СИНТЕЗ ТА АНАЛІЗ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН

Recommended by Doctor of Pharmacy, professor S.V.Kolisnyk

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SYNTHESIS AND THE ANTIMICROBIAL ACTIVITY OF 5-METHYL-6-(2-METHYL-1,3-THIAZOL-4-YL)-3-PHENYLTHIENO[2,3-*d*]PYRIMIDINE-2,4(1*H*,3*H*)-DIONES

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Key words: thiophene; pyrimidine; alkylation; thiazole

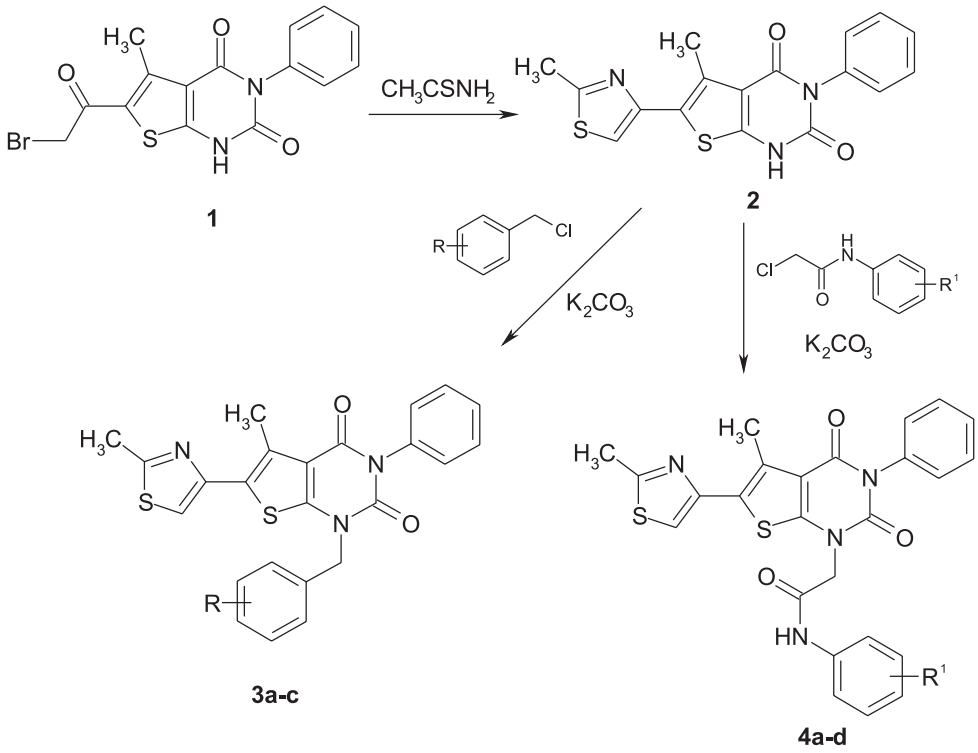
By the reaction of 3-phenyl-6-(α-bromoacetyl)-5-methylthieno[2,3-d]pyrimidine-2,4(1H,3H)-dione with thioacetamide 5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-d]pyrimidine-2,4(1H,3H)-dione was obtained; further the compound was modified by alkylation of its position 1 with benzyl chlorides and chloroacetamides. The structures of the compounds obtained have been confirmed by ^{1}H NMR and mass-spectral data. All the ^{1}H NMR spectra of the compounds obtained have the signals of thiazole cycle at 7.62-7.57 ppm, for 1-alkyl-5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-d] pyrimidine-2,4(1H,3H)-diones the signals of the methylene group protons are observed in the range of 5.13-5.21 ppm for benzyl substituted derivatives and 4.78-4.82 ppm for the compounds with the acetamide fragment; the last ones also contain the sharp signal of NH proton in the range of 9.68-10.41 ppm. 5-Methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-d]pyrimidine-2,4(1H,3H)-dione showed the antimicrobial activity against Staphylococcus aureus higher than the reference drugs Metronidazole and Streptomycin, it also appeared to be moderately active against Pseudomonas aeruginosa and Candida albicans fungi. The antimicrobial activity of 1-alkyl-5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-d]pyrimidine-2,4(1H,3H)-diones is inferior to the activity of the compound with the hydrogen atom in position 1; the highest activity has been determined for the derivative with 4-methylbenzyl substituent in position 1, which inhibits the growth of Staphylococcus aureus and Candida albicans.

In the last years the interest to derivatives of 6-hetarylthieno[2,3-*d*]pyrimidines has grown. They were reported as adenosine A_{2A} receptors antagonists [8], as the compounds with antioxidant [10] activity and acetyl-CoA carboxylase (ACC) inhibitors [7]. Some compounds of the similar structure are potential anti-viral agents [9]. Our last investigations also confirm the expediency of searching novel antimicrobial agents in the range of thieno [2,3-*d*]pyrimidine derivatives modified in position 6 with the aromatic heterocyclic substituents [2, 3, 4, 12]. We have also found that in some cases the presence of the electron-withdrawing substituent in position 6 of thieno [2,3-*d*]pyrimidine together with alkylation of the nitrogen atom in position 1 promotes the antimicrobial activity of the compounds [11].

In order to construct the novel 6-hetarylthieno[2,3-*d*] pyrimidine-2,4(1*H*,3*H*)-diones we performed the reaction of previously reported 3-phenyl-6-(α-bromoacetyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione **1** [4] with thioacetamide in the acetic acid medium and as the result 5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione **2** was obtained. Further alkylation of compound **2** was performed with benzyl chlorides and chloroacetamides in the DMF medium promoted with potassium carbonate (Scheme). The series of 1-alkyl-5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones **3** and **4** were isolated as white crystalline solids (Tab. 1).

In the ^{1}H NMR spectrum of compound **2** the signals of thiazole and thiophene methyl groups are very close to each other 2.57 (3H, s, CH_3) and 2.67 (3H, s, CH_3); the signal of aromatic proton of thiazole is observed at 7.57 ppm. In all of the ^{1}H NMR spectra of compounds **3** and **4** the signals of the methylene group protons are observed in the range of 5.13-5.21 ppm for compounds **3**, and 4.78-4.82 ppm for derivatives **4**. The sharp signals of acetamide NH are present in the spectra of compounds **4** in the region of 9.68-10.41 ppm.

For all of the compounds obtained the screening of the antimicrobial activity by the agar diffusion method has been performed; the results are given in Tab. 3. The screening showed that 5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione **2** displayed the highest antimicrobial activity; its acti-



Scheme

vity against *Staphylococcus aureus* was higher than that of the reference drugs Metronidazole and Streptomycin; it also appeared to be moderately active against *Pseudomonas aeruginosa* and *Candida albicans* fungi. Alkylation of position 1 of the thieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione fragment decreases the antimicrobial activity.

Among 1-alkyl-5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones **3** and **4** the highest activity was revealed by derivative **3b** with the 4-methylbenzyl substituent in position 1, which moderately inhibited the growth of *Staphylococcus aureus* and *Candida albicans*. It is noteworthy that none of the compounds tested inhibited the growth of *Bacillus subtilis* strain.

Materials and Methods

Chemical Part

The melting points (°C) were measured with a Kofler melting point apparatus and were not corrected. The IR spectrum was recorded on a Bruker Tensor 27 spectrometer in KBr. ^{1}H NMR spectra were recorded on a Varian Mercury (200 MHz) spectrometer in DMSO-d_6 using TMS as an internal standard (chemical shifts are in ppm). LC/MS was recorded with a PE SCIEX API

Table 1

Physicochemical properties of 1-alkyl-5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones **3** and **4**

Compd. No.	R	R^1	Mol. formula M.w.	Yield %, in the alkylation step	M.p., °C	N% calc. / found
3a	H	–	$C_{24}H_{19}N_3O_2S_2$ 445.57	76	279-280	9.43 / 9.67
3b	4-Me	–	$C_{25}H_{21}N_3O_2S_2$ 459.59	83	272-274	9.14 / 9.18
3c	4-F	–	$C_{24}H_{18}FN_3O_2S_2$ 463.56	71	253-255	9.06 / 9.32
4a	–	4-Me	$C_{26}H_{22}N_4O_3S_2$ 502.62	71	> 300	11.15 / 11.29
4b	–	2,4-diMe	$C_{27}H_{24}N_4O_3S_2$ 516.65	79	>300	10.84 / 10.97
4c	–	4-OEt	$C_{27}H_{24}N_4O_4S_2$ 532.64	86	>300	10.52 / 10.79
4d	–	3,5-diOMe	$C_{27}H_{24}N_4O_5S_2$ 548.64	77	293-295	10.21 / 10.32

Table 2

Data of ^{1}H NMR-spectra of 1-alkyl-5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones 3 and 4

Compd. No.	Chemical shift, δ, ppm.			
	NH	Thiophene CH_3 (3H, s.) Thiazole CH_3 (3H, s.)	Aliphatic protons	Aromatic protons
3a	–	2.52 + 2.64	5.21 (2H, s, CH_2);	7.25-7.53 (10H, m, Ar-H); 7.62 (1H, s, CH thiazole)
3b	–	2.58 + 2.64	2.26 (3H, s, CH_3); 5.13 (2H, s, CH_2);	7.1-7.5 (9H, m, Ar-H); 7.62 (1H, s, CH thiazole)
3c	–	2.52 + 2.64	5.13 (2H, s, CH_2);	7.05-7.5 (9H, m, Ar-H); 7.62 (1H, s, CH thiazole)
4a	10.21 (1H, s)	2.64+2.66	2.23 (3H, s, CH_3); 4.80 (2H, s, CH_2);	7.0-7.5 (9H, m, Ar-H); 7.67 (1H, s, CH thiazole)
4b	9.68 (1H, s)	2.63+2.66	2.15 (3H, s, CH_3); 2.22 (3H, s, CH_3); 4.82 (2H, s, CH_2);	6.8-7.5 (9H, m, Ar-H); 7.67 (1H, s, CH thiazole)
4c	10.18 (1H, s)	2.64+2.66	1.28 (3H, t, CH_3); 3.97 (2H, q, CH_2); 4.78 (2H, s, CH_2);	6.86 (2H, d, Ar-H); 7.27 (2H, d, Ar-H); 7.46 (5H, m, Ar-H); 7.64 (1H, s, CH thiazole)
4d	10.41 (1H, s)	2.63+2.66	3.67 (6H, s, $2OCH_3$); 4.80 (2H, s, CH_2);	6.23 (1H, m, Ar-H); 6.79 (2H, d, Ar-H); 7.27 (2H, d, Ar-H); 7.44 (3H, m, Ar-H); 7.65 (1H, s, CH thiazole)

150EX chromatograph equipped with a mass-spectrometer using the column C18 (100×4 мм), the cycle of analysis was 25 min.

The method for preparation of 5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione 2

To 2 g of thioacetamide in glacial acetic acid add 9 g of 3-phenyl-6-(α-bromoacetyl)-5-methylthieno[2,3-*d*]pyrimidin-2,4(1*H*,3*H*)-dione **1** and reflux the mixture till formation of a blue-violet precipitate and then additionally for 2-3 hours. After cooling dilute the mixture with water, filter the precipitate formed. Next suspend the precipitate in water, alkalify with the concentrated ammonia solution, and boil the suspension for 1 hour. Filter the precipitate formed, wash with plenty of water and dry.

Table 3

The antimicrobial activity of 5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones **2**, **3** and **4**

Compnd. No.	Diameter of the growth inhibition zone*, mm					
	Staphylococcus aureus ATCC 25923	Escherichia coli ATCC 25922	Proteus vulgaris ATCC 4636	Pseudomonas aeruginosa ATCC 27853	Bacillus subtilis ATCC 6633	Candida albicans ATCC 653/885
2	+++	+	+	+	–	++
3a	–	–	–	–	–	–
3b	++	+	+	+	–	++
3c	–	–	–	–	–	–
4a	+	–	–	–	–	–
4b	–	–	–	–	–	+
4c	–	–	–	–	–	–
4d	+	–	–	–	–	+
Metr. **	+	+	–	–	++	+
Strept.***	++	++	–	–	++	–

* "–" – diameter of the growth inhibition zone is less than 10 mm; "+" – diameter of the growth inhibition zone is 10-15 mm; "++" – diameter of the growth inhibition zone is 15-20 mm; "+++" – diameter of the growth inhibition zone is more than 20 mm;
** Metr. – Metronidazole DMSO solution (the concentration is 30 μg/ml);
*** Strept. – Streptomycin H_2O solution (the concentration is 30 μg/ml).

M.p. > 300°C.

Yield – 75%.

IR (KBr): 3435, 3191, 3062, 2911, 1719, 1654, 1567, 1529, 1503, 1454, 1437, 1375, 1352, 1297, 1273, 1173, 1155, 1121, 1058, 1028, 986, 961, 911, 851, 766, 734, 711, 700, 686, 664, 617, 579, 535, 495 cm^{-1}.

^1H NMR (200 MHz, DMSO-d_6): 2.57 (3H, s, CH_3), 2.67 (3H, s, CH_3), 7.27 (2H, m, Ar-H), 7.42 (3H, m, Ar-H), 7.57 (1H, s, CH thiazole), 12.36 (1H, br. s, NH).

LC/MS: m/z (MH^+) 356.

Found, %: N 11.99. $C_{17}H_{13}N_3O_2S_2$. Calculated, %: N 11.82. M. 355.44.

The general method for synthesis of 1-alkyl-5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno [2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones 3 and 4.

To the suspension of 0.15 g of 5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione and 0.06 g of potassium carbonate add 0.00045 mole of the corresponding alkylating agents and stir the mixture when heating (60-80°C) for 5-7 hours. After cooling dilute the reaction mixture with water and filter the precipitate formed.

The study of the antimicrobial activity

According to the WHO recommendations [1, 5, 6] the following test-strains have been used: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 4636, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC653/885. The bacterial concentration was 10^7 CFU/ml (determined by McFarland standard). Overnight cultures kept for 18-24 h at 36ºC±1ºC were used. The bacterial suspension was inoculated onto the entire surface of Mueller-Hinton agar (Dagestan Scientific Research Institute of Nutrient Media). The compounds were introduced to the wells in the form of DMSO solution in the concentrations of 100 μg/ml; the open wells were filled with 0.3 ml of the solution.

CONCLUSIONS

By the reaction of 3-phenyl-6-(α-bromoacetyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione with thioacetamide 5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione was obtained; further the compound was modified by alkylation of its position 1 with benzyl chlorides and chloroacetamides. 5-Methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione showed the antimicrobial activity against *Staphylococcus aureus* higher than the reference drugs Metronidazole and Streptomycin, it also appeared to be moderately active against *Pseudomonas aeruginosa* and *Candida albicans* fungi. For 1-alkyl-5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones the lower antimicrobial activity was found than that for the compound with unsubstituted position 1; the highest activity in this range was exhibited by compound **3b** with 4-methylbenzyl substituent at position 1. It moderately inhibits the growth of *Staphylococcus aureus* and *Candida albicans*.

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СИНТЕЗ ТА АНТИМІКРОБНА АКТИВНІСТЬ 5-МЕТИЛ-6-(2-МЕТИЛ-1,3-ТІАЗОЛ-4-ІЛ)-3-ФЕНІЛТІЄНО[2,3-d]ПІРИМІДИН-2,4(1H,3H)-ДІОНІВ

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Ключові слова: *тіофен; піримідин; алкілування; тіазол*

Шляхом реакції 3-феніл-6-(α-бромацетил)-5-метилтієно[2,3-d]піримідин-2,4(1H,3H)-діону з тіоацетамідом отримано 5-метил-6-(2-метил-1,3-тіазол-4-іл)-3-фенілтієно[2,3-d]піримідин-2,4(1H,3H)-діон, який далі модифікували шляхом алкілювання положення 1 бензилхлоридами та хлороацетамідами. Структура одержаних сполук була підтверджена даними ^{1}H ЯМР та масспектроскопії. Для одержаних сполук у спектрах ^{1}H ЯМР спостерігається сигнал протону тіазолу біля 7.62-7.67 м.ч., для 1-алкіл-5-метил-6-(2-метил-1,3-тіазол-4-іл)-3-фенілтієно[2,3-d]піримідин-2,4(1H,3H)-діонів присутні сигнали протонів метиленових груп у діапазоні 5.13-5.21 м.ч. для 1-бензилпохідних та при 4.78-4.82 м.ч. для сполук із ацетамідним фрагментом, для останніх також характерні чіткі сигнали протонів NH в діапазоні 9.68-10.41 м.ч. 5-Метил-6-(2-метил-1,3-тіазол-4-іл)-3-фенілтієно[2,3-d]піримідин-2,4(1H,3H)-діон виявив значну антимікробну активність, яка перевищила препарати порівняння метронідазол та стрептоміцин по відношенню до штаму Staphylococcus aureus, також він показав помірну пригнічуючу активність до росту Pseudomonas aeruginosa та грибів Candida albicans. Антимікробна активність 1-алкіл-5-метил-6-(2-метил-1,3-тіазол-4-іл)-3-фенілтієно[2,3-d]піримідин-2,4(1H,3H)-діонів поступається активності сполуки із незаміщеним положенням 1; найбільшу активність у цьому ряду проявила сполука, яка містить 4-метилбензильний замісник у положенні 1; вона помірно пригнічує ріст Staphylococcus aureus та Candida albicans.

СИНТЕЗ И ПРОТИВОМИКРОБНАЯ АКТИВНОСТЬ 5-МЕТИЛ-6-(2-МЕТИЛ-1,3-ТИАЗОЛ-4-ИЛ)-3-ФЕНИЛТИЕНО[2,3-d]ПИРИМИДИН-2,4(1H,3H)-ДИОНОВ

С.В.Власов, Т.П.Осолодченко, С.Н.Коваленко, В.П.Черных

Ключевые слова: *тиофен; пиримидин; алкилирование; тиазол*

Путем реакции 3-фенил-6-(α-бромацетил)-5-метилтиено[2,3-d]пиримидин-2,4(1H,3H)-диона с тиоацетамидом получен 5-метил-6-(2-метил-1,3-тиазол-4-ил)-3-фенилтиено[2,3-d]пиримидин-2,4(1H,3H)-дион, который в дальнейшем модифицировали с помощью алкилирования положения 1 бензилхлоридами и хлорацетамидами. Структура полученных соединений была подтверждена данными ^{1}H ЯМР и масс-спектроскопии. Для полученных соединений в спектрах ^{1}H ЯМР наблюдается сигнал протона тиазола при 7.62-7.67 м.д., для 1-алкил-5-метил-6-(2-метил-1,3-тиазол-4-ил)-3-фенилтиено[2,3-d]пиримидин-2,4(1H,3H)-дионов присутствуют сигналы протонов метиленовых групп в диапазоне 5.13-5.21 м.д. для 1-бензил производных и при 4.78-4.82 м.д. для соединений с ацетамидным фрагментом, для них также характерны четкие сигналы протонов NH в диапазоне 9.68-10.41 м.д. 5-Метил-6-(2-метил-1,3-тиазол-4-ил)-3-фенилтиено[2,3-d]пиримидин-2,4(1H,3H)-дион проявил значительную противомикробную активность, которая превысила препараты сравнения метронидазол и стрептомицин по отношению к штамму Staphylococcus aureus, также он показал умеренную подавляющую активность роста Pseudomonas aeruginosa и грибов Candida albicans. Противомикробная активность 1-алкил-5-метил-6-(2-метил-1,3-тиазол-4-ил)-3-фенилтиено[2,3-d]пиримидин-2,4(1H,3H)-дионов уступает активности соединения с незамещенным положением 1; наибольшую активность в этом ряду проявило соединение, которое содержит 4-метилбензильный заместитель в положении 1; оно умеренно угнетает рост Staphylococcus aureus и Candida albicans.

Recommended by Doctor of Pharmacy, professor I.V.Ukrainets

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SYNTHESIS AND EVALUATION OF THE ANTIOXIDANT ACTIVITY OF {[1-ARYL-4-CHLORO-1*H*-IMIDAZOLE-5-YL) METHYL]THIO}ALKANE CARBOXYLIC ACIDS

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Key words: synthesis; imidazole; [(1-aryl-5-formyl-1H-imidazol-4-yl)thio]acetic acids; {[1-aryl-4-chloro-1H-imidazole-5-yl)methyl]thio}alkanecarboxylic acids; antioxidant activity

This study is devoted to development of the optimal conditions for synthesis and the study of some "structure – antioxidant activity" regularities of [(1-arylimidazole-5-yl)methylthio]alkane carboxylic acids, which structural analogues have found an application as medicinal products with a wide range of biological activities. The methodology of interaction between 4-cloro-5-chloromethylimidazoles with thioglycolic and thiopropionic acids has been used to obtain these compounds. Selection of the optimal reaction conditions has allowed to obtain target compounds in a dry dimethylformamide in the presence of potash at 50°C with yields of 75-82%. The compounds synthesized are high-melting crystalline substances that dissolve well in polar organic solvents and aqueous alkaline solutions. Their composition and structure have been confirmed by the results of elemental analysis and measurement data of IR-, ^{1}H NMR- and chromatography mass-spectra. The study of the compounds synthesized has been conducted in vitro on biological samples. The antioxidant activity has been determined by the inhibition value of the ascorbate-dependent endogenous lipid peroxidation rate in rats' liver found by the concentration of one of the final products of free-radical lipid oxidation processes – malonaldehyde in the test sample. The results of the biological activity screening of the compounds synthesized show that all imidazole derivatives studied in the final concentration ranges of 10^{-3}-10^{-1} M exhibit a high antioxidant action in the system in vitro. It has been found that the value of the antioxidant activity is influenced by the nature and position of the substituent in position 1 of imidazole. In particular, the presence of electron-acceptor substituents in the aryl fragment decreases the molecule activity in comparison with electron-donor substituents, wherein increase of the methylene groups quantity in the carboxyalkylthiol fragment does not significantly impact the antioxidant effect of the compounds synthesized.

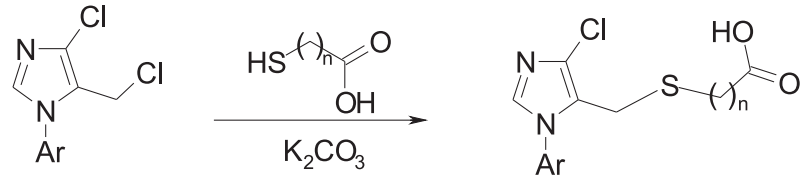
The process of free-radical lipid oxidation (FRLO) plays a significant role in development of the most diseases of the liver, cardiovascular, respiratory and nervous systems [1, 16]. Antioxidants are widely used to normalize the basic organism functions as a part of complex therapy of such diseases [7]. It should be noted that at present there is insufficient amount of medicines with the antioxidant mechanism of action offered for clinical use, and those that are in use have many adverse effects and high toxicity [4]. Taking this into account, the search of compounds that are able to inhibit the FRLO processes effectively is of high interest nowadays.

Design and synthesis of new compounds with antioxidant properties is a subject of many studies as they are of high interest in prophylactics and therapy of many diseases that have FRLO in their pathogenesis [3, 9]. From this aspect the derivatives of imidazole are not left aside too; according to the literature data they are characterized by a wide spectrum of pharmacological properties, among which the antioxidant effect is particularly noteworthy [10, 11, 15].

Earlier we found the antioxidant activity in the series of [(1-phenyl-5-formyl-1*H*-imidazole-4-yl)thio]acetic acids and their derivatives [5, 6, 8, 13]. It was to be expected that the change in the position of the thioalkanecarboxylic acid fragment in the structure of the imidazole cycle would allow a much bigger quantity of potentially active compounds with the antioxidant action, and also give an opportunity to approach to the solution of the topical problem of pharmaceutical chemistry – establishing of the structure – activity relationship. With this purpose we have synthesized [(1-arylimidazole-5-yl)methylthio]alkanecarboxylic acids (**2a-g**) and carried out the screening of their antioxidant properties.

The reaction of easily available 4-chloro-5-chloromethylimidazoles (**1a-d**) [12] with thioglycolic and thiopropionic acids, which occurs selectively at 50°C in a dry DMFA in the presence of potash, has been used to obtain compounds of such kind. The reaction gives the target compounds (**2a-g**) with yields of 75-82%.

The compounds **(2a-g)** synthesized (Tab. 1, 2) are light-yellow, high-melting crystalline compounds, readily soluble in polar organic solvents and aqueous alkaline solutions. Their composition and structure were confirmed by elemental analysis and by the results of IR-, ^{1}H NMR and chromatography mass-spectra measurements. IR-spectra, in particular, are characterized



1 a-d **2 a-g**

I, Ar = Ph (**a**), 4-FC_6H_4 (**b**), 4-ClC_6H_4 (**c**), 4-MeC_6H_4 (**d**); **II**, n=1, Ar = 4-FC_6H_4 (**a**), 4-ClC_6H_4 (**b**), 4-MeC_6H_4 (**c**); n=2, Ar = Ph (**d**), 4-FC_6H_4 (**e**), 4-ClC_6H_4 (**f**), 4-MeC_6H_4 (**g**)

Scheme

Table 1

Physical and chemical characteristics, data of the elemental analysis and chromatography mass spectra of {[(1-aryl-4-chloro-1*H*-imidazole-5-yl)methyl]thio}alkanecarboxylic acids **(2a-g)**

Compound	Ar	Yield, %	T melt., °C	Empiric formula	Found, %			Calculated, %			$[M+1]^+$	M
					C	H	N	C	H	N		
2a	4-FC_6H_4	75	139-140	$C_{12}H_{10}ClFN_2O_2S$	47.82	3.28	9.14	47.93	3.35	9.31	301	300.74
2b	4-ClC_6H_4	81	86-87	$C_{12}H_{10}Cl_2N_2O_2S$	45.64	3.28	9.01	45.44	3.18	8.83	318	317.20
2c	4-MeC_6H_4	76	111-113	$C_{13}H_{13}ClN_2O_2S$	52.42	4.33	9.30	52.61	4.42	9.44	297	296.78
2d	Ph	82	105-107	$C_{13}H_{13}ClN_2O_2S$	52.34	4.33	9.42	52.61	4.42	9.44	297	296.78
2e	4-FC_6H_4	75	132-134	$C_{13}H_{12}ClFN_2O_2S$	49.34	3.79	8.77	49.61	3.84	8.90	315	314.77
2f	4-ClC_6H_4	79	100-102	$C_{13}H_{12}Cl_2N_2O_2S$	47.07	3.62	8.33	47.14	3.65	8.46	332	331.22
2g	4-MeC_6H_4	75	142-144	$C_{14}H_{15}ClN_2O_2S$	53.88	4.75	8.87	54.10	4.86	9.01	311	310.80

by intensive absorption bands of carbonyl groups at 1675-1685 cm^{-1} and by a wide absorption range (2430-2850 cm^{-1}) of carboxyl groups, and it indicates a dimeric character of the acids obtained in their solid state. In ^1H NMR spectra of all compounds the most illustrative are H^2 proton singlets of the imidazole cycle at 7.83-7.95 ppm and the singlets of methylene groups bound with the imidazole cycle at 3.72-3.85 ppm. In their turn, the methylene protons of thioacetic acid fragments are visible as singlets at 3.17 ppm, and those of thiopropionic acid – as triplets in the range of 2.30-2.35 and 2.49-2.54 ppm.

Experimental Part (Chemistry)

IR-spectra of the compounds synthesized were recorded on a UR-20 spectrophotometer in KBr tablets. ^1H NMR spectra were recorded on a Varian-Mercu-

Table 2

IR and ^1H NMR spectra of {[(1-aryl-4-chloro-1*H*-imidazole-5-yl)methyl]thio}alkanecarboxylic acids

Compound	IR-spectrum, cm^{-1}		^1H NMR, δ, ppm
	C=O	COOH	
2a	1685	2430-2850	3.17 s (2H, CH_2), 3.82 s (2H, CH_2), 7.36-7.43 m (2$H_{arom.}$), 7.60-7.65 m (2$H_{arom.}$), 7.87 c (1H, $H^2_{imidazole}$), 12.55 br.s. (1H, COOH)
2b	1680	2450-2840	3.17 s (2H, CH_2), 3.85 s (2H, CH_2), 7.57-7.63 m (4$H_{arom.}$), 7.90 s (1H, $H^2_{imidazole}$), 12.60 br.s. (1H, COOH)
2c	1680	2450-2820	2.38 s (3H, CH_3), 3.17 s (2H, CH_2), 3.82 s (2H, CH_2), 7.36 d (2$H_{arom.}$, *J* 8.0 Hz), 7.41 d (2$H_{arom.}$, *J* 8.0 Hz), 7.83 s (1H, $H^2_{imidazole}$), 12.54 br.s. (1H, COOH)
2d	1675	2435-2830	2.34 t (2H, CH_2, *J* 6.8 Hz), 2.54 t (2H, CH_2, *J* 6.8 Hz), 3.75 s (2H, CH_2), 7.49-7.55 m (5$H_{arom.}$), 7.87 s (1H, $H^2_{imidazole}$), 12.24 s. (1H, COOH)
2e	1680	2440-2835	2.32 t (2H, CH_2, *J* 6.8 Hz), 2.50 t (2H, CH_2, *J* 6.8 Hz), 3.77 s (2H, CH_2), 7.40-7.48 m (2$H_{arom.}$), 7.62-7.69 m (2$H_{arom.}$), 7.90 s (1H, $H^2_{imidazole}$), 12.28 br.s. (1H, COOH)
2f	1680	2435-2850	2.35 t (2H, CH_2, *J* 6.8 Hz), 2.49 t (2H, CH_2, *J* 6.8 Hz), 3.76 s (2H, CH_2), 7.56 d (2$H_{arom.}$, *J* 8.0 Hz), 7.64 d (2$H_{arom.}$, *J* 8.0 Hz), 7.94 s (1H, $H^2_{imidazole}$), 12.20 br.s. (1H, COOH)
2g	1685	2445-2840	2.30 t (2H, CH_2, *J* 6.8 Hz), 2.38 s (3H, CH_3), 2.53 t (2H, CH_2, *J* 6.8 Hz), 3.72 s (2H, CH_2), 7.36 d (2$H_{arom.}$, *J* 7.0 Hz), 7.42 d (2$H_{arom.}$, *J* 7.0 Hz), 7.95 s (1H, $H^2_{imidazole}$), 12.40 br.s. (1H, COOH)

Table 3

The antioxidant activity of {[(1-aryl-4-chloro-1*H*-imidazole-5-yl)methyl]thio}alkanecarboxylic acids *in vitro*

Compound	Concentration, mole/l									
	10^{-1}		5×10^{-2}		10^{-2}		5×10^{-3}		10^{-3}	
	MA, μmole/g of the tissue	AOA, %	MA, μmole/g of the tissue	AOA, %	MA, μmole/g of the tissue	AOA, %	MA, μmole/g of the tissue	AOA, %	MA, μmole/g of the tissue	AOA, %
2a	45.68±0.19*	60.8	46.32±0.12*	60.2	44.39±0.12*	61.9	46.19±0.07*	60.3	45.16±0.12*	61.2
2b	66.26±0.25*	43.1	53.14±0.19*	54.4	44.78±0.32*	61.5	43.36±0.19*	62.8	50.31±0.31*	56.8
2c	50.44±0.19*	56.7	53.01±0.07*	54.5	44.39±0.12*	61.9	49.79±0.21*	57.2	45.42±0.19*	61.0
Control 1	100.36±0.37	–	100.36±0.37	–	100.36±0.37	–	100.36±0.37	–	100.36±0.37	–
2d	24.19±0.07*	59.0	16.73±0.07*	71.6	14.02±0.07*	76.2	13.90±0.12*	76.4	16.60±0.12*	71.9
2e	28.95±0.12*	50.9	20.07±0.12*	66.0	16.98±0.32*	71.2	29.34±0.12*	50.3	20.46±0.12*	65.3
2f	23.93±0.12*	59.4	24.58±0.19*	58.3	20.84±0.24*	64.7	35.13±0.12*	40.5	35.13±0.12*	40.5
2g	17.37±0.12*	70.5	19.56±0.07*	66.8	23.03±0.14*	61.0	26.25±0.12*	55.5	24.70±0.12*	58.1
Control 2	59.06±0.12	–	59.06±0.12	–	59.06±0.12	–	59.06±0.12	–	59.06±0.12	–
Thiotriazoline	70.64±0.56*	38.5	73.98±0.19*	35.6	77.33±0.25*	32.7	76.43±0.24*	33.5	79.13±0.12*	31.2
Control 4	115.03±0.24	–	115.03±0.24	–	115.03±0.24	–	115.03±0.24	–	115.03±0.24	–

* – valid in relation to control ($p \le 0.05$)

ry-400 spectrophotometer (400 MHz) in the solution of DMSO-d_6, the inner standard – tetramethylsilane. Chromatography mass-spectra were recorded by a PE SCXAPI 150 EX device, UV (250 nm) and ELSOJ detectors.

{[(1-Aryl-4-chloro-1*H*-imidazole-5-yl)methyl]thio}acetic (propionic) acids (2 a-g). To the solution of 2 mmoles of 5-chloromethylimidazole (**1a-d**) in 20 ml of a dry DMFA 0.55 g (4 mmoles) of potash and 0.19 g (2 mmoles) of thioglycolic acid (in cases of **2a-c**) or 0.21 g (2 mmoles) of thiopropionic acid (in cases of **2d-g**) were added; the mixture was stirred at 50°C for 2 hours. After that the reaction mixture was poured into 20 ml of water, and acidified with diluted hydrochloric acid to pH 4-5. The precipitate formed was filtered, washed with water, dried and crystallized from 70% aqueous solution of ethanol.

Experimental Part (Biology)

The research of the antioxidant activity of the compounds **(2a-g)** synthesized was performed *in vitro* [14] and determined by the inhibition value of the ascorbate-dependent endogenous lipid peroxidation rate in rats' liver found by the concentration of one of the final products of free-radical lipid oxidation processes – malonaldehyde (MA) in the test sample. The content of MA was determined by the reaction with thiobarbituric acid (TBA) and was calculated in μmoles/g of the tissue. Statistical analysis of the results obtained was performed using the parametric Student's t-test [2]. The value of ascorbate-induced FRLO inhibition was calculated in percents, with MA concentration in the control samples being equal to 100%.

The range of concentrations of the compounds synthesized was chosen within the limits of concentrations already researched for their structural analogue, thiotriazoline (**T**), (the manufacturer is "Arterium" corporation, Ukraine, solution for injections, 25 mg/ml), which has the proven antioxidant activity [7].

Results and Discussion

The results of the antioxidant activity screening of the compounds synthesized *in vitro* (Tab. 3) show that all compounds are able to inhibit Fe^{2+}-ascorbate initiated FRLO in these conditions in the final concentration ranges of 10^{-3}-10^{-1} M studied. Thus, the degree of Fe^{2+}-ascorbate initiated FRLO inhibition *in vitro* of all original compounds synthesized was higher than the antioxidant activity of thiotriazoline in the same final concentrations. The antioxidant activity of thiotriazoline in the specified range of final concentrations *in vitro* varied between 31.32% and 38.59% and was the highest with the final drug concentration of 10^{-1} M. With the same final concentration the highest inhibiting effect on the initiated FRLO was shown by compound **2g** (the degree of FRLO inhibition was 70.59%). Most compounds (**2a**, **2c, 2e, 2f**) have shown the highest antioxidant activity *in vitro* in the concentrations of 10^{-2} M. The highest antioxidant effect *in vitro* was recorded for compound **2d** in the range of the final concentrations of $5\cdot10^{-3}$-10^{-2} M: the degree of FRLO inhibition was 76.47-76.25%. On average, it is 43% higher than the results shown by thiotriazoline in the same range of the final concentrations, and 37% higher than the maximum effect of thiotriazoline recorded *in vitro*.

