochloride API against the background of medicine components.

The purpose of this study is to develop and validate a method for determining sibutramine in medicinal products by HPLC-UV.

MATERIALS AND METHODS

The objects of the study were a standard sample of sibutramine hydrochloride (Tocris, UK), microcrystalline cellulose (Sigma Aldrich, USA), metformin hydrochloride (Supelco, USA).

RESULTS AND DISCUSSION

The optimal solvent was selected experimentally [9, 10]. Methanol of various concentrations was used as an extraction solvent, and the dependence of the sibutramine peak area on the solvent concentration was studied, as a result of which we

selected anhydrous methanol (J.T. Baker, Poland) as the solvent.

The chromatographic determination of sibutramine was performed using a high-performance liquid chromatograph Agilent 1100 (Agilent Technologies, USA) with a UV detector. A chromatographic column made of stainless steel C18 NUCLEOSIL (Macherry-Nagel, Germany) with dimensions of 4.6 mm × 150 mm and sorbent particles of 5 μ was used as a stationary phase; the temperature of the column thermostat was 40°C; the flow rate is 1 ml/min.

Various mobile phases were tested to obtain the best separations in the shortest possible time in order to optimize the analytical procedure. Mobile phases of acetonitrile buffer (pH 3–7) were tested at different ratios of organic and aqueous components (20–80%). The results that meet the requirements of the regulatory documentation were achieved using the mobile phase components in mixture of 0.05 M formate buffer pH=4.0 (ammonium formate-Honeywell, Germany, formic acid-Sigma-Aldrich, USA) and acetonitrile (PanReac AppliChem, Germany) at the ratio of 40:60 (by volume).

The analytical wavelength was chosen based on the obtained spectrum of methanol (Fig. 2) – 225 nm.

The completeness of sibutramine extraction was determined from the dosage form on model mixtures of the sibutramine substance and microcrystalline cellulose at the ratio corresponding to medicine (10 mg (15 mg) + 158.5 mg).

For the preparation of 9 model mixtures, about 0.010 g of the sibutramine hydrochloride substance (exact sample weight) and 0.159 g of microcrystalline cellulose were placed in measuring flasks with a volume of 10 ml, then, 8 ml of methanol was added, put into an ultrasonic bath for 10 minutes, the volume was brought to the mark, mixed, filtered through a membrane filter with a size of 0.2 microns (nylon). Then 1 ml of the resulting solution was placed into a 10 ml flask, 10 ml of methanol was added, and mixed.

The solution of the Reference Standard (RS) of sibutramine hydrochloride was prepared in a similar way for the model mixtures (the concentration of sibutramine hydrochloride was 0.10 mg/ml).

The method of quantitative determination of sibutramine using the HPLC-UV method was performed according to GPM 1.1.0012.15 SP XIV "Validation of analytical methods".

The specificity was determined based on the resulted chromatograms of the solvent, Reference Standard, placebo (microcrystalline cellulose, metformin), and model mixtures (Fig. 3–7).

The retention time of sibutramine is 5 minutes. Excipients do not interfere with the determination of API, therefore, the method meets the value of the specificity of the analytical method.

The linearity was determined on 9 model mixtures (the content of sibutramine hydrochloride from 80 to 120%) (Fig. 8).

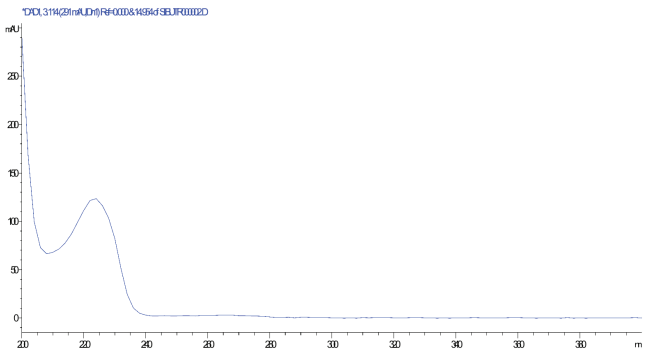


FIG. 2. *Sibutramine spectrum*

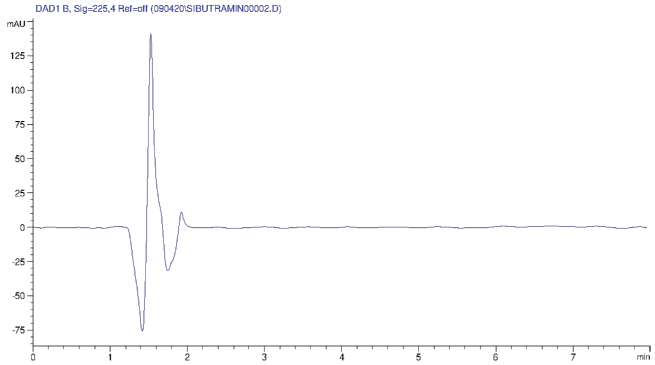


FIG. 3. *Solvent chromatogram*

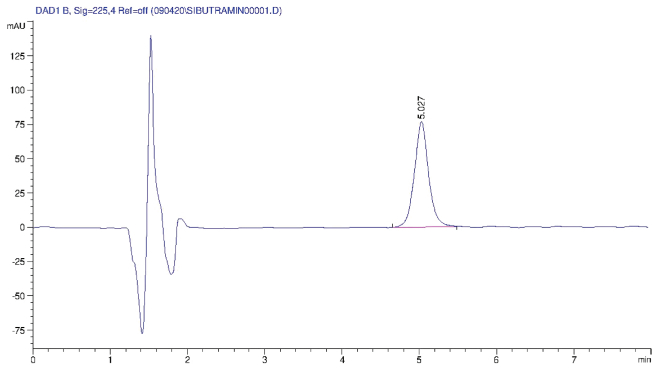


FIG. 4. *Chromatogram of the Reference Standard of sibutramine hydrochloride*

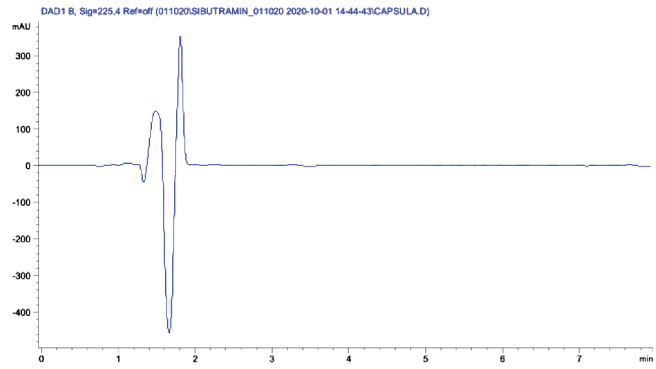


FIG. 5. *Chromatogram of microcrystalline cellulose*

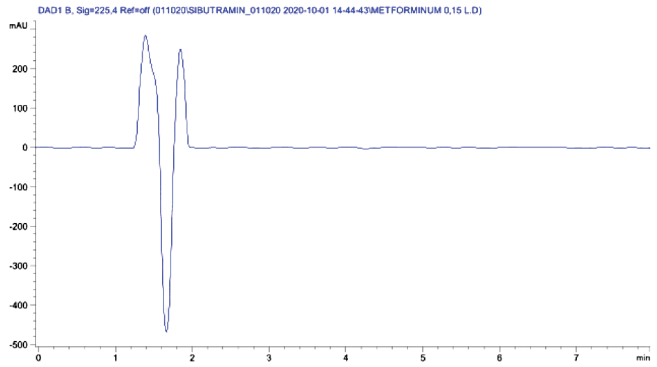


FIG. 6. *Chromatogram of metformin hydrochloride*

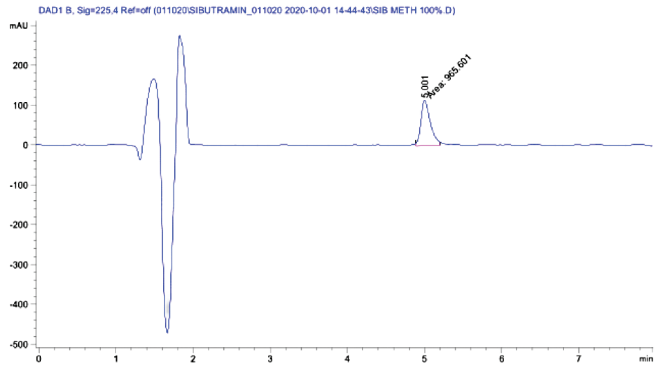


FIG. 7. *Chromatogram of the model mixture*

The value of the correlation coefficient R2 is 0.9992 (more than 0.9950) at y = 9672.9 x – – 18.066 (x – concentration (mg/ml), y – peak area).

The correctness of the method was proved by determining the degree of extraction of sibutramine introduced into the model mixtures.

Table 1 shows that the extraction of sibutramine from the model mixtures under experimental conditions is complete, the relative error of a single determination does not exceed 1.5% (0.70%). The confidence interval $\bar{X} \pm \Delta\bar{X}$ (100.22% ± 0.70%) includes 100%, the calculated value of the Student's test t_{calc}(2.14) is less than the tabular t_{tabl}(2.36), therefore, the method is correct and there is no systematic error.

To assess the *precision (convergence)* of the results, a coefficient of variation (RSD) not exceeding 1% (0.93%) was calculated.

In the study of two solutions prepared similarly to the model mixtures (the mass of microcrystalline cellulose is 0.159 g) by different analytical chemists, the *intermediate precision* was determined. The first chemist had the sample weight of the Reference Standard (RS) of sibutramine equal to 10.0 mg, the second chemist had 10.1 mg. Each

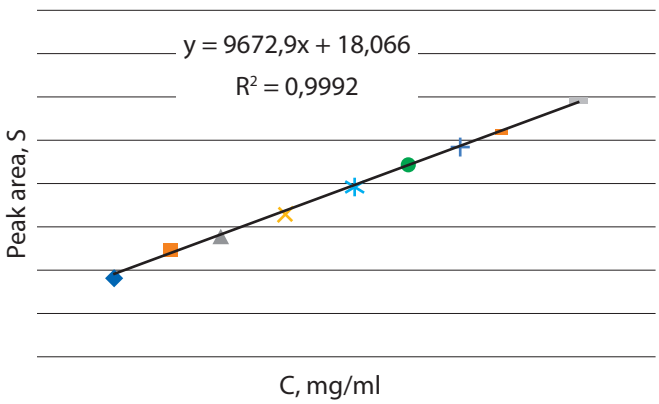


FIG. 8. *Graph of the linear dependence of the peak area (S) on the concentration (C) of the sibutramine solution*

Table 1

RESULTS OF QUANTITATIVE DETERMINATION OF SIBUTRAMINE IN MODEL MIXTURES

No.	Taken, mg/ml (C_1)	Determined, mg/ml (C_2)	Absolute error, mg/ml ($d = C_2 - C_1$)	Relative error, % ($Y = d \times 100/C_1$)	Determined, %	Metrological characteristics (P = 95%, n = 9)
1	0.0800	0.0796	-0.0004	-0.50	99.50	n = 9 $\bar{X} = 100.22$ S = 0.93 $S\bar{x} = 0.31$ $\Delta\bar{X} = 0.70$ $\bar{\varepsilon} = 0.70\%$ $t_{calc} = 2.14$ $t_{tabl} = 2.36$ RSD = 0.93%
2	0.0847	0.0851	0.0004	0.47	100.47	
3	0.0900	0.0893	-0.0007	-0.78	99.22	
4	0.0949	0.0947	-0.0002	-0.21	99.79	
5	0.0999	0.1010	0.0011	1.10	101.10	
6	0.1052	0.1061	0.0009	0.86	100.86	
7	0.1101	0.1105	0.0004	0.36	100.36	
8	0.1151	0.1139	-0.0012	-1.04	98.96	
9	0.1200	0.1221	0.0021	1.75	101.75	

solution was chromatographed 5 times in three repetitions.

The average sibutramine content for 10 measurements is 100.14%, the standard deviation is 0.16%, and the relative standard deviation of RSD is 0.16%. The results obtained meet the acceptance criteria for the *intermediate precision* value (the RSD value should be no more than 3%) for 10 parallel measurements.

The limit of detection of sibutramine using this validated technique is 0.004 mg /ml, the limit of quantification is 0.01 mg/ml.

CONCLUSION

The developed method meets the requirements of GPM 1.1.0012.15 SP XIV "Validation of analytical methods" according to the following parameters: specificity, linearity, correctness, convergence and intermediate precision.

The method is suitable for the determination of sibutramine in medicinal products and can be included in the regulatory documentation for dosage forms (capsules, tablets) containing sibutramine, for quality control of medicines on the pharmaceutical market.

REFERENCES

1. *Healthcare in Russia 2019. Statistical book. Federal State Statistics Service (Rosstat). – Moscow, 2019.*
2. *Yershova E.V., Koshmilova K.A., Galieva M.O. Sibutramin: myths and reality // Obesity and metabolism. – 2014. – No.4. – Pp. 12–17.*
3. *Astashkin E.I., Glezer M.G. Obesity and arterial hypertension // Women's health issues. – 2008. – No. 3 (4). – Pp. 23–33.*
4. *Melnichenko G.A., Romantsova T.I., Zhuravleva M.V. All-Russian program of safe weight loss "PrimaVera" // Results of the first year of the program. – 2014. – No. 1. – Pp. 62–68.*
5. *Register of Medicines of Russia. URL: https://www.rlsnet.ru/ (access date: 06.08.2020).*

6. *Lean M.E. Sibutramine – a review of clinical efficacy // Int.J. Obes. Relat. Metab. Disord. 1997. – Vol. 30. – №6. – P. 37–39.*
7. *The United States Pharmacopeia 36. The National Formulary 31. V. 1, 2. 2013.*
8. *Decree of the Government of the Russian Federation No. 964 of December 29, 2007 "On Approval of Lists of Potent and Toxic Substances for the Purposes of Article 234 and Other Articles of the Criminal Code of the Russian Federation, as Well as Large-Scale Potent Substances for the Purposes of Article 234 of the Criminal Code of the Russian Federation" (as amended).*
9. *Zhong Y., Sun C., Xiong J., Shi Y. Simultaneous determination of eight adulterants in weight management supplements and herbs by HPLC-DAD and LC-MS/MS // Journal of Liquid Chromatography and Related Technologies. – 2017. – №12. – P. 640–648.*

UDC 615.074
https://www.doi.org/510.34907/JPQAI.2020.60.39.005

STUDY OF ECDISTEROIDS FROM SERRATULA CORONATA L. HERB

A.I. Radimich, *Senior Researcher of the Department of Phytochemistry and Standardization, All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), Moscow, Russia, vilarnii.radimich@mail.ru*
V.I. Sheychenko, *Candidate of Physical and Mathematical Sciences, All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), Moscow, Russia, vilarnii.sheychenko@mail.ru*
O.Yu. Kulyak, *Candidate of Pharmaceutical Sciences, Research Scientist at the Laboratory of Atomic-molecular Bioregulation and Selection, All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), Assistant Professor of the Department of Pharmaceutical Chemistry, Pharmacognosy and Organization of Pharmaceutical Business, Lomonosov Moscow State University, Moscow, Russia, Kulyak-Olesya@mail.ru*
O.L. Saibel, *Candidate of Pharmaceutical Sciences, Head of the Center for Chemistry and Pharmaceutical Technology, All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), Moscow, Russia, olster@mail.ru*
I.N. Korotkich, *Master of Agriculture, Senior Research Scientist of the Department of Agrobiology and Breeding All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), Moscow, Russia, slavnica241270@yandex.ru*

Serratula coronata is a plant-producer of ecdysteroids, which is of interest for the introduction into pharmaceutical practice and the development of adaptogenes. As a result of chromatographic separation, five main ecdysteroids were isolated from the aboveground part of Serratula coronata L., introduced in the Botanical Garden of VILAR and identified on the basis of NMR spectroscopy data. The presence of four previously identified compounds in Serratula coronata L. was confirmed: 20-hydroxyecdysone (1), ayugasterone C (2), α-ecdysone (3) and taxisterone (4). For the first time, a new compound – 20-hydroxyecdysone 20,22-propylidene (5) was isolated.

