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 (34) 2021

Dear friends and colleagues!

The scientific and practical journal "Journal of Pharmaceutical Quality Assurance Issue" has been published since 2013 with a frequency of 4 issues per year. Despite the difficult period associated with the spread of coronavirus infection, we managed to maintain a high level of publication activity and selection of high-quality materials for inclusion into the periodical edition. We are included in the list of the Higher Attestation Commission of the Ministry of Science and Higher Education of the Russian Federation, are reflected in the RSCI, and are gradually moving towards indexing in well-known international databases. Starting from 2019, an English-language version of the journal is published for entry into the international scientific space. We invite all interested parties to cooperate for productive interaction and filling the content of the publication.

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

© JOURNAL OF RESEARCH AND PRACTICE "JOURNAL OF PHARMACEUTICALS QUALITY ASSURANCE ISSUES"

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.

Reprint of materials published in the Journal is allowed only with the written consent of the Editorial Board

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

Circulation 3000 copies
Order №2602-03

ISSN 2309-6039 №4 (34) 2021

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

Chief Editor

A.A. Markaryan,
PharmDr., Professor

Deputy chief editors

I.V. Maev, Doctor of Medicine, Professor, Member of RAS

E.I. Sakanyan,
PharmDr., Professor

Executive Editor – K.A. Krasilnikova, Associate Professor of Pharmaceutical Sciences

EDITORIAL BOARD

Arzamastsev E.B., Doctor of Medicine, Professor (Moscow)
Volskaya E.A., Candidate of Historical Sciences (Moscow)
Glazkova T.Yu., Candidate of Engineering Sciences, Assistant Professor (Moscow)
Dargaeva T.D., Dr. Phil., Professor (Moscow)
Durnev A.D., Doctor of Medicine, Professor, Corresponding member of RAS (Moscow)
Evdokimova O.V., Dr. Phil., (Moscow)
Zaborovskiy A.V., Doctor of Medicine, (Moscow)
Kosova I.V., Dr. Phil., Professor (Moscow)
Loskutova E.E., Dr. Phil., Professor (Moscow)
Lyakina M.N., Dr. Phil. (Moscow)
Maksimkina E.A., Dr. Phil., Professor (Moscow)
Sokolskaya T.A., Dr. Phil., Professor (Moscow)
Soloninina A.V., Dr. Phil., Professor (Perm)
Tsyndymeev A.G., Candidate of philosophical sciences (Moscow)
Shchekin D.A. (Moscow)
Eigel M.Ya., Candidate of economic sciences (Moscow)
Yagudina R.I., Dr. Phil., Professor (Moscow)

CONTENTS

REVIEWS

THEORETICAL JUSTIFICATION OF THE CHOICE OF MEDICINAL PLANT RAW MATERIALS FOR THE CREATION OF A HERBAL TEA INTENDED FOR THE TREATMENT OF TYPE 2 DIABETES MELLITUS 3
M.A. Javakhyan, N.R. Pavets, O.K. Pavelieva

PHARMACEUTICAL ANALYSIS AND QUALITY CONTROL OF PHARMACEUTICALS

STUDY OF TOTAL MINERAL COMPLEX OF MEDICINAL PLANT RAW MATERIALS OF SYNANTHROPIC FLORA IN THE VORONEZH REGION 13
N.A. Dyakova

DEVELOPMENT AND VALIDATION OF A PROCEDURE FOR QUANTITATIVE ANALYSIS OF MOXIFLOXACIN IN THE "MOXIFLOXAZOL" MEDICINE 21
A.I. Zamaraeva, T.A. Kobeleva, A.I. Sichko, N.S. Bessonova, E.M. Shapovalova

CONTENT OF ORGANIC ACIDS IN PORTULACA OLERACEA L. HERB 29
R.A. Nasser, O.G. Potanina, A.V. Nikulin

FORMULATION OF MEDICINES

THE ASPECTS OF PHARMACEUTICAL DEVELOPMENT OF THE TABLETS BASED ON DRY EXTRACTS 38
A.V. Foteeva, M.P. Chugunova, N.A. Prozorova, P.N. Vshivkov

PHARMACY MANAGEMENT AND ECONOMICS

THE ROLE OF THE AUTHORIZED PERSON RESPONSIBLE FOR PHARMACOVIGILANCE IN THE ORGANIZATION OF THE PHARMACOVIGILANCE SYSTEM OF THE LICENSE HOLDER 44
E.Yu. Kurganova, A.V. Soloninina

PHARMACOLOGY. CLINICAL PHARMACOLOGY

STUDY OF COMPARATIVE PHARMACOKINETICS AND BIOEQUIVALENCE OF URSODEOXYCHOLIC ACID MEDICATIONS 49
E.V. Glushko, S.G. Mardanly, T.A. Koroleva

UDC 615.322,542.943
https://www.doi.org/10.34907/JPQAI.2021.71.31.001

THEORETICAL JUSTIFICATION OF THE CHOICE OF MEDICINAL PLANT RAW MATERIALS FOR THE CREATION OF A HERBAL TEA INTENDED FOR THE TREATMENT OF TYPE 2 DIABETES MELLITUS

M.A. Javakhyan, *Doctor of Pharmaceutical Sciences, Associate Professor, Chief Researcher of the Experimental and Technological Department, All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), Moscow, Russia, ORCID 0000 0003 2673 6203.*
N.R. Pavets, *Post-Graduate Student, All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), Moscow, Russia, ORCID 0000 0003 4953 8859.*
O.K. Pavelieva, *Junior Research Assistant of the Experimental and Technological Department, All-Russian Research Institute of Medicinal and Aromatic Plants (VILAR), Moscow, Russia, pavelieva.olesya@yandex.ru, ORCID 0000-0002-2397-8920.*

The incidence of diabetes mellitus of the second type is growing every year, and the search for medicines for supporting therapy of patients is urgent. Ideally, the treatment program should include exposure in several directions: to have hypoglycemic, hypolipidemic effects, to prevent the progression of vascular complications, etc. The fact is that the medicines having only a hypoglycemic effect are not able to prevent the development of complications and normalize metabolism in patients with diabetes mellitus. The article highlights the issues related to the state of morbidity of the population with diabetes mellitus. A conceptual model of maintaining glucose homeostasis during and after meals, proposed by Mukhamedzhanov E.K., is presented. The characteristics of plants and the mechanism of action of biologically active substances (BAS) that contribute to a complex effect on the pathological process in the treatment of type 2 diabetes mellitus are given. Among them there are roots and rhizomes of elecampane inula, sunflower family (Inula helenium L., Asteraceae family), that are rich in inulin, which can be used as a prebiotic to modulate the gut microbiota, potentially affecting glucose homeostasis and lipid profile; leaves of cowberry, heath family (Vaccinium vitis-idaea L., Ericaceae family), containing tannins and arbutin, which in its individual form is currently a promising substance for the diabetic nephropathy control; dog rose fruits, Rose family (Rosa canina L., Rosaceae family) having a large set of biologically active substances (studies have shown that rosehips prevent obesity and diabetes); motherwort herb, mint family (Leonurus cardiaca L., Lamiaceae family), that is rich in flavonoids such as rutin, apigenin and others. Rutin reduces the absorption of carbohydrates from the small intestine, stimulates the secretion of insulin by beta cells, protects the islet of Langerhans from degeneration, increases the absorption of glucose by tissues and suppresses gluconeogenesis in the liver.

The expediency of using a plant composition made of the roots and rhizomes of elecampane inula, lingonberry leaves, rosehips and motherwort herb, which are sources of inulin and inulicin, arbutin, vitamin C and organic acids, rutin and apigenin, is shown.

Keywords: diabetes mellitus, mechanism of action, plant raw materials, hypoglycemic tea

A chronic disease in which the pancreas does not produce enough insulin or the body itself is unable to use it is called diabetes. In the first

case, it is type 1 diabetes mellitus (DM1) and as a treatment, regular insulin is injected into the patient's body. In the second case it is type 2 diabetes mellitus (DM2).

Significantly more people suffer from type 2 diabetes than from the first one. It is often called as a "disease of civilization" because the incidence of it is growing every year [1]. According to the World Health Organization, the number of cases increased from 108 million in 1980 to 422 million in 2014, and premature mortality increased by 5% for the period from 2000 to 2016.

For the first time, the term "insensitivity to insulin" was introduced in 1939 by Himsworth and Kerr to define the lack of an organism's response to insulin administration in diabetic patients. Type 2 diabetes is characterized by impaired glucose tolerance, which is caused by insensitivity of cells and tissues of the body to insulin [2]. Insulin resistance can lead to a number of pathological processes. All together it is known as "insulin resistance syndrome". This syndrome includes the following disorders: obesity, increased triglyceride levels, arterial hypertension, impaired fasting glycemia, increased levels of thrombic and antifibrinolytic factors, impaired glucose tolerance. All this can eventually lead to cardiovascular diseases [3].

One of the most popular ways to manage DM2 is to reduce glycemia. Glucose homeostasis can be maintained by autoregulation of enzymes responsible for glucose breakdown, but such regulation has limited possibilities. In the case when enzymes fail to cope with the specified task, complex mechanisms for maintaining glucose homeostasis are activated. A conceptual model of maintaining glucose homeostasis during and after meals was proposed by the scientist Mukhamedzhanov E.K. (Fig. 1) [4].

Glucose is a source of energy for the brain and blood cells. Proteins act as a coordinator of carbohydrate and fat metabolism when using exogenous and endogenous food flows. Excess exogenous food flows are converted into fats. Glucose catabolism consumes ATP energy for protein synthesis. That is, with a decrease in carbohydrates in the diet, the amount of energy that is released for protein synthesis decreases. If, on the contrary, the food is relatively poor in protein and excessively rich in carbohydrates, then protein synthesis decreases due to insufficient substrate support, as a result of which less ATP is released for protein biosynthesis and the formation of ATP in the system is reduced in principle.

Inhibition of glucose uptake leads to its accumulation in the blood, which, in turn, forces the pancreas to secrete more insulin, as a result of which the discharge of the carbon skeleton of glucose into fats accelerates and hyperlipidemia develops.

In the Russian Federation, the tactics of managing a patient with diabetes mellitus were specified in the Consensus of the Council of experts of the Russian Association of Endocrinologists on the initiation and intensification of hypoglycemic therapy [5]. Ideally, the treatment program should include effects in several directions, have hypoglycemic, hypolipidemic effects, prevent the progression of vascular complications, etc. The fact is that the medicines that have only a hypoglycemic effect are not able to prevent the development

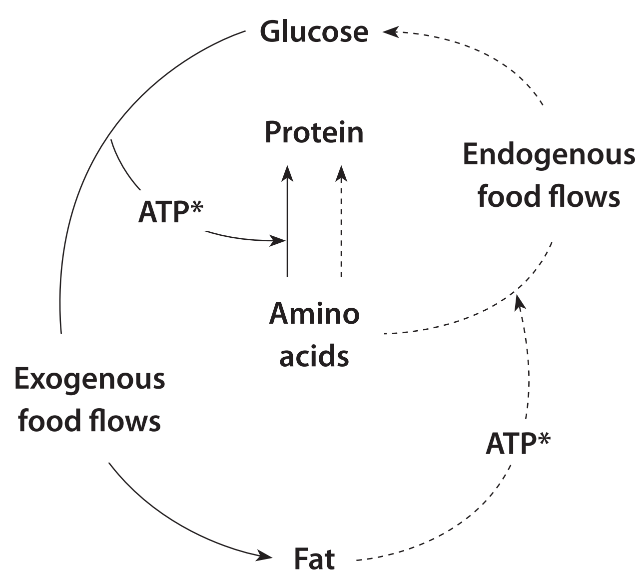


FIG. 1. *Model of glucose homeostasis*

of complications and normalize metabolism in patients with diabetes mellitus. Based on this, it can be concluded that it is necessary to bring to the market safe and effective complex preparations for the treatment of diabetes of type 2.

Among the numerous active substances that affect the reduction of glucose tolerance, it is necessary to note substances of plant origin that have a wide spectrum of action and a complex effect on the body. In addition, they have a number of obvious advantages over synthetic molecules, for example, they have low toxicity, which allows them to be used for a long time without pronounced side effects, have a mild effect, are combined with medicinal substances, enhancing their therapeutic effect [6].

The glucose homeostasis model presented in Fig. 1 demonstrates the influence of exogenous and endogenous factors, some of which can be controlled, including in pharmacotherapy the groups of substances that contribute to the normalization of glucose uptake and transport into cells, its excretion from the body and reduction of insulin synthesis from proinsulin.

For example, S.A. Kalmykov, when developing a tea for the treatment of diabetes mellitus, proposed an algorithm for selecting medicinal plant raw materials for the tea, including [6]:

- substances that promote the activation of hexokinase, which is necessary for the phosphorylation of glucose, or substances that promote the conversion of glucose into mannose and fructose, for the uptake of which insulin is not required (for example, substances of the guanidoisoamylene group);
- substances containing chromium, which provides glucose transport to cells. It has a hypolipidemic effect, prevents the development of cardiovascular diseases;
- substances with antioxidant activity. They protect β-cells by neutralizing reactive oxygen intermediates, causing a violation in the structure of their DNA, which ultimately leads to decrease in proinsulin synthesis;
- substances that have a diuretic effect, due to which the removal of excess glucose from the body is ensured, and substances that improve the functioning of the links of the immune system.

Promising objects with the above properties, as well as having an extensive domestic raw material base, are the following plants: motherwort, mint family (*Leonurus cardiaca* L., Lamiaceae family), cowberry, heath family (*Vaccinium vitis-idaea* L., Ericaceae family), elecampane inula, sunflower family (*Inula helenium* L., Asteraceae family), dog rose fruits, rose family (*Rosa canina* L., Rosaceae family).

The roots and rhizomes of elecampane inula are rich in inulin (up to 44%), inulicin (Fig. 2), as well as polysaccharides, for example pseudo-inulin, in addition, saponins, resins, gums were found in the extracts [7].

Inulin is a fermentable indigestible carbohydrate, and its effect on the lipid profile and glucose level has been studied for a long time (8). Inulin has a long chain of polymer residues (n=10-60), which allows it to remain in the human body and have a positive effect on it for a longer time until it reaches the colon (9). In recent years, there has been more and more evidence that the gut microbiota plays a crucial role in the development of inflammation and metabolic disorders, such as obesity, insulin resistance and type 2 diabetes mellitus [10,11]. Inulin can be used as a probiotic to modulate the gut microbiota, which potentially affects glucose homeostasis and lipid profile due to the balance of satiety hormones, regulation of lipid synthesis and reduction of insulin resistance [12].

In the studies of scientists Dehgan P. and Beylot M., aimed at studying the effect of inulin on the state of people with obesity or DM2, there was a significant decrease in total plasma cholesterol during 6–8 weeks of inulin treatment compared with the control group [13,14]. In addition, the analysis of subgroups by types of insulin-type fructosans showed that the consumption of inulin alone without additives of other polysaccharides

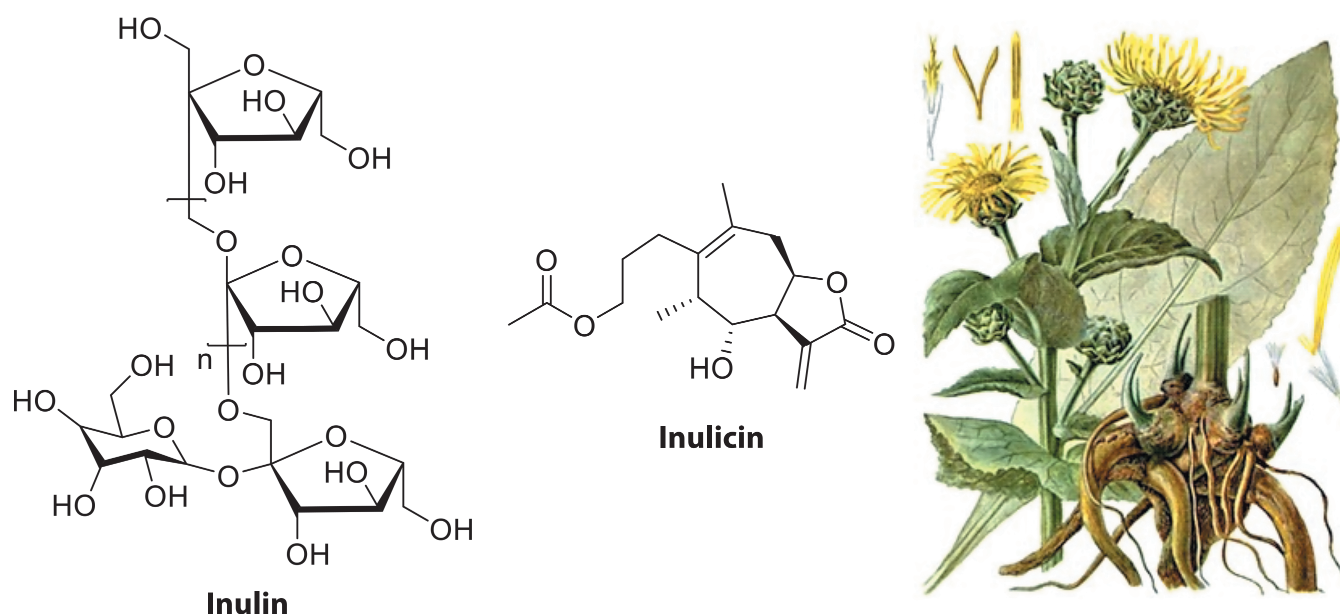


FIG. 2. *Structural formulas of inulin and inulicin and the structure of elecampane inula*

improved the lipid profile and reduced total cholesterol. The mechanism of action of inulin-type fructosans on glucose and lipid metabolism remains unclear. A number of mechanisms have been proposed concerning the effect of inulin on lowering the cholesterol levels. One of them was considered as a possible way to reduce cholesterol absorption through intestinal epithelial cells [15]. Inulin is a soluble and viscous compound that increases the thickness of the unmixed layer of the small intestine, thus inhibiting the absorption of cholesterol [16]. Inulin does not bind to bile acid in the upper digestive tract, however, it can help bile acid interact with bacteria or insoluble compounds such as calcium phosphate by lowering the pH of the cecum [17]. As a result of increased excretion of bile acids with feces, the use of cholesterol to restore bile acid in the liver increases, which, in turn, reduces the concentration of cholesterol in it

Another potential mechanism of action is that changes in the composition of the intestinal microbiota after the intake of short-chain inulin-type fructosans lead to an improvement in glucose and lipid metabolism and reduce the level of lipopolysaccharides in plasma, as shown by the example of mice [18]. In the colon, inulin is broken down by the gut microbiota into short fatty acid residues such as acetate, propionate and butyrate [19]. As a rule, propionate and butyrate are metabolized in the colon and liver, which mainly affects local intestinal and liver functions. They also cause gluconeogenesis and sympathetic activity in the intestine, which improves glucose homeostasis. In addition, circulating acetate can be absorbed by the brain and subsequently regulate the feeling of satiety through a central homeostatic mechanism [20,21]. Butyrate suppresses cholesterol synthesis in the liver by suppressing lipogenic genes in it and provides energy to epithelial cells of the human colon [22,23].

The roots and rhizomes of elecampane can serve as a source of inulin both as part of the tea and as a result of extraction.

The leaves of cowberry contain tannins and arbutin (Fig. 3) – a glycoside of the phenolic type [24].

Arbutin in its individual form is currently a promising substance for the diabetic nephropathy management. Diabetic nephropathy is a complication in patients with diabetes mellitus, which in 80% of cases leads to renal failure [25,26]. Recently, in the treatment of diabetic nephropathy,

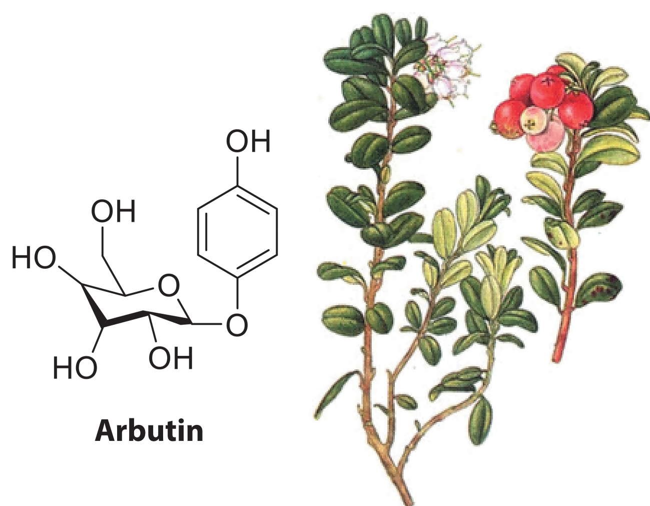


FIG. 3. *Structural formula of arbutin and the structure of cowberry*

emphasis has been placed on blood sugar control, regulation of blood lipids, diet compliance [27]. Compliance with the doctor's recommendations can slow down the development of the disease, but in the long term it still leads to unpleasant consequences. One of these consequences is tubular necrosis, i.e. the death of tubular epithelial cells forming renal tubules. Tubule damage is particularly active in an environment with high glucose content [28]. Arbutin has been shown to inhibit the apoptosis of tubular epithelial cells induced by increased glucose content.