Analysis of the data obtained shows that the value of the antioxidant activity is influenced by the nature and position of the substituent in position 1 of imidazole. In particular, the presence of electron-acceptor substituents in the aryl fragment decreases the molecule activity in comparison with electron-donor substituents, wherein increase of the methylene groups quantity in the carboxyalkylthiol fragment does not significantly impact the antioxidant effect of the compounds synthesized.

CONCLUSIONS

1. By interaction of 5-chlorometylimidazoles with thioglycolic and thiopropionic acids new {[(1-Aryl-4-

chloro-1*H*-imidazole-5-yl)methyl]thio}alkanecarboxylic acids have been synthesized.

2. All derivatives of imidazole studied in the range of concentrations of 10^{-3}-10^{-1}M show a high antioxidant activity in the system *in vitro.* The highest activity was recorded for compound **2d** in the final concentration of $5 \cdot 10^{-3}$ M.

3. Increase of the methylene groups quantity in the carboxyalkylthiol fragment does not significantly affect the antioxidant effect of the compounds synthesized.

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СИНТЕЗ ТА ОЦІНКА АНТИОКСИДАНТНОЇ АКТИВНОСТІ {[(1-АРИЛ-4-ХЛОРО-1*H*-ІМІДАЗОЛ-5-ІЛ)МЕТИЛ]ТІО}АЛКАНКАРБОНОВИХ КИСЛОТ

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Ключові слова: *синтез; імідазол; [(1-арил-5-форміл-1Н-імідазол-4-іл)тіо]оцтові кислоти; {[(1-арил-4-хлоро-1Н-імідазол-5-іл)метил]тіо}алканкарбонові кислоти; антиоксидантна активність*

Дослідження присвячене розробці оптимальних умов синтезу та вивченню деяких закономірностей «структура-антиоксидантна активність» [(1-арилімідазол-5-іл)метилтіо]алканкарбонових кислот, структурні аналоги яких знайшли застосування в ролі лікарських засобів із широким спектром біологічної дії. Для отримання зазначених сполук було використано методологію, що полягає у взаємодії 4-хлоро-5-хлорометилімідазолів з тіогліколевою та тіопропановою кислотами. Підбір оптимальних умов перебігу реакції дозволив отримати цільові сполуки в сухому ДМФА в присутності поташу при 50°С з виходами 75-82%. Синтезовані сполуки – високоплавкі кристалічні речовини, що добре розчиняються в полярних органічних розчинниках та водних розчинах лугів. Їх склад та структура підтверджені результатами елементного аналізу і даними вимірювань ІЧ-, ЯМР ^{1}Н- та хроматомас-спектрів. Дослідження синтезованих сполук проводили in vitro на біологічних зразках. Антиоксидантну активність визначали за величиною інгібування швидкості аскорбатзалежного пероксидного окиснення ендогенних ліпідів у печінці щурів, яку встановлювали за концентрацією одного з кінцевих продуктів процесів ВРОЛ – малонового альдегіду у досліджуваному зразку. Результати скринінгу біологічної активності синтезованих сполук свідчать про те, що всі досліджені похідні імідазолу в діапазоні кінцевих концентрацій 10^{-3}-10^{-1} М проявляють високу антиоксидантну дію в системі in vitro. Встановлено, що на величину антиоксидантної активності впливає характер та положення замісника в положенні 1 імідазолу. Зокрема, присутність в арильному фрагменті електроноакцепторних замісників знижує активність порівняно з електронодонорними. При цьому збільшення кількості метиленових груп в карбоксіалкілтіольному фрагменті суттєво не впливає на антиоксидантний ефект синтезованих сполук.

СИНТЕЗ И ОЦЕНКА АНТИОКСИДАНТНОЙ АКТИВНОСТИ {[(1-АРИЛ-4-ХЛОР-1*Н*-ИМИДАЗОЛ-5-ИЛ)МЕТИЛ]ТИО}АЛКАНКАРБОНОВЫХ КИСЛОТ

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Ключевые слова: *синтез; имидазол; [(1-арил-5-формил-1Н-имидазол-4-ил)тио]уксусные кислоты; {[(1-арил-4-хлоро-1Н-имидазол-5-ил)метил]тио}алканкарбоновые кислоты; антиоксидантная активность*

Исследование посвящено разработке оптимальных условий синтеза и изучению некоторых закономерностей «структура-антиоксидантная активность» [(1-арилимидазол-5-ил)метилтио]алканкарбоновых кислот, структурные аналоги которых нашли применение в качестве лекарственных средств с широким спектром биологического действия. Для получения указанных соединений использована методология, которая заключается во взаимодействии 4-хлор-5-хлорметилимидазолов с тиогликолевой и тиопропановой кислотами. Подбор оптимальных условий протекания реакции позволил получить целевые соединения в сухом ДМФА в присутствии поташа при 50°С с выходами 75-82%. Синтезированные соединения – высокоплавкие кристаллические вещества, хорошо растворимые в полярных органических растворителях и водных растворах щелочей. Их состав и структура подтверждены результатами элементного анализа и данными измерений ИК-, ЯМР ^{1}Н- и хроматомасс-спектров. Исследование синтезированных соединений проводили in vitro на биологических образцах. Антиоксидантную активность определяли по величине ингибирования скорости аскорбат-зависимого перекисного окисления эндогенных липидов в печени крыс, которую устанавливали по концентрации одного из конечных продуктов процессов СРОЛ – малонового альдегида в исследуемом образце. Результаты скрининга биологической активности синтезированных соединений свидетельствуют о том, что все исследованные производные имидазола в диапазоне конечных концентраций 10^{-3}-10^{-1} М проявляют высокое антиоксидантное действие в системе in vitro. Установлено, что на величину антиоксидантной активности влияет характер и положение заместителя в положении 1 имидазола. В частности, присутствие в арильном фрагменте электроноакцепторных заместителей снижает активность по сравнению с электронодонорными. При этом увеличение количества метиленовых групп в карбоксиалкилтиольном фрагменте существенно не влияет на антиоксидантный эффект синтезированных соединений.

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CAFFEIC AND ROSMARINIC ACIDS IN THYME SPECIES

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Key words: thyme species; herb; caffeic acid; rosmarinic acid; hydroxycinnamic acids

Hydroxycinnamic acids undoubtedly contribute to the wide range of pharmacological effects of the species of Thyme genus. Rosmarinic acid has antioxidant, hepatoprotective, cardioprotective, nephroprotective, anti-inflammatory, immunomodulatory, anti-allergic and anti-tumor properties. Caffeic acid has the antimicrobial, fungistatic activity, produces a choleretic effect. Accumulation of these acids is specific for Lamiaceae family. The aim of our research was to determine the type of hydroxycinnamic acids, the concentration of rosemarinic and caffeic acids and the total content of hydroxycinnamic acids, also to determine parameters of perfect extraction of this type of biological active compounds in the herb of four species of Thyme genus of the European flora. Identification of hydroxycinnamic acids has been carried out using paper and thin layer chromatography in different systems of solvents in comparison with standard compounds. Using TLC and HPLC caffeic and rosmarinic acids have been identified in the herb samples of Thymus Marshallianus, Thymus pulegioides, Thymus crenulatus, Thymus dimorphus . The results of HPLC analysis of caffeic and rosmarinic acids in the herb of Thymus species show that the concentration of rosmarinic acid ranges from 2343.40 mg/kg to 14351.74 mg/kg; the content of caffeic acid ranges from 74.41 mg/kg to 93.86 mg/kg. The highest concentration of rosmarinic acid, as well as caffeic acid was in Thymus pulegioides. The total content of hydroxycinnamic acids has been determined by spectrophotometry, and it ranges from 3.27 to 19.28%. Thymus crenulatus is the richest species by accumulation of the total content hydroxycinnamic acids. The optimal parameters for herb extraction such as the size of the crushed herb particles, extragent and extraction time have been found.

Hydroxycinnamic acids are one of the most wide spread classes of natural compounds containing in herbal drugs. Among them rosemarinic and caffeic acid are the most representative compounds in species of Thyme genus [2, 5, 10, 11, 15]. Hydroxycinnamic acids exhibit a high antioxidant activity, have also the anti-inflammatory, antiviral, immunostimulatory effects [2, 6, 11, 14].

For the first time rosmarinic acid was isolated from plants of *Lamiaceae* family, namely from rosemary by the Italian chemists M.Skorpati and G.Oriente, who offered its name [5, 11, 14]. Rosmarinic acid has the antioxidant, hepatoprotective, cardioprotective, nephroprotective, anti-inflammatory, immunomodulatory, anti-allergic and antitumor properties. It inhibits the activity of acetylcholinesterase, glutathionereductase, aldose-reductase, angiotensin-converting enzyme and reduces genotoxic and cytotoxic effects of ionizing radiation [2, 11].

Caffeic acid has the antimicrobial, fungistatic activity, produces a choleretic effect [7, 8, 9].

Rosmarinic and caffeic acids have been found in many types of thyme [2, 5, 6, 9-13, 15]. They undoubtedly contribute to the pharmacological effects of Thyme genus plants. Therefore, research aimed at studying of rosmarinic and caffeic acids in Thyme genus plants, as well as methods for their analysis are highly actual.

Materials and Methods

The objects of study were herb of species of Thyme genus of Central Russia flora: *Thymus Marshallianus*, *Thymus dimorphus*, *Thymus crenulatus*, *Thymus pulegioides*. Herb samples were harvested at full flowering time in 2011-2012 in different regions: Belgorod (*Th. Marshallianus*), Bryansk (*Th. pulegioides*), Voronezh (*Th. crenulatus*) and Kursk region (*Th. dimorphus*). After gathering herb samples were dried in proper condition and standardized.

The qualitative composition of hydroxycinnamic acids was analysed using paper chromatography and thin layer chromatography. For this purpose Filtrak No.1, No.5 chromatographic paper, as well as "Silufol" and "Sorbfil" chromatographic plates were applied. The analysis was carried out in the solvent system: chloroform – methanol – water (24:14:3); butanol – acetic acid – water (4:1:2); 2% and 15% acetic acid. After developing the chromatograms were analysed in the UV-light before and after spraying with specific reagents (ammonia vapours; solution of sodium hydroxide) [5, 6, 12, 13].

To confirm the presence of hydroxycinnamic acids in the herbal raw material the HPLC method was used. The herbal raw accurately weighed (approx. 1.0 g) was ground to a particle size of 2 mm, placed in a 100 ml flask; then 50 ml of 40% ethyl alcohol was added. The alcohol-water mixture with the herbal raw material was attached to a reflux condenser and heated on a boiling water bath for 1 hour after its boiling. After cooling the extract was filtered through a filter paper into a 100 ml volumetric flask and diluted to the volume with 40% ethyl alcohol.

In parallel 0.02% solutions of reference standards of rosmarinic and caffeic acid were prepared in 40% ethyl alcohol. Then the resulting extract (1 ml) was passed

Table 1

Chromatographic characteristics of hydroxycinnamic acids

Acid	Colour		Rf value	
	UV light	+ ammonia vapour	2% acetic acid	Butanol – acetic acid – water (4:1:2)
Rosmarinic	blue	blue-green	0.42	0.89
Caffeic	blue	blue	0.30	0.92

through the sorbent (1 g octadecyl silica gel with the particle size of 10 micron) and eluted with 40% ethyl alcohol to obtain 10 ml of the eluate. The content and composition of acids in the sample were determined by HPLC on a Shimadzu LC 20 Prominence chromatograph. For analysis a column of Macherey-Nagel Company with the size of 150 X 3 mm filled with the reversed-phase sorbent Nucleosil C 18 AB with 3 micron grain and porosity of 100 A was used. The sample volume – 2 μl, detection at λ=280 nm, 330 nm, 360 nm, with the scanning frequency of 3 Hz. Elution was performed in a gradient mode of increasing the proportion of solution B (acetonitrile : methyl alcohol : water, 40:40:20, pH 2.5) mixed with solution A (an aqueous solution of $HClO_4$ pH 1.8) from 0% to 100% within 80 minutes at the temperature of 30°C. Identification of the peaks was performed by comparison of UV spectra with the spectra from databases and by retention times with the reference standards. The mass concentration was determined from calibration curves using reference standards and LC Solutions programme (Shimadzu) [4].

The method of direct spectrophotometry is in the basis of the assay of the total amount of hydroxycinnamic acids [3].

Method for the assay of the total amount of hydroxycinnamic acids

Grind the analytical sample of the herbal raw material to a particle size passing through a 2 mm sieve. Place approximately 0.5 g of the crushed raw material (accurately weighed) in a 250 ml flask, add 90 ml of 50% ethyl alcohol and weigh with an accuracy of ±0.01 g. Attach the flask to reflux and extract in a boiling water bath for 75 minutes. After cooling weigh the flask with its contents, if necessary, dilute with 50% ethyl alcohol to the initial mass. Filter the extract through a folded filter paper discarding the first 10 ml of the filtrate.

Transfer 1.5 ml of the extract obtained into a 25 ml volumetric flask and dilute to the volume with 50% ethyl alcohol. Measure the absorbance of the test solution at the wavelength of 328 nm. Use 50% ethyl alcohol as a compensation solution.

Calculate the percentage of the total content of hydroxycinnamic acids, equivalent to rosmarinic acid according to the following formula:

$$X = \frac{A \cdot V_1 \cdot V_2}{E^{1\%}_{1cm} \cdot m \cdot V_3},$$

where: A – is the absorbance of the test solution at 328 nm; V_1 – is the volume of the volumetric flask used for the extract collection, ml; V_2 – is the volume of the volumetric flask used for dilution and analysis; V_3 – is the volume of the test solution, ml; $E^{1\%}_{1cm}$ – is the specific absorbance of rosmarinic acid equal to 500 at λ=328 nm; m – is the weighed quantity of the herbal raw material, g.

Table 2

The content of caffeic and rosmarinic acids in the herb of Thymus species

Thymus species	Rosmarinic acid, mg/kg	Caffeic acid, mg/kg
Thymus Marshallianus	5740.66	58.39
Thymus dimorphus	2343.40	93.86
Thymus crenulatus	10202.46	74.41
Thymus pulegioides	14351.74	80.18

Results and Discussion

The presence of caffeic and rosmarinic acids in thyme species under study was determined. Chromatographic characteristics of caffic and rosmarinic acid are presented in Tab. 1.

Rosmarinic acid was pronounced the most intensely in *Th. pulegioides* and *Th. cretaceous*, and caffeic acid was in *Th. dimorphus* and *Th. pulegioides*.

The analysis by HPLC has confirmed the results of the chromatographic analysis and allowed us to determine the content of rosmarinic and caffeic acids in the herb of Thymus species (Tab. 2).

The results show that the rosmarinic acid content ranges from 2343.40 mg/kg to 14351.74 mg/kg, its highest concentration is in *Th. pulegioides* herb; the caffeic acid content ranges from 74.41 mg/kg to 93.86 mg/kg, the maximum amount of it accumulates in *Th. dimorphus* herb.

When studying the UV-absorption spectrum of the alcohol extract of the thyme species herb it has been found that the absorption maximum of the alcohol extract is at the wavelength of λ=325-330 nm; it suggests that the nature of the absorption curve is determined mainly by hydroxycinnamic acids contained in them, and it gives us the opportunity to use this wavelength for spectrophotometric determination of the total content of acids.

When developing the method for quantitative determination of the total content of hydroxycinnamic acids the following conditions were studied: the herb fineness (the particle size), the type of the solvent for extraction, the ratio of extraction and the time of extraction. The study was carried out using *Th. Marshallianus* herb. The research results are presented in Tab. 3.

From the given data it follows that the optimal parameters are the fineness degree of the raw material – 2 mm,

Table 3

The effect of extraction conditions on isolation of hydroxycinnamic acids from *Thymus Marshallianus* herb

Extraction conditions	Total content of hydroxycinnamic acids, %
Particles size, mm	
1	4.34±0.07
2	5.35±0.10
3	4.64±0.11
Solvent for extraction: alcohol, %	
30	5.32±0.07
50	5.34±0.06
70	5.05±0.07
96	1.97±0.06
Extraction time (50% alcohol, the herb: extract ratio – 1:100)	
30	4.70±0.11
45	4.67±0.06
60	5.01±0.09
75	5.34±0.05
90	5.39±0.11

the extragent – 50% ethyl alcohol, the extraction time – 75 minutes before the extraction equilibrium. To determine the total content of hydroxycinnamic acids we proposed to use rosmarinic acid as a standard sample because it is a dominant compound and accumulates significantly in *Thymus* species herb. The amount of total content of hydroxycinnamic acids in the herb samples was calculated using the specific absorption of rosmarinic acid in 50% ethyl alcohol equal to 500 at the wavelength of 328 nm. The conditions described above allowed us to develop a method for quantitative determination of the total content of hydroxycinnamic acids.

Table 4

The total content of hydroxycinnamic acids in *Thymus species* herb

Thymus species herb	Total content of hydroxycinnamic acids, %
Thymus Marshallianus	4.51±0.17
Thymus pulegioides	10.83±0.34
Thymus crenulatus	19.28±0.50
Thymus dimorphus	3.27±0.14

Several sample of *Thymus* species herb have been analyzed by this method (see results in Tab. 4).

The results show that the total content of hydroxycinnamic acids in *Thymus* species herb ranges from 3.27 to 19.28%. *Thymus crenulatus* herb demonstrates the highest concentration of hydroxycinnamic acids.

CONCLUSIONS

1. Using TLC and HPLC the presence of caffeic and rosmarinic acids in the herb samples of *Thymus Marshallianus*, *Thymus pulegioides*, *Thymus crenulatus*, *Thymus dimorphus* has been determined.

2. Using HPLC the content of caffeic and rosmarinic acids in the herb of *Thymus* species has been determined. It has been found that the concentration of rosmarinic acid ranges from 2343.40 mg/kg to 14351.74 mg/kg, the content of caffeic acid ranges from 74.41 mg/kg to 93.86 mg/kg.

3. The method for spectrophotometric determination of the total content of hydroxycinnamic acid in the herb of *Thymus* species has been developed. The results show that the total content of hydroxycinnamic acids in *Thymus* species herb ranges from 3.27 to 19.28%.

4. The optimal parameters for the herb extraction have been found: the size of crushed herb particles – 2 mm, the extragent – 50% alcohol, the extraction time – 75 min.

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КОФЕЙНА ТА РОЗМАРИНОВА КИСЛОТИ В РОСЛИНАХ РОДУ ЧЕБРЕЦЬ

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Ключові слова: *види чебрецю; кофейна, розмаринова кислоти; гідроксикоричні кислоти*

Гідроксикоричні кислоти обумовлюють широкий спектр фармакологічної активності рослин роду чебрець. Розмаринова кислота продукує антиоксидантну, гепатопротекторну, кардіопротекторну, нефропротекторну, протизапальну, імуномодулюючу, протиалергічну та протипухлинні властивості. Кофейна кислота чинить антимікробну, фунгістатичну, жовчогінну дію. Накопичення цих кислот специфічне для рослин родини Ясноткові. Метою дослідження було визначення похідних гідроксикоричної кислоти, концентрації розмаринової та кофейної кислот, а також суми гідроксикоричних кислот, визначення параметрів досконалої екстракції цього класу біологічно активних сполук у траві чотирьох видів роду чебрець європейської флори. Ідентифікацію похідних гідроксикоричної кислоти визначали за допомогою паперової та тонкошарової хроматографії у ряду систем розчинників у порівнянні з вірогідними речовинами. За допомогою ТШХ і ВЕРХ аналізу ідентифікували кофейну і розмаринову кислоти у траві видів чебрецю Thymus Marshallianus, Thymus pulegioides, Thymus crenulatus, Thymus dimorphus. Результати ВЕРХ аналізу показують, що вміст розмаринової кислоти варіює від 2343,40 мг/кг до 14351,74 мг/кг, а кофейної – від 74,41 мг/кг до 93,86 мг/кг. Найвища концентрація розмаринової кислоти, також як і кофейної була відзначена у траві Thymus pulegioides. Вміст суми гідроксикоричних кислот встановлювали спектрофотометричним методом і він складав 3,27-19,28%. Найвищий рівень суми гідроксикоричних кислот спостерегається у траві Thymus crenulatus. Визначені оптимальні параметри екстракції трави чебрецю: розмір частинок, тип екстрагенту і час екстракції.

КОФЕЙНАЯ И РОЗМАРИНОВАЯ КИСЛОТЫ В РАСТЕНИЯХ РОДА ТИМЬЯН

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Ключевые слова: *виды тимьяна; кофейная, розмариновая кислоты; гидроксикоричные кислоты*

Гидроксикоричные кислоты несомненно обусловливают широкий спектр фармакологической активности растений рода тимьян. Розмариновая кислота проявляет антиоксидантные, гепатопротекторные, кардиопротекторные, нефропротекторные, противовоспалительные, иммуномодулирующие, противоаллергические и противоопухолевые свойства. Кофейная кислота обладает противомикробным, фунгистатическим и желчегонным действием. Накопление этих кислот специфично для растений семейства Ясноткловые. Целью исследований было определение производных гидроксикоричной кислоты, содержания розмариновой и кофейной кислот, а также суммы гидроксикоричных кислот, анализ параметров оптимальной экстракции этого класса биологически активных соединений травы четырех видов рода тимьян европейской флоры. Идентификацию производных гидроксикоричной кислоты проводили с помощью бумажной и тонкослойной хроматографии в ряде систем растворителей в сравнении с достоверными веществами. С помощью ТСХ и ВЭЖХ анализа идентифицировали кофейную и розмариновую кислоты в траве видов тимьяна Thymus Marshallianus, Thymus pulegioides, Thymus crenulatus, Thymus dimorphus. Результаты ВЭЖХ анализа показывают, что содержание розмариновой кислоты варьирует от 2343,40 мг/кг до 14351,74 мг/кг, а кофейной – от 74,41 мг/кг до 93,86 мг/кг. Самое высокое содержание розмариновой кислоты, так же как и кофейной было характерно для травы Thymus pulegioides. Содержание суммы гидроксикоричных кислот устанавливали спектрофотометрически и оно составляло 3,27-19,28%. Наивысший уровень суммы гидроксикоричных кислот наблюдался в траве Thymus crenulatus. Определены оптимальные параметры экстракции травы тимьяна: размер частиц, тип экстрагента и время экстракции.

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DEVELOPMENT OF THE METHOD FOR QUANTITATIVE DETERMINATION OF PHENYLEPHRINE HYDROCHLORIDE IN THE COMBINED DROPS

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Key words: phenylephrine hydrochloride; validation; quantitative determination; spectrophotometry; combined nasal drops

The work is devoted to development of the method for quantitative determination of phenylephrine hydrochloride in the combined nasal drops Gripocitron Rinis. Combination of phenylephrine hydrochloride possessing the vasoconstrictor action with dimetindene maleate having the antihistaminic action formulated into drops decreases nasal discharge and helps to clear the nasal passages without disturbing physiological functions of the ciliated epithelium and mucous membrane. It has been found that the quantitative content of phenylephrine hydrochloride in the combined nasal drops can be determined by spectrophotometry in the ultraviolet spectrum at the wavelength of 296 nm. The method proposed demonstrates the possibility of phenylephrine hydrochloride determination in combined drops in the presence of another active pharmaceutical ingredient – dimetindene maleate, which has the absorption minimum in sodium hydroxide solution at this wavelength. The influence of the additional ingredient – benzalkonium chloride drops on the nature of the absorption spectrum of phenylephrine hydrochloride is recommended to eliminate by the action of potassium dichromate solution. The optimal amount and concentration of solutions of sodium hydroxide and potassium dichromate, the analytical band have been determined. Validation of the given method has been carried out according to the following validation characteristics: linearity (a = 4.34 ≤ maxa 5.10%, b = 1.04), accuracy (0.52 ≤ maxδ 1.02%), convergence (1.07% ≤ max Δas 3.20%) and the correlation coefficient r, which is 0.9997. It has been found that the method suggested for determination of phenylephrine hydrochloride in nasal drops is precise, accurate, reproducible and linear, and it allows recommending it for using in pharmaceutical analysis.

Phenylephrine hydrochloride is a known sympathomimetic providing a selective stimulating effect mainly on postsynaptic α-adrenergic receptors [4, 5]. Being a decongestant with a moderate vasoconstrictor action phenylephrine contains in a number of nasal drugs for local application, such as Nasol Baby (phenylephrine hydrochloride), Nasol Kids (phenylephrine hydrochloride with eucalyptol and benzalkonium chloride), Vibrocil and Gripocitron Rinis (phenylephrine hydrochloride with dimetindene maleate and benzalkonium chloride), which are used for symptomatic treatment of nasal congestion, acute and chronic rhinitis, allergic rhinitis, sinusitis, acute otitis.

The search of safe and effective monocomponent and combined decongestant nasal drops for local application with phenylephrine hydrochloride actualizes development and standardization of methods for quality control of this substance in the presence of other active pharmaceutical ingredients (API) [3, 6, 7, 9].

Materials and Methods

The object of the research was Gripocitron Rinis, nasal drops, 15 ml. The active substances were phenylephrine hydrochloride equivalent to 2.5 mg of phenylephrine, dimetindene maleate 0.25 mg; excipients were citric acid, monohydrate; anhydrous sodium hydrogen phosphate; sorbitol (E 420); benzalkonium chloride; peppermint oil; purified water as needed. The reference standard (RS) of the substance of phenylephrine hydrochloride (Unichem laboratories Ltd, India) is batch PPPPH/1104 from 01.10.10.

The analytical study was performed by the method of absorption spectroscopy on an Evolution 60S spectrophotometer v4.003. During the work "AXIS" electronic laboratory balances, measuring glassware of class A were used. Reagents and titrants used in tests met the requirements of the State Pharmacopoeia of Ukraine [3, 8].

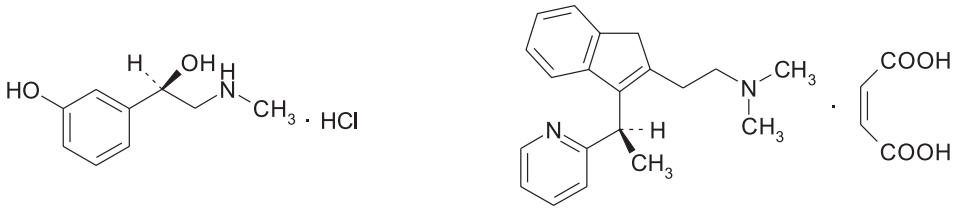
Experimental Part

Test solution. To the accurately weighed volume of the drug, which is equivalent to 2.5 mg of phenylephrine hydrochloride, add 0.25 ml of 5% solution of potassium dichromate, 5 ml 1 *M* solution of sodium hydroxide and dilute with water to the volume of 50.0 ml.

Reference solution (a). Dissolve 0.05 g of phenylephrine hydrochloride RS in 30 ml of 0.1 *M* solution of sodium hydroxide, dilute the volume of the solution with the same solvent to 50.0 ml. To 2.5 ml of the solution obtained add 0.25 ml of 5% solution of potassium dichromate, 5 ml of 1 *M* solution of sodium hydroxide and dilute with water to the volume of 50.0 ml.

Compensation solution. Dilute 0.25 ml of 5% solution of potassium dichromate and 5 ml of 1 *M* solution of sodium hydroxide with water to the volume of 50 ml.

The optical density of the test solution and the reference solution is measured at the wavelength of 296 nm with respect to the compensation solution.



I II

Scheme

Results and Discussion

To develop the method for quantitative determination of phenylephrine hydrochloride in Gripocitron Rinis drops the parameters of active ingredients solubility [3, 5] were studied, and their UV-spectra were investigated. Phenylephrine hydrochloride ((1*R*)-1-(3-hydroxyphenyl)-2-(methylamino)ethanol hydrochloride) (I) and dimetindene maleate (*N,N*-dimethyl-2-[3-[(*RS*)-1-(pyridine-2-yl)ethyl]-1*H*-indene-2-yl]ethanamine (*Z*)-butenedioate) (II) are readily soluble in diluted acids and alkalis, in alcohols their solubility is slightly different (Scheme).

At the same time it was found that when using 0.1 *M* solution of hydrochloric acid or alcohol as solvents the maxima of the UV-spectra of 0.005% solution of phenylephrine hydrochloride were observed at the wavelengths of 275 nm or 272 nm [1], and in 0.0005% solution of dimetindene maleate in the acidic medium at 260 nm and the alcoholic one – at 258 nm. Therefore, under these conditions dimetindene maleate has impact on the character of the ultraviolet spectrum of phenylephrine hydrochloride.

When replacing the solvent on 0.1 *M* solution of sodium hydroxide the absorption spectra of 0.005% solution of phenylephrine hydrochloride is characterized by the presence of two absorption maxima at the wavelengths of 239 nm and 292 nm (Fig. 1).

The UV-spectrum of 0.0005% alkaline solution of dimetindene maleate has the absorption maximum at the wavelength of 263 nm, which corresponds to the UV-spectrum minimum of phenylephrine hydrochloride, and at the wavelength of 292 nm the substance does not practically absorb. It indicates that dimetindene maleate does not interfere with detection of phenylephrine hydrochloride under such conditions. The alkaline solutions of Gripocitron Rinis drug and the test mixture containing the active substances in the same concentrations as in the dosage form were also studied. It has been found in the analysis of UV-spectra in the range from 220 to 320 nm that the spectrum of the test solution is characterized by the absorption maxima at the same wavelengths and practically overlaps with the absorption spectrum of phenylephrine hydrochloride solution. The maximum of the absorption spectrum of the solution prepared from the drops under research is also observed at the wavelength of 292 nm, but it is more intensive. Therefore, excipients containing in the drops influence on the character of the drug absorption spectrum (Fig. 1).

Our assumption has been made that the presence of benzalkonium chloride included into the composition of the drops as a preservative and antiseptic has an impact on the character of the UV absorption spectrum of the drops. From literature data it is known that the al-

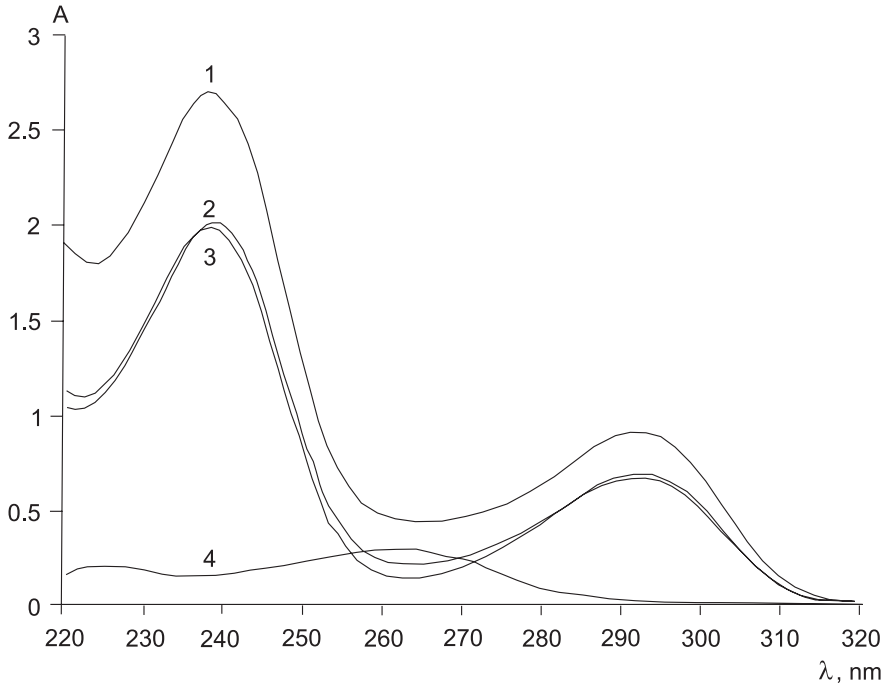


Fig. 1. The UV absorption spectra in 0.1*M* solution of sodium hydroxide: 1 –Gripocitron Rinis drug; 2 – test solution; 3 – 0.005% solution of phenylephrine hydrochloride; 4 – 0.0005% solution of dimetindene maleate.

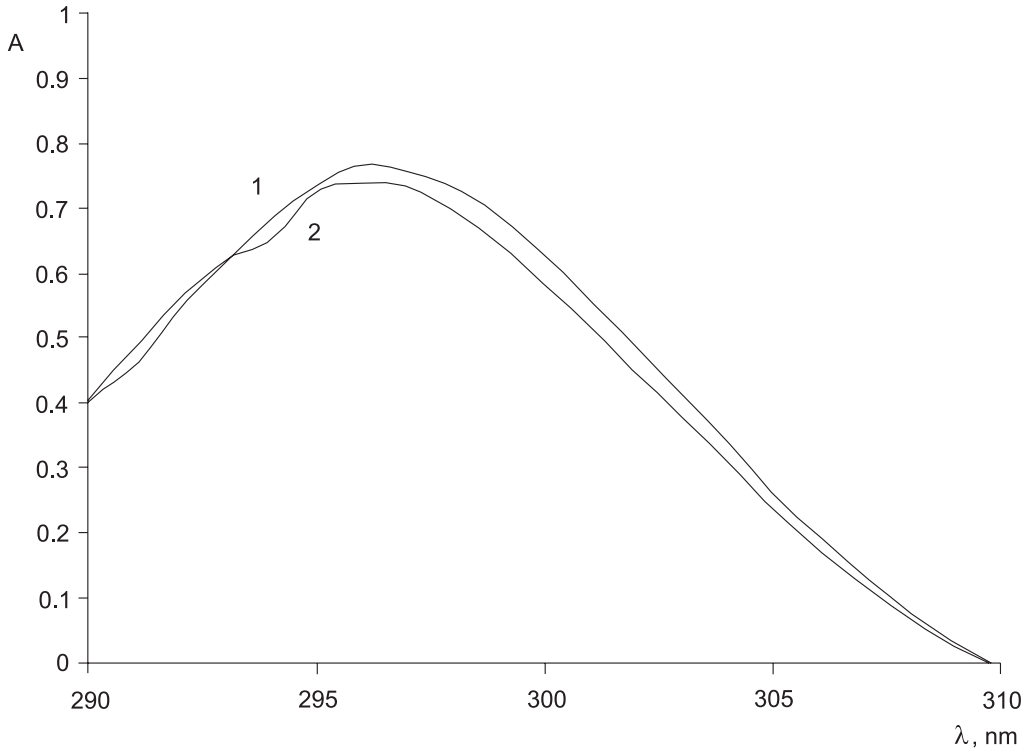


Fig. 2. The UV absorption spectra in 0.1*M* solution of sodium hydroxide with addition of 0.25 ml of 5% solution of potassium dichromate: 1 – Gripocitron Rinis drug; 2 – 0.005% of phenylephrine hydrochloride solution.

Table 1

Dependence of the optical density on the amount of potassium dichromate solution

Optical density	The amount of 5% solution of potassium dichromate, ml			
	0.15	0.20	0.25	0.30
A	0.716	0.845	0.706	0.938

coholic solution of benzalkonium chloride in the ultraviolet light has absorption maxima at 257, 263 and 269 nm [10]. To precipitate benzalkonium chloride its ability to interact with potassium dichromate was used. When studying the UV absorption spectra of Gripocitron Rinis drug in 0.1 *M* solution of sodium hydroxide with addition of 5% solution of potassium dichromate and phenylephrine hydrochloride solution under the same conditions in the region from 290 to 310 nm it has been found that their intensity is practically the same (Fig. 2).

Since the solution of potassium dichromate is coloured in an orange-yellow colour, the solution containing the same amount of potassium dichromate as in the test and reference solutions is used as a compensation solution in order to obtain more reliable data.

Table 2

The results of the linearity study

Parameters	Values	Values according to the SPhU
b	1.0425	
s_b	0.0049	
a	| -4.3372 |	max, a=4.80%
s_0	0.6585	max, S_0=1.69%
s_Y	13.0741	
r	0.9997	min, r= 0.99236

To determine the amount of potassium dichromate required for benzalkonium chloride binding the model solutions of drops were prepared; different amounts of 5% solution of potassium dichromate (Tab. 1) were added to them.