Keywords: Serratula coronate L., phytoecdysteroids, 20-hydroxyecdysone, Ajugasterone C, α-ecdysone, taxisterone, 20-hydroxyecdysone 20,22-propylidene, NMR spectroscopy

Ecdysteroids are triterpene compounds consisted of four condensed rings having 27 or 28–29 carbon atoms. Ecdysteroids are widespread in the flora and fauna and have a wide spectrum of pharmacological activity. The source for their synthesis is cholesterol or other plant sterols [1]. More than 510 ecdysteroids are known, most of which are phytoecdysteroids [2] isolated from plants. Ecdysteroids have a wide range of pharmacological activity, exhibit an anabolic effect [3], which is used not only in the pharmaceutical industry, but also in the agricultural industry [4], they reduce cholesterol, relieve the symptoms of osteoporosis, improve skin regeneration, exhibit hepatoprotective, anti-inflammatory, adaptogenic effects [5–9]. The most common phytoecdysone is 20-hydroxyecdysone (ecdysterone, β-ecdysone) [10,11], which is a white crystalline powder that is poorly soluble in water [12].

To this date, two ecdysteroid-containing medicines are produced in Russia: Ecdisten® (containing 20-hydroxyecdysone [13], made of Rhaponticum carthamoides) and a liquid extract of Leuzea carthamoides roots [13]. Low toxicity and high biological activity are the main criteria of prospectivity for the use of medicines based on phytoecdysteroids. Thus, the LD50 for 20-hydroxyecdysone in case of abdominal and oral administration to mice is 6 g/kg [14] and 9 g/kg [15], respectively. The limiting factor in the production of these medicines is the low content of target substances in medicinal plant raw materials and a poor raw material base. As alternative objects containing ecdysteroids, a plant of the Asteraceae family, Serratula coronata L. (synonyms – S. wolfii Andrae, S. manshurica Kitag), is of particular interest [9]. Thus, in the herb of this plant, the total content of ecdysteroids can exceed 2%, which makes it promising for use as a source for obtaining these compounds [16].

Various researchers have found more than 50 phytoecdysteroids [17] and a wide variety of flavonoids (3-methylquercetin, 3-methylquercetin-3′-O-β-D-glucuronopyranoside, 3-methylquercetin-4′-O-β-D-glucuronopyranoside, 3-methylcampferol, apigenin, isocampferide, quercetin, quercetin-3′-O-β-D-glucuronopyranoside, quercetin-4′-β-D-glucoside, kaempferol, luteolin, luteolin-4′-β-D-glucoside, rutin), phenolcarboxylic acids (caffeic, ferulic, chlorogenic, neochlorogenic acids), higher fatty acids and their derivatives (linoleic, linolenic, palmitic and their methyl esters), sesquiterpenoids (caryophyllene, caryophyllene oxide, germacrene D), cyclitols ((-)-inositol) [18–23].

The effective doses of the main phytoecdysteroids isolated from Serratula coronata are presented in Table. 1. The content of the studied substances is subject to significant fluctuations in different parts of the plant, and also depends on the phase of its development [24,25]. In addition, scientists have shown that the Serratula coronata growing in different ecological and geographical conditions has a different qualitative and quantitative composition of ecdysteroids, which, in turn, indicates the presence of different chemotaxonomic races of this plant in nature [24].

In this regard, the **purpose** of this work was to isolate and identify the main (dominant) phytoecdysteroids of the Serratula coronata herb growing in the experimental field of the Botanical Garden of the All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), in order to detect differences in the chemical composition of this plant population in comparison with the information previously described in the literature.

MATERIALS AND METHODS

The object of the study was the dried aboveground part (herb) of the Serratula coronata (2018), introduced in the VILAR Botanical Garden

Table 1

EFFECTIVE DOSES OF THE MAIN PHYTOECDYSTEROIDS ISOLATED FROM SERRATULA CORONATA [26,27]

Ecdysteroids	ED50	Reference
20-hydroxyecdysone	$7{,}5 \cdot 10^{-9}$M	[27]
ecdysone	$1{,}1 \cdot 10^{-6}$M	[27]
polipodin B	$1{,}0 \cdot 10^{-9}$M	[27]
inocosteron	–	
Ajugasterone C	$6{,}2 \cdot 10^{-8}$M	[26]
22-O- acetyl 20-hydroxyecdysone	–	
taxisterone	$9{,}5 \cdot 10^{-8}$M	[26]
3-эпи-20-гидроксиэкдизон	$1{,}6 \cdot 10^{-7}$M	[26]

(Moscow). Raw materials were harvested in the 3–5-th year of the growing season in the phase of the beginning of flowering, the length of the shoots was 45–65 cm and the herb was dried in natural conditions at temperature of 28–34°C and at relative humidity of 52–65% for 10 days. The dried raw materials were ground to the size of particles passing through a 3 mm mesh screen (Kraft, Russia).

Extracts were prepared by three-time dynamic maceration of raw materials with 70% ethyl alcohol (vol.) at a temperature of (50±2) °C for 60 minutes. Raw materials/ extraction solvent ratio was 1:10 (by weight). The obtained water-alcohol extracts were combined and evaporated in the vacuum-rotary evaporator Heidolph Basis Hei-VAP ML (Germany) at a temperature of (50±2) °C until the distillation residue was obtained, which was quantitatively transferred to a separating funnel for sequential triple extraction with chloroform and n-butanol in a ratio of 1:1. The chloroform and n-butanol extracts were combined and evaporated in a rotary evaporator under vacuum at a temperature of 50±2°C until the solvents were completely removed. Thus, the target chloroform (non-polar) and n-butanol (polar) fractions were extracted.

To extract the individual compounds from the chloroform fraction, column chromatography was used, the column diameter was 1.0 cm and the height of the sorbent layer was 10 cm, on a silica gel made by Woelm (Germany) with a particle size of 80 microns. The "cyclohexane – isopropanol" system from 95:5 to 50:50 (vol.) was used as the mobile phase. The assessment of the phytoecdysteroid content in each fraction was analyzed by TLC on Sorbfil ПТСХ-АФ-УФ 20x20 plates in the "chloroform – methanol – water" system at the ratio of 26: 14: 3. The target fractions were combined and evaporated using a vacuum-rotary evaporator and re-chromatographed in the "chloroform-methanol" system from 98: 2 to 80:20 (vol.).

To remove the accompanying phenolic compounds, the n-butanol fraction was chromatographed on the Woelm (Germany) neutral aluminum oxide – II degree of activity (according to Brockman). The column diameter is 7 cm, the height of the sorbent layer is 30 cm and the eluent is "chloroform-methanol" with an increase in the gradient of the latter from 2 to 50%. Eluates from the column were analyzed by TLC on Sorbfil ПТСХ-АФ-УФ 20x20 plates in the systems "chloroform-methanol" at ratio of 90:10 and "chloroform-methanol-water" at ratio of 26:14: 3 (vol.). The plates were viewed in UV light at 254 nm and developed with a 25% solution of phosphoric-molybdenum acid. Fractions containing 20-hydroxyecdysone and other minor ecdysteroids were combined and evaporated using a rotary evaporator under vacuum to dry. The extracted fractions were re-chromatographed on silica gel made by Woelm (Germany) with a particle size of 80 microns. The column diameter is 1.5 cm, the height of the sorbent layer is 20 cm, the "chloroform – methanol" systems from 98:2 to 70:30 (vol.) were used as the mobile phase. The assessment of the phytoecdysteroid content in each fraction was analyzed by TLC on Sorbfil ПТСХ-АФ-УФ 20x20 plates in the "chloroform-methanol-water" system at the ratio of 26: 14: 3 (vol.). The target fractions containing individual compounds were combined and evaporated using a vacuum-rotary evaporator.

To determine the chemical structure of the isolated substances, ^{1}H – and ^{13}C-NMR spectra were taken in CD_3OD and DMSO with the Gemini 200 NMR spectrometer (Varian, USA).

RESULTS AND DISCUSSION

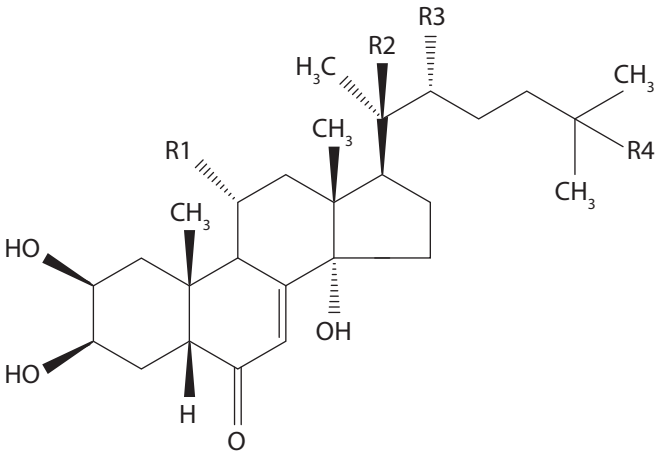
As a result of the study, five phytoecdysteroids were isolated from the Serratula coronata herb – four from the polar (n-butanol) fraction and one from the non-polar (chloroform) fraction. Using NMR spectroscopy (Table. 2) it was found that

Table 2

DATA OF ^{13}C NMR SPECTRA OF COMPOUNDS 1–4 (125 MHZ)

Number of a carbon atom	1		2		3		4	
	CD_3OD	DMSO-D^6	CD_3OD	DMSO-D^6	CD_3OD	DMSO-D^6	CD_3OD	DMSO-D^6
1	37.41	36.61	37.39	36.57	37.35	36.61	40.19	40.76
2	68.73	66.76	68.71	66.70	68.69	66.74	69.22	66.87
3	68.54	66.58	68.51	66.54	68.49	66.56	68.85	66.65
4	32.84	31.51	32.86	31.48	32.84	31.51	33.57	31.97
5	51.80	50.07	51.78	50.02	53.35	51.44	53.06	51.04
6	206.44	202.66	206.51	202.64	206.41	202.57	206.93	203.07
7	122.15	120.44	122.01	120.31	121.99	120.33	123.01	120.82
8	167.96	165.21	167.59	164.79	168.06	165.32	165.99	163.30
9	35.13	33.19	35.30	33.26	35.07	33.11	43.23	41.21
10	39.28	37.61	39.25	37.57	39.23	37.61	39.38	38.15
11	21.52	20.07	21.60	20.08	21.58	20.10	69.79	67.34
12	32.53	30.84	32.06	30.27	32.38	30.61	44.08	42.45
13	48.58	46.85	48.12	46.41	48.11	46.29	*	46.74
14	85.25	82.99	85.09	82.66	85.49	83.27	85.16	82.81
15	31.79	30.29	32.06	30.53	31.59	30.11	32.13	30.29
16	21.52	20.25	27.00	25.63	21.98	20.63	21.81	20.79
17	50.51	48.69	48.82	47.03	51.77	50.06	50.45	48.38
18	18.05	17.10	16.19	15.22	18.12	17.14	19.16	17.97
19	24.42	23.83	24.45	23.81	24.47	23.83	24.90	24.05
20	77.92	75.71	43.43	41.74	75.95	73.17	78.05	75.38
21	21.07	20.94	13.32	12.84	26.47	26.45	21.26	20.21
22	78.44	76.22	75.26	72.33	45.87	44.91	78.24	75.49
23	27.36	26.09	25.38	24.07	20.08	18.72	30.77	29.09
24	42.41	41.36	42.25	41.19	45.47	44.49	37.94	36.10
25	71.29	68.71	71.40	68.74	71.38	68.78	29.50	27.44
26	28.98	28.98	29.15	29.05	29.35	29.22	23.69	22.94
27	29.69	29.91	29.57	29.87	29.11	29.56	23.03	22.21

** Signal under solvent*



	R1	R2	R3	R4
1	H	OH	OH	OH
2	H	H	OH	OH
3	H	OH	H	OH
4	OH	OH	OH	H

FIG. 1. *Structural formulas of isolated phytoecdysteroids (1-4)*

the isolated phytoecdysteroids are 20-hydroxyecdysone (1), Ajugasterone C (2), α-ecdysone (3) and taxisterone (4) (Fig. 1). The obtained NMR spectra of the isolated substances correspond to the available literature data [28,29]. In Tables 2 and 3, along with the values of chemical shifts in methanol, the values of chemical shifts in dimethyl sulfoxide (DMSO) are given. In DMSO, the relaxation time of carbon atoms is significantly lower than in other solvents, due to its higher viscosity, which significantly reduces the time to provide ^{13}C NMR spectra. To a greater extent, this applies to quaternary carbon and carbon of the carbonyl group, the relaxation time of which can exceed 10 seconds.

A new, previously unidentified ecdysteroid (5) $C_{31}H_{50}O_7$ with molar mass of 534.74 Da was isolated from the low-polar (chloroform) fraction of the Serratula coronata herb.

In the ^{13}C NMR spectra (CD_3OD, DMSO-D^6) of substance 5, 31 signals appear. The chemical shifts of 25 of them almost completely coincide with the similar signals in the spectra of 20-hydroxyecdysone (Table. 2 and 3). The results indicate that substance 5 is a derivative of 20-hydroxyecdysone. The two signals in substance 5 and 20-hydroxyecdysone, the chemical shift of which is significantly different, apply to the carbons at position 20 and 22. A signal the chemical shift of which in substance 5 has a value of 109.92 ppm (CD_3OD), according to the literature data [20,31], is manifested in the spectrum of 20-hydroxyecdysone acetonides. The calculated 13C-NMR spectrum for the but-2-dioxyylidene fragment $CH_3C(O)_2CH_2CH_3$ gives the following values: 23,3 (1), 110,5 (2), 32,2 (3) and 8.3 (4) ppm, which are close to the values of carbon atoms in substance 5. It should be noted that in the ^1H-NMR spectrum of substance 5, the fourth methyl group of the aliphatic chain provides a signal in the form of a triplet at 0.86 ppm (DMSO-D^6), which also confirms the presence of a butylidene fragment in the test substance.

When analyzing the resulting data, it can be assumed that the isolated new phytoecdysteroid corresponds to 20,22-(but-2-ylidene) 20-hydroxyecdysone (Fig. 2).