In addition, Yuritsa K. and colleagues studied the role of arbutin in human peripheral blood lymphocytes and found that arbutin can inhibit lymphocyte proliferation [29]. In B. Zhang 's studies, the bactericidal [30], anti-inflammatory [31] and anti-glycation effects of arbutin in vitro have been confirmed.

In the work of Farzanegi and co-authors, data on the study of the effect of arbutin on the protection of cardiac tissue in experimental animals with diabetes-induced oxidative stress are presented [32]. And the antioxidant activity of arbutin can reduce the risk of DM2 by improving protection against oxidative stress [33,34].

Dog rosehips have a large set of biologically active substances. It is known for having the highest vitamin C content compared to other plants and is a source of organic acids. In addition, carotenoids, vitamins P, B1, K, E, polyphenolic substances, flavonoids, catechins, anthocyanins, tannins and chlorophylls can be found in its composition. The percentage of certain substances depends on the soil, the season and even the height of the shrub growth above sea level. Rosehips contain vitamin C, citric acid, malic acid (Fig. 4) [35,36].

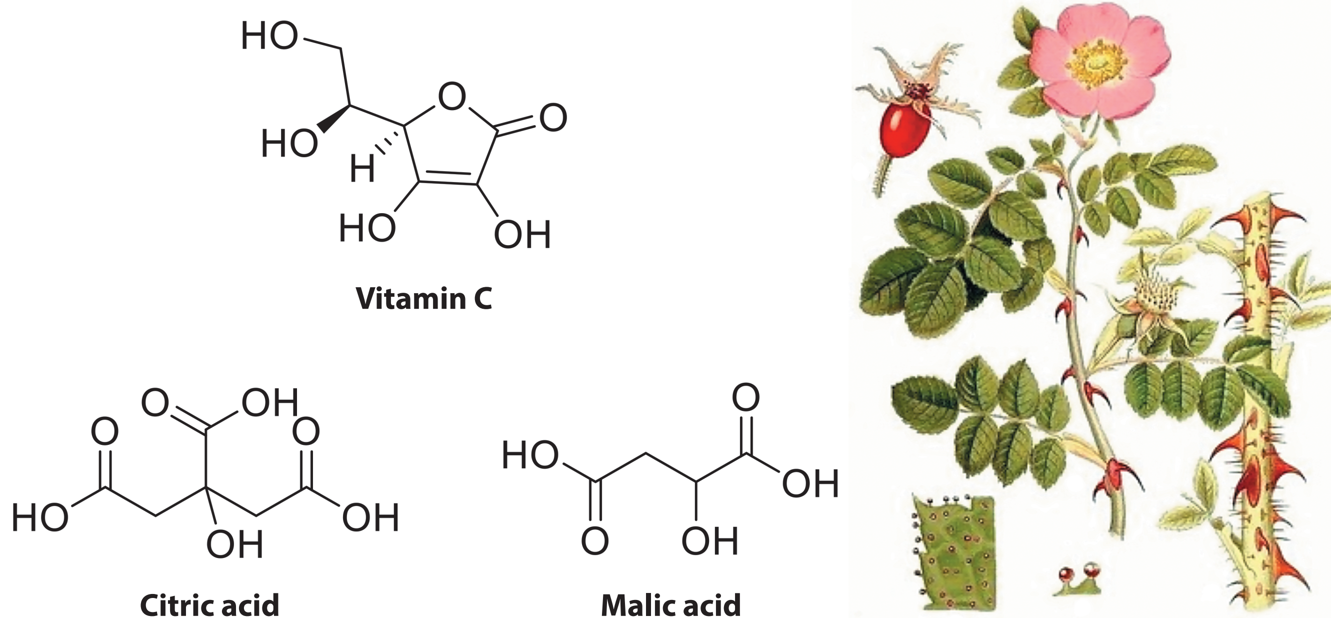


FIG. 4. *Structural formulas of vitamin C, citric acid, malic acid and the structure of rose hips*

During the last decade, the anti-inflammatory properties of rosehip have been documented in several studies, and it has been successfully used to relieve symptoms in patients suffering from osteoarthritis, rheumatoid arthritis and lumbar pain [37-41]. Two independent studies conducted on two different mouse lines have shown that rosehips prevent obesity and diabetes. In one of them [42], mice were injected with acetone extract of rosehip fruits and seeds, which helped prevent weight gain in mice that did not follow a diet. Another used a line of mice simulating obesity and human insulin resistance. During the study, they were injected with rosehip fruit powder, which made it possible to stop weight gain and reduce insulin resistance [43]. The mechanism of lowering the level of cholesterol in plasma could not be determined, there was a decrease in the level of cholesterol in both plasma and liver, although it was recorded that there was no effect on the biosynthesis of cholesterol. Afterwards, the same group of scientists provided a human subject research to study the metabolic effects of administration of rosehip (44). As a result of daily consumption of rosehip fruits, a significant decrease in cholesterol levels was observed, but unlike rats, there was no effect on body weight, glucose tolerance and markers of inflammation.

The motherwort herb is rich in flavonoids such as rutin, apigenin (Fig. 5) and others [45].

The data show that glucose transporters play a key role in the absorption of sugar, which makes them attractive targets for the discovery of antidiabetic agents [46,47]. Glucose is a hydrophilic compound and cannot pass through the lipid bilayer due to passive diffusion, therefore, specific carrier proteins are required for its transport to the cytosol. The small intestine and kidneys express several isoforms of glucose transporters, such as GLUT1, GLUT2, GLUT5 and Na+-dependent glucose transporter 1 (SGLT1). Consequently, new strategies for the prevention and treatment of diabetes and obesity can be achieved due to reducing glucose absorption by inhibiting these glucose transporters in the intestine or due to stimulating the renal glycosuria.

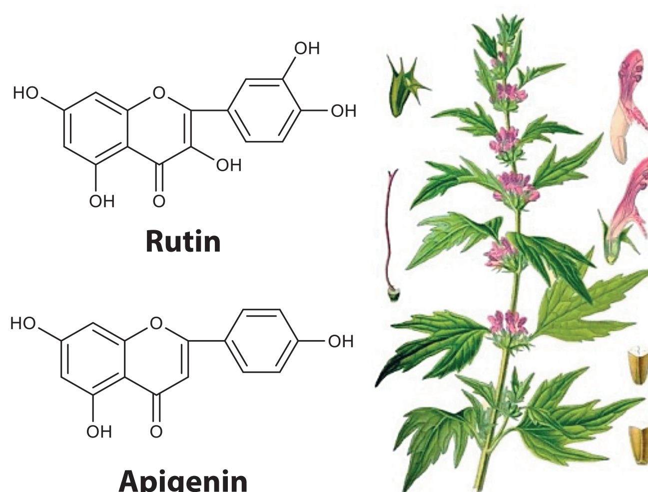


FIG. 5. *Structural formulas of rutin, apigenin and the structure of motherwort herb*

A research was conducted to study the effect of flavonoids on glucose transport via human SGLT1 [48]. Various subclasses of flavonoids (flavones, isoflavones, flavonols, flavanones) were studied by measuring the glucose transport in oocytes expressing the human SGLT1. As a result, apigenin showed good activity.

Diabetic cardiomyopathy is an independent ischemic heart disease that develops in diabetics, characterized by changes in the structure and function of the myocardium. It has been shown [49] that rutin protects and improves myocardial dysfunction by preventing oxidative stress, apoptosis and inflammation in the hearts of diabetic rats. Rutin can protect the liver in diabetes by alleviating inflammation, steatosis, fibrosis, as well as rutin promotes signal transmission along the insulin signaling pathway in the liver and hepatocyte proliferation [50].

Rutin reduces the absorption of carbohydrates from the small intestine, stimulates the secretion of insulin by beta cells, protects the islets of Langerhans from degeneration, increases the absorption of glucose by tissues and suppresses gluconeogenesis in the liver [51].

Rutin reduces glucose absorption from the small intestine by inhibiting α-glucosidases and α-amylases involved in the digestion of carbohydrates. Suppression of glucose absorption in the intestine prevents sharp increase in blood glucose levels after eating. Lowering blood glucose levels can also be achieved due to stimulating the insulin secretion by beta cells and increasing the glucose uptake by tissues. In isolated islets of the pancreas of rats, rutin significantly increases insulin secretion. In rat beta cells, rutin increased glucose-induced insulin secretion and maintained glucose sensitivity under conditions of high glucose levels. Rutin also showed the role of the insulin mimetic in the soleus muscle of rats and the muscles of the diaphragm. It stimulated the glucose transport into the muscles by activating the synthesis and translocation of the GLUT-4 transporter. Rutin also increases the expression of PPARy, which improves insulin resistance and glucose uptake in skeletal muscles and adipose tissue.

Histopathological studies in vitro on rats with diabetes have shown that rutin improves the histoarchitectures of the islets of Langerhans. Treatment of rats with STZ-induced diabetes with 50 mg/kg and 100 mg/kg rutin prevented a reduction in the size of the pancreas and a reduction in the number of islets. Rutin has also been shown to suppress glucolipotoxicity in rat pancreatic beta cells by activating the insulin receptor substrate-2 and signaling of AMP-activated protein kinase.

CONCLUSION

As a result of the analysis of the literature data, the expediency of the integrated use of motherwort, mint family (Leonurus cardiaca L., Lamiaceae family), cowberry, heath family (*Vaccinium Vitis-idaea* L., Ericaceae family), elecampane inula, sunflower family (*Inula helenium* L., Asteraceae family), dog rose, rose family (*Rosa canina* L., Rosaceae family) has been shown as rich sources of biologically active substances that are active participants in biochemical processes aimed at counteracting of the mechanism of the pathological process in type 2 diabetes mellitus.

REFERENCES

1. *Feudtner C. Diabetes: the sweet irony of modern technology // Bulletin of the World Health Organization. 2011; 89(2): 90–91. DOI: 10.2471/BLT.11.040211.*
2. *Himsworth H.P., Kerr R.B. Insulin-sensitive and insulin-insensitive types of diabetes mellitus // Clinical Science. 1939; 4: 119–152.*
3. *Nathan D.M., Davidson M.B., DeFronzo R. A., Heine R.J., Henry R.R., Pratley R., et al. Impaired fasting glucose and impaired glucose tolerance: implications for care // Diabetes care. 2007; 30(3): 753–759. DOI: 10.2337/dc07-9920.*
4. *Mukhamedzhanov E.K. Type 2 diabetes mellitus: new aspects of the pathogenesis of the disease // Diabetes mellitus. 2013; 4: 49–51.*
5. *Ametov A.S. Modern approaches to the treatment of type 2 diabetes mellitus and its complications // Problems of endocrinology. 2012; 58(3): 61–64.*
6. *Kalmykov S., Kalmykova Yu. Characteristics of medicinal plants used in phytotherapy of type 2 diabetes mellitus // Slobozhansky scientific and sports bulletin. 2016(3): 53–58.*
7. *Afemei M.G. E., Boz I., Toma C., Zamfirache M.M. Aspectes regarding the qualitative and quantitative phitochemical analysis of the inula helenium L. species // Vegetal Biology. 2012; 58(1): 29–34.*
8. *Liu F., Prabhakar M., Ju J., Long H., Zhou H.W. Effect of inulin-type fructans on blood lipid profile and glucose level: a systematic review and meta-analysis of randomized controlled trials // European Journal of Clinical Nutrition. 2017; 71(1): 9–20. DOI: 10.1038/ejcn.2016.156.*

9. Liu T.-W., Cephas K.D., Holscher H.D., Kerr K.R., Mangian H.F., Tappenden K.A., et al. Nondigestible Fructans Alter Gastrointestinal Barrier Function, Gene Expression, Histomorphology, and the Microbiota Profiles of Diet-Induced Obese C57BL/6J Mice // The Journal of Nutrition. 2016; 146(5): 949–956. DOI: 10.3945/jn.115.227504.
10. Karlsson F.H., Tremaroli V., Nookaew I., Bergström G., Behre C.J., Fagerberg B., et al. Gut metagenome in European women with normal, impaired and diabetic glucose control // Nature. 2013; 498(7452): 99–103. DOI: 10.1038/nature12198.
11. Cani P.D., Bibiloni R., Knauf C., Waget A., Neyrinck A.M., Delzenne N.M., et al. Changes in Gut Microbiota Control Metabolic Endotoxemia-Induced Inflammation in High-Fat Diet-Induced Obesity and Diabetes in Mice. 2008; 57(6): 1470–1481. DOI: 10.2337/db07–1403.
12. Roberfroid M., Gibson G.R., Hoyles L., McCartney A.L., Rastall R., Rowland I., et al. Prebiotic effects: metabolic and health benefits // The British journal of nutrition. 2010; 104 Suppl. 2: 1–63. DOI: 10.1017/S0007114510003363.
13. Dehghan P., Gargari B.P., Jafar-Abadi M.A., Aliasgharzadeh A. Inulin controls inflammation and metabolic endotoxemia in women with type 2 diabetes mellitus: a randomized-controlled clinical trial // International Journal of Food Sciences and Nutrition. 2014; 65(1): 117–123. DOI: 10.3109/09637486.2013.836738.
14. Beylot M. Effects of inulin-type fructans on lipid metabolism in man and in animal models // British Journal of Nutrition. 2005; 93(S1): 163–168. DOI: 10.1079/bjn20041339.
15. Ooi L.G., Liong M.T. Cholesterol-lowering effects of probiotics and prebiotics: a review of in vivo and in vitro findings. International journal of molecular sciences. 2010; 11(6): 2499–2522. DOI: 10.3390/ijms11062499.
16. Dikeman C.L., Murphy M.R., Fahey G.C., Jr. Dietary Fibers Affect Viscosity of Solutions and Simulated Human Gastric and Small Intestinal Digesta // The Journal of Nutrition. 2006; 136(4): 913–919. DOI: 10.1093/jn/136.4.913.
17. Levrat M.-A., Rémésy C., Demigné C. High Propionic Acid Fermentations and Mineral Accumulation in the Cecum of Rats Adapted to Different Levels of Inulin // The Journal of Nutrition. 1991; 121(11): 1730–1737. DOI: 10.1093/jn/121.11.1730.
18. Delzenne N.M., Daubioul C., Neyrinck A., Lasa M., Taper H.S. Inulin and oligofructose modulate lipid metabolism in animals: review of biochemical events and future prospects // Br.J. Nutr. 2002; 87 Suppl. 2: 255–259. DOI: 10.1079/BJNBJN/2002545.
19. Wolever T.M., Brighenti F., Royall D., Jenkins A.L., Jenkins D.J. Effect of rectal infusion of short chain fatty acids in human subjects // Am.J. Gastroenterol. 1989; 84(9): 1027–1033.
20. Kimura I., Inoue D., Maeda T., Hara T., Ichimura A., Miyauchi S., et al. Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). 2011; 108(19): 8030–8035. DOI: 10.1073/pnas.1016088108.
21. Gérard C., Vidal H. Impact of Gut Microbiota on Host Glycemic Control // Frontiers in Endocrinology. 2019; 10(29).
22. Rossi M., Corradini C., Amaretti A., Nicolini M., Pompei A., Zanoni S., et al. Fermentation of fructooligosaccharides and inulin by bifidobacteria: a comparative study of pure and fecal cultures // Appl. Environ. Microbiol. 2005; 71(10): 6150–6158. DOI: 10.1128/AEM.71.10.6150–6158.2005.
23. Trautwein E.A., Rieckhoff Dr., Erbersdobler H.F. Dietary Inulin Lowers Plasma Cholesterol and Triacylglycerol and Alters Biliary Bile Acid Profile in Hamsters // The Journal of Nutrition. 1998; 128(11): 1937–1943.
24. Safronova I.V., Gaidul K.V., Kozlov V.A. Features of the chemical composition of cowberry and prospects of its use in medicine and healthy nutrition // Innovations and food security. 2015; 4: 63–73.
25. Ma Z.-J., Sun P., Guo G., Zhang R., Chen L.-M. Association of the HLA-DQA1 and HLA-DQB1

Alleles in Type 2 Diabetes Mellitus and Diabetic Nephropathy in the Han Ethnicity of China // Journal of Diabetes Research. 2013; 2013: 452537. DOI: 10.1155/2013/452537.

26. *Senthilkumar G.P., Anithalekshmi M.S., Yasir M., Parameswaran S., Packirisamy Rm., Bobby Z. Role of omentin 1 and IL-6 in type 2 diabetes mellitus patients with diabetic nephropathy // Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2018; 12(1): 23–26. DOI: 10.1016/j.dsx.2017.08.005.*
27. *Lv M., Chen Z., Hu G., Li Q. Therapeutic strategies of diabetic nephropathy: recent progress and future perspectives // Drug Discov. Today. 2015; 20(3): 332–346. DOI: 10.1016/j.drudis.2014.10.007.*
28. *Lv L., Zhang J., Tian F., Li X., Li D., Yu X. Arbutin protects HK-2 cells against high glucose-induced apoptosis and autophagy by up-regulating microRNA-27a // Artificial Cells, Nanomedicine, and Biotechnology. 2019; 47(1): 2940–2947. DOI: 10.1080/21691401.2019.1640231.*
29. *Jurica K., Brčić Karačonji I., Mikolić A., Milojković-Opsenica D., Benković V, Kopjar N. In vitro safety assessment of the strawberry tree (Arbutus unedo L.) water leaf extract and arbutin in human peripheral blood lymphocytes // Cytotechnology. 2018; 70(4): 1261–1278. DOI: 10.1007/s10616-018-0218-4.*
30. *Jurica K., Gobin I., Kremer D., Čepo D.V., Grubešić R.J., Karačonji I.B., et al. Arbutin and its metabolite hydroquinone as the main factors in the antimicrobial effect of strawberry tree (Arbutus unedo L.) leaves // Journal of Herbal Medicine. 2017; 8: 17–23. DOI: 10.1016/j.hermed.2017.03.006.*
31. *Zhang B., Zeng M., Li B., Wang Y., Kan Y., Wang S., et al. Inhibition of oxidative stress and autophagy by arbutin in lipopolysaccharide-induced myocardial injury. 2019; 15(63): 507–513. DOI: 10.4103/pm.pm_552_18.*
32. *Farzanegi P., Habibian M., Anvari S.M. Effect of swimming training and arbutin supplement on cardiac antioxidant enzymes and oxidative stress in diabetic rats // Journal of Gorgan University of Medical Sciences. 2015; 17(3): 39–44.*
33. *Bang S.-H., Han S.-J., Kim D.-H.. Hydrolysis of arbutin to hydroquinone by human skin bacteria and its effect on antioxidant activity // Journal of Cosmetic Dermatology. 2008; 7(3): 189–193. DOI: 10.1111/j.1473-2165.2008.00387.x.*
34. *Guo X.-f., Yang B., Tang J., Jiang J.-J., Li D. Apple and pear consumption and type 2 diabetes mellitus risk: a meta-analysis of prospective cohort studies // Food & Function. 2017; 8(3): 927–934. DOI: 10.1039/c6fo01378c.*
35. *Mihaylova D., Vrancheva R., Petkova N., Ognyanov M., Desseva I., Ivanov I., et al. Carotenoids, tocopherols, organic acids, charbohydrate and mineral content in different medicinal plant extracts // J. Zeitschrift für Naturforschung C. 2018; 73(11–12): 439–448. DOI: 10.1515/znc-2018-0057.*
36. *Kostik V.B. B. Bioactive compounds of Rosa canina L. biotypes from spotaneouse flora of Republic of Macedonia. 2nd International Conference on Natural Products Utilization and safety. 2015: 25.*
37. *Kharazmi A., Winther K. Rose hip inhibits chemotaxis and chemiluminescence of human peripheral blood neutrophils in vitro and reduces certain inflammatory parameters in vivo // InflammoPharmacology. 1999; 7(4): 377–386. DOI: 10.1007/s10787-999-0031-y.*
38. *Winther K., Apel K., Thamsborg G. A powder made from seeds and shells of a rose-hip subspecies (Rosa canina) reduces symptoms of knee and hip osteoarthritis: a randomized, double-blind, placebo-controlled clinical trial // Scandinavian Journal of Rheumatology. 2005; 34(4): 302–308. DOI: 10.1080/03009740510018624.*
39. *Winther K., Rein E., Kharazmi A. The anti-inflammatory properties of rose-hip // InflammoPharmacology. 1999; 7(1): 63–68. DOI: 10.1007/s10787-999-0026-8.*
40. *Jäger A.K., Eldeen I.M. S., van Staden J. COX-1 and -2 activity of rose hip // Phytotherapy*

Research. 2007; 21 (12): 1251–1252. DOI: 10.1002/ptr.2236.