It has been found that to carry out the precipitation reaction with benzalkonium chloride 0.25 ml of 5% solution of potassium dichromate is required since with this amount of the solution the spectrum is the most similar to the spectrum of phenylephrine hydrochloride.

Thus, the research conducted allows to propose the quantitative determination of phenylephrine hydrochlo-

Table 3

The results of analysis for test solutions and their statistical processing

Mean, Z%	99.84
Relative standard deviation, Sz%	0.16
Relative confidence interval Δas%=t(95%.8)·Sz=1.860·Sz=	0.29
Critical value for convergence of results Δas%	3.20
Systematic error δ	0.05
Criterion of the systematic error insignificance 1) δ≤Δ/√9=0.05/3=0.016 2) if it is not satisfied 1), then δ≤0.75	satisfied
The overall conclusion of the method	correct

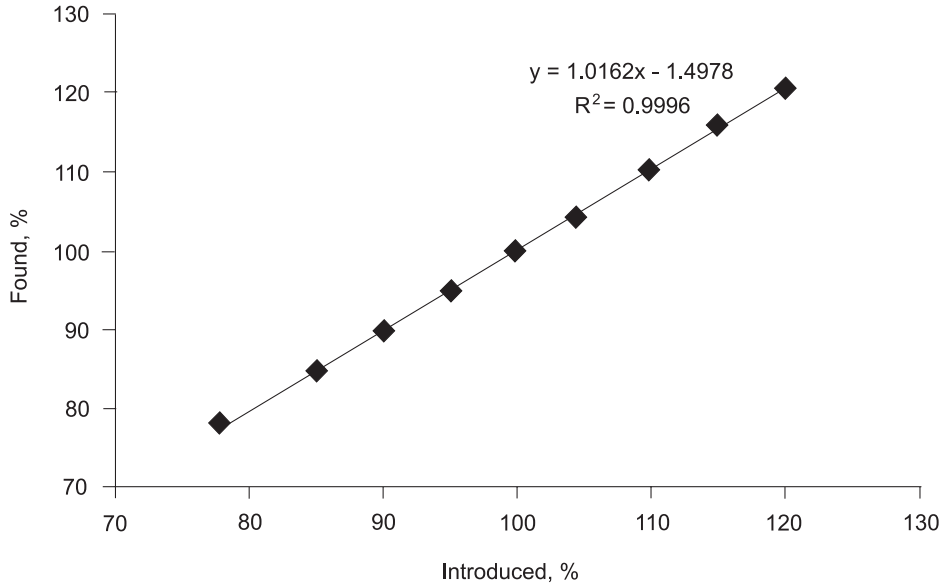


Fig. 3. The linear dependence of the optical density on the concentration of phenylephrine hydrochloride solutions in the normalized coordinates.

Table 4

The results of quantitative determination of phenylephrine hydrochloride in drops

No.	The volume of a dosage form, ml	A	A_o	Found phenylephrine hydrochloride, mg	Metrological characteristics
1	1.00	0.589	0.589	2.4975	$\bar{x}$ = 2.4939 S^2 = 0.0001 S = 0.0112 $\Delta\bar{x}$ = 0.0048 ε% = 0.47
2		0.590		2.5017	
3		0.586		2.4848	
4		0.585		2.4805	
5		0.592		2.5102	
6		0.587		2.4890	

ride in the combined nasal drops can be determined by spectrophotometry after preliminary transfer of dimetindene maleate into the base and precipitation of the excipient benzalkonium chloride with potassium dichromate solution.

To use the method for analysis of phenylephrine hydrochloride in the combined drops some validation characteristics such as linearity, precision, accuracy and convergence were studied (Tab. 2).

Linearity of the method was studied on the model solutions within the range of concentrations of 80-120%. It corresponds to the range of use relative to the nominal content of phenylephrine hydrochloride in nasal drops (Fig. 3, Tab. 2) [2].

The method of analysis is characterized by sufficient convergence and accuracy within the whole range of concentrations of 80-120%; it can be seen from the results obtained that are presented in Tab. 3.

The results of quantitative determination of phenylephrine hydrochloride in nasal drops by spectrophotometry are given in Tab. 4.

It has been found that relative uncertainty of the individual result is ±0.47%.

CONCLUSIONS

1. The method for quantitative determination of the active substance – phenylephrine hydrochloride in Gripocitron Rinis nasal drops has been developed by UV-spectroscopy.

2. The validation characteristics of the method proposed (accuracy and convergence, linearity, precision) have been studied. According to the results this method can be recommended for using in analysis of the given dosage form.

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РОЗРОБКА МЕТОДИКИ КІЛЬКІСНОГО ВИЗНАЧЕННЯ ФЕНІЛЕФРИНУ ГІДРОХЛОРИДУ В КОМБІНОВАНИХ КРАПЛЯХ

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Ключові слова: *фенілефрину гідрохлорид; валідація; кількісне визначення; спектрофотометрія; комбіновані назальні краплі*

Робота присвячена розробці методики кількісного визначення фенілефрину гідрохлориду у комбінованих назальних краплях Грипоцитрон Риніс. Поєднання у складі крапель фенілефрину гідрохлориду, який чинить судинозвужуючу дію, та диметиндену малеату, що має антигістамінну дію, зменшує виділення з носа і сприяє очищенню носових ходів, не порушуючи при цьому фізіологічних функцій миготливого епітелію та слизової оболонки. Встановлено, що кількісний вміст фенілефрину гідрохлориду в комбінованих назальних краплях можна визначати методом спектрофотометрії в ультрафіолетовій області спектра за довжини хвилі 296 нм. Запропонована методика доводить можливість визначення фенілефрину гідрохлориду в складних краплях у присутності іншого активного фармацевтичного інгредієнта диметиндену малеату, який у розчині натрію гідроксиду за цієї довжини хвилі має мінімум поглинання. Вплив допоміжного компонента крапель – бензалконію хлориду на характер спектра поглинання фенілефрину гідрохлориду рекомендовано усунути дією розчину калію дихромату. Встановлені оптимальна кількість та концентрація розчинів натрію гідроксиду та калію дихромату, аналітична хвиля дослідження. Проведена валідація зазначеної методики за такими валідаційними характеристиками: лінійність (a = 4.34 ≤ maxa 5,10%, b = 1,04), правильність (0,52 ≤ maxδ 1,02%), збіжність (1.07% ≤ max Δas 3,20%) та коефіцієнт кореляції r, який становить 0.9997. Встановлено, що запропонована методика визначення фенілефрину гідрохлориду в назальних краплях є точною, правильною, відтворюваною і лінійною, що дозволяє рекомендувати її для використання у фармацевтичному аналізі.

РАЗРАБОТКА МЕТОДИКИ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ФЕНИЛЭФРИНА ГИДРОХЛОРИДА В КОМБИНИРОВАННЫХ КАПЛЯХ

А.В.Криванич, Н.Ю.Бевз, В.А.Георгиянц

Ключевые слова: *фенилэфрина гидрохлорид; валидация; количественное определение; спектрофотометрия; комбинированные назальные капли*

Работа посвящена разработке спектрофотометрической методики количественного определения фенилэфрина гидрохлорида в комбинированных назальных каплях Гриппоцитрон Ринос. Сочетание в составе капель фенилэфрина гидрохлорида, обладающего сосудосуживающим действием, и диметиндена малеата с антигистаминным эффектом уменьшает выделения из носа и способствует очищению носовых ходов, не нарушая при этом физиологических функций мерцательного эпителия и слизистой оболочки. Установлено, что количественное содержание фенилэфрина гидрохлорида в комбинированных назальных каплях можно определять методом спектрофотометрии в ультрафиолетовой области спектра при длине волны 296 нм. Предложенная методика доказывает возможность определения фенилэфрина гидрохлорида в сложных каплях в присутствии другого активного фармацевтического ингредиента диметиндена малеата, который в растворе натрия гидроксида при указанной длине волны имеет минимум поглощения. Влияние вспомогательного компонента капель бензалкония хлорида на характер спектра поглощения фенилэфрина гидрохлорида рекомендовано устранять действием раствора калия дихромата. Установлены оптимальное количество и концентрация растворов натрия гидроксида и калия дихромата. Проведена валидация данной методики по следующим валидационным характеристикам: линейность (a = 4.34 ≤ maxa 5,10%, b = 1,04), правильность(0,52 ≤ maxδ 1,02%), сходимость(1,07% ≤ max Δas 3,20%) и коэффициент корреляции r, равный 0,9997. Установлено, что предлагаемая методика определения фенилэфрина гидрохлорида в назальных каплях является точной, правильной, воспроизводимой и линейной, что позволяет рекомендовать ее для использования в фармацевтическом анализе.

Recommended by Doctor of Pharmacy S.V.Garna

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THE QUANTITATIVE CONTENT OF THE MAIN GROUPS OF BIOLOGICALLY ACTIVE SUBSTANCES IN THE BAY LAUREL RAW MATERIAL

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Key words: biologically active substances; bay laurel; quantitative content

The study of the chemical composition of the bay laurel raw material has shown that the plant raw material contains such groups of biologically active substances (BAS) as carbohydrates, fatty and amino acids, phenolic compounds revealing a wide spectrum of the biological activity. The data concerning the chemical composition of the bay laurel raw material is not enough described in the literature sources. The content of the main BAS in different samples of bay laurel shoots and leaves has been determined. The content of the total amount of organic acids, the total amount of oxidizable phenols, the total amount of hydroxycinnamic acids and the total amount of flavonoids in the bay laurel leaves samples are slightly higher. The content of the total amount of organic acids in shoots is not less than 1.7%, in leaves – not less than 1.9%. The content of the total amount of oxidizable phenols in both types of the raw material is not less than 4.5%, the total amount of hydroxycinnamic acids – not less than 1.3%, the total amount of flavonoids – not less than 0.8%. The standardization parameters for the bay laurel raw material have been determined on the basis of the research conducted. The results obtained will be used for developing the project of quality control methods "Lauri Cormus".

The profound research of the medicinal plant raw material previously studied for the potential expansion of the aspects of its application is a topical task of modern pharmacy. Our attention was attracted to plants of the *Lauraceae* family, *Laurus* L. genus combining 3 species – *Laurus azorica* (Seub.) Franco, *Laurus nobilis* L., *Laurus porrecta* Roxb. (the latter species is related to *Cinnamomum* genus, *Cinnamomum porrectum* (Roxb.) Kosterm. species) [3]). The most wide-spread species is bay laurel – an evergreen tree or a shrub up to 10 m high. Bay laurel fruits were included into 6 pre-revolutionary editions of the Russian Pharmacopoeia, and the State Pharmacopoeia of the USSR, the 1st ed. Its leaves are used in the treatment of throat cancer, psoriasis, arthritis, pain in joints, cramps, diabetes. The bay laurel oil obtained with flax or sunflower oil is used in the cases of paralysis, rheumatism, etc. The essential oil from leaves contains 1,8-cineol, terpinen-4-ol, α-pinene, β-pinene, linalool acetate [15], as well as β-cymene, β-longipirene, cadinene, α-terpinyl acetate, α-balnesene [12]. BAS of bay laurel are considered to reveal wound-healing [14], anti-inflammatory [10], antinociceptive [10], immune stimulating [5], neuroprotective [11, 14], anticholinergic [14], anti-oxidant [6, 8, 9, 13], anti-ulcer, anti-convulsant, antimutagenic [14], insecticidal [4], antibacterial [7, 14], antiviral, antifungal [14], larvicidal [15] activities. At the same time bay laurel is contraindicated in the cases of glomerulonephritis, pregnancy, tendency to bleedings, amyloidosis. Moreover, medicines produced from this plant are absent nowadays. Thus, the profound studies of the bay laurel raw material and creation of new substances on its basis are up to date.

The study of the chemical composition of the bay laurel plant raw material has shown that the plant contains such groups of BAS as carbohydrates, fatty and amino acids, phenolic compounds. The data concerning the chemical composition of the bay laurel raw material is not enough described in the literature sources. Thus, the aim of our research was to determine the quantitative content of the main BAS groups in the bay laurel raw material.

Materials and Methods

The objects of the study were the bay laurel shoots and leaves collected in November, 2013 in Alushta (Sample 1) and Rybachye (Crimea) (Sample 2).

The method described in the State Pharmacopoeia of the USSR, the XI-th ed., art. 38 "Rosae fructus" [2] was used to determine the total amount of organic acids calculated with reference to malic acid. The total amount of oxidizable phenols was determined using the method described in the State Pharmacopoeia of the USSR, the XI-th ed. [2].

The quantitative content the total amount of hydroxycinnamic acids in the bay laurel raw material was determined by the spectrophotometric method calculated with reference to chlorogenic acid [1]. For this purpose, place 2.0 g (accurate weight) of the cut plant raw material passing through the sieves with the size of 1 mm in diameter into a 200 ml conical flask and add 60 ml of 50% ethanol. Attach the flask to a reflux condenser and heat on a boiling water bath for 15 min. After cooling filter the content of the flask through a paper filter into a 100 ml conical flask. Repeat the extraction twice under the same conditions.

Transfer the extracts obtained quantitatively into a 200 ml volumetric flask and dilute the solution to the volume with 50% ethanol (Solution A). Place 1 ml of Solution A into a 50 ml volumetric flask and dilute the solution to the volume with 50% ethanol.

Table

Results of the quantitative determination of the main BAS in the bay laurel raw material (m=5, P≥0.95, % calculated with reference to the dry raw material)

No. of the sample	Quantitative content of, X±ΔX			
	organic acids	oxidizable polyphenols	hydroxycinnamic acids	flavonoids
Shoots				
1	1.75±0.01	4.80±0.12	1.35±0.08	0.85±0.03
2	1.81±0.01	4.54±0.17	1.29±0.07	0.81±0.07
Leaves				
1	2.14±0.09	5.25±0.16	1.73±0.05	0.95±0.06
2	1.98±0.02	5.04±0.11	1.71±0.05	0.91±0.05

The optical density of the solution obtained was measured using a Lomo SF – 46 spectrophotometer at the wavelength of 327 nm. As the reference solution 50% ethanol was used. The content of the total amount of hydroxycinnamic acids in per cents calculated with reference to chlorogenic acid was calculated using the formula:

$$X = \frac{A \cdot 200 \cdot 50 \cdot 100 \cdot 100}{E_{1cm}^{1\%} \cdot m \cdot (100 - W)},$$

where: A – is the optical density of the solution studied; m – is the raw material weight, g; *W* – is the weight loss on drying, %; $E_{1cm}^{1\%}$ – is the specific absorption coefficient of chlorogenic acid (531).

The content of the total amount of flavonoid glycosides was determined using the method described in literature [1]. Place 4 ml of Solution A (obtained by the method described above) into a 50 ml volumetric flask, add 4 ml of 3% ethanol solution of aluminium chloride and dilute the solution to the volume with 50% ethanol. Place the solution containing 4 ml of Solution A into a 50 ml volumetric flask and dilute the solution to the volume with 50% ethanol. The solution obtained was used as the reference solution.

Both solutions were filtered through a "blue strip" paper filter discarding the first filtrate portions, and the solutions were analyzed in 30 min after preparation.

The optical density was determined at the wavelength of 417 nm. The content of the total amount of flavonoids (*X*) was calculated with reference to rutin using the formula:

$$x = \frac{A \cdot 2500 \cdot 100 \cdot 100}{E_{1cm}^{1\%} \cdot m \cdot (100 - W)},$$

where: *A* – is the optical density of the solution studied; $E_{1cm}^{1\%}$ – is the specific absorption coefficient of rutin (257); m – is the raw material weight, g; *W* – is the weight loss on drying, %.

Results and Discussion

The data obtained are generalized in the Table. The content of the total amount of organic acids, of the total amount of oxidizable phenols, of the total amount of hydroxycinnamic acids and of the total amount of flavonoids was found to be slightly higher in samples of the bay laurel leaves than in shoots.

The highest content of the total amount of organic acids was found in leaves of Sample 1 (2.14±0.09%). The content of this group of BAS in leaves was not less than 1.9%, in shoots – not less than 1.7%.

The content of the total amount of oxidizable phenols was not less than 4.5% calculated with reference to the absolutely dry plant raw material independently from its type and the place of gathering. The highest content of this group of BAS was determined in the bay laurel leaves (Sample 1) – 5.25±0.16%, which was only 1.2 times higher than in shoots of Sample 2 (the lowest value – 4.54±0.17%).

According to the data obtained the content of the total amount of hydroxycinnamic acids in all types of the raw material was not less than 1.3%. The variability of this value was insignificant. The highest content was determined in the bay laurel leaves of Sample 1 (1.73±0.05%). The lowest content of this group of BAS (1.34 times lower than the highest value) was found in shoots of Sample 2 (1.29±0.07%).

The quantitative content of the total amount of flavonoids was not less than 0.8% in all types of the raw material. The results 1.2 times varied depending on the place of collection and the type of the raw material: from 0.95±0.06% (leaves of Sample 1) up to 0.81±0.07% (shoots of Sample 2).

CONCLUSIONS

1. The quantitative content has been determined in 2 samples of the bay laurel shoots and leaves; the lower content values of the main groups of BAS – the total amount of organic acids, as well as different phenolic compounds (the total amount of oxidizable phenols, the total amount of hydroxycinnamic acids and the total amount of flavonoids) have been found. The content of the total amount of organic acids in shoots was not less than 1.7%, in leaves – not less than 1.9%. The content of the total amount of oxidizable phenols in both types of the raw material was not less than 4.5%, the total amount of hydroxycinnamic acids – not less than 1.3%, the total amount of flavonoids – not less than 0.8%.

2. The standardization parameters for the bay laurel plant raw material have been determined on the basis of the research conducted. The results obtained are necessary for developing the project of quality control methods "Lauri Cormus".

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КІЛЬКІСНИЙ ВМІСТ ОСНОВНИХ ГРУП БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН У СИРОВИНІ ЛАВРА БЛАГОРОДНОГО

С.Г.Мусієнко, В.С.Кисличенко

Ключові слова: *біологічно активні речовини; лавр благородний; кількісний вміст*

Вивчення хімічного складу сировини лавра благородного показало, що сировина рослини містить такі групи БАР, як вуглеводи, кислоти жирні, амінокислоти, фенольні сполуки, що проявляють широкий спектр біологічної дії. В літературних джерелах відомості про хімічний склад сировини лавра благородного не достатньо повні. Визначено вміст основних груп БАР у різних зразках пагонів та листя лавра благородного. Вміст БАР, що досліджувалися, майже не відрізняється в обох видах сировини, що вивчалася. При цьому вміст суми органічних кислот становить у пагонах не менше 1,7%, в листі – не менше 1,9%, вміст суми окиснюваних фенолів становить для обох видів сировини не менше 4,5%, суми кислот гідроксикоричних – не менше 1,3%, суми флавоноїдів – не менше 0,8%. На підставі проведених досліджень визначені показники доброякісності сировини, які будуть використані при розробці відповідних розділів проекту МКЯ «Lauri cormus».

КОЛИЧЕСТВЕННОЕ СОДЕРЖАНИЕ ОСНОВНЫХ ГРУПП БИОЛОГИЧЕСКИ АКТИВНЫХ ВЕЩЕСТВ В СЫРЬЕ ЛАВРА БЛАГОРОДНОГО

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Ключевые слова: *биологически активные вещества; лавр благородный; количественное содержание*

Изучение химического состава сырья лавра благородного показало, что сырье содержит такие группы БАВ, как углеводы, жирные кислоты, аминокислоты, фенольные вещества, которые проявляют широкий спектр биологической активности. В литературных источниках сведения о химическом составе сырья лавра благородного недостаточно полные. Определено содержание основных групп БАВ в разных образцах побегов и листьев лавра благородного. Содержание суммы органических кислот, суммы окисляемых фенолов, суммы оксикоричных кислот и суммы флавоноидов в образцах листьев незначительно выше. При этом содержание суммы органических кислот составляет в побегах не меньше 1,7%, в листьях – не меньше 1,9%, содержание суммы окисляемых фенолов составило для обоих видов сырья не меньше 4,5%, суммы оксикоричных кислот – не меньше 1,3%, суммы флавоноидов – не меньше 0,8%. На основании проведенных исследований определены показатели доброкачественности сырья, которые будут использованы при разработке соответствующих разделов проекта МКК «Lauri cormus».

Recommended by Doctor of Chemistry, professor A.I.Gryzodub

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EVALUATION OF METROLOGICAL CHARACTERISTICS OF SPECTROPHOTOMETRIC QUANTITATIVE DETERMINATION OF PARACETAMOL IN TABLETS BY SPECIFIC ABSORBANCE

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Key words: paracetamol; quantitative determination; spectrophotometry; specific absorbance method; validation

The validation characteristics of the spectrophotometric quantitative determination of paracetamol in tablets by specific absorbance according to the British Pharmacopoeia (BPh) have been evaluated. The results of paracetamol content of 83.59% and 84.39% in terms of the average mass of one tablet do not meet the permissible limits B ±5.0%. The peculiarities of the sample preparation method for quantitative determination of the active pharmaceutical ingredient in tablets has been discussed, and comparative analysis of "Dissolution" and "Assay" tests for paracetamol tablets according to the BPh has been conducted. We have suggested to make such changes at the stage of the sample preparation as "… place the flask in an ultrasonic bath for 30 min…" instead of "…shake for 15 minutes…". The acceptance criteria of the assay method for paracetamol tablets have been calculated for permissible limits of ±5.0%, ±7.5%, ±10.0%. The results of the convergence and linearity research of the method meet requirements for the permissible limits of ±5.0%. The results of the intermediate precision research of the method meet requirements for the permissible limits of ±7.5%. The results of the accuracy research of the method meet requirements for the permissible limits of ±10.0%. Taking into account the technical capabilities of the Ukrainian producers and a diverse list of excipients used in the manufacture of the drug, the spectrophotometric quantitative determination of paracetamol tablets by specific absorbance is recommended to use with the permissible limits of ±10.0%. The prognosis of the total uncertainty of the analysis results is consistent with requirements to the maximum permissible uncertainty of the analysis $\Delta_{As}^{10.0\%} = 2.6 \leq max\ \Delta_{As}^{10.0\%} = 3.2\%$ *and with results of the 3rd round of the Professional Testing Programme (PTP) of "Pharma-test" laboratories in the system of the State Inspection for Medication Quality Control of the Ministry of Public Health of Ukraine.*

Paracetamol belongs to the group of non-steroidal anti-inflammatory drugs, it is a nonselective COX inhibitor, and over 50 years it has already been used as an antipyretic and analgesic [10]. Monocomponent formulations based on paracetamol tablets, capsules, solutions, suppositories, suspensions, granules, gel are produced by pharmaceutical industry. Paracetamol is part of many combined medicines with antipyretic and analgesic effects. The research concerning the use of paracetamol to treat pain in neonates as an alternative to opiates is being performed [12].

Quantitative determination of paracetamol in the substance according to the monographs of the State Pharmacopoeia of Ukraine (SPhU) [7], European [11], British [9] pharmacopoeias and Pharmacopoeia of the Republic of Belarus [3] is carried out by the ceriometry method, American [16], Japan [14] Korea [15] pharmacopoeias by the spectrophotometric method (by standard), China [13] – by specific absorbance.

UV-spectrophotometry by standard [7] and specific absorbance methods [9, 13], HPLC [16] are used for pharmacopoeial quantitative assessment of paracetamol tablets.

Thanks to the introduction of quality assurance systems for results of analysis, equipment qualification the specific absorbance method has been widely used in pharmacopoeial analysis. At present the specific absorbance method is recommended by the SPhU not only for quantitative determination of 10 substances [7], but also for 21 types of medicinal plants [6]. A standardized procedure of validation of spectrophotometric methods for quantitative determination of drugs by specific absorbance has been developed [4] and successfully approved on the quantitative determination methods for prednisolone and riboflavin substances [18].

The aim of this research is to evaluate the metrological characteristics of spectrophotometric quantitative determination of paracetamol in tablets by specific absorbance, which is recommended by the British Pharmacopoeia (BPh) and to determine acceptable permissible limits for this method.

Experimental Part

Tablets "Paracetamol", 200 mg, manufactured by the pharmaceutical company "Darnitsya", batch UA / 4369/01/01 were chosen as an object of the research.

The following analytical equipment was used: a "SPECORD 200" spectrophotometer, AV 204 S / A METTLER TOLEDO analytical balance. Reagents, measuring glassware of class A (first class) and excipients meeting the requirements of the SPhU were used for the work.

The assay method for paracetamol in tablets according to the British Pharmacopoeia [9]: weigh and powder 20 tablets.

Table 1

The critical values of the systematic error ($max\ \delta_{tot}$), total uncertainty of the analysis ($max\Delta_{As}$) and parameters of the linear dependence $Y_i = b \cdot X_i + a^*$

Permissible limits, *B*%	λ, nm	$A_{1cm}^{1\%}$	C_{nom}, mg/100 ml	A_{nom}	max$\Delta_{,As}$%	maxδ_{tot} = max$\Delta_{prec,}$%	$RSD_{o,}$ %	min R^2_c	max*a*, %	max$\delta_{A,}$%
±5.0%	257	715	0.75	0.536	1.6	1.15	0.60	0.9981	2.34	2.6
±7.5%					2.4	1.7	0.90	0.9957	3.5	
±10.0%					3.2	2.3	1.20	0.9924	4.7	

* the number of points 9, for the range of 80-120%.

Add an accurately weighed powder containing 0.15 g of paracetamol to 50 ml of 0.1 M sodium hydroxide, dilute with 100 ml of water, shake for 15 minutes and dilute to 200 ml with a sufficient amount of water. Mix, filter and dilute 10 ml of the filtrate to 100 ml with water. Add 10 ml of the solution obtained to 10 ml of 0.1M sodium hydroxide, dilute to 100 ml with water and measure the absorbance of the solution obtained at the maximum at 257 nm. Calculate the content of $C_8H_9NO_2$ taking 715 as the value of A ($A_{1cm}^{1\%}$) at the maximum at 257 nm.

The nominal content of paracetamol b_{nom} is 200 mg; the average weight of one tablet is 256.02 mg. The content of paracetamol in one tablet in terms of the average weight of one tablet in percentage of the prescribed amount was calculated by the formula:

$$X\ (\%) = \frac{10 \cdot A_1}{A_{1cm}^{1\%}} \cdot D \cdot m_t \cdot \frac{100}{b_{nom}}\ ;\ D = \frac{V_D}{m}\ ,$$

where: D – is dilution of the sample analyzed, m – is the mass of the sample for analysis. In our case, dilution is:

$$D = \frac{V_D}{m} = \frac{200}{0.1952} \times \frac{100}{10} \times \frac{100}{10} = \frac{20000}{0.1952} \cdot$$

Results and Discussion

According to the specific absorbance method it is possible to obtain the correct results using a high level of equipment, its qualification and compliance with the requirements of the SPhU [7]. Taking this into account the qualification spectrophotometer characteristics were evaluated before the experiment. The control of cells, absorbance accuracy, absorbance convergence with removing cells, the limit of stray light have been carried out. The results obtained meet requirements of the SPhU.

The acceptance criteria of the assay method for paracetamol tablets was calculated considering the peculiarities of spectrophotometry by the specific absorbance method [4] for permissible limits of 95-105% (B = ±5.0%) and ±7.5%, ±10.0% according to the monograph (Tab. 1).

At first quantitative determination of paracetamol tablets in the concentration of 100% in accordance with the prescribed amount was carried out. To control correctness of the results and accuracy of the sample preparation two parallel studies of the tablet powder were conducted. Immediately after preparing analytical solutions according to the method, absorbance (A) was measured at the absorbance maximum of 257 nm three times with removing the cells. The results of the paracetamol content of 83.59% and 84.39% in terms of the average mass of one tablet do not meet the permissible limits (Tab. 2).

According to the results of the accuracy control of the sample preparation $|X_1\text{-}X_2| = 0.80\% < \Delta_{As}1.60\%$; the negative result cannot be associated with the analyst's errors.

Peculiarities of the sample preparation of quantitative determination methods for the active pharmaceutical ingredient (API) in tablets. Determination of the quantitative content of the API in tablets has certain features that must be considered in standardization of methods. An accurately weighed quantity is dissolved in a suitable solvent in one or several steps using measuring glassware in quantitative determination of substances. Each step of the sample preparation is a part of uncertainty, which is calculated from the values of permissible uncertainty of measuring glassware and weighing according to the SPhU. In addition to the abovementioned sample preparation steps the method includes such additional operations as weighing of 20 tablets, powdering, dissolving and filtering, which bring more uncertainty to the total uncertainty of the sample preparation in quantitative determination of the API in tablets.

The relationship of "Dissolution" and "Assay" tests for paracetamol tablets according to the BPh. It should be noted that according to the BPh monograph control of dissolution of paracetamol tablets and "Assay" test for the API in tablets are carried out by UV-spectrophotometry using specific absorbance [9]. "Dissolution" and "Assay" tests are quite similar in operations, but differ in terms of dissolution, the purpose and test evaluation.

"Dissolution" test determines the minimum quality requirements for pharmaco-technological properties of paracetamol tablets regardless of the manufacturer (the composition of excipients, technology (Tab. 3)) based on the API quantitative determination after dissolution.

Conditions for "Dissolution" test: place one tablet in Apparatus II (paddle apparatus), rotate the paddle at 50 rpm (tolerance ±4%); the medium is phosphate buffer, pH 5.8 (±0.05 units), carry out dissolution at a temperature from 36.5° to 37.5°C; assess the API release in 45 minutes; dilute 20 ml of the filtrate with 0.1M sodium hydroxide to the concentration of 0.00075% (w/v); measure the absorbance of this solution; the amount of

Table 2

The results of the spectrophotometric quantitative determination of paracetamol tablets by specific absorbance

Description / parameter	The BPh method			
	sample preparation without changes		sample preparation with changes	
	Test 1	Test 2	Test 1	Test 2
The nominal content of paracetamol in one tablet of the prescribed amount b_{nom}, mg	200		200	
Permissible limits of paracetamol, %	95.0-105.0			
B, %	5			
The average mass of one tablet m_t, mg	256.02			
The mass of the tablet powder for analysis $m_н$, mg	195.2	196.8	196.3	195.9
Mean absorbance, A_i	0.4557	0.4638	0.5358	0.5363
Standard deviation, $S_{as,}$ %	0.0006	0.0010	0.0003	0.0002
Relative standard deviation, $S_{as,r,}$ %	0.12	0.21	0.06	0.04
The paracetamol content in terms of the average mass of one tablet, $X_{mean,}$ %	83.59	84.39	97.74	98.03
Control of accuracy of the sample preparation, % $\|X_1-X_2\|<\Delta_{As}$	0.80		0.29	

the active ingredient in the solution should be not less than 70% of the prescribed amount.

Conditions for "Assay" test: add an accurately weighed quantity of the powder to 50 ml of 0.1 M sodium hydroxide, dilute with 100 ml of water, shake for 15 minutes; dilute 10 ml of the filtrate with 0.1 M sodium hydroxide to the concentration of 0.00075% (w/v); measure the absorbance of this solution; the amount of the active ingredient in the solution should be within the range of 95%-105% of the prescribed amount.

The question is if the API of paracetamol can be completely released under the following conditions for 15 minutes. Paracetamol belongs to the 1st class of the biopharmaceutical classification system (BCS) and is considered to be very instant (at least 85% of the prescribed amount of the API passes into the solution for 15 min when using the paddle apparatus (50 or 75 rpm) or basket apparatus (100 rpm)) [2, 17]. The quality of 17 batches of 10 names of paracetamol tablets made in Russia and Western Europe was comparatively assessed in terms of the content of the API and the rate of dissolution (quantitative determination of the API for each batch was performed by HPLC (n = 10) according to the EuPh monograph "Paracetamol"). The results show that in 30 min 44.0±3.3% of the API of paracetamol was released for one batch; for 6 batches the dissolution percentage was in the range of 88.0±1.3% – 94.0±1.1%; for 10 batches it was 96.0±0.7% – 100.0±0.4% [1]. Thus, the difference in the release of the API may be associated with the composition of the excipients of tablets and their different formulations that each manufacturer sets independently.

Considering the facts described above the stage of the sample preparation of the method – "…shake for 15 minutes…" should be changed to "… place the flask in an ultrasonic bath for 30 min…". Two parallel experiments were conducted with the tablet powder. The results concerning the paracetamol content in terms of the average mass of one tablet of 97.74% and 98.03% meet the permissible limits (Tab. 2). Thus, too low results ob-

Table 3

Comparative analysis of the excipients when producing paracetamol tablets by the Ukrainian manufacturers

Manufacturer	How supplied	Excipients
"Lubnyfarm" JSC, Lubni, Poltava region	Tablets, 0.2 g No.10 in the blister card	Potato starch, calcium stearate, colloidal anhydrous silica, methylcellulose
"Lugansk Pharmaceutical Plant" JSC, Lugansk	Tablets, 0.2 g No. 10 in the strip	Sugar, corn starch, stearic acid, gelatin
"Agrofarm" LLC, Irpin, Kyiv region	Tablets, 0.2 g No.10	Potato starch, corn syrup, calcium stearate
"Styrolbiofarm" Ltd., Gorlovka, Donetsk region	Tablets, 0.325 g No.6 in the blister card	Croscarmellose sodium, povidone, pregelatinized starch, corn starch, stearic acid
"Pharmaceutical company" Darnitsa" PJSC, Kiev	Tablets, 0.2 g No.10 in the blister card	Potato starch, povidone, calcium stearate, aerosil
"Galychpharm", JSC, Lviv	Tablets, 0.2 g No.10 in the blister card	Sodium carboxymethyl starch, low molecular weight polyvinyl pyrrolidone, calcium stearate

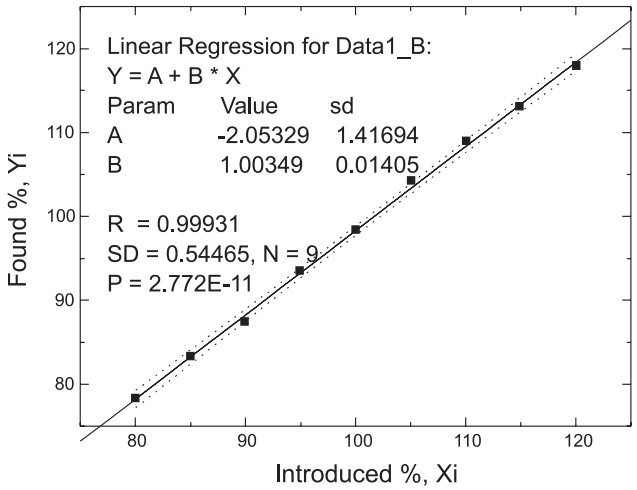

Fig. 1. The plot of linear dependence of absorbance on the concentration of paracetamol in the normalized coordinates.

tained in the first experiment caused by incomplete release of the active substance when dissolving.

Further research and evaluation of validation characteristics of quantitative determination methods for paracetamol tablets by specific absorbance (the sample preparation with changes) were performed according to the standardized procedure of validation of spectrophotometric methods of quantitative determination of drugs by specific absorbance [4].