20-hydroxyecdysone (1) is dominant in terms of the content of the isolated ecdysteroids that is 78%, other ecdysteroids are, respectively:

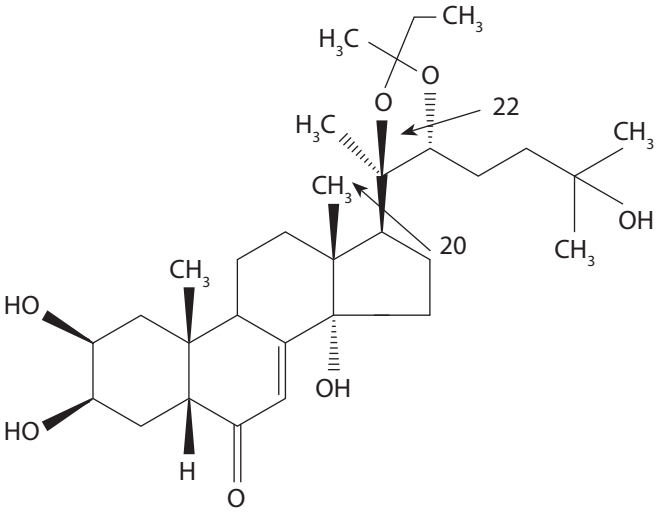


FIG. 2. *Structural formula of 20,22-(but-2-ylidene) 20-hydroxyecdysone*

Table 3

DATA OF ^{13}C NMR –SPECTRA OF COMPOUND 5 (125 MHZ)

Number of a carbon atom	5	
	CD_3OD	DMSO-D^6
1	37.43	36.57
2	68.73	66.72
3	68.53	66.54
4	32.86	31.48
5	51.78	50.02
6	206.39	202.57
7	122.12	120.50
8	167.61	164.57
9	35.19	33.15
10	39.23	37.53
11	21.54	20.01
12	32.39	30.58
13	*	46.67
14	85.50	83.61
15	31.75	30.15
16	22.47	20.92
17	50.69	48.80
18	17.66	16.54
19	24.43	23.80
20	85.32	82.95
21	22.91	21.98
22	83.03	81.14
23	24.74	23.14
24	42.23	40.99
25	71.11	68.40
26	29.07	28.91
27	29.44	29.64
28 O-**C**-O	109.92	107.78
29 OC**CH_3**	24.11	23.63
30 C**CH_2**CH_3	36.12	34.50
31 CCH_2**CH_3**	9.42	8.98

** Signal under solvent*

Ajugasterone C (2) – 11%, α-ecdysone (3) – 6%, taxisterone (4) – 1.2% of the total amount of isolated compounds by weight.

CONCLUSIONS

The results of the phytochemical study of the Serratula coronata herb growing on the territory of the Botanical Garden of the All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR) confirm the available literature data on the qualitative composition of ecdysteroids and the dominant share of 20-hydroxyecdysone among these compounds.

Along with this, the chemical structure of a new phytoecdysone of the Serratula coronata herb – 20-hydroxyecdysone 20,22-propylidene – was first isolated and identified.

The study was carried out within the framework of the implementation of the VILAR research plan on the subject No. 0576-2019-0010 "Search for active fractions of natural compounds, development of methods for their production from plant raw materials, standardization methods and creation of modern dosage forms based on them"

REFERENCES

1. *Tarkowská D., Strnad M. Plant ecdysteroids: plant sterols with intriguing distributions, biological effects and relations to plant hormones // Planta. – 2016. – 244 (3). – Pp. 545–555. doi:10.1007/s00425-016-2561-z*
2. *The Ecdysone Handbook [Electronic resource]. URL: http://ecdybase.org*
3. *Bathori M., Toth N., Hunyadi A., Marki A., Zador E. Phytoecdysteroids and anabolicandrogenic steroids: structure and effects on humans // Curr.Med. Chem. – 2008. – 15. – Pp. 75–91.*

4. *Khaziev D., Galina C., Gadiev R., Valitov F., Gumarova G., Galyautdinov I. Phytoecdisteroids from Serratula coronata when growing ducklings // Res.Vet. Sci. – 2020. – 128. – Pp. 170–176. doi: 10.1016/j.rvsc.2019.11.012*
5. *Catalan K.D., Martinez A.M., Aragones M.D., Miguel B.G., Robles A., Godoy J.E. Alterations in rat lipid metabolism following ecdysterone treatment // Comp. Biochem. Physiol. (B). – 1985. – 81. – Pp. 771–775.*
6. *Gao L., Cai G., Shi X. b-Ecdysterone induces osteogenic differentiation in mouse mesenchymal stem cells and relieves osteoporosis // Biol. Pharm. Bull. – 2008. – 31. – Pp. 2245–2249.*
7. *Tang Y.H., Yue Z.S., Li G.S. et al. Effect of β-ecdysterone on glucocorticoid-induced apoptosis and autophagy in osteoblasts // Mol. Med. Rep. – 2018. – 17 (1). – Pp. 158–164. doi: 10.3892/mmr.2017.7840*
8. *Zhang X., Xu X., Xu T., Qin S. β-Ecdysterone suppresses interleukin-1β-induced apoptosis and inflammation in rat chondrocytes via inhibition of NF-κB signaling pathway // Drug Dev.Res. – 2014. – 75 (3). – Pp. 195–201. doi: 10.1002/ddr.21170*
9. *Punegov V.V., Sychov R.L., Zainullin V.G., Fedorov V.N., Punegova N.V. Extraction of ecdysteron-80 from Serratula coronata L. and assessment of its pharmacological action. Part I. Adaptogenic, gastroprotective, thermoprotective, and antihypoxic activity // Pharmaceutical Chemistry Journal. – 2008. – 42. – Pp. 446–451. doi: 10.1007/s11094-008-0159-x.*
10. *Dinan L. The Karlson Lecture. Phytoecdysteroids: What use are they? // Arch. Insect. Biochem. Physiol. – 2008. – 72. – Pp. 126–141. doi: 10.1002/arch.20334*
11. *Bathori M., Pongracz Z. Phytoecdysteroids – from isolation to their effects on humans // Current Medicinal Chemistry. – 2005. – 12. – Pp. 153–172.*
12. *Cahlíková L., Macáková K., Chlebek J., Host'álková A., Kulhánková A., Opletal L. Ecdysterone and its activity on some degenerative diseases // Nat. Prod. Commun. – 2011. – 6 (5). – Pp. 707–718.*
13. *Register of Medicines (electronic resource) – https://grls.rosminzdrav.ru/*
14. *Dinan L., Lafont R. Effects and applications of arthropod steroid hormones (ecdysteroids) in mammals // J. Endocrinol. – 2006. – 191 (1). – Pp. 1–8. doi: 10.1677/joe.1.06900*
15. *Báthori M., Tóth N., Hunyadi A., Márki A., Zádor E. Phytoecdysteroids and anabolic-androgenic steroids-structure and effects on humans // Curr.Med. Chem. – 2008. – 15 (1). – Pp. 75–91. doi: 10.2174/092986708783330674*
16. *Volodin V.V. Phytoecdysteroids // Edited by V.V. Volodin. – St. Petersburg: Nauka. – 2003. – 293 p.*
17. *Olennikov D.N., Kashchenko N.I. Phytoecdy steroids of the Serratula L. and Klasea Cas. (Asteraceae) genera: chemo diversity, methods of isolation and analysis // Chemistry of plant raw materials. – 2017. – No.4. – Pp. 123–135.*
18. *Báthori M., Zupkó I., Hunyadi A., Gácsné-Baitz E., Dinya Z., Forgó P. Monitoring the antioxidant activity of extracts originated from various Serratula species and isolation of flavonoids from Serratula coronata // Fitoterapia. – 2004. – 75 (2). – Pp. 162–167. doi: 10.1016/j.fitote.2003.12.009*
19. *Angaskieva A.S., Andreeva V.Yu., Kalinkina G.I., Salnikova E.N., Borodyshena E.A., Kharina T.G. Study of the chemical composition of Serratula coronata cultivated in Siberia. // Chemistry of plant raw materials. – 2003. – No. 4. – Pp. 47–50.*
20. *Flavonoids of common buckwheat (Fagopyrum sagittatum Gilib.) and Serratula coronata L. (methods of isolation, identification of substances, prospects of use) / Myagchilov A.V. // Abstract of the dissertation for the degree of Candidate of Biological Sciences. – Vladivostok. – 2015. – P. 21.*
21. *Myagchilov A.V., Sokolova L.I., Gorovoy P.G. New flavonoids of the Serratula coronata L. plant //*

Chemical and Pharmaceutical Journal. – 2017. – Vol. 51 (2). – Pp. 23–27.

22. *Myagchilov A.V., Sokolova L.I., Gorovoy P.G. 3-methylquercetin-4'-O-β-D-glucuronopyranoside – a new flavonoid isolated from Serratula coronata L. // Chemistry of plant raw materials. – 2016. – No. 4. – Pp. 77–81.*
23. *Angaskieva A.S. Pharmacognostic study of Serratula coronata cultivated in Siberia / Abstract of the dissertation for the degree of Candidate of Pharmaceutical Sciences. – Tomsk. – 2006. – P. 19.*
24. *Khanumidi E.I., Korotkikh I.N., Ilyin M.M., Meshkov A.I. Determination of ecdysterone (20E) content in the aboveground part of the Serratula coronata L.) // Issues of ensuring the quality of medicines. – 2017. – No.1 (15). – Pp. 11–16.*
25. *Báthori M., Kalász H., Csikkelné S.A., Máthé I. Serratula fajok 20-hidroxiekdizon és szervetlen elem tartalmának vizsgálata (Components of Serratula species; screening for ecdysteroid and inorganic constituents of some Serratula plants) // Acta. Pharm. Hung. – 1999. – 69 (2). – Pp. 72–76.*
26. *Galyautdinov I.V., Sadretdinova Z.R., Muslimov Z.S., Gareev V.F., Khalilov L.M., Odinokov V.N. New minor phytoecdysteroids from the juice of Serratula coronata L. (Asteraceae) // Journal of Medicinal Plants Studies. – 2016. – 4 (5). – Pp. 30–34.*
27. *Timofeev N.P. Achievements and problems in the field of studying, using and predicting the biological activity of ecdysteroids (review). // Butler's messages. – 2006. – No. 8 (2). – Pp. 7–34.*
28. *Odinokov V.N., Galyautdinov I.V., Nedopekin D.V., Khalilov L.M., Shashkov A.S., Kachala V.V., Dinan L., Lafont R. Phytoecdysteroids from the juice of Serratula coronata L. (Asteraceae) // Insect. Biochemistry and Molecular Biology. – 2002. – 32. – Pp. 161–165.*
29. *Odinokov V.N., Kumpun S., Galyautdinov I.V., Evrard-Todeschi N., Veskina N.A., Khalilov L.M. et al. Low polarity phytoecdysteroids from the juice of Serratula coronata L. (Asteraceae) // Collect. Czech. Chem. Commun. – 2005. – 70. – Pp. 2038–2052.*
30. *Olennikov D.N. Makisterone C-20,22-Acetonide from Rhaponticum uniflorum // Chem.Nat. Compd. – 2018. – 54. – Pp. 930–933. https://doi.org/10.1007/s10600-018-2515–8*
31. *Pis J., Budesinsky M., Vokac K., Laudova V., Harmatha J. Ecdysteroids from the roots of Leuzea carthamoides // Phytochemistry. – 1994. – 37 (3). – Pp. 707–711.*

UDC 547.398 + 547.583
https://www.doi.org/610.34907/JPQAI.2020.20.33.006

INFLUENCE OF A SERIES OF 1,4-DICARBONIC ACID DERIVATIVES ON THE BLOOD CLOTTING TIME

N.V. Kolotova, *Candidate of Chemical Sciences, Associate Professor of the Department of Analytical Chemistry, Perm State Pharmaceutical Academy, Perm, Russia, nina.kolotowa2015@yandex.ru*
A.V. Starkova, *Candidate of Medicine, Associate Professor of the Department of Physiology, Perm State Pharmaceutical Academy, Perm, Russia, allaperm@list.ru*

The effect of six monosubstituted amides and eight acylhydrazides of succinic, maleic, citraconic and phthalic acids on hemostasis on the APG4-02-P coagulometer was studied. The experiments were performed on citrated (3.8%) blood (9:1) of rabbits. To determine the activity, 50 µl of blood was placed in the cuvette and 50 µl of 0.2% solution of the test compound was added. In the control, instead of the substance, 50 µl of isotonic sodium chloride solution was added. As the reference preparation, 50 µl of heparin was used at a concentration of 1 U/ml of blood or 50 µl of the solution of the Ethamsylate preparation at concentration of 0.2%. The degree of influence of the compounds on hemostasis was determined by the change in the clotting time of citrated blood in the control and experiment. Among the fourteen studied derivatives of 1,4-dicarboxylic acids, ten compounds had an effect on hemostasis. Five compounds exhibited anticoagulant effects. Five compounds exhibited a hemostatic effect. The effect of the two compounds is similar to that of Ethamsylate.

Keywords: amides and hydrazides of 1,4-dicarboxylic acids, direct anticoagulant and emostatic activity

Medicines that affect hemostasis are used in various fields of medicine. Anticoagulants are widely used in surgical and therapeutic practice for prevention and treatment of diseases that cause thrombosis. However, the currently available direct anticoagulants have a number of disadvantages: reactions at the injection sites, the development of thrombocytopenia, the risk of hemorrhagic complications, increased thrombus formation after withdrawal of medication, and the high cost of medications [1,2].

Prevention and control of bleeding are critical for hematology, surgery, traumatology, oncology and obstetrics, since the use of hemostatic agents is necessary to stop serious bleeding. For this purpose, the medicines that have different mechanisms of influence on blood clotting are used. The hemostatics currently used have a number of disadvantages that limit their use [3–5].

All of this determines the relevance of the search and study of new compounds that affect hemostasis. There is evidence in the literature that amides and hydrazides of 1,4-dicarboxylic acids have hemostatic and anticoagulant activities [6–13].

The purpose of our study is to continue studying the effect on hemostasis of monosubstituted amides and hydrazides of succinic, maleic, citraconic and phthalic acids obtained by known methods [12–14], as well as to identify the biological action – structure of compounds relationship.

MATERIALS AND METHODS

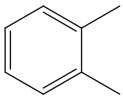
The objects of study of biological activity were monosubstituted amides and hydrazides

of succinic, maleic and citraconic acids. General formula of the studied compounds:

RCOX-YCOOH,

where

X-Y: -CH_2-CH_2-, -CH=CH-, -CH=C(CH_3)-,



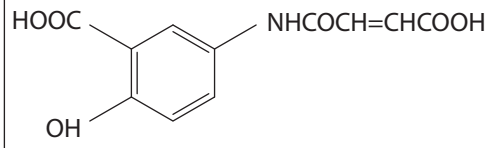
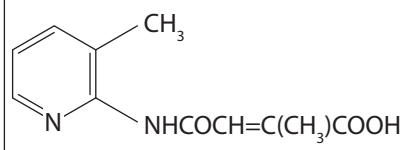
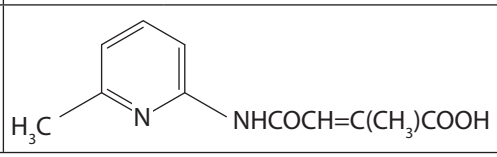
R: acyl-NH-, acyl-CONHNH- (**acyl:** aliphatic, aromatic, and heterocyclic substituents, see Table).