41. *Willich S.N., Rossnagel K., Roll S., Wagner A., Mune O., Erlendson J., et al. Rose hip herbal remedy in patients with rheumatoid arthritis – a randomised controlled trial // Phytomedicine. 2010; 17(2): 87–93. DOI: 10.1016/j.phymed.2009.09.003.*
42. *Ninomiya K., Matsuda H., Kubo M., Morikawa T., Nishida N., Yoshikawa M. Potent anti-obese principle from Rosa canina: Structural requirements and mode of action of trans-tiliroside // Bioorganic & Medicinal Chemistry Letters. 2007; 17(11): 3059–3064. DOI: 10.1016/j.bmcl.2007.03.051.*
43. *Andersson U., Henriksson E., Ström K., Alenfall J., Göransson O., Holm C. Rose hip exerts antidiabetic effects via a mechanism involving down-regulation of the hepatic lipogenic program // American Journal of Physiology-Endocrinology and Metabolism. 2010; 300(1): 111–121. DOI: 10.1152/ajpendo.00268.2010.*
44. *Andersson U., Berger K., Högberg A., Landin-Olsson M., Holm C. Effects of rose hip intake on risk markers of type 2 diabetes and cardiovascular disease: a randomized, double-blind, cross-over investigation in obese persons // European Journal of Clinical Nutrition. 2012; 66(5): 585–590. DOI: 10.1038/ejcn.2011.203.*
45. *Wojtyniak K., Szymański M., Matławska I. Leonurus cardiaca L. (Motherwort): A Review of its Phytochemistry and Pharmacology. 2013; 27(8): 1115–1120. DOI: 10.1002/ptr.4850.*
46. *Kellett G., Brot-Laroche E. Apical GLUT2: A Major Pathway of Intestinal Sugar Absorption // Diabetes. 2005; 54: 3056–3062. DOI: 10.2337/diabetes.54.10.3056.*
47. *Sato S., Takeo J., Aoyama C., Kawahara H. Na+-Glucose cotransporter (SGLT) inhibitory flavonoids from the roots of Sophora flavescens // Bioorganic & Medicinal Chemistry. 2007; 15(10): 3445–3449. DOI: 10.1016/j.bmc.2007.03.011.*
48. *Kottra G., Daniel H. Flavonoid Glycosides Are Not Transported by the Human Na+/Glucose Transporter When Expressed in Xenopus laevis Oocytes, but Effectively Inhibit Electrogenic Glucose Uptake // Journal of Pharmacology and Experimental Therapeutics. 2007; 322(2): 829–835. DOI: 10.1124/jpet.107.124040.*
49. *Wang Y.B., Ge Z.M., Kang W.Q., Lian Z.X., Yao J., Zhou C.Y. Rutin alleviates diabetic cardiomyopathy in a rat model of type 2 diabetes // Exp. Ther. Med. 2015; 9(2): 451–455. DOI: 10.3892/etm.2014.2090.*
50. *Liang W., Zhang D., Kang J., Meng X., Yang J., Yang L., et al. Protective effects of rutin on liver injury in type 2 diabetic db/db mice // Biomedicine & Pharmacotherapy. 2018; 107: 721–728. DOI: 10.1016/j.biopha.2018.08.046.*
51. *Ghorbani A. Mechanisms of antidiabetic effects of flavonoid rutin // Biomedicine & Pharmacotherapy. 2017; 96: 305–312. DOI: 10.1016/j.biopha.2017.10.001.*

UDC 615.322:574.2
https://www.doi.org/10.34907/JPQAI.2021.45.57.002

STUDY OF TOTAL MINERAL COMPLEX OF MEDICINAL PLANT RAW MATERIALS OF SYNANTHROPIC FLORA IN THE VORONEZH REGION

N.A. Dyakova, *Candidate of Biological Science, Associate Professor of the Department of Pharmaceutical Chemistry and Pharmaceutical Engineering, Voronezh State University, Voronezh, Russia, Ninochka_V89@mail.ru*

On the basis of ten plants, the content of the total mineral complex of medicinal plant raw materials of agro- and urban cenoses of the Voronezh region was studied. The study was provided on the example of medicinal plant raw materials harvested within the time limits according to the regulatory documentation. The most frequent excess of the standard values for the "total ash" numerical indicator was noted for samples of quinquelobate motherwort (Leonurus quinquelobatus) herb and common tansy (Tanacetum vulgare) flowers (in 16 of 51 samples), which can be explained by pubescence of these types of raw materials absorbing well the pollutants suspended in the air, as well as by sufficiently high requirements of the relevant pharmacopoeial monographs for these types of raw materials. In 15 samples of common plantain leaves out of 51 studied samples, the total content of mineral substances was also exceeded, which is caused by the large area of the leaf blade located mainly in the horizontal plane, as well as its squat growth, which creates good conditions for the deposition of dust particles on the raw material surface. The analysis of the average values of the total ash content makes it possible to build a series of reductions in the content of the total mineral complex from the analyzed types in medicinal plant raw materials, which looks as follows: leaves of common plantain (Plantago major L.) > leaves of stinging nettle (Urtica dioica L.) > knotgrass (Polygonum aviculare L.) herb > common yarrow (Achillea millefolium L.) herb > absinthium (Artemisia absinthium L.) herb> quinquelobate motherwort (Leonurus quinquelobatus Gilib.) herb > flowers of common tansy (Tanacetum vulgare L.) > flowers of tillet (Tilia cordata Mill.), > roots of common burdock (Arctium lappa L.) > roots of dandelion (Taraxacum officinale F.H. Wigg).

Keywords: ash total, Voronezh region, *Polygonum aviculare* L., *Artemisia absinthium* L., *Achillea millefolium* L., *Leonurus quinquelobatus* Gilib., *Plantago major* L., *Urtica dioica* L., *Tilia cordata* Mill., *Tanacetum vulgare* L., *Taraxacum officinale* F.H. Wigg, *Arctium lappa* L.

Herbal medicinal products in the domestic pharmaceutical market have always been very popular, which is explained by their good therapeutic effect and relative harmlessness. Thus, according to the data of the Register of Medicines of Russia for July 2021, there are more than 2.1 thousand herbal medicinal products, and the number of biologically active supplements based on medicinal plant raw materials (MPRM) exceeds 7.9 thousand [1]. At the same time, a large share of medicinal plant raw materials collected falls on the European part of the Russian Federation, which is characterized by significant population density, high economic activity, and dynamic development of transport highways [2,3]. In this regard, the threat of harvesting the medicinal

plant raw materials in ecologically unfavorable areas increases and the relevance of identifying the influence of anthropogenic pollution on the chemical composition of plants increases [2].

Currently, there are about 200 kg of suspended dust particles for every inhabitant of Russia. According to the data of the Federal Service for Hydrometeorology and Environmental Monitoring as of 01.01.2019, Voronezh belongs to the four cities of Russia in which the average annual concentrations of suspended solids in the air exceed the MPC by more than twice, and the city is the first in this negative rating (the excess of the MPC of suspended substances by 3.1 times). At the same time, according to the Hydrometeorological Center, in especially dry summers and autumns, the dust MPC in Voronezh is exceeded by 3.3 times [4].

In pharmacopoeia analysis, the "total ash" indicator allows to judge about the total mineral complex of the medicinal plant raw materials. The "total ash" is the residue of inorganic substances, which is obtained as a result of burning medicinal substances or medicinal plant raw materials and subsequent calcination up to constant weight. The definition is based on the fact that some analyzed objects do not contain elements which are capable of producing ash residue. Other objects burn up leaving a mineral residue that has a more or less definite value. The total ash content makes it possible to judge about the mineral residue associated with the presence of inorganic substances in the plant, as well as with the content of impurities in it that got into the raw material from the outside. Deviations in the amount of the ash residue in comparison with the natural ash content indicate contamination of the analyzed object with mineralizing impurities, in particular dust particles [5,6].

The purpose of the research is to study the content of the total mineral complex of medicinal plant raw materials of agro- and urban cenoses of the Voronezh region.

MATERIALS AND METHODS

As study objects quinquelobate motherwort (*Leonurus quinquelobatus* Gilib.) herb, knotgrass (*Polygonum aviculare* L.) herb, absinthium (*Artemisia absinthium* L.) herb, common yarrow (*Achillea millefolium* L.) herb, leaves of stinging nettle (*Urtica dioica* L.), leaves of common plantain (*Plantago major* L.), flowers of tillet (*Tilia cordata* Mill.), flowers of common tansy (*Tanacetum vulgare* L.), roots of dandelion (*Taraxacum officinale* F.H. Wigg), roots of common burdock (*Arctium lappa* L.) were used. When choosing the study objects, researchers were guided by several conditions: different types of medicinal plant raw materials were presented, including different organs or groups of plant organs (leaves, flowers, herb) from different forms of source plants – herbaceous and woody forms of vegetation. In addition, the selected objects are representatives of both natural plant communities and synanthropic vegetation. They are harvested mainly from wild raw materials in central Russia, including in the Voronezh region.

The choice of the studied areas is determined by the nature of the specific anthropogenic impact on them (Fig. 1, Table 1): combined heat power plant (CHP) (Fig. 1: 27); nuclear power plant (NPP) (Fig. 1: 8); chemical industrial enterprises (Fig. 1: 23, 24, 28); international airport (Fig. 1: 30); Voronezh street (Fig. 1: 31); high-voltage power transmission lines (Fig. 1: 9); Voronezh reservoir (Fig. 1: 29); small towns – Borisoglebsk (Fig. 1: 25), Kalach (Fig. 1: 26); nickel ore deposit zone (Fig. 1: 4); zones of radioactive contamination after the Chernobyl NPP accident (Fig. 1: 5–7); areas of active crop production using chemicals (Fig. 1: 10–22); control (for comparison) – protected areas (Fig. 1: 1–3). Sampling was also carried out along roads of varying degrees of load: forest zone (Fig. 1: 32–35) – highway M-4, forest-steppe zone (Fig. 1: 36–39) – highway A-144, steppe zone (Fig. 1: 40–43) – highway M-4, country road (Fig. 1: 44–47) and railway (Fig. 1:

48–51). The order of sampling from highways was determined in increments of one hundred meters (0, 100, 200, 300 m).

The total ash content in the tested samples of medicinal plant raw materials, characterizing the content of mineral substances which are peculiar to raw materials, and foreign mineral impurities, was determined in accordance with the OFS.1.2.2.2.0013.15 “General ash” [6]. The comparison was carried out with numerical indicators specified in individual pharmacopoeia monographs for these types of raw materials [7]. Each determination was carried out three times. The data obtained during the study was statistically processed in Microsoft Excel.

RESULTS AND DISCUSSION

The obtained average values of the results of determining the total ash content in the studied samples of medicinal plant raw materials are shown in Table 1.

All samples of medicinal plant raw materials harvested in control territories and in conditions of agrobiocenoses meet the requirements of pharmacopoeia monographs for the studied types of medicinal plant raw materials according to the “total ash” indicator [7].

In a number of samples of raw materials harvested in urban cenoses of the Voronezh region, an excess of the numerical parameters of the total

FIG. 1. *Map of sampling (numbers are deciphered in the text)*

Table 1

TOTAL ASH CONTENT, %

№	Area of harvesting	Type of medicinal plant raw materials									
		Knotgrass (*Polygonum aviculare* L.) herb	Absinthium (*Artemisia absinthium* L.) herb	Common yarrow (*Achillea millefolium* L.) herb	Quinquelobate motherwort (*Leonurus quinquelobatus* Gilib) herb	Leaves of common plantain (*Plantago major* L.)	Leaves of stinging nettle (*Urtica dioica* L.)	Flowers of tillet (*Tilia cordata* Mill.)	Flowers of common tansy (*Tanacetum vulgare* L.)	Roots of dandelion (*Taraxacum officinale* F.H. Wigg)	Roots of common burdock (*Arctium lappa* L.)
1.	Voronezh Biosphere Reserve	7.72	8.42	9.34	8.01	11.23	12.73	5.92	6.27	4.74	6.86
2.	Khopersky Nature Reserve (Novokhopersky district)	6.19	6.90	8.67	7.34	13.85	14.09	6.05	5.07	4.90	7.22
3.	Khopersky Nature Reserve (Borisoglebsky district)	9.42	7.99	7.44	6.06	12.09	11.85	4.82	7.30	3.88	5.30
4.	Village settlement of Elan-Koleno	8.60	9.05	10.40	8.90	10.11	16.73	3.22	5.33	5.10	6.17
5.	Village settlement of Nizhnedevitsk	9.25	8.03	8.55	6.55	9.85	17.05	5.08	6.86	6.12	7.88
6.	Town of Ostrogozhsk	7.32	8.44	9.42	10.08	14.88	19.00	6.93	7.45	4.90	7.15
7.	Town of Semiluki	9.90	10.28	10.97	9.65	13.05	18.41	7.30	8.22	5.31	4.99
8.	Town of Novovoronezh	10.74	11.09	8.08	8.07	16.72	15.38	6.59	8.00	4.89	6.12
9.	High-voltage power transmission line	12.61	13.50	8.12	14.55	17.21	20.67	9.03	10.44	5.09	5.52
10.	Liskinsky district	7.94	6.59	6.55	7.54	9.55	12.09	8.35	6.19	5.12	6.78
11.	Olkhovatsky district	10.73	7.02	7.09	6.49	14.52	14.44	7.43	7.02	4.90	5.55
12.	Podgorensky district	11.68	7.88	6.02	8.05	17.43	17.02	6.08	8.21	5.33	6.19
13.	Petropavlovsk district	9.21	8.90	6.90	8.39	14.08	16.71	5.21	6.23	6.17	6.97
14.	Gribanovsky district	10.00	9.39	8.16	7.62	10.12	12.34	8.53	3.78	3.90	5.76
15.	Khokholsky district	7.55	7.21	5.41	7.37	15.62	10.06	4.94	4.90	4.76	7.09
16.	Novokhopersky district	11.07	10.05	7.08	9.05	16.02	8.32	3.86	7.29	5.95	8.09
17.	Repyevsky district	6.82	7.03	5.32	8.88	9.00	9.07	7.34	6.55	6.41	7.22
18.	Vorobyevsky district	8.74	7.53	7.22	5.66	12.85	12.89	6.66	5.90	5.08	6.77
19.	Paninsky district	9.05	8.90	6.07	7.79	10.64	13.06	5.39	6.29	5.37	7.39
20.	Verkhnekhavsky district	6.86	8.05	9.07	8.40	13.99	15.28	4.28	5.89	5.62	6.84
21.	The town of Ertil	10.43	9.32	10.21	10.42	17.32	13.75	4.23	7.77	6.82	7.03
22.	Rossoshansky district	7.60	7.96	8.09	7.56	12.48	9.45	5.28	7.95	4.08	8.67
23.	Near Minudobreniya JSC	12.33	11.67	14.19	11.05	19.81	17.82	8.94	8.32	6.02	7.09
24.	near Bormash LLC	11.02	12.05	13.05	14.09	23.62	18.90	11.53	9.90	5.76	7.22

Table continuation 1

№	Area of harvesting	Type of medicinal plant raw materials									
		Knotgrass (*Polygonum aviculare* L.) herb	Absinthium (*Artemisia absinthium* L.) herb	Common yarrow (*Achillea millefolium* L.) herb	Quinquelobate motherwort (*Leonurus quinquelobatus* Gilib) herb	Leaves of common plantain (*Plantago major* L.)	Leaves of stinging nettle (*Urtica dioica* L.)	Flowers of tillet (*Tilia cordata* Mill.)	Flowers of common tansy (*Tanacetum vulgare* L.)	Roots of dandelion (*Taraxacum officinale* F.H. Wigg)	Roots of common burdock (*Arctium lappa* L.)
25.	The town of Borisoglebsk	15.74	14.17	14.11	10.98	28.26	23.94	12.40	7.40	3.87	8.95
26.	The town of Kalach	17.40	15.90	13.79	12.66	22.91	20.65	9.42	8.29	4.10	6.43
27.	near the TPP "VOGRES"	15.97	14.86	14.21	13.09	25.05	19.53	13.72	9.55	7.27	7.55
28.	near Sibur LLC	12.56	11.09	15.76	13.36	28.04	25.97	12.07	10.55	5.08	7.78
29.	along the Voronezh reservoir	9.96	7.45	9.02	7.73	17.93	15.51	6.12	7.33	6.25	7.37
30.	airport named after Peter I	11.88	9.04	10.44	11.26	19.42	18.57	8.03	8.20	6.11	7.99
31.	Street of Voronezh (Dimitrova Street)	19.74	18.09	18.77	19.04	31.74	29.15	15.94	16.03	8.53	9.88
32.	along the M-4 highway (Ramonsky district)	21.94	19.55	19.99	18.90	29.52	30.41	15.09	18.12	9.01	10.04
33.	100 m from the M-4 highway (Ramonsky district)	17.42	16.32	17.08	15.53	25.71	23.91	12.07	10.67	5.55	8.27
34.	200 m from the M-4 highway (Ramonsky district)	11.80	10.07	13.11	11.08	18.64	15.06	7.90	8.43	4.89	6.12
35.	300 m from the M-4 highway (Ramonsky district)	10.78	10.29	11.98	9.06	17.93	16.38	6.08	6.22	3.98	6.08
36.	along the A-144 highway	16.06	17.46	18.90	17.51	30.06	26.41	12.85	15.35	8.31	9.51
37.	100 m from the A-144 highway	15.33	15.40	16.52	15.30	27.42	23.06	12.09	12.22	5.78	7.22
38.	200 m from the A-144 highway	13.84	14.80	15.08	11.22	20.65	17.41	9.69	11.08	6.17	7.08
39.	300 m from the A-144 highway	12.55	11.06	10.12	9.60	17.32	13.09	7.53	7.45	4.21	6.41
40.	along the M-4 highway (Pavlovsky district)	18.94	17.46	18.89	19.05	28.51	32.62	14.98	14.87	7.77	7.90
41.	100 m from the M-4 highway (Pavlovsky district)	16.45	15.21	16.33	17.43	26.12	28.06	11.08	13.88	6.33	7.33
42.	200 m from the M-4 highway (Pavlovsky district)	16.73	13.75	13.16	13.75	23.33	23.95	9.34	13.07	6.04	7.97
43.	300 m from the M-4 highway (Pavlovsky district)	12.60	11.87	12.87	12.66	19.38	18.04	6.38	12.08	5.28	7.90
44.	along a non-high-speed road	13.65	12.33	14.21	13.88	19.08	19.05	9.04	10.55	4.39	8.89

End of table 1

№	Area of harvesting	Type of medicinal plant raw materials									
		Knotgrass (*Polygonum aviculare* L.) herb	Absinthium (*Artemisia absinthium* L.) herb	Common yarrow (*Achillea millefolium* L.) herb	Quinquelobate motherwort (*Leonurus quinquelobatus* Gilib) herb	Leaves of common plantain (*Plantago major* L.)	Leaves of stinging nettle (*Urtica dioica* L.)	Flowers of tillet (*Tilia cordata* Mill.)	Flowers of common tansy (*Tanacetum vulgare* L.)	Roots of dandelion (*Taraxacum officinale* F.H. Wigg)	Roots of common burdock (*Arctium lappa* L.)
45.	100 m from a non-high-speed road	10.53	10.89	12.04	10.62	19.57	15.98	8.34	8.90	5.99	6.80
46.	200 m from a non-high-speed road	9.05	9.06	12.14	8.09	17.31	16.62	7.09	6.00	4.65	7.31
47.	300 m from a non-high-speed road	7.41	8.21	11.08	8.50	14.02	10.09	7.66	6.27	3.90	6.91
48.	along the railway	15.62	14.22	16.78	14.74	24.63	20.43	12.87	11.98	6.59	7.90
49.	100 m from the railway	12.04	11.75	12.09	10.89	15.98	13.84	7.90	8.35	4.29	7.23
50.	200 m from the railway	11.49	9.56	11.20	8.44	13.80	11.08	6.94	5.08	5.22	6.47
51.	300 m from the railway	10.07	8.11	8.23	8.68	14.66	9.05	4.12	5.66	4.90	6.17
Average		11.77	11.00	11.36	10.80	18.29	17.35	8.23	8.64	5.50	7.20
Numerical indicator under the pharmacopoeial monograph, no more than		13	13	15	12	20	20	10	9	8	11

ash specified in the pharmacopoeia monograph was noted. Thus, the total content of mineral substances was exceeded in the absinthium (*Artemisia absinthium* L.) herb, quinquelobate motherwort (*Leonurus quinquelobatus* Gilib.) herb, leaves of stinging nettle (*Urtica dioica* L.), flowers of common tansy (*Tanacetum vulgare* L.) harvested under high-voltage power lines, characterized by the occurrence of corona discharges, accompanied by air ionization in an electric field with high intensity and the movement of gas particles and impurities contained therein from the corona electrode to the neutral power lead, that is, from high-voltage power lines to the ground, which contributes to the deposition of dust particles and other airborne pollutants on plants. The excess of the numerical indicator for total ash was noted in quinquelobate motherwort herb, common plantain leaves, flowers of tillet and flowers of common tansy harvested near Bormash LLC, as well as in the quinquelobate motherwort herb, common yarrow herb, stinging nettle leaves, common plantain leaves, tillet flowers and common tansy flowers harvested near Voronezhsintezkauchuk JSC.