The prognosis of the total uncertainty of the analysis results (Δ_{As})

The prognosis of uncertainty of the sample preparation. The approach and requirements for maximum permissible errors for volumetric glassware, balances and devices were used to assess uncertainty of the sample preparation [7, 8]:

$$\Delta_{Sp} = \sqrt{0.10^2 + 0.10^2 + 0.5^2 + 0.12^2 + 0.5^2 + 0.12^2} = 0.74\%.$$

The prognosis of the total uncertainty of the analysis result for the permissible limits of ±5.0%; ±7.5%; ±10.0% was conducted according to the standardized procedure of validation of spectrophotometric methods by specific absorbance [4]:

$$\Delta_{As}^{5.0\%} = \sqrt{\max\delta_{tot}^2 + \Delta_{SP}^2 + \Delta_{FAO}^2} =$$

$$= \sqrt{1.15^2 + 0.74^2 + 0.49^2} = 1.7\%;$$

$$\Delta_{As}^{7.5\%} = 2.1\%; \ \Delta_{As}^{10.0\%} = 2.6\%.$$

The total uncertainty should be insignificant compared with the maximum permissible uncertainty of the analysis results:

$$\Delta_{As} \le \max \Delta_{As}^{5.0\%} = 1.6\% : \ \Delta_{As}^{5.0\%} = 1.7 \ge 1.6\%;$$

$$\Delta_{As}^{7.5\%} = 2.1 \le \max \Delta_{As}^{7.5\%} = 2.4\%;$$

$$\Delta_{As}^{10.0\%} = 2.6 \le \max \Delta_{As}^{10.0\%} = 3.2\%.$$

The total uncertainty of the analysis results exceeds the maximum permissible uncertainty for the permissible limits of ±5.0% and meets requirements for the permissible limits of ±7.5% and ±10.0%.

Accuracy, linearity, repeatability, intermediate precision were investigated using 9 model solutions within the whole range of the method application from 80 to 120% of the prescribed amount. The assessment of linearity was performed in the normalized coordinate system (Fig. 1). The results are shown in Tab. 4. The Table shows that the requirements for the parameters of the linear dependence are performed for permissible limits of ±5.0%.

The assessment of the validation parameters of the method is given in Tab. 5. Parameters of the accuracy and convergence are shown graphically in Fig. 2.

The results of the convergence research of the method meet requirements for the permissible limits of ±5.0%. The results of the intermediate precision research of the method meet requirements for the permissible limits of ±7.5%. The results of the accuracy research of the method meet requirements for the permissible limits of ±10.0%. In this case without the other tests results (e.g. Art. 2.9.3. "Dissolution", Art. 2.9.6. "Uniformity of the content of the active ingredient per unit dosage of a medicinal pro-

Table 4

The metrological characteristics of the linear dependence

Parameters	Value		Criteria (for tolerances of 95-105%, the number of points 9)	Conclusion
	test 1	test 2		
b	1.0034	0.9798	–	–
s_b	0.0141	0.0077	–	–
a	-2.05	-0.36	statistical insignificance $a \le t(95\%, g-2) \cdot s_a = 1.89 \cdot s_a = 2.67\%$ $a \le t(95\%, g-2) \cdot s_a = 1.89 \cdot s_a = 1.47\%$	satisfied
			practical insignificance $\lvert a_{\delta A} \rvert \le \max \delta A = 0.71 \cdot \max \Delta_{As} = 1.15\%$	satisfied
			$\max a = 2.34\%$	satisfied
s_a	1.4172	0.7787	–	–
RSD_0	0.54	0.30	$RSD_o \le 0.60\%$	satisfied
r	1.0000	1.0000	$\min R^2_c = 0.9981$	satisfied

Table 5

The results of the accuracy and convergence research of the spectrophotometric quantitative determination of paracetamol tablets by specific absorbance

Validation parameters	Research 1	Research 2
$\overline{X}$%	**98.27**	**97.61**
RSD_x%	0.60	0.31
$\Delta_{prec}\% = t(95\%,8)\cdot RSD_x =$	**1.12**	**0.59**
Critical value for $\Delta_{prec} \le 1.15\%$	satisfied	satisfied
$\delta = \lvert X - 100 \rvert$	**1.73**	**2.39**
Criterion of the systematic error insignificance $\delta \le \Delta_{prec}/3$ if it is not satisfied 1), then 2) $\delta \le max\delta_{tot} = 1.15$ *for permissible limits ±7.5%* $\delta \le max\delta_{tot} = 1.7$; *for permissible limits ±10.0%* $\delta \le max\delta_{tot} = 2.3$	$\delta \le 0.37$ unsatisfied satisfied satisfied	$\delta \le 0.20$ unsatisfied unsatisfied satisfied
The conclusion of the method	**correct**	**correct**
Intermediate precision		
$Z_{intra}\% =$	**97.94**	
$SD_{z\text{-}intra}\% =$	**1.07**	
$\Delta_{intra}\% = t(95\%,n\cdot m\text{-}1)\cdot SD_{z\text{-}intra}\% = 1.75\, SD_{z\text{-}intra}\%$	**1.87**	
Critical value for $\Delta_{prec} \le 1.15\%$	unsatisfied	
Intermediate systematic error $\delta =$	**2.06**	
Criterion of the systematic error insignificance $\delta \le \Delta_{prec}/\sqrt{18}$ if it is not satisfied 1), then 2) $\delta \le max\delta_{tot} = 1.15$ *for permissible limits ±7.5%* $\delta \le max\delta_{tot} = 1.7$; *for permissible limits ±10.0%* $\delta \le max\delta_{tot} = 2.3$	$\delta \le 0.24$ unsatisfied unsatisfied satisfied	
The overall conclusion of the method	**correct**	

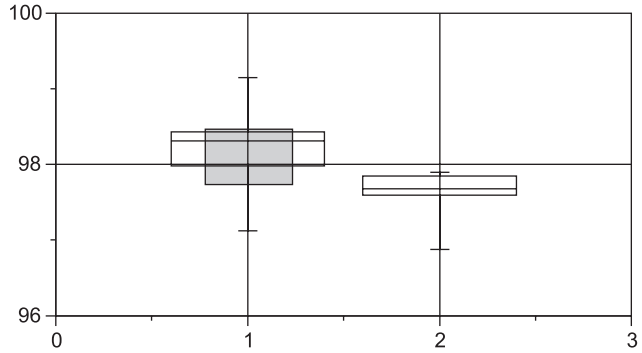

Fig. 2. The plot of the accuracy and convergence research results of the spectrophotometric quantitative determination of paracetamol tablets by specific absorbance.

duct") conclusions about the quality of the tablets cannot be done. Taking into account the technical capabilities of the Ukrainian producers and a diverse list of excipients used in the manufacture of the drug, the spectrophotometric quantitative determination of paracetamol tablets by specific absorbance is recommended to use for quantitative determination of the API in the drug with the permissible limits of ±10.0%, while the permissible limits of ±7.5% results may be doubtful. The prognosis of the total uncertainty of the analysis results is consistent with requirements to the maximum permissible uncertainty of the analysis $\Delta_{As}^{10.0\%} = 2.6 \le \max\ \Delta_{As}^{10.0\%}\ 3.2\%$ and with results of the 3rd round of the Professional Testing Programme (PTP) of "Pharma-test" laboratories in the system of the State Inspection for Medication Quality Control of the Ministry of Public Health of Ukraine [5].

CONCLUSIONS

The validation characteristics of the spectrophotometric quantitative determination of paracetamol in tablets by specific absorbance according to the British Pharmacopoeia have been evaluated. We have suggested to make such changes at the stage of the sample preparation as "… place the flask in an ultrasonic bath for 30 min…" instead of "…shake for 15 minutes…". Taking into account the technical capabilities of the Ukrainian producers and a diverse list of excipients used in the manufacture of the drug, the spectrophotometric quantitative determination of paracetamol tablets by specific absorbance is recommended to use for quantitative determination of API in the drug with the permissible limits of ±10.0%.

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ОЦІНКА МЕТРОЛОГІЧНИХ ХАРАКТЕРИСТИК МЕТОДИКИ СПЕКТРОФОТОМЕТРИЧНОГО КІЛЬКІСНОГО ВИЗНАЧЕННЯ ПАРАЦЕТАМОЛУ У ТАБЛЕТКАХ МЕТОДОМ ПОКАЗНИКА ПОГЛИНАННЯ

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Ключові слова: *парацетамол; кількісне визначення; спектрофотометрія; метод показника поглинання; валідація*

Здійснено оцінку метрологічних характеристик методики кількісного визначення парацетамолу у таблетках методом показника поглинання (МПП), яка рекомендується Британською фармакопеєю (БФ). Отримані результати у перерахунку на середню масу однієї таблетки 83.59% та 84.39% не відповідають допускам вмісту 95-105% (В ±5.0%). При встановленні можливих причин негативного результату обговорені особливості пробопідготовки методик кількісного визначення активного фармацевтичного інгредієнта у таблетках та здійснено порівняльний аналіз випробувань «Розчинення» та «Кількісний вміст» парацетамолу у таблетках відповідно до монографії БФ. Запропоновано внести зміни в етап пробопідготовки: «…перемішувати на протязі 15 хв…» змінити на «помістити колбу в ультразвукову баню на 30 хв». З метою визначення прийнятних допусків розраховані критерії прийнятності для В ±5.0%, ±7.5% та ±10.0%. Результати вивчення лінійності та збіжності відповідають вимогам при В±5,0%; внутрішньолабораторної прецизійності для В ±7.5%. Результати правильності методики обох дослідів перевищують критерії для В ±5.0%; результати досліду 1 відповідають критеріям для В ±7.5%; результати досліду 2 та результат внутрішньолабораторної правильності відповідають критеріям для В ±10.0%. Враховуючи технічні можливості українських виробників та різноманітний перелік допоміжних речовин, які застосовуються при виробництві препарату, рекомендується використовувати методику кількісного визначення парацетамолу у таблетках за МПП при В ±10.0%. Прогноз невизначеності результатів аналізу узгоджується з вимогами до максимально припустимої невизначеності аналізу $\Delta_{As}^{10.0\%} = 2.6 \leq max\ \Delta_{As}^{10.0\%} = 3.2$ *та з результатами 3-го раунду Програми професійного тестування лабораторій «Фарма-тест».*

ОЦЕНКА МЕТРОЛОГИЧЕСКИХ ХАРАКТЕРИСТИК МЕТОДИКИ СПЕКТРОФОТОМЕТРИЧЕСКОГО КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ПАРАЦЕТАМОЛА В ТАБЛЕТКАХ МЕТОДОМ ПОКАЗАТЕЛЯ ПОГЛОЩЕНИЯ

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Ключевые слова: *парацетамол; количественное определение; спектрофотометрия; метод показателя поглощения; валидация*

Осуществлена оценка метрологических характеристик методики количественного определения парацетамола в таблетках методом показателя поглощения (МПП), которая ре-

комендуется Британской фармакопеей (БФ). Полученные результаты в пересчете на среднюю массу одной таблетки 83.59% и 84.39% не соответствуют допускам содержания 95-105% (В ±5.0%). При установлении возможных причин отрицательного результата обсуждены особенности пробоподготовки методик количественного определения активного фармацевтического ингредиента в таблетках и осуществлен сравнительный анализ испытаний «Растворение» и «Количественное определение» парацетамола в таблетках согласно монографии БФ. Предложено внести изменения в этап пробоподготовки: «... перемешивать в течение 15 мин...» изменить на «поместить колбу в ультразвуковую баню на 30 мин». С целью определения приемлемых допусков рассчитаны критерии приемлемости для В ±5.0%, ±7.5% и ±10.0%. Результаты изучения линейности и сходимости соответствуют требованиям при В ±5.0%; внутрилабораторной прецизионности – для В ±7.5%. Результаты правильности методики обоих опытов превышают критерии для В ±5.0%; результаты опыта 1 соответствуют критериям В ±7.5%; результаты опыта 2 и результат внутрилабораторной правильности соответствуют критериям В ±10.0%. Учитывая технические возможности украинских производителей и разнообразный перечень вспомогательных веществ, которые применяются при производстве препарата, рекомендуется использовать методику количественного определения парацетамола в таблетках МПП при В ±10.0%. Прогноз неопределенности результатов анализа согласовывается с требованиями к максимально допустимой неопределенности анализа $\Delta_{As}^{10.0\%} = 2.6 \leq max\ \Delta_{As}^{10.0\%} = 3.2\%$ *и с результатами 3-го раунда Программы профессионального тестирования лабораторий «Фарма-тест».*

ТЕХНОЛОГІЯ ЛІКАРСЬКИХ ПРЕПАРАТІВ

Recommended by Doctor of Pharmacy, professor V.I.Chuyeshov

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TECHNOLOGICAL PECULIARITIES FOR OBTAINING OF MEDICATED CHEWING GUMS

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Key words: medicated chewing gum; technology; direct compression; Health in gum compositions

A medicated chewing gum (MCG) is a new alternative solid dosage form for oral application, which is used for the delivery of a great number of active pharmaceutical ingredients. Monographs for this dosage form are introduced in the SPhU, European Pharmacopoeia and United States Pharmacopoeia. But to date, domestic manufacturers do not produce preparations in the form of medicated chewing gums. This is primarily due to the complexity of the equipment applied and the absence of appropriate legal documentation for MCG manufacture, which limited the introduction of this dosage form in pharmaceutical companies of Ukraine. We have analyzed the literature data on the methods of obtaining a medicated chewing gum, as well as the basic technological aspects and the equipment applied have been considered. This allowed us to conclude that with development of compositions for obtaining chewing gums by direct compression Pharmagum® (SPI Pharma, USA) and Health in gum® (Cafosa, Spain) the possibility of production of this dosage form in domestic enterprises has increased and is promising to date. Chemically, they are a mixture of polyols (sorbitol/xylitol/mannitol) and of sugars with gum bases, plasticizers and anti-caking agents. Chewing gums made by these compositions give faster release of drugs than conventional methods owing to lower binding of the medicinal substance with the gum base and can be directly compressed on a conventional tablet machine.

Nowadays, a chewing gum is part of daily life of many people [1, 5, 8, 9]. The SPhU and European Pharmacopoeia contain a monograph "Chewing gums, medicated", according to which they are "solid preparations containing one or more active substances and a base consisting mainly of a gum that are intended to be chewed but not swallowed. They are intended to be used for local treatment of mouth diseases, systemic drug delivery after absorption through the buccal mucosa or from the gastrointestinal tract" [2, 6-11]. That is, a medicated chewing gum (MCG) is a new alternative solid dosage form for oral application, which is used for the delivery of a great number of active pharmaceutical ingredients [3, 5, 8, 10, 11].

A medicated chewing gum has several advantages over other solid dosage forms for application in the mouth. This is, first of all:

- innovation and modernity;
- better perception by the patient and a pleasant way to introduction of drugs, especially for children;
- it is not required to use water, i.e. it is possible to use it in any convenient place for the patient;
- there is no need to swallow, it is important for children and people who have problems with swallowing of drugs;
- providing a rapid effect;
- fewer side effects and others [4, 5, 8, 9].

Unfortunately, due to the absence of the corresponding regulatory documentation, which controls the production of drugs, and because of the complexity of equipment required for production of MCG, current domestic drugs in the form of medicated chewing gums are absent at the pharmaceutical market of Ukraine.

The aim of this work is to characterize the methods for obtaining of medicated chewing gums and consider their main technological aspects.

Experimental Part

When developing a medicated chewing gum, only if there is a correct selection of active substances and excipients, the rational technology and storage conditions can provide the desired therapeutic effect.

Besides active pharmaceutical ingredients (API) and the base, MCGs contain excipients, which type and amount depend on the method of the gum obtaining. During chewing a medicated gum does almost not decrease in the volume, but the active substances and excipients are dissolved or dispersed gradually in saliva, then the base becomes tasteless and thrown away [5, 7, 8, 10, 11].

Methods employed for manufacturing of a chewing gum can be classified into three main classes [8, 10, 11]:

1. Conventional / traditional method (Melting method).
2. Freezing, grinding and tabletting method.
3. Direct compression method.

Results and Discussion

The traditional method is the previous melting or softening of the gum base (butadiene-styrene-like basic copolymer, isobutylene-isoprene copolymer (butyl

rubber), polyvinyl acetate and identical polymers) and subsequent mixing with the desired active substances and excipients. The mixture obtained is then sent through a series of rollers for obtaining a thin, wide ribbon. During this process a light coating of finely powdered sugar or sugar substitutes is added to enhance the flavour and to keep the gum away from sticking. In a carefully controlled room, the gum is cooled for 48 hours, after that it is cut to the desired size and cooled at a controlled temperature and humidity. If it is necessary for obtaining of the desired appearance of the product, then MCG is further processed (coating, glossing, etc.).

However, this method has several disadvantages: elevated temperature used in melting restricts the use of this method for thermosensitive ingredients; melting and mixing of a highly viscous gum mass makes controlling of accuracy and uniformity of drug dose difficult; there is a risk of providing an inaccurate form, shape or weight of the dosage form; because of the complexity of the equipment (extrusion and rolled lines) and facilities involving hot-melt and cool processers the technology itself is not so easily adaptable to incorporate the manufacturing conditions required for production of pharmaceutical products. In addition, such a chewing gum composition is difficult to form into chewing gum tablets because of their moisture content (2-8%); if attempted to grind and tablet such a composition would jam the grinding machine, stick to blades, screens adhere to punches and would be difficult to compress [8, 10, 11].

The method of freezing (cooling), grinding and tabletting has been developed with an attempt to decrease the moisture content and alleviate the problems of the conventional method mentioned above.

In this method the gum base is cooled to a temperature, at which the composition is sufficiently brittle and will remain brittle during the subsequent grinding step without adhesion to the grinding apparatus. The temperature required for cooling is determined in part by the composition of the chewing gum. Generally, the temperatures of the cooled mixture are around -15°C or lower. As coolants a liquid nitrogen, hydrocarbon slush are used; solid carbon dioxide is preferred as it can give low temperatures (up to -78.5°C), sublimes readily on warming the mixture, is not absorbed by the chewing gum composition, does not interact adversely with the processing apparatus and does not leave behind any residue, which may be undesirable or potentially hazardous.

To facilitate cooling, grinding and to achieve the desired properties of a chewing gum some excipients such as a grinding agent and an anti-caking agent can be added to the composition.

An anti-caking agent such as precipitated silicon dioxide can be mixed with the chewing gum composition and solid carbon dioxide prior to grinding. It helps to prevent agglomeration of the subsequently ground chewing gum particles.

To prevent the gum from sticking to the grinding apparatus the grinding auxiliary substance can be incorporated (in the amount of 2-8%): alkaline metal phosphate, an alkaline earth metal phosphate or maltodextrin. However, the practical use of these substances is limited because these substances are highly alkaline and, therefore, will be incompatible with acidic ionisable therapeutic agents. They also tend to remain in the composition of the final chewing gum, and it may be problematic for therapeutic and safety point of view.

After the composition is ground to a powder, the coolant is removed by allowing the coolant to evaporate. After that the powder is mixed in a suitable mixer (sigma mill, high shear mixer or fluidized bed reactor) with other ingredients. The resulting mixture is transferred to the stage of pressing, which can be carried out by any conventional process like punching on a tablet machine.

However, this method has several disadvantages such as a large number of the equipment applied; careful monitoring of moisture during the tabletting process [8, 10, 11].

The manufacturing process can be accelerated, and the above-mentioned disadvantages are excluded using compositions for obtaining chewing gums by direct compression *Pharmagum*® (SPI Pharma, USA) and *Health in gum*® (Cafosa, Spain) [4, 8, 10, 11]. These compositions are manufactured under GMP conditions, comply with food chemical specifications and are "generally regarded as safe" (GRAS), regulated by FDA title 21 C.F.R Section 172.615 [4, 11]. A chewing gum made by these gum compositions can be directly compressed on a conventional tablet machine, which enables rapid and low-cost development of medicated chewing gums. As it does not require high temperature, this method is also suitable for thermosensitive and water-sensitive APIs.

Medicated chewing gums made with *Pharmagum* and *Health in gum* compositions are similar to tablets in appearance and give faster release of drugs than conventional methods owing to lower binding of the medicinal substance with the gum base [8, 10, 11].

Last years the *Health in gum*® composition has gained widespread, the advantages of which are, above all, homogeneity and simplicity of manufacture, so that to work with a single elastic base is difficult and it requires additional equipment. Chemically, *Health in gum* compositions are a mixture of polyols (sorbitol/xylitol/mannitol/isomalt) or of sugars with gum bases (elastomers), plasticizers and anti-caking agents. Depending on the percentage of the elastic base and the type of polyols that are included in the composition 3 types of *Health in Gum* are produced such as HiG PWD 01, HiG PWD 03, HiG PWD 04 [4, 10].

Obtaining of chewing gums with *Health in gum* lies in mixing of this composition, the active substance and flavourings in the mixer; after adding an anti-caking agent and a lubricant the resulting mass is sent to direct compression. If necessary, for protection from moisture and providing additional external characteristics the finished product can be covered with a film or coated with sugar [4, 10].

CONCLUSIONS

The analysis of possible technologies of medicated chewing gums manufacture conducted has allowed to determine that the use of *Health in gum* compositions provides an easy and rapid obtaining of MCG by direct compression without purchasing and installation of sophisticated technological equipment, which, in turn, will make this dosage form more promising for introduction into industrial production of Ukraine.

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ТЕХНОЛОГІЧНІ ОСОБЛИВОСТІ ОТРИМАННЯ МЕДИЧНИХ ЖУВАЛЬНИХ ГУМОК

О.А.Рубан, Ю.С.Маслій

Ключові слова: *медичні жувальні гумки; технологія; пряме пресування; композиції Health in gum*

Медична жувальна гумка (МЖГ) – нова альтернативна тверда лікарська форма для орального застосування, яка використовується для доставки великої кількості активних компонентів. Статті на дану лікарську форму введені у ДФУ, Європейську Фармакопею та Фармакопею США. Однак на сьогоднішній день препарати у формі медичних жувальних гумок не виробляються вітчизняними виробниками. Це пов'язано насамперед зі складністю використовуваного обладнання та відсутністю відповідної нормативної документації на виробництво МЖГ, що обмежує впровадження даної лікарської форми у виробництво фармацевтичних підприємств України. Нами був проведений аналіз літературних даних відносно методів отримання медичних жувальних гумок та основних технологічних аспектів, а також обладнання, що використовується. Це дозволило зробити висновок, що з розробкою композицій для отримання жувальних гумок методом прямого пресування Pharmagum® (SPI Pharma, США) і Health in gum® (Cafosa, Іспанія) можливість виробництва даної лікарської форми на вітчизняних підприємствах зросла і є перспективною на теперішній час. Хімічно вони являють собою суміш поліолів (сорбіту/ксиліту/маніту) та цукрів з жувальними основами, пластифікаторами і антизлежувальними агентами. Жувальні гумки, виготовлені за допомогою цих композицій, забезпечують більш швидке вивільнення лікарських речовин, ніж МЖГ, отримані традиційними методами, внаслідок більш низького зв'язування лікарської речовини з жувальною основою та можуть бути безпосередньо спресовані на звичайній таблетковій машині.

ТЕХНОЛОГИЧЕСКИЕ ОСОБЕННОСТИ ПОЛУЧЕНИЯ МЕДИЦИНСКИХ ЖЕВАТЕЛЬНЫХ РЕЗИНОК

Е.А.Рубан, Ю.С.Маслий

Ключевые слова: *медицинские жевательные резинки; технология; прямое прессование; композиции Health in gum*

Медицинская жевательная резинка (МЖР) – новая альтернативная твердая лекарственная форма для орального применения, которая применяется для доставки большого количества активных компонентов. Статьи на данную лекарственную форму введены в ГФУ, Европейскую Фармакопею и Фармакопею США. Однако на сегодняшний день препараты в форме медицинских жевательных резинок не производятся отечественными производителями. Это связано, прежде всего, со сложностью используемого оборудования и отсутствием соответствующей нормативной документации на производство МЖР, что ограничивает внедрение данной лекарственной формы в производство фармацевтических предприятий Украины. Нами был проведен анализ литературных данных относительно методов получения медицинских жевательных резинок, раскрыты основные технологические аспекты, а также рассмотрены виды используемого оборудования. Это позволило сделать вывод, что с разработкой композиций для получения жевательных резинок методом прямого прессования Pharmagum® (SPI Pharma, США) и Health in gum® (Cafosa, Испания) возможность производства данной лекарственной формы на отечественных предприятиях возросла и является перспективной в настоящее время. Химически они представляют собой смесь полиолов (сорбита/ксилита/маннита) и сахаров с жевательными основами, пластификаторами и антислеживающими агентами. Жевательные резинки, полученные с помощью этих композиций, обеспечивают более быстрое высвобождение лекарственных веществ, чем МЖР, полученные традиционными методами, вследствие более низкого связывания лекарственного вещества с жевательной основой и могут быть непосредственно спрессованы на обычной таблеточной машине.

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THE STUDY OF CRYSTALLOGRAPHIC AND THERMOGRAVIMETRIC CHARACTERISTICS OF RECTAL SUPPOSITORIES WITH DIACAMPH

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Key words: proctology diseases; suppositories; diacamph; thermogravimetric analysis

At the Department of Industrial Technology of Drugs suppositories for the treatment of degenerative and inflammatory diseases of the rectum have been developed; the active substance with the reparative activity – diacamph is included in their composition. The crystallographic characteristics of the diacamph original powder crystals, crushed diacamph crystals and suspensions of diacamph in propylene glycol have been studied. The optimal technology of diacamph introduction to the dosage form has been selected. Based on the thermogravimetric analysis the maximum heating temperature of the suppository mass has been determined, and the absence of interaction between the active substance and excipients in this dosage form has been proven. The research results will be used when developing the industrial technology for manufacturing suppositories.

Proctology diseases remain an urgent problem of modern medicine due to the prevalence of destructive inflammatory processes in the rectum and the complexity of the course of pharmacotherapy of these pathologies [7, 9-12]. Taking into account that the range of domestic medicines for treating diseases of the rectum is insufficient there is a need to create new drugs in the most rational and effective dosage form in proctology – suppositories [8].

At the National University of Pharmacy (Kharkiv) under the supervision of prof. S.I.Merzlikin (±)-cys-3-(2´-benzoimidazolyl)-1,2,2-trimethyl-cyclopentancarboxylic acid has been synthesized; on its basis the original antidiabetic drug "Diacamph" has been developed. The results further pharmacological studies have shown that diacamph reveals a pronounced reparative action [5]. Taking into account that diseases of the rectum are accompanied by inflammatory and destructive processes the use of diacamph in proctology is appropriate.

At the Department of Industrial Technology of Drugs of the National University of Pharmacy a new drug in the form of rectal suppositories with diacamph as an active substance has been developed [6]. Based on the biopharmaceutical research it has been determined that the rational suppository base is an alloy of proxanol-268 with PEO-400 and propylene glycol [2].

The aim of this work is to study the crystallographic and thermogravimetric characteristics of the active substance and the full composition of suppositories for selecting the rational conditions of the technological process.

Materials and Methods

Crystallographic properties of the powder was determined by optical crystallography and microphotography using a Kruss MBL 2100 microscope (Germany) with an eyepiece micrometer with magnification of 150 times. The object of the study was the active ingredient – diacamph. Crystals of diacamph original powder, crushed pure diacamph and suspensions of crushed diacamph in propylene glycol were studied.

The thermogravimetric analysis was performed on a Q – 1000 derivatograph of the system by F.Paulik, J.Paulik, L.Yerdey with a platinum to platinum/rhodium thermocouple by heating samples in ceramic crucibles from 20 to 250°C. Aluminum oxide served as a standard. The sample weight was 30 mg. Such curves as T (temperature changes), W (weight change), DTF (differential curve of thermal factors change), DW (differential curve of weight change) were recorded. The active substance – diacamph, the suppository base and the full composition of suppositories were subjected to thermogravimetric analysis.

Results and Discussion

To select the rational technology of introduction of the active ingredient diacamph to the dosage form its crystallographic characteristics were studied.

The visual examination under a microscope allows to observe the shape and structure of particles to obtain the preliminary data concerning their maximal and minimal sizes, and it is especially important when developing the technology of new dosage forms [3-4]. Taking into account that diacamph is in the suppository base as a suspension we conducted the study in order to select the rational technology of its introduction into the composition of suppositories.

The substance of diacamph is rod-shaped crystals with the size of 100-400 microns (Fig. 1). Crystals of diacamph is quite large, so a uniform distribution of the active ingredient in the base without crushing is impossible. When crushing diacamph in a dry form a mixture of particles with an uneven particle size was obtained (Fig. 2). And when crushing diacamph with propylene glycol (a liquid, in which the substance is in a semi-soluble state) a fine homogeneous suspension was ob-

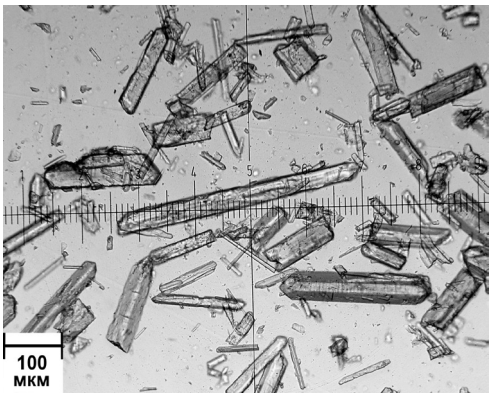


Fig. 1. The original powder of diacamph.

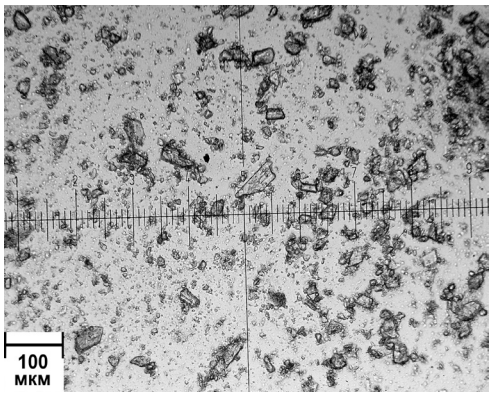


Fig. 2. The crushed powder of diacamph.

tained. Therefore, in the manufacture of suppositories diacamph must first triturated in a dry form and then with propylene glycol; it will provide partial dissolution of the substance at first, and when exceeding the limit of solubility there will be an even distribution of it in the base (Fig. 3).

In order to determine the optimal technology of the drug preparation we studied the decomposition tempera-

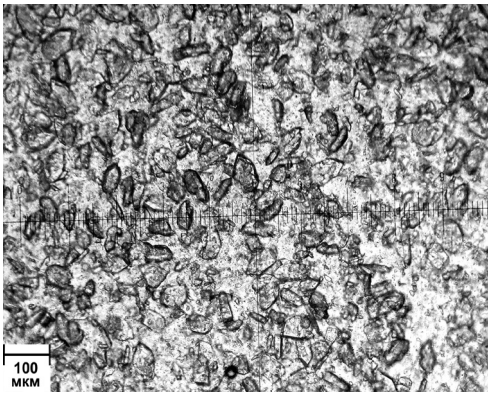


Fig. 3. The suspension of diacamph in propylene glycol.

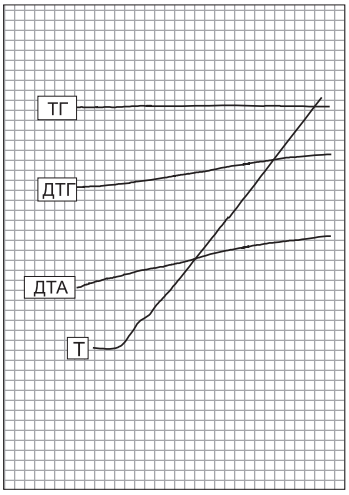
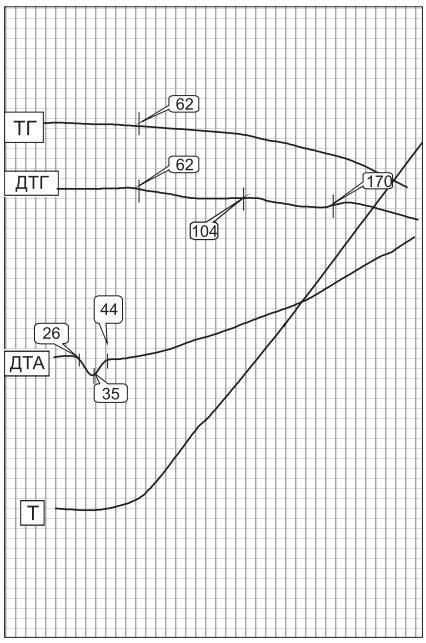
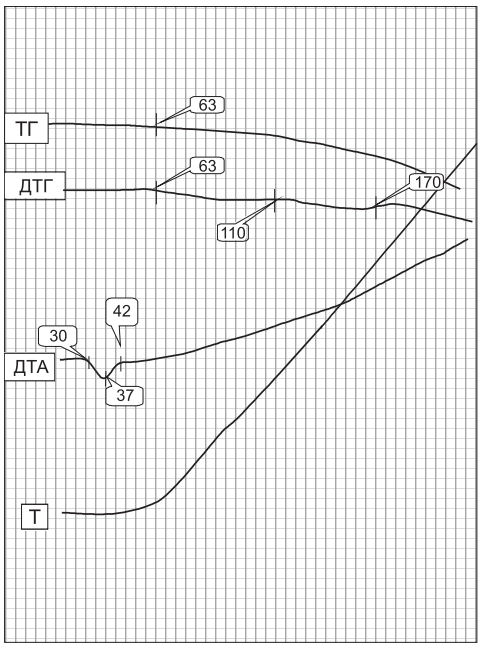


Fig. 4. The thermogram of diacamph.

ture of diacamph and the suppository base included in the suppositories. These parameters allow to select the optimal temperature mode for the suppository base preparation and introduction of the active substance into its composition without destroying the structure of the



A



B

Fig. 5. The thermograms of the suppository base (A), the full composition of suppositories (B).

active substance and changes of its pharmacological effect [1, 3]. The thermograms of the test samples are given in Fig. 4-5.

Our studies show that diacamph (Fig. 4) does not undergo any change when heated to the temperature of 250°C: thermal effects and loss in weight are absent. This substance is stable, therefore, it does not require special temperature conditions when preparing suppositories.

The suppository base (Fig. 5, A) is melted at the temperature of 35°C. When heated above 62°C a gradual loss in weight starts; at first, it is characterized by evaporation of the liquid components, and then the thermal degradation of proxanol occurs.

A test sample of the full composition of rectal suppositories (Fig. 5, B) melts at 37°C. When increasing the temperature from 63°C to 110 C there is a gradual loss in weight due to evaporation of the liquid components of the base. In the temperature range of 110-170°C the thermooxidative degradation of proxanol begins.

Thus, on the basis of the research conducted a combined suppository base (60°C) appeared to be the least heat-resistant because it is composed of liquid components in large quantities. Therefore, when preparing the suppository mass it is important not to allow its heating above 60°C. The thermogram of the full composition of suppositories has shown the complete identity of thermal effects of individual substances, indicating the absence of interaction between the active substance and excipients in the dosage form proposed.

CONCLUSIONS

1. The crystallographic characteristics of the diacamph original powder crystals, crushed diacamph crystals and the suspension of diacamph in propylene glycol have been studied, and the optimal technology of its introduction to the suppositories has been selected.

2. The thermogravimetric analysis of suppositories and individual components of the suppository base has been performed, the maximum heating temperature of the suppository mass has been determined. The thermogram of the full composition of suppositories has shown the complete identity of thermal effects of individual substances, indicating the absence of interaction between the active substance and excipients in this dosage form when manufacturing suppositories.

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ВИВЧЕННЯ КРИСТАЛОГРАФІЧНИХ ТА ТЕРМОГРАВІМЕТРИЧНИХ ХАРАКТЕРИСТИК РЕКТАЛЬНИХ СУПОЗИТОРІЇВ З ДІАКАМФОМ

Н.А.Гербіна

Ключові слова: *проктологічні захворювання; супозиторії; діакамф; термогравіметричний аналіз*

На кафедрі заводської технології ліків НФаУ розроблені супозиторії для лікування деструктивно-запальних захворювань прямої кишки, до складу яких включено діючу речовину з репаративною активністю – діакамф. Вивчені кристалографічні характеристики кристалів вихідного порошку діакамфу, подрібненого у чистому вигляді, та суспензії подрібненого діакамфу у пропіленгліколі. Підібрано оптимальну технологію введення діакамфу до складу лікарської форми. На основі термогравіметричного аналізу визначено максимально допустиму температуру нагрівання супозиторної маси та доведено відсутність взаємодії діючої і допоміжних речовин у складі лікарської форми. Результати дослідження будуть використані при розробці промислової технології виробництва супозиторіїв.