The effect of the compounds on hemostasis was studied using an APG4-02-P coagulometer. For the study, citrated (3.8%) blood (9:1) of rabbits was used. To determine the activity, 50 µl of blood was placed in the cuvette and 50 µl of a 0.2% solution of the test compound was added; for control, 50 µl of isotonic sodium chloride solution was added instead of the substance. As reference drugs, 50 µl of heparin was added at concentration of 1 U/ml of blood or 50 µl of solution of the Ethamsylate medicine at concentration of 0.2%. The samples were then incubated for 60 seconds, 50 µl of 1% calcium chloride solution was added, and blood clotting time was measured. Each compound was studied using the blood of 10 animals.

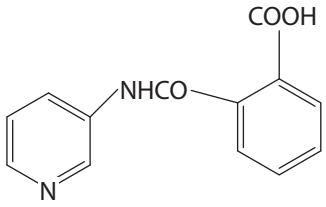
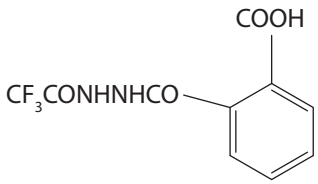
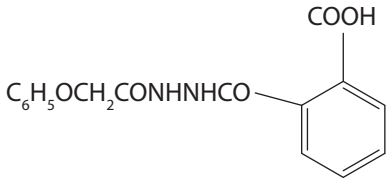
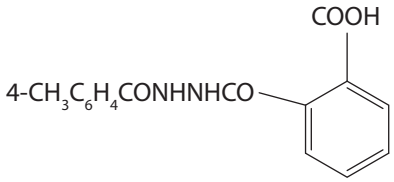
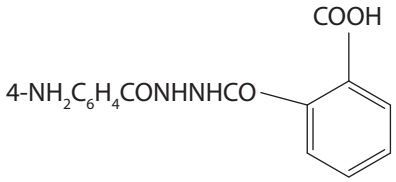
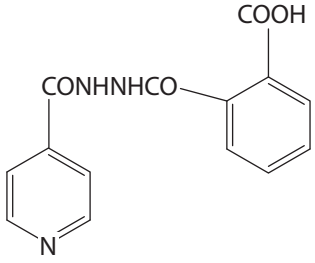
The results of the study of the effect of compounds on blood clotting were processed by the method of variation statistics according to the Fisher-Student method using the statistical

Table

EFFECT OF COMPOUNDS ON THE BLOOD CLOTTING TIME

No.	Compound formulas	Number of rabbits, control	Clotting time, sec control	Number of rabbits, experiment	Clotting time, sec experiment	% coagulability change	P
1.	4-$CH_3CONHC_6H_4NHCO\ CH_2$-H_2COOH	10	163.1±4.88	10	185.1±7.59	-13.3	<0.05
2.	$(C_6H_5)_2C$=NNHCOCH_2-CH_2COOH	10	127.9±4.93	10	103.2±5.84	**+19.3**	<0.02
3.	4-$CH_3C_6H_4CONHNHCO\ CH_2$-H_2COOH	10	116.3±4.80	10	118.2±4.07	-1.6	>0.05
4.	2-$CF_3C_6H_5NHCOCOOH$	10	95.3±7.14	10	101.1±4.64	-6.1	>0.05
5.		10	125.1±2.30	10	135.9±3.98	-8.6	<0.05
6.		10	121.5±4.07	10	91.9±2.98	**+24.4**	<0.02
7.		10	110.8±3.80	10	125.4±5.13	-13.2	<0.05

End of the table

No.	Compound formulas	Number of rabbits, control	Clotting time, sec control	Number of rabbits, experiment	Clotting time, sec experiment	% coagulability change	P
8.	2-OH-$C_6H_5CONHNHCOCH{=}C(CH_3)COOH$	10	105.1±4.70	10	138.7±3.97	-31.9	<0.001
9.			145.1±3.08	10	119.1±3.29	**+17.9**	<0.001
10.		10	98.1±7.39	10	100.5±6.77	-2.4	>0.05
11.		10	115.4±5.14	10	99.6±5.48	+13.7	>0.05
12.		10	141.6±5.09	10	120.2±4.23	**+15.1**	<0.01
13.		10	92.7±3.05	10	72.6±3.80	**+21.7**	<0.01
14.		10	122.1±4.46	10	138.1±3.94	-13.6	<0.05
References	Ethamsylates	10	144.1±7.83	10	121.0±7.20	+16.0	<0.05
	Heparin	10	145.7±9.64	10	618.3±55.88	-324.4	<0.001

processing program StatBase [15] and are shown in the table.

The assessment of biological activity in animal experiments was carried out in accordance with the requirements of the Pharmacological Committee set out in the Guidelines for Preclinical Studies of Medicines [16]. Animal management was in compliance with the rules of good laboratory practice in preclinical studies in the Russian Federation (GOST R 51000.3–96, General requirements for testing laboratories) and the Order of the Ministry of the Russian Federation No. 267 dated 19.06.2003 "On approval of the rules of good laboratory practice «(GLP), in compliance with international recommendations of the European Convention for protection of vertebrate animals used in experimental studies (1997).

RESULTS AND DISCUSSION

Among 14 compounds studied, five derivatives of 1,4-dicarboxylic acids have a hemostatic effect, two of them (compounds 6, 13) exceed the activity of the Ethamsylate medicine, two compounds (2 and 9) effect similarly to the reference drug. The activity of 3-Methyl-2-pyridylamide citraconic acid (compound 6) is 1.5 times higher than the activity of the Ethamsylate medicine, while the same derivatives of maleic and phthalic acids have an anticoagulant effect, and the derivative of succinic acid does not affect hemostasis [8,10]. Transfer of the methyl group from position 3 (compound 6) to position 6 (compound 7) leads to change from the hemostatic effect on blood clotting to anticoagulant one. 6-Methyl-2-pyridylamide of phthalic acid has an anticoagulant effect, and the same amide of succinic acid is inactive [8]. 4-Aminobenzoylhydrazide of phthalic acid (compound 13) is 1.35 times higher than the activity of Ethamsylate, the same derivative of maleic acid is less active [8], and potassium, sodium and ammonium salts of phthalic and succinic acids do not affect hemostasis [8,10]. Five compounds (1, 5, 7, 8, 14) showed an anticoagulant effect, significantly inferior to the action of heparin. 2-hydroxybenzoylhydrazide of citraconic acid (compound 8) has the greatest anticoagulant activity although previously only a hemostatic effect was found for citraconic acid derivatives [11]. Isonicotinoylhydrazide of phthalic acid (compound 14) showed a slight anticoagulant effect, the same maleic acid derivative is inactive, and isonicotinoylhydrazide of citraconic acid showed a hemostatic effect exceeding the activity of Ethamsylate [8,10]. Phenoxyacetylhydrazide of phthalic acid (compound 11) does not affect hemostasis, whereas methoxyacetylhydrazide of this acid has a hemostatic effect [10], that is, the replacement of the methoxyl group with the phenoxy group leads to a loss of activity.

CONCLUSIONS

1. Monosubstituted amides and hydrazides of 1,4-dicarboxylic acids are promising compounds that affect hemostasis, and are characterized by anticoagulant and hemostatic activity.

2. Among the substances studied, five compounds with hemostatic effect (two of them exceed the effect of Ethamsylate) and five compounds with direct anticoagulant activity were found.

REFERENCES

1. *Blommel M.L., Blommel A.L. Dabigatran etexilate: a novel oral direct thrombin inhibitor // Am.J. Health Syst. Pharm. – 2011. – Vol. 68 (16). – Pp. 1506–1519.*
2. *Mak K.-H. // European heart journal. – 2011. – Vol. 32. – Pp. 20–22.*
3. *V.V. Boyarintsev, V.B. Nazarov, E.V. Fronchek, A.S. Samoilov, A.B. Yudin, R.A. Kovalenko Preclinical evaluation of the effectiveness of local hemostatic medicines (experimental*

study). // Emergency medicine. – 2010. – No.3. – pp. 23–25.

4. *Pusateri A., McCarthy S., Gregory K. et al. Effect of a Chitosan-Based Hemostatic Dressing on Blood Loss and Survival in a Model of Severe Venous Hemorrhage and Hepatic Injury in Swine // Journal of Trauma-Injury Infection & Critical Care. – 2003. – Vol. 54. – Pp. 177–182.*
5. *Alam Hasan B., Chen Z., Miller D. // Ibid. – 2004. – Vol. 56 (5). – Pp. 974–983.*
6. *D´Angelo J. // Isr.J. Cyem. – 1997. – Vol. 37. – Pp. 81–85.*
7. *V.O. Kozminykh, B.Ya. Syropyatov. Substituted amides and hydrazides of maleic acid. Journal of Pharmacy and Chemistry. – 1993. – Vol. 27. – No.2. – Pp. 43–47.*
8. *B.Ya. Syropyatov, N.V. Kolotova, A.V. Dolzhenko, Effect of succinic and maleic acid derivatives on hemostasis // Questions of biological, medical and pharmaceutical chemistry. – 2012. – No.5. – Pp. 31–36.*
9. *N.V. Kolotova, A.V. Starkova, S.V. Chashchina. Synthesis and biological activity of monosubstituted hydrazides of itaconic and dimethylmaleic acids // Issues of ensuring the quality of medicines. – 2016. – No.3 (13). – Pp. 15–23.*
10. *B.Ya. Syropyatov, N.V. Kolotova. Influence of phthalic acid derivatives on hemostasis // Innovations in science, Proceedings of the XVI International Virtual Scientific and Practical Conference, January 28, 2013, Moscow. – 2013. – Pp. 139–147.*
11. *B.Ya. Syropyatov, N.V. Kolotova. Influence of citraconic acid derivatives on hemostasis // Questions of biological, medical, and pharmaceutical chemistry. – 2015. – No. 6. – Pp. 54–56.*
12. *N.V. Kolotova, A.V. Starkova. Biological activity of series of acylhydrazides of 1,4-dicarboxylic acids // Bulletin of Voronezh State University. – 2018. –No. 1. – Pp. 131–137.*
13. *A.V. Dolzhenko // Pharmaceutical Chemistry Journal. – 2002. – Vol. 36. – Pp. 174–176.*
14. *A.V. Dolzhenko // Pharmaceutical Chemistry Journal. – 2003. – Vol. 37. – Pp. 229–231.*
15. *V.V. Prozorovsky. Statistical processing of the results of pharmacological studies // Psychopharmacology and biological narcology. 2007. – Vol. 7. – Issue 3–4. – Pp. 2090–2120.*
16. *A.N. Mironov. Guidelines for preclinical studies of medicinal products. – Moscow: GrificK, 2012, Part 1. – 197 p.*

UDC 616-092
https://www.doi.org/710.34907/JPQAI.2020.49.88.007

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF HAIR-VEIN ARGIMONY (AGRIMONIA PILOSA) EXTRACT ON TOXIC HEPATITIS MODEL

E.N. Kurmanova, *Research Scientist of the Department of Experimental and Clinical Pharmacology, All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), Moscow, Russia, kurmanova1968@yandex.ru*
L.B. Strelkova, *Candidate of Medicine, All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), Moscow, Russia*
E.V. Ferubko, *Candidate of Medicine, Head of the Department of Experimental and Clinical Pharmacology, All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), Moscow, Russia,*
O.P. Sheichenko, *Candidate of Chemical Sciences, Leading Researcher of the Department of Phytochemistry and Standardization, All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), Moscow, Russia*

The effect of Hair-Vein Argimony herb dry extract on the state of the rat liver in experimental toxic hepatitis was studied. The pharmacological properties of the extract were studied during its intragastric administration to laboratory animals for 3 days. Experimental hepatitis in rats was caused by a single subcutaneous injection of 50% oil solution of carbon tetrachloride an hour after the last administration of the test substance and the Carsil reference medication. Microsomal fraction was isolated from the liver of animals and the content of microsomal protein, as well as the rate of monooxygenase reactions catalyzed by cytochrome P450 – p-hydroxylation of aniline, N-demethylation of dimethylaniline reflecting the activity of the detoxifying microsomal system of the liver were determined. Taking into account all the studied parameters, the hepatoprotective and detoxifying properties of the extract were evaluated in comparison with the Carsil standard medication. As a result of the study, it was found that the treatment of hepatitis with the extract of Hair-Vein Argimony at doses of 50 mg/kg and 100 mg/kg normalizes the content of microsomal protein, and cytochrome P450 content and anilination and demethylation rates in liver microsomes increase compared to control. Therefore, dry purified extract of Hair-Vein Argimony at the studied doses has a hepatoprotective effect against detoxifying enzyme systems of cytochrome P450 in the rat liver under the conditions of a model of carbon tetrachloride hepatitis.

Keywords: *Hair-Vein Argimony* herb dry extract, experimental toxic hepatitis, hepatoprotective activity, antitoxic effect

The significant prevalence of liver diseases, the special severity of their course, the hepatotoxic effect of a number of medicines, and the insufficient nomenclature of domestic hepatotropic agents used in medical practice, specify the significance of the search for new medicines that have hepatoprotective effect [1].

Currently, there is a search for sources of new effective biologically active substances from plant raw materials for creation of medicines based on them with a wide spectrum of action, low toxicity and the associated possibility of their long-term use [2].

A promising object for the development of new medicines for the prevention and treatment of liver lesions is the genus *Agrimonia* of the *Rosaceae* family. The representative of this genus is the *Hair-Vein Argimony* (Agrimonia pilosa Ledeb.). Extracts from the aboveground part of the plant have anti-inflammatory, antimicrobial, tonic, diuretic, hemostatic for uterine and pulmonary bleeding, astringent, anthelmintic, antitumor effects, regulate the function of the liver and gallbladder [3]. The chemical composition of the aboveground part of the *Hair-Vein Argimony* (herb) includes flavonoids (rutin, quercetin, kaempferol, hyperoside, luteolin, luteolin-7-glucoside), phenol-carboxylic acids (caffeic, chlorogenic, ellagic) [4].

Purpose of the study: to study the effect of *Hair-Vein Argimony* herb dry extract on the liver of rats in experimental toxic hepatitis to create a new herbal medicine.

MATERIALS AND METHODS

Hair-Vein Argimony herb dry extract was prepared in the All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR). The Department of Experimental and Clinical Pharmacology conducted its pharmacological study. The effect of the extract on the state of the rat liver under experimental toxic hepatitis was studied. Experimental toxic hepatitis caused by a single subcutaneous injection of carbon tetrachloride to rats was used [5]. The study was performed according to the Rules of laboratory practice in the Russian Federation (the Order of the Ministry of Health of Russia No. 199н dated 01.04.2016, the National Standard of the Russian Federation GOST 33044-2014 "Principles of good laboratory practice"), the Guidelines for Preclinical Studies of Medicines (2012) and in accordance with the Federal law No.61-FZ dated 12.04.2010 (as amended on 28.11.2018) "On circulation of medicines". The studies were approved by the Bioethical Commission (Protocol No. 4 of 24.09.2018). When conducting an experiment on a model of carbon tetrachloride hepatitis, white non-linear male rats with body weight of 200–250 g in the number of 50 individuals were used. Animal producer is Andreevka Branch of the BTSC (Biomedical Technology Science Center) of the FMBA (Federal Medical and Biological Agency) of Russia (Moscow region). The animals were kept in the vivarium of the VILAR on a standard diet.