The total content of mineral substances does not meet the requirements of the regulation documents in the knotgrass herb, absinthium herb, common plantain leaves, stinging nettle leaves, tillet flowers growing in Borisoglebsk, as well as in the knotgrass herb, absinthium herb, quinquelobate motherwort herb, stinging nettle leaves, common plantain leaves harvested in the town of Kalach. In the samples harvested near the TPP-1

"VOGRES", an excess of total ash was found in the grass of the knotgrass herb, absinthium herb, quinquelobate motherwort herb, common plantain leaves, tillet flowers and common tansy flowers.

In all samples of medicinal plant raw materials, except for the roots of common burdock, harvested on the street of Voronezh, along the highways M-4 "Don" in Ramonsky district and A-144 in Anninsky district, the excess of permissible levels for the "total ash" indicator was found. All the studied samples of herbs, leaves and flowers that grew along the railway tracks, along and at a distance of 100 m from the M-4 "Don" highway in the Pavlovsky district, at a distance of 100 m from the M-4 "Don" highway in the Ramonsky district and A-144 highway in the Anninsky district, also turned out to be of poor quality according to this numerical indicator. At a distance of 200 m from the highway A-144, the knotgrass herb, absinthium herb, common yarrow herb, common plantain leaves, common tansy flowers; at a distance of 200 m from the highway M-4 "Don" in the Pavlovsky district – the knotgrass herb, absinthium herb, quinquelobate motherwort herb, common plantain leaves, stinging nettle leaves, common tansy flowers; at a distance of 300 m from the highway M-4 in the Pavlovsky district – quinquelobate motherwort herb and flowers of common tansy were harvested that did not meet the requirements of the pharmacopoeial monograph.

Thus, the roots of common burdock can be recognized as the most prosperous in terms of "total ash" because all the harvested samples of this type of medicinal plant raw materials meet the requirements of the pharmacopoeia monograph, as well as the roots of dandelion, in which only three samples were found to be substandard, can be considered as the most successful. The results obtained can be explained by the absence of aerosol contamination of plant roots by airborne particles from motor transport and emissions from industrial enterprises.

The most frequent exceedance of statutory limits for the "total ash" numerical indicator was noted for samples of quinquelobate motherwort herb and common tansy flowers (in 16 of 51 samples), which can be explained by the pubescence of these types of raw materials that well absorb airborne pollutants, as well as sufficiently high requirements of the relevant pharmacopoeia monographs for these types of medicinal plant raw materials. In 15 samples of common plantain leaves out of 51 studied ones, the total content of mineral substances was also exceeded, which is caused by the large leaf blade of the plant located mainly in the horizontal plane, as well as its squat growth, which creates good conditions for the deposition of dust particles on the surface of the medicinal plant raw materials.

CONCLUSION

The content of total ash was determined as an indicator of the content of the total mineral complex and an indicator of contamination of medicinal plant raw materials by dust particles in ten objects collected within the periods of harvesting regulated by regulatory documentation in various agro- and urban cenoses of the Voronezh region. The analysis of the average values of the total ash content makes it possible to build a series of reductions in the content of the total mineral complex in the analyzed types of medicinal plant raw materials, which looks as follows: leaves of common plantain (*Plantago major* L.) > leaves of stinging nettle (*Urtica dioica* L.) > knotgrass (*Polygonum aviculare* L.) herb > common yarrow (*Achillea millefolium* L.) herb > absinthium (*Artemisia absinthium* L.) herb> quinquelobate motherwort (*Leonurus quinquelobatus* Gilib.) herb > flowers of common tansy (*Tanacetum vulgare* L.) > flowers of tillet (*Tilia cordata* Mill.) > roots of common burdock (*Arctium lappa* L.) > roots of dandelion (*Taraxacum officinale* F.H. Wigg).

REFERENCES

1. *Dyakova N.A. Ecological and hygienic assessment of the state of soils of anthropogenic ecosystems of the Voronezh region / N.A. Dyakova, A.I. Slivkin, S.P. Gaponov // Bulletin of Kaliningrad State Technical University. – 2020. – No. 59. – pp. 61–72.*
2. *Dyakova N.A. Accumulation of heavy metals and arsenic by absinthium herb / Dyakova N.A. // Bulletin of Saratov University. A new series. The series "Chemistry. Biology. Ecology". – 2020. – Vol. 20, No. 4. – pp. 445–453. DOI: https://doi. org/10.18500/1816-9775-2020-20-4-445-453*
3. *Dyakova N.A. Features of accumulation of biologically active substances in the roots of common burdock of the synanthropic flora in the Voronezh region / Dyakova N.A. // Conventional medicine. – 2021. – No. 2(65). – pp. 47–52.*
4. *Dyakova N.A. Identification of permissible zones of harvesting of medicinal plant raw materials near major transport routes / N.A. Dyakova, A.I. Slivkin, E.E. Chupandina, S.P. Gaponov // Chemistry of plant raw materials. – 2020. – No. 4. – pp. 5–13. DOI: 10.14258/jcprm.2020047609*
5. *The State Pharmacopoeia of the Russian Federation. Edition XIV. Vol. 2. – Moscow: Federal Electronic Medical Library (FEML), 2018. – 2303–2323 pp.*
6. *Kurkin V.A. Pharmacognosy / V.A. Kurkin. – Samara: Etching, 2004. – 46–50 pp.*
7. *The State Pharmacopoeia of the Russian Federation. Edition XIV. Vol. 4. – Moscow: Federal Electronic Medical Library (FEML), 2018. – 6251–6389 pp.*

UDC 615.072
https://www.doi.org/10.34907/JPQAI.2021.51.17.003

DEVELOPMENT AND VALIDATION OF A PROCEDURE FOR QUANTITATIVE ANALYSIS OF MOXIFLOXACIN IN THE "MOXIFLOXAZOL" MEDICINE

A.I. Zamaraeva, *Postgraduate Student of the Department of Chemistry, Tyumen State Medical University, Tyumen, Russia, anyuta.zamaraeva@yandex.ru*
T.A. Kobeleva, *Doctor of Pharmaceutical Sciences, Professor, Head of the Department of Chemistry, Tyumen State Medical University, Tyumen, Russia, kobeleva57@yandex.ru, kobeleva@tyumsmu.ru*
A.I. Sichko, *Doctor of Pharmaceutical Sciences, Professor of the Department of Chemistry, Tyumen State Medical University, Tyumen, Russia, sichko@tyumsmu.ru*
N.S. Bessonova, *Candidate of Biological Sciences, Associate Professor of the Department of Chemistry, Tyumen State Medical University, Tyumen, Russia, bessonov21@mail.ru*
E.M. Shapovalova, *Doctor of Biological Sciences, Associate Professor, Professor of the Department of Chemistry, Tyumen State Medical University, Tyumen, Russia, shapovalova@tyumsmu.ru*

In this work, the absorption spectra of moxifloxacin in ethanol were studied and it was found that quantitative analysis of the medical product should be rationally carried out at λmax = 296 nm. Experimental data on the quantitative analysis of moxifloxacin in the "Moxifloxazol" medicine are presented. The relative error of the analysis is not higher than ±1.82%. The sensitivity of the determination of moxifloxacin is 0.213 μg /ml. The developed procedure was validated according to the following validation characteristics: specificity, linearity, correctness and precision. The moxifloxacin content in the ointment is calculated by the method of the calibration graph equation. The calculated content is within the range of 0.0482–0.0544 g and corresponds to the standards of permissible deviations.

Keywords: moxifloxacin, Tizol gel, quantitative analysis, UV spectrophotometry, validation

Moxifloxacin is a chemotherapeutic agent of the fluoroquinolone group with a wide spectrum of bactericidal action. Due to the ability to suppress microorganisms which are resistant to other antibiotics, the relevance of expanding the nomenclature of dosage forms that include it increases [8,9]. The substance of moxifloxacin hydrochloride is represented by crystalline powder from light yellow to yellow and characterized by moderate solubility in water, low solubility or very low solubility in 96% alcohol. Currently, the European Pharmacopoeia recommends using the high-performance liquid chromatography (HPLC) analysis for quantitative determination of the moxifloxacin hydrochloride substance and its dosage forms [10]. This method is also regulated by the draft pharmacopoeial monograph "PM Moxifloxacin hydrochloride", considered at the Council of the Ministry of Health of the Russian Federation on the State Pharmacopoeia. In addition, a number of studies describe the possibility of using the UV spectrophotometry method for quantitative analysis of moxifloxacin [2,6].

We offer an ointment under the conventional name "Moxifloxazol" containing 0.05 g of moxifloxacin and Tizol gel up to 10.0 g. The medicine may be in demand in the treatment of a number of dermatological, dental and ophthalmological

diseases caused by pathogenic microflora. The modern low-toxic base "Tizol" will contribute to the increased conductivity of the medicine to the lesion, as well as provide anti-inflammatory, antiseptic, antipruritic and analgesic effects [5]. When developing new medicines, the "Quantitative Determination" test is important, which allows assessing the quality of the finished medical product [3,4].

The purpose of this work is to develop and validate the procedures of quantitative analysis of moxifloxacin in the "Moxifloxazol" medicine.

MATERIALS AND METHODS

The moxifloxacin hydrochloride substance (Neuland Laboratories Limited, India, PM 000-715-081118, 2018), titanium-containing Tizol gel (Olympus LLC, Yekaterinburg, Russia, FSP 3157-06), solutions of moxifloxacin in 95% ethyl alcohol (RFK CJSC, Russia, FS.2.1.0105.18), hydrochloric acid 0.01 mol/l (Bashkir Soda Company, Russia), ointment under the conventional name "Moxifloxazol" containing 0.5% of the medicine in the Tizol gel were used in this study. The study was carried out using a spectrophotometer SF-2000 (OKB SPECTRUM CJSC, St. Petersburg, Russia).

Quantitative determination of moxifloxacin was carried out by an easy-to-perform UV spectrophotometric method, which is used in the analysis of the medicine in tablets and is not inferior to chromatography in accuracy. The weight percent and the weight of moxifloxacin in grams were calculated using a calibration graph.

When constructing the calibration graph, a 0.02% standard solution (reference standard) of the medicine in ethyl alcohol was prepared. Then a variable amount of milliliters (from 0.2 ml to 1.2 ml) of the test solution was transferred to a measuring flask with capacity of 25 ml and the volume of liquid in the flask was brought to the mark with ethanol. The optical densities of the obtained solutions were measured using a spectrophotometer at a wavelength of 296 nm. Based on experimental data, a calibration line was constructed in coordinates A – C, µg/ml (Fig. 2). To obtain objective results of the analysis, eight parallel experiments were conducted using 0.6 ml of the initial solution. The moxifloxacin content as a percentage was calculated using the formula (1):

$$W = \frac{C(mox) \cdot V(total) \cdot V_2 \cdot 100}{10^6 \cdot a(mox) \cdot V_1}, \quad (1)$$

where C(mox) – the concentration of moxifloxacin, calculated by the equation of the calibration graph, µg /ml; V(total) – the volume of ethyl alcohol containing the weight of moxifloxacin, 100 ml; V_1, V_2 – dilution factor, 0.6 ml and 25 ml; a(mox) – moxifloxacin weighed sample, 0.02 g.

The model mixture was prepared taking into account the solubility of moxifloxacin in ethyl alcohol (moxifloxacin 0.05 g, ethanol 200 ml). Research procedure: 4 ml of ethanol solution is introduced into a measuring flask and the volume of liquid in the flask is brought to 25 ml with ethanol. Next, ethanol is added to 3 ml of the resulting solution to a total volume of 25 ml. The optical density of the solution is measured with respect to ethanol at a wavelength of 296 nm. The weight of moxifloxacin in the simulated solution is found by the formula (2):

$$m(mox) = \frac{C(mox) \cdot V(total) \cdot V_2 \cdot V_3}{10^6 \cdot V \cdot V_1}, \quad (2)$$

where m(mox) – the weight of moxifloxacin, g; V(total) – the volume of the simulated solution, 200 ml; V – the volume of the simulated solution taken for analysis, 4 ml; V1, V2, V3 – reciprocal dilution, 3 ml, 25 ml, 25 ml, respectively.

Quantitative analysis of moxifloxacin in the ointment "Moxifloxazol" was carried out as follows: 4 ml of 0.01 mol/l hydrochloric acid

solution and ethanol were added to the ointment weighed sample (about 0.10 g) to obtain a total volume of 30 ml. The mixture was stirred and filtered through a folded filter with discarding the first portion of the filtrate. Then 7 ml of ethanol was added to 3 ml of the resulting solution and the mixture was scanned photometrically at a wavelength of 296 nm. The compensation solution was an ethanol extract from 0.10 g of Tizol gel, obtained similarly to the study of moxifloxacin. The concentration of the medicine in the sample (μg/ml) was found by the equation of the calibration graph, and the weight percent and the weight in ointment were calculated by the formulas (3, 4):

$$m(mox) = \frac{C(mox) \cdot V(total) \cdot V_2 \cdot P}{10^6 \cdot a(ointment) \cdot V_1}, \quad (3)$$

$$W = \frac{C(mox) \cdot V(total) \cdot 100 \cdot V_2}{10^6 \cdot a(ointment) \cdot V_1}, \quad (4)$$

where a(ointment) is the weighed sample of ointment taken for analysis, g; P is the weight of the dosage form, 10.0 g; V_1, V_2 is the dilution factor, 3 ml and 10 ml, respectively; V(total) is the volume of ethyl alcohol containing the weighed sample of ointment, 30 ml.

RESULTS AND DISCUSSION

The conducted spectral analysis showed that for quantitative spectrophotometric determination of moxifloxacin in the ointment "Moxifloxazol" it is rationally to use absorption bands within the wavelengths of 280–310 nm with maximum absorption λ = 296 nm (Fig. 1)

As experimental data have shown, the similar maxima and minima are observed on the ethanol absorption spectra of moxifloxacin together with the Tizol gel, as in the absence of a base (Fig. 1, curves 2, 3). Extreme points also coincide on the absorption spectrum of the ethanol extract of moxifloxacin from ointment (curve 4).

Validation of the procedure was carried out according to the State Pharmacopoeia of the Russian Federation of the XIV edition, OFS.1.1.0012.15 "Validation of analytical procedures".

Specificity

To determine the specificity using a spectrophotometer, the spectral data on placebo solutions (ethanol extract of Tizol gel and 95% ethanol) and a standard moxifloxacin solution were sequentially collected, according to the quantitative analysis procedure. The obtained spectra did not contain the peaks which were characteristic of the standard moxifloxacin solution (Fig. 1, curves 5 and 6).

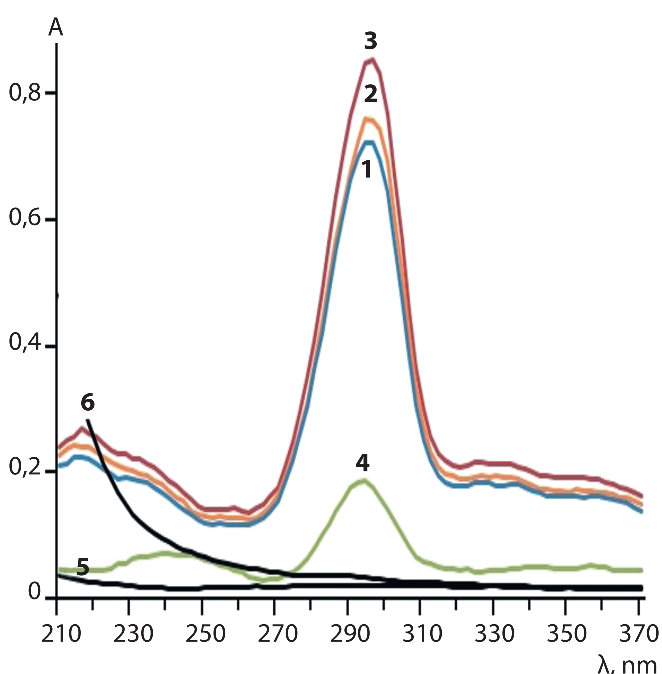


FIG. 1. *Dependence of light absorption of ethanol solutions of moxifloxacin and placebo solutions on wavelength:*
1 – moxifloxacin concentration 2.0×10⁻⁵ mol/L;
2 – moxifloxacin concentration 2.0×10⁻⁵ mol/L; Tizol gel 5.0×10⁻⁵ mol/L;
3 – moxifloxacin concentration 2.0×10⁻⁵ mol/L; Tizol gel 1.0×10⁻⁴ mol/L;
4 – ethanol extract of moxifloxacin from ointment (C = 4.3×10⁻⁶ mol/L);
5 – ethanol extract of Tizol gel 4×10⁻⁵ mol/L;
6 – 95% ethanol

Table 1

RESULTS OF THE REGRESSION ANALYSIS OF MOXIFLOXACIN

x_i, μg/ml	y_i	$x_i y_i$	x_i^2	y_i^2	b	C, μg/ml
1.6	0.15	0.24	2.56	0.023	0.0938	0.213
3.2	0.30	0.96	10.24	0.090		
4.8	0.45	2.16	23.04	0.203		
6.4	0.60	3.84	40.96	0.360		
8.0	0.75	6.00	64.00	0.563		
9.6	0.90	8.64	92.16	0.810		
33.6	3.15	21.84	232.96	2.049		

Linearity

In order to establish the linearity of the procedure, the optical densities of moxifloxacin solutions were experimentally measured in the range of 1.6 μg/ml – 9.6 μg/ml. At least five parallel experiments were carried out, on the basis of which the regression analysis indicators were calculated (Table 1). The statistical insignificance of the free term of the linear dependence was estimated. Linearity was considered optimal at values of the correlation coefficient $|r| \geq 0.99$ (Table 2).

It was found that the sensitivity of the moxifloxacin assay is 0.245 μg/ml, the value of the correlation coefficient satisfies the condition $|r| \geq 0.99$. The value of the free term of the linear dependence is less than its confidence interval, which gives reason to proceed to the equation of a straight line passing through the origin (Table 2).

According to the data obtained (Table. 1) we built a calibration graph. There is a direct relationship between the concentration of moxifloxacin and the optical density (Fig. 2). This indicates the possibility of analyzing moxifloxacin by UV spectrophotometry in ointment.

Accuracy and precision

The repeatability (convergence) of the validated procedure was evaluated using model mixtures of moxifloxacin under the same laboratory conditions during a short period of time based on the results of eight parallel experiments. Intra-laboratory precision was determined on different days with the participation of two researchers (analysts). The obtained data were statistically processed (Table 3).

The obtained values of the standard deviation (precision) and relative error (accuracy) do not exceed the limits of 100±2,0%.

Analytical range

The interval between the upper and lower values of the moxifloxacin concentration, within which the acceptable accuracy, precision

Table 2

MOXIFLOXACIN REGRESSION EQUATION IN UV SPECTROPHOTOMETRY

Regression equation	Correlation coefficient	$\|a\| \leq t(P; f) \cdot S_a$ at P = 95%	Equation of a straight line
y = 0.0938x – 0.00028	0.9981	0.00028 < 0.043	y = 0.0938x

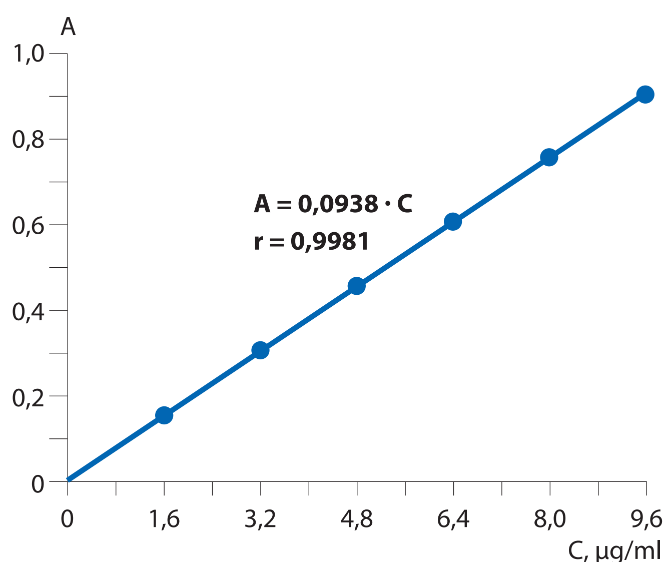


FIG. 2. *Calibration graph for the analysis of moxifloxacin*

and linearity of the procedure have been proven, ranges from 1.6 μg/ml to 9.6 μg/ml.