ИЗУЧЕНИЕ КРИСТАЛЛОГРАФИЧЕСКИХ И ТЕРМОГРАВИМЕТРИЧЕСКИХ ХАРАКТЕРИСТИК РЕКТАЛЬНЫХ СУППОЗИТОРИЕВ С ДИАКАМФОМ

Н.А.Гербина

Ключевые слова: *проктологические заболевания; суппозитории; диакамф; термогравиметрический анализ*

На кафедре заводской технологии лекарств НФаУ разработаны суппозитории для лечения деструктивно-воспалительных заболеваний прямой кишки, в состав которых включено действующее вещество с репаративной активностью – диакамф. Изучены кристаллографические характеристики кристаллов исходного порошка диакамфа, измельченного в чистом виде, и суспензии измельченного диакамфа в пропиленгликоле. Подобрана оптимальная технология введения диакамфа в состав лекарственной формы. На основе термогравиметрического анализа определена максимально допустимая температура нагревания суппозиторной массы и доказано отсутствие взаимодействия действующего и вспомогательных веществ в составе лекарственной формы. Результаты исследования будут использованы при разработке промышленной технологии производства суппозиториев.

Recommended by Doctor of Pharmacy, professor Ye.V.Gladukh

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SUBSTANTIATION OF THE CHOICE OF EXCIPIENTS WHEN DEVELOPING THE COMPOSITION OF "APISED" CAPSULES

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Key words: hard gelatin capsules; excipients; natural powder-like honey; medicinal plant raw material; pharmaceutical and technological properties

The aim of this work is to substantiate the qualitative composition and quantitative content of excipients; it will allow to provide optimal properties of the mass for capsulation when producing "Apised" capsules – a new original medicine developed on the basis of the standardized substance of natural powder-like honey and the medicinal plant raw material (the herb of garden balm, the cones of hop, the inflorescences of spike lavender). The results of the study of pharmaceutical and technological properties of active substances and their mixture have shown that it is impossible to obtain the mass for capsulation with satisfactory values of flowability and ability to settle without additional introduction of excipients. Antifriction substances were added to the mixture of active substances to improve these parameters. It has been found that addition of aerosil or its mixture with calcium stearate (3:1) in the amount of 2% is the most expedient for improvement of flowability of the mixture of active substances. To reduce ability to settle and prevent stratification of the mixture we have suggested to carry out pregranulation of the mixture of active substances. Starch (in the form of 5% solution) and polyvinylpyrrolidone, Plasdone K29/32 and Plasdone S629 in different amounts were used as wetting agents. The best values of flowability have been obtained when using Plasdone K29/32 in the amount of 5%; at the same time ability to settle achieve acceptable values and the disintegration time of granules does not exceed acceptable limits. The optimal procedure of preparation of the mass for capsulation has been grounded: 5% Plasdone K29/32 solution is added to the mixture of the medicinal plant raw material, and granulation is carried out, then natural powder-like honey (NPH) and antiantifriction substances (aerosil or its mixture with calcium stearate (3:1)) are added to the granulate obtained. It is recommended to use in future when developing the technology of "Apised" capsules. The absence of necessity of adding humidity regulators into the composition of the mass for capsulation has been shown. The complex of the pharmaceutical and technological research conducted has allowed to substantiate the qualitative composition and quantitative content of excipients in the composition of the mass for capsulation under the conditions of pharmacy and industrial production of "Apised" capsules developed, as well as the procedure of obtaining the mass for capsulation.

To date physical overloads, tension of life rhythm, emotional stress, etc., can not be excluded from the sphere of the human professional activity. It quite often results in the partial loss of their working efficiency, the organism overexcitation, worsening of sleep and the general state and, therefore, in extended psychological and neurotic disorders [1]. Top records sport is not exception – solving the problem of treatment and rehabilitation of sportsmen with overload and the overtraining syndrome continues to remain one of key tasks in sport medicine [2, 10].

Under present-day conditions the training process of most of professional sportsmen takes place in the mode of increased tension and is characterized by continuous physical loads, which results in increasing the frequency of psychological, stress and psychotraumatic situations. In the period of intensive training there is also weakening of the immune system and the organism exhaustion being often the cause of change of its immunological reactivity. All mentioned above results in worsening of the sportsmen effectiveness.

In literature sources there is the information that in intensive sports activities the immune system is subjected, on the one hand, to training impact of physical and emotional load that extends its functionality, and on the other hand – to distress effect of extreme irritants occurring at the excessive loading [7].

To prevent these phenomena it is reasonable to use medicines, which are able to strengthen the body's immune system and reduce the irritating and psychoemotional loading without a doping effect.

It should be noted that medicines of the natural origin, in particular on the basis of beekeeping products [5], as well as herbal medicines (HM), are widely used lately in pharmaceutical practice; it is conditioned by their relative availability, efficiency and harmlessness, and in some cases by their competitiveness relative to their synthetic analogues.

Besides, apiproducts and components of medicinal plant raw material (MPR) are derivatives of the body natural metabolites by their chemical structure; by low toxicity and the minimal number of complications in therapeutic doses they show numerous pharmacological properties [5].

Therefore, along with hygienical and technological measures one of urgent tasks of sport medicine is the

Table 1

The pharmaceutical and technological parameters of the active substances of the "Apised" capsules and their mixture (n = 5, P = 95%)

The name of the parameter	Garden balm herb	Hop cones	Spike lavender inflorescences	NPH	The mixture of APhI	The dried mixture of APhI
Flowability, s/100 g	81±3	108±20	112±21	27.2±0.9	92±1	62±1
Angle of natural slip, °	43-44	52-53	45-46	36±37	47-48	45-46
Bulk volume V_0, ml	352±2	451±2	371±2	263±2	365±2	395±1
Settled volume V_{10}, ml	281±2	364±2	287±2	151±2	275±2	348±2
Settled volume V_{500}, ml	236±2	318±2	244±2	115±2	238±2	274±1
Settled volume V_{1250}, ml	231±2	311±2	236±2	110±2	224±2	265±1
Ability to settle $V_{10} - V_{500}$, ml	45	46	43	36	37	74
Density of bulk product m/V_0, g/ml	0.284±0.002	0.222±0.002	0.270±0.002	0.38±0.03	0.274±0.002	0.253±0.002
Density of settled product m/V_{1250}, g/ml	0.433±0.002	0.322±0.002	0.424±0.002	0.91±0.05	0.446±0.002	0.377±0.002

search, development and manufacturing application of natural medicines, which are able to increase the body's resistance to extreme influences and psychological disorders.

The studies on development of the composition and technology of medicines for application in sport medicine, mainly on the basis of the standardized substances of beekeeping products are carried out. One of these medicines is the original medicine in the form of capsules under the conditional name "Apised" developed on the basis of natural powder-like honey (NPH) and the medicinal plant raw material (the herb of garden balm, the cones of hop, the inflorescences of spike lavender). It is recommended to application with the purpose of rehabilitation acceleration of the body's functions of sportsmen worsened as the result of overstrain and overtraining, and also as the additional source of vitamins, organic and mineral acids, essential oils, alkaloids, flavonoids, phenolic compounds, antioxidants, etc. [6, 8].

The choice of capsules as a dosage form with oral application, in the first place, is related to such advantages as accuracy of dose, comfort in application and storage, portability, etc., as well as high bioavailability of active pharmaceutical ingredients (APhI), which are the components of the medicine developed.

The aim of this work is to substantiate the qualitative composition and quantitative content of excipients; it will allow to provide optimal pharmaceutical and technological properties of the mass for capsulation when producing "Apised" capsules developed under conditions of pharmacy and industrial production.

Materials and Methods

The objects of the research were the following samples of APhI of "Apised" capsules: the herb of garden balm – *Herba Melissa officinalis L.* (the registration certificate No.UA/8919/01/01, batch 60612) manufactured by "Liktravy" PJSC (Zhytomyr, Ukraine); the cones of hop – *Strobuli Humuli lupuli L.* (the registration certificate No.UA/11477/01/01, batch 003) manufactured by "Liktravy" PJSC (Zhytomyr, Ukraine); the inflorescences of spike lavender – *Flores Lavandulae angustifolia Mill.* cultivated on the territory of Nikitsky Botanical Garden of UAAS; NPH (the Ukrainian specification 01.2-02010936-001:2007; the changes No.1:2013 to the Ukrainian specification 01.2-02010936-001:2007) obtained by freeze drying under conditions of "Biolik" JSC (Kharkiv) using "Virtis" production equipment (USA) [6].

Our research was carried out on the basis that one capsule would contain 0.225 g of active substances [6]. Talc, aerosil of A-380 grade (the State Standard 14922-77), starch (the State Standard 7699-78), calcium stearate (the Ukrainian specification 22942814.003-2000), Plasdone K29/32, Plasdone S629 and polyvinylpyrrolidone (PVP) [9, 11-19] were used as excipients.

The flowability was studied for APhI, their mixture and the masses for capsulation obtained, as well the bulk volume, ability to settle, density of bulk and settled product were determined according to the procedure of SPhU (SPhU 1th ed., art. 2.9.15, art. 2.9.16) [3, 4]. Besides, the value of the angle of natural slip was measured by means of a goniometer.

When studying the moisture absorption APhI, their mixture and masses for capsulation were introduced into the preliminary weighted weighing bottles with the diameter of 29±0.5 mm and the height of 35±0.5 mm, the weighing bottles were placed into the desiccator with the diameter of 140 mm; the permanent relative air humidity at the level of 100% created with purified water was supported in the desiccator at the temperature of 20°C. In a certain period of time the samples of the substances studied were taken from weighing bottles, and the moisture content was determined in them by means of a "Sartorius"humidity analyser of MA-150 grade produced by "Sartorius" AG group company, Germany.

Results and Discussion

The results of studying the pharmaceutical and technological properties of APhI and their mixtures (flowability, angle of natural slip, bulk volume, ability to settle, density of bulk product) indicate that it is impossible to obtain the mass for capsulation with satisfactory values of flowability and ability to settle without additional introduction of excipients (Tab. 1).

Therefore, to improve these parameters such antifriction substances as talc, starch, Plasdone K29/32, as

Table 2

The pharmaceutical and technological parameters of the masses for capsulation of "Apised" capsules developed (n = 5, P = 95%)

The name of parameter	The mixture of APhI, talc and starch	The mixture of APhI, 5% of Plasdone K29/32, 1% of calcium stearate	The mixture of APhI, 2% of aerosil	The mixture of APhI, 2% of calcium stearate	The mixture of APhI, 0.5% of calcium stearate, 1.5% of aerosil
Flowability, s/100 g	115.5±0.6	33±6	25±2	33±2	24±2
Angle of natural slip, °	45-46	38-39	35-36	38-39	34-35
Bulk volume V_0, ml	378±2	381±2	398±2	396±2	394±2
Settled volume V_{10}, ml	265±2	273±2	276±2	278±2	275±2
Settled volume V_{500}, ml	234±2	239±2	241±2	245±2	240±2
Settled volume V_{1250}, ml	214±2	220±2	221±2	218±2	218±2
Ability to settle $V_{10} - V_{500}$, ml	31	34	35	33	35
Density of bulk product m/V_0, g/ml	0.265±0.002	0.262±0.002	0.251±0.002	0.253±0.002	0.254±0.002
Density of settled product m/V_{1250}, g/ml	0.467±0.002	0.455±0.002	0.452±0.002	0.459±0.002	0.459±0.002

well as calcium stearate and aerosil in the amount of 0.5-2.0% were added to the mixture of active substances. The results obtained are given in Tab. 2 and Fig. 1.

The results of the pharmaceutical and technological research show that addition of aerosil or its mixture with calcium stearate (3:1) in the amount of 2% is the most expedient for improvement of flowability of the mixture of active substances. Addition of calcium stearate or its mixture with Plasdone K29/32 also increases flowability of the mass for capsulation, however, less effectively. Further increase of the content of antifriction substances is not expedient as the parameters of flowability do not significantly improve. It should be noted that introduction of calcium stearate also allows to avoid adhesion of a powder-like mixture to the capsule-filling machine in the process of capsulation.

The results of the experimental research indicate that in spite of improvement of flowability when adding the excipients to the mixture of APhI, ability to settle of the masses for capsulation obtained continues to be unsatisfactory large. Moreover, stratification of the mixture is fixed visually, it is confirmed by the considerable differences of pharmaceutical and technological parameters of APhI. Therefore, in order to prevent occurrence of this phenomenon we have suggested to carry out pregranulation of the mixture of active substances. Thus, starch (in the form of 5% solution) and PVP, Plasdone K29/32 and Plasdone S629 in different amounts were used as wetting agents. The results of the flowability research of the granular masses for capsulation obtained are given in Fig. 2, they indicate that the best values of flowability were obtained when using Plasdone K29/32 as a wetting agent in the amount of 5%. Thus, ability to settle decrease and achieve acceptable values. It is also necessary to note that the disintegration time of granules does not exceed acceptable limits.

The use of 5% starch solution as a wetting agent results in adherence of the granulate obtained, and it makes impossible its application for manufacturing of "Apised" capsules developed.

The next stage of our research was the choice of the optimal order of mixing and granulation of active and excipients. Three variants of the procedure of obtaining the mass for capsulation were studied:

1) 5% solution of Plasdone K29/32 was added to the mixture of APhI and antifriction substances (aerosil or its mixture with calcium stearate (3:1)), and granulation was carried out;

2) 5% solution of Plasdone K29/32 was added to the mixture of MPR and antifriction substances (aerosil or its mixture with calcium stearate (3:1)), and granula-

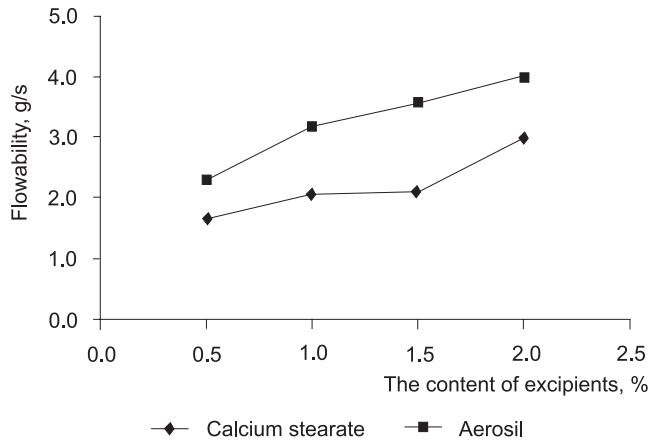

Fig. 1. Dependence of flowability of the mass for capsulation on the calcium stearate and aerosil content.

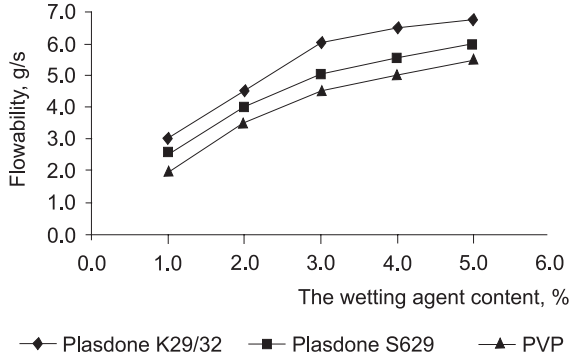

Fig. 2. Dependence of flowability of the mass for capsulation on the wetting agents content.

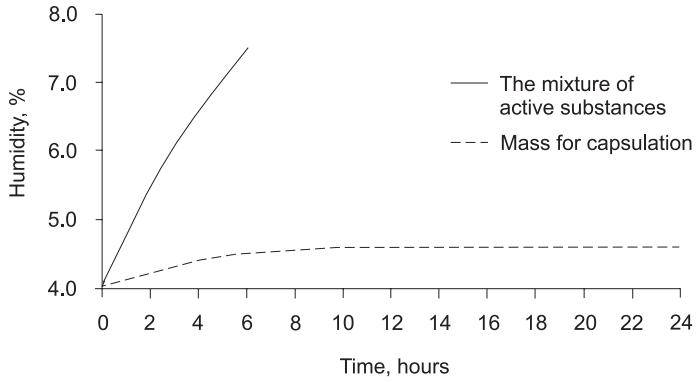

Fig. 3. Dependence of moisture absorption on time for "Apised" capsules developed.

tion was carried out, then NPH was added to the granulate obtained;

3) 5% solution of Plasdone K29/32 was added to the mixture of MPR, and granulation was carried out, then NPH and antifriction substances (aerosil or its mixture with calcium stearate (3:1)) were added to the granulate obtained.

It has been found that optimal pharmaceutical and technological parameters were obtained under conditions of application of the third variant of the procedure of preparing the mass for capsulation. It is recommended to use in future when developing the technology of "Apised" capsules.

The results of the experiment has also shown that the mixture of APhI has unsatisfactory parameters of moisture absorption (Fig. 3). Therefore, it is necessary to introduce a humidity regulator to the composition of the mass for capsulation. One of the most used humidity regulators is aerosil. We suggested it for introduction to the composition of the mass for capsulation to improve its flowability at the previous stage of our research. In this connection, moisture absorption of the masses obtained for capsulation was also studied. The results obtained indicate that there is no need of further addition of humidity regulators (Fig. 3).

Thus, the complex of pharmaceutical and technological research conducted has allowed to substantiate the qualitative composition and quantitative content of excipients in the composition of the mass for capsulation under the conditions of pharmacy and industrial production of "Apised" capsules developed (aerosil – 1.5%, calcium stearate – 0.5%, Plasdone K29/32 – 5% or aerosil – 2%, Plasdone K29/32 – 5%), as well as the procedure of obtaining the mass for capsulation.

CONCLUSIONS

1. The complex of the pharmaceutical and technological research has been carried out in order to substantiate the qualitative composition and quantitative content of excipients under the conditions of pharmacy and industrial production of "Apised" capsules developed.

2. The expediency of introduction of antifriction substances into the composition of the mass for capsulation has been determined, and their optimal amount in the process of production of "Apised" capsules: aerosil – 1.5%, calcium stearate – 0.5% or aerosil – 2% has been experimentally substantiated.

3. The necessity of pre-granulation of the mass for capsulation has been shown, the choice of a wetting agent, its amount and the procedure of obtaining the granular mass for capsulation in the course of production of the medicine developed have been substantiated.

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ОБҐРУНТУВАННЯ ВИБОРУ ДОПОМІЖНИХ РЕЧОВИН ПРИ РОЗРОБЦІ СКЛАДУ КАПСУЛ «АПІСЕД»

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Ключові слова: *тверді желатинові капсули; допоміжні речовини; мед натуральний порошкоподібний; лікарська рослинна сировина; фармакотехнологічні властивості*

Метою даної роботи було обґрунтування якісного складу та кількісного вмісту допоміжних речовин, що дозволить забезпечити оптимальні властивості маси для капсулювання при виробництві капсул «Апісед» – нового оригінального лікарського препарату, розробленого на основі стандартизованої субстанції меду натурального порошкоподібного та лікарської рослинної сировини (трави меліси лікарської, шишок хмелю звичайного, суцвіть лаванди вузьколистої). Результати вивчення фармакотехнологічних властивостей діючих речовин та їх суміші показали, що без додаткового введення допоміжних речовин неможливо отримати масу для капсулювання із задовільними показниками плинності та здатності до усадки. З метою покращення плинності додавали антифрикційні речовини, встановлено, що найбільш доцільним для поліпшення плинності суміші діючих речовин є додавання аеросилу або його суміші з кальцію стеаратом (3:1) у кількості 2%. З метою зменшення здатності до усадки та запобігання розшаруванню суміші запропоновано проводити попереднє гранулювання суміші діючих речовин. Як зволожувачі було використано крохмаль (у вигляді 5% розчину) та полівінілпіролідон, Plasdone K29/32 і Plasdone S629 у різних кількостях. Найбільш оптимальні показники плинності були отримані у випадку використання Plasdone K29/32 у кількості 5%, при цьому здатність до усадки досягає прийнятних значень, а час розпадання гранул не перевищує допустимих меж. Обґрунтовано оптимальну процедуру приготування маси для капсулювання – до суміші лікарської рослинної сировини додають 5% розчин Plasdone K29/32 та проводять гранулювання, потім до отриманого грануляту додають мед натуральний порошкоподібний та антифрикційні речовини, яку рекомендовано використовувати надалі при розробці технології капсул «Апісед». Показано відсутність необхідності додавання вологорегуляторів до складу маси для капсулювання. Комплекс проведених фармакотехнологічних досліджень дозволив обґрунтувати якісний склад та кількісний вміст допоміжних речовин у складі маси для капсулювання при виготовленні в аптечних та виробництві в промислових умовах розроблених капсул «Апісед», а також процедуру отримання маси для капсулювання.

ОБОСНОВАНИЕ ВЫБОРА ВСПОМОГАТЕЛЬНЫХ ВЕЩЕСТВ ПРИ РАЗРАБОТКЕ СОСТАВА КАПСУЛ «АПИСЕД»

О.С.Шпичак, А.И.Тихонов

Ключевые слова: *твердые желатиновые капсулы; вспомогательные вещества; мед натуральный порошкообразный; лекарственное растительное сырье; фармакотехнологические свойства*

Целью данной работы было обоснование качественного состава и количественного содержания вспомогательных веществ, что позволит обеспечить оптимальные свойства массы для капсулирования при производстве капсул «Аписед» – нового оригинального лекар-

ственного препарата, разработанного на основе стандартизированной субстанции меда натурального порошкообразного и лекарственного растительного сырья (травы мелиссы лекарственной, шишек хмеля обыкновенного, соцветий лаванды узколистной). Результаты изучения фармакотехнологических свойств действующих веществ и их смеси показали, что без дополнительного введения вспомогательных веществ невозможно получить массу для капсулирования с удовлетворительными показателями текучести и способности к усадке. С целью улучшения текучести добавляли антифрикционные вещества, установлено, что наиболее целесообразным для улучшения текучести смеси действующих веществ является добавление аэросила или его смеси с кальция стеаратом (3:1) в количестве 2%. С целью уменьшения способности к усадке и предотвращения расслоения смеси предложено проводить предварительное гранулирование смеси действующих веществ. В качестве увлажнителей были использованы крахмал (в виде 5% раствора) и поливинилпирролидон, Plasdone K29/32 и Plasdone S629 в различных количествах. Наиболее оптимальные показатели текучести были получены в случае использования Plasdone K29/32 в количестве 5%, при этом способность к усадке достигает приемлемых значений, а время распада гранул не превышает допустимых пределов. Обоснована оптимальная процедура приготовления массы для капсулирования – к смеси лекарственного растительного сырья добавляют 5% раствор Plasdone K29/32 и проводят гранулирование, затем к полученному грануляту добавляют мед натуральный порошкообразный и антифрикционные вещества, которую рекомендуется использовать в дальнейшем при разработке технологии капсул «Аписед». Показано отсутствие необходимости добавления влагорегуляторов в состав массы для капсулирования. Комплекс проведенных фармакотехнологических исследований позволил обосновать качественный состав и количественное содержание вспомогательных веществ в составе массы для капсулирования при изготовлении в аптечных и промышленных условиях разработанных капсул «Аписед», а также процедуру получения массы для капсулирования.

Recommended by Doctor of Pharmacy, professor O.A.Ruban

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THE STUDY OF RISKS OF HERBAL MEDICINES PRODUCTION BY THE FMEA-ANALYSIS METHOD

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Key words: risk assessment of quality; FMEA-analysis; herbal medicines; technological process

Identification and assessment of risks arising in the process of manufacture of extraction herbal medicines have been performed. The object of research was the technological process of manufacture of multicomponent tinctures and liquid extracts. Risk analysis of production is based on the results of retrospective validation of manufacture of multicomponent extraction herbal medicines, in particular "Climased" oral drops. When conducting validation the critical stages and parameters of the technological process have been determined, acceptance criteria have been specified. Identification of the possible risks for each critical stage and critical parameter of the technological process has been conducted. Expert assessments have been recorded to the form of quality risk assessment. The results of calculations of the risk priority number (RPN) have allowed to refer possible inconsistencies of the technological process at the stage of extraction to unacceptable risks. Risks arising at the stages of the raw material preparation, purification and filtration of extracts have a great impact. In the process of the risk management at the enterprise the categorization of risks (assessed level of risk) has been carried out, the methods of monitoring, prevention and risk response have been proposed; preventive measures, as well as measures in case of risk occurrence have been developed. To prevent and minimize the possible risks of pharmaceutical production the methodological approaches to functioning of the system of the risk management have been developed taking into account the manufacture of drugs based on the medicinal plant raw material.

Under market conditions any enterprise inevitably faces with extraordinary situations and unforeseen events, which should be prevented, or which should be timely responded to. Regardless of the reasons of occurrence of risk situations the desire to reduce the threat and minimize the possible financial losses is natural [9, 10].

In this regard, at present the problem of developing the management concept based on the risk management is particularly acute for all departments of a pharmaceutical enterprise, and in particular a manufacturing enterprise; this concept would take into account the latest world achievements, accumulated personal experience and the peculiarities of the domestic business environment. In this case efficient management of the organizational structure is impossible without special methods of analysis based on the theory and practice of the risk management. Therefore, it is very important to take fully into account external and internal factors affecting the nature of the risk management organization at the enterprise, as well as to focus on some directions of development of the risk management for a particular enterprise (with consideration for business profile) [1, 7, 9].

The ISO standards and the corresponding national standards concerning risk analysis contain several tens of methods of their evaluation. The choice and application of specific methods depend on a number of factors: complexity of the problem, degree of risk uncertainty, the possibility of obtaining quantitative estimates of the input data, etc. [2, 4].

The aim of our research was to identify and analyze the risks arising in the process of manufacture of extraction herbal medicines.

Materials and Methods

The process of identification and assessment of risk situations at pharmaceutical manufacturing enterprises should have a strategic level and be permanent. Timely monitoring of potential threats allows to avoid great losses in the material, financial and labour resources [5].

The object of the study was the technological process of manufacture of multicomponent tinctures and liquid extracts at the premises of Zhytomyr "SCE "Pharmaceutical factory" JSC.

In our study the method of failure modes and effect analysis (FMEA) was used. It is one of the most popular tool of risk assessment included in the general list of tools of Guidelines CT-H. the Ministry of Public Health of Ukraine 42-4.2: 2011 "Medicines. Quality Risk Management (ICH Q9)", and corresponds to the task set. FMEA is intended for potential failure mode evaluation of the process, as well as their potential consequences on the result of the process or product characteristics [4, 11].

When processing the quantitative results of control of intermediate and finished products statistical methods of quality control of manufacturing processes were used [3].

Results and Discussion

Risk analysis of production was based on the results of retrospective validation of manufacture of multicomponent extraction herbal medicines, in particular "Climased" oral drops. When conducting validation the critical stages and parameters of the technological process were determined, acceptance criteria were specified. Control charts were composed. The reproducibili-

Table 1

Risk analysis in the manufacture of liquid extracts and tinctures (FMEA method)

The process stage	Controlled indicator	Consequences of noncompliance	Seriousness of consequences	A possible cause of noncompliance	Probability	The method of determination or control	Complexity of identification	RPN
Grinding and sieving of the raw material	Grinding speed	Particle size	3	Malfunction of equipment	1	Monitoring	3	9
	Integrity of the sieve	Particle size	3	Malfunction of equipment	2	Visual control	4	24
Preparation of the extractant	Time of mixing	Uniformity of the extractant concentration	1	Operator's error	1	Alcoholometry	1	1
	Mixing speed	Uniformity of the extractant concentration	1	Malfunction of equipment	1	Alcoholometry	1	1
Mixing of the raw material	Time of mixing	Uniformity of the raw material	1	Operator's error	3	Visual control	1	3
	Mixing speed	Uniformity of the raw material	1	Malfunction of equipment	3	Visual control	1	3
Extraction	Duration of extraction	The content of extractive substances	5	Operator's error	2	Monitoring	3	30
	Temperature of extraction	The content of extractive substances, the concentration of ethanol	5	Malfunction of the ventilation and air conditioning system	3	Monitoring	4	60
	Frequency and duration of circulation	The content of extractive substances	4	Operator's error	3	Monitoring	4	48
Purification of the extract	Temperature	Stability of the drug	4	Malfunction of the ventilation and air conditioning system	2	Monitoring	3	24
	Time	Stability of the drug	4	Operator's error	2	Monitoring	3	24
Filtration	Pressure	Specification noncompliance	2	Malfunction of equipment	2	Monitoring	1	4
	Filter material	Change of qualitative and quantitative indicators	4	Violation of the procedure	1	Visual control	4	16

ty index calculated and the process capability index indicate potential risks to product quality required identification and decision making for their reduction [6].

The FMEA method is based on listing all potential effects (possible failures) with its subsequent analysis and numerical evaluation. Either heads of departments or a dedicated team are engaged in risk management. At "SCE "Pharmaceutical factory" JSC a permanent group of experienced professionals was formed, their official duties referred to the activities of the entire enterprise. The main fixed team included the production manager, the quality system coordinator, the head of Quality Control Department, the head of Research Centre, the process quality control engineer, the mechanic engineer of Production Supporting Services. Additionally the workshop supervisor and foremen of the phytochemical department were included in the team.

In the process of the risk management at the enterprise the following activities were implemented: identification of risks (the fullest list of possible risks being specific to each production stage was determined) was conducted; categorization of risks (assessed level of risk) was carried out when each risk was assigned one of three categories that affect the monitoring method, prevention and response to risk; preventive measures and actions in case of risk occurrence were developed.

After the initial compilation of the list of risks they were classified and evaluated. Each failure was estimated according to three criteria: severity (criticality) of failure, frequency (probability) of occurrence of this defect, possibility (complexity) of identification. To assess these criteria the 5-point rating system was used. The final result is the composite index – risk priority number (RPN), which is equal to the product of scores of

Table 2

Efficiency criteria of the risk management

Project class	Effective	Poorly effective	Ineffective
High-risk	Less than 10% of risks are implemented	Less than 25% of risks are implemented	More than25 % of risks are implemented
Risks	Less than 20% of risks are implemented	Less than 50% of risks are implemented	More than 50% of risks are implemented
Medium risks	Less than 30% of risks are implemented	Less than 60% of risks are implemented	More than 60% of risks are implemented
Low risks	Less than 50% of risks are implemented	Less than 75% of risks are implemented	More than 75% of risks are implemented

three specified criteria. At the same time the enterprise sets for itself the limits (norms, level) of risk that can be taken to achieve the desired parameters [4, 8].

Guided by the knowledge and experience the team performed identification of possible defects for each critical stage and the critical parameter of the technological process. Expert assessments were recorded to the form of the quality risk assessment (Tab. 1).

The quantitative risk assessment is more acceptable, but it also requires determination of the risk assessment scale for decision making on further action [1]. In accordance with the 5-point rating system chosen the risk priority number can vary from 1 to 125. The results of the calculations presented in Table allow to refer possible inconsistencies of the technological process at the stage of extraction to unacceptable risks (RPN > 40). Risks arising at the stages of the raw material preparation, purification and filtration of extracts have a great impact (39 > RPN > 15).

To prevent and minimize the possible risks of pharmaceutical production the methodological approaches to functioning of the system of the risk management of a pharmaceutical manufacturing enterprise (the manufacture of a drug based on the medicinal plant raw material) have been developed.

In the process of manufacture the staff that is responsible for risk monitors the criteria, which may indicate approaching to the conditions of the risk occurrence. When determining the risk factors the current values are checked and compared with the threshold values signaling the risk occurrence. When revealing a trend in a particular indicator and approaching to the limited value the staff that is responsible for risk conducts preventive measures. If measures to prevent the risk are not effective and the risk occurs, the staff carries out the planned actions of risk response, and further monitoring of the problem is carried out on a daily basis.

To understand and perceive the level (efficiency) of the risk management activities organized the efficiency criteria of the risk management are given in Tab. 2.

In addition to monitoring the risk management efficiency the work on updating the database of the known risks is in progress. For this purpose any information that can help in the procedure of the risk management in future projects is added to the database. If risks, which descriptions are not in the database, are identified in the production process, then a full description of these risks are entered into the database.

If the project is successfully used information on the known risks and improvement of the procedures for preventing and responding have also been made, or any other significant corrections have been introduced in the risk passport led to more effective risk management (the risk should not be appeared on the project), then all changes are added to the database risks.

If changes were made, however, the risk in the process of work on the project still appeared, a more thorough analysis of the causes of risk should be conducted with a critical assessment of the changes in standard procedures.

CONCLUSIONS

The research conducted has allowed to identify and classify the main types of risks, determine the probability of their occurrence, assess the degree of their influence on the production process and parameters of work of a pharmaceutical enterprise: falling beyond the budget, failure to meet the deadline, reduction in the quality of the products produced. The results obtained has allowed to improve the risk management system at the enterprise, namely to develop the basic measures for prevention of risks, algorithms of control conducting, points of tighten control. The measures proposed has allowed to prevent or reduce the possible losses, as well as improve one of the main indicators of competitiveness of a pharmaceutical enterprise – the quality of the final product. The risk management framework developed (sequence of operations) at the stage of manufacture of a drug based on the medicinal plant raw material is in the basis of functions of persons or department responsible for the risk management at the enterprise and for the quality of products.

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ДОСЛІДЖЕННЯ РИЗИКІВ ВИРОБНИЦТВА РОСЛИННИХ ЛІКАРСЬКИХ ЗАСОБІВ МЕТОДОМ FMEA-АНАЛІЗУ

В.К.Яковенко

Ключові слова: *оцінка ризиків якості; FMEA-аналіз; рослинні лікарські засоби; технологічний процес*

Проведено ідентифікацію та оцінку ризиків, що виникають у процесі виробництва екстракційних рослинних лікарських засобів. Об'єктом досліджень був технологічний процес виробництва багатокомпонентних настойок та рідких екстрактів. Аналіз ризиків виробництва базувався на результатах проведеної ретроспективної валідації виробництва багатокомпонентних екстракційних рослинних препаратів, зокрема крапель оральних «Клімасед». При проведенні валідації були визначені критичні стадії та параметри технологічного процесу, встановлені критерії прийнятності. Була проведена ідентифікація можливих ризиків для кожної критичної стадії та критичного параметра технологічного процесу. Експертні оцінки заносились до формуляру оцінки ризиків якості. Результати розрахунків підсумкового коефіцієнту ризику (RPN-risk priority number) дозволили віднести до неприйнятних ризиків можливі невідповідності технологічного процесу на стадії екстрагування. Суттєвий вплив мають ризики, що виникають на стадії підготовки сировини, очистки та фільтрування екстрактів. У процесі управління ризиками на підприємстві було здійснено категоризацію ризиків (оцінка рівня ризику), запропоновані методи моніторингу, запобігання і реагування на ризик; розроблені профілактичні заходи, а також заходи на випадок реалізації ризику. З метою профілактики та мінімізації можливих ризиків фармацевтичного виробництва розроблені методологічні підходи до функціонування системи ризик-менеджменту з урахуванням особливостей виробництва лікарських препаратів на основі лікарської рослинної сировини.