The pharmacological properties of the *Hair-Vein Argimony* extract were studied during its intragastric administration to laboratory rats. The experimental animals were divided into 5 groups of 10 individuals: the first group – intact animals; the second group – control animals, in which an experimental toxic hepatitis was reproduced; the third, fourth and fifth groups – experimental animals, which additionally received the *Hair-Vein Argimony* extract at doses of 50 mg/kg, 100 mg/kg and the Carsil standard medication at a dose of 100 mg/kg, suspended in 1% starch suspension. The extract and Carsil were pre-administered once a day for 3 days to experimental groups of laboratory animals, at the same time the control rats received an equivalent volume of 1% starch suspension. Experimental hepatitis in animals was caused by a single subcutaneous injection of 50% carbon tetrachloride oil solution at a dose of 0.4 ml per 100 g of animal weight an hour after the last administration of the test substance and the reference medicine. After 48 hours, the rats were euthanized in a CO_2 chamber and the liver was removed for further examination.

Microsomal fraction was isolated from the liver of animals using the method of differential centrifugation [6]. In the microsomal fractions of the liver of experimental groups of animals, the content of microsomal protein was determined [7], as well as the rate of monooxygenase reactions catalyzed by cytochrome P_{450} – p-hydroxylation of aniline, N-demethylation of dimethylaniline (DMA), reflecting the activity of the detoxifying microsomal system of the liver. Taking into account

all the studied parameters, the hepatoprotective and detoxifying properties of the *Hair-Vein Argimony* extract were evaluated in comparison with the Carsil standard medication.

Statistical processing of the obtained data was performed using the Statistica 10.0 software package (USA). The sampling is symmetric. The significance of differences between samples with a distribution approaching the normal one was assessed using the Student's t-test. The differences were considered as significant at $p \leq 0,05$.

The work was carried out on the subject: "Preclinical studies of individual fractions, substances and medicinal products made of medicinal plant raw materials", topic code No. 0576-2019-0009.

RESULTS AND DISCUSSION

The results of the study of the hepatoprotective and detoxifying properties of the *Hair-Vein Argimony* extract in comparison with the Carsil standard medication in experiments on animals with reproduced CCl4 hepatitis are shown in Tables 1 and 2.

Table 1 contains the results on the effect of the *Hair-Vein Argimony* extract on the content of cytochrome P450 microsomal protein in the suspension of rat liver microsomes in toxic hepatitis.

Based on the results obtained, it was found that in experimental carbon tetrachloride acute hepatitis, the content of microsomal protein in the liver microsomes definitely increased by 28%, but the content of cytochrome P_{450} decreased by 53%. It is not impossible that the compensatory mechanisms of toxic damage to liver tissue influence on the protein content in microsomes. It is known that hepatitis negatively affects the liver cells due to the formation of lipid peroxidation products, which has an effect on the content of cytochrome P_{450} [1,2].

Table 1

EFFECT OF *HAIR-VEIN ARGIMONY* EXTRACT ON THE CONTENT OF MICROSOMAL PROTEIN IN TOXIC HEPATITIS

Groups of animals, n= 10	Content of microsomal protein, protein mg/liver g, M±m	Experiment/ control, %	Content of cytochrome P_{450}, cyt. P_{450} nM/protein mg, M±m	Experiment/ control, %
Intact	2.25±0.14	72	0.544±0.020	153
Control (experimental CCl_4 hepatitis)	3.11±0.11	100	0.355±0.018	100
Experimental 1 (*Hair-Vein Argimony* extract, 50 mg/kg)	2.10±0.13*	67	0.471±0.014*	133
Experimental 2 (*Hair-Vein Argimony* extract, 100 mg/kg)	2.08±0.09*	68	0.557±0.010	156
Experimental 3 (Carsil, 100 mg/kg)	1.88±0.07*	60	0.784±0.022*	220

*Note: here and hereinafter * – the differences between the data of the control and experimental groups at P≤0,05 are statistically significant*

The results of the study have revealed that the treatment of hepatitis with *Hair-Vein Argimony* extract at doses of 50 mg/kg and 100 mg/kg normalizes the content of microsomal protein, and the content of cytochrome P_{450} in liver microsomes increases by 33% and 56%, respectively, compared to control. When the Carsil standard medication is administered to animals, the content of microsomal protein is normalized, and the content of cytochrome P_{450} increases by 2.2 times.

Table 2 presents the results of the evaluation of the effect of the *Hair-Vein Argimony* extract and the Carsil standard medication on the hepatoprotective and detoxifying function of the liver microsomes. The activity of the detoxifying microsomal system of the liver was evaluated by the rate of enzymatic reactions catalyzed by cytochrome P_{450}, namely, by the reaction of demethylation with the type I substrate – dimethylaniline and by the reaction of p-hydroxylation with the type II substrate – aniline.

As we can see from the presented data, in the control, as a result of the toxic effect of carbon tetrachloride on the liver, the specific enzymatic aniline hydroxylase and demethylase activity of cytochrome P_{450} decreased.

The administration of the *Hair-Vein Argimony* extract to animals at a dose of 50 mg/ml and 100 mg/ml against the background of acute toxic hepatitis showed the hepatoprotective activity of the *Hair-Vein Argimony* herb extract against detoxifying enzyme systems of rat liver cytochrome P450. It was found that in the treatment of hepatitis with the *Hair-Vein Argimony* herb extract at a dose of 50 mg /kg and 100 mg/kg, the anilination

Table 2

EVALUATION OF THE EFFECT OF THE *HAIR-VEIN ARGIMONY* EXTRACT ON THE ACTIVITY OF CYTOCHROME P450 IN MICROSOMAL FRACTIONS OF THE LIVER IN EXPERIMENTAL HEPATITIS

Groups of animals, n=10	Activity of cytochrome $P_{450,}$ M±m			
	Aniline hydroxylation NADPH nM/cyt. P_{450} nM per minute	Experiment/ control, %	Demethylation of DMA, NADPH nM/cyt. P_{450} nM per minute	Experiment/ control, %
Intact	1.84±0.04	127	1.67±0.05	123
Control (experimental CCl_4 hepatitis)	1.45±0.03	100	1.35±0.04	100
Experimental 1 (*Hair-Vein Argimony* extract, 50 mg/kg)	1.68±0.04*	116	1.90±0.04*	141
Experimental 2 (*Hair-Vein Argimony* extract, 100 mg/kg)	1.75±0.05*	121	2.00±0.06*	148
Experimental 3 (Carsil, 100 mg/kg)	1.92±0.03*	132	2.33±0.06*	172

*Note: here and hereinafter * – the differences between the data of the control and experimental groups at P≤0,05 are statistically significant*

rate increases by 16% and 21%, respectively, and the demethylation rate increases by 41% and 48%, respectively. This indicates the enzymatic activity of the cytochrome P450 demethylation centers and the inducing effect of BAS contained in the *Hair-Vein Argimony* extract on the liver monooxygenase system. In this experiment, the Carsil standard medication (100 mg/kg) showed hydroxylase and demethylase activity exceeding the activity of the *Hair-Vein Argimony* extract.

Thus, the *Hair-Vein Argimony* dry extract at the studied doses has the less pronounced hepatoprotective effect against detoxifying enzyme systems of cytochrome P450 of the rat liver in the conditions of the experimental carbon tetrachloride hepatitis, than that of the Carsil standard medication.

CONCLUSIONS

It was experimentally concluded that the *Hair-Vein Argimony* herb dry extract in the conditions of simulation of carbon tetrachloride hepatitis in rats has hepatoprotective and detoxifying properties at doses of 50 and 100 mg/kg; at the same time, the dose-dependent effect of the extract was noted. The activating effect of the *Hair-Vein Argimony* dry extract at a dose of 100 mg/kg on the detoxifying microsomal systems enzymes of cytochrome P450 of the rat liver was established.

REFERENCES

1. *V.T. Ivashkin. Gastroenterology: national leadership / V.T. Ivashkin. – M.: GEOTAR-Media, 2008, – 704 p.*
2. *A.R. Reizis, S.N. Borzakova, V.A. Aksenova. Drug-induced liver injuries – an actual problem of modern medicine // Gastroenterology. 2010. – No.3. – pp. 49–54.*
3. *M.G. Khanina, M.A. Khanina. Pharmacognostic research of the hairy agrimony // Development, research and marketing of new pharmaceutical products / Ed. by E.N. Vergeychik, N.N. Kareva. – Pyatigorsk: Pyatigorsk SPhA, 2007, No. 62. – Pp. 119–122.*
4. *V.V. Dudko, N.Yu. Degil, E.V. Saprykina, E.A. Krasnov, V.Yu. Babikov. Hepatoprotective activity of the Hair-Vein Argimony herb extract // Pharmacy. 2009, No.3. – Pp. 41–43.*
5. *L.S. Polyanskikh, M.A. Petrosyan, N.V. Zhestkova, N.N. Balashova Experimental models of liver pathology // Experimental and clinical pharmacology. 2017, No. 2. – p. 41.*
6. *I.I. Karuzina, G.I. Bachmanova, D.E. Mengazetdinov, K.N. Myasoedova, V.O. Zhikhareva, G.I. Kuznetsova, A.I. Archakov. Isolation and properties of cytochrome P450 from rabbit liver microsomes // Biochemistry. 1979, No. 6. – pp. 1049–1057.*
7. *V.G. Avdeev. Methods for determining the protein concentration // Medical Chemistry Issues. 1977, No.4. – pp. 562–571.*

UDC 615.014
https://www.doi.org/810.34907/JPQAI.2020.53.96.008

CO-PROCESSED EXCIPIENTS, THEIR CHARACTERISTICS AND ACTUAL RANGE

K.V. Aleksyev, *Doctor of Pharmacy, Professor, Deputy Director for Innovation, V.V. Zakusov Research Institute of Pharmacology; Head of the Department of Pharmacy, Medical Institute REAVIZ, Moscow, Russia*
E.V. Blynskaya, *Candidate of Pharmaceutical Sciences, Head of the Laboratory of Ready-made Dosage Forms of the Experimental and Technological Department, V.V. Zakusov Research Institute of Pharmacology, Moscow, Russia*
D.V. Yudina, *Junior Research Scientist, V.V. Zakusov Research Institute of Pharmacology, Moscow, Russia, una_1093@mail.ru*
V.V. Bueva, *Junior Research Scientist, V.V. Zakusov Research Institute of Pharmacology, Moscow, Russia*
V.K. Aleksyev, *Junior Research Scientist, V.V. Zakusov Research Institute of Pharmacology, Moscow, Russia*
V.V. Adzhiyenko, *Postgraduate Student, V.V. Zakusov Research Institute of Pharmacology, Moscow, Russia*
S.V. Tishkov, *Junior Research Scientist, V.V. Zakusov Research Institute of Pharmacology, Moscow, Russia*
A.A. Ivanov, *Senior Mechanical Engineer, V.V. Zakusov Research Institute of Pharmacology, Moscow, Russia*
S.V. Minaev, *Candidate of Pharmaceutical Sciences, Head of the Experimental and Technological Department, V.V. Zakusov Research Institute of Pharmacology, Moscow, Russia*

The article covers a new generation of excipients such as multifunctional co-processed excipients, manufactured by coprocessing. The article shows the results of the search for co-processed excipients presented in the modern pharmaceutical market, as well as scientific studies reflecting the main aspects of their application.

Keywords: co-processed excipients, coprocessing, orally disintegrated tablets, chewing tablets, solid dosage forms

Current trends in the development of solid dosage forms (DF) are reduced to the choice of technologies that ensure the consistency of the quality of the medicine. Simplification of the production process eliminates the need to validate additional production stages, thus eliminating the risk of their impact on quality. Co-processed excipients are increasingly widely being used which are a mixture of two or more excipients of different functional classes, produced by co-processing, most often by spray drying technology, in which there is no change in the chemical structure of the initial components and the formation of new substances [1,2]. The development and implementation of co-processed excipients will simultaneously solve a number of problems related to both the medicine quality assurance and the logistics of the production process. The main prerequisites for the development of co-processed excipients were the increasing tendency for developers to choose the direct compression technology (DC) as the cheapest and easiest to perform, and the lack of a "universal" excipient, which would have all the necessary properties to produce the dosage form with the characteristics required by the direct compression technology. Co-processed excipients are a separate generation of excipients produced

using well-known and proven technologies already widely used in the production of dosage forms of excipients, such as spray drying, melt granulation, wet granulation, fluidized bed granulation, etc.

In addition, due to the growing interest in the opportunities that open up with the use of 2D and 3D technologies in the development of solid dosage forms, co-processed excipients can become excipients for tablets produced by three-dimensional printing [3].

LACTOSE-BASED CO-PROCESSED EXCIPIENTS

Lactose is the most common diluent in solid dosage forms due to its low cost, availability, physicochemical stability, and good solubility. Lactose exists in several forms, which differ in physical and processing characteristics. For example, β-lactose is found only in anhydrous form, whereas α-lactose can be obtained both in anhydrous form and as a monohydrate.

Crystalline α-lactose monohydrate (α-LM) has a poor binding capacity compared to lactose produced by spray drying, or β-lactose, which is characterized by plastic deformation due to the predominance of the amorphous fraction. In addition, the use of α-LM as a diluent in direct compression technology is limited by its poor flowability and compressibility, which is due to the insufficient cohesive ability of the particles. The use of spray drying technology contributed to the improvement of processing characteristics, as a result of which Tablettose® and Pharmatose® DCL 15 (DFE Pharma, Germany) were produced. However, the ability to press these products is limited by a certain limit, above which the tablet has insufficient crushing strength.

Low *dilution potential* (dilution capacity) is also a limitation in the use of α-LM in direct compression technology. Dilution capacity is the ability of a diluent to retain its compressibility properties when mixed with another component with poor compressibility. The concept of dilution capacity is applied to diluents for direct compression and is estimated by increasing the value of the area parameter under the curve (AUC) of the tablet strength dependence on the compression pressure. The method for evaluating the dilution capacity is proposed by *Minchom & Armstrong* (1987) and consists in mixing the test filler with a gradually increasing amount of the second component with poor compressibility and following measuring the area under the AUC curve of the tablet strength dependence on the compression pressure. The curve allows us to calculate the dilution capacity index (DCI) for further use as a comparison value for two fillers [4].

The solution to the problem of poor compressibility was found in the combination of α-LM with microcrystalline cellulose (MCC), povidone (PVP) or starch, but this did not significantly affect the flowability, which became a prerequisite for the creation of co-processed excipients produced by the method of co-processing.

Ludipress® (BASF, Germany) is white free-flowing lactose granules (93%), covered with a film of povidone (3.5%) (Kollidon® 30) and crospovidone (Kollidon® CL). There is also Ludipress® LCE without disintegrant (Table 3). Ludipress® is odorless and tasteless and is used in the development of tablets and gelatin capsules. The process of such a product also allowed to reduce the hygroscopicity of lactose and to ensure the independence of the strength of the tablets from the pressing speed.

It is noted that, despite the presence of a disintegrant, tablets with Ludipress® disintegrate longer than tablets based on α-LM, anhydrous β-lactose, lactose produced by spray drying, or Tablettose®, which is explained by increase of PVP in proportion to increase of Ludipress®. Ludipress® proves to be a more effective disintegrant (4–8%) compared to croscarmellose sodium and povidone, which provides high release of API (active pharmaceutical ingredient) of class II

according to the Biopharmaceutical classification system – up to 99% [5].