During the study, the moxifloxacin quantitative analysis procedure in a model dosage form was tested. Experimental data are specified in Table 4.

The weight of moxifloxacin in the simulated solution is from 0.0483 g to 0.0510 g with an acceptable range of 0.040–0.060 g [7].

Quantitative analysis of moxifloxacin in the ointment "Moxifloxazol" was carried out in an ethanol extract obtained from an exact weighted sample. Experiments have shown that about 22% of the medicine passes into the organic phase due to its low solubility in ethanol, while the weight fraction (%) increases in the presence of acid. To determine the optimal conditions for quantitative analysis, a model ointment with an exact content of moxifloxacin and a base was prepared. The studies were carried out in the presence of various volumes of 0.01 mol/l hydrochloric acid solution injected into ethanol. The results of the experiments are shown in Table 5.

Table 3

RESULTS OF THE EVALUATION OF THE ACCURACY AND PRECISION OF THE MOXIFLOXACIN SPECTROPHOTOMETRIC ANALYSIS PROCEDURE

First day			Second day			Metrological characteristics
A	Found		A	Found		
	C, μg/ml	x_i (W), %		C, μg/ml	x_i (W), %	
0.460	4.90	102.08	0.445	4.74	98.75	**First day** x = 100.05% S = 2.182 Sx = 0.772 ε_α = 1.82 A = ±1.82% Δ = 100.05 ± 1.82% **Second day** x = 100.08% S = 1.984 Sx = 0.702 ε_α = 1.66 A = ±1.66% Δ = 100.08 ± 1.66%
0.445	4.74	98.75	0.460	4.90	102.08	
0.450	4.80	100.0	0.440	4.69	97.71	
0.440	4.69	97.71	0.460	4.90	102.08	
0.445	4.74	98.75	0.440	4.69	97.71	
0.440	4.69	97.71	0.445	4.74	98.75	
0.465	4.96	103.33	0.460	4.90	102.08	
0.460	4.90	102.08	0.455	4.85	100.97	

Table 4

THE RESULTS OF THE ANALYSIS OF MOXIFLOXACIN IN A MODEL DOSAGE FORM BY THE METHOD OF THE EQUATION OF THE CALIBRATION GRAPH (A = 0,0938 · C)

№	Optical density	Weight, µg/ml	Found		Acceptance criteria	
			%	g	%	g
1	0.440	4.69	0.49	0.0489	±20.0	0.040–0.060
2	0.450	4.80	0.50	0.0500		
3	0.435	4.64	0.48	0.0483		
4	0.460	4.90	0.51	0.0510		
5	0.445	4.74	0.49	0.0494		
6	0.455	4.85	0.51	0.0505		

It has been found that when 4 ml of 0.01 mol /l hydrochloric acid solution is injected into the test solution, about 100% of moxifloxacin passes from the Tizol gel into an aqueous ethanol medium. We have accepted these analysis conditions as optimal.

According to the conducted research, a procedure of quantitative analysis of moxifloxacin in the ointment "Moxifloxazol" has been developed. The content of moxifloxacin in the ointment is in the range of 0.0482–0.0544 g (Table 6).

Table 5

DATA ON THE CHOICE OF OPTIMAL CONDITIONS FOR THE ANALYSIS OF MOXIFLOXACIN (A = 0,0938 · C)

№	Sampled				Optical density	Found W, %
	m (ointment), g	m (Tizol), g	Volume of 0,01 mol/L HCl, mL	Volume of ethanol, mL		
1	0.1033	0.1041	0.0	30.0	0.11 0.12	22.60 24.76
2	0.1023	0.1041	1.0	29.0	0.32 0.33	66.60 68.80
3	0.1047	0.1041	2.0	28.0	0.42 0.44	85.60 89.60
4	0.1056	0.1041	3.0	27.0	0.46 0.46	92.80 92.80
5	0.1030	0.1041	4.0	26.0	0.48 0.50	99.40 103.60
6	0.1017	0.1041	5.0	25.0	0.62 0.60	129.80 125.80

Table 6

RESULTS OF QUANTITATIVE ANALYSIS OF MOXIFLOXACIN IN OINTMENT BY THE METHOD OF EQUATION OF THE CALIBRATION GRAPH (A = 0,0938·C)

Sampled, g		Test results				Standard deviations	
ointment	Tizol	A	C, µg/ml	m, g	W, %	g	%
0.1038	0.1015	0.53	5.65	0.0544	0.54	0.040–0.060	±20.0
0.1038	0.1015	0.52	5.54	0.0534	0.53		
0.1038	0.1015	0.48	5.12	0.0493	0.49		
0.1038	0.1015	0.50	5.33	0.0513	0.51		
0.1038	0.1015	0.47	5.01	0.0482	0.48		
0.1038	0.1015	0.49	5.22	0.0503	0.50		

CONCLUSIONS

As a result of studying the ethanol absorption spectra of moxifloxacin, the optimal conditions for quantitative analysis of the semi-solid medicine have been established.

Based on experimental data, a procedure for quantitative analysis of moxifloxacin in a model mixture with a relative error of ± 1.82% is proposed.

The developed procedure for quantitative analysis of moxifloxacin in ointment based on the Tizol gel was validated according to the following validation characteristics: specificity, linearity, accuracy and precision. The studied validation characteristics meet the acceptance criteria.

The proposed procedure of quantitative analysis of moxifloxacin in the ointment "Moxifloxazol" shall be used rationally for inclusion in the regulatory documentation to assess the quality of the finished medicine.

REFERENCES

1. *Baranov Yu.N., Shormanov V.K., Nesterova A.V., Kovalenko E.A. Development and validation of a procedure for determining 2-dimethylamino-1,3-bis (phenylsulfonylthio) propane in the tissue of a putrefactively altered liver // Kursk Scientific and Practical Bulletin "Man and his health". 2018. – No.1. – pp. 121–127.*
2. *Dorofeev V.L., Titov I.V., Arzamastsev A.P. The use of the UV spectrophotometry method for the quantitative determination of medicines of the fluoroquinolone group // Bulletin of the Voronezh State University. Series: Chemistry. Biology. Pharmacy. – 2004. – No.2. – pp. 205–209.*
3. *Zamaraeva A.I., Bessonova N.S., Kobeleva T.A., Sichko A.I. Quantitative analysis and stability of a new dosage form "Metronidazol" // Bulletin of the Smolensk State Medical Academy. – 2020. – Vol. 19. – No. 2. – pp. 155–162.*
4. *Zamaraeva A.I., Bessonova N.S., Kobeleva T.A., Sichko A.I. Quantitative spectrophotometric analysis of the medicine "Metroketoconazol" // Issues of quality assurance of medicines. – 2020. – No. 4(30). – pp. 21–27.*
5. *Makhotina M.V., Sysuev B.B., Petrov A.Yu., Emelyanova I.V. Research of rheological characteristics of the original basis of Tizol-gel and medical compositions based on it according to manual prescriptions // Development and registration of medicines. – 2016. – No. 3(16). – pp. 44–47.*

6. *Menshikova L.A., Lvova A.A., Shokhin I.E., Boldina Yu.E., Komarov T.N., Medvedev Yu.V. Validation of the procedure for quantitative determination of moxifloxacin for the "Dissolution" test by UV spectrophotometry // Development and registration of medicines. – 2016. – No. 2(15). – pp. 94–97.*
7. *Order of the Ministry of Health of the Russian Federation No. 751n dated 26.10.2015 "On approval of the rules for the manufacture and release of medicines for medical use by pharmacy organizations, individual entrepreneurs licensed for pharmaceutical activities".*
8. *Sidorenko S.V. Fluoroquinolones: properties and clinical use // Difficult patient. – 2011. – No. 5. – pp. 21–27.*
9. *Sinopalnikov A.I. Moxifloxacin: focus on the safety profile // Medical Board. – 2013. – No. 11. – pp. 82–87.*
10. *European Pharmacopoeia, Strasbourg. – 2019. – 10th Ed. – P. 3306–3308.*

UDC: 615.074
https://www.doi.org/10.34907/JPQAI.2021.94.10.004

CONTENT OF ORGANIC ACIDS IN *PORTULACA OLERACEA* L. HERB

R.A. Nasser, *Postgraduate Student, pharmacist-analyst, Common Use Center (CUC) (REC), Peoples' Friendship University of Russia (RUDN University), Moscow, Russia*
O.G. Potanina, *Doctor of Pharmaceutical Sciences, Professor of the Faculty of Fundamental Medicine, Lomonosov Moscow State University, Moscow, Russia*
A.V. Nikulin, *Candidate of Chemical Sciences, Head of the Laboratory of Physical and Chemical methods of medicines research, Common Use Center (CUC) (REC), Peoples' Friendship University of Russia (RUDN University), Moscow, Russia*

Medicinal herbs are popular medicines because they are available, cheap, effective and low-toxic in most cases. People have been using them since ancient times to obtain medicines, food, insecticides, etc. One of these plants is Portulaca oleracea L. which is well known in folk medicine and widely used in food. Free organic acids are one of the important biologically active groups as a part of this plant, which are known for their numerous medicinal properties.

The purpose of this work is to study the content of the sum of free organic acids in the Green Purslane herb (Portulaca oleracea L.) using the pharmacopoeial procedure for determining the sum of organic acids in fresh viburnum berries, for which it is necessary to carry out qualitative confirmation of the content of free organic acids; to select the conditions and to develop a procedure for determining the sum of free organic acids as well as to provide the validation evaluation of the developed procedure in accordance with compendial requirements.

The scope of this study includes the qualitative and quantitative determination of the sum of organic acids in aqueous extracts from the Portulaca oleracea L. herb using the titration and TLC method; the main metrological characteristics are evaluated.

The procedures of qualitative and quantitative determination of the sum of organic acids in the Portulaca oleracea L. herb are proposed. The content of the sum of organic acids in the studied form of medicinal plant raw materials has been established. The relative error in determining the sum of organic acids is not higher than 5%. The content of the sum of organic acids in the Portulaca oleracea L. herb is at least 2%.

Conditions have been selected and methods have been developed for qualitative (using TLC) and quantitative (by titrometry) determination of the sum of organic acids in the Portulaca oleracea L. herb. The developed methods for determining the sum of organic acids in the Portulaca oleracea L. herb can be recommended for inclusion into the regulatory documentation for this type of raw material. The statistical characteristics obtained when determining repeatability correspond to the established criteria for the acceptability of the analytical procedure for determining the sum of free organic acids, which allows us to conclude that it is valid.

Keywords: standardization, titration, *Portulaca oleracea* L. herb, free organic acids, TLC

Green Purslane herb (*Portulaca oleracea* L.) is an herbaceous annual plant, which is a Eurasian species. It is found in Eastern and Western India, China, Japan, Ascension Island, as well as

in the British Isles [1]. On the territory of Russia it grows in the European part, the Caucasus, the Far East [2].

Since ancient times, the *Portulaca oleracea* L. herb was used for food in raw form in salads. The whole plant is edible. It is considered a valuable salad-spinach vegetable in most of Europe and Asia, in many parts of the United States, in developing countries. Over time, several different varieties of *portulaca* have been bred. It was noted that feeding of pets and birds with the leaves of *Portulaca oleracea* L. are useful for their immune system and as a prevention of diarrhea [3]. The widespread use of this plant in food can serve as a confirmation of the safety of its use. The possibility of cultivation will provide a sufficient raw material base.

Portulaca oleracea L. is used in folk medicine in many countries of the world as an antipyretic, antiseptic, anthelmintic agent and is included in the PRC SP [4,5]. It has a wide range of pharmacological activities, including antibacterial, antiulcer, anti-inflammatory, antioxidant and wound healing ones [6–10]. Russian scientists estimate it as a high vitamin plant [11].

Portulaca oleracea L. contains 3.5% lipids in terms of dry weight, 25% of which are free fatty acids. *Portulaca oleracea* L. is one of the richest sources of omega-3 polyunsaturated fatty acids at a level of 4 mg/g of wet weight [12–14]. When studying atherosclerosis, a proposal was made to use *Portulaca oleracea* L. as an alternative to fish oil in relation to omega-3 fatty acids [15]. *Portulaca* has an anti-atherosclerotic effect, increases blood clotting and lowers blood pressure [16]. According to other data, *Portulaca* contains nutrients in high percentages of recommended dietary intake such as alpha-linolenic acid, beta-carotene, tocopherol, magnesium and potassium [17–18].

Phenolic components, namely scopoletin, bergapten, isopimpinellin, lonchocarpic acid, lonchocarpenin, robustin and genistein, which have antimicrobial activity, were isolated from *Portulaca oleracea* [19].

Carotenoids are present in an amount of 89 mg/g. Beta-carotene is contained in significant amounts, but is lost up to 43% due to incorrect processing methods [20–21]. The level of a-tocopherol in the leaves of *Portulaca oleracea* L. is seven times higher than in spinach (1.71 mg per 100 g) [22]. Phylloquinone, or vitamin K1, is present in an amount of 381 mg per 100 g and is quite resistant to cooking [23].

A polysaccharide complex in the form of transparent and viscous mucus, having physicochemical properties suitable for industrial use as food fillers and thickeners, was extracted from the leaves of *Portulaca oleracea* L. It has been previously established that it is a neutral arabinogalactan and a polydisperse pectin-like polysaccharide [24]. We have identified a significant content of the sum of reducing sugars (at least 9%) in the Green Purslane herb [25].

Malic and citric acids, coumarins, flavonoids, alkaloids and saponins are also noted as ingredients of the Green Purslane (*Portulaca oleracea* L.) herb [26–27]. *Portulaca oleracea* L. contains 3-quinoline carboxylic acid, p-coumaric acid, ferulic acid, catechol, caffeic acid and oxalic acid [28–30]. The content of the sum of flavonoids in the aboveground part of *Portulaca oleracea* L. is at least 0.3% [31]. Rutin was found to be the main leaf flavonoid, and the highest myricetin content was in flowers and stems [32]. Both of these flavonoids are powerful antioxidants and have been found to have antimutagenic properties in laboratory studies [33].

The anti-inflammatory activity of *Portulaca oleracea* L. was confirmed in the Common Use Center (CUC) (REC) of the RUDN on the model of "acute formalin paw edema" in rats in comparison with carprofen. In more detail, the chemical composition, pharmacological properties and application of the Green Purslane herb were presented earlier [34].

Thus, *Portulaca oleracea* L. is a potentially valuable medicinal plant raw material with a sufficient raw material base. It is actively studied

abroad in various directions, which is due to the diversity of its chemical composition and the manifestation of a whole range of activities. However, this plant is not pharmacopoeial, there is no standardization of raw materials. The latter makes it much more difficult to obtain medicines based on this medicinal plant raw material. In this regard, it was interesting to study the chemical composition of the *Portulaca oleracea* L. herb for the purpose of its further standardization and introduction into medical practice in the Russian Federation and Syria. Previously, we studied the reducing sugars and flavonoids that provide anti-inflammatory, antioxidant and expectorative properties of the studied raw materials [25,31].

Organic acids are pharmacologically active substances (citric, nicotinic, ascorbic acids). They delay the growth of bacteria, exhibit anti-inflammatory properties and have a positive effect on functioning the gastrointestinal tract and other body systems [11,37]. Since the literature data indicate that the herb of the Green Purslane contains a significant amount of organic acids, their study in the herb of this type of plant was also interesting.

Volumetric, enzymatic, colorimetric, spectrophotometric, microfluorimetric, polarographic, chromatographic methods are used to determine the content of organic acids. One of the classical methods included in the SP is titration [35].

The purpose of this work is to study the content of the sum of free organic acids in the Green Purslane (*Portulaca oleracea* L.) herb using a Compendial Procedure for determining the sum of organic acids in fresh viburnum berries [36].

To achieve this purpose, it is necessary to provide qualitative confirmation of the content of free organic acids; selection of conditions and development of a procedure for determining the sum of free organic acids; validation evaluation of the developed procedure in accordance with compendial requirements.

MATERIALS AND METHODS

The object of the study was samples of the *Portulaca oleracea* L. herb harvested in different districts of the Voronezh region in the period from July to September and in Syria (Latakia province).

Water was used as an extractant for the extraction of acids from plant material [8].

In [9], a titer of 0.0067 was established that is the amount of malic acid corresponding to 1 ml of caustic soda solution (0.1 mol/l), in grams.

The following reference standards (RS) were used in the TLC research process:

- citric acid, substance – powder, citric acid content 99%, Sigma-Aldrich, USA, series 77-92-9, valid until 02/19/2022;
- oxalic acid, substance – powder, oxalic acid content 99%, chemically-pure, Himmed, Russia, series 392/10, valid until 02.03.2021.

Qualitative analysis procedure

TLC analysis was used to prove the presence of free organic acids. During the analysis, an aqueous extraction from the *Portulaca oleracea* L. herb was used. Extracts were prepared according to the following procedure: 5 g of crushed raw materials to the size of particles passing through a sieve with holes of 1 mm, placed in a flask with a slice with capacity of 100 ml, 50 ml of extractant (water) was added. The flask was attached to a backflow condenser and heated in a water bath for 60 minutes, with periodical shaking to wash off the raw material particles from the walls. The extract was then cooled to room temperature and filtered through cotton wool and a folded paper filter. In the analysis the “Sorbfil PTSH AF-UV” plates, on which 5 µl of each extract was applied, and aqueous 0.1% solutions of reference standards of citric acid and oxalic acid were used. Chromatography was carried out by an ascending method in the “ethyl acetate – acetic acid – formic acid – water” solvent system (100:11:11:25). Saturation time of

the chamber: 30–40 min. After chromatography, the plate was removed from the chamber, dried in air for 5 min., detection was performed at a wavelength of 365 nm. Solutions of reference standards of citric acid and oxalic acid were used as "witnesses".

Quantitative analysis procedure

The content of sum of free organic acids in *Portulaca oleracea* L. was determined using a compendial procedure for determining the sum of organic acids in fresh viburnum berries [34].

The analytical sample of raw materials is crushed to the size of particles passing through a sieve mesh with a diameter of 2 mm. Then 2.5 g of crushed raw materials are placed in a flask with capacity of 250 ml, is poured with 200 ml of water and kept for 2 hours in a boiling water bath, cooled and quantitatively transferred to a measuring flask with capacity of 250 ml. After cooling to room temperature, the mixture is filtered through a paper filter "red ribbon", brought the volume of extract with water to the mark and mixed. 10 ml of extract is taken and placed in a flask with capacity of 100 ml, then, 40–50 ml of freshly boiled water, 0.2 ml of 1% alcohol solution of phenolphthalein, 0.4 ml of 0.1% methylene blue solution are added and the mixture is titrated with a solution of caustic soda (0.1 mol/l) until a purple-red color appears in the foam.

The content of free organic acids in terms of malic acid in absolutely dry raw materials as a percentage (X) is calculated by the formula:

$$X = \frac{v \cdot 0{,}0067 \cdot 250 \cdot 100}{m \cdot 10 \cdot (100 - W)} \cdot 100 = \frac{v \cdot 1675}{m \cdot (100 - W)},$$

where 0.0067 is the amount of malic acid corresponding to 1 ml of caustic soda solution (0.1 mol/l), in grams; v – volume of caustic soda solution (0.1 mol/l) used for titration, in milliliters; m – mass of raw materials in grams; W – mass loss during drying of raw materials as a percentage.

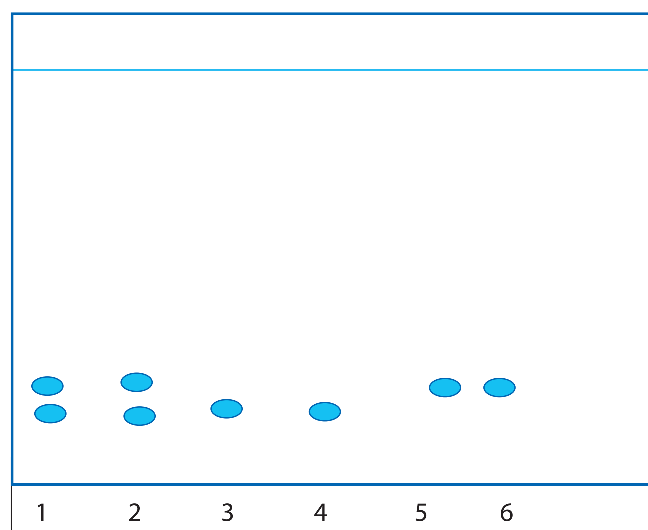
Statistical analysis

To determine the repeatability, the coefficient of variation was calculated based on the results of quantitative determination of the sum of free organic acids (n=6) in the test solution.