ИССЛЕДОВАНИЕ РИСКОВ В ПРОИЗВОДСТВЕ РАСТИТЕЛЬНЫХ ЛЕКАРСТВЕННЫХ СРЕДСТВ МЕТОДОМ FMEA-АНАЛИЗА

В.К.Яковенко

Ключевые слова: *оценка рисков качества; FMEA-анализ; растительные лекарственные средства; технологический процесс*

Проведена идентификация и оценка рисков, возникающих в процессе производства экстракционных растительных лекарственных средств. Объектом исследований был технологический процесс производства многокомпонентных настоек и жидких экстрактов. Анализ рисков производства базировался на результатах проведенной ретроспективной валидации производства многокомпонентных экстракционных растительных препаратов, в частности капель оральных «Климасед». При проведении валидации были определены критические стадии и параметры технологического процесса, установлены критерии приемлемости. Была проведена идентификация возможных рисков для каждой критической стадии и критического параметра технологического процесса. Экспертные оценки заносились в формуляр оценки рисков качества. По результатам расчетов ранга приоритетности риска (RPN-risk priority number) к неприемлемым рискам отнесены возможные нарушения технологического процесса на стадии экстрагирования. Существенное влияние на качество имеют риски, возникающие на стадии подготовки сырья, очистки и фильтрации экстрактов. В процессе управления рисками на предприятии была осуществлена категоризация рисков (оценка уровня риска), предложены методы мониторинга, предотвращения и реагирования на риск; разработаны профилактические мероприятия, а также мероприятия на случай реализации риска. С целью профилактики и минимизации возможных рисков фармацевтического производства разработаны методологические подходы к функционированию системы риск-менеджмента с учетом особенностей производства лекарственных препаратов на основе лекарственного растительного сырья.

ОРГАНІЗАЦІЯ ТА ЕКОНОМІКА ФАРМАЦІЇ

Recommended by Doctor of Pharmacy, professor A.A.Kotvitska

UDC 338.24:005.34:615.1

MANAGEMENT DECISION AS A COMPONENT OF EFFECTIVE ORGANIZATION MANAGEMENT

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Key words: management decision; models of management decision; methods; management; pharmaceutical management

The article is devoted to substantiation of the theoretical aspects of management decision, its modern methods and adaptation of classical theories to pharmaceutical management. The efficiency of enterprises depends on the level of the enterprise management organization and decision-making efficiency. Most management decisions are made under uncertainty, which causes risk in the organizations activity. Each problem of decision-making is different. Some initial situation, alternative solutions, implications of different options are common to all the problems. With these components any problem decision can be described. The requirements for effective management decisions have been analyzed. The algorithm for management decision making when applying different types of management at the enterprise.

The current state of the market economy requires adequate types of management to these processes at all levels and sectors of the economy. This problem is of particular importance at the level of pharmaceutical companies because the market orientation of the pharmaceutical sector increasingly requires the ability to see perspectives and make strategic management decisions by managers for the effective performance of both commercial and social functions.

Providing market stability and success of pharmaceutical functioning in a competitive environment depends on effective management. The changes occurred in the economy and politics of Ukraine revealed a number of controversial and topical issues that are of theoretical and applied nature and are essential for the sustainable functioning and development of the pharmaceutical sector of the economy. Therefore, the process of conceptual and practically significant developments of management decisions making problems considering risk factors and uncertainties of the environment and healthcare reforms, including the pharmaceutical area, is of special importance.

A significant contribution to the development of the conceptual foundations of the decision making theory was made by such domestic and foreign authors as V.P.Halushko, E.P.Golubkov, I.B.Oleksiv, S.M.Zadorozhna, M.V.Tulenkov, H.Rayfa, G.Simon, E.Hunt, G.Hale, etc. To study management decisions is impossible without the theoretical background and practical experience in management. The studies of R.Ackoff, I.Ansoff, W.Morris, I.Parsons, T.Peters were devoted to the theory and practice of management aspects.

The works of such leading Ukrainian scientists as Z.M.Mnushko, A.S.Nemchenko, M.S.Ponomarenko, V.M.Tolochko, V.A.Zagory, D.S.Volokh, B.L.Parnovsky, O.L.Grom et al., are devoted to problems in the theory of pharmaceutical needs adequate management practices and realities of the national pharmaceutical market.

However, the task of improving the efficiency of the development process and decision-making requires a systematic approach to the study of the nature, specific characteristics and procedural organization.

The aim of the study is development and updating of theoretical ideas about the nature and procedural organization of management decisions and substantiation of the efficiency improvement of enterprises in the pharmaceutical industry.

Materials and Methods

The methodological and theoretical framework for the study was the papers of domestic and foreign scholars and experts in the field of management.

While carrying out the research such general scientific methods as systematic, and comprehensive situational approaches, as well as the general theory of management were used.

Today's unpredictable environmental factors significantly affect the operation of business. In these circumstances compliance with the requirements of the enterprise's manageability is one of the most important criteria for evaluating the effectiveness of its activities. Thus, the more the environmental effect is, the more attention managers at all levels of government must pay to the study of the processes occurring in the environment of the organization. The primary means of the enterprise

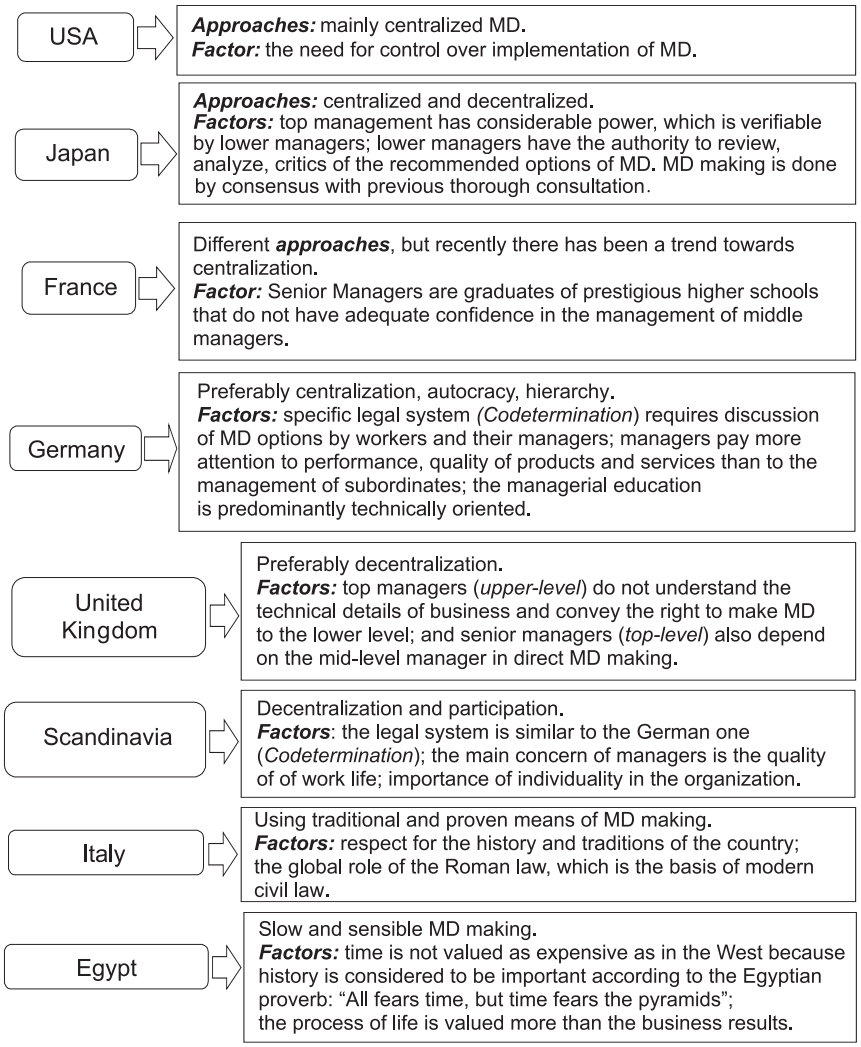


Fig. 1. Foreign practice of MD making.

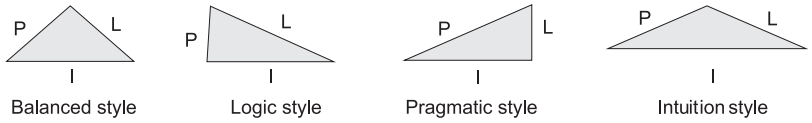
adaptation to uncertain and rapidly changing environmental conditions is an effective management system with the appropriate set of methods, tools, styles, providing formation and implementation of the optimal variant of the entity [1, 3]. According to experts in the field of management today's leaders (regardless of their hierarchy) are under pressure of such environmental factors as increased competition; limited time, increase of the information flow, error rates and the level of uncertainty of market dynamics changing market needs and expectations, falling demand and its solvency, the complexity of the structure of business and its operating environment in general [2, 7, 8]. Diversity of styles and management practices, variability of personal characteristics of the head, as well as a direct impact on his/her duties are the core of factors of the internal environment of the organization. Combination of external and internal factors creates a cumulative effect on the head of any rank in making his/her managerial decisions (MD). The study of the works of the leading experts in management, economics, sociology, psychology and other research areas that influenced on the formation of the modern model of MD is of a great importance for understanding the nature and characteristics of MD making in organizations, including those of the pharmaceutical branch [4-6, 10] (Table).

It is also important to pay attention to peculiarities of making MD in terms of individual national cultures. International experience suggests that there are different approaches in the process of MD making. Thus, management organization in Western European companies compared with the American ones has its specific features due to historical conditions of the theory and practice of management in these countries. Leading American companies had in its basis the trust structure, and branches of large firms were not endowed with self-sufficiency, especially in dealing with strategic issues. At the same time the majority of large European companies were focused on decentralization of management, and their subsidiaries had financial and legal independence. But, both in Europe and the United States, small and medium enterprises play a very important role in the economy. This caused some features of their management (Fig. 1).

Based on the national and international experience, as well as the experience of operating businesses in the pharmacy branch we have concluded that the new economic relations, forms of organization and management methods cannot be implemented directively. Therefore, a necessary condition for formation and stable functioning of the pharmaceutical market is review of the style and methods of business management, and above all,

Table

Basic classical models of management decision making

MODELS OF G. SIMON	
The concept of Bounded Rationality	*Process of MD making is* represented by three stages: 1. Search of the reasons for the need to make MD 2. Modelling of possible situations and analysis of activities 3. The choice of a particular course of activity
The model of "economic man"	The behaviour of an "economic man" provides the best selection of possible courses of action for making MD. It is assumed that an "economic man" knows all the choices and is able to foresee all the possible consequences of each alternative MD in advance and use practice (P), logic (L), intuition (I) 
MODEL OF J. MARCH	
Garbage Can Model	Management decision making in organizations and development of the concept of bounded rationality Three types of constraints that are inherent to managers and affect the MD are identified: ***cognitive; political; organization***. According to him the process of MD has four features: **1. Conflict quasi-resolution** (special measures of quasi-resolution of conflicts that weaken, reduce them and make it possible to coexist with them. Such measures include mechanisms of "local rationality", "acceptable level of decisions" and "consistent achievement of objectives") **2. Avoiding uncertainty.** (Reducing the impact of uncertain factors of the environment by signing exclusive contracts with partners and customers, cooperation with authorities, negotiation, gathering marketing information) **3. Problem search** ("localization" of searching the options around some well-known solution used in the past. Innovative, radical solutions are usually ignored in order not to make changes and violate the "established order of things") **4. Organisational learning** (any MD making is a learning process. Acting by trial and error, people learn, knowing from their own experience which MD is permissible or effective and which is not, what under the present circumstances is permitted and what is forbidden, etc. The knowledge gained adapts in future to new situations and objectives of activity)
MODELS OF CH. LINDBLOM	
Synoptic approach	*Deductive scheme of adoption of MD, which provides:*. determination of all the factors that affect the adoption of MD; their ranking in order of priority; determination of information and measures to compare each alternative with any other; obtaining complete information about all the factors in order to determine the best alternative by "logical deductive calculations"
Strategy of local increases	Decision-making is aimed to have slight changes being made in small increments ("small change of important value"). *Characteristics of the strategy:* ***limitation*** (problem simplification, consideration of a limited number of alternatives and a limited number of their consequences), ***orientation to the means*** (adaptation of purposes to means), ***reconstruction*** (MD making is accompanied by continuous changes ("reconstructions") of factors affecting the outcome of the choice), ***seriation*** (MD making is a series of attacks on persistent problems for their detection and solving), ***practicality*** (small improvements according to the principle "here and now" in practice are better than a planned move to distant targets), ***fragmentation*** (different participants in the MD making process at different times may make different estimates about the same problems)
MODEL OF V. VROOM	
Management decisions by a leader	According to him there are *five styles of MD:* A1 + A2 – ***autocratic style,*** self acceptance of MD; C1 + C2 – ***consultative style***, MD is taken independently, they only reflect the opinion of subordinates. G2 – ***group style***, MD is made during the joint discussion without the administrative influence over the Group; (G1 – corresponds to the extreme case where there is only one subordinate)
The normative model of MD	It provides determination of criteria for assessing the problem situation, which include: • significance of the MD quality and availability of sufficient information or experience of the head for making a high-quality MD; • degree of structuring of the problem; • significance of the consent of subordinates with the MD accepted for its effective implementation; • probability that the autocratic decision of the head will be supported by subordinates; • motivation of subordinates to achieve organizational goals that are pursued in solving this problem; • likelihood of conflicts between subordinates in making MD
MODEL OF M. CROZIER	
Conflict-game model	It specifies that the organization is a collection of interacting "power" games or the so-called ensemble of games. Game is a certain type of relations that arise in any organization. The "players" are heads and subordinates, managers and workers, divisions and groups. The game rules are not formally established, and gradually formed through evolution by analyzing the behaviour of players in similar situations

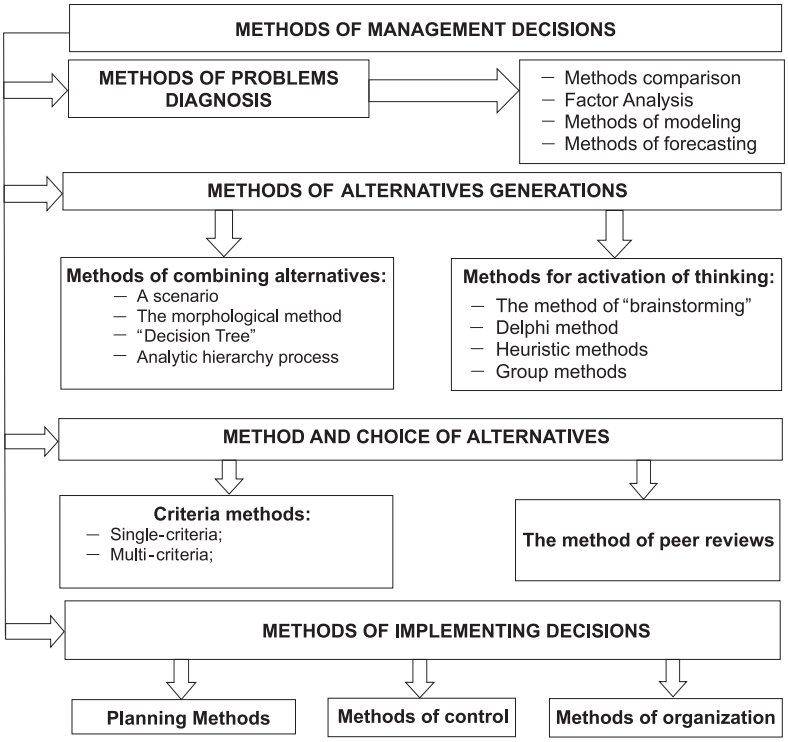


Fig. 2. Classification of the main methods of MD making.

through the practical implementation of scientifically based methods of decision-making (Fig. 2).

However, it seems clear that the choice of MD method is defined both by objective factors and the specific subject of application, which can be managers of the certain management level. But usually they (methods of MD) must be *accurate, reliable, motivated, determined both in time, methods, and in choosing the executive; they should minimize the uncertainty of the external and internal environment, and thus, reduce or completely avoid the risk in MD making* [12-16]. An important component of effective management of the organization is the ***quality of MD***.

The main conditions to ensure high quality and efficiency of implementation of MD at pharmaceutical companies include:

- application of scientific approaches to MD development;
- the study of the effect of socio-economic laws on the effectiveness of MD;
- providing a manager with a qualitative information that characterizes the parameters of "input", "output", "environment" and "process" in the system of management decision implementation;
- automation of continuous data collection and processing in the process of MD implementation;
- application of the methods of functional – cost analysis, forecasting, modeling and economic feasibility of implementation of each decision;
- structuring of the problem and building a "tree" of goals;
- providing comparability of alternatives of MD and their multiple variants;
- compliance with legal validity of MD;
- development and functioning of the system for responsibility and motivation of qualitative and effective implementation of this decision;
- a clear mechanism for implementing solutions based on the performance discipline.

The most important aspect of organizational development and implementation of MD is organization of the sequence of work required to complete this process. We believe that for practical implementation of this process the type of management that managers of different levels of management use in the company regardless of the industry sector, legal status and ownership may be of special importance. Within the framework of the research conducted we detailed the possible algorithms for developing and making MD in certain types of management, which are now commonly used in organizations of the pharmaceutical branch (Fig. 3).

Thus, MD making in ***traditional management*** is based on the study of the past experience in solving similar problems in a particular organization, and predicting the effects of their analogy. These features have their impact on the MD algorithm comprising the steps related to identification of the similar problems and prediction of results similar to the results already obtained.

The principal difference of the algorithm of MD making in ***systemic management*** is implementation of stages of gathering information about the system and relations of its elements, determination of objectives for management of the element in solving the problem at the ***system*** level, generation of the list of possible control actions in relation to the system – the source of the problem, and prediction of the consequences of these impacts for the system level.

The ***situational approach*** focuses on the fact that suitability of different management practices is determined by the situation, therefore, the decision-making algorithm includes the stages of collecting and analyzing information concerning the ***situation***, detremination of

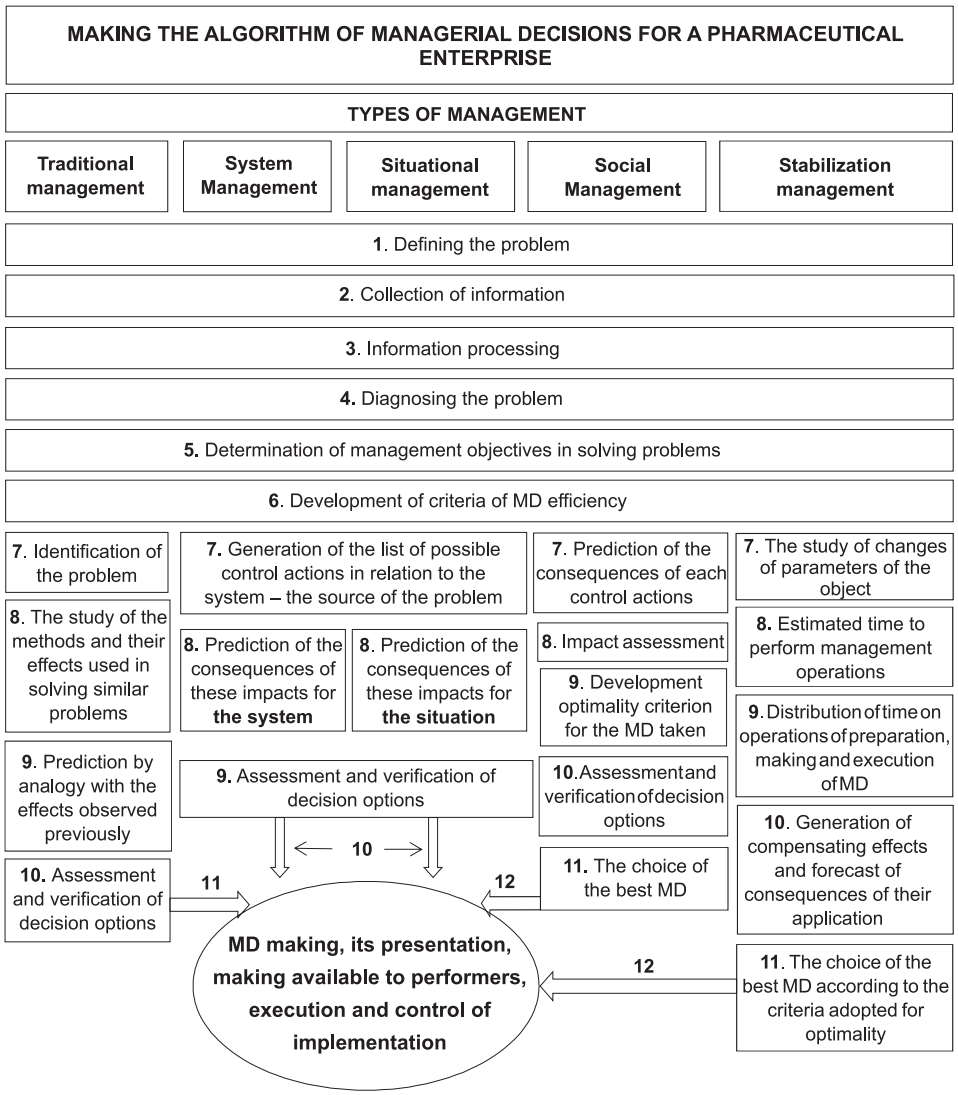


Fig. 3. The algorithm of MD depending on the type of organization management.

objectives for management of the situation when solving the problem and prediction of the consequences of control actions for the situation.

In ***social management*** when making MD special attention is paid to consideration of permissible and impermissible implications of options of control actions according to various parameters. It should be emphasized that social and stabilization types of management have the longest chain of the manager's operations concerning MD. It can be explained, firstly, by the social orientation of the subjects of the pharmaceutical industry (manufacturers, wholesale management, retail sector), and secondly, the current instability and uncertainty of environmental factors, increased competition at the pharmaceutical market with simultaneous reduction of consumer demand for pharmaceutical products and solvency.

For specific tasks using a particular type of management the algorithms may vary according to the specificity of a particular task.

CONCLUSIONS

The effectiveness of enterprises in a competitive environment depends on the level of the enterprise management efficiency and decision-making. The vast majority of administrative decisions have been taken under conditions of uncertainty, which causes risk of organizations. Uncertainty in decision-making is understood as availability of several possible outcomes and alternatives, and arises because of the influence of uncertain factors, including factors of personal uncertainty, and uncertainties of the environment, including natural and behavioral uncertainty.

Each problem of decision-making is different. Some initial situation, alternative solutions, implications of different options are common to all the problems. With these components any problem decision can be described. Taking all these into account the algorithm in the managerial decision has been developed. MD is quite complex and cannot always take place in accordance with the stages mentioned above. Some procedures, stages may be absent or occur in parallel, and sometimes they need to be repeated. The quality of the decision making process is directly dependent on the completeness of all factors that significantly affect the consequences of the decision.

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УПРАВЛІНСЬКЕ РІШЕННЯ ЯК СКЛАДОВА ЕФЕКТИВНОГО МЕНЕДЖМЕНТУ ОРГАНІЗАЦІЇ

В.В.Малий

Ключові слова: *управлінське рішення; моделі управлінського рішення; методи; менеджмент; фармацевтичний менеджмент*

Стаття присвячена обґрунтуванню теоретичних аспектів управлінського рішення, його сучасних методів та адаптації класичних теорій до фармацевтичного менеджменту. Ефективність діяльності підприємств залежить від рівня організації управління підприємством та оперативності прийняття управлінських рішень. Більшість управлінських рішень приймається в умовах невизначеності, яка служить причиною ризику в діяльності організацій. Кожна проблема прийняття рішення має свої особливості. Загальним для всіх проблем є певна вихідна ситуація, альтернативні варіанти рішення, певні наслідки різних варіантів. За допомогою цих компонентів можна охарактеризувати будь-яку проблему прийняття рішення. Проаналізовані вимоги до прийняття ефективного управлінського рішення. Розроблено алгоритм прийняття управлінського рішення при застосуванні на підприємстві різних видів менеджменту.

УПРАВЛЕНЧЕСКОЕ РЕШЕНИЕ КАК СОСТАВЛЯЮЩАЯ ЭФФЕКТИВНОГО МЕНЕДЖМЕНТА

В.В.Малый

Ключевые слова: *управленческое решение; модели управленческого решения; методы; менеджмент; фармацевтический менеджмент*

Статья посвящена обоснованию теоретических аспектов управленческого решения, его современных методов и адаптации классических теорий к фармацевтическому менеджменту. Эффективность деятельности предприятий зависит от уровня организации управления предприятием и оперативности принятия управленческих решений. Большинство управленческих решений принимается в условиях неопределенности, которая служит причиной риска в деятельности организаций. Каждая проблема принятия решения имеет свои особенности. Общим для всех проблем являются определенная исходная ситуация, альтернативные варианты решения, определенные последствия различных вариантов. С помощью этих компонентов можно охарактеризовать любую проблему принятия решения. Проанализированы требования к принятию эффективного управленческого решения. Разработан алгоритм принятия управленческого решения при применении на предприятии различных видов менеджмента.

Recommended by Doctor of Pharmacy, professor O.A.Ruban

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THE STUDY OF THE CURRENT STATE OF DISPENSING NARCOTIC, PSYCHOTROPIC DRUGS AND PRECURSORS BY PRESCRIPTION IN UKRAINE

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Key words: dispensing by prescription; expert assessment; narcotic and psychotropic substances; precursors; drug abuse

The analysis and generalization of the provisions of normative legal acts (NLA) regulating prescription and OTC drugs circulation in Ukraine have been conducted. The causes of violations in dispensing drugs by prescription are the problem of the healthcare system on the whole and the problem of the lack of control over the professional activities of doctors and pharmacists, and it requires a comprehensive approach to this issue by the government. It has been determined that the current legislation requires a clear regulation of the issues dealing with prescribing. In order to regulate the circulation of prescription narcotic drugs, psychotropic substances and their precursors it is necessary to strengthen the responsibility of pharmacists for dispensing of prescription drugs without prescriptions, and the responsibility of doctors for prescriptions written incorrectly. The attitude of health professionals to dispensing drugs by prescription in Ukraine has been studied in the article. According to the questioning of pharmaceutists (pharmacists) it has been found that the vast majority of professionals are bound constantly to dispense prescription drugs without prescriptions during their work. At the same time they understand the social danger that is caused by breaking rules of dispensing drugs, which are excessively used by addicts, from pharmacies. The expert opinions concerning the causes of breaking rules in dispensing drugs from pharmacies and the frequency of inspections of pharmacies by law enforcement authorities have been assessed.

Narcotic drugs, psychotropic substances and their precursors (NPP) are substances of the natural and synthetic origin. They are dangerous for human health and can cause the state of dependence, a depressing or stimulating effect on the CNS, perception and behaviour disorders, etc. [1, 14, 19].

At the same time it is impossible to identify the concept of narcotic (psychotropic) drugs [15]. According to the reference book written by M.D.Mashkovsky (1977), only 5 subgroups can be referred to the main group of "Psychotropic drugs": neuroleptic and sedative medicines, tranquilizers, antidepressants and medicines stimulating the central nervous system.

Circulation of prescription and OTC drugs are an integral part of pharmaceutical provision of the population [20].

Unfortunately, nowadays commercial activities of the pharmacy dominates in Ukraine. Physicians seldom write out a prescription, and pharmaceutists get a list of medicines written by the physician on a sheet of paper, but not on the prescription form or simply a patient tells them what drug he wants. Taking this into consideration pharmaceutists often offer the most advertised and expensive medicine [16-18].

That is why unreasonable prescription and uncontrolled drug intake is an important current socio-economic and healthcare problem in Ukraine.

Analysis of the latest investigations and publications. Many Ukrainian scientists such as A.S.Nemchenko [5, 6], A.A.Kotvitska [2], A.P.Gudzenko [5] and others paid their attention to different directions as to the problems of dispensing of prescription and OTC drugs.

Highlighting the issues unsolved previously. A comprehensive consideration of the problem is practically absent.

The aim of our research was generalization of the provisions of normative legal acts (NLA) concerning the regulation of prescription and OTC drugs circulation and the study of healthcare professionals' attitude to dispensing of prescription drugs in Ukraine with subsequent determination of efficient ways of solution.

Materials and Methods

Analysis and generalization of NLA regulating prescription and OTC drugs circulation in Ukraine; the questionnaire survey of professionals (pharmaceutists, pharmacists), statistical, mathematical and graphical analysis were used in the research.

Results and Discussion

The list of NPP was approved by the resolution of the Cabinet of Ministers of Ukraine (CMU) from 06.05.2000, No. 770 [11] (with amendments) in order to regulate the activity and control the circulation of this group of substances, including volume of quotas, within which manufacturing, storing, import into Ukraine and export from Ukraine of narcotic drugs and psychotropic substances for medical and scientific purposes and medicines containing NPP in the amount that does not exceed the permissible limit.

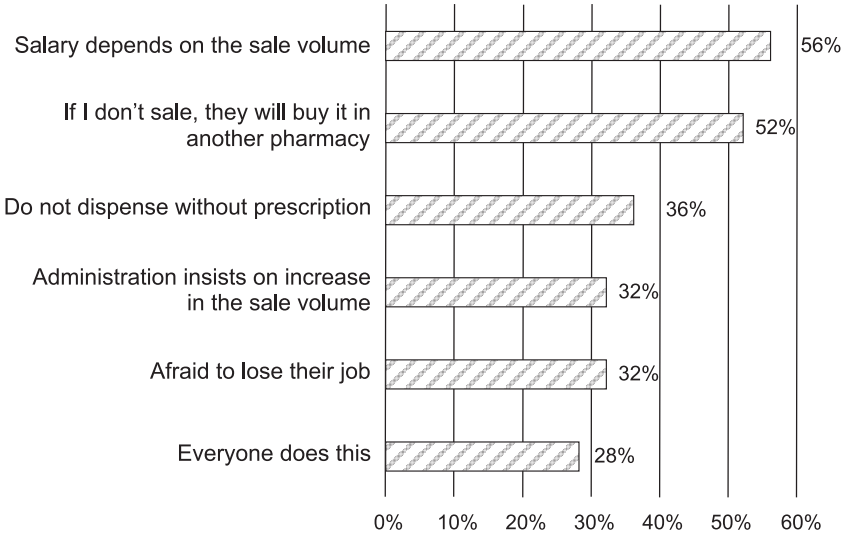

Fig. 1. The answers of professionals concerning the causes of breaking rules in dispensing drugs.

The volume of quotas for NPP is regulated by the resolution of CMU: from 25 June 2014 No. 209 (not yet in force), from 29 April 2013 No. 313 (in force), from 22.02.2012 No. 128 with amendments (in force) [8-10].

Comparing NPP quotas within the period from 2012 to 2014 the conclusion can be made that, in general, within the period from 2012 to 2013 the volume of quotas for NPP manufacturing increased. Within the period from 2013 to 2014 the volume of quotas, on the contrary, decreased. This will help to reduce the level of narcomania in the country.

According to order of the Ministry of Health of the USSR No. 175 (1982) "On measures on further improvement of drug provision of the population and medical preventive institutions" for the first time 3 types of prescription forms were introduced: form No. 1 – for dispensing drugs at full cost (for psychotropic drugs) valid for 10 days and stored 1 month at the pharmacy; form No. 2 – for prescribing drugs free of charge or on preferential terms; form No. 3 – a special prescription form of a pink colour for dispensing narcotic drugs, which were additionally certified by the signature of the head physician of the medical preventive institutions (MPI) or by the head of the department and by the round stamp of the hospital valid for 5 days from the day of prescribing and stored at the pharmacy for 1 year, later the date of expiry increased to 5 years.

After the expiry date of storing prescriptions of form 3 and orders (requests) for narcotic (psychotropic) drugs they are destroyed according to the procedure established by the legislation.

The circulation of prescription and OTC drugs in Ukraine is regulated according to the following NLA:

- the Order of the Ministry of Public Health of Ukraine from 19.07.2005 No. 360 "On approval of the Rules of prescribing and order requirements for medicines and medical products, the Order of dispensing medicines and medical products from pharmacies and their structural subdivisions, Instructions on the procedure for keeping, accounting and destruction of prescription forms and order requirements" [7];
- Order of the Ministry of Public Health of Ukraine from 11.29.2013 No.1034 «On amendments to the Order of the Ministry of Public of Ukraine from July, 19, 2005 No. 360» [4];
- the Order of the Ministry of Public Health of Ukraine from 7.09.2012 No. 708 "On amendments to the Order of the Ministry of Public Health of Ukraine from May, 14, 2003 No. 210 "On approval of criteria for referring narcotic (psychotropic) drugs containing a small quantity of narcotic drugs or psychotropic substances and precursors to the category of drugs, which are dispensed without prescription, and the List of these drugs" [3];
- the Resolution of CMU from 6.04.2000 No. 770 "On approval of the list of narcotic drugs, psychotropic substances and precursors" [11];
- the Resolution of CMU from 3.06.2009 No. 589 "Approving the activities related to narcotic drugs, psychotropic substances and precursors, and control over their turnover" [12];
- the Law of Ukraine from 15.02.1995 No. 60/95-BP "On narcotic drugs, psychotropic substances and precursors" with amendments [13], etc.

Unfortunately, at present the most pharmacies are bound to sell prescription drugs without prescription. The budgets of MPI do not include costs for making prescription forms. Physicians have to write out prescription drugs not on the special forms according to the Order of the Ministry of Public Health of Ukraine "On approval of the Rules of prescribing and order requirements for medicines and medical products …" from 19.07.2005 No. 360 [7], but on ordinary paper sheets certified by the doctor's personal seal or advertising samples in the form of prescriptions. The exception is prescription of drugs subjected to control, physicians always write out prescriptions on them according to the current legislation. Thus, physicians, pharmacists and pharmaceutists become hostages of the situation (a physician is bound to prescribe treatment, and a pharmacist can not refuse a patient in purchasing the necessary drugs).

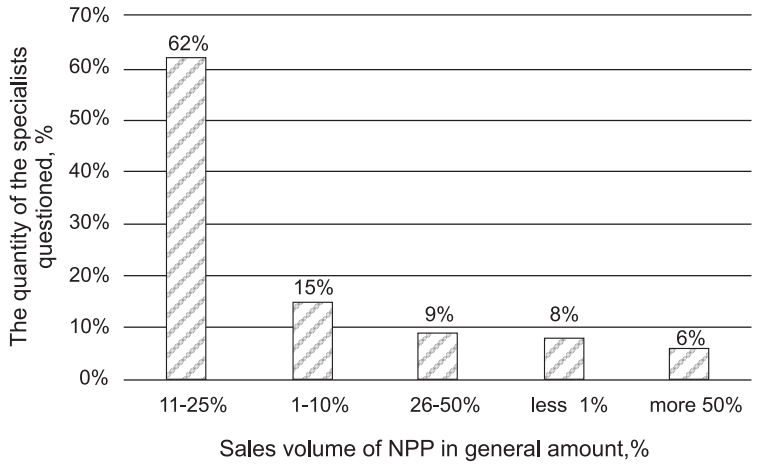

Fig. 2. The answers of professionals concerning the NPP volume of sales in goods circulation of a pharmacy.

According to the Order [4] doctors should not specify trade names (TN) of drugs in prescriptions; they should prescribe drugs according to international non-proprietary names (INN). A pharmacist when dispensing prescription drugs should offer the patient the existing names of the drugs prescribed by INN considering different price groups.

With the aim of determination of the professionals' (pharmaceutists, pharmacists) attitude to the current problems of dispensing NPP by prescription the questioning of pharmaceutists one of the cities of Ukraine has been carried out. In this questioning 98 specialists of all qualification levels with the length of work from 1 to 39 years took part. The most respondents (73%) have a higher pharmaceutical education.

The questionnaire developed has met all modern ethical requirements, namely confidentiality of the information received; the questions, which do not affect the respondents' dignity; appeal to a respondent with the specific goal of the research; the absence of questions with confusing statements.

According to results of the research conducted it has been found that 95% of professionals are bound constantly to dispense prescription drugs without prescriptions during their professional activity. The moral principles of specialists, management and owners of pharmacies are one of the most important factors concerning dispensing drugs abused by drug addicts without prescription.

Administration of 60% of the pharmacists questioned reminds the staff about their responsibility of breaking rules of dispensing prescription drugs, but in most cases attention is focused on drugs that cause drug addiction.

96% of the respondents understand the social dangers, which breaking rules of dispensing drugs abused by drug addicts from the pharmacy cause.

At the next stage of our research the causes of breaking rules of dispensing drugs by pharmacists were determined (Fig. 1).