During the disintegration test, tablets with Ludipress® produced at compression pressure of 100 MPa showed a minimum value of the disintegration time, which did not change with increase in the compression pressure, which was not observed in tablets with Cellactose® (>20min.). It is noted that the use of Ludipress® as a diluent ensures the weight variation during continuous tablet forming. It was found that the strength of tablets increases in proportion to the compression pressure up to 300 MPa and does not depend on their diameter, thickness and shape [6,7].

Cellactose® 80 (Meggle Pharma, Germany) due to the uniformity of the fraction composition has good flowability and was developed for the direct compression process. Cellactose® has greater dilution capacity compared to a physical mixture of similar composition and less hygroscopicity due to the inclusion of MCC. The plasticity of microcrystalline cellulose (MCC) particles helps to improve the particle adhesion and compressibility of lactose monohydrate [8]. It is suitable for tablets with a high dosage and has a good adhesive ability, which is important when applying coatings [9].

MicroceLac® 100 (Meggle Pharma, Germany) is a co-processed excipient in which the embedding of MCC (25%) in α-LM (75%) is carried out by spray drying process. MicroceLac® 100 is characterized by a lower tendency to delamination compared to a similar physical mixture, and also allows to significantly increase the compressibility of a tablet mixture with API with unsatisfactory processing characteristics [10].

CombiLac® (Meggle Pharma, Germany) is another multifunctional diluent based on α-LM (70%), MCC (20%) and native corn starch (10%). Tablets made of CombiLac® show significantly higher values of crushing strength compared to MicroceLac® 100. When pressing, CombiLac® and MicroceLac® 100 exhibit predominantly elastic deformation. The disadvantage of both co-processed excipients is poor microbiological stability, confirmed by experiment, *Eline Byl et al.* [11].

Starlac® (Meggle Pharma, Germany) is a bifunctional co-processed excipient prepared from α-LM (85%) and corn starch (15%). The compressibility of lactose is increased by starch fibers, which provide a binding and disintegrating action when swollen in water. Starlac® is recommended for development of tablets with rapid release of API, since the distinctive feature of Starlac® – based dosage form is the independence of the disintegration time from the strength of the tablets and the absence of the influence of the amount of antifriction substance on the compressibility. In comparison with the rest of the above mentioned co-processed excipienты, Meggle Pharma is the least suitable for high-dose formulations [12].

A comparison of the suitability of Meggle Pharma co-processed diluents for direct compression process by the SeDeM diagram method allows us to conclude that Starlac® and MicroceLac® 100 are significantly inferior in compressibility to Cellactose® 80. However, due to the fibers of the MCC, Cellactose® 80 is more hygroscopic compared to Starlac® and MicroceLac® 100 [13]. Starlac®, MicroceLac® 100, and Cellactose® 80 are used as diluents in the development of microcapsule tablets for oral use [14].

RetaLac® (Meggle Pharma, Germany) is a co-processed diluent based on α-LM (50%) and hypromellose of type K (viscosity 4000 MPa *s) (50%) for development of the dosage form with modified pH-independent release of API. The co-processed excipient is produced by fluidized bed process. Due to the inclusion of α-LM, the wettability of hypromellose is improved and the disintegration of the tablet is accelerated.

Anhydrous lactose has a higher flowability value and is suitable for direct compression, but this value is lower than optimal one due to the high content of the dust fraction. In addition, at high humidity, there is a noticeable increase in

the weight of the tablet. Co-processed excipient **Pharmatose® DC DCL 40** (DFE Pharma, Germany), consisting of anhydrous lactose (95%) and anhydrous lactitol (5%), was developed. Among all the lactose-based composite diluents, Pharmatose® DC DCL 40 has the best binding and diluting properties in comparison with the other co-processed excipients and is characterized by low hygroscopity.

Among the monosaccharides, fructose and sucrose are also distinguished as a diluent. The main limitations of fructose as a diluent are poor compressibility and formation of too strong granules when moistened with water. Improvement in the processing characteristics of fructose was found in the preparation of **Advantose® FS 95** (SPI Pharma, France) by joint spray drying with starch (5%). In addition, Advantose® FS 95 is superior in taste to sucrose (20% sweeter) and is approved for use in patients with diabetes. Advantose® FS 95 is used as a diluent for tablets from microcapsules for oral administration [14].

Di-Pac® (Domino Spec. Ingredients, USA) is a diluent for direct compression based on sucrose 97% and dextrin 3%, which has high porosity, due to which a uniform distribution of API in the volume is ensured. Porous Di-Pac® co-crystals are produced by cooling a supersaturated solution with continuous stirring. Excellent solubility allows the use of Di-Pac® for the accelerated release dosage form. Low hygroscopicity (up to 1%) is also called the advantage of Di-Pac®.

MCC-BASED CO-PROCESSED EXCIPIENTS

MCC has the greatest dilution capacity among the diluents used in the solid dosage form process. The disadvantage of the MCC is decrease in compressibility when interacting with water. The loss of the functional properties of excipients as a result of interaction with water in the foreign scientific literature is called as "quasihornification", which is literally translated as "the effect of quasihornification", and co-processing allows us to reduce this effect. An example of improving the compressibility of the MCC can be its co-processing with silicon dioxide. Thus, the **Prosolv®** line of diluents (JRS Pharma, Germany) was developed.

The Prosolv® line is represented by three types of co-processed excipients: Prosolv® SMCC 50, Prosolv® SMCC 90 and Prosolv® SMCC HD 90. The difference between the latter two excipients is connected with the different bulk density and the ability to maintain the uniformity of the mass of the tablets during the compression process. The introduction of silicon dioxide provides a barrier to moisture, which is sorbed by particles at a humidity value of up to 52%. At higher humidity values (≥72%), the absorbed moisture reduces the deformability of the particles and can lead to increase in the disintegration time of the tablet. There is evidence of decrease in the absorption of API of derivatives of amines (tacrine hydrochloride) from aqueous solutions of MCC. The disadvantage of Prosolv® SMCC HD 90 is its high sensitivity to anti-friction substances. Comparative study of the mutual effect of Prosolv® 90 HD / Prosolv® 50 and low-compressible API (such as ibuprofen (50 microns) and acetaminophen) on the compressibility of the binary mixture showed that the ratio of the API and diluent particle sizes affects the functionality of the diluent in case of the increase of API loading (up to 60%) [15].

FMC Health Nutrition (USA) is a manufacturer of a multifunctional Avicel® diluent based on MCC, prepared by the method of joint spray drying (Table. 1) [16].

SUGARS AND POLYOLS -BASED CO-PROCESSED EXCIPIENTS

Polyols are widely used in the development of oral dispersible (ODT) and chewable tablets as diluents and sweeteners, since they have

suitable organoleptic properties and quickly dissolve when interacting with the dissolution medium [17]. However, as mono diluents, they do not have the processing properties necessary for the direct compression process.

Thus, the process of sorbitol compression is complicated by high hygroscopicity, which is the reason for poor compressibility and caking as well as affects the properties of the finished tablets (crushing strength, dissolution kinetics, bioavailability of API).

Compressol™ SM (SPI Pharma, France) is a mixture of mannitol and sorbitol for direct compression, high compressibility of which is provided by sorbitol, and almost 300 times decrease in sensitivity to moisture is provided by mannitol. Therefore, Compressol™ SM is recommended primarily for formulations with moisture-sensitive and poorly pressed API. Tablets with Compressol™ SM show good disintegration. SPI Pharma also produces spray-dried co-processed excipients for ODT **PharmaBurst® 500**

Table 1

THE RESULTS OF THE COMPARISON OF PROPERTIES OF DIFFERENT TYPES OF AVICEL® (FMC HEALTH NUTRITION, USA)

Trademark	Composition	Features highlighted by the manufacturer	Properties [16]
Avicel® CE-15	MCC (85%), guar gum (15%)	Development of chewable tablets (suitable organoleptic properties, creamy structure, without graininess)	The particles accumulate a negative charge on the surface, but are more easily powdered. Compared to the other types, the composition shows a lower value of the disintegration time and crushing strength of the tablets as well as greater hygroscopicity. Due to guar gum, it has characteristic organoleptic properties. It is not recommended to use as a diluent with pH-sensitive API.
Avicel® HFE 102	Avicel PH 102, mannitol (10%)	Improved flowability due to the inclusion of mannitol	It is characterized by better flowability due to large symmetrical sphere-like particles; moderate hygroscopicity, worse exposed to powdering due to the constantly changing charge on the surface of the particles. Tablets have a higher value of crushing strength and a longer disintegration time.
Avicel® DG	MCC (75%), dicalcium phosphate (25%)	It is recommended for compaction process. Possibility of repeated compression without loss of compressibility properties	The lowest value of flowability due to asymmetric and small particles with a stable charge; has a low hygroscopicity. It shows a relatively good ability to powder and the crushing strength of the tablets, while the tablets quickly disintegrate. It is suitable for wet granulation technology in formulations with moisture-sensitive API.

tablets based on D-mannitol (85%), silicon dioxide (<10%), sorbitol (<10%) and crospovidone (5%).

Ludiflash (BASF, Germany) consists of 90% mannitol, 5% crospovidone (Kollidon CL-SF) and 5% polyvinyl acetate (Kollicoat SR30D). Co-processed excipient is characterized by low sensitivity to moisture and reduces the probability of delamination of the tablet mixture with API. Ludiflash® is used in the development of ODT tablets [18,19], and also as component that accelerates the disintegration of tablets with API having poor solubility in water [20].

Parteck® ODT (Merck KGaA, Germany) is a co-processed excipient based on joint spray-dried D-mannitol (95%) with croscarmellose sodium (5%) for development of ODT tablets. Mannitol is in its most stable polymorphic modification i.e. the β-crystalline form, which has a melting point of 155–156°C [21,22].

A comparison of the two diluents on the example of metformin tablets showed that at a pressure value of 5.0 and 7.5 kN and 40–50% concentration of Parteck® ODT in the tablet, they delaminate in contrast to tablets based on Ludiflash® [23].

F-MELT® (Fuji Chemical Industry Co., Ltd, Japan) is a coprocessor diluent consisting of mannitol, xylitol, MCC, crospovidone disintegrant and one of the inorganic substances such as magnesium aluminosilicate (Neusilin ®) or dicalcium phosphate (Fujicalin ®). There are three types of F-MELT: M, C, and F1, depending on the functional features (Table. 3) [24]. A study by Karolina Dziemidowicz et al. demonstrated that patients prefer the tablets with F-MELT® diluent of type C in terms of taste sensations, although there is no significant difference between types C and M [25].

SmartEx™ (Shin-Etsu Chemical, Japan) is a three-component co-processed excipient based on mannitol, hypromellose with a low degree of substitution as a disintegrant and polyvinyl alcohol as a binder. Two types of SmartEx™, differing in particle sizes, have been developed (Table. 2) [26].

A study by *Karolina Dziemidowicz et al.,* which aimed to identify a co-processed diluent for ODT tablets with the best organoleptic profile, identified SmartEx™ QD-100 as the most preferred among patients in the following sequence: SmartEx™ QD-100 > F-MELT C > F-MELT M > MicroceLac > Ludiflash [25].

Xylitab® (Danisco A/S, Denmark) co-processed excipient is xylitol (98%), treated with sodium carboxymethylcellulose (2%), can be used in direct compression process in the development of tableted dosage forms for various applications, especially chewable tablets due to the cooling effect [26].

CO-PROCESSED EXCIPIENTS WITH INTEGRATED LUBRICANTS

The study of the effect of a lubricant such as magnesium stearate on the processing parameters of tablets and API release from a tablet [27],

Table 2

CHARACTERISTICS OF SMARTEX™ (SHIN-ETSU CHEMICAL, JAPAN)

Type	The range of the spread of the particle size, microns	Average particle size, microns	Recommendations
QD-50	45–75	51.6	To reduce the desintegration time
QD-100	85–125	85.3	To increase the compressibility

Table 3

A SUMMARY TABLE OF CO-PROCESSED EXCIPIENTS OF THE VARIOUS GROUPS

Trade name	Manufacturer	Composition	
		Brittle component	Plastic component
Sugar based			
Ludipress®	BASF, Germany	Lactose 93,5%	Povidone (Kollidon 30) 3,5%, Crospovidone (Kollidon CL) 3%
Ludipress® LCE		Lactose 96,5%	Povidone (Kollidon 30) 3,5%
Cellactose® 80	Meggle Pharma, Germany	α-Lactose 75%	MCC 25%
Microcelac® 100	Meggle Pharma, Germany	Lactose 75%	MCC 25%
CombiLac®	Meggle Pharma, Germany	α-Lactose 70%	MCC 20%, Native corn starch 10%
Starlac®	Meggle Pharma, Germany	α-Lactose 85%	Corn starch 15%
RetaLac®	Meggle Pharma, Germany	α-LM 50%	Hypromellose, Type K (50%)
Pharmatose® DCL 40	DFE Pharma, Germany	Lactose ahydrous 95%	Lactitol anhydrous 5%
Disintequik ODT	Kerry Ingredients & Flavours, USA	Lactose	Desintegrant (?)
Disintequik MCC 25	Kerry Ingredients & Flavours, USA	α-LM	MCC
Di-Pac®	Domino Spec. Ingredients, USA	Saccharose 97% Dextrine 3%	–
Advantose® FS 95	SPI Pharma, France	Fructose 90%	Starch 5%
MCC based			
Prosolv® SMCC 50/90/HD 90	JRS Pharma, Germany	Silicon dioxide 2%,	MCC 98%
Prosolv® ODT		Silicon dioxide, Fructose	MCC, mannitol, crospovidone
Prosolv® EastTab		Silicon dioxide	MCC, Sodium starch glycolate, Sodium stearyl fumarate

End of the table 3

Trade name	Manufacturer	Composition	
		Brittle component	Plastic component
Avicel CE-15	FMC Corporation, USA	–	MCC 85%, guar gum 15%
Avicel® HFE 102		–	Avicel PH 102 90%, mannitol 10%
Avicel® DG		Calcium hydrogen phosphate 25%	MCC 75%
Polyol based			
CompressolTM SM	SPI Pharma, France	–	Mannitol, sorbitol
Ludiflash®	BASF, Germany	–	Mannitol 90% Polyvinylacetate 5% Crospovidone 5%
Parteck® ODT	Merck KGaA, Germany	–	Mannitol 95% Cross-carmellose sodium 5%
F-MELT® type C	Fuji Chemical Industry Co., Ltd, Japan	Fujicalin® 2–9%	D- mannitol 55–70% Xylitol 2–9% MCC 10–25% Crospovidone 5–13%
F-MELT® type M		Neusilin® 2–9%	
F-MELT® type F1		Fujicalin®	Waxy Rice Starch MCC
SmartEx™ QD-50/100	Shin-Etsu Chemical, Japan	–	Mannitol Low-substituted hypromellose
PharmaBurst™500	SPI Pharma, France	Silicon dioxide <10%	Mannitol 85% Sorbitol <10% Crospovidone 5%
Xylitab® 100/200	Danisco A/S, Denmark	–	Xylitol 98% Sodium carboxylmethylcellulose 2%
With integrated lubricants			
LubriTose™ MCC	Kerry Ingredients & Flavours, USA	LM 98%	Glyceryl monostearate 2%
LubriTose™ AM			Glyceryl monostearate 4%

as well as its toxicological properties (for example, irritating effects on the epithelium of the gastrointestinal tract) excites especial interest [28,29]. Manufacturers are faced with the urgent task of finding alternatives to lubricants that are not inferior in functionality to the classic derivatives of stearic acid.