RESULTS AND DISCUSSION

As a result of the TLC analysis, the content of free organic acids corresponding to the reference standard was established (Fig. 1): reference standard of citric acid (Rf= 0.24±0.03); reference standard of oxalic acid (Rf=0.21±0.02).

In order to develop a procedure for quantifying the sum of organic acids in this work, the influence of the degree of grinding of raw materials, the ratio "raw material: extractant", the multiplicity of extraction on the yield of free organic acids was studied. The results are presented in Table 1.



Chromatography conditions:
Stationary phase: "Sorbfil PTSH AF-UV" plate
Mobile phase: ethyl acetate – acetic acid – formic acid – water (100:11:11:25)
Reference Standards: solutions of reference standards of oxalic and citric acids
1–2 water extraction
3–4 reference standard of citric acid, Rf=0.24±0.03
5–6 reference standard of oxalic acid, Rf=0.21±0.02

FIG. 1. *Chromatogram diagram for determination of organic acids*

Table 1

THE INFLUENCE OF VARIOUS FACTORS ON THE COMPLETENESS OF THE EXTRACTION OF THE SUM OF FREE ORGANIC ACIDS FROM THE *PORTULACA OLERACEA* L. HERB (N=3, P=0,95)

Unchangeable parameter	Variable parameter	Content of free organic acids, %
Grinding degree, mm		
	7	3.2±0.1
	5	3.1±0.1
	3	3.1±0.1
	2	**3.3±0.1**
	1	2.7±0.1
The ratio of the weight of raw materials to the volume of the extractant		
Grinding degree 2 mm	1:10	0.9±0.2
	1:20	2.1±0.1
	1:40	2.4±0.1
	1:80	**2.8±0.1**
	1:100	2.6±0.1
Extraction time (min)		
Ratio of Raw material: extractant 1:80, grinding degree 2 mm	30	2.9±0.1
	60	3.1±0.2
	90	3.2±0.1
	120	**3.5±0.1**
	180	3.4±0.1
Multiplicity of Extraction		
Ratio of Raw material: extractant 1:80, grinding degree 2 mm, extraction time 120 min	1	**2.7±0.1**
	2	2.6±0.1
	3	2.6±0.2

From the data presented in Table 1, it can be seen that the greatest completeness of the extraction of the sum of free organic acids can be achieved by a single extraction with purified water in 120 minutes when the degree of grinding of raw materials is 2 mm and the ratio of "raw material: extractant" is 1:80.

As a result of the conducted research, a procedure for quantifying the amount of organic acids of the *Portulaca oleracea* L. herb has been developed.

Validation of the procedure was carried out in accordance with the requirements of the OFS.1.1.0012.15 "Validation of analytical procedures" according to the parameters: specificity, linearity, correctness, repeatability, precision, stability of solutions. This study was conducted within the framework of the verification

Table 2

METROLOGICAL CHARACTERISTICS OF THE PROCEDURE OF QUANTITATIVE DETERMINATION OF THE SUM OF FREE ORGANIC ACIDS IN THE *PORTULACA OLERACEA* L. HERB

n	f	P	t (P,f)	Xav, %	S²	S	ΔX	E, %
5	4	0.95	2.78	2.97	0.01	0.1	0.13	3.23

requirements for the following parameters: specificity and repeatability, since the compendial procedure was used [11]. The metrological characteristics of the procedure are presented in Table 2.

The error in quantifying the content of organic acids in (n= 5) is not higher than 5.0%.

Using the developed procedure, the content of the sum of free organic acids in samples of the Green Purslane (*Portulaca oleracea* L.) herb harvested in Syria and Voronezh region was determined. The results are presented in Table 3.

Thus, it was found that the Green Purslane (*Portulaca oleracea* L.) herb contains at least 2% of the sum of free organic acids. At the same time, the highest content of the studied substances is observed in samples harvested in Voronezh region in 2017, 2018 and 2019, and the lowest content is in the samples harvested in Syria in 2019.

Table 3

CONTENT OF THE SUM OF FREE ORGANIC ACIDS IN THE *PORTULACA OLERACEA* L. HERB HARVESTED IN SYRIA AND VORONEZH REGION

Place of harvesting of *Portulaca oleracea* / year of harvesting	Content of the sum of free organic acids
Voronezh region, 2017	2.2±0.2
Voronezh region, 2018	2.9±0.2
Voronezh region, 2019	2.3±0.1
Syria, Latakia, 2019	2.5±0.3

The amount of organic acids found is comparable to the amount established in medicinal plant raw materials containing organic acids as the main active substances (cranberry berries (2–5%), raspberry berries (up to 2%), fresh viburnum berries (at least 6%), rosehip fruits (at least 2.6%), mountain ash fruits (at least 3.2%) [11,36].

Specificity

The color of the test solution corresponded to the color of the standard solution, which is specified in the reference standard procedure after titration with a solution of caustic soda (0.1 mol/l) when the end point of titration is reached. The color of the placebo solution indicated that there was no placebo effect on the results of determining the quantitative content of organic acids at the titration point corresponding to the color change after adding one drop of caustic soda solution (0.1 mol/l).

Repeatability

The results are shown in Table 4.

CONCLUSIONS

1. Conditions have been selected and procedures have been developed for qualitative (TLC method) and quantitative determination (titrometry method) of the total content of organic acids in the *Portulaca oleracea* L. herb.

2. The content of the sum of organic acids in the *Portulaca oleracea* L. herb is at least 2%.

Table 4

EVALUATION OF THE REPEATABILITY OF THE PROCEDURE OF QUANTITATIVE DETERMINATION OF THE SUM OF FREE ORGANIC ACIDS

Name	1	2	3	4	5	6
Sample volume, ml	10.00	10.00	10.00	10.00	10.00	10.00
V, ml	0.30	0.35	0.35	0.30	0.35	0.35
Content, mg/ml	0.216	0.252	0.252	0.216	0.252	0.252
V_0, ml	0					
Xav, mg/ml	0.240					
Standard deviation, S	0.01					
Standard deviation of the average result, S_0	0.007					
Coefficient of variation, S_0, %	7.75					
Confidence interval (P=0.95), µg/ml	0.02 (от 0.22 до 0.26)					

Relative error in determining the sum of organic acids in the *Portulaca oleracea* L. herb is not higher than 5%.

3. Verification of the developed procedure for the quantitative determination of the sum of organic acids in terms of specificity and repeatability was carried out. The statistical characteristics obtained in determining repeatability correspond to the established criteria for the acceptability of the analytical procedure for determining the sum of free organic acids, which allows us to conclude that it is valid.

4. Developed procedures of qualitative and quantitative determination of the sum of organic acids in the *Portulaca oleracea* L. herb may be recommended for inclusion in the regulatory documentation for this type of medicinal plant raw materials.

REFERENCES

1. *Ahmad M., Alireza G., Mahboobeh V. Hypocholesterolemic effects of purslane extracts on serum lipids in rabbits fed with high cholesterol levels // International Journal of Pharmacology. – 2007; 3: pp. 285–289. DOI: 10.3923/ijp.2007.285.289*
2. *Illustrated guide to plants of Central Russia / I.A. Gubanov, K.V. Kiseleva, V.S. Novikov, V.N. Tikhomirov. – M.: KMK Scientific Press Ltd.: In-t technol. research, 2004. – Vol. 3. – 519 p.*
3. *Okafor Izuchukwu Azuka, Ayalokunrin Mary B. and Orachu Lovina Abu. A review on Portulaca oleracea (Purslane) plant – Its nature and biomedical benefits // International Journal of Biomedical Research. – 2014; 5(2), pp. 75–80. DOI:10.7439/ijbr.v5i2.462*
4. *Pharmacopoeia of the People's Republic of China, v. I, 2005. / Chinese Pharmacopoeia Commission – 2005.*
5. *Lee A.S., Kim J.S., Lee Y.J., Kang D.G., Lee H.S. AntiTNF-activity of Portulaca oleracea in vascular endothelial cells // International Journal of Molecular Sciences, vol. 13, № 5, pp. 5628–5644, 2012. DOI: 10.3390/ijms13055628*
6. *Soliman et al. Assessment of herbal drugs for promising anti-Candida activity // BMC Complementary and Alternative Medicine, pp. 17:257. 2017. DOI 10.1186/s12906-017-1760-x*

7. *Karimi G., Hosseinzadeh H., Ettehad N. Evaluation of the gastric antiulcerogenic effects of Portulaca oleracea L. extracts in mice // Phytotherapy Research, vol. 18, № 6, pp. 484–487, 2004. DOI: 10.1002/ptr.1463*
8. *K. Chan, M.W. Islam, M. Kamil et al. The analgesic and anti-inflammatory effects of Portulaca oleracea L. subsp. Sativa (Haw.) Celak // Journal of Ethnopharmacology, vol. 73, №3, pp. 445–451, 2000. DOI: 10.1016/S0378-8741(00)00318-4*
9. *9. Chen B., Zhou H., Zhao W., Zhou W., Yuan Q., and Yang G. Effects of aqueous extract of Portulaca oleracea L. on oxidative stress and liver, spleen leptin, PAR and FAS mRNA expression in high-fat diet induced mice // Molecular Biology Reports, vol. 39, № 8, pp. 7981–7988, 2012. DOI: 10.1007/s11033-012-1644-6*
10. *Rashed A.N., Afifi F.U., and Disi A.M. Simple evaluation of the wound healing activity of a crude extract of Portulaca oleracea L. (growing in Jordan) in Mus musculus JVI-1 // Journal of Ethnopharmacology, vol. 88, № 2–3, pp. 131–136, 2003. DOI:10.1016/S0378-8741(03)00194-6*
11. *Pharmacognosy. Medicinal raw materials of plant and animal origin: textbook / Edited by G.P. Yakovlev. – St. Petersburg: SpecLit, 2013. – 847 p.*
12. *Omara T., Mebrhatu T., Prior D., Ezekwe M. Omega-three fatty acids in purslane (Portulaca oleracea) tissues // JAOCS. Vol. 68, № 3. 1991, pp. 198–199.*
13. *Koch H. Purslane. Omega-3 fatty acids in an old medicinal plant // Dtsch. Apoth. Ztg. – 1988. – P. 47.*
14. *Simopoulos A. Terrestrial sources of omega-3 fatty acids: Purslane // N. Engl.J. Med. 1986, pp. 315:833. DOI: 10.1056/NEJM198609253151313*
15. *Waleed A.K., Hu Chun-Mei., Nadeem K., Amjad I., Shan-Wu L., Farooq S. Bioengineered Plants Can Be a Useful Source of Omega-3 Fatty Acids // BioMed Research International. Vol. 2017, pp. 9. ID 7348919.*
16. *Hunter J. n-3 Fatty acids from vegetable oils // Am.J. Clin. Nutr. 1990 May; 51(5): 809–814. DOI: 10.1093/ajcn/51.5.809*
17. *Caballero-Salazar S., Riveron-Negrete L., Ordaz-Tellez M., Abdullaev F., Espinosa-Aguirre J. Evaluation of The Antimutagenic Activity of Different Vegetable Extracts Using an In Vitro Screening Test // West. Pharmacol. Soc. 45. 2002, pp. 101–103.*
18. *18. Amirul Alam M., Abdul Shukor J., Rafii M., Azizah Abdul H., Farzad A., Hasan M., Mohd Asraf Mohd Z., Kamal Uddin M. Evaluation of Antioxidant Compounds, Antioxidant Activities, and Mineral Composition of 13 Collected Purslane (Portulaca oleracea L.) Accessions // Hindawi Publishing Corporation BioMed Research International. 2014, pp. 10. ID 296063.*
19. *Awad N. Lipid content and antimicrobial activity of phenolic constituents of cultivated Portulaca oleracea L. // Bull.Fac. Pharm. 1994, pp. 1.*
20. *Rahman M., Wahed M., M. Akbar Ali M. b-Carotene losses during different methods of cooking green leafy vegetables in Bangladesh / Rahman M. // J. Food Comp. Anal. Vol. 3, Issue 1. 1990, pp. 47–53. DOI: 10.1016/0889-1575(90)90008-A*
21. *Plant life. Education. 1974-1981/. Vol. 1–6. USSR – 1974. (in Russ.)*
22. *Simopoulos A., Norman H., Gillaspy J., Duke J. Common purslane: a source of omega-3 – fatty acids and antioxidants. Published online: 02 Sep 2013, pp. 374–382. DOI: 10.1080/07315724.1992.10718240*
23. *Langenberg J., Tjaden U., De Vogel E., Langerak D. Determination of phylloquinone (vitamin K1) in raw and processed vegetables using reversed phase HPLC with electrofluorometric detection // Acta Aliment. 1986, pp. 3.*
24. *Wenzel G., Fontana G., Correa J. The viscous mucilage from the weed Portulaca oleracea / L.G. Wenzel // Appl. Biotechnol. Biotechnol. 24.1990, pp. 341–353.*
25. *Nasser R.A., Nikulin A.V., Yamshchikova S.I., Potanina O.G. The content of reducing sugars*

in medicinal plant raw materials Portulaca oleracea L. Proceedings of the 7th scientific conference with international participation "Modern trends in the development of health-saving technologies". Collection of scientific papers, – M.: VILAR, 2019, p. 247–253.

26. *Leung A., Foster Steven. Encyclopedia of Common Natural Ingredients used in food, drugs and cosmetics. 2nd. Edition. John Wiley. 1996, pp. 649. ISBN-13: 978–0471508267.*
27. *Roshchina V.V. Biomediators in plants. Acetylcholine and biogenic amines. – Pushchino: Pushchinsky Scientific Center of the USSR Academy of Sciences. 1991. – 193 p.*
28. *Yan-Xi Zhou, Hai-Liang Xin, Khalid Rahman, Su-Juan Wang, Cheng Peng and Hong Zhang. Portulaca oleracea L.: A Review of Phytochemistry and Pharmacological Effects // BioMed Research International. Vol. 2015, pp. 11. ID 925631.*
29. *Vafa B.R., Farideh A., Hasan R., Vahid R.A. A Pharmacological Review on Portulaca oleracea L.: Focusing on Anti-Inflammatory, Anti- Oxidant, Immuno-Modulatory and Antitumor Activities // Journal of Pharmacopuncture. 22 [1]. 2019, pp. 007–015. DOI: https://doi.org/10.3831/KPI.2019.22.001*
30. *Mou-Tuan Huang, Robert C. Smart, Ching-Quo Wong and Allan H. Conney. Inhibitory Effect of Curcumin, Chlorogenic Acid, Caffeic Acid, and Ferulic Acid on Tumor Promotion in Mouse Skin by 12-O-Tetradecanoylphorbol-13-acetate // Cancer Research. 48. 1988, pp. 5941–5946.*
31. *Nasser R.A., Nikulin A.V., Potanina O.G. Content of flavonoids in medicinal plant raw materials Portulaca oleracea L. / Proceedings of the International scientific conference of young scientists "Modern trends in the development of health-saving technologies", VILAR. 2020. pp. 245–249. DOI: 10.52101/9785870190921_2021_8_245*
32. *Sirithon S., Maitree S. Microchemical Components and Antioxidant Activity of Different Morphological Parts of Thai Wild Purslane (Portulaca oleracea) // Weed Science, 58(3). 2010, pp. 182–188. https://doi.org/10.1614/WS-D-09-00073.1*
33. *Port Portulaca L. purslane [Electronic resource] / NRCS. – 2003. – Available at: https://plants.usda.gov core/profile?symbol=PORTU"*
34. *Nasser R.A., Potanina O.G. Pharmacognostic characteristics of Green Purslane (Portulaca oleracea L.) (literature review) / II International Scientific Conference "The role of metabolomics in the improvement of biotechnological means of production" in the direction of "Metabolomics and quality of life", VILAR. – 2019. – pp. 197–194.*
35. *Romero Rodriguez M.A., Vazquez Oderiz M.L., Lopez Hernandez J., and Simal Lozano J. Determination of Vitamin C and Organic Acids in Various Fruits by HPLC // Journal of Chromatographic Science 30(11):433–7. DOI: 10.1093/chromsci/30.11.433*
36. *State Pharmacopoeia of the Russian Federation XIV, vol. 4 // Ministry of Health of the Russian Federation 2018; pp. 6124–6128.*
37. *Logvinova E.E. Study of groups of biologically active substances of fruits of mountain ash of various varieties / Thesis for a Candidate Degree in Pharmaceutical Sciences. – Voronezh: Voronezh State University, 2016. – 162 p.*

UDC 615.014.4
https://www.doi.org/10.34907/JPQAI.2021.62.85.005

THE ASPECTS OF PHARMACEUTICAL DEVELOPMENT OF THE TABLETS BASED ON DRY EXTRACTS

A.V. Foteeva, *General Director of Parma Clinical LLC, Perm, a.foteeva@parmaclinical.ru*
M.P. Chugunova, *Leading Process Engineer of the Technological Laboratory of Parma Clinical LLC, Perm, m.chugunova@parmaclinical.ru*
N.A. Prozorova, *Deputy General Director for Pharmaceutical Development and Registration of Parma Clinical LLC, Perm, n.prozorova@parmaclinical.ru*
P.N. Vshivkov, *Process Engineer of the Technological Laboratory of Parma Clinical LLC, Perm, p.vshivkov@parmaclinical.ru*

Determination of critical process parameters is one of the important aspects of pharmaceutical development of the consistent high-quality medicines.

The purpose of this work is to identify factors that can potentially effect on the quality and stability of a tableted medicinal product containing althea dry extract such as "Mukaltin, 50 mg tablets".

To determine the parameters that are critical for the manufacturing process of Mukaltin tablets, the influence of a number of factors on the stability of the medicine during storage was studied. These factors include humidity of the starting materials; residual moisture of the finished tablet; physical and chemical properties of the excipients (tartaric acid isomerism); primary packaging material.

During the study, it was found that the critical parameters of the process of Mukaltin tablet manufacturing are the humidity of the intermediates and the finished product and the packaging material.

Keywords: Mukaltin, dry extract, tablets, stability, storage

Herbal medicinal products, including tableted medicines based on plant extracts, are widely used in the pharmaceutical industry and have been used for a long time due to their high efficiency, accessibility, ease of use and low toxicity [1,8].

In addition to the obvious pharmacological advantages, this dosage form has certain technological characteristics. Dry extracts, for the most part, have high hygroscopicity, as a result of which the pharmaceutical development of a tablet dosage form based on them requires a special approach [5,7,9].

One of the important aspects of the pharmaceutical development of consistent high-quality medicines is the determination of critical parameters of the technological process. [10].

The purpose of this work is to identify the factors which are potentially capable of influencing on the quality and stability of a tableted medicinal product containing althea dry extract – "Mukaltin, 50 mg tablets" (hereinafter referred to as Mukaltin tablets) [2,6].

In order to determine as accurately as possible the factor or factors that are critical, that is, having the most pronounced effect on the stability of the medicine "Mukaltin, 50 mg tablets", and in order to distinguish them (since a combination of factors may participate in this process), the following assumptions were made:

1. The effect of the residual moisture of the tablet. Assuming that the packaging

insulates the tablets as much as possible from the effects of external moisture, it is possible that the residual moisture of the tablets may be sufficient for the occurrence and course of a gas formation reaction [4, 5].

2. The influence of the packaging material. The permeability of the packaging material may well provoke a gas formation reaction in the tablet, taking into account that most of the components are hygroscopic substances [11].

3. Influence of physical and chemical properties of excipients (isomerization of tartaric acid). The composition of Mukaltin tablets includes tartaric acid as an excipient. Tartaric acid has isomers with different properties [5,6].

Based on the above assumptions, the influence of the following factors on the stability of Mukaltin tablets during storage was studied:

- humidity of the starting materials;
- residual moisture of the finished tablet;
- physical and chemical properties of excipient (isomerism of tartaric acid);
- packaging material.

MATERIALS AND METHODS

The following components were used for the manufacture of Mukaltin tablets [2,6]:

- althea dry extract produced by Harms, Russia;
- tartaric acid D- isomer produced by Rono Chem Co. Ltd, China;
- tartaric acid D–L-isomer produced by KONO, China;
- sodium bicarbonate produced by Bashkir Soda Company JSC, Russia;
- calcium stearate produced by Chemresource LLC, Russia.

As a reference product, Mukaltin tablets produced by Obnovlenie JSC, Russia were taken.

- Packaging material:
- polyvinyl chloride (PVC) 200 microns thick;
- polyvinylidene chloride (PVDC) 200 microns thick.

The following methods were used to determine the qualitative parameters [3].

Description of the appearance of tablets in a package during storage (scoring method):

a) *the appearance of the tablets* was assessed by the intensity of color change – darkening – in comparison with a tablet stored at a constant humidity of 10 ± 5% and a temperature of 30 °C (0 according to a point scale), and a tablet stored at a constant humidity of 75 ± 5% and a temperature of 30 °C (3 according to a point scale);

b) *the appearance of the primary packaging* was assessed by the presence or absence of a blister bulge (where, according to a point scale, 0 is the absence of a bulge, and 4 is a condition in which a slight compression of the packaging blister causes it to rupture) [4].