Unfortunately, according to the data obtained 56% of the respondents answer that their salary depends on the sale volume, and they don't care if a patient buys drugs causing narcotic dependence without a prescription.

52% of the respondents motivate their actions in such a way: "if I don't sell – they will buy it in another pharmacy and I will lose a part of my salary". During the research it has been found that 36% of the respondents refuse to dispense socially dangerous drugs without the physician's prescription. 28% of the pharmacists questioned justify their actions saying that everyone does this.

It should be noted that in goods in stock of every pharmacy there are some drugs, which are within the area of interest of drug users, but these drugs are different and depend on such factors as the chemist's loca-

Fig. 3. The frequency of inspections from law enforcement authorities.

tion, management and owner's attitude to this problem, changes in legislation, etc.

The most of the professionals questioned (62%) work at the pharmacies where NPP sales are 10-25% (Fig. 2).

Taking into consideration a high social danger of narcomania and toxicomania pharmacies as one of the source of suppling potential dangerous drugs are subject to various inspections by law enforcement authorities. These inspections are aimed at revealing violations of the current legislation concerning the order of dispensing prescription drugs. It can be control purchase, drugs circulation check, check the availability and storage rules of prescriptions of the drugs dispensed, compliance with the rules for storing these drugs, accordance of actual availability of drugs to report, compliance with license terms, etc.

As to periodicity of such inspections the respondents answer as follows: 60% – checks are once a year, 28% – they occur once in three month (Fig. 3).

CONCLUSIONS

1. NLA regulating prescription and OTC drugs circulation have been analyzed. It has been determined that the current legislation requires a clear regulation of the issues dealing with prescribing. In order to regulate the circulation of prescription narcotic drugs, psychotropic substances and their precursors it is necessary to strengthen the responsibility of pharmacists for dispensing of prescription drugs without prescriptions, and the responsibility of doctors for prescriptions written incorrectly.

2. With the aim of determination of the professionals' (pharmaceutists, pharmacists) attitude to the current problems of dispensing NPP by prescription the questioning of pharmaceutists in one of the cities of Ukraine has been carried out. According to results of the research conducted it has been found that 95% of professionals are bound constantly to dispense prescription drugs without prescriptions during their work, and 96% of the respondents understand the social danger that is caused by breaking rules of dispensing drugs, which are excessively used by addicts, from pharmacies.

3. The expert opinions concerning the causes of breaking rules in dispensing drugs and the frequency of inspections of pharmacies by law enforcement authorities have been assessed.

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ДОСЛІДЖЕННЯ СУЧАСНОГО СТАНУ РЕЦЕПТУРНОГО ВІДПУСКУ НАРКОТИЧНИХ, ПСИХОТРОПНИХ ЗАСОБІВ ТА ПРЕКУРСОРІВ В УКРАЇНІ

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Ключові слова: *рецептурний відпуск; експертна оцінка; наркотичні та психотропні засоби; прекурсори; наркотична залежність*

Проведено аналіз та узагальнені положення нормативно-правових актів, що регулюють рецептурний та безрецептурний обіг лікарських засобів в Україні. Причинами порушень рецептурного відпуску препаратів є проблема всієї системи охорони здоров'я та проблема недостатнього контролю за професійною діяльністю лікарів та фармацевтичних працівників, що потребує комплексного підходу до цього питання з боку уряду. Встановлено, що чинне законодавство потребує чіткого врегулювання питань виписування рецептів. З метою регулювання рецептурного обігу наркотичних, психотропних засобів та прекурсорів необхідно посилити відповідальність фармацевтичних працівників за відпуск рецептурних лікарських засобів без рецептів та відповідальність лікарів за неправильно виписані рецепти. Вивчене ставлення фахівців охорони здоров'я до рецептурного відпуску лікарських засобів в Україні. За результатами опитування провізорів (фармацевтів) встановлено, що переважна більшість фахівців змушена постійно в своїй професійній діяльності відпускати рецептурні лікарські засоби без рецептів. При цьому вони розуміють соціальну небезпеку, яку викликають порушення правил відпуску з аптек лікарських засобів, якими зловживають наркозалежні. Зроблено оцінку думок фахівців щодо причин порушень правил відпуску лікарських засобів та частоти перевірок аптек з боку правоохоронних органів.

ИССЛЕДОВАНИЯ СОВРЕМЕННОГО СОСТОЯНИЯ РЕЦЕПТУРНОГО ОТПУСКА НАРКОТИЧЕСКИХ, ПСИХОТРОПНЫХ СРЕДСТВ И ПРЕКУРСОРОВ В УКРАИНЕ

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Ключевые слова: *рецептурный отпуск; экспертная оценка; наркотические и психотропные средства; прекурсоры; наркотическая зависимость*

Проведен анализ и обобщены положения нормативно-правовых актов, регулирующих рецептурный и безрецептурный оборот лекарственных средств в Украине. Причинами нарушений рецептурного отпуска препаратов являются проблемы всей системы здравоохранения и недостаточного контроля за профессиональной деятельностью врачей и фармацевтических работников, что требует комплексного подхода к этому вопросу со стороны правительства. Установлено, что действующее законодательство требует четкого урегулирования вопросов выписывания рецептов. С целью регулирования рецептурного оборота наркотических, психотропных средств и прекурсоров необходимо усилить ответственность фармацевтических работников за отпуск рецептурных лекарственных средств без рецептов, усилить ответственность врачей за неправильно выписанные рецепты. Изучено отношение специалистов здравоохранения к рецептурному отпуску лекарственных средств в Украине. По результатам опроса провизоров (фармацевтов) установлено, что подавляющее большинство специалистов вынуждено постоянно в своей профессиональной деятельности отпускать рецептурные лекарственные средства без рецептов. При этом они понимают социальную опасность, которую вызывают нарушения правил отпуска из аптек лекарственных средств, которыми злоупотребляют наркозависимые. Обобщена оценка мнений специалистов о причинах нарушений правил отпуска лекарственных средств и о частоте проверок аптек со стороны правоохранительных органов.

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ORGANIZATION OF THE QUALITY ASSURANCE SYSTEM OF COMPOUNDING PHARMACIES IN UKRAINE: RESULTS OF THE SURVEY

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Key words: compounding preparations; quality assurance system; survey

To meet the needs of patients for treatment of a number of diseases compounding preparations are often used. The aim of our study was to determine the possibility of introduction of the international experience of forming the quality assurance system for compounding preparations in conditions of compounding pharmacies of Ukraine. For this purpose the survey of employees of compounding pharmacies regarding organization of the quality assurance system for compounding preparations has been conducted. In the survey 93 employees of compounding pharmacies took part. The research was conducted in 12 regions of Ukraine in 2013-2014. The questionnaire contained 44 questions, which represented the statements of organization of the quality assurance system of compounding pharmacies of Ukraine and compounding preparations. The statements were divided into 10 groups: personnel and training, premises and equipment, documentation, production, complaints and product recalls, self audits, in-pharmacy quality control, work contracted out, the necessary requirements of the State Pharmacopoeia, overarching statements. For the collection and processing of data 4-point Likert scale was used. The statement was considered to be accepted if more than 50% of respondents chose "A – strongly agree" or "B – agree". The survey of employees of compounding pharmacies concerning the possibility of introducing the international experience of the quality assurance system for compounding preparations has allowed to determine and estimate their attitude to the statements proposed, to identify the points of discussion. Processing of the results of the survey has shown that all 44 statements were approved by more than 71.0% of the respondents. Each statement received an average of 94.9% of "A" or "B". And 22 statements (50.0%) had 100.0% agreement "A" + "B".

To meet the needs of patients for treatment of a number of diseases the magistral formulas are often used together with manufactured medicines. Worldwide compounding preparations are applied in various fields of medicine: hormone replacement therapy, dentistry, dermatology, oncology, pediatrics, geriatrics, physiotherapy, medical cosmetology, sports medicine, allergies treatment, and others; as well as in veterinary medicine [15, 17, 19, 21, 22, 24].

It is known that quality assurance is the combination of organizational arrangements taken to ensure quality conformance of drugs with their purpose [11, 23]. The quality assurance system of compounding preparations is the system of steps and corresponding actions, which guarantee the necessary quality for drug preparation. Nowadays creation of programmes and implementation of quality assurance systems for compounding preparations are urgent in the USA, EU, Japan, UK, etc. [9, 14, 16, 23].

The quality assurance system for compounding preparations must ensure development and technology of compounding preparations in accordance with the latest achievements of science; preparation and control operations are clearly specified and implemented according to the principles of Good Preparation Practice; dispensing only those compounding preparations, which are correctly prepared, checked and stored in accordance with the procedures approved and prepared by a competent person; the use of appropriate equipment, containers, active substances and excipients, packaging material, it allows preparation, storage, use of compounding preparations in such a way as to provide the required quality throughout the shelf-life and in-use experity dates; good documentation practice [23].

Taking into consideration the experience of Ukraine regarding the implementation of appropriate Good Practices (G_XP) [12] for manufactured drugs it is obvious that some difficulties can appear when introducing the quality assurance system for compounding preparations; therefore, when organizing standards it is necessary to consider the legislation and experience of compounding pharmacies of the USSR, and harmonization with the international standards.

The aim of our study was to determine the possibility of introduction of the international experience of forming the quality assurance system for compounding preparations in conditions of compounding pharmacies of Ukraine.

Materials and Methods

For this purpose the survey of employees of compounding pharmacies regarding organization of the quality assurance system for compounding preparations was conducted. The questionnaire was developed taking into account the main statements of the PIC/S documents related to preparation of drugs in conditions of pharmacies, modern legislative base of compounding

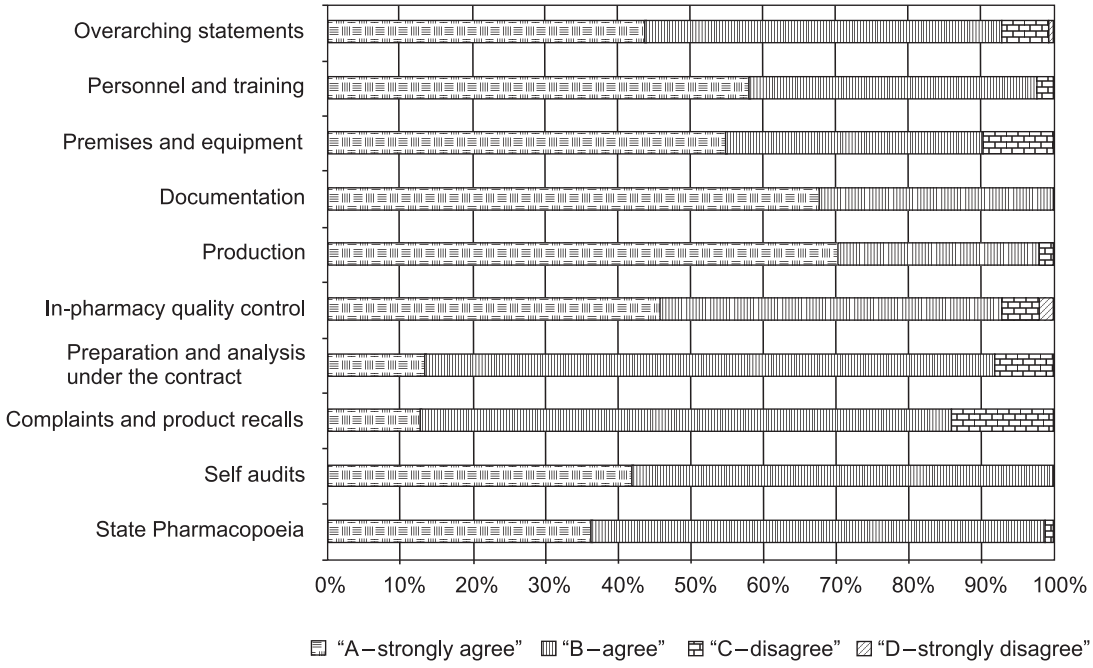

Fig. Distribution of the respondents' answers concerning the statements proposed for the quality assurance system of compounding preparations.

preparations in Ukraine, the experience of US and British Pharmacopoeias [11, 13, 20]. The experience of survey hospital pharmacists from 85 countries of the world was also used, its results were presented at the International Congress of FIP "Global Conference Future of Hospital Pharmacy" (Switzerland, Basel, 2008). The Ukrainian pharmacists did not participate in this questioning [18].

The questionary method was used in our study, 93 employees who worked or had experience of work in compounding pharmacies responded to the survey. The average work experience of the respondents in the compounding pharmacy was 18 years. The research was conducted in 12 regions of Ukraine (Volyn, Dnipropetrovsk, Donetsk, Zakarpattia, Kyiv, Luhansk, Rivne, Sumy, Ternopil, Kharkiv, Cherkasy, Chernihiv) in 2013-2014.

There were 44 questions in the questionnaire, which represented the statements of organization of the quality assurance system of compounding pharmacies of Ukraine and compounding preparations. The statements were divided into 10 groups: personnel and training (7 questions), premises and equipment (1 question), documentation (2 questions), production (5 questions), complaints and product recalls (3 questions), self audits (2 questions), in-pharmacy quality control (5 questions), preparation and analysis under the contract (2 questions), the necessary requirements of the State Pharmacopoeia (8 questions) and overarching statements containing questions (9 questions), which were not included in other groups.

For the collection and processing of data 4-point Likert scale was used: "A – strongly agree with statement"; "B – agree"; "C – disagree"; "D – strongly disagree with statement". The statement was considered to be accepted if more than 50% of respondents chose "A – strongly agree" or "B – agree".

Results and Discussion

Processing of the results of the survey has shown that all 44 statements were approved by more than 71.0% of the respondents: "A – strongly agree" or "B – agree" (Fig.). Each statement received an average of 94.9% of "A" or "B". Only 174 (4.3%) of 4092 answers were "C – disagree" and 15 (0.4%) "D – totally disagree"; at the same time there were 1870 positive answers (45.7%) "A – strongly agree", and 2033 (49.6%) "B – agree". And 22 statements (50.0%) had 100.0% agreement "A" + "B". The minimum level of consensus A + B in all statements was 71.0%.

According to the results of the survey the revival of practical preparation of compounding preparations and use new active ingredients when preparing compounding and creation of programme of extension use of compounding preparations for oncology, pediatrics, geriatrics, veterinary are urgent (93.6%). At present there is a necessity to update formulations of compounding preparations and use new active ingredients when preparing compounding preparations (87.1%), although during the survey 12 respondents noticed that this task should be solved with participation of scientists and physicians, as well as research for improving methods and systems of preparation and quality assurance of compounding preparations.

Personnel and training. According to the survey 100.0% of the respondents approved education and advanced training of pharmacists based on last scientific research; the curriculum should include the course of quality assurance of compounding preparations. The cases of the staff incompetence in compounding pharmacies should be investigated, solved through strategic research and made public for the purpose of prevention of reappearance (93.6%). 90.3% of the respondents

agreed with the statement concerning participation of pharmacists in improvement of methods of preparation and quality control of compounding preparations, it reflected the experience of the quality assurance system introduced in compounding pharmacies of the USA.

During the research challenges of implementation of requirements for *premises and equipment* and *preparation process* in compounding pharmacies were identified. The preparation process should be based on the requirements of GMP. Based on the results obtained it has been found that these statements require significant investment and should be harmonized with the existing ones in Ukraine [2, 3].

According to the results of the survey 100.0% of the respondents supported the need for good *documentation* practice and development and implementation of documented standard operating procedures (SOP) for all activities in the compounding pharmacy in preparation, quality control, labeling, storage, purification, disinfection, qualification, and work with the equipment, etc.

Appreciation of the majority of pharmacists concerning creation of the reporting system about rejected low-quality compounding preparations (80.7%), *product recalls* and *self audits* in order to improve the quality and safety of drug treatment practice and prevent re-occurrence of such error (100.0%), the risk assessment of preparation and quality control of compounding preparations is important [6].

Since one of the ways of quality assurance of extemporaneous medicines is development and introduction of general articles and monographs on officinal formulas to the National Pharmacopoeia [8], the questions related to development and improvement of general articles on sterile and nonsterile compounding preparations, dose regimen [4, 5], introduction of monographs on officinal formulas of compounding preparations taking into account the needs of modern compounding pharmacies were included into the questionnaire. 96.8% of the respondents were agreed with the statement about urgency of forming the conception of determination of shelf life and stability for sterile and nonsterile compounding preparations [7]; 100% of the respondents were agreed with the statement about the order of conducting experimental research.

According to the results of the questionnaire, the statements about *preparation and analysis under the contract* were not fully supported by the respondents since to conduct analysis in a certified laboratory for drug quality control will lead to significant increase of costs.

In-pharmacy quality control. According to the respondents all prescriptions and orders of medical preventive institutions must be double checked before preparation and dispensing (100.0%).

A physician and a clinical pharmacist should take into account the compatibility of the compounding preparations prescribed with the manufactured drug, and, if necessary, with herbal drugs and diets (71.0%).

The statements about development and validation of analytical methods taking into account the capabilities of the material and technical base of compounding pharmacies (96.8%), substantiation of the possibility of using non-pharmacopeial methods, qualification of analytical equipment (photocolorimetry, refractometry and polarimetry for assay) are very urgent [1, 10]. Development of such methods will contribute to save funds of compounding pharmacies. In case of external inspections of pharmacies or conducting the stability studies of compounding preparations other specific developed and validated methods, which can be reproduced in laboratories for drug quality control, should be used (100.0%).

CONCLUSIONS

1. The survey of employees of compounding pharmacies concerning the possibility of introducing of the international experience of the quality assurance system of compounding preparations has allowed to determine and estimate their attitude to the statements proposed, to identify the points of discussion.

2. Processing of the results of the survey has shown that all 44 statements proposed concerning the quality assurance system of compounding preparations were approved by more than 71.0% of the respondents: “A – strongly agree” or “B – agree”. Each statement received an average of 94.9% of “A” or “B”. And 22 statements (50.0%) had 100.0% agreement “A” + ”B”.

3. According to the results of the survey it has been found that the question of material costs is an important factor for implementation of the quality assurance system of compounding preparations.

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ОРГАНІЗАЦІЯ СИСТЕМИ ЗАБЕЗПЕЧЕННЯ ЯКОСТІ ВИРОБНИЧИХ АПТЕК В УКРАЇНІ: РЕЗУЛЬТАТИ АНКЕТУВАННЯ

О.А.Здорик

Ключові слова: *екстемпоральні лікарські засоби; система забезпечення якості; анкетування*

Для задоволення потреб пацієнтів при низці захворювань використовуються екстемпоральні лікарські засоби (ЕЛЗ). Метою дослідження було визначення можливості впровадження міжнародного досвіду формування системи забезпечення якості ЕЛЗ в умовах виробничих аптек України. Для досягнення мети було проведено анкетування співробітників виробничих аптек України щодо організації системи забезпечення якості ЕЛЗ. В опитуванні взяли участь 93 співробітники виробничих аптек. Дослідження проводили в 12 областях України у 2013-14 рр. Анкета включала 44 питання, які висвітлювали положення організації системи забезпечення якості виробничих аптек України та ЕЛЗ. Наведені положення були розподілені на 10 груп: персонал та навчання, приміщення та обладнання, документація, виготовлення, скарги та відкликання продукції, самоінспекція, внутрішньоаптечний контроль якості, виготовлення та аналіз за контрактом, необхідні вимоги Державної фармакопеї, загальні положення. Для збору та обробки голосів використовували 4-и бальну шкалу Лайкерта. Положення вважали прийнятими, якщо більше 50% голосів відповідали варіантам «А – абсолютно згоден» і «В – згоден». Анкетування співробітників виробничих аптек щодо визначення можливості впровадження міжнародного досвіду системи забезпечення якості ЕЛЗ дозволило встановити і оцінити їх ставлення до запропонованих положень, виявити дискусійні питання. Обробка результатів опитування показала, що всі 44 запропонованих положення отримали більше 71,0% схвальних голосів респондентів. Кожне положення отримало у середньому 94,9% голосів «А» або «В». 22 положення (50,0%) отримали 100,0% схвалення «А» + «В».

ОРГАНИЗАЦИЯ СИСТЕМЫ ОБЕСПЕЧЕНИЯ КАЧЕСТВА ПРОИЗВОДСТВЕННЫХ АПТЕК В УКРАИНЕ: РЕЗУЛЬТАТЫ АНКЕТИРОВАНИЯ

А.А.Здорик

Ключевые слова: *экстемпоральные лекарственные средства; система обеспечения качества; анкетирование*

Для удовлетворения потребностей пациентов при ряде заболеваний используются экстемпоральные лекарственные средства (ЭЛС). Целью исследования было определение возможности внедрения международного опыта формирования системы обеспечения качества ЭЛС в условиях производственных аптек Украины. Для достижения цели было проведено анкетирование сотрудников производственных аптек касательно организации системы обеспечения качества ЭЛС. В опросе приняли участие 93 сотрудника производственных аптек. Исследование проводили в 12 областях Украины в 2013-14 гг. Анкета содержала 44 вопроса, которые отображали положения по организации системы обеспечения качества

производственных аптек Украины и ЭЛС. Приведенные положения были распределены на 10 групп: персонал и обучение, помещение и оборудование, документация, изготовление, жалобы и отзыв продукции, самоинспекция, внутриаптечный контроль качества, изготовление и анализ по контракту, необходимые требования Государственной фармакопеи, общие положения. Для сбора и обработки голосов использовали 4-х балльную шкалу Лайкерта. Положения считали принятыми, если более 50% голосов отвечали вариантам «А – абсолютно согласен» и «В – согласен». Анкетирование сотрудников производственных аптек по определению возможности внедрения международного опыта системы обеспечения качества ЭЛС позволило установить и оценить их отношение к предложенным положениям, выявить дискуссионные вопросы. Обработка результатов опроса показала, что все 44 предложенные положения получили более 71,0% согласия респондентов. Каждое положение получило в среднем 94,9% голосов «А» или «В». 22 положения (50,0%) получили 100,0% согласия «А» + «В».

ЕКСПЕРИМЕНТАЛЬНА ТА КЛІНІЧНА ФАРМАКОЛОГІЯ

Recommended by Doctor of Medicine, professor V.A.Volkovoy

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THE STUDY OF TOXICOLOGICAL PROPERTIES OF A NEW COMBINED CREAM "DERMALIPOIN"

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Key words: toxicological properties; combined cream "Dermalipoin"

The aim of the work was to study acute toxicity, the irritating and allergic effects of a new combined cream "Dermalipoin" consisting of α-lipoic acid, urea, olive oil, tea tree oil, PEG-400. It has been found that the LD_{50} of the cream with intragastric introduction is greater than 5000 mg/kg, and when applied to the skin LD_{50} > 2810 mg/kg; in accordance with the classification of K.K.Sidorov it allows to refer this cream to the class of almost nontoxic substances (the V-th toxicity class). "Dermalipoin" cream shows no irritative action in contact with the mucous membrane of the eye and sensitizing actions in external application.

Treatment of wounds remains one of the most important problems of modern medicine [13]. The analysis conducted has shown that at the pharmaceutical market out of 12 medicines intended for the I-st phase of the wound process only one combined drug – "Inflarax" ointment ("Zdorovya", Ukraine) has a multidirectional and pronounced pharmacological action [3]. But one drug cannot completely satisfy the needs of the treatment of purulent wounds, therefore, there is the necessity of development and introduction of new medicines with a wide range of the pharmacological action.

Nowadays one of the most promising soft medical forms are creams as they more fully and smoothly release the medicinal substances, they are easily applied and absorbed by the skin, they do not leave on it greasy luster, they are economically available, etc. [5].

At the National University of Pharmacy (NUPh) a new combined product in the form of a cream has been developed for treating inflammatory and microbial skin diseases conventionally named "Dermalipoin" containing α-lipoic acid, urea, olive oil, tea tree oil, PEG-400 (polyethylene glycol). PEG-400 is a solvent with a pronounced osmotic activity, which causes its wide application in the production of creams for the treatment of infected wounds where it provides the osmotic and dehydration effects, which accelerates the term of wound healing [12]. Medicines containing PEG have high efficiency, especially in the treatment of exudative dermatoses when fat and carbohydrate carriers can not be used [1]. PEG well dissolves a great number of medicinal substances, easily release them providing a good contact with the skin or the wound surface tissue, which, in turn, significantly increases their absorption and activity [7].

One of the main stages of introduction of medicines is to determine their toxicity [2]. The study of acute toxicity along with other indicators (possible local irritative and allergic effects) that characterize the toxicological properties of medicinal products is a mandatory requirement of the State Pharmacological Centre (SPhC) of the Ministry of Public Health of Ukraine in preclinical study of new medicines [7].

The aim of the work was to study acute toxicity, the irritating and allergic effects of a new combined cream "Dermalipoin".

Materials and Methods

Preclinical study of toxicological properties of "Dermalipoin" cream was conducted at the premises of the Central Scientific Research Laboratory of the NUPh. In preclinical studies the experimental animals grown in vivarium of the Central Research Laboratory equipped according to the sanitary-and-hygienic norms were used. All animals were kept under the standard sanitary conditions [8]. During the experiment the animals were kept in vivarium at 19-24°C, with humidity of not more than 50%, with the natural light mode "day-night" in plastic cages on a balanced food diet. Before the experiment the animals acclimatized themselves under experimental room conditions within 7 days. The experiments were carried out in accordance with the national "General ethical principles of experiments on animals" (Ukraine, 2001), which are consistent with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 18.03.86).

Acute toxicity of the cream was studied according to Pastushenko G.V. method in nonlinear sexually mature male white mice with the body weight of 18-25 g and in nonlinear sexually mature male white rats with the body weight of 180-200 g in a single intragastric introduction and skin application. Toxicity was assessed according to the generally accepted classification of K.K.Sidorov [10]. When studying acute toxicity of drugs

Table 1

The study of acute toxicity of "Dermalipoin" cream in mice in intragastric introduction (n=6)

Group	Dose, mg/kg	Animals that died/ the total number of animals
1	500	0/6
2	1000	0/6
3	3000	0/6
4	4000	0/6
5	5000	0/6

the survival/mortality of animals is an integral indicator, which allows calculating the drug medium lethal dose (LD_{50}). It is recommended to use 5 levels of doses. When it is impossible to introduce the drug in doses that cause death of animals, then in order to study acute toxicity the SPhC of the Ministry of Public Health of Ukraine recommends to use the maximal dose of the IV-th toxicity class (low toxic substances) taking into account the way of introduction [2]. Therefore, when determining nontoxicity of the cream in the dose of 2810 mg/kg in application on the skin and in the dose of 5000 mg/kg in intragastric introduction the further research may be considered inexpedient. In accordance with the recommendations of the SPhC of the Ministry of Public Health of Ukraine the number of animals was 6 in each experimental group [2]. Acute toxicity of the experimental samples was studied when applying the cream on a skin area of the back previously clipped, which was not less than 10% of the total surface area of the animal. Test samples were applied with a thin layer in the dose of 2810 mg/kg. The monitoring of survival, clinical signs and condition of the skin (with the skin application) was carried out within 14 days.

The local irritative effect was studied in guinea pigs with the body weight of 400-450 g using the conjunctival test [9]. For this purpose the cream was introduced into the conjuctival ocular vesicle of the right eye of the experimental animals. The left eye served as the control. The reaction of the mucous membranes of the eye was observed in 15 min, one hour and a day after introduction of the cream. In the experiment the degree of hyperemia, swelling and the amount of discharge were considered. Assessment of the damaging effect was conducted by the rating scale:

0 – the absence of the response of the mucous membrane of the eye;

1 – a slight redness of the lacrimal ducts;

2 – the redness of the lacrimal ducts and the sclera in the cornea;

3 – the redness throughout the conjunctiva and sclera.

The study of the possible allergic effect of the cream was performed using the method of skin applications [6, 11]. Experiments were conducted in guinea pigs with the body weight of 400-450 g. The experimental animals were divided into 2 groups: the first group was the control, in the animals of the second group cream was applied on the skin daily in the dose of 0.01 ml/cm^2. The test was performed by applying the decoupling dose on the 10-th and the 20-th day of each sensibilization. In the case of the absence of sensibilization signs at the 1-st phase the application of the cream under research was continued to the 2-nd phase. On the 20-th day the test was repeated. The skin condition was assessed in 1 hour and 24 hours after applying the decoupling dose and the changes were reflected by the rating scale: 1 point – punctate weak hyperemia; 2 points – punctate intense hyperemia; 3 points – continuous weak hyperemia; 4 points – continuous intense hyperemia and infiltration hyperemia. In order to evaluate the expression of inflammation after application of the decoupling dose the thickness of skin folds was measured.

Table 2

The study of acute toxicity of "Dermalipoin" cream in rats after application on the skin (n=6)

The drug under study	Dose, mg/kg	Died	Survived	The reaction of the skin
"Dermalipoin" cream	2810	0	6	without changes

Table 3

The effect of "Dermalipoin" cream on the condition of the skin cover of guinea pigs (n=6)

Conditions of the experiment	The thickness of skin folds, mm		
	The time frame of the study		
	Initial data	10-th day	20-th day
Control	3.2±0.2	3.0±0.1	3.1±0.14
"Dermalipoin" cream	3.0±0.1	3.1±0.2	3.0±0.15

The data obtained was processed by the method of variation statistics at the significance level $p<0.05$ (taking into account the arithmetic mean and its standard error) [4].

Results and Discussion

As mentioned, the integral indicator in the study of acute toxicity of drugs is the survival/mortality of animals, it allows to calculate the medium lethal dose (LD_{50}) that is the main toxicological characteristics of a medicine. The research results are presented in Tab. 1, they indicate the absence of animals' deaths in a single intragastric introduction of the cream in all doses.

Thus, LD_{50} of the cream analyzed lies outside the IV-th class of toxicity (LD_{50} > 5000 mg/kg). It allows to refer this cream to the class of practically nontoxic substances (the V-th toxicity class) according to K.K.Sidorov classification.

Since "Dermalipoin" cream is offered as a potential drug for topical treatment of skin lesions, it was necessary to study its possible negative effects on the body of experimental animals in a single skin application.

The results of the experimental studies are pesented in Tab. 2 and indicate the absence of toxic effects in the maximal dose (2810 mg/kg).

When studying the possible local irritative effect it was found that introduction of the cream into the conjuctival ocular vesicle of the experimental animals caused a slight redness during the first 5-8 min, but in 15 min any noticeable reaction in the mucous membrane of the eye was not observed.

Analysis of the results of the experiment conducted allows to draw a conclusion about the absence of the ir-

ritative action of the cream in contact with the mucous membrane of the eye.

The results of the study of possible allergic effects of the cream are given in Tab. 3. On the 10-th day the signs of sensibilization were not detected. When applying the cream within 20 days any notable changes from the skin cover was not observed. Application of the decoupling dose of the cream did not lead to development of hyperemia, infiltration and edema. The thickness of skin folds after application of the decoupling dose did not change in relation to the control.

CONCLUSIONS

1. It has been found that the LD_{50} of the cream with intragastric introduction is greater than 5000 mg/kg, and when applied to the skin LD_{50} > 2810 mg/kg; in accordance with the classification of K.K. Sidorov it allows to refer this cream to the class of almost nontoxic substances (the V-th toxicity class).

2. "Dermalipoin" cream shows no irritative action in contact with the mucous membrane of the eye and sensitizing actions when applying on the skin.

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ДОСЛІДЖЕННЯ ТОКСИКОЛОГІЧНИХ ВЛАСТИВОСТЕЙ НОВОГО КОМБІНОВАНОГО КРЕМУ «ДЕРМАЛІПОЇН»

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Ключові слова: *токсикологічні властивості; комбінований крем «Дермаліпоїн»*

Досліджена гостра токсичність, місцевоподразнювальна та алергізуюча дія нового комбінованого крему «Дермаліпоїн», до складу якого увійшли: α-ліпоєва кислота, сечовина, оливова олія, олія чайного дерева, ПЕГ-400. Встановлено, що LD_{50} крему при внутрішньошлунковому введенні перевищує 5000 мг/кг, при нанесенні на шкіру – LD_{50} > 2810 мг/кг, що дозволяє віднести його у відповідності до класифікації К.К.Сидорова до класу практично нетоксичних речовин (V клас токсичності). Крем «Дермаліпоїн» не виявляє іритативної дії при контакті зі слизовою оболонкою ока та не чинить сенсибілізуючої дії при нашкірному нанесенні.

ИССЛЕДОВАНИЕ ТОКСИКОЛОГИЧЕСКИХ СВОЙСТВ НОВОГО КОМБИНИРОВАННОГО КРЕМА «ДЕРМАЛИПОИН»

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Ключевые слова: *токсикологические свойства; комбинированный крем «Дермалипоин»*

Изучена острая токсичность, местнораздражающее и аллергизирующее действие нового комбинированного крема «Дермалипоин», в состав которого вошли: α-липоевая кислота, мочевина, оливковое масло, масло чайного дерева, ПЭГ-400. Установлено, что LD_{50} крема при внутрижелудочном введении превышает 5000 мг/кг, при нанесении на кожу – LD_{50} > 2810 мг/кг, что позволяет отнести его в соответствии с классификацией К.К.Сидорова к классу практически нетоксичных веществ (V класс токсичности). Крем «Дермалипоин» не проявляет ирритативного действия при контакте со слизистой оболочкой глаза и не оказывает сенсибилизирующего действия при наружном нанесении.

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MICROBIOLOGICAL STUDIES OF THE CREAM FOR USE IN THE DIABETIC FOOT SYNDROME

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Key words: tea tree oil; preservative; microbiological stability; microbiological research; diabetic foot

Microbiological stability is an important requirement to the medicines developed. In order to prevent microbial contamination of medicines it is necessary to use effective antimicrobial preservatives or active substances with a high preservative effect. Tea tree oil (Melaleuca alternifolia Maid) is a promising antimicrobial substance in development of drugs for use in the diabetic foot syndrom. The microbiological study of the cream with α-lipoic acid, urea, and tea tree oil developed for use in the diabetic foot syndrome has been carried out. The study of the preservative action efficiency of tea tree oil was conducted according to the method of the SPhU. The criterion for efficiency evaluation of the preservative action of the samples under research was decrease of the number of viable cells of microorganisms in the medicines for the certain periods of time after inoculation. The data obtained have shown that the test samples with different concentrations of tea tree oil provide the antimicrobial effect in relation to all test strains of the microorganisms used. The number of microorganisms decreased during storage. It has been found that introduction of tea tree essential oil in the concentration of 3% provides microbiological stability of the medicine developed according to the requirements of the SPhU. By efficiency of its preservative action the essential oil of tea tree meets the requirements of criterion "A" according to the SPhU, and it allows not to add extra preservatives to the cream developed.

Infectious inflammatory skin disease in patients with diabetes mellitus can be attributed to its non-specific complications. As a result of neuropathy, micro- and macroangiopathy there are disorders of innervation and microcirculation, redox processes change, and it leads to decrease of local and general immunity. On the skin surface of patients with diabetes approximately 2-3 times more microorganisms than in healthy people, including pathogenic and opportunistic microorganisms, are usually detected. It is associated with the decreased bactericidal activity of the skin in patients with diabetes that aggravates specific complications, such as the diabetic foot syndrome [8, 12].

An essential tea tree oil (*Melaleuca alternifolia Maid*) is a promising antimicrobial substance in development of drugs for use in the diabetic foot syndrom. Numerous investigations confirm the healing properties of tea tree oil in parasitic and fungal skin diseases, acute necrosis of diabetes, as well as other infectious diseases (dental, otorhinolaryngological, gynecological diseases) [6, 7, 9-11, 13-16]. Tea tree oil can be also used as a preservative [1, 4].

Microbiological stability is an important requirement to the medicines developed. Microbiological stability control is particularly important for soft drugs based on the emulsion, which are exposed to contamination.

The aim of research was to investigate the microbiological purity of the cream with α-lipoic acid, urea, and tea tree oil developed for use in the diabetic foot syndrome [2].