The **LubriTose™** line of fillers (Kerry Ingredients & Flavors, USA) was developed to simplify the production process by introducing a lubricant into one of the most commonly used fillers such as lactose anhydrous (96%), MCC (98%) or mannitol (96%). Glyceryl monostearate (2% – LubriTose™ MCC; LubriTose™ AM – 4%) is used as a lubricant. Thus, comparative studies of the co-processed diluent LubriTose™ MCC and a mixture of Vuvapur® 12 glyceryl monostearate showed a lower value of the friction force energy and the maximum plastic deformation of the co-processed diluent, which indicates the elimination of the supermixing effect [30,31]. However, there is evidence that LubriTose™ MCC is inferior in the time of disintegration of ibuprofen tablets in comparison with magnesium stearate, while tablets based on the LubriTose™ MCC co-processed excipient and a physical mixture of MCC with glyceryl monostearate did not show a significant difference in the time of disintegration [32].

OTHER CO-PROCESSED EXCIPIENTS

The range of co-processed excipients for direct compression process is constantly expanding, and pharmaceutical manufacturing companies offer new multifunctional platforms and delivery systems.

Advantol™ 300 (SPI Pharma, USA) Sof™elt delivery system is a ready-made powder mixture for direct compression, prepared by the method of co-processing, which has suitable organoleptic properties and quickly disintegrates upon contact with aqueous medium. This system is suitable for development of chewable tablets for pharmaceutical and nutraceutical applications. Advantol™ 300 is a relatively new product on the pharmaceutical market and its composition and process are not disclosed by the manufacturer.

Pharmasperse® 416 (SPI Pharma, USA) is a platform for oral dispersed powders (ODP) which is an alternative to tablets and is available in the form of sachets or stick bags. Pharmasperse® 416 has a refreshing taste with a cooling effect, which allows it to be used to mask the taste of API. The diluent has low hygroscopicity and uniform fractional composition, due to which it has an excellent flowability characteristic.

New co-processed excipients **Disintequik™ ODT** and **Disintequik™ MCC 25** produced by Kerry Ingredients & Flavours (USA) based on LM and disintegrant have been developed for use as diluents in ODT tablets.

REGULATORY STATUS OF CO-PROCESSED EXCIPIENTS IN USP/NF

Co-processed excipients are also subject to the registration process. The main parameter by which the quality and safety of co-processed excipients is checked is confirmation of the absence of chemical interaction between the components of the co-processed excipient. In this case, the composite diluent does not require additional toxicological studies and can be recognized as safe and approved for pharmaceutical use – GRAS (generally recognized as safe). To assign the GRAS status, it is also necessary that all components of the co-processed excipient have the GRAS status.

For each new co-processed excipient, a monograph is required; since the process for their production involves physical interaction, analytical methods for evaluating the quality of the initial components are not quite suitable for quality control of a co-processed diluent. To confirm the fact that a co-processed excipient is composite, at least

one analytical qualitative experiment is required, the results of which distinguish it from a physical mixture with the similar qualitative and quantitative composition.

The classifier of functional assignments of excipients in the Eurasian Economic Union does not contain any references to co-processed excipient. In December 2015, a draft monograph "Co-processed Excipients" was included into the European Pharmacopoeia (Ph. Eur.) 27.4, which states that the components of the co-processed excipient must meet the requirements of the monographs for each specific substance. In 2016, an updated draft of the USP Guideline for Submitting Requests for Revision to *USP-NF,* Submission Guideline for Excipients, was submitted for review for inclusion into the USP, which notes the key parameters for which excipients can be classified as co-processed, as well as the requirements for them. The Guideline fundamentally distinguishes a co-processed excipient from a physical mixture and focuses on the need for analytical tests aimed at confirming the absence of covalent bond formation during their production and storage.

IPEC, International Council of Manufacturers, Distributors and Consumers of Active Pharmaceutical Ingredients (Excipients) of the United States and Europe have jointly developed and published the first edition of the Co – Processed Excipient Guide (2017). The Guide allow manufacturers and consumers of co-processed excipients to come to an understanding in resolving the issues related to providing the latter with information on the safety of co-processed excipient to provide data to regulatory authorities when registering medicines included in co-processed excipients. Each co-processed excipient is accompanied by a master file that meets the requirements of the Food and Drug Administration (FDA) and the European Medicines Agency (EMA), so, as a rule, there are no difficulties in the process of registering a medicine containing a co-processed excipient.

As analytical methods for the determination of co-processed excipient, the Guide suggests taking into account the methods recommended by the IPEC Excipient Qualification Guide (2008) and the USP monograph "Monograph Submission Guidelines for Excipients", including testing for such indicators as "Mass loss during drying", "Residual organic solvents", "Elemental impurities" (ICH Q3D), particle shape and size distribution. When choosing or developing a method for defining the co-processed excipient, you should choose those that allow you to detect covalent bonds, so it is most rational to recommend several different analytical methods to ensure the accuracy of the results.

CONCLUSIONS

Engineering of new multifunctional co-processed excipients is a promising direction in the field of solid dosage form development due to the demand for direct compression process. The undeniable advantages of using the co-processed excipients, such as the simplification of the technological production flow diagram, the reduction of the stages at which the quality control and validation are required, as well as the time of the production cycle itself, allow the co-processed excipients to firmly occupy their pharmaceutical business segment.

An obvious limitation of the use of co-processed excipients is the fixed proportion of components in the composition of the co-processed excipient, which can significantly reduce the number of APIs and their dosages suitable for inclusion into a pharmaceutical composition based on the co-processed excipient. It should also be noted that it is necessary to develop more specific principles of the methodology and clarify the requirements for them to confirm the stability of the co-processed excipient structure as part of the medicine and during storage.

REFERENCES

1. *Mayur A. Deorel et al. Novel co-process excipients used to improve tabletting performance // EJBPS, 2017; 4 (3): 114–121. DOI: 10.1517/17425240802708978*
2. *Liew Kai Bin, Anand Gaurav, Uttam Kumar Mandal. A review on co-processed excipients: current and future trend of excipient technology // Int.J. Pharm. Sci. 2018; 11 (1): 1–9. DOI: http://doi.org/10.22159/ijpps.2019v11i1.29265*
3. *E.V. Blynskaya Technologies of three-dimensional printing for the production of dosage forms / E.V. Blynskaya, S.V. Tishkov, K.V. Alekseev // Development and registration of medicinal products. – 2018. – №3 (24). – Pp. 10–19.*
4. *Olowosulu et al. Dilution potential and filler-binder functionality of starch-based co-processed excipients (starac) in the formulation of metronidazole tablets // Nig. Journ. Pharm. Sci., September 2014. Vol. 13 (2): 44–53.*
5. *Suresh Kumar R., Sai Ratan S., Nethravathi P. Development and Characterization of Oral Disintegrating Tablet Containing Nanosuspension of Lurasidone Hydrochloride Antipsychotic Drug // Asian Journal of Pharmaceutics. 2017; 11 (2): 102–111. DOI: http://dx.doi.org/10.22377/ajp.v11i02.1153*
6. *N.V. Tikhonova Dispersion analysis in the selection of excipients for Dilept tablets / N.V. Tikhonova, E.V. Blynskaya, K.V. Alekseev // Pharmacy. – 2011. – №8. – pp. 11–14.*
7. *E. Yu. Karbusheva The selection of excipients in the development of tropoxin tablets / E.Yu. Karbusheva, E.V. Blynskaya, K.V. Alekseev // Pharmacy. – 2012. – №3. – pp. 38–40.*
8. *Arida Adi I., Al-Tabakha M.M. Cellactose® a Co-processed Excipient: A Comparison 60. Study // Pharm. Dev. Technol. 2008. – Vol. 13, – P. 165–175. DOI: 10.1080/10837450701831294*
9. *Meggle Pharma Technical brochure: Cellactose® 80 http://www.meggle-pharma.com/en/viewDocuments/upload/89/cellactose80.pdf (2014).*
10. *Sebastian Escotet-Espinoza M. et al. Using a material property library to find surrogate materials for pharmaceutical process development // Powder Technology, 2018. – Vol. 339. – P. 659–676. DOI: 10.1016/j.powtec.2018.08.042*
11. *Eline Byl et al. Elastic recovery of filler-binders to safeguard viability of Lactobacillus rhamnosus GG during direct compression // European Journal of Pharmaceutics and Biopharmaceutics, February 2019. – Vol. 135. – P. 36–43. DOI: https://doi.org/10.1016/j.ejpb.2018.12.005*
12. *Meggle Pharma Technical brochure: StarLac® http://www.meggle-pharma.com/en/viewDocuments/upload/91/starlac.pdf (2014).*
13. *Jacques C. Scholtz et al. The SeDeM Expert Diagram System: Its performance and predictability in direct compressible formulations containing novel excipients and different types of active ingredients // Powder Technology, May 2017. – Vol. 312. – P. 222–236. DOI: https://doi.org/10.1016/j.powtec.2017.02.019*
14. *Tongkai Chen et al. Tablets of multi-unit pellet system for controlled drug delivery // Journal of Controlled Release, September 2017. – Vol. 262. – P. 222–231. DOI: https://doi.org/10.1016/j.jconrel.2017.07.043*
15. *Liang Chen et al. Surface engineered excipients: III. Facilitating direct compaction tableting of binary blends containing fine cohesive poorly-compactable APIs // International Journal of Pharmaceutics, 25 February 2019. – Vol. 557. – P. 354–365. DOI: https://doi.org/10.1016/j.ijpharm.2018.12.055*
16. *Pavlína Vodálková et al. Evaluation and Comparison of Three Types of Spray Dried Co-processed Excipient Avicel® for Direct Compression // BioMed Research International. – Vol. 2018. – 15 p. DOI: https://doi.org/10.1155/2018/2739428*
17. *Shaikh Siraj Nawaj*, Shaikh Heena Kausar, G.J. Khan, Tajammul Khan. Insight in to use of co-process excipients in oral disintegrating tablets –*

A review // IAJPS. 2017, 4 (08): 2622–2626. DOI: http://doi.org/10.5281/zenodo.858669

18. *Sipos E. et al. Application of SeDeM expert systems in preformulation studies of pediatric ibuprofen ODT tablets // Acta Pharm. 2017, – Vol. 67. – P. 237–246. DOI: https://doi.org/10.1515/acph-2017–0017*
19. *Patrick Hooper, Jason Lasher, Kenneth S. Alexander, Gabriella Baki. A new modified wetting test and an alternative disintegration test for orally disintegrating tablets // Journal of Pharmaceutical and Biomedical Analysis. 20 February 2016. – Vol. 120. – P. 391–396. DOI: https://doi.org/10.1016/j.jpba.2015.12.046*
20. *Deepali B. Rajpure et al. Assessment of Ludiflash as a Superdisintegrant in Formulation and Evaluation of Fast Disintegrating Tablet of Febuxostat // World Journal of Pharmaceutical Research. 2017. – Vol. 6 (4): 1576–1585. DOI: https://doi.org/10.20959/wjpr20174–8277*
21. *Aleksandra Amelian et al. Preparation and Characterization of Orally Disintegrating Loratadine Tablets Manufactured with Co-processed Mixtures // Acta Poloniae Pharmaceutica and Drug Research, 2016; 73 (2): 453–460.*
22. *Tugba Gulsun et al. Development and evaluation of terbutaline sulfate orally disintegrating tablets by direct compression and freeze drying methods // Journal of Drug Delivery Science and Technology. – Vol. 46, August 2018. – P. 251–258. DOI: https://doi.org/10.1016/j.jddst.2018.05.014*
23. *Gustavo Freire Petrovick, Peter Kleinebudde, Jörg Breitkreutz. Orodispersible tablets containing taste-masked solid lipid pellets with metformin hydrochloride: Influence of process parameters on tablet properties // European Journal of Pharmaceutics and Biopharmaceutics. – Vol. 122, January 2018. – P. 137–145. DOI: https://doi.org/10.1016/j.ejpb.2017.10.018*
24. *Witold Brniak, Renata Jachowicz, Przemyslaw Pelka. The practical approach to the evaluation of methods used to determine the disintegration time of orally disintegrating tablets (ODTs) // Saudi Pharmaceutical Journal. September 2015; 23 (4). – P. 437–443.*
25. *Karolina Dziemidowicz et al. Co-processed Excipients for Dispersible Tablets. Part 2: Patient Acceptability // APPS Pharm. Sci. Tech., 2018. 19 (6); 2646–2657. DOI: https://doi.org/10.1208/s12249-018-1104–2*
26. *Yoshiko Takeuchi et al. Characterization of mannitol granules and powder: A comparative study using two flowability testers // International Journal of Pharmaceutics. 5 February 2018; 536 (2). – P. 506–507.*
27. *Shipar et al. Affect of Granule Sizes, Types and Concentrations of Lubricants and Compression Forces on Tablet Properties // IJPSR, 2014. – Vol. 5 (11): 4893–4901. DOI: 10.13040/IJPSR.0975-8232.5 (11).4893–01.*
28. *Cheryl A. Hobbs, Kazuhiko Saigo, Mihoko Koyanagi, Shim-mo Hayashi. Magnesium stearate, a widely-used food additive, exhibits a lack of in vitro and in vivo genotoxic potential // Toxicology Reports. – Vol. 4, 2017. – P. 554–559. DOI: https://doi.org/10.1016/j.toxrep.2017.10.003*
29. *Lizbeth Martínez-Acevedo et al. Evaluation of the lubricating effect of magnesium stearate and glyceryl behenate solid lipid nanoparticles in a direct compression process // International Journal of Pharmaceutics. – Vol. 545. – Issues 1–2, 10 July 2018. – P. 170–175. DOI: https://doi.org/10.1016/j.ijpharm.2018.05.002*
30. *Mužíková J1, Muchová S. A study of a co-processed dry binder composed of microcrystalline cellulose and glycerol monostearate // Ceska Slov. Farm. 2012 Oct; 61 (5): 229–233.*
31. *Xiaochi Xua, Chaitanya Krishna Prasad Vallabh, Stephen W. Hoag, Vivek S. Dave, Cetin Cetinkaya. Early detection of capping risk in pharmaceutical compacts // International Journal of Pharmaceutics. – Vol. 553. – Issues 1–2, 20 December 2018. – P. 338–348.*
32. *Claudia Al-Karawi et al. Novel aspects on the direct compaction of ibuprofen with special focus on sticking // Powder Technology. – Vol. 317, 15 July2017. - P. 370-380.*

UDC 615.1
https://www.doi.org/910.34907/JPQAI.2020.88.43.009

STATE-OF-THE ART TECHNOLOGIES IN PHARMACY

A.V. Anuchina, *Candidate of Pharmaceutical Sciences, Faculty of Fundamental Medicine, Moscow State University, Moscow, Russia, davydova.anna91@gmail.com*
M.A. Djavakhyan, *Doctor of Pharmacy, All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), Moscow, Russia, akopovamarina13@mail.ru*

Modern technologies are being actively introduced into various spheres of human activity, including the pharmaceutical industry. Today, in the process of developing and manufacturing the medicines, new approaches are being explored and applied, such as computer modeling, the creation of modern systems for delivering medicines to organs and tissues, three-dimensional printing of medicines, the use of supercritical fluids at various stages of production, etc. They allow manufacturers to improve the final product, adapt it to the clinical characteristics and needs of patients, as well as optimize the production processes

Keywords: state-of-the art technologies, pharmacy, three-dimension printing

Three-dimensional printing, (3DP) is a form of additive manufacturing (by layer-by-layer building up of raw materials), in which a structure is created by depositing or binding the materials in successive layers to create a 3D object.