The sum of the points characterizes the overall assessment of the appearance of the sample (assessment of the appearance of tablets and assessment of the appearance of the primary packaging) in various storage conditions;

- weight loss during drying;
- measurement of the weight of tablets during storage;
- tablet breaking force.

Equipment:

- climate chamber BINDER KBF1020 with operation temperature 30°C and humidity 75±5%;
- dry-air thermostat TC-80M-2 with operation temperature 40°C;
- tester for determining the strength of tablets Erweka THB 125;
- scales of OKB "Vesta" BM2202;

weight humidity analyzer "Gosmeter" AVG-60.

RESULTS AND DISCUSSION

Immediately before the manufacture of tablets and during the technological process, the weight loss of both components separately (Table. 1), and the tablet mixture during drying was determined (Table. 2) [10].

Table 1

WEIGHT LOSS OF MUKALTIN TABLET COMPONENTS DURING DRYING

Component	Drying temperature	Weight loss during drying, %
Tartaric acid D	100°C	0.77
Tartaric acid D crushed		0.78
Tartaric acid (D,L)		0.56
Sodium hydrocarbonate		1.22
Althea extract dry		2.05
Calcium stearate		1.06

Note: climatic conditions in the laboratory room: t=22,7°C, humidity =20,6%

Further, 8 types of experimental samples of Mukaltin tablets were manufactured and packaged for the experiment, differing in the physical and chemical properties of the excipient (tartaric acid), manufacturing process (with and without drying stage) and primary packaging material. The reference product was repackaged into a PVC blister. More detailed characteristics of the samples are presented in Table 3.

All tablets after packaging had the following parameters:

- tablet weight – 0,300±0,005 g;
- tablet breaking strength – 110±10 N;
- tablet height – 3,82±0,1 mm;
- weight loss during drying of tablets that have not passed the drying stage – 1,75±0,1%;
- weight loss during drying of tablets that have passed the drying stage – 0,94±0,1%.

The tablets were stored under 4 different conditions [7]:

1) at 25°C and humidity 40±5% ("at room temperature");

2) at 40°C and humidity 40±5% ("in thermostat");

3) at 30°C and humidity 75±5% ("in a climate chamber simulating zone 4B");

4) in a double dense polyethylene bag at 25°C and humidity 40±5%.

Every week the appearance was visually evaluated, every month a part of the blisters were selected for testing.

The results of observations (after 3 months) are shown in Tables 4 and 5.

After 3 months of testing, all the samples in the climate chamber changed their appearance: the packaging blisters swelled, the tablets turned very dark. The average weight of the samples also decreased due to the gas formation

Table 2

WEIGHT LOSS OF TABLET MIXTURE DURING DRYING AT DIFFERENT STAGES OF MANUFACTURE

Tartaric acid isomer	Weight loss during drying, %				Note
	Tablet mixture	Tablet mixture after moistening	Tablets after tabletizing	Tablets after drying	
Racemate (D,L)	1.60	2.08	1.75	0.94	Some of the tablets were selected before drying and packed separately
D-isomer	1.47	2.08	1.59	0.80	

Table 3

CHARACTERISTICS OF THE STUDIED SAMPLES OF MUKALTIN TABLETS

Sample number	Tartaric acid isomer	Availability of the drying stage	Primary packaging material
1	Reference product		PVC
2	Racemic mixture	–	PVC
3	Racemic mixture	–	PVDC
4	D-isomer	–	PVDC
5	D-isomer	–	PVC
6	Racemic mixture	+	PVC
7	Racemic mixture	+	PVDC
8	D-isomer	+	PVDC
9	D-isomer	+	PVC

reaction (the weight loss was about 20%). None of the studied samples showed stability in the conditions of the climatic chamber.

In a double plastic bag and a thermostat, the samples were preserved better than in the climate chamber, but also had an unsatisfactory appearance (the packaging was swollen).

When stored at room temperature, the samples of tablets No. 7 and No. 8 showed the best stability. The appearance of these samples has not changed.

Tablets produced by Obnovlenie JSC, repackaged in a PVC blister, darkened and swollen in all storage conditions, while there was no change in appearance of the tablets in the original packaging.

The study allows us to make a number of conclusions.

The residual moisture content of the finished tablet plays an important role in ensuring the stability of Mukaltin tablets. Mukaltin tablets that have passed the drying stage demonstrate higher stability.

Table 4

EVALUATION OF THE APPEARANCE OF MUKALTIN TABLETS

Number of a sample	Appearance								Sum of indicators
	Discoloration				Bulging				
	cc*	therm*	room*	PE bag	cc	therm	room	PE bag	
1	3	1	2	2	4	4	3	2	21
2	3	2	2	3	4	4	2	3	23
3	3	2	2	2	4	4	2	3	22
4	3	1	2	2	4	4	3	4	23
5	3	1	2	3	4	3	3	3	22
6	3	0	1	3	4	2	3	4	20
7	3	0	0	1	4	2	0	2	12
8	3	0	0	1	4	2	0	2	12
9	3	0	1	2	4	3	3	4	20

Note: cc – climatic chamber; therm* – thermostat; room* – room temperature*

Table 5

SUMMARY OF TABLET CHARACTERISTICS AFTER 3 MONTHS OF TESTING

Number of a sample	Appearance of tablets, total score	Average tablet weight, g	Breaking strength, N	Weight loss during drying, %
1	21	0.254	33	3.93
2	23	0.236	28	3.80
3	22	0.242	30	3.11
4	23	0.217	26	7.01
5	22	0.254	31	3.93
6	20	0.233	28	7.15
7	12	0.280	35	2.27
8	12	0.291	38	2.04
9	20	0.262	33	3.74

The breaking strength of the tablets after storage in the climatic chamber decreased by 3 times, when stored at room temperature, the strength practically did not change, and at 40°C it increased by 1.5 times.

According to experience, PVDC packaging has satisfactory barrier parameters. Only in this package the tablets were preserved for 3 months. The original packaging of the tablets produced by Obnovlenie JSC turned out to be thinner compared to the one used in this experiment (150 microns versus 200 microns). Probably, this thickness allows the package to remain permeable to the products of the gas formation reaction, therefore, the resulting carbon dioxide can escape through the pores of the material without damaging the blister, thereby preserving the marketable appearance of the medicine.

CONCLUSIONS

Based on the studies conducted, it can be concluded that from the studied factors, the critical parameters of the production process of Mukaltin tablets include the following:

1. Humidity of semi-finished products and finished product. To achieve the best stability of the medicine under the stated conditions, it is recommended to include the drying stage of finished tablets into the production process.

2. Packing material. Primary blister packaging made of PVDC provides better stability of the medicine.

3. Storage conditions. Mukaltin tablets are best stored at a temperature of up to 25 °C and a relative humidity of 40 ± 5%.

Factors that do not have a pronounced effect on the quality and stability of Mukaltin tablets and are not related to critical parameters:

- humidity of the staring materials;
- isomerism of tartaric acid.

This study shows that various production factors can have a pronounced effect on the stability of tablets containing dry extracts. Failure to comply with these requirements may result in the rejects of the entire batch. Therefore, when developing medicines of this group, it is necessary to carefully approach the definition of critical parameters and strictly control them throughout the entire process

REFERENCES

1. *Val E. Medicines prepared of plant raw materials: an industry problem / E. Val / Remedium, 2001. – Pp. 20–40.*
2. *State Register of Medicines [Electronic resource] / URL: https://grls.rosminzdrav.ru/ (accessed date: 07.09.2020).*
3. *The State Pharmacopoeia of the XIV edition [Electronic resource] / URL: https://pharmacopoeia.ru/ (accessed date: 07.09.2020).*
4. *Gumerov R.H. Effervescent tablets in the range of medicines / R.H. Gumerov, T.N. Galiullina, S.N. Egorova / New pharmacy, 2002. – Pp. 53–55.*
5. *Kachalina T.V. Development of technology for obtaining solid dosage forms containing plant extracts // Dissertation of Candidate of Pharmaceutical Sciences. – Moscow. – 2005. – Pp. 154.*
6. *Soluble effervescent composition containing plant extract // Russian Patent No. 99124707/14, 18.11.1999 / Pozharitskaya O.N., Shikov A.N., Makarov V.G.*
7. *Redchenkova V.M., Hishova O.M. Analysis of the requirements of some pharmacopoeias for extracts / Chemical.-pharm. magazine, volume 40, No.1, 2006. – Pp. 37–40.*
8. *Sambukova T.V., Ovchinnikov B.V., Ganapolsky V.P., etc. Prospects for the use of herbal medicinal products in modern pharmacology // Reviews on clinical pharmacology and medical therapy. – 2017. – Vol. 15. – No. 2. – Pp. 56–63.*
9. *Sorokin V.V. Extraction of plant raw materials by systems of limited miscible solvents in the technology of dry extracts on the example of St. John's wort and meadow clover // Dissertation of Candidate of Pharmaceutical Sciences. – Saint Petersburg. – 2009. – P. 230.*
10. *Pharmaceutical Development (ICH Q8). Translation: PharmAdvisor, version of translation dated 11.05.2020. URL: https://pharmadvisor.ru/document/tr3614/*
11. *Chistyakova T.B., Polosin A.N., Software package and mathematical models for designing pharmaceutical blister packs with the specified barrier characteristics / T.B. Chistyakova, A.N. Polosin / SPSTU, 2013*

UDC 614.2
https://www.doi.org/10.34907/JPQAI.2021.25.24.006

THE ROLE OF THE AUTHORIZED PERSON RESPONSIBLE FOR PHARMACOVIGILANCE IN THE ORGANIZATION OF THE PHARMACOVIGILANCE SYSTEM OF THE LICENSE HOLDER

E.Yu. Kurganova, *postgraduate student of the 3rd year of study, Perm State Pharmaceutical Academy of Health Ministry of Russia, Perm, Russia, authorized person for pharmacovigilance, Medisorb JSC, Perm, e.kurganova@medisorb.ru*
A.V. Soloninina, *Doctor of Pharmaceutical Sciences, Professor, Head of the Department of Pharmacy Management and Economics, Perm State Pharmaceutical Academy of Health Ministry of Russia, Perm, Russia, soloninina@mail.ru*

The pharmacovigilance system, organized by the holder of the license to control the safety of manufactured medicines, is a necessary function of the healthcare system and is aimed at identifying potential safety hazards associated with the use of medicines. The regulatory authorities of the Russian Federation in the field of healthcare pay special attention to monitoring the safety and effectiveness of the use of medicines not only at the stages of their obtaining of marketing authorization and production, but also at all stages of civil circulation. A key role in the organization of the pharmacovigilance system in a pharmaceutical company holding a license is played by a Qualified Person Responsible for Pharmacovigilance.

Keywords: pharmaceutical company, Qualified Person Responsible for Pharmacovigilance, medicine safety, Pharmacovigilance

The extent of the problem of the safety of the use of the medicines brought to the pharmaceutical market in the Russian Federation defines the development and updating of legislation in the field of pharmacovigilance regulating the involvement of all subject of circulation of medicinal products into the work of the pharmacovigilance system [1,3]. In accordance with international standards, the responsibility for the safety of manufactured medicines is borne by the license holder (LH) [4,7]. The importance of studying the issue of medicine safety of medicines defines the need to study the causes and mechanisms of adverse reactions at all stages of circulation of medicinal products.

According to the rules of good practice of pharmacovigilance (GPvP), the license holder has a special role as the main participant in the monitoring of adverse reactions of medicines, which must control the safety by monitoring the information, assessing the benefit-risk ratio of manufactured medicines, training the pharmaceutical company employees, ensuring effective communication with medical organizations, pharmacy organizations, consumers, regulatory authorities [4]. Safety should be ensured at all stages of the life cycle of the medicine [3]. Also the license holder must organize pre-licensure safety monitoring activities at all stages of clinical trials of medicines [1,3].

The purpose of our study was to specify the key tasks, ways of organizing and maintaining the system of pharmacovigilance of the license holder within the framework of the performance of the duties of the Qualified Person Responsible

for Pharmacovigilance in the process of pharmacovigilance in a pharmaceutical company. In order to ensure timely monitoring of pharmacovigilance activities, according to GPvP, the license holder must appoint and have at its disposition a Qualified Person Responsible for Pharmacovigilance (QPRPV).

RESULTS AND DISCUSSION

The criteria for the appointment of the Qualified Person Responsible for Pharmacovigilance in a pharmaceutical company holding the license include:

- pharmacovigilance systems management skills;
- expertise skills / access to expertise in areas such as medicine, pharmaceutical sciences, epidemiology and biostatistics [4,7].

Responsibility for training and retraining of the Qualified Person Responsible for Pharmacovigilance in the field of its pharmacovigilance system is laid on the senior management of the license holder [4]. Training of the Qualified Person Responsible for Pharmacovigilance and its results are documented properly. The Qualified Person Responsible for Pharmacovigilance has the authority to manage and make changes to the pharmacovigilance system, risk management plans, preparation of regulatory actions in response to emergencies to change the safety profile of medicines [7]. The areas of work of the Qualified Person Responsible for Pharmacovigilance are extensive and are specified by the job description [4].

Based on the requirements of regulatory acts [4,6,7] and the experience of the Qualified Person Responsible for Pharmacovigilance of the license holder, we have specified the following key tasks of the Qualified Person Responsible for Pharmacovigilance (see Figure):

1) review of medicine safety profiles and emergency situations connected with changing the medicine safety profiles;

2) organization of work with information on safety and efficacy in relation to medicines covered by the system of pharmacovigilance of the license holder, accounting, reporting on adverse events;

3) identification of new safety and efficacy data concerning the use of medicines at the pre-licensure and post-licensure stages;

4) development and updating of standard operating procedures of the pharmacovigilance system of license holders;

5) working with the master file of the pharmacovigilance system, its development and updating;

6) collection and systematization of comprehensive information on risk minimization measures;

7) advanced training and continuous professional development on the issues of improving the system of pharmacovigilance of license holders and ensuring the safety of medicine;

8) training of license holder's employees on medicines safety, collecting spontaneous reports and transmitting them to the Qualified Person Responsible for Pharmacovigilance; documenting the results of training;

9) functioning as a contact person for authorized bodies with 24-hour access.

In order to solve the identified key tasks, the Qualified Person Responsible for Pharmacovigilance must permanently interact:

1) with employees of a pharmaceutical company involved in receiving spontaneous reports on the safety and efficacy of medicines;

2) with regulatory authorities on medicine safety issues, ensuring timely preparation and provision of reports, risk management plans, provision of complete and timely responses to requests.

High-quality and timely fulfillment of the assigned tasks allows the Qualified Person Responsible for Pharmacovigilance to avoid errors in the operation of the pharmacovigilance system of the license holder. The Qualified

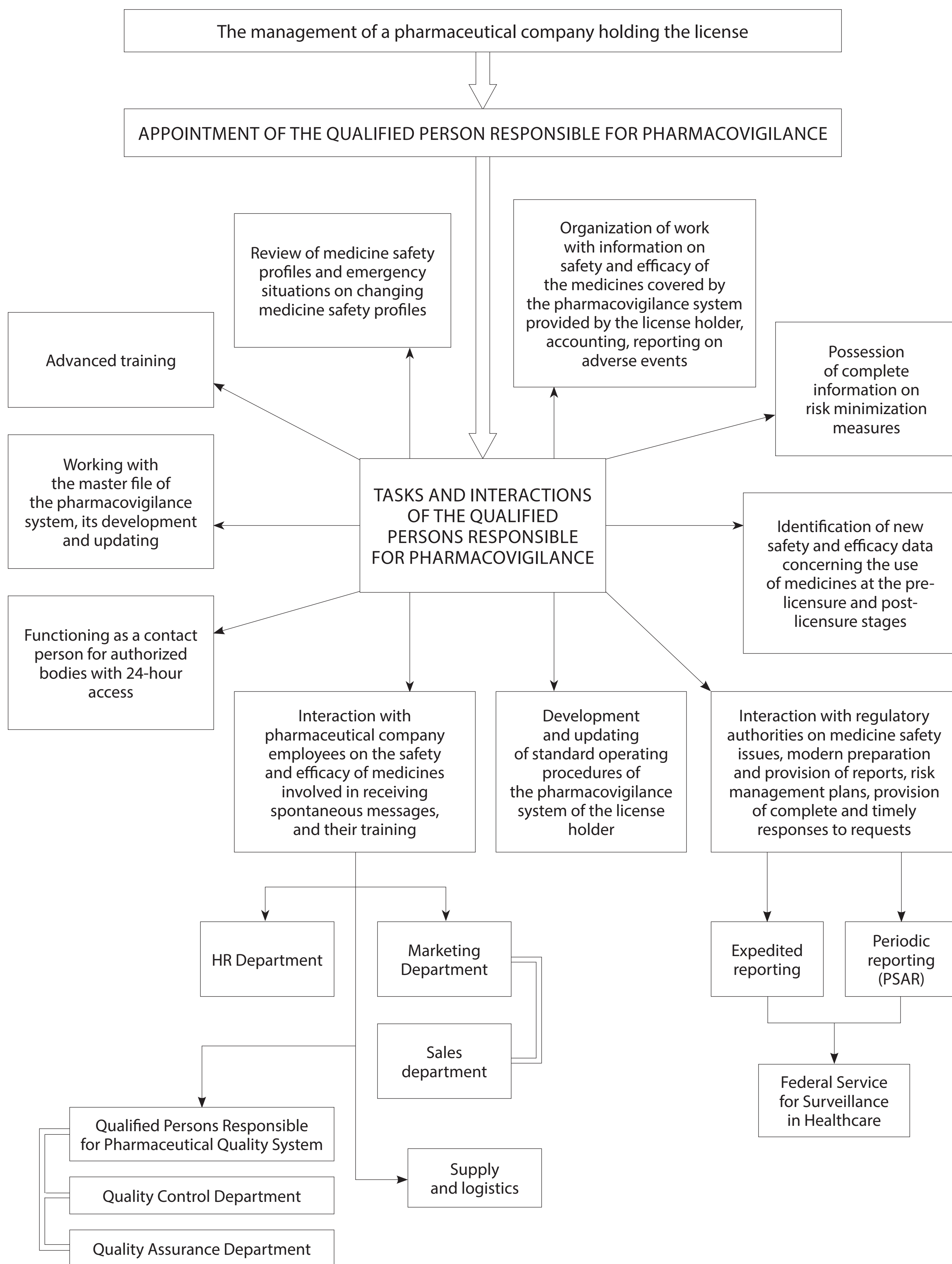


FIG. *The main tasks and ways of interaction of the Qualified Persons Responsible for Pharmacovigilance in the pharmaceutical company holding the license*

Person Responsible for Pharmacovigilance should have information about the validation status of the database of adverse reactions to medicine, including all shortcomings identified during validation and corrective actions taken [4].

In addition, the Qualified Person Responsible for Pharmacovigilance interacts on a daily basis with various divisions (departments) of the pharmaceutical company such as departments of sales, marketing, HR, quality control and assurance, supply and logistics to coordinate monitoring and evaluation of spontaneous reports on adverse reactions

Employees of departments that closely interact with the pharmacovigilance system and participate in monitoring spontaneous reports undergo introductory training at the workplace, and then at least once a year update their knowledge of the basics of good practice according to the developed internal plan and training program. At the workplaces of employees of a pharmaceutical company, there is always a protocol for transmitting data on safety, efficacy or quality according to an internal form. The manager or specialist who received the spontaneous report fills in the internal form of the data transmission protocol on safety, efficacy or quality and sends it to the Qualified Person Responsible for Pharmacovigilance by e-mail or in paper format within one calendar day. Then, the employees of the Pharmacovigilance and Medical Information Department carry out an assessment, analysis and registration of reports on suspected adverse reactions according to internal procedures. The license holder pharmacovigilance system is designed in such a way as to ensure a proper assessment of the quality of the collected reports on adverse reactions in terms of authenticity, legibility, accuracy, consistency and the possibility of verifying the maximum completeness of the data for their clinical judgement [4].

The Qualified Person Responsible for Pharmacovigilance can delegate to trained persons with appropriate qualifications the performance of specific tasks under their supervision, for example, the performance of activities as specific medicine safety experts, provided that the Qualified Person Responsible for Pharmacovigilance will monitor the functioning of the entire system and the safety profiles of medicines [4,7].

CONCLUSIONS

The tasks of the Qualified Person Responsible for Pharmacovigilance are associated with high responsibility, therefore, for their successful implementation, the Qualified Person Responsible for Pharmacovigilance shall have extensive knowledge in the field of medicine and pharmacy, analytical abilities, the ability to maintain documentation and process a large amount of data.