Materials and Methods

The study of efficiency of tea tree oil was carried out on clinical strains of *Pseudomonas aeruginosa ATCC 9027*, *Staphylococcus aureus ATCC 6538, Candida albicans ATCC 10231* by the method 5.1.3 of the State Pharmacopoeia of Ukraine (SPhU) for medicinal products for topical use [3]. For analysis six samples of the cream with different concentrations of tea tree oil (1%, 2%, 3%, 4%, 5%) and the cream sample without essential oil were prepared.

The samples were inoculated with a suspension of one of the test microorganisms selected. The inoculated samples of the cream were kept at a temperature from 20°C to 25°C and protected from light. After contamination by microorganisms at certain time intervals (days 2, 7, 14, 28) the samples were placed on nutrient media to determine the viable cells count.

Dense nutrient media (Sabouraud's medium, egg yolk high salt agar culture medium, nutrient agar, thioglycollate medium) used in the research were standard. They were prepared in accordance with the manufacturer's requirements (the amount of powder per litre, pH, autoclaving conditions, etc.). Each batch used in the experiment was tested on the growth quality according to regulatory documents (inoculation of test strains of microorganisms taking into account 24-120-hour growth of the corresponding strains).

Table 1

Efficiency of the antimicrobial preservative action of the cream containing 3% tea tree oil

Exposition	Requirements of the SPhU		Number of microorganisms (CFU/ml) * lg of reduction		
	the bacterial count CFU/ml, lg of reduction	the number of fungi CFU/ml, lg of reduction	*Staphylococcus aureus ATCC 6538*	*Pseudomonas aeruginosa ATCC 9027*	*Candida albicans ATCC 885/653*
Microbial load	10^6	10^6	2×10^5 *5.30	2.5×10^5 *5.39	2.5×10^5 *5.39
Primary inoculation, lg	–	–	5.1×10^4 *0.60	5.1×10^4 *0.69	5.1×10^4 *0.69
2 days	2.00	–	2.3×10^2 *2.94	2.5×10^3 *2.00	2.7×10^3 *1.96
7 days	3.00	–	NI	0.9×10^2 *3.44	1.1×10^2 *3.35
14 days	–	2	NI	NI	0.3×10^2 *3.92
28 days	NR	NR	NI	NI	NI

Notes: NR – the number of viable cells of microorganisms or fungi does not rise; NI – viable cells of microorganisms or fungi are not isolated.

The criterion for efficiency evaluation was reduction of the number of viable cell colonies of microorganisms for a certain period after contamination [3]. In accordance with the requirements of the SPhU in medicinal products for topical application logarithms of reduction in viable bacterial colonies in 2 days should be not less than 2, in 7 days – not less than 3, and further the number of viable bacterial cells should not increase. Logarithms of reduction of the number of fungi viable cells in 14 days should be not less than 2. These values correspond to criterion "A". In accordance with criterion "B" in formulations for topical application logarithms of reduction in viable bacterial colonies in 14 days must be at least 3, and further the number of viable colonies should not increase. Logarithms of reduction of viable fungi in 14 days should be not less than 1 and there is no further increase. The presence of viable cells of microorganisms and fungi on the 28-th day of studies indicate that the drug does not meet criteria "A" or "B" and does not meet the requirements of the SPhU.

Results and Discussion

The data obtained experimentally show that the test samples with tea tree oil exhibit the antimicrobial activity against all the microorganisms used. The number of microorganisms during storage decreased (Tab. 1).

The data obtained showed that the samples with the concentration of tea tree oil of 3%, 4% and 5% on day 2 had logarithms of reduction for bacteria more than 2, and in 7 days – more than 3. On day 14 and 28 of incubation microorganisms were not registered. Logarithms

Table 2

Efficiency of the antimicrobial preservative action of the cream without tea tree oil

Exposition	Requirements of the SPhU		Number of microorganisms (CFU/ml) * lg of reduction		
	the bacterial count CFU/ml, lg of reduction	the number of fungi CFU/ml, lg of reduction	*Staphylococcus aureus ATCC 6538*	*Pseudomonas aeruginosa ATCC 9027*	*Candida albicans ATCC 885/653*
Microbial load	10^6	10^6	2×10^5 *5.30	2.5×10^5 *5.39	2.5×10^5 *5.39
Primary inoculation, lg	–	–	5.1×10^4 *0.60	5.1×10^4 *0.69	5.3×10^4 *0.67
2 days	2	–	4.3×10^3 *1.67	4.9×10^3 *1.70	2.4×10^4 *1.01
7 days	3	–	1.5×10^2 *3.13	2.1×10^2 *3.07	3.1×10^3 *1.90
14 days	–	2	NI	NI	0.9×10^2 *3.44
28 days	NR	NR	NI	NI	0.2×10^2 *4.09 NR

Notes: NR – the number of viable cells of microorganisms or fungi does not rise; NI – viable cells of microorganisms or fungi are not isolated.

of the viable fungal cells number after 14 days of cultivation were more than 3. Further fungal cells were not isolated. It indicates that these samples correspond to the efficiency criterion "A".

For samples with the concentration of tea tree oil of 1% and 2% logarithms of reduction for bacteria were less than 2 on the 2nd day; in 7 days they were less than 3. In 14 days logarithms of reduction for bacteria and fungi were more than 3. The logarithm of viable fungal cells after 28 days of cultivation did not increase, but single cells continued to register. This indicates that the samples satisfy the efficiency criterion "B" according to the requirements of the SPhU.

It should be noted that decrease in the number of viable organisms was also observed in the sample of the cream without tea tree oil comparing to the initial microbial load (Table 2). After 28 days of cultivation single fungal cells were registered. The sample corresponds to the criterion of efficiency "B"; it can be associated with the low value of pH (5.0-5.5) due to introduction of α-lipoic acid and another active component (urea) [5, 12].

It has been found that three of the six samples studied correspond to the criterion of efficiency "A" in accordance with the requirements of the SPhU (the cream with concentrations of essential tea tree oil of 3%, 4% and 5%), and another three samples meet criterion "B" (the cream without the essential oil, the sample of the cream with 1%, 2% concentration of tea tree oil).

Thus, inroduction of 3% tea tree oil provides the microbiological stability of the composition according to the requirements of the SPhU. To increase the concentration (4%, 5%) is not expedient from the economic point of view. The results obtained in the research indicate that addition of extra preservatives are not required. As a preservative it is advisable to use tea tree oil in the concentration of 3%.

CONCLUSIONS

Microbiological studies of the cream with α-lipoic acid, urea, and tea tree oil have been carried out. It has been found that introduction of tea tree essential oil (*Melaleuca alternifolia Maid*) in the concentration of 3% provides microbiological stability of the formulation developed; and therefore, introduction of additional preservatives is not required. By efficiency of its preservative action the cream meets the requirements of criterion "A" according to the State Pharmacopoeia of Ukraine.

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МІКРОБІОЛОГІЧНІ ДОСЛІДЖЕННЯ КРЕМУ ДЛЯ ЗАСТОСУВАННЯ ПРИ СИНДРОМІ ДІАБЕТИЧНОЇ СТОПИ

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Ключові слова: *олія чайного дерева; консервант; мікробіологічні дослідження; мікробіологічна стабільність; діабетична стопа*

Важливою вимогою до розроблюваних лікарських препаратів є мікробіологічна стабільність. З метою запобігання мікробній контамінації препаратів необхідно використовувати ефективні антимікробні консерванти або діючі речовини з високою консервуючою дією. Перспективною

антимікробною субстанцією при розробці засобів для застосування при синдромі діабетичної стопи є ефірна олія чайного дерева (Melaleuca alternifolia Maid). Нами було проведено мікробіологічне вивчення розробленого крему з α-ліпоєвою кислотою, сечовиною і олією чайного дерева для застосування при синдромі діабетичної стопи. Дослідження ефективності консервуючої дії ефірної олії чайного дерева проводилося за методикою ДФУ. Критерієм оцінки ефективності консервуючої дії досліджуваних зразків було зниження числа життєздатних клітин мікроорганізмів у препаратах за певний період часу після їх інокуляції. Отримані дані показали, що досліджувані зразки з різною концентрацією олії чайного дерева проявляють антимікробну дію по відношенню до всіх використаних тест-штамів мікроорганізмів. Кількість життєздатних клітин бактерій та грибів зменшувалася в процесі зберігання. Встановлено, що введення олії чайного дерева в концентрації 3% забезпечує мікробіологічну стабільність препарату згідно з вимогами ДФУ. Ефірна олія чайного дерева в концентрації 3% за ефективністю консервуючої дії відповідає вимогам критерію «А» ДФУ, що дозволяє не включати до складу розроблюваного препарату додаткові консерванти.

МИКРОБИОЛОГИЧЕСКИЕ ИССЛЕДОВАНИЯ КРЕМА ДЛЯ ПРИМЕНЕНИЯ ПРИ СИНДРОМЕ ДИАБЕТИЧЕСКОЙ СТОПЫ

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Ключевые слова: *масло чайного дерева; консервант; микробиологические исследования; микробиологическая стабильность; диабетическая стопа*

Важным требованием к разрабатываемым лекарственным препаратам является микробиологическая стабильность. С целью предотвращения микробной контаминации препаратов необходимо использовать эффективные антимикробные консерванты либо действующие вещества с высоким консервирующим действием. Перспективной антимикробной субстанцией при разработке средств для применения при синдроме диабетической стопы является эфирное масло чайного дерева (Melaleuca alternifolia Maid). Нами было проведено микробиологическое изучение разработанного крема с α-липоевой кислотой, мочевиной и маслом чайного дерева для применения при синдроме диабетической стопы. Исследование эффективности консервирующего действия эфирного масла чайного дерева проводилось по методике ГФУ. Критерием оценки эффективности консервирующего действия исследуемых образцов было снижение числа жизнеспособных клеток микроорганизмов в препаратах за определенный период времени после их инокуляции. Полученные данные показали, что исследуемые образцы с различной концентрацией эфирного масла чайного дерева проявляют противомикробное действие по отношению ко всем использованным тест-штаммам микроорганизмов. Количество жизнеспособных клеток бактерий и грибов уменьшалось в процессе хранения. Установлено, что введение масла чайного дерева в концентрации 3% обеспечивает микробиологическую стабильность препарата согласно требованиям ГФУ. Эфирное масло чайного дерева в концентрации 3% по эффективности консервирующего действия отвечает требованиям критерия «А» ГФУ, что позволяет не включать в состав разрабатываемого препарата дополнительные консерванты.

Recommended by Doctor of Medicine, professor O.I.Zalyubovs'ka

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DETERMINATION OF REACTOGENICITY AND ALLERGENICITY OF THE IMMUNOBIOLOGICAL DRUG FOR PREVENTION AND TREATMENT OF CANDIDIASIS

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Key words: candidiasis; antigen; vaccine; immunity; therapy

It is known that the use of drugs, which are capable to stimulate a protective immune response against candidal infection, i.e. immunobiological drugs stimulating protective functions, is a promising direction in the fight against candidiasis. The aim of this work was to study reactogenicity and allergenicity of a new immunobiological drug based on the antigens of C. albicans and C. tropicalis fungi. The study of reactogenicity and allergenicity of the solution of the immunobiological drug for prevention and treatment of candidal infection was carried out in healthy guinea pigs weighing 300-400 g; there were 3 animals in the control and experimental groups each. The experimental animals were dehaired on the sides. To determine reactogenicity the solution of the immunobiological drug in the volume of 0.2 ml was injected intradermally on one side of the body. To determine allergenicity the immunobiological drug in the volume of 0.2 ml was injected intradermally to the experimental animals three times at 14 days intervals, and in 14 days after the last injection the immunobiological drug in the volume of 0.2 ml was injected intradermally to guinea pigs. The animals of the control group were injected with the sterile 0.9% isotonic saline solution. Observations for the presence of local reactions occurrence at the site of injection of the immunobiological drug were performed the first 5 minutes and every 2 hours for 24 hours. As a result of the research conducted it has been found that the immunobiological drug based on the antigens of fungi of C. albicans with the protein concentration of 3 mg/ml and C. tropicalis with the protein concentration of 5 mg/ml in the ratio of 1:1 for prevention and treatment of candidal infection is not reactogenic and allergenic.

Fungi of *Candida* genus are the most widespread causative agents of fungal infections. They can cause both minor skin lesions and mucous membranes, as well as very serious diseases of almost all organs of the body [1]. Such wide range of infections requires a significant range of diagnostic and therapeutic strategies.

It is known that the use of vaccines for treating candidiasis is considered to be a promising direction. Such studies are carried out actively both on the territory of the former Soviet Union, and in Europe and America [4, 7, 8]. Development of subunit combined vaccines is a priority area [2, 5, 6]. It should be noted that currently no domestic vaccine is produced in Ukraine and no imported vaccines have been registered. Therefore, development of a vaccine against candidiasis is the topical issue of modern pharmacy and medicine.

According to our previous studies the immunobiological drug developed at the National University of Pharmacy and based on the associated antigens of fungi of *C. albicans* with the protein concentration of 3 mg/ml and *C. tropicalis* with the protein concentration of 5 mg/ml in the ratio of 1:1 is capable of providing the prophylactic and therapeutic effect with two intramuscular injections in the volume of 0.2 ml [9, 10].

At the next stage of our research the immunobiological drug developed must be checked on reactogenicity and allergenicity [3, 5, 6].

The aim of this work is to study reactogenicity and allergenicity of a new immunobiological drug based on the associated antigens of *C. albicans* and *C. tropicalis* fungi.

Materials and Methods

The study of reactogenicity of a new immunobiological drug for prevention and treatment of candidal infection was carried out in healthy guinea pigs weighing 300-400 g. There were 3 animals in the control and experimental groups each; they were kept in the same conditions on a standard diet. Before the research the animals acclimatized themselves under experimental room conditions.

The experimental animals were dehaired on the sides, and the solution of the immunobiological drug in the volume of 0.2 ml was injected intradermally on one side of the body. The animals of the control group were injected with the sterile 0.9% isotonic saline solution. Observations for the presence of local reactions occurrence at the site of injection of the immunobiological drug were performed every 2 hours for 24 hours. In case of the skin redness at the site of injection with the size of more than 5 mm the drug under study is considered to be reactogenic. The measurement is performed in two perpendicular directions.

The study of allergenicity of the drug was carried out in healthy guinea pigs weighing 300-400 g. There were 3 animals in the control and experimental groups each. The experimental animals were dehaired on the sides and the immunobiological drug in the volume of 0.2 ml was injected intradermally three times at 14 days

Table 1

The study of reactogenicity of a new immunobiological drug

Groups of animals	Animals		
	1	2	3
Test group	–	–	–
Control group	–	–	–

Note: «–» – the absence of redness of the skin with the size of 5 mm and more.

Table 2

The study of allergenicity of a new immunobiological drug

Groups of animals	Animals		
	1	2	3
Test group	–	–	–
Control group	–	–	–

Note: «–» – the absence of redness of the skin with the size of 5 mm and more.

intervals. In 14 days after the last injection the immunobiological drug in the volume of 0.2 ml was injected intradermally to guinea pigs. The animals of the control group were injected with the sterile 0.9% isotonic saline solution. Sensitizing properties are absent if the body response to the introduction of the drug in guinea pigs is absent, i.e. local reactions, such as redness, are not observed. The body response of the experimental animals was examined in 1-5 min and in 24-48 hours after injection. In case of the skin redness at the site of injection with the size of more than 5 mm the drug under study is considered to be allergenic. The measurement is performed in two perpendicular directions.

Results and Discussion

The observation period for the animals was 24 hours. The research results are given in Tab. 1. In the control group of the animals there was a slight redness of the skin when introducing the isotonic saline solution at the site of injection; its sizes did not exceed 3 mm, being a normal skin reaction for the injection.

After introduction of the immunobiological drug to the animals of the test group the skin redness gradually began to appear at the site of injection. The fixed size of the sites with the skin redness in animals did not exceed 3 mm, i.e. research results on reactogenicity were within tolerable limits. Therefore, one may state that the immunobiological drug under study on the basis of the associated antigens of *C. albicans* and *C. tropicalis* fungi for prevention and treatment of candidal infection is not reactogenic.

The research results on allergenicity showed that there were no local responses at the site of injection in animals of the control group injected with the isotonic saline solution within the first 5 minutes after introduction of the immunobiological drug (Tab. 2). The size of the site with the skin redness in animals did not exceed 3 mm, being a normal skin reaction for the injection. In 24 hours the skin redness in animals almost disappeared at the site of introduction of the immunobiological drug. The research results are given in Tab. 2.

In the animals of the test group after introduction of the immunobiological drug the size of the skin redness did not also exceed 3 mm at the site of injection for the first 5 minutes, and in 24 hours the skin redness in animals almost disappeared. Therefore, the research results on allergenicity were within the normal range. It indicates that the immunobiological drug under study on the basis of the antigens of *C. albicans* and *C. tropicalis* fungi for prevention and treatment of candidal infection is non-allergenic.

CONCLUSIONS

1. The immunobiological drug based on the antigens of fungi of *C. albicans* with the protein concentration of 3 mg/ml and *C. tropicalis* with the protein concentration of 5 mg/ml in the ratio of 1:1 is not reactogenic.

2. The immunobiological drug developed for prevention and treatment of candidal infection is non-allergenic.

3. Taking into account the proven action of the immunobiological drug in preventing and treating candidal infection and the data obtained the absence of reactogenic and allergenic properties of this drug is background for creation of an effective and safe medicine.

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ВИЗНАЧЕННЯ РЕАКТОГЕННОСТІ ТА АЛЕРГЕННОСТІ ІМУНОБІОЛОГІЧНОГО ЛІКАРСЬКОГО ЗАСОБУ ДЛЯ ПОПЕРЕДЖЕННЯ ТА ЛІКУВАННЯ КАНДИДАМІКОЗІВ

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Ключові слова: *кандидамікоз; антиген; вакцина; імунітет; терапія*

Відомо, що використання препаратів, здатних стимулювати захисні імунні реакції проти кандидозної інфекції, тобто імунобіологічні препарати, які стимулюють протективні функції, є перспективним напрямком у боротьбі з кандидозом. Метою даної роботи було дослідження реактогенності та алергенності імунобіологічного лікарського засобу на основі антигенів грибів C. albicans та C. tropicalis. Дослідження з реактогенності та алергенності розчину імунобіологічного лікарського засобу для попередження та лікування кандидозної інфекції проводили на здорових мурчаках масою 300-400 г по 3 тварини у контрольних та дослідних групах. У дослідних тварин депілювали на боках хутро. Для визначення реактогенності вводили внутрішньошкірно з одного боку тіла розчин імунобіологічного лікарського засобу в об'ємі 0,2 мл. Для визначення алергенності дослідним тваринам вводили внутрішньошкірно трикратно імунобіологічний лікарський засіб у об'ємі 0,2 мл з інтервалом у 14 діб, а через 14 діб після останньої ін'єкції мурчакам уводили внутрішньошкірно імунобіологічний лікарський засіб у об'ємі 0,2 мл. Тваринам контрольної групи вводили фізіологічний розчин. Проводили спостереження за місцем введення імунобіологічного лікарського засобу на наявність виникнення місцевих реакцій у перші 5 хв та через кожні 2 год протягом 24 годин. У результаті проведених досліджень встановлено, що імунобіологічний лікарський засіб на основі антигенів грибів C. albicans з концентрацією білка 3 мг/мл та C. tropicalis з концентрацією білка 5 мг/мл у співвідношенні 1:1 для попередження та лікування кандидозної інфекції не є реактогенним та алергенним.

ОПРЕДЕЛЕНИЕ РЕАКТОГЕННОСТИ И АЛЛЕРГЕННОСТИ ИММУНОБИОЛОГИЧЕСКОГО ЛЕКАРСТВЕННОГО СРЕДСТВА ДЛЯ ПРЕДУПРЕЖДЕНИЯ И ЛЕЧЕНИЯ КАНДИДАМИКОЗОВ

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Ключевые слова: *кандидамикоз; антиген; вакцина; иммунитет; терапия*

Многие исследователи считают, что использование препаратов, которые способны стимулировать защитные иммунные реакции против кандидозной инфекции, т.е. иммунобиологические препараты, которые стимулируют протективные функции, является перспективным направлением в борьбе с кандидозом. Целью данной работы было исследование реактогенности и аллергенности иммунобиологического лекарственного средства на основе ассоциированных антигенов грибов C. albicans и C. tropicalis. Исследования по реактогенности и аллергенности раствора иммунобиологического лекарственного средства для предупреждения и лечения кандидозной инфекции проводили на здоровых морских свинках массой 300-400 г по 3 животных в контрольных и опытных группах. В опытных животных депилировали на боках мех. Для определения реактогенности вводили внутрикожно с одной стороны тела раствор иммунобиологического лекарственного средства в объеме 0,2 мл. Для определения аллергенности опытным животным вводили внутрикожно трехкратно иммунобиологическое лекарственное средство в объеме 0,2 мл с интервалом в 14 дней, а через 14 дней после последней инъекции морским свинкам вводили внутрикожно иммунобиологическое лекарственное средство в объеме 0,2 мл. Животным контрольной группы вводили физиологический раствор. Проводили наблюдение за местом введения иммунобиологического лекарственного средства на наличие возникновения местных реакций в первые 5 мин и через каждые 2 ч в течение 24 часов. Допускается покраснение кожи в месте инъекции на участке не более 5 мм. В результате проведенных исследований установлено, что иммунобиологическое лекарственное средство на основе ассоциированных антигенов грибов C. albicans с концентрацией белка 3 мг/мл и C. tropicalis с концентрацией белка 5 мг/мл в соотношении 1:1 для предупреждения и лечения кандидозной инфекции не является реактогенным и аллергенным.

Recommended by Doctor of Pharmacy, professor A.G.Serbin

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THE STUDY OF THE CHEMICAL COMPOSITION, EFFECTS ON BEHAVIOURAL RESPONSES AND THE ANTIHYPOXIC ACTIVITY OF THE HERBAL TEA AND THE DRY EXTRACT ON ITS BASIS

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Key words: black horehound; white nettle; wormwood; herbal tea; substance; behavioural responses; antihypoxic activity

The chemical composition and pharmacological activity of the dry extract based on the herbal tea composed of black horehound (Ballota nigra) herb, white nettle (Lamium album) herb, wormwood (Artemisiae absinthii) herb in equal amounts have been studied. As a result of the chemical study of the main groups of biologically active substances in the herbal tea and the extract on its basis the presence of phenolic compounds such as derivatives of hydroxycinnamic acid, hydroxycoumarins, flavonoids and their quantitative content have been determined. In the research objects aglycons of flavonoids (luteolin, kaempferol, quercetin, apigenin) have been idenified. 70% ethanol provides a more complete extraction of phenolic compounds from the herbal tea: the content of hydroxycinnamic acid and flavonoids is higher than in the infusion of the herbal tea, it is more than 60% and 80% respectively; the total amount of phenolic compounds in the dry extract is greater than in the infusion – more than 10% (calculated with reference to a dry residue). The results of the pharmacological research suggest the presence of the sedative effect of the dry extract from the herbal tea in the dose of 400 mg/kg. The dry extract from the herbal tea exhibits the antihypoxic activity that exceeds the activity of Piracetam, thus proving its cerebroprotective action.

In the last decades diseases associated with the human higher nervous dysfunctions remain one of the most important medical and social problems of most countries in the world, first of all, due to high prevalence and severe consequences for public health.

According to the World Health Organization (WHO), approximately 6.7 mln. people die from cerebrovascular diseases annually in the world. Insult is the second most common cause of death; 6150000 inhabitants of the Earth (92 per 100 000) died from insult in 2008, i.e. 10.8% of the total mortality. In Ukraine 754 461 patients died in 2008: of them from insult – 42 422 (91.8 per 100 000 of the population, 5.6% of all the dead) [9, 10, 12].

For the treatment of cerebrovascular diseases, in which pathogenesis hypoxia is of key importance, medicines of various groups are used: nootropic (piracetam), amino acid and peptidergic, acetylcholinergic (donepezil, galantamine, rivastigmine), glutamatergic; when using them a number of side effects appears. The use of herbal drugs in patients with cerebrovascular disease is one of the alternative therapies accelerating normalization of the functions of the nervous system [11]. Therefore, development of effective herbal medicines in cerebrovascular diseases is an important task.

The aim of the work was to study the chemical composition, identify the effects on behavioural responses and the possible antihypoxic activity of the herbal tea and the dry extract on its basis.

Materials and Methods

The objects of the research were the herbal tea composed of black horehound (*Ballota nigra*) herb, white nettle (*Lamium album*) herb, wormwood (*Artemisiae absinthii*) herb in equal amounts and the dry extract on its basis.

To obtain the herbal tea the mixture of black horehound herb, white nettle herb, wormwood herb in the ratio of 1:1:1 was used. From 10 g of the herbal tea the infusion was obtained by the standard technology, and it was further studied [1].

To obtain the dry extract, 100 g of the herbal tea was poured with 1000 ml of 70% ethanol and infused for a week, filtered, allowed to settle within 24 hours, evaporated under vacuum to a dry extract. The extract yield was 7.8%. To determine the qualitative composition of the infusion and the extract obtained conventional research methods – qualitative reactions, paper chromatography (PC) and thin-layer chromatography (TLC) were used. Hydroxycinnamic acids and flavonoids were studied by the method of two-dimensional PC in comparison with authentic samples of hydroxycitric acids in the systems: (direction I) *n*-butanol – acetic acid – water (4:1:2) and (direction II) 5% acetic acid with the subsequent processing of chromatograms with ammonia vapour. To identify hydroxycoumarins the infusion and the extract were chromatographed (PC) in the systems of chloroform (25% formamide) and hexane (25% formamide) with the subsequent examination of chromatograms in the filtered UV light before and after processing with 10% alcoholic solution of potassium hydroxide.

The quantitative determination of derivatives of hydroxycinnamic acid, flavonoids and phenolic compounds was

Table 1

The quantitative content of the main groups of BAS in the infusion and the extract of the herbal tea

Object	Yield, %	Quantitative content, %		
		hydroxycinnamic acids	flavonoids	the total amount of phenolic compounds
Infusion from the herbal tea*	8.39	9.02	0.35	14.84
Dry extract from the herbal tea	7.82	14.81	0.64	16.83

Note: * – the content in a dry residue.

conducted by the spectrophotometric method. The optical density was measured in a cell with the layer thickness of 10 mm on a Specol 1500 spectrophotometer (Switzerland) at an appropriate wavelength. The content of derivatives of hydroxycinnamic acid was calculated with reference to chlorogenic acid at 327 nm, the content of the total amount of flavonoids was calculated with reference to rutin – at the wavelength of 417 nm after forming a complex with aluminium chloride, the content of the total amount of polyphenolic compounds was calculated with reference to gallic acid – at 270 nm [3-8].

For statistical significance the experiments were repeated at least five times.

Pharmacological properties of the dry extract from the herbal tea were studied at the Pharmacology Department of the National University of Pharmacy under the supervision of professor Shtrygol S.Yu. To assess the nature of the psychotropic action the standard screening open field test was used.

The antihypoxic activity as a component of the cerebroprotective action was studied on the model of normobaric hypoxic hypoxia with hypercapnia. The doses of the extract were chosen empirically – 200 and 400 mg/kg. The reference drug was piracetam (b. 321113, Galychpharm, JSC) in the conditionally therapeutic dose of 400 mg/kg. The experiments were conducted in white mice with the body weight of 16-18 g. Five experimental groups of animals were formed. The reference drug and the dry extract were introduced intragastrically 30 min before the test [2]. The results are presented in the form of M±m. Statistical processing was performed using Student t-test.

Results and Discussion

As a result of the chemical study of the main groups of biologically active substances containing in the herbal tea and the extract on its basis the presence of derivatives of hydroxycinnamic acid, hydroxycoumarins, phenolic compounds and flavonoids have been determined. Aglycons of flavonoids – luteolin, kaempferol, quercetin, apigenin were idenified in the research objects by the method of paper chromatography.

The results of determination of the quantitative content of the main groups of BAS identified in the objects of the research are given in Tab. 1.

70% ethanol provides a more complete extraction of phenolic compounds from the herbal tea: the content of hydroxycinnamic acid and flavonoids is higher than in the infusion of the herbal tea, it is 64% and 82% respectively; the total amount of phenolic compounds in the dry extract is 13% greater than in the infusion (calculated with reference to a dry residue).

The results of the pharmacological research of the extract are given in Tab. 2 and Tab. 3.

The results of the open field test indicate the presence of the expressed sedative effect of the dry extract from the herbal tea under study in the dose of 400 mg/kg.

Table 2

The effect of the dry extract from the herbal tea on the indices of the open field test

Indices for 3 min	Control (n=10)	Piracetam, 400 mg/kg (n=10)	Dry extract, 200 mg/kg (n=9)	Dry extract, 400 mg/kg (n=9)
Locomotor activity (the number of squares)	53.7±7.7	65.0±9.9 (+21.0%)	51.3±4.4 (-4.5%)	29.3±10.3! (-45.4%)
Indicative exploration activity				
1. Rearing	2.4±0.9	6.6±1.8* (+175%)	5.9±1.6 (+146%)	2.6±1.6 (+13.0%)
2. "Looking" into the holes	27.3±5.0	36.9±4.5 (+35.2%)	40.9±4.9 (+50.0)	10.0±4.4*! (-63.4%)
3. The sum	29.7±5.6	43.5±3.5* (+46.5%)	46.8±5.2* (+57.6)	12.6±5.5*! (-57.6%)
Emotional reactions:				
1. Boluses	0.4±0.17	0.6±0.2	0.11±0.02	0*
2. Urination	0	0.2±0.1	0	0.1±0.1
3. Grooming	2.4±0.5	1.5±0.4	4.33±1.2	2.2±1.0
4. The sum	2.8±0.6	2.3±0.6	4.7±1.3	2.33±1.0
The sum of all activities	86.2±10.0	110.8±9.2 (+28.5%)	102.8±10.1 (+19.3)	44.2±16.59* (48.7%)

Note: * – significant changes in the control group ($p<0.05$); ! – significant changes in the piracetam group ($p<0.05$).

Table 3

The antihypoxic activity of the dry extract from the herbal tea on the model of normobaric hypoxic hypoxia with hypercapnia in mice

Group	Dose mg/kg	n	Survival time, min	% changes comparing to the control
Control	–	10	43.0±3.27	–
Piracetam	400	10	61.15±4.02*	+42.2
Dry extract	400	9	74.2±7.63*	+72.6

Note: * – significant changes in the control group ($p<0.05$).

It is manifested by inhibition of all types of behavioural activity: locomotor (the number of crossed squares), indicative exploration activity (rearing and "looking" into the holes) and their sum compared to the effects of piracetam, which caused statically significant intensification of manifestations of indicative exploration behaviour, first of all, rearing being a typical manifestation of cognitive functions enhancement for a nootropic drug. The sedative effect of the dry extract studied is confirmed by a clear tendency to the locomotor activity reducing with authentic decrease of the number of the holes explored, the sum of indices of exploration responses and reducing to 0 of the number of fecal boluses. The latter is inhibition of the vegetative support of emotional reactions that is typical for the sedative action.

When using in the dose of 200 mg/kg the dry extract affected the behaviour of animals in a different way: comparing to the control it increased the indicative exploration activity at the level of the reference drug piracetam without a considerable impact on the locomotor activity.

These results demonstrate the dose-dependent effect of the dry extract under study on the behavioural responses: stimulation of exploration responses is revealed in a lower dose, and with the dose increase the sedative action appears.

As can be seen from Tab. 3, the dry extract in the dose of 400 mg/kg increases more than one-half the survival time of the mice comparing to the control exceeding piracetam. It indicates a protective effect of the extract primarily on the brain – the organ that is most sensitive to hypoxia.

Therefore, the results of the pharmacological research suggest the presence of the sedative and antihypoxic activity proving the cerebroprotective action of the herbal medicinal product studied.

CONCLUSIONS

The chemical composition, the influence on the behaviour of animals in the open field test and the antihypoxic activity of the dry extract from the herbal tea composed of black horehound herb, white nettle herb, wormwood herb have been studied.

As a result of the chemical study of the main groups of biologically active substances in the herbal tea with the antioxidant effect and the extracts on its basis the presence of derivatives of hydroxycinnamic acid, hydroxycoumarins, phenolic compounds and flavonoids, as well as their quantitative content have been determined.

The dry extract on the basis of the herbal tea exhibits the sedative and antihypoxic activity confirming its cerebroprotective action.

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ДОСЛІДЖЕННЯ ХІМІЧНОГО СКЛАДУ, ВПЛИВУ НА ПОВЕДІНКОВІ РЕАКЦІЇ ТА АНТИГІПОКСИЧНОЇ АКТИВНОСТІ ФІТОЗБОРУ ТА СУХОГО ЕКСТРАКТУ НА ЙОГО ОСНОВІ

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Ключові слова: *м'яточник чорний; кропива глуха; полин гіркий; фітозбір; субстанція; поведінкові реакції; антигіпоксична активність*

Досліджено хімічний склад та фармакологічну активність сухого екстракту на основі збору, до складу якого входять трава м'яточника чорного, трава кропиви глухої та трава полину гіркого у рівних кількостях. В результаті хімічного вивчення основних груп біологічно активних речовин у зборі та екстракті зі збору встановлено наявність фенольних сполук: похідних гідроксикоричної кислоти, гідроксикумаринів, флавоноїдів, та їх кількісний вміст. В об'єктах дослідження були ідентифіковані аглікони флавоноїдів (лютеолін, кемпферол, кверцетин, апігенін). 70% етанол забезпечує більш повну екстракцію фенольних сполук зі збору: вміст гідроксикоричних кислот та флавоноїдів більший, ніж у настої збору понад 60% та понад 80% відповідно, вміст суми фенольних сполук у сухому екстракті більший, ніж у настої понад 10% (у перерахунку на сухий залишок). Результати фармакологічних досліджень свідчать про наявність седативного ефекту сухого екстракту зі збору в дозі 400 мг/кг. Сухий екстракт зі збору виявляє антигіпоксичну активність, перевершуючи пірацетам, що свідчить про церебропротекторну дію.

ИССЛЕДОВАНИЕ ХИМИЧЕСКОГО СОСТАВА, ВЛИЯНИЯ НА ПОВЕДЕНЧЕСКИЕ РЕАКЦИИ И АНТИГИПОКСИЧЕСКОЙ АКТИВНОСТИ ФИТОСБОРА И СУХОГО ЭКСТРАКТА НА ЕГО ОСНОВЕ

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Ключевые слова: *белокудренник черный; яснотка белая; полынь горькая; фитосбор; субстанция; поведенческие реакции; антигипоксическая активность*

Исследованы химический состав и фармакологическая активность сухого экстракта на основе сбора, в состав которого входят трава белокудренника чёрного, трава яснотки, трава полыни горькой в равных количествах. В результате химического изучения основных групп биологически активных веществ в сборе и экстракте на его основе установлено наличие фенольных соединений: производных гидроксикоричной кислоты, гидроксикумаринов, флавоноидов и их количественное содержание. В объектах исследования были идентифицированы агликоны флавоноидов (лютеолин, кемпферол, кверцетин, апигенин). 70% этанол обеспечивает более полную экстракцию фенольных соединений из сбора: содержание гидроксикоричных кислот и флавоноидов больше, чем в настое сбора свыше 60% и 80% соответственно, содержание суммы фенольных соединений в сухом экстракте больше, чем в настое свыше 10% (в пересчете на сухой остаток). Результаты фармакологических исследований свидетельствуют о наличии седативного эффекта сухого экстракта из сбора в дозе 400 мг/кг. Сухой экстракт из сбора проявляет антигипоксическую активность, по которой он превосходит пирацетам, что свидетельствует о церебропротекторном действии.

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СИНТЕЗ ТА АНАЛІЗ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН

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ЕКСПЕРИМЕНТАЛЬНА ТА КЛІНІЧНА ФАРМАКОЛОГІЯ

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