3D printing technologies have been used in various industries for several decades. Today, articulation joints and implants for cranio-cerebral and maxillofacial surgery are printed by a 3D printer. Printers print buildings, tools, food, and parts used in aircraft and mechanical engineering. It is known that the annual growth rate of the world market for additive technologies is 15% and by 2025, the market volume is expected to increase fourfold: from 5 to 20 billion dollars per year [1].

In medicine, the use of these technologies began with the production of anatomical models used for the diagnosis and planning of various operations. Then they began to be used in dentistry for the manufacture of individual tooth crowns, dental bridges, etc. After that, they also began to print individual prostheses, orthopedic implants, various cellular tissues, personalized hearing devices and medicines [2].

Three-dimensional printing as a revolutionary method of medicine production is a potential tool for personalized medicine and makes it possible to take into account age, weight, comorbidity, pharmacokinetic characteristics. This approach is especially important for children and elderly patients. Dose adaptation according to pharmacokinetic characteristics and age is a key way to achieve the desired therapeutic effect and improve the balance between efficacy and safety. In addition, changes in color, smell, and even the configuration of dosage forms can significantly increase adherence to treatment in both children and elderly patients. It is worth noting that at the moment, the selection of the dose is mostly based on empirical methods, and therefore the probability of side effects increases significantly. At the same time, the side effects even became part of the therapeutic process [3].

Not only for children and the elderly, it is important to adapt the medicines to individual characteristics but personal approach is also required for the patients who must take 5 drugs a day at the same time. The therapy simplification, which is based on taking a fixed combination in one tablet, improves the patient persistence to the prescribed treatment. By the method of three-dimensional printing, it is possible to create fixed combinations of complex composition with an optimal release profile [3,4].

In production of medicines using 3D printing, the changes are simplified and easily introduced at the design development stage. It is possible to modify the product by releasing a series to meet the customer needs. Also an important advantage of 3D printing technology is the ability to produce complex dosage forms from the point of view of release. For example, it is possible to provide precise location of the active component (one or more) and excipients in the medicine for the purpose of modified release to provide several "sites" with different composition, release rate and mechanism of action [5,6].

There are various types of 3D printing, but only some approaches are most applicable in pharmaceutical technology. For example, printing on the basis of inkjet systems: solid-based deposition (DOS-drop on solid), drip deposition (DOD – drop on drop). Also selective laser melting (SLL – selective laser sintering or melting), semi-liquid material deposition technology (FDM-fused deposition modeling), pressure micro-spraying (PAM-pressure-assisted microsyringe), stereolithography are used (Table. 1) [6,7].

It is necessary to note that the methods presented in Table 1 include such technological processes as bonding, solidification, and melting, which occur at certain temperatures and these temperatures can be significantly higher than under conditions of granulation, microencapsulation, and tableting used in classical medicine

Table 1

THREE-DIMENSIONAL PRINTING METHODS USED TO CREATE MEDICINES

Material used	Method	Processes
Printing with the use of powder	DOS	Bonding of powder particles by liquid: the head of the printer ejects a drop on a solid material.
	SLL	Solidification of the molten powder after exposure to a laser beam
Printing with the use of liquid	DOD	Solidification of dropы: the printer head ejects the drops on one another
	stereo-lithography	Solidification of the photosensitive liquid. The geometric pattern is transferred to photosensitive liquid polymer on a substrate using UV light
Printing with the use of extrusion process	FDM	Solidification of the molten material. The molten thermoplastic polymer thread is extruded by two rollers through a high-temperature nozzle, and then solidified on the assembly platform
	PAM	Extrusion of a viscous semi-liquid material from an extrusion syringe to create the required three-dimensional shape, solidification

production technology. Therefore, the question arises about the introduction of additional requirements for the substance and the determination of critical points in the technological process.

Special attention should be paid to the fact that the first medicine produced using 3D printing for commercial purposes was the antiepileptic drug Spritam (Aprecia Pharmaceuticals), which contains levetiracetam as an active substance.

The drug was approved by the FDA in 2015. In the production of Spritam, the DOS method was used, which made it possible to achieve a porous structure of the tablet, capable of dissolving in the oral cavity in no time. It is important that the dose of levetiracetam in a tablet was 1000 mg and there were technological problems with the production of the orodisperse form of the drug by the traditional way, since the tablet was large, including due to a significant amount of excipients, and did not dissolve properly in the oral cavity [3,6,8].

After the first medicine was "printed", research in this area began to be conducted even more actively. Today, there are a large number of developments for printing various medicines (Table 2).

To date, a large number of studies on the production of medicines using 3D printing are based on the study and development of such medicines based on polymers. The polymers that are most suitable for certain methods of 3D printing of medicines are studied separately. Thus, the technology of three-dimensional modeling uses polyvinyl alcohol (a thermolabile synthetic polymer with high solubility in water, low solubility in ethanol and insoluble in many organic solvents),

Table 2

SOME DEVELOPMENTS OF DOSAGE FORMS PRODUCED BY A 3D PRINTER

Type of 3D-printing	Dosage form	Active substance/ auxiliary polymer	Author
Stereolithography	Hydrogel	Ibuprofen, riboflavin, PEG, diacrylate	Martinez et al.
FDM	Tablets	Felodipine, PEG, Tween 80, Eudragit EPO	Alhijjaj et al.
UV-jet printing	Tablets	Ropinirole, PEGDA	Clark et al.
PAM in combination with UV crosslinking	Tablets	Prednisolone, polydimethylsiloxane	Hollander et al.
FDM	Tablets	Haloperidol	Solanki et al.
FDM and hot melt extrusion	Tablets	Domperidone, hydroxypropyl cellulose	Chai et al.
FDM	Tablets	Hydrochlorothiazide	Sadia et al.
FDM and hot melt extrusion	Suppositories	Indometacin, ethylenevinyl acetate copolymers	Genina et al.
FDM	Tablets	Furadantin, polylactide, HPMC	Boetker et al.

** PEG – polyethyleneglycol, PEGDA – polyethylene glycol diacrylate, HPMC – hydroxypropyl methylcellulose*

polylactide, polycaprolactone, etc. [3]. However, there are studies that also study the «printing» of dosage forms based on lipids, for example, self-emulsifying medicine delivery systems [10].

One of the state-of-the-art processing method used in the pharmaceutical industry is also the use of supercritical liquid at various stages of production. A supercritical solvent is a state of matter in which its temperature and pressure exceed critical parameters. At the critical point, the liquid and gas phases become indistinguishable. Supercritical solvents include: carbon dioxide, n-pentane, ethanol, water, etc. [11]. Using supercritical fluids, the pharmaceutical industry produces nano-and microparticles which are carriers of active pharmaceutical ingredients and systems for prolonged medicine release [12].

Supercritical fluids are also actively used for the micronization of substances. One of the methods of micronization in the production of pharmaceuticals is RESS (Rapid Expansion of Supercritical Solutions): a solution of a substance in a supercritical fluid is sprayed through a nozzle. When the pressure decreases, the solvent converts into a gaseous state, and the dissolved substance is deposited in the form of a fine powder. The use of supercritical fluids provides opportunities for creation of water-soluble, fat-soluble substances, as well as polymers. By changing the temperature, pressure, and configuration of the dispersing nozzle, it is possible to produce powders with the specified particle size [13]. In addition, supercritical fluid technology is an alternative approach for increasing the solubility of water-insoluble substances [11].

In addition, in recent years, the scientific community has focused on the use of biodegradable solvents. They include deep eutectic solvents (DES), which are a mixture of solid compounds, such as choline chloride and sugar, whose melting point is significantly lower than that of individual components [14]. They are characterized by the formation of strong hydrogen bonds and, due to the extremely low vapor pressure, are widely used in polymer chemistry and synthetic organic chemistry, as well as for the extraction of biologically active substances [15,16]

For example, when using a mixture of "urea-choline chloride" and "choline chloride-malonic acid", the solubility of poorly soluble molecules, such as benzoic acid, griseofulfine, danazole and itraconazole, in DES is 5–22000 times higher than in water [17].

It is also worth noting computational simulation as one of the up-to-date methods that is actively used in various industries. Computational simulation is the construction of symbolic and physical models of objects studied in science, created in technology, medicine, art, and other areas of human activity using computers and computer devices [18].

Currently, computational simulation is used to predict the physiological activity, the compatibility of pharmaceutical substances and excipients, and to determine the relationship between the structure and properties of substances. Important advantages of computational simulation, which specify the efficiency of its use by scientists, are the possibility of its repetition for the required number of times, the ability to simulate such experimental parameters that cannot be created in the laboratory, the ability to study fast-flowing processes, the safety of virtual research, which excludes harm to humans and the environment, significant savings and economic benefits compared to in vivo and in vitro experiments. In addition, using the mathematical simulation, scientists study the functioning of various human organs in normal and pathological conditions. So, in 2016, researchers from the University of York for the first time created a three-dimensional model of heart tissue, which is able to pulse like a real heart. Scientists were able to include three types of tissues in this virtual model. This three-dimensional model can be used to predict the toxic effect of the studied medicines on the heart or to study the problems that arise during heart tissue transplantation [19,20].

CONCLUSIONS

Today, everywhere in the world, new processes are being actively introduced into the pharmaceutical industry, speeding up and improving the production process and the final product, as well as introducing the principles of personalized medicine, which in the future can make the production of medicines more accessible and adapted to a specific patient. In this regard, analyzing the possibilities of introducing 3D printers into domestic use, the humanities are faced with the question "Can consumers be trusted with the means of producing an unlimited range of things?". The production and distribution of pharmaceutical products, especially narcotic drugs and strong drugs, should undoubtedly be subject to strict control, which means that the introduction of new processes, such as three-dimensional printing, should be carefully developed and regulated in the future [1]

REFERENCES

1. *O.V. Letunova, N.A. Ermienko, I.Yu. Ermienko, V.G. Sherstyukov. Additive processes and their influence on the market of personalized goods // Collection of articles of the International Scientific and Practical Conference "Economics, Management, Law: current issues and vectors of development". – Petrozavodsk, 2019. – pp. 109–113.*
2. *D.V. Yakovleva, E.V. Verkhoturova. 3D printing processes in the production of pharmaceutical products // Collection of scientific papers under the general editorship of E.G. Privalova "Innovative processes in pharmacy". – Irkutsk, 2019. – pp. 401–404.*
3. *Konta A.A., García-Piña M., Serrano D.R. Personalised 3D-Printed Medicines: Which Techniques and Polymers Are More Successful? // Bioengineering. – 2017. – №4. – Vol. 79.*
4. *Nishimura R., Kato H., Kisanuki K., Oh A., Onishi Y., Guelfucci F., Shimasaki Y. Comparison of persistence and adherence between fixed-dose combinations and two-pill combinations in Japanese patients with type 2 diabetes // Current Medical Research and Opinion. – 2019. – №5. – Vol. 35.*
5. *Hsiao W.K., Lorber B., Reitsamer H., Khinast J. 3D-printing of oral drugs: a new reality or hype? // Expert Opinion on Drug Delivery. – 2018. – №1 (15). – P. 1–4.*
6. *Jamróz W., Szafraniec J., Kurek M., Jachowicz R. 3D-Printing in Pharmaceutical and Medical Applications – Recent Achievements and Challenges // Pharm. Res. – 2018. – №9. – Vol. 35. – P. 176.*
7. *E.V. Blynskaya, S.V. Tishkov, K.V. Alekseev. Processes of three-dimensional printing for the production of dosage forms // Development and registration of medicinal products. – 2018. – №3 (24). – pp. 10–19.*
8. *Norman J., Madurawe R.D., Moore C.M.V., Khan A., Khairuzzaman A.K.M. A new chapter in pharmaceutical manufacturing: 3D-printed drug products // Advanced Drug Delivery Reviews. – 2017. – V. 108. – P. 39–50.*
9. *Diogo J H. 3D-Printing of Pharmaceutical Drug Delivery Systems // Arc.Org. Inorg. Chem. Sci. – 2018. – №2. – Vol. 1.*
10. *Vithani K., Goyanes A., Jannin V. et al. A Proof of Concept for 3D-Printing of Solid Lipid-Based Formulations of Poorly Water-Soluble Drugs to Control Formulation Dispersion Kinetics // Pharm. Res. – 2019. – №7. – Vol. 36. – P. 102.*
11. *Kaur T., Sharma P., Kumar S. Supercritical fluid technology: an innovative approach used to enhance solubility of water insoluble drugs // Journal of Drug Delivery & Therapeutics. – 2017. – №7 (5). – P. 22–26.*
12. *S.E. Bogorodsky, L.I. Krotova, S.A. Minaeva, G.V. Mishakov, V.K. Popov, Yu.B. Basok, V.I. Sevastyanova. Supercritical fluid micronization and encapsulation of ibuprofen*

in microparticles of aliphatic polyesters. // Advanced Materials. – 2013. – №1. – pp. 23–32.

13. *D.Yu. Zalepugin, N.A. Tilkunova, I.V. Chernyshova, V.S. Polyakov. Development of processes based on the use of supercritical fluids // Supercritical fluids: theory and practice. – 2006. – №1. – Vol. 1. – pp. 27–51.*
14. *Abbott A.P. et al. Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids // Journal of the American Chemical Society. – 2004. №29. – V.126. – P. 9142–9147.*
15. *Dai Y. et al. Ionic Liquids and Deep Eutectic Solvents in Natural Products Research: Mixtures of Solids as Extraction Solvents // Journal of Natural Products. – 2013. – №11. – Vol. 76. – P. 2162–2173.*
16. *Tang B., Zhang H., Row K.H. Application of deep eutectic solvents in the extraction and separation of target compounds from various samples // Journal of Separation Science. – 2015. – №6. – Vol. 38. – P. 1053–1064.*
17. *Morrison H.G., Sun C.C., Neervannan S. Characterization of thermal behavior of deep eutectic solvents and their potential as drug solubilization vehicles // International Journal of Pharmaceutics. – 2009. – №1. – Vol. 378. – P. 136–139.*
18. *URL: https://bigenc.ru/technology_and_technique/text/4010577 (access date 03.06.2020).*
19. *S.O. Losenkova, A.V. Pogrebnyak, Yu.A. Morozov, E.F. Stepanova. Computational simulation as one of the up-to-date methods of forecasting in pharmaceutical technology. // Pharmacy and Pharmacology. – 2014. – №6 (7). – pp. 105–113.*
20. *S.A. Chiryapkin, A.A. Glushko Mathematical simulation in medicine and pharmacy // Rostov Scientific Journal. – 2019. – №3. – pp. 343–351.*

ARTICLE SUBMISSION GUIDELINES

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