For all medicines, there is a certain balance between the benefits they bring and the potential risk they can cause. The effectiveness of the pharmacovigilance system directly depends on the level of responsibility and competence of the Qualified Person Responsible for Pharmacovigilance. The potential risks of the use of medicines can be minimized due to the Qualified Person Responsible for Pharmacovigilance, which in the pharmaceutical company holding the license performs the functions, on the one hand, of a safety expert, and on the other hand – coordinator of the correct operation of the pharmacovigilance system at the pre-licensure and post-licensure stages of medicine safety monitoring. The priority of the work of the Qualified Person Responsible for Pharmacovigilance is the timely and accurate fulfillment of the tasks set, the preservation of the safety of medicines when received by consumers, as well as the improvement of the quality of products

REFERENCES

1. *Belousov B.Yu., Kolbin A.S., Burbello A.T., Zagorodnikova K.A. Specialist in pharmacological safety in a pharmaceutical company // Good clinical practice. – 2010. –No. 1. – p. 81–86.*
2. *Gildeeva G.N., Glagolev S.V., Yurkov V.I. Problems of medicine safety control in the Russian Federation: the role of pharmacovigilance specialists // Bulletin of Roszdravnadzor. – 2016. – No. 5. – p. 114–118.*
3. *Krasheninnikov A.E., Matveev A.V., Egorova E.A. Qualified Person Responsible for Pharmacovigilance in the medicines quality management system // Remedium. – 2017. – No. 11. – p. 53–55.*
4. *Decision No. 87 "On approval of the Rules of Good Practice of Pharmacovigilance of the Eurasian Economic Union" dated 03.11.2016.*
5. *Guideline on the organization of a medicine safety monitoring system (pharmacovigilance) in medicine manufacturing companies or license holders (05.10.2009).*
6. *Federal Law No. 61-FZ dated 12.04.2010 "On Circulation of Medicines".*
7. *Guideline on good pharmacovigilance practices (GPvP) European Medicines Agency and Heads of Medicines Agencies, 2012.*

UDC 615.038
https://www.doi.org/10.34907/JPQAI.2021.66.67.007

STUDY OF COMPARATIVE PHARMACOKINETICS AND BIOEQUIVALENCE OF URSODEOXYCHOLIC ACID MEDICATIONS

E.V. Glushko, *Regulatory Affairs Manager of NPO GLS, EKOlab CJSC, Elektrogorsk, Russia, ekolab-glushko@mail.ru*
S.G. Mardanly, *Doctor of Medical Sciences, Associate Professor, Professor of the Department of Epidemiology, Professor of the Department of Pharmacology and Pharmaceutical Disciplines, State Humanitarian and Technological University (SHTU), Orekhovo-Zuevo, Russia, ekolab-president@mail.ru*
T.A. Koroleva, *Head of NPO GLS, EKOlab CJSC, Elektrogorsk, Russia, ekolab-koroleva.t@mail.ru*

One of the most common diseases of mankind is cholelithiasis (GSD). Currently, there is only one existing proven on various links of biliary lithogenesis – ursodeoxycholic acid (UDCA). Its therapeutic area is extensive and includes various diseases of the liver and biliary tract. To confirm the efficacy and safety of the medicine Ursolab, registered by EKOlab CJSC, the main active ingredient of which is ursodeoxycholic acid, the comparative pharmacokinetics and bioequivalence of this medicine and the medicine Ursofalk (Dr. Falk Pharma GmbH, Germany) was studies in 28 healthy volunteers.

Keywords: ursodeoxycholic acid (UDCA), bioequivalence, pharmacokinetics, Ursolab, liver and gall bladder diseases

According to the World Health Organization, more than 2 billion people worldwide suffer from liver diseases. In the CIS countries, from 500 thousand to 1 million medical care encounters related to liver pathology are registered annually. One of the most common diseases of mankind is cholelithiasis (GSD) that is a multifactorial and multi-stage disease of the hepatobiliary system, characterized by a certain clinical picture such as a violation of cholesterol and/or bilirubin metabolism with the formation of gallstones in the gallbladder (GB) and/or bile ducts [1]. Epidemiological data show that 10% of the world's population suffers from GSD and for every decade the number of patients increases by about 2 times [2–5]. At the same time, in developed countries, the number of patients with GSD is 10–40% of the adult population. In Russia, the number of patients with GSD is 5–20% [2,6]. Cholecystectomy, unfortunately, remains to this day the "gold standard" of GSD treatment [3,7,8].

Currently, there is only one substance with a proven effect on various links of biliary lithogenesis – ursodeoxycholic acid (UDCA) [9, 10], respectively, its use for dissolving cholesterol stones today is an alternative to cholecystectomy [11,12].

Ursodeoxycholic acid is a tertiary bile acid formed in hepatocytes and intestines. Unlike its predecessors – primary and secondary bile acids – this acid is hydrophilic and, therefore, non-toxic. The scope of its therapeutic use is very extensive and includes various diseases of the liver and biliary tract such as chronic active hepatitis with cholestatic syndrome, acute hepatitis, toxic liver lesions of various genesis, primary biliary cirrhosis of the liver, primary sclerosing cholangitis, biliary dyskinesia, etc.

Normally, the content of UDCA in human bile is no more than 5% of the total pool of bile acids;

cholic acid – 26–39%, deoxycholic – 16–33%, litocholic – 0.5–5%. When administered at a dose of 13–15 mg/kg per day (orally), the content of UDCA in bile approaches 50%, which makes it the main one among bile acids, and the content of toxic bile acids (cholic, deoxycholic, litocholic, etc.) decreases. The absence of toxicity of UDCA is explained by its higher polarity and, accordingly, hydrophilicity [13,14].

It was found that the cytoprotective effect of UDCA on cholangiocytes and hepatocytes is due to the prevention of the release of cytochrome C from mitochondria, which, in turn, blocks the activation of caspases and apoptosis (programmed cell death). UDCA, embedded in the hepatocyte membrane, improves the fluidity of the phospholipid bilayer, stabilizing the cell structure and protecting them from damage.

In addition, UDCA has an immunomodulatory effect, reducing the production of proinflammatory cytokines (interleukins 1, 2, 6, gamma interferon, etc.), the level of the immune complexes of IdM and autoantibodies, the expression of histocompatibility antigens on hepatocytes (HLA I and II classes), which, in turn, prevents the activation of cytotoxic T-lymphocytes, normalizes the ratio of CD4/CD8 immunocompetent cells and promotes the suppression of immunopathological reactions. Stimulating exocytosis in hepatocytes during cholestasis by activating Ca++-dependent alpha-protein kinase, UDCA reduces the concentration of bile acids (cholic, litocholic, deoxycholic, etc.) which are toxic to the liver cell.

UDCA inhibits the absorption of lipophilic bile acids in the intestine, induces bicarbonates-rich choleresis, which leads to increase in bile passage and stimulates the excretion of toxic bile acids through the intestine. Replacing nonpolar bile acids, UDCA forms nontoxic mixed micelles (liquid crystals with cholesterol molecules). By reducing the synthesis of cholesterol in the liver, its secretion into bile, as well as absorption in the intestine, UDCA reduces the lithogenicity of bile, reduces the cholato-cholesterol index, promotes the dissolution of cholesterol stones (macrolites) and prevents the formation of new crystals (microlites). UDCA is well absorbed in the small intestine and is almost completely bound to serum proteins. In the liver, UDCA is rapidly and actively conjugated with glycine, taurine, N-acetylglucosamine, glucuronic acid and sulfate, which specifies its low level in plasma. In conjugated form, UDCA is released into the bile, where its concentration specifies the effectiveness of the medicine [15].

The main active ingredient of the medicine Ursolab, to which this study is devoted, is ursodeoxycholic acid. Ursolab is a reproduced medicinal product. The equivalence of the reproduced medicine to the reference medicine is usually proved in the framework of a bioequivalence study, which demonstrates that both medicines have the same rate and degree of absorption [16]. Such studies are intended to confirm that the reproduced medicines have the same efficacy and safety as the reference medicine. In this work, bioequivalence was studied for the medicine Ursolab (manufactured by ECOlab CJSC) relative to the medicine Ursofalk (Dr. Falk Pharma GmbH, Germany).

The purpose of this study is to evaluate the pharmacokinetic parameters of bioequivalence of the studied medicine Ursolab, oral suspension 250 mg /5 ml (ECOlab CJSC, Russia), and the medicine Ursofalk, oral suspension 250 mg /5 ml (Dr. Falk Pharma GmbH, Germany), in healthy volunteers after an acute administration under fasting conditions.

MATERIALS AND METHODS

The study was planned as a randomized, open, comparative, cross-sectional, two-period bioequivalence study in healthy volunteers under fasting conditions.

The study determined the concentration of ursodeoxycholic acid in the blood plasma of volunteers after acute administration of 250 mg (1 dose-measuring cup of 5 ml) of each of the medicines under fasting conditions. Based on the data on the concentration of ursodeoxycholic acid in blood plasma, the pharmacokinetic parameters were calculated.

The volunteers, after signing a written form of informed consent, were examined at the clinical center of Clinic "Bessalar" LLC.

The volunteers included in the study were randomized into two groups in a ratio of 1:1. The study plan is presented in Table 1.

The study consisted of screening, two study periods and a "cleaning" period. The duration

Table 1

STUDY PLAN

Study stages/procedures	Screening	Study periods			Final evaluation
		I	"cleaning"	II	
Period duration (days)	up to 10	4	14	4	
Informed consent	X				
Inclusion criteria	X				
Exclusion criteria	X	X		X	
Demographic and anthropometric data	X				
Medical history	X				
Physical examination	X	X		X	X
Laboratory examination (clinical blood analysis, biochemical blood test, clinical urinalysis)	X			X	X
Serology (HIV blood test, syphilis, hepatitis B and C markers)	X				
Urine test for pregnancy	X	X		X	
Breath test for alcohol	X	X		X	
Urine test for narcotic drugs and psychotropic substances, psychoactive drugs	X	X		X	
hospitalization		X		X	
Blood pressure, heart rate, body temperature	X	X		X	X
ECG	X			X	X
Randomization		X			
Administration of the studied medicinal product or the reference medicinal product		X		X	
Blood sampling for PK		X		X	
Registration AEs (adverse events) or SAEs (serious adverse events)		X	X	X	X

of screening was up to 10 days. The duration of each period was 4 days, the "cleaning" period was 14 days. The total duration of the study for one volunteer was no more than 28 days.

The study was planned to include no more than 32 healthy volunteers. As a result, 31 healthy volunteers were included in the study, of which 28 volunteers underwent all screening procedures, were randomized and completed the study in accordance with the protocol

The studied medications were taken by 28 volunteers, 21 of them were men and 7 were women. The average age of the volunteers was 30.82±6.42 years (M±SD). The average height of volunteers was 175.29±6.33 cm. The average weight of volunteers was 72.05±10.13 kg.

Blood sampling to determine the concentration of ursodeoxycholic acid from a cubital catheter or by direct venipuncture was carried out during the periods of administration of the studied medicines according to the schedule: before administration of the medicine a sample of 12.0 h and a sample of 0 (15 minutes before administration of the medicine) will be taken, then in 0,25, 0,5, 0,75, 1,0, 1,5, 2,0, 2,5, 3,0, 3,5, 4,0, 5,0, 6,0, 8,0, 12,0, 24,0, 48,0 and 72.0 h.

Since ursodeoxycholic acid is an endogenous compound, the protocol provides for taking two blood samples 12 hours and immediately before administration of the medicine to assess the endogenous concentration. In this study, the duration of observation of the concentration of the active ingredient was 72 hours, since such a time interval overlaps 4 T1/2 for ursodeoxycholic acid from blood plasma, the value of the parameter T1/2 was about 13–14 hours.

Analytical procedures were carried out in a specialized analytical laboratory.

All blood plasma samples were subject to analytical study. To determine the concentration of ursodeoxycholic acid in blood plasma, a bioanalytical method was developed and validated using HPLC–MS/MS of the Agilent 1260 Infinity system with a mass-selective detector G6125B.

RESULTS AND DISCUSSION

Ursodeoxycholic acid was absorbed into the blood with a Tmax value of 1.5 [1.0; 2.125] hours (Me [Q25; Q75]) for the tested medicine Ursolab, oral suspension 250 mg / 5 ml (ECOlab CJSC, Russia), and 2.0 [1.0; 3.5] for the reference medicinal product Ursofalk, oral suspension 250 mg /5 ml (Dr. Falk Pharma GmbH, Germany). The average (Mean) maximum concentration of the studied medicines (Cmax) was 4184.59±1974.53 ng/ml (Mean±SD) for the tested medicinal product and 4025.77±1768.49 ng/ml for the reference medicinal product. The average AUC0-t for the tested medicinal product was 16070.7=7220.96 h·ng/ml and 14155.2=4273.44 h·ng/ml for the reference medicinal product. The average pharmacokinetic profiles of the studied medicinal product are shown in Fig. 1.

Safety parameters included physical and systemic examinations, measurements of basic vital signs, clinical laboratory tests and control of adverse events.

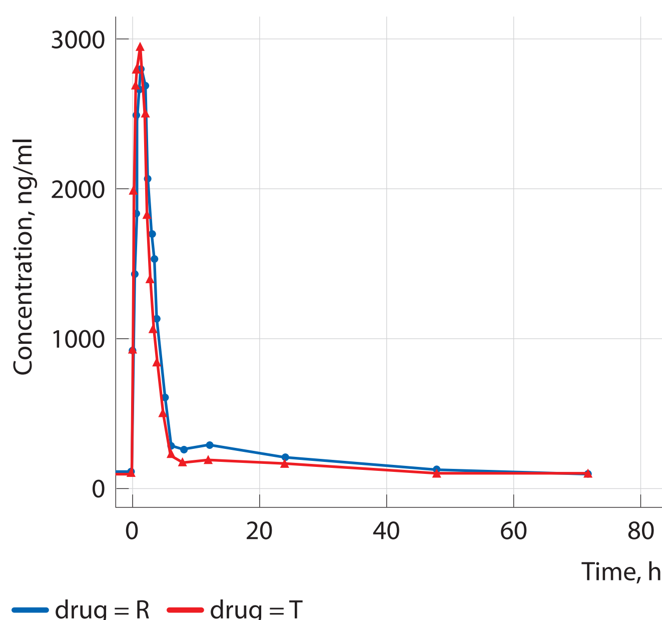


FIG. 1. *Averaged pharmacokinetic profiles: R – Ursofalk, oral suspension, 250 mg / 5 ml (Dr. Falk Pharma GmbH, Germany); T – Ursolab, oral suspension 250 mg /5 ml (ECOlab CJSC, Russia)*

When examined after the study at the end of the clinical part, none of the participants expressed any complaints and they were all physically healthy. The main vital signs of all participants did not change during the study. The vital functions of the body (blood pressure, heart rate) were assessed 12 hours before administration of the studied medicinal product or a reference medicinal product and 1,0, 2,0, 3,0, 4,0, 5,0, 6,0, 7,0, 8,0, 12,0, 24,0, 48,0 and 72.0 hours after administration of the studied medicinal product or a reference medicinal product. Thermometry was performed in the evening when a volunteer was admitted to the hospital at the beginning of the first study period.

During the registration of the main vital signs, each participant was asked about his/her health.

During the entire study, there were no clinically significant changes in the measured parameters, such as blood pressure, heart rate and body temperature.

The bioequivalence of the compared medicinal products was evaluated using an approach based on the assessment of 90% confidence intervals for the ratio of geometric averages for AUC0-t (correct) and Cmax (correct), where AUC0-t (correct) is the area under the pharmacokinetic curve "concentration – time", and Cmax (correct) is the value of the maximum concentration of ursodeoxycholic acid in blood plasma adjusted for the endogenous concentration of ursodeoxycholic acid.

The 90% confidence interval for the geometric mean ratios for the Cmax parameter was 88.49–119.37 (LSM T Geo/R Geo = 102.78), for the AUC0-t parameter 81.35–106.96 (LSM T Geo/R Geo = 93.28). The specified confidence intervals are within the limits of 80.00–125.00%. According to the protocol, the medicinal products are considered bioequivalent if the boundaries of the estimated confidence interval for AUC0-t and Cmax are in the range of 80.00–125.00%. In accordance with this, the tested medicinal product Ursolab, oral suspension 250 mg /5 ml (ECOlab CJSC, Russia), is recognized as bioequivalent to the reference medicinal product Ursofalk, oral suspension 250 mg /5 ml (Dr. Falk Pharma GmbH, Germany).

CONCLUSIONS

As part of the registration of the medicine "Ursolab", its bioequivalence relative to the reference medicine "Ursofalk" was studied with an acute administration by healthy volunteers under fasting conditions. Based on the data obtained, it can be affirmed that the studied medicines are characterized by a high degree of similarity in pharmacokinetics. The individual and averaged profiles of the pharmacokinetic curves of the studied and reference medicines have the same forms. The confidence intervals for the ratios of the geometric mean values of the estimated parameters of the studied and reference medicines fully correspond to the established limits.

Thus, the performed study allows us to state the bioequivalence of the medicine Ursolab, oral suspension 250 mg /5 ml (ECOlab CJSC, Russia), relative to the medicine Ursofalk, oral suspension 250 mg /5 ml (Dr. Falk Pharma GmbH, Germany).

REFERENCES

1. *Selezneva E.Ya., Bystrovskaya E.V., Orlova Yu.N. et al. Algorithm of diagnosis and treatment of gallstone disease // Russian Medical Journal. – 2015. – No. 13. – P. 730–737.*
2. *Ivashkin V.T. Diseases of the liver and biliary tract: guidelines for Physicians. – M.: M-Vesti, 2002. – 416 p.*
3. *Ilchenko A.A. Cholelithiasis. – M.: Anacharsis, 2004. – 200 p.*
4. *Simanenkov V.I., Sablin O.A., Lutaenko E.A., Ilchishina T.A. The possibilities of using ursodeoxycholic acid (medicine Urdoxa) for biliary*

dyskinesia // Gastroenterology of St. Petersburg. – 2010. – No. 2–3. – P. 23–26.

5. *Simanenkov V.I., Lutaenko E.A. Methodological recommendations for the use of ursodeoxycholic acid (Urdoxa) in patients with biliary dyskinesia. Available by: http://medznate.ru/docs/index-35443.html.*
6. *Drapkina O.M. Diseases of the biliary tract: new methods of prevention and treatment // Effective pharmacotherapy. – 2011. – No. 9. – P. 44–49.*
7. *Petukhov V.A. Cholelithiasis and the syndrome of impaired digestion. – M.: Vedi, 2003. – 128 p.*
8. *8. Skvortsova T.E. Litolytic therapy with Ursofalk in patients with cholelithiasis and its effect on the state of the hepatobiliary system and intestinal microbiocenosis // Gastroenterology of St. Petersburg. – 2009. – No. 4. – P. 11–13.*
9. *Minushkin O.N. Ursodeoxycholic acid in gastroenterology // Effective pharmacotherapy in gastroenterology. – 2008. – No. 2. – P. 18–24.*
10. *Minushkin O.N., Elizavetina G.A., Ivanova O.I., Shaposhnikova O.F. Ursodeoxycholic acid in the treatment of patients with biliary sludge // Effective pharmacotherapy. Gastroenterology. – 2012. – No. 3. – P. 10–12.*
11. *Roma M.G., Toledo F.D., Boaglio A.C., et al. Ursodeoxycholic acid in cholestasis: linking action mechanisms to therapeutic applications // Clin. Sci. (Lond). – 2011. – No. 21. – P. 523–544.*
12. *Portincasa P., Ciaula A.D., Bonfrate L., Wang D.Q. Therapy of gallstone disease: What it was, what it is, what it will be // World J. Gastrointest. Pharmacol. Ther. – 2012. – № 3. – P. 7–20.*
13. *Bouscarel B., Nussbaum R., Dubner H., Fromm H. The role of sodium in the uptake of ursodeoxycholic acid in isolated hamster hepatocytes // Hepatology. – 1995. – № 21(1). – P. 145–154.*
14. *Day D., Meyer D., Johnson S., Weisbrode S., Thudium D., Rhodes D. Evaluation of total serum bile acids concentration and bile acid profiles in healthy cats after oral administration of ursodeoxycholic acid // Am.J. Vet. Res. – 1994. – № 55(10). – P. 1474–8.*
15. *Raicht R., Cohen B., Sarwal A., Takahasln M. Ursodeoxycholic acid. Effects on sterol metabolism in rats // Biochim. Biophys. Acta. – 1978. – № 531(1). – P. 1–8.*
16. *Ushkalova E.A., Zyryanov S.K., Ushkalova A.V. Reproduced medicinal products and features of their regulation // Neurology, neuropsychiatry, psychosomatics. – 2016. – № 8(3). – P. 82–87.*

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
9 772309 